IMPAIRED SYNAPTIC SIGNALLING INVOLVED IN FRAGILE X SYNDROME

Literature Review

Writing Assignment
Master Programme Neuroscience & Cognition, Utrecht University, 4/1/2023

Examiner: Dr. Fleur Velders (F.P.Velders@umcutrecht.nl)
Second reviewer: Dr. Harold MacGillavry (H.D.MacGillavry@uu.nl)

Kloosterhuis, K. (Kes)
k.kloosterhuis@students.uu.nl

Abstract

Fragile X syndrome (FXS) is a monogenetic disorder caused by loss of function of the Fmr1 gene encoding the RNA-binding protein FMRP, which is known to associate with ribosomes and act as a translational regulator. FXS shows a lot of overlapping cellular features with autism spectrum disorders (ASDs), including aberrancies in synapse number, morphology and function. This review aims to provide an overview of synaptic signalling pathways involved in FXS in the context of recent findings, and increase the understanding of the interplay of synaptic processes at hand to aid future research and FXS treatment development. Important mechanisms dysregulated in FXS synapses include metabotropic glutamate receptor (mGluR) mediated long-term depression (LTD), which is the focus of the popular mGluR theory of FXS, association and functioning of FMRP-containing translation complexes and competing regulatory complexes including the WAVE complex, various forms of synaptic plasticity involving mGluRs, N-methyl-D-aspartate receptors (NMDARs), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) and γ-aminobutyric acid receptors (GABARs) leading to a disturbed excitation-inhibition balance and actin cytoskeleton remodelling. FMRP deficiency leads to exaggerated dendritic synthesis of proteins involved in these processes which underly functional and structural synaptic plasticity, inducing FXS features including an increase in immature thin long spines with a lower postsynaptic density. Current FXS treatment development shows limited results, illustrating the need for a shift towards iPSC-derived models that can account for heterogeneity among patients and improve drug screenings, development of new treatment strategies and fundamental research on synaptic disease mechanisms underlying FXS.

Keywords: Fragile X syndrome, FMRP, Fmr1, synapse, synaptic plasticity, cytoskeleton remodelling

Inhoud

Introduction

Autism Spectrum Disorders (ASDs) are very complex and highly heterogenous developmental conditions. This heterogeneity makes it complicated to define a generalized clinical picture. However, a set of core symptoms has been identified, including deficits in social interaction and communication and repetitive and restrictive behaviours. Furthermore, many patients also show symptoms like sensory and motor impairment, developmental delay, sleep abnormalities, epilepsy and comorbidity with a wide range of psychiatric disorders (Bellosta & Soldano, 2019; Sledziowska, Galloway & Baudouin, 2020). Part of this heterogeneity can be explained by the variety of genetic and non-genetic causes that can underly the specific combination of symptoms for each patient (Park et al., 2016). However, in some cases of this type of neurodevelopmental disorders, a specific monogenetic cause can be determined, as is the case for fragile X syndrome (FXS).

FXS is a condition caused by mutations in the Fmr1 gene (bagni & Zukin, 2019). Symptoms of FXS resemble those described for ASDs and include reduced social interactions, repetitive behaviour, avoidant eye gaze, speech perseverations and comorbidity with attention-deficit/hyperactivity disorder (ADHD) in most of the younger patients. In the more extreme cases, patients can also be completely non-verbal and show aggressive and self-injurious behaviour (Bagni et al., 2012; Roberts et al., 2018). A lot of comorbidity occurs for FXS and ASDs, with 40 to 60% of male FXS patients and 20% of female patients meeting the criteria for diagnosis with ASD (Kaufman et al., 2017). This indicates the possibility of a shared molecular pathway.

The most well-known genetic defect causing FXS is an unstable CGG trinucleotide expansion in the 5'untranslated region (UTR) of the Fmr1 gene, leading to hypermethylation and transcriptional silencing (Bagni & Zukin, 2019; Clifton et al., 2020 & Khlebodarova et al., 2018). This causes a deficiency in FMRP protein, encoded by the Fmr1 gene, which is the basis of molecular pathway dysregulation involved in FXS. While FMRP has two paralogs in mammals, Fragile X Related 1 (FXR1) and Fragile X Related 2 (FXR2), only FRMP loss is known to cause the disordered phenotype (Coffee et al., 2010). In addition to transcriptional silencing, a few cases of point mutations located in the RNA-binding domains (RBDs) of FMRP were also found, impairing its functioning (Starke et al., 2022).

FMRP binds RNA and thereby controls localisation (to dendrites), stability and translation of various RNAs which are mostly involved in developmental processes which determine dendritic spine density and morphology, but also in maintaining and reforming neuronal connections via processes like synaptic plasticity (Bagni & Zukin, 2019; Pfeiffer & Huber, 2009). FMRP is believed to target as many as 400 to 600 mRNAs, including its own mRNA (Pfeiffer & Huber, 2009). Through its interaction with these mRNAs, FMRP mostly acts as a repressor of translation initiation, thereby regulating cellular protein levels in the synapse (Khlebodarova et al., 2018). In excitatory glutamatergic synapses, activity of FMRP itself is regulated by metabotropic glutamate receptor (mGluR) activity via a dynamic interplay between FMRP activating and inactivating agents (Khlebodarova et al., 2018). mGluR-activity activates both S6 kinase and PP2A phosphatase which respectively phosphorylate and dephosphorylate FMRP in a different time rate so that the activity window of FMRP is strictly regulated (Khlebodarova et al., 2018). In a phosphorylated state, FMRP forms a complex with its target mRNAs, ribosomes and translation initiation factor eIF4E, thereby preventing translation of mTOR signalling pathway proteins, various receptors (including mGluR, NMDAR and AMPAR) and postsynaptic proteins (including NLGN, SHANK and PSD-95) among others, demonstrating its key role in regulating the synaptic proteome (Iacoangeli & Tiedge, 2013). Since many FMRP targets are known to be involved in synaptopathies like ASDs, FMRP deficiency can cause a "multiple hit effect" and induce autism-like features (Guang et al., 2018), which include alterations in dendritic spine number, shape and function (Pfeiffer & Huber, 2009).

ASDs, similarly to other neurodevelopmental disorders, can be hard to study because of the involvement of several biological pathways which are affected in different forms of autism (Sledziowska, Galloway & Baudouin, 2020). On top of that, many reviews on the topic of ASDs, and neurodevelopmental disorders in general, show that main actors involved in these pathways can also interact with each other, creating a very complex interplay of synaptic protein functions (Bagni & Zukin, 2019; Clifton et al., 2020; Guang et al., 2018; Song & Broadie, 2022). This creates a complicated starting point for unravelling the molecular mechanisms at play and linking functions of individual proteins to certain symptoms and phenotypes. This review therefore aims at identifying which pathways are involved in FXS because of the monogenetic nature of the disorder. By creating an overview of the biological pathways and neuronal functions specifically regulated by FMRP, this subset of the molecular pathways involved in synaptopathies can be singled out and studied and more insight will be gained in the mechanisms underlying FXS.

Hence, first the regulation of FMRP activity itself is discussed, followed by mGluR-mediated long term depression (mGluR-LTD), regulation and functioning of FMRP containing protein complexes, synaptic plasticity mediated via NMDARs, AMPARs and γ-Aminobutyric acid receptors (GABARs), cytoskeleton remodelling and lastly treatment strategies. From this FXS pathology basis, insights could eventually be extended to biological pathways proven to be involved in related disorders, creating a complete overview of synaptic disease mechanisms involved in neurodevelopmental disorders.

Methods

For this literature review, recent studies and review articles from up to 5 years old were searched in order to extract information for providing an overarching summary of the current knowledge on molecular mechanisms involved in FXS, and to find novel exciting results which can add to this knowledge base. The studies obtained from this literature search formed the basis for the topics discussed in this review. Based on the information extracted from the included articles, further additional literature searches were performed to provide a complete background on FXS and to include critical information from previous research on the molecular signalling pathways discussed in the various chapters.

Literature search design

The initial literature search was performed using Pubmed and Embase because of the focus of these databases on biomedical research. A systematic search was performed in both search engines to ensure all studies and review articles combining FXS and synaptic pathology were included. The databases were both last consulted on 28-10-22. At this moment in time, no literature review focussed on studies of the last 2 years on molecular signalling in FXS was published yet. The following search strings were used:

- PubMed: *(fmrp protein OR fmr1) [Title/Abstract] AND synaps* [Title/Abstract] AND (y_5[Filter])*
- Embase: *(fmrp:ti,ab,kw OR fmr1:ti,ab,kw) AND(combine) synaps*:ti,ab,kw (filter 2022 t/m 2018)*

Both keywords 'fmrp' and 'fmr1' and truncation of synaps* were used to optimize the thorough search and find articles using variations of these search terms. Using the terms 'fmr1' and 'fmrp' rather than 'FXS' or 'Fragile X Syndrome' was decided upon, to specifically search for articles focussing on the genetic and molecular level rather than on the more general pathology of the disorder. The search strings were formulated in collaboration with a librarian proficient in systematic search techniques connected to Utrecht University.

Article selection

All articles obtained from this literature search were uploaded into Rayyan QCRI (Qatar Computing Research Institute, Data Analytics) (Ouzzani et al., 2016). Part of the duplicates were removed automatically by the program, while the remaining possible duplicates were manually evaluated. Subsequently, the remaining studies were screened on relevance and incorporated based on the following inclusion criteria:

- Only articles written in English or Dutch*
- Only articles published in 2018 or more recent
- Only studies focussed on neurons, not on astroglia
- Only animal model (mice/rat/drosophila/zebrafish) or cell culture (animal or human) studies
- Only studies focussed on molecular pathways related to synapses, so no studies evaluating behavioural phenotypes or only including electrophysiology data.

**no studies in Dutch were found using the described search strategies*

First a selection was made based on the title and abstract resulting in a collection of 69 articles. The full text of these publications was then evaluated, yielding a final selection of 45 articles to be included in the review. An overview of the selection process according to Systematic reviews and Meta-Analyses (PRISMA) guidelines of 2020 (Page et al., 2021) can be found in figure 1.

*Figure 1: Flowchart of article selection process according to Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines.*Part of the duplications of search results uploaded were automatically removed by Rayyan QCRI while the remaining duplications were manually checked and discarded.*

Additional articles on background information regarding FXS, basic molecular pathways involved in FXS and further elaboration regarding topics touched upon in the selected literature were found via PubMed, Embase or Google Scholar through snowballing (Greenhalgh, Peacock, 2005). For these searches, no articles were excluded based on publication date.

Results

1. Regulation of FMRP activity

In the human brain, healthy neuronal networks are formed and maintained via dynamic processes controlling the formation, maturation and elimination of synaptic connections (Prieto, Folci & Martin 2019). Disbalances in synaptic communication, specifically in the ratio between excitation and inhibition (E/I balance), have been found in many neurodevelopmental disorders including autism and FXS (Bagni & Zukin, 2019; Li & Pozzo-Miller, 2020). Development, functionality and adaptation of synapses all depend on the strict regulation of synaptic protein levels (Khlebodarova et al., 2018). One way to control the synaptic proteome is by regulation of RNA translation, which ensures the desired levels of proteins crucial for synaptic interaction, like scaffolding proteins, neurotransmitter receptors and messenger proteins (Khlebodarova et al., 2018; Prieto, Folci & Martin 2019).

FMRP, the protein which is deficient in FXS, has been found to negatively regulate dendritic mRNA translation following mGluR stimulation, thereby controlling activity-dependent protein translation in excitatory synapses (Chen & Joseph, 2015). To be able to regulate RNA translation, activity of FMRP has to be tightly regulated itself. This happens by means of phosphorylation in addition to other post-translational modifications (Prieto, Folci & Martin 2019).

When postsynaptic receptors, including mGluRs, NMDA-typeglutamate receptors (NMDARs) and tyrosine kinase (TRK) receptors, are activated upon neuronal activity, this leads to activation of intracellular signalling pathways including the PI3K/Akt/mTOR and MAPK/ERK pathway, which eventually lead to the phosphorylation and dephosphorylation of FMRP (Bagni & Zukin, 2019). An overview of these pathways combined with various other processes discussed in this review can be found in figure 2. This figure serves to provide an overview of the most important pathways, and show how they are interrelated. The PI3K/Akt/mTOR pathway is known to regulate several processes like cell growth and proliferation, cell cycle mechanisms, protein synthesis and autophagy (Costa-Mattioli & Monteggia, 2013). Meanwhile, The MAPK/ERK pathway plays a key role in inducing neuronal progenitors from pluripotent stem cells and consolidation of memory (Zoghbi & Bear, 2012). Both pathways converge on protein synthesis and play a role in regulating FMRP activity (Bagni & Zukin, 2019). Therefore, it is not surprising that actors in these pathways have been found to be dysregulated in FXS and related disorders (Borrie et al., 2017).

Within the PI3K/Akt/mTOR pathway, Phosphoinositide 3-kinases (P13Ks) are activated upon postsynaptic receptor activation via scaffolding proteins like PI 3-Kinase Enhancer (PIKE), HOMER and SHANK proteins. This subsequently leads to the activation of Protein kinase B (PKB/Akt), which inhibits the tuberous sclerosis complex 1 and 2 (TSC1 and TSC2) complex, which is also known to be dysregulated in autism related syndrome tuberous sclerosis complex (TSC) (Bagni & Zukin, 2019). In turn, this relieves inhibition of Ras homolog enriched in brain (RHEB) which leads to elevation of mammalian target of rapamycin complex (mTORC) activity and causes S6K to phosphorylate FMRP (Bagni & Zukin, 2019). Additionally, mTORC activity can inhibit PP2A, which can causes dephosphorylation of FMRP (Prieto, Folci & Martin 2019). This signalling cascade is known as the PI3K/Akt/mTOR pathway. Further regulation takes place via the MAPK/ERK pathway, which can also lead to inhibition of the TSC1-TSC2 complex and cause phosphorylation of FMRP binding partners, including eIF4E (Bagni & Zukin, 2019). An overview of the described pathways can be found in figure 2.

Important to note is that while activation of mGluRs and related receptors leads to phosphorylation of FMRP by SK6 which inhibits translation, activity of these receptors also causes dephosphorylation

of FMRP by PP2A phosphatase which in turn promotes translation (Khlebodarova et al., 2018). The right balance between these processes of activation and inactivation of FMRP results in the appropriate amount of protein synthesis and is created by varying thresholds of glutamate receptor activation for both mechanisms (Prieto, Folci & Martin 2019) (Figure 2).

