

# RNA-Mediated Neurodegeneration in Repeat Expansion Disorders

Peter K. Todd, MD, PhD and Henry L. Paulson, MD, PhD

Most neurodegenerative disorders are thought to result primarily from the accumulation of misfolded proteins, which interfere with protein homeostasis in neurons. For a subset of diseases, however, noncoding regions of RNAs assume a primary toxic gain-of-function, leading to degeneration in many tissues, including the nervous system. Here we review a series of proposed mechanisms by which noncoding repeat expansions give rise to nervous system degeneration and dysfunction. These mechanisms include transcriptional alterations and the generation of antisense transcripts, sequestration of mRNA-associated protein complexes that lead to aberrant mRNA splicing and processing, and alterations in cellular processes, including activation of abnormal signaling cascades and failure of protein quality control pathways. We place these potential mechanisms in the context of known RNA-mediated disorders, including the myotonic dystrophies and fragile X tremor ataxia syndrome, and discuss recent results suggesting that mRNA toxicity may also play a role in some presumably protein-mediated neurodegenerative disorders. Lastly, we comment on recent progress in therapeutic development for these RNA-dominant diseases.

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Nucleotide repeat expansion disorders comprise a heterogeneous group of diseases that result from expansion of specific repetitive DNA microsatellite sequences. Pathogenic expansions can occur in coding or noncoding regions of genes, and were initially believed to act in 2 dichotomous ways. In disorders such as Friedreich ataxia, expansions in noncoding regions cause transcriptional silencing or downregulation of the associated gene, and therefore act as recessively inherited, loss-of-function mutations. In contrast, in disorders such as Huntington disease, trinucleotide expansions in the protein coding region introduce an abnormally long stretch of a single amino acid (often glutamine) into the associated protein, which leads to a dominantly inherited, gain-of function mutation. In the 9 known polyglutamine diseases, the mutant proteins accumulate in ubiquitin-positive inclusions and interfere with cellular homeostasis through several different mechanisms (for a recent review, see Williams and Paulson<sup>1</sup>).

Many nucleotide repeat expansion disorders, however, do not fit neatly into either category. In myotonic dystrophy (DM1), an expanded CTG repeat sequence in the 3' untranslated region (UTR) of *DMPK* causes disease

in a dominantly inherited manner. After studies failed to reveal a significant role for *DMPK* haploinsufficiency in DM1 disease pathogenesis, evidence emerged supporting a toxic gain of function mechanism at the RNA level. Over the past 10 years, at least 7 other neurological disorders have been identified that likely share this new pathogenic mechanism, each with its own nuances (Table and recent reviews<sup>2–5</sup>). This review addresses how these nucleotide repeat expansions are thought to cause toxicity and dysfunction by affecting (1) transcriptional regulation, (2) mRNA splicing and metabolism, (3) RNA-binding protein distribution, and (4) signal transduction and cellular homeostatic pathways, with an eye toward potential sites of therapeutic intervention.

## A Primer on RNA Processing in Neurologic Disease

To explain how repeat expansions in a noncoding region of mRNA can lead to a multisystemic disease and neuronal dysfunction, it is important that we first review recent advances in understanding how RNA participates in gene regulation, RNA processing, and protein translation. The human transcriptome is made up of protein-coding

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Address correspondence to Dr Todd, 109 Zina Pitcher Place, 4188 BSRB, Ann Arbor, MI 48109-2200. E-mail: petertod@med.umich.edu, henryp@med.umich.edu

From the Department of Neurology, University of Michigan, Ann Arbor, MI.

TABLE 1: RNA Mediated Neurological Disorders

Disease	Repeat	Gene	Normal Repeat	Disease-Causing Repeat	Antisense Transcript?	CNS Phenotype
DM1	CTG	3'UTR of DMPK	5-38	50~1500: adult onset DM1; ~1500+: congenital DM1	Yes; no ORF	Adult onset: neuropsychiatric symptoms, executive dysfunction; congenital DM1: mental retardation in >50%
DM2	CCTG	Intron 1 of ZNF9	Up to 30	75-11,000	Unknown	Neuropsychiatric symptoms common; no congenital onset or mental retardation
FXTAS	CGG	5'UTR of FMR1	20-45	55-200; incomplete penetrance at all repeat lengths	Yes; possible ORF for polyproline	Late onset; cerebellar ataxia, action tremor, dementia parkinsonism; neuropsychiatric symptoms in females
SCA3*	CAG	Exon 10 of ATXN3	Up to 44	~45-51: reduced penetrance; ~52-86: fully penetrant	Unknown	Ataxia; parkinsonism, dystonia; dementia uncommon
SCA8	CTG	5'UTR of KLH1	15-50	~71-1,300; incomplete penetrance at all repeat lengths	Yes, with an ORF for polyglutamine	Cerebellar ataxia; dementia uncommon
SCA10	ATTCT	3'UTR of E46L	10-29	800-4,500	Unknown	Cerebellar ataxia; seizures; cognitive decline
SCA12	CAG	5'UTR/promoter of PPP2R2B	Up to 32	51-78	Unknown	Action tremor; cerebellar ataxia; dementia uncommon
HDL2	CTG	3'UTR of JPH-3	6-28	>41	Yes; possible ORF for polyglutamine	Clinically similar to Huntington disease

