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Role of noncoding RNAs in Trinucleotide repeat neurodegenerative disorders

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Abstract

Increasingly complex networks of noncoding RNAs are being found to play important and diverse roles in the regulation of gene expression throughout the genome. Many lines of evidence are linking mutations and dysregulations of noncoding RNAs to a host of human diseases, and noncoding RNAs have been implicated in the molecular pathogenesis of some neurodegenerative disorders. The expansion of trinucleotide repeats is now recognized as a major cause of neurological disorders. Here we will review our current knowledge of the proposed mechanisms behind the involvement of noncoding RNAs in the molecular pathogenesis of neurodegenerative disorders, particularly the sequestration of specific RNA-binding proteins, the regulation of antisense transcripts, and the role of the microRNA pathway in the context of known neurodegenerative disorders caused by the expansion of trinucleotide repeats.

Introduction

Recent genome-wide studies have shown that only 2% of the mammalian genome encodes mRNAs. The vast majority of the genome is transcribed and produces many thousands of regulatory non-protein-coding RNAs (ncRNAs), including tRNA, rRNA, snRNA, snoRNA, miRNA, siRNA, piRNA, natural antisense transcripts, and long noncoding RNA (Birney et al., 2007; Carninci et al., 2005; Core et al., 2008). A series of findings suggests that these RNAs act through complex networks to fulfill important and diverse roles as transcriptional and post-transcriptional regulators and as guides of chromatin-modifying complexes. Since biological complexity generally correlates with the proportion of the genome that is non-protein-coding (Taft et al., 2007), uncovering the functions of these noncoding RNAs could significantly improve our understanding of gene regulatory networks and the molecular pathogenesis of human diseases.

Neurodegenerative disorders are caused by a wide range of genetic mutations and epigenetic and environmental factors, and trinucleotide repeat expansion is increasingly recognized as the cause of many neurodegenerative diseases. To date, trinucleotide repeat expansions account for more than 30 neurological and neuromuscular diseases (Lopez Castel et al.,

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2010; Mirkin, 2007). Pathogenic expansions can occur in coding or noncoding regions of genes. In disorders such as Huntington's disease (HD) and six spinocerebellar ataxias (SCA1, 2, 3, 6, 7, and 17), trinucleotide expansions in the protein-coding region result in the synthesis of polyglutamine (poly Q) expansions, which accumulate in ubiquitin-positive inclusions and interfere with cellular homeostasis, leading to a protein gain-of-function mutation (Brouwer et al., 2009; Orr and Zoghbi, 2007). In contrast, in disorders such as Fragile X-associated Tremor Ataxia Syndrome (FXTAS), expansions in noncoding regions give rise to pathogenic RNA foci that sequester the RNA-binding proteins from their normal cellular functions. Toxicity is thought to ensue via an RNA gain-of-function mechanism (Iwahashi et al., 2006; Khalili et al., 2003). In this review, we will focus on the involvement and function of noncoding RNAs in the context of the neurodegenerative disorders caused by trinucleotide repeat expansion. We will discuss how these noncoding RNAs cause toxicity and dysfunction through multiple mechanisms.

Sequestration of RNA-binding proteins by pathogenic RNA with expanded trinucleotide repeats

