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Regulation of molecular pathways in the Fragile X Syndrome: insights into Autism Spectrum Disorders

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Abstract The Fragile X syndrome (FXS) is a leading cause of intellectual disability (ID) and autism. The disease is caused by mutations or loss of the Fragile X Mental Retardation Protein (FMRP), an RNA-binding protein playing multiple functions in RNA metabolism. The expression of a large set of neuronal mRNAs is altered when FMRP is lost, thus causing defects in neuronal morphology and physiology. FMRP regulates mRNA stability, dendritic targeting, and protein synthesis. At synapses, FMRP represses protein synthesis by forming a complex with the Cytoplasmic FMRP Interacting Protein 1 (CYFIP1) and the cap-binding protein eIF4E. Here, we review the clinical, genetic, and molecular aspects of FXS with a special focus on the receptor signaling that regulates FMRP-dependent protein synthesis. We further discuss the FMRP–CYFIP1 complex and its potential relevance for ID and autism.

Keywords Fragile X syndrome · Autism · FMRP· Protein synthesis · mRNPs · mRNA metabolism · Synaptic plasticity

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Fragile X and fragile X tremor ataxia syndromes: clinical phenotypes

The Fragile X syndrome (FXS) is the most frequent form of inherited intellectual disability (Jacquemont et al. 2007). Patients with FXS have physical features, such as large ears, an elongated face, and a high arched palate, which have been reported in 60% of prepubertal FXS boys. Other symptoms include connective tissue anomalies, which can lead to a prolapsed mitral valve, scoliosis, flat feet, and joint laxity and macro-orchidism in boys. Macro-orchidism affects about 90% of boys with FXS (Jacquemont et al. 2007) but the causes are still largely unknown. In addition, subtle dysfunctions of the hypothalamic-pituitary-thyroid axis have been reported in FXS patients (Bregman et al. 1990). Studies performed in *Fmr1* KO animals did not reveal altered levels of the follicle-stimulating hormone, but an increase of Sertoli cell proliferation during testis development (Slegtenhorst-Eegdeman et al. 1998).

Patients display a broad spectrum of cognitive and behavioral deficits. The developmental delay is the most consistent feature, with a mean IQ of 42 in boys and severe mental retardation in about 25% of cases. Since the disorder is X-linked and the penetrance is variable, females are usually in a low-normal range, with an IQ ranging from 70 to 90 (Jacquemont et al. 2007). Moreover, epilepsy has been described in 13–18% of boys and 4% in girls, but the seizures tend to resolve during childhood (Berry-Kravis 2002). Despite the severe neurobehavioral symptoms, the anatomical studies only revealed minor abnormalities in post mortem brains from FXS patients (Hallahan et al. 2011; Reiss et al. 1995). The most prominent neuroanatomical feature is the dysgenesis of dendritic spines that appear longer and thinner than normal, likely due to a developmental delay in spine dynamics and in transition from immature

to mature spines (Cruz-Martin et al. 2010; Irwin et al. 2001). FXS is also considered the most common monogenic cause of Autism Spectrum Disorder, ASD (Hatton et al. 2006) (see below).

FXS is mainly due to a triplet repeat expansion in the 5′ untranslated region (5′UTR) of the Fragile X mental retardation 1 (FMR1) gene, located on chromosome Xq27-3. In over 90% of patients, a CGG triplet in the 5′ UTR of the gene is expanded to over 200 copies, leading to hypermethylation of the CGG, transcriptional silencing, and abolished production of the Fragile X Mental Retardation Protein (FMRP) (Jacquemont et al. 2007). Cases of FXS due to point mutations or microdeletions are very rare. A severe FXS form has been documented in a patient with a mutation in the coding region, leading to the substitution of Isoleucine 304 to Asparagine (Ile304Asn) (De Boulle et al. 1993). A few cases of deletions in the coding regions have also been identified (Gedeon et al. 1992; Meijer et al. 1994; Mila et al. 2000; Wohrle et al. 1992).

The CGG triplet region is highly polymorphic in the population. Normal alleles (5–44 CGG copies) are stably transmitted to the offspring; "grey-zone" alleles (45–54 copies), and "premutation" alleles (55–200 copies) are rather unstable and can evolve into a "full mutation" (>200 repeats) during the maternal transmission. The risk of transmitting a full mutation allele is a function of the repeat length (Hagerman and Hagerman 2002).

Premutation alleles do not lead to FXS, but can cause two distinct pathologies, namely the Fragile X-associated Premature Ovarian Insufficiency (FX-POI) and the Fragile X-associated Tremor/Ataxia Syndrome (FXTAS). FX-POI is defined as menopause or hypoestrogenic amenorrhea occurring prior to age 40. Premature ovarian failure occurs in about 1% of the general population, and about 6% of women with this disease are positive for premutation alleles (Hagerman and Hagerman 2002). FXTAS is a neurodegenerative disorder mainly featured by progressive cerebellar ataxia and intention tremor. The patients also show neuropsychiatric alterations (anxiety, hostility, depression) and cognitive dysfunctions, ranging from mild frontal executive and memory deficits to global dementia (Hagerman and Hagerman 2002; Jacquemont et al. 2007). Although FXTAS mainly affects men, clinical cases of women with FXTAS have also been reported (Hagerman et al. 2004).

