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RNA-binding proteins in microsatellite expansion disorders: mediators of RNA toxicity

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Abstract

Although protein-mediated toxicity in neurological disease has been extensively characterized, RNA-mediated toxicity is an emerging mechanism of pathogenesis. In microsatellite expansion disorders, expansion of repeated sequences in noncoding regions gives rise to RNA that produces a toxic gain of function, while expansions in coding regions can disrupt protein function as well as produce toxic RNA. The toxic RNA typically aggregates into nuclear foci and contributes to disease pathogenesis. In many cases, toxicity of the RNA is caused by the disrupted functions of RNA-binding proteins. We will discuss evidence for RNA-mediated toxicity in microsatellite expansion disorders. Different microsatellite expansion disorders are linked with alterations in the same as well as disease-specific RNA binding proteins. Recent studies have shown that microsatellite expansions can encode multiple repeat-containing toxic RNAs through bidirectional transcription and protein species through repeat-associated non-ATG translation. We will discuss approaches that have characterized the toxic contributions of these various factors.

Keywords

Microsatellite expansion; Neuromuscular disorder; RNA toxicity; RNA processing; RNA-binding protein; RNA foci; Alternative splicing

1. Introduction

Neuromuscular disease arises from complex pathological mechanisms, many of which are not yet fully understood. Microsatellite expansion disorders result from the expansion of short repeated nucleotide sequences in transcribed DNA. The effects of these repeated units is heterogeneous among microsatellite expansion disorders and depends on the location of the expansion within the transcript (Orr and Zoghbi, 2007). Expansions in transcribed regions produce expanded repeat-containing RNA transcripts (RNAexp). Expansions within protein-coding regions can produce repeated residues in both the RNA and protein, both of which can contribute to pathogenesis. Repeats within open reading frames are typically

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CAGexp, encoding polyglutamine which can result in loss of protein function as well as toxic gain-of-function effects. Bidirectional transcription across repeat expansions can result in the production of multiple toxic repeat-containing transcripts and/or proteins. Furthermore, the recent observation of repeat-associated non-ATG (RAN) translation producing proteins from all three reading frames from the sense and antisense transcripts of repeat expansions (Zu et al., 2011) greatly increases the pathological potential of microsatellite expansions.

Pathogenic molecular events initiated by RNAexp are often mediated through functional disruption of RNA-binding proteins that interact with the repeats. RNAexp is known to aggregate into nuclear RNA foci. These are dynamic, protein-containing structures that are hallmarks of myotonic dystrophy (DM) and may play important roles in other RNA-toxic disorders (recently reviewed in (Wojciechowska and Krzyzosiak, 2011a)). This review will focus on the dominant role of toxic RNAexp and the disruption of RNA-binding protein function in neuromuscular diseases (Fig. 1). We begin with a discussion of myotonic dystrophy, a neuromuscular disorder which has established a paradigm for the toxic contribution of repeat-containing RNA. In DM, some of the molecular mechanisms leading to disrupted RNA-binding protein function, as well as disruption of their downstream functions, have been well characterized. We will also discuss evidence for RNAexp-dominant mechanisms of pathogenesis in other neurological disorders including fragile X tremor-ataxia syndrome (FXTAS), Huntington disease-like 2 (HDL2), spino-cerebellar ataxia 8 (SCA8), SCA3, SCA10, and amyotrophic lateral sclerosis (ALS) / frontotemporal dementia (FTD).

2. DM

Myotonic dystrophy type 1 (DM1) is caused by expansion of a CTG triplet in the 3' UTR of the dystrophin myotonia protein kinase (*DMPK*) gene (Brook et al., 1992; Fu et al., 1992). Unaffected alleles contain 5–30 CTG repeats, while disease-causing alleles contain 50 to several thousand repeats (Brook et al., 1992). The disease has autosomal dominant inheritance and the transcribed expanded CUG repeat (CUGexp) RNA is retained in the nucleus as RNA foci (Davis et al., 1997; Taneja et al., 1995). DM affects multiple organ systems, and many studies have been aimed at understanding the toxic effects of the CUGexp RNA. While results from mouse and fly models of DM1 demonstrated that a gain-of-function of the CUGexp RNA is the primary cause of DM1 pathogenesis (de Haro et al., 2006; Mankodi et al., 2000; Orengo et al., 2008), *DMPK* dosage alterations cause cardiac abnormalities in mice (Berul et al., 1999). CUGexp RNA forms a stable hairpin structure containing an A-type helix with a U-U bulge that is electronegative, providing a surface for interactions with RNA-binding proteins (Mooers et al., 2005; Tian et al., 2000). A second form of DM, DM type 2 (DM2) is caused by an expanded CCTG tetranucleotide repeat in the first intron of the zinc finger protein 9 (*ZNF9*) gene. DM2 shares clinical and molecular features with DM1, including the presence of nuclear RNA foci (Liquori et al., 2001; Mankodi et al., 2001), highlighting the dominant role of RNAexp in pathogenesis.

DM1 affects most organ systems. The central nervous system features of DM1 include cognitive impairment, hypersomnolence, mental retardation, and personality effects (Modoni et al., 2004; Tanaka et al., 2011). DM1 brain tissue exhibits CUGexp RNA foci in neuronal nuclei (Dhaenens et al., 2008; Jiang et al., 2004). Similarly, motoneurons generated from human DM1 embryonic stem cell lines exhibited nuclear RNA foci, aberrant neurite outgrowth, and impaired formation of neuromuscular junctions when co-cultured with primary myotubes. A gene involved in neurite outgrowth, *SLITRK4*, was shown to be down-regulated in DM1 motor neuron cultures and brain samples. Interestingly, overexpression of *SLITRK4* in DM1 motor neurons rescued the neurite outgrowth defect

(Marteyn et al., 2011). Disruption of neurite outgrowth also results from expression of CUGexp in neuronal cell culture, suggesting that RNA toxicity may mediate these effects (Quintero-Mora et al., 2002). Whether disruption of SLITRK4 is a result of toxic RNA remains to be determined. These data suggest that DM1 causes disruption of a neuronal development program in the CNS that is mediated by loss of SLITRK4 expression.

2.1 RNA-binding proteins involved in DM

It is evident that functional disruption of RNA-binding proteins by CUGexp and CCUGexp RNA causes mis-regulation of RNA processing, particularly developmentally-regulated alternative pre-mRNA splicing (Kalsotra et al., 2008; Lin et al., 2006). The best-characterized alterations are for two RNA-binding proteins that act antagonistically to regulate alternative splicing during development. CUGBP and ETR3-like factor (CELF) and muscleblind-like (MBNL) splicing regulatory proteins regulate a subset of splicing events that occur in postnatal development (Kalsotra et al., 2008). Disruption of splicing regulated by these two proteins contributes to disease features.

2.1.a MBNL proteins in DM—Muscleblind (mb1) was initially discovered to be a nuclear protein containing CCCH-type zinc finger motifs that is involved in photoreceptor and muscle differentiation in *D. melanogaster* (Artero et al., 1998; Begemann et al., 1997). Human MBNL is a double-stranded RNA-binding protein whose specific binding to CUGexp RNA positively correlates with repeat length (Miller et al., 2000). There are three MBNL paralogues in mammals and the role of MBNL1 in normal development and DM pathogenesis has been the best characterized. During postnatal skeletal muscle development, MBNL1 activity is controlled by a switch in its localization from cytoplasmic to nuclear (Lin et al., 2006), while MBNL1 is up-regulated 4-fold during postnatal heart development, coinciding with multiple splicing transitions in both tissues (Kalsotra et al., 2008). It was demonstrated that in skeletal muscle of a DM1 mouse model (HSA^{LR}) MBNL1 and MBNL2, but not MBNL3, co-localize with and are sequestered on CUGexp RNA foci (Fardaei et al., 2001; Jiang et al., 2004; Mankodi et al., 2001; Miller et al., 2000). Up-regulation of MBNL3 was observed in DM1 skeletal muscle, however the contribution of this to pathogenesis has not been determined (Lee et al., 2010). MBNL sequestration was also demonstrated in a fly model of DM1 in which MBNL1 over-expression rescued the muscle and eye phenotype (de Haro et al., 2006). These results indicate that MBNL is sequestered on CUGexp RNA, resulting in a loss of MBNL1 function, contributing to the RNA-dominant mechanism of pathogenesis. MBNL is also sequestered on RNA foci formed in DM2 (Mankodi et al., 2001), indicating that MBNL sequestration contributes to common pathogenic aspects of the two diseases. Direct evidence for the role of MBNL in DM came from a Mbnl1 knockout mouse which exhibited phenotypic features and splicing abnormalities seen in DM1 and DM2 (Kanadia et al., 2003).

A comparison of mis-splicing events in two mouse models of DM1, the Mbnl1 isoform knockout and the HSA(LR) mouse revealed that over 120 events are attributable to Mbnl1 loss-of-function in skeletal muscle (Du et al., 2010). Myotonia in DM patients and the Mbnl1 knockout mouse is due to mis-splicing of Clcn1 exon 7a (Kanadia et al., 2003). Aberrant splicing of Clcn1 results in the inclusion of a premature termination codon and subsequent loss of Clcn1 expression in DM1 skeletal muscle. Over-expression of MBNL1 in a DM1 mouse model expressing CUGexp in skeletal muscle reverted Clcn1 splicing to the adult pattern and rescued myotonia (Kanadia et al., 2006). In addition, correction of full-length Clcn1 splicing using morpholino oligonucleotides increased Clcn1 RNA and protein levels and reduced myotonia in DM mouse models (Wheeler et al., 2007).

