

## REVIEW ARTICLE

# Myotonic Dystrophy: RNA Pathogenesis Comes into Focus

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Myotonic dystrophy (DM)—the most common form of muscular dystrophy in adults, affecting 1/8,000 individuals—is a dominantly inherited disorder with a peculiar and rare pattern of multisystemic clinical features affecting skeletal muscle, the heart, the eye, and the endocrine system. Two genetic loci have been associated with the DM phenotype: *DM1*, on chromosome 19, and *DM2*, on chromosome 3. In 1992, the mutation responsible for DM1 was identified as a CTG expansion located in the 3' untranslated region of the dystrophin protein kinase gene (*DMPK*). How this untranslated CTG expansion causes myotonic dystrophy type 1 (DM1) has been controversial. The recent discovery that myotonic dystrophy type 2 (DM2) is caused by an untranslated CCTG expansion, along with other discoveries on DM1 pathogenesis, indicate that the clinical features common to both diseases are caused by a gain-of-function RNA mechanism in which the CUG and CCUG repeats alter cellular function, including alternative splicing of various genes. We discuss the pathogenic mechanisms that have been proposed for the myotonic dystrophies, the clinical and molecular features of DM1 and DM2, and the characterization of murine and cell-culture models that have been generated to better understand these diseases.

## Introduction

Steinert (1909) and Batten and Gibb (1909) identified myotonic dystrophy (DM [MIM 160900 and MIM 602668]) as a multisystemic disorder that is now recognized as one of the most common forms of muscular dystrophy in adults. In addition to muscular dystrophy and myotonia (involuntary persistence of muscle contraction), DM causes a consistent constellation of seemingly unrelated and rare clinical features, including: cardiac conduction defects, posterior subcapsular iridescent cataracts, and a peculiar and specific set of endocrine changes (Harper 2001). The 90 years between the identification of myotonic dystrophy as a clinical disorder and the identification of the first gene were important for the development of a detailed description of the clinical features, inheritance, and epidemiology of myotonic dystrophy. Clinical neurologists and geneticists identified non-Mendelian features of myotonic dystrophy type 1 (DM1) inheritance, including variable penetrance, anticipation (a tendency for the disease to worsen in subsequent generations), and a maternal transmission bias for congenital forms (Harper 2001), all of which preceded the identification of the CTG expansion respon-

sible for DM1. The genetic cause of DM1 was identified in 1992 as a (CTG)*n* repeat in the 3'-UTR of a protein kinase gene (Brook et al. 1992; Buxton et al. 1992; Fu et al. 1992; Harley et al. 1992; Mahadevan et al. 1992).

In 1995, several reports described families with dominantly inherited multisystemic myotonic disorders that were genetically distinct from DM1 (Ricker et al. 1994; Rowland 1994; Thornton et al. 1994). Ricker and colleagues (1994) most thoroughly evaluated this new disorder and showed that the genetic cause in these families was distinct from the known causes of myotonia congenita, paramyotonia, and DM1, demonstrating the existence of a novel genetic disorder. Because affected family members often came to medical attention because of pelvic girdle weakness, he referred to the disorder as “proximal myotonic myopathy” (PROMM). A broader phenotype was described as “proximal myotonic dystrophy” in 1997 (Udd et al. 1997). Subsequently, we reported a large Minnesota kindred with a second form of myotonic dystrophy (DM2) that closely mimics the phenotype of adult-onset DM1, and we localized the disease gene to a 10-cM region on chromosome 3q (Ranum et al. 1998; Day et al. 1999). Ricker and colleagues then reported that the gene for PROMM in many families also mapped to the *DM2* locus (Ricker et al. 1999). The International Myotonic Dystrophy Consortium (IDMC 2000) and the Online Mendelian Inheritance in Man (OMIM) database updated the clinical and scientific nomenclature so that the chromosome 19 locus is now referred to as “myotonic dystrophy type

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1" (*DM1*) and the locus on chromosome 3 to which *DM2* and many *PROMM* families map is the *DM2* locus. The diseases are collectively referred to as "the myotonic dystrophies" (*DM*), and each individual genetic disorder is referred to by the same name as the locus name (*DM1*, *DM2*), whereas "*PROMM*" can be used to describe the clinical presentation but is not restricted to any specific genetic cause or locus.

## Clinical Features

### Muscle Dysfunction

The multisystemic nature of *DM* results in varied symptoms in affected individuals (table 1), although almost all affected individuals have clinically identifiable muscle involvement at the time of diagnosis. Muscle dysfunction is the most common presenting complaint in both *DM1* and *DM2* (Moxley 1996; Day et al. 1999, 2003; Ricker 1999; Thornton 1999; Harper 2001), with symptomatic weakness, pain and myotonia. Myotonia is involuntary muscle contraction and delayed relaxation due to muscle hyperexcitability.