The molecular mechanisms underlying the premutation pathologies are not fully understood. A consistent molecular feature is the elevation of *FMR1* mRNA levels (Allen et al. 2004; Kenneson et al. 2001; Tassone et al. 2000) due to increased transcription (Tassone et al. 2007). Nevertheless, carriers of premutation alleles show decreased levels of FMRP (Brouwer et al. 2008; Entezam et al. 2007; Kenneson et al. 2001; Primerano et al. 2002) caused by reduced translational efficiency of the *FMR1* mRNA due to the CGG expansion in the 5′UTR (Feng et al. 1995; Primerano et al. 2002) as well as polyadenylation (Tassone et al. 2011). Additionally, *FMR1* mRNA has been detected in the ubiquitin-positive intranuclear inclusions found in neurons and astrocytes throughout the brain of FXTAS patients (Greco et al. 2002; Tassone et al. 2004). The intranuclear foci were consistently observed in model organisms for FXTAS, both mouse (Berman and Willemsen 2009) and *Drosophila* (Jin et al. 2007; Sofola et al. 2007). Remarkably, the number of inclusions correlates with the size of the CGG expansion (Greco et al. 2006; Greco et al. 2002). These observations led to the hypothesis that the premutation causes a gain-of-function phenotype due to RNA toxicity (Brouwer et al. 2009; Garcia-Arocena and Hagerman 2010; Tan et al. 2009).

Fragile X syndrome and Autism Spectrum Disorder FXS is the most common monogenic cause of Autism Spectrum Disorder (ASD), a heterogenous group of neurodevelopmental pathologies affecting approximately 37 individuals in 10,000 (Fombonne 2005) and observed in more than 40% of patients with intellectual disability (Moss and Howlin 2009). ASD is diagnosed by clinical assessment of three core dysfunctions before 3 years of age: atypical social behavior, deficits in verbal and non-verbal communication, and presence of repetitive and highly restricted interests (Geschwind and Levitt 2007). These disturbances range from the Autistic Disorder (13 in 10000), through a Pervasive Developmental Disorder Not Otherwise Specified (PDD-NOS, 20.8 in 10000), to a less frequent form, the Asperger syndrome (2.6 in 10000) (Fombonne 2005). However, while children with Autistic Disorder and PDD-NOS have specific impairments in the three domains (language, social and interests) and can be mentally retarded, individuals with Asperger Syndrome have a proper use of language and are not affected by cognitive delay (Abrahams and Geschwind 2008).

About 25% of FXS boys and 6% of girls have been reported to meet criteria for ASD, while 1–2% of patients affected by ASD have FXS (Abrahams and Geschwind 2008; Hatton et al. 2006). Recent reports estimated that about 30% of FXS subjects meet criteria for Autistic Disorder and 30% for PDD-NOS (Harris et al. 2008). However, up to 90% of children with Fragile X display behavioral alterations which resemble ASD, such as social anxiety, gaze avoidance, sensory hypersensitivity, tactile defensiveness, stereotypic movements, poor motor coordination, delayed speech development, and echolalia (Belmonte and Bourgeron 2006; Hernandez et al. 2009). The basis of reduced penetrance of ASD in patients with FXS is still unclear; nevertheless, the cognitive delay is more severe in FXS children with ASD, and children with FXS and ASD are more likely to have a secondary medical problem compared to those with FXS alone (Garcia-Nonell et al. 2008).

Molecular functions of FMRP

Fragile X syndrome is caused by the absence of the Fragile X Mental Retardation Protein (FMRP). *FMR1* mRNA is ubiquitously expressed, and high levels have been detected in brain and gonads—the organs mainly affected in FXS patients (Tamanini et al. 1997). Considering the correlation between FMRP expression and cognitive abilities, the molecular role of this protein has been deeply investigated in brain.

Functionally, FMRP belongs to the family of RNA binding proteins (RBPs), which shuttle between nucleus and cytoplasm. As other RBPs, FMRP interacts with protein partners and RNAs forming large messenger ribonucleoparticles (mRNPs). FMRP has four RNA binding domains and can associate with messenger RNAs, as well as noncoding RNAs, such as the Brain Cytoplasmic 1 (*BC1*) RNA and microRNAs (Edbauer et al. 2010; Gabus et al. 2004; Johnson et al. 2006; Zalfa and Bagni 2005; Zalfa et al. 2003). Among the best characterized FMRP targets are *α-CaMKII*, *Arc*, *Map1b*, *Sapap4*, and *PSD-95* mRNAs (Bassell and Warren 2008) (Table 1). While some mRNAs such as *PSD-95* are directly recognized by FMRP (Zalfa et al. 2007), some others are recruited through base-pairing with noncoding RNAs such as *BC1* (Zalfa et al. 2005; Zalfa et al. 2003) and microRNAs (Edbauer et al. 2010). Furthermore, FMRP mRNPs orchestrate the posttranscriptional destiny of bound mRNAs by regulating their stability, localization, or translation (Bagni and Greenough 2005; De Rubeis and Bagni 2010) (Table 1).

Effects of FMRP on mRNA stability FMRP can regulate mRNAs half-life, either by favoring or preventing mRNA decay (De Rubeis and Bagni 2010). Two high-throughput screenings revealed that FMRP affects the expression of target mRNAs. First, Warren and colleagues found that the levels of 144 target mRNAs were changed in lymphoblastoid cells from FXS patients (Brown et al. 2001). In a second study, Eberwine and collaborators reported decreased expression levels of at least 2 mRNAs (*p40/LRP* and *GRK4* mRNAs) in the hippocampus of *Fmr1* KO mice (Miyashiro et al. 2003). Furthermore, loss of FMRP may affect the expression of mRNAs encoding $GABA_A$ receptors. The expression of *δ subunit* mRNA, previously identified as FMRP target by Miyashiro et al. (2003), was found to be reduced in FMRP-deficient neurons in a