Studies of Fragile X-associated Tremor Ataxia Syndrome (FXTAS), an age-dependent progressive intention tremor and ataxia disorder affecting up to 1:3,000 males (Jacquemont et al., 2004), have established that the sequestration of RNA-binding proteins due to the expression of pathogenic RNA with expanded repeats is involved in disease pathogenesis. FXTAS is the result of a massive CGG trinucleotide repeat expansion within the 5' UTR of the *FMR1* gene on the X chromosome. People in the general population carry between five and 54 CGG repeats. Expansions of over 200 CGG repeats (known as the full mutation) lead to transcriptional silencing of *FMR1* transcript and the loss of the encoded fragile X mental retardation protein (FMRP), eventually causing fragile X syndrome (FXS), the most common form of inherited mental retardation (Kremer et al., 1991; Oberle et al., 1991; Pieretti et al., 1991; Verkerk et al., 1991). By contrast, patients with FXTAS have a repeat between 55 and 200 CGG repeats (known as the premutation). The premutation is different at the molecular level from either the normal or full mutation alleles. Based on the significantly elevated levels of rCGG-containing *FMR1* mRNA seen in FXTAS, along with either no detectable change in FMRP or slightly reduced FMRP levels in premutation carriers, an RNA-mediated gain-of-function toxicity model has been proposed for FXTAS (Figure 1 and Table). Several lines of evidence in mouse and *Drosophila* models support the notion that transcription of the CGG repeats leads to this RNA-mediated neurodegenerative disorder (Arocena et al., 2005; Greco et al., 2006; Greco et al., 2002; Hashem et al., 2009; Jacquemont et al., 2004; Jin et al., 2003; Tassone et al., 2000; Willemsen et al., 2003). The major neuropathological hallmark of FXTAS is ubiquitin-positive intranuclear inclusions located in broad distribution throughout the brain in neurons, astrocytes, and in the spinal column (Greco et al., 2006). These inclusions contain the expanded *FMR1* mRNA, as well as an array of proteins, including Pur α , hnRNPA2/B1, MBNL1, CUGBP1, and Sam68 (Iwahashi et al., 2006; Khalili et al., 2003; Sellier et al., 2010). Experimental findings suggest that over-produced fragile X premutation rCGG repeats may sequester the rCGG-binding proteins from their normal cellular functions, thereby contributing to the pathogenesis of FXTAS. These proteins are RNA-binding proteins and are known to play a role in transcription, mRNA trafficking, splicing, and translation. Pur α and hnRNPA2/B1 have been shown to interact directly with rCGG; however, the interaction of CUGBP1 with rCGG specifically requires an association with hnRNP A2/B1 (Jin et al., 2007; Sofola et al., 2007). In addition, Pur α was found to be present in the inclusions of both human and fly tissues. Moreover, Pur α knockout mice appear normal at birth, but develop severe tremor and spontaneous seizures at two weeks of age, possibly due to substantially lower numbers of neurons in the hippocampus and cerebellum (Khalili et al., 2003). Overexpression of

either Pur α or hnRNP A2/B1 or CUGBP1 can also ameliorate neurodegeneration in the fly model of FXTAS (Jin et al., 2007; Sofola et al., 2007). These findings together suggest that fragile X premutation rCGG repeats could sequester specific RNA-binding proteins, and the depletion of these proteins could lead to neuronal cell death and neurodegeneration.