There are similarities and differences in the muscle features of *DM1* and *DM2*. Both diseases result in early clinically detectable weakness of neck flexors and lateral deep finger flexors (Moxley 1996; Day et al. 1999; Mathieu et al. 1999; Ricker 1999; Thornton 1999; Harper 2001; Day et al. 2003), although initial symptoms in *DM2* often involve proximal lower extremity musculature, whereas *DM1* is more apt to be associated with severe muscle atrophy and symptomatic finger flexor weakness. *DM2* is not associated with severely atrophic facial and forearm muscles. Electrical myotonia is detectable by electromyography in almost all individuals with *DM1* or *DM2*; although clinical symptoms of myotonia are not typically severe in either disease, patients with *DM1* are often more symptomatic than those with *DM2*.

The histological features of *DM* muscle are distinct, though not pathognomonic, with dystrophic features accompanied by atrophic fibers, scattered severely atrophic fibers with pyknotic myonuclei, and a marked proliferation of fibers with central nuclei. There is one report of differences in fiber-type involvement in the two diseases, with more type 1 involvement in *DM1* and more type 2 involvement in *DM2* (Vihola et al. 2003).

### Multisystemic Features

*DM1* and *DM2* have similar disease-specific effects on the eye, heart, and endocrine systems. Ocular involvement results in almost all adults with *DM1* or *DM2* having posterior subcapsular cataracts, with distinctive red and green iridescent opacities on slitlamp examination. Some adults are unaffected by these cataracts,

**Table 1**

**Clinical Features of the Myotonic Dystrophies**

FEATURE	FINDING FOR <sup>a</sup>	
	DM1	DM2
Myotonia	+	+
Facial weakness	+	+
Proximal weakness	+	+
Distal weakness	+	+
Sternocleidomastoid atrophy	+	+
Iridescent cataracts	+	+
Cardiac arrhythmias	+	+
Testicular failure	+	+
Hyperinsulinemia	+	+
Hypogammaglobulinemia	+	+
Elevated creatine kinase	+	+
Retardation/congenital abnormalities	+	-

<sup>a</sup> + = present; - = absent.

but other subjects with *DM1* or *DM2* may require lens removal as early as the 2nd decade of life (Harper 2001; Day et al. 2003). Cardiac involvement leads to conduction defects, arrhythmias, and sudden death in both *DM1* and *DM2* (Nguyen et al. 1988; Colleran et al. 1997; Merino et al. 1998; Philips et al. 1998; Day et al. 2003) but does not correlate with severity of skeletal muscle involvement or repeat length (Lazarus et al. 1999). Endocrine abnormalities in both *DM1* and *DM2* result in hyperinsulinemia, hyperglycemia, and insulin insensitivity, with type 2 diabetes occurring in each disorder (Harper 2001; Day et al. 2003). Testicular failure is also common in both disorders, with associated hypotestosteronism, elevated follicle-stimulating hormone (FSH) levels, and oligospermia. Other serological abnormalities in both disorders include reduced levels of immunoglobulin G and M (Harper 2001; Day et al. 2003).

### Congenital Myotonic Dystrophy

A primary difference between *DM1* and *DM2* is that *DM1* can result in a severe congenital form that has not been reported for *DM2*. The congenital form of *DM1* involves marked developmental abnormalities that can be present to a lesser degree in patients with *DM1* who become symptomatic during adulthood; the hallmark developmental features, including craniofacial skeletal abnormalities, characteristic dysmorphic features, and mental retardation, have not been reported in *DM2*.

### CNS Involvement

CNS involvement in *DM1* involves both (1) developmental abnormalities with retardation and (2) degenerative changes, exemplified by abnormal white matter changes on MRI scanning, memory deficits, and personality abnormalities (Rubinsztein et al. 1997; Bun-

gener et al. 1998; Delaporte 1998; Ogata et al. 1998; Ono et al. 1998; Wilson et al. 1999). The CNS effects of DM2 mirror the degenerative changes of DM1, including the MRI abnormalities (Hund et al. 1997), but developmental abnormalities and retardation are not recognized features of DM2 (Day et al. 2003).

### DM1 (CTG)*n* Repeat Expansion and Instability

In 1992, the chromosome 19 form of DM was shown to be caused by an expanded CTG repeat located in the 3' UTR of the *myotonia-dystrophica protein kinase* (*DMPK* [MIM 605377]) gene (Brook et al. 1992; Buxton et al. 1992; Fu et al. 1992; Harley et al. 1992; Mahadevan et al. 1992) and the promoter region of the immediately adjacent homeodomain gene *SIX5* (MIM 600963) (Boucher et al. 1995). DM1 was the first autosomal dominant disease found to be caused by an untranslated trinucleotide repeat expansion, in which the mutation is transcribed into RNA but not translated into protein. The CTG expansion in *DM1* can vary from 50 to >4,000 repeats in affected individuals. Both intergenerational and somatic instability are observed with increases in repeat size on the order of ~50–80 repeats per year (Monckton et al. 1995; Martorell et al. 1998). For DM1, there are reasonably good correlations of repeat size and age at onset for CTGs <400 repeats (Hamshire et al. 1999).