Aberrant skipping of insulin receptor (IR) exon 11 in DM1 skeletal muscle produces an isoform with lower signaling capacity, which is hypothesized to contribute to insulin insensitivity in DM1 skeletal muscle (Moxley et al., 1978; Savkur et al., 2001; Savkur et al., 2004). Inclusion of IR exon 11 normally increases during development due to the combined activity of CELF1, which represses exon 11 inclusion through binding to an intronic splicing silencer, and MBNL1, which enhances exon 11 inclusion through binding a downstream intronic splicing enhancer (Grammatikakis et al., 2010; Nezu et al., 2007; Savkur et al., 2001; Sen et al., 2010). Through reversal of MBNL1 and CELF1 expression in DM1 and DM2, IR exon 11 is aberrantly skipped in adult skeletal muscle (Savkur et al., 2001).

Recently, mis-splicing of bridging integrator 1 (BIN1) exon 11, which correlates with disrupted T-tubule formation, was observed in DM1 and DM2 skeletal muscle. MBNL1 activates BIN1 exon 11 inclusion through intronic binding sites downstream of exon 11. BIN1 exon 11 encodes a phosphoinositide binding domain which is required for the ability of BIN1 to promote T-tubule biogenesis, a membrane structure involved in the coupling of muscle excitation and contraction (Lee et al., 2002). Strikingly, transduction of DM1 myotubes with the BIN1 isoform including exon 11 restored T-tubule biogenesis, while this did not occur with the BIN1 isoform excluding exon 11. Furthermore, forced missplicing of BIN1 exon 11 in wild type mice was sufficient to disrupt T-tubule biogenesis and cause muscle weakness (Fugier et al., 2011). These studies indicate that disruption of MBNL proteins in DM results in mis-regulation of alternative splicing events that contribute to disease symptoms. In addition to regulating alternative splicing, MBNL2 is required for proper integrin α_3 protein localization to adhesion complexes via binding to the 3' UTR of its mRNA (Adereth et al., 2005). The relevance of this function to the mechanism to DM pathogenesis remains to be experimentally investigated.

MBNL1's RNA-binding properties are flexible, as it can bind CUG and CCUG repeats, as well as CAG repeats (Kino et al., 2004). MBNL1 binds pyrimidine-rich motifs, YGCY or YGCY(U/G)Y near the alternative exon within pre-mRNAs (Goers et al., 2010; Grammatikakis et al., 2010; Ho et al., 2004). These sequences are commonly found in introns surrounding exons mis-spliced in DM1, indicating that MBNL1 regulates many of these events. These intronic motifs form hairpins that resemble the tertiary structures of expanded CUGexp and CCUGexp RNA (Warf and Berglund, 2007; Yuan et al., 2007). Interestingly, the position of the MBNL1 binding sequences (upstream or downstream of the alternative exon) correlates with whether its silencer or enhancer activity is promoted (Du et al., 2010; Goers et al., 2010). This phenomenon has been noted with other splicing regulators, such as NOVA1 (Ule et al., 2006), and may contribute to future predictions of mis-regulated splicing events in disease such as DM. Binding to these regions is mediated by the four CCCH-type zinc finger motifs (Warf and Berglund, 2007). The four zinc finger domains of MBNL1 are arranged into two pairs, and the crystal structure of one of these pairs suggests that the protein binds looped RNA, since the bound RNA elements were in an antiparallel orientation (Teplova and Patel, 2008). Other protein regions outside of the 4 zinc finger motifs have been shown to be required for splicing activity and are hypothesized to mediate protein-protein interactions that are involved in splicing activation or repression (Grammatikakis et al., 2010).

2.1.b CELF proteins in DM—CELF1 was initially discovered as a protein that bound CUG₈ *in vitro* (Timchenko et al., 1996). There are six CELF paralogues which are related to *Drosophila* Bruno RNA-binding proteins and are widely expressed in humans with highest expression in skeletal muscle, cardiac muscle, and brain (Good et al., 2000; Ladd et al., 2001; Ladd et al., 2004). CELF protein expression is developmentally-regulated subsequently controlling multiple splicing events during postnatal development (Kalsotra et al., 2008; Ladd et al., 2001). Disruption of CELF activity in the mouse heart by transgenic

expression of a protein with dominant negative activity causes cardiac hypertrophy, dilated cardiomyopathy, and aberrant splicing of CELF target exons. These defects were restored by crossing these mice to mice with heart-specific CELF1 over-expression, indicating that CELF proteins play an important role in heart development (Ladd et al., 2005). Interestingly, CELF1 does not co-localize with CUGexp RNA foci in DM1 cells (Fardaei et al., 2001), and several lines of evidence indicate that a gain of CELF1 function is pathogenic in DM1. First, DM1 heart and skeletal muscle have increased levels of CELF1 protein (Charlet et al., 2002; Savkur et al., 2001; Timchenko et al., 2001). Second, a mouse model in which CUGexp is expressed specifically in the heart has up-regulation of CELF1 protein levels (Wang et al., 2007). Third, heart and skeletal muscle-specific over-expression of CELF1 in transgenic mice recapitulates muscle histopathology and mis-splicing events characteristic of DM (Ho et al., 2005; Ladd et al., 2005). Evidence for the mechanistic basis of CELF1 up-regulation in DM came from the observation that CUGexp RNA induces hyperphosphorylation of CELF1 protein that correlates with increased nuclear CELF1 steady state levels. This phosphorylation is mediated by protein kinase C (PKC) in cell culture, mouse DM models, and human DM patients (Kuyumcu-Martinez et al., 2007). The signaling pathway that responds to expression of CUGexp RNA and causes PKC-mediated hyperphosphorylation of CELF1 is yet unknown.

Mis-splicing events may also contribute to neurological aspects of the DM phenotype. MBNL1 and MBNL2 are sequestered on CUGexp RNA foci in neuronal nuclei in the brains of DM1 patients (Jiang et al., 2004). DM1 and DM2 patients exhibit neurofibrillary tangles (NFT) which consist of aggregated pathological isoforms of the tau protein (Maurage et al., 2005), which is attributed to aberrant splicing of tau isoforms (Dhaenens et al., 2011; Jiang et al., 2004; Sergeant et al., 2001; Seznec et al., 2001; Vermersch et al., 1996). Whether the tau splicing defect in DM1 is due to altered functions of MBNL1 or CELF1 is unclear (Chapple et al., 2007; Dhaenens et al., 2008; Dhaenens et al., 2011; Leroy et al., 2006). Additional mis-regulated splicing events were identified in DM1 brain samples: decreased amyloid precursor protein (APP) exon 7 inclusion and increased N-methyl D-aspartate receptor 1 (NMDA-R1) exon 5 inclusion (Jiang et al., 2004). This suggests that mis-regulation of alternative splicing may be a unifying mechanism that contributes to the multi-systemic nature of DM pathogenesis. The specific roles of these splicing events in neurological features of the DM phenotype remain to be evaluated.

An RNA gain-of-function mechanism in DM1 disrupts a network of alternative splicing developmentally regulated by CELF and MBNL proteins. Specific disease features have been attributed to disruption of individual splicing events. This link between mutant RNA and DM pathogenesis has set a paradigm for the study of other RNA gain-of-function diseases. As discussed in the following sections, there are several shared molecular markers of RNA gain-of-function diseases such as formation of nuclear aggregates and subsequent disruption of protein function. Although these mechanisms have been demonstrated for several RNA-dominant microsatellite expansion disorders, disruption of other cellular events may play a yet unknown role.

3. FXTAS

Fragile X disorders result from CGG repeats of variable length within a methylated CpG island in the 5' UTR of the fragile X mental retardation 1 (*FMR1*) gene (Oberle et al., 1991; Verkerk et al., 1991). Crystal structures of short CGG repeats reveal that they form A-helices and are more thermodynamically stable than CCG, CUG, or CAG repeats, suggesting that interactions between CGG repeats and RNA-binding proteins could be very specific (Kiliszek et al., 2011). Premutation alleles (55–200 CGG repeats) result in fragile X-associated tremor/ataxia syndrome (FXTAS), characterized by gait ataxia, tremor, and

neurodegeneration, while full expansion alleles give rise to fragile X syndrome (FXS), which is the most common form of inherited mental retardation (Jacquemont et al., 2003; Verkerk et al., 1991). Clinical manifestations of full mutation alleles are due to loss of FMR1 protein (FMRP) levels due to methylation of the *FMR1* locus (Sutcliffe et al., 1992). Paradoxically, in premutation allele carriers FMR1 RNA levels are up to five-fold higher than normal individuals due to increased transcription, while FMRP is at normal levels (Kenneson et al., 2001; Tassone et al., 2000; Tassone et al., 2007). This is highly suggestive of an RNA gain-of-function pathogenic mechanism in FXTAS.

3.1 Evidence for an RNA gain-of-function mechanism in FXTAS

Fly and mouse models expressing premutation FMR1 alleles do not express detectable protein, but exhibit progressive neurodegeneration, implicating CGGexp RNA as a pathogenic agent (Bontekoe et al., 2001; Todd et al., 2010; Willemssen et al., 2003). This is consistent with the observation that neurodegeneration is found in FXTAS but not FXS. The toxicity of CGGexp is independent of its context in the FMR1 transcript, as a CGGexp-containing reporter elicited neurotoxicity in mice (Hashem et al., 2009). CGG repeat-containing transcripts are found in nuclear inclusions in FXTAS patients and mammalian cell lines transiently transfected with 60 CGG repeats (Sellier et al., 2010; Tassone et al., 2004). Further evidence for RNA-mediated toxicity was discovered when FXTAS CGG permutation repeats were co-expressed with 90 CCG repeats in *Drosophila*. Co-expression of CGG and CCG repeats caused decreased repeat RNA levels, suppressed the individual neuro-toxicities of the repeats, and was dependent on argonaute-2 (*ago2*) (Sofola et al., 2007a). Thus, it appears that expression of RNA antisense to CGG repeats leads to their silencing and/or degradation via the RNAi pathway, further emphasizing the role of CGG repeat RNA in the toxicity of FXTAS. In fact, CGG repeat hairpins are cleaved, although inefficiently, by Dicer *in vitro* (Handa et al., 2003). Transcripts antisense to the FMR1 CGG repeats (ASFMR1) have been detected specifically in premutation alleles, but not full mutation alleles. ASFMR1 transcript levels match those of FMR1 premutation alleles, suggesting a role for antisense transcription in FXTAS pathogenesis (Ladd et al., 2007). Furthermore, phenotypic severity of FXTAS positively correlates with CGG repeat length (within the premutation range) (Jin et al., 2003). Taken together, these studies suggest that premutation CGGexp RNA has toxic effects in FXTAS.