Suppression of RNA translation by FRMP is caused by association of phosphorylated FMRP with the target RNA, ribosomes and translation initiation factor elF4E (Khlebodarova et al., 2018; Prieto, Folci & Martin 2019). This RNA granule formation blocks the possibility of mRNA translation. Initially, in case of neuronal activity, short mGluR activation of less than 1 minute induces PP2A-dependent dephosphorylation of FMRP, causing granule disassembly which promotes translation. Via this process translation of FMRP targets, including the Fmr1 gene itself, is rapidly increased, resulting in an increase of FMRP and other proteins. However, when mGluR activity is persistent, rephosphorylation of FMRP by S6K is promoted and PP2A activity is inhibited in an mTOR-dependent manner, reducing mRNA translation again. This rephosphorylation restores FMRP activity to baseline levels and prevents excessive protein synthesis (Prieto, Folci & Martin 2019) (Figure 2).

In addition phosphorylation, activity of FMRP can also be controlled by other post-translational modifications, including sumoylation and ubiquitination. Sumoylation of FMRP following mGluR activation increases homomerization of FMRP, promoting the disassembly of RNA granules and enabling protein synthesis (Khayachi et al., 2018). Furthermore, dephosphorylation of FMRP can trigger its ubiquitination which leads to its degradation. This process ensures that FMRP levels are not exaggerated as a consequence of short mGluR activation and subsequent Fmr1 translation (Prieto, Folci & Martin 2019).

To complicate the regulatory function of FMRP even more, Greenblatt & Spradling (2018) have recently shown that FMRP regulation of translation might be dependent on protein size, with FMRP upregulating rather than downregulating protein synthesis of large autism-related proteins. This emphasizes the importance of unravelling the exact molecular pathways involved in FXS and understanding how these in turn are regulated.

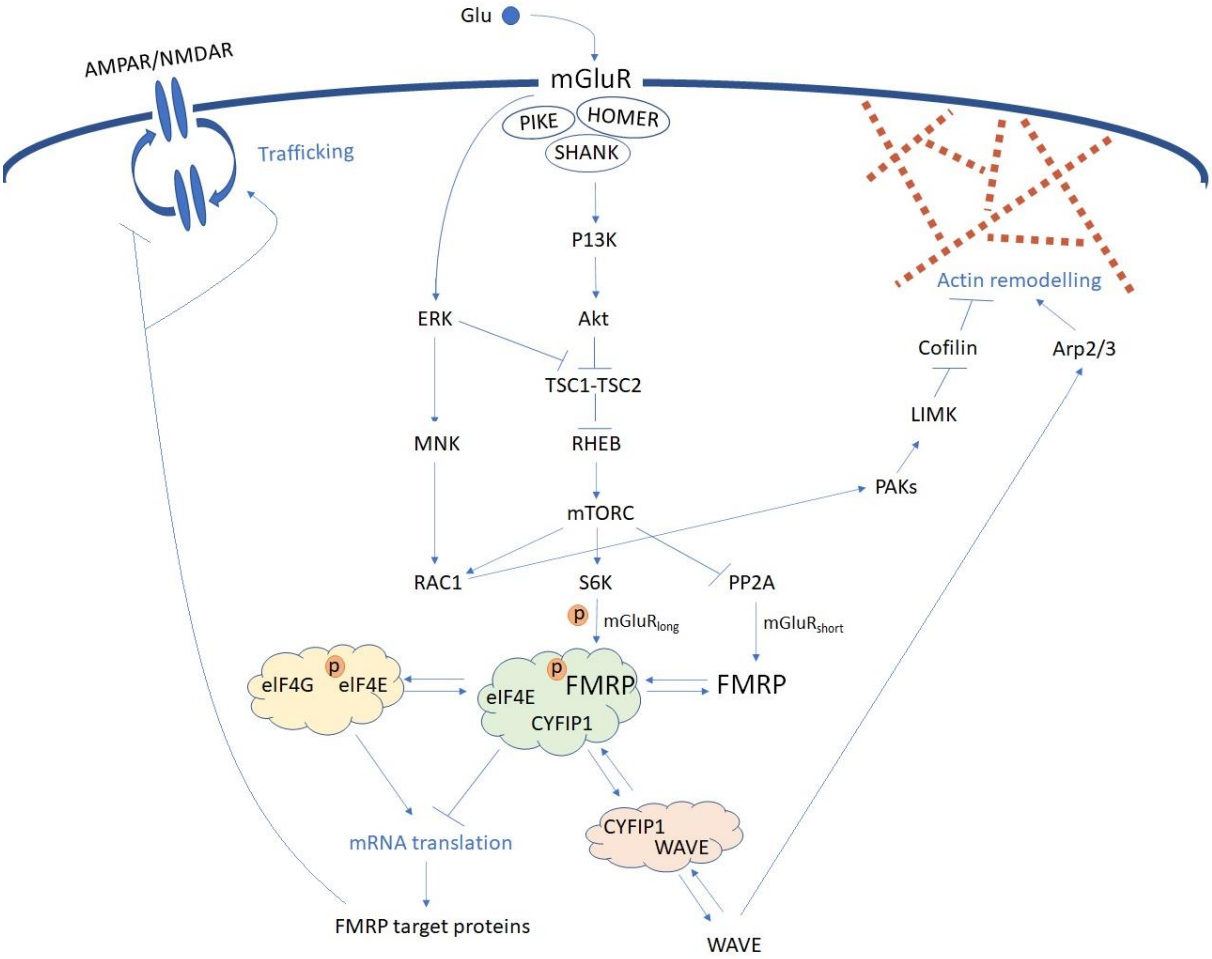

Figure 2: Overview of the most important signalling pathways and cellular processes discussed in this review, including the PI3K/Akt/mTOR and MAPK/ERK pathway, the CYFIP1-FMRP-eIF4E translation inhibition complex, the eIF4E-eIF4G translation initiation complex, the WAVE complex, actin remodelling and mGluR-dependent glutamate receptor endocytosis as part of synaptic plasticity.

2. The mGluR theory

One of the most well-known theories for explaining the phenotypes associated with FXS is the mGluR theory. According to this theory, mGluR activaty stimulates local protein synthesis, which is normally controlled via mRNA translation inhibition by FMRP, but is exaggerated in the case of FXS (Bagni & Zukin, 2019; Bear, Huber & Warren 2004; Bellosta & Soldana, 2019; Dölen & Bear, 2008). Via upregulation of dendritic proteins mediated by mGluR signalling, long-lasting functional and structural changes of the synapse can be accomplished to adequately adapt to the input the postsynaptic cell receives (Zeidler et al., 2017). These processes are known as long-term potentiation (LTP), which increases the cellular response to incoming signals, and long-term depression (LTD), which decreases the response to incoming signals (Bear, Huber & Warren 2004). Various forms of LTP and LTD are known to be involved in formation and adaptation of neuronal networks, of which the most well-known forms are NMDAR-mediated (Oliet, Malenka & Nicoll, 1997). However in FXS, one of the most clear instances of synaptic plasticity affected by absence of FMRP, is mGluR-mediated, protein synthesis dependent LTD (Bagni & Zukin, 2019; Bear, Huber & Warren 2004). Therefore, this deficit is thought to underly many of the FXS related symptoms according to the mGluR theory.

Describing the basic theory from a synaptic perspective, mGluRs are activated in response to neuronal activity and trigger a postsynaptic signalling pathway including extracellular signal-regulated kinase (ERK) and mitogen-activated protein kinases (MAPK). This eventually leads to an increase in local protein synthesis and AMPAR internalisation, which are processes involved in LTD (Bellosta & Soldana, 2019) (Figure 2). One of the upregulated genes is Fmr1 itself, resulting in an increase in FMRP protein. This FMRP increase subsequently results in more repression of mRNA translation, thereby correcting the initial increase in protein synthesis and bringing protein levels back to baseline. In this way, mGluR-mediated LTD can be regulated so that neuronal networks are precisely fine-tuned (Bear, Huber & Warren 2004; Bear, Huber & Warren 2004). However, in the case of FXS, FMRP is absent, leading to uncontrolled upregulation of mGluR-mediated processes. These processes include the already mentioned internalisation of AMPARs but also NMDARs, rendering the neuron to be less able to respond to preseynaptically released glutamate, thereby reducing the synaptic connection between the pre- and postsynaptic neuron (Snyder et al., 2001). When mGluR signalling is sustained, this can create an irreversible loss of glutamate receptors and eventually lead to synapse elimination (Bellosta & Soldano, 2019; Snyder et al., 2001). Additionally, glutamate transmission itself is also impaired by a reduction of presynaptic vesicle release (Zakharenko, Zablow & Siegelbaum, 2002). These exaggerated mGluR-mediated processes linked to FMRP depletion match the synaptic features of FXS, which include an increase in immature thin long spines with a lower postsynaptic density, less AMPARs and reduced presynaptic vesicle docking (Bear, Huber & Warren 2004).

Recent research has added to this general model of mGluR activity and FMRP-controlled protein synthesis and has identified more players involved in the regulatory network of proteins controlling mRNA translation at dendritic spines. Within these pathways, multiple components have been found to be dysregulated in FXS and ASDs.

One example is the HOMER scaffolding protein family, to which a short Homer 1a and longer Homer 1b and 1c variants belong amongst other isoforms (Ronesi et al., 2012). The longer Homer forms interact with SHANK proteins and target mGluRs to the membrane while the short Homer 1a form uncouples glutamate receptors from the membrane (Kammermeier & Worley, 2007). In absence of FMRP, mGluR5 associates more with the shorter isoform, causing an increase in the mobility of mGluRs resulting in co-clustering with NMDARs (Aloisi et al., 2017). The resulting dysregulation of

NMDARs might contribute to the exaggerated mGluR-mediated LTD that is observed in FXS, because of the reduction of postsynaptic glutamate signalling (Guang et al., 2018). Supporting this Homer isoform explanation, Guo et al. (2016) show that genetic deletion of the short Homer 1a protein restores interactions of mGluRs with Homer 1b/c and rescues FXS phenotypes.

Furthermore, dysregulation of PIKE has been observed in both mouse and fly models of FXS (Gross et al., 2015). PIKE links P13K to mGluRs and helps activate downstream signalling pathways including Akt and mTOR signalling, eventually controlling FMRP activity (Sharma et al., 2010). However, PIKE itself is also a target of FMRP, leading to elevated PIKE levels in the absence of FMRP-dependent inhibition of translation (Darnell et al., 2011). In turn, this leads to increased mGluR-mediated mTOR signalling and aberrant protein synthesis. To confirm the role of PIKE in these signalling pathways, Gross et al. reduced PIKE levels in knock-out mice and fly models (Centg1 heterozygous Fmr1KO mice; and Centaurin Gamma-1A (CenG1A; the invertebrate Centg1 homolog) heterozygous dFmr1 mutant Drosophila). They found PIKE to be a crucial contributor to mGluR-mediated signalling and could rescue synaptic plasticity and cognitive deficits in both animal models by reducing PIKE levels (Gross et al., 2015).

Outside of these MAPK/ERK and PI3K/Akt/mTOR pathways, the FMRP protein has been found to form a complex with Cdh1-APC as part of a novel ubiquitin signalling pathway, regulating mGluR-LTD in the hippocampus (Huang et al., 2015). This separate function of FMRP highlights that the complete network of processes which contribute to the strict regulation of protein synthesis is hard to capture in an overview of signalling pathways. To complicate the process even further, research has shown that while regulation of translation by FMRP usually downregulates translation, it can also upregulate translation for some mRNAs (Bear, Huber & Warren 2004). As discussed in the previous chapter, this is usually the case for bigger proteins (Greenblatt & Spradling, 2018).

Together these examples illustrate the point that was also made by a large amount of review articles, namely that the complete overview of which processes are involved in the aberrant mGluR-mediated regulation of protein transcription has not been agreed upon yet (Telias, 2019). In addition to the exact mechanisms part of the mGluR theory remaining to be further discovered, more criticism has been provided on this theory as a means to explain the FXS phenotype. One of the most important points is that findings on the mGluR theory in animals can differ from those in human models. Providing MPEP, a selective mGluR5 antagonist, to mouse Fmr1 KO cells and human FXS-hiPSCs derived neural progenitors did not affect the mouse cells, while tripling the number of human cells responding to DPHG, an agonist for mGluR1/5 (Achuta et al., 2017). Furthermore, while compensation for FMRP function loss by genetic or pharmacological reduction of mGluR signalling was shown to be effective in restoring part of FXS related defects, including synaptic plasticity and mushroom body impairments, not all symptoms could be relieved in this way (Gross, Berry-Kravis & Bassell, 2012; McBride et al., 2005). This demonstrates that more signalling pathways may be involved in FXS pathology.

3. FMRP interacting proteins

As was previously touched upon, FMRP regulates mRNA translation by interacting with target mRNAs. FMRP can bind RNA directly through its RNA-binding motifs, which include two hnRNP-K homology domains (KH domains): KH1 and KH2, an arginine/glycine-rich RNA-binding motif (RGG box) and an N-terminal domain (NDF) (Pfeiffer & Huber, 2009). However, often these regulatory mechanisms include interaction with other proteins to form inhibitory complexes. A subset of the most important and newly found interacting partners will be discussed in this chapter.

RNA Granules

In addition to binding target mRNAs, FMRP can associate with ribosomes in an RNA-dependent manner (Khandjian et al., 2004). Ribosomes are responsible for mRNA translation and their heterogenous composition was shown to allow for selective translation of proteins necessary for different cellular functions, depending on cellular localisation and developmental state amongst other factors (Shi et al., 2017). Interaction between FMRP and ribosomes can provide another regulatory step in the process of protein synthesis, which is thought to be particularly important for controlling dendritic mRNA translation (Antar et al., 2004). This step proves to be very influencial since repression of translation was found to mainly occur at the level of initiation, via inhibition of pre-initiation complexes including these ribosomal subunits (Sossin & Costa-Mattioli, 2019).