### The *DM1* CTG Expansion: Proposed Mechanisms of Pathogenesis

#### *Haploinsufficiency of DMPK*

Because most dominant disorders are caused by the expression of an abnormal protein with an altered function, it has not been clear how the multisystemic clinical features of dominantly inherited DM1 could be caused by a trinucleotide repeat that did not affect the protein coding portion of a gene (Tapscott 2000). Initially, the location of the mutation at the 3' end of a kinase gene suggested that alterations in *DMPK* expression might cause the multisystemic clinical features of the disease. Most of the early expression studies were consistent with this hypothesis, indicating that *DMPK* mRNA and protein levels were reduced in patient muscle and cell culture (Fu et al. 1992; Hoffmann-Radvanyi et al. 1993; Novelli et al. 1993). However, *DMPK* knockout mice generated to test this hypothesis did not have the typical multisystemic features of the disease. Initial reports of these mice showed only a very mild, late-onset myopathy that is not typical of DM1 (Jansen et al. 1996; Reddy et al. 1996). More recently, both hemizygous and homozygous *DMPK* knockout mice have been reported to have cardiac conduction abnormalities. When considered to-

gether, these results suggested that *DMPK* may contribute to the cardiac features of DM1, but haploinsufficiency of *DMPK* does not cause the multisystemic clinical features of DM1. The fact that there are no reported cases of DM1 caused by point mutations in the *DMPK* gene further suggests that the multisystemic features of DM1 are not caused simply by *DMPK* haploinsufficiency.

#### *Haploinsufficiency of SIX5 and Neighboring Genes*

A second proposed mechanism is that the expanded repeat affects the expression of multiple genes in the region. Support for this hypothesis comes from the observation that the CTG expansion is a strong nucleosome-binding site that could potentially alter chromatin structure and have regional effects on the expression of multiple genes (Wang et al. 1994; Otten and Tapscott 1995). The expansion overlaps not only the 3' end of *DMPK*, but the 5' promoter region of the neighboring gene *SIX5*. *SIX5* has a strong resemblance to the fruit fly gene *sine oculis* (needed for eye development) and to a family of mouse genes that regulate distal limb muscle development. Because cataracts and distal muscle wasting are common in DM1, haploinsufficiency of *SIX5* was suggested as a possible contributor to DM1 pathogenesis (Shaw et al. 1993; Boucher et al. 1995; Jansen et al. 1995). In addition to *DMPK* and *SIX5*, other neighboring genes suggested to be involved in aspects of DM1 pathogenesis included the *DMWD* gene immediately upstream of *DMPK*, which is expressed in the testis and is proposed to play a role in male infertility, and *FCGRT* (MIM 601437), an IgG receptor gene located 4 Mb from the CTG expansion, was proposed to underlie the low IgG levels in DM (Junghans et al. 2001). In this model, the multisystemic features of DM1 would be explained by haploinsufficiency of a number of neighboring genes, with expression level—and, hence, disease severity—dependent on repeat length.

Expression studies have focused on examining the transcript levels of *DMPK*, *DMWD*, and *SIX5* in both individuals with DM1 and normal individuals. In the case of *DMPK*, an equal amount of unprocessed transcripts are produced from both the normal and the mutant *DMPK* genes (Krahe et al. 1995), indicating that there is no interference with *DMPK* transcription caused by the CTG expansion. Studies involving *SIX5* and *DMWD* have given conflicting results. Thornton and colleagues (1997) used an allele-specific RT-PCR assay and found that the expression of the CTG repeat expansion-linked *SIX5* allele was reduced—in some cases approaching inactivation—compared with the other *SIX5* allele. This reduction was seen with pre-mRNA, as well as with processed mRNA. Klesert and colleagues (1997) demonstrated that a DNase I hypersensitive site,

shown by Otten and Tapscott (1995) to be eliminated by the CTG repeat expansion, contains an enhancer sequence for the *SIX5* gene but not for the *DMPK* gene, and that expansion of the CTG reduces the transcription of the *SIX5* allele located *cis* to the expansion. Data generated by Hamshere and colleagues (1997), indicated that the cytoplasmic expression of both *SIX5* and *DMWD* was the same in both normal and DM1 cells.

The most compelling support for the involvement of neighboring genes in DM pathogenesis came from the fact that *Six5* knockout mice develop cataracts (Klesert et al. 2000; Sarkar et al. 2000). Although cataracts are one of the clinical features of DM, the cataracts in the *Six5* knockouts do not have the distinctive iridescent opacities or the posterior location that is associated with myotonic dystrophy in humans.

