MENTAL RETARDATION AND DEVELOPMENTAL DISABILITIES
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RNA Interference: A New Mechanism by which FMRP Acts in the Normal Brain? What Can Drosophila Teach Us?

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Fragile X syndrome is the most common heritable form of mental retardation caused by loss-of-function mutations in the *FMR1* gene. The *FMR1* gene encodes an RNA-binding protein that associates with translating ribosomes and acts as a negative translational regulator. Recent work in *Drosophila melanogaster* has shown that the fly homolog of *FMR1* (*dFMR1*) plays an important role in regulating neuronal morphology, which may underlie the observed deficits in behaviors of *dFMR1* mutant flies. Biochemical analysis has revealed that dFMR1 forms a complex that includes ribosomal proteins and, surprisingly, Argonaute2 (AGO2), an essential component of the RNA-induced silencing complex (RISC) that mediates RNA interference (RNAi) in *Drosophila*. dFMR1 also associates with Dicer, another essential processing enzyme of the RNAi pathway. Moreover, both a micro-RNA (miRNA) and short interfering RNAs (siRNAs) can coimmunoprecipitate with dFMR1. Together these findings suggest that dFMR1 functions in an RNAi-related apparatus to regulate the expression of its target genes at the level of translation. These findings raise the possibility that Fragile X syndrome may be the result of a protein synthesis abnormality caused by a defect in an RNAi-related apparatus. Because the core mechanisms of complex behaviors such as learning and memory and circadian rhythms appear to be conserved, studies of Fragile X syndrome using *Drosophila* as a model provide an economy-of-scale for identifying biological processes that likely underlie the abnormal morphology of dendritic spines and behavioral disturbances observed in Fragile X patients.
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Key Words: FMR1; *Drosophila;* RNAi; miRNA; translational repression

In most cases, fragile X syndrome is caused by a trinucleotide repeat expansion in the 5′ untranslated region of the fragile X mental retardation 1 gene (*FMR1*) [Imbert et al., 1998; O'Donnell and Warren, 2002]. An expansion of the CGG repeat is associated with abnormal DNA methylation of both a nearby CpG island and the repeat itself. As a result, the *FMR1* locus becomes silent at the transcriptional level and thus no translation occurs [Verheij et al., 1993; Siomi et al., 1993]. It is clear that fragile X syndrome results from the lack of FMR1 expression. In other words, the cause of the disease is a loss-of-function of *FMR1*. This has been confirmed by the Dutch–Belgian Consortium [1994], which generated mouse models by the targeted disruption (knockout) of the mouse *Fmr1* gene. The *Fmr1* knockout mouse reproduces a subtle phenotype reminiscent of the human phenotype [Dutch–Belgian Consortium, 1994]. It is therefore clear that the pathophysiological mechanisms leading to the symptoms in fragile X syndrome can be elucidated by studying the function of the *FMR1* gene.

Although this disease is caused by the expansion of a triplet repeat, it is not associated with neurodegeneration, as is often the case for other "triplet repeat" diseases. Rather, the pathology of brains of fragile X patients and *Fmr1* knockout mice show the presence of abnormal dendritic spines reminiscent of a maturation delay [Greenough et al., 2001; Irwin et al., 2001; see Beckel-Mitchener and Greenough, this issue]. Because spines are specialized regions where dendrites receive synaptic input from other neurons, abnormal dendritic spines observed in fragile X patients may be the basis of their mental retardation. A link between abnormal dendritic spines (dendritic spine "dysgenesis") and mental retardation has been suggested for many other mental retardation disorders, including trisomy 21 (Down syndrome) [Purpura, 1974; Nimchinsky et al., 2002]. Because the FMR1 protein (FMRP) is a cytoplasmic RNA-binding protein that associates with polyribosomes as part of large ribonucleoprotein complexes (mRNP), it is suspected that it participates in the translational regulation of target mRNAs in a manner that is critical for the correct development of neurons [Inoue et al., 2000; O'Donnell and Warren 2002]. Indeed, biochemical studies suggest that FMRP acts as a negative regulator of translation [Laggerbauer et al., 2000; Li et al., 2000; Schaeffer et al., 2001]. These observations suggest that by modulating RNA translation, and consequently protein synthesis, FMRP is important for the formation and function of synapses. Recently, a number of mammalian FMRP mRNA targets have been identified [Schaeffer et al., 2001; Brown et al., 2001; Darnell et al., 2001; Miyashiro et al., 2003; see Darnell, Warren, and Darnell, this issue]. Interestingly, a number of these RNA molecules have peculiar RNA sequences capable of adopting a unique secondary structure forming G-quartets and code for important neuronal proteins [Schaeffer et al., 2001; Darnell et al., 2001; Brown et al., 2001]. However, to what extent and how FMRP might affect translation in vivo is unknown. Fur-

