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Myotonic dystrophy: is a narrow focus obscuring the rest of the field?

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Abstract

Purpose of review—The myotonic dystrophies (DM1 and DM2) are the paradigm for RNA toxicity in disease pathogenesis. The emphasis of this review will be on recent developments and issues in understanding the pathogenesis of DM1 and how this is driving the accelerated pace of translational and therapeutic developments.

Recent findings—RNA toxicity in myotonic dystrophy is now associated with bi-directional antisense transcription, dysregulation of microRNAs and potentially non-ATG-mediated translation of homopolymeric toxic proteins. The role of other RNA-binding proteins beyond MBNL1 and CUGBP1, such as Staufen 1 and DDX5, are being identified and studied with respect to their role in myotonic dystrophy. New functions for MBNL1 in miR-1 biogenesis might have a clinically relevant role in myotonic dystrophy cardiac conduction defects and pathology. Advances are being made in identifying and characterizing small molecules with the potential to disrupt CUG-MBNL1 interactions.

Summary—Mechanisms of RNA toxicity are moving beyond a simplistic ‘foci-centric’ view of DM1 pathogenesis as a spliceopathy due to MBNL1 sequestration. Therapeutic development for myotonic dystrophy is moving rapidly with the development of antisense and small molecule therapies. Clinically, significant emphasis is being placed on biomarker discovery and outcome measures as an essential prelude to clinical trials.

Keywords

MBNL1; myotonic dystrophy; RNA foci; RNA toxicity; therapeutic development

INTRODUCTION

Myotonic dystrophy (DM1 and DM2) is the most common muscular dystrophy in adults with an incidence of about 1 : 8000. Both DM1 and DM2 are autosomal dominant disorders with highly variable clinical presentations affecting multiple systems including skeletal, cardiac and smooth muscles, the brain, the lens, endocrine and immunological functions.

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Conflicts of interest

There are no conflicts of interest.

The most frequent manifestations are cataracts, progressive skeletal muscle wasting, myotonia, smooth muscle dysfunction (e.g. irregular bowel function, swallowing difficulties, cholelithiasis), cardiac conduction defects and a potpourri of central nervous system manifestations (including hypersomnia, vague personality and cognitive changes, dementia and apathy). Adult DM1 typically presents in the late teens to early twenties and progresses over two to four decades. Patients with DM1 have a shortened lifespan of about 55 years [1], with death often caused by unrecognized cardiac arrhythmias and respiratory failure. DM2 is usually of later onset and often follows a less severe course [2]. In most ways, DM2 can be considered a milder clinical form of DM1.

Major clinical distinctions between DM1 and DM2 are the lack of a congenital form and lack of genetic anticipation in DM2. This is likely related to the nature and genetic behavior of the causative mutations. DM1 is caused by an expansion of the (CTG)_n triplet repeat in the 3' untranslated region (3'UTR) of the myotonic dystrophy protein kinase (*DMPK*) gene on chromosome 19q [3], whereas, DM2 is caused by a (CCTG)_n repeat within the first intron of the zinc finger-9 (*ZNF9*) gene on chromosome 3 [4]. Unlike the DM2 mutation, the DM1 mutation shows a propensity to expand through successive generations, providing a molecular basis for the observed increase in severity and earlier age of onset within a family (termed genetic anticipation) and for the congenital form of DM1 that is associated with large (usually >1000) (CTG) expansions [3,5].

MOLECULAR PATHOGENESIS OF MYOTONIC DYSTROPHY: CLINGING TO FOCI

The DM1 and DM2 mutations are transcribed into mRNA, but do not affect the encoded protein sequence. In 1995, Robert Singer's group discovered that the mutant *DMPK* mRNA aggregated within the nucleus in 'RNA foci' [6]. This led to a fervent search for RNA-binding proteins interacting with the CUG repeats [7–9] and experiments designed to address the role of the mutant mRNA in disease. In 1998, Tom Copper's group published a seminal study identifying RNA-splicing defects caused by expression of RNAs containing expanded CUGs [10], and we reported the first clear cellular evidence of RNA toxicity due to mutant *DMPK* 3'UTR mRNAs [11]. Eventually, the toxicity of the expanded CUG mRNAs was confirmed in a mouse model, termed *HSA*^{LR}, which expressed an RNA with (CUG)₂₅₀ within the context of a human skeletal actin (*HSA*) gene [12]. Subsequently, RNA foci and splicing defects were found in DM2 [4,13], thereby cementing the concept of RNA toxicity and establishing DM as the first example of an inherited disease caused by RNA toxicity.