#### RNA Pathogenesis

A third hypothesized mechanism is that the enlarged CUG-containing transcripts that accumulate as foci in the nuclei of both cultured cells and biopsied tissue (Taneja et al. 1995; Davis et al. 1997) exert a *trans*-dominant effect that disrupts splicing and possibly other cellular functions (Timchenko et al. 1996a; Philips et al. 1998; Lu et al. 1999; Miller et al. 2000; Savkur et al. 2001). Strong experimental support for the involvement of an RNA mechanism in skeletal muscle pathology came from a mouse model developed by Mankodi and colleagues (2000). Initial attempts to develop animal models to study the effects of the expression of elongated CUG expansions in transgenic mice were hampered by infertility of the mice, which prevented successful breeding (Monckton et al. 1998). In 2000, Mankodi and colleagues avoided this problem by developing a mouse model in which the CTG expansion was inserted into the 3' end of the human skeletal actin gene, a gene not thought to be involved in DM1 but the expression of which is limited to skeletal muscle (Mankodi et al. 2000). This mouse model, which expressed an mRNA with a CUG repeat tract of ~250 repeats was the first to develop the myotonia and the myopathic features characteristic of DM1 (Mankodi et al. 2000). However, because the expression of the CUG-containing transcripts in this model was limited to skeletal muscle, the role of the CUG expansion in the multisystemic features of DM was not addressed.

#### Additive Model of DM1 Pathogenesis

Subsequently, an additive model was proposed in which each of the above mechanisms contributes to DM1 pathogenesis (Groenen and Wieringa 1998; Tapscott 2000; Filippova et al. 2001; Larkin and Fardeai 2001), with some aspects of the disease caused by haploinsufficiency of *DMPK*, *SIX5*, and other neighboring

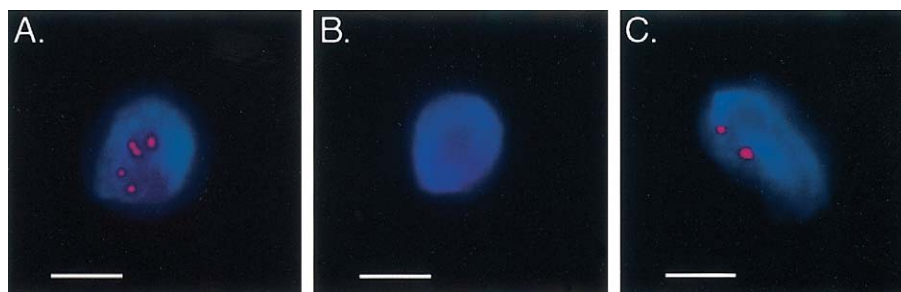
genes and other clinical features resulting from effects of the CUG expansion in RNA. An inconsistency with the additive model of DM1 pathogenesis was that the genetic locus for DM2 maps to a different chromosome, with no known conservation between the two chromosomal regions (Ranum et al. 1998).

#### DM2 Caused by CCTG Expansion in Intron 1 of *ZNF9*

Given the confusion in understanding the molecular pathogenesis of DM1, the identification of a second human mutation that causes the multisystemic effects of DM and a determination of what is common to these diseases at the molecular level, provided an independent means of evaluating the pathogenic pathway(s) of DM. In 2001, we demonstrated that DM2 is also caused by a transcribed but untranslated repeat expansion—but this time a CCTG repeat expansion located in intron 1 of the *zinc finger protein 9* (*ZNF9*) gene (MIM 116955). Although DM2 is generally a milder disease than DM1, the DM2 CCTG expansions can be much larger than DM1 CTG expansions, with alleles ranging in size from ~75 to 11,000 CCTG repeats (mean ~5,000 CCTGs). The smallest pathogenic size is not yet clear, because somatic instability has resulted in individuals with uncommon shorter expansions also having larger allele sizes in lymphocyte DNA (Liquori et al. 2001).

The DM2 CCTG expansion mutations show both somatic and intergenerational instability. In peripheral blood samples, the degree of somatic instability is so extreme that one in five DM2 expansions is not detectable by Southern analysis because of size heterogeneity. This causes a diagnostic challenge for DM2 not previously encountered with DM1 or any of the other reported microsatellite expansion disorders (Liquori et al. 2001; Day et al. 2003). Although anticipation has been reported in DM2/PROMM families, on the basis of clinical criteria (Schneider et al. 2000; Day et al. 2003), the expected trend of longer repeat expansions in patients with earlier ages at onset was not observed (Schneider et al. 2000; Day et al. 2003), with the somatic instability of the repeat clearly complicating this analysis (Liquori et al. 2001).

