Unfolding neurodevelopmental disorders

The complexity of the brain adds another level of difficulty to our understanding of how the brain develops, matures and functions. Both structural and molecular components define brain functional connectivity, and its alteration may result in developmental, behavioral and social deficits. Uncovering the roots and mechanisms behind neurodevelopmental disorders, such as fragile X syndrome or autism, is the goal of several lines of research. Despite the challenges associated with studying these diseases, new advances are linking pathological genetic changes with mechanisms in the brain. In Bench to Bedside, Guoping Feng and Jonathan Ting peruse a study that uncovers how fragile X syndrome–causing gene mutations unleash a translation break that finally leads to overexpression of synaptic proteins that alter the proper transmission of signals at the synapse. Furthermore, changes in the brain during the development of a person can also provide information about when and where the diseased brain loses functional connectivity. In Bedside to Bench, Jeffrey Neul proposes that studying the functional networks in people with autism and other neurodevelopmental disorders, and correlating changes with functional connectivity in animal models of these diseases, will uncover the mechanisms of normal and abnormal development and suggest possible treatment strategies.

■ BENCH TO BEDSIDE

Found in translation

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Fragile X syndrome (FXS) is the most common form of heritable intellectual disability in humans, affecting approximately 1 in 4,000 males and 1 in 8,000 females[1]. Individuals with FXS may also have dysmorphic facial features, low muscle tone, enlarged testes in males, stereotyped and repetitive behaviors and deficits in social interaction. The disorder is caused by trinucleotide repeat expansions in the promoter region of the fragile X mental retardation 1 (*Fmr1*) gene, which leads to transcriptional silencing and severely diminished production of the encoded neuronal mRNA binding protein fragile X mental retardation protein (FMRP). *Fmr1* is located on the X chromosome, and the presence of extensive repeat expansions in *Fmr1* causes a constricted appearance of the chromosome due to hypermethylation at this site—hence the name 'fragile X' syndrome. In the normal brain, FMRP is thought to negatively regulate the translation of proteins important for development and function of excitatory synapses. Yet the detailed molecular mechanisms of this putative function are not well established.

Previous studies have shown that the majority of FMRP protein is associated with polyribosomes, linking FMRP to the translational machinery[2,3]. Genetically modified mice harboring a missense mutation in FMRP that disrupts mRNA binding and polyribosome association show many features reminiscent of people with FXS[4], thereby establishing the functional importance of these physical associations *in vivo*. Notably, polyribosomes are found in dendritic shaft regions at the base of dendritic spines, and the association of FMRP with polyribosomes and mRNA places this protein in a prime position to regulate neuronal translation in response to ongoing synaptic activity. However, it is not clear how FMRP negatively regulates the translational machinery. Furthermore, although previous studies have identified some mRNAs as potential targets of FMRP[5,6], the full extent of FMRP targets is not known. Filling these knowledge gaps is crucial to our quest to develop effective treatments for the disorder. A recent study by Darnell *et al.*[7] provides important evidence that sheds new light on the detailed molecular function of FMRP protein in the brain.

The authors previously developed a method for identifying RNA-protein interaction sites *in vivo*—crosslinking-immunoprecipitation (CLIP)[8]—which uses ultraviolet irradiation of brain tissue to covalently crosslink protein and RNA species that are in direct physical association, followed by immunoprecipitation of proteins with bound RNAs. By introducing additional steps to enrich for polyribosomes and then immunoprecipitate the FMRP with covalently bound transcripts, the authors were able to identify and validate a large set of stringent FMRP target transcripts from wild-type mouse brain tissue[7]. The FMRP transcripts were processed into DNA for high-throughput sequencing, mapping to the mouse genome and bioinformatics analysis to determine functional clustering of FMRP target transcripts. Notably, this unbiased approach has provided a comprehensive list of predominantly new FMRP targets. A large portion of the targets cluster in groups directly related with synaptic development and function (**Fig. 1**), suggesting that synaptic dysfunction may be a key neural substrate for cognitive and behavioral impairments in FXS.

A novel 'brain-programmed' *in vitro* translation assay has been developed to probe the molecular mechanism of FMRP action in the translational control of identified targets[7]. In this assay, translation initiation was acutely inhibited with the antibiotic puromycin to enable a biochemical analysis of the ensuing ribosomal runoff as a readout of dynamic protein synthesis. This led to the discovery that FMRP is preferentially associated with transcripts that are stalled on ribosomes.

