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The MicroRNA Pathway and Fragile X Mental Retardation Protein

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Abstract

Fragile X syndrome, one of the most common forms of inherited mental retardation, is caused by the functional loss of fragile X mental retardation protein (FMRP). MicroRNAs (miRNAs), a newly discovered class of small noncoding RNAs, have been implicated in multiple biological processes through posttranscriptional gene regulation. Recent evidence supports this view in terms of the biochemical and genetic interaction found between FMRP and the miRNA pathway, providing deeper insight into the molecular pathogenesis of mental retardation. This review briefly summarizes the progress towards an understanding of the role miRNAs play in neurological disorders, with a focus on the mechanism of interaction between FMRP and the miRNA pathway in the context of fragile X syndrome. In addition, we go on to discuss how the miRNA pathway may be involved in mental retardation.

Keywords

microRNA pathway; mental retardation; fragile X syndrome; fragile X mental retardation protein (FMRP); synaptic plasticity

Fragile X syndrome is one of the most common forms of inherited mental retardation, with an estimated prevalence of approximately 1 in 4000 males and 1 in 8000 females [1]. The syndrome is transmitted as an X-linked dominant trait with reduced penetrance (80% in males and 30% in females) [1,2]. The clinical presentations of fragile X syndrome include mild to severe mental retardation, with IQs between 20 and 70, mild abnormal facial features that include a prominent jaw and large ears, mainly in males, and macroorchidism in postpubescent males [2]. In 1991, the gene responsible for fragile X syndrome, *Fragile X Mental Retardation 1* (*FMR1*), was identified by positional cloning [3–5].

FMR1 is a highly conserved gene that consists of 17 exons spanning around 38 kilobases (kb) to Xq27.3 [6]. Within the 4.4 kb of *FMR1* transcript containing a 190-bp 5' untranslated region (5'UTR) and a 2281-bp 3'UTR, a CGG trinucleotide repeat is located in the 5'UTR [7]. Among normal individuals, this CGG repeat is highly polymorphic in length and content and is often interrupted by AGG trinucleotides. The normal repeat size ranges from 7 to 40, with 30 repeats found on the most common allele [8]. A massive CGG repeat expansion has been associated with fragile X syndrome [8]. In most affected fragile X individuals, the CGG repeats expand to more than 230 (full mutation) and become abnormally hypermethylated, which results in

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Abbreviations: FMR1, FMRP, FXTAS, miRNA, mRNA, mRNP, pre-miRNA, pri-miRNA, RISC.

silencing of the *Fmr1* gene. Identification of other mutations, such as deletions and point mutations among patients with typical fragile X phenotypes, strongly suggests that *FMR1* is the only gene involved in the pathogenesis of fragile X syndrome and that the functional loss of fragile X mental retardation protein (FMRP) is the cause of fragile X syndrome [1]. Alleles with between 60 and 230 CGG repeats are called premutation alleles, and they are generally unmethylated. It has recently been appreciated that some premutation carriers develop fragile X-associated tremor and ataxia syndrome (FXTAS), a progressive neurodegenerative disease [9]. FXTAS is not due to the alteration of FMRP function, but rather is caused by the excess of *FMR1* mRNAs containing long CGG repeats [9]. The elevated *FMR1* mRNA level in premutation carriers is not the consequence of increased stability of the mRNAs but due to increased transcription [10]. *FMR1* mRNA with lengthy CGG repeats has been suggested to cause the toxicity responsible for FXTAS [11]. The possible mechanism of the toxicity is the rCGG RNA-mediated sequestration of the rCGG-repeat-binding proteins (RBPs), such as Pur α and hnRNP A2/B1, leading to cell death evidenced by the fact that overexpression of these RBPs could suppress the rCGG-mediated neurodegeneration in fly models [12,13].

FMRP, as an RNA-binding protein, has been implicated in mRNA transport and translational control [1]. Recently, FMRP has also been linked to the microRNA pathway that is involved in translational suppression. Here we will review the current knowledge on the roles of the microRNA pathway in neural development and discuss the involvement of the microRNA pathway in the pathogenesis of fragile X syndrome and mental retardation more generally.

