

Translational Complexity of the Fragile X Mental Retardation Protein: Insights from the Fly

Through the awesome power of *Drosophila* genetics, two recent studies (Costa et al., 2005; Zarnescu et al., 2005) reveal novel mechanisms by which the Fragile X Mental Retardation protein regulates selective mRNA translation, controlling key steps of germline development during oogenesis and neuronal development during synaptogenesis.

Fragile X syndrome is the most common form of inherited mental retardation, with nonneuronal symptoms including prominent gonadal defects. The disease is caused by loss of the RNA binding FMR1 protein (FMRP), which participates in a large ribonucleoprotein complex with multiple other RNA binding proteins to regulate translation of specific mRNAs. Over the past three years, a *Drosophila* disease model has been particularly important in defining the molecular bases of *Drosophila* FMRP (dFMRP) function during germ cell development important to both male and female fertility and during nerve cell development and synaptogenesis/plasticity critical for the manifestation of normal behaviors (Zhang and Broadie, 2005). Two particularly active arenas of study are defining the mechanisms by which dFMRP regulates translation initiation and identifying dFMRP's *in vivo* mRNA targets.

dFMRP is suggested to regulate translation via several mechanisms (all quite different), with, possibly, multiple *in vivo* pathways (Zhang and Broadie, 2005). In general, most translational control during development is mediated by 3' UTR sequences, determining translation initiation (e.g., cytoplasmic polyadenylation; modulation of poly(A) tail length), ribosome binding, and post-initiation events. Cytoplasmic polyadenylation-induced mRNA translation is a hallmark of early development, especially in oogenesis, but is also important in synaptic plasticity in mature brain (Barnard et al., 2004). Regulated polyadenylation activates localized mRNA translation via an AAUAAA sequence bound by the cleavage and polyadenylation specificity factor (CPSF) and an upstream, U rich cytoplasmic polyadenylation element (CPE) recognized by a CPE binding (CPEB) protein. Phosphorylation of CPEB activates the polyadenylation machinery (Barnard et al., 2004; Figure 1). The single characterized *Drosophila* CPEB, Orb, is a germline-specific RNA binding protein that mediates translation activation in oocytes. Orb is required for multiple steps in oogenesis, including formation of the 16-cell cyst, oocyte differentiation, and establishing both A-P and D-V axes in the egg. Costa et al. (2005) use anti-Orb immunoprecipitation to identify associated proteins, isolating a number of new Orb interactors, including dFMRP.

Loss of *dfmr1* causes mild *orb*-like defects in a proportion of egg chambers, which may have either too many or too few germline cells. dFMRP appears to antagonize Orb function during oogenesis; *dfmr1* mutants suppress *orb* D-V defects, whereas dFMRP overexpression enhances defects. The root of these interac-

tions is that dFMRP negatively regulates Orb translation and, thus, acts as a negative regulator in an Orb autoregulatory circuit. Costa et al. (2005) showed that constructs that contain Orb 3' UTR, but not FMRP binding sites, are insensitive to regulation. However, Orb itself, which contains both Orb and FMRP recognition sites, is very sensitive to both Orb and FMRP dose (Figure 1). Oogenesis defects in *dfmr1* are suppressed by reducing Orb levels, albeit only suppressing the excess germ cell phenotype and not other phenotypes. Consistently, dFMRP also regulates Orb-mediated translation of only a subset of its targets, likely only the subset that contains dFMRP recognition motifs, explaining why *dfmr1* phenotypes are so mild compared to *orb* phenotypes. Orb only associates with a small proportion of dFMRP, defining a small percentage of mRNA target overlap (Figure 1). This result suggests that the temporal/spatial role of dFMRP in translation regulation appears dependent on complexing with a number of different, specific mRNA binding partners. Costa et al. (2005) propose that a similar mechanism occurs in the nervous system.

