Fragile X Mental Retardation Protein controls Ion Channel Expression and Activity

Laurent Ferron

Department of Neuroscience, Physiology and Pharmacology, University College London, Gower St.

London WC1E 6BT, UK

l.ferron@ucl.ac.uk

Abstract

Fragile X-associated disorders are a family of genetic conditions resulting from the partial or complete loss of fragile X mental retardation protein (FMRP). Among these disorders is fragile X syndrome, the most common cause of inherited intellectual disability and autism. FMRP is an RNA-binding protein involved in the control of local translation, which has pleiotropic effects, in particular on synaptic function. Analysis of the brain FMRP transcriptome has revealed hundreds of potential mRNA targets encoding postsynaptic and presynaptic proteins, including a number of ion channels. FMRP has been confirmed to bind voltage-gated potassium channels (Kv3.1 & Kv4.2) mRNAs and regulates their expression in somatodendritic compartments of neurons. Recent studies have uncovered a number of additional roles for FMRP besides RNA-regulation. FMRP was shown to directly interact with, and modulate, a number of ion channel complexes. The sodium-activated potassium (Slack) channel was the first ion channel shown to directly interact with FMRP; this interaction alters the single-channel properties of Slack channel. FMRP was also shown to interact with the auxiliary β4 subunit of the calcium-activated potassium (BK) channel; this interaction increases calcium-dependent activation of the BK channel. More recently, FMRP was shown to directly interact with the voltage-gated calcium channel, Cav2.2, and reduce its trafficking to the plasma membrane. Studies performed on animal models of fragile X syndrome have revealed links between modifications of ion channel activity and changes in neuronal excitability, suggesting that these modifications could contribute to the phenotypes observed in patients with fragile X-associated disorders.

Abstract Figure legend

Fragile X mental retardation protein (FMRP) interacts with voltage-gated potassium channels (Kv3.1 & Kv4.2) mRNAs and regulates their expression in somatodendritic compartments of neurons. FMRP also directly interacts with Slack, BK and Cav2.2 channel complexes and alters their activity in the soma and presynaptic terminals. Overall, FMRP modulates neuronal excitability by controlling ion channel expression and activity.

Abbreviations

FMR1 fragile X mental retardation 1 gene

FMRP fragile X mental retardation protein

FXS fragile X syndrome

FXTAS fragile X-associated tremor/ataxia syndrome

Slack sodium-activated potassium channel

Kv voltage-gated potassium channel

BK large conductance Ca^{2+}- activated potassium channel

$Ca_V2.2$ voltage-gated calcium channel

PP2A protein phosphatase 2A

S6K ribosomal protein S6 kinase

The fragile X mental retardation protein (FMRP) is an RNA-binding protein encoded by the fragile X mental retardation 1 (FMR1) gene located on the chromosome X (Bhakar *et al.*, 2012). A variety of disorders are associated with mutation in the FMR1 gene including fragile X syndrome (FXS) and fragile X-associated tremor/ataxia syndrome (FXTAS) (Lozano *et al.*, 2014).

FXS is the most common heritable form of intellectual disability and is the leading known monogenic cause for autism spectrum disorders (Bhakar *et al.*, 2012). The FMR1 gene contains an unstable CGG-repeat in the 5' untranslated region which is normally 5-44 repeats long. FXS is caused by a CGG expansion of more than 200 repeats (called full mutation) which induces methylation of the gene and leads to the partial or complete absence of FMRP. Rarely, FXS can also be caused by point mutations or deletions (Bassell & Warren, 2008; Myrick *et al.*, 2015). FXS has a prevalence of 1 in 2500-4000 males and 1 in 7000-8000 females. The prevalence of carrier status has been estimated to be up to 1 in 130-250 of females. People with FXS show mild to moderate cognitive dysfunction, attention deficits and hyperactivity, anxiety, autistic behaviours, sensory integration problems (such as hypersensitivity to loud noises, bright lights and heightened tactile sensitivity) and they are often also affected by seizures.

