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Minireview

Molecules and Cells

Fragile X Mental Retardation Protein in Learning-Related Synaptic Plasticity

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Fragile X syndrome (FXS) is caused by a lack of the fragile X mental retardation protein (FMRP) due to silencing of the *Fmr1* gene. As an RNA binding protein, FMRP is thought to contribute to synaptic plasticity by regulating plasticity-related protein synthesis and other signaling pathways. Previous studies have mostly focused on the roles of FMRP within the hippocampus - a key structure for spatial memory. However, recent studies indicate that FMRP may have a more general contribution to brain functions, including synaptic plasticity and modulation within the prefrontal cortex. In this brief review, we will focus on recent studies reported in the prefrontal cortex, including the anterior cingulate cortex (ACC). We hypothesize that alterations in ACC-related plasticity and synaptic modulation may contribute to various forms of cognitive deficits associated with FXS.

INTRODUCTION

Fragile X syndrome (FXS) is the most common inherited form of mental retardation, with a population prevalence of about 1/4,000 males and females (Hagerman, 2008; Turner et al., 1996). FXS results from an unstable trinucleotide (CGG) repeat expansion mutation in the *Fmr1* gene (Verkerk et al., 1991), located on the long arm of the X chromosome (xq 27.3). This mutation leads to hypermethylation and results in transcriptional silencing of *Fmr1*; thus, the gene product (FMRP, Fragile X Mental Retardation Protein) is reduced or absent in FXS (Devys et al., 1993) (Fig. 1). The most prominent physical features of FXS include macroorchidism, an elongated face, and low muscle tone (Hagerman, 2002). Additionally, FXS is characterized by intellectual disabilities including attention deficits and behavioural stereotypic movements (e.g. hand flapping, hand biting) (Hagerman, 2002; Reiss and Dant, 2003). Some FXS patients present attention deficit hyperactivity disorder (ADHD), anxiety, and autism, combined with language and other learning impairments (Hagerman et al., 2009). Furthermore, many FXS patients also show high susceptibility to epilepsy (Qiu et al., 2008).

The cerebral cortex and related structures are responsible for key cognitive functions such as attention, decision-making, sensory perception, learning and memory (see Zhuo, 2008 for review). Thus the cognitive impairments observed in FXS patients are likely mediated through alterations of FMRP related mechanisms within these brain areas. At the cellular level, activity-dependent plasticity, such as long-term potentiation (LTP) and long-term depression (LTD), is thought to be critical for long-term changes in synaptic transmission (Bliss and Collingridge, 1993; Kandel, 2001; Nicoll and Malenka, 1995; Zhuo, 2009). Gene regulation and new protein synthesis play important roles in long-term plasticity, including long-term alterations of cortical circuits (Bear, 1996; Kandel, 2001; Klann and Dever, 2004). Thus, the loss of brain functions may result from different genetic mutations, including the expression of mutated proteins or the deletion of key proteins. In this review, we will discuss recent progress of basic investigations of FMRP in central synaptic plasticity and related behaviours using gene knockout mice. We propose that FMRP acts as a key intracellular messenger for cortical plasticity. Characterisation of the molecular mechanisms involved in the modulation of FMRP may lead to future treatments for patients with FXS.

Fmr1-knockout (KO) mice: a mouse model for FXS

Fmr1 is the only gene known to be associated with *Fmr1*-related disorders. In 1994, a mouse model of FXS was developed through the inactivation of the *Fmr1* gene by homologous recombination (Bakker et al., 1994), interrupting exon 5 with a neomycin cassette, resulting in the absence of FMRP protein in adults. *Fmr1* "knockout" (KO) mice allowed rapid advancement in our understanding of the pathophysiology of FXS, offering a model system in which it may be possible to dissect the cascade of events leading from FMRP deficiency to synaptic plasticity and functional impairments (Bear et al., 2004).

