Am. J. Hum. Genet. 64:360–364, 1999

HUMAN GENETICS '99: TRINUCLEOTIDE REPEATS
Myotonic Dystrophy: The Role of RNA CUG Triplet Repeats

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Myotonic dystrophy (DM) is an autosomal dominant neuromuscular disorder associated with defects in many tissues, including skeletal muscle myotonia, progressive myopathy, and abnormalities in the heart, the brain, and the endocrine system (Harper 1995). The clinical phenotype, which is notably variable, is subject to genetic anticipation, the progressive worsening of symptoms, and/or presentation of symptoms at an earlier age in successive generations (Harper 1995). Both genetic anticipation and variability of phenotype in patients with DM were explained when an unstable CTG triplet–repeat expansion was identified in the myotonin protein kinase (*DMPK*) gene and shown to be responsible for the disease (Aslanidis et al. 1992; Brook et al. 1992; Buxton et al. 1992; Fu et al. 1992; Harley et al. 1992; Mahadevan et al. 1992). Unaffected individuals usually have 5–40 CTG repeats in the *DMPK* gene, but in an affected population the number of repeats is dramatically higher, sometimes even up to several hundred or thousands of repeats. Six years after the discovery of unstable CTG triplet–repeat expansion on chromosome 19q, significant amounts of experimental data have been collected, but the molecular mechanisms involved in the induction of the disease are still uncertain.

DM is unusual among triplet-repeat disorders in that it is associated with a trinucleotide repeat occurring in the 3′ UTR of a disease gene. On the basis of the dominant transmission of DM, one would ordinarily expect the disease allele mutation to affect protein structure, but it is difficult to see how a CTG triplet repeat outside the gene's open reading frame (ORF) could affect the DMPK protein. Thus, it has been hypothesized that CTG expansion might affect DMPK expression at the transcriptional or posttranscriptional level (Fu et al. 1993; Krahe et al. 1995; Wang et al. 1995), and, indeed, DMPK protein levels are reduced in DM, with some apparent differences between the adult and congenital forms of the disease (Timchenko et al. 1995). However, even if we assume that alleles with long CTG tracts are null, haploinsufficiency does not seem to be an adequate model for the transmission of DM. Analysis of *Dmpk* knockout mice shows that even complete deletion of the gene does not confer the DM phenotype (Jansen et al. 1996; Reddy et al. 1996). Rather, mice homozygous for the *Dmpk* deletion show some abnormalities, such as skeletal muscle weakness and myopathy, but do not have the myotonia that is a hallmark of DM disease (Jansen et al. 1996; Reddy et al. 1996). Thus, although DMPK is clearly an important component of muscle, loss of its expression cannot explain the complete DM phenotype. These results prompted us to reevaluate our knowledge of the basis for DM pathogenesis and to develop new strategies for studies of molecular mechanisms of DM.

The overall correlation between CTG repeat length and severity of the disease suggests that these repeats represent the major cause of DM pathogenesis. Two new hypotheses that proceed from this assumption are now under intensive investigations. The first suggests that expansion of CTG repeats leads to the change in expression of a neighboring gene, in particular, the myotonic dystrophy–associated homeodomain gene, which maps immediately downstream of *DMPK* (Boucher et al. 1995; Steinbach et al. 1998; Tapscott et al. 1998). The second hypothesis, concerning the role of RNA CUG repeats in DM pathogenesis, is the focus of this review.

RNA CUG Repeats in DM

The first experimental data suggesting a role for DMPK mRNA containing CUG repeats in DM pathology were obtained in the laboratory of Dr. E. P. Hoffman (Wang et al. 1995). The authors showed that DMPK polyA$^+$ RNA is significantly reduced in muscle biopsy samples from DM patients but that total RNA from these same samples contains essentially normal levels of DMPK transcripts (Krahe et al. 1995; Wang et al. 1995). This effect of the repeat expansion on RNA processing seems to act in *trans* on transcripts derived from the wild-type allele of heterozygous DM individuals. Such an explanation can account for the dominant transmis-

Received December 3, 1998; accepted for publication December 17, 1998; electronically published January 27, 1999.