RNA-mediated protein sequestration events like those seen in FXTAS have also been studied in-depth in myotonic dystrophy type 1 (DM1), a multi-systemic disease affecting mainly the muscle but also the brain (Table). DM1 is caused by the expansion of an unstable CTG trinucleotide repeat located within the 3'UTR (3 untranslated region) of the *DMPK* (dystrophia myotonica protein kinase) gene on chromosome 19 (Brook et al., 1992; Mahadevan et al., 1992). An RNA gain-of-function mechanism may be the culprit behind the pathogenesis of DM1 (Amack et al., 1999). This is supported by several experiments showing that mutant *DMPK* transcripts with expanded CUG trinucleotide repeats accumulate in the nucleus as discrete aggregates or foci (Davis et al., 1997; Taneja et al., 1995). Transgenic mice that express CUG trinucleotide repeat expansion either in the 3'UTR of the human skeletal muscle alpha actin (HSA-LR) mRNA or in its natural context within the 3'UTR of the human *DMPK* transcript exhibited several features of DM1, including nuclear aggregates of CUG trinucleotide repeats, myotonia, and muscle abnormalities (Mankodi et al., 2000; Seznec et al., 2001). Altogether, these observations demonstrate that expanded CUG trinucleotide repeats alone, regardless of the gene context, are sufficient to induce pathogenic features of DM1. One explanation of how expanded CUG-containing transcripts can cause disease is via interference with RNA-protein interactions, resulting in altered activity of the mRNA splicing regulators decreased muscleblind-like 1 (MBNL1) and increased CUG-binding protein 1 (CUGBP1). This leads to the misregulation of alternative splicing events in DM1, such as increased inclusion of exons containing premature stop codons in the skeletal muscle-specific chloride channel 1 (CIC-1), which is sufficient to cause myotonia (Charlet et al., 2002; Mankodi et al., 2002), increased skipping of exon 11 in the insulin receptor (IR), which correlates with insulin resistance in DM1 individuals (Savkur et al., 2001), and increased inclusion of cardiac troponin T (cTNT) exon 5, which contributes to the cardiac conduction defects (Philips et al., 1998). MBNL1 and CUGBP1 have been identified as regulators of more than half of the splicing transitions tested (Kalsotra et al., 2008). Many of the regulated splicing events are modulated exclusively by MBNL1 or CUGBP1, but all events regulated by both proteins exhibit antagonistic responses (Kalsotra et al., 2008). In the above three samples (CIC-1, IR, and cTNT), when MBNL1 induces exclusion of an exon, CUGBP1 increases inclusion of the same exon and vice versa. A combined effect of decreased MBNL1 and increased CUGBP1 activity promotes the expression of a fetal splicing pattern in adult tissues (Kalsotra et al., 2008; Lin et al., 2006), leading to the formation of the abnormal splicing event in DM1. Although both disrupted splicing regulatory functions are affected by CUG trinucleotide repeat expansions, the mechanisms underlying each are distinct. MBNL1 is found to bind, in a length-dependent manner, expanded CUG repeats with high affinity and to colocalize to CUG-containing nuclear foci in DM1 cells (Miller et al., 2000). Sequestration of MBNL1 within the nuclear aggregates of expanded CUG repeats and the subsequent involvement of MBNL1 loss of function in DM1 pathogenesis are supported by several observations. First, a knockout *Mbnl1* mouse model (*Mbnl1* ^{Δ E3/ Δ E3}) has shown a DM-like phenotype, as well as the same splicing defects observed in DM1 (Kanadia et al., 2003). In addition, AAV (adeno-associated virus)-mediated delivery of *Mbnl1* to HAS-LR mice skeletal muscle reversed myotonia and restored normal CIC-1 (Kanadia et al., 2006). Furthermore, some recent studies show that the introduction of antisense CAG oligonucleotides (morpholinos) (Mulders et al., 2009; Wheeler et al., 2009), or small chemical compounds like pentamidine (Warf et al., 2009) that are able to target the expanded CUG-repeat RNA, can block the pathological RNA interactions with MBNL1 and prevent the formation of new foci. This leads to replenishment of MBNL1 levels and reverses the abnormal splicing defects,

rescuing muscle symptoms in DM1 cellular or mouse models. On the other hand, CUGBP1 does not bind to double-stranded CUG repeats or co-localize with the nuclear aggregates of CUG repeat expansions in DM1 cells, meaning it is not sequestered like MBNL1 (Timchenko et al., 2001). In contrast, the CUGBP1 protein steady-state levels are increased in DM1 tissues, leading to a gain of CUGBP1 activity (Philips et al., 1998; Timchenko et al., 2001). It has been proposed that the expanded CUG repeat activates the PKC signaling pathway through an unknown mechanism, leading to hyperphosphorylation and stabilization of the CUGBP1 protein (Kuyumcu-Martinez et al., 2007; Wang et al., 2009). The pathogenic role of CUGBP1 in DM1 is supported by evidence that several DM1 mouse models with repeats located within the *DMPK* 3'-UTR had increased CUGBP1 levels (Mahadevan et al., 2006; Orengo et al., 2008; Wang et al., 2007). Furthermore, transgenic mice overexpressing CUGBP1 displayed muscle abnormalities, as well as splicing misregulation similar to DM1 (Koshelev et al., 2010; Ward et al., 2010), suggesting that the elevation of CUGBP1 could contribute to the DM1 phenotype. Taken together, these studies indicate that expanded CUG-repeat RNA induces toxicity through dominant effects on MBNL1 and CUGBP1 proteins. This also raises the possibility that preventing abnormal RNA-protein interactions could be a good target for intervention in this disorder.

