Transcription, translation and fragile X syndrome
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The fragile X mental retardation protein (FMRP) plays a role in the control of local protein synthesis in the dendrites. Loss of its production in fragile X syndrome is associated with transcriptional dysregulation of the gene. Recent work demonstrates that Sp1 and NRF1 transcriptionally control this gene. Other studies reveal how the microRNA pathway and signaling are related to FMRP function through the metabotropic glutamate receptor. These studies provide new insights through which we can better understand the inactivation of the FMR1 gene and, in turn, the consequence of FMRP loss.

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Introduction
The FMR1 (FRAGILE X MENTAL RETARDATION 1) gene is directly associated with three distinct diseases: fragile X syndrome, fragile X-associated tremor/ataxia syndrome, and premature ovarian failure [1,2] (see also review by D Toniolo [3], this issue). All are caused by FMR1 alleles with expanded CGG trinucleotide repeats in the 5ᵗʰ untranslated region. Although normal alleles contain, on average, 30 repeats, fragile X syndrome is caused by a massive expansion beyond 200 repeats (i.e. the full mutation), and both fragile X-associated tremor/ataxia syndrome and premature ovarian failure are associated with premutation alleles (i.e. 55–200 repeats). Both abnormal alleles result in transcriptional dysregulation of the FMR1 gene. Whereas fragile X syndrome is almost exclusively caused by a complete transcriptional shutdown of the gene, the premutation-associated diseases are caused by excess transcript levels leading to — at least for fragile X-associated tremor/ataxia syndrome — toxic effects of repeat-containing mRNA [1,4,5]. Given that mutations in FMR1 manifest two vastly different transcriptional defects, much work has gone into understanding the cis sequences and trans-acting proteins that normally influence this gene in addition to its chromatin structure.

Fragile X syndrome occurs in approximately 1 in 4000 males and in 1 in 8,000 females and presents as developmental delay around 36 months of age. Speech delay is frequent, along with behavior problems such as overactivity and anxiety. Many parallel phenotypes with autism are seen, such as gaze avoidance, stereotyped repetitive behavior, resistance to change in routines or environment, and preservation. Premutation males, and to a lesser degree females, can have fragile X-associated tremor ataxia syndrome with cerebellar tremor/ataxia, cognitive decline and generalized brain atrophy presenting beyond the fifth decade of life. Approximately 24% of premutation females also experience premature ovarian failure (i.e. cessation of menses at <40 years).

FMRP, the protein encoded by FMR1, is an RNA binding protein involved in the control of local protein synthesis. FMRP shuttles from the nucleus to the cytoplasm, where it associates with polyribosomes through large mRNP particles [6] and suppresses translation of a selective group of mRNAs to which it binds [7,8]. In vivo, the lack of Fmrp in mice is associated with elevations in the rates of protein synthesis in certain regions of the brain [9]. Various approaches have been taken to identify the mRNAs associated with FMRP and its homologs, and these mRNAs include, among others, MAP1B (MICROTUBULE-ASSOCIATED PROTEIN 1B), the FMR1 message itself, and others involved in neuronal development and plasticity [10–12]. FMRP recognizes two three-dimensional structures in the RNAs: an intramolecular G-quartet and an intricate tertiary structure termed an FMRP-kissing complex [11,13]. Interactions have also been discovered between FMRP and components of the microRNA pathway in addition to the microRNAs themselves, suggesting a mechanistic link in the regulation of protein synthesis [14–15]. Current theories suggest that FMRP is involved in the control of local translation within dendrites in response to synaptic activity, and that loss of FMRP results in defects in protein synthesis-dependent plasticity [16].

Two key aspects of FMR1 biology are in need of further mechanistic insight. One involves the FMR1 promoter and the process of transcriptional silencing in the full mutation and transcriptional enhancement in the premutation. The second is the precise function of FMRP in the
neuron and the neuronal consequence of its loss in fragile X syndrome.

In this review, we focus on recent advances in our understanding of the transcription of the FMR1 gene and the influence of FMRP on translation.

**FMR1 promoter function and chromatin structure**

Loss of transcription of FMR1 in fragile X syndrome is the best understood of the FMR1-related disease processes. Repeat expansion results in cytosine methylation of the repeats in addition to the CpG island in the promoter. It appears that the full mutation-bearing FMR1 is recognized as repeated DNA and subjected to ‘heterochromatinization’, much as is transposon or centromeric DNA. Interestingly, it has been shown that long CGG-repeat tracts, as RNA, are substrates for the binding of the RNase III enzyme Dicer [17]. This suggests a possible scenario whereby transient expression of the fully mutant FMR1 in the developing embryo results in microRNA-like CGG fragments that, in turn, recruit chromatin modification activities back to the FMR1 locus [18].

