



Publications by Research Fellows

Though the research fellowship program, Myotonic strives to increase the scope and quality of publications in DM research. Since 2009, Myotonic has committed over \$3M in total research funding to 34 fellows from 17 different institutions in five countries. Research has spanned a variety of areas, including exploring the design of synthetic proteins with potential therapeutic value, improving the current understanding of molecular events contributing to the cardiac symptoms in DM1 patients, generating a mouse model of DM2 to better understand the contribution of both the mutant Ribonucleic acid (RNA)s and mutant RAN proteins, and providing new mechanistic insights into central nervous system-associated behavioral symptoms in DM. This research has garnered a range of publications, including:

1. Kalsotra A, Wang K, Li PF, Cooper TA. MicroRNAs coordinate an alternative splicing network during mouse postnatal heart development. *Genes & Development*. 2010;24(7):653-658. doi:10.1101/gad.1894310

ABSTRACT: Alternative splicing transitions have been identified recently as a conserved component of vertebrate heart remodeling during postnatal development. Here we report that the targeted deletion of Dicer, specifically in adult mouse myocardium, reveals the role of microRNAs (miRNAs) in regulating networks of postnatal splicing transitions and in maintaining adult splicing programs. We demonstrate a direct role for miR-23a/b in the dramatic postnatal down-regulation of CUGBP and ETR-3-like factor (CELF) proteins that regulate nearly half of developmentally regulated splicing transitions in the heart. These findings define a hierarchy in which rapid postnatal up-regulation of specific miRNAs controls expression of alternative splicing regulators and the subsequent splicing transitions of their downstream targets.

2. Kalsotra A, Cooper TA. Functional consequences of developmentally regulated alternative splicing. *Nat Rev Genet*. 2011;12(10):715-729. doi:10.1038/nrg3052

ABSTRACT: Genome-wide analyses of metazoan transcriptomes have revealed an unexpected level of mRNA diversity that is generated by alternative splicing. Recently, regulatory networks have been identified through which splicing promotes dynamic remodelling of the transcriptome to promote physiological changes, which involve robust and coordinated alternative splicing transitions. The regulation of splicing in yeast, worms, flies and vertebrates affects a variety of biological processes. The functional classes of genes that are regulated by alternative splicing include both those with widespread homeostatic activities and those with cell-type-specific functions. Alternative splicing can drive determinative physiological change or can have a permissive role by providing mRNA variability that is used by other regulatory mechanisms.



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- Purcell J, Oddo JC, Wang ET, Berglund JA. Combinatorial Mutagenesis of MBNL1 Zinc Fingers Elucidates Distinct Classes of Regulatory Events. *Molecular and Cellular Biology*. 2012;32(20):4155-4167. doi:10.1128/MCB.00274-12

ABSTRACT: The RNA binding protein and alternative splicing factor Muscleblind-like 1 (MBNL1) has been a topic of intense study due to its role in myotonic dystrophy (DM) pathogenesis. MBNL1 contains four zinc finger (ZF) RNA binding domains arranged in two pairs. Through combinatorial mutagenesis of the ZF domains, we demonstrate that the pairs of ZFs have differential affinity for RNA and subsequently differential splicing activities. We evaluated splicing and binding activity for six MBNL1-mediated splicing events and found that the splicing activity profiles for the ZF mutants vary among transcripts. Clustering analysis of splicing profiles revealed that two distinct classes of MBNL1 pre-mRNA substrates exist. For some of the RNA transcripts tested, binding and splicing activity of the ZF mutants correlated. However, for some transcripts it appears that MBNL1 exerts robust splicing activity in the absence of RNA binding. We demonstrate that functionally distinct classes of MBNL1-mediated splicing events exist as defined by requirements for ZF-RNA interactions.

- Coonrod LA, Nakamori M, Wang W, et al. Reducing Levels of Toxic RNA with Small Molecules. *ACS Chem Biol*. 2013;8(11):2528-2537. doi:10.1021/cb400431f

ABSTRACT: Myotonic dystrophy (DM) is one of the most common forms of muscular dystrophy. DM is an autosomal dominant disease caused by a toxic gain of function RNA. The toxic RNA is produced from expanded noncoding CTG/CCTG repeats, and these CUG/CCUG repeats sequester the Muscleblind-like (MBNL) family of RNA binding proteins. The MBNL proteins are regulators of alternative splicing, and their sequestration has been linked with mis-splicing events in DM. A previously reported screen for small molecules found that pentamidine was able to improve splicing defects associated with DM. Biochemical experiments and cell and mouse model studies of the disease indicate that pentamidine and related compounds may work through binding the CTG*CAG repeat DNA to inhibit transcription. Analysis of a series of methylene linker analogues of pentamidine revealed that heptamidine reverses splicing defects and rescues myotonia in a DM1 mouse model.

- Du J, Campau E, Soragni E, Jespersen C, Gottesfeld JM. Length-dependent CTG·CAG triplet-repeat expansion in myotonic dystrophy patient-derived induced pluripotent stem cells. *Hum Mol Genet*. 2013;22(25):5276-5287. doi:10.1093/hmg/ddt386

ABSTRACT: Myotonic dystrophy type 1 (DM1) is an inherited dominant muscular dystrophy caused by expanded CTG·CAG triplet repeats in the 3' untranslated region of



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the DMPK1 gene, which produces a toxic gain-of-function CUG RNA. It has been shown that the severity of disease symptoms, age of onset and progression are related to the length of the triplet repeats. However, the mechanism(s) of CTG·CAG triplet-repeat instability is not fully understood. Herein, induced pluripotent stem cells (iPSCs) were generated from DM1 and Huntington's disease patient fibroblasts. We isolated 41 iPSC clones from DM1 fibroblasts, all showing different CTG·CAG repeat lengths, thus demonstrating somatic instability within the initial fibroblast population. During propagation of the iPSCs, the repeats expanded in a manner analogous to the expansion seen in somatic cells from DM1 patients. The correlation between repeat length and expansion rate identified the interval between 57 and 126 repeats as being an important length threshold where expansion rates dramatically increased. Moreover, longer repeats showed faster triplet-repeat expansion. However, the overall tendency of triplet repeats to expand ceased on differentiation into differentiated embryoid body or neurospheres. The mismatch repair components MSH2, MSH3 and MSH6 were highly expressed in iPSCs compared with fibroblasts, and only occupied the DMPK1 gene harboring longer CTG·CAG triplet repeats. In addition, shRNA silencing of MSH2 impeded CTG·CAG triplet-repeat expansion. The information gained from these studies provides new insight into a general mechanism of triplet-repeat expansion in iPSCs.