In combination, the above studies point to expanded trinucleotide repeat RNAs of disease genes having dominant effects on a variety of proteins due to aberrant RNA-protein interactions, such that these RNA-binding proteins become functionally limited by their sequestration to lengthy trinucleotide repeats (Table). Previous studies have shown that blocking such aberrant RNA-protein interactions can release sequestered proteins and restore their function, thereby reducing RNA-mediated toxicity. Thus, therapeutics targeted at interfering with the aberrant RNA-protein interactions could make promising treatments for these RNA-dominant disorders.

Antisense transcript-mediated transcriptional regulation

Recent evaluations of genome-wide transcription have revealed that antisense transcripts are a pervasive feature of mammalian genomes and exert positive or negative regulation at different levels of sense mRNA expression (Faghihi and Wahlestedt, 2009). Bidirectional transcription at repeat loci is an important emerging theme in repeat expansion diseases. The most compelling evidence implicating bidirectional transcription in disease pathogenesis comes from cellular and animal models of spinocerebellar ataxia type 8 (SCA8), as well as pathological samples from SCA8 patients. SCA8 is a dominantly inherited, slowly progressive neurodegenerative disorder caused by a CTG-CAG repeat expansion located on chromosome 13q21 (Day et al., 2000; Koob et al., 1999). Bidirectional expression of the SCA8 CTG-CAG expansion produces toxic noncoding CUG expansion RNAs from the ataxin 8 opposite strand (*ATXN8OS*) and a nearly pure polyglutamine expansion protein encoded by *ATXN8*. Initially, SCA8 was thought to be exclusively a CUG expansion RNA-mediated disease reminiscent of DM1, because the uncovered mutation was a CUG repeat expansion transcript in the 3' region of an untranslated gene, *ATXN8OS* (Koob et al., 1999). Furthermore, BAC transgenic mice expressing a human AXTN8/*ATXN8OS* gene with a (CTG)₁₁₆ expansion mutation but not a control repeat (CTG)₁₁ from the endogenous human promoter develop a progressive neurological phenotype, striking gait disturbances, and a loss of cerebellar GABAergic inhibition (Moseley et al., 2006). Moreover, SCA8 CUG repeat transcripts accumulate as ribonuclear inclusions that co-localize with MBNL1 in selected neurons of both SCA8 patients and transgenic mice, and loss of Mbnl1 enhances rotarod deficits in SCA8 BAC-EXP mice. Additionally, SCA8 CUG repeat transcripts trigger alternative splicing changes, as a CUGBP1-MBNL1-regulated CNS target, the GABA-A transporter 4 (GAT4) transcript, shows upregulation and a shift in alternative splicing favoring exon 7 insertion in SCA8 mouse and patient brains (Daughters et al.,

2009). Taken together, these data suggest that a toxic RNA gain of function contributes to the pathophysiology of SCA8. SCA8 BAC mice were generated to test the RNA gain-of-function effects of CUG expansion transcripts in SCA8; however, intranuclear inclusions, detectable by antibody 1C2, which specifically recognizes polyQ expansions, were found unexpectedly in Purkinje cells and brainstem neurons in the SCA8 BAC-EXP mice and SCA8 patients (Moseley et al., 2006). The novel gene ataxin8 (*ATXN8*), spanning the repeat in the opposite CAG direction, is predicted to encode the nearly pure polyQ protein that formed the typical neuronal nuclear inclusions. Therefore, the expression of noncoding CUG expansion transcripts from *ATXN8OS* and the polyQ protein translated by CAG expansion transcripts from *ATXN8* suggests that SCA8 pathogenesis involves toxic gain-of-function mechanisms at both the RNA (CUG expansion transcripts) and protein (polyQ inclusions) levels.