FXTAS is caused by an expansion of 55-200 CGG-repeats (called premutation) inducing an elevation in FMR1 mRNA transcript levels (Lozano *et al.*, 2014). The leading molecular mechanism proposed for these disorders involves elevated levels of mRNA containing the expanded CGG repeats. This is thought to sequester RNA binding proteins and as a consequence affect their normal functions (Hagerman & Hagerman, 2013). However, a recent study investigating FMR1 splice variants in brain samples of premutation carriers has shown that mRNA isoforms lacking the C-terminal of FMRP are the most increased (Pretto *et al.*, 2015). The fact that FMRP C-terminus contains important functional domains (Bagni & Greenough, 2005; Bassell & Warren, 2008; Ferron *et al.*, 2014) led the authors of the study to suggest that the overexpression of these truncated FMRP isoforms could inhibit FMRP function and contribute to the pathology of premutation disorders. People with the premutation expansions can present with a wide range of clinical phenotypes, from mild cognitive problems during childhood (attention deficit hyperactivity disorder, autism spectrum disorder) to psychiatric disorders in adulthood (anxiety & depression), motor symptoms (tremor, ataxia, muscle weakness and Parkinsonism), neuropathy and chronic pain. FXTAS has a prevalence of 1 in 260 to 814 males and 1 in 100-260 females indicating that 1 in 3000 men and 1 in 5200 women in the general population will develop symptoms of FXTAS.

FMRP is expressed in the nucleus and the cytoplasm, and is part of cytoplasmic RNA granules, where it plays a role in both the trafficking of specific mRNAs to sites of translation, and the stalling of their translation (Bassell & Warren, 2008; Darnell *et al.*, 2011). FMRP has been shown to bind a large number of mRNAs, also called the FMRP transcriptome, and many of them code for proteins involved in neuronal excitability and synaptic transmission (Darnell *et al.*, 2011). In *fmr1* knockout mice, the loss of FMRP results in an excessive and unregulated dendritic mRNA translation (Antar *et al.*, 2004; Bassell & Warren, 2008), and an alteration of synapse number and shape (Antar *et al.*, 2006). Consequently, research has concentrated particularly on the dendritic/postsynaptic role of FMRP (Ronesi & Huber, 2008; Krueger & Bear, 2011). However, there is now growing evidence for a presynaptic role of FMRP. Loss of presynaptic FMRP reduces the formation of functional synapse (Hanson & Madison, 2007) and modifies presynaptic protein levels (Liao *et al.*, 2008; Klemmer *et al.*, 2011). Moreover, electron microscopy studies of the ultrastructure of the synapses of CA3 pyramidal neurons onto CA1 pyramidal neurons in the hippocampus of *fmr1* knockout mice have revealed an increase of the number of docked vesicles at the active zones compare with control animals (Deng *et al.*, 2011; Klemmer *et al.*, 2011). In central neurons, granules containing FMRP are present in presynaptic terminals and axons and they are mostly prominent during synapse maturation (Christie *et al.*, 2009; Akins *et al.*, 2012). Studies also show a role for FMRP in local protein synthesis in peripheral sensory axons (Price *et al.*, 2006). While *fmr1* knockout mice present normal acute nociceptive responses, they show modifications of the chronic responses, both in the peripheral and central nervous system (Price *et al.*, 2007). Heightened tactile sensitivity and self-injurious behavior is described in some FXS patients, and this could be linked to dysregulation of nocifensive behaviour (Price *et al.*, 2007).

The analysis of the brain FMRP transcriptome have revealed that, among the mRNA coding for proteins involved in excitability and synaptic transmission, a number of target mRNAs code for ion channels (Brown *et al.*, 2001; Darnell *et al.*, 2011; Brager & Johnston, 2014). Voltage-gated potassium channels Kv3.1b and Kv4.2 mRNA have been confirmed as targets of FMRP (Darnell *et al.*, 2001; Darnell *et al.*, 2011; Gross *et al.*, 2011; Lee *et al.*, 2011). Kv3.1 channels play a critical role in auditory brainstem sound localisation circuit in rodents (Brown & Kaczmarek, 2011). In *fmr1* knockout mice, the normal gradient of Kv3.1 in the medial nucleus of the trapezoid body is flattened and the activity-dependent increase of Kv3.1 expression is abolished damaging encoding and processing of auditory information (Strumbos *et al.*, 2010). In hippocampal neurons, the A-type potassium channel Kv4.2 is the major potassium channel regulating neuronal excitability, and it has been confirmed that FMRP binds Kv4.2 mRNAs (Gross *et al.*, 2011; Lee *et al.*, 2011). However, the impact of FMRP on Kv4.2 expression is still a matter of debate. Indeed, two studies have investigated the level of Kv4.2 expression in *fmr1* knockout mice and their results point towards opposite conclusions: Gross et al. concluded that FMRP act as a positive regulator of Kv4.2 whereas Lee et al. found that FMRP acts as a repressor of Kv4.2 expression (Gross *et al.*, 2011; Lee *et al.*, 2011). The reason for this discrepancy has not been elucidated but the use of two different mouse strains has been suggested as a possible explanation (Brager & Johnston, 2014).