This analysis was also applied to brain samples lacking FMRP either due to genetic deletion or pharmacological displacement of FMRP from polyribosomes[7]. FMRP loss of function relieved ribosomal stalling in the runoff assay, and this effect was specific for the select set of FMRP target transcripts tested. This mechanism implies that loss of a translational pause in the synthesis of specific transcripts, in particular those encoding proteins at the synapse, can lead to altered protein levels and synaptic dysfunction that is crucial for the development of FXS and autistic features (**Fig. 1**). One caveat of this hypothesis is that limited evidence was provided to substantiate the overexpression of specific synaptic proteins from

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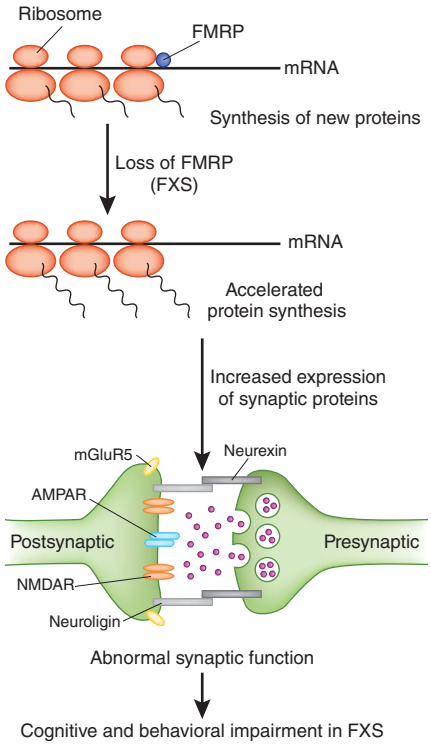

Figure 1 Synaptic dysfunction in FXS. FMRP can function as a translational break to stall new protein synthesis. Many of the targets of FMRP are mRNAs encoding synaptic proteins. In the absence of FMRP in FXS, the translation of many synaptic proteins are therefore accelerated, leading to aberrant synaptic function that contributes to cognitive and behavioral impairment in FXS. Some of the synaptic proteins identified as FMRP targets are listed. AMPAR, AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid)-type glutamate receptor; NMDAR, NMDA receptor.

FMRP-deficient brain by Darnell *et al.*[7]. In addition, no evidence was provided to directly link the loss of FMRP-mediated ribosomal stalling specifically to synaptic dysfunction in FXS. These topics represent important new directions for future investigations.

At present there are no specific treatments available for FXS in humans. One prominent hypothesis derived from animal model studies—the 'mGluR theory of fragile X'—posits that excessive protein synthesis of synaptic plasticity-gating proteins (of unknown number and identity) occurs in response to group I metabotropic glutamate receptor (mGluR) activation in the absence of FMRP[9,10]. The excessive production of these proteins is proposed to underpin aberrant neuronal function in FXS. In fact, treatment with mGluR5 negative allosteric modulators such as MPEP and genetic reduction of mGluR5 levels can ameliorate some but not all symptoms in FXS mouse models[11,12]. On the heels of this promising work in animal models, various new selective mGluR5 negative allosteric modulators are currently in clinical trials for people with FXS[13].

The study by Darnell *et al.*[7] identified mGluR5 as a key target of FMRP, thus providing strong support for the mGluR theory. More important, the identification of numerous FMRP targets involved in synaptic development and function strongly suggests a broader synaptic function and/or plasticity defect mechanism for FXS (**Fig. 1**) and may open new avenues for developing effective treatments for FXS. For several other key targets identified by Darnell *et al.*[7] such as NMDA (*N*-methyl-D-aspartate) receptors and GABA (γ-aminobutyric acid) receptors, which have crucial roles in regulating synaptic plasticity and neuronal activity, drugs modulating their function are already available or under development for other nervous system conditions. In light of this study, these existing compounds may find new indications for FXS and related disorders.

Interestingly, approximately 25% of individuals with FXS also show some degree of autistic behaviors[14]. As such, FXS is the leading known cause of autism linked to dysfunction of a single gene. Thus, efforts aimed at understanding the nature of brain dysfunction in FXS can provide valuable insights concerning several poorly understood neurodevelopmental disorders comprising a group termed the autism spectrum disorders (ASDs). Recently, synaptic dysfunction has emerged as a leading hypothesis for neural mechanisms of ASDs[15]. A comparison of the newly identified set of FMRP target transcripts with more than one hundred autism and ASD candidate genes from the Simons Foundation Autism Research Initiative (SFARI) database revealed significant overlap with several prominent autism and ASD candidate genes, including those encoding Shank3, neurexin-1 and neuroligin-3 (ref. 7).

It is also striking that the three most commonly detected duplications linked to ASDs were each found to encompass at least one or more of the FMRP gene targets identified by the CLIP methodology—despite spanning variable numbers of genes[7]. These findings provide a clear molecular mechanism linking FMRP function to the development of autistic symptoms common to FXS and ASDs. Such insights have provided clear avenues for continued basic research and may hasten progress in translational research efforts aimed at filling the important unmet clinical need of providing effective treatments for these debilitating neurodevelopmental disorders.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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■ BEDSIDE TO BENCH

The mystery of developing connections

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Knowledge of brain development has advanced with detailed understanding about neuronal birth and specification, axonal extension and synapse formation, dendritic pruning, myelination and regional brain specification. These events are characterized by both progressive and regressive processes, which occur in a

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