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Epigenetic changes and non-coding expanded repeats

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Abstract

Many neurogenetic disorders are caused by unstable expansions of tandem repeats. Some of the causal mutations are located in non-protein-coding regions of genes. When pathologically expanded, these repeats can trigger focal epigenetic changes that repress the expression of the mutant allele. When the mutant gene is not repressed, the transcripts containing the expanded repeat can give rise to a toxic gain-of-function by the mutant RNA. These two mechanisms, heterochromatin-mediated gene repression and RNA dominance, produce a wide range of neurodevelopmental and neurodegenerative abnormalities. Here we review the mechanisms of gene dysregulation induced by non-coding repeat expansions, and early indications that some of these disorders may prove to be responsive to therapeutic intervention.

Keywords

myotonic dystrophy; RNA dominance; epigenetics; Friedreich ataxia; fragile X syndrome; MBNL; trinucleotide repeat

More than 20 human neuropsychiatric diseases are caused by genomic expansions of simple tandem repeats [reviewed by (Gatchel and Zoghbi, 2005)]. When these mutations were initially discovered it quickly became apparent that they exhibit an unprecedented degree of genetic instability [reviewed by (Pearson et al., 2005)]. For example, the size of the repeat is likely to change when transmitted from one generation to the next. In fact, intergenerational increments of hundreds of repeats are routinely seen in some of these disorders. These mutations are also unstable in somatic cells, leading to age-dependent growth of the expansion during the life of an individual. The net effect is to create large tandem arrays of simple repeats, a type of genomic element that previously has been associated with focal epigenetic changes (Martens et al., 2005).

The unstable repeat expansion disorders fall in two broad categories according to the position of the repeat element within the mutant gene. In most cases the repeat tract is located in protein coding sequences. For these conditions the repeat is invariably a triplet, the motif is CAG, and the orientation in the reading frame specifies a run of glutamine codons. [Polyglutamine disorders are discussed in recent reviews by (Shao and Diamond, 2007; Orr and Zoghbi, 2007; Williams and Paulson, 2008; Bauer and Nukina, 2009)]. In the normal population the CAG repeats at these loci are polymorphic, and differences in the number of consecutive

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glutamines may subtly modulate function of the encoded protein. When the repeat length exceeds a threshold, usually of around 40 units, neurodegeneration will result, with the age of onset determined, at least in part, by the extent of the pathological expansion. While these expanded polyglutamine proteins generally are widely expressed, the associated phenotypes are mainly neurodegenerative.

The second major group of unstable repeat expansions disorders are caused by repeats in non-protein-coding regions of genes. For these disorders the genetic features and mechanisms are more diverse. The non-coding expansions typically cause multisystem disease and the phenotypes can be developmental, degenerative, or both. The purpose of this review is to summarize evidence that epigenetic mechanisms are fundamentally involved in pathogenesis of non-coding repeat expansion disorders, and to consider how these mechanisms may create opportunities for novel therapy.

Expanded repeats in non-coding regions

The non-coding repeat expansions involve various sequence motifs in different intragenic positions (Fig. 1, Table 1). For example, the expansion is a CGG repeat in the 5' untranslated region (UTR) of *FMRI* in fragile X syndrome (FXS) (Verkerk et al., 1991), a GAA repeat in the first intron of *Frataxin* in Friedreich's ataxia (FRDA) (Campuzano et al., 1996), and a CTG repeat in the 3' UTR of *DMPK* in myotonic dystrophy type 1 (Brook et al., 1992) (Figure 1). The resulting phenotypes also are quite diverse (Table 1). FXS is the most common cause of hereditary mental retardation (Garber et al., 2008). Friedreich's ataxia is the most common cause of hereditary ataxia in Indo-European populations, and is often accompanied by cardiomyopathy (Pandolfo, 2008). Myotonic dystrophy type 1 (DM1) is the most common cause of muscular dystrophy in adults, and is characterized by CNS involvement (hypersomnolence and cognitive dysfunction), cataracts, and cardiac dysrhythmias (Harper, 2001).

