PERSPECTIVES: BIOMEDICINE

Deconstructing Myotonic Dystrophy

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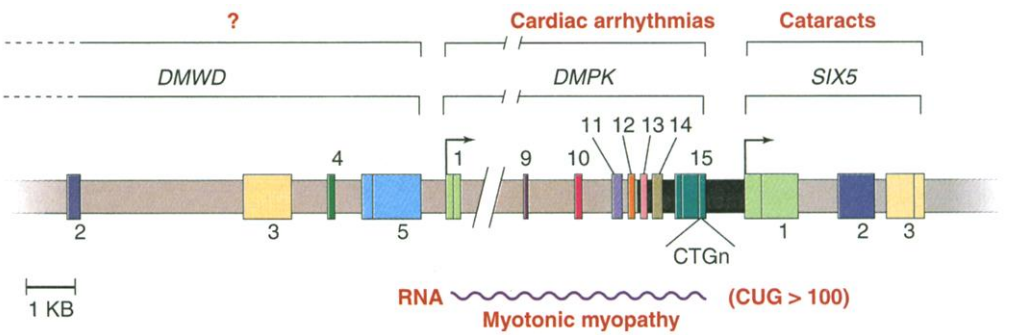
Myotonic dystrophy (DM), the most common muscular dystrophy in adults, is characterized by hyperexcitability of skeletal muscle (myotonia) and muscle degeneration (myopathy), a conduction defect in cardiac muscle cells, and cataracts. Inheritance of DM is autosomal dominant (that is, a mutation in one copy of the affected gene causes disease). There is also evidence of anticipation (with each generation disease severity increases and age of onset decreases) and a tendency for maternal transmission of a severe congenital form of the disease. An unstable repeat sequence of triplet nucleotides (CTG) at the DM1 locus on chromosome 19q13.3 is the cause of DM in the majority of families. The number of triplet repeats can increase with each generation, and disease severity correlates with the size of the expansion (providing a molecular basis for anticipation). Unlike many other triplet repeat diseases such as Huntington's disease where the repeats are in the coding region of the affected gene and result in an altered protein product, the CTG expansion at the DM1 locus is in the 3′ noncoding region of the *DMPK* gene (which encodes a serine-threonine protein kinase).

Three distinct hypotheses have been put forward to explain how a triplet repeat expansion in a noncoding region of a gene could cause DM. The first hypothesis postulates that the expanded repeat in the *DMPK* transcript (present as a repetitive CUG sequence in the RNA) alters processing of the *DMPK* RNA, resulting in a deficiency in the DMPK protein (see the figure). In the second hypothesis, the expanded CTG repeat in the *DMPK* gene is proposed to alter regional chromatin structure, resulting in misexpression of neighboring genes. The third hypothesis argues that CUG repeats in the *DMPK* RNA interact with RNA-binding proteins blocking their normal activity (termed a toxic gain-of-function), which results in disruption of cellular metabolism. Now, new evidence from the DM mouse model reported by Mankodi *et al.* on page 1769 of this issue (*1*), together with findings from several other mouse models, suggest that each hypothesis may be right.

It has been established that the expanded triplet repeat in the *DMPK* gene results in altered splicing of the *DMPK* transcript and retention of the transcript in the nucleus, preventing its translocation to the cytoplasm where it would normally be translated into protein (*2*). To test whether a decrease in the DMPK protein contributes to DM, two groups used homologous recombination to disrupt the *DMPK* gene in mice (*3*). Their analyses showed that mice carrying one defective copy of the gene (heterozygous) had no muscle pathology, whereas older mice with two defective copies of the gene (homozygous) developed a mild myopathy. As the skeletal muscles of these older mice did not show the characteristic electrophysiological and pathological changes associated with DM, it was presumed that lack of DMPK protein was not the principal cause of the myopathy in DM patients. In contrast, a more recent analysis of cardiac muscle conduction in these heterozygous and homozygous knockout mice revealed a striking similarity between the cardiac conduction defects in these animals and those seen in human DM (*4*).

The second hypothesis postulates that the expanded CTG repeat might alter expression of neighboring genes in a manner similar to that seen at loci near the repetitive sequences of telomeres and centromeres (position effect variegation). Indeed, an expansion of CTG repeats in the *DMPK* gene alters the local chromatin structure and suppresses the expression of the adjacent *SIX5* gene (see the figure) (*5*). The SIX5 protein belongs to a family of homeobox proteins that have been implicated in the regulation of muscle cell differentiation and sodium ion homeostasis, both of which are disrupted in DM. Formation of cataracts in mice with either one or two defective copies of the *SIX5* gene suggests that the cataracts in human DM might result from decreased expression of *SIX5* (*6*).

The DM mouse model described by Mankodi *et al.* (*1*) provides critical support for the third hypothesis that the CUG repeat in *DMPK* RNA exerts a toxic gain-of-function effect on cellular metabolism. These investigators show that expression of an RNA containing about 250 CUG repeats produced the myopathy and myotonia characteristic of DM. The result was surprising in view of the fact that the repeat was placed in the 3′ noncoding region of a skeletal actin transgene, which is unrelated to the *DMPK* gene. As the pathology was dependent on expression of the actin transgene RNA containing the expanded repeat, they concluded that it was the toxic effect of the expanded CUG repeat in the RNA that caused the myotonic myopathy. Together with work showing that expression of transcripts containing expanded CUG repeats inhibits differentiation of cultured muscle cells, these find-



Trouble comes in threes. In many patients with DM, the *DMPK* gene carries a CTG nucleotide triplet repeat (which has expanded from fewer than 38 repeats to more than 100) in the untranslated region of its terminal exon. The expression of other genes at the DM1 locus such as *DMWD* and *SIX5* may be affected by the expanded triplet repeat in the *DMPK* gene. The various pathological features of DM can be explained in an additive model in which decreased expression of the *DMPK* and *SIX5* genes causes heart arrhythmias and cataracts, and the toxicity of the CUG repeats in the *DMPK* transcript causes myotonic myopathy. Shown in black is a CpG island within the *DMPK* gene that is partially hypermethylated in congenital DM.