Table 1 FMRP functions and target mRNAs

FMRP Function	Regulation	Target MRNAS	Where FMRP affects their regulation	How FMRP affects their regulation	References
Stability	+	PSD-95	Mouse hippocampus	Activity-dependent	Zalfa et al. 2007
	+	GABA subunits	Mouse cortex; Drosophila.	mRNA levels are reduced but a direct evidence on their different stability is not available	Gantois et al. 2006; D'Hulst et al. 2006; Dictenberg et al. 2008
Transport	+	Map1B	Primary hippocampal neurons	Activity-driven transport	Dictenberg et al. 2008
	+	α-CaMKII	Primary hippocampal neuron	Activity-driven transport	Dictenberg et al. 2008; Kao et al. 2010
	+	Sapap4	Primary hippocampal neurons	Activity-driven transport	Dictenberg et al. 2008
	+	Rgs5	Hippocampal slices	Basal mRNA localization	Miyashiro et al. 2003
Translation	−	Map1B	Mouse brain and hippocampal slices; human FXS lymphoblastoid cells;	Basal and activity-dependent	Brown et al. 2001; Zalfa et al. 2003; Lu et al. 2004; Hou et al. 2006
	−	Arc	Mouse brain; hippocampal slices; synaptoneurosomes	Basal and activity-dependent	Zalfa et al. 2003; Park et al. 2008
	−	α-CaMKII	Mouse brain; hippocampal slices; synaptoneurosomes	Basal and activity-dependent	Zalfa et al. 2003; Hou et al. 2006; Muddashetty et al. 2007; Kao et al. 2010
	−	App	Cortical synaptoneurosomes	Basal and activity-dependent; antagonistic effects with HnRNPC	Westmark and Malter 2007; Lee et al. 2010
	−	PSD-95	Cortical synaptoneurosomes	Activity-dependent	Todd et al. 2003; Muddashetty et al. 2007

Three well-characterized FMRP functions, namely mRNA stability, transport and translation, are indicated. Established FMRP target mRNAs are listed. Other studies on additional putative mRNA targets are cited in the text

genome-wide expression profiling study (Gantois et al. 2006) as well as by in situ hybridization studies (Dictenberg et al. 2008). Additionally, the mRNAs encoding 8 out of 18 known GABA subunits (α1, α3, α4, β1, β2, γ1, γ2 as well as the above mentioned δ) are significantly reduced in cortex, but not in cerebellum, of *Fmr1* KO mice (D'Hulst et al. 2006). Similar results were obtained for the three subunits conserved in *Drosophila* (D'Hulst et al. 2006). However, further studies are required to address if this regulation is directly or indirectly mediated by FMRP.

Finally, two recent reports have implicated FMRP as a direct modulator of mRNA turnover. First, FMRP through its C terminus domain recognizes a G-rich structure in the 3′UTR of *PSD-95* mRNA and protects it from decay. This effect, observed only in hippocampus and not in cortex, leads to decreased *PSD-95* mRNA expression in *Fmr1* KO animals. Of note, the stabilizing function of FMRP is promoted after the activation of the group I metabotropic glutamate receptors (mGluRs) with the agonist (S)-3,5-dihydroxyphenylglycine (DHPG) (Zalfa et al. 2007). Furthermore, the region-specific effect of FMRP on *PSD-95* mRNA stability is consistent with other reports showing that *PSD-95* mRNA synaptic translation is affected in cortical synaptoneurosomes of FMRP-lacking mice (Muddashetty et al. 2007; Todd et al. 2003). This leads to the hypothesis that (1) different molecular complexes act, together with FMRP, in cortex and hippocampus; (2) FMRP regulates mRNAs differentially in cell soma and at synapses. Second, in a mouse neuroblastoma cell line, FMRP favors the decay of *Nxf1* mRNA acting in concert with the nuclear export factor NXF2 (Zhang et al. 2007). Upon NXF2 overexpression, *Nxf1* mRNA is rapidly degraded, but this effect is obstructed by silencing FMRP; this would suggest that FMRP mediates the degradation of *Nxf1* mRNA induced by NXF2 (Zhang et al. 2007).

Effects of FMRP on mRNA dendritic transport FMRP can also modulate the targeting of mRNAs in subcellular domains far away from the cell body, such as dendrites and spines. While travelling along the dendrites, the mRNAs are thought to be translationally silent; after synaptic stimulation, the mRNPs can be docked to the spines and protein synthesis ensues (Bramham and Wells 2007). FMRP is present along the dendritic shaft, at the basis of the spines (Antar et al. 2004; Feng et al. 1997; Ferrari et al. 2007), in growth cones and mature axons (Antar et al. 2006; Centonze et al. 2008; Price et al. 2006).

The synaptic distribution of FMRP increases upon the activation of the group I mGluRs. In fact, in response to DHPG, the dendritic transport of *Fmr1* mRNA is enhanced, and FMRP is newly synthesized in close proximity to mGluR5 (Antar et al. 2004; Ferrari et al. 2007; Kao et al. 2010); in addition, FMRP is further recruited by travelling along the microtubules (Antar et al. 2004; Antar et al. 2005; Dictenberg et al. 2008; Ferrari et al. 2007; Kanai et al. 2004). In mammals, FMRP has been reported to interact with the motor proteins kinesin 5 (KIF5) and 3C (Dictenberg et al. 2008; Kanai et al. 2004; Davidovic et al. 2007); in *Drosophila*, FMRP has been found with kinesin and dynein (Ling et al. 2004). In the model proposed by Dictenberg and colleagues, upon DHPG stimulation, FMRP would interact more efficiently with the kinesin light chain, a major cargo-binding subunit of KIF5, thus promoting the activity-dependent localization of bound mRNAs. Therefore, the stimulus-induced dendritic targeting of some mRNAs such as *Map1b*, *α-CaMKII*, and *Sapap4* is compromised in *Fmr1* KO hippocampal neurons (Dictenberg et al. 2008). This report is in agreement with previous in vivo studies by Steward and colleagues showing that mRNA transport is not affected in basal condition (Steward et al. 1998).

Recently, a time-lapse imaging study revealed that the dendritic granules containing *Fmr1* and *α-CaMKII* mRNAs undergo decelerated motion within 0–40 min after DHPG stimulation, likely due to docking of these mRNAs to the spines. Consistently, *α-CaMKII* mRNA distribution in spines increases upon DHPG application to adjacent dendrites. Both effects are abolished in FMRP deficient neurons (Kao et al. 2010). This piece of evidence suggests that FMRP not only contributes to mRNA transport along dendrites, but also to activity-induced docking of the mRNAs in the spines.