Similar to the polyglutamine diseases, the size of a non-coding expanded repeat may correlate with disease severity, as observed in DM1 and FRDA (Hunter et al., 1992; Filla et al., 1996). However, expansion length can also modulate phenotype in a qualitative, discontinuous fashion. For example, the multisystem degenerative features of classical DM1 are usually associated with several hundred CTG repeats in the 3' UTR of *DMPK*, as determined by genetic analysis of peripheral blood. When the expansion exceeds a threshold of around 1000 repeats, however, it often impacts fetal development, leading to impaired muscle differentiation and mental retardation (congenital DM1) (Tsiflidis et al., 1992). A similar shift between developmental and degenerative features occurs in FXS (Hagerman and Hagerman, 2004). The CNS dysfunction and maldevelopment in FXS result from expansions of 200 or more CGG repeats in the 5' UTR of *FMRI*. In contrast, individuals with smaller expansions, in the range of 55 to 200 CGG repeats, are at risk for a late-onset neurodegenerative syndrome of ataxia, tremor, and cognitive impairment (fraxile X tremor ataxia syndrome, or FXTAS).

The wide spectrum of phenotypes in non-coding repeat expansion disorders reflects the diversity of underlying molecular mechanisms. Two broad categories of biologic effects have emerged: (1) gene silencing, which can affect the gene containing the repeat or flanking genes; and (2) RNA dominance, which entails a deleterious gain-of-function by transcripts that contain an expanded repeat. Furthermore, the epigenetic changes that are triggered by expanded repeats can, in turn, modulate the genetic stability of the repeat tract. We will consider these mechanisms in the context of the three commonest non-coding repeat expansion disorders, FXS, FRDA, and myotonic dystrophy.

Gene silencing

The critical effect of the expanded repeat in fragile X syndrome (FXS) is to induce transcriptional silencing of *FMR1*, a gene encoding an mRNA binding protein. FMR1 protein regulates translation at the synapse, and its loss is believed to compromise synapse formation and neuronal plasticity (Darnell et al., 2001; Huber et al., 2002). The location of the CGG repeat in the 5' UTR is close to the *FMR1* promoter (~ 100 bp). Expansions of more than 200 repeats induce cytosine methylation of CG dinucleotides within the repeat tract and also in the adjacent promoter region (Pieretti et al., 1991; Sutcliffe et al., 1992). The CGG expansion also triggers other epigenetic modifications that are characteristic of heterochromatin, such as, histone H3 lysine 9 (H3K9) methylation and histone deacetylation (Coffee et al., 1999; Coffee et al., 2002) [histone modifications are reviewed in another article in this issue (Huynh & Casaccia)]. Although DNA methylation and histone modifications have a complex role in gene silencing, it has been suggested that DNA methylation is the key event that silences transcription of *FMR1* (Pietrobono et al., 2002; Pietrobono et al., 2005). It is clear that loss of FMR1 activity is the primary mechanism responsible for mental retardation in FXS because a similar phenotype is produced by conventional mutations, such as, deletions or point mutations, that inactivate *FMR1* (Gedeon et al., 1992; De Boulle et al., 1993). However, CGG expansion is by far the commonest genetic lesion causing loss of FMR1 protein. Because *FMR1* is on the X chromosome, the phenotype is less severe in females, depending on the ratio of inactivation for the wild-type versus the mutant X chromosome.

Both strands of the *FMR1* 5' UTR are transcribed, as is true for many 5' UTRs and for several other, if not all repeat expansion loci (Ladd et al., 2007) [discussed further below]. One possible mechanism for silencing is that bidirectional transcription of the expanded repeat leads to formation of double-stranded repeat-containing RNAs. Processing of these dsRNAs to small RNAs may recruit protein complexes that generate a repressive chromatin imprint (Martens et al., 2005; Ladd et al., 2007), although it remains to be established whether this mechanism of small RNA-mediated chromatin modification, initially characterized in yeast, is conserved in mammals. In addition to its effects on transcription, the expanded repeat also has an important post-transcriptional effect, which is to reduce the translational efficiency of *FMR1* mRNA. The expanded CGG repeat may form a stable RNA secondary structure that inhibits ribosomal scanning of the 5' UTR (Feng et al., 1995). While this effect may not be pertinent to FXS, where *FMR1* transcripts are hardly produced, it may play a greater role in FXTAS, as discussed below.