In order to inhibit translation, FMRP forms complexes called 'RNA granules' which include mRNA, ribosomes and other interacting proteins (Prieto, Folci & Martin, 2019). According to a recent study, RNA granules are formed by phase separation, which is a process that concentrates RNA and its binding proteins into condensed membraneless compartments that prevent translation due to constraint (Tsang et al., 2019). This study finds that this process is mediated by interaction between RNA and specific RNA binding and low-complexity disordered regions of FMRP. Phosphorylation of these regions further increases the tendency for phase separation and thereby favours granule formation. As part of activity-dependent translation, mGluR activity can regulate phosphorylation of FMRP, and thereby RNA granule formation, so that mRNAs can be transported to dendrites via microtubuli without being translated (Tsang et al., 2019). In this way, FMRP can control mRNA translation and transport to synaptic sites and accordingly, deficits in transport and translation observed for FXS can be explained by FMRP deficiency. Furthermore, this study by Tsang et al. (2019) found that this process of phase separation can be further modulated by other post-translational modifications of FMRP, including methylation which was found to decrease granule formation. This results in promotion instead of reduction of mRNA translation. Moreover, phosphorylation of FMRP by kinases involved in different pathways was shown, proving the network of regulatory processes to be even more complex. Future research could help to further dissect this interplay of signalling pathways involved in regulation of this phase separation process, which provides a very interesting angle to study FMRP and understand its complete cellular function.

The CYFIP1-FMRP-eIF4E complex

In addition to Granule formation, which serves to inhibit ribosomal RNA translation, FMRP also interacts with other proteins to control protein synthesis. One of these proteins is cytoplasmic Fmr1-interacting protein 1 (CYFIP1), which is bound by FMRP and in turn binds the 5'cap binding protein eukaryotic translation initiation factor 4E (eIF4E). In this CYFIP1-FMRP-eIF4E complex, CYFIP1 functions as a non-canonical 4E binding partner and occludes the eukaryotic translation initiation factor 4G (eIF4G) binding site on eIF4E (Napoli et al., 2008). Via this interaction, formation of eIF4E- and eIF4G-containing translation initiation complexes is prevented, which inhibits the induction of translation of dendritically localized FMRP targets (Bagni & Zukin, 2019; Pfeiffer & Huber, 2009; Napoli et al., 2008). In this way, the CYFIP1-FMRP-eIF4E complex exerts a controlling function and ensures that translation can be locally regulated in response to neuronal activity, which is a crucial part of long-term synaptic plasticity at glutaminergic synapses (Clifton et al., 2020). Additionally,

translation initiation factors like eIF4E can be phosphorylated via the MAPK/ERK and the PI3K/Akt/mTOR pathway described in the previous chapter, which enables interaction with ElF4G and formation of the translation initiation complex (Pfeiffer & Huber, 2009). In this way, neuronal activity and mGluR activation can drive protein synthesis by regulating other components of translation complexes next to FMRP (Figure 2).

The WAVE complex

CYFIP1 also associates with the WAVE regulatory complex, which consists of WAVE1/2/3, CYFIP1/2, ABI1/2, NCKAP1, and HPSC300 (Clifton et al., 2020) and regulates actin dynamics via interaction with the Arp2/3 complex (Chen et al., 2010). When CYFIP1 is bound to the WAVE complex, it inhibits its function and actin cytoskeleton rearrangements are prevented (Clifton et al., 2020). Since both the CYFIP1-FMRP-eIF4E complex and the WAVE complex bind CYFIP1, this creates competition which is functional for formation and maintenance of synapses (Figure 2). When FMRP is not bound by CYFIP1, its mRNA targets are translated, including ARC, which drives synaptic plasticity via regulation of AMPAR trafficking and increases actin skeleton stability. At the same time, free CYFIP1 can bind WAVE complexes, inhibiting actin cytoskeleton rearrangement, thereby contributing to the stability of synapses. Conversely, when CYFIP1 forms the translation inhibiting complex with FMRP and eIF4E, the WAVE complex is free to induce actin remodelling. Under basal conditions, 30% of CYFIP1 is found as part of the CYFIP1-FMRP-eIF4E complex and 70% as part of the WAVE complex (De Rubeis et al., 2013), which determines the balance between protein translation and cytoskeleton remodelling (Clifton et al., 2020). Upon mGluR activity, this balance is shifted and CYFIP1 is located to the WAVE complex (Santini et al., 2017). By strictly coordinating cytoskeleton skeleton remodelling and protein synthesis like this, proper dendritic spines can be formed.

In accordance with its cellular functions, heterozygous CYFIP1 deletion (CYFIP1 +/-) mouse models show increased protein translation, an increase in mGluR-mediated LTD, immature spine morphology and increased dendritic branching (Clifton et al., 2020). Similar features were observed for X-linked Fmr1 deletion (Fmr1-/y) models, where the Fmr1 copy on the X-chomosome is deleted in male mice. These results confirm the interaction between FMRP and CYFIP1 (Clifton et al., 2020). Conversely, overexpression of eIF4E-binding protein, 4EBP, which leads to inhibition of translation by blocking the translation initiation complex, induces opposite features including decreased dendritic complexity (Jaworski et al., 2005).

The distribution of CYFIP1 between the CYFIP1-FMRP-eIF4E complex and the WAVE complex can be regulated by synaptic activity induced brain-derived neurotrophic factor (BDNF), mGluR or NMDAR signalling and is mediated by a conformational change in CYFIP1 (Di Marino en at., 2015). This change in conformation results in the release of CYFIP1 from eIF4E, so that it is able to bind eIF4G and form the translation initiation complex which enables ribosomes to translate mRNA (Pfeiffer & Huber, 2009). The small Rho GTPase Ras-related C3 botulinum toxin substrate 1 (Rac1), which is also involved in both MAPK/ERK and PI3K/Akt/mTOR signalling, can help induce this conformational change in CYFIP1 (De Rubeis et al., 2013; Napoli et al., 2008). On top of this, Panja et al. (2014) showed that MNKs can phosphorylate CYFIP1 and trigger the release of CYFIP1 and FMRP from target mRNA, which they link to increased mRNA translation in the dendrites. A recent study showed that FXS treatments focussing on restoring the balance between CYFIP1 involvement in the CYFIP1-FMRP-eIF4E complex and the WAVE complex can correct spine morphology, enhanced mGluR-LTD and aberrant actin dynamics among other symptoms in FXS mouse models (Santini et al., 2017). For this research 4EGI-1 was used, which is an inhibitor of the translation initiation complex containing eIF4E and eIF4G. 4EGI-1 prevents eIF4E from binding eIF4G, thereby inhibiting translation. In addition, free eIF4E can now bind CYFIP1, releasing its inhibition on the WAVE complex and actin remodelling (Santini et al., 2017). This treatment is a clear example of how disproportionate signalling as a consequence of FMRP loss can be corrected to resolve FXS phenotypes.

miRNAs and the RISC-complex

Another mechanism via which FMRP might induce translational repression is by association with the RNA-induced silencing complex (RISC) and short noncoding RNAs or microRNAs (miRNAs). These RNAs in turn will bind complementary sequences in target mRNAs, thereby preventing their translation (Valdez-Sinon & Bassell, 2020). This process is also regulated via phosphorylation of FMRP downstream of mGluR signalling, which allows for the assembly of the inhibitory complex, and dephosphorylation of FMRP, which releases the miRNA-RISC complex from target mRNAs. One of the mRNAs targeted by this inhibitory complex is the well-known FMRP regulated protein PSD-95, which plays an important role in morphology and plasticity of synapses. In Fmr1 KO synapses, interaction between miR-125a and Argonaute-2 (AGO2), which is a protein that also associates with FMRP and the RISC complex, is lost, resulting in dysregulated control of synaptic protein synthesis and aberrant morphology and density of dendritic spines (Muddashetty et al., 2011).

MOV10-AGO2 inhibitory complex

In addition to translation regulated via mGluR signalling, FMRP is also part of other complexes regulated via other signalling pathways as a response to neuronal activity (Kute et al., 2019). Similarly to mGluR signalling, NMDAR activity can control synaptic plasticity and protein synthesis via inhibitory complexes. Under basal conditions, FMRP associates with Moloney Leukemia Virus 10 (MOV10) protein and AGO2 and inhibits translation of a group of NMDA responsive mRNAs. Upon NMDAR stimulation, the complex disassociates and translation of this group of NMDAR responsive mRNAs is induced. The disassociation process is thought to be controlled via FMRP activity, since the response to NMDA stimulation is not found in the absence of FMRP. The switch is thought to occur through phosphorylation of FMRP (Kute et al., 2019). This example of the role of FMRP in translation controlling complexes illustrates the importance of FMRP functioning for regulation of cellular protein levels in response to various signalling pathways.

FUS

In addition to the before mentioned FMRP interacting proteins, there are many more suspected interaction partners of FMRP which might help regulate mRNA translation. One of these is the fused in sarcoma (FUS) protein, which is a heterogeneous nuclear ribonucleoprotein (Imperatore et al., 2020). FUS has been shown to localise to the post-synaptic density as well as the nucleus and pre-synapse. FUS is thought to play an important role in synaptic functioning via processes like mRNA localisation and translation, since it's deletion resulted in abnormal spine & dendrite morphology in cultured hippocampal cells, resembling the effects of FMRP deletion. In addition to this, Imperatore et al. (2020) showed that FUS binds G quadruplex structures in 3'-untranslated regions (3'UTR) regions of mRNA with its RGG boxes, just like FMRP. Lastly, both FUS and FMRP target PSD-95 and Shank1 mRNAs, which are known to be dysregulated in FXS (Bagni & Zukin, 2019). Based on these findings, interaction between FUS and FMRP to regulate local translation of mRNAs involved in synapse functioning and plasticity seems very plausible.

Combined, these studies demonstrate that initiation of mRNA translation induced by mGluR activity and other signalling pathways can be regulated via different mechanisms. Previous chapters showed that FMRP is first dephosphorylated by PP2A upon short mGluR activity, promoting protein synthesis, and later rephosphorylated by S6K in case of prolonged mGluR activity, reducing protein synthesis back to baseline levels. Research discussed in this chapter adds to the understanding of mRNA translation regulation by showing that this process can also be regulated by controlling FMRP interacting proteins. Components of the MAPK/ERK and PI3K/Akt/mTOR pathways, which regulate PP2A and SK6 activity to control activity of FMRP, can also interact with other components of translation complexes. An example of this is MNKs phosphorylating CYFIP1 to induce mRNA translation (Panja et al., 2014). This increase in protein synthesis contributes to the initial increase in protein levels stimulated by short mGluR activity. In addition to these regulatory mechanisms controlled by mGluR signalling, FMRP activity and protein synthesis can also be controlled via other

pathways, including NMDAR signalling. Lastly, many FMRP interacting proteins are most likely yet to be identified. This results in an even more extensive network of interacting signalling pathways than is classified yet.

4. Synaptic plasticity

Neuronal networks are reformed and maintained by coördination of synaptic connections via synaptic plasticity. Many forms of synaptic plasticity require synthesis of new proteins. As was previously touched upon in chapter 1 about FMRP regulation, mRNA trafficking to dendrites and activity-dependent translation are controlled by FMRP, indicating an important role for FMRP in synaptic plasticity (Sidorov, Auerbach & Bear, 2013). Indeed, dysregulated plasticity at the synapse as a consequence of uncontrolled protein synthesis has been shown to be at the core of FXS pathophysiology (Bagni & Zukin, 2019). In this chapter, four forms of synaptic plasticity involved in FXS will be discussed using recent literature, adding to our current understanding of synaptic imbalances in excitation and inhibition observed in FXS. An overview of these forms of synaptic plasticity and the receptors and subunits involved can be found in figure 3.

mGluR-mediated LTD

The most clearly dysregulated form of synaptic plasticity involved in FXS is mGluR-mediated LTD (Erickson et al., 2017; Franchini et al., 2020), which is more extensively discussed in previous chapters. In short, the mGluR theory entails that in absence of FMRP, mGluR activity induced translation of a subset of mRNAs that regulates AMPAR endocytosis is increased, resulting in a higher rate of AMPAR internalisation (Yang et al., 2016) (Figure 2). This process of excessive endocytosis is mediated by ARC, which is also translated in dendrites upon mGluR activity. In ARC KO mice, mGluR-LTD is abolished, proving that ARC functioning is crucial for inducing mGluR-LTD by regulation of AMPAR incorporation in the membrane (Pfeiffer & Huber 2009). In addition to AMPARs, NMDARs were shown to also be involved in mGluR-LTD. Toft, Lundbye and Banke (2016) demonstrated that enhanced mGluR-LTD is caused at least in part by dysregulated NMDAR signalling, using NMDAR blocker PVA. Furthermore, they demonstrated that specifically GluN2B-containing NMDARs were involved using NMDAR-subunit-specific antagonists.

NMDAR-mediated synaptic plasticity

While various studies have demonstrated enhanced mGluR-LTD for FXS, research on other types of synaptic plasticity renders less clear results (Bostrom et al., 2013; Eadie et al., 2012; Lundbye, Toft & Banke, 2018). Effects of FMRP loss on NMDAR-mediated synaptic plasticity could differ between brain areas, based on results from Bostrom et al. (2013). They show impaired NMDAR dependent synaptic plasticity in the dentate gyrus while no such effects were found for the cornu ammonis area 1 (CA1). of the hippocampus in adult mice lacking FMRP. These results are in accordance with a study by Eadie et al. (2012), who observe impaired bidirectional synaptic plasticity in the dentate gyrus of Fmr1 KO mice, associated with a reduction in functional NMDARs. Furthermore, additional studies report similar impaired NMDAR-dependent synaptic plasticity in the dentate gyrus (Yun & Trommer, 2011), while studies in the CA1 area show no such effects (Huber et al., Pilpal et al., 2009). However, several more studies have reported impaired LTP in various brain regions (Desai et al., 2006; Meredith et al., 2007; Shang et al., 2009), including the CA1 area (Lauterborn et al. 2007; Lee et al. 2011). Lundbye, Toft and Banke (2018) recently showed that impaired NMDAR dependent LTP in the CA1 as a result of elevated GluN2A-containing NMDAR levels could be restored to baseline levels by selectively inhibiting GluN2A subunits. These experiments were carried out in Fmr1(-/y) FXS mouse models using GluN2A antagonists and negative allosteric modulators, and by crossing Fmr1(-/y) and Grin2a(-/-) mice, with Grin2A being the gene coding GluN2A subunits. Interestingly, GluN2A inhibition restored LTP but also recovered mGluR-mediated LTD, making it a very interesting target for FXS treatments. Together, these studies strongly indicate involvement of abnormalities in NMDAR-mediated synaptic plasticity in FXS.