Effects of FMRP on protein synthesis In highly polarized cells like neurons, protein synthesis occurs not only in the soma, but also along dendrites, axons, and at synapses. While polyribosomes, translational factors, and specific mRNAs have been detected at post-synaptic sites (Bramham and Wells 2007; Steward and Schuman 2003), considerable evidence indicates regulation of axonal protein synthesis as well (Holt and Bullock 2009). De novo protein synthesis at synapses is a critical event underlying long-lasting forms of synaptic plasticity, required for consolidation and storage of long-term memories. In fact, BDNF-induced Long Term Potentiation (LTP) in hippocampal brain slices can be blocked by translational inhibitors, even when the pre- and post-synapses are severed from the soma (Kang and Schuman 1996). Another form of synaptic plasticity, mGluR-dependent Long Term Depression (mGluR-LTD), also depends on local protein synthesis (Huber et al. 2000).

Remarkably, FMRP is implicated in both basal and activity-dependent local protein synthesis (Hou et al. 2006; Kao et al. 2010; Lu et al. 2004; Muddashetty et al. 2007; Napoli et al. 2008; Park et al. 2008; Zalfa et al. 2003). FMRP represses translation both in vitro (Laggerbauer et al.

2001; Li et al. 2001) and in vivo (Hou et al. 2006; Lu et al. 2004; Muddashetty et al. 2007; Napoli et al. 2008; Narayanan et al. 2007; Park et al. 2008; Zalfa et al. 2003). The properties of FMRP as translational regulator have been widely investigated through the biochemical fractionation of brain extracts in actively translating ribosomes (polysomes) and translationally silent particles (mRNPs). These studies revealed that more than 200 FMRP target mRNAs display an abnormal polysome/mRNP distribution in lymphoblastoid cells from individuals with FXS, indicative of altered translation (Brown et al. 2001). In the brain from *Fmr1* KO mice, several mRNAs including *α-CaMKII*, *Arc*, *Map1b* are preferentially distributed on polysomes rather than on mRNPs as result of excessive translation. Accordingly, the levels of the proteins encoded by those mRNAs are significantly increased in the absence of FMRP (Zalfa et al. 2003). In addition, these changes are also present in purified synaptoneurosomes (Muddashetty et al. 2007; Zalfa et al. 2003), extending the function of FMRP as a repressor to synapses. In response to DHPG stimulation, however, FMRP-mediated inhibition of *α-CaMKII* mRNA is rapidly released; this activity-dependent response is abolished in the absence of FMRP (Hou et al. 2006; Kao et al. 2010; Muddashetty et al. 2007). Finally, a high-throughput proteomic study recently showed that the expression of over 100 proteins is altered in synaptoneurosomes isolated from *Fmr1* KO neurons, possibly due to affected dendritic mRNA localization and protein synthesis (Liao et al. 2008). At the level of the synaptic compartment, local protein synthesis can be coupled with membrane receptors. It has been demonstrated that DCC, the receptor for the axonal guidance factor netrin, anchors components of the translational machinery in growth cones, filopodial tips, and at synapses (Tcherkezian et al. 2010). In addition, FMRP interacts with the sodium-activated potassium channel Slack-B and recruits some co-interacting mRNAs (*Map1b* and *Arc* mRNAs) to the channel (Brown et al. 2010). Therefore, it is tempting to hypothesize that FMRP is not only implicated in the regulation of local translation, but also couples it to synaptic membrane receptors.

The complex FMRP regulation downstream receptor activation has been investigated by several laboratories and is summarized in Fig. 1.

FMRP form different mRNP particles The mechanisms responsible for FMRP-dependent protein synthesis are still debated. To investigate this aspect, the polysome/mRNPs sedimentation of protein extracts along sucrose gradients has been used by different laboratories delivering different results (Zalfa et al. 2006). Initial studies from Dreyfuss' laboratory showed that in mammalian cells, FMRP is mainly associated with mRNPs (Siomi et al. 1996). Following studies detected FMRP either co-fractionating with polysomes (Ceman et al. 2003; Khandjian et al. 2004; Stefani et al. 2004) or with mRNPs (Ishizuka et al. 2002; Monzo et al. 2006; Napoli et al. 2008; Papoulas et al. 2010; Siomi et al. 1996; Zalfa et al. 2003) or equally distributed between polysomes and mRNPs (Brown et al. 2001). FMRP can associate with many nuclear and cytoplasmic partners forming different neuronal granules, including P bodies, stress granules, and transport granules (Anderson and Kedersha 2006; Kanai et al. 2004; Zalfa et al. 2006). This suggests that FMRP can take part in a variety of mRNPs and is possibly influenced by its phosphorylation state (Ceman et al. 2003). Importantly for the physiology of the FMRP complexes, both P bodies and stress granules do not contain large ribosomal subunits and polysomes (Anderson and Kedersha 2006). Furthermore, the distribution of FMRP on both mRNPs and polysomes could account for different functions of FMRP in either repressing or activating translation (Zalfa et al. 2006). In agreement with this hypothesis, Brown and colleagues found that out of 251 mRNAs displaying different polysomal distribution in cells from FXS patients, 136 were increased on polysomes and 115 decreased (Brown et al. 2001), suggesting that FMRP might have a dual role in translation.

The FMRP-CYFIP1-eIF4E complex: regulation of translational initiation

The hypothesis that FMRP is implicated in the repression of translational initiation is supported by the recent discovery that the Cytoplasmic FMRP Interacting Protein 1 (CYFIP1) acts as an eIF4E-binding protein (Napoli et al. 2008). CYFIP1, also known as Specifically Rac1 Activated protein 1 (SRA-1), was identified early as a partner of FMRP in neurons (Schenck et al. 2003; Schenck et al. 2001). CYFIP1 is also a component of a signaling machinery controlling the actin cytoskeleton, formed by the WASP-family verprolin-homologous (WAVE) (Takenawa and Suetsugu 2007).