\* = Proposed based on work in drosophila. Role of SCA3 RNA in mammalian models and patients is unknown.

CNS = central nervous system; DM1 = myotonic dystrophy type 1; DMPK = dystrophin myotonica protein kinase; ORF = open reading frame; DM2 = myotonic dystrophy type 2; ZNF9 = zinc finger 9; FXTAS = fragile X tremor ataxia syndrome; UTR = untranslated region; FMR1 = fragile X mental retardation gene 1; SCA = spinocerebellar ataxia; ATXN3 = ataxin-3; KLH1 = Kelch-like 1; PPP2R2B = protein phosphatase 2, regulatory subunit B, beta isoform; HDL-2 = Huntington disease-like 2; JPH-3 = junctophilin 3.

mRNA and multiple different classes of noncoding RNAs, including ribosomal RNAs, transfer RNAs, small nuclear and nucleolar RNAs, microRNAs, and a host of recently described RNA species whose functions are less clear (eg, vault RNAs, Y RNAs, piwi-interacting RNAs, large intervening noncoding RNAs; see Cooper et al<sup>4</sup> for a detailed review). Furthermore, many genes are transcribed in both the sense direction (yielding a protein-encoding mRNA) and the antisense direction (usually producing a shorter noncoding sequence), often such that increased production of the sense transcript is associated with similar increases in the antisense transcript. The roles of antisense transcripts are still incompletely defined, but

likely include regulation of transcription, stability, and translation of the sense mRNA.

Messenger RNAs are initially transcribed as pre-mRNAs that contain a 5'UTR, a 3'UTR, and numerous noncoding intronic regions (introns) between the protein-coding regions of the mRNA (exons). A pre-mRNA is then spliced such that introns are removed, and a variable subset of exons are compiled into a single linear sequence for translation. For each pre-mRNA, usually multiple alternatively spliced mature mRNAs are produced, with different mature mRNAs being favored in different tissue and cell types or in response to different environmental cues. The mature mRNA, still containing the 5'UTR and

3'UTR, is then transported from the nucleus to the cytoplasm as part of a large ribonucleoprotein complex known as an RNP. In the cytoplasm, it associates with ribosomal components and is translated in a regulated fashion. From the beginning of transcription through splicing and translation, the mRNA is associated with numerous RNA binding proteins and noncoding RNAs that regulate its processing, stability, transport, and translation.

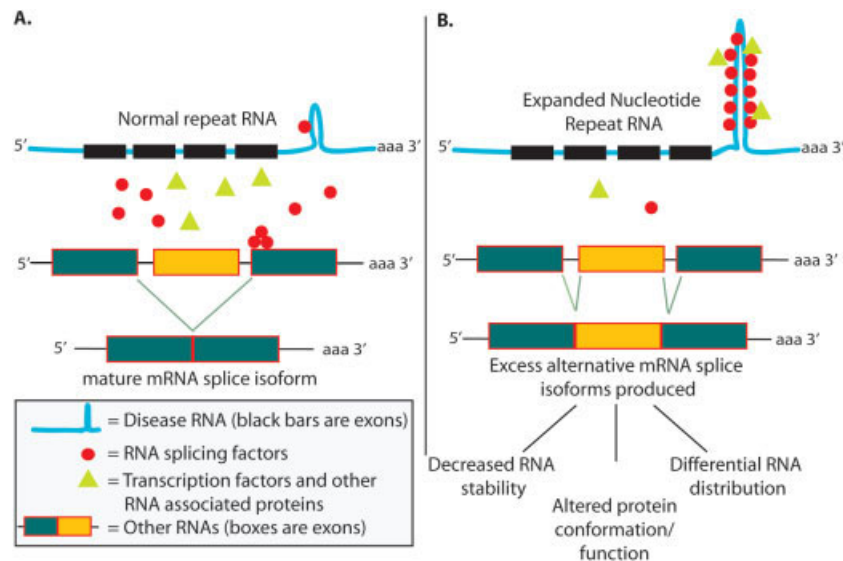
Both the coding and noncoding RNAs and their associated binding proteins are involved in numerous cellular pathways. These pathways, which include RNA processing and the regulation of transcription and translation, are critical determinants of neuronal differentiation and plasticity. Perhaps not surprisingly, alterations in these pathways have now been identified that contribute to a wide variety of neurologic and non-neurologic disorders, including a number of neurodegenerative diseases.<sup>4</sup> For example, mutations in two RNA-binding proteins involved in RNA splicing, the Tar DNA-binding protein of 43kd (TDP-43) and fused in sarcoma (FUS), can cause amyotrophic lateral sclerosis (ALS). Redistribution of TDP-43 from the nucleus into cytoplasmic aggregates is a common feature in sporadic ALS and frontotemporal dementia.<sup>6</sup>