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thermore, the discovery of the existence of two FMR1-related genes, *FXR1* and *FXR2*, has revealed an additional level of complexity in the study of FMR1 functions in vertebrates [Siomi et al., 1995; Zhang et al., 1995]. For many human disorders, redundancy of gene function due to gene duplication in the same species makes it difficult to interpret clearly the effects of loss-of-function mutations on cellular physiology and biochemistry.

DROSOPHILA AS A MODEL FOR FRAGILE X SYNDROME

With forward genetics or reverse genetics, the fruit fly *D. melanogaster* has proven to be a powerful tool for the genetic dissection of biochemical pathways. Many genes are conserved between flies and humans, including genes that regulate complex behaviors such as learning and memory and circadian rhythms, as well as entire pathways of development and oncogenesis [Patel, 1994; Miklos and Rubin, 1996; Rubin et al., 2000]. Although the fly's nervous system differs vastly from ours, it does work via neurons, synapses, and transmitter molecules, and accumulating molecular biological evidence suggests a staggering level of evolutionary conservation, although there are, of course, variations in detail [Kammermeier and Reichert, 2001; Cayouette and Raff, 2002]. In addition, due to its small size, yet behavioral complexity, the brain of the fruit fly is largely accepted as an important model for brain function. *Drosophila* could be used, therefore, to define the molecular pathways leading to human neurological diseases and to identify the genes involved [Fortini and Bonini, 2000; Muqit and Feany, 2002]. Conversely, studies on human diseases using *Drosophila* as a model offer new insights into normal developmental processes. In fact, *Drosophila* has revealed much about the mysteries of development and provided hints about the elements directing complex behavior [Waddell and Quinn, 2001; Dubnau and Tully, 1998; Dunlap, 1999; Hall, 1994]. Furthermore, building on years of experience, a number of fly researchers are also using *Drosophila* as a model for designing new therapeutic strategies for treating human diseases [Steffan et al., 2001; Kazantsev et al., 2002; Auluck et al., 2002].

The *Drosophila* genome contains a single gene that is homologous to *FMR1*, the *Drosophila FMR1 related gene* (*dFMR1*, also referred to as *dfxr*) [Wan et al., 2000]. *Drosophila* and vertebrate FMR1 proteins share a number of topographical landmarks [Wan et al., 2000], including two types of RNA-binding motifs, namely two KH domains and an RGG box. Moreover, they show similar biochemical properties, such as RNA binding and ribosome association [Wan et al., 2000], and similar expression patterns with high levels of expression in neurons [Zhang et al., 2001; Dockendorff et al., 2002; Morales et al., 2002]. dFMR1 also has a high affinity for G-rich RNA sequences (MC Siomi and H Siomi, unpublished results). Importantly, genetic studies have demonstrated that dFMR1 has a role in the regulation of neuronal morphology and functions [Zhang et al., 2001; Dockendorff et al., 2002; Morales et al., 2002; Inoue et al., 2002]. Examining the role of FMRP in the fruit fly is, therefore, a promising approach for providing significant insights into the function of FMRP.