In contrast to SCA8, SCA7 is caused by a CAG/polyglutamine (polyQ) repeat expansion in the ataxin-7 gene and is considered one of the nine polyQ neurodegenerative disorders (La Spada and Taylor, 2010). A recent study has implicated regulatory bidirectional transcription at repeat loci in SCA7 (Sopher et al., 2011). The ataxin-7 CAG repeat tract and the translation start site are both located in exon 3, flanked by two functional binding sites for CTCF, a highly conserved, multi-functional transcription regulator. An adjacent alternative promoter and an antisense noncoding RNA, *SCAANTI* (spinocerebellar ataxia-7 antisense noncoding transcript 1), which is convergently transcribed across exon 4 and exon 3, were discovered when the ataxin-7 repeat region was analyzed. Studies on the ataxin-7 transgenic mouse revealed that CTCF binding is required for the production of *SCAANTI* and that loss of *SCAANTI* expression de-repressed ataxin-7 sense transcription from the alternative promoter. *SCAANTI* regulates the corresponding ataxin-7 sense expression in cis by convergent transcription. Together, these studies reveal a regulatory pathway that links CTCF transactivation of antisense noncoding RNA with repression of the corresponding sense transcript. Clearly, regulatory bidirectional transcription in association with CTCF binding contributes to the pathogenesis of SCA7.

In DM1, discussed above, the major clinical features are believed to be caused by expression of the CUG repeat-containing RNA through its interaction with the RNA-binding proteins, MBNL1 and CUGBP1. However, an anti-sense *DMPK* transcript emanating from the adjacent *SIX5* regulatory region is found to contribute to the earlier disease phenotype in congenital DM1 (Cho et al., 2005). The bidirectional transcripts are converted into 21-nucleotide (nt) RNA fragments associated with local chromatin modifications and gene silencing. At the wild-type DM1 locus, CTCF restricts the extent of the antisense RNA and constrains the H3-K9 methylation to the nucleosome associated with the CTG repeat. However, the expanded allele in congenital DM1 is associated with the loss of CTCF binding, spread of heterochromatin, and regional CpG methylation. It has been proposed that impaired CTCF binding between *DMPK* and *SIX5* might result in higher *DMPK* expression late in embryogenesis as a consequence of the high *SIX5* enhancer activity, leading to the earlier disease phenotype in DM1. Other recent work using a *Drosophila* model of DM1 found that CTG and CAG bidirectional transcripts may interact, leading to the generation of dcr-2 and ago2-dependent ~21-nt triplet repeat-derived siRNAs, which may target other transcripts that contain CAG repeat stretches through the RNA interference pathway and which are highly toxic to the animal (Yu et al., 2011). These findings indicate that repeat-derived small RNAs generated from bidirectional transcription may contribute novel pathogenic components in which anti-sense transcription occurs.

Bidirectional transcription across an expanded CTG-CAG repeat is also present in Huntington's disease-like 2 (HDL2). HDL2 is a recently described autosomal dominant neurodegenerative disorder with features similar to Huntington's disease (HD). It is caused

by CTG-CAG repeat expansion at the Juncophilin-3 (*JPH3*) locus. The CTG repeat expansion is located within the variably spliced exon 2A of *JPH3*. The existence of a *JPH3* splice variant with the CTG repeat in the 3'UTR suggests that transcripts containing an expanded CUG repeat could play a role in the pathogenesis of HDL2, similar to DM1. Indeed, RNA foci resembling DM1 foci were detected in HDL2 cortex and other brain regions. The foci contain *JPH3* transcript with an expanded CUG repeat and colocalize with muscleblind-like protein 1 (MBNL1). Furthermore, nuclear MBNL1 in HDL2 cortical neurons is decreased relative to controls. Abnormal splicing of several MBNL1 targets, MAPT exon 2 and APP exon 7, is also seen in the HDL2 brain. In addition, cell experiments suggest that the *JPH3* transcript with an expanded repeat is toxic (Rudnicki et al., 2007). These results imply that CUG repeat RNA toxicity may contribute to the pathogenesis of HDL2. Given the clinical and pathological similarity between HDL2 and HD, protein gain-of-function mechanisms to explain HDL2 have also been evaluated. Recently, a BAC transgenic mouse model of HDL2 (BAC-HDL2) containing an expanded CTG-CAG repeat in the human *JPH3* was developed to explore the plausibility of this protein gain of function (Wilburn et al., 2011). Indeed, BAC-HDL2 mice, but not control BAC mice, recapitulated age-dependent motor deficits, selective neurodegenerative pathology, and ubiquitin-positive nuclear inclusions. Molecular analyses revealed that nuclear inclusions in HDL2 result from the expression of a polyQ protein encoded by a *JPH3* antisense transcript containing an expanded CAG repeat. This expanded CAG transcript is driven by a promoter located upstream of the polyQ ORF and is translated into an expanded polyQ protein. Moreover, the selective expression of mutant CAG transcripts, but not CUG transcripts, is sufficient to manifest polyQ pathogenesis. Taken together, a combination of protein and RNA gain-of-function mechanisms could be involved in HDL2 pathogenesis.