AMPAR-mediated synaptic plasticity

In addition to glutamate signalling via NMDARs, AMPAR-mediated synaptic plasticity is also involved in FXS as was already demonstrated by the mGluR theory (Erickson et al., 2017; Yang et al., 2016). A study performed by Yun et al. (2011) found impaired LTP in medial perforant path-granule cells in Fmr1 KO mice. Subsequently, they measured smaller peak amplitudes of NMDAR-mediated excitatory postsynaptic currents (EPSCs) in Fmr1 KO mice compared to control, while AMPAR-mediated EPSCs were similar. The higher AMPAR/NMDAR ratio resulting from this can explain in part how a loss of FMRP can reduce LTP. While this change in ratio seems to be caused by altered NMDAR functioning, other studies show AMPARs to be involved. Banke & Barria (2020) also find a higher AMPAR-NMDAR ratio in hippocampal CA1 pyramidal neurons of Fmr1-KO mice during early development, which is corrected after postnatal day 13. Additionally a lack of LTP and upregulation of GluA2 subunits of AMPARs was found, affecting Ca^{2+} permeability and formation of neuronal circuits. As expected, altered morphological dendritic branching was observed in Fmr1 KO mice similar to features typically observed for FXS. Interestingly, these AMPA receptor abnormalities were absent in adult animals, indicating that successful treatment should take place during early development. Determining the exact moment in human development at which interference should take place proves to be difficult however, since alignment of developmental stages between mice and humans differs for each neurodevelopmental process (Watson et al., 2006). Therefore, more research on this process of GluA2 upregulation in FXS mouse models and translation to humans should be carried out to answer this type of questions. Research by Hwang et al. (2022) adds to these findings and highlights the selective upregulation of GluA2 but not GluA1 subunits. Furthermore they confirm that this causes a switch from Ca^{2+}-permeable to Ca^{2+}-impermeable AMPARs, which reduces inhibitory synaptic transmission and results in a loss of NMDAR-independent LTP at glutamatergic synapses projecting to inhibitory interneurons in the CA1. These imbalances is synaptic plasticity can contribute to the excitatory/inhibitory balance commonly found in FXS.

Furthermore, research by Zhou et al. (2017) links regulation of synaptic plasticity via control of AMPAR implementation into the membrane to processes controlling synapse structure, including actin reorganisation and spine enlargement. This study shows that blocking synaptic activity by use of TTX results in postsynaptic AMPAR accumulation affecting synaptic plasticity. TTX application induces FMRP-dependent translation of NGPF2, which activates (ALK)-LIMK-cofilin signalling which is known to control actin remodelling (Bagni & Zukin, 2019). Via this pathway the processes of actin reorganization, spine enlargement, and stabilization of AMPARs at the synapse can be controlled simultaneously, all contributing to synaptic scaling up. Another recent study on structural and functional synaptic plasticity shows that FMRP can also regulate these processes by controlling protein synthesis in the presynapse (Monday et al., 2022). Here LTP was associated with translation-dependent enlargement of mossy fiber boutons, which was prevented in the absence of FMRP. Together, these studies demonstrate that FMRP controls multiple processes involved in synapse maintenance and remodelling, which work together to form healthy neuronal networks. By aberrant functioning of one component in this signalling network, various interplaying processes controlling synaptic structure and plasticity become dysregulated, resulting in a variety of symptoms.

GABA signalling

Another important factor to mention is that aberrant inhibitory, GABAergic signalling has also been demonstrated in multiple brain areas and contributes to the disturbed E/I balance in FXS (Erickson et al., 2017). GABAergic deficits found for FXS mostly involve reduced expression of $GABA_A$-subunit receptors ($GABA_A$Rs), but synthesis and release of GABA may also be impaired (Olmos-Serrano et al., 2010). Zhang et al. (2017) show that decreased surface expression of $GABA_A$-receptor δ subunits leads to reduced tonic inhibition in FXS. This reduction in inhibition contributes to the distortion of the E/I balance. Zeidler et al., (2017) point out that to most effectively treat FXS symptoms, multiple

pathways should be targeted at the same time, and provide the example of interfering with mGluR signalling and GABA signalling simultaneously.

In conclusion, these four forms of signalling all seem to play an important part in aberrant synaptic communication and plasticity involved in FXS. This highlights that FXS features cannot be attributed to deficiencies related to one singled out receptor or signalling pathway, and that we should use a holistic approach to understand and target all mechanisms at play to successfully relieve FXS symptoms.

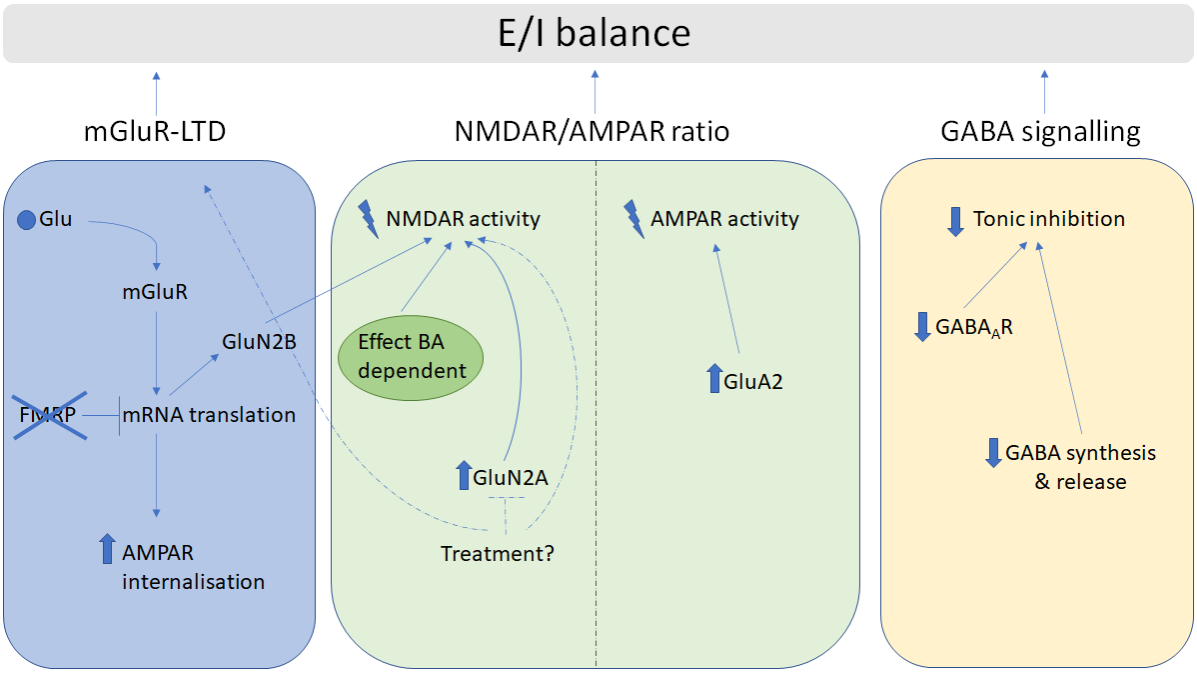

Figure 3: Overview of forms of synaptic plasticity involved in FXS, including mGluR-LTD, NMDAR and AMPAR dependent plasticity and GABA signalling.

5. Cytoskeleton remodelling

Many neurodevelopmental disorders, including FXS, show dysregulation of the synaptic cytoskeleton, leading to altered spine morphology (Bagni & Zukin, 2019; Michaelsen-Preusse, Feuge & Korte, 2018). Together with the strong structure-to-function relationship found for dendritic spines (Holtmaat & Svoboda, 2009), this indicates that aberrant modulation of actin dynamics could be one of the key neuropathological mechanisms involved in FXS.

Rac1-induced actin remodelling

As was mentioned already in chapter 3 on FMRP interacting proteins, CYFIP1 not only cooperates with FMRP to regulate mRNA translation, it also associates with the WAVE regulatory complex which regulates actin remodelling. When CYFIP1 is bound to this complex, it inhibits its function and actin polymerisation is prevented (Clifton et al., 2020). Rac1 is part of the Rho-family of small GTPases, which are signalling molecules that respond to synaptic activity and can regulate actin dynamics (Santini et al., 2017). In its activated state, when Rac1 is bound to GTP instead of GDP, a process that is controlled by guanine exchange factors (GEFs) and GTPase-activating proteins (GAPs) (Santini et al., 2017), Rac1-GTP can interact with the WAVE complex and induce an activating conformational change (Yelland et al., 2021). This relieves inhibition by CYFIP1, upon which the WAVE complex is able to promote actin remodelling via the Arp2/3 complex (Clifton et al., 2020)(Figure 2).

In addition to this form of actin remodelling involving the WAVE regulatory complex, Rac1 also controls actin turnover by regulating Cofilin activity (Bagni & Zukin 2019; Santini et al., 2017). GTP-bound Rac1 activates Rho-associated kinases (ROCK), including p21-activated kinases (PAKs). These kinases then proceed to activate LIM motif-containing protein kinases 1 and 2 (LIMK1/2), which phosphorylate and thereby inactivate Cofilin (Figure 2). Cofilin is known to bind actin and induce depolymerization (Mizuno, 2013). Consequently, by regulation of cofilin, changes is spine morphology can be controlled, as well as structural plasticity (Bosch & Hayashi, 2012).

In FXS patients, increased Rac1 levels are found (Fatemi et al., 2013) and impaired Rac1 signalling was shown to either result in abnormally weak or strong glutamatergic synapses (Sadybekov et al., 2017). Furthermore, formation and maintenance of spines are regulated by PAK1 and deficits in various PAK isoforms were shown to be involved in intellectual disabilities (Bagni & Zukin, 2019). FXS mouse models show elevated neuronal levels and activity of Rac1 and PAK1 (Bongmba et al., 2011; Castets et al., 2005; Chen et al., 2010a, Dolan et al., 2013) and PAK1 inhibition helps to reduce increased spine density and length (Dolan et al., 2013; Hayashi et al., 2007; Pyronneau et al., 2017). Moreover, PAK2 haplo-insufficiency was shown to lead to a decrease in spine density and deficits in LTP due to reduced LIMK1 and cofilin activity (wang et al., 2018). Together, these studies show that dysregulation of proteins involved in actin dynamics leads to synaptic features observed in FXS, supporting the idea that aberrant actin remodelling is key to FXS pathology.

Alternative cytoskeleton regulation

In addition to these relatively well studied mechanisms of actin regulation, new research discovering more regulatory proteins involved in regulation of spine structure adds to our understanding of synapse formation and maintenance. A number of recent studies on such processes will be discussed in the next section of this chapter.

Firstly, Sears and Broadie (2018) describe how FMRP is involved in synaptic pruning deficits in FXS. They report that Drosophila models show that FMRP binds and prevents translation of Shrub (human CHMP4 homolog) mRNA in an activity-dependent manner. This results in disruption of endosomal membrane trafficking within synaptic boutons, which impairs synaptic pruning necessary for formation of healthy neuronal networks. In addition to this, they also describe the processes of

synaptogenesis and activity-dependent synaptic remodelling, which depend on extracellular matrix metalloproteinase (MMP) and the heparan sulfate proteoglycan (HSPG) glypican dally-like protein (Dlp). A study by Dear, Shilts & Broadie (2017) confirms that neuronal activity induces FMRP- and HSPG-dependent functioning of MMP, which drives synaptogenesis. Deficits in this type of signalling are present in FXS and lead to impaired restriction of trans-synaptic Wnt signalling, involved in tissue self-renewal and cell death (Song & Broadie, 2022). Reduction of both MMP and HSPG were shown to alleviate synaptic defects of FXS (Sears & Broadie, 2018).

As was touched upon already in the previous chapter, synaptic plasticity and cytoskeleton remodelling are interconnected and can be regulated by overlapping signalling pathways (Zhou et al., 2017). Song et al. (2022) find that FMRP and Staufen co-regulate Coracle expression, which controls GluR2A levels in the postsynaptic membrane and bouton development. Accordingly, they find that FMRP, Staufen and Coracle are all suppressors of presynaptic pMad activity, providing a trans-synaptic signalling pathway that links postsynaptic glutamate receptor abundance to development of presynaptic boutons. Furthermore, Briševac et al. (2021) find that impaired functioning of a small GTPase called Arf6, which is a regulator of actin and thereby controls glutaminergic synapse and dendritic spine development, is involved in FXS. In mature Fmr1 KO neurons they observe increased Arf6 activity, a loss of Arf6 response to synaptic stimulation and increased Arf6-mediated actin polymerization in dendritic areas. Similar impairments were found for RNAi-mediated depletion of postsynaptic Arf6 guanylate exchange factors IQSEC1 (BRAG2) or IQSEC2 (BRAG1) in wild-type neurons. Furthermore, depletion of IQSEC1 affected mGluR-LTD in wild-type mice, but not in Fmr1 KO animals. Together these findings indicate that Arf6 is dysregulated in FXS, resulting in aberrant synaptic plasticity, actin dynamics and spine morphology.

Lastly, as was previously demonstrated by research on FXS and other neurodevelopmental disorders, a correct synaptic membrane protein composition is crucial for healthy neuronal network development and maintenance (Bagni & Zukin, 2019). Two recent studies illustrate the importance of this principle in the light of FXS. Cheng et al. (2019) find that the process of dendritic spine maturation, which is crucial for forming functional synaptic connections, is influenced by synaptic ICAM5 levels. Fmr1 KO mice show reduced levels of the CLSTN1 protein, resulting in ICAM5 accumulation in the postsynaptic membrane. Reduced CLSTN1 levels lead to increased surface expression of ICAM5, since CLSTN1 is a negative regulator, and result in immature filopodia-like spines. Normalization of CLSTN1 levels rescued impaired dendritic feautures in Fmr1 KO mice. Additionally, Parvin et al. (2019) find enhanced presynaptic accumulation of active zone protein Munc18-1, of which translation is regulated by FMRP, in Fmr1 KO axons. Together, these studies demonstrate that normal protein levels in both pre- and postsynaptic compartments are crucial for healthy synaptic development and maturation, and that cellular levels of multiple proteins are dysregulated in FXS, contributing to impaired synaptic morphology and function observed in FXS.

