RNA Modification Enzymes are Mis-regulated in Myotonic Dystrophy Type 1 (DM1).

THE RNA INSTITUTE
COLLEGE OF ARTS & SCIENCES UNIVERSITY AT ALBANY

Emily Davey[1], Carl Shotwell[1], J. Andrew Berglund[1]

[1]RNA Institute, Department of Biological Sciences, University at Albany, Albany, NY

UNIVERSITY AT ALBANY
State University of New York

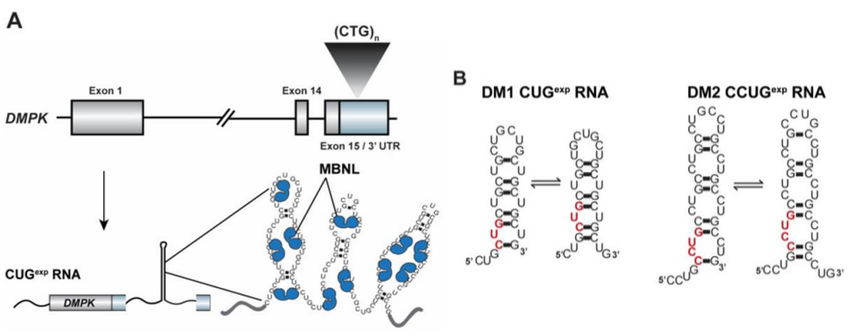
1. Abstract:

Myotonic dystrophy type 1 (DM1) is the most common form of adult onset muscular dystrophy. Individuals affected by this disease are impacted by muscle weakness, muscle wasting, heart issues, cognitive impairment, gastrointestinal issues, cataracts and many other health challenges. DM1 is a microsatellite repeat disorder characterized by expanded CTG repeats in the 3' UTR of the DMPK gene. The expanded CUG repeats have a toxic RNA gain of function as they sequester the MBNL family of proteins, which normally act as master regulators of RNA processing including alternative splicing, RNA localization, and RNA stability. A layer of RNA processing yet to be explored in DM1 are RNA modifications, which have been shown to be important for RNA regulation at nearly every level. The enzymes that perform RNA modifications may be of interest in DM1 as their transcripts have the potential to be regulated by the MBNL proteins. Using RNA-sequencing data, we looked at a MBNL1 dosing cell line to determine if modification enzymes exhibit differing levels of alternative splicing with increasing levels of MBNL1 to predict whether MBNL1 sequestration in DM1 may affect the ability of RNA modification enzymes to perform their normal cellular functions. By using replicate multivariate analysis of transcript splicing (rMATS), we found numerous modification enzymes that showed significant changes in alternative splicing, quantified by change in percent spliced in (PSI), with MBNL1 dosing, suggesting mis-regulation of MBNL might cause mis-regulation of RNA modifications. We also performed motif analysis, looking for MBNL's binding site, YGCY. Numerous modification enzymes showed increased numbers of YGCY motifs compared to expected numbers, suggesting MBNL1 likely binds these RNA sequences within these modification enzymes. Understanding the effect MBNL1 has on RNA modification enzymes may aid our understanding of how these enzymes are affected in DM1 due to MBNL1 sequestration.

2. Project:

Due to MBNL's role in regulating RNA processing, we hypothesized that MBNL may also have the ability to regulate RNA modifications. In order to determine whether MBNL proteins may regulate the transcripts of RNA modification enzymes, we mined RNA-Sequencing data from a MBNL1 dosing cell line to determine whether differing levels of MBNL1 lead to changes in alternative splicing or differential gene expression of RNA modification enzymes. Next, we performed motif analysis to determine whether MBNL1 has the ability to bind to the transcripts of these modification enzymes. Finally, we also analyzed RNA-Sequencing data from two DM1 patient-derived cell lines to determine whether this mis-regulation of RNA modification enzymes is also present in patient cells. Analyzing the potential mis-regulation of RNA modification enzymes will help us to further understand MBNL's role as a master regulator of RNA processing.

