Editor's Note: These short, critical reviews of recent papers in the *Journal*, written exclusively by graduate students or postdoctoral fellows, are intended to summarize the important findings of the paper and provide additional insight and commentary. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

A Fragile Balance at Synapses: New Evidence Supporting a Role for FMRP in Homeostatic Plasticity

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Review of Soden and Chen

Homeostatic synaptic scaling operates in tandem with forms of neuronal plasticity such as long-term potentiation and long-term depression to maintain neural circuit activity within a stable range. Experimentally, homeostatic adaptations at synapses are usually induced by prolonged spike blockade [with tetrodotoxin (TTX)], yielding a form of synaptic scaling thought to involve cell-wide, transcription-dependent changes that affect every synapse on a given neuron (Turrigiano, 2008). However, more recent evidence has also identified locally mediated adaptive mechanisms that allow spatially discrete synaptic compensation within a neuron's highly compartmentalized dendritic arbor (Sutton et al., 2006; Rabinowitch and Segev, 2008). Early work in this area demonstrated that concurrent action potential and NMDA receptor (NMDAR) blockade (TTX + the NMDAR antagonist APV) promotes dendritic translation of GluA1 AMPAR subunits, leading to localized enhancement of synaptic strength (Sutton et al., 2006). Other work demonstrated that TTX and APV treatment drives intracellular synthesis of retinoic acid (RA), which reduces

the association of the RNA-binding protein RAR α with select mRNAs such as GluA1, thereby removing their translational block (Aoto et al., 2008).

Another RNA-binding protein that regulates dendritic translation is the fragile X mental retardation protein (FMRP) (Zalfa et al., 2003; Muddashetty et al., 2007). As evidenced by the devastating symptoms in fragile X syndrome (FXS), mutations in the *Fmr1* gene result in severe disruptions in cognitive and social abilities, suggesting a pivotal role for FMRP in basic learning and memory processes (Bassell and Warren, 2008). In a recent article published in *The Journal of Neuroscience*, Soden and Chen (2010) demonstrate that FMRP is directly involved in homeostatic plasticity, and provide evidence to support it acting within dendrites to regulate the translation of specific mRNAs in response to increases in RA signaling.

To investigate a potential role for FMRP in local synaptic scaling, Soden and Chen first examined the effects of prolonged action potential and NMDAR blockade (36 h TTX + APV) in organotypic hippocampal slices prepared from wild-type (WT) or *Fmr1* knock-out (KO) mice. Whereas CA1 pyramidal neurons exhibited enhanced mEPSC amplitude following TTX+APV treatment, no such scaling response was evident in CA1 neurons from *Fmr1* KO mice (their Fig. 1). Because this group had previously shown a role for RA in homeostatic scaling induced by TTX+APV treatment (Aoto et

al., 2008), the authors next examined whether FMRP was required upstream or downstream of activity-dependent RA synthesis. Using a fluorescent reporter in which three tandem retinoic acid response elements (RAREs) control GFP transcription, the authors found that cultured WT and *Fmr1* KO hippocampal neurons displayed equivalent levels of RARE-GFP reporter expression after TTX+APV treatment (their Fig. 2). However, the protein synthesis-dependent increase in mEPSC amplitude induced by direct RA treatment (4 h) was lost in *Fmr1* KO neurons. In addition, Soden and Chen found that RA application induced a translation-dependent increase in the levels of GluA1, GluA2, and eEF2 in synaptoneurosomes in organotypic slices from WT, but not *Fmr1* KO, slices (their Fig. 5). Finally, in studies where specific proteins were immunoprecipitated following metabolic labeling with radiolabeled amino acids, the increase in GluA1 synthesis driven by RA treatment was also lost in *Fmr1* KO neurons. Together, these results suggest that FMRP plays a role downstream of RA in dendritic protein synthesis, where it is required for the translation of specific mRNAs in response to RA signaling.

Although Soden and Chen (2010) demonstrated a critical role for FMRP in homeostatic plasticity dependent on RA synthesis, interestingly, they found that neither FMRP nor RA was required for homeostatic enhancement of synaptic strength induced by TTX alone [Soden and Chen (2010), their Fig. 4]. In WT

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and *Fmr1* KO neurons, 60 h of TTX treatment induced a similar increase in mEPSC amplitude, and in each case the enhanced mEPSCs were resistant to inhibition with the polyamine philanthotoxin, which targets GluA2-lacking AMPARs. In contrast, scaled mEPSCs following TTX+APV treatment are specifically reversed by polyamine toxins [Sutton et al. (2006); Aoto et al. (2008); Soden and Chen (2010), their Fig. 2]. These observations underscore the notion that multiple forms of homeostatic plasticity exist in hippocampal neurons, and suggest that FMRP plays a specific role in synaptic adaptations that require local translation near synaptic sites.