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0002-9297/99/6402-0006$02.00

sion of DM. By extension, we have proposed that CUG repeat–containing DMPK RNA might also affect processing of other mRNAs, perhaps by sequestering specific RNA-binding proteins. According to this model (fig. 1), altered processing of different CUG-containing mRNAs results in the tissue-specific features of DM.

In agreement with this hypothesis, several CUG-binding proteins have been identified (Timchenko et al. 1996*b*). The first of these proteins, now named CUGBP1, has been shown to bind to CUG repeats, but not to single-stranded DNA CTG repeats (Timchenko et al. 1996*a*, 1996*b*). CUGBP1 is identical to a novel human nuclear heterogeneous RNA-binding protein, hNab50 (Timchenko et al. 1996*a*), which has been found to interact with an RNA-binding protein from yeast, which regulates RNA polyadenylation and transport (Caskey et al. 1996; Timchenko et al. 1996*a*). CUGBP1 belongs to a family of RNA-binding proteins that contain three RNA-binding domains (Caskey et al. 1996; Timchenko et al. 1996*a*), and it is highly homologous to several other RNA-binding proteins expressed in a variety of animal species. In flies, these binding proteins localize to the nucleus, where they regulate RNA splicing (Bell et al. 1991), but in vertebrates, they may also act in the cytoplasm to regulate translation and RNA stability (Levine et al. 1993; Myer et al. 1997). CUGBP1, in particular, plays an important role in the splicing of certain pre-mRNAs that contain CUG repeats in their regulatory elements (Philips et al. 1998). Several such repeats occur in regulatory elements that are needed for the inclusion of an alternative exon 5 in cardiac troponin T (cTnT). Philips et al. (1998) demonstrated that CUGBP1 binds to CUG repeats in this pre-mRNA and positively regulates inclusion of alternative exon that is usually absent in the adult form of the protein. Furthermore, splicing of cTnT RNA is indeed abnormal in hearts of DM patients, with an increase of the inclusion of alternative exon 5, as might be expected given that CUGBP1 RNA-binding activity is increased in nuclei of DM patients (Timchenko et al. 1996*a*; Roberts et al. 1997). These findings confirm the importance, for regulation of muscle cell gene expression, of interactions between CUG elements and specific RNA-binding proteins. However, as discussed below, they are not compatible with a simple protein-sequestration model for DM pathogenesis.

CUG-Binding Proteins in Translational Regulation

Since a significant amount of CUGBP1 is found in cytoplasm, it has been suggested that this protein also acts in postprocessing RNA regulation. Recently, we found that CUGBP1 is associated with polyribosomes and regulates translation of the transcription factor C/EBPβ (Timchenko et al. 1998). CUG-binding proteins bind to CUG repeats located in the 5′ region of C/EBPβ mRNA and affect the use of three alternative ribosomal initiation codons (Descombes and Schibler 1991), leading to the translation of different set of C/EBPβ isoforms.

CUGBP1 also binds to the 3′ UTR of DMPK RNA (Timchenko et al. 1996*a*) and may regulate its splicing or translation. We suggest that CUGBP1, in addition to other CUG-binding proteins, regulates a broad spectrum of RNAs with CUG repeats. It is interesting to note that Hoffman's group found a reduction of insulin-receptor mRNA and protein in muscle biopsy samples from patients with DM (Morrone et al. 1997). Although the mechanism of reduction of insulin receptor is unknown, it is possible that alteration of RNA processing in DM cells affects expression of the insulin receptor consistent with abnormal insulin resistance seen in patients with DM (Harper 1995).

Recently, two other nuclear proteins with molecular weights 25 and 35 kDa were isolated from human brain by affinity purification with RNA (CUG)10 (Bhagwati et al. 1996). Although the function of these proteins is unknown, they might be affected by expansion of CUG repeats in DM. We recently found that an elav-type RNA-binding protein, ETR-3, also binds specifically to expanded CUG repeats (Lu et al. 1999). This protein exhibits 75% homology to CUGBP1 and has three RNA-binding domains. Expression studies indicate that ETR-3 is abundant in the human heart and therefore could be involved in the regulation of RNA metabolism of cardiac pre-mRNAs and mRNAs (Lu et al. 1999). The identification of several proteins with CUG-binding activity raises the question whether all or only a few of the members of the CUG-binding protein family are affected by CUG repeats. It is also important to know whether CUG-binding proteins can be affected by any CUG repeats or only by CUG repeats placed into a DMPK environment.