The DM2 repeat tract contains the complex repeat motif (TG)<sub>n</sub> (TCTG)<sub>n</sub> (CCTG)<sub>n</sub>. The CCTG portion of the repeat tract is interrupted on normal alleles, but as in other expansion disorders these interruptions are lost on affected alleles. Haplotype analysis of 228 control chromosomes identified a potential premutation allele with an uninterrupted (CCTG)<sub>20</sub> on a haplotype that was identical to the most common affected haplotype. Similar to some of the other microsatellite expansion disorders (Chung et al. 1993; Kunst and Warren 1994; Pulst et al. 1996; Gunter et al. 1998), the loss of se-



**Figure 1** RNA in situ hybridization of the expansion. *A*, In situ hybridization of CAGG probe to DM2 muscle. *B*, In situ hybridization of CAGG probe to normal muscle. *C*, In situ hybridization of CAG probe to DM1 muscle. The scale bar is 5  $\mu$ M. (Reprinted from *Science* 293:864–867.)

quence interruptions within the CCTG portion of the *DM2* repeat tract may predispose alleles to further expansion (Liquori et al. 2003).

Our 2001 discovery of the *DM2* CCTG expansion allowed for further analysis of the genetic heterogeneity of DM. It now appears that most if not all families with dominant multisystemic myotonic disorders have either *DM1* or *DM2* expansions, with no convincing examples that would suggest the existence of a third mutation that causes a similar disorder—that is, *DM3* (Day et al. 2003). Families previously reported to have a form of DM or PROMM not linked to either the *DM1* or *DM2* loci were subsequently shown to carry the *DM2* CCTG expansion (Day et al. 2003). Linkage disequilibrium and haplotype analysis suggest that single founder mutations led to the CTG and CCTG expansions responsible for both *DM1* and *DM2* (Imbert et al. 1993; Neville et al. 1994; Chakraborty et al. 1996; Bachinski et al. 2003; Liquori et al. 2003).

### **DM2: Evidence that RNA Pathogenesis Causes Multisystemic Clinical Features**

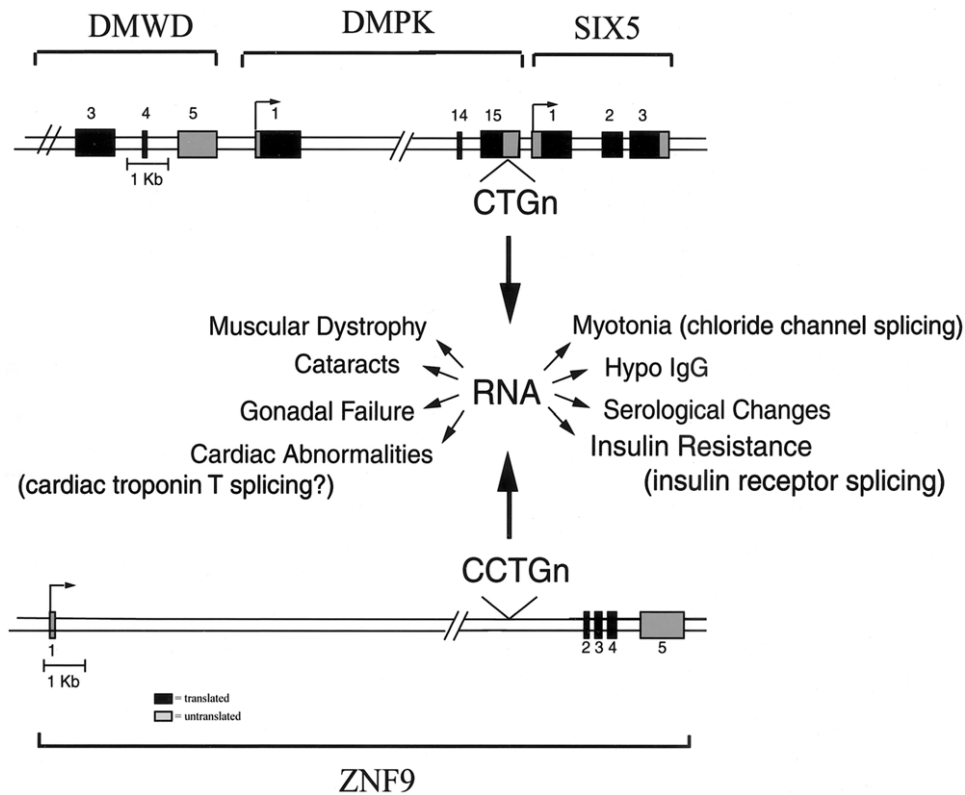
The location of the CCTG expansion within intron 1 of *ZNF9* is similar to the *DM1* CTG expansion, in that both repeats are transcribed into RNA but do not alter the protein coding portion of a gene (Liquori et al. 2001). The normal function of *ZNF9* as a nucleic acid-binding protein (Pellizzoni et al. 1997, 1998; Shimizu et al. 2003) appears unrelated to any of the proteins encoded in the *DM1* region of chromosome 19. Similarly, genes in the *DM2* region (*KIAA1160*, *Rab 11B*, *glycoprotein IX*, *FLJ11631*, and *FLJ12057*) bear no obvious relationship to genes near the *DM1* locus (*DMPK*, *SIX5*, *DMWD*, and *FCGRT*). Even if the *DM2* expansion alters the regulation of *ZNF9* and other genes in the *DM2* region, it would be unlikely that alterations in the regulation of different sets of proteins at the *DM1*

and *DM2* loci would result in diseases with such strikingly similar multisystemic features.

