**Myotonic dystrophy type 2 (DM2)** is characterized by progressive muscle wasting, myotonia, cataracts, and cardiac problems [1], and there is currently no cure. DM2 is caused by a repeating nucleotide motif that is found within the first intron of the *CNBP* gene, thereby resulting in up to 11,000 nucleotide repeats for individuals with DM2 [2]. These excessive repeats result in mutant, double stranded, *CNBP* pre-mRNA transcripts that sequester and bind to critical RNA-binding proteins (RBPs), thereby causing changes in skeletal muscle function [3]. In addition, reduced translation of functional CNBP protein, an RBP itself for over 4000 transcripts, is believed to contribute to many of the detrimental skeletal muscle changes associated with DM2 [4].These two disease mechanisms likely work in conjunction with one another, *but* *the complete profile of dysregulated proteins caused by mutant CNBP pre-mRNA, as well as the specific mRNA targets affected by the loss of functional CNBP protein have yet to be determined.*

 The **objective** of this study is to characterize the CNBP protein domains, and to understand how both mutant *CNBP* pre-mRNA transcripts and the resulting loss of cellular CNBP protein contributes to muscle wasting and myotonia in DM2 patients. I **hypothesize** that insufficient CNBP levels leads to diminished translation of transcripts essential to skeletal muscle function, and that the mutant *CNBP* pre-mRNA transcripts lead to the dysregulation and sequestering of a wide range of skeletal muscle proteins. The **long-term goal** of this research is to better characterize the specific skeletal muscle genes and pathways affected by both CNBP loss and mutant *CNBP* transcripts, so that therapies aimed at restoring their function in skeletal muscle can be developed. *Mus musculus* and *Drosophila melanogaster* will be used as a model organisms because their *CNBP* mutants both replicate detrimental DM2 muscle phenotypes, like muscle wasting.

***Aim 1- Identify conserved protein domains of CNBP critical to proper muscle function.***

**Approach:** Using sequence data from the NCBI’s BLAST database, I will search for *CNBP* homologs in various species to identify conserved protein domains using the SMART database. Using *Mus musculus* as a model organism, I will individually make targeted amino acid substitutions using CRISPR/Cas9 within each conserved protein domain. Following this, I will screen for mice that exhibit muscle wasting and myotonia DM2 phenotypes to determine which conserved domains of CNBP are most critical to the proper functioning of skeletal muscle tissue. **Rationale:** The domains of CNBP that are most vital to the regulation of skeletal muscle can be identified using individual CRISPR/Cas9 analyses. The mouse model is advantageous because it allows for measurements of grip and muscular strength. **Hypothesis:** I hypothesize that the conserved zinc-finger domains of CNBP are critical to the regulation of genes specific to skeletal muscle, and that disruption of at least one of these domains with result in both myotonia and muscle wasting.

***Aim 2- Identify differentially expressed transcripts in CNBP mutants important for skeletal muscle function.***

**Approach:** I will use RNA-seq on wild-type (WT) and mutant *CNBP* *Drosophila melanogaster* muscle tissue to identify differences in RNA transcript profiles and determine differentially expressed genes in *CNBP* mutant flies. Using Gene Ontology (GO), I will sort expressed genes by function and identify any differences in skeletal muscle genes between the normal and disease states. I will then knock out these genes using CRISPR to determine if they show similar muscle wasting phenotypes as the *CNBP* mutants. **Rationale:** By using flies with mutant *CNBP* DNA, I will be able to identify the differentially expressed transcript profiles that arise from both a lack of CNBP protein and the presence of mutant pre-mRNA *CNBP* transcripts. This screen will also identify which genes specific to skeletal muscle are up-regulated and down-regulated in the WT vs. mutant state. **Hypothesis:** I hypothesize that the *CNBP* mutants will have fewer properly spliced transcripts and a lower amount of mRNA transcripts overall within their skeletal muscle tissue.

***Aim 3- Characterize the dysregulation of proteins involved in skeletal muscle function.***

**Approach:** Using an iTRAQ method of proteomic analysis, I will isolate proteins found within the skeletal muscle of WT and *CNBP*-knock-out flies in order to quantify the up-regulated and down-regulated proteins in each state. Using PANTHER, I will classify these proteins by their GO terms to determine what protein classes and biological processes are affected in the disease state. **Rationale:** Performing a proteomic screen on *CNBP*-KO mutants will identify proteins that are dysregulated in the absence of CNBP, and GO will identify biological processes that may be linked to skeletal muscle dysfunction. This screen eliminates the possibility of dysregulation due to mutant *CNBP* pre-mRNAs, and will reveal any dysregulation in skeletal muscle at the post-translational level caused by decreased CNBP levels. **Hypothesis:** I hypothesize that *CNBP*-KO mutants will have highly down-regulated DNA and RNA-binding proteins, and that low levels of these proteins will lead to dysregulation of the other proteins critical to skeletal muscle function.

**References:**

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