CUGBP1 Function in DM

An outstanding question for the RNA-based model of DM is how CUGBP1 expression and function are affected by the *DMPK* mutation. Our original model held that expanded CUG repeats in the mutant DMPK mRNA sequester CUGBP1, causing reduced binding of CUGBP1 to other RNAs (Caskey et al. 1996; Timchenko et al. 1996*a*). However, analysis of CUGBP1 expression in DM patients shows that the increase in CUG repeat length correlates with a 3–4-fold induction of the CUGBP1 protein (Timchenko et al. 1998). Similar activation was found in tissues from transgenic mice overexpressing CUG repeats (author's unpublished data). The nature of this feedback mechanism is unknown, but CUGBP1 phosphorylation may be involved (fig. 1) (Roberts et al. 1997).

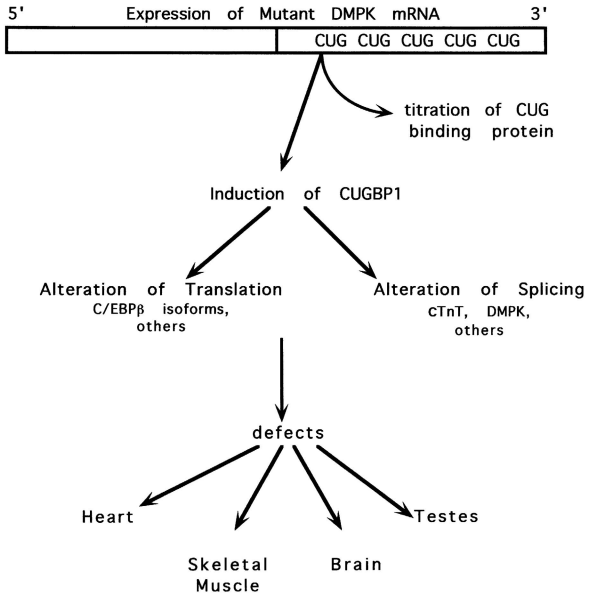

Figure 1 The role of RNA CUG repeats in DM pathogenesis. Overexpression of CUG repeats leads to overproduction of RNA-binding sites for specific RNA-binding proteins. These proteins are sequestered by expanded CUG repeats. Such sequestration results in the altered expression of specific RNA CUG-binding proteins. One such protein, CUGBP1, is induced, leading to altered splicing and translation of CUG-containing mRNAs and causing abnormalities in a variety of tissues, including the brain, heart, testes, and skeletal muscle.

Another important question concerns the tissue-specific regulation of CUGBP1 target mRNAs. DM is primarily a muscular disorder characterized by marked atrophy in the adult form of the disease and by the presence of immature myofibers in the congenital condition (Harper 1995). It is likely that long CUG repeats in the 3′ UTR of DMPK affect the maintenance of muscle differentiation status via CUGBP1 or other related proteins. A similar situation has been described for the 3′ UTRs of muscle-specific genes, such as troponin I, tropomyosin, and α-cardiac actin (Rastinejad and Blau 1993). It has been demonstrated that the 3′ UTRs of each of these mRNAs can rescue the mutant phenotype of differentiation-defective myoblast mutant cell lines, permitting these cells to reach their differentiated state in culture. The mechanism of this effect on muscle-cell phenotype remains uncertain, but because there is no common ORF in these various 3′ UTRs, it is unlikely that the mechanism requires the synthesis of any peptides that might be encoded in these sequences. Rastinejad and Blau (1993) suggest several models by which an untranslated RNA could affect cell physiology, such as by sequestering, by activating specific proteins or other RNA species, or by acting as a ribozyme.

