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Review

Fragile X family members have important and non-overlapping functions

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Abstract

The fragile X family of genes encodes a small family of RNA binding proteins including FMRP, FXR1P and FXR2P that were identified in the 1990s. All three members are encoded by 17 exons and show alternative splicing at the 3′ ends of their respective transcripts. They share significant homology in the protein functional domains, including the Tudor domains, the nuclear localization sequence, a protein-protein interaction domain, the KH1 and KH2 domains and the nuclear export sequence. Fragile X family members are found throughout the animal kingdom, although all three members are not consistently present in species outside of mammals: only two family members are present in the avian species examined, *Gallus gallus* and *Taeniopygia guttata,* and in the frog *Xenopus tropicalis*. Although present in many tissues, the functions of the fragile X family members differ, which are particularly evident in knockout studies performed in animals. The fragile X family members play roles in normal neuronal function and in the case of FXR1, in muscle function.

Keywords: fragile X; FMR1; FXR1; FXR2; RNA binding proteins.

List of abbreviations

cc, coiled-coil domain; microRNA, miRNA; NLS, nuclear localization sequence; NoS, nucleolar targeting sequence; nt, nucleotide; RGG, arginine glycine glycine; siRNA, silencing RNA; Td, Tudor domain; UTR, untranslated region of mRNA; WT, wild-type.

Introduction

The fragile X family of genes consists of three family members FMR1, FXR1 and FXR2 based on homology of the conserved domains. The gene encoding the first family member, FMR1, was isolated by positional cloning of the X chromosomal region containing the inducible fragile site in individuals with fragile X syndrome (1). Cloning of the gene revealed the molecular defect to be a trinucleotide (CGG) repeat expansion in exon 1 (2). Normally, individuals have less than 45 repeats with an average around 30 repeats; however, expansion to greater than 200 repeats leads to aberrant methylation of the cytosines, leading to recruitment of histone deacetylases with consequent transcriptional silencing of the FMR1 locus (3). Thus, individuals with fragile X syndrome do not express transcript from the FMR1 locus. To identify the *Xenopus laevis* ortholog of FMR1 for further use in developmental studies, the human FMR1 gene was used to screen a cDNA library prepared from *Xenopus laevis* ovary. In addition to identifying the *Xenopus laevis* ortholog of FMR1, the first autosomal paralog FXR1 was discovered because of its sequence similarity to FMR1 (4).

In contrast to FMR1, which is encoded on the X chromosome, FXR1 is encoded on human chromosome 3 and an FXR1 pseudogene is on human chromosome 12 (5, 6). The third family member FXR2 was discovered in a yeast two-hybrid screen devised to identify proteins that interacted with the protein product of FMR1, FMRP (7). FXR2 is encoded on human chromosome 17 (7). The protein products of the FXR1 and FXR2 loci, FXR1P and FXR2P, respectively, are very similar in overall structure to FMRP with approximately 60% amino acid identity (8). In addition, all three family members share 73%−90% amino acid identity over the first half of the protein with greater divergence in the C-terminal regions encoded by exons 14−17 (6).

Genomic sequence and protein domain structures of the fragile X family of proteins, from 5′ to 3′

The genes encoding the fragile X family members are comprised of 17 exons (6) (Figure 1). The first exon of the FMR1 gene encodes the CGG repeat upstream of the start site of translation (1). Both FMR1 and FXR2 have CGG repeats; however, the repeat tracts are much shorter in FXR2 (6). The average size of 5′UTRs among human genes has been estimated to be 200 nt (19), although an average size of 125 nt has also been suggested (20). By either estimate, the 5′UTRs of the fragile X family members are relatively large: 840 nt for FXR1 (NM_001013439), 366 nt for FXR2 (NM_004860) and 230 for FMR1 (NM_002024.5), although it is important to note that this size will vary based on the number of repeats. In addition, there are alternative start sites for tran-