6. Wang ET, Cody NAL, Jog S, et al. Transcriptome-wide Regulation of Pre-mRNA Splicing and mRNA Localization by Muscleblind Proteins. *Cell*. 2012;150(4):710-724. doi:10.1016/j.cell.2012.06.041

ABSTRACT: The muscleblind-like (Mbnl) family of RNA-binding proteins plays important roles in muscle and eye development and in myotonic dystrophy (DM), in which expanded CUG or CCUG repeats functionally deplete Mbnl proteins. We identified transcriptome-wide functional and biophysical targets of Mbnl proteins in brain, heart, muscle, and myoblasts by using RNA-seq and CLIP-seq approaches. This analysis identified several hundred splicing events whose regulation depended on Mbnl function in a pattern indicating functional interchangeability between Mbnl1 and Mbnl2. A nucleotide resolution RNA map associated repression or activation of exon splicing with Mbnl binding near either 3' splice site or near the downstream 5' splice site, respectively. Transcriptomic analysis of subcellular compartments uncovered a global role for Mbnls in regulating localization of mRNAs in both mouse and *Drosophila* cells, and Mbnl-dependent translation and protein secretion were observed for a subset of mRNAs with Mbnl-dependent localization. These findings hold several new implications for DM pathogenesis.



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7. Tabebordbar M, Wang ET, Wagers AJ. Skeletal Muscle Degenerative Diseases and Strategies for Therapeutic Muscle Repair. *Annu Rev Pathol Mech Dis*. 2013;8(1):441-475. doi:10.1146/annurev-pathol-011811-132450

ABSTRACT: Skeletal muscle is a highly specialized, postmitotic tissue that must withstand chronic mechanical and physiological stress throughout life to maintain proper contractile function. Muscle damage or disease leads to progressive weakness and disability, and manifests in more than 100 different human disorders. Current therapies to treat muscle degenerative diseases are limited mostly to the amelioration of symptoms, although promising new therapeutic directions are emerging. In this review, we discuss the pathological basis for the most common muscle degenerative diseases and highlight new and encouraging experimental and clinical opportunities to prevent or reverse these afflictions.

8. van Blitterswijk M, Wang ET, Friedman BA, et al. Characterization of FUS Mutations in Amyotrophic Lateral Sclerosis Using RNA-Seq. Cai H, ed. *PLoS ONE*. 2013;8(4):e60788. doi:10.1371/journal.pone.0060788

ABSTRACT: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease resulting in severe muscle weakness and eventual death by respiratory failure. Although little is known about its pathogenesis, mutations in fused in sarcoma/translated in liposarcoma (FUS) are causative for familial ALS. FUS is a multifunctional protein that is involved in many aspects of RNA processing. To elucidate the role of FUS in ALS, we overexpressed wild-type and two mutant forms of FUS in HEK-293T cells, as well as knocked-down FUS expression. This was followed by RNA-Seq to identify genes which displayed differential expression or altered splicing patterns. Pathway analysis revealed that overexpression of wild-type FUS regulates ribosomal genes, whereas knock-down of FUS additionally affects expression of spliceosome related genes. Furthermore, cells expressing mutant FUS displayed global transcription patterns more similar to cells overexpressing wild-type FUS than to the knock-down condition. This observation suggests that FUS mutants do not contribute to the pathogenesis of ALS through a loss-of-function. Finally, our results demonstrate that the R521G and R522G mutations display differences in their influence on transcription and splicing. Taken together, these results provide additional insights into the function of FUS and how mutations contribute to the development of ALS.

9. Gao Z, Cooper TA. Antisense oligonucleotides: rising stars in eliminating RNA toxicity in myotonic dystrophy. *Hum Gene Ther*. 2013;24(5):499-507. doi:10.1089/hum.2012.212

ABSTRACT: Myotonic dystrophy (DM) is a dominantly inherited, multisystemic disease caused by expanded CTG (type 1, DM1) or CCTG (type 2, DM2) repeats in untranslated



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regions of the mutated genes. Pathogenesis results from expression of RNAs from the mutated alleles that are toxic because of the expanded CUG or CCUG repeats. Increased understanding of the repeat-containing RNA (C/CUG(exp) RNA)-induced toxicity has led to the development of multiple strategies targeting the toxic RNA. Among these approaches, antisense oligonucleotides (ASOs) have demonstrated high potency in reversing the RNA toxicity in both cultured DM1 cells and DM1 animal models, thus offering great promise for the potential treatment of DM1. ASO targeting approaches will also provide avenues for the treatment of other repeat RNA-mediated diseases.

10. Han H, Irimia M, Ross PJ, et al. MBNL proteins repress ES-cell-specific alternative splicing and reprogramming. *Nature*. 2013;498(7453):241-245. doi:10.1038/nature12270

ABSTRACT: Previous investigations of the core gene regulatory circuitry that controls the pluripotency of embryonic stem (ES) cells have largely focused on the roles of transcription, chromatin and non-coding RNA regulators. Alternative splicing represents a widely acting mode of gene regulation, yet its role in regulating ES-cell pluripotency and differentiation is poorly understood. Here we identify the muscleblind-like RNA binding proteins, MBNL1 and MBNL2, as conserved and direct negative regulators of a large program of cassette exon alternative splicing events that are differentially regulated between ES cells and other cell types. Knockdown of MBNL proteins in differentiated cells causes switching to an ES-cell-like alternative splicing pattern for approximately half of these events, whereas overexpression of MBNL proteins in ES cells promotes differentiated-cell-like alternative splicing patterns. Among the MBNL-regulated events is an ES-cell-specific alternative splicing switch in the forkhead family transcription factor FOXP1 that controls pluripotency. Consistent with a central and negative regulatory role for MBNL proteins in pluripotency, their knockdown significantly enhances the expression of key pluripotency genes and the formation of induced pluripotent stem cells during somatic cell reprogramming.