MicroRNA biogenesis

MicroRNAs (miRNAs) are a class of endogenous noncoding RNAs 18–25 nucleotides (nt) in length [14]. Three RNA processing events lead to the production of the mature form of miRNAs [15,16]. The enzymes and accessory elements involved in the biosynthesis are well conserved from plants to animals. RNA polymerase II is usually responsible for the transcription of miRNA genes to generate primary miRNAs (pri-miRNAs) (Figure 1). The RNase III endonuclease Drosha, along with its partner DGCR8, cleaves the pri-miRNAs to generate precursors (pre-miRNAs) with a short hairpin structure and a length of 60–75 nt. Unlike the first two steps, which occur in the nucleus, the final processing step is in the cytoplasm, after the pre-miRNAs are transported out of the nucleus by Exportin 5 (Figure 1). In cytoplasm, pre-miRNA is further processed by Dicer, the other RNase III enzyme involved in the miRNA pathway, to produce mature miRNA duplexes. Mature miRNAs are then loaded onto the effector complex or the RNA-induced silencing complex (RISC), which function as negative posttranscriptional regulators of gene expression in plants and animals through guide strand-directed, sequence-specific mRNA cleavage or through translational repression by the AGO protein family, or both (reviewed in [17,18]).

MiRNAs in neural development and human diseases

MiRNAs are known to be involved in diverse biological pathways, including development timing, differentiation, apoptosis, myogenesis, and glucose homeostasis [19]. MiRNAs are predicted to potentially regulate the expression of a large number of protein-coding genes [20]. Among more than 3800 unique mature miRNAs listed in the miRNA database of the Sanger Center, 462 are of human origin and a large number of these are present in the brain at different levels [21–23]. About 20–40% of these miRNAs in the brain appear to be developmentally regulated. Many of these small miRNAs expressed in the mammalian brain seem to be critical for dictating neuronal cell identity during development and to play a pivotal role in neurite growth, synaptic development, neuronal plasticity, and possibly in learning and memory [24]. Some miRNAs (such as miR-124 and miR-128) are preferentially expressed in neurons, some (such as miR-23, miR-26, and miR-29) are restricted to or are more strongly

expressed in astrocytes, and some (such as miR-9 and miR-125) are evenly distributed between these cell types [25]. Another study has reported that 450 distinct miRNAs could be detected with specific or enriched expression in mammalian brain, which account for ~70% of experimentally detectable miRNAs [17]. Spatial and temporal expression of these miRNAs should play significant roles in neural development.

In recent years, a growing number of studies have focused on the link between miRNAs and neurological disorders. Abeliovich and colleagues demonstrated that the specific expression of miR-133b in midbrain dopaminergic neurons (DNs) plays an important role in the negative feedback circuit to regulate the maturation and function of midbrain DN. Interestingly, the expression of miR-133b is altered in patients with Parkinson's disease [26]. Furthermore, a recent genetic study has revealed that variation in the miR-433 binding site of the *FGF20* gene could raise the risk of Parkinson's disease via overexpression of α -Synuclein [27]. In addition, the loss of Dicer in fly and human cells strikingly enhances polyglutamine (polyQ) toxicity, suggesting an important role for the miRNA pathway in the modulation of polyQ-induced neurodegeneration [28]. More recently, Nelson and his colleagues have shown that the expression of miR-107 decreases in Alzheimer's disease and could accelerate disease progression through regulation of beta-site amyloid precursor protein-cleaving enzyme 1 (BACE1) [29]. These observations together suggest that miRNAs could play roles in the disease pathogenesis of multiple neurological disorders.

