Trends in Genetics

CellPress REVIEWS

Review

Multifarious Functions of the Fragile X Mental Retardation Protein

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Fragile X syndrome (FXS), a heritable intellectual and autism spectrum disorder (ASD), results from the loss of Fragile X mental retardation protein (FMRP). This neurodevelopmental disease state exhibits neural circuit hyperconnectivity and hyperexcitability. Canonically, FMRP functions as an mRNA-binding translation suppressor, but recent findings have enormously expanded its proposed roles. Although connections between burgeoning FMRP functions remain unknown, recent advances have extended understanding of its involvement in RNA, channel, and protein binding that modulate calcium signaling, activity-dependent critical period development, and the excitation–inhibition (E/I) neural circuitry balance. In this review, we contextualize 3 years of FXS model research. Future directions extrapolated from recent advances focus on discovering links between FMRP roles to determine whether FMRP has a multitude of unrelated functions or whether combinatorial mechanisms can explain its multifaceted existence.

Trends

FXS, a leading heritable autism, is caused by a 5′ untranslated region (UTR) trinucleotide repeat expansion in the gene encoding FMRP.

The disease presents with stereotypical hyperexcitability and synaptic overelaboration, which are well replicated across a range of neural circuits in genetic models.

Canonically, FMRP is a mRNA-binding translational suppressor, but genetic roles range from chromatin binding to mRNA splicing and/or editing to other forms of translation control.

Additional FMRP roles include direct ion channel binding to regulate neural excitability, which may be an independent function or linked to activity-dependent translation control.

FXS cell type-specific defects in neurons and glia include altered calcium signaling, critical period synapse refinement, and E/I imbalance in neural circuitry.

Overview of Expanding FMRP Functions

FXS (see Glossary), a common genetic root of both intellectual disorders and ASD, is usually caused by a 5′ untranslated region (UTR) trinucleotide repeat expansion in the *FMR1* gene, resulting in loss of **FMRP**. FMRP functions as a master regulator of activity-dependent neurodevelopment, with null mutants manifesting hyperexcitability and reduced activity-dependent modulation of synapse maturation, refinement, and plasticity [1]. FMRP is canonically defined as an mRNA-binding translational repressor, with a broad but largely indeterminate range of transcript targets [2], although the scope of FMRP genetic functions continues to expand (Table 1). From the cytosol, FMRP is classically described to shuttle to and from the nucleus, with a recent *Drosophila* study mapping a novel C terminus mutation to this nuclear export function [3]. In the nucleus, recent work indicates that FMRP binds chromatin through tandem Tudor (Agenet) domains during the **DNA damage response** (DDR) to regulate genome stability in mice (Table 1) [4]. The involvement with DDR machinery has been shown to be important during spermatogenesis, but a requirement in neurodevelopment has not yet been established. In the *Drosophila* FXS model, the FMRP chromatin-binding function mediates replication stress-induced H2av phosphorylation, one of the earliest DDR responses to double-stranded breaks and replication stress [5], although a requirement in neurodevelopment has not yet been shown. Downstream of DNA interactions, but before canonical translational regulation roles, FMRP is proposed to act at multiple RNA life stages, in: mRNA editing, pre-mRNA splicing, and the miRNA pathway (Table 1). Recent work using zebrafish and mouse FXS models showed that FMRP alters RNA editing via interaction with the **adenosine deaminase ADAR**, supporting earlier *Drosophila* studies (Table 1) [6,7]. In the mouse FXS model, FMRP also works with RNA-binding protein 14 (RBM14) in **pre-mRNA alternative**

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Table 1. Recently Defined Genetic Functions of FMRP[a]

Proposed FMRP roles	FXS model	Refs
mRNA-binding translational regulator; canonically suppressing translation	Mouse and *Drosophila*	[2,33,34,46]
Chromatin binding; regulates genome stability, required for early DDR	*Drosophila* and mouse	[4,5]
ADAR-binding RNA-editing regulator; alters RNA editing of neural genes	*Drosophila*, zebrafish, mouse	[6,7]
RBM14 binding in pre-mRNA splicing; promotes mRNA target binding	Mouse	[8]
miRNA pathway regulation; neural circuit plasticity and behavioral output	*Drosophila*	[9,10]
Regulation of cell differentiation kinetics; affects both neurons and glia	Mouse	[14]
Ion channel binding to regulate gating; circuit excitability and plasticity	Mouse	[16,17]

[a]The table lists recently validated roles of FMRP (column 1), the FXS model system of the work (column 2), and the primary reference(s) discussed in the main text (column 3).

splicing (Table 1) [8]. FMRP has long been associated with the miRNA pathway, and recent studies suggest key interactions in circuit plasticity and behavioral output in *Drosophila* (Table 1) [9,10]. In both canonical and newly discovered RNA-binding functions of FMRP, the mechanism of FMRP mRNA-binding specificity has long been a conundrum, but recent insights into mRNA diversification provide further possible means for FMRP to recognize and regulate target transcripts [11,12].