The prevailing model of myotonic dystrophy pathogenesis could be termed 'foci-centric'. It is posited that the RNA aggregates sequester RNA-binding proteins, primarily the Muscleblind-like family (MBNL1–3), leading to a functional deficiency of these proteins; and that the plethora of resulting splicing defects result in a 'spliceopathy' that manifests clinically as myotonic dystrophy [14]. Experimental evidence supporting this includes the colocalization of MBNL1 with RNA foci in cells, mouse models, and a variety of affected tissues from patients [15–17]. There is also a growing list of splicing defects caused by overexpression of RNAs with (CUG)_n expansions or depletion of MBNL1, that is found in

model systems and in patient cells and tissues [18]. This model of myotonic dystrophy pathogenesis has driven most of the research and therapeutic developments in the past decade, leading to a portfolio of publications narrowly and repeatedly focused on reinforcing the model. However, until recently, only one splicing defect, that for muscle-specific chloride channel (*CLCN1*) had been clearly shown to cause DM1 pathology [19]. In the past year, a French group provided substantial evidence that the splicing defect in *BIN1* (bridging integrator-1), a protein implicated in T-tubule biosynthesis, is associated with muscle weakness and myopathy in DM1 [20[■]]. Nevertheless, in a recent study most of the DM1-associated splicing defects were present in a number of mouse models of other muscular dystrophies and myotoxin-mediated damage/regeneration and are likely secondary to the regenerative response in damaged muscle [21[■]]. Thus, ‘spliceopathies’ exist in most conditions associated with muscle regeneration and the authors provide a cautionary statement about assigning cause and effect.

Evidence for MBNL1 sequestration is based on: MBNL1 deficiency in mice resulting in several DM1 phenotypes including myotonia, splicing defects and cataracts [22]; increasing MBNL1 levels by gene therapy lessens splicing defects and myotonia in the tibialis anterior muscle of *HSA^{LR}* mice [23]; and (CAG) morpholino oligonucleotides designed to compete with MBNL1 for binding to the (CUG) RNAs are similarly effective in *HSA^{LR}* mice [24]. However, studies with a panel of monoclonal antibodies against MBNL1–3 in patient cells and tissues found only partial sequestration of MBNL1 [25]. In a recent elegant study using advanced microscopy and RNA labeling designed to track single RNAs in living cells, Querido *et al.* [26[■]] studied the kinetics of (CUG)_n RNA aggregation. Interestingly, they found that the interaction between an RNA with (CUG)₁₄₅ and MBNL1 was not static, but actually a stochastic process in which the RNA–MBNL1 aggregates are constantly forming and dissolving. This suggests that *in vivo*, MBNL1 sequestration is actually a transient state. In addition, the mutant *DMPK* mRNA likely exists in a ‘free state’ as well as in RNA foci.

So what? The natural assumption since the discovery of RNA foci in DM1 is that RNA aggregates must be bad. But, if the mutant *DMPK* mRNA exists in a free state and in RNA foci, which state is toxic or more toxic? Or, do the different states cause distinct kinds of RNA toxicity (that is different molecular mechanisms) leading to different aspects of DM1 pathology? Why ask such questions, when after all, the *HSA^{LR}* mice, and MBNL1 and MBNL2 deficient mice all have myotonic dystrophy? But, they actually express only a limited aspect of the myotonic dystrophy phenotype (namely myotonia and RNA splicing defects), and do not exhibit other key features such as progressive muscle wasting and weakness. In the past year, Tom Cooper’s group using a muscle-specific inducible mouse model of CUG-binding protein (CUGBP1) overexpression showed that this leads to significant muscle weakness, wasting and histopathology along with some RNA-splicing defects [27]. CUGBP1 was the first RNA-binding protein implicated in DM1 [7] and though it does not colocalize with the RNA foci, it has been proposed that it interacts with the ‘free’ mutant *DMPK* transcript [28]. Several studies in DM1 patients and RNA-toxicity mice exhibiting muscle weakness and pathology [29,30] have shown increased levels of CUGBP1 in affected muscles. Notably, the *HSA^{LR}* mice do not have elevated CUGBP1 levels [31]. The major difference is that the *HSA^{LR}* mice express a toxic RNA with only an expanded