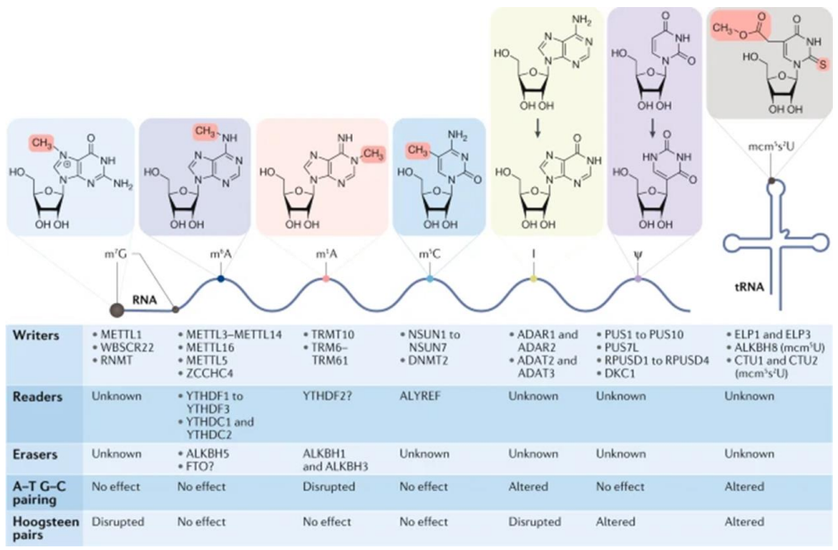
3. Mechanism of Myotonic Dystrophy (DM)



- Most common form of adult-onset muscular dystrophy
- Prevalence of DM1 ~1 in 2100 in NY state
- CUG or CCUG repeats have a toxic RNA gain-of-function
  - Repeats sequester MBNL proteins, forming nuclear foci
- **MBNL proteins are master regulators of RNA processing**

Hale, Melissa A., et al. "Repeat-associated RNA structure and aberrant splicing." *Biochimica et Biophysica acta* (2019).

4. RNA modification enzymes

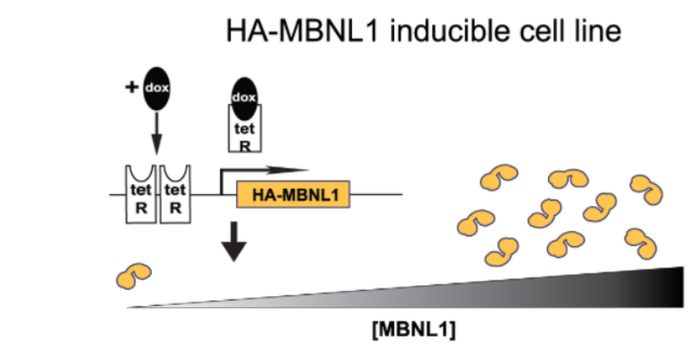


Writers	• METTL1 • WBSCR22 • RNMT	• METTL3–METTL14 • METTL16 • METTL5 • ZCCHC4	• TRMT10 • TRM6–TRM61	• NSUN1 to NSUN7 • DNMT2	• ADAR1 and ADAR2 • ADAT2 and ADAT3	• PUS1 to PUS10 • PUS7L • RPUSD1 to RPUSD4 • DKC1	• ELP1 and ELP3 • ALKBH8 (mcm^5U) • CTU1 and CTU2 (mcm^5s^2U)
Readers	Unknown	• YTHDF1 to YTHDF3 • YTHDC1 and YTHDC2	YTHDF2?	ALYREF	Unknown	Unknown	Unknown
Erasers	Unknown	• ALKBH5 • FTO?	ALKBH1 and ALKBH3	Unknown	Unknown	Unknown	Unknown
A–T G–C pairing	No effect	No effect	Disrupted	No effect	Altered	No effect	Altered
Hoogsteen pairs	Disrupted	No effect	No effect	No effect	Disrupted	Altered	Altered

- RNA modifications allow for posttranscriptional regulation of gene expression
- There are various types of RNA modification enzymes, such as writers, readers and erasers
- **RNA modifications are another level of RNA misprocessing yet to be explored in the context of DM1**

Barbieri, Isaiai. et al. "Role of RNA modifications in cancer." *Nat Rev Cancer* (2020).