The RNA-dominant disorders discussed in this review can result from expansions of repetitive sequences in the 5'UTR, the 3'UTR, or intronic sequences of protein-

encoding mRNAs and can also occur as microsatellite expansions in nonprotein coding RNAs. In many cases, evidence suggests the repeats are transcribed in both the sense and antisense directions. Once transcribed, the pre-mRNA containing these repeat expansions forms complex secondary structures, including hairpin loops that can alter their processing, transport, translation, and interactions with RNA-binding proteins. In many cases, these expanded repeat-containing RNAs accumulate and form aggregates with a subset of RNA-binding proteins implicated in regulating RNA splicing and transcription. To date, most work in this field has focused on the concept that formation of these nuclear aggregates, termed RNA foci, drives pathogenesis via sequestration of specific RNA-binding proteins. The relative absence of the RNA-binding proteins then leads to misregulated processing of numerous other non-repeat-containing RNA transcripts, resulting in altered patterns of mRNA splicing and hence alterations in protein isoform frequency. This sequestration hypothesis can explain many, but not all aspects, of neurodegeneration in these disorders (Fig 1).

### The Sequestration Hypothesis of RNA Toxicity

The most compelling evidence that sequestration of RNA-interacting proteins by the expanded repeat-containing mRNA causes neurological disease comes from cellular and animal models of DM1 and pathological



**FIGURE 1:** The sequestration hypothesis of RNA-dominant disorders. Noncoding nucleotide repeat expansions can elicit cellular dysfunction in many tissues, including the nervous system. (A) Under normal conditions, numerous RNA binding proteins are involved in RNA splicing and processing, as well as in other cellular functions. (B) Expanded nucleotide repeat sequences in RNA induce secondary hairpin structures that bind to and sequester numerous RNA-binding proteins, including splicing factors (red circles), as well as factors involved in transcription and RNA trafficking (represented by green triangles). The sequestration of these factors by the toxic RNA prevents the normal splicing and processing of other mRNAs, leading to retention of rare splice isoforms. These isoforms are either less stable and thus rapidly degraded, or they encode proteins with different functional characteristics from the major isoforms usually produced. In the central nervous system, splicing errors can also interfere with proper distribution of the mRNA within neurons, especially dendrites.

samples from patients with type I and type II myotonic dystrophy. The following has been established:

- An expanded CTG repeat placed into the 3'UTR of an unrelated gene is sufficient to induce toxicity in mouse and *drosophila* models, reproducing many aspects of the human disease.<sup>7,8</sup>
- In DM1 patients and various model systems, CUG repeat-containing mRNA forms aberrant RNA foci in the nucleus.<sup>9</sup> These mRNA foci contain members of the muscleblind-like family of RNA splicing proteins, MBNL1, 2, and 3,<sup>10</sup> and disrupt their normal nuclear distribution.
- Overexpression of MBNL1 in *drosophila* and mouse models rescues the CTG repeat-induced muscle phenotype, and MBNL1 knockout mice recapitulate key aspects of the adult form of the human disease, suggesting that reduced MBNL1 splicing activity contributes directly to DM1 pathophysiology.<sup>11,12</sup>
- Consistent with a major role for MBNL proteins in DM1 pathogenesis, aberrant splicing occurs in several key genes in DM1 muscle and brain.<sup>5</sup>