Indeed, commensurate with the shut down of the FMR1 full mutation is loss of acetylation of histone H3 and H4, a reduction in methylation of H3 (lysine 4), and a large increase in methylation of H3 (lysine 9) [19,20]. Acetylation of H3 is more tightly associated with FMR1 transcriptional activity than is acetylation of H4; the latter is clearly insufficient for transcription or an open chromatin configuration of FMR1 [19,20]. Pharmacological demethylation of FMR1 DNA results in a reversal of the histone modifications and a resumption of transcription [20,21]. Whether DNA methylation and histone methylation are directly linked, however, is an important, unresolved mechanistic question.

The change in the FMR1 local environment from transcriptionally active chromatin to inactive heterochromatin correlates with the loss of four in vivo footprints normally found on the active FMR1 promoter [22,23]. The four occupied sites in the promoter include a palindrome, two GC-like boxes, and an overlapping E-box–CRE (cAMP response element) site. Identification of the proteins residing at these sites, and their roles in FMR1 transcription have been addressed in several studies [22–27,28,29,30]. Perhaps the two most important factors are nuclear respiratory factor 1 (NRF1) and Sp1. NRF1 specifically binds to the GC-rich palindrome in vitro and in vivo and is a strong activator of FMR1 transcription [27,28]. The functionally related nuclear respiratory factor 2 (NRF2) also controls FMR1 expression [30]. NRF1 and NRF2 are best known for regulating transcription of nuclear genes involved in mitochondrial function [31]. However, recognition sites for both of these factors are also present in promoters of several genes encoding proteins that bind RNA or are associated with translation, including FMR1, eIF2-a, eIF2-b and hnRNP-A2 [22,30,32]. Therefore, FMR1 might fit into a potential NRF1- and NRF2-controlled regulon of RNA metabolism-related genes. This may be of particular importance in the brain, because NRF1 and NRF2 have suspected roles in neuronal function, and the tight control of translation is especially crucial in neurons [33–35].

Sp1 can bind to and activate 100-fold increased levels of FMR1 transcription from two recognition sites in the promoter, both in vitro and in vivo [24,26,28]. Sp1 activity is the strongest of the factors involved in FMR1 transcription and is the most resistant to DNA methylation [28]. Given that Sp1 can still activate transcription in the presence of DNA methylation, but that its binding is lost at the fragile X allele, complete silencing of FMR1 undoubtedly requires an additional event [20,28]. The presence of the methyl-CpG-binding protein, MeCP2, and the chromatin-remodeling protein Brahma might be one of the factors preventing Sp1’s occupancy on the FMR1 promoter in fragile X patient cells [36].

The identity of the transcription factor binding the overlapping E-box and CRE has raised some controversy [25,27]. In vivo studies reveal that both USF (upstream stimulatory factor) and CREB bind to the FMR1 promoter region; however, only CREB acts positively though the E-box–CRE site [28,30]. Regulation of CREB transcriptional activity through cAMP has been well studied in brain, particularly in regards to learning and memory [37]. Early work showed that a transfected FMR1-driven reporter could be activated by a cAMP agonist [25]. However, using the PC12 model system, Smith et al. [30] were unable to show induction of endogenous FMR1 in response to cAMP agonists under conditions in which c-fos was responsive.

These results suggest that transcriptional induction of FMR1 does not normally appear to regulate synaptic functioning in adult neurons in culture, at least not in response to cAMP or depolarization [30]. However, inducible regulation of FMR1 transcription during development cannot be excluded. In fact, a role for AP2 in controlling FMR1 expression during development has been suggested, but the signals that might stimulate FMR1 developmentally are unknown [29].

It may be that FMR1 does not need to be induced at the transcriptional level in adult neurons, because post-transcriptional events for FMR1 are responsive to certain stimuli. Treatment of primary neurons with KCl rapidly induces localization of both FMR1 mRNA and FMRP to dendrites [38]. In addition, FMRP is quickly translated within five minutes of glutamate receptor stimulation of neurons [39]. These stimulations can also activate CREB; however, it would take longer to make FMRP through a
transcriptional induction pathway than to directly increase FMRP levels by post-transcriptional processes [40]. Hence, mobilization of pre-existing FMR1 mRNA and protein, along with rapid local translation, may be more important for the neuronal function of FMRP than new FMR1 mRNA synthesis. In support of this hypothesis, FMRP levels increase in cortical neurons following stimulation by light exposure, but mRNA levels do not [41]. FMR1 mRNA may be produced in sufficient amounts on a continuous basis, supporting the dynamic control of FMRP expression in adults at or near the synapse through post-transcriptional processes. For this predominantly cytoplasmic protein, the time saved by not having to signal to the nucleus could be crucial for its neuronal function.