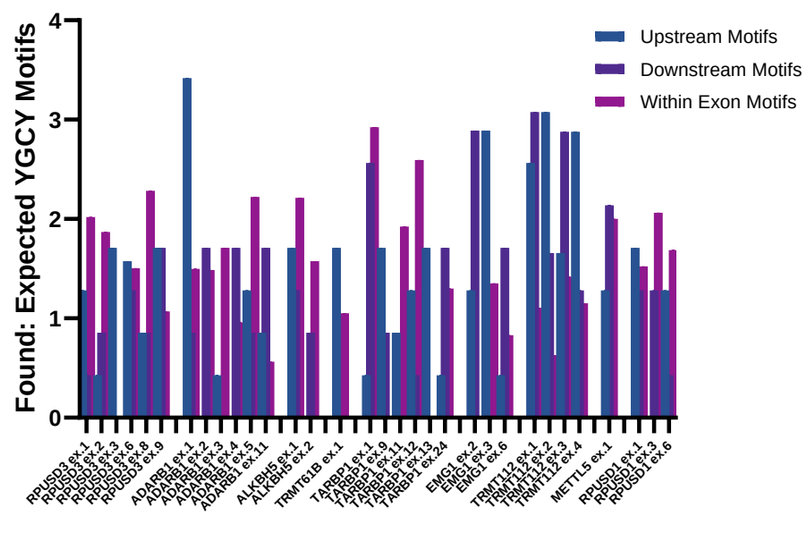
5. Mining RNA-Seq data from an MBNL1 dosing cell line



- RNA-Sequencing data from an MBNL1 dosing cell line was generated by previous work in the Berglund lab
- We re-analyzed this data for our analysis, utilizing alternative splicing and differential gene expression pipelines to look at transcriptomic changes in RNA modification enzymes with increasing levels of MBNL1

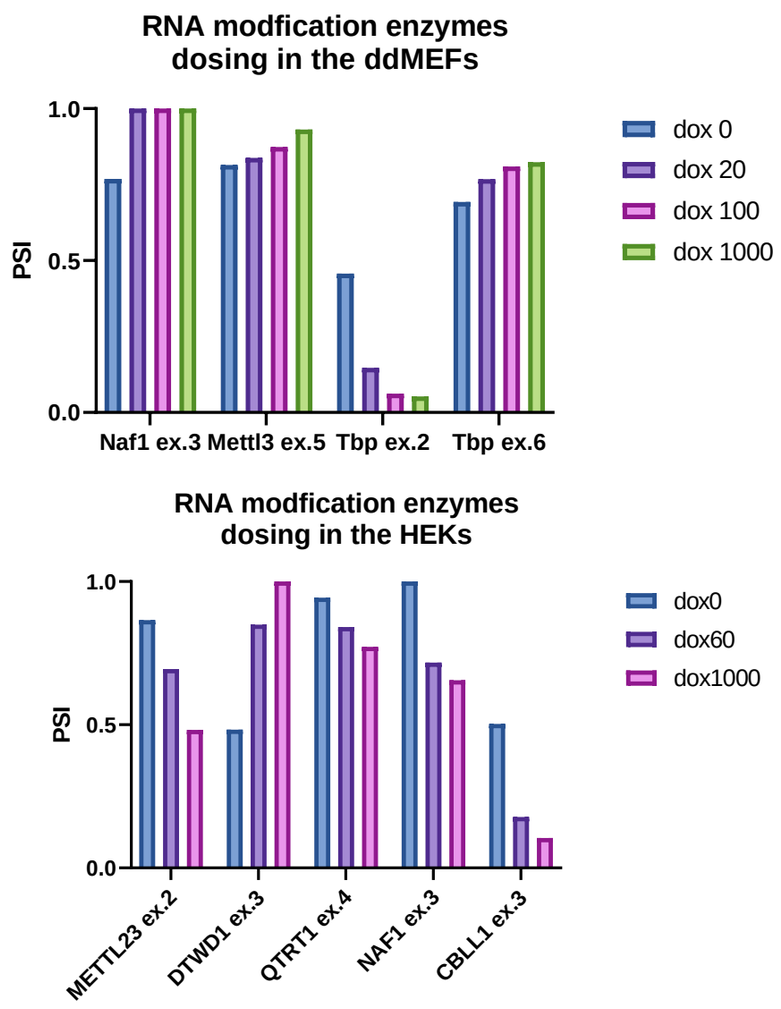
Wager, Stacey D. et al. "Dose-Dependent Regulation of Alternative Splicing by MBNL Proteins Reveals Biomarkers for Myotonic Dystrophy." *PLoS genetics* vol. 12,9 (2018).

