J Neurodevelop Disord
DOI 10.1007/s11689-009-9015-x

Fragile x syndrome and autism: from disease model to therapeutic targets

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Received: 7 February 2009 / Accepted: 29 April 2009

Abstract Autism is an umbrella diagnosis with several different etiologies. Fragile X syndrome (FXS), one of the first identified and leading causes of autism, has been modeled in mice using molecular genetic manipulation. These *Fmr1* knockout mice have recently been used to identify a new putative therapeutic target, the metabotropic glutamate receptor 5 (mGluR5), for the treatment of FXS. Moreover, mGluR5 signaling cascades interact with a number of synaptic proteins, many of which have been implicated in autism, raising the possibility that therapeutic targets identified for FXS may have efficacy in treating multiple other causes of autism.

Keywords Fragile X · FXS · Metabotropic · Glutamate · Receptor · mglur · mglur5 · FMRP · Fragile x mental retardation protein · Synaptic plasticity · Long term depression · LTD · Protein synthesis · Translation · Ocular · Dominance · Plasticity · Visual · Cortex · Hippocampus · Inhibitory avoidance · Passive avoidance · Extinction · Autism · HOMER · SHANK · Neuroligin · Neurexin · Tuberous sclerosis · TSC · TSC1 · TSC2 · Rett · MeCP · BDNF · PTEN · Hamartoma · Angelman · UBE3 · Dendritic spine · Synapse · Development · Synapsopathy · Audiogenic seizure · Seizure · Mental retardation · Cognitive · Impairment

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Introduction

Leo Kanner first described autism in 1943 [1]. It wasn't until 1980 however, that autism was formally recognized in the Diagnostic and Statistical Manual of Mental disorders (DSM-III), and included as part of a new class, the Pervasive Developmental Disorders (PDD) [2]. At the same time, early psychodynamic theories of the etiology of autism [3] were being abandoned in favor of genetic ones. As early as 1975, case reports of monozygotic twins concordant for autism [4], followed by several systematic twin studies [5–10] substantiated the strong heritability of autism [11–13].

Standardization of diagnostic criteria [2], and improvements in our ability to reliably detect chromosomal abnormalities [14] allowed for the identification in the early 1980's of the first genetic cause of autism—Fragile X syndrome (FXS) [15–17]. Subsequently, the Fragile X gene (*FMR1*) was discovered [18], and by 1994 the first animal model became available [19]. This genetically engineered *Fmr1* knockout mouse (*Fmr1* KO), has been validated for FXS, and is currently one of the leading animal models of autism [20].

Using this mutant mouse, we have been able to address the role of the *FMR1* gene and the protein it encodes (fragile X mental retardation protein, FMRP) in brain development. Now, over 25 years since FXS was identified as a cause of autism, a new putative therapy has been proposed based on our understanding of the function of FMRP.

Modeling autism: a derailment of synaptic plasticity

Inherited mutations have the potential to disrupt brain development from the moment of fertilization onward; however, a genetic etiology does not preclude pathogenesis

Published online: 12 May 2009

involving regulated processes later in development. Symptoms of autism typically present during the early postnatal period, usually between ages 1–3 years [20]. This epoch, the so-called 'critical period' [21], corresponds to a dynamic phase of brain development in which neurite outgrowth, maturation of inhibition and signaling, axon myelination, and synaptic plasticity are set in motion by the complex interplay of molecular genetic programs and experience [22]. Disruption of any of one of these processes could hypothetically lead to the characteristic symptoms of autism, which include abnormal social interaction and communication, stereotyped repetitive behaviors, often with co-morbid mental retardation, epilepsy, sleep disturbances, attention deficit and hyperactivity [23]. Thus, it has been tempting to speculate that the pathogenesis of autism involves a derailment of at least one of these developmental processes [24–26]. Given this framework, studies of synaptic plasticity in the *Fmr1* KO mouse have been an obvious priority.