6. Treatments

While increasing advances are made in elucidating the molecular mechanisms underlying FXS pathology, clinical therapies still mostly focus on treating symptoms and tackling comorbid behaviours and psychiatric problems, instead of providing an mechanism-based cure for the disorder (Erickson et al., 2017; Protic et al., 2019). For example, FXS patients are prescribed selective serotonin reuptake inhibitors (SSRIs), stimulants, and (atypical) antipsychotics (Erickson et al., 2017). The difficulty to find a treatment that interferes with aberrant molecular mechanisms underlying FXS phenotypes can be explained in part by the fact that FMRP regulates various types of signalling, including glutaminergic and GABAergic signalling, via multiple receptors and numerous signalling pathways, like the MAPK/ERK and PI3K/Akt/mTOR pathways discussed in previous chapters. Development of treatments mostly focusses on correcting increased glutaminergic or impaired GABAergic signalling (Erickson et al., 2017). However, while this strategy has shown some promising results in animal models, successful translation to treatments for FXS patients is still lacking (Zeidler et al., 2017). Most likely, this is a result of oversimplification of disease mechanisms as a result of focussing on singled out pathways or a lack of understanding of the interplay of processes at hand (D'Incal et al., 2022; Erickson et al., 2017; Zeidler et al., 2017). However, the broad array of FMRP targets and interacting proteins can also be used to our advantage to develop treatments that tackle dysregulation of multiple signalling pathways at the same time. In this chapter, a short overview will be presented of the types of treatments and molecular targets that have been the focus of FXS drug development in recent years.

Glutamate and GABA signalling

As was already mentioned, most treatments so far have focussed on correcting the E/I balance by either downregulation of glutamate signalling or upregulation of GABA signalling. This can be explained by the popularity of the mGluR theory, which entails that FXS behavioural and cellular features, including electrophysiological and molecular dysfunction, are caused by excessive mGluR signalling (Bear, Huber & Warren, 2004). Researchers have tried to interfere with this type of dysregulation and correct synaptic deficiencies via mGluR-antagonists, including mavoglurant and basimglurant, and GABAR-agonists, including $GABA_BR$-agonist arbaclofen and $GABA_AR$-agonist ganaxolone (Erickson et al., 2017). Stimulation of GABARs improved dendritic spine morphology, seizures and other cellular abnormalities in mouse and fly models (Chang et al., 2008; Henderson et al., 2012; Heulens et al., 2012; Pacey et al., 2009).

mGluR antagonist

Subsequent to proving efficacy of these type of drugs in animal models, clinical trials were started to evaluate if these findings could lead to new FXS treatments (Erickson et al., 2017; Protic et al., 2019). For three specific mGluR antagonists, fenobam, mavoglurant and basimglurant, trials have been completed in humans. For fenobam, initially promising results were found with 50% of subjects showing at least 20% improvement on one of the measured outcomes; prepulse inhibition. However, due to financial problems of the original producers and quite serious side effects found, including hallucinations, vertigo, paraesthesias, and insomnia, fenobam studies did not result in a successful FXS treatment (Berry-Kravis et al., 2009). For mavoglurant and basimglurant, improvements in FXS patients were not extensive enough to continue treatment development (Berry-Kravis et al., 2016; Erickson et al., 2017).

Targeting NMDARs and AMPARs

In addition to targeting mGluRs, trials targeting NMDARs and AMPARs were also initiated. One of the drugs involved was Memantine, a non-competitive NMDAR-antagonist approved by the US Food and Drug Administration (FDA) for treating Alzheimer's disease. Results were varying for FXS patients,

with 4 subjects showing clinical improvement while 2 subjects had to stop the treatment due irritability during the single memantine trial in FXS patients that was published (Erickson et al., 2009). For AMPAR targeting, a positive allosteric modulator CX516 was used in a double-blind, placebo-controlled trial of 4 weeks in FXS patients. The study showed no significant improvement in memory, language skills and behavioural measurements, indicating unsuccessful treatment of FXS symptoms (Berry-Kravis et al., 2006).

Targeting multiple pathways

As was emphasized earlier, targeting multiple forms of signalling simultaneously is expected to improve efficacy of the treatment. In line with this reasoning, multiple treatments have been developed that affect both glutaminergic and GABAergic neurotransmission. Firstly, Riluzole, which is already FDA-approved for treatment of amyotrophic lateral sclerosis (ALS) and could potentially be used for treating anxiety and depression (Grant et al., 2007; Zarate et al., 2004), might also be suitable for treatment of FXS via inhibition of glutamate release (Martin, Thompson, & Nadler, 1993) and activation of $GABA_A$Rs (Jahn et al., 2008). The same rationale applies to Acamprosate, which is an FDA-approved drug used in treatment of alcohol addiction, that can potentially increase $GABA_A$R activity and reduce mGluR- and NMDAR activity in FXS (Mann et al., 2008). Lastly, Arbaclofen, which functions as an $GABA_B$R agonist and is thought to decrease glutamate release, is acknowledged as a potential treatment for FXS (Henderson et al., 2012).

Another strategy is to directly interfere with synaptic signalling pathways dysregulated in FXS. One example following this principle is metformin, an FDA-approved drug for treatment of type II diabetes. metformin inhibits MAPK/ERK and PI3K/Akt/mTOR pathways and can therefore compensate for overactivation of these pathways in FXS (Banerjee et al., 2018). In FXS mouse models, metformin successfully relieved FXS symptoms, including increased mGluR-LTD, aberrant dendritic spine morphology and social behaviour deficits, after 10 days of treatment (Gantois et al., 2017). Similarly, potential Attention Deficit Hyperactivity Disorder (ADHD) drug metadoxine can normalize Akt and ERK activity and is therefore an interesting possible treatment for FXS. A clinical trial in FXS patients rendered varying results however, indicating that replication and further research is necessary to determine whether metadoxine could be a suitable treatment for FXS (Alcobra et al., 2015). Furthermore, lovastatin, a drug prescribed for management of familial hyper-cholesterolemia, was shown to inhibit Ras signalling, leading to a reduction of downstream ERK signalling in Fmr1 KO mice (Osterweil et al., 2013). Reduction of excessive ERK activity was also observed in a clinical trial involving FXS patients (Çaku et al., 2014). An extensive overview of treatments focussing on different kinase inhibitors is provided by D'Incal et al. (2022). They reason that many proteins affected by FMRP absence are kinases and phosphatases, indicating the potential for kinase inhibitor therapies.

In addition to treatments targeting receptors and signalling proteins discussed in this review, other treatments are being developed that interact with other players involved in FXS pathology, of which discussion was beyond the scope of this study. Examples are dysregulation of BDNF, GSK3 and MMP9, which are targeted by SSRI Sertraline, mood stabilizer Lithium and Minocycline respectively (Erickson et al., 2017). Furthermore, since this study focusses on dysregulation in FXS at the level of molecular mechanisms, extensive details of the treatments described in this chapter were not provided. For more information on pharmacological details, a complete overview of the history of drugs developed for treatment of FXS and the process of clinical trials started for various treatments, including details like the number of participants, reasons for discontinuing drug development etc., the original reports of the trials referenced in this review or literature reviews by Banerjee et al. (2018), D'Incal et al. (2022), Erickson et al. (2017) and Protic et al. (2019) can be consulted.

Discussion

This review discussed recent advances made in understanding synaptic dysregulation underlying FXS, a monogenetic condition showing a lot of resemblance to ASDs (Kaufman et al., 2017), caused by loss of function of the Fmr1 gene, encoding the protein FMRP (bagni & Zukin, 2019). In contrast to ASDs which are linked to a variety of genetic and non-genetic causes resulting in very heterogeneous phenotypes (Park et al., 2016), FXS being specifically linked to FMRP dysfunction introduces a valuable opportunity to study signalling pathways involved in FXS and ASD phenotypes. FMRP was shown to regulate transport, translation and stability of many mRNAs encoding proteins crucial to synaptic development and function (Pfeiffer & Huber 2009), proving FMRP to be a very important key regulator for development and maintenance of neuronal networks. Consequently, absence of FMRP results in dysregulation of various signalling pathways and causes FXS features like increased spine density and immature morphology (Pfeiffer & Huber, 2009). However, mapping of the complete pool of FMRP targets and identification of their exact cellular function is still a work in progress (Bagni & Zukin; Clifton et al., 2020), resulting in an incomplete overview of downstream FMRP effects. To complicate the picture even further, activity of FMRP itself is regulated via various post-translational modifications and the relation between these various types of modifications and their activity-dependent interconnected regulation of FMRP is not fully explored yet (Prieto, Folci & Martin 2019). Additionally, it might be possible that some forms of FXS are caused by dysregulation of these post-translational modifications, rather than of FMRP itself. While this complicates the disease mechanism of FXS, it also provides opportunities for treatments targeting these modifications for patients carrying these specific missense mutations. Adding one more layer of complexity, regulation of synaptic processes by FMRP might vary for different brain areas. An example of this is studies showing different results for interference with LTP in absence of FMRP, depending on the exact brain area (Bostrom et al., 2013; Eadie et al., 2012; Lundbye, Toft & Banke, 2018), as was discussed in chapter 4 on synaptic plasticity. This principle might also apply to other mechanisms involved in FXS and should be taken into account before results are generalized across the whole brain.

Furthermore, some additional remarks should be taken into consideration to put this work into perspective. As was described in the method section, certain filters were applied when selecting articles to be included in this review. Of specific importance is the choice for a literature search including studies of the last 5 years, which yielded recent findings in the FXS research field to be used for the basis of this work. While older papers important for the foundation of our understanding of FXS pathology were found through snowballing and included to be able to explain the processes in question and provide a general overview of our current knowledge on FXS, not all work related to FXS from before 2017 could be discussed. Due to the scope of this research, choices had to be made on which topics from within the field of FXS research to discuss, and therefore papers were selected that could best introduce the processes and background knowledge that recent papers build upon. This was touched upon already in the treatments section, where dysregulation of BDNF, GSK3 and MMP9 were mentioned, which were investigated in research prior to 2017 but not focussed on in the selection of recent studies included in this review, and therefore not discussed here. Another choice made that is worth elaborating on is the decision to focus on cellular models (derived from humans and/or animals) and organism level animal models, and not research including human participants. While studies using cell lines and animal models of FXS, including for example Fmr1 KO mice, are most suitable for elucidating disease mechanisms at the level of synaptic processes, translation of these findings to humans should not be automatically assumed.

Future research

Development of more accurate and suitable models for FXS can help drive advances in our understanding of the molecular pathology, but also translation to humans and development of successful treatments. Right now, the most important animal models for FXS are the fruit fly *(Drosophila)* (Banerjee et al., 2007; Drozd, Bardoni & Capovilla, 2018; Zarnescu et al., 2005), Mouse *(Mus)* (Dahlhaus, 2018), rat *(Rattus)* (Tian et al., 2017) and zebrafish *(Danio)* (den Broeder et al., 2009), which are all models based on loss of function of FMRP via Fmr1 homologue disruption or knockout. As a result of the high degree of brain homology, mouse and rat models have been able to provide a deeper understanding of complex FXS symptoms, including learning, motor, cognitive, and behavioural impairments (Musumeci et al., 2000; Spencer et al., 2005; de Vrij et al., 2008). However, rodent models can only provide a certain level of complexity, limiting the degree to which we can evaluate higher cognitive functions using these types of models. Furthermore, breeding and housing of rodents is relatively time-consuming and expensive, resulting in a demand for alternatives. Based on the research question, other models might suffice. While fly and zebrafish models show limited homology and are not suitable for studying complex behavioural phenotypes (Kalueff, Stewart & Gerlai, 2014), their high-throughput genetic and pharmacological screening capabilities and relatively low costs make them a valuable addition to techniques at hand for studying FXS pathology and developing treatments. In addition to these conventional models, new animal models are being developed with unique molecular and phenotypic features that can help us answer specific research questions. Examples of these novel models include Mongolian gerbils, which can more accurately recapitulate sensory (Including auditory and visual) and social behaviour deficits, and chicken embryos, which provide high temporal and spatial resolutions for in-depth characterization of FMRP functions during formation of neuronal networks (Curnow & Wang, 2022). By combining several models in FXS research, we can make use of each models' specific advantages and optimize cost-benefit ratios to grow our understanding of FXS.

However, the use of animal models always comes with certain challenges. Ideally, an animal model should mimic both human disease phenotypes and underlying biological processes as close as possible. Furthermore, animal models should be predictive and reliable for providing insights into efficacy of newly developed drugs and their mechanisms of action. This principle can be described as a challenge in validation, for which a set of criteria can be of help to evaluate the translation capability of a certain model. These include face validity, entailing similar symptom manifestations in animals and humans; construct validity, entailing similar underlying biology; and predictive validity, entailing similar response to clinically effective therapeutic agents (McGonigle and Ruggeri, 2014). However, due to insurmountable differences between model animals and humans in terms of alignment of developmental processes (Rabadan-Diehl & Nathanielsz, 2013; Watson et al., 2006), drug pharmacodynamics and -kinetics (Jansen et al., 2020) and complexity of behaviour (van der Staay, 2006), a demand for human based models is created.

Therefore, FXS modelling using patient derived material could be the next big step for unravelling disease mechanisms of FXS and provides a novel more reliable method for development and screening of treatments. The recent popularity of induced pluripotent stem cell (iPSC) use in FXS and ASD research illustrates the paradigm shift from rodent models to human based models (Bhattacharyya & Zhao, 2016; Linda, Fiuza, & Kasri, 2018; Sacco, Cacci & Novarino, 2018). By using patient-derived cells, the individual genetic and epigenetic background of the patient can be contained and treatment development can be personalized. This method allows to correct for individual variability and may therefore provide a solution for dealing with the high levels of heterogeneity found for ASDs and to a lesser extend for FXS. Furthermore, patients could be classified based on newly identified biomarkers. Research has already shown that levels of protein synthesis can vary a lot between FXS patients (Jacquemont et al., 2018). This type of information can

help identify subgroups within the population of FXS patients and develop personalized medicine, which in turn may increase the percentage of successful clinical trials. Research using iPSCs has already shown promising results for interfering with aberrant epigenetic modifications as a possibility to treat FXS. Bhattacharyya & Zhao (2016) and Liu et al. (2018) demonstrated that demethylation of the promoter region of FMRP can induce the proteins' expression and relieve FXS phenotypes. Perhaps similar techniques could be used to regulate post-translational modifications of FMRP for patients carrying missense mutations affecting these modifications and disrupting FMRP function rather than expression. The main disadvantage to iPSC models is the inability to evaluate behavioural phenotypes, resulting in the ongoing need for animal models alongside these animal free alternatives.