6. Can MBNL1 bind the RNA sequences within RNA modification enzymes?



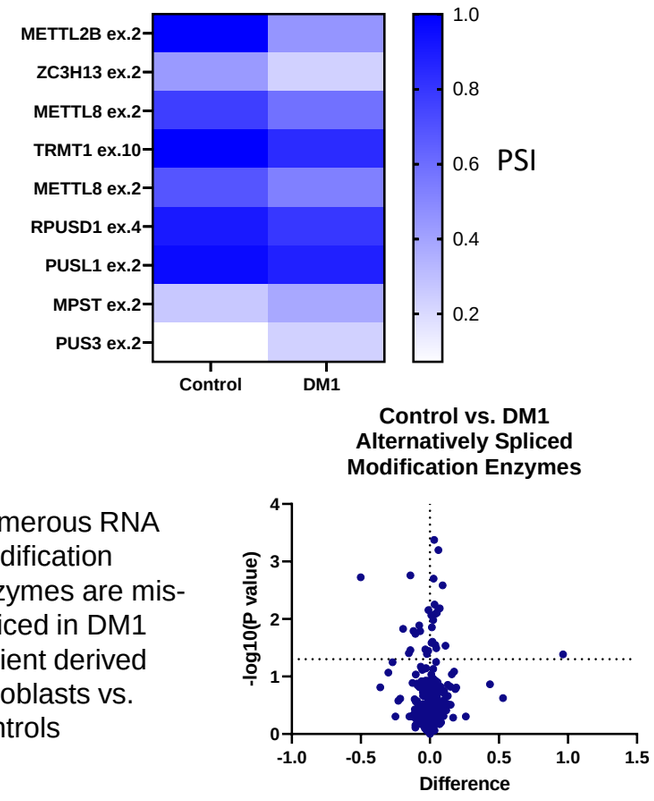
- MBNL1 binds to YGCY motifs
- We found the number of YGCY motifs in the upstream, downstream, and within exon regions of the RNA sequences of a select set of RNA modification enzymes
- **The number of MBNL1 binding motifs was several-fold higher than the expected number of YGCY motifs in the RNA sequences of numerous modification enzymes**

7. Do RNA modification enzymes dose with MBNL1?



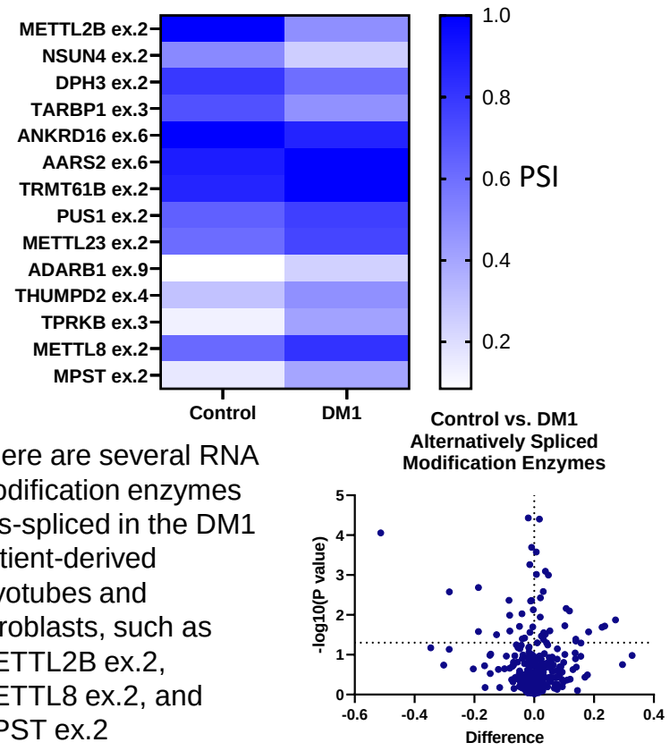
- Numerous modification enzymes appear to be dosing with MBNL1, suggesting that MBNL may regulate the transcripts of these RNA modification enzymes
- **This suggests that the mis-regulation of MBNL in DM1 may cause the mis-regulation of RNA modification enzymes**