11. Gao Z, Cooper TA. Reexpression of pyruvate kinase M2 in type 1 myofibers correlates with altered glucose metabolism in myotonic dystrophy. *Proceedings of the National Academy of Sciences*. 2013;110(33):13570-13575. doi:10.1073/pnas.1308806110

ABSTRACT: Myotonic dystrophy type 1 (DM1) is caused by expansion of CTG repeats in the 3' UTR of the DMPK gene. Expression of CUG expansion (CUG(exp)) RNA produces a toxic gain of function by disrupting the functions of RNA splicing factors, such as MBNL1 and CELF1, leading to splicing changes associated with clinical abnormalities. Progressive skeletal muscle weakness and wasting is one of the most prominent clinical features in DM1; however, the underlying mechanisms remain unclear. Here we report that the embryonic M2 isoform of pyruvate kinase (PKM2), a key enzyme contributing to the



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Warburg effect in cancer, is significantly induced in DM1 tissue and mouse models owing to aberrant splicing. Expression of PKM2 in DM1 skeletal muscle is restricted to the type 1 fibers, which are particularly susceptible to wasting in DM1. Using antisense oligonucleotides to shift PKM splicing toward increased PKM2 expression, we observed increased glucose consumption with reduced oxidative metabolism in cell culture and increased respiratory exchange ratio in mice, suggesting defects in energy metabolism conferred by PKM2 expression. We propose that PKM2 expression induces changes in type 1 fibers associated with muscle atrophy and muscle weakness in DM1.

12. Kalsotra A, Singh RK, Gurha P, Ward AJ, Creighton CJ, Cooper TA. The Mef2 Transcription Network Is Disrupted in Myotonic Dystrophy Heart Tissue, Dramatically Altering miRNA and mRNA Expression. *Cell Reports*. 2014;6(2):336-345. doi:10.1016/j.celrep.2013.12.025

ABSTRACT: Cardiac dysfunction is the second leading cause of death in myotonic dystrophy type 1 (DM1), primarily because of arrhythmias and cardiac conduction defects. A screen of more than 500 microRNAs (miRNAs) in a DM1 mouse model identified 54 miRNAs that were differentially expressed in heart. More than 80% exhibited downregulation toward the embryonic expression pattern and showed a DM1-specific response. A total of 20 of 22 miRNAs tested were also significantly downregulated in human DM1 heart tissue. We demonstrate that many of these miRNAs are direct MEF2 transcriptional targets, including miRNAs for which depletion is associated with arrhythmias or fibrosis. MEF2 protein is significantly reduced in both DM1 and mouse model heart samples, and exogenous MEF2C restores normal levels of MEF2 target miRNAs and mRNAs in a DM1 cardiac cell culture model. We conclude that loss of MEF2 in DM1 heart causes pathogenic features through aberrant expression of both miRNA and mRNA targets. Copyright © 2014 The Authors. Published by Elsevier Inc. All rights reserved.

13. Giudice J, Xia Z, Wang ET, et al. Alternative splicing regulates vesicular trafficking genes in cardiomyocytes during postnatal heart development. *Nat Commun*. 2014;5(1):3603. doi:10.1038/ncomms4603

ABSTRACT: During postnatal development the heart undergoes a rapid and dramatic transition to adult function through transcriptional and post-transcriptional mechanisms, including alternative splicing (AS). Here we perform deep RNA-sequencing on RNA from cardiomyocytes and cardiac fibroblasts to conduct a high-resolution analysis of transcriptome changes during postnatal mouse heart development. We reveal extensive changes in gene expression and AS that occur primarily between postnatal days 1 and 28. Cardiomyocytes and cardiac fibroblasts show reciprocal regulation of gene expression reflecting differences in proliferative capacity, cell adhesion functions and mitochondrial



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metabolism. We further demonstrate that AS plays a role in vesicular trafficking and membrane organization. These AS transitions are enriched among targets of two RNA-binding proteins, Celf1 and Mbnl1, which undergo developmentally regulated changes in expression. Vesicular trafficking genes affected by AS during normal development (when Celf1 is downregulated) show a reversion to neonatal splicing patterns after Celf1 re-expression in adults. Short-term Celf1 induction in adult animals results in disrupted transverse tubule organization and calcium handling. These results identify potential roles for AS in multiple aspects of postnatal heart maturation, including vesicular trafficking and intracellular membrane dynamics.

14. Khalifa MM, Bodner MJ, Berglund JA, Haley MM. Synthesis of N-substituted aryl amidines by strong base activation of amines. *Tetrahedron Lett.* 2015;56(27):4109-4111. doi:10.1016/j.tetlet.2015.05.029

ABSTRACT: We describe an efficient method for the direct preparation of N-substituted aryl amidines from nitriles and primary amines. The protocol employs activation of amines by a strong base and provides greater access to a pharmaceutically relevant functional group. This synthetic approach tolerates deactivated nitriles, nitriles with competing substitution sites, and aryl amines.

15. Katz Y, Wang ET, Silterra J, et al. Quantitative visualization of alternative exon expression from RNA-seq data. *Bioinformatics.* 2015;31(14):2400-2402. doi:10.1093/bioinformatics/btv034

ABSTRACT: MOTIVATION: Analysis of RNA sequencing (RNA-Seq) data revealed that the vast majority of human genes express multiple mRNA isoforms, produced by alternative pre-mRNA splicing and other mechanisms, and that most alternative isoforms vary in expression between human tissues. As RNA-Seq datasets grow in size, it remains challenging to visualize isoform expression across multiple samples. RESULTS: To help address this problem, we present Sashimi plots, a quantitative visualization of aligned RNA-Seq reads that enables quantitative comparison of exon usage across samples or experimental conditions. Sashimi plots can be made using the Broad Integrated Genome Viewer or with a stand-alone command line program. AVAILABILITY AND IMPLEMENTATION: Software code and documentation freely available here: <http://miso.readthedocs.org/en/fastmiso/sashimi.html> © The Author 2015. Published by Oxford University Press. All rights reserved. For Permissions, please email: journals.permissions@oup.com.