We suggest that a CUG repeat in the 3′ UTR of DMPK also can play such a role. Sabourin et al. (1997) demonstrated that the 3′ UTR of DMPK mRNA is important for muscle differentiation. In contrast to the 3′ UTRs of troponin I, tropomyosin, and α-cardiac actin, the 3′ UTR of DMPK represses differentiation of mouse C2C12 cells, possibly by inhibiting myogenin expression. The 3′ UTR sequences important for the delay in muscle maturation do not include CTG repeats, but the authors speculate that CUG repeat expansion alters the structure of DMPK mRNA and that this alteration affects binding of RNA-binding proteins to the regions outside CUG repeats. If so, expanded CUG repeats might affect not only CUG-binding proteins, but also other RNA-binding proteins that interact with other elements of the DMPK 3′ UTR.

Further investigations are necessary to identify and characterize these proteins, but if this model is correct, proteins that bind the 3′ UTR of DMPK RNA will emerge as strong candidates for regulators of DM pathogenesis. Furthermore, changes in DMPK protein are known to alter the expression of skeletal muscle–specific genes, such as myogenin and the differentiation-dependent isoform of β-tropomyosin, which are induced by DMPK, and vimentin and retinoblastoma (Rb), which are repressed (Bush et al. 1996). This pattern of gene expression agrees with the positive role of DMPK in muscle differentiation. Myopathy in homozygous DMPK-knockout mice also shows that DMPK is important for muscle function (Reddy et al. 1996).

Our data thus suggest a complex relationship between DMPK and CUGBP1. CUGBP1 regulates DMPK RNA processing (X. Lu, L. Timchenko, unpublished data), so abnormalities in CUGBP1 should affect DMPK expression. DMPK, in turn, affects the expression of muscle-specific genes dependent on DMPK, some of which appear to be required to establish or maintain normal muscle cell differentiation. In addition, it has been suggested that CUGBP1 could be regulated by DMPK via phosphorylation (Roberts et al. 1997). Hence, both proteins might contribute to the muscle function. The evidence that CUGBP1 regulates patterns of C/EBPβ synthesis suggests that other transcription factors implicated in the muscle differentiation may also be affected. Thus, alteration of CUGBP1 expression in patients with DM could affect transcription, translation, and processing of muscle RNAs (fig. 1). Similar abnormalities of RNA metabolism could occur in other affected tissues, including the heart, brain, and testes. Identification of tissue-specific RNA-binding proteins interacting with the 3′ UTR of DMPK and CUG repeats will be required for the understanding of the role of RNA mechanism in manifestation of disease.

Future Prospects

It is clear that the molecular basis for DM is complex and consists of several steps. If, as I have argued, RNA

CUG repeats are the major factor in DM pathogenesis, several important issues must still be resolved. First, other CUG repeat–binding proteins should be identified. Second, various tissue-specific responses to CUG repeats expansion should be analyzed. Third, the functions of different CUG-binding proteins and the effect of overexpression of CUG repeats on their activities should be examined. Finally, that some cases of DM may be associated not with DMPK but with the mutation or deletion of some CUG-binding proteins, or with the expansion of CUG repeats in other RNAs, should be anticipated.

Regarding the last point, a new locus for DM (DM2), which was recently mapped to 3q (Ranum et al. 1998), appears to cause classical features of DM, including myotonia, myopathy, cataracts, and cardiac abnormalities. Clinical evaluation of a five-generation family shows that the phenotype is consistent with anticipation. I suggest that DM2 could be associated with CTG repeats in a gene different from DMPK or with another type of triplet-repeat expansion. Our data also suggest that an RNA-based mechanism of pathogenesis may also contribute to other neurologic disorders. For example, a molecular analysis of a new type of spinocerebellar ataxia (SCA 8) showed that the disease might be associated with long CTG expansions in the 3′ UTR of a novel gene with unknown function (Koob et al. 1998). It will be interesting to examine whether CUG-binding proteins are affected in this disease. If so, it is possible that common, RNA-based pathways lead to each of these disorders.

Acknowledgments

The author is supported by grants from the National Institutes of Health and the Muscular Dystrophy Association.

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