The first suggestion that CUG-containing transcripts were involved in *DM1* pathogenesis came from FISH experiments demonstrating that CUG-containing nuclear RNA foci accumulate in *DM1* cells (Taneja et al. 1995). Evidence that *DM1* and *DM2* share a common pathogenic RNA mechanism comes from experiments showing that similar CCUG-containing RNA foci are found in *DM2* muscle (fig. 1) (Liquori et al. 2001). These results demonstrate that the CCTG expansion is expressed at the RNA level, but additional experiments are needed to determine if the RNA foci contain the entire unprocessed *ZNF9* transcript or if the transcript is normally processed but the intron or the repeat tract alone resists degradation forming the RNA foci. Additional evidence that RNA foci containing the *DM1* and *DM2* repeat motifs behave in a similar manner is that several forms of the RNA-binding protein muscleblind (MBNL, MBLL, and MBXL) colocalize to the repeat-containing foci in both diseases (Mankodi et al. 2001; Fardaei et al. 2002).

Although the additive model of *DM1* suggested that CUG repeats in RNA cause the myotonia and muscular dystrophy of *DM1*, the causes of other DM features—including cardiac conduction defects and cataracts—had been ascribed to haploinsufficiency of genes in the *DM1* region. The clinical and molecular parallels between *DM1* and *DM2* suggest a simpler model of DM pathogenesis (figs. 2 and 3), in which the clinical features common to both diseases—including myotonia, muscular dystrophy, cataracts, cardiac arrhythmias, insulin insensitivity and diabetes, hypogammaglobulinemia, and testicular failure—are caused by the pathogenic effects of RNA containing the CUG and CCUG expansions (Day et al. 1999; Liquori et al. 2001)

Although *DM1* and *DM2* phenotypes are strikingly similar, they are not identical. *DM2* does not show a



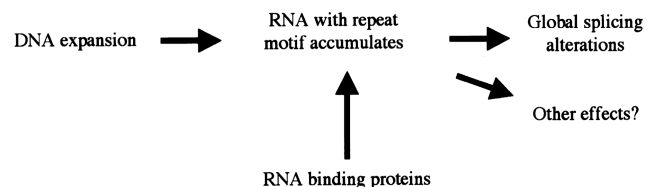
**Figure 2** RNA model of DM pathogenesis. The unusual and rare multisystemic clinical parallels between DM1 and DM2 suggest a similar pathogenic mechanism. The discovery that *DM2* mapped to chromosome 3 and not the *DM1* region of chromosome 19 make it unlikely that regional effects of gene expression play a major role in causing the common clinical features of the disease because regional dysregulation would affect different sets of genes. The discovery that a CCTG repeat expansion located on chromosome 3 which is expressed at the RNA but not the protein level causes DM2, and the observation that both CUG and CCUG repeat-containing foci accumulate in affected muscle nuclei suggests that a gain-of-function RNA mechanism underlies the clinical features common to both diseases. Specific changes in pre-mRNA splicing have been that have been associated with several genes, including the insulin receptor, the chloride channel, and cardiac troponin T, are correlated with insulin resistance, myotonia, and possibly cardiac abnormalities. (Adapted from Curr Opin Genet Dev 12:266–271.)

congenital form or mental retardation that can occur in DM1 (Liquori et al. 2001). Downstream differences of the CUG vs. CCUG expansions could be responsible for the clinical distinctions between these diseases as could differences in temporal or spatial levels of transcripts containing the expanded repeats. A possible mechanism for congenital DM1 has been suggested by Filippova and colleagues, who showed that methylation at the *DM1* locus in congenital cases can increase expression of *DMPK*, resulting in higher levels of CUG-containing transcripts and the more severe congenital phenotype (Filippova et al. 2001). Alternatively, the differences between DM1 and DM2 could involve locus-specific genes such as *DMPK*, *SIX5*, or *DMWD* for DM1, and *ZNF9* for DM2.

### Dominant RNA Mechanism: Specific Targets of Abnormal Pre-mRNA Splicing

Evidence that a gain-of-function alteration at the RNA level plays a role in DM1 pathogenesis includes (i) the

observation that an expanded repeat in the 3' UTR of *DMPK* mRNA inhibits myoblast differentiation (Amack et al. 1999); (ii) transgenic models in which >250 CTG repeats expressed at the RNA level cause myotonia and muscular dystrophy (Mankodi et al. 2000; Seznec et al.