Perhaps the most convincing evidence for a primary role of MBNL sequestration in DM1 RNA pathogenesis came when the genetic cause for myotonic dystrophy type 2 was found to be another noncoding nucleotide expansion in an unrelated gene, zinc finger 9. This expanded CCUG repeat-containing RNA sequesters MBNL1 proteins in the nucleus in RNA foci that are very similar to those seen in DM1. Moreover, many of the same missplicing events are shared between these two clinically similar disorders.<sup>13</sup>

The sequestration model for RNA pathogenesis is not limited to CUG/CCUG repeat expansions. It may also apply to fragile X tremor ataxia syndrome (FXTAS), a recently described cause of late onset dementia gait disorder and tremor that potentially affects upwards of 1:3,000 males.<sup>14</sup> FXTAS is caused by an expanded CGG repeat in the 5'UTR of the *FMR-1* gene on the X chromosome. Normally, the sequence is <50 CGG repeats. Expansion to >200 CGG repeats (a full mutation) leads to transcriptional silencing of the *FMR-1* gene and causes fragile X syndrome, the most common inherited cause of mental retardation. By contrast, patients with FXTAS have a repeat between 50 and 200 CGG repeats. This premutation range repeat is transcribed efficiently, and there is near-normal expression of the fragile X mental retardation protein, FMRP. Intriguingly, the translational efficiency of a premutation CGG repeat mRNA is actually quite poor, but is offset by a 5- to 8-fold increase in *FMR1* mRNA levels.<sup>15,16</sup>

Pathologically, FXTAS is associated with neurode-

generation throughout the cortex and cerebellum. In addition, ubiquitin-positive inclusions accumulate in neuronal and glial nuclei in these brain regions. These inclusions contain the expanded *FMR1* mRNA as well as a host of proteins. Experimental evidence now suggests that two RNA-binding proteins, Pur  $\alpha$  and hnRNPA2/B1, are sequestered to a degree that impairs their function.<sup>17,18</sup> Pur  $\alpha$  is a ubiquitously expressed RNA- and DNA-binding protein that binds avidly to expanded rCGG repeats in vitro and in vivo. Moreover, Pur  $\alpha$  localizes to inclusions in the *drosophila* model of FXTAS and, when overexpressed, mitigates CGG repeat-mediated neurodegeneration. Similarly, hnRNPA2/B1, interacts directly with rCGG repeats and may act to recruit the CUG-binding protein 1 (CUGBP1) into RNA inclusions.<sup>18</sup> Both CUGBP1 and hnRNP A2/B1 play multiple roles in RNA processing, including RNA splicing, and CUGBP1 has been implicated in the splicing abnormalities in myotonic dystrophy (see below).<sup>19,20</sup> In support of a role for these two molecules in FXTAS, overexpression of either hnRNP A2/B1 or CUGBP1 in a *drosophila* FXTAS model rescues the phenotype.<sup>18</sup>

### Abnormal Activation of Signaling Cascades

Sequestration of MBNL into nuclear foci by CTG repeats does not explain certain aspects of the phenotype in myotonic dystrophy. In addition to the role of MBNL proteins, there appears to be accumulation and aberrant subcellular distribution of another splicing regulatory protein family typified by the CUG-binding protein, CUGBP1. This protein binds CUG RNA sequences, but is not present in CUG RNA nuclear foci. Overexpression of CUGBP1 recapitulates key features of the human disease, and its induction may contribute to muscular atrophy and aberrant differentiation in certain animal models of DM1.<sup>21,22</sup> The toxic consequences of CUGBP1 redistribution are presumably mediated through altered splicing of a different set of mRNAs than those targeted by MBNL proteins.<sup>19</sup> CUGBP1 is also known to impact translational regulation, and thus its effects may be more pleiotropic.<sup>23</sup>

CUGBP1 is stabilized via phosphorylation by protein kinase C and is hyperphosphorylated in DM1 patient tissues and in some animal models of DM1.<sup>24</sup> Precisely how an expanded CUG repeat leads to this phosphorylation is unknown. Some evidence suggests that expanded nucleotide repeats can activate protein kinase cascades by forming hairpin loops that trigger double-stranded RNA-dependent protein kinase.<sup>25</sup> How important such signaling pathways are in other RNA-mediated disorders remains to be seen, but overexpression of CUGBP actually rescued CGG repeat dependent toxicity in a *drosophila*

model of FXTAS, the opposite of the predicted effects in DM1. Thus, the relevant signaling pathways may be affected differently in each disorder.