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16. Siboni RB, Bodner MJ, Khalifa MM, et al. Biological Efficacy and Toxicity of Diamidines in Myotonic Dystrophy Type 1 Models. *J Med Chem.* 2015;58(15):5770-5780. doi:10.1021/acs.jmedchem.5b00356

ABSTRACT: Myotonic Dystrophy type 1 (DM1) is a disease characterized by errors in alternative splicing, or “mis-splicing”. The causative agent of mis-splicing in DM1 is an inherited CTG repeat expansion located in the 3’ untranslated region of the DM protein kinase gene. When transcribed, CUG repeat expansion RNA sequester MBNL proteins, which constitute an important family of alternative splicing regulators. Sequestration of MBNL proteins results in the mis-splicing of its regulated transcripts. Previous work has demonstrated that pentamidine, a diamidine which is currently FDA-approved as an anti-parasitic agent, was able to partially reverse mis-splicing in multiple DM1 models, albeit at toxic concentrations. In this study, we characterized a series of pentamidine analogues in order to determine their ability to reverse mis-splicing and their toxicity in vivo. Experiments in cell and mouse models demonstrated that compound 13, also known as furamidine, effectively reversed mis-splicing with equal efficacy and reduced toxicity compared to pentamidine.

17. Gould GM, Paggi JM, Guo Y, et al. Identification of new branch points and unconventional introns in *Saccharomyces cerevisiae*. *RNA.* 2016;22(10):1522-1534. doi:10.1261/rna.057216.116

ABSTRACT: Spliced messages constitute one-fourth of expressed mRNAs in the yeast *Saccharomyces cerevisiae*, and most mRNAs in metazoans. Splicing requires 5' splice site (5'SS), branch point (BP), and 3' splice site (3'SS) elements, but the role of the BP in splicing control is poorly understood because BP identification remains difficult. We developed a high-throughput method, Branch-seq, to map BPs and 5'SSs of isolated RNA lariats. Applied to *S. cerevisiae*, Branch-seq detected 76% of expressed, annotated BPs and identified a comparable number of novel BPs. We performed RNA-seq to confirm associated 3'SS locations, identifying some 200 novel splice junctions, including an AT-AC intron. We show that several yeast introns use two or even three different BPs, with effects on 3'SS choice, protein coding potential, or RNA stability, and identify novel introns whose splicing changes during meiosis or in response to stress. Together, these findings show unanticipated complexity of splicing in yeast. © 2016 Gould et al.; Published by Cold Spring Harbor Laboratory Press for the RNA Society.



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18. Thomas JD, Sznajder ŁJ, Bardhi O, et al. Disrupted prenatal RNA processing and myogenesis in congenital myotonic dystrophy. *Genes Dev.* 2017;31(11):1122-1133. doi:10.1101/gad.300590.117

ABSTRACT: Myotonic dystrophy type 1 (DM1) is a CTG microsatellite expansion (CTGexp) disorder caused by expression of CUGexp RNAs. These mutant RNAs alter the activities of RNA processing factors, including MBNL proteins, leading to re-expression of fetal isoforms in adult tissues and DM1 pathology. While this pathogenesis model accounts for adult-onset disease, the molecular basis of congenital DM (CDM) is unknown. Here, we test the hypothesis that disruption of developmentally regulated RNA alternative processing pathways contributes to CDM disease. We identify prominent alternative splicing and polyadenylation abnormalities in infant CDM muscle, and, although most are also misregulated in adult-onset DM1, dysregulation is significantly more severe in CDM. Furthermore, analysis of alternative splicing during human myogenesis reveals that CDM-relevant exons undergo prenatal RNA isoform transitions and are predicted to be disrupted by CUGexp-associated mechanisms in utero. To test this possibility and the contribution of MBNLs to CDM pathogenesis, we generated mouse Mbnl double (Mbnl1; Mbnl2) and triple (Mbnl1; Mbnl2; Mbnl3) muscle-specific knockout models that recapitulate the congenital myopathy, gene expression, and spliceopathy defects characteristic of CDM. This study demonstrates that RNA misprocessing is a major pathogenic factor in CDM and provides novel mouse models to further examine roles for cotranscriptional/post-transcriptional gene regulation during development.

19. deLorimier E, Hinman MN, Copperman J, Datta K, Guenza M, Berglund JA. Pseudouridine Modification Inhibits Muscleblind-like 1 (MBNL1) Binding to CCUG Repeats and Minimally Structured RNA through Reduced RNA Flexibility. *J Biol Chem.* 2017;292(10):4350-4357. doi:10.1074/jbc.M116.770768

ABSTRACT: Myotonic dystrophy type 2 is a genetic neuromuscular disease caused by the expression of expanded CCUG repeat RNAs from the non-coding region of the CCHC-type zinc finger nucleic acid-binding protein (CNBP) gene. These CCUG repeats bind and sequester a family of RNA-binding proteins known as Muscleblind-like 1, 2, and 3 (MBNL1, MBNL2, and MBNL3), and sequestration plays a significant role in pathogenicity. MBNL proteins are alternative splicing regulators that bind to the consensus RNA sequence YGCY (Y = pyrimidine). This consensus sequence is found in the toxic RNAs (CCUG repeats) and in cellular RNA substrates that MBNL proteins have been shown to bind. Replacing the uridine in CCUG repeats with pseudouridine (Ψ) resulted in a modest reduction of MBNL1 binding. Interestingly, Ψ modification of a minimally structured RNA containing YGCY motifs resulted in more robust inhibition of MBNL1 binding. The different levels of inhibition between CCUG repeat and minimally structured RNA binding appear to be due to the ability to modify both pyrimidines in the YGCY motif, which is not possible in the



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CCUG repeats. Molecular dynamic studies of unmodified and pseudouridylated minimally structured RNAs suggest that reducing the flexibility of the minimally structured RNA leads to reduced binding by MBNL1.

20. Cheng AW, Shi J, Wong P, et al. Muscleblind-like 1 (Mbnl1) regulates pre-mRNA alternative splicing during terminal erythropoiesis. *Blood*. 2014;124(4):598-610. doi:10.1182/blood-2013-12-542209

ABSTRACT: The scope and roles of regulated isoform gene expression during erythroid terminal development are poorly understood. We identified hundreds of differentiation-associated isoform changes during terminal erythropoiesis. Sequences surrounding cassette exons of skipped exon events are enriched for motifs bound by the Muscleblind-like (MBNL) family of splicing factors. Knockdown of Mbnl1 in cultured murine fetal liver erythroid progenitors resulted in a strong block in erythroid differentiation and disrupted the developmentally regulated exon skipping of Ndel1 mRNA, which is bound by MBNL1 and critical for erythroid terminal proliferation. These findings reveal an unanticipated scope of the alternative splicing program and the importance of Mbnl1 during erythroid terminal differentiation. © 2014 by The American Society of Hematology.