Friedreich's ataxia (FRDA) is an autosomal recessive disorder that also involves gene silencing. Expansion of the GAA repeat causes a graded reduction of frataxin, a protein involved in iron homeostasis in mitochondria (Campuzano et al., 1997). Modest reductions of frataxin expression are well tolerated in individuals who are heterozygous for the GAA expansion (frequency ~1 in 120 in European populations), whereas homozygotes exhibit a critical reduction of frataxin that leads to mitochondrial impairment, juvenile-onset neurodegeneration, and cardiomyopathy [reviewed by (Pandolfo, 2008)].

The silencing of *Frataxin* by GAA expansion evidently occurs through two mechanisms. The repeat is positioned in intron 1, ~ 2 kilobases from the *Frataxin* promoter. When expanded, this repeat has a unique capability to form triplex DNA structures that inhibit transcriptional elongation (Bidichandani et al., 1998; Sakamoto et al., 1999; Grabczyk and Usdin, 2000). In parallel, it also has become clear that GAA expansions induce epigenetic changes that silence *Frataxin* expression (Saveliev et al., 2003; Herman et al., 2006; Greene et al., 2007; Al-Mahdawi et al., 2008). A recent study of FRDA cells has shown an interesting constellation of increased H3K9 trimethylation, recruitment of heterochromatin protein 1, reduced binding of the chromatin insulator protein CTCF, and increased antisense transcription, all in the region

immediately downstream from the transcription start site (De Biase et al., 2009). Studies showing reversibility of *Frataxin* silencing with histone deacetylase inhibitors suggest that these epigenetic mechanisms have a critical role in causing the frataxin deficiency [discussed further below].

The expanded repeat in myotonic dystrophy is also associated with gene silencing. Highly expanded CTG repeats trigger DNA and histone H3K9 methylation in regions of *DMPK* that immediately flank the repeat tract (Otten and Tapscott, 1995; Filippova et al., 2001; Cho et al., 2005). In this case, however, the repeat is located in the final exon of *DMPK*, 13 kilobase pairs distal to the promoter, and the expansion does not alter the activity of the *DMPK* promoter (Krahe et al., 1995). Moreover, despite the extreme length of DM1 expansions, which often extend for 5 to 15 kilobase pairs in affected tissues, the expansion does not block transcription elongation across the repeat (Krahe et al., 1995; Davis et al., 1997). Notably, because the density of genes in this region of chromosome 19 is high, the CTG repeat is close to regulatory elements that control a neighboring gene, which encodes the *Six5* transcription factor. When expanded, the repeat does cause silencing of *Six5* (Klesert et al., 1997; Thornton et al., 1997), and the mechanism once again appears to involve bidirectional transcription followed by endonucleolytic cleavage of dsRNAs to short RNAs (Cho et al., 2005). However, the studies of heterozygous *Six5* knockout mice indicate that mono-allelic reduction of *SIX5* expression is unlikely to have a major role in DM1 pathogenesis, although it may contribute to premature formation of ocular cataracts (Klesert et al., 2000; Sarkar et al., 2000).

The more important effect of the expanded DM1 repeat is post-transcriptional, in that nuclear export of mRNA with an expanded CUG repeat (CUG^{exp}) is blocked. Although fully processed and polyadenylated, the mutant transcript is retained in the nucleus, where it accumulates in discrete foci at the edge of SC-35 domains (Taneja et al., 1995; Davis et al., 1997; Hamshire et al., 1997; Smith et al., 2007). A similar phenomenon of nuclear retention can be induced for other transcripts by inserting a CUG^{exp} tract in the 3' UTR (Amack et al., 1999; Mankodi et al., 2000). Notably, the formation of nuclear foci is markedly reduced by knock down of Muscleblind 1 (MBNL1) (Dansithong et al., 2005), the major CUG^{exp} binding protein in muscle nuclei (Miller et al., 2000). This suggests that interaction of CUG^{exp} RNA with MBNL1 protein is fundamentally involved in tethering the mutant RNA within the nucleus. However, the resulting decrease in translation of the mutant *DMPK* mRNA has a limited role, if any, in the pathogenesis of DM1. Indeed, the more important consequence is the effect on MBNL1 activity.