### Aberrant mRNA Splicing

The mis-spliced mRNAs in DM1 offer insight into how a multisystemic disease can arise from a single noncoding mutation. For example, misregulated splicing of *CIC-1*, a chloride channel, leads to decreased expression of the mature channel in muscle fibers.<sup>20,26</sup> This results in the muscle hyperexcitability underlying myotonia. Importantly, correction of the *CIC-1* mis-splicing event alleviates the myotonia in mouse models of DM1.<sup>27</sup> Mis-splicing events may also contribute to cardiac abnormalities and muscle wasting seen in DM1.

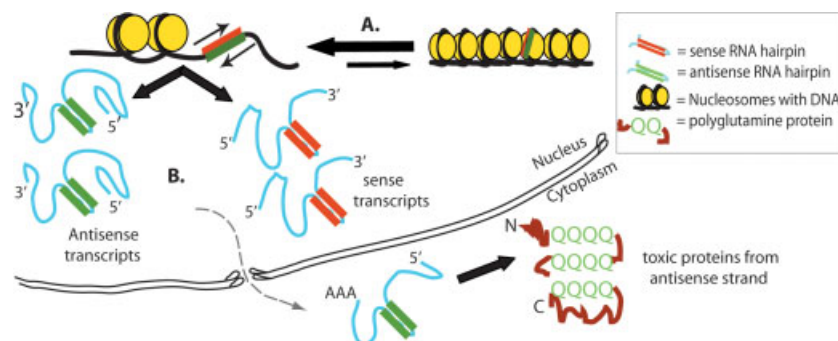
Less is known about which mis-splicing events might underlie central nervous system dysfunction in DM1. To date, screens of a limited number of candidate genes have identified 3 mis-spliced genes in the brains of adult DM1 patients: abnormal splicing of exon 5 in glutamate receptor *NMDAR1*, multiple exons in microtubule-associated protein *tau* (*MAPT*), and exon 7 in the amyloid precursor protein (*APP*).<sup>28</sup> These same mis-splicing events were recently reported in a mouse model of DM1.<sup>29</sup> Of these 3, evidence best supports a direct role for *MAPT* mis-splicing in the clinical symptoms of patients. There is accumulation of neurofibrillary tangles and intranuclear inclusions that contain *tau* in adult DM1 brains at autopsy, suggesting that DM1 is partly a neurodegenerative disorder.<sup>30</sup> In addition, both *APP* and *NMDAR1* are critically involved in synaptic plasticity and neuronal function, and thus their mis-splicing may explain some of the cognitive symptoms seen in DM1, especially in the congenital form of the disorder.

These 3 mRNAs likely represent only a small subset of the transcripts mis-spliced in neurons in this disease. The identity of these additional mRNAs will be critical for understanding the effects of DM1 on the nervous system in adults and children.

Given the complexity of RNA splicing and processing in neurons, it is perhaps not surprising that all of the RNA-dominant disorders uncovered to date have some central nervous system effects, although the specific effects are highly variable between the disorders, and more subtle in some than others. The exact effect of each repeat expansion on splicing and RNA processing in specific cell types and regions in the brain at least partially explains how a single mechanism can lead to such different phenotypes across these diseases. However, exactly what drives the disease and tissue specificity of neurodegeneration in RNA-dominant diseases (and protein mediated neurodegenerative diseases, for that matter) remains largely unknown.

### Antisense Transcripts and Aberrant Transcriptional Regulation

One of the most intriguing recent developments in repeat expansion disorders is the emerging role of antisense transcripts in disease pathogenesis (Fig 2). As discussed above, antisense transcripts are very common in the genome and likely play important roles in RNA stability and transcriptional activity. A role for toxicity associated with these transcripts is best established for spinocerebellar ataxia type 8 (*SCA8*). In this disorder, a CTG repeat expansion in a nonprotein coding gene is associated with disease. Normal individuals typically have upward of 50 repeats, whereas patients with *SCA8* typically have between 70 and 250 repeats. This CTG repeat is transcribed as



**FIGURE 2: Transcriptional dysregulation and antisense transcripts in repeat expansion diseases.** In addition to effects on RNA splicing, expanded noncoding nucleotide repeats likely lead to neurodegeneration via a number of overlapping mechanisms. (A) At the level of DNA, the repeats themselves may impact both the temporal expression (as in congenital myotonic dystrophy) and the level of expression of the expanded repeat containing mRNAs (as in fragile X tremor ataxia syndrome) by altering local chromatin structure. (B) In many cases, antisense transcripts through the repeat sequence are also produced. These antisense transcripts may be translated into poly-amino acid-containing proteins (eg, polyglutamine proteins in spinocerebellar ataxia type 8), elicit toxicity directly at the RNA level, or both. They also may play a role in regulating the stability of the expanded repeat-containing sense transcripts.