(CUG) tract, whereas the other mouse models express the entire *DMPK* 3' UTR mRNA. After all, the (CUG) expansion does not exist in isolation in DM1 cells; it exists as part of a processed mutant *DMPK* mRNA, in which the sequences flanking the (CUG) tract likely impart a three-dimensional structure to the toxic RNA that is not recapitulated by expressing only the (CUG) sequences. This fits with previous work that showed RNA foci were insufficient to cause myogenic defects and that the entire *DMPK* 3' UTR was also necessary [32]. In fact, the *HSA^{LR}* mouse model may be a better model of DM2, in which only the expanded (CCUG) RNA is found in the RNA foci [33].

THINGS ARE NEVER AS EASY AS THEY SEEM

To complicate matters, my laboratory developed the first inducible/reversible mouse model of RNA toxicity in which key aspects of DM1 pathology including cardiac conduction defects, skeletal muscle pathology and RNA-splicing defects are seen in the absence of any discernible RNA foci or MBNL1 sequestration [29]. Surprisingly, these mice develop the phenotypes while overexpressing a normal *DMPK* 3'UTR mRNA (i.e. with (CUG)₅) and they are all reversible upon silencing of toxic RNA expression. Our ongoing studies show that these mice have relevant functional deficits (e.g. running, grip strength), muscle wasting and histopathology (unpublished). These mice bring into question the role of RNA foci in DM1 pathogenesis, and some have speculated that MBNL1 is still able to interact with the (CUG)₅ *DMPK* 3'UTR transcript resulting in MBNL1 sequestration on submicroscopic 'foci' [34]. Indeed, using RNA-IP, we find that MBNL1 interacts with the normal *DMPK* mRNA in DM1 cells and the transgene mRNA from our mice (unpublished). Despite the robust nature of the model and its utility in uncovering novel mechanisms of RNA toxicity, such as the ectopic expression of NKX2-5 and increased levels of Staufien-1 in skeletal muscle [35,36^{*}], skepticism abounds amongst devotees, who are stuck on RNA foci.

Though MBNL1 and CUGBP1 are characterized primarily as RNA splicing factors, they may have additional functions that may be relevant to DM1 pathogenesis. Recently, Rau *et al.* [37^{**}] reported that MBNL1 binds to premiR-1 and blocks the binding of LIN28, a protein involved in miRNA biogenesis. They proposed that MBNL1 sequestration in DM1 allowed LIN28 to bind and repress synthesis of miR-1 in DM1 cardiac tissues and increased expression of miR-1 targets such as connexin 43 resulting in cardiac pathology. As yet, there is no consensus with regard to miRNA levels in DM1 skeletal muscles [38^{*},39^{*}]. Nevertheless, these studies highlight the potential role of miRNAs in DM1.

In addition, a number of other RNA-binding proteins have now been implicated in DM1. Studies of proteins interacting with MBNL1 have revealed a large complex of RNA-binding proteins (e.g. hnRNP-H, DDX5 and hnRNP-F) whose levels increased in DM1 myoblasts with the potential to reinforce the splicing defects associated with MBNL1 deficiency [40^{**}, 41^{*}]. In contrast, it was recently reported that Staufien-1, an RNA-binding protein normally associated with RNA translation, was increased in DM1 tissues and RNA toxicity models, and that it increases cytoplasmic export of mutant *DMPK* 3'UTR mRNAs and has a novel function as a splicing factor that might play a positive role in modulating some RNA splicing defects seen in DM1 [36^{*}].

