

Review

Come FLY with us: toward understanding fragile X syndrome

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The past few years have seen an increased number of articles using *Drosophila* as a model system to study fragile X syndrome. Phenotypic analyses have demonstrated an array of neuronal and behavioral defects similar to the phenotypes reported in mouse models as well as human patients. The availability of both cellular and molecular tools along with the power of genetics makes the tiny fruit fly a premiere model in elucidating the molecular basis of fragile X syndrome. Here, we summarize the advances made in recent years in the characterization of fragile X *Drosophila* models and the identification of new molecular partners in neural development.

Keywords: dFmr1, *Drosophila*, FMRP, fragile X syndrome

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Fragile X syndrome is the most common form of inherited mental retardation, with the estimated prevalence of one in 4000 males and one in 8000 females (Warren & Sherman 2001). In addition to cognitive deficits, the phenotype of fragile X syndrome includes mild facial dysmorphology (prominent jaw, high forehead and large ears), machroorchidism in postpubescent males and subtle connective tissue abnormalities (Warren & Sherman 2001). Many patients also manifest attention-deficit hyperactivity disorder and autistic-like behaviors. As one of the first identified human disorders caused by trinucleotide repeat expansion, fragile X syndrome is typically caused by a massive CGG trinucleotide repeat expansion within the 5′ untranslated region (UTR) of the fragile X mental retardation 1 gene (*FMR1*), which results in transcriptional silencing of *FMR1* (Fu *et al*. 1991; Kremer *et al*. 1991; Oberle *et al*. 1991; Pieretti *et al*. 1991; Verkerk *et al*. 1991). Identification of other mutations (e.g. deletions in patients with the typical phenotype) has confirmed that *FMR1* is the only gene involved in the pathogenesis of fragile X syndrome, and the loss of *FMR1* product – fragile X mental retardation protein (FMRP) – causes fragile X syndrome (De Boulle *et al*. 1993; Lugenbeel *et al*. 1995; Wohrle *et al*. 1992).

FMRP, along with its autosomal paralogs, the fragile-X-related proteins FXR1P and FXR2P, compose a well conserved, small family of RNA-binding proteins (fragile X-related gene family) that share over 60% amino acid identity and contain two types of RNA-binding motifs: two ribonucleoprotein K homology (KH) domains and a cluster of arginine and glycine residues (RGG box) (Siomi *et al*. 1995; Zhang *et al*. 1995). How the loss of a single protein, FMRP, leads to mental retardation and a plethora of behavioral problems has been intensively investigated since the cloning of *FMR1* gene in 1991. FMRP was found to form a messenger ribonucleoprotein (mRNP) complex that associates with translating polyribosomes (Feng *et al*. 1997). It has been proposed that FMRP is involved in synaptic plasticity through the regulation of mRNA transportation and translation (Fig. 1).

Given the power of *Drosophila* genetics in dissecting biological pathways, within the last several years, the fruit fly has been increasingly used to gain insights into the physiological roles of FMRP. Here, we are reviewing the role of *Drosophila* fragile X mental retardation gene (*dFmr1*) in development, synaptogenesis and behavior as well as discuss new functional partners revealed by genetic studies.

dFmr1 protein is highly similar to mammalian *Fmrp* and is ubiquitously expressed during development

Unlike their mammalian counterparts, the fly genome harbors a single *Fmr1* gene homolog, also referred to as *dFmr1* or *dfxr* (*dFmr1* here, as per Flybase annotation). Sequence comparisons show a high level of similarity between the functional domains of fly and mammalian *Fmrp*, with overall 56% similarity and 35% identity (Gao 2002; Zhang *et al*. 2001). Thus, *dFmr1* is comprised of two KH domains, ribosomal- and self-association domains, an RGG box as well as a nuclear localization signal (NLS) and nuclear export signal (NES), although it remains to be determined whether the