Beside its role as an RNA binding protein and translation modulator, FMRP has recently been shown to directly interact with ion channels. The first ion channel to be identified that interacts with FMRP was the sodium activated potassium channel Slack (Brown *et al.*, 2010). In this study, Brown and co-workers used biochemical techniques and single channel recordings to demonstrate that FMRP directly interacts with the cytoplasmic carboxy - terminal tail of Slack channel and increases

the channel mean open time (Brown *et al.*, 2010). FMRP has also been shown to interact with endogenous Slack channels and modulate their activity in bag cell neurons of the *Aplysia* (Zhang *et al.*, 2012). Slack channels contribute to the firing patterns of a variety of neurons (Yang *et al.*, 2007; Zhang *et al.*, 2012) and it has been suggested that some of the neuronal defects observed in FXS patients could be link to the alteration of Slack channel activity (Kim & Kaczmarek, 2014).

A second type of potassium channel has been shown to be modulated by FMRP: the large conductance Ca^{2+}- activated potassium BK channel (Deng *et al.*, 2013). The modulation of BK channel function by FMRP does not occur directly with the pore forming subunits of BK channel but involves an interaction with the auxiliary $\beta 4$ subunit. $\beta 4$ subunits have been described as a negative modulator of BK channel (Brenner *et al.*, 2000; Torres *et al.*, 2007). The proposed mechanism of action is that the binding of FMRP to the auxiliary $\beta 4$ subunit alters the interaction of $\beta 4$ subunit with the pore-forming subunits and consequently reduces its sensitivity to Ca^{2+} (Deng *et al.*, 2013). BK channels are important regulators of action potential duration by driving both the phases of repolarisation and afterhyperpolarisation (Bean, 2007). In hippocampal and cortical pyramidal neurons of knockout *fmr1* knockout mice, Deng et al. have shown a reduction of BK channel activity that leads to the elongation of the action potential duration and an increase in presynaptic calcium influx (Deng *et al.*, 2013). As a direct consequence, glutamate release and short-term synaptic plasticity is affected between CA3 and CA1 pyramidal neurons of the hippocampus of *fmr1* knockout mice. Interestingly, a recent study has shown that the genetic upregulation of BK channel activity normalizes a number of neuronal defects in a mouse model of fragile X syndrome (Deng & Klyachko, 2015). In this latter study, the authors have crossed *fmr1* knockout mice with slo$\beta 4$ knockout mice (slo$\beta 4$ gene codes for the BK channel auxiliary $\beta 4$ subunit) to genetically upregulate BK channel in the absence of FMRP and they show that BK single-channel properties, action potential duration, glutamate release and presynaptic short-term plasticity in hippocampal pyramidal neurons are similar to those in control animals (Deng & Klyachko, 2015).

In addition to potassium channels, FMRP has also been shown to directly interact with N-type voltage gated calcium channels (Ferron *et al.*, 2014). These channels ($Ca_V2.2$) are critical for neurotransmission both in central neurons, particularly early in development, and in the autonomic and sensory nervous system (Hirning *et al.*, 1988; Turner *et al.*, 1993; Catterall & Few, 2008). Thus they are the main mediators of neurotransmission between primary sensory afferent neurons involved in nociception and other sensory modalities, and the spinal cord (Bowersox *et al.*, 1996; Altier *et al.*, 2007). $Ca_V2.2$ channels are formed of a main pore forming $\alpha 1$ subunit and auxiliary $\alpha_2\delta$ and β subunits (Dolphin, 2012). FMRP has been shown to interact with the $\alpha 1$ subunit of $Ca_V2.2$ channels (Ferron *et al.*, 2014). The interaction with FMRP occurs between two cytoplasmic domains of the $Ca_V2.2$ $\alpha 1$ subunit: the cytoplasmic loop between the transmembrane domains II and III and the carboxy terminal tail. These intracellular domains of the $Ca_V2.2$ channel are important for the targeting to the presynaptic terminals (Mochida *et al.*, 2003; Szabo *et al.*, 2006; Kaeser *et al.*, 2011) and they have been described to functionally interact with presynaptic proteins (Sheng *et al.*, 1994; Bezprozvanny *et al.*, 1995; Mochida *et al.*, 1996; Maximov *et al.*, 1999; Coppola *et al.*, 2001; Kaeser *et al.*, 2011). In peripheral neurons, the loss of FMRP induces an increase $Ca_V2.2$ channel cell surface expression and an increase of neurotransmitter release (Ferron *et al.*, 2014).