Novel concepts for RNA toxicity continue to be proposed. For instance, experiments in *Drosophila* have shown that triplet repeat derived small interfering RNAs (siRNAs) enhance RNA toxicity [42^{***}]. This builds on the concept that bi-directional transcription at loci associated with repeat disorders is the norm. Another study proposed that these loci, including the DM1 locus, may be associated with non-ATG-mediated translation of the repeat sequences in all six potential reading frames (both sense and antisense) resulting in toxic homopolymeric proteins that might contribute to disease pathogenesis [43^{***}]. For myotonic dystrophy it is questionable how significant this is, as the mutant transcripts in both DM1 and DM2 are stuck in the nucleus and thus not available to the translational machinery, unless one were to speculate that the antisense transcripts from these loci are not subject to nuclear retention.

There is no perfect animal model for myotonic dystrophy. They all have limitations, whether it be the number of repeats [29] or nature of the repeats [30] or toxic RNA [12], level of transgene expression [44], or limited tissue-specific expression [12,30]. But each may prove useful in its own right. These studies only highlight the fact that the ‘foci-centric’, spliceopathy view of DM1 pathogenesis may have limitations and that an objective examination of new observations might provide a truer, nuanced understanding of RNA toxicity in DM1.

CLINICAL RESEARCH AND THERAPEUTICS DEVELOPMENT

On the clinical front a significant and spirited debate is being waged about the merits of prophylactic implantable cardioverter defibrillators (ICDs) [45^{***}] versus pacemaker devices for DM1 patients [46^{*}]. This is an important issue as sudden arrhythmias are one of the principal causes of death in myotonic dystrophy patients. As developments in therapeutics have been promising, the need for biomarkers and outcome measures is imperative. A number are being assessed including grip strength meters and RNA-splicing targets. A recent study highlighted the utility of MRI showing that residual muscle volume in tibialis anterior muscles correlated well ($r = 0.91$) with maximal ankle dorsiflexor strength [47^{***}].

In recent years, work in myotonic dystrophy therapeutics has been driven by the ‘foci-centric’ view with the goal of displacing MBNL1 from the RNA foci. Two general approaches have been taken using oligonucleotides and development of small molecules. The first successful approach was a CAG morpholino, which displaced MBNL1 from the mutant *DMPK* transcript and corrected myotonia and RNA-splicing defects [24]. The first small molecule was pentamidine, an antimicrobial that bound to CUG RNAs and displaced MBNL1 [48]. However, its effects were modest due to its high toxicity and narrow therapeutic window. More recently ABP1, a hexapeptide composed of D-amino acids, showed substantial benefit in both *Drosophila* and mouse models with improvement in muscle histology, splicing and *Clcn1* expression [49^{***}]. Several other groups are also identifying compounds and promising leads [50^{*},51^{*},52]. One nagging concern about these strategies is that we don’t know if foci are playing a protective role. What if the trade-off for RNA foci is myotonia and RNA-splicing defects? What if freeing mutant *DMPK* RNAs from the shackles of MBNL1 worsens RNA toxicity in other ways?

Perhaps the best way to treat RNA toxicity is to just get rid of the ‘poison’. This is clearly what our mouse model demonstrated [29]. This strategy is being actively pursued using antisense oligonucleotides (ASOs) [53,54^{***}], and gene therapy vectors expressing antisense RNAs [55^{*}]. The issues and concerns with gene therapy are well known, and the primary issue with ASOs is delivery into tissues and concerns about the unknown aspects of long-term toxicity.

CONCLUSION

RNA toxicity in DM1 is likely more complex and multifaceted than just the ‘foci-centric’ view that is commonly perpetuated. Nonetheless, significant progress is being made and continued investigation of RNA toxicity in myotonic dystrophy is providing new leads for therapeutic development.

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REFERENCES AND RECOMMENDED READING

References

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 646).