21. Taliaferro JM, Wang ET, Burge CB. Genomic analysis of RNA localization. *RNA Biology*. 2014;11(8):1040-1050. doi:10.4161/rna.32146

ABSTRACT: The localization of mRNAs to specific subcellular sites is widespread, allowing cells to spatially restrict and regulate protein production, and playing important roles in development and cellular physiology. This process has been studied in mechanistic detail for several RNAs. However, the generality or specificity of RNA localization systems and mechanisms that impact the many thousands of localized mRNAs has been difficult to assess. In this review, we discuss the current state of the field in determining which RNAs localize, which RNA sequences mediate localization, the protein factors involved, and the biological implications of localization. For each question, we examine prominent systems and techniques that are used to study individual messages, highlight recent genome-wide studies of RNA localization, and discuss the potential for adapting other high-throughput approaches to the study of localization.



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22. Wang ET, Ward AJ, Cherone JM, et al. Antagonistic regulation of mRNA expression and splicing by CELF and MBNL proteins. *Genome Res.* 2015;25(6):858-871. doi:10.1101/gr.184390.114

ABSTRACT: RNA binding proteins of the conserved CUGBP1, Elav-like factor (CELF) family contribute to heart and skeletal muscle development and are implicated in myotonic dystrophy (DM). To understand their genome-wide functions, we analyzed the transcriptome dynamics following induction of CELF1 or CELF2 in adult mouse heart and of CELF1 in muscle by RNA-seq, complemented by crosslinking/immunoprecipitation-sequencing (CLIP-seq) analysis of mouse cells and tissues to distinguish direct from indirect regulatory targets. We identified hundreds of mRNAs bound in their 3' UTRs by both CELF1 and the developmentally induced MBNL1 protein, a threefold greater overlap in target messages than expected, including messages involved in development and cell differentiation. The extent of 3' UTR binding by CELF1 and MBNL1 predicted the degree of mRNA repression or stabilization, respectively, following CELF1 induction. However, CELF1's RNA binding specificity in vitro was not detectably altered by coincubation with recombinant MBNL1. These findings support a model in which CELF and MBNL proteins bind independently to mRNAs but functionally compete to specify down-regulation or localization/stabilization, respectively, of hundreds of mRNA targets. Expression of many alternative 3' UTR isoforms was altered following CELF1 induction, with 3' UTR binding associated with down-regulation of isoforms and genes. The splicing of hundreds of alternative exons was oppositely regulated by these proteins, confirming an additional layer of regulatory antagonism previously observed in a handful of cases. The regulatory relationships between CELFs and MBNLs in control of both mRNA abundance and splicing appear to have evolved to enhance developmental transitions in major classes of heart and muscle genes. © 2015 Wang et al.; Published by Cold Spring Harbor Laboratory Press.

23. Siboni RB, Nakamori M, Wagner SD, et al. Actinomycin D Specifically Reduces Expanded CUG Repeat RNA in Myotonic Dystrophy Models. *Cell Rep.* 2015;13(11):2386-2394. doi:10.1016/j.celrep.2015.11.028

ABSTRACT: Myotonic dystrophy type 1 (DM1) is an inherited disease characterized by the inability to relax contracted muscles. Affected individuals carry large CTG expansions that are toxic when transcribed. One possible treatment approach is to reduce or eliminate transcription of CTG repeats. Actinomycin D (ActD) is a potent transcription inhibitor and FDA-approved chemotherapeutic that binds GC-rich DNA with high affinity. Here, we report that ActD decreased CUG transcript levels in a dose-dependent manner in DM1 cell and mouse models at significantly lower concentrations (nanomolar) compared to its use as a general transcription inhibitor or chemotherapeutic. ActD also significantly reversed DM1-associated splicing defects in a DM1 mouse model, and did so within the currently approved human treatment range. RNA-seq analyses showed that low



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concentrations of ActD did not globally inhibit transcription in a DM1 mouse model. These results indicate that transcription inhibition of CTG expansions is a promising treatment approach for DM1.

24. Johnson NE, Ekstrom A-B, Campbell C, et al. Parent-reported multi-national study of the impact of congenital and childhood onset myotonic dystrophy. *Dev Med Child Neurol*. 2016;58(7):698-705. doi:10.1111/dmcn.12948

ABSTRACT: AIM: The frequency and impact of symptoms experienced by patients with congenital, childhood, and juvenile-onset myotonic dystrophy (CDM/ChDM/JDM) is not documented. This report identifies symptomatic areas with the greatest disease burden in an international population of patients with early-onset myotonic dystrophy type-1 (DM1). METHOD: We distributed surveys to parents of patients with CDM/ChDM/JDM. Patients with CDM/ChDM/JDM were members of the US National Registry of DM1 Patients and Family Members, the Canadian Neuromuscular Disease Registry, or the Swedish Health System. Surveys inquired about 325 symptoms and 20 themes associated with CDM/ChDM/JDM. Parents identified the importance of each symptom and theme to their affected child. The prevalence of each symptom and theme were compared across subgroups of patients. The statistical analysis was performed using Fisher's exact and Kruskal-Wallis tests. RESULTS: One hundred and fifty parents returned surveys. The most frequently reported symptomatic themes in children were issues involving communication (81.7%) and problems with hands or fingers (79.6%). Problems with communication and fatigue were the issues that were reported to have the greatest impact on children's lives, while 24.1% of children reported cardiac disorders and 15.8% had problems with anesthesia. INTERPRETATION: A range of symptoms contribute to the burden of disease faced by children with DM1. Many of these symptoms are under-recognized. © 2015 Mac Keith Press.