8. Mis-regulation of RNA modification enzymes in DM1 patient-derived fibroblasts



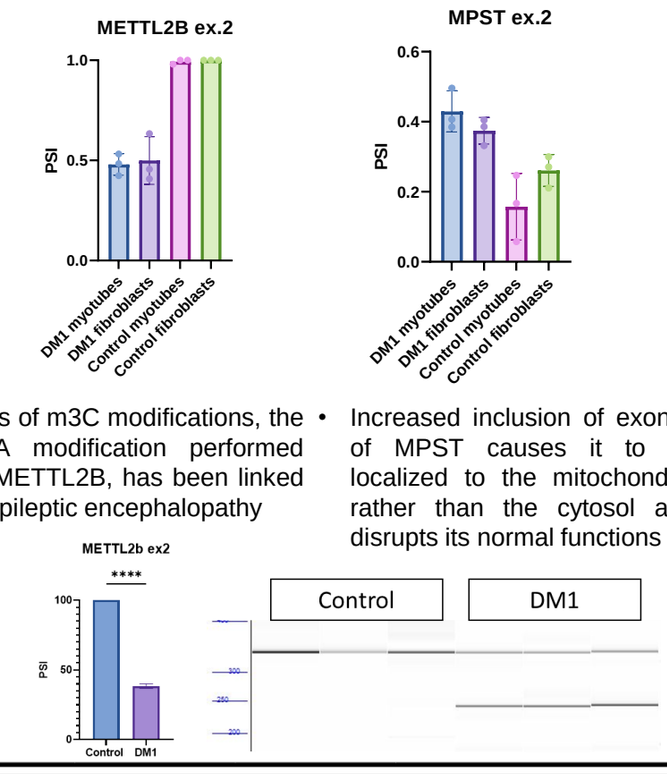
- Numerous RNA modification enzymes are mis-spliced in DM1 patient derived fibroblasts vs. controls

9. Mis-regulation of RNA modification enzymes in DM1 patient-derived myotubes



- There are several RNA modification enzymes mis-spliced in the DM1 patient-derived myotubes and fibroblasts, such as METTL2B ex.2, METTL8 ex.2, and MPST ex.2

10. RNA modification enzymes are mis-regulated across multiple tissue types



- Loss of m3C modifications, the RNA modification performed by METTL2B, has been linked to epileptic encephalopathy
- Increased inclusion of exon 2 of MPST causes it to be localized to the mitochondria rather than the cytosol and disrupts its normal functions

11. Conclusions and future experiments

- RNA modification enzymes are mis-regulated across multiple tissue types in DM1; however, the mis-regulation of some modification enzymes may not be MBNL1-dependent
- The mis-splicing of some of these modification enzymes may have detrimental effects and should be further investigated
- Validate the mis-splicing and differential expression of these RNA modification enzymes in patient-derived myotubes and fibroblasts using RT-qPCR and qPCR, respectively
- Look into the overlap in mis-splicing and differential expression with other tissue types from DM1 patients

11. Acknowledgements

I would like to thank everyone in the Berglund lab for their feedback and support on this project, as well as the patients and their families.