**FMRP and translational control**

Synaptic transmission occurs at the dendritic spines, and in individuals with fragile X syndrome these spines are abnormally long and appear to be immature [42,43]. This has led to the notion that FMRP is involved in synaptic maturation and spine-pruning. In the dendritic spines, long-term potentiation (LTP) and long-term depression (LTD) — two forms of synaptic plasticity — are triggered by synaptic activity through processes that require local protein synthesis [44]. The messages that are rapidly translated at the synapse in response to stimulation by metabotropic glutamate receptors (mGlurRs) — and which may, therefore, have a role in LTP and LTD — include FMR1 [38,45]. Supporting a role for FMRP in LTD is the fact that LTD triggered by mGlurRs is selectively enhanced in mice that do not express Fmrp [46,47]. Together with the evidence for FMRP’s role in suppression of protein translation, these findings led to the proposal that FMRP suppresses mGlur-stimulated local protein synthesis at the dendrites, and that mGlur-stimulated processes are exaggerated in fragile X syndrome [16]. This model is supported by the fact that treatment with mGlur antagonists rescues behavioral and neurological defects in Drosophila strains lacking the FMR1 homolog dfmr1, as well as in mice that have a disruption in Fmr1 [48,49].

More recent data suggest that, perhaps in the absence of FMRP, protein synthesis is not stimulated by mGlur, because protein synthesis is already enhanced to a maximal level and cannot be further stimulated through the receptor [54]. If this is the case, then prior accumulation of the proteins whose production would normally be stimulated by mGlur could circumvent the requirement for protein synthesis in mGlur-mediated LTD. Indeed, this is true in Fmr1 knockout mice; unlike in wild type mice, the presence of translation inhibitors in these mice does not prevent mGlur-mediated LTD [54]. Protein synthesis-independent LTD in Fmr1 knockout mice is not generated through an alternative pathway, because, as in wild type mice, mGlur-stimulated LTD is associated with decreases in cell surface expression of AMPA receptors, and this decrease in receptors also loses its protein synthesis-dependence in Fmr1 knockout mice [54]. Production of a different set of proteins or the use of a different pathway may be involved in other aspect of fragile X syndrome pathogenesis, however, because mGlur-stimulated protein synthesis is still required for the enhanced epileptiform bursts that are seen in hippocampal slices from Fmr1 knockout mice [55]. The development of prolonged epileptiform discharges in the mutant mouse is blocked by treatment with ERK1 and/or ERK2 (extracellular signal-regulated kinases) inhibitors, implicating ERK-containing signal transduction pathways in this process [55].

One factor as yet unknown is the mechanism by which FMRP activity is regulated. FMRP phosphorylation suppresses translation in FMRP-associated polyribosomes [56], mGlur5 stimulation is known to decrease the activity of the phosphatase PP2A [57]. One could speculate that PP2A is the phosphatase that acts on FMRP to release its translation-suppressing effect. The fact that FMRP binds to pp2acbd mRNA and represses its translation [58] suggests an interesting regulatory loop whereby FMRP could control its own activity by modulating levels of its activating phosphatase.

**FMRP and miRNAs work together to inhibit translation**

MicroRNAs (miRNAs) are a class of non-coding RNAs that control translation of their target mRNAs by basepairing with partially complementary transcript sequences [59]. miRNAs use the RNA-induced silencing complex (RISC) to effect their function. FMRP interacts with the Argonaute proteinsAGO1 and AGO2, which are components of RISC, and with miRNAs [14,15,60], so it has been proposed that the translational suppression associated with FMRP occurs through miRNAs. In support of this idea, AGO1 was shown to be crucial for the effects of dfmr1 on synaptic plasticity [15].

New reports provide further detail on the regulation of synaptic protein synthesis by RISC and FMRP. This
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regulation includes an mRNA transport component and a translational silencing component, as was shown for the synthesis of the calcium–calmodulin-dependent kinase II (CaMKII), a protein required for memory formation [61]. Upon neural stimulation, CaMKII mRNA is transported to the dendrites in a process that requires protein synthesis and sequences in the 3' UTR of CaMKII. Within this 3’ UTR are predicted binding sites for the miRNAs miR-280 and miR-289. RISC inhibits translation of the CaMKII message, and this inhibition is relieved upon neural stimulation, at which time components of RISC, such as Armitage, are degraded by the proteasome. Armitage further controls expression of Stau1 and the kinesin heavy chain (Khc), which, along with the CaMKII mRNA and FMRP, are found in RNA transport granules [62].