25. Freyermuth F, Rau F, Kokunai Y, et al. Splicing misregulation of SCN5A contributes to cardiac-conduction delay and heart arrhythmia in myotonic dystrophy. *Nature Communications*. 2016;7(1):1-14. doi:10.1038/ncomms11067

ABSTRACT: Myotonic dystrophy (DM) is caused by the expression of mutant RNAs containing expanded CUG repeats that sequester muscleblind-like (MBNL) proteins, leading to alternative splicing changes. Cardiac alterations, characterized by conduction delays and arrhythmia, are the second most common cause of death in DM. Using RNA sequencing, here we identify novel splicing alterations in DM heart samples, including a switch from adult exon 6B towards fetal exon 6A in the cardiac sodium channel, SCN5A. We find that MBNL1 regulates alternative splicing of SCN5A mRNA and that the splicing variant of SCN5A produced in DM presents a reduced excitability compared with the



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control adult isoform. Importantly, reproducing splicing alteration of Scn5a in mice is sufficient to promote heart arrhythmia and cardiac-conduction delay, two predominant features of myotonic dystrophy. In conclusion, misregulation of the alternative splicing of SCN5A may contribute to a subset of the cardiac dysfunctions observed in myotonic dystrophy.

26. Wagner SD, Struck AJ, Gupta R, et al. Dose-Dependent Regulation of Alternative Splicing by MBNL Proteins Reveals Biomarkers for Myotonic Dystrophy. *PLOS Genetics*. 2016;12(9):e1006316. doi:10.1371/journal.pgen.1006316

ABSTRACT: Alternative splicing is a regulated process that results in expression of specific mRNA and protein isoforms. Alternative splicing factors determine the relative abundance of each isoform. Here we focus on MBNL1, a splicing factor misregulated in the disease myotonic dystrophy. By altering the concentration of MBNL1 in cells across a broad dynamic range, we show that different splicing events require different amounts of MBNL1 for half-maximal response, and respond more or less steeply to MBNL1. Motifs around MBNL1 exon 5 were studied to assess how cis-elements mediate the MBNL1 dose-dependent splicing response. A framework was developed to estimate MBNL concentration using splicing responses alone, validated in the cell-based model, and applied to myotonic dystrophy patient muscle. Using this framework, we evaluated the ability of individual and combinations of splicing events to predict functional MBNL concentration in human biopsies, as well as their performance as biomarkers to assay mild, moderate, and severe cases of DM.

27. Gallais B, Gagnon C, Forgues G, Côté I, Laberge L. Further evidence for the reliability and validity of the Fatigue and Daytime Sleepiness Scale. *J Neurol Sci*. 2017;375:23-26. doi:10.1016/j.jns.2017.01.032

ABSTRACT: BACKGROUND: Myotonic dystrophy type 1 (DM1) is an inherited neuromuscular disease causing, among other symptoms, fatigue and excessive daytime sleepiness, which are frequently undifferentiated by patients and/or clinicians. The Fatigue and Daytime Sleepiness Scale (FDSS) has been devised to measure these two overlapping symptoms as a single clinical entity. **OBJECTIVE:** To further examine the reliability and the construct validity of the FDSS in patients with DM1. **METHODS:** The scale was administered to 48 DM1 patients on two occasions at a 2week-interval. Intra-rater reliability and internal consistency were established by calculating the intraclass correlation coefficient (ICC) and Cronbach's alpha, respectively. Construct validity was assessed by using the known-group method. More precisely, the mean FDSS score of patients with and without subjective complaints of fatigue and/or sleepiness was compared. **RESULTS:** The FDSS showed good intra-rater reliability (ICC=0.83) and



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acceptable internal consistency (Cronbach's alpha =0.6). Also, the FDSS was able to distinguish between patients who had fatigue and sleepiness complaints from those who did not (mean FDSS score of 10.6 vs 8.0, $p=0.01$), suggesting good construct validity. CONCLUSION: Overall, the present study supports the continued use of the FDSS as a reliable and valid instrument to measure fatigue and daytime sleepiness in patients with DM1 for either clinical or research purposes.

28. Gallais B, Gagnon C, Côté I, Forgues G, Laberge L. Reliability of the Apathy Evaluation Scale in Myotonic Dystrophy Type 1. *J Neuromuscul Dis.* 2018;5(1):39-46. doi:10.3233/JND-170274

ABSTRACT: BACKGROUND: Apathy is a common debilitating symptom of myotonic dystrophy type 1 (DM1). The Apathy Evaluation Scale (AES) has been identified as a promising measurement instrument to be used in DM1 but its metrological properties must be further documented. OBJECTIVE: To determine the internal consistency of the Self (AES-S), Informant (AES-I), and Clinician (AES-C) versions of the AES and to assess the test-retest reliability, standard error of measurement, and minimal detectable change of the AES-S and AES-I in a sample of DM1 patients and their related informants. RESULTS: All scales showed good internal consistency (Cronbach's alpha: 0.83-0.87) and the AES-S and AES-I showed good test-retest reliability (ICC = 0.79-0.91). Additionally, clinicians and informants had a tendency to overestimate DM1 patients' level of apathy compared to patients' self-ratings, suggesting potentially impaired self-awareness in DM1 patients. CONCLUSIONS: The present results advocate the use of the AES-I as a reliable instrument to estimate apathy in DM1 patients for either clinical or research purposes, and support the relevance to pursue the assessment of metrological properties of the AES as a tool of great value for the development of outcomes for clinical trial readiness in DM1.

29. Morriss GR, Rajapakshe K, Huang S, Coarfa C, Cooper TA. Mechanisms of skeletal muscle wasting in a mouse model for myotonic dystrophy type 1. *Hum Mol Genet.* 2018;27(16):2789-2804. doi:10.1093/hmg/ddy192

ABSTRACT: Myotonic dystrophy type 1 (DM1) is a multi-systemic disease resulting in severe muscle weakening and wasting. DM1 is caused by expansion of CTG repeats in the 3' untranslated region of the dystrophin myotonia protein kinase (DMPK) gene. We have developed an inducible, skeletal muscle-specific mouse model of DM1 (CUG960) that expresses 960 CUG repeat-expressing animals (CUG960) in the context of human DMPK exons 11-15. CUG960 RNA-expressing mice induced at postnatal day 1, as well as adult-onset animals, show clear, measurable muscle wasting accompanied by severe histological defects including central myonuclei, reduced fiber cross-sectional area, increased percentage of oxidative myofibers, the presence of nuclear RNA foci that



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colocalize with Mbnl1 protein, and increased Celf1 protein in severely affected muscles. Importantly, muscle loss, histological abnormalities and RNA foci are reversible, demonstrating recovery upon removal of toxic RNA. RNA-seq and protein array analysis indicate that the balance between anabolic and catabolic pathways that normally regulate muscle mass may be disrupted by deregulation of platelet derived growth factor receptor β signaling and the PI3K/AKT pathways, along with prolonged activation of AMP-activated protein kinase α signaling. Similar changes were detected in DM1 skeletal muscle compared with unaffected controls. The mouse model presented in this paper shows progressive skeletal muscle wasting and has been used to identify potential molecular mechanisms underlying skeletal muscle loss. The reversibility of the phenotype establishes a baseline response for testing therapeutic approaches.