Antisense transcription may also contribute to the pathogenesis of FXTAS. *ASFMR1*, an antisense transcript driven by the promoter located in the second intron of *FMR1*, spans the CGG repeat of the *FMR1* gene in the CCG orientation and exhibits premutation-specific alternative splicing and contains an ORF with the CCG repeat encoding a polyproline peptide (Ladd et al., 2007). Similar to *FMR1*, *ASFMR1* transcript was found to be upregulated in FXTAS premutation carriers and shut down in full mutation (FXS) patients. Together, these findings suggest complex transcription within the *FMR1* locus and that *ASFMR1* expression from the expanded allele might contribute to the variable clinical phenotypes associated with the CGG repeat expansion.

An auxiliary toxic RNA in polyglutamine disorders

Polyglutamine (polyQ) disorders are a group of dominantly inherited neurodegenerative disorders caused by the expansion of an existing CAG trinucleotide repeat encoding glutamine in the open reading frame of their respective disease genes (Orr and Zoghbi, 2007). The widely held view is that the mutant expanded polyglutamine tracts underlie the pathogenesis of polyQ disease. However, recent studies from several laboratories suggest the mutant repeat RNA play a pathogenic role in polyQ toxicity. Studies in cultured human HD and SCA3 fibroblast cells found that the expanded transcripts are retained in the nucleus, where they form RNA foci and colocalize with MBNL1 protein (de Mezer et al., 2011). Later reports using transgenic SCA3 *Drosophila* and mutant CAG repeat *Caenorhabditis elegans* and mouse models have yielded evidence that mutant CAG repeat RNAs can be toxic at the RNA level, contributing to polyQ-induced degeneration (Hsu et al., 2011; Li et al., 2008; Wang et al., 2011). For example, transgenic flies bearing an untranslatable pathogenic-length CAG repeat within the 3'UTR of a reporter gene exhibited late-onset progressive neurodegeneration. This neurodegeneration did not appear to be due to translation of CAG repeat-containing protein or transcription of an antisense CUG-containing mRNA (Li et al., 2008). However, a previous study with a long RNA with a 960

CAG repeat RNA expressed in COSM6 cells showed that the toxic effects of the MBNL1-positive CAG foci were milder compared with CUG foci; CAG foci did not induce the misregulation of alternative splicing events typical for CUG repeats (Ho et al., 2005). These studies demonstrate that CAG repeats can partially mimic the pathogenic activity of CUG repeats. The pathogenic-length CAG repeat RNA may contribute to polyQ disorders beyond coding for a pathogenic polyQ protein.