RNA dominance

DM1 is an autosomal dominant disease. Accordingly, the expected effect of retaining mutant mRNA in the nucleus, for affected individuals who are heterozygous for the expanded repeat, would approach a 50% reduction of DMPK protein. However, in knockout mice the DM protein kinase can be completely eliminated with only minor consequences for skeletal muscle (Jansen et al., 1996; Reddy et al., 1996), leaving geneticists to ponder how a non-coding repeat expansion could give rise to dominant disease if not by haploinsufficiency. Two lines of evidence now support the conclusion that symptoms of DM1 result from a toxic gain-of-function by transcripts that contain an expanded CUG repeat. First were observations that muscle, cardiac, and CNS features of DM1 could be reproduced by expression of CUG^{exp} RNA in transgenic animals (Mankodi et al., 2000; Seznec et al., 2001; de Haro et al., 2006; Wang et al., 2007; Orengo et al., 2008). Second was the discovery that a second form of myotonic dystrophy, caused by a non-coding repeat expansion in a different gene, also involves the accumulation of mutant RNA in nuclear foci. The expanded repeat in myotonic dystrophy type 2 (DM2) is a CCTG tetramer in the first intron of *ZNF9*, a gene encoding a multi-functional DNA and RNA binding protein (Liquori et al., 2001). Remarkably, DM2 expansions can attain

lengths of up to 44 kilobases. The DM2 repeat is fully transcribed and the intron containing the CCUG expansion is properly excised (Margolis et al., 2006) but not rapidly degraded, producing toxic effects and having protein interactions similar to those observed with CUG repeats.

Mechanisms of RNA dominance in DM1

As mentioned above, the RNA binding protein MBNL1 has strong affinity for CUG^{exp} RNA (Miller et al., 2000; Yuan et al., 2007; Warf and Berglund, 2007). When CUG^{exp} RNA accumulates in the nucleus, MBNL1 is heavily recruited into RNA foci and depleted from the nucleoplasm (Miller et al., 2000; Jiang et al., 2004; Lin et al., 2006). Because MBNL1 is a regulator of alternative splicing (Ho et al., 2004), its sequestration in foci leads to misregulated splicing for the exons that it normally controls. One example with a clear functional consequence relates to alternative splicing of *Clcn1*. This gene encodes a chloride ion channel that stabilizes the transmembrane potential during bouts of muscle activity. Alternative splicing of *Clcn1* is misregulated in response to expression of CUG^{exp} RNA or ablation of MBNL1, causing loss of channel activity (Mankodi et al., 2002; Charlet-B et al., 2002; Lueck et al., 2007). Loss of this channel causes membrane depolarization during muscle activity, triggering volleys of involuntary action potentials that cause a delay of muscle relaxation (myotonia). MBNL1 also regulates the alternative splicing of more than 50 other muscle-expressed genes (Du et al., 2010), though for many of these changes the effects of MBNL1 loss are not pronounced and the biological consequences of inappropriate exon skipping or inclusion are difficult to determine. Loss of MBNL1 also has other effects on gene expression, including alterations of transcription and possibly mRNA decay (Osborne et al., 2009). A plausible but still-unproven hypothesis is that the cumulative alterations of transcription, alternative splicing, and decay of many different MBNL1-dependent transcripts has a major contribution to the muscle and cardiac features of DM1.

While the hypothesis of MBNL1 sequestration has considerable experimental support, it clearly does not provide a unitary explanation for the disease. Most strikingly, MBNL1 knockout mice do not reproduce the developmental features of congenital DM1, nor do they display the severe muscle degeneration that occurs in individuals with adult-onset disease (Kanadia et al., 2003a). Also, it is clear that some transcripts whose expression is impacted by CUG^{exp} RNA are neither direct nor indirect targets of MBNL1 (Osborne et al., 2009). Other candidates for involvement in DM1 include the two other members of the muscleblind-like family, MBNL2 and MBNL3 (Fardaei et al., 2002; Kanadia et al., 2003b). These proteins are closely related to MBNL1 and display similar CUG^{exp}-binding activity. MBNL2 and MBNL3 can also become sequestered in nuclear RNA foci, making it likely that DM1 can produce combinatorial deficiency of all three muscleblind family members, depending on the amount of CUG^{exp} RNA in relation to the supply of MBNL proteins in different cells and tissues.