30. Berggren KN, Hung M, Dixon MM, et al. Orofacial strength, dysarthria, and dysphagia in congenital myotonic dystrophy: Orofacial Function in CDM. *Muscle Nerve*. 2018;58(3):413-417. doi:10.1002/mus.26176

ABSTRACT: INTRODUCTION: Herein we present an exploratory study of orofacial function in children with congenital myotonic dystrophy (CDM) vs. healthy controls. **METHODS:** We evaluated 41 children with CDM and 29 healthy controls for speech and swallow function and for lingual and labial strength. **RESULTS:** The Iowa Oral Performance Instrument (IOPI), measuring tongue strength, and a lip force meter (LFM), measuring lip strength, had excellent interrater reliability with intraclass correlation coefficients (ICCs) of 0.75 ($n = 19$, $P < 0.001$) and 0.96 ($n = 20$, $P < 0.001$), respectively. Mean overall lingual strength was 3.5-fold less and labial strength was about 7-fold less in CDM patients than in healthy controls. Eighteen of 24 children with CDM demonstrated dysarthria and an additional 11 participants were nonverbal. Dysarthria correlated moderately with lingual strength, age, and dysphagia. Strength measures correlated moderately with dysphagia. **DISCUSSION:** Children with CDM have impaired orofacial functioning that affects communication and swallowing. Reliability of strength measures may be useful for future therapeutic trials.

31. Minier L, Lignier B, Bouvet C, Gallais B, Camart N. A Review of Psychopathology Features, Personality, and Coping in Myotonic Dystrophy Type 1. *J Neuromuscul Dis*. 2018;5(3):279-294. doi:10.3233/JND-180310

ABSTRACT: BACKGROUND: The last literature review on psychopathological features in Myotonic Dystrophy type 1 had been conducted by Ambrosini and Nurnberg in 1979. Since that date, many researches had been carried out. **OBJECTIVE:** The aim of this study is (i) to systematically obtain and evaluate the relevant literature on psychopathological features, personality, and coping in individuals with adult phenotypes of Myotonic



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Dystrophy type 1. (ii) To summarize current research findings and draw conclusions for future research. METHODS: A systematic search was conducted on Pubmed, PubPsych, PsycInfo, Science Direct, and Scopus covering the period of January 1979 to July 2017. RESULTS: In view of our literature review, patients show mild psychopathological problems, such as interpersonal difficulties, lack of interest, dysphoria, concern about bodily functioning, and hypersensitivity. However, they do not experience more psychiatric disorder in comparison to the general population, except for personality disorders and depression. We discussed problems concerning depression's assessment tool. Patients also present symptoms of several personality disorders: avoidant personality disorder was the most common. Finally, coping strategies relative to limitations resulting from their disease have a negative impact on their quality of life. CONCLUSIONS: In conclusion, Myotonic Dystrophy type 1 patients did not present homogeneous psychopathological and psychological features. However, based on tendencies observed among Myotonic Dystrophy type 1 patients, elements to conceptualize their social difficulties are provided.

32. Thomas JD, Oliveira R, Sznajder ŁJ, Swanson MS. Myotonic Dystrophy and Developmental Regulation of RNA Processing. *Compr Physiol.* 2018;8(2):509-553. doi:10.1002/cphy.c170002

ABSTRACT: Myotonic dystrophy (DM) is a multisystemic disorder caused by microsatellite expansion mutations in two unrelated genes leading to similar, yet distinct, diseases. DM disease presentation is highly variable and distinguished by differences in age-of-onset and symptom severity. In the most severe form, DM presents with congenital onset and profound developmental defects. At the molecular level, DM pathogenesis is characterized by a toxic RNA gain-of-function mechanism that involves the transcription of noncoding microsatellite expansions. These mutant RNAs disrupt key cellular pathways, including RNA processing, localization, and translation. In DM, these toxic RNA effects are predominantly mediated through the modulation of the muscleblind-like and CUGBP and ETR-3-like factor families of RNA binding proteins (RBPs). Dysfunction of these RBPs results in widespread RNA processing defects culminating in the expression of developmentally inappropriate protein isoforms in adult tissues. The tissue that is the focus of this review, skeletal muscle, is particularly sensitive to mutant RNA-responsive perturbations, as patients display a variety of developmental, structural, and functional defects in muscle. Here, we provide a comprehensive overview of DM1 and DM2 clinical presentation and pathology as well as the underlying cellular and molecular defects associated with DM disease onset and progression. Additionally, fundamental aspects of skeletal muscle development altered in DM are highlighted together with ongoing and potential therapeutic avenues to treat this muscular dystrophy. © 2018 American Physiological Society. *Compr Physiol* 8:509-553, 2018.



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33. Taylor K, Sznajder LJ, Cywoniuk P, Thomas JD, Swanson MS, Sobczak K. MBNL splicing activity depends on RNA binding site structural context. *Nucleic Acids Res.* 2018;46(17):9119-9133. doi:10.1093/nar/gky565

ABSTRACT: Muscleblind-like (MBNL) proteins are conserved RNA-binding factors involved in alternative splicing (AS) regulation during development. While AS is controlled by distribution of MBNL paralogs and isoforms, the affinity of these proteins for specific RNA-binding regions and their location within transcripts, it is currently unclear how RNA structure impacts MBNL-mediated AS regulation. Here, we defined the RNA structural determinants affecting MBNL-dependent AS activity using both cellular and biochemical assays. While enhanced inclusion of MBNL-regulated alternative exons is controlled by the arrangement and number of MBNL binding sites within unstructured RNA, when these sites are embedded in a RNA hairpin MBNL binds preferentially to one side of stem region. Surprisingly, binding of MBNL proteins to RNA targets did not entirely correlate with AS efficiency. Moreover, comparison of MBNL proteins revealed structure-dependent competitive behavior between the paralogs. Our results showed that the structure of targeted RNAs is a prevalent component of the mechanism of alternative splicing regulation by MBNLs.