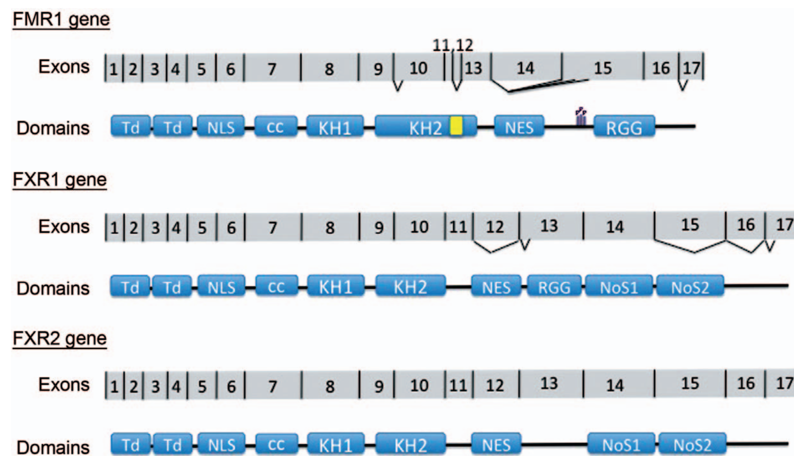

Figure 1 Gene structures and corresponding protein domains of the fragile X family members.
Exons are numbered 1–17 and reported splice site usage is indicated by lines below the exons (9–11). Exons 1–10 of all three proteins encode for similar domain structures. Since FXR1 and FXR2 do not have sequence equivalent to exons 11 and 12 of FMR1, the numbering diverges: in FXR1 and FXR2, the NES is encoded by exon 12; the RGG box in FXR1 is encoded by exon 13 and the nucleolar targeting sequences by exons 14 and 15 (6). Td- Tudor domains (12–14), NLS-nuclear localization sequence (15), cc- coiled-coil domain (8), P-phosphorylation sites (16), NES-nuclear export sequence (15, 17), NoS-nucleolar targeting sequence (11, 18).

scription of the FMR1 mRNA, which will affect the size and sequence of the 5′UTR (21). Initial examination of the predicted amino acid sequence of FMRP indicated that it contained two KH domains and an RGG box, suggesting that it was an RNA binding protein (22, 23) (Figure 1).

Structural studies of the N-terminal domain in all three family members revealed a repeat of two domains closely resembling the Tudor domain of the SMN protein (12, 14, 24). Tudor domains have many functions, including protein-protein interactions (25) and binding methylated lysines, which might facilitate targeting of the fragile X family proteins within the nucleus (12). Immediately C-terminal to the Tudor domains is a non-classical nuclear localization sequence (NLS) that is able to direct entry into the nucleus (22, 15). All three proteins have been observed in the nucleus of hippocampal neurons (26).

The fragile X family of proteins have a coiled-coil domain that mediates homo-dimerization, as well as heterodimerization among the three family members (8). This same region has also been described as containing a helix-loop-helix motif (24). In addition, all three proteins have a KH1 domain and a KH2 domain, which are very distinct from one another (4, 23, 27, 28) (Figure 1).

Interestingly, exons 11 and 12 are unique to mammalian FMR1: exon 11 is constitutively included in all transcripts while exon 12 is alternatively spliced (9, 29). Accordingly, sequence corresponding to exons 11 and 12 is NOT found in FXR1 and FXR2 (4). Nor are Exons 11 and 12 present in the following FMR1 orthologs: *Drosophila* (30); zebra finch FMR1 (31), chicken FMR1 (32), zebra fish FMR1 (33) and *Xenopus tropicalis* FMR1 (34).

The fragile X family members are all primarily cytoplasmic; thus, it is not surprising that in addition to an NLS, they have a Rev-like nuclear export sequence (NES) (15, 17, 35). Upon removal of the NES or by blocking RNA export, FMRP can be observed accumulating in the nucleus (15, 17, 35, 36). Treatment of transfected cells with leptomycin B to block nuclear export through the CMR1/exportin 1 pathway, resulted in the nuclear accumulation of all three fragile X family proteins, suggesting that they use the same mechanism for nucleocytoplasmic shuttling (37).