**Figure 3** Schematic diagram of steps involved in RNA gain-of-function mechanism. CTG and CCTG expansions at the DM1 or DM2 loci result in the accumulation of CUG or CCUG repeat-containing transcripts as nuclear RNA foci. RNA-binding proteins, including CUG-BP and the muscleblind family of proteins bind to or are dysregulated by the repeat-containing RNA transcripts resulting in specific *trans*-alterations in pre-mRNA splicing.

2001); (iii) CUG- and CCUG-containing transcripts that accumulate as RNA foci alter the regulation or localization of CUG-binding proteins, including CUG-BP (Timchenko et al. 1996a) and three different forms of muscleblind (Miller et al. 2000; Fardaei et al. 2002); (iv) altered CUG- and CCUG-induced splicing changes that are directly related to phenotypic features of DM. A recent report using cell-culture models suggests that CUG RNA expansions bind and sequester transcription factors in DM1 and may disrupt gene regulation including the chloride channel *ClC-1* (Ebraldidze et al. 2004), but the role of altered gene transcription in DM pathogenesis is not clear and will need further investigation.

The currently identified splicing alterations are described in further detail below.

#### *Cardiac Troponin T (cTNT)*

Initial efforts to understand how the *DM1* CUG expansion expressed at the RNA level could mediate a *trans*-dominant effect focused on the identification of RNA-binding proteins that could bind to CUG repeat motifs. The first of these proteins to be isolated and described in detail was CUG-binding protein (CUG-BP) (Timchenko et al. 1996a, 1996b). In a landmark discovery in 1998, Phillips and colleagues (1998) reported that CUG-BP, a conserved heterogeneous nuclear ribonucleoprotein, could mediate a *trans*-dominant effect of excess CUG repeat-containing RNA by dysregulating the alternative splicing of cardiac troponin T (cTNT). CUG-BP was shown to bind to intronic CUG repeat sequences in human cardiac troponin T (cTNT) pre-mRNA, which are normally used as splicing signals. The presence of excess numbers of CUG repeats in transfection studies and in *DM1* cardiac and skeletal muscle cultures cause the preferential inclusion of exon 5, which is a splice form produced during the early development of heart and skeletal muscle but not in adult heart. This typical splicing pattern of cTNT is disrupted in *DM1* striated muscle and in transfected cells expressing either CUG-BP protein or elongated CUG-containing transcripts. This was the first demonstrated splicing target of CUG-BP and the first demonstration that the presence of elongated CUG repeat motifs lead to *trans* alterations in gene splicing (Phillips et al. 1998).

#### *Insulin Receptor (IR)*

A classic clinical feature in both *DM1* and *DM2* patients is insulin resistance; the reduced insulin response in skeletal muscle predisposes *DM* patients to diabetes. Savkur et al. (2001) demonstrated that alternative splicing of the insulin receptor (IR) pre-mRNA is aberrantly regulated in *DM1* skeletal muscle tissue, resulting in predominant expression of the insulin insensitive nonmuscle splice form lacking exon 11. Steady state levels of CUG-

BP are increased in skeletal muscle of *DM1* patients and dysregulation of CUG-BP is thought to mediate an IR alternative splicing switch by the binding to an intronic element upstream of the alternatively spliced exon 11. These results support a model in which increased expression of a splicing regulator contributes to insulin resistance in *DM1* by affecting IR alternative splicing (Savkur et al. 2001).

#### *Muscle-Specific Chloride Channel*

A classic feature of both *DM1* and *DM2* is myotonia, in which voluntary muscle contraction is followed by involuntary repetitive firing of action potentials that delay a patient's ability to relax muscle (Harper 2001). Using skeletal muscle from a transgenic mouse model of *DM1*, Mankodi and colleagues (2000, 2002) showed that expression of expanded CUG repeats in skeletal muscle reduces the transmembrane chloride conductance to levels consistent with those expected to cause myotonia. Additional studies determined that aberrant splicing of *Clc-1*, the main chloride channel in muscle, resulting in loss of *Clc-1* protein from the surface membrane (Mankodi et al. 2002). Similar splicing alterations were observed in skeletal muscle from both *DM1* and *DM2* patients (Charlet et al. 2002; Mankodi et al. 2002). Charlet and colleagues (2002) demonstrated that CUG-BP, which is elevated in *DM1* skeletal muscle, binds to the *ClC-1* pre-mRNA, and overexpression of CUG-BP in transfected cells reproduces the aberrant pattern of *ClC-1* splicing. Both groups propose that disruptions in alternative splicing regulation of *ClC-1* causes a channelopathy and membrane hyperexcitability, leading to the classic *DM* feature of myotonia (Charlet et al. 2002; Mankodi et al. 2002).