Ataxin8OS (ataxin-8 opposite strand), a noncoding mRNA expressed in the brain and cerebellum.<sup>31</sup> Initial work on SCA8 suggested that this CTG repeat acts via a dominant mechanism at the mRNA level similar to that seen in DM1.<sup>32</sup> In line with this hypothesis, a recent study demonstrates mis-splicing of a GABA transporter in the cerebellum in SCA8 that is dependent on sequestration of MBNL1 and results in cerebellar dysfunction.<sup>33</sup> However, there is also evidence that an antisense transcript is produced that includes the expansion.<sup>34</sup> This transcript contains a short open reading frame that would translate the repeat in the CAG direction into an expanded polyglutamine polypeptide. Moreover, nuclear inclusions of aggregated protein that immunostain for polyglutamine have been noted in the cerebellum of an SCA8 patient. It remains unclear, however, if this CAG transcript and resultant polyglutamine protein contribute significantly to SCA8 pathogenesis.

A similar mixed mechanism may be present in Huntington disease-like 2 (HDL2). HDL2 is a rare disorder clinically similar to Huntington disease that results from a CTG repeat expansion in the Juncophilin-3 gene. Pathologic samples from patients with HDL2 demonstrate neurodegeneration in the striatum and the formation of RNA foci that contain expanded CUG repeat sequences and MBNL1.<sup>35,36</sup> However, these same pathologic samples demonstrate ubiquitin-positive inclusions that stain for the presence of a polyglutamine protein.<sup>36</sup> Thus, an antisense transcript encoding for a glutamine stretch may also be present in this disorder and contribute to pathogenesis.

In addition to possibly encoding toxic proteins, antisense transcripts also have potential roles in modifying RNA stability of the toxic mRNA product. Two different antisense transcripts have been described in FXTAS, 1 of which begins in the first exon (ASFMR1) and extends through the CGG:CCG repeat with multiple splice variants, the other of which overlaps only the very 5' end of the sense transcript start site (FMR4).<sup>37,38</sup> At least 1 isoform of ASFMR1 contains an open reading frame that would be predicted to lead to translation of a polyproline-containing protein through the CCG repeat sequence, but to date there is no evidence that this actually occurs. However, these transcripts potentially play important roles in RNA toxicity and in the stability of the sense transcript. Along this line, 1 intriguing experiment in a fly model of FXTAS demonstrated that expression of a CGG or CCG repeat mRNA can induce toxicity in flies, but that coexpression of both mRNAs leads to phenotypic rescue via an Argonaute 2 (ie, RNA interference)-dependent pathway.<sup>39</sup> In lymphoblasts derived from FXTAS patients, both the sense and antisense FMR1

transcripts are upregulated in cells, but toxicity could result from an imbalance of sense and antisense mRNA expression levels in affected tissues.

The expanded nucleotide repeats also have important effects *in cis* at the gene level. In FXTAS, the expanded CGG repeat may contribute directly to increased transcription of the FMR1 mRNA by effecting local chromatin structure.<sup>40,41</sup> Similarly, the more severe phenotype seen in congenital DM1 may result not only from a larger CTG expansion at the mRNA level, but also from effects of the CTG expansion on local chromatin regulation and transcription, both of itself and of surrounding genes. In congenital DM1, unlike adult DM1, the *DMPK* locus becomes methylated. This methylation inhibits CTCF binding at sites surrounding the *DMPK* gene, leading to transcriptional activation of *DMPK* concurrent with that of a nearby homeodomain gene, *Six5*.<sup>42</sup> As *Six5* is expressed at high levels during early neuronal and muscular development,<sup>43</sup> temporally aberrant expression of toxic *DMPK* mRNA could lead to a developmental phenotype that would be absent in adult onset DM1. However, this hypothesis has not yet been tested *in vivo*.

In addition to altered mRNA splicing, significant effects on the transcriptional regulation of many other genes have also been reported for both FXTAS and DM1.<sup>44,45</sup> In DM1, some of these changes may still be explained by sequestration of MBNL1, although it remains unclear if this reflects a direct role of MBNL1 in transcriptional regulation or an indirect effect through altered splicing of critical transcription factors.<sup>44</sup> In FXTAS, some of these effects may be mediated by Pur  $\alpha$ , which can act as a transcriptional repressor.<sup>46,47</sup>