3.2 RNA-binding proteins involved in FXTAS

The RNA-binding proteins heterogeneous ribonucleoprotein A2/B1 (hnRNP A2/B1), MBNL1, and purine-rich binding protein (Pur α) are found in FXTAS nuclear inclusions and have been suggested as contributing to CGGexp RNA toxicity (Iwahashi et al., 2006). Pur α interacts with CGGexp RNA in fly and mammalian brain extracts, is a component of nuclear inclusions in flies, and its over-expression suppresses CGGexp-induced neurodegeneration in a *Drosophila* FXTAS model (Jin et al., 2007). Pur α is involved in regulation of transcription, RNA transport, and translation, and disruption of its expression in mice causes neurodegeneration and tremors (Khalili et al., 2003). Further studies are needed to elucidate the downstream functions of Pur α that are disrupted in FXTAS. hnRNP A2/B1 binds directly to CGG repeat RNA and anchors CELF1 to the repeats. Over-expression of either hnRNP A2/B1 or CELF1 suppresses FXTAS-mediated neurodegeneration in a *Drosophila* FXTAS model (Sofola et al., 2007b). How the downstream functions of these RNA-binding proteins are relevant to the FXTAS phenotype remain to be explored. A critical question is whether localization of any of these proteins to CGG repeat RNA results in loss of their function, analogous to sequestration of MBNL in DM.

The first direct evidence for sequestration of an RNA-binding protein on premutation CGGexp RNA and its subsequent loss-of-function was for Src-associated substrate during mitosis of 68 kDa (Sam68). Sam68 is an RNA-binding splicing regulatory protein that is highly expressed in the brain. Sam68 knockout mice exhibit motor coordination defects (Lukong and Richard, 2008). Sam68 co-localizes with CGGexp RNA in patient brain samples and in a variety of cell types expressing 60 CGG RNA repeats following transient transfection. Sam68 is recruited to the CGG repeats early after transfection and is proposed to recruit MBNL1 and hnRNP G to the repeats. Expanded CGG repeats resulted in loss of Sam68 function, indicated by disrupted splicing of two targets, Bcl-X long isoform and ATP11B exon 28B, in transfected cells and patient cells. Over-expression of a Sam68 mutant that no longer bound CGG repeats but retained splicing activity rescued the splicing alterations, indicating that Sam68 sequestration caused the splicing defects (Sellier et al., 2010). It is unknown whether Sam68-regulated mis-splicing events are relevant to disease symptoms. In addition, the potential roles of MBNL1 and hnRNP G-regulated splicing events in FXTAS have not been evaluated.

4. DNA microsatellites encode multiple toxic units

It is well established that bi-directional transcription is prevalent in mammals (Katayama et al., 2005). Bidirectional transcription across expanded microsatellites has been observed in several disorders. This presents additional challenges for elucidating disease mechanisms, since a single expanded repeat DNA sequence has the potential to encode multiple toxic units: sense transcripts and protein translated from those transcripts, as well as antisense transcripts and their translated proteins [recently reviewed in (Batra et al., 2010; Krzyzosiak et al., 2011; Wojciechowska and Krzyzosiak, 2011b)]. Many groups have worked to carefully identify the pathogenic contribution of each of these entities to neurological disease. Antisense transcripts containing CGG repeats have been detected in FXTAS, suggestive of a role in RNA-mediated toxicity (Ladd et al., 2007). In addition, antisense transcripts adjacent to the CUGexp region in the DMPK transcript have been identified and may play a role in local chromatin remodeling in DM1 (Cho et al., 2005). In addition, antisense transcription across the CTG expansion results in the expression of CAGexp RNA which is translated into polyglutamine protein (Zu et al., 2011). The roles of CAGexp RNA and polyglutamine protein in DM will be interesting topics for future studies. Further studies will reveal the role of these antisense transcripts in FXTAS and DM1 pathogenesis. Outlined below are three diseases, HDL2, SCA8, and SCA3, which express repeat-containing RNA transcripts (sense and antisense) and polyglutamine protein. The roles of these factors in disease are discussed.

4.1 HDL2

Huntington disease-like 2 (HDL2) is an adult-onset autosomal dominant disorder, originally described as a CTG repeat expansion within alternative exon 2a of the junctophilin-3 (*JPH3*) gene. Alternative splicing of JPH exon 2a can result in transcripts with the expansion in the ORF or in the 3' UTR depending on the splicing pattern, suggesting that both CUGexp RNA and protein may mediate pathogenesis (Holmes et al., 2001). The repeat expansion in HDL2 causes similar symptoms to those in Huntington's disease such as motor coordination defects, neurodegeneration, dementia, and eventually death (Greenstein et al., 2007; Margolis et al., 2001; Rudnicki et al., 2008). Affected individuals have the expanded allele (51–57 repeats) while unaffected individuals carry the unexpanded allele (6–27 repeats) and longer expansions correlate with earlier age-of-onset (Holmes et al., 2001). *JPH3* loss of function may also contribute to pathogenesis, as *JPH3* expression is brain-specific and *JPH3* knockout mice have coordination defects. However, *JPH3* knock out mice show no neurodegenerative phenotype, suggesting that other molecular events may mediate this feature of HDL2 (Nishi et al., 2002).

There are several lines of evidence for the contribution of toxic CUGexp RNA to HDL2 pathogenesis. Similar to DM1, human HDL2 neurons have nuclear RNA foci that contain JPH3 transcripts with CUG repeats that co-localize with MBNL1 (Rudnicki et al., 2007). The localization of MBNL1 to the repeats was correlated with disrupted splicing of APP exon 7 and MAPT exon 2, as seen in DM1. Expression of a non-translatable expanded CTG repeat in HEK293 and HT22 mouse hippocampal cells resulted in RNA foci that co-localize with MBNL, as well as cytotoxicity (Rudnicki et al., 2007). These studies suggest that CUGexp RNA results in MBNL1 loss-of-function, analogous to DM1.

Evidence for polyglutamine protein-mediated pathogenicity in HDL2 has been recently established. The polyglutamine protein is encoded by a CAGexp-containing ORF in a *JPH3* antisense RNA. Cortical neurons in HDL2 BAC mice, as well as human patients, have ubiquitin-positive nuclear inclusions that stain using antibodies to polyglutamine protein which are independent from the CUG repeat RNA foci (Greenstein et al., 2007; Rudnicki et al., 2007; Rudnicki et al., 2008; Wilburn et al., 2011). The mice exhibit age-dependent neurodegeneration and motor defects. To test whether polyglutamine-mediated pathogenesis can occur without the presence of CUG repeat RNA, a transcription termination sequence was placed upstream of the CTG expansion in the JPH3 strand. These mice showed nuclear inclusions and neuronal dysfunction, but no neurodegenerative phenotype (Wilburn et al., 2011) indicating that expanded polyglutamine protein is sufficient to induce certain features of HDL2 phenotype, excluding neurodegeneration. Neurodegeneration may require CUGexp RNA-mediated toxicity. However, whether the sense-strand CTG expansion causes JPH3 loss-of-function and whether this contributes to neurodegeneration in HDL2 has not been explored.

4.2 SCA8

Spinocerebellar ataxia 8 (SCA8) is an adult-onset neurodegenerative disease characterized by cerebellar atrophy and coordination defects. An expansion of 107–127 CTG repeats in the *ataxin 8* (*ATXN8*) gene produces a central nervous system phenotype (Day et al., 2000). The pathogenesis of SCA8 appears to result from at least three factors: polyglutamine protein translated from CAG repeat RNA, polyalanine protein arising from RAN translation, and CUG repeat RNA transcribed in the antisense direction from the *ataxin 8 opposite strand* (*AXN8OS*) locus (Daughters et al., 2009; Koob et al., 1999; Moseley et al., 2006; Nemes et al., 2000; Zu et al., 2011). SCA8 transgenic mice expressing 118 CTG/CAG repeats displayed neurodegeneration, motor coordination defects, and cerebellar inhibition defects, consistent with features of SCA8. Transgenic mice and patient brain samples have nuclear inclusions which contain polyglutamine protein in Purkinjee and brain stem neurons (Moseley et al., 2006). Interestingly, non-ATG-initiated translation of the CAGexp results in polyalanine protein (Zu et al., 2011). It is yet to be determined whether the polyglutamine and polyalanine proteins contribute to the pathogenesis of SCA8.