As proposed by Napoli et al. (2008), CYFIP1 is a neuronal eIF4E-binding protein (4E-BP). The molecular function of 4E-BPs is to repress mRNA translation by sequestering the cap-binding protein eIF4E. The 4E-BPs and eIF4G, the scaffolding protein required for the assembly of the active initiation complex, share a canonical eIF4E-binding site (YXXXXLΦ, where X is any amino acid and Φ is a hydrophobic amino acid) (Costa-Mattioli et al. 2009; Marcotrigiano et al. 1999), therefore competing for the binding to eIF4E. As a consequence, the interaction of the 4E-BPs with eIF4E blocks the translation initiation (Fig. 2).

Three canonical 4E-BPs have been identified in mammals. 4E-BP1 is mostly present in adipose tissues and in pancreas,

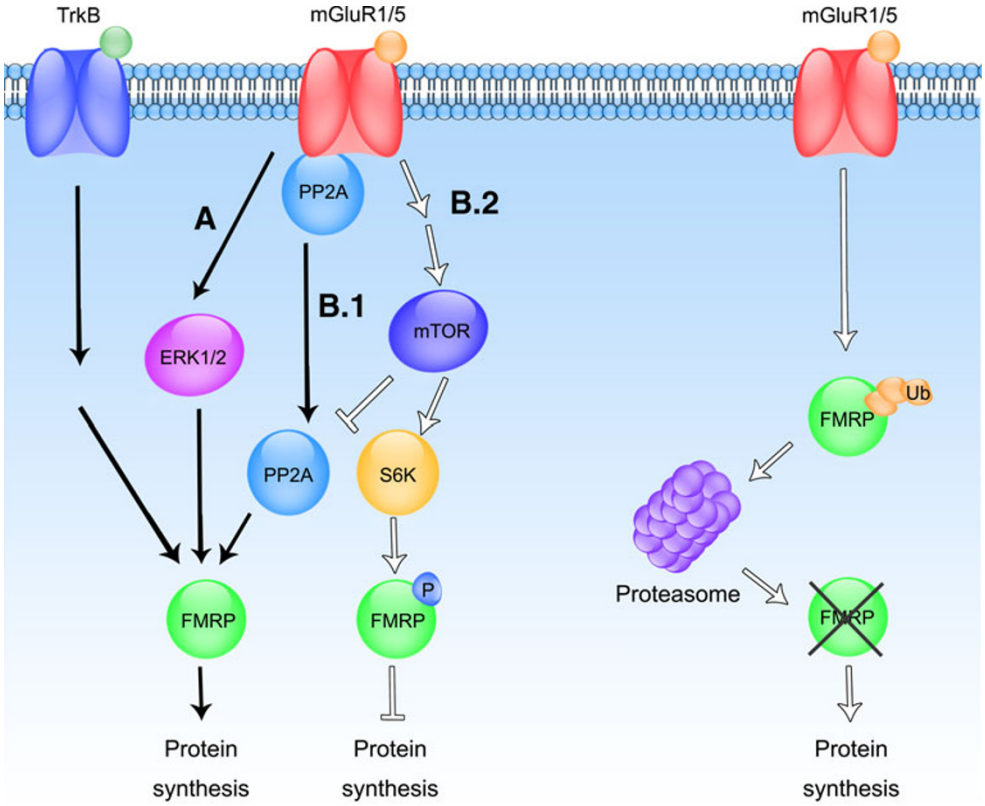

Fig. 1 Model for a postsynaptic FMRP signaling. At synapses, FMRP translational control is released upon TrkB tyrosin kinase signaling activated by BDNF (Napoli et al. 2008) and/or group I mGluRs cascade activated by DHPG (Napoli et al. 2008; Narayanan et al. 2007; Narayanan et al. 2008; Osterweil et al. 2010). Two alternative models have been proposed for the mGluR signaling upstream FMRP. The first one (*A*) proposes that the kinase ERK1/2 activation releases FMRP translational inhibition (Osterweil et al. 2010). The second one (*B*) implicates a bimodal PP2A/S6K signaling: an early dephosphorylation by PP2A (*B.1*) activates translation; sustained DHPG stimulation activates mTOR pathway (*B.2*), which suppresses PP2A activity and stimulates S6K, thus leading to FMRP phosphorylation and translational block (Narayanan et al. 2007; Narayanan et al. 2008). Furthermore, activation of group I mGluRs induces an early raise of FMRP due to protein synthesis (Antar et al. 2004; Ferrari et al. 2007; Kao et al. 2010) followed by a proteasome-dependent degradation of the protein (Hou et al. 2006; Zhao et al. 2011), restoring normal FMRP levels. Early events are indicated with black arrows, while late events induced by sustained stimulation are indicated by white arrows

4E-BP3 in the liver and 4E-BP2 in brain, which expresses little or no 4E-BP1 and 3 (Banko et al. 2005; Klann and Dever 2004). These proteins regulate a large subset of cellular mRNAs. The translation of a small number of specific mRNAs can be regulated by other non-canonical 4E-BPs. Two examples have been so far reported: the *Drosophila* Cup and the vertebrate Maskin/Neuroguidin. In such cases, the 4E-BP (Maskin or Neuroguidin, Cup) sequesters eIF4E and relies on a specific RNA-binding protein (CPEB, and Bruno, respectively) to inhibit the translation of associated mRNAs (Richter and Klann 2009) (Fig. 2). The CYFIP1-FMRP complex closely resembles these complexes: in brain and at synapses, CYFIP1 tethers the mRNAs associated with FMRP on the eIF4E, thus repressing their translation (Napoli et al. 2008) (Fig. 2). CYFIP1, as well as FMRP and eIF4E, co-fractionates with mRNPs along sucrose gradients from mouse brain lysates. Moreover, CYFIP1 associates with the cap-binding complex in an FMRP-independent manner. Although CYFIP1 does not have a canonical eIF4E-binding sequence (YXXXXLΦ), it directly binds eIF4E. In fact, CYFIP1 possesses a "non canonical" sequence predicted to form a peculiar "reverse L shaped" structure, which overlaps with the region of the 4E-BPs fitting into the eIF4E pocket (Napoli et al. 2008).