Further support for the role of miRNAs in dendritic spine development comes through the finding that miR-134 inhibits translation of Lim-domain-containing protein kinase 1 (LimK1), which has a role in dendritic spine structure [63]. Stimulation of rat hippocampal neurons using brain-derived neurotrophic factor (BDNF) relieves the inhibition of LimK1 translation, thereby promoting dendritic spine growth. As in the previous experiments, these authors also believe that the miRNA maintains mRNAs in a translationally suppressed state during transport of the messages to the dendrites within granules. Once there, they are ready to be translated in an activity-dependent manner, as is required for synaptic plasticity.

FMRP is another factor that has a role in maintaining translational suppression during RNA transport. The level of granules in the brains of Fmr1 knockout mice is reduced; although granules can form in the absence of FMRP, their disassembly in response to mGluR stimulation is exaggerated [64]. Stimulation of mGluRs increases the transport of FMRP-containing granules to the dendrites [65]. This stimulation also increases the level of Fmr1 mRNA and reduces the level of FMRP at synapses, perhaps reflecting the process of granule disassembly [65].

Current model of FMRP function

Synthesizing the current data on translational suppression by FMRP, we propose a model in which FMRP is transported into the nucleus, where it associates with specific RNA transcripts and forms messenger ribonucleoprotein complexes. These complexes are transported out of the nucleus, enabling them to interact with components of the RISC complex, thereby inhibiting the translation of the messages therein. Using kinesin as its motor, these translationally silent complexes can be transported to the dendrites as granules. Stimulation of neurons increases the transport of messages to the dendrites and it also activates the proteasome to degrade RISC, thus enabling rapid translation of the messages that were contained therein. The disassembly of granules in response to mGluR stimulation could perhaps reflect the degradation of RISC components, whereas the reduction of FMRP at the synapse could reflect the recycling of some granule components back to the cell body for further use. In the absence of FMRP, the cell lacks the opposing effect to mGluR stimulation, and this leads to increased levels of the proteins normally stimulated by this system. These increased protein levels result in the abnormal dendritic spine morphology observed in people and animal models that lack FMRP.

Conclusions

We now understand in detail the consequence of repeat-mediated transcriptional shut-off of FMR1, and this knowledge, especially in comparison with transcriptional upregulation of premutation-sized repeats at this locus, will add to our basic understanding of gene regulation. Because this transcriptional shut-off causes fragile X syndrome through the loss of a single protein, it places FMRP in a central role for learning and memory. Synaptic plasticity requires tightly controlled and highly orchestrated protein synthesis that can occur immediately upon stimulation of a synapse. It is becoming clear that FMRP is a crucial component of this process and is involved in the transport of the appropriate messages to the synapse and in holding them in their silent state until the precise time that they are needed. As is abundantly clear from individuals with fragile X syndrome, the lack of this protein has severe ramifications for the development and function of the brain.

Acknowledgements

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- outstanding interest


The authors demonstrate biochemical interactions among human FMRP, mRNAs and elf2C, a member of the Argonaute family of proteins. They also use a Drosophila model to demonstrate a genetic interaction between dmar1 and the Argonaute gene AGO1.


This is the first direct demonstration of specific transcription factors residing at and regulating FMR1 in vivo and shows that DNA methylation alone is insufficient for complete transcription factor loss at FMR1.


The authors show that the methyl-CpG-binding protein McP2 and the chromatin remodeling protein Brahma reside at expanded, silenced FMR1 and that abrogation of these proteins in cell culture can reactivate FMR1 transcription.


Using a mouse model of fragile X syndrome, the authors show that protein synthesis is no longer required for mGluR-dependent LTD. This supports the theory that FMRP opposes the action of mGluR and that, in its absence, proteins required for mGluR dependent LTD are translated in the absence of receptor signaling.


mRNA transport and synaptic protein synthesis are shown in a Drosophila model to be regulated by components of the RISC pathway. The patterns of synaptic protein synthesis required for memory formation are under the control of RISC, which uses a post-transcriptional mechanism to control gene expression. Degradation by the proteasome of the RISC component Armitage releases this repression. This work ties the mRNA pathway more intimately to control of local protein synthesis in neurons.


A miRNA, miR134, is shown to inhibit translation of a Limk1, a protein that controls spine development. Signaling through BDNF relieves this inhibition. This report links translational control through the miRNA pathway more intimately to control of local protein synthesis in neurons.