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PHENOTYPE

Fragile X patients exhibit elongated dendritic spines, a higher density of spines along the dendrites, and a more immature morphology compared with a matched sample of unaffected individuals [Greenough et al., 2001; Irwin et al., 2001]. *Fmr1* knockout mice also show an increase in spine density and spine length on pyramidal cell dendrites compared with wild-type mice [Kooy, 2003]. These findings suggest an impairment of spine maturation and pruning in fragile X patients [Greenough et al., 2001; Irwin et al., 2001].

Recently, several groups have independently generated *dFMR1* null alleles. *dFMR1* null mutants proceed into adulthood and appear to be morphologically normal in external appearance. However, *dFMR1* has a role in the regulation of neuronal morphology and function as is the case for mammalian *FMR1*. Zhang et al. [2001] found that *dFMR1* null mutant flies display enlarged synaptic terminals at the neuromuscular junction (NMJ). In addition, the loss of *dFMR1* resulted in an altered neurotransmission with synapse type-specific regulation in the central and peripheral synapses. This finding suggests that dFMR1 is required for the differential regulation of synaptic neurotransmission. Interestingly, *dFMR1* mutations caused elevated spontaneous vesicle fusion at the NMJ, suggesting that the role of *dFMR1* is primarily presynaptic, mediating synaptic vesicle fusion probability. Because spontaneous neurotransmitter release is crucial for organizing sites of neuronal communication in the developing fly NMJ, but probably also at other synapses [Saitoe et al., 2001; Broadie and Richmond, 2002], the possible role of *dFMR1* in synaptic vesicle fusion may underlie the observed deficits in the neuronal morphology of *dFMR1* mutant flies. Because the role of FMR1 in mammals appears to be primarily postsynaptic as judged by abnormal dendritic spines observed in fragile X patients and *Fmr1* knockout mice, the presynaptic deficits observed in *dFMR1* mutant flies suggest that the cellular functions to which FMR1 contribute may change through evolution, although the molecular mechanisms may be conserved. Zhang et al. [2001] also found that, taking a clue from the considerable similarity in phenotype between the *dFMR1* mutants and *futsch* mutants, the introduction of the *futsch* mutation rescued the structural and functional defects at the NMJ observed in *dFMR1* mutants. Together with their finding that *futsch* mRNA is associated with dFMR1, and that Futsch expression is higher in *dFMR1* mutants, these results suggest that dFMR1 regulates the synaptic structure and function, probably by acting as a translational repressor of an mRNA encoding Futsch, which is the fly homolog of the microtubule-associated protein MAP1B [Zhang et al., 2001].

Dockendorff et al. [2002] and Morales et al. [2002] examined neurons in the brain of *dFMR1* mutants and found abnormal axon branching and an overgrowth of terminal arborizations in dorsal cluster (DC) neurons and ventral lateral neurons (LNv), but not in other neurons. This suggests that different neuronal subtypes are affected differentially by the loss of *dFMR1*. Although observations of ax-

onal defects in *dFMR1* mutants suggest a possible local function for dFMR1 in the regulation of axonal outgrowth and/or presynaptic structure, it remains to be determined whether dendritic (postsynaptic) abnormalities are found in *dFMR1* mutants such as in fragile X patients and *Fmr1* knockout mice. Most multiple dendritic (MD) neurons in the peripheral nervous system in *Drosophila* larvae develop highly diverse dendritic branching patterns with spinelike structures and share morphological similarities with dendrites of the mammalian central nervous system (CNS) [Sweeney et al., 2002]. These neurons thus could be used as a model system for addressing this issue.