1. Mathieu J, Allard P, Potvin L, et al. A 10-year study of mortality in a cohort of patients with myotonic dystrophy. *Neurology*. 1999; 52:1658–1662. [PubMed: 10331695]
2. Ranum LP, Day JW. Myotonic dystrophy: clinical and molecular parallels between myotonic dystrophy type 1 and type 2. *Curr Neurol Neurosci Rep*. 2002; 2:465–470. [PubMed: 12169228]
3. Mahadevan M, Tsilfidis C, Sabourin L, et al. Myotonic dystrophy mutation: an unstable CTG repeat in the 3' untranslated region of the gene. *Science*. 1992; 255:1253–1255. [PubMed: 1546325]
4. Liquori CL, Ricker K, Moseley ML, et al. Myotonic dystrophy type 2 caused by a CCTG expansion in intron 1 of ZNF9. *Science*. 2001; 293:864–867. [PubMed: 11486088]
5. Tsilfidis C, MacKenzie AE, Mettler G, et al. Correlation between CTG trinucleotide repeat length and frequency of severe congenital myotonic dystrophy. *Nat Genet*. 1992; 1:192–195. [PubMed: 1303233]
6. Taneja KL, McCurrach M, Schalling M, et al. Foci of trinucleotide repeat transcripts in nuclei of myotonic dystrophy cells and tissues. *J Cell Biol*. 1995; 128:995–1002. [PubMed: 7896884]
7. Timchenko LT, Miller JW, Timchenko NA, et al. Identification of a (CUG)_n triplet repeat RNA-binding protein and its expression in myotonic dystrophy. *Nucleic Acids Res*. 1996; 24:4407–4414. [PubMed: 8948631]
8. Tiscornia G, Mahadevan MS. Myotonic dystrophy: the role of the CUG triplet repeats in splicing of a novel DMPK exon and altered cytoplasmic DMPK mRNA isoform ratios. *Mol Cell*. 2000; 5:959–967. [PubMed: 10911990]

9. Miller JW, Urbinati CR, Teng-Ummuay P, et al. Recruitment of human muscleblind proteins to (CUG)_n expansions associated with myotonic dystrophy. *Embo J*. 2000; 19:4439–4448. [PubMed: 10970838]
10. Philips AV, Timchenko LT, Cooper TA. Disruption of splicing regulated by a CUG-binding protein in myotonic dystrophy. *Science*. 1998; 280:737–741. [PubMed: 9563950]
11. Amack JD, Paguio AP, Mahadevan MS. Cis and trans effects of the myotonic dystrophy (DM) mutation in a cell culture model. *Hum Mol Genet*. 1999; 8:1975–1984. [PubMed: 10484765]
12. Mankodi A, Logigian E, Callahan L, et al. Myotonic dystrophy in transgenic mice expressing an expanded CUG repeat. *Science*. 2000; 289:1769–1773. [PubMed: 10976074]
13. Savkur RS, Philips AV, Cooper TA, et al. Insulin receptor splicing alteration in myotonic dystrophy type 2. *Am J Hum Genet*. 2004; 74:1309–1313. [PubMed: 15114529]
14. Ranum LP, Day JW. Myotonic dystrophy: RNA pathogenesis comes into focus. *Am J Hum Genet*. 2004; 74:793–804. [PubMed: 15065017]
15. Mankodi A, Urbinati CR, Yuan QP, et al. Muscleblind localizes to nuclear foci of aberrant RNA in myotonic dystrophy types 1 and 2. *Hum Mol Genet*. 2001; 10:2165–2170. [PubMed: 11590133]
16. Jiang H, Mankodi A, Swanson MS, et al. Myotonic dystrophy type 1 is associated with nuclear foci of mutant RNA, sequestration of muscleblind proteins and deregulated alternative splicing in neurons. *Hum Mol Genet*. 2004; 13:3079–3088. [PubMed: 15496431]