There is evidence that CUGexp RNA transcribed from *AXN8OS* plays a pathogenic role in SCA8. RNA-binding proteins have been implicated in the RNA-mediated toxicity of SCA8. In a *D. melanogaster* screen in photoreceptor neurons, *mbf* loss-of-function enhanced the CUGexp-induced rough eye phenotype (Mutsuddi et al., 2004). SCA8 human and mouse cerebella, as well as stable HEK293 cells expressing expanded CUGexp, have RNA foci that co-localize with MBNL1 (Chen et al., 2009; Daughters et al., 2009). Crossing MBNL1 knockout mice (Kanadia et al., 2003) with SCA8 transgenic mice resulted in worsened rotarod performance, suggesting that MBNL1 loss of function contributes to RNA toxicity in SCA8. Furthermore, the splicing pattern of gamma-aminobutyric acid (GABA-A) transporter 4 (*Gabt4* in mice, or *GAT3* in human) exon 7 is disrupted in SCA8 patients. The misregulation of *GAT3* splicing is caused only by expanded CUG, not CAG, repeats, and is rescued by over-expression of MBNL1 in SCA8 CUGexp-expressing SK-N-SH human

neuroblastoma cells (Daughters et al., 2009). Taken together, these studies indicate CUGexp RNA contributes to the SCA8 phenotype through its ability to form RNA foci, sequester MBNL1 protein, and disrupt alternative splicing.

4.3 SCA3

In SCA3, or Machado-Joseph disease, both CAGexp RNA and the encoded polyglutamine protein contribute to pathogenesis. SCA3 was originally characterized as a polyglutamine protein expansion disorder in which a CAG trinucleotide encoding the C-terminal region of the ataxin-3 protein expands from 12–37 repeats in unaffected individuals to 61–84 repeats in affected individuals, causing late-onset ataxia and neurodegeneration. As is typical with polyglutamine expansion disorders, nuclear inclusions are found in SCA3 neurons (Paulson et al., 1997; Warrick et al., 1998). There are several lines of evidence for the toxicity of CAGexp RNA in SCA3. Untranslated CAG repeats elicit disease features and RNA foci in transgenic mice, *C. elegans*, and *D. melanogaster* models of SCA3 (Hsu et al., 2011; Li et al., 2008; Wang et al., 2010). In flies and worms expressing CAGexp, disease features are late-onset and phenotypic severity correlates with repeat length (Li et al., 2008; Wang et al., 2010). Interruption of the CAG repeats with CAA repeats (which still encode glutamine) dramatically diminished the SCA3 phenotype in flies, suggesting that the CAG repeat RNA, not the protein, is intrinsically toxic (Li et al., 2008).

MBNL1 co-localizes with CAGexp RNA foci in these three animal models of SCA3. In *C. elegans*, CeMbl over-expression partially rescued the phenotype, suggesting that its localization to CAG repeat RNA foci results in loss of its function (Wang et al., 2010). However, over-expression of mbl enhanced SCA3-mediated neurodegeneration in the SCA3 *D. melanogaster* model (Li et al., 2008). This suggests that factors other than MBNL proteins may contribute to CAG repeat RNA-mediated pathogenesis in SCA3. The role of MBNL in SCA3 remains unclear, as several splicing events known to be disrupted in DM are unaffected in SCA3 mouse and fly models (Hsu et al., 2011; Li et al., 2008). Further studies in stable HeLa and SK-N-MC cell lines revealed that untranslated CAG repeats form RNA foci that co-localize with MBNL1 protein and elicit splicing defects similar to those seen with CUG repeats in DM1 (Mykowska et al., 2011). Additionally, the RNA-binding protein orb2 in *Drosophila* was recently found to rescue CAGexp RNA-induced defects, indicative of roles for other RNA-binding proteins in SCA3 pathogenesis. Mutation of the CAG repeats to contain interruptions of CAA (which still encodes glutamine) revealed that orb2 can still modify toxicity of the polyglutamine protein. Therefore, orb2 is involved in toxicity mediated by CAGexp-containing RNA and protein (Shieh and Bonini, 2011). Taken together, these studies reveal the ability of CAGexp RNA to form foci that co-localize with MBNL1 protein. While the effects on alternative splicing events are less clear, these studies provide evidence that CAG repeat RNA contributes to neuropathology. Further studies could include in situ analysis for accumulation of CAG RNA in SCA3 tissue samples as well as studies to directly investigate the roles of MBNL and MBNL-regulated splicing events in pathogenesis.

5. Recent findings: RNA dominance in penta- and hexanucleotide RNA repeat disorders

5.1 SCA10

SCA10, an autosomal dominant neurodegenerative disorder, results from expansion of a pentanucleotide ATTCT repeat within an intron of the ataxin 10 (*ATXN10*) gene from about 15 repeats in unaffected individuals to 800–4,500 repeats in affected individuals. The toxicity of the AUUCUexp is suggested by the fact that longer expansion sizes correlate with earlier age of disease onset (Matsuura et al., 2000). The expanded repeat does not affect

ATXN10 expression, since RNA steady state levels and splicing are unaltered in cells from SCA10 patients (Wakamiya et al., 2006). The spliced intron containing AUUCUexp forms nuclear and cytoplasmic foci that co-localize with hnRNP K in SCA10 transgenic mouse brain and fibroblasts derived from SCA10 patients. The RNA foci are proposed to sequester hnRNP K, resulting in the loss of its ability to regulate splicing of β -tropomyosin. hnRNP K binds RNA through three K-homology (KH) domains and has roles in splicing, transcription, signaling, and apoptosis (reviewed in (Bomsztyk et al., 2004)). Interestingly, expression of AUUCU repeats or siRNA against hnRNP K activates caspase 3-mediated apoptosis in Sy5y neuroblastoma cells. Furthermore, over-expression of hnRNP K in AUUCUexp-expressing cells rescued the apoptotic phenotype induced by expression of the repeats, suggesting that loss of hnRNP K function mediates AUUCUexp-induced apoptosis (White et al., 2010).

5.2 ALS/FTD

Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are fatal neurodegenerative diseases that are proposed to share a common pathogenic mechanism, in that the trans-active response DNA-binding protein with M_r43kD (TDP-43) forms pathological cytoplasmic inclusions in nerve tissue in both disorders (Neumann et al., 2006). Two recent studies identified an expanded hexanucleotide repeat, GGGGCC, in an intronic region of chromosome 9 ORF 72 (C9ORF72) that is the most common cause of familial ALS/FTD (DeJesus-Hernandez et al., 2011; Renton et al., 2011). Affected patients carry approximately 700–1600 hexanucleotide repeats. The GGGGCCexp is found within an intron and forms nuclear RNA foci in neurons strongly suggesting the potential for an RNA gain-of-function mechanism. Furthermore, the presence of the GGGGCCexp RNA alters the splicing pattern of the C9ORF72 transcript. Further studies to elucidate the downstream consequences of GGGGCCexp RNA will provide more insight into pathogenic mechanisms of the disease.

6. Conclusion

The neuromuscular diseases highlighted here share several common themes. DM established a paradigm for RNA-dominant microsatellite expansion disorders. In DM, as in several other disorders discussed in this review, expansions are expressed as RNAexp which form nuclear foci. Formation of these foci exclusively in affected patients has established them as a molecular marker of disease. In some cases, these RNA foci sequester RNA-binding proteins, leading to loss of their function. In the case of DM and FXTAS, sequestration and loss of function of MBNL and Sam68, respectively, results in disruption of splicing events. In DM, several of these splicing events have been directly linked to disease features. RNAexp can also alter RNA-binding protein function through other signaling pathways, such as PKC-mediated CELF1 stabilization in DM1. RNA-binding protein sequestration and mis-splicing events have been detected in other disorders, as described above. Future studies will provide a clearer picture of the connection between expression of RNAexp and disease symptoms.

Although RNA toxicity occurs in all of the diseases described here, other factors are likely to contribute to pathogenesis as well. Mechanisms such as bidirectional transcription of repeat expansions and RAN translation of RNAexp generate repeat-containing RNA and protein. Many studies have provided evidence that several factors can mediate toxicity. This has notable importance for therapeutics, as disruption of just one toxic entity could have limited effectiveness. A thorough understanding of the toxic contributions from both repeat-containing RNA and protein, as well as the respective disruption of downstream pathways will enhance the success of targeted therapies.

Research Highlights

We do not require research highlights for this review article. Please contact the authors if a highlights section is necessary.