Several anatomical features of FXS, such as macroorchidism (Bakker et al., 1994) and abnormal dendritic and spine development (Comery et al., 1997; Mineur et al., 2002; Restivo et al., 2005), have been observed both in FXS patients and in *Fmr1*-KO mice. However, the complexity of spine maturation processes indicate that alterations observed in *Fmr1*-KO mice vary according to mouse strain, brain regions investigated (Irwin et al., 2002)

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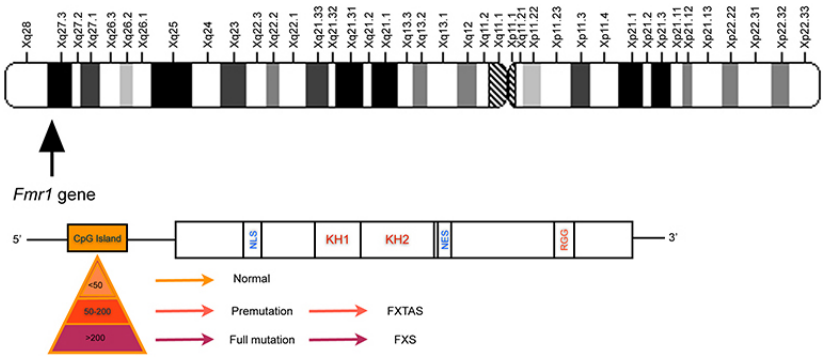

Fig. 1. (Top) X chromosome and Fmr1 gene location. (Middle) Untranslated region of *Fmr1* gene and CpG island (orange). Domains of FMRP: NLS, nuclear localization signal; KH1 and KH2, RNA binding domains; NES, nuclear export signal; RGG, RGG box. (Bottom) CpG island expansion leads to different phenotype: < 50 CGG-repeats are present in normal individuals. 55 < CGG-repeats < 200 are the premutation carrier alleles; > 200 CGG-repeats are the full mutation FXS alleles.

and across developmental stages (Galvez and Greenough, 2005). Furthermore, it is still unclear whether hippocampus-dependent learning impairments are present in *Fmr1*-KO mice (Bakker et al., 1994; Dobkin et al., 2000; Frankland et al., 2004; Paradee et al., 1999) (see Table 1). Recent work in the prefrontal cortex of *Fmr1*-KO mice however showed a consistent loss of cortical LTP in the ACC region and an attenuation of ACC-related trace fear memory (Zhao et al., 2005).

Distribution of FMRP

Central nervous system

FMRP is an ubiquitously expressed RNA-binding protein that is highly expressed within the central nervous system. Specifically, FMRP is present throughout the cerebral cortex, and has been observed in the thalamic and subthalamic nuclei. A high level of FMRP expression has been reported in the granular layer of the hippocampus and the cerebellum (Nimchinsky et al., 2001). More recently, immunostaing observations have shown that FMRP is located within the dorsal root ganglion and spinal cord neurons (Price et al., 2007). Additional studies have demonstrated the presence of FMRP in glial cells, particularly in oligodendrocytes during their early developmental stage (Wang et al., 2004a). Moreover, recent immunocytochemical studies of embryonic and postnatal mouse brains revealed co-expression of FMRP and GFAP (a glial cell marker) within the hippocampus during development but not in adult brains (Pacey and Doering, 2007; Price et al., 2007). These findings suggest a role of FMRP during development, and further indicate that FMRP may contribute to the developmental regulation of oligodendrocyte-specific mRNAs, such as the message encoded by myelin basic protein (MBP).

Subcellular location

FMRP is mostly localized in the cytoplasm of neurons. Through its four RNA binding domains it can aggregate with multiple RNAs and proteins to form messenger ribonucleoproteins (mRNP) that travel along the dendrites and spines. Various mechanisms of target recognition for different messages have been characterized (Bagni and Greenough, 2005), and thus a variety of different complexes can be formed with FMRP. These complexes have been reported to localize mainly in the cytosol, at branching points of the neuritis, and at bases of dendritic spines. In addition to cytoplasmic localization, immunogold techniques have revealed that FMRP is located within the nucleus of neurons during development and within nuclear pores (Feng et al., 1997). Further, although FMRP is mainly localized within the cytoplasm, upon synaptic stimulation it can travel as a part of an mRNP through microtubules along dendrites, axons and spines (Devys et al., 1993; Feng et al., 1997; Ferrari et al., 2007; Napoli et al., 2008). As a result, FMRP is able to contribute locally to events involved in synaptic plasticity.