17. Mankodi A, Lin X, Blaxall BC, et al. Nuclear RNA foci in the heart in myotonic dystrophy. *Circ Res*. 2005; 97:1152–1155. [PubMed: 16254211]
18. Du H, Cline MS, Osborne RJ, et al. Aberrant alternative splicing and extracellular matrix gene expression in mouse models of myotonic dystrophy. *Nat Struct Mol Biol*. 2010; 17:187–193. [PubMed: 20098426]
19. Wheeler TM, Lueck JD, Swanson MS, et al. Correction of CIC-1 splicing eliminates chloride channelopathy and myotonia in mouse models of myotonic dystrophy. *J Clin Invest*. 2007; 117:3952–3957. [PubMed: 18008009]
20. Fugier C, Klein AF, Hammer C, et al. Misregulated alternative splicing of BIN1 is associated with T tubule alterations and muscle weakness in myotonic dystrophy. *Nat Med*. 2011; 17:720–725. This study identified BIN1 splicing was affected in DM1 and importantly, using mouse models they showed that altering BIN1 splicing led to muscle weakness. [PubMed: 21623381]
21. Orengo JP, Ward AJ, Cooper TA. Alternative splicing dysregulation secondary to skeletal muscle regeneration. *Ann Neurol*. 2011; 69:681–690. Using a number of mouse models and a couple of myotoxins, this study provided the cautionary message that splicing defects in muscle need to be interpreted carefully in the context of muscle regeneration. [PubMed: 21400563]
22. Kanadia RN, Johnstone KA, Mankodi A, et al. A muscleblind knockout model for myotonic dystrophy. *Science*. 2003; 302:1978–1980. [PubMed: 14671308]
23. Kanadia RN, Shin J, Yuan Y, et al. Reversal of RNA missplicing and myotonia after muscleblind overexpression in a mouse poly(CUG) model for myotonic dystrophy. *Proc Natl Acad Sci U S A*. 2006; 103:11748–11753. [PubMed: 16864772]
24. Wheeler TM, Sobczak K, Lueck JD, et al. Reversal of RNA dominance by displacement of protein sequestered on triplet repeat RNA. *Science*. 2009; 325:336–339. [PubMed: 19608921]
25. Holt I, Jacquemin V, Fardaei M, et al. Muscleblind-like proteins: similarities and differences in normal and myotonic dystrophy muscle. *Am J Pathol*. 2009; 174:216–227. [PubMed: 19095965]
26. Querido E, Gallardo F, Beaudoin M, et al. Stochastic and reversible aggregation of mRNA with expanded CUG-triplet repeats. *J Cell Sci*. 2011; 124(Pt 10):1703–1714. This work offers new insights into the behaviour of the mutant *DMPK* transcript in living cells and demonstrates that RNA foci are dynamic structures. [PubMed: 21511730]
27. Ward AJ, Rimer M, Killian JM, et al. CUGBP1 overexpression in mouse skeletal muscle reproduces features of myotonic dystrophy type 1. *Hum Mol Genet*. 2010; 19:3614–3622. [PubMed: 20603324]
28. Timchenko NA, Cai ZJ, Welm AL, et al. RNA CUG repeats sequester CUGBP1 and alter protein levels and activity of CUGBP1. *J Biol Chem*. 2001; 276:7820–7826. [PubMed: 11124939]
29. Mahadevan MS, Yadava RS, Yu Q, et al. Reversible model of RNA toxicity and cardiac conduction defects in myotonic dystrophy. *Nat Genet*. 2006; 38:1066–1070. [PubMed: 16878132]