Although we state early in this article that dFMR1 is homologous to FMR1, the fact that the role of dFMR1 appears to be primarily presynaptic may also cast this in a new light. The amino acid sequence of dFMR1 is equally similar, if not more so, to FXR1 and FXR2. The loss of FMR1 in fragile X patients cannot be compensated by the presence of FXR1 and FXR2. Therefore, there is a functionally unique region in FMR1 or the protein is localized to an exclusive region in the cell. In this regard, it will be particularly interesting to ask the question of which one of the human *FMR1/FXR* family genes can ameliorate or even rescue neuronal morphology and/or behavioral abnormalities (see below) in *dFMR1*-null flies.

BEHAVIOR

Fragile X patients are known to exhibit a more or less specific complex of behavioral disturbances [Hagerman, 2002]. These include social and attention deficits, autisticlike behaviors, unusual responses to sensory stimuli, hyperactivity, and abnormal sleep patterns. *Fmr1* knockout mice show mild but consistent abnormalities, analogous to the clinical symptoms observed in human patients [Kooy, 2003].

Although in many behavioral tests the *dFMR1* mutant flies did not differ from wild-type flies, three research groups found independently that a loss of *dFMR1* affects circadian behavior [Dockendorff et al., 2002; Morales et al., 2002; Inoue et al., 2002]. In a 24-h light:dark (LD) cycle, wild-type flies are entrained to (or synchronized with) LD cycling and exhibit a substantial locomotor activity rise during the second half of the day. Rhythmic behavior persists when wild-type flies proceed from LD into constant darkness (DD). *dFMR1* mutant flies were well entrained during LD cycles and exhibited normal ~24-h periodicity under such conditions. However, the locomotor behavior of *dFMR1* mutant flies in DD was arrhythmic. The arrhythmic locomotor activity phenotype in DD could be rescued by the introduction of a genomic fragment containing the wild-type *dFMR1* gene, demonstrating that the disruption of the *dFMR1* gene is directly responsible for the circadian phenotype in mutant flies. This finding is consistent with the observation that dFMR1 is required for the normal extension and guidance of the lateral neurons in which the clock cells that control the circadian rhythms are known to be located in the brain of *Drosophila* [Kaneko et al., 1997]. However, because the circadian locomotor defects are not rescued by the introduction of a *futsch* loss-of-function mutation into the *dFMR1* mutant background [Dockendorff et al., 2002], these behavioral defects are not due to the same synaptic defects caused by Futsch misregulation at the NMJ, suggesting that *dFMR1* differentially regulates diverse targets in the brain.

Among the behavioral disturbances observed in fragile X children, sleep problems are, according to parental reports, especially difficult to manage [Gould et al., 2000]. These include shorter sleep durations, greater variations in sleep duration, longer night waking episodes, and sleep timing problems. It is tempting to speculate that these aspects of sleep are attributable to alterations in circadian rhythmicity because sleep propensity is modulated by a circadian clock [Klein et al., 1991]. Most organisms contain molecular time-keepers known as circadian clocks, which drive daily variations in many physiological and behavioral processes, such as the wake/sleep rhythm and daily variations in body temperature, hormone levels, cognition, and memory [Klein et al., 1991; Hall, 1998]. *Drosophila* has the best understood molecular clock [Dunlap, 1999; Young, 1998]. In addition, recent experiments have revealed that the molecular mechanisms involved in the generation of circadian rhythms are remarkably similar between *Drosophila* and mammals [Dunlap, 1999]. Therefore, a *Drosophila* model of fragile X syndrome provides insight into the sleep/wake cycles of mammals. The discovery of modifiers involved in the dFMR1-mediated regulation of circadian rhythms reveals additional molecular mechanisms in the fragile X syndrome.