At the time that FMRP was characterized, several RNA binding proteins were found to contain an arginine- and glycine-rich domain comprised of a cluster of the tripeptide repeat called the RGG box (38, 39). The RGG box of FMRP was described as bearing a striking similarity to those found in the RNA binding proteins fibrillarin (40) and hnRNP A1 (23, 41). FXR1P was also described as having an RGG box (4), although FXR2 does not (7). Two groups working independently found the RGG box to be the high affinity RNA binding site, recognizing intramolecular G-quadruplexes that were present in brain mRNAs, including the FMR1 mRNA (42, 43). G-quadruplex-like structures were subsequently found in FMRP-associated mRNAs encoding amyloid precursor protein (44), MAP1B (45, 42), PSD95 (46–48) and semaphorin 3A (42, 49). Darnell and colleagues found that FMRP was unique in its ability to bind G-quadruplex RNA and that FXR1P, FXR2P and the *Drosophila* ortholog of FMRP could not, suggesting that the RGG box plays a non-redundant role in the pathophysiology of the disease (28). Since the RGG box plays a critical role in FMRP function, it is important to understand how it is regulated. Stetler and colleagues showed that the RGG box of FMRP is methylated on four of the arginines comprising the RGG box and that *in vitro* methylation with protein arginine methyl transferase 1 (PRMT1) inhibited binding to G-quadruplex RNA (50, 51), suggesting one mechanism for regulating RGG box function.

Evidence of the divergence between FMRP and the autosomal paralogs is apparent in the identification of two nucle-

olar targeting sequences (NoS) in the C-terminal regions of FXR1P and FXR2P that are not present in FMRP (18) (Figure 1). The Rev-like, arginine-rich nucleolar targeting sequence of FXR1P was able to target to the nucleolus (18). In addition, FXR2P had been demonstrated to shuttle between the cytoplasm and the nucleolus (37).

All three fragile X family members contain 3'untranslated regions (3′UTRs). In FMR1, the coding sequence is ~1.9 kb [(9) and NM_002024.5] and the 3′UTR is ~2.3 kb in size, suggesting an important function. 3′UTRs have been shown to direct subcellular localization (52) or facilitate microRNA (miRNA)-mediated regulation (53). The FMR1 transcript has been reported to be regulated by miRNAs (54), although this was not observed in other systems (55, 56). In contrast, there is strong support for miRNA-mediated regulation of the autosomal paralog FXR1 (55). In the initial characterization of FXR1, the 3′UTR of human FXR1 was determined to be 90% identical to the 3′UTR of FXR1 in *Xenopus*, over the 238 nucleotides examined, suggesting that this region may have been conserved for an important function (4). In contrast, comparison of the 3′UTRs of human and *Xenopus* FMR1 transcripts over 280 nucleotides showed them to be only 42% identical (4). Subsequently, Cheever and colleagues found FXR1 expression to be regulated at the level of translation by miRNAs 92b, 363 and 367, which bind the same site in the 3′UTR of FXR1 (55).

Spliceforms of the fragile X family members

FMR1 isoforms

Extensive alternative splicing has been demonstrated for the FMR1 transcript that could potentially generate 20 protein isoforms (9, 29). Only the isoforms that have been extensively characterized will be explored here. The full length transcript including all exons is described as isoform 1; removal of exon 12 leads to creation of isoform 7 (9, 29). As described above, exon 12 is present only in the mammalian homologs of FMR1. However, approximately 80% of mature brain FMR1 transcripts lack exon 12 due to alternative splicing (29). At the biochemical level, exon 12 encodes the most hydrophobic region of FMRP (9) and its presence in recombinant proteins leads to marked instability (28), although what this means in a cell is unknown.

Isoforms lacking exon 14 of the FMR1 mRNA have been described in brain. Because exon 14 encodes the NES, its removal from FMRP leads to increased nuclear retention (15, 17, 36, 35), although the role of such a nuclear-retained FMRP is unknown at this time. Interestingly, it should also be noted that exclusion of exon 14 causes a +1 frameshift, leading to a novel but truncated C-terminus that no longer encodes an RGG box (15, 17, 36, 35).