30. Orengo JP, Chambon P, Metzger D, et al. Expanded CTG repeats within the DMPK 3' UTR causes severe skeletal muscle wasting in an inducible mouse model for myotonic dystrophy. *Proc Natl Acad Sci U S A*. 2008; 105:2646–2651. [PubMed: 18272483]
31. Lin X, Miller JW, Mankodi A, et al. Failure of MBNL1-dependent postnatal splicing transitions in myotonic dystrophy. *Hum Mol Genet*. 2006; 15:2087–2097. [PubMed: 16717059]
32. Amack JD, Mahadevan MS. The myotonic dystrophy expanded CUG repeat tract is necessary but not sufficient to disrupt C2C12 myoblast differentiation. *Hum Mol Genet*. 2001; 10:1879–1887. [PubMed: 11555624]
33. Margolis JM, Schoser BG, Moseley ML, et al. DM2 intronic expansions: evidence for CCUG accumulation without flanking sequence or effects on ZNF9 mRNA processing or protein expression. *Hum Mol Genet*. 2006; 15:1808–1815. [PubMed: 16624843]
34. Wheeler TM, Thornton CA. Myotonic dystrophy: RNA-mediated muscle disease. *Curr Opin Neurol*. 2007; 20:572–576. [PubMed: 17885447]
35. Yadava RS, Frenzel-McCardell CD, Yu Q, et al. RNA toxicity in myotonic muscular dystrophy induces NKX2-5 expression. *Nat Genet*. 2008; 40:61–68. [PubMed: 18084293]
36. Ravel-Chapuis A, Belanger G, Yadava RS, et al. The RNA-binding protein Stau1 is increased in DM1 skeletal muscle and promotes alternative premRNA splicing. *J Cell Biol*. 2012; 196:699–712. This intriguing study identified a new player, the RNA-binding protein, Stau1, is increased in DM1 skeletal muscles, HASLR mice, and a mouse model overexpressing the *DMPK* 3'UTR(CTG)₅ and (CTG)₂₀₀. It also reported on new functions for Stau1 that may be relevant to DM1 pathology. [PubMed: 22431750]
37. Rau F, Freyermuth F, Fugier C, et al. Misregulation of miR-1 processing is associated with heart defects in myotonic dystrophy. *Nat Struct Mol Biol*. 2011; 18:840–845. This study suggests that MBNL1 may have important roles in miRNA biogenesis and perturbations in miRNAs could affect major phenotypes (e.g., cardiac function) in DM1. [PubMed: 21685920]
38. Perbellini R, Greco S, Sarra-Ferraris G, et al. Dysregulation and cellular mislocalization of specific miRNAs in myotonic dystrophy type 1. *Neuromuscul Disord*. 2011; 21:81–88. This report showed that a number of miRNAs were altered in DM1 skeletal muscles, but differed in the effect on miR-1 as compared to reference [37]. [PubMed: 21169019]
39. Gambardella S, Rinaldi F, Lepore SM, et al. Overexpression of microRNA-206 in the skeletal muscle from myotonic dystrophy type 1 patients. *J Transl Med*. 2010; 8:48. This report also looked at a number of miRNA targets in DM1 muscles and found no effect on miR-1, but significantly elevated miR-206, another key miRNA in skeletal muscle. [PubMed: 20487562]
40. Paul S, Dansithong W, Jog SP, et al. Expanded CUG repeats dysregulate RNA splicing by altering the stoichiometry of the muscleblind 1 complex. *J Biol Chem*. 2011; 286:38427–38438. This is probably the most comprehensive attempt to identify proteins interacting with MBNL1 that may be affected by the toxic RNA in DM1 cells. The authors identified a number of interesting RNA-binding proteins. [PubMed: 21900255]
41. Laurent FX, Sureau A, Klein AF, et al. New function for the RNA helicase p68/DDX5 as a modifier of MBNL1 activity on expanded CUG repeats. *Nucleic Acids Res*. 2012; 40:3159–3171. This study identified the RNA helicase, DDX5, colocalized with RNA foci and that it may be a positive regulator of MBNL1 interactions with the toxic RNA. Perhaps a new target for therapeutic intervention? [PubMed: 22156369]
42. Yu Z, Teng X, Bonini NM. Triplet repeat-derived siRNAs enhance RNA-mediated toxicity in a *Drosophila* model for myotonic dystrophy. *PLoS Genet*. 2011; 7:e1001340. This study expanded the mechanisms of RNA toxicity by showing that siRNAs could be generated from the bi-directional transcription of CTG repeat sequences and that this had significant toxicity by targeting genes that normally contain repeat sequences. [PubMed: 21437269]
43. Zu T, Gibbens B, Doty NS, et al. Non-ATG-initiated translation directed by microsatellite expansions. *Proc Natl Acad Sci U S A*. 2011; 108:260–265. This study suggested that repeat sequences are able to generate toxic homopolymeric proteins by breaking one of the fundamental concepts in molecular biology (i.e. the need for an ATG start codon). Suggests an additional layer of triplet repeat toxicity. [PubMed: 21173221]