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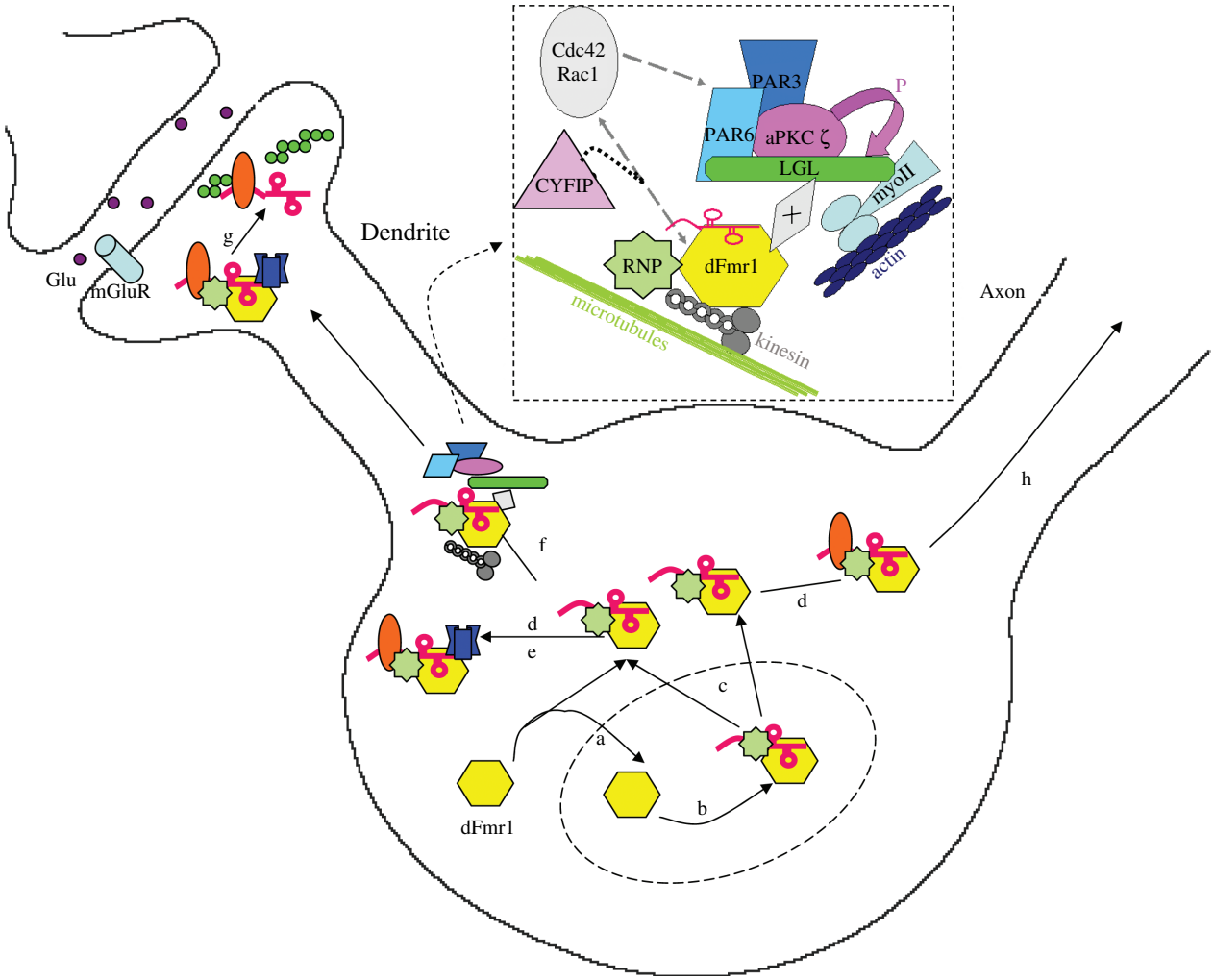