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References

- Adereth Y, Dammai V, Kose N, Li R, Hsu T. RNA-dependent integrin alpha3 protein localization regulated by the Muscleblind-like protein MLP1. *Nat Cell Biol.* 2005; 7:1240–1247. [PubMed: 16273094]
- Artero R, Prokop A, Paricio N, Begemann G, Pueyo I, Mlodzik M, Perez-Alonso M, Baylies MK. The muscleblind Gene Participates in the Organization of Z-Bands and Epidermal Attachments of *Drosophila* Muscles and Is Regulated by Dmef2. *Dev Biol.* 1998; 195:131–143. [PubMed: 9520330]
- Batra R, Charizanis K, Swanson MS. Partners in crime: bidirectional transcription in unstable microsatellite disease. *Hum Mol Genet.* 2010; 19:R77–R82. [PubMed: 20368264]
- Begemann G, Paricio N, Artero R, Kiss I, Perez-Alonso M, Mlodzik M. muscleblind, a gene required for photoreceptor differentiation in *Drosophila*, encodes novel nuclear Cys3His-type zinc-finger-containing proteins. *Development.* 1997; 124:4321–4331. [PubMed: 9334280]
- Berul CI, Maguire CT, Aronovitz MJ, Greenwood J, Miller C, Gehrmann J, Housman D, Mendelsohn ME, Reddy S. DMPK dosage alterations result in atrioventricular conduction abnormalities in a mouse myotonic dystrophy model. *J Clin Invest.* 1999; 103:R1–R7. [PubMed: 10021468]
- Bomsztyk K, Denisenko O, Ostrowski J. hnRNP K: One protein multiple processes. *BioEssays.* 2004; 26:629–638. [PubMed: 15170860]
- Bontekoe CJM, Bakker CE, Nieuwenhuizen IM, van der Linde H, Lans H, de Lange D, Hirst MC, Oostra BA. Instability of a (CGG)₉₈ repeat in the *Fmr1* promoter. *Hum Mol Genet.* 2001; 10:1693–1699. [PubMed: 11487573]
- Brook JD, McCurrach ME, Harley HG, Buckler AJ, Church D, Aburatani H, Hunter K, Stanton VP, Thirion JP, Hudson T, et al. Molecular basis of myotonic dystrophy: expansion of a trinucleotide (CTG) repeat at the 3' end of a transcript encoding a protein kinase family member. *Cell.* 1992; 68:799–808. [PubMed: 1310900]
- Chapple JP, Anthony K, Martin TR, Dev A, Cooper TA, Gallo JM. Expression, localization and tau exon 10 splicing activity of the brain RNA-binding protein TNRC4. *Hum Mol Genet.* 2007; 16:2760–2769. [PubMed: 17725984]
- Charlet BN, Savkur RS, Singh G, Philips AV, Grice EA, Cooper TA. Loss of the muscle-specific chloride channel in type 1 myotonic dystrophy due to misregulated alternative splicing. *Mol Cell.* 2002; 10:45–53. [PubMed: 12150906]
- Chen IC, Lin HY, Lee GC, Kao SH, Chen CM, Wu YR, Hsieh-Li HM, Su MT, Lee-Chen GJ. Spinocerebellar ataxia type 8 larger triplet expansion alters histone modification and induces RNA foci. *BMC Mol Biol.* 2009; 10:9. [PubMed: 19203395]
- Cho DH, Thienes CP, Mahoney SE, Analau E, Filippova GN, Tapscott SJ. Antisense Transcription and Heterochromatin at the DM1 CTG Repeats Are Constrained by CTCF. *Mol Cell.* 2005; 20:483–489. [PubMed: 16285929]
- Daughters RS, Tuttle DL, Gao W, Ikeda Y, Moseley ML, Ebner TJ, Swanson MS, Ranum LP. RNA gain-of-function in spinocerebellar ataxia type 8. *PLoS Genet.* 2009; 5:e1000600. [PubMed: 19680539]
- Davis BM, McCurrach ME, Taneja KL, Singer RH, Housman DE. Expansion of a CUG trinucleotide repeat in the 3' untranslated region of myotonic dystrophy protein kinase transcripts results in

- nuclear retention of transcripts. *Proc Natl Acad Sci U S A*. 1997; 94:7388–7393. [PubMed: 9207101]
- Day JW, Schut LJ, Moseley ML, Durand AC, Ranum LP. Spinocerebellar ataxia type 8: clinical features in a large family. *Neurology*. 2000; 55:649–657. [PubMed: 10980728]
- de Haro M, Al-Ramahi I, De Gouyon B, Ukani L, Rosa A, Faustino NA, Ashizawa T, Cooper TA, Botas J. MBNL1 and CUGBP1 modify expanded CUG-induced toxicity in a *Drosophila* model of myotonic dystrophy type 1. *Hum Mol Genet*. 2006; 15:2138–2145. [PubMed: 16723374]
- Dejesus-Hernandez M, Mackenzie IR, Boeve BF, Boxer AL, Baker M, Rutherford NJ, Nicholson AM, Finch NA, Flynn H, Adamson J, Kouri N, Wojtas A, Sengdy P, Hsiung GY, Karydas A, Seeley WW, Josephs KA, Coppola G, Geschwind DH, Wszolek ZK, Feldman H, Knopman DS, Petersen RC, Miller BL, Dickson DW, Boylan KB, Graff-Radford NR, Rademakers R. Expanded GGGGCC Hexanucleotide Repeat in Noncoding Region of C9ORF72 Causes Chromosome 9p-Linked FTD and ALS. *Neuron*. 2011; 72:245–256. [PubMed: 21944778]
- Dhaenens CM, Schraen-Maschke S, Tran H, Vingtdoux V, Ghanem D, Leroy O, Delplanque J, Vanbrussel E, Delacourte A, Vermersch P, Maurage CA, Gruffat H, Sergeant A, Mahadevan MS, Ishiura S, Buee L, Cooper TA, Caillet-Boudin ML, Charlet-Berguerand N, Sablonniere B, Sergeant N. Overexpression of MBNL1 fetal isoforms and modified splicing of Tau in the DM1 brain: two individual consequences of CUG trinucleotide repeats. *Exp Neurol*. 2008; 210:467–478. [PubMed: 18177861]
- Dhaenens CM, Tran H, Frandemichie ML, Carpentier C, Schraen-Maschke S, Sistiaga A, Goicoechea M, Eddarkaoui S, Van Brussels E, Obriot H, Labudeck A, Gevaert MH, Fernandez-Gomez F, Charlet-Berguerand N, Deramecourt V, Maurage CA, Buee L, de Munain AL, Sablonniere B, Caillet-Boudin ML, Sergeant N. Mis-splicing of Tau exon 10 in myotonic dystrophy type 1 is reproduced by overexpression of CELF2 but not by MBNL1 silencing. *Biochim Biophys Acta*. 2011; 1812:732–742. [PubMed: 21439371]
- Du H, Cline MS, Osborne RJ, Tuttle DL, Clark TA, Donohue JP, Hall MP, Shiue L, Swanson MS, Thornton CA, Ares M Jr. Aberrant alternative splicing and extracellular matrix gene expression in mouse models of myotonic dystrophy. *Nat Struct Mol Biol*. 2010; 17:187–193. [PubMed: 20098426]
- Fardaei M, Larkin K, Brook JD, Hamshire MG. In vivo co-localisation of MBNL protein with DMPK expanded-repeat transcripts. *Nucleic Acids Res*. 2001; 29:2766–2771. [PubMed: 11433021]
- Fu YH, Pizzuti A, Fenwick RG Jr, King J, Rajnarayan S, Dunne PW, Dubel J, Nasser GA, Ashizawa T, de Jong P, et al. An unstable triplet repeat in a gene related to myotonic muscular dystrophy. *Science*. 1992; 255:1256–1258. [PubMed: 1546326]
- Fugier C, Klein AF, Hammer C, Vassilopoulos S, Ivarsson Y, Toussaint A, Tosch V, Vignaud A, Ferry A, Messaddeq N, Kokunai Y, Tsuburaya R, de la Grange P, Dembele D, Francois V, Precigout G, Boulade-Ladame C, Hummel MC, de Munain AL, Sergeant N, Laquerriere A, Thibault C, Deryckere F, Auboeuf D, Garcia L, Zimmermann P, Udd B, Schoser B, Takahashi MP, Nishino I, Bassez G, Laporte J, Furling D, Charlet-Berguerand N. Misregulated alternative splicing of BIN1 is associated with T tubule alterations and muscle weakness in myotonic dystrophy. *Nat Med*. 2011; 17:720–725. [PubMed: 21623381]
- Goers ES, Purcell J, Voelker RB, Gates DP, Berglund JA. MBNL1 binds GC motifs embedded in pyrimidines to regulate alternative splicing. *Nucleic Acids Res*. 2010; 38:2467–2484. [PubMed: 20071745]
- Good PJ, Chen Q, Warner SJ, Herring DC. A family of human RNA-binding proteins related to the *Drosophila* Bruno translational regulator. *J Biol Chem*. 2000; 275:28583–28592. [PubMed: 10893231]
- Grammatikakis I, Goo YH, Echeverria GV, Cooper TA. Identification of MBNL1 and MBNL3 domains required for splicing activation and repression. *Nucleic Acids Res*. 2010; 39:2769–2780. [PubMed: 21109529]
- Greenstein PE, Vonsattel JP, Margolis RL, Joseph JT. Huntington's disease like-2 neuropathology. *Mov Disord*. 2007; 22:1416–1423. [PubMed: 17516481]
- Handa V, Saha T, Usdin K. The fragile X syndrome repeats form RNA hairpins that do not activate the interferon-inducible protein kinase, PKR, but are cut by Dicer. *Nucleic Acids Res*. 2003; 31:6243–6248. [PubMed: 14576312]