The FMRP-CYFIP1-eIF4E complex contains *BC1* RNA, as well as several known FMRP target mRNAs, including *Map1b, αCaMKII*, and *App* mRNAs. Since *BC1* RNA can mediate the association of specific mRNAs to FMRP, loss of *BC1* affects, to differing extents, the recruitment of those mRNAs. Consistent with this evidence, the downregulation of CYFIP1 in cultured neurons, as well as its genetic depletion in mouse, causes a significant increase in α-CaMKII, MAP1B, and APP protein levels (Napoli et al. 2008).

Remarkably, the inhibitory FMRP-CYFIP1-eIF4E complex is present along dendrites and at synapses, but its formation can be reversed upon neuronal activity (Fig. 3). The stimulation of synaptoneurosomes with either BDNF or DHPG favors the release of eIF4E from CYFIP1, thus

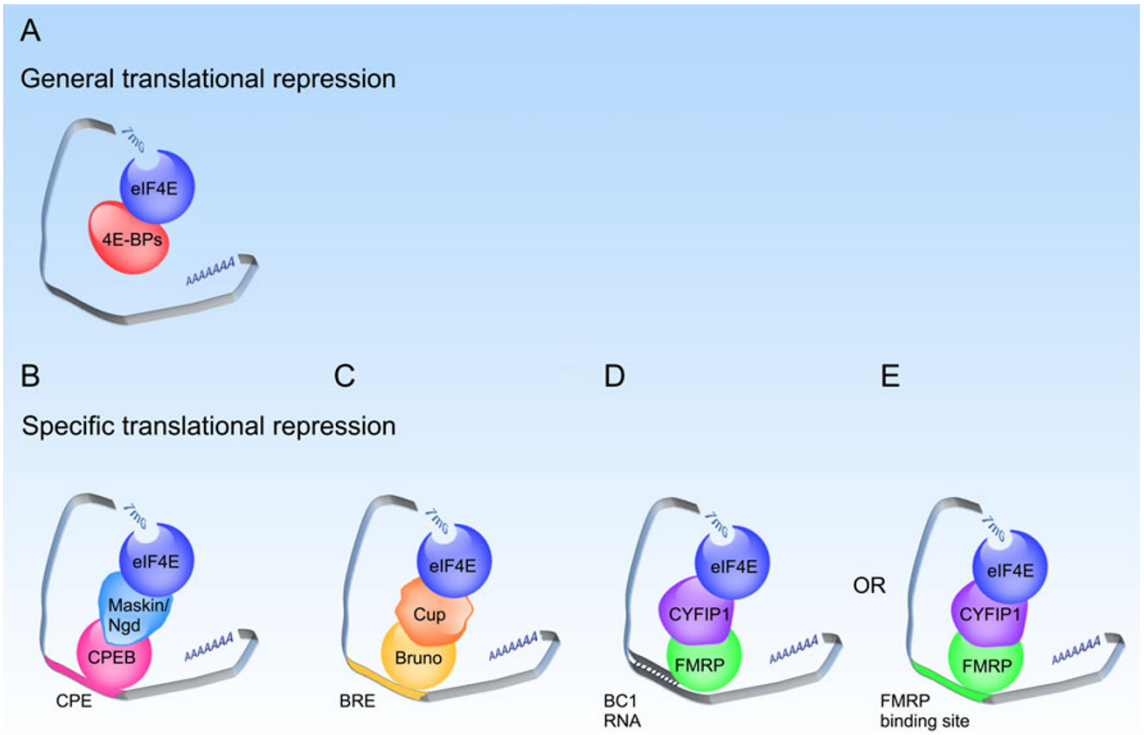

Fig. 2 mRNA translational repression. **a** General mRNA translation is repressed by binding of 4E-BP1/2/3 (*red*) to the cap-binding protein eIF4E (*blue*). **b** Specific translational regulation through sequence specific regulatory elements. mRNAs harboring the Cytoplasmic Polyadenylation Element (CPE) are recruited by CPEB (*purple*) and their translation repressed by Maskin/Neuroguidin (*light blue*); **c** mRNAs carrying the Bruno-Responsive Element (BRE) are bound by Bruno (*yellow*) and their translation inhibited by Cup (*orange*). **d** FMRP (*green*) can either directly interact with the mRNAs or **e** recruit them to the inhibitory complex by base-pairing with the non coding RNA *BC1*. In this case, the translation repression occurs via the eIF4E-BP CYFIP1 (*violet*)

alleviating the translational block (Napoli et al. 2008). In conclusion, these data are consistent with a model whereby FMRP tethers specific mRNAs on CYFIP1, which in turn sequesters eIF4E and represses translation initiation. Upon synaptic stimuli, FMRP-CYFIP1 dissociates from eIF4E and translation is activated (Fig. 3).

The mechanisms reverting the CYFIP1-mediated repression are unknown. In several cell types, 4E-BPs are regulated through the extracellular signal-regulated kinase (ERK), phosphoinositide 3-kinase (PI3K) and mammalian target of rapamycin (mTOR) pathways (Fig. 1). Upon an array of stimuli, the activation of the Ser/Thr kinase mTOR leads to the phosphorylation of several targets, including the kinase responsible for FMRP phosphorylation, S6K, and the 4E-BPs. The phosphorylation of the 4E-BPs disrupts the interaction with eIF4E, therefore relieving the translational inhibition (Richter and Klann 2009). Further studies are necessary to determine if CYFIP1 activity can also be modulated by post-translational modifications.