A potential breakthrough in understanding the pathogenesis of fragile X came from studies of group 1 metabotropic glutamate receptors (Gp1 mGluR) [27–31]. Gp1 mGluRs (which are further subdivided into mGluR1 and mGluR5 subtypes) couple to postsynaptic Gq-like G-proteins and phospholipase C (PLC) [32] as well as to extracellular signal-regulated kinase (ERK) transduction pathways [33, 34]. Their activation leads to the synthesis of new protein at the synapse [28, 35, 36], likely through the ERK signaling cascade [37, 38]. A functional consequence of Gp 1 mGluR-dependent protein synthesis in the hippocampus is long-term depression (LTD), a form of synaptic plasticity [29]. In the *Fmr1* KO mouse, this mGluR-LTD is exaggerated and no longer protein synthesis-dependent [31, 39].

Meanwhile, studies of FMRP revealed that the expression of the protein is developmentally regulated [40, 41], such that in the post-natal brain it is largely cytoplasmic [42, 43], predominantly expressed in neurons [44, 45] and enriched postsynaptically at glutamatergic synapses [46]. Furthermore, FMRP is an RNA binding protein that co-localizes with polyribosomes [44, 47–55] which are found at the base of dendritic spines where they are thought to mediate local translational control of the synapse [56]. Indeed, both in vitro and in vivo metabolic labeling studies have now directly shown that FMRP functions as a repressor of protein synthesis [57–60].

Taken together, these findings led to the hypothesis that Gp1 mGluRs and FMRP might work in functional opposition to regulate mRNA translation at the synapse, and that in the absence of FMRP, unchecked mGluR-dependent protein synthesis leads to the pathogenesis of the disease (Fig. 1) [61]. We have recently tested this so-called 'mGluR theory' and shown that increased levels of protein synthesis in the *Fmr1* KO mouse [59, 60], are restored to wild type (WT) levels by selective reduction of mGluR5 signaling [60]. This manipulation also significantly decreases the magnitude of Gp1 mGluR-LTD in *Fmr1* KO mice, confirming the role of mGluR5 in producing the exaggerated synaptic plasticity phenotype [60].

The synapse is too small to be directly visualized by light microscopy. However, dendritic spines (the postsynaptic half of an excitatory synapse) can be visualized, and are used to estimate the number of excitatory synapses in the brain. Dendritic spines are highly modifiable structures,