FMRP in learning-related plasticity: hippocampus and cortex

Long term potentiation (LTP)

LTP is the key cellular model for learning, memory and persistent cortical changes related to chronic pain (Bliss and Collingridge, 1993; Kandel, 2001; Nicoll and Malenka, 1995; Zhuo, 2008). However, several studies have reported that LTP is unaltered in the hippocampus of *Fmr1*-KO mice. Four independent studies have shown that LTP within the (Cornus Ammonis 1) CA1 region of the hippocampus is unaltered in *Fmr1*-KO mice (Godfraind et al., 1996; Huber et al., 2002; Li et al., 2002; Paradee et al., 1999). However, different protocols can trigger different forms of LTP within the hippocampus (Hu et al., 2008; Lauterborn et al., 2007; Meredith et al., 2007; Shang et al., 2009), and may expose subtle differences in LTP. For instance, although both tetanic stimulation (Godfraind et al., 1996; Li et al., 2002) and conventional 10 burst theta trains (Larson et al., 2005) produce normal LTP in knockout hippocampal slices, changing the threshold levels of theta burst afferent stimulation revealed altered LTP in *Fmr1*-KO mice (Lauterborn et al., 2007). Similarly, Meredith and others reported that deficits in spike timing potentiation in the frontal cortex of *Fmr1*-KO mice are only evident with low threshold levels of stimulation and can be overcome with stronger stimulation (Meredith et al., 2007).

Using the pairing protocol Hu et al. (2008) reported a selective impairment of synaptic trafficking of GluR1-containing AMPA receptors that yielded a 50% reduction of LTP within the CA1 area of the hippocampus of *Fmr1*-KO mice. Recently, we reported that FMRP is also required for glycine induced LTP (Gly-LTP) in the CA1 region of the hippocampus through the activation of postsynaptic NMDA receptors (Shang et al., 2009). We found that there was a decrease in the expression of FMRP during the Gly-LTP, elicited by the activation of the glycine site of NMDA receptors, which can trigger mitogen-activated protein kinase (MAPK) signaling (Igartua et al., 2007; Shang et al., 2009). The activation of the MAPK cascade, together with a reduction in FMRP expression, seems to play a prominent role during the Gly-LTP process within the CA1 region of the hippocampus, which is impaired in *Fmr1*-KO mice. The prominent deficit found in *Fmr1*-KO mice is thus relayed through a reduction of MAPK pathway activation (Shang et al., 2009).

Abnormal plasticity of excitatory transmission has also been reported in the cortex of *Fmr1*-KO mice. For example, in both the somatosensory and neo-cortex of *Fmr1*-KO mice, LTP is reduced or absent (Desai et al., 2006; Larson et al., 2005; Li et al., 2002; Wilson and Cox, 2007; Zhao et al., 2005), however

Table 1. *In vivo* and *in vitro* studies on *Fmr1*-KO mouse

Methods	References
In vivo	
Anatomical features	Bakker et al. (1994)
Neuronal dysmorphogenesis	Comery et al. (1997); Galvez and Greenough (2005); Irwin et al. (2002); Mineur et al. (2002); Restivo et al. (2005)
Excitability phenotype	Chen and Toth (2001); Musumeci et al. (2000)
Learning impairments	Bakker et al. (1994); Dobkin et al. (2000); Paradee et al. (1999); Zhao et al. (2005)
No deficit or minor learning impairments	Frankland et al. (2004); Van Dam et al. (2000)
In vitro	
Cortex	
- LTP impairment	Larson et al. (2005); Li et al. (2002); Meredith et al. (2007); Wilson and Cox (2007); Zhao et al. (2005)
- Normal LTD	Desai et al. (2006)
CA1 of Hippocampus	
- LTP impairment	Hu et al. (2008); Lauterborn et al. (2007); Shang et al. (2009)
- Normal LTP	Godfraind et al. (1996); Huber et al. (2002); Li et al. (2002); Paradee et al. (1999)
- LTD enhancement	Dolen et al. (2007); Hou et al. (2006); Huber et al. (2002); Volk et al. (2007)

basal transmission (AMPA and NMDA receptor mediated) is unaltered in *Fmr1*-KO mice (Huber et al., 2002; Pfeiffer and Huber, 2009). Furthermore, we recently found that FMRP is required for NMDA receptor-dependent LTP in the cingulate region of the prefrontal cortex (Zhao et al., 2005). We also observed that LTP was reduced or blocked in the ACC of *Fmr1*-KO mice. This deficit is relatively selective, and presynaptic mechanisms are not likely the cause of the impairment. Furthermore, basal transmission within the ACC of *Fmr1*-KO mice is unaltered. The impact of FMRP in ACC LTP is likely mediated through postsynaptic FMRP regulation of AMPA receptor trafficking (Zhao et al., 2005). Although the molecular mechanisms mediating FMRP regulation of ACC LTP are still unknown, these observations link FMRP to LTP within the ACC, and suggest that this area be further investigated to understand specific forms of cognitive deficits associated with FXS.