The FXS field is rife with debates about the roles of FMRP, including cellular locations, temporal timing, and FMRP functions beyond DNA and/or RNA regulation. A long-term question concerns roles in neurons versus glia. A recent study of patients with FXS showed that FMR1 epigenetic alterations silenced FMRP specifically in neurons, but not in glia or neurons obtained from reprogrammed pluripotent stem cells [13]. In the mouse FXS model, recent work showed that FMRP loss changed the cell differentiation kinetics of both neurons and glia (Table 1) [14], and astrocyte-specific FMRP knockout in mice increased neuronal dendritic spine density similar to the global FXS condition [15]. Within neurons in all model systems, the soma contains the majority of FMRP, yet the lion's share of research and discussion focuses on local FMRP functions at the synapse [2,16–19]. Most studies have focused on postsynaptic mechanisms, but there appears to be at least as many presynaptic mRNA targets and presynaptic defects in mutants [2,16,20,21]. Yet another long-term debate concerns the timing of FMRP requirements [2]. Although FXS defects persist throughout life in both patients and animal models, this does not necessarily require continuous, maintained FMRP function [22–24]. Indeed, peak FMRP levels in both mouse and *Drosophila* FXS models occur during development, and prominent defects restricted to critical period neural circuit refinement may drive mature dysfunction [1,9,25–27]. Finally, the central role of FMRP mRNA binding itself has been strongly challenged. In addition to the range of newly discovered FMRP functions discussed above, including nuclear roles, such as chromatin binding, mRNA splicing, and mRNA editing (Table 1) [9,28], FMRP shows direct ion channel binding that modulates pore conductivity properties, and it is argued that many core FXS neurological defects may be explained by channel binding alone [20]. These two widely separated biological roles could represent completely divergent FMRP functions in neurons (Figure 1). Alternatively, FMRP RNA- and channel-binding functions could be linked in a common mechanism controlling activity-dependent protein synthesis (Figure 1).

In this review, we provide an update on major advances in the FXS field over the past 3 years; we group our discussions based on the biological functions of FMRP. While FXS phenotypes present similarly in different genetic models, we carefully define the animal system used in each body of work, and note when any contradictory findings have been found between models or

Glossary

Activity-regulated cytoskeleton-associated protein (ARC): an immediate-early gene (IEG) displaying activity-dependent mRNA localization to the synapse, where local translation is involved in synaptic plasticity, learning and memory.
Adenosine deaminase acting on RNA (ADAR): a class of RNA-editing enzymes that bind double-stranded RNA to convert adenosine to inosine by direct deamination.
Adenylyl cyclase (ADCY1): converts ATP into the second messenger cAMP.
Amyloid precursor protein (APP): an integral membrane protein concentrated at neuronal synapses.
Bone morphogenetic protein receptor type 2 (BMPR2): a serine-threonine receptor kinase of the transforming growth factor-β superfamily.
Calcium/calmodulin-dependent protein kinase II (CAMKII): a serine-threonine protein kinase regulated by calcium-calmodulin complexes, involved in many signaling cascades at synapses.
cAMP: derivative of ATP and a common second messenger in intracellular signaling at synapses.
Cytoplasmic FMR1-interacting protein 1 (CYFIP1): serves as a Rac-1 (an activating Ras GTPase) binding site in the WAVE complex to facilitate actin nucleation through ARP2/3 complex activation.
Diacylglycerol (DAG) kinase: converts DAG into phosphatidic acid (PA) during lipid signaling.
DNA damage response (DDR): phosphatidylinositide-3-kinases are activated by double-stranded DNA breaks and open replication forks to enable histone phosphorylation driving chromatin folding changes.
Down syndrome cell adhesion molecule (DSCAM): a transmembrane protein regulating synaptic growth. In patients with Down Syndrome, DSCAM is overexpressed owing to chromosome 21 trisomy.
Extracellular signal-regulated kinases (ERK): a core component of the Ras-Raf-MEK-ERK signaling pathway that regulates many functions; also known as mitogen-activated protein kinase.
Fragile X mental retardation protein (FMRP): classically defined as an mRNA-binding translation