44. Seznec H, Agbulut O, Sergeant N, et al. Mice transgenic for the human myotonic dystrophy region with expanded CTG repeats display muscular and brain abnormalities. *Hum Mol Genet.* 2001; 10:2717–2726. [PubMed: 11726559]
- 45■. Bhakta D, Shen C, Kron J, et al. Pacemaker and implantable cardioverter-defibrillator use in a US myotonic dystrophy type 1 population. *J Cardiovasc Electrophysiol.* 2011; 22:1369–1375. This prospective multicenter, 10-year clinical study of patients with implantable devices found decreased mortality rates in patients with ICDs and suggested that ICDs should be considered instead of pacemakers for DM1 patients. [PubMed: 22035077]
- 46■. Laurent V, Pellieux S, Corcia P, et al. Mortality in myotonic dystrophy patients in the area of prophylactic pacing devices. *Int J Cardiol.* 2011; 150:54–58. This study of 100 myotonic dystrophy patients followed over 14 years found a very low sudden death frequency, suggesting that aggressive implantation of ICDs may not be warranted. [PubMed: 20227121]
- 47■. Hiba B, Richard N, Hebert LJ, et al. Quantitative assessment of skeletal muscle degeneration in patients with myotonic dystrophy type 1 using MRI. *J Magn Reson Imaging.* 2012; 35:678–685. This study found that MRI of tibialis anterior muscles could be used as a biomarker for evaluating muscle degeneration with consistency. This may be a valuable tool in clinical trials. [PubMed: 22069222]
48. Warf MB, Nakamori M, Matthys CM, et al. Pentamidine reverses the splicing defects associated with myotonic dystrophy. *Proc Natl Acad Sci U S A.* 2009; 106:18551–18556. [PubMed: 19822739]
- 49■. Garcia-Lopez A, Llamusi B, Orzaez M, et al. In vivo discovery of a peptide that prevents CUG-RNA hairpin formation and reverses RNA toxicity in myotonic dystrophy models. *Proc Natl Acad Sci U S A.* 2011; 108:11866–11871. This study used a very interesting approach to identify small peptides capable of disrupting MBNL1–toxic RNA interactions. The potential advantage of these molecules with regard to drug development and toxicities is significant. [PubMed: 21730182]
- 50■. Childs-Disney JL, Hoskins J, Rzuczek SG, et al. Rationally designed small molecules targeting the RNA that causes myotonic dystrophy type 1 are potently bioactive. *ACS Chem Biol.* 2012; 7:856–862. This group is using rational drug design to identify a number of small molecules capable of disrupting MBNL1–RNA interactions and showed their benefit in disrupting RNA foci. [PubMed: 22332923]
- 51■. Wong CH, Fu Y, Ramisetty SR, et al. Selective inhibition of MBNL1–CCUG interaction by small molecules toward potential therapeutic agents for myotonic dystrophy type 2 (DM2). *Nucleic Acids Res.* 2011; 39:8881–8890. Another article from Matt Disney’s group using RNA structure analysis for rational drug design. These approaches are yielding new compounds that are under-represented in the usual small molecule libraries used for high-throughput screens. [PubMed: 21768123]
52. Parkesh R, Childs-Disney JL, Nakamori M, et al. Design of a bioactive small molecule that targets the myotonic dystrophy type 1 RNA via an RNA motif–ligand database and chemical similarity searching. *J Am Chem Soc.* 2012; 134:4731–4742. [PubMed: 22300544]
53. Mulders SA, van den Broek WJ, Wheeler TM, et al. Triplet-repeat oligonucleotide-mediated reversal of RNA toxicity in myotonic dystrophy. *Proc Natl Acad Sci U S A.* 2009; 106:13915–13920. [PubMed: 19667189]
- 54■. Lee JE, Bennett CF, Cooper TA. RNase H-mediated degradation of toxic RNA in myotonic dystrophy type 1. *Proc Natl Acad Sci U S A.* 2012; 109:4221–4226. This article reports on the use of ‘gapmers’ as an antisense oligonucleotide therapy strategy to target the mutant *DMPK* for degradation. The gapmers are able to recruit the endogenous RNase-H pathway to target the mutant *DMPK* mRNA. Gapmers are becoming a standard design for ASOs. [PubMed: 22371589]
- 55■. Francois V, Klein AF, Beley C, et al. Selective silencing of mutated mRNAs in DM1 by using modified hU7-siRNAs. *Nat Struct Mol Biol.* 2011; 18:85–87. This article reports on the use of lentiviral vectors in gene therapy for DM1. They demonstrate selective targeting of the mutant *DMPK* mRNA; a strategy to avoid effects on DMPK protein. [PubMed: 21186365]