Small noncoding RNAs in trinucleotide repeat neurodegenerative disorders

Small regulatory RNAs, particularly miRNAs, are known to be dynamically regulated in neurogenesis and brain development (Kosik and Krichevsky, 2005; Krichevsky et al., 2006). Some recent studies have suggested that the alterations in small regulatory RNAs could contribute to the pathogenesis of several neurodevelopmental disorders. Here we focus on the role(s) of the miRNA pathway in several well-defined trinucleotide repeat neurodegenerative disorders. Huntington's disease (HD) is caused by an expanded CAG repeat leading to a polyglutamine strand at the N-terminus of the huntingtin (HTT) gene that encodes mutant HTT (Walker, 2007). HD is characterized by widespread mRNA misregulation, especially in the striatum and cortical regions (Hodges et al., 2006). Alterations in miRNA-mediated post-transcriptional regulation could be an important mechanism contributing to the deregulation of mRNA expression in HD. Studies have shown that small-RNA silencing-dependent mechanisms play a part in HD neuropathology. First, huntingtin interacts with transcriptional repressor RE1-silencing transcription factor (REST/NRSF) to modulate the transcription of NRSE-controlled neuronal genes (Zuccato et al., 2003). The increased repression by REST/NRSF leads to changes in the expression of specific neuronal miRNAs (miR-124, miR-29a, miR-29b, miR9/9*, miR-132 and miR-330-3p) in HD patients and HD mouse models (Johnson et al., 2008; Packer et al., 2008). Furthermore, HTT interacts with Argonaute proteins. This interaction with the mutant HTT leads to reduced reporter gene silencing activity compared with the wild-type HTT (Savas et al., 2008). Additionally, miRNA biogenesis is altered during the pathogenesis of HD. Reduced expression of Dicer in the HD transgenic mouse model YAC128 and Drosha in R6/2 corresponds with the reduced amount of total miRNA in these HD models (Lee et al.). The Lee group has found that nine miRNAs (miR-22, miR-29c, miR-128, miR-132, miR-138, miR-218, miR-222, miR-344, and miR-674*) are commonly downregulated in different HD transgenic mice, especially at the age when the mice present phenotypes. Although the roles of these miRNAs in HD are not fully understood yet, the work published to date suggests their involvement in HD pathogenesis (Lee et al., 2011). For example, downregulation of miR-218 is associated with the activation of nuclear factor kappa B (Gao et al., 2010), which might be linked to the activation of nitric oxide synthase in HD models (Napolitano et al., 2008). Previous studies have shown that miR-29c upregulates p53 levels (Park et al., 2009), and increased p53 activity is seen in HD (Bae et al., 2005).

An altered miRNA pathway has also been observed in the spinocerebellar ataxias (SCAs). SCA1 is caused by expansion of a translated CAG repeat in ataxin1. miR-19, miR-101, and miR-130 directly bind to the ATXN1 3'UTR to suppress the translation of ATXN1, and the expression of these miRNAs in cerebellar Purkinje cells could play a role in modulating the toxicity of mutant ataxin1 (Lee et al., 2008). In SCA3, reduced miRNA processing with the loss of DCR-1 and R3D1 dramatically enhances ataxin 3-induced neurodegeneration in *Drosophila*. In contrast, loss of DCR-2, which reduces siRNAs, had little or no effect on polyQ-induced neurodegeneration. Moreover, the miRNA bantam (ban) is found to prevent SCA3-associated neurodegeneration by suppressing SCA3 degeneration and functions downstream of the toxicity of the SCA3 protein (Bilen et al., 2006). These findings indicate

that miRNA pathways dramatically modulate polyQ-induced neurodegeneration, giving us fresh insight to guide our pursuit of new therapeutics.

Summary

Recent discoveries of dysregulated noncoding RNAs have uncovered a new layer of complexity in the molecular pathogenesis of human diseases. Emerging work suggests that the involvement of noncoding RNAs in human diseases could be far more prevalent than previously appreciated, and untangling the functions of these RNAs could vastly improve our understanding and treatment of human diseases. In this review, we have summarized the roles that different noncoding RNAs might play in the molecular pathogenesis of multiple neurodegenerative disorders: RNA toxicity is probably mediated by several parallel mechanisms, including sequestration of RNA-binding proteins, which activates abnormal signaling cascades; aberrant expression of antisense transcripts, which has deleterious consequences at multiple levels; accumulation of mRNA/protein aggregates, which may have detrimental effects on cellular homeostasis; and an altered microRNA pathway, which influences target(s) transcription or translation. However, we suspect these findings are just the tip of the iceberg, with these and other noncoding RNAs possibly being involved in disease pathogenesis at different levels and via multiple distinct mechanisms. Future studies are needed to elucidate the mechanism by which dysregulated noncoding RNA functional motifs can affect regulatory domains and compromise its ability to interact with other molecules, thereby contributing to the pathogenesis of disease. Based on all the current evidence, the investigation of noncoding RNA biogenesis and function will be necessary for a comprehensive understanding of human disease. It is therefore important to take noncoding RNAs into account when trying to identify disease-causing gene(s) and dissect the biological pathway(s) altered in the pathogenesis of neurodegenerative disorders.

