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RNA Interference: The Fragile X Syndrome Connection

Dispatch

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Fragile X syndrome is caused by loss of expression of FMRP, a protein proposed to act as a regulator of mRNA translation which promotes synaptic maturation and function. Now FMRP has been found to associate with the RNP complex that mediates post-transcriptional silencing by RNAi.

Fragile X syndrome is one of the most common forms of inherited mental retardation. In most cases, the disease is caused by the methylation-induced transcriptional silencing of the *FMR1* gene which occurs as a result of the expansion of a CGG repeat in the gene's 5′ untranslated region (reviewed in [1]). This leads to the loss of production of gene product FMRP, an RNA-binding protein that contains several structural motifs implicated in RNA interactions — two KH domains and one RGG motif. FMRP associates with translating polyribosomes as part of a large ribonucleoprotein (RNP) complex [2], suggesting a role in regulating translation. Indeed, hundreds of mRNA transcripts exhibit abnormal polyribosome profiles in cells derived from patients with fragile X syndrome [3], and biochemical studies of FMRP suggest that it inhibits translation [1].

Pathological studies of the brains from fragile X syndrome patients and *Fmr1* knockout mice have revealed abnormal dendritic spines on neurons, implicating FMRP in synapse formation and function [4]. Both *in vitro* and *in vivo* neuronal studies have shown that FMRP is located at the synapse, and the loss of FMRP alters synaptic plasticity [1], which is thought to underlie learning and memory. Interestingly, there is increasing evidence that synaptic efficacy is controlled in part by the regulated translation of mRNAs stored in dendrites [5]. It is tempting to speculate on a potential link between FMRP-mediated translation control and synapse function, more so since FMRP is associated with ribosomes localized to postsynaptic dendritic spines [6].

Ishizuka *et al.* [7] purified the *Drosophila* homolog of FMRP (dFMR1) as a complex from cell lysates to find what proteins stably associate with it. They used dFMR1 in the hope of applying genetic analysis to associated proteins, a fortunate choice. *Drosophila* has a single *FMR1* homolog, and the *dFMR1* gene product has similar biochemical properties to mammalian FMRP [8]. Moreover, mutation studies indicated that dFMR1 regulates synapse growth and function in the fly [9,10]. Ishizuka *et al.* [7] purified from *Drosophila* cell lysates a dFMR1 complex that contained two ribosomal proteins, L5 and L11, 5S rRNA and, surprisingly, AGO2, a protein implicated in RNA interference (RNAi).

RNAi is a conserved gene-silencing response to double-stranded RNA (dsRNA), which mediates resistance to both endogenous and exogenous parasitic nucleic acids, encoded by transposons and viruses, respectively (reviewed in [11]). RNAi also regulates the expression of cellular protein-coding genes. A signature feature of RNAi is the formation of small RNAs, 21–23 nucleotides in length, which are generated by a class of conserved RNase III enzymes called the DICER family (Figure 1).

DICER recognizes and cleaves dsRNA molecules to produce 21–23 nucleotide fragments [12]. It has been shown that these fragments, called short interfering RNAs (siRNAs), can substitute for dsRNA in stimulating degradation of homologous mRNAs, the primary mechanism of gene silencing in RNAi [13]. Thus, siRNAs are thought to be the direct guides that identify homologous mRNA substrates for degradation. DICER also processes short hairpin RNAs transcribed from endogenous genes [11], producing 21 nucleotide single-stranded micro-RNAs (miRNAs). In contrast to the siRNA pathway, miRNAs can silence gene expression by repressing translation of complementary mRNA transcripts [14,15]. There are estimated to be possibly hundreds of miRNA-encoding genes in the genome [16].

Biochemical analysis using *Drosophila* extracts has revealed how siRNAs interact with mRNA substrates (Figure 1A). It was observed that siRNAs associate into a nuclease complex dubbed RISC which rec-ognizes and cleaves homologous mRNAs [17]. The cleavage reaction is endonucleolytic and occurs at a central site in the region homologous to the siRNA [13]. One of the subunits of RISC is AGO2 [18], the same protein found by Ishizuka *et al.* [7] to associate with dFMR1. At the same time, Caudy *et al.* [19] were identifying proteins associated with RISC activity from partially purified *Drosophila* preparations, and found that dFMR1 is one such associated factor. Together, these initial observations suggested that dFMR1 may be involved in RNAi.

The two groups [7,19] report a variety of experimental evidence in support of this idea. dFMR1 co-immunoprecipitates with AGO2 and another RISC protein (VIG) from *Drosophila* cell lysates. Moreover, both a miRNA (miR-2b) and siRNAs can co-immunoprecipitate with dFMR1, consistent with the view that dFMR1 associates with RISC. The most compelling biochemical evidence, provided by Caudy *et al.* [19], is that immunoprecipitates of dFMR1 from dsRNA-challenged cells have RISC activity.