Long term depression (LTD)

Hippocampal LTD is an important form of synaptic plasticity, in addition to LTP. Several studies investigating the role of FMRP in LTD have observed its involvement in the regulation of local activity-dependent protein synthesis, which is absent in *Fmr1*-KO mice. Most observations have focused on the mGluR-LTD pathway, which appears to be the most compromised in *Fmr1*-KO mice, and has lead to the formulation of an mGluR theory of FXS (Bear et al., 2004). The mGluR theory of FXS states that FMRP normally acts as a repressor of protein synthesis downstream of Gp1 coupled to mGluRs activation. In the absence of FMRP, this pathway is altered and in FXS exaggerated mGluR signalling is observed (Bear et al., 2004; Ronesi and Huber, 2008). As a consequence, LTD is modestly enhanced in the CA1 neurons of *Fmr1*-KO mice (Hou et al., 2006; Huber et al., 2002). The overactivity of the mGluR pathway is believed to cause many symptoms of FXS, including hyperactivity and anxiety. Importantly, administration of mGluR antagonist (MPEP) (McBride et al., 2005; Nakamoto et al., 2007; Tucker et al., 2006; Yan et al., 2005) or the genetic reduction of group I mGluR (Bassell and Gross, 2008; Dolen et al., 2007) can correct the exaggerated mGluR pathway observed in the absence of FMRP.

Possible roles of FMRP in cortical LTD have, thus far, not been investigated, although a developmentally transient circuit defect in the barrel cortex of *Fmr1*-KO mice has been observed, which may suggest effects on other cortical circuits (Bureau et al., 2008).

GABAergic transmission

Alterations of inhibitory GABAergic transmission have also been postulated in morphological, neurochemical, and molecular studies of *Fmr1*-KO mice (D'Antuono et al., 2003; D'Hulst et al., 2006; El Idrissi et al., 2005). Reduced activation of frontostriatal circuits has also been reported in FXS which is correlated with levels of FMRP expression (Menon et al., 2004). A recent study found that the absence of FMRP is associated with normal glutamate-mediated transmission but abnormal GABA transmission in the striatum, due to an increase in transmitter release from GABAergic nerve terminals (Centonze et al., 2007). In addition a reduced tonic $GABA_A$ current and an altered expression of $GABA_A$ receptor subunits $\alpha 5$ and δ were found in the subiculum of *Fmr1*-KO mice (Curia et al., 2009). However, it must be noted that such possible inhibitory alterations seem to cause undetectable changes in excitatory synaptic transmission in several forebrain regions, including the hippocampus and cortex. For example, excitatory transmissions recorded from brain slices of *Fmr1* KO mice appear to be quite normal (Centonze et al., 2007). Additionally, it has been hypothesized that the altered composition of $GABA_A$ receptor subunits could be related to the behavioural and neurological phenotypes of FXS, including epilepsy, anxiety, depression and learning and memory defects (D'Hulst and Kooy, 2007). Future studies are clearly needed to confirm and identify molecular details.

FMRP links to dopamine

Neuronal function within the prefrontal cortex is strongly influenced by dopamine signaling through D1 and D2 receptors (Sawaguchi and Goldman-Rakic, 1991; Wang et al., 2004b; Yuen and Yan, 2009). Although mechanisms underlying AMPA receptor trafficking have been well studied in the hippocampus, cerebellum, and cortex (Collingridge et al., 2004), little is known about how synaptic AMPA receptor levels are regulated by activation of dopamine receptors. Nevertheless, it is well known that D1 receptors preferentially link to Gs proteins, and subsequent activation of adenylyl cyclases (ACs) and protein kinase A (PKA) pathways (Huang and Kandel, 1995; Missale et al., 1998). Furthermore, evidence of a clear pathway that links