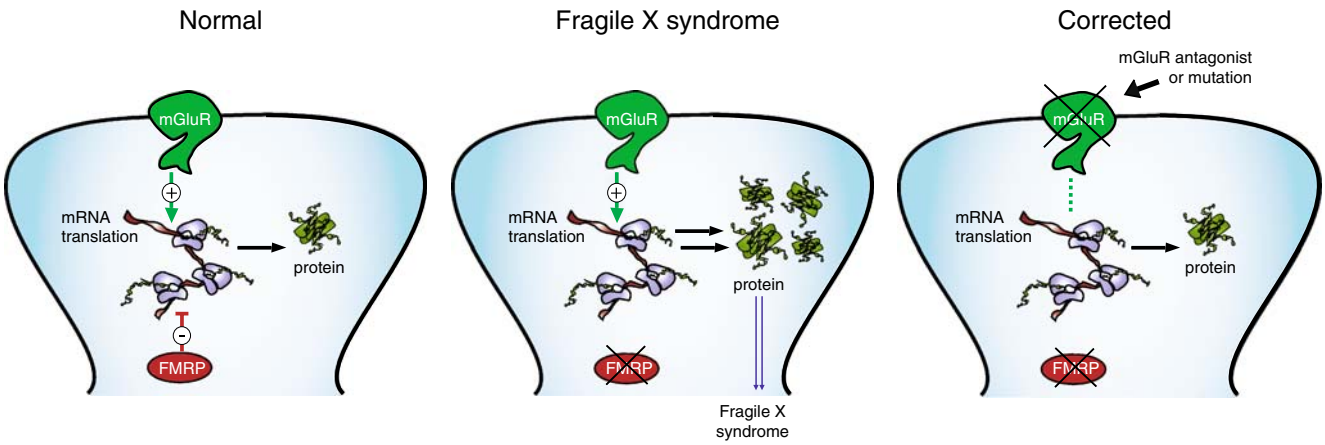

Fig. 1 Opponent regulation of protein synthesis by FMRP and GpI mGluRs. FMRP is a negative regulator of translation at the synapse. Stimulation of GpI mGluRs with DHPG leads to the synthesis of proteins. Furthermore, many of the long-term consequences of Gp1 mGluR activation are protein synthesis dependent. The mGluR theory posits that in the absence of FMRP, as is the case in Fragile X syndrome, this balance between FMRP and Gp1 mGluRs is lost, and unchecked protein synthesis at the synapse leads to the characteristic features of the disease. Furthermore, this balance could be restored by reducing Gp1 mGluR activity at the synapse, by either knockdown or pharmacological blockade of the receptor. The therapeutic implication of the theory is that symptoms of FXS syndrome could be corrected by appropriate modulation of GpI mGluR signaling

and changes in spine density and morphology have been correlated with synaptic plasticity [62]. Furthermore, abnormalities in dendritic spine morphology have long been associated with human mental retardation of unknown etiology [63], as well as with XLMR (x-linked mental retardation) [64], Down [65], Patau [65], Rett [66] and Fragile X syndromes [67, 68].

Dendritic spine structure is regulated by Gp1 mGluRs. Application of the selective mGluR5 agonist, DHPG, to cultured hippocampal neurons induces a protein synthesis dependent increase in the density of long thin spines [69]. Because DHPG application in cell culture also induces rapid protein synthesis dependent internalization of AMPA and NMDA receptors [70], receptor internalization may be the prelude to morphologic remodeling in response to plasticity inducing stimuli.

This response to stimulation with DHPG parallels spine changes seen in the *Fmr1* KO mice, which lent support to the theory that exaggerated signaling through mGluR5 in the absence of FMRP could account for this morphologic correlate of synaptic plasticity [61]. Consistent with this idea, recent studies have shown that that AMPA receptor internalization is exaggerated in the absence of FMRP [71] and both this and the increased spine density phenotype seen in *Fmr1* KO mice [60, 72–78] are rescued by selective reduction in mGluR5 signaling [60, 71].

Modeling autism: plasticity in vivo

While these in vitro and ex vivo demonstrations of opponent regulation by FMRP and mGluR5 provided the necessary foundation for identifying and correcting synaptic abnormalities, we also wanted to determine whether these interactions regulate circuit-level responses in the intact animal. Landmark studies of in vivo ocular dominance plasticity (ODP) in monkeys and cats [79–81] established a role for experience dependent plasticity in shaping the circuitry of the brain during the critical period. Moreover, because ODP occurs on the biologically relevant timescale, in response to perturbations of environmental stimuli using intrinsic patterns of neuronal activity, this paradigm is more readily translated to future studies in human patients (e.g. using visually evoked potentials [82] or transcranial magnetic stimulation [83]).

The development of transgenic technologies [19, 84, 85] and adaptation of the ODP paradigm to rodents [86–91] has allowed us to answer mechanistic questions about experience dependent plasticity in vivo. For example, ODP is in-part mGluR5 dependent [60], requires protein synthesis [92], and signals through ERK transduction [93]. In the *Fmr1* KO mouse, this plasticity is exaggerated, such that bidirectional modifications that require 7 days of monocular deprivation (MD) in WT mice [91], occur after only 3 days in the absence of FMRP [60]. Significantly this hyperplastic response is reminiscent of the exaggerated synaptic plasticity phenotype seen in the hippocampal slice [31], and is likewise restored to WT levels by 50% reduction of mGluR5 signaling [60].

Modeling autism: behavioral phenotypes

As mentioned above, epilepsy and mental retardation are both co-morbid features of autism [23]—an estimated 5–38% of autistic patients have seizure or subclinical epileptiform activity [94] while 70% have cognitive impairment [95, 96](but see, [97][98]). Thus, an important goal for modeling the disease is to establish behavioral tasks that recapitulate these symptoms in the *Fmr1* KO mouse.