### **Cellular and Protein Homeostasis in RNA-Mediated Disease**

The culmination of the diverse pathogenic mechanisms discussed above is neuronal dysfunction and death. The degree of mechanistic overlap between mRNA and protein-mediated disorders is unknown, but early evidence suggests some commonality in these neurodegenerative pathways. For example, the intranuclear inclusions in FXTAS contain many proteins that are not thought to interact directly with mRNA, including nuclear lamin proteins that function in nuclear envelope formation and structure. Intriguingly, the nuclear envelope structure in cultured cells transfected with an expanded CGG repeat mRNA is abnormal.<sup>48</sup> Beyond sequestration of specific proteins, the biological impact of inclusions is also unclear. The inclusions in FXTAS are ubiquitinated, and both the neuronal inclusions in FXTAS and the RNA foci in DM1 contain components of the proteasome.<sup>28,49</sup> In addition, overexpression of chaperone proteins (eg, HSP-

70) alleviates aspects of the FXTAS phenotype in a *drosophila* model, and cells overexpressing an expanded CGG repeat-containing mRNA display increased sensitivity to impairment of the ubiquitin-proteasome system.<sup>45,50</sup> These results support a model of disease in which aberrant mRNA-protein interactions can lead to degradation-resistant complexes that may function as a toxic species in these disorders, interfering with a host of cellular processes in a manner similar to that proposed for polyglutamine proteins.

### **RNA Toxicity in Polyglutamine Disorders**

Until recently, mRNA transcripts were not thought to contribute significantly to toxicity in repeat expansion disorders where the repeat is translated into protein. However, a recent experiment questions this dogma for at least some polyglutamine disorders. Spinocerebellar ataxia type 3, also known as Machado-Joseph disease, results from a CAG expansion that encodes an abnormally long polyglutamine stretch in the disease protein, ataxin-3. Using a genetic modifier screen in a *drosophila* model of SCA3, Li et al found that the muscleblind protein family implicated in DM1 pathogenesis also modified the ataxin-3 expanded repeat phenotype.<sup>51</sup> Modification of the mRNA sequence from a pure CAG repeat to CAACAG (which still encodes glutamine) reduced the toxicity of the repeat in flies, despite similar expression of the polyglutamine protein. Moreover, expression of a long, untranslatable CAG repeat by itself in the 3'UTR of a reporter gene caused slowly progressive neurodegeneration. This neurodegeneration did not appear to be due to transcription of an antisense CUG-containing mRNA. Taken together, these findings suggest that large CAG repeats could contribute to disease processes at the mRNA level in some polyglutamine disorders. However, whether these findings are relevant to mammalian systems and patients with polyglutamine disorders is still unknown. Given the requirement for very large repeats to achieve toxicity in the *drosophila* model system, it will likely only be significant in humans if there is dramatic somatic repeat instability in affected tissues. This possibility needs to be investigated.

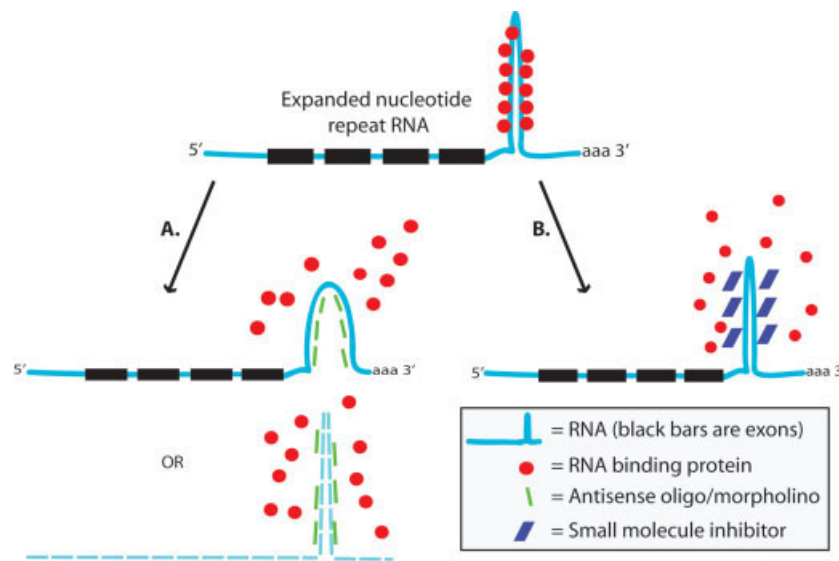
In some polyglutamine disorders, however, it seems unlikely that mRNA-mediated neurodegeneration plays a prominent role. For example, in spinal and bulbar muscular atrophy (also known as Kennedy disease), a pathogenic expanded CAG repeat that is translated into a polyglutamine tract is present in the androgen receptor gene. There is clear evidence from *drosophila* and mouse models that translocation of the disease protein to the nucleus in response to ligand binding is required for disease pathogenesis, precluding a primary role for mRNA toxicity.<sup>52</sup> Moreover, single nonconservative nucleotide changes dis-