Translational control and Autism Spectrum Disorder As discussed, de novo local protein synthesis is required for synaptic plasticity; in particular, the control of translational initiation mediated by mTOR signal cascade and 4E-BPs is critical for the long-lasting changes underlying the consolidation and storage of long-term memories. In fact, both late phase LTP (L-LTP) and mGluR-dependent LTD trigger mTOR activation, resulting in enhanced 4E-BP2 phosphorylation and increased initiation complex formation (Costa-Mattioli et al. 2009). Additionally, the mTOR inhibitor rapamycin blocks long-lasting changes and memory consolidation in mammals (Costa-Mattioli et al. 2009). Mice lacking 4E-BP2 show impaired hippocampal LTP: early LTP (E-LTP) is converted in late LTP (LTP) and mice present memory deficits in several behavioral tests (Banko et al. 2007; Banko et al. 2005). Likewise, mice lacking FMRP, used as model of FXS, display numerous neurobehavioral defects, including enhanced mGluR-dependent LTD and defective LTP (Hou et al. 2006; Huber et al. 2002; Meredith et al. 2007; Pfeiffer and Huber 2009). According to the so-called mGluR theory, the increased LTD in FMRP deficient mice is due to exaggerated mGluR-dependent translation; when FMRP is lost, uncontrolled protein synthesis would result in excessive AMPA internalization and increased LTD (Bear et al. 2004). A plausible candidate in this process is *Arc* mRNA, whose de novo synthesis is

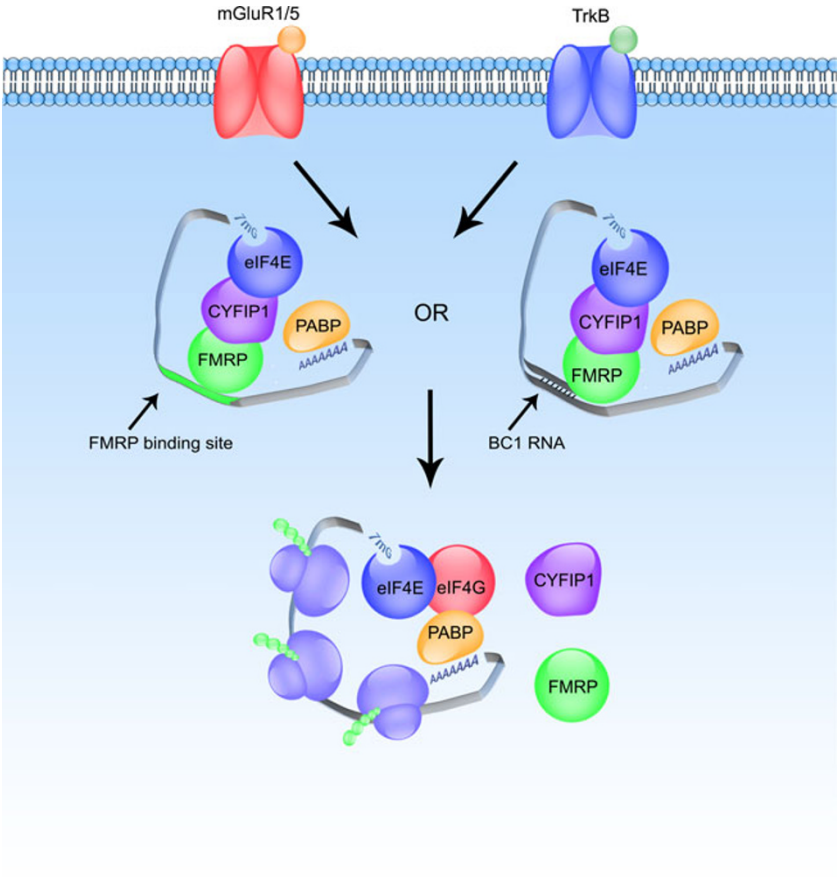

Fig. 3 Regulation of the FMRP-CYFIP1-eIF4E complex. FMRP (*green*) binds the mRNAs in a *BC1*-dependent or independent manner and anchors them to CYFIP1 (*violet*). CYFIP1 binds eIF4E (*blue*), preventing the formation of active translational initiation complexes. Upon synaptic stimuli, i.e., activation of the TrkB (BDNF) or mGluR (DHPG) receptors, CYFIP1 and FMRP are released from eIF4E, which then binds eIF4G allowing protein synthesis to occur

excessive in the absence of FMRP and has been implicated in AMPA receptors trafficking in response to mGluR-LTD (Park et al. 2008; Waung et al. 2008; Zalfa et al. 2003). In support of the mGluR theory, some morphological, physiological, and behavioral features of FXS can be rescued in model organisms either by administration of a mGluR antagonist (MPEP) (McBride et al. 2005; Tucker et al. 2006; Yan et al. 2005) or genetic reduction of mGluR5 (Dolen et al. 2007).

The mTOR pathway could play a role in FXS. First, the fine tuning of the PP2A/S6K activity through the mTOR signaling modulates the phosphorylation state of FMRP (Narayanan et al. 2007; Narayanan et al. 2008) (Fig. 1). Second, elevated activity of mTOR pathway has been found in *Fmr1* KO mice (Sharma et al. 2010). In FMRP-lacking hippocampi, both mTOR phosphorylation and activity are enhanced, resulting in increased phosphorylation of S6K, 4E-BPs, and consequently eIF4E-eIF4G interaction (Sharma et al. 2010). This evidence further indicates that FMRP is involved in modulating translational initiation.