34. Sznajder Ł, Thomas JD, Carrell EM, et al. Intron retention induced by microsatellite expansions as a disease biomarker. *Proc Natl Acad Sci USA.* 2018;115(16):4234-4239. doi:10.1073/pnas.1716617115

ABSTRACT: Expansions of simple sequence repeats, or microsatellites, have been linked to ~30 neurological–neuromuscular diseases. While these expansions occur in coding and noncoding regions, microsatellite sequence and repeat length diversity is more prominent in introns with eight different trinucleotide to hexanucleotide repeats, causing hereditary diseases such as myotonic dystrophy type 2 (DM2), Fuchs endothelial corneal dystrophy (FECD), and C9orf72 amyotrophic lateral sclerosis and frontotemporal dementia (C9-ALS/FTD). Here, we test the hypothesis that these GC-rich intronic microsatellite expansions selectively trigger host intron retention (IR). Using DM2, FECD, and C9-ALS/FTD as examples, we demonstrate that retention is readily detectable in affected tissues and peripheral blood lymphocytes and conclude that IR screening constitutes a rapid and inexpensive biomarker for intronic repeat expansion disease.



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35. Ashizawa T, Gagnon C, Groh WJ, et al. Consensus-based care recommendations for adults with myotonic dystrophy type 1. *Neurol Clin Pract.* 2018;8(6):507-520. doi:10.1212/CPJ.0000000000000531

ABSTRACT: Purpose of review: Myotonic dystrophy type 1 (DM1) is a severe, progressive genetic disease that affects between 1 in 3,000 and 8,000 individuals globally. No evidence-based guideline exists to inform the care of these patients, and most do not have access to multidisciplinary care centers staffed by experienced professionals, creating a clinical care deficit. Recent findings: The Myotonic Dystrophy Foundation (MDF) recruited 66 international clinicians experienced in DM1 patient care to develop consensus-based care recommendations. MDF created a 2-step methodology for the project using elements of the Single Text Procedure and the Nominal Group Technique. The process generated a 4-page Quick Reference Guide and a comprehensive, 55-page document that provides clinical care recommendations for 19 discrete body systems and/or care considerations. Summary: The resulting recommendations are intended to help standardize and elevate care for this patient population and reduce variability in clinical trial and study environments.

36. Nutter CA, Bubenik JL, Oliveira R, et al. Cell-type-specific dysregulation of RNA alternative splicing in short tandem repeat mouse knockin models of myotonic dystrophy. *Genes Dev.* 2019;33(23-24):1635-1640. doi:10.1101/gad.328963.119

ABSTRACT: Short tandem repeats (STRs) are prone to expansion mutations that cause multiple hereditary neurological and neuromuscular diseases. To study pathomechanisms using mouse models that recapitulate the tissue specificity and developmental timing of an STR expansion gene, we used rolling circle amplification and CRISPR/Cas9-mediated genome editing to generate Dmpk CTG expansion (CTGexp) knockin models of myotonic dystrophy type 1 (DM1). We demonstrate that skeletal muscle myoblasts and brain choroid plexus epithelial cells are particularly susceptible to Dmpk CTGexp mutations and RNA missplicing. Our results implicate dysregulation of muscle regeneration and cerebrospinal fluid homeostasis as early pathogenic events in DM1.

37. Shotwell CR, Cleary JD, Berglund JA. The potential of engineered eukaryotic RNA-binding proteins as molecular tools and therapeutics. *WIREs RNA.* 2020;11(1):e1573. doi:10.1002/wrna.1573

ABSTRACT: Eukaryotic RNA-binding proteins (RBPs) recognize and process RNAs through recognition of their sequence motifs via RNA-binding domains (RBDs). RBPs usually consist of one or more RBDs and can include additional functional domains that modify



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or cleave RNA. Engineered RBPs have been used to answer basic biology questions, control gene expression, locate viral RNA in vivo, as well as many other tasks. Given the growing number of diseases associated with RNA and RBPs, engineered RBPs also have the potential to serve as therapeutics. This review provides an in depth description of recent advances in engineered RBPs and discusses opportunities and challenges in the field. This article is categorized under: RNA Interactions with Proteins and Other Molecules > Protein–RNA Recognition RNA Methods > RNA Nanotechnology RNA in Disease and Development > RNA in Disease.

38. Li M, Zhuang Y, Batra R, et al. HNRNPA1-induced spliceopathy in a transgenic mouse model of myotonic dystrophy. *Proc Natl Acad Sci USA*. 2020;117(10):5472-5477. doi:10.1073/pnas.1907297117

ABSTRACT: Studies on myotonic dystrophy type 1 (DM1) have led to the RNA-mediated disease model for hereditary disorders caused by noncoding microsatellite expansions. This model proposes that DM1 disease manifestations are caused by a reversion to fetal RNA processing patterns in adult tissues due to the expression of toxic CUG RNA expansions (CUG exp) leading to decreased muscleblind-like, but increased CUGBP1/ETR3-like factor 1 (CELF1), alternative splicing activities. Here, we test this model in vivo, using the mouse HSA LR poly (CUG) model for DM1 and recombinant adeno-associated virus (rAAV)-mediated transduction of specific splicing factors. Surprisingly, systemic overexpression of HNRNPA1, not previously linked to DM1, also shifted DM1-relevant splicing targets to fetal isoforms, resulting in more severe muscle weakness/myopathy as early as 4 to 6 wk posttransduction, whereas rAAV controls were unaffected. Overexpression of HNRNPA1 promotes fetal exon inclusion of representative DM1-relevant splicing targets in differentiated myoblasts, and HITS-CLIP of rAAV-mycHnrnpa1-injected muscle revealed direct interactions of HNRNPA1 with these targets in vivo. Similar to CELF1, HNRNPA1 protein levels decrease during postnatal development, but are elevated in both regenerating mouse muscle and DM1 skeletal muscle. Our studies suggest that CUG exp RNA triggers abnormal expression of multiple nuclear RNA binding proteins, including CELF1 and HNRNPA1, that antagonize MBNL activity to promote fetal splicing patterns.