MiRNA pathway in learning and memory

The link between the miRNA pathway and learning and memory has been established in both fly and mammals [17,30–32]. MiRNAs could modulate the activities of learning and memory by participating in the regulation of local protein synthesis. Flies trained by olfactory learning show increased transport of calcium-calmodulin-dependent kinase II (CamKII) mRNA to dendrites, as well as CamKII protein synthesis [31]. This localization and translational regulation are mediated by the 3' UTR of CamKII mRNA, which harbors several predicted binding sites for multiple miRNAs [31]. Mutations of the RISC components lead to dramatically increased CamKII mRNA transport and protein synthesis [31]. In addition, synaptic activity is closely associated with proteasome-mediated destruction of RISC. In this model, synaptic activity provides a signal to destroy RISC, and these mRNAs are subsequently used for local protein synthesis [30]. Several specific mammalian miRNAs have also proved to be involved in the regulation of local protein synthesis. For example, the brain-specific miRNA, miR-134, is localized to the synaptodendritic compartment of rat hippocampal neurons and negatively regulates the size of dendritic spines via inhibition of the translation of an mRNA encoding a protein kinase, *Limk1*, that controls spine development [32]. Similarly, miR-132 was found to target to and repress P250GAP, a Rho/Rac regulator [33]. The activities of both miR-132 and miR-134 were regulated in response to synaptic activity. Similarly, miR-125b and its homolog, miR-125a, concordantly play important roles in mammalian neuronal differentiation through downregulation of their target *lin-28* mRNA [34]. Given that the control of local protein synthesis in mammalian dendrites is essential for the synaptic plasticity phenomena (e.g., long-term potentiation, LTP) that contribute to the molecular basis of learning and memory formation, miRNAs could participate in learning and memory by modulating local translation in response to changes in synaptic activity, which would in turn regulate synaptic strength and the growth of spines. Indeed, the presence of abnormal dendritic spines is a hallmark of mental retardation.

FMRP and Fragile X Syndrome

Understanding the molecular pathogenesis of fragile X syndrome, one of the most common inherited forms of mental retardation, has yielded insight into the molecular basis of mental

retardation in general. FMRP and its autosomal paralogs, the *Fragile X-Related* proteins *FXR1P* and *FXR2P*, make up a small family of RNA-binding proteins (the fragile X-related gene family) [35,36]. These proteins share over 60% amino acid identity and contain two types of RNA-binding motif: two ribonucleoprotein K homology domains (KH domains) and a cluster of arginine and glycine residues (RGG box). FMRP is an RNA-binding protein associated with polyribosomes in an RNA-dependent manner via messenger ribonucleoprotein (mRNP) particles [37,38]. FMRP is involved in translational control and could suppress translation both *in vitro* and *in vivo* [39,40]. FMRP also contains a functional nuclear localization signal (NLS) and a nuclear export signal (NES) and has been found to shuttle between the nucleus and cytoplasm [41]. The phosphorylation status of FMRP can also regulate FMRP itself activity [42]. At the cellular level, abnormal dendritic spines were found in the brains of both human patients with fragile X syndrome and *Fmr1* knockout (KO) mice, implying that synaptic plasticity is affected in the absence of FMRP [43,44]. Further, FMRP was found to be associated with polyribosomes in the synapses of wild-type neuron [45]. Based on these observations, the current working model is that FMRP is involved in synaptic plasticity via regulation of mRNA transport and local protein synthesis at synapses.

In 2002, two groups independently reported the possible link between FMRP and the RNA interference (RNAi) pathway in *Drosophila* [46,47]. In efforts to identify the protein components of both Fmrp-containing complexes and the RISC in *Drosophila*, *Drosophila Fmrp* was found to be a component of the RISC [46,47]. Subsequent studies showed that FMRP interacts with both Argonaute proteins and Dicer in mammals [48]. Further studies in both mammals and flies demonstrated that FMRP is associated with endogenous miRNAs. Considering the model whereby FMRP initially selects its targets through low-affinity interactions with G-quartets and/or kissing complexes, it seems plausible to speculate that FMRP may function as a precise translational repressor with the aid of the associated miRNAs [48–50].

In addition to the biochemical evidence, genetic interactions have been demonstrated between *dFmr1* and components of the miRNA pathway, including *dAGO1* and *dAGO2* [48,51]. It was found that *AGO1* is required for *dFmr1*-mediated regulation of synaptic plasticity. Moreover, partial loss of *AGO1* could suppress the neuronal apoptosis caused by the overexpression of *dFmr1* [48]. Together these data suggest that *AGO1* is critical for the biological functions of FMRP in neural development and synaptogenesis [48]. *dFmr1* has also been found to interact genetically with *AGO2*, and the *ppk1* mRNA level appears to be regulated by *dFmr1* and *AGO2* [51]. Recent studies provide further evidence of the involvement of Fmrp in miRNA-containing RISC and P body-like granule in *Drosophila* neurons [52]. The protein identities of the Staufen- and Fmrp-containing RNPs in *Drosophila* neurons include Dcp1p and Xrn1p/Pacman (RNA degradation pathway), Argonaute (miRNA pathway), NMD (Upf1p) (nonsense-mediated decay), and Me31B/Dhh1p (general translational repression pathway). Among these protein components, Me31B is shown to be involved in translational repression together with an Fmrp-associated P-body protein (Scd6p/trailer hitch) in an FMRP-driven and Argonaute-dependent manner in developing eye imaginal discs. In addition, Me31B protein functions as a translational repressor that is mediated by *bantam* miRNA together with Fmrp and Scd6p/trailer hitch in wing imaginal discs. These results strongly suggest that the miRNA pathway is involved in regulation mediated by FMRP.