Exon 15 of the FMR1 mRNA can also be alternatively spliced because there are alternate 3' acceptor sites present (9, 10). Exon 15 encodes the highly conserved phosphorylation sites (16, 57) and the RGG box. Three alternative splice acceptor sites are upstream of the RGG box (Figure 1). Consequently, their usage maintains the RGG box but truncates the N-terminally encoded region of exon 15 (Figure 1). All three exon 15 splice forms have been characterized at the biochemical level (58) but not in cells. The RGG box is part of a 3–4 strand antiparallel beta sheet which functions as a platform for nucleic acid interactions in other RNA binding proteins (58). Isoform 3, the isoform with the largest N-terminal deletion in exon 15, is predicted to have a truncated first strand, significantly perturbing the side chain conformations of the RGG box arginines (58) and likely modulating RGG box function. FMRP has been shown to associate with its own mRNA (27), specifically binding the G-rich region of the RNA, which encodes the RGG box through its RGG domain (43). Binding of the G-rich RNA regulates splice site usage in exon 15 (59). Thus, there is evidence that FMRP regulates splicing of its own mRNA.

FXR1 isoforms

Regarding the autosomal paralogs, there have been no reports of alternative splicing of FXR2. In contrast, evidence for alternative splicing of the FXR1 transcript was presented in the first description of the gene in 1995 (4). FXR1P has seven isoforms (6, 60) including one cardiac-specific isoform (61) and three muscle-specific isoforms (60). Studies in muscle tissues as well as in murine myoblastic cell lines that were induced to differentiate into myotubules, demonstrated that the common isoforms of FXR1P that are present in neurons and cell lines were replaced by novel longer isoforms of 81 and 84 kilodaltons in size (61). The longer isoforms contain the NOS2 sequence indicated in Figure 1, although a specific role for FXR1P in the nucleolus has yet to be determined. For a more detailed description of the isoforms of FXR1 and their tissue specific expression, please see (18).

Fragile X family members in non-mammalian species

Proteins in the fragile X family are not unique to mammals, but seem to be unique to animals. An ortholog was searched for in the yeast *Saccharomyces cerevisiae*; one yeast protein was shown to have a KH domain with 50% homology to the second KH domain of FMRP but no yeast protein has yet been named an FMR ortholog (62). Non-mammalian species expressing an FMRP ortholog include marine snails *Aplysia californica* (63) and a Cnidarian species *Hydractinia echinata* (64), although *Caenorhabditis elegans* appears to have lost the gene, as no FMRP ortholog is present (64). An FMRP ortholog has been found in the planarian species *Dugesia japonica* (65, 66) and *Schmidtea mediterranea* (Winograd Zayas and Newmark, unpublished observations). Two avian species have been shown to express FMRP orthologs as well, the chicken *Gallus gallus* (32) and zebra finch *Taeniopygia guttata* (31).

Examination of FMRP orthologs in non-mammalian species has helped to elucidate the role of this protein in regulation of cellular and developmental function. A key feature of this protein is the conservation of the KH and RGG domains, establishing FMRP's evolutionarily conserved role

of RNA binding (28). The chicken ortholog was shown to bind RNA, a function that required the integrity of the FMRP RGG domain (32). The planarian *D. japonica* FMRP has been localized to ribonucleoprotein-containing stress granules and neuronal granules in an RNA-binding fashion, suggesting the protein is conserved functionally in a larger scale, as well, in neuronal regulation (65).

Intriguingly, FMRP expression has been observed to change during development in a cerebral neural network critical for song learning in the zebra finch *T. guttata* (31). A role for FMRP in learning and memory is corroborated in *A. californica*, where FMRP affects both pre- and postsynaptic regulation of long-term depression (LTD), suggesting its function in establishment of long-term memory (63). These studies suggest that just as in mammals, FMRP is critical for normal learning and memory, possibly by precise spatio-temporal regulation of its bound RNAs, particularly during development and maintenance of neural circuits. Excitingly, the expression of FMRP can be observed during development in the zebra fish *Danio rerio*, an optimal organism for monitoring genetic changes during development (33); a knockout animal has recently been generated and promises further insight (67).

Focusing more on the cellular localization of FMRP, the NLS of FMRP is conserved across multiple species, albeit with a missense alteration in the pufferfish *Takifugu rubripes* and *Tetraodon nigroviridis*, which may or may not affect the protein's function (68). In the frog *Xenopus laevis*, FMRP can bind mRNA while still in the nucleus (36).