- Hashem V, Galloway JN, Mori M, Willemsen R, Oostra BA, Paylor R, Nelson DL. Ectopic expression of CGG containing mRNA is neurotoxic in mammals. *Hum Mol Genet.* 2009; 18:2443–2451. [PubMed: 19377084]
- Ho TH, Charlet BN, Poulos MG, Singh G, Swanson MS, Cooper TA. Muscleblind proteins regulate alternative splicing. *EMBO J.* 2004; 23:3103–3112. [PubMed: 15257297]
- Ho TH, Bundman D, Armstrong DL, Cooper TA. Transgenic mice expressing CUG-BP1 reproduce splicing mis-regulation observed in myotonic dystrophy. *Hum Mol Genet.* 2005; 14:1539–1547. [PubMed: 15843400]
- Holmes SE, O'Hearn E, Rosenblatt A, Callahan C, Hwang HS, Ingersoll-Ashworth RG, Fleisher A, Stevanin G, Brice A, Potter NT, Ross CA, Margolis RL. A repeat expansion in the gene encoding junctophilin-3 is associated with Huntington disease-like 2. *Nat Genet.* 2001; 29:377–378. [PubMed: 11694876]
- Hsu RJ, Hsiao KM, Lin MJ, Li CY, Wang LC, Chen LK, Pan H. Long tract of untranslated CAG repeats is deleterious in transgenic mice. *PLoS One.* 2011; 6:e16417. [PubMed: 21283659]
- Iwahashi CK, Yasui DH, An HJ, Greco CM, Tassone F, Nannen K, Babineau B, Lebrilla CB, Hagerman RJ, Hagerman PJ. Protein composition of the intranuclear inclusions of FXTAS. *Brain.* 2006; 129:256–271. [PubMed: 16246864]
- Jacquemont, Sb; Hagerman, RJ.; Leehey, M.; Grigsby, J.; Zhang, L.; Brunberg, JA.; Greco, C.; Des Portes, V.; Jardini, T.; Levine, R.; Berry-Kravis, E.; Brown, WT.; Schaeffer, S.; Kissel, J.; Tassone, F.; Hagerman, PJ. Fragile X Premutation Tremor/Ataxia Syndrome: Molecular, Clinical, and Neuroimaging Correlates. *Am J Hum Genet.* 2003; 72:869–878. [PubMed: 12638084]
- Jiang H, Mankodi A, Swanson MS, Moxley RT, Thornton CA. Myotonic dystrophy type 1 is associated with nuclear foci of mutant RNA sequestration of muscleblind proteins and deregulated alternative splicing in neurons. *Hum Mol Genet.* 2004; 13:3079–3088. [PubMed: 15496431]
- Jin P, Zarnescu DC, Zhang F, Pearson CE, Lucchesi JC, Moses K, Warren ST. RNA-mediated neurodegeneration caused by the fragile X premutation rCGG repeats in *Drosophila*. *Neuron.* 2003; 39:739–747. [PubMed: 12948442]
- Jin P, Duan R, Qurashi A, Qin Y, Tian D, Rosser TC, Liu H, Feng Y, Warren ST. Pur alpha binds to rCGG repeats and modulates repeat-mediated neurodegeneration in a *Drosophila* model of fragile X tremor/ataxia syndrome. *Neuron.* 2007; 55:556–564. [PubMed: 17698009]
- Kalsotra A, Xiao X, Ward AJ, Castle JC, Johnson JM, Burge CB, Cooper TA. A postnatal switch of CELF and MBNL proteins reprograms alternative splicing in the developing heart. *Proc Natl Acad Sci U S A.* 2008; 105:20333–20338. [PubMed: 19075228]
- Kanadia RN, Johnstone KA, Mankodi A, Lungu C, Thornton CA, Esson D, Timmers AM, Hauswirth WW, Swanson MS. A muscleblind knockout model for myotonic dystrophy. *Science.* 2003; 302:1978–1980. [PubMed: 14671308]
- Kanadia RN, Shin J, Yuan Y, Beattie SG, Wheeler TM, Thornton CA, Swanson MS. Reversal of RNA missplicing and myotonia after muscleblind overexpression in a mouse poly(CUG) model for myotonic dystrophy. *Proc Natl Acad Sci U S A.* 2006; 103:11748–11753. [PubMed: 16864772]
- Katayama S, Tomaru Y, Kasukawa T, Waki K, Nakanishi M, Nakamura M, Nishida H, Yap CC, Suzuki M, Kawai J, Suzuki H, Carninci P, Hayashizaki Y, Wells C, Frith M, Ravasi T, Pang KC, Hallinan J, Mattick J, Hume DA, Lipovich L, Batalov S, Engstrom PG, Mizuno Y, Faghihi MA, Sandelin A, Chalk AM, Mottagui-Tabar S, Liang Z, Lenhard B, Wahlestedt C. Antisense transcription in the mammalian transcriptome. *Science.* 2005; 309:1564–1566. [PubMed: 16141073]
- Kenneson A, Zhang F, Hagedorn CH, Warren ST. Reduced FMRP and increased FMR1 transcription is proportionally associated with CGG repeat number in intermediate-length and premutation carriers. *Hum Mol Genet.* 2001; 10:1449–1454. [PubMed: 11448936]
- Khalili K, Del Valle L, Muralidharan V, Gault WJ, Darbinian N, Otte J, Meier E, Johnson EM, Daniel DC, Kinoshita Y, Amini S, Gordon J. Puralpha is essential for postnatal brain development and developmentally coupled cellular proliferation as revealed by genetic inactivation in the mouse. *Mol Cell Biol.* 2003; 23:6857–6875. [PubMed: 12972605]

- Kiliszek A, Kierzek R, Krzyzosiak WJ, Rypniewski W. Crystal structures of CGG RNA repeats with implications for fragile X-associated tremor ataxia syndrome. *Nucleic Acids Res.* 2011; 39:7308–7315. [PubMed: 21596781]
- Kino Y, Mori D, Oma Y, Takeshita Y, Sasagawa N, Ishiura S. Muscleblind protein, MBNL1/EXP, binds specifically to CHHG repeats. *Hum Mol Genet.* 2004; 13:495–507. [PubMed: 14722159]
- Koob MD, Moseley ML, Schut LJ, Benzow KA, Bird TD, Day JW, Ranum LP. An untranslated CTG expansion causes a novel form of spinocerebellar ataxia (SCA8). *Nat Genet.* 1999; 21:379–384. [PubMed: 10192387]
- Krzyzosiak WJ, Sobczak K, Wojciechowska M, Fiszler A, Mykowska A, Kozlowski P. Triplet repeat RNA structure and its role as pathogenic agent and therapeutic target. *Nucleic Acids Res.* 2011
- Kuyumcu-Martinez NM, Wang GS, Cooper TA. Increased steady-state levels of CUGBP1 in myotonic dystrophy 1 are due to PKC-mediated hyperphosphorylation. *Mol Cell.* 2007; 28:68–78. [PubMed: 17936705]
- Ladd AN, Charlet-B N, Cooper TA. The CELF Family of RNA Binding Proteins Is Implicated in Cell-Specific and Developmentally Regulated Alternative Splicing. *Mol. Cell. Biol.* 2001; 21:1285–1296. [PubMed: 11158314]
- Ladd AN, Nguyen NH, Malhotra K, Cooper TA. CELF6, a member of the CELF family of RNA-binding proteins, regulates muscle-specific splicing enhancer-dependent alternative splicing. *J Biol Chem.* 2004; 279:17756–17764. [PubMed: 14761971]
- Ladd AN, Taffet G, Hartley C, Kearney DL, Cooper TA. Cardiac Tissue-Specific Repression of CELF Activity Disrupts Alternative Splicing and Causes Cardiomyopathy. *Mol. Cell. Biol.* 2005; 25:6267–6278. [PubMed: 15988035]
- Ladd PD, Smith LE, Rabaia NA, Moore JM, Georges SA, Hansen RS, Hagerman RJ, Tassone F, Tapscott SJ, Filippova GN. An antisense transcript spanning the CGG repeat region of FMR1 is upregulated in premutation carriers but silenced in full mutation individuals. *Hum Mol Genet.* 2007; 16:3174–3187. [PubMed: 17921506]
- Lee E, Marcucci M, Daniell L, Pypaert M, Weisz OA, Ochoa G-C, Farsad K, Wenk MR, De Camilli P. Amphiphysin 2 (Bin1) and T-Tubule Biogenesis in Muscle. *Science.* 2002; 297:1193–1196. [PubMed: 12183633]
- Lee KS, Cao Y, Witwicka HE, Tom S, Tapscott SJ, Wang EH. RNA-binding protein Muscleblindlike 3 (MBNL3) disrupts myocyte enhancer factor 2 (Mef2) β -exon splicing. *J Biol Chem.* 2010; 285:33779–33787. [PubMed: 20709755]
- Leroy O, Dhaenens CM, Schraen-Maschke S, Belarbi K, Delacourte A, Andreadis A, Sablonniere B, Buee L, Sergeant N, Caillet-Boudin ML. ETR-3 represses Tau exons 2/3 inclusion, a splicing event abnormally enhanced in myotonic dystrophy type I. *J Neurosci Res.* 2006; 84:852–859. [PubMed: 16862542]
- Li LB, Yu Z, Teng X, Bonini NM. RNA toxicity is a component of ataxin-3 degeneration in *Drosophila*. *Nature.* 2008; 453:1107–1111. [PubMed: 18449188]
- Lin X, Miller JW, Mankodi A, Kanadia RN, Yuan Y, Moxley RT, Swanson MS, Thornton CA. Failure of MBNL1-dependent post-natal splicing transitions in myotonic dystrophy. *Hum Mol Genet.* 2006; 15:2087–2097. [PubMed: 16717059]
- Liquori CL, Ricker K, Moseley ML, Jacobsen JF, Kress W, Naylor SL, Day JW, Ranum LP. Myotonic dystrophy type 2 caused by a CCTG expansion in intron 1 of ZNF9. *Science.* 2001; 293:864–867. [PubMed: 11486088]
- Lukong KE, Richard Sp. Motor coordination defects in mice deficient for the Sam68 RNA-binding protein. *Behavioural Brain Research.* 2008; 189:357–363. [PubMed: 18325609]
- Mankodi A, Logigian E, Callahan L, McClain C, White R, Henderson D, Krym M, Thornton CA. Myotonic dystrophy in transgenic mice expressing an expanded CUG repeat. *Science.* 2000; 289:1769–1773. [PubMed: 10976074]
- Mankodi A, Urbinati CR, Yuan QP, Moxley RT, Sansone V, Krym M, Henderson D, Schalling M, Swanson MS, Thornton CA. Muscleblind localizes to nuclear foci of aberrant RNA in myotonic dystrophy types 1 and 2. *Hum Mol Genet.* 2001; 10:2165–2170. [PubMed: 11590133]