Protein synthesis, including highly local protein synthesis at synapses, is required for synaptogenesis and many forms of synaptic plasticity. Regulation of polyadenylation-dependent translation initiation is critical for both synaptic structural changes and modulating synaptic strength. In mammals, FMRP has been shown to act as a negative translational regulator in neurons, locally within dendrites, and CPEB also regulates localized translation within dendrites and axonal growth cones (Brittis et al. 2002; Wells et al., 2001). Importantly, translation of α -calcium/calmodulin-dependent protein kinase II (α -CaMKII) mRNA, whose product is critical for synaptic development and plasticity, is antagonistically regulated by CPEB and FMRP. The 3' UTR of α -CaMKII mRNA, as well as other synaptic mRNAs, contains CPE binding domains, and activity-dependent polyadenylation and translation of α -CaMKII mRNA occurs during synaptic plasticity (Wells et al., 2001). CPEB-dependent polyadenylation underlies translational activation of α -CaMKII in dendrites, whereas FMR1 knockout mice overexpress α -CaMKII in synaptoneuroosomes (Zalfa et al., 2003). α -CaMKII-mediated CPEB phosphorylation drives protein synthesis in hippocampal dendrites, generating a positive feedback loop involving FMRP (Atkins et al., 2004; Figure 1). These observations suggest that the regulatory mechanism revealed above could also be relevant to translational misregulation underlying cognitive/behavioral symptoms in fragile X patients. Interestingly, the *Drosophila* genome encodes another CPEB-like protein suggested to have specific expression in the nervous system, implying that tissue-specific CPEBs may subdivide dFMRP-mediated translation regulation between germline and neuronal development (Figure 1).

To identify additional dFMRP-interacting genes, Zarnescu et al. (2005) conducted a saturating genetic screen for suppressors of a rough eye phenotype induced by dFMRP overexpression. A screen of 50,000 mutagenized flies identified 63 single gene hits and 5 larger complementation groups. Of the genes hit multiple times, four remain unidentified, but the gene with the highest hit rate (19/109 suppressors) was the *lethal large larvae (lgl)* gene. Lgl is a RNA binding, cytoskele-

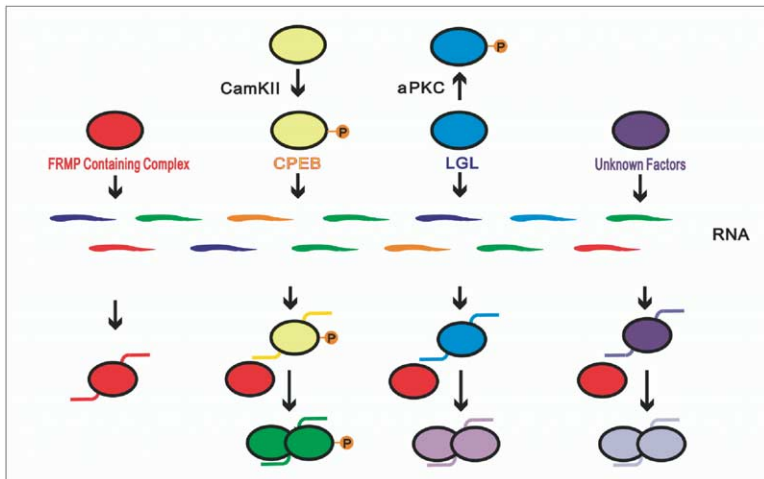


Figure 1. Multiple, Context-Specific Protein Partners May Regulate the Specificity of FMRP Translation Regulation

FMRP has a broad mRNA binding spectrum, but cooperative interactions with other mRNA binding proteins, such as CPEB/Orb and Lgl, may specify select mRNA targets temporally and spatially. Both CPEB/Orb and Lgl are regulated by kinase phosphorylation. When complexed with FMRP, these proteins likely define mRNA intersection targets for translation regulation. There are likely additional partners of FMRP, which further hone temporal or spatial functions of FMRP.

tal protein implicated in cell polarity and transport (Betschinger et al., 2005). Lgl is one of three “neoplastic tumor suppressor genes” (nTSGs), including the membrane-associated, multi-PDZ scaffolding proteins Scribble (Scrib) and Discs-large(Dlg)/PSD-95—two proteins with known critical roles in synaptic development/function at the neuromuscular junction (NMJ; Ruiz-Canada et al., 2004). Consistently, Zarnescu et al. (2005) show that *lgl* is a dominant enhancer of the *dfmr1* mutant NMJ structural overgrowth phenotype, with a 2-fold increase in synaptic bouton number in the double heterozygote. Unlike Scrib and Dlg, Lgl is not an obvious scaffold and does not display polarized localization. However, Lgl is at the plasma membrane, together with Scrib and Dlg, and this localization is critical for Lgl function (Betschinger et al., 2005). In *scrib* and *dlg* mutants, Lgl is not localized to plasma membrane, suggesting that Scrib and Dlg recruit Lgl to the membrane. Lgl may regulate FMRP function during sorting, transport, and/or retention of specific cotarget mRNAs (Figure 1). Zarnescu et al. (2005) propose that Lgl regulates the polarized delivery and/or retention of specific target mRNAs during synaptic development/plasticity. Lgl assembles with dFMRP in a common complex (Figure 1). Immunoprecipitation experiments combined with Affymetrix microarrays were used to identify mRNAs that associate with this complex, revealing 83 dFMRP-associated transcripts, 78 Lgl-associated transcripts, and an overlap of nine potential joint targets. Thus, only a few mRNAs bind dFMRP and Lgl in common, defining a small percentage of mRNA cotarget overlap (Figure 1). Importantly, Lgl association with all nine transcripts is lost in *dfmr1* mutants, showing that Lgl interaction depends genetically on dFMRP. All nine mRNAs encode proteins of unknown function, although two display a circadian cycle, which may be of interest owing to dFMRP’s requirement for some circadian rhythms (Zhang and Broadie, 2005).