Figure 1: Model of *Drosophila* fragile X mental retardation protein (*dFmrp*) function in the neuron. *dFmrp* (yellow hexagon) enters into the nucleus (a) via its NLS and forms a messenger ribonucleoprotein (mRNP) complex (b) by interacting with specific RNA transcripts (red hairpin structure) and proteins (green eight-point star). *dFmrp* could also form a mRNP complex in cytoplasm without entering into the nucleus. The *dFmrp*–mRNP complexes are then transported out of the nucleus (c) via its NES. In cytoplasm, the *dFmrp*–mRNP complex can associate with ribosomes (orange oval) and interact with the RNA-induced silencing complex [RNA-induced silencing complex (RISC); blue ribbon)] (d & e). The *dFmrp*–mRNP complex could be transported (f) into dendrites after the *dFmrp*-mRNP has been assembled into a large transport complex which is shown with details in the dashed square. Once transported into the postsynaptic region, both complexes can be regulated to modulate (g) local protein synthesis (strings of green circles) from specific mRNAs in response to synaptic stimulation signals such as activation of the metabotropic glutamate receptor (mGluR). Certain *dFmrp*-mRNP complexes may also be transported into axon (h) and function presynaptically. Components in the *dFmrp*-mRNP transport complex; the LGL protein encoded by *lethal(2) giant larvae* (*dlgl*) interacts with both *dFmrp*-mRNP (probably via other unidentified proteins) and the PAR cell polarity complex (PAR3 and PAR6). The whole complex can be transported along microtubles via motor protein kinesin. αPKCζ leads to the phosphorylation of LGL, and this could modulate its interaction with nonmuscle myosin II. It could provide an interesting mechanism that enables the switch of *dFmrp*–mRNP cargos from dendritic microtuble to postsynaptic actin. Cdc42 and Rac1 are two proteins participating in the trafficking in polarized cells. *Rac1* could interact with *dFmrp*, and this interaction may be antagonized by cytoplasmic FMRP interacting protein (*CYFIP*), which has been shown to be an interactor for both *Rac1* and *Drosophila* fragile X mental retardation gene (*dFmr1*).

latter domain functions as an export sequence (an essential leucine has been substituted to glutamine) (Wan *et al.* 2000). It has been shown that both mammalian Fmrp and dFmr1 protein are phosphorylated *in vivo* on conserved serine residues, and this might regulate their activities (Ceman *et al.* 2003; Siomi *et al.* 2002). Not only is dFmr1 highly homologous to mammalian Fmrp but also it exhibits similar homopolymer RNA-binding properties. *In vitro* translated dFmr1 protein can bind strongly to poly(G), weakly to poly(U) but not to poly(A)/(C) (Wan *et al.* 2000). Mutations in each of the

KH domains abolish homopolymer binding, consistent with a functional role for these motifs as suggested from human genetics studies (De Boulle *et al*. 1993). This level of conservation taken together with the genetic tools available in *Drosophila* makes the fly an unparalleled model system for fragile X syndrome.

Sequence analyses of available expressed sequence tags (ESTs) suggest that *dFmr1* possesses alternative splicing and polyadenylation sites, which is consistent with Northern blots showing the presence of more than one transcript (Dockendorff *et al*. 2002; Zhang *et al*. 2001). Immunohistochemical data show that *dFmr1* is ubiquitously expressed during the early stages of embryogenesis, with strong expression in the mesoderm, the brain lobes and ventral ganglia developing at later stages (Dockendorff *et al*. 2002; Wan *et al*. 2000; Zhang *et al*. 2001). Other tissues where *dFmr1* has been detected are the developing imaginal discs, testes, ovaries and the ring gland (Zarnescu *et al*. 2005; Zhang *et al*. 2001). Just like its mammalian counterpart(s), *dFmr1* is enriched in all neurons and with low or absent levels in glia. In addition, the protein is detected largely in the cytoplasm and not in the nuclei of all cells examined to date (Morales *et al*. 2002; Wan *et al*. 2000; Zhang *et al*. 2001).