FMRP interaction with $Ca_V2.2$ does not affect the biophysical properties of the channel which contrast with the interaction of FMRP with Slack and BK channels. Another noticeable

difference resides in the domain of FMRP that is involved in the interaction with the channel. The amino terminal domain of FMRP is a well-described platform for protein-protein interactions (Bagni & Greenough, 2005; Ramos *et al.*, 2006; Bassell & Warren, 2008) and this domain interacts with Slack channels and the β4 subunit of BK channels (Brown *et al.*, 2010; Deng *et al.*, 2013). Interestingly, it is the carboxy terminal domain of FMRP that has been shown to interact with voltage gated calcium channels (Ferron *et al.*, 2014). The carboxy terminal domain of FMRP is a non - conserved region in the related FXR1P and FXR2P (Bassell & Warren, 2008) and only two other protein / protein interactions have been described (Dictenberg *et al.*, 2008; Menon *et al.*, 2004). The carboxy terminal domain of FMRP was then suggested to contribute to the specificity of FMRP function (Menon *et al.*, 2004). This idea is supported by a recent study performed on premutation carriers that suggests a potential link between the overexpression of an FMRP mRNA splicing variant lacking the carboxy terminal domain and the pathology of premutation disorders (Pretto *et al.*, 2015).

One can speculate on the function of the direct interaction between FMRP and ion channels. It has been hypothesized that the interaction of an ion channel with part of the biochemical machinery that regulates translation of mRNAs suggests that changes in channel activity may contribute to the regulation of activity-dependent protein synthesis in neurons (Zhang *et al.*, 2012; Lee *et al.*, 2014). FMRP has been shown to modulate postsynaptic local protein synthesis in dendrites of hippocampal neurons (Muddashetty *et al.*, 2007). FMRP phosphorylation status, controlled by protein phosphatase 2A (PP2A) and ribosomal protein S6 kinase (S6K), determines the switch between translational activation and repression of mRNA targets of FMRP (Narayanan *et al.*, 2007; Narayanan *et al.*, 2008). Local protein synthesis also occurs in presynaptic terminals (Akins *et al.*, 2009) and PP2A and S6K are expressed in presynaptic terminals (Viquez *et al.*, 2009; Cheng *et al.*, 2011). Moreover, a recent study identified a subset of mRNAs encoding presynaptic proteins as targets of FMRP (Darnell *et al.*, 2011). FMRP has been shown to form protein complexes with $Ca_V2.2$ channels in the soma and also in the presynaptic terminals of neurons (Ferron *et al.*, 2014). Therefore, FMRP tethering to the vicinity of $Ca_V2.2$ may localize it to sites where local activity-dependent presynaptic protein synthesis may occur. Moreover, PP2A activity can be modulated by Ca^{2+} influx through voltage gated calcium channels (Ferron *et al.*, 2011), which suggests that presynaptic Ca^{2+} influx resulting from $Ca_V2.2$ channel activation may activate PPA2, which in turn would dephosphorylate FMRP and affect local translation. Determining the mechanisms that control FMRP function will be an important issue for future investigations. Indeed, a study has recently shown that the deletion of S6K1 in *fmr1* knockout mice partially corrected the phenotypes associated with FXS (Bhattacharya *et al.*, 2012).

In conclusion, FMRP can regulate ion channel activity (Figure 1) either by controlling the stability and trafficking of the mRNA encoding particular channels (Kv3.1b & Kv4.2) or by a new and unconventional way, by directly binding to a channel subunit (Slack, BK and $Ca_V2.2$ channels). Several other ion channels have been reported to be altered in different parts of the brain of animal models of fragile X syndrome but the mechanism of regulation have not been identified yet (Brager & Johnston, 2014; Contractor *et al.*, 2015). All those modifications of ion channel expression contribute to the modification of neuronal excitability and could account for the alterations observed in fragile X-associated disorders (Figure1).

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Competing Interests

The author has no conflicts of interests.

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Figure 1 legend:

Diagram illustrating the interaction between FMRP and ion channels in neurons. A) In wild type neurons (WT), FMRP interacts with voltage-gated potassium channels (Kv3.1 & Kv4.2) mRNAs and regulates their expression in somatodendritic compartments of neurons. In the soma and presynaptic terminals, FMRP directly interacts with Slack, BK and Cav2.2 channel complexes and regulates their activity. B) In neurons lacking FMRP (no FMRP), like in models of fragile X syndrome, ion channels expression and activity is modified inducing alteration of excitability and neurotransmitter release.