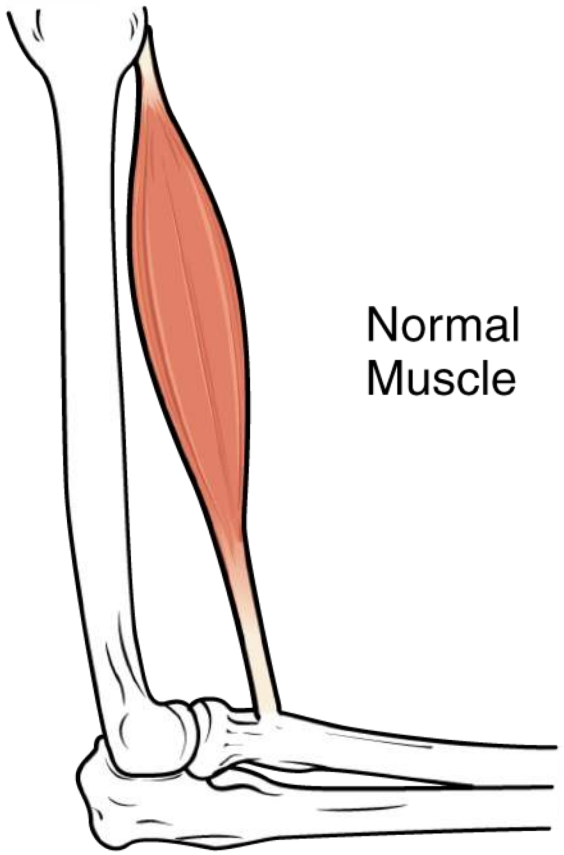