The loss of *dFmr1* leads to the defects in behavior, synaptogenesis and spermatogenesis

To characterize the physiological functions of *dFmr1*, several loss-of-function mutations ranging in strength from weak hypomorphs to nulls have been isolated in the *dFmr1* locus (Dockendorff *et al*. 2002; Inoue *et al*. 2002; Lee *et al*. 2003; Morales *et al*. 2002; Zhang *et al*. 2001). *dFmr1* is not essential for viability, although some variability has been reported in the numbers of adult homozygotes (Dockendorff *et al*. 2002; Morales *et al*. 2002). Such differences in the numbers of viable homozygotes could be due to genetic background effects. Homozygous mutant adults appear morphologically normal but display abnormalities in behavior, synaptogenesis and spermatogenesis, some of which may be viewed as resembling the phenotypes observed in fragile X patients.

The loss of dFmr1 leads to several behavioral defects

Examination of locomotor activity in adult flies lacking *dFmr1* revealed a statistically significant arrhythmic behavior (Dockendorff *et al*. 2002; Morales *et al*. 2002). *dFmr1* mutants exhibit erratic activity patterns with brief periods of high activity. As the overall activity of *dFmr1* nulls is unchanged, this suggests that the arrhythmicity observed is not due to defects in motor function and locomotion ability, but rather in the circadian clock. Interestingly, just like mutants lacking normal circadian function, *dFmr1* nulls can be driven to display normal rhythms and even anticipate lights turning on and off when trained in light/dark cycles (Dockendorff *et al*. 2002; Inoue *et al*. 2002). This suggests that the molecular clock in itself is intact, and the defects observed may be due to downstream effectors of the clock. To address the first possibility, the expression of known molecular components of the circadian clock, such as *timeless* and *period*, were examined in dFmr1 mutants; however, no significant changes have been found (Dockendorff *et al*. 2002; Inoue *et al*. 2002; Morales *et al*. 2002). To address the possibility of downstream effects, using a reporter construct (CRE-luciferase) to monitor the downstream activity of the molecular clock, it was found that the amplitude of oscillations was reduced, suggesting that at least the CREB (cAMP response element binding) protein, a known molecular output of the clock, is controlled by *dFmr1* function (Dockendorff *et al*. 2002). Indeed, fragile X patients have shorter sleep duration, greater variation in sleep duration and sleep timing problems, which might be related to the disturbance of circadian rhythms (Hagerman & Hagerman 2002).

Other tested behaviors included phototactic, geotactic and chemotactic abilities, which appear unaffected (Dockendorff *et al*. 2002; Morales *et al*. 2002; Zhang *et al*. 2001). Male courtship activity was found reduced in *dFmr1* mutants at the level of maintaining courtship and was not due to a specific sensory deficit. In addition, *dFmr1* mutant larvae were shown to exhibit altered crawling behavior, with shorter linear paths and more frequent turns in environments controlled for geotactic, phototactic and chemotactic cues (Xu *et al*. 2004). Taken together, these data suggest that *dFmr1* loss-of-function mutants can execute simple behavioral tasks but exhibit deficits in the more complex behaviors analyzed to date.

dFmr1 regulates synaptic morphology and function

Using *Drosophila* larval neuromuscular junction (NMJ, a metabotropic type of synapse), it was shown that the loss of *dFmr1* resulted in an increased number of synaptic boutons and overelaboration of synaptic terminals, similar to the dendritic overgrowth phenotype reported in the *Fmr1* knockout mouse as well as fragile X patients (Hinton *et al*. 1991; Nimchinsky *et al*. 2001; Zhang *et al*. 2001). As expected, *dFmr1* gain of function results in underelaborated synaptic terminals with enlarged synaptic boutons (Zhang *et al*. 2001). Using tissue-specific drivers to overexpress the protein either presynaptically or postsynaptically, it was found that *dFmr1* functions on both sides of the synapse, but is predominantly presynaptic (Zhang *et al*. 2001). Electrophysiological studies found that evoked synaptic neurotransmission is significantly increased at NMJ in *dFmr1* mutants, suggesting that the average synaptic efficacy is upregulated in these mutants (Zhang *et al*. 2001). In addition, miniature excitatory junctional currents had a mildly increased frequency in nulls compared with controls and also showed a significant increase in frequency when *dFmr1* was overexpressed on the presynaptic but not postsynaptic side (Zhang *et al*. 2001). This result was surprising in that both loss-of-function and gain-of-function conditions resulted in increased efficacy of synaptic transmission, suggesting that the physiology of the