Another role for FMRP may be in orchestrating neurons required for motor effects. The brain region aforementioned in *T. guttata* is also critical for birdsong production, as a premotor nucleus; it would be interesting to characterize motor changes in a finch that does not express FMRP in this brain region. In fact, a motor role for FMRP has been shown in the cricket *Gryllus bimaculatus*. Absence of the protein has no effect on wing morphology but does result in atypical wing stridulation (wing rubbing to produce calls), likely due to abnormal wing posture (69). This study additionally showed altered circadian locomotor activity in the knock-down cricket; a circadian role for FMRP has also been observed in regulation of locomotor activity of the fly *D. melanogaster* (70). The *D. melanogaster* ortholog of FMRP is intriguing to study because the fly has one FMR family member that comprises features of all three human family members, FMRP, FXR1, and FXR2 (30). Studies in *Drosophila* were the first to show that FMRP associates with components of the RNA interference (RNAi) pathway, a gene silencing mechanism triggered by the presence of double-stranded RNA (71, 72). Interestingly, FMRP has only subtle effects on RNAi pathway efficiency and is not required for small interfering RNA (siRNA) biogenesis (71, 72). Genetic evidence in fly suggests that the siRNA pathway and FMRP converge to regulate larval crawling behavior and synaptic growth during development (73, 74). Studies in *Drosophila* also gave insight into the role of FMRP at the neuromuscular junction, demonstrating that in the absence of FMRP, the axons in the neuromuscular junction display overelaboration and an increase in synaptic boutons and branching (75). In addition, loss of FMRP results in a midline-crossing defect in the beta-lobe of the mushroom body structure, which functions analogously to the hippocampus (76, 77). More discussion of the specific contributions made by work in *Drosophila* is reviewed elsewhere (78).

Functions of the fragile X family members

All of the family members

At the molecular level, FMRP, FXR1P and FXR2P form homo- and hetero-dimers (8). In addition, all three are primarily cytoplasmic, associate with ribosomes by immuno-electron microscopy (26) and are also found in granules (79). All three family members also associate with microRNA (miRNA) pathway components, including precursor miRNAs, mature miRNAs, argonaute proteins, which are key for mediating miRNA-regulated expression and Dicer, an endonuclease that processes precursor miRNAs (80).

Regarding cell type specificity, there is overlapping expression in human tissues, especially the nervous system (81). In differentiated neurons in adult human brain, a common expression pattern was observed for FMRP and FXR1P in the cytoplasm. In contrast, differential distribution of the family members was found in fetal brain, suggesting that the fragile X family of proteins, although structurally very similar, may have independent functions during embryonic and adult life (82).

FMRP

Although each of the family members is predicted to be an RNA binding protein, it is only FMRP whose bound mRNAs have been extensively characterized (45, 83). FMRP is estimated to bind 4% of brain mRNAs (27, 45), activating the translation of some mRNAs, while suppressing the translation of others. The role of FMRP as a regulator of translation was first suggested when FMRP was observed on polyribosomes (84, 85). Subsequent identification of the mRNAs on polyribosomes showed that their levels varied significantly depending on whether FMRP was present or absent (45), strongly suggesting that FMRP both suppresses and enhances the translation of subsets of mRNAs. Importantly, a mechanism for how FMRP modulates translation had been lacking until recently. FMRP has been shown to activate the translation of some of its bound mRNAs (86, 87). In one example, binding of FMRP to the 5′UTR of the SOD1 mRNA stabilizes a novel secondary structure (SoSLIP) to expose the initiator ATG codon, leading to enhanced translation (86). However, it is unknown how FMRP activates the translation of mRNAs that do not contain the SoSLIP motif.

FMRP also suppresses translation of some of its bound mRNAs; *in vitro* binding of FMRP to mRNAs repressed protein synthesis in translation assays through an unknown mechanism (88, 89). One way to mediate translation repression is through the miRNA pathway (90). FMRP was recently been shown to be required for miRNA-mediated

suppression of NR2A (91). However, the molecular basis for how FMRP utilizes the miRNA pathway for translation regulation is unknown.