tant from the CAG repeat sequence in *Atn1*, the disease gene in spinocerebellar ataxia type 1, can abrogate toxicity in a mouse model of the disease without affecting mRNA levels.<sup>53</sup> Lastly, an earlier study that formally tested for toxicity from untranslated CAG repeats failed to show an effect, although the expansion size of the tested repeat was smaller.<sup>54</sup> Thus, further research is needed to determine the impact of this finding that very long CAG repeats can lead to RNA-mediated toxicity in polyglutamine disorders, and in turn on our understanding of protein-mediated neurodegeneration.

### **Therapeutic Development in RNA-Mediated Diseases**

As RNA-mediated disorders do not change the sequence or function of the protein associated with the mutated gene, they may be particularly amenable to curative therapeutic development. Moreover, in DM1 in particular, there is fairly strong evidence that haploinsufficiency for the associated protein, DMPK, is not associated with significant dysfunction in animal models. Thus, therapeutics targeted at the elimination of the toxic mRNA hold great promise (Fig 3). Two recent papers using antisense technology in animal models of DM1 have provided proof of principle for this technique. In the first of these, Wheeler et al used a morpholino synthetic oligonucleotide made up of 8 CAG repeats to interfere with the interaction of the expanded CUG repeat containing mRNA and MBNL1 in a mouse model of DM1.<sup>55</sup> Delivery of this morpholino into affected muscles led to elimination of RNA foci, correction of splicing abnormalities, and a reversal of clinical myotonia. In a second related paper, Mulders et al utilized a modified antisense oligonucleotide in two mouse models of DM1 and also demonstrated resolution of RNA foci and splicing abnormalities, although myotonia persisted.<sup>56</sup> Importantly, nonspecific toxicity was not observed in either study.

A second line of therapeutic development involves identification of small molecules that directly interfere with the interaction of expanded nucleotide repeats and their connate RNA-binding protein partners. Three different groups have used various rational compound screening approaches to identify molecules as inhibitors of the interaction of expanded CUG repeats with MBNL1.<sup>57-59</sup> Warf et al have gone on to demonstrate that their identified compound, pentamidine, is capable of reversing splicing defects in cell culture and in a mouse model of DM1.<sup>58</sup> Similar chemical studies have also been done to target CAG and CCUG mRNA repeats, suggesting that this technique may have broader application to all RNA-dominant disorders.<sup>59,60</sup> Other recent studies have utilized high-throughput drug screens in either cell culture or invertebrate models of DM1. A recent screen in



**FIGURE 3:** Therapeutic development in RNA-mediated diseases. Recent studies support at least 2 therapeutic approaches to RNA-dominant diseases. (A) Eliminating the toxic RNA hairpin structure can be achieved using short antisense oligonucleotide transcripts. Depending on how they are constructed, the oligonucleotides can either lead to the degradation of the toxic RNA or interfere with the formation of the hairpin structure that is thought to elicit toxicity. (B) Small molecules can be used to interfere directly with the interaction between toxic RNA and its binding proteins. Both treatments free the sequestered RNA-binding proteins to perform their normal functions, thus restoring RNA-processing homeostasis.

a *drosophila* model of DM1 identified 10 compounds, most of which have been approved by the US Food and Drug Administration for other indications, that suppress CUG RNA toxicity.<sup>61</sup>

### Conclusions and Future Directions

It is now clear that mRNA plays a primary pathogenic role in several disorders that involve the central nervous system. Moreover, this toxicity is probably mediated via several parallel mechanisms, including sequestration of RNA-splicing factors and RNA-binding proteins, altered production of coding and noncoding antisense transcripts that themselves exert deleterious consequences at multiple levels, and accumulation of mRNA/protein aggregates that may have detrimental effects on cellular homeostasis. If anything, these multiple mechanisms are likely to become more complicated in the next few years, as explorations of the roles played by microRNAs and other noncoding RNAs are considered in these diseases. Sorting out which mechanistic components are critical mediators of toxicity in each disorder will be required to understand the pathogenesis of these diseases. Yet, based on our understanding of these mechanisms to date, early preclinical studies aimed at eliminating or neutralizing the toxic mRNA species are raising hope that therapeutic approaches to these diseases will be available for clinical application in the not too distant future.

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