FMRP belongs to a small family of RNA-binding proteins, which also includes FXR1P and FXR2P. These three proteins have also been associated with each other biochemically and were believed to be involved in translational suppression together. Surprisingly, in a recent studies, FXR1P together with AGO2, factors associated with miRNPs, are shown to be recruited by AU-rich elements (AREs) in 3'UTR of cytokine mRNAs, such as TNF α , contributing significantly to translation upregulation of these mRNAs upon cell cycle arrest

[53,54]. Further studies indicate that two with flanks of the three AUUA motifs in ARE is responsible for recruiting FXR1P and AGO2 via bas -pairing with seed region of miR369-3, which is required for miRNA-dependent translation activation under cell cycle arrest conditions [54]. Thus, a novel role for miRNAs and versatile miRNP function has been proposed in response to cell cycle. Switch of AGO2 from repressor to activator may, at least, partially alter its interactions with FXR1P as well as expression levels of FXR1P, maintaining the oscillation between translational repression and activation mediated by miRNAs in coordination with cell cycle [54]. It remains to be seen how widespread this phenomenon is, and whether FMRP or FXR2P also participate in this process.

Concluding Remarks

Studies have clearly demonstrated that the miRNA pathway is involved in the regulation of synaptogenesis. Given that the interaction between FMRP and the miRNA pathway contributes to translational regulation of target mRNAs, particularly at dendrites, it is logical to wonder whether alterations in miRNA-mediated gene regulation could contribute to the development of mental retardation in general. It would be interesting and important to ascertain whether FMRP only uses the miRNA pathway to more precisely regulate the translation of its target mRNAs, or whether it functions as an indispensable component of the miRNA pathway in general. In addition, several questions remain to be answered before we can fully understand the FMRP-miRNA pathway interaction, including: 1) How does FMRP interact with the miRNA pathway *in vivo*, particularly in mammals; 2) Are there any other cellular proteins and/or cofactors that facilitate the interaction between FMRP and the miRNA pathway; and 3) Does phosphorylation status affect FMRP to relieve miRNA-guided mRNA translation repression and, if so, what are the mechanisms and what signal pathway is involved (phosphorylation/dephosphorylation) (Figure 1). Finally, it would be intriguing to investigate the potential involvement of the miRNA pathway in other types of inherited mental retardation, such as Rett syndrome and Down syndrome.

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Figure 1. Proposed model for FMRP-mediated translational regulation of its mRNA targets via the miRNA pathway

The nascent pri-miRNAs transcribed from miRNA genes are first processed into pre-miRNAs by Drosha together with DGCR8 inside the nucleus. After being exported into the cytoplasm by Exportin 5, the pre-miRNAs are further processed into the mature form of miRNA:miRNA* duplex by Dicer. The guide strand of the miRNA:miRNA* duplex is assembled into miRNP, which contains FMRP and subsequently functions as a translational repressor. Through the affinity recognition of G-quartet structures by FMRP, the FMRP-bound mRNAs may involve the concerted assembly of the miRNAs in RISC onto their binding sites, possibly under phosphorylated conditions. Thus, the precise translational suppression is carried out with the aid of the enhanced assembly of the target mRNAs and the associated miRNAs in RISC. The translational suppression is reversible, possibly under dephosphorylation conditions, through disassociation of target mRNAs from RISC. The disassociated mRNAs could then be actively translated. The potential involvement of phosphorylation in FMRP-mediated translational regulation of its mRNA targets via the miRNA pathway was proposed previously [49,55].