#### *Tau and Myotubularin*

Splicing alterations in the microtubule-associated tau pre-mRNA have been observed in CNS tissue from patients with *DM1* (Sergeant et al. 2001) and in a murine model described by Seznec and colleagues (2001), which may underlie various CNS alterations in both diseases. Also, muscle-specific changes in alternative splicing of the myotubularin-related 1 (*MTMR1*) gene are observed in congenital *DM1* muscle cells in culture and in skeletal muscle samples from patients with congenital-onset *DM1*. These results suggest that *MTMR1* plays a role in muscle formation and represents a novel target for abnormal mRNA splicing in myotonic dystrophy that may be responsible for the profound muscle atrophy of congenital *DM1* (Buj-Bello et al. 2002).

#### **Are RNA Foci Pathogenic or Protective?**

Neurodegenerative diseases, such as Alzheimer disease and those caused by polyglutamine expansions, result in

protein aggregates or plaques and tangles that accumulate in affected tissues and have led investigators to debate their role in disease pathogenesis (Hsiao et al. 1995; Klement et al. 1998). Similar discussions in the DM field revolve around the role of RNA foci in disease pathogenesis. CUG-BP, the first RNA-binding protein with a demonstrated pathogenic effect, has been shown to bind to single-stranded CUG repeats. In contrast with the muscleblind family of proteins, CUG-BP does not colocalize with the nuclear RNA foci in DM1 and DM2. Although it appeared possible that both groups of proteins play a role in the pathogenic effects of DM, colocalization of a protein to the ribonuclear inclusions did not appear to be necessary to cause a pathogenic effect.

Direct evidence supporting the hypothesis that sequestration of muscleblind in CUG and CCUG containing RNA foci plays a central role in disease pathogenesis comes from a recently developed muscleblind knockout model of myotonic dystrophy. Kanadia and colleagues showed that disruption of the mouse *Mbnl1* gene leads to muscle, eye, and RNA-splicing abnormalities that are characteristic of DM1 and DM2 in humans (Kanadia et al. 2003).

Data now indicate that overexpression of CUG-BP and depletion of *Mbnl1* both can cause specific alternative-splicing changes. Previous studies have reported that expression of elongated CUG-containing transcripts result in increased levels of CUG-BP through an unknown mechanism. In contrast, the sequestration of *Mbnl1* in CUG and CCUG RNA foci has been proposed as a mechanism that results in *Mbnl1* depletion. It now appears that both of these proteins play a role in affecting alternative splicing and that, in the case of *Clc1*, changes through antagonistic effects in which CUG-BP promotes the inclusion of exons that are normally favored during fetal development, whereas the normal function of *Mbnl1* is to favor splice forms expressed in adults. These data predict that overexpression of CUG-BP or depletion of *Mbnl1* would both result in the splicing alterations and that changes in the regulation of either these proteins can cause downstream splicing changes that have now been shown to be characteristic of the myotonic dystrophies.

### Other Noncoding Disorders

Although the striking clinical and molecular parallels between DM1 and DM2 demonstrate that RNA pathogenesis plays a much broader role in the multisystemic features of myotonic dystrophy than previously suspected, noncoding microsatellite expansions for *SCA8* (Koob et al. 1999) and *SCA10* (Matsuura et al. 2000) were identified before the *DM2* expansion. The pathogenic mechanism of the *DM1* and *DM2* expansions are evident only because they cause such a distinctive phe-

notype. Molecular parallels between the *SCA8* CUG repeat and the *DM1* and *DM2* repeats suggest the possibility that the known toxic properties of transcripts with elongated CUGs underlie the cerebellar degeneration in *SCA8* (Ranum and Day 2002). Although, in *SCA10*, it is possible that the enormous ATTCT expansion in an intron of a gene may cause disease through haploinsufficiency, the dominant inheritance pattern and the fact the expression of the *SCA10* transcript does not appear to be reduced in affected individuals (Matsuura et al. 2000) make this potential mechanism less likely. Parallels between *SCA10* and the RNA mechanism involved in *DM1*, *DM2*, and possibly *SCA8* suggest a gain-of-function mechanism at the RNA level could be involved. Unlike *SCA8*, the gene that harbors the mutation for *SCA10* is ubiquitously expressed, indicating that, if a toxic RNA mechanism is involved, secondary proteins that interact with the AUUCU repeat motif may confer organ-specific pathogenicity (Matsuura et al. 2000). *FMR* premutation alleles that have recently been reported to be associated with a tremor-ataxia syndrome may involve a similar RNA mechanism (Brunberg et al. 2002; Aziz et al. 2003; Jacquemont et al. 2003).

### Conclusions

The clinical and molecular parallels of *DM1* and *DM2* demonstrate the multisystemic effects of CUG and CCUG expansions. A detailed description of *trans*-dominant splicing alterations of genes relevant to the DM phenotype is emerging, providing a convincing model of how these RNA expansions cause myotonic dystrophy. In other microsatellite-expansion diseases, including *SCA8* and *SCA10*, transcribed noncoding expansions cause very different CNS phenotypes, suggesting that dominant RNA mechanisms may be a general cause of disease.

### Electronic-Database Information

The URL for data presented herein is as follows:

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/>

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