synapse is highly sensitive to the level of dFmr1 protein. A similar effect was observed with electroretinograms recorded at the histaminergic photoreceptor synapse, although in this case the transmission efficacy was decreased by modulating the level of dFmr1 protein (Morales *et al*. 2002). These results are similar to the observation that in mouse the level of *Fmrp* is critical and overexpression of *Fmrp* could overcorrect the behavioral phenotypes affected in *Fmr1* knockout mice (Peier *et al*. 2000).

dFmr1 is a negative regulator of neurite extension and branching

Just like its mammalian counterpart, dFmr1 protein plays a role in dendrite morphogenesis. A detailed developmental analysis of multiple dendritic (MD) neurons in *dFmr1* mutants showed that dFmr1 protein is a negative regulator of neurite extension (Lee *et al*. 2003). In contrast, overexpression of *dFmr1* allows the extension of the major dendritic branches but blocks the formation of higher order structures thus reducing the overall dendritic complexity (Lee *et al*. 2003).

Other studies focused on the dorsal cluster neurons (DC), which have been implicated in the control of eclosion and the lateral (LNv) neurons, which control circadian rhythms (Dockendorff *et al*. 2002; Morales *et al*. 2002). In the absence of *dFmr1*, DC neurons exhibit a failure of axon extension, while LNv neurons show overextended axons. This suggests that although *dFmr1* controls at least some aspects of their cellular architecture, it may have distinct functions in various neurons, perhaps by regulating different mRNA targets. Interestingly, overexpression of *dFmr1* in both wildtype and mutant backgrounds results in failure of axonal extension, suggesting once again that dosage is critical for normal functions (Morales *et al*. 2002).

Recently, the mushroom body (MB), a highly plastic brain region, essential for many forms of learning and memory, was also studied (Michel *et al*. 2004; Pan *et al*. 2004). Phenotypic analyses showed that, in the absence of *dFmr1*, MB neurons display a more complex architecture, including overgrowth, overbranching and abnormal synapse formation (Michel *et al*. 2004; Pan *et al*. 2004). Interestingly, whole brains mutant for *dFmr1* exhibit a more severe MB phenotype (Michel *et al*. 2004) compared with brains where only subsets of MB neurons lack *dFmr1* (Pan *et al*. 2004). These phenotypes are consistent with a cell non-autonomous function for *dFmr1*. Taken together, these data showed that *dFmr1* is a potent negative regulator of neuronal architecture and synaptic differentiation in the nervous system.

The loss of dFmr1 leads to abnormal spermatogenesis and oogenesis

Although *dFmr1* mutants are viable and lack obvious morphological abnormalities, they cannot be maintained as a stock, using standard fly husbandry (Zhang *et al*. 2004). A detailed analysis of *dFmr1* expression during spermatogenesis showed that the protein is upregulated in the late and larger spermatocytes (first four stages of spermatogenesis) compared with the more mature, elongated spermatids (last two stages of spermatogenesis) (Zhang *et al*. 2004). Consistent with this expression pattern, an age-dependent enlargement (100% penetrant in newly eclosed, but insignificant in 3-day-old males) in the middle region of the testes was observed. This enlargement is not due to an overproliferation of spermatids but rather due to the accumulation of misarranged spermatid bundles. Moreover, at the next developmental stage, coiled spermatid bundles appear to be degenerating in *dFmr1* mutant testes, and thus very few individual spermatozoa are present in the mutant seminal vesicles (Zhang *et al*. 2004). The studies using electron microscopy showed that the basis of this degenerative phenotype is the loss of the central pair of microtubules without effects on the overall integrity of the axoneme (Zhang *et al*. 2004).