- Margolis RL, O'Hearn E, Rosenblatt A, Willour V, Holmes SE, Franz ML, Callahan C, Hwang HS, Troncoso JC, Ross CA. A disorder similar to Huntington's disease is associated with a novel CAG repeat expansion. *Ann Neurol*. 2001; 50:373–380.
- Marteyn A, Maury Y, Gauthier MM, Lecuyer C, Vernet R, Denis JA, Pietu G, Peschanski M, Martinat C. Mutant human embryonic stem cells reveal neurite and synapse formation defects in type 1 myotonic dystrophy. *Cell Stem Cell*. 2011; 8:434–444. [PubMed: 21458401]
- Matsuura T, Yamagata T, Burgess DL, Rasmussen A, Grewal RP, Watase K, Khajavi M, McCall AE, Davis CF, Zu L, Achari M, Pulst SM, Alonso E, Noebels JL, Nelson DL, Zoghbi HY, Ashizawa T. Large expansion of the ATTCT pentanucleotide repeat in spinocerebellar ataxia type 10. *Nat Genet*. 2000; 26:191–194. [PubMed: 11017075]
- Maurage CA, Udd B, Ruchoux MM, Vermersch P, Kalimo H, Krahe R, Delacourte A, Sergeant N. Similar brain tau pathology in DM2/PROMM and DM1/Steinert disease. *Neurology*. 2005; 65:1636–1638. [PubMed: 16301494]
- Miller JW, Urbinati CR, Teng-Umnuy P, Stenberg MG, Byrne BJ, Thornton CA, Swanson MS. Recruitment of human muscleblind proteins to (CUG)(n) expansions associated with myotonic dystrophy. *EMBO J*. 2000; 19:4439–4448. [PubMed: 10970838]
- Modoni A, Silvestri G, Grazia Pomponi M, Mangiola F, Tonali PA, Marra C. Characterization of the Pattern of Cognitive Impairment in Myotonic Dystrophy Type 1. *Arch Neurol*. 2004; 61:1943–1947. [PubMed: 15596617]
- Mooers BH, Logue JS, Berglund JA. The structural basis of myotonic dystrophy from the crystal structure of CUG repeats. *Proc Natl Acad Sci U S A*. 2005; 102:16626–16631. [PubMed: 16269545]
- Moseley ML, Zu T, Ikeda Y, Gao W, Mosemiller AK, Daughters RS, Chen G, Weatherspoon MR, Clark HB, Ebner TJ, Day JW, Ranum LP. Bidirectional expression of CUG and CAG expansion transcripts and intranuclear polyglutamine inclusions in spinocerebellar ataxia type 8. *Nat Genet*. 2006; 38:758–769. [PubMed: 16804541]
- Moxley RT, Griggs RC, Goldblatt D, VanGelder V, Herr BE, Thiel R. Decreased insulin sensitivity of forearm muscle in myotonic dystrophy. *J Clin Invest*. 1978; 62:857–867. [PubMed: 701484]
- Mutsuddi M, Marshall CM, Benzow KA, Koob MD, Rebay I. The spinocerebellar ataxia 8 noncoding RNA causes neurodegeneration and associates with staufen in *Drosophila*. *Curr Biol*. 2004; 14:302–308. [PubMed: 14972680]
- Mykowska A, Sobczak K, Wojciechowska M, Kozłowski P, Krzyżosiak WJ. CAG repeats mimic CUG repeats in the misregulation of alternative splicing. *Nucleic Acids Res*. 2011; 39:8938–8951. [PubMed: 21795378]
- Nemes JP, Benzow KA, Moseley ML, Ranum LP, Koob MD. The SCA8 transcript is an antisense RNA to a brain-specific transcript encoding a novel actin-binding protein (KLHL1). *Hum Mol Genet*. 2000; 9:1543–1551. [PubMed: 10888605]
- Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, Bruce J, Schuck T, Grossman M, Clark CM, McCluskey LF, Miller BL, Masliah E, Mackenzie IR, Feldman H, Feiden W, Kretschmar HA, Trojanowski JQ, Lee VMY. Ubiquitinated TDP-43 in Frontotemporal Lobar Degeneration and Amyotrophic Lateral Sclerosis. *Science*. 2006; 314:130–133. [PubMed: 17023659]
- Nezu Y, Kino Y, Sasagawa N, Nishino I, Ishiura S. Expression of MBNL and CELF mRNA transcripts in muscles with myotonic dystrophy. *Neuromuscul Disord*. 2007; 17:306–312. [PubMed: 17331722]
- Nishi M, Hashimoto K, Kuriyama K, Komazaki S, Kano M, Shibata S, Takeshima H. Motor Discoordination in Mutant Mice Lacking Junctophilin Type 3. *Biochem Biophys Res Commun*. 2002; 292:318–324. [PubMed: 11906164]
- Oberle I, Rousseau F, Heitz D, Kretz C, Devys D, Hanauer A, Boue J, Bertheas M, Mandel J. Instability of a 550-base pair DNA segment and abnormal methylation in fragile X syndrome. *Science*. 1991; 252:1097–1102.
- Orengo JP, Chambon P, Metzger D, Mosier DR, Snipes GJ, Cooper TA. Expanded CTG repeats within the DMPK 3' UTR causes severe skeletal muscle wasting in an inducible mouse model for myotonic dystrophy. *Proc Natl Acad Sci U S A*. 2008; 105:2646–2651. [PubMed: 18272483]

- Orr HT, Zoghbi HY. Trinucleotide repeat disorders. *Annu Rev Neurosci.* 2007; 30:575–621. [PubMed: 17417937]
- Paulson HL, Perez MK, Trotter Y, Trojanowski JQ, Subramony SH, Das SS, Vig P, Mandel JL, Fischbeck KH, Pittman RN. Intranuclear Inclusions of Expanded Polyglutamine Protein in Spinocerebellar Ataxia Type 3. *Neuron.* 1997; 19:333–344. [PubMed: 9292723]
- Quintero-Mora ML, Depardon F, Waring James, Robert GK, Cisneros B. Expanded CTG repeats inhibit neuronal differentiation of the PC12 cell line. *Biochem Biophys Res Commun.* 2002; 295:289–294. [PubMed: 12150945]
- Renton AE, Majounie E, Waite A, Simon-Sanchez J, Rollinson S, Gibbs JR, Schymick JC, Laaksovirta H, van Swieten JC, Myllykangas L, Kalimo H, Paetau A, Abramzon Y, Remes AM, Kaganovich A, Scholz SW, Duckworth J, Ding J, Harmer DW, Hernandez DG, Johnson JO, Mok K, Ryten M, Trabzuni D, Guerreiro RJ, Orrell RW, Neal J, Murray A, Pearson J, Jansen IE, Sondervan D, Seelaar H, Blake D, Young K, Halliwell N, Callister JB, Toulson G, Richardson A, Gerhard A, Snowden J, Mann D, Neary D, Nalls MA, Peuralinna T, Jansson L, Isoviiita VM, Kaivorinne AL, Holtta-Vuori M, Ikonen E, Sulkava R, Benatar M, Wu J, Chio A, Restagno G, Borghero G, Sabatelli M, Heckerman D, Rogaeva E, Zinman L, Rothstein JD, Sendtner M, Drepper C, Eichler EE, Alkan C, Abdullaev Z, Pack SD, Dutra A, Pak E, Hardy J, Singleton A, Williams NM, Heutink P, Pickering-Brown S, Morris HR, Tienari PJ, Traynor BJ. A Hexanucleotide Repeat Expansion in C9ORF72 Is the Cause of Chromosome 9p21-Linked ALS-FTD. *Neuron.* 2011; 72:257–268. [PubMed: 21944779]
- Rudnicki DD, Holmes SE, Lin MW, Thornton CA, Ross CA, Margolis RL. Huntington's disease-- like 2 is associated with CUG repeat-containing RNA foci. *Ann Neurol.* 2007; 61:272–282. [PubMed: 17387722]
- Rudnicki DD, Pletnikova O, Vonsattel JP, Ross CA, Margolis RL. A comparison of huntington disease and huntington disease-like 2 neuropathology. *J Neuropathol Exp Neurol.* 2008; 67:366–374. [PubMed: 18379432]
- Savkur RS, Philips AV, Cooper TA. Aberrant regulation of insulin receptor alternative splicing is associated with insulin resistance in myotonic dystrophy. *Nat Genet.* 2001; 29:40–47. [PubMed: 11528389]
- Savkur RS, Philips AV, Cooper TA, Dalton JC, Moseley ML, Ranum LP, Day JW. Insulin receptor splicing alteration in myotonic dystrophy type 2. *Am J Hum Genet.* 2004; 74:1309–1313. [PubMed: 15114529]
- Sellier C, Rau F, Liu Y, Tassone F, Hukema RK, Gattoni R, Schneider A, Richard S, Willemssen R, Elliott DJ, Hagerman PJ, Charlet-Berguerand N. Sam68 sequestration and partial loss of function are associated with splicing alterations in FXTAS patients. *EMBO J.* 2010; 29:1248–1261. [PubMed: 20186122]
- Sen S, Talukdar I, Liu Y, Tam J, Reddy S, Webster NJG. Muscleblind-like 1 (Mbnl1) Promotes Insulin Receptor Exon 11 Inclusion via Binding to a Downstream Evolutionarily Conserved Intronic Enhancer. *J Biol Chem.* 2010; 285:25426–25437. [PubMed: 20519504]
- Sergeant N, Sablonniere B, Schraen-Maschke S, Ghestem A, Maurage CA, Wattez A, Vermersch P, Delacourte A. Dysregulation of human brain microtubule-associated tau mRNA maturation in myotonic dystrophy type 1. *Hum Mol Genet.* 2001; 10:2143–2155. [PubMed: 11590131]
- Seznec H, Agbulut O, Sergeant N, Savouret C, Ghestem A, Tabti N, Willer JC, Ourth L, Duros C, Brisson E, Fouquet C, Butler-Browne G, Delacourte A, Junien C, Gourdon G. Mice transgenic for the human myotonic dystrophy region with expanded CTG repeats display muscular and brain abnormalities. *Hum Mol Genet.* 2001; 10:2717–2726. [PubMed: 11726559]
- Shieh SY, Bonini NM. Genes and pathways affected by CAG-repeat RNA-based toxicity in *Drosophila*. *Hum Mol Genet.* 2011 Epub 2011/9/22.
- Sofola OA, Jin P, Botas J, Nelson DL. Argonaute-2-dependent rescue of a *Drosophila* model of FXTAS by FRAXE premutation repeat. *Hum Mol Genet.* 2007a; 16:2326–2332. [PubMed: 17635840]
- Sofola OA, Jin P, Qin Y, Duan R, Liu H, de Haro M, Nelson DL, Botas J. RNA-binding proteins hnRNP A2/B1 and CUGBP1 suppress fragile X CGG premutation repeat-induced neurodegeneration in a *Drosophila* model of FXTAS. *Neuron.* 2007b; 55:565–571. [PubMed: 17698010]