FMRP and the dopamine system has been recently presented (Wang et al., 2008). A possible contribution of FMRP to dopamine-induced GluR1 synaptic insertion and dopaminergic facilitation of LTP was observed, as potentiation-induced increases of GluR1 surface expression in the prefrontal cortex via D1 receptor activation was significantly attenuated in *Fmr1*-KO neurons. Moreover, D1 receptor inhibition is regulated by its phosphorylated state, which is modulated by translocation of G-coupled receptor kinase 2 (GRK2) to the membrane (Wang et al., 2008). We have shown that the interaction between FMRP and GRK2 can regulate D1 receptor inhibition through a subcellular re-distribution of GRK2. In *Fmr1*-KO mice, the absence of the FMRP-GRK2 coupling results in constitutive phosphorylation of D1 receptors and uncoupling from G protein signaling-pathways. These findings suggest that FMRP plays a role in NMDA receptor-dependent LTP and DA receptor-mediated facilitation (Wang et al., 2008).

Additionally, in the Drosophila model for FXS, it has been observed that dopamine synthesis is upregulated. As a consequence, a high rate of this neurotransmitter is available to be secreted at synapses (Zhang et al., 2005). It has been reported that *Fmr1*-KO females present age-dependent, region-specific alterations in brain amino acids (Gruss and Braun, 2004). As dopamine receptors mediate the regulation of PKA dependent modulations of K^+ and Ca^{2+} currents to the overall excitability of the prefrontal cortex, dopamine and serotonin provide a mechanistic explanation for some aspects of cognitive and behavioral deficits in FXS patients.

Activity-dependent translation of *Fmr1*

One of the major components of long-term structural and functional changes in the synaptic plasticity process is elicited by the activity-dependent gene expression of critical genes involved in synaptic maturation and cytoskeletal rearrangement -- processes that are partially absent and/or miss-regulated in FXS. Several lines of evidence suggest that FMRP has an active role in mRNA transport and translation, and its own message, *Fmr1*, is found in both the soma and dendritic processes, where it is locally translated (Antar et al., 2004; Ferrari et al., 2007; Weiler et al., 1997). The activity-dependent translation of the *Fmr1* gene has been established since 1997, when the role of FMRP was still largely unknown. Eventually it became clear that a rapid translation of *Fmr1* occurs in the cortex upon activation of mGluR1 (Weiler et al., 1997). These observations were soon followed by a model of experience-dependent plasticity that confirmed the activity-dependent expression of the *Fmr1* gene within the somatosensory cortex (Todd and Mack, 2000).

How activity-dependent *Fmr1* translation is involved in the modulation of synaptic plasticity and how it is regulated is now partially established. It has been largely accepted that genetic deletion of FMRP enhances mGluR-dependent LTD. For example, FMRP seems to be critically involved in the biochemical regulation of the translation of several dendritic mRNAs during mGluR-LTD, and particularly its own message *Fmr1* (Hou et al., 2006). It has been shown that a dynamic translation and proteasoma-degradation of FMRP is required during mGluR-dependent LTD in the cortex (Hou et al., 2006). More recently, Wang and colleagues exposed the signaling cascade that leads to *Fmr1* expression increases, which involve the Ca^{2+}/calmodulin-dependent protein kinase IV (CaMKIV), a key molecule involved in the regulation of transcription of FMRP. The activation of mGluR1 through 3,5-dihydroxyphenylglycine (DHPG) can trigger the CREB signaling pathway, which is Ca^{2+} dependent, within ACC neurons (Wang et al., 2009). This mechanism also involves adenylyl cyclase 1 (AC1), which, together with calcium/calmodulin-dependent protein kinase type IV (CaMKIV), is a Ca^{2+} sensor leading to FMRP transcription activation through CREB phosphorylation in ACC neurons (Hao et al., 2008; Wang et al., 2009).