An estimated 20% of human patients with FXS have epileptiform activity or generalized seizure [99, 100]. Audiogenic seizure (AGS) is a robust paradigm for inducing seizure in the *Fmr1* KO [60, 101–105] and recapitulates this neurologic feature of FXS and autism. Previous studies have not been able to account for increased epileptiform activity in *Fmr1*-KO mice by any of the anticipated mechanisms. For example, no differences have been observed between WT and *Fmr1*-KO mice in basal synaptic transmission, excitability, paired pulse facilitation, and long-term potentiation in the CA1 region of the hippocampus [106, 107].

Interestingly, it has been shown that agonists of group I mGluRs act as convulsants in rodents [32, 108] while selective Gp I mGluR antagonists block seizures in a range of rodent models of epilepsy [105, 109, 110]. Increases in epileptiform activity in response to mGluR5 stimulation are protein synthesis dependent [111, 112], suggesting that in addition to synapse specific changes, circuit level modulation of excitability is sensitive to the state of mGluR5 dependent protein synthesis [113]. Consistent with this idea, AGS seen in the *Fmr1* KO is attenuated by 50% reduction of mGluR5 signaling [60].

Despite the moderate to severe mental retardation seen in human patients with FXS [114], cognitive phenotypes in the *Fmr1* KO mice have been difficult to model [107, 115–117]. Inhibitory avoidance (IA) is a contextual (fear) conditioning paradigm used in animals to test hippocampus-based associative learning and memory [118]. IA extinction (IAE) is a paradigm that tests those conditioned responses in the face of contradictory contextual (safe) conditioning [119]. While IA learning is normal in *Fmr1* KO mice on the C57-Bl6 background [19, 60], we have recently identified an IAE phenotype in the *Fmr1* KO [60].

Although the synaptic mechanisms underlying IAE are not currently known, this behavior, like mGluR5-LTD, is

protein synthesis dependent [119]. Furthermore, since both mGluR5-LTD and IAE are exaggerated in the *Fmr1* KO mice and rescued by reduction of mGluR5 signaling [60], one interesting possibility is that mGluR5 LTD is the cellular mechanism subserving IAE learning. This mechanism is likely distinct from that which subserves IA, since IA training induces NMDA-LTP [120]and neither IA nor NMDA-LTP [106, 107] is disrupted in the *Fmr1* KO on the C57-Bl6 background.

Therapeutic implications

In summary, we have discovered that FMRP is a protein that acts to regulate protein synthesis and synaptic plasticity triggered by Gp1 mGluRs. Understanding this balance between FMRP and mGluR-5 has allowed us to restore normal function in the *Fmr1* KO model of autism—metabolic, morphologic, synaptic, circuit, and behavioral disruptions can all be corrected by reducing mGluR5 signaling by 50% [60]. Currently clinical trials based these and related findings are under way to determine safety and efficacy of mGluR modifying drugs in human patients with FXS and autism.

To put these findings in context, it is important to remember that mGluRs and FMRP do not exist in isolation at the synapse. As shown in Fig. 2, a number of other synaptic proteins that interact with the mGluRs either by direct physical contact or biochemical cascades, have also been identified as autism candidate genes [121–126] or single gene disorders associated with autism [127–134].

For example, Gp1 mGluR signaling converges on transduction cascades also implicated in PTEN hamartoma syndrome and Tuberous sclerosis complex (TSC), which are other single gene causes of autism. PTEN inhibits PI3K-dependent signaling, which couples Gq signaling to the mTOR/S6K pathway for protein synthesis [128]. TSC 1/2 inhibits this same mTOR pathway, by acting as a GTPase-activating protein for the Ras-related small G protein Rheb [135].