Studies indicate that RNA dominance also causes important perturbations of cell signaling. For example, protein kinase C (PKC) is activated within 6 hours of expressing a non-coding CUG^{exp} in mice (Kuyumcu-Martinez et al., 2007; Wang et al., 2007; Orengo et al., 2008). While the mechanism responsible for sensing the repeat RNA and activating PKC have not been determined, one important downstream target has been identified – the RNA binding protein CUG-binding protein 1 (CUG-BP1) (Philips et al., 1998). This protein is subject to strong post-translational controls, in that phosphorylation can extend its half-life from 1 hr to > 4 hrs (Kuyumcu-Martinez et al., 2007). CUG-BP1 phosphorylation and stabilization has several consequences, including misregulated alternative splicing of several pre-mRNAs, as well as altered translation and decay of mRNAs that are CUG-BP1 binding targets (Savkur et al., 2001; Timchenko et al., 2001; Charlet-B et al., 2002; Timchenko et al., 2004; Ho et al.,

2005). The complex effects of CUG-BP1 upregulation may be particularly important for the cardiac and developmental problems of DM1.

RNA dominance in Fragile X Tremor Ataxia Syndrome (FXTAS)

In contrast to heterochromatin-mediated repression that is associated with “full mutations” of *FMRI*, shorter expansions of 55 to 200 repeats, and especially those in the range of 60 to 80 repeats, do not cause methylation or silencing, but instead are associated with production of a neurotoxic mRNA [reviewed by (Jacquemont et al., 2007)]. Thus, depending on the length of the repeat, CGG expansions in *FMRI* can produce two neurological syndromes, FXS or FXTAS, whose clinical signs, neuropathologic features, and mechanistic bases seem entirely distinct. The late-onset neurodegenerative syndrome, FXTAS, is associated with increased levels of *FMR1* mRNA, whereas levels of *FMR1* protein are either unchanged or slightly reduced (Tassone et al., 2000; Kenneson et al., 2001; Tassone et al., 2004a; Tassone et al., 2004b). The altered relationship between transcript and protein levels is likely to result from the decreased translational efficiency of the CGG-expanded transcript.

The mechanism for increased accumulation of *FMRI* mRNA in FXTAS is unknown. Possibly this alteration may represent a physiological compensation for decreased translational efficiency of CGG-expanded mRNA (Tassone et al., 2000; Kenneson et al., 2001).

Alternatively, the expanded CGG repeat may act directly as an enhancer of *FMRI* transcription. Whichever mechanism applies, it appears that the increased expression of the CGG-expanded transcript is deleterious in neurons and glia. FXTAS symptoms of tremor and ataxia typically develop in men after the age of 50, and are associated with ubiquitinated nuclear inclusions in specific populations of neurons and glia (Hagerman et al., 2001; Greco et al., 2002; Jacquemont et al., 2003). Similar inclusions are found in knockin mice that express *Fmr1* with ~105 CGG repeats (Willemsen et al., 2003). Inclusion formation and degeneration can also be induced in *Drosophila* by expression of an untranslated (CGG)₉₀ in retinal cells or neurons (Jin et al., 2003). In the latter model a CGG-repeat binding protein, Pur α , is recruited into nuclear inclusions, and the neurodegenerative process is alleviated by increased expression of Pur α protein (Jin et al., 2007). These observations raise the possibility of a protein sequestration mechanism, similar to that postulated for DM1.

Modulation of repeat instability by epigenetic changes

As discussed above, expanded repeats induce focal epigenetic changes that may repress the mutant allele or other genes in the vicinity. Studies of FXS suggest that the epigenetic changes, in turn, can modify the genetic stability of the expanded repeat. For example, an individual with FXS will typically show variability of repeat length in different somatic cells, but the instability that underlies this mosaicism occurs early in development. Later in fetal development the CGG expansion and flanking sequences become heavily methylated, and from this point the repeat length remains stable, even in clonal isolates of fibroblasts during extended periods of propagation (Wohrle et al., 1993). In contrast, individuals harboring a “premutation” allele of >130 repeats, or rare individuals with full expansions that remain unmethylated, will continue to show extensive somatic instability during postnatal life (Wohrle et al., 1998; Taylor et al., 1999). One possible explanation is that methylation has directly suppressed somatic instability by improving the fidelity of DNA replication or repair (Nichol and Pearson, 2002). Alternatively, somatic instability may depend, at least in part, on transcription of the repeat tract in one or both directions, in which case the epigenetic mark may stabilize the repeat by silencing the bidirectional transcription of *FMRI*. Whether DNA methylation has a general stabilizing influence on other repeat-containing loci has not been determined.