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Figure 1. RNA-binding proteins may become functionally limited by their sequestration to lengthy fragile X premutation rCGG repeats, thereby contributing to the pathogenesis of FXTAS

Three RNA-binding proteins, Pur α , hnRNP A2/B1, and CUGBP1, were found to bind rCGG repeats either directly (Pur α and hnRNP A2/B1) or indirectly (CUGBP1, through the interaction with hnRNA A2/B1). Overexpression of these proteins suppresses the neurodegenerative phenotype of flies expressing massive expansion of rCGG repeats, which supports the model that sequestration of these proteins by the over-produced rCGG repeats in FXTAS prevents them from carrying out their normal functions, leading to neurodegeneration.

Figure 2. Bidirectional transcription in SCA8 pathogenesis

Transcripts from the *ATXN8* sense strand (blue) results in production of polyQ protein-forming neuronal nuclear inclusions. In contrast, toxic noncoding CUG expansion RNAs from the *ATXN8OS* strand accumulate in nuclear RNA foci (red). Sequestration of MBNL1 within the nuclear aggregates and/or an increase in the expression or activity of CUGBP1 trigger alternative splicing changes of CUGBP1-MBNL1-regulated CNS targets that are important for GABAergic inhibition. These results indicate that both toxic-protein (polyQ) and toxic-RNA gains of function might underlie SCA8 pathogenesis.

Table 1

Neurodegenerative Disorders associated with Trinucleotide-Repeat Noncoding RNAs.

Disorders	Repeat type	Location of the repeat	Normal repeat	Disorder repeat	Gene affected	The proposed mechanisms associated with Noncoding g RNAs
FXTAS	CGG	5'UTR	5-54	55-200	FMR1; FMR4	Sequestration of RNA-binding proteins: Pur α, hnRNP A2/B1, CUGBP1; Antisense transcript: CCG repeat encoding a polyproline peptide;
DM1	CUG	3'UTR	5-37	>50	DMPK	Sequestration of RNA-binding proteins: MBNL1, CUGBP1; Antisense transcript: ~21 nt CAG repeat siRNA
SCA1	CAG	Exon 8	6-39	>39	ATXN1	Altered miRNA pathway
SCA3	CAG	Exon 8	13-44	>55	ATXN3	An auxiliary toxic long CAG repeat RNA; Altered miRNA pathway
SCA7	CAG	Exon 3	4-35	>37	ATXN7	Antisense transcript: SCAANT1 (spinocerebellar ataxia-7 antisense noncoding transcript 1) repress ataxin-7 sense transcript on
SCA8	CUG	3'UTR	16-34	>74	ATXN8OS; ATXN8	Sequestration of RNA-binding proteins: MBNL1, CUGBP1; Antisense transcript: CUG repeat RNA toxicity and polyQ toxicity
HDL2	CUG	Exon 2A	6-28	>41	JPH3	Antisense transcript: CUG repeat RNA toxicity and polyQ toxicity
HD	CAG	Exon 1	10-34	>35	HTT	An auxiliary toxic long CAG repeat RNA; Altered miRNA pathway

FXTAS = Fragile X-associated Tremor/Ataxia Syndrome; DM1 = myotonic dystrophy type 1; SCA = spinocerebellar ataxia; HDL2 = Huntington disease like 2; HD = Huntington disease; UTR = untranslated region; FMR1 = fragile X mental retardation gene 1; DMPK = dystrophin myotonia protein kinase; JPH3 = junctophilin 3; HTT = huntingtin; MBNL1 = muscleblind-like 1; CUGBP1 = CUG-binding protein 1