Although FMRP binds a large collection of mRNAs, it does not regulate the translation of all of them (45). In fact one of the first mRNAs identified as being bound by FMRP was its own mRNA, FMR1 (27); however, FMRP does not regulate the translation of that mRNA (59). FMRP has also been shown to regulate the stability of one of its bound mRNAs PSD-95 (92).

With regard to function, in the case of fragile X syndrome, FMRP is clearly required for the development of normal cognition. Although the autosomal paralogs are present in brain, they cannot compensate for the absence of FMRP (81). This was recently demonstrated again in the *Drosophila* model where loss of the single fragile X family member dFMRP resulted in elevated protein levels that altered the central brain and neuromuscular junction synaptic structure. Introduction of human FMR1 fully rescued the molecular and cellular defects in neurons, in contrast to when either of the autosomal paralogs was introduced, supporting the idea that FMRP has a unique function in neurons (93). Interestingly, all three human genes fully rescued the testes deficits in fly. Thus, FMR1 has a neuron-specific function that is distinct from its autosomal paralogs and FMRP functions to regulate neuron protein expression and synaptic connectivity (93).

The specific role of FMRP in mammalian neuronal development has been reviewed extensively elsewhere (3, 94); however, it is important to note that in the absence of FMRP, neurons have more dendritic spines that are longer and thinner, consistent with immaturity (95). Spines are dynamic structures that regulate many neurochemical events related to synaptic transmission (96). At the functional level, FMRP is required for normal long-term depression (LTD), a form of synaptic plasticity, because in its absence, there is enhanced LTD in the FMR1 knockout mouse (97).

A relatively new role for FMRP in neural stem and progenitor cells has been elucidated from studies in fruit fly, mouse and humans (98–101). Collectively, these works suggest that FMRP plays a critical role in embryonic and adult neurogenesis, as comprehensively reviewed in (102).

FXR1P

FXR1P is found throughout the body; however, it is highly expressed in muscle and heart tissue where FMRP and FXR2P are mostly absent (5, 103, 104). FXR1P is critical for postnatal viability; inactivation of FXR1P in mice leads to impaired myogenesis resulting in death of neonates shortly after birth, most likely due to cardiac or respiratory failure (60). Both skeletal and cardiac muscles showed disrupted cellular architecture and overall structure in the *FXR1* knockout mice compared to wild-type (WT) littermates (60).

Further evidence for a key role of FXR1P in normal muscle development comes from studies of other model organisms. Reduction of FXR1P in *Xenopus laevis* disrupted MyoD expression and somite formation, while re-introduction of long and short *FXR1* mRNA isoforms rescued these muscle-specific effects (105). In zebrafish, knockdown of *FXR1* caused abnormalities in striated muscle and severe cardiomyopathy that resulted in heart failure in embryos (106). Lastly, in humans, altered expression of muscle specific isoforms of FXR1P has been implicated in facioscapulohumeral muscular dystrophy (FSHD) because patients have abnormal expression patterns of three different FXR1P isoforms in myoblasts and myotubes (107).

FXR1P also has a novel role in miRNA-mediated translation regulation, although it is not as a translation suppressor. Elements in the 3′UTR of TNFα enhance translation relative to mRNA levels upon serum starvation, which subsequently leads to cell cycle arrest. To determine the molecular mechanism driving this phenomenon, the protein complex assembled on the 3′UTR was analyzed and found to contain FXR1P (108, 109). FXR1P was then shown to be recruited with Argonaute 2 by miRNAs bound to the 3′UTR of TNFα in quiescent cells to activate translation. These results suggest that FXR1P plays a role in translation activation (108, 109).

Recently, both FMR1 and FXR1, as well as specific miRNAs were shown to have a role in eye development in *Xenopus laevis* (110). Knockdown of FMR1, FXR1 or Dicer led to abnormal eye development and defects in cranial cartilage derived from cranial neural crest cells. These results suggest an essential role for the fragile X family of proteins through an interaction with the miRNA pathway.