Opportunities for therapeutic intervention

Options for treating non-coding repeat expansion disorders are limited at present. No treatments are currently available that can delay the onset of symptoms or slow the disease progression. However, the elucidation of epigenetic and RNA dominant mechanisms has led to several novel therapeutic approaches [reviewed by (Di Prospero and Fischbeck, 2005)]. In the case of FRDA, histone deacetylase (HDAC) inhibitors offer one approach to reverse the epigenetic change, revert chromatin to an active conformation, and restore frataxin expression to levels that sustain neuronal survival. In support of this idea, histone H3 and H4 acetylation in chromatin near the GAA repeat was increased by HDAC inhibitor BML-210 (Herman et al., 2006). This compound and its derivatives were able to upregulate frataxin mRNA and protein in FRDA cells. Moreover, treatment of FRDA knockin mice with BML-210 derivatives resulted in increased histone H3 and H4 acetylation in chromatin near the expanded GAA repeat, accompanied by increased expression of frataxin mRNA and protein (Rai et al., 2008). While the pharmacologic manipulation of histone acetylation throughout the genome understandably raises concerns of toxicity, it appeared that short-term treatment was well tolerated in mice, perhaps because the effect of these compounds were relatively specific for HDAC3, an HDAC that may have a unique role in mediating the silencing of *Frataxin* (Xu et al., 2009).

Recently, a metabotropic glutamate receptor (mGluR) theory has been proposed in FXS pathogenesis (Bear et al., 2004). According to this theory, mGluR and FMR1 protein (FMRP) have antagonistic effects on activity-dependent translation at the synapse, with FMRP acting as a repressor of translation whereas mGluR signaling has the opposite effect of stimulating the translation of proteins involved in long term depression, a physiological marker of synaptic remodeling. Loss of FMRP would thus give rise to unopposed action of mGluR. Consistent with this theory, pharmacologic inhibitors of mGluR, or genetic knockdown of mGluR5, have favorably impacted the structural and behavioral features of FXS in animal models. Clinical trials are currently underway to test this strategy in patients with FXS [reviewed in (D'Hulst and Kooy, 2009)].

The therapeutic goal in DM1, in contrast, is to reduce the cellular accumulation of a toxic RNA or mitigate its harmful effects. For example, if the toxicity of DMPK transcripts is proportional to length of the expanded CUG repeat, then disease onset and progression may depend on growth of the CTG repeat in somatic cells of an individual. While this assumption has not been formally tested, if correct it would predict that methods to stabilize the repeat at a pre-symptomatic length, or to promote contraction rather than expansion events, would be preventative or therapeutic (Yang et al., 2003; Gomes-Pereira and Monckton, 2004). Agents that selectively alter the dynamics of repeat instability, however, have not yet been developed.

Another potential approach is to silence the transcription of the mutant *DMPK* allele. Theoretically this could be achieved by augmenting the expansion-induced epigenetic modifications, so that the heterochromatinization spreads to include the elements that control DMPK transcription. However, this approach has not yet been tested.

In terms of technologies that are currently available, a more feasible approach is to reduce RNA toxicity by accelerating the turnover of mutant *DMPK* mRNA. This has been achieved in cell culture systems by viral-mediated expression of antisense RNAs, short hairpin RNAs, or ribozymes, resulting in beneficial effects on cellular phenotypes (Furling et al., 2003; Langlois et al., 2005; Langlois et al., 2003). The obvious challenge for this approach is to avoid destruction of transcripts from the wild-type *DMPK* allele, which would further exacerbate the partial DMPK deficiency state that already exists in DM1. Another gene-therapy approach is to overexpress MBNL1 to levels that exceed the protein binding capacity of the toxic RNA