In a recent study, it was found that loss of *dFmr1* function also leads to defects in oogenesis (Costa *et al*. 2005). *Drosophila* oocytes develop at the posterior end of egg chambers consisting of 16 germ cells surrounded by a monolayer of follicle cells (Spradling *et al*. 1997). dFmr1 protein is upregulated in the developing oocyte, and phenotypic analyses of null ovaries show that *dFmr1* plays a role in the formation of the 16-cell germ cell cyst (Costa *et al*. 2005). Thus egg chambers lacking *dFmr1* function have either too many or too few cells, suggesting a possible role for *dFmr1* in cell division. In addition, *dFmr1* loss of function results in egg chambers containing either no or an extra oocyte, suggesting that *dFmr1* functions in oocyte differentiation (Costa *et al*. 2005).

mRNA targets and genetic interactors of *dFmr1*

Given that FMRP is involved in the translational control of specific mRNAs in mammals, it is important to identify the *Drosophila* mRNA targets of dFmr1 protein. Several mRNA targets of dFmr1 protein also have been identified, including *futsch*, *rac1*, *pickpocket1* (*ppk1*) and *orb* (Costa *et al*. 2005; Lee *et al*. 2003; Xu *et al*. 2004; Zhang *et al*. 2001). Most recently, we also conducted a microarray analysis of the mRNA targets associated with dFmr1 protein and found some but not all of the reported dFmr1 protein-associated mRNAs (Zarnescu *et al*. 2005). This is not surprising, as experimental conditions and the choice of biological material differ among the various reports, and thus, each identified target requires individual validation. Interestingly, two of the mRNA targets we identified to be associated with the lethal(2) giant larvae (dlgl)/dFmr1 complex have been previously implicated in circadian rhythms (Zarnescu *et al*. 2005), a biological process controlled by *dFmr1* (Dockendorff *et al*. 2002; Morales *et al*. 2002).

Gain of function for *dFmr1* at the NMJ results in a smaller number of large synaptic boutons, which faithfully resembles

the loss of function phenotype for *futsch*, the *Drosophila* ortholog of microtubule-associated protein MAP1B (Zhang *et al*. 2001). Moreover, loss-of-function *futsch* is sufficient to rescue the synaptic hyperplasia phenotype due to loss of function for *dFmr1*. Taken together, these genetic data suggest that *dFmr1* and *futsch* are functionally antagonistic. Furthermore, immunoprecipitation experiments show that *futsch* mRNA associates with dFmr1 protein and more importantly the latter controls the levels of futsch protein, presumably at the level of translation (Zhang *et al*. 2001). This regulation is also conserved in mouse where the translation of MAP1B was found to be negatively regulated by FMRP (Lu *et al*. in press).

Interestingly, the loss of *futsch* is not sufficient to rescue other *dFmr1*-associated phenotypes, such as circadian rhythm defects and male infertility, suggesting that dFmr1 protein might act on specific targets in different tissues and distinct developmental contexts (Dockendorff *et al*. 2002; Zhang *et al*. 2001). These data underscore the importance of comprehensive phenotypic studies and the identification of dFmr1 protein-associated mRNA targets as well as its functional partners.