BIOCHEMISTRY

How might dFMR1 regulate synapse growth and function in the fly brain? Because dFMR1 is likely to function in cytoplasmic mRNP particles associated with ribosomes, as is the case for mammalian FMR1 [Imbert et al., 1998; Inoue et al., 2000], Ishizuka et al. [2002] conducted affinity purification of a dFMR1-associated complex from cultured *Drosophila* S2 cell lysates to find what proteins and RNAs are stably associated with it in the hope of applying genetic analysis to the associated components. Consistent with previous findings implicating FMRP in translation inhibition and ribosomal association, two ribosomal proteins, L5 and L11, along with 5S ribosomal RNA (rRNA) were identified in the dFMR1-associated complex. Surprisingly, Ishizuka et al. [2002] discovered that in addition to ribosomal proteins, dFMR1 associates with components of RNA interference (RNAi). These include AGO2, p68 RNA helicase (Dmp68), and Dicer.

RNAi/miRNA PATHWAYS

The RNAi pathway was first discovered in the nematode worm *Caenorhabditis elegans* and is widely used as an experimental tool for uncovering the function of genes of interest in a variety of organisms. RNAi is the process of sequence-specific posttranscriptional gene silencing (PTGS) in a variety of organisms, initiated by the introduction of double-stranded (ds) RNA that is homologous in sequence to the silenced gene [Fire et al., 1998; Cogoni and Macino, 2000; Vance and Vaucheret, 2001; Waterhouse et al., 2001; Hutvagner and Zamore, 2002a; Hannon, 2002]. During RNAi, the introduced dsRNAs are processed into small dsRNAs of approximately 21–22 nucleotides (nt) through the action of Dicer, an RNase III family enzyme [Hammond et al., 2000]. These processed small dsRNAs have been termed small interfering RNAs (siRNAs) because they assemble into a multiprotein RNase enzyme called an RNA-induced silencing complex (RISC) and act as guides for the complex that uses the siRNA to identify and destroy complementary mRNA, thus silencing gene expression in a sequence-specific manner (Fig. 1) [Zamore et al., 2000; Elbashir et al., 2001]. Although it was initially thought to be an immune system that protects against transposones and viruses, RNAi is emerging as a fundamental regulatory process that is likely to affect many layers of endogenous gene expressions in eukaryotes. For example,