Rescue experiments using viral expression of FMRP in organotypic hippocampal slices from *Fmr1* KO mice indicated that proper RA-induced scaling in CA1 neurons can be restored by returning FMRP to appropriate levels (their Fig. 7). That acute restoration of FMRP expression can restore RA-induced scaling argues against the possibility that defects in synapse development or maturation in *Fmr1* KO neurons accounts for the absence of homeostatic control, and supports a specific requirement for FMRP in RA-induced scaling. To elucidate FMRP's mechanism of action in RA-induced scaling, Soden and Chen assessed the efficacy of two FMRP mutants in additional rescue experiments. One mutant, FMRP Δ RGG, lacked the RGG box necessary for binding to G-quartet structures on select target mRNAs. The other mutant, FMRP(I304N), carried a point mutation in a crucial KH domain necessary for binding to mRNAs with a "kissing complex" motif. Neither of these mutants had an effect on AMPAR levels or basal synaptic transmission when expressed in WT neurons (their supplemental Fig. 5). Likewise, expression of FMRP Δ RGG-GFP in slices from *Fmr1* KO mice had no effect on baseline mEPSC amplitude (their Fig. 7) or GluA1 levels as assessed via Western blotting (their Fig. 8). In contrast, expression of FMRP(I304N)-GFP decreased basal mEPSC amplitude and reduced GluA1 protein levels in *Fmr1* KO cells (their Fig. 7, Fig. 8). Given previous work showing an interaction between FMRP and GluA1 mRNA in synaptoneurosomes (Muddashetty et al., 2007), these results raise the possibility that FMRP may normally associate with and suppress the translation of GluA1 mRNA via its RGG box and not its KH domain, though Soden and Chen did not directly examine the mRNA binding properties of either mutant. Importantly, neither mutant rescued scaling induced by RA or TTX+APV treatment, indicating

that recovery of FMRP's ability to suppress GluA1 synthesis may not be sufficient to restore its role in RA-induced dendritic translation.

The differential effect of the FMRP mutants on baseline mEPSC amplitude and GluA1 levels suggests that their inability to rescue synaptic scaling results from distinct impairments. The inability of FMRP Δ RGG to rescue synaptic scaling in *Fmr1* KO cells may stem from the possibility that the RGG box is necessary for FMRP's interaction with GluA1 mRNA. If this is the case, why then is expression of the FMRP(I304N) mutant, which contains an intact RGG box and clearly exerts a suppressive effect on GluA1 expression, not successful in restoring RA-induced scaling? Previous reports have shown that this mutation blocks the ability of FMRP to associate with translating polyribosomes, as well as with its autosomal paralog FXR2P (Feng et al., 1997). It may be that mRNP particles containing FMRP must be associated with actively translating ribosomes for RA-induced translational regulation to occur, so disrupting this interaction renders FMRP(I304N) unable to rescue the scaling effect in *Fmr1* KO cells. On the other hand, given recent data suggesting that FMRP and FXR2P play unique roles during the expression of mGluR-LTD (Zhang et al., 2009), it is possible that FXR2P may also serve an important function during RA-induced synaptic scaling, and that this function requires its interaction with FMRP. Future experiments should clarify the precise aspect of FMRP function that is necessary for homeostatic plasticity.

In the experiments detailed above, Soden and Chen have provided compelling evidence that FMRP plays an important role in RA-induced synaptic scaling. However, one remaining unanswered issue concerns the mechanism by which FMRP regulates the effects of RA on dendritic protein synthesis. In coimmunoprecipitation studies, Soden and Chen found no evidence for a direct physical interaction between FMRP and RAR α (their Fig. 6), yet FMRP was clearly required for increased translation signaled through RAR α . The authors suggest that FMRP and RAR α need to be bound to the same mRNAs for RA-induced translational changes to occur. Indeed, two of the RAR α targets highlighted in this study, GluA1 and GluA2 mRNA, are also known targets of FMRP (Muddashetty et al., 2007), suggesting that combinatorial action of these two RNA-binding proteins might be responsible for mediating the specific alterations in

translational efficiency necessary for local homeostatic plasticity. While this idea is attractive, it is also possible that FMRP is necessary for the appropriate targeting of GluA1 mRNA to specific RA-response sites near synapses. Another possibility is that FMRP, through an allosteric mechanism, operates in concert with RA to induce the conformational changes in RAR α necessary for translational disinhibition of specific mRNAs. In the Chen laboratory's initial series of studies of RA-induced synaptic scaling, they found that treatment with the RAR α -specific agonist AM580 was sufficient to stimulate local synthesis of GluA1 and cause an enhancement of mEPSC amplitude (Aoto et al., 2008). Although the authors clearly demonstrate that scaling induced by direct RA application is lost in FMR1 KO neurons, it would be informative to know whether this same effect is observed following RAR α activation with AM580.

Although a number of important questions remain, Soden and Chen's work clearly establishes a role for FMRP in homeostatic control of excitatory synapse function and serves to further our understanding of the synaptic etiology of FXS. To this point, the abnormal mGluR-LTD observed in FMR1 KO mice has received considerable attention as a potential link to the cognitive dysfunction associated with this disorder. These new results raise the intriguing possibility that dysfunctional homeostatic regulation, perhaps in concert with defects in mGluR-dependent signaling in neurons, plays an important role in the devastating symptoms of FXS. The extent to which altered homeostatic plasticity contributes to the pathogenesis of FXS and other such disorders is an important area of future study.

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