In addition, immunoprecipitation experiments showed that *Rac1* mRNA specifically associates with *dFmr1*/mRNP complexes (Lee *et al*. 2003). The removal of *Rac1* function in MD neurons during development resulted in underelaborated higher order dendritic branches, a phenotype opposite to the loss of *dFmr1*. Gain-of-function *Rac1* in a subset of MD neurons increases dendritic branching, while dFmr1 overexpression results in reduced arborization (Lee *et al*. 2003). Furthermore, concomitant overexpression of *Rac1* and *dFmr1* in the MD neurons partially restores the reduced branching phenotype produced by *dFmr1* overexpression alone. Taken together, these data suggest that *dFmr1* mediates dendritic elaboration and branching, in part via regulating *Rac1* mRNA. *Rac1* could also function with *dFmr1* at the protein level, and this interaction is modulated by cytoplasmic FMRP interacting protein (CYFIP), a previously known Rac1 interactor (Kobayashi *et al*. 1998; Schenck *et al*. 2003). CYFIP associates with either constitutively active Rac1V12 or dFmr1 in mutually exclusive complexes. Genetic interactions in *Drosophila* eye and central nervous system provided further evidence for *CYFIP* acting as an antagonist of *Rac1* as well as *dFmr1* (Schenck *et al*. 2003). This study provided evidence to support the possibility that *CYFIP*, which interacts with *Rac1* in an activity-dependent manner, acts as a link between two processes underlying synaptic remodeling: cytoskeleton reorganization regulated by *Rac1* and control of local protein translation via dFmr1 protein (Fig. 1).

In order to identify novel genetic interactors of *dFmr1*, we took a forward genetic approach using the fly eye as a model system (Zarnescu *et al*. 2005). We used an overexpression paradigm, whereby ectopic expression of *dFmr1* under the control of an eye-specific promoter results in a visible rough eye phenotype, which we used as a basis for a saturating genetic screen (Wan *et al*. 2000; Zarnescu *et al*. 2005). In screening over 51 000 progeny, we isolated a total of 109 mutants of which 19 fell into a single complementation group, *dlgl*. *dlgl* encodes a tumor-suppressor gene with a documented role in cell polarity (Bilder 2004). Taken together, our genetic interactions, colocalization and biochemical data suggest that *dlgl* functions with *dFmr1* in neural development at three possible levels: (1) sorting, (2) transport and/or (3) anchoring of mRNA. In addition, the dlgl/dFmr1 complex is regulated by the PAR cell polarity complex, including atypical PKC-ζ (Zarnescu *et al*. 2005) (Fig. 1). Importantly, mammalian Lgl (mlgl) forms a developmentally regulated complex with *Fmrp* in the mouse brain, suggesting that this interaction is conserved in mammals and that mlgl may function with FMRP in synaptic maturation and/or plasticity. This demonstrates that taking a forward genetic approach in *Drosophila* is a powerful and fruitful tool for identifying novel functional partners of FMRP.

dFmr1 protein-mediated translation control and microRNA pathway

Studies performed in mammalian systems found that FMRP was associated with polyribosomes in an RNA-dependent manner (Corbin *et al*. 1997; Eberhart *et al*. 1996; Feng *et al*. 1997; Khandjian *et al*. 1996; Stefani *et al*. 2004). FMRP could act as a translational repressor of reporter constructs both *in vitro* and in transfected cells. In *Drosophila*, the dFmr1 protein was also found to function as a translational suppressor as well (Lee *et al*. 2003; Zhang *et al*. 2001).

The accumulation of work from several groups is now suggesting that the RNA interference (RNAi) pathway is the major molecular mechanism by which FMRP regulates translation. The initial critical observation came from biochemical studies in *Drosophila* cell culture. There, it was demonstrated that the dFmr1 protein associates with Argonaute 2 (*AGO2*) and the RNA-induced silencing complex (RISC), which mediate RNAi (Caudy *et al*. 2002; Ishizuka *et al*. 2002). RNAi, now a widely used experimental tool, is a conserved gene-silencing response to double-stranded RNA (dsRNA) (Novina & Sharp 2004). Silencing is initiated when dsRNA triggers are processed into small interfering RNAs (siRNAs). This is catalyzed by a group of related RNase III enzymes known as the Dicer family. The siRNAs are incorporated into the effector complex, RISC, which uses siRNA as a guide to select complementary mRNA substrates (Novina & Sharp 2004). Most components of RISC also can be utilized by endogenous microRNAs (miRNAs) (Bartel 2004) which are a new class of non-coding RNAs that are believed to control translation of specific target mRNAs by base pairing with complementary sequences in the 3′ UTR of these messages (Bartel 2004). The functions of miRNAs and siRNAs are facilitated by members of the PIWI/PAZ-domain protein (Argonaute) family (Bartel 2004).