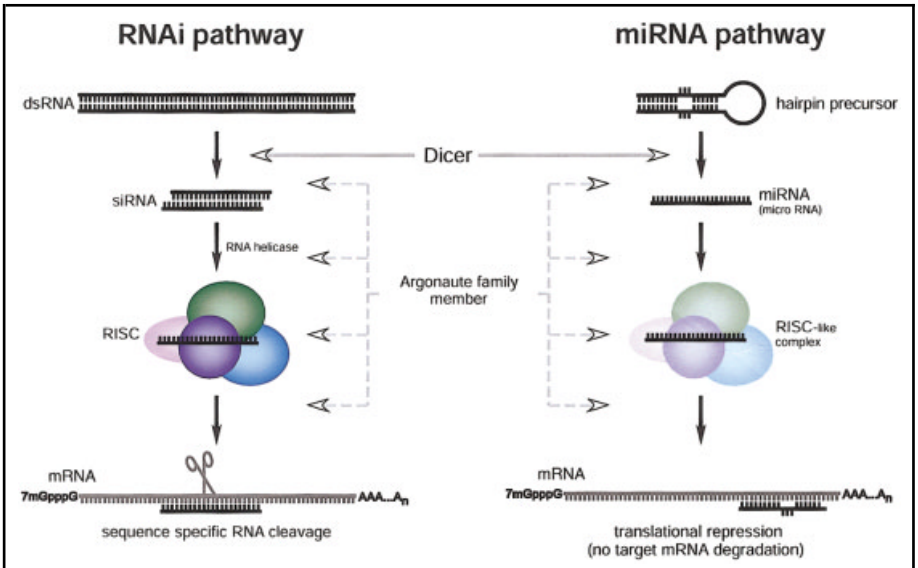

Fig. 1. RNAi and miRNA pathways. Two distinct pathways exist in eukaryotes in which ~22-nucleotide (nt) RNAs function as posttranscriptional regulators of gene expression. RNA interference (RNAi) is the process by which doubl-stranded (ds) RNA triggers the destruction of cognate mRNAs. An RNase III enzyme, Dicer, cleaves the dsRNA into duplexes of 21–23 nt, termed short interfering RNAs (siRNA), which then guide a multicomponent complex known as RISC to mRNAs sharing perfect complementarity and target cleavage. A related short RNA species, microRNA (miRNA), is endogenously transcribed as a long RNA and then processed to a premiRNA of ~70 nt. This premiRNA forms an imperfect hairpin structure, which is processed by Dicer to produce the mature, single-stranded ~22-nt miRNA. Some of the miRNAs are known to regulate developmental timing by mediating sequence-specific repression of mRNA translation. This translational repression is achieved by partial base pairing between miRNAs and the 3′ UTR of target mRNAs. Although the mechanism of translational repression remains to be elucidated, miRNAs are known to be incorporated into RISC. Thus a RISC core might interact with different combinations of short RNA and accessory proteins to carry out either transcript cleavage or translational repression (see text). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com]

components of RNAi are involved in heterochromatin formation, thus suggesting that RNAi processes appear to be associated with the chromatin-silencing machinery [Volpe et al., 2002; Hall et al., 2002].

RNAi pathways share features with a developmental gene regulatory pathway that involves natural dsRNA-encoding genes, recently named microRNA (miRNA) genes (Fig. 1) [Lee and Ambros, 2001; Lau et al., 2001; Lagos-Quintana et al., 2001]. miRNA genes encode approximately 70 nt hairpin precursor RNAs that are processed into mature 22 nt miRNAs. Dicer is also required for processing miRNAs, thus making it essential for both the siRNA pathway and the miRNA pathway [Bernstein et al., 2001; Ketting et al., 2001; Grishok et al., 2001; Hutvagner et al., 2001]. miRNAs are thought to regulate mRNA translation because the founding members of the miRNA gene family, *lin-4* and *let-7*, encode miRNA products that repress translation during *C. elegans* development by base pairing with complementary sequences located in the 3′UTRs of their target mRNAs [Lee et al., 1993; Wightman et al., 1993; Reinhart et al., 2000]. Both the siRNA pathway and the miRNA pathway require members of the Argonaute family (also referred to as the PAZ-and-Piwi domain [PPD] family or the RDE-1 family) [Tabara et al., 1999; Catalanotto et al., 2000; Fagard et al., 2000; Hammond et al., 2001; Mourelatos et al., 2002]. Recently, it has been shown that in addition to siRNAs, miRNAs can also be incorporated into RISCs [Hutvagner and Zamore, 2002b]. Thus these observations suggest that miRNA functions through RNAi-related pathways to regulate the expression of target genes and that the distinct Argonaute family protein associated with siRNAs or miRNAs regulates the function of these small RNAs in a variety of homology-dependent mechanisms that involve base pairing between small guide RNAs and target mRNAs.

Recently, Ishizuka et al. [2002] found that a dFMR1-associated complex contains AGO2, an RNA helicase Dmp68, Dicer, and miRNAs. AGO2 is an Argonaute family protein that is an essential component of the RISC nuclease in *Drosophila* and copurifys with siRNAs and Dicer [Hammond et al., 2001]. Studies in other organisms have also shown that Argonaute family proteins essential for RNAi are in complexes that contain an RNA helicase(s), Dicer, and small guide RNAs [Tabara et al., 2002; Mourelatos et al., 2002; Doi et al., 2003]. Dmp68 belongs to a large family of highly evolutionarily conserved proteins, the so-called DEAD-box family of putative ATPases and helicases [de la Cruz et al., 1999], and is required for efficient RNAi [Ishizuka et al., 2002]. Because ATP-dependent unwinding of the siRNA duplex remodels the RISC to generate an active RISC in the RNAi pathway [Nykanen et al., 2002], Dmp68 may mediate the unwinding process.