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ings support the conclusion that expanded CUG repeats in the noncoding region of RNA have a toxic gain-of-function effect on cell metabolism, regardless of whether the repeats are in the *DMPK* transcript or in some other transcript expressed in skeletal muscle.

There are several intriguing possibilities that could explain how RNA transcripts with expanded CUG repeats alter cell metabolism. The accumulation of these transcripts in numerous intranuclear foci has led to the search for proteins that interact with the repeats. One such protein, the CUG binding protein (CUGBP1), has been implicated in regulating the processing of messenger RNA (*7*). Recent in vitro studies have shown that the expanded repeat forms a single extended hairpin that binds and activates a kinase activated by double-stranded RNA (called PKR) (*8*). Additional studies are needed to determine whether CUGBP1, PKR, or other proteins that bind to triplet repeats are involved in DM.

Thus, three different mechanisms might account for the three cardinal features of DM. Cardiac conduction defects could be explained by a decrease in DMPK protein due to aberrant processing of the *DMPK* transcript. A decrease in SIX5 protein due to suppression of *SIX5* gene expression by the CTG repeat expansion in the *DMPK* gene could explain the formation of cataracts. Myotonic myopathy could be explained by the toxic gain-of-function of the expanded CUG repeats in the *DMPK* transcript. It has been further suggested that the triplet repeat expansion in the *DMPK* gene might affect expression of additional genes in this gene-rich region. For example, abnormal processing of the transcript of a nearby gene (*DMWD*) has also been shown in DM cells (*9*); as this gene is highly expressed in testis and brain, it may be implicated in the testicular atrophy and cognitive disturbances that are also associated with DM. The CpG island within the *DMPK* and *SIX5* genes is hypermethylated only in the severe congenital form of DM (*10*), further indicating that additional mechanisms might contribute to specific aspects of the disease. An attractive possibility is that the complex and variable phenotype of DM is caused by the additive effects of the toxicity of the CUG repeat in the *DMPK* transcript, decreased *DMPK* and *SIX5* gene expression, and perhaps altered expression of other genes in the vicinity of *DMPK*.

There is, however, a troublesome problem with this additive model of DM. A subset of families with the clinical characteristics of DM do not carry a triplet repeat expansion in the *DMPK* gene at the DM1 locus on chromosome 19q13.3; instead, they show linkage to a second locus (DM2) on chromosome 3q (*11*). The DM2 families have a myotonic myopathy, cataracts, cardiac conduction defects, and most of the other features characteristic of DM1. If the complex features of DM1 are caused by the additive effects of a toxic gain-of-function of the *DMPK* transcript and altered expression of neighboring genes (certainly *SIX5* and *DMPK* itself, but possibly also *DMWD* and others), then how can a mutation on another chromosome so accurately reproduce the clinical phenotype? The mutation at the DM2 locus has yet to be identified, but evidence that DM2 families show anticipation argues for another repeat expansion disease. Unless there is a parallel genetic universe with a near repetition of the DM1 locus at DM2, the unifying hypothesis must be that expressing an RNA with an expanded repeat can cause the symptoms shared by the DM1 and DM2 families. Given that Mankodi *et al.* used a skeletal muscle–specific promoter to drive the actin transgene containing the expanded triplet repeat, it is possible that expression of this transgene in other tissues would induce still other features of DM. With the Mankodi work another important piece has been added to the DM puzzle, inevitably drawing our attention to the pieces yet to be placed.

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PERSPECTIVES: SOLID STATE PHYSICS

A Question of Dimensions

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Carbon is the element of extremes and opposites and appears to be inexhaustible in providing new insights into the properties of matter. In the last decade, we have seen a great surge in research activity inspired by the discovery of fullerenes and subsequently of carbon nanotubes. On page 1730 of this issue, Hone *et al.* (*1*) investigate the quantized vibrations of carbon nanotubes through their heat capacities. Historically, the explanation of the temperature dependence of the heat capacity in terms of quantized vibrations remains one of the most important turning points in modern physics, ultimately culminating in the formulation of quantum mechanics.

In 1907, Einstein was unifying the nascent theory of radiation quanta, introduced by Planck, with the thermodynamics of solids. This led him to conclude that the vibrations in solids must be quantized as well, as he explained in his groundbreaking paper on "Planck's theory of radiation and the theory of specific heat" (*2*). In this paper, Einstein demonstrated that if the atomic vibrations are quantized according to Planck's relation between the quantum of energy and the vibrational energy, then the heat capacity of a solid will be temperature dependent rather than constant, as given by the Dulong-Petit law of classical thermodynamics. The temperature dependence arises essentially because at low temperatures, thermal motion is insufficient to provide the atoms with the quantum of energy needed to set them in motion. Hence, at low temperatures, they are frozen. In Einstein's picture, the heat capacity increases monotonically from zero to the classical value with increasing temperature. Einstein demonstrated the validity of the theory by comparing it with the available heat capacity data of diamond (see the figure), which, because of its hardness, exhibits these quantum effects compellingly even at elevated temperatures.

Although the trend is obviously reproduced, the theory is not complete: It can be seen already from the figure that Einstein's prediction falls short at low temperatures. Debye generalized the quantization rules to include all lattice vibrations, like standing waves and other normal modes of the system (*3*). This modified the low-temperature behavior such that in simple sys-

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