(Kanadia et al., 2006). This method has shown promising effects in mouse models of DM1. A third approach is to neutralize the toxic RNA, using agents that inhibit the interaction of CUG expansion RNA with binding proteins (Gareiss et al., 2008; Pushechnikov et al., 2009; Warf et al., 2009). For example, morpholino oligomers are DNA analogues that bind to RNA targets with high affinity and specificity, without inducing target cleavage by RNase H (Summerton and Weller, 1997). A morpholino oligonucleotide consisting of CAG repeats was able to bind to CUG^{exp} RNA and disrupt CUG^{exp}-MBNL1 complexes *in vitro* (Wheeler et al., 2009). When injected into muscle tissue, this oligonucleotide dispersed the nuclear foci of CUG^{exp} RNA, reversed the abnormalities of alternative splicing, and eliminated the myotonia in a transgenic mouse model of DM1. Interestingly, an oligonucleotide of similar sequence but utilizing a different chemistry (2'-O-methyl-phosphorothioate-modified RNA) showed remarkably selective knock down of transcripts from the mutant *DMPK* allele (Mulders et al., 2009). While the exact mechanism of this effect has not been determined, the therapeutic potential seems high, if it can be systemically delivered.

Conclusion

Short tandem repeats are ubiquitous features in the human genome. A tiny fraction of these repetitive elements are prone to become hypermutable and highly expanded. Determinants of this propensity are not understood, but may include length and sequence of the repeat, and genomic context in which it occurs (e.g., proximity to origins of replication, bidirectional transcription, and other factors). When located in non-coding sequence, these hyperexpanded repeats lead to neuropsychiatric symptoms through repeat-induced epigenetic silencing or RNA dominance. As the mechanisms underlying these disorders are clarified, the opportunity for therapeutic intervention becomes apparent. For many recessive disorders the primary focus of therapeutic development is on gene or protein replacement therapy. By contrast, the epigenetic mechanism for loss of function in *FRDA* is amenable to therapy by non-genomic methods. In addition, the RNA gain-of-function mechanism in DM1 may prove responsive to RNA targeted therapeutics.

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Figure 1. Location of unstable non-coding repeats within their respective genes

The sequences and locations of non-coding expanded repeats are shown. The figure does not include expanded tandem repeats in which there is uncertainty whether the primary pathogenic effect is related to expression of a coding or non-coding repeat, such as, spinocerebellar ataxia type 8 or Huntington's disease-like 2.

Figure 2. Antisense oligonucleotides (AONs) rescue RNA-mediated toxicity in DM1

Expanded CUG repeats in the mutant RNA form hairpin structures and sequester MBNL1 in the nucleus. Loss of functional MBNL1 causes misregulation of alternative splicing. AONs bind to expanded CUG repeats and release MBNL1, thereby restoring its function in splicing regulation.

Table 1

Unstable non-coding repeats and associated diseases. Note that this table does not include disorders in which repeats may lie in coding or non-coding sequence, depends on which strand is transcribed (Huntington disease-like 2 or spinocerebellar ataxia type 8). AR, autosomal recessive; XL, X-linked; AD, autosomal dominant; FRAXE, fragile X syndrome E; FXTAS, fragile X tremor and ataxia syndrome; SCA10, spinocerebellar ataxia type 10.

Disease	Inheritance	Gene	Location	Repeat motif	Normal repeat size	Expanded repeat size	Symptoms	Epigenetic effect
Friedreich ataxia	AR	<i>FXN</i>	Intron 1	(GAA) _n	6-32	>200	Ataxia, cardiomyopathy, diabetes	CpG methylation, histone modification, transcriptional silencing
Fragile X syndrome A (FRAXA)	XL	<i>FMR1</i>	5'-UTR	(CGG) _n	6-52	>200	Mental retardation	CpG methylation, histone modification, transcriptional silencing, bi-directional transcription
FRAXE	XL	<i>FMR2, FMR3</i>	5'-UTR	(CGG) _n	4-39	200-900	Mental retardation	CpG methylation
FXTAS	XL	<i>FMR1</i>	5'-UTR	(CGG) _n	6-52	55-200	Tremor, ataxia, cognitive dysfunction	CpG methylation, bi-directional transcription
Myotonic dystrophy type 1	AD	<i>DMPK</i>	3'-UTR	(CTG) _n	5-37	>50	Muscle weakness, myotonia, cardiac conduction deficit, cognitive dysfunction	RNA dominance, CpG methylation, histone modification, bi-directional transcription
Myotonic dystrophy type 2	AD	<i>ZNF9</i>	Intron 1	(CCTG) _n	10-26	>75	Muscle weakness, myotonia, cardiac conduction deficit	RNA dominance
SCA10	AD	<i>ATXN10</i>	Intron 9	(AATCT) _n	10-29	800-4500	Ataxia, seizure	Unknown