Caudy et al. [2002] isolated the endogenous RISC complex from S2 cells to identify the associated proteins and found that dFMR1 and a novel RNA-binding protein termed VIG are such associated factors. These, together with the findings by Ishizuka et al. [2002], suggest that dFMR1 may associate with RISC and thus be involved in RNAi. Supporting this idea, siRNAs can coimmunoprecipitate with dFMR1 [Caudy et al., 2002]. Moreover, Caudy et al. [2002] were able to show that immunoprecipitates of dFMR1 from dsRNA-challenged cells have RISC activity. These observations strongly suggest that dFMR1 is part of RISC in *Drosophila*. Given the observed defects in neuronal morphology and behaviors of *dFMR1* mutant flies, these new findings further suggest the possibility that RNAi-related processes may allow neurons to fine-tune the expression of mRNAs that are critical for morphology and the function of neurons.

POSSIBLE MECHANISM OF ACTION

At present, the role of dFMR1 in RISC is not clear. Two groups performed similar experiments to determine the role of dFMR1 in mRNA degradation by RNAi and found that dFMR1 is not an essential factor for RNAi-mediated degradation, at least in cultured S2 cells [Caudy et al., 2002, Ishizuka et al., 2002]. However, the association of dFMR1 with miRNA, as well as with RISC, may provide a means of linking the RNAi pathways to dFMR1-mediated translational control pathways, as dFMR1 and FMRP have been shown to repress the translation of select mRNAs, as do some miRNAs. These new findings suggest a model in which dFMR1is targeted to its mRNA substrates as part of RISC, which are guided by miRNAs (Fig. 2). Thus, RISC could provide a

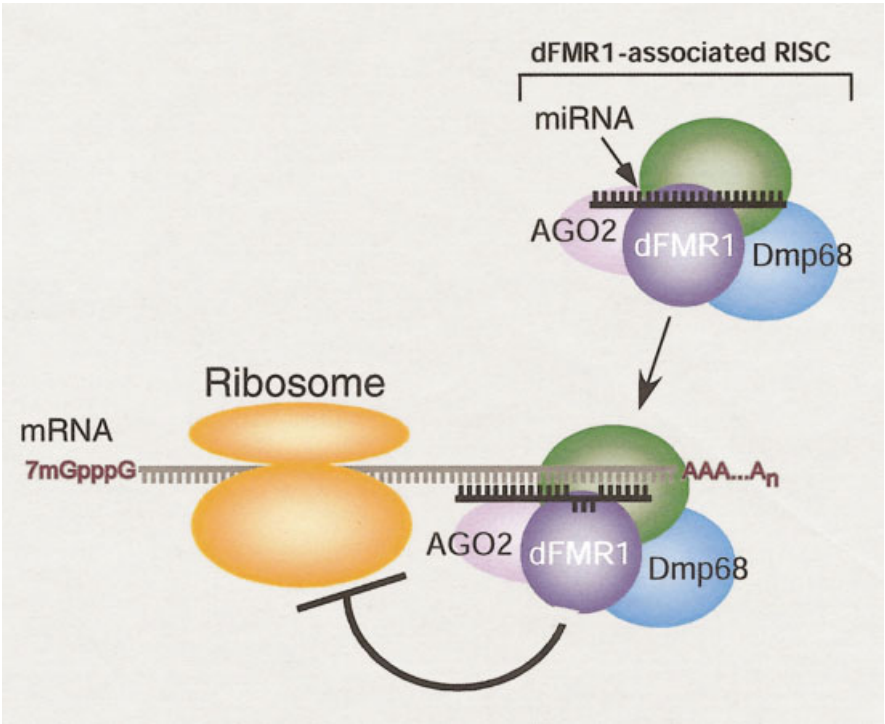

Fig. 2. A model for dFMR1's role in RISC. The association of dFMR1 with miRNA as well as with RISC suggests a model in which dFMR1is targeted to its mRNA substrates as part of RISC, which are guided by miRNAs. Thus, RISC could provide a platform through which dFMR1 can act. dFMR1 could act as a chaperone or selector for recruiting specific miRNAs onto RISC. The interaction between the dFMR1-associated RISC and components of ribosome such as L5 and L11 may inhibit translation at one or more postinitiation steps (see text). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com]