Lgl has recently been shown to be regulated by the PAR complex via aPKC-zeta phosphorylation, promoting its dissociation from the actin cytoskeleton and plasma membrane and rendering it inactive (Betschinger et al., 2005; Figure 1). Zarnescu et al. (2005) show that

both aPKC-zeta and PAR-6 interact with dFMRP, either alone or in complex with Lgl, and dFMRP is coenriched in oocytes with Bazooka/PAR-3 and aPKC-zeta. Both PAR-3 and PAR-6 mutants act as dominant enhancers of the dFMRP overexpression-induced rough eye phenotype, suggesting that the Par complex antagonizes dFMRP function. In this context, it is particularly interesting to note that aPKC-zeta as a key regulator of the PAR complex was recently shown to regulate microtubule dynamics to control NMJ structural morphogenesis (Ruiz-Canada et al., 2004). The *aPKC-zeta* mutant suppresses the dFMRP overexpression rough eye phenotype, and *aPKC-zeta* dominantly suppresses the NMJ synaptic overgrowth in *dfmr1* mutants. These results strongly suggest that aPKC-zeta and dFMRP function together to regulate structural synaptogenesis. Immunoprecipitation experiments indicate the conserved presence of an aPKC-zeta/FMRP/mLgl complex in the mouse brain, where it is developmentally regulated in a manner consistent with the hypothesis that the temporal/spatial role of dFMRP in selective translation regulation is dependent on complexing with mLgl (Figure 1). In mammals, FMRP function is regulated by phosphorylation at three conserved serine sites. This phosphorylation is associated with stalled polyribosomes and, so, is believed to modulate translation regulation of target mRNAs. The interaction of dFMRP with the PAR complex is particularly interesting given the role of aPKC-zeta in learning and memory and its proposed function as a synaptic tag.

Kendal Broadie and Luyuan Pan

Department of Biological Sciences
Kennedy Center for Research on Human Development
Vanderbilt University
Nashville, Tennessee 37232

Selected Reading

- Atkins, C.M., Nozaki, N., Shigeri, Y., and Soderling, T.R. (2004). *J. Neurosci.* 24, 5193–5201.
Barnard, D.C., Ryan, K., Manley, J.L., and Richter, J.D. (2004). *Cell* 119, 641–651.

- Betschinger, J., Eisenhaber, F., and Knoblich, J.A. (2005). *Curr. Biol.* *15*, 276–282.
- Brittis, P.A., Lu, Q., and Flanagan, J.G. (2002). *Cell* *110*, 223–235.
- Costa, A., Wang, Y., Dockendorff, T.C., Erdjument-Bromage, H., Tempst, P., Schedl, P., and Jongens, T.A. (2005). *Dev. Cell* *8*, 331–342.
- Ruiz-Canada, C., Ashley, J., Moeckel-Cole, S., Drier, E., Yin, J., and Budnik, V. (2004). *Neuron* *42*, 567–580.
- Wells, D.G., Dong, X., Quinlan, E.M., Huang, Y.S., Bear, M.F., Richter, J.D., and Fallon, J.R. (2001). *J. Neurosci.* *21*, 9541–9548.
- Zalfa, F., Giogi, M., Primerano, B., Moro, A., Di Penta, A., Reis, S., Oostra, B., and Bagni, C. (2003). *Cell* *112*, 317–327.
- Zarnescu, D.C., Jin, P., Betschinger, J., Nakamoto, M., Wang, Y., Dockendorff, T.C., Feng, Y., Jongens, T.A., Sisson, J.C., Knoblich, J.A., et al. (2005). *Dev. Cell* *8*, 43–52.
- Zhang, Y.Q., and Broadie, K. (2005). *Trends Genet.* *21*, 37–45.