While recent data in *Drosophila* suggest that *AGO1* is mainly involved in the endogenous miRNA pathway and that *AGO2* is required for siRNA-mediated gene silencing, the loss of *dFmr1* or FMRP does not seem to affect the siRNA pathway (Caudy *et al*. 2002; Ishizuka *et al*. 2002; Okamura *et al*. 2004). Therefore, it is still unclear what role, if any, FMRP plays in siRNA-mediated gene silencing. The association of the dFmr1 protein with RISC raises the possibility that FMRP may regulate the translation of its target genes through miRNAs. Indeed, FMRP was found to be associated with miRNAs in both *Drosophila* and mammals (Caudy *et al*. 2002; Ishizuka *et al*. 2002; Jin *et al*. 2004). To further test the functional importance of these interactions, our group examined the genetic interaction between *dFmr1* and *AGO1*. We 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* (Jin *et al*. 2004). Together, these data suggest that *AGO1* is critical for the biological functions of FMRP in neural development and synaptogenesis (Jin *et al*. 2004). It recently has been found that *dFmr1* also interacts genetically with *AGO2*, and the *ppk1* mRNA level appears to be regulated by *dFmr1* and *AGO2* (Xu *et al*. 2004).

These observations strongly support the idea that dFmr1 protein might regulate the translation of its mRNA via miRNA interaction. A likely scenario is that once dFmr1 protein binds to its specific mRNA ligands, it recruits RISC along with miRNAs and facilitates the recognition between miRNAs and their mRNA ligands. Thus, dFmr1 protein might modulate the efficiency of translation of its mRNA targets using miRNAs. This mechanism would allow this activity to be rapid and reversible, as would be needed in protein synthesis-dependent synaptic plasticity.

Drug discovery for fragile X syndrome in *Drosophila*

In addition to the typical use of *Drosophila* (i.e. screening for novel genes and their mutations), the fruit fly is becoming the model of choice when a combination of gene alteration, pharmacological and functional assays of a phenotype is needed. Such a combined approach is particularly valuable in studies of complex systems such as the CNS (Manev *et al*. 2003). It was discovered a few years ago that one of the phenotypes in *Fmr1* knockout mice is the enhanced metabotropic glutamate receptor (mGluR) activity (Huber *et al*. 2002). This led to the proposition of the 'mGluR hypothesis' as the underlying mechanism for cognitive deficits present in fragile X patients (Bear *et al*. 2004). Recently, it has been shown that the enhanced mGluR activity is a conserved feature of fly *dFmr1* mutant as well (McBride *et al*. 2005). More importantly, it was demonstrated that administration of various mGluR antagonists rescues the behavioral phenotypes previously reported in the fly (McBride *et al*. 2005). These findings are opening the exciting possibility that a similar approach might work to ameliorate some of the cognitive and behavioral deficits in human patients.

Concluding remarks

Recent developments of *Drosophila* models for fragile X syndrome have provided new avenues to understand the molecular pathogenesis of this disease. Despite that the fly genome only harbors a single *Fmr1* gene homolog and some of the functions ascribed to *dFmr1* in fly might be carried out by the paralogs in mammals, the power of fly genetics should enable the field to identify and dissect biological pathways regulated by FMRP. The next exciting step will be taking the discoveries made in the fly and apply them towards a better understanding of fragile X syndrome.

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