- Sutcliffe JS, Nelson DL, Zhang F, Pieretti M, Caskey CT, Saxe D, Warren ST. DNA methylation represses FMR-1 transcription in fragile X syndrome. *Hum Mol Genet.* 1992; 1:397–400. [PubMed: 1301913]
- Tanaka H, Arai M, Harada M, Hozumi A, Hirata K. Cognition and event-related potentials in adult-onset non-demented myotonic dystrophy type 1. *Clin Neurophysiol.* 2011 Epub 2011/07/12.
- Taneja KL, McCurrach M, Schalling M, Housman D, Singer RH. Foci of trinucleotide repeat transcripts in nuclei of myotonic dystrophy cells and tissues. *J Cell Biol.* 1995; 128:995–1002. [PubMed: 7896884]
- Tassone F, Hagerman RJ, Taylor AK, Gane LW, Godfrey TE, Hagerman PJ. Elevated Levels of FMR1 mRNA in Carrier Males: A New Mechanism of Involvement in the Fragile-X Syndrome. *Am J Hum Genet.* 2000; 66:6–15. [PubMed: 10631132]
- Tassone F, Iwahashi C, Hagerman PJ. FMR1 RNA within the intranuclear inclusions of fragile X-associated tremor/ataxia syndrome (FXTAS). *RNA Biol.* 2004; 1:103–105. [PubMed: 17179750]
- Tassone F, Beilina A, Carosi C, Albertosi S, Bagni C, Li L, Glover K, Bentley D, Hagerman PJ. Elevated FMR1 mRNA in premutation carriers is due to increased transcription. *RNA.* 2007; 13:555–562. [PubMed: 17283214]
- Teplova M, Patel DJ. Structural insights into RNA recognition by the alternative-splicing regulator muscleblind-like MBNL1. *Nat Struct Mol Biol.* 2008; 15:1343–1351. [PubMed: 19043415]
- Tian B, White RJ, Xia T, Welle S, Turner DH, Mathews MB, Thornton CA. Expanded CUG repeat RNAs form hairpins that activate the double-stranded RNA-dependent protein kinase PKR. *RNA.* 2000; 6:79–87. [PubMed: 10668800]
- Timchenko LT, Miller JW, Timchenko NA, DeVore DR, Datar KV, Lin L, Roberts R, Caskey CT, Swanson MS. Identification of a (CUG)_n triplet repeat RNA-binding protein and its expression in myotonic dystrophy. *Nucleic Acids Res.* 1996; 24:4407–4414. [PubMed: 8948631]
- Timchenko NA, Cai ZJ, Welm AL, Reddy S, Ashizawa T, Timchenko LT. RNA CUG repeats sequester CUGBP1 and alter protein levels and activity of CUGBP1. *J Biol Chem.* 2001; 276:7820–7826. [PubMed: 11124939]
- Todd PK, Oh SY, Krans A, Pandey UB, Di Prospero NA, Min KT, Taylor JP, Paulson HL. Histone deacetylases suppress CGG repeat-induced neurodegeneration via transcriptional silencing in models of fragile X tremor ataxia syndrome. *PLoS Genet.* 2010; 6:e1001240. [PubMed: 21170301]
- Ule J, Stefani G, Mele A, Ruggiu M, Wang X, Taneri B, Gaasterland T, Blencowe BJ, Darnell RB. An RNA map predicting Nova-dependent splicing regulation. *Nature.* 2006; 444:580–586. [PubMed: 17065982]
- Verkerk AJ, Pieretti M, Sutcliffe JS, Fu YH, Kuhl DP, Pizzuti A, Reiner O, Richards S, Victoria MF, Zhang FP, et al. Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell.* 1991; 65:905–914. [PubMed: 1710175]
- Vermersch P, Sergeant N, Ruchoux MM, Hofmann-Radvanyi H, Wattez A, Petit H, Dwailly P, Delacourte A. Specific tau variants in the brains of patients with myotonic dystrophy. *Neurology.* 1996; 47:711–717. [PubMed: 8797469]
- Wakamiya M, Matsuura T, Liu Y, Schuster GC, Gao R, Xu W, Sarkar PS, Lin X, Ashizawa T. The role of ataxin 10 in the pathogenesis of spinocerebellar ataxia type 10. *Neurology.* 2006; 67:607–613. [PubMed: 16924013]
- Wang GS, Kearney DL, De Biasi M, Taffet G, Cooper TA. Elevation of RNA-binding protein CUGBP1 is an early event in an inducible heart-specific mouse model of myotonic dystrophy. *J Clin Invest.* 2007; 117:2802–2811. [PubMed: 17823658]
- Wang LC, Chen KY, Pan H, Wu CC, Chen PH, Liao YT, Li C, Huang ML, Hsiao KM. Muscleblind participates in RNA toxicity of expanded CAG and CUG repeats in *Caenorhabditis elegans*. *Cell Mol Life Sci.* 2010; 68:1255–1267. [PubMed: 20848157]
- Warf MB, Berglund JA. MBNL binds similar RNA structures in the CUG repeats of myotonic dystrophy and its pre-mRNA substrate cardiac troponin T. *TRNA.* 2007; 13:2238–2251.

- Warrick JM, Paulson HL, Gray-Board GL, Bui QT, Fischbeck KH, Pittman RN, Bonini NM. Expanded Polyglutamine Protein Forms Nuclear Inclusions and Causes Neural Degeneration in *Drosophila*. *Cell*. 1998; 93:939–949. [PubMed: 9635424]
- Wheeler TM, Lueck JD, Swanson MS, Dirksen RT, Thornton CA. Correction of CIC-1 splicing eliminates chloride channelopathy and myotonia in mouse models of myotonic dystrophy. *J Clin Invest*. 2007; 117:3952–3957. [PubMed: 18008009]
- White MC, Gao R, Xu W, Mandal SM, Lim JG, Hazra TK, Wakamiya M, Edwards SF, Raskin S, Teive HLAG, Zoghbi HY, Sarkar PS, Ashizawa T. Inactivation of hnRNP K by Expanded Intronic AUUCU Repeat Induces Apoptosis Via Translocation of PKC δ to Mitochondria in Spinocerebellar Ataxia 10. *PLoS Genet*. 2010; 6:e1000984. [PubMed: 20548952]
- Wilburn B, Rudnicki DD, Zhao J, Weitz TM, Cheng Y, Gu X, Greiner E, Park CS, Wang N, Sopher BL, La Spada AR, Osmund A, Margolis RL, Sun YE, Yang XW. An antisense CAG repeat transcript at JPH3 locus mediates expanded polyglutamine protein toxicity in Huntington's disease-like 2 mice. *Neuron*. 2011; 70:427–440. [PubMed: 21555070]
- Willemsen R, Hoogeveen-Westerveld M, Reis S, Holstege J, Severijnen L-AWFM, Nieuwenhuizen IM, Schrier M, van Unen L, Tassone F, Hoogeveen AT, Hagerman PJ, Mientjes EJ, Oostra BA. The FMR1 CGG repeat mouse displays ubiquitin-positive intranuclear neuronal inclusions; implications for the cerebellar tremor/ataxia syndrome. *Hum Mol Genet*. 2003; 12:949–959. [PubMed: 12700164]
- Wojciechowska M, Krzyzosiak WJ. Cellular toxicity of expanded RNA repeats: focus on RNA foci. *Hum Mol Genet*. 2011a; 20:3811–3821. [PubMed: 21729883]
- Wojciechowska M, Krzyzosiak WJ. CAG repeat RNA as an auxiliary toxic agent in polyglutamine disorders. *RNA Biol*. 2011b; 8
- Yuan Y, Compton SA, Sobczak K, Stenberg MG, Thornton CA, Griffith JD, Swanson MS. Muscblind-like 1 interacts with RNA hairpins in splicing target and pathogenic RNAs. *Nucleic Acids Res*. 2007; 35:5474–5486. [PubMed: 17702765]
- Zu T, Gibbens B, Doty NS, Gomes-Pereira Mr, Huguet A, Stone MD, Margolis J, Peterson M, Markowski TW, Ingram MAC, Nan Z, Forster C, Low WC, Schoser B, Somia NV, Clark HB, Schmechel S, Bitterman PB, Gourdon Gv, Swanson MS, Moseley M, Ranum LPW. Non-ATG-initiated translation directed by microsatellite expansions. *Proc Natl Acad Sci U S A*. 2011; 108:260–265. [PubMed: 21173221]

Figure 1. RNA-binding protein (RBP) function is disrupted in RNAexp-dominant disorders

Microsatellite expansion disorders that produce repeat-containing RNA, or RNAexp, are shown. The relative location within the gene of the repeat expansion is depicted. While other toxic RNAs are generated from the opposite strand in the case of several diseases, only toxic entities known to be related to the disruption of RBP function are depicted here. The sequence of the RNAexp is drawn for each disorder. This toxic RNA is thought to contribute to pathogenesis through its ability to disrupt one or more RBPs. In some cases, disruption of the downstream function of an RBP has been observed. These mechanisms will be discussed in detail in this review.