Genetic manipulation of some components of mTOR signal cascade, such as TSC2, results in synaptic plasticity defects and behavioral anomalies in mice (Ehninger et al. 2008a; Hoeffer et al. 2008). Remarkably, mutations in components of mTOR signaling have been linked to learning disabilities and ASD (Ehninger et al. 2008b).

Likewise, both CYFIP1 and eIF4E, key components for the FMRP-regulated protein synthesis, have been correlated with ASD. *CYFIP1* gene is located on a hot spot chromosomal region for ASD, the 15q11-13 chromosome. This region lies within the breakpoints (BP) 1 and 3; it is characterized by a proximal not imprinted region (within BP1 and BP2), a large imprinted region with several paternally imprinted and two maternally imprinted genes, and a distal non-imprinted region. *CYFIP1* gene does not undergo imprinting and, together with other three genes, is located in the proximal not imprinted region (BP1–BP2). Altered imprinting of the genes distally to the BP2 can give rise to three ASD-related syndromes, namely the 15q duplication syndrome, Angelman Syndrome, and Prader-Willi Syndrome (Chamberlain and Lalande 2010).

Angelman Syndrome (AS) is due to loss of function of the maternal copy of the imprinted gene *UBE3A*. In normal individuals, only the maternally inherited allele is expressed in a brain-specific manner, while the paternal copy is silenced; in patients with AS, the maternal *UBE3A* is not longer expressed. AS is characterized by intellectual disability, deficits in verbal communication, motor dysfunction, ataxic gait, seizures, cognitive impairment, epilepsy,

behavioral features (excitability, frequent smiling, hand-flapping movements), and sometimes hyperactivity. On the other hand, loss of expression of the paternal copy of several genes paternally imprinted leads to Prader-Willi Syndrome (PWS). This disease is featured by mild to moderate cognitive deficit, behavioral problems, and hyperphagia leading to morbid obesity.

Of interest, a subgroup of patients with FXS shows a PWS-like phenotype with severe hyperphagia, obesity, short stature, short fingers and toes, and hypogonadism (de Vries et al. 1993; Nowicki et al. 2007). FXS children with Prader-Willi phenotype do not have cytogenetic alterations of 15q11–13 region, but have a significant reduction in *CYFIP1* mRNA levels compared to FXS individuals without PWS-like phenotype or unaffected individuals (Nowicki et al. 2007). As mentioned, *CYFIP1* lies within the proximal not imprinted gene, which includes three other genes, *TUBGPC5*, *NIPA2*, and *NIPA1*. Large deletions of the 15q11–13 region including these four genes lead to more severe neurobehavioral phenotype in both PWS and AS, underlining the potential role of these genes in ASD (Butler et al. 2004; Sahoo et al. 2006). Furthermore, microdeletions or microduplications of the proximal non-imprinted genes have been found to co-segregate with cognitive disabilities and ASD (Doornbos et al. 2009; Murthy et al. 2007; van der Zwaag et al. 2010). In particular, BP1–BP2 microduplications are associated with developmental delay, mental retardation, speech impairment, and behavioral alterations (ADHD, autism, obsessive-compulsive behavior) (Doornbos et al. 2009; Murthy et al. 2007). These observations, together with the functional linkage of CYFIP1 to FXS, point towards a role of *CYFIP1* in behavioral anomalies and ASD. The other key partner of the functional FMRP complex, eIF4E, has been recently implicated in autism as well. Genome-wide association studies in patients with ASD have indicated linkage to a region containing the *eIF4E* gene on chromosome 4q (Trikalinos et al. 2006; Yonan et al. 2003). Moreover, a de novo translocation involving the chromosomal region containing eIF4E has been found in a boy affected by Autistic Disorder (Neves-Pereira et al. 2009). Moreover, a heterozygous nucleotide insertion in the *eIF4E* promoter has been detected in two unrelated families with two autistic siblings, likely leading to downregulation of eIF4E (Neves-Pereira et al. 2009).

Future perspectives

In conclusion, we propose that both CYFIP1 and eIF4E could contribute to the ASD phenotypes observed in FXS. Genetic alterations of the FMRP-CYFIP1-eIF4E complex, as well as other modulators of local protein synthesis, lead to cognitive delay and behavioral disturbances, thus emphasizing that defective local translation may underlie intellectual disability and autism. Although the mRNAs deregulated in the absence of FMRP have been deeply investigated so far, little information is available concerning FMRP-CYFIP1 interactome networks. We believe that future studies should be addressed to define FMRP and CYFIP1-related proteins. In fact, alterations in the proteins co-interacting with FMRP may explain the reduced penetrance of ASD phenotype of FXS. Moreover, since common pathways may be implicated in related neurodevelopmental disorders, these studies may lay the ground work for the study of novel susceptibility genes for ID and ASD.

Acronyms

4E-BPs	4E binding proteins
AS	Angelman syndrome
ASD	Autism spectrum disorder
BC1	Brain cytoplasmic 1
BDNF	Brain derived neurotrophic factor
CYFIP1	Cytoplasmic FMRP interacting protein 1
DHPG	(*S*)-3,5-dihydroxyphenylglycine
eIF4E	Eukaryotic initiation factor 4E
eIF4G	Eukaryotic initiation factor 4 G
ERK	Extracellular signal-regulated kinases
FMR1	Fragile X mental retardation 1
FMRP	Fragile X mental retardation protein
FX-POI	Fragile X-associated primary ovarian insufficiency
FXS	Fragile X syndrome
FXTAS	Fragile X-associated tremor/ataxia syndrome
LTD	Long-term depression
LTP	Long-term potentiation
mGluRs	Metabotropic glutamate receptors
mRNP	Messenger ribonucleoparticle
mTOR	Mammalian target of rapamycin
PP2A	Protein phosphatase 2A
PWS	Prader-Willi syndrome
RBP	RNA binding protein
S6K	Ribosomal protein S6 kinase
TSC2	Tuberous Sclerosis protein 2

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