platform through which dFMR1 can act. Because dFMR1 appears to have an intrinsic ability to discriminate among different RNAs [Wan et al., 2000, MC Siomi unpublished results], as is the case for mammalian FMRP, it is also possible that dFMR1 may act as a chaperone or selector for recruiting specific miRNAs onto RISC. This scenario further envisions that a RISC core might interact with different combinations of RNA and protein accessory factors to carry out either mRNA degradation or translation control.

How then might dFMR1 repress the translation of its mRNA targets? *lin-4* and *let-7*, prototypes of miRNAs, regulate endogenous genes involved in developmental timing in *C. elegans* by partially base pairing to the 3′ UTR of target mRNAs such as *lin-14* and *lin-41*, respectively [Lee et al., 1993; Wightman et al., 1993; Ha et al., 1996; Reinhart et al., 2000; Slack et al., 2000]. This interaction does not affect the stability of the target mRNA but rather represses gene expression through an unknown mechanism known as translational repression. The polysome profile of the target mRNA does not change on gene silencing [Olson and Ambros, 1999; Seggerson et al., 2002], suggesting a block at a step after translation initiation. dFMR1 may form a RISC complex containing specific miRNAs on its mRNA targets, and the association of this complex with components of ribosome such as L5 and L11 may inhibit translation at one or more postinitiation steps, including elongation, termination, or the release of functional proteins (Fig. 2). The formation of the RISC on its mRNA targets could be regulated by phosphorylation of dFMR1 because the protein/protein interaction and RNA-binding activities of dFMR1 are drastically modulated by phosphorylation [Siomi et al., 2002], thereby making possible the on-and-off regulation of translational repression of target mRNAs.

Although dFMR1 has multiple RNA-binding domains, it appears to be in RISC complexes through protein/protein interactions [Ishizuka et al., 2002]. So what is the role of RNA binding of FMRP? Although it was originally proposed that FMRP binds directly to its mRNA targets with G-quartet motifs [Schaeffer et al., 2001; Brown et al., 2001; Darnell et al., 2001], FMRP may instead recognize specific miRNAs with these features, which then guide FMRP to its targets. It is also conceivable that the recognition of target mRNAs by the FMRP-associated RISC takes place in two steps. Initially, the RISC may target mRNAs, which are guided by miRNAs. This binding may then be stabilized by the attachment of FMRP to the G-quartet motif found in these target mRNAs. Alternatively, there could be two pathways in which FMRP recognizes target mRNAs: one is the direct binding of FMRP to the G-quartet motif that is found in a number of potential target mRNAs, and the other is the indirect binding of FMRP to the non-G-quartet mRNAs via complementary miRNAs.

Whatever the mechanism of action of FMRP in translation, the association of dFMR1 with components of RNAi and miRNAs [Caudy et al., 2002; Ishizuka et al., 2002] has the implication that defects in an RNAi-related process may cause human disease. Of course, one must be cautious in making connections such as this, because, needless to say, a fly is not a human. An important step toward establishing the importance of an FMRP/RNAi link in humans will be to determine if any or all of the FMR1/FXR family members interact with a RISC and function in an RNAi-related process in mammalian cells. Studies of disease-causing proteins have often provided the first glimpses into hitherto-unknown cellular regulatory mechanisms. If history repeats itself, FMRP and dFMR1 are the harbingers of regulatory mechanisms of gene expression waiting to be discovered. Further studies of dFMR1 aimed at, for example, the identification of miRNAs that specifically associate with a dFMR1-associated RISC in the fly brain will shed light on the mechanism of action of dFMR1 and provide further clues about how the lack of FMRP expression leads to fragile X syndrome. ■

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