Several studies performed in the hippocampus region or in hippocampal primary neurons confirmed the upregulation of the *Fmr1* gene during mGluR-LTD processes (Hou et al., 2006). Specifically, an increase of *Fmr1* mRNA was observed in the dentate gyrus of the hippocampus after electroconvulsive shock, whereas typical LTP protocols do not elicit any detectable changes (Valentine et al., 2000). More recently our lab showed that Gly-LTP is strongly compromised in the CA1 region of the hippocampus of *Fmr1*-KO mice (Shang et al., 2009). Gly-LTP is largely due to the activation of the glycine site of NMDA receptors, and surprisingly FMRP expression is decreased upon Gly-LTP elicitation (Igartua et al., 2007; Shang et al., 2009). Another treatment that leads to a deregulation of *Fmr1* mRNA is the application of brain derived neurotrophic factor (BDNF), which can trigger an increase in TrkB signaling in both hippocampus (Castren et al., 2002) and cortex (Todd and Mack, 2000). Finally, most observations suggest that FMRP might regulate mRNA translation, acting as a link between transport and gene expression regulation, and its own level might be a functional player in the modulation of synaptic plasticity processes. Moreover different levels of FMRP expression deficits correlate with the severity of FXS (Tassone et al., 1999). Thus it is evident that a lack of FMRP in FXS results in abnormal plasticity. A clear understanding of these alterations will provide possible therapeutic avenues for FXS patients.

Biphasic regulation of FMRP

It is clear that the expression level of FMRP can be regulated by neuronal activities within adult neurons in a biphasic manner (see Fig. 2). Pharmacological and genetic studies have revealed the pivotal role of FMRP in the modulation of synaptic plasticity and learning and memory. Intriguingly, FMRP might also regulate synaptic transmission through the auto-regulation of FMRP levels (Antar et al., 2004; Ferrari et al., 2007; Weiler et al., 1997) and phosphorylation (Bassell and Warren, 2008). Future studies are clearly needed to dissect molecular pathways for FMRP related synaptic plasticity (LTP and LTD), and their contribution to behavioral functions under physiological and pathological conditions.

Conclusions and future directions

In summary, we described some of the prominent features of FXS, and in particular how cortical circuits, both excitatory and inhibitory, can be affected through the absence of FMRP. We propose a biphasic modulation of FMRP in central neurons by activity-dependent mechanisms and G protein coupled signaling pathways. Because FMRP is an RNA binding protein that can form different complexes, which can travel within the neuronal cell, it will be interesting to understand if different FMRP complexes might differently modulate the strength of the synaptic response. It is possible that different complex compositions and functions can give rise to activity-dependent specificity, different subcellular functions, and activation of different pathways, which may underlie different forms of behavior. Combining all the different aspects - from the molecular level to the behavioral - of specific impairments found in the *Fmr1*-KO mouse model might help in a better understanding of the molecular mechanisms involved in prominent and severe features

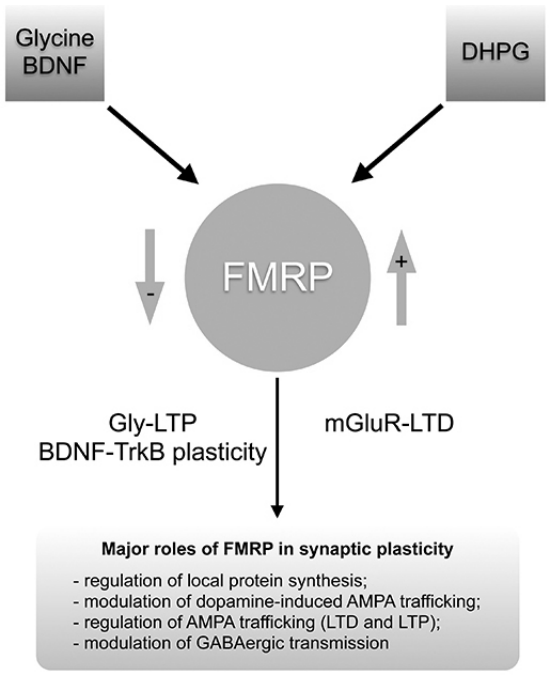

Fig. 2. Biphasic regulation of FMRP in the central synapses. The activity-dependent regulation of FMRP modulates synaptic plasticity events. Drug applications such as Glycine or BDNF can reduce the expression of FMRP within neurons, whereas DHPG application positively regulates the expression of FMRP at both dendrites and synapses. FMRP levels are critical for the regulation of synaptic transmission in LTP and LTD processes. Moreover, FMRP plays an important role in many aspects of neuronal activities.

of FXS. Ultimately, the final goal is to invest part of this precious knowledge to a preclinical trial focusing on specific FXS aspects, such as anxiety, autistic-like behavior, and learning impairments; and eventually translate these basic discoveries into effective clinical treatments in the near future.

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