Structural proteins within the synapse also interconnect Gp1 mGluRs to various autism candidate genes. For example, both Shank and Homer proteins crosslink mGluR5 to the postsynaptic density [136], and misregulated Homer1b and PSD-95 have been implicated in the pathogenesis of FXS [137, 138]. The Neuroligin/Neurexin complex, important for synapse formation and implicated in autism, is in turn tethered to the synapse via its interaction with PSD-95 [125]. AlphaCaMKII, a major regulatory protein in synaptic plasticity [139] is also tethered to the synapse by PSD-95; absence of inhibitory phosphorylation of alphaCaMKII by UBE3a, has been implicated in Angelman syndrome [132]. Interestingly, mGluR5 stimulated protein synthesis of alphaCaMKII and PSD-95 are impaired in synaptoneurosomes from *Fmr1* KO mice [140]. Furthermore, CAMKII dependent phosphorylation of MeCP2 links

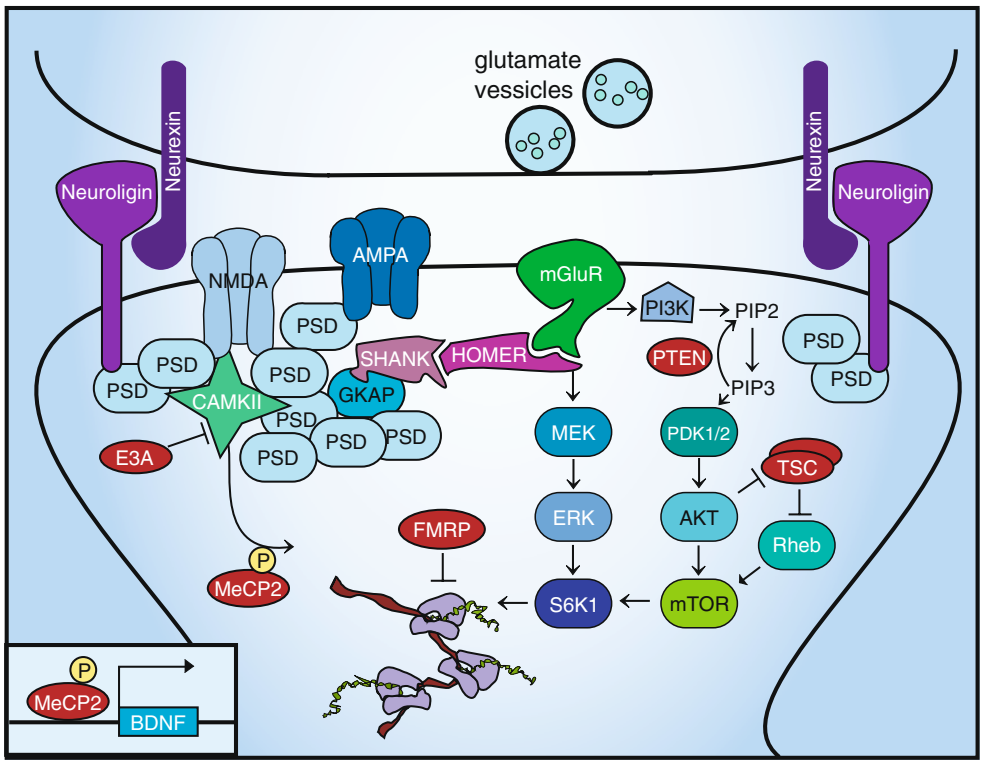

Fig. 2 Autism as a synapsopathy. mGluR5 interacts with a number of postsynaptic proteins. Some of these have been identified as autism candidate genes (shown in purple; HOMER, SHANK, Neuroligin, Neurexin); others are proteins associated with single gene causes of autism (shown in red: FMRP/FXS, TSC/Tuberous Sclerosis, PTEN/Hamartoma syndrome, MeCP2/Rett syndrome, E3A/Angelman's syndrome)

these synaptic proteins to Rett syndrome, another single gene disorder associated with autism, and transcriptional regulation of brain derived nerve growth factor (BDNF) [141]. In turn, TrkB mediated BDNF signals through ERK, regulates dendritic spine formation [142], and has also been implicated in the pathogenesis of FXS [143].

Together, these results suggest it may be useful to think of autism as a synapsopathy [144]—a disease where disruption of the synapse during development produces a common clinical picture, despite a heterogeneity of interconnected causes. It also raises the interesting possibility that treatments for one cause, such as fragile X, may have efficacy in treating other causes of autism.

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