All this suggests that dFMR1 is intimately associated with RISC in *Drosophila* cells; but is it required for mRNA degradation? The matter is unclear. Both groups [7,19] performed a similar experiment to determine the function of dFMR1. They used RNAi to silence the endogenous *dFMR1* gene in cells, and

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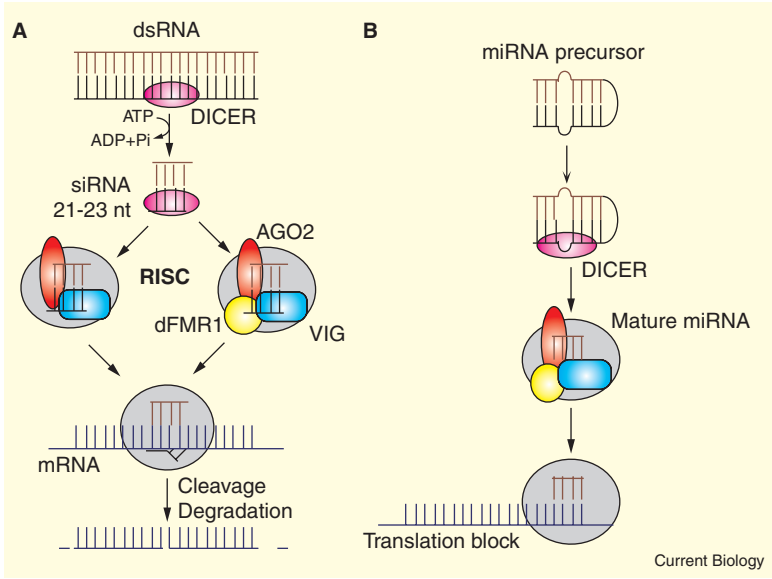

Figure 1. A model for dFMR1's role in RNAi.

(A) The mRNA cleavage/degradation pathway is initiated by dsRNA conversion to siRNAs, which associate with RISC complexes. The proteins AGO2, VIG and dFMR1 also associate with RISC. dFMR1, and possibly VIG, are not found in all RISC complexes, as they appear not to be absolutely essential for the degradation reaction. These assembled complexes are competent to carry out target mRNA recognition and cleavage from immunoprecipitates, though it is unclear whether the complexes remain assembled during the cleavage reaction. (B) The translation repression pathway is initiated by miRNA precursor processing to mature miRNAs, which associate with related RISC complexes. AGO2, VIG, and dFMR1 are also associated with these complexes. The relationship between assembled miRNA complexes and mRNA translation control is surmised by a variety of circumstantial evidence implicating some complex components — miRNAs and dFMR1 — in translation control.

then challenged these cells with a heterologous dsRNA complementary to a reporter gene as a way of measuring RNAi activity in the absence of *dFMR1*. An earlier application of this approach established a role for AGO2 in mRNA degradation by RNAi [18], suggesting it can detect strong functional interactions. However, Caudy *et al.* [19] detected only a weak requirement for *dFMR1*, and Ishizuka *et al.* [7] detected none. But each group measured RNAi activity semi-quantitatively by reporter-protein levels. Direct analysis of reporter mRNA levels might have revealed similarities or differences that were not detected at the protein level. Clearly more work is needed to resolve this issue.

What is clear is that dFMR1 is not an essential factor for RNAi-mediated degradation. Rather than being a constitutive component of transcript-cleaving RISC, dFMR1 might be an accessory factor that associates with RISC but uses another mechanism to regulate its targets. This interpretation fits nicely with the other mechanism being translational repression, as dFMR1 and FMRP have been shown to repress translation of select mRNAs, as do some miRNAs [3,9,14,15,20]. Because miRNAs and dFMR1 interact with each other as well as with RISC, it is quite possible that dFMR1 is targeted to substrate transcripts as part of a RISC complex that is guided by miRNAs. Thus, a RISC core might interact with different combinations of RNA and protein accessory factors to carry out either transcript cleavage or translation control.

An alternative scenario is that dFMR1-associated RISC complexes may only use a subset of trigger siRNAs or miRNAs available. dFMR1 itself may contribute to such RNA specificity, as FMRP was found to selectively bind RNA oligonucleotides with G-quartet structures adjacent to short RNA helices [3,20]. While it was originally proposed that FMRP directly binds to mRNA substrates with these structural features, FMRP may instead recognize specific miRNAs with these features, which then guide FMRP to its correct targets. It is possible that some, but not all, siRNAs associate with FMRP and guide RISC complexes that are competent to degrade mRNA as well.

The connection between the RNAi and fragile X fields is likely to grow as we find out which small RNAs mediate translational repression by dFMR1 and FMRP, and how the repressive mechanism operates. Nevertheless, it is worth pondering that fragile X syndrome may be the result of a defect in the RNAi activity within neurons, making its molecular nature a RISCy business.

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