FXR2P

The role of FXR2P has been more difficult to elucidate than that of its other family members. Individuals with mutations exclusively in their FXR2 genes have not been documented, although there is at least one report of an individual with mild developmental delay and other abnormal features who has a microdeletion on chromosome 17 that includes FXR2 (111). Since there are many other genes in this region, and the patient still has one normal copy of FXR2, it is difficult to know how much of the patient's phenotype is caused by haploinsufficiency for FXR2. In contrast, an FXR2 knockout mouse was created and characterized (112). FXR2-knockout mice have increased hyperactivity and impaired performance on the rotorod test, as is observed in FMR1-knockout mice. In contrast, the FXR2 knockout mouse had reduced levels of prepulse inhibition, while the FMR1 knockout mouse had increased levels of prepulse inhibition. Significantly, a role for FXR2 in cognition was suspected when the knockout mouse had difficulty locating the hidden platform in the Morris water task, suggesting mild cognitive impairment (112).

To gain insight into the functional relationship between FMRP and FXR2P, a FMR1/FXR2 double knockout was created and examined in behavior assays (113). As might be expected if the proteins work together, the double knockout mice have exaggerated phenotypes in open-field activity, prepulse inhibition of acoustic startle response and contextual fear conditioning when compared to the single knockouts or to wild-type mice. These findings suggest that FMR1 and FXR2 genes contribute in a cooperative manner to pathways controlling locomotor activity, sensorigating and cognitive processes (113). An additional role for both proteins in

circadian rhythm behavior was uncovered in the double knockout mice. In both the FMR1/FXR2 double knockout mouse and in the FMR1 knockout/FXR2 heterozygous mouse, a loss of rhythmic activity in a light:dark cycle was observed (114).

Because FXR2P has RNA binding domains, RNA expression was examined and compared in the FXR2 knockout brain and WT (115). 52 RNAs were identified as significantly changed and the proteins they encode are related to memory or cognition disorders. Interestingly, the 52 RNAs were unique to FXR2P and not found among those co-immunoprecipitated with FMRP (45), nor are misregulated in the FMR1 knockout mouse (113, 116), suggesting that FXR2P regulates a unique subset of RNAs.

Expert opinion

This review strives to comprehensively cover knowledge of the fragile X family members, from the genomic organization to the mRNA spliceforms to the structure and function of the protein domains. Cellular expression patterns were explored, as well as the functional significance of each of the family members and the contributions gained from studying the orthologs in other species. Continuing to analyze the role of the individual fragile X family members in nonhuman species will give greater insight into their specific functions in humans.

In addition to identifying the mRNAs and perhaps miRNAs that are directly bound by each fragile X family member, the functional outcome of RNA binding needs to be determined. Is RNA binding merely for incorporation into a transport granule, whose localization determines where the mRNA is translated? Or, does binding by one of the fragile X family members play a more mechanistic role in the translational fate of the mRNA by either exposing a ribosome binding site or by recruiting proteins like helicases to effect translation?

It also remains to be determined how the fragile X family members are regulated. Post-translational modifications offer an obvious means of regulating these proteins. FMRP is phosphorylated, which regulates its translation state through an unknown mechanism. In addition, FMRP is methylated on its RGG box and is also ubiquitinated. Both FXR1 and FXR2 are methylated but it is not known where in the protein this occurs or what the consequence on function will be.

Outlook

In the future, the neurophysiological roles of these proteins will be determined, specifically, what their specific function is in the developing nervous system and at the synapse.

Highlights

- First fragile X family member was identified in the molecular characterization of the most common form of inherited mental retardation, fragile X syndrome.
- Subsequent family members (FXR1 and FXR2) were identified by their sequence similarity to FMR1 or by their ability to interact with FMRP.
- All three family members have similar domain structures and are RNA binding proteins found in brain and other tissues, although alternative splicing may lead to more specialized functions.
- All three family members are associated with the miRNA pathway although it is not yet clear how they function there.
- FXR1P participates in translation activation under conditions of serum starvation.
- Studies of fragile X family members in other species gives specific insights into their specialized functions.
- FMRP levels increase in the premotor cortex of zebra finch during a critical phase in vocal learning.
- Knockout studies in mouse and zebra fish indicate a critical role of FXR1 in cardiac and striatal muscle development and function.
- FXR2 knockout mice are mildly cognitively impaired.
- Knocking out both FMR1 and FXR2 revealed a role of the fragile X family members in circadian rhythms.

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