To aid the development of novel treatments, most of all a more in depth understanding of biological FXS mechanisms and FMRP functions is needed. While we can now identify FXS features in various animal models and even patient derived cells, we still lack insight into the causality of symptoms. For example, many of our knowledge of FXS pathology is derived from observations in FXS KO animals and cell lines, but this does not provide us with an explanation of how exactly these aberrancies arise. Similarly, correcting for dysregulation of signalling in FXS, by for example inhibition of mGluR5 activity, demonstrates that mGluR signalling is involved in FXS and that its downregulation can rescue FXS features, but does not indicate whether this is due to compensation for lost FMRP translational control or more direct functions of mGluR activity itself. An approach that could be considered is studying KO models of either FMRP interacting proteins or proteins involved in FMRP signalling instead of FMRP itself. In their review, Clifton et al. (2020) describe that FMRP and CYFIP1 KO models show similar phenotypes, which is to be expected since the proteins function together to inhibit translation. However, it is emphasized that these phenotypes do not align exactly. These subtle differences could help us understand better how proteins and more precisely the signalling pathways involved in FXS pathology interact, and how they are modulated by related proteins and cellular conditions. Furthermore, techniques like RNA-sequencing and proteomic tools can help us further identify the pool of FMRP target mRNAs and understand their regulation patterns. As was shown by Greenblatt and Spradling (2018), FMRP can promote or suppress translation depending of protein size. Therefore, to fully understand the consequences of FMRP deficiency, the regulation and function of each mRNA target has to be studied individually, and its contribution to FXS phenotypes has to be determined.

Lastly, an interesting point to examine is the effect of new knowledge on neurobiological processes underlying FXS on FXS diagnosis and treatments. As was touched upon already, FXS pathology shares a lot of overlap with ASDs and to some extend other neurodevelopmental diseases. Furthermore, treatments officially developed for other disorders can in some cases be used to treat FXS due to overlapping symptoms and/or shared dysregulation of biological mechanisms. According to these findings, an increased biological understanding of a variety of related neurodevelopmental diseases might call for a re-evaluation of our current system of classification and diagnosis, improving personalized treatment in the future.

Conclusion

While research has focussed on elucidating dysregulated signalling pathways underlying FXS pathology, a complete understanding of the interplay between these processes is still not reached, resulting in an ongoing search for effective treatments. In this review, the most important theories on which signalling mechanisms underly synaptic phenotypes observed in FXS are discussed in light of recent findings. Based on this, an overview of the current knowledge on these processes is provided and insights are combined to discuss future research opportunities. Topics discussed include the mGluR theory based on elevated mGluR-mediated LTD, composition and regulation of FMRP-containing protein complexes and competition with other regulatory complexes to balance activation-induced synaptic protein translation and cytoskeleton remodelling, the role of various forms of synaptic plasticity involving mGluR, NMDAR, AMPAR and GABAR signalling, mechanisms of actin remodelling and their consequences for spine morphology and the current state of treatment development, including the biological mechanisms targeted and progression towards clinical trials. While animal models have helped reach the current understanding of these processes and new animal models are being developed to better imitate and study specific FXS symptoms, the future of FXS research most likely lies with patient derived models. Drug development and screening using iPSCs from patients can help account for heterogeneity and drive personalized medicine, hopefully increasing the number of successful clinical trials. Furthermore, these models provide interesting opportunities to evaluate effects of epigenetic interference to regulate protein expression and knockout of FMRP-interacting proteins and -targets in human cells. These novel techniques, including patient-derived models, RNA sequencing and proteomic tools, can help us understand the interplay of signalling pathways involved in development and functioning of synapses, and aberrancies herein causing FXS phenotypes.

Layman's summary

Fragile X syndrome (FXS) is a disease caused by a mutation in a single gene and involves abnormal development of connections between brain cells, which are called synapses. This results in synapses with an abnormal shape which indicates that they are underdeveloped and hinders communication between brain cells. This type of abnormalities in brain cell connections is also often found for patients with autism. In the case of FXS, the abnormalities are caused by a missing protein, namely the protein FMRP that was made using the gene that is mutated. A protein is a machine that carries out a specific function in the cell. In the case of FMRP this function is to bind to RNA and prevent that this RNA can be used to make other proteins that are needed for functioning of healthy synapses. This may seem contradictory because the absence of FMRP means that there are extra proteins which are needed for healthy synapses. However, it is important that all proteins are present in the right amount and FMRP normally acts as a control protein to make sure that there are not too many proteins. When FMRP is thus not present, the process of protein production is out of control and this causes the abnormalities in the synapses. In this review the most important processes that cause these abnormalities are discussed and information from new research is added. The first of these processes that is not functioning well in FXS is the adjusting of the strength of connections between brain cells when these braincells are active. Normally, the synapses between these brain cells can respond to brain activity and become stronger or weaker so that there remains a balance in communication in the total network of brain cells. When FMRP is not present, the production of proteins that cause the synapses to become weaker or stronger is not under control and the synapses become too weak or too strong. The proteins that are part of this process are receptors, which are proteins on one of the brain cells that can receive signals from the other brain cell. This is the way in which the brain cells communicate. Specifically metabotropic glutamate receptors (mGluRs), N-methyl-D-aspartate receptors (NMDARs), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) and γ-aminobutyric acid receptors (GABARs) are involved in this process. These are different types of receptors, meaning that they receive different types of signals from other brain cells which can stimulate or suppress activity of the receiving brain cell. Because this process of creating weaker or stronger synapses using these receptors does not work well, there is an imbalance in stimulating and suppressing signals in the brains of FXS patients. In addition to this, missing FMRP causes problems with processes that create the shape of the synapses which causes the abnormal form. This happens because the building blocks of the skeleton of the synapse, which are called actin, are not put together in the right way because this process is normally controlled by FMRP. Lastly, because FMRP is not present, it can also not bind other proteins which means that functions carried out by FMRP and these proteins together are halted. Right now, developing successful cures for FXS is difficult because we don't know all of the processes yet that cannot function well because FMRP is not present, and how these processes influence each other. One factor that may be a problem for the development of successful treatments is differences between individual patients. Using models that are made using cells of the patients themselves instead of models using animals can help resolve this problem because the models would contain all the information of the cells of the patients. Therefore, patient based models can hopefully help increase development of successful treatments in the future.

References

1. *Achuta, V. S., Grym, H., Putkonen, N., Louhivuori, V., Kärkkäinen, V., Koistinaho, J., ... & Castren, M. L. (2017). Metabotropic glutamate receptor 5 responses dictate differentiation of neural progenitors to NMDA-responsive cells in fragile X syndrome. Developmental neurobiology, 77(4), 438-453.*
2. *Alcobra announces results from phase 2 clinical trial of MDX for fragile X syndrome. http://www.alcobra-pharma.com/releasedetail.*
3. *cfm?ReleaseID=919218. Aloisi, E., Le Corf, K., Dupuis, J., Zhang, P., Ginger, M., Labrousse, V., ... & Frick, A. (2017). Altered surface mGluR5 dynamics provoke synaptic NMDAR dysfunction and cognitive defects in Fmr1 knockout mice. Nature communications, 8(1), 1-14.*
4. *Antar, L. N., Afroz, R., Dictenberg, J. B., Carroll, R. C., & Bassell, G. J. (2004). Metabotropic glutamate receptor activation regulates fragile x mental retardation protein and FMR1 mRNA localization differentially in dendrites and at synapses. Journal of Neuroscience, 24(11), 2648-2655.*
5. *Antar, L. N., Dictenberg, J. B., Plociniak, M., Afroz, R., & Bassell, G. J. (2005). Localization of FMRP-associated mRNA granules and requirement of microtubules for activity-dependent trafficking in hippocampal neurons. Genes, Brain and Behavior, 4(6), 350-359.*
6. *Bagni, C., Tassone, F., Neri, G., & Hagerman, R. (2012). Fragile X syndrome: causes, diagnosis, mechanisms, and therapeutics. The Journal of clinical investigation, 122(12), 4314-4322.*
7. *Bagni, C., & Zukin, R. S. (2019). A synaptic perspective of fragile X syndrome and autism spectrum disorders. Neuron, 101(6), 1070-1088.*
8. *Banerjee, A., Ifrim, M. F., Valdez, A. N., Raj, N., & Bassell, G. J. (2018). Aberrant RNA translation in fragile X syndrome: From FMRP mechanisms to emerging therapeutic strategies. Brain research, 1693, 24-36.*
9. *Banerjee, P., Nayar, S., Hebbar, S., Fox, C. F., Jacobs, M. C., Park, J. H., ... & Dockendorff, T. C. (2007). Substitution of critical isoleucines in the KH domains of Drosophila fragile X protein results in partial loss-of-function phenotypes. Genetics, 175(3), 1241-1250.*
10. *Banke, T. G., & Barria, A. (2020). Transient enhanced glua2 expression in young hippocampal neurons of a fragile X mouse model. Frontiers in synaptic neuroscience, 12, 588295.*
11. *Bellosta, P., & Soldano, A. (2019). Dissecting the genetics of autism spectrum disorders: A Drosophila perspective. Frontiers in physiology, 10, 987.*
12. *Bear, M. F., Huber, K. M., & Warren, S. T. (2004). The mGluR theory of fragile X mental retardation. Trends in neurosciences, 27(7), 370-377.*
13. *Bellosta, P., & Soldano, A. (2019). Dissecting the genetics of autism spectrum disorders: A Drosophila perspective. Frontiers in physiology, 10, 987.*
14. *Berry-Kravis, E., Des Portes, V., Hagerman, R., Jacquemont, S., Charles, P., Visootsak, J., ... & Von Raison, F. (2016). Mavoglurant in fragile X syndrome: Results of two randomized, double-blind, placebo-controlled trials. Science translational medicine, 8(321), 321ra5-321ra5.*
15. *Berry-Kravis, E., Hessl, D., Coffey, S., Hervey, C., Schneider, A., Yuhas, J., ... & Hagerman, R. (2009). A pilot open label, single dose trial of fenobam in adults with fragile X syndrome. Journal of medical genetics, 46(4), 266-271.*
16. *Berry-Kravis, E., Krause, S. E., Block, S. S., Guter, S., Wuu, J., Leurgans, S., ... & Hagerman, R. (2006). Effect of CX516, an AMPA-modulating compound, on cognition and behavior in fragile X syndrome: Acontrolled trial. Journal of Child & Adolescent Psychopharmacology, 16(5), 525-540.*
17. *Bhattacharyya, A., & Zhao, X. (2016). Human pluripotent stem cell models of Fragile X syndrome. Molecular and Cellular Neuroscience, 73, 43-51.*

18. *Boismare, F., Daoust, M., Moore, N. D., Saligaut, C., Lhuintre, J. P., Chretien, P., & Durlach, J. 1. (1984). A homotaurine derivative reduces the voluntary intake of ethanol by rats: are cerebral GABA receptors involved?. Pharmacology Biochemistry and Behavior, 21(5), 787-789.*
19. *Bongmba, O. Y., Martinez, L. A., Elhardt, M. E., Butler, K., & Tejada-Simon, M. V. (2011). Modulation of dendritic spines and synaptic function by Rac1: a possible link to Fragile X syndrome pathology. Brain research, 1399, 79-95.*
20. *Borrie, S. C., Brems, H., Legius, E., & Bagni, C. (2017). Cognitive dysfunctions in intellectual disabilities: the contributions of the Ras-MAPK and PI3K-AKT-mTOR pathways. Annual review of genomics and human genetics, 18, 115-142.*
21. *Bosch, M., & Hayashi, Y. (2012). Structural plasticity of dendritic spines. Current opinion in neurobiology, 22(3), 383-388.*
22. *Bostrom, C. A., Majaess, N. M., Morch, K., White, E., Eadie, B. D., & Christie, B. R. (2015). Rescue of NMDAR-dependent synaptic plasticity in Fmr1 knock-out mice. Cerebral cortex, 25(1), 271-279.*
23. *Briševac, D., Scholz, R., Du, D., Elagabani, M. N., Köhr, G., & Kornau, H. C. (2021). The small GTPase Arf6 is dysregulated in a mouse model for fragile X syndrome. Journal of neurochemistry, 157(3), 666-683.*
24. *den Broeder, M. J., van der Linde, H., Brouwer, J. R., Oostra, B. A., Willemsen, R., & Ketting, R. F. (2009). Generation and characterization of FMR1 knockout zebrafish. PloS one, 4(11), e7910.*
25. *Çaku, A., Pellerin, D., Bouvier, P., Riou, E., & Corbin, F. (2014). Effect of lovastatin on behavior in children and adults with fragile X syndrome: An open-label study. American Journal of Medical Genetics Part A, 164(11), 2834-2842.*
26. *Castets, M., Schaeffer, C., Bechara, E., Schenck, A., Khandjian, E. W., Luche, S., ... & Bardoni, B. (2005). FMRP interferes with the Rac1 pathway and controls actin cytoskeleton dynamics in murine fibroblasts. Human Molecular Genetics, 14(6), 835-844.*
27. *Chang, S., Bray, S. M., Li, Z., Zarnescu, D. C., He, C., Jin, P., & Warren, S. T. (2008). Identification of small molecules rescuing fragile X syndrome phenotypes in Drosophila. Nature chemical biology, 4(4), 256-263.*
28. *Chen, Z., Borek, D., Padrick, S. B., Gomez, T. S., Metlagel, Z., Ismail, A. M., ... & Rosen, M. K. (2010). Structure and control of the actin regulatory WAVE complex. Nature, 468(7323), 533-538.*
29. *Chen, E., & Joseph, S. (2015). Fragile X mental retardation protein: A paradigm for translational control by RNA-binding proteins. Biochimie, 114, 147-154.*
30. *Chen, L. Y., Rex, C. S., Babayan, A. H., Kramár, E. A., Lynch, G., Gall, C. M., & Lauterborn, J. C. (2010). Physiological activation of synaptic Rac> PAK (p-21 activated kinase) signaling is defective in a mouse model of fragile X syndrome. Journal of Neuroscience, 30(33), 10977-10984.*
31. *Cheng, K., Chen, Y. S., Yue, C. X., Zhang, S. M., Pei, Y. P., Cheng, G. R., ... & Zeng, Y. (2019). Calsyntenin-1 negatively regulates ICAM5 accumulation in postsynaptic membrane and influences dendritic spine maturation in a mouse model of fragile X syndrome. Frontiers in neuroscience, 13, 1098.*
32. *Clifton, N. E., Thomas, K. L., Wilkinson, L. S., Hall, J., & Trent, S. (2020). FMRP and CYFIP1 at the synapse and their role in psychiatric vulnerability. Complex Psychiatry, 6(1-2), 5-19.*
33. *Coffee Jr, R. L., Tessier, C. R., Woodruff III, E. A., & Broadie, K. (2010). Fragile X mental retardation protein has a unique, evolutionarily conserved neuronal function not shared with FXR1P or FXR2P. Disease models & mechanisms, 3(7-8), 471-485.*

34. *Costa-Mattioli, M., & Monteggia, L. M. (2013). mTOR complexes in neurodevelopmental and neuropsychiatric disorders. Nature neuroscience, 16(11), 1537-1543.*
35. *Curnow, E., & Wang, Y. (2022). New Animal Models for Understanding FMRP Functions and FXS Pathology. Cells, 11(10), 1628.*
36. *Dahlhaus, R. (2018). Of men and mice: modeling the fragile X syndrome. Frontiers in molecular neuroscience, 11, 41.*
37. *Darnell, J. C., Van Driesche, S. J., Zhang, C., Hung, K. Y. S., Mele, A., Fraser, C. E., ... & Darnell, R. B. (2011). FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism. Cell, 146(2), 247-261.*
38. *De Rubeis, S., Pasciuto, E., Li, K. W., Fernández, E., Di Marino, D., Buzzi, A., ... & Bagni, C. (2013). CYFIP1 coordinates mRNA translation and cytoskeleton remodeling to ensure proper dendritic spine formation. Neuron, 79(6), 1169-1182.*
39. *Desai, N. S., Casimiro, T. M., Gruber, S. M., & Vanderklish, P. W. (2006). Early postnatal plasticity in neocortex of Fmr1 knockout mice. Journal of neurophysiology, 96(4), 1734-1745.*
40. *Di Marino, D., Chillemi, G., De Rubeis, S., Tramontano, A., Achsel, T., & Bagni, C. (2015). MD and docking studies reveal that the functional switch of CYFIP1 is mediated by a butterfly-like motion. Journal of chemical theory and computation, 11(7), 3401-3410.*
41. *D'Incal, C., Broos, J., Torfs, T., Kooy, R. F., & Vanden Berghe, W. (2022). Towards Kinase Inhibitor Therapies for Fragile X Syndrome: Tweaking Twists in the Autism Spectrum Kinase Signaling Network. Cells, 11(8), 1325.*
42. *Dolan, B. M., Duron, S. G., Campbell, D. A., Vollrath, B., Rao, B. S., Ko, H. Y., ... & Tonegawa, S. (2013). Rescue of fragile X syndrome phenotypes in Fmr1 KO mice by the small-molecule PAK inhibitor FRAX486. Proceedings of the National Academy of Sciences, 110(14), 5671-5676.*
43. *Dölen, G., & Bear, M. F. (2008). Role for metabotropic glutamate receptor 5 (mGluR5) in the pathogenesis of fragile X syndrome. The Journal of physiology, 586(6), 1503-1508.*
44. *Drozd, M., Bardoni, B., & Capovilla, M. (2018). Modeling fragile X syndrome in Drosophila. Frontiers in Molecular Neuroscience, 11, 124.*
45. *Eadie, B. D., Cushman, J., Kannangara, T. S., Fanselow, M. S., & Christie, B. R. (2012). NMDA receptor hypofunction in the dentate gyrus and impaired context discrimination in adult Fmr1 knockout mice. Hippocampus, 22(2), 241-254.*
46. *Erickson, C. A., Davenport, M. H., Schaefer, T. L., Wink, L. K., Pedapati, E. V., Sweeney, J. A., ... & Berry-Kravis, E. (2017). Fragile X targeted pharmacotherapy: lessons learned and future directions. Journal of neurodevelopmental disorders, 9(1), 1-14.*
47. *Erickson, C. A., Mullett, J. E., & McDougle, C. J. (2009). Open-label memantine in fragile X syndrome. Journal of autism and developmental disorders, 39(12), 1629-1635.*
48. *Fatemi, S., Folsom, T. D., Kneeland, R. E., Yousefi, M. K., Liesch, S. B., & Thuras, P. D. (2013). Impairment of fragile X mental retardation protein-metabotropic glutamate receptor 5 signaling and its downstream cognates ras-related C3 botulinum toxin substrate 1, amyloid beta A4 precursor protein, striatal-enriched protein tyrosine phosphatase, and homer 1, in autism: a postmortem study in cerebellar vermis and superior frontal cortex. Molecular autism, 4(1), 1-19.*
49. *Franchini, L., Carrano, N., Di Luca, M., & Gardoni, F. (2020). Synaptic GluN2A-containing NMDA receptors: from physiology to pathological synaptic plasticity. International journal of molecular sciences, 21(4), 1538.*
50. *Friedmann, C. T., LJ, D., PE, C., & RT, R. (1980). Phase II double-blind controlled study of a new anxiolytic, fenobam (McN-3377) vs placebo.*
51. *Gantois, I., Khoutorsky, A., Popic, J., Aguilar-Valles, A., Freemantle, E., Cao, R., ... & Sonenberg, N. (2017). Metformin ameliorates core deficits in a mouse model of fragile X syndrome. Nature medicine, 23(6), 674-677.*

52. *Grant, P., Lougee, L., Hirschtritt, M., & Swedo, S. E. (2007). An open-label trial of riluzole, a glutamate antagonist, in children with treatment-resistant obsessive-compulsive disorder. Journal of child and adolescent psychopharmacology, 17(6), 761-767.*
53. *Greenblatt, E. J., & Spradling, A. C. (2018). Fragile X mental retardation 1 gene enhances the translation of large autism-related proteins. Science, 361(6403), 709-712.*
54. *Greenhalgh, T., & Peacock, R. (2005). Effectiveness and efficiency of search methods in systematic reviews of complex evidence: audit of primary sources. Bmj, 331(7524), 1064-1065.*
55. *Gross, C., Chang, C. W., Kelly, S. M., Bhattacharya, A., McBride, S. M., Danielson, S. W., ... & Bassell, G. J. (2015). Increased expression of the PI3K enhancer PIKE mediates deficits in synaptic plasticity and behavior in fragile X syndrome. Cell reports, 11(5), 727-736.*
56. *Guang, S., Pang, N., Deng, X., Yang, L., He, F., Wu, L., ... & Peng, J. (2018). Synaptopathology involved in autism spectrum disorder. Frontiers in cellular neuroscience, 12, 470.*
57. *Guo, W., Molinaro, G., Collins, K. A., Hays, S. A., Paylor, R., Worley, P. F., ... & Huber, K. M. (2016). Selective disruption of metabotropic glutamate receptor 5-homer interactions mimics phenotypes of fragile X syndrome in mice. Journal of Neuroscience, 36(7), 2131-2147.*
58. *Hayashi, M. L., Rao, B. S., Seo, J. S., Choi, H. S., Dolan, B. M., Choi, S. Y., ... & Tonegawa, S. (2007). Inhibition of p21-activated kinase rescues symptoms of fragile X syndrome in mice. Proceedings of the national academy of sciences, 104(27), 11489-11494.*
59. *Henderson, C., Wijetunge, L., Kinoshita, M. N., Shumway, M., Hammond, R. S., Postma, F. R., ... & Healy, A. M. (2012). Reversal of disease-related pathologies in the fragile X mouse model by selective activation of GABAB receptors with arbaclofen. Science translational medicine, 4(152), 152ra128-152ra128.*
60. *Heulens, I., D'Hulst, C., Van Dam, D., De Deyn, P. P., & Kooy, R. F. (2012). Pharmacological treatment of fragile X syndrome with GABAergic drugs in a knockout mouse model. Behavioural brain research, 229(1), 244-249.*
61. *Holtmaat, A., & Svoboda, K. (2009). Experience-dependent structural synaptic plasticity in the mammalian brain. Nature Reviews Neuroscience, 10(9), 647-658.*
62. *Huang, J., Ikeuchi, Y., Malumbres, M., & Bonni, A. (2015). A Cdh1-APC/FMRP ubiquitin signaling link drives mGluR-dependent synaptic plasticity in the mammalian brain. Neuron, 86(3), 726-739.*
63. *Huber, K. M., Gallagher, S. M., Warren, S. T., & Bear, M. F. (2002). Altered synaptic plasticity in a mouse model of fragile X mental retardation. Proceedings of the National Academy of Sciences, 99(11), 7746-7750.*
64. *Hwang, J. Y., Monday, H. R., Yan, J., Gompers, A., Buxbaum, A. R., Sawicka, K. J., ... & Zukin, R. S. (2022). CPEB3-dependent increase in GluA2 subunits impairs excitatory transmission onto inhibitory interneurons in a mouse model of fragile X. Cell Reports, 39(10), 110853.*
65. *Iacoangeli, A., & Tiedge, H. (2013). Translational control at the synapse: role of RNA regulators. Trends in biochemical sciences, 38(1), 47-55.*
66. *Jacquemont, S., Pacini, L., Jønch, A. E., Cencelli, G., Rozenberg, I., He, Y., ... & Bagni, C. (2018). Protein synthesis levels are increased in a subset of individuals with fragile X syndrome. Human molecular genetics, 27(12), 2039-2051.*
67. *Jahn, K., Schlesinger, F., Jin, L. J., Dengler, R., Bufler, J., & Krampfl, K. (2008). Molecular mechanisms of interaction between the neuroprotective substance riluzole and GABAA-receptors. Naunyn-Schmiedeberg's archives of pharmacology, 378(1), 53-63.*
68. *Jansen, K., Casellas, C. P., Groenink, L., Wever, K. E., & Masereeuw, R. (2020). Humans are animals, but are animals human enough? A systematic review and meta-analysis on interspecies differences in renal drug clearance. Drug Discovery Today, 25(4), 706-717.*

69. *Jaworski, J., Spangler, S., Seeburg, D. P., Hoogenraad, C. C., & Sheng, M. (2005). Control of dendritic arborization by the phosphoinositide-3'-kinase–Akt–mammalian target of rapamycin pathway. Journal of Neuroscience, 25(49), 11300-11312.*
70. *Kalueff, A. V., Stewart, A. M., & Gerlai, R. (2014). Zebrafish as an emerging model for studying complex brain disorders. Trends in pharmacological sciences, 35(2), 63-75.*
71. *Kammermeier, P. J., & Worley, P. F. (2007). Homer 1a uncouples metabotropic glutamate receptor 5 from postsynaptic effectors. Proceedings of the National Academy of Sciences, 104(14), 6055-6060.*
72. *Kaufmann, W. E., Kidd, S. A., Andrews, H. F., Budimirovic, D. B., Esler, A., Haas-Givler, B., ... & Berry-Kravis, E. (2017). Autism spectrum disorder in fragile X syndrome: cooccurring conditions and current treatment. Pediatrics, 139(Supplement_3), S194-S206.*
73. *Khandjian, E. W., Huot, M. E., Tremblay, S., Davidovic, L., Mazroui, R., & Bardoni, B. (2004). Biochemical evidence for the association of fragile X mental retardation protein with brain polyribosomal ribonucleoparticles. Proceedings of the National Academy of Sciences, 101(36), 13357-13362.*
74. *Khayachi, A., Gwizdek, C., Poupon, G., Alcor, D., Chafai, M., Cassé, F., ... & Martin, S. (2018). Sumoylation regulates FMRP-mediated dendritic spine elimination and maturation. Nature communications, 9(1), 1-17.*
75. *Khlebodarova, T. M., Kogai, V. V., Trifonova, E. A., & Likhoshvai, V. A. (2018). Dynamic landscape of the local translation at activated synapses. Molecular psychiatry, 23(1), 107-114.*
76. *Lai, A., Valdez-Sinon, A. N., & Bassell, G. J. (2020). Regulation of RNA granules by FMRP and implications for neurological diseases. Traffic, 21(7), 454-462.*
77. *Lauterborn, J. C., Rex, C. S., Kramár, E., Chen, L. Y., Pandyarajan, V., Lynch, G., & Gall, C. M. (2007). Brain-derived neurotrophic factor rescues synaptic plasticity in a mouse model of fragile X syndrome. Journal of neuroscience, 27(40), 10685-10694.*
78. *Lee, H. Y., Ge, W. P., Huang, W., He, Y., Wang, G. X., Rowson-Baldwin, A., ... & Jan, L. Y. (2011). Bidirectional regulation of dendritic voltage-gated potassium channels by the fragile X mental retardation protein. Neuron, 72(4), 630-642.*
79. *Li, W., & Pozzo-Miller, L. (2020). Dysfunction of the corticostriatal pathway in autism spectrum disorders. Journal of neuroscience research, 98(11), 2130-2147.*
80. *Linda, K., Fiuza, C., & Kasri, N. N. (2018). The promise of induced pluripotent stem cells for neurodevelopmental disorders. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 84, 382-391.*
81. *Liu, X. S., Wu, H., Krzisch, M., Wu, X., Graef, J., Muffat, J., ... & Jaenisch, R. (2018). Rescue of fragile X syndrome neurons by DNA methylation editing of the FMR1 gene. Cell, 172(5), 979-992.*
82. *Lundbye, C. J., Toft, A. K. H., & Banke, T. G. (2018). Inhibition of GluN2A NMDA receptors ameliorates synaptic plasticity deficits in the Fmr1−/y mouse model. The Journal of Physiology, 596(20), 5017-5031.*
83. *Mann, K., Kiefer, F., Spanagel, R., & Littleton, J. (2008). Acamprosate: recent findings and future research directions. Alcoholism: Clinical and Experimental Research, 32(7), 1105-1110.*
84. *Martin, D., Thompson, M. A., & Nadler, J. V. (1993). The neuroprotective agent riluzole inhibits release of glutamate and aspartate from slices of hippocampal area CA1. European journal of pharmacology, 250(3), 473-476.*
85. *McGonigle, P., & Ruggeri, B. (2014). Animal models of human disease: challenges in enabling translation. Biochemical pharmacology, 87(1), 162-171.*

86. *Michaelsen-Preusse, K., Feuge, J., & Korte, M. (2018). Imbalance of synaptic actin dynamics as a key to fragile X syndrome?. The Journal of physiology, 596(14), 2773-2782.*
87. *Mizuno, K. (2013). Signaling mechanisms and functional roles of cofilin phosphorylation and dephosphorylation. Cellular signalling, 25(2), 457-469.*
88. *Monday, H. R., Kharod, S. C., Yoon, Y. J., Singer, R. H., & Castillo, P. E. (2022). Presynaptic FMRP and local protein synthesis support structural and functional plasticity of glutamatergic axon terminals. Neuron, 110(16), 2588-2606.*
89. *McBride, S. M., Choi, C. H., Wang, Y., Liebelt, D., Braunstein, E., Ferreiro, D., ... & Jongens, T. A. (2005). Pharmacological rescue of synaptic plasticity, courtship behavior, and mushroom body defects in a Drosophila model of fragile X syndrome. Neuron, 45(5), 753-764.*
90. *Meredith, R. M., Holmgren, C. D., Weidum, M., Burnashev, N., & Mansvelder, H. D. (2007). Increased threshold for spike-timing-dependent plasticity is caused by unreliable calcium signaling in mice lacking fragile X gene FMR1. Neuron, 54(4), 627-638.*
91. *Muddashetty, R. S., Nalavadi, V. C., Gross, C., Yao, X., Xing, L., Laur, O., ... & Bassell, G. J. (2011). Reversible inhibition of PSD-95 mRNA translation by miR-125a, FMRP phosphorylation, and mGluR signaling. Molecular cell, 42(5), 673-688.*
92. *Musumeci, S. A., Bosco, P., Calabrese, G., Bakker, C., De Sarro, G. B., Elia, M., ... & Oostra, B. A. (2000). Audiogenic seizures susceptibility in transgenic mice with fragile X syndrome. Epilepsia, 41(1), 19-23.*
93. *Napoli, I., Mercaldo, V., Boyl, P. P., Eleuteri, B., Zalfa, F., De Rubeis, S., ... & Bagni, C. (2008). The fragile X syndrome protein represses activity-dependent translation through CYFIP1, a new 4E-BP. Cell, 134(6), 1042-1054.*
94. *Oliet, S. H., Malenka, R. C., & Nicoll, R. A. (1997). Two distinct forms of long-term depression coexist in CA1 hippocampal pyramidal cells. Neuron, 18(6), 969-982.*
95. *Olmos-Serrano, J. L., Paluszkiewicz, S. M., Martin, B. S., Kaufmann, W. E., Corbin, J. G., & Huntsman, M. M. (2010). Defective GABAergic neurotransmission and pharmacological rescue of neuronal hyperexcitability in the amygdala in a mouse model of fragile X syndrome. Journal of Neuroscience, 30(29), 9929-9938.*
96. *Osterweil, E. K., Chuang, S. C., Chubykin, A. A., Sidorov, M., Bianchi, R., Wong, R. K., & Bear, M. F. (2013). Lovastatin corrects excess protein synthesis and prevents epileptogenesis in a mouse model of fragile X syndrome. Neuron, 77(2), 243-250.*
97. *Pacey, L. K., Heximer, S. P., & Hampson, D. R. (2009). Increased GABAB receptor-mediated signaling reduces the susceptibility of fragile X knockout mice to audiogenic seizures. Molecular pharmacology, 76(1), 18-24.*
98. *Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., ... & Moher, D. (2021). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. Systematic reviews, 10(1), 1-11.*
99. *Panja, D., Kenney, J. W., D'Andrea, L., Zalfa, F., Vedeler, A., Wibrand, K., ... & Bramham, C. R. (2014). Two-stage translational control of dentate gyrus LTP consolidation is mediated by sustained BDNF-TrkB signaling to MNK. Cell reports, 9(4), 1430-1445.*
100. *Park, H. R., Lee, J. M., Moon, H. E., Lee, D. S., Kim, B. N., Kim, J., ... & Paek, S. H. (2016). A short review on the current understanding of autism spectrum disorders. Experimental neurobiology, 25(1), 1.*
101. *Parvin, S., Takeda, R., Sugiura, Y., Neyazaki, M., Nogi, T., & Sasaki, Y. (2019). Fragile X mental retardation protein regulates accumulation of the active zone protein Munc18-1 in presynapses via local translation in axons during synaptogenesis. Neuroscience Research, 146, 36-47.*
102. *Pfeiffer, B. E., & Huber, K. M. (2009). The state of synapses in fragile X syndrome. The Neuroscientist, 15(5), 549-567.*

103. *Prieto, M., Folci, A., & Martin, S. (2020). Post-translational modifications of the Fragile X Mental Retardation Protein in neuronal function and dysfunction. Molecular Psychiatry, 25(8), 1688-1703.*
104. *Pyronneau, A., He, Q., Hwang, J. Y., Porch, M., Contractor, A., & Zukin, R. S. (2017). Aberrant Rac1-cofilin signaling mediates defects in dendritic spines, synaptic function, and sensory perception in fragile X syndrome. Science signaling, 10(504), eaan0852.*
105. *Rabadan-Diehl, C., & Nathanielsz, P. (2013). From mice to men: research models of developmental programming. Journal of developmental origins of health and disease, 4(1), 3-9.*
106. *Roberts, J. E., Ezell, J. E., Fairchild, A. J., Klusek, J., Thurman, A. J., McDuffie, A., & Abbeduto, L. (2018). Biobehavioral composite of social aspects of anxiety in young adults with fragile X syndrome contrasted to autism spectrum disorder. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics, 177(7), 665-675.*
107. *Ronesi, J. A., Collins, K. A., Hays, S. A., Tsai, N. P., Guo, W., Birnbaum, S. G., ... & Huber, K. M. (2012). Disrupted Homer scaffolds mediate abnormal mGluR5 function in a mouse model of fragile X syndrome. Nature neuroscience, 15(3), 431-440.*
108. *Pyronneau, A., He, Q., Hwang, J. Y., Porch, M., Contractor, A., & Zukin, R. S. (2017). Aberrant Rac1-cofilin signaling mediates defects in dendritic spines, synaptic function, and sensory perception in fragile X syndrome. Science signaling, 10(504), eaan0852.*
109. *Sacco, R., Cacci, E., & Novarino, G. (2018). Neural stem cells in neuropsychiatric disorders. Current opinion in neurobiology, 48, 131-138.*
110. *Sadybekov, A., Tian, C., Arnesano, C., Katritch, V., & Herring, B. E. (2017). An autism spectrum disorder-related de novo mutation hotspot discovered in the GEF1 domain of Trio. Nature communications, 8(1), 1-13.*
111. *Santini, E., Huynh, T. N., Longo, F., Koo, S. Y., Mojica, E., D'Andrea, L., ... & Klann, E. (2017). Reducing eIF4E-eIF4G interactions restores the balance between protein synthesis and actin dynamics in fragile X syndrome model mice. Science signaling, 10(504), eaan0665.*
112. *Sears, J. C., & Broadie, K. (2018). Fragile X mental retardation protein regulates activity-dependent membrane trafficking and trans-synaptic signaling mediating synaptic remodeling. Frontiers in Molecular Neuroscience, 10, 440.*
113. *Shang, Y., Wang, H., Mercaldo, V., Li, X., Chen, T., & Zhuo, M. (2009). Fragile X mental retardation protein is required for chemically-induced long-term potentiation of the hippocampus in adult mice. Journal of neurochemistry, 111(3), 635-646.*
114. *Sharma, A., Hoeffer, C. A., Takayasu, Y., Miyawaki, T., McBride, S. M., Klann, E., & Zukin, R. S. (2010). Dysregulation of mTOR signaling in fragile X syndrome. Journal of neuroscience, 30(2), 694-702.*
115. *Shi, Z., Fujii, K., Kovary, K. M., Genuth, N. R., Röst, H. L., Teruel, M. N., & Barna, M. (2017). Heterogeneous ribosomes preferentially translate distinct subpools of mRNAs genome-wide. Molecular cell, 67(1), 71-83.*
116. *Sledziowska, M., Galloway, J., & Baudouin, S. J. (2020). Evidence for a contribution of the Nlgn3/Cyfip1/Fmr1 pathway in the pathophysiology of autism spectrum disorders. Neuroscience, 445, 31-41.*
117. *Snyder, E. M., Philpot, B. D., Huber, K. M., Dong, X., Fallon, J. R., & Bear, M. F. (2001). Internalization of ionotropic glutamate receptors in response to mGluR activation. Nature neuroscience, 4(11), 1079-1085.*
118. *Song, C., & Broadie, K. (2022). Dysregulation of BMP, Wnt, and Insulin Signaling in Fragile X Syndrome. Frontiers in Cell and Developmental Biology, 10.*

119. *Song, C., Leahy, S. N., Rushton, E. M., & Broadie, K. (2022). RNA-binding FMRP and Staufen sequentially regulate the Coracle scaffold to control synaptic glutamate receptor and bouton development. Development, 149(9), dev200045.*
120. *Sossin, W. S., & Costa-Mattioli, M. (2019). Translational control in the brain in health and disease. Cold Spring Harbor Perspectives in Biology, 11(8), a032912.*
121. *Spencer, C. M., Alekseyenko, O., Serysheva, E., Yuva-Paylor, L. A., & Paylor, R. (2005). Altered anxiety-related and social behaviors in the Fmr1 knockout mouse model of fragile X syndrome. Genes, Brain and Behavior, 4(7), 420-430.*
122. *van der Staay, F. J. (2006). Animal models of behavioral dysfunctions: basic concepts and classifications, and an evaluation strategy. Brain research reviews, 52(1), 131-159.*
123. *Starke, E. L., Zius, K., & Barbee, S. A. (2022). FXS causing missense mutations disrupt FMRP granule formation, dynamics, and function. PLoS genetics, 18(2), e1010084.*
124. *Telias, M. (2019). Molecular mechanisms of synaptic dysregulation in fragile X syndrome and autism spectrum disorders. Frontiers in molecular neuroscience, 12, 51.*
125. *Tian, Y., Yang, C., Shang, S., Cai, Y., Deng, X., Zhang, J., ... & Zhang, C. (2017). Loss of FMRP impaired hippocampal long-term plasticity and spatial learning in rats. Frontiers in molecular neuroscience, 10, 269.*
126. *Toft, A. K. H., Lundbye, C. J., & Banke, T. G. (2016). Dysregulated NMDA-receptor signaling inhibits long-term depression in a mouse model of fragile X syndrome. Journal of Neuroscience, 36(38), 9817-9827.*
127. *Tsang, B., Arsenault, J., Vernon, R. M., Lin, H., Sonenberg, N., Wang, L. Y., ... & Forman-Kay, J. D. (2019). Phosphoregulated FMRP phase separation models activity-dependent translation through bidirectional control of mRNA granule formation. Proceedings of the National Academy of Sciences, 116(10), 4218-4227.*
128. *Ouzzani, M., Hammady, H., Fedorowicz, Z., & Elmagarmid, A. (2016). Rayyan—a web and mobile app for systematic reviews. Systematic reviews, 5(1), 1-10.*
129. *de Vrij, F. M., Levenga, J., Van der Linde, H. C., Koekkoek, S. K., De Zeeuw, C. I., Nelson, D. L., ... & Willemsen, R. (2008). Rescue of behavioral phenotype and neuronal protrusion morphology in Fmr1 KO mice. Neurobiology of disease, 31(1), 127-132.*
130. *Wang, Y., Zeng, C., Li, J., Zhou, Z., Ju, X., Xia, S., ... & Sun, Z. S. (2018). PAK2 haploinsufficiency results in synaptic cytoskeleton impairment and autism-related behavior. Cell reports, 24(8), 2029-2041.*
131. *Watson, R. E., DeSesso, J. M., Hurtt, M. E., & Cappon, G. D. (2006). Postnatal growth and morphological development of the brain: a species comparison. Birth Defects Research Part B: Developmental and Reproductive Toxicology, 77(5), 471-484.*
132. *Yang, T., Zhao, H., Lu, C., Li, X., Xie, Y., Fu, H., & Xu, H. (2016). Synaptic plasticity, a prominent contributor to the anxiety in fragile X syndrome. Neural Plasticity.*
133. *Yun, S. H., & Trommer, B. L. (2011). Fragile X mice: Reduced long-term potentiation and N-Methyl-D-Aspartate receptor-mediated neurotransmission in dentate gyrus. Journal of neuroscience research, 89(2), 176-182.*
134. *Zakharenko, S. S., Zablow, L., & Siegelbaum, S. A. (2002). Altered presynaptic vesicle release and cycling during mGluR-dependent LTD. Neuron, 35(6), 1099-1110.*
135. *Zarate Jr, C. A., Payne, J. L., Quiroz, J., Sporn, J., Denicoff, K. K., Luckenbaugh, D., ... & Manji, H. K. (2004). An open-label trial of riluzole in patients with treatment-resistant major depression. American Journal of Psychiatry, 161(1), 171-174.*
136. *Zarnescu, D. C., Shan, G., Warren, S. T., & Jin, P. (2005). Come FLY with us: toward understanding fragile X syndrome. Genes, Brain and Behavior, 4(6), 385-392.*

137. *Zeidler, S., De Boer, H., Hukema, R. K., & Willemsen, R. (2017). Combination therapy in fragile X syndrome; possibilities and pitfalls illustrated by targeting the mGluR5 and GABA pathway simultaneously. Frontiers in molecular neuroscience, 10, 368.*
138. *Zoghbi, H. Y., & Bear, M. F. (2012). Synaptic dysfunction in neurodevelopmental disorders associated with autism and intellectual disabilities. Cold Spring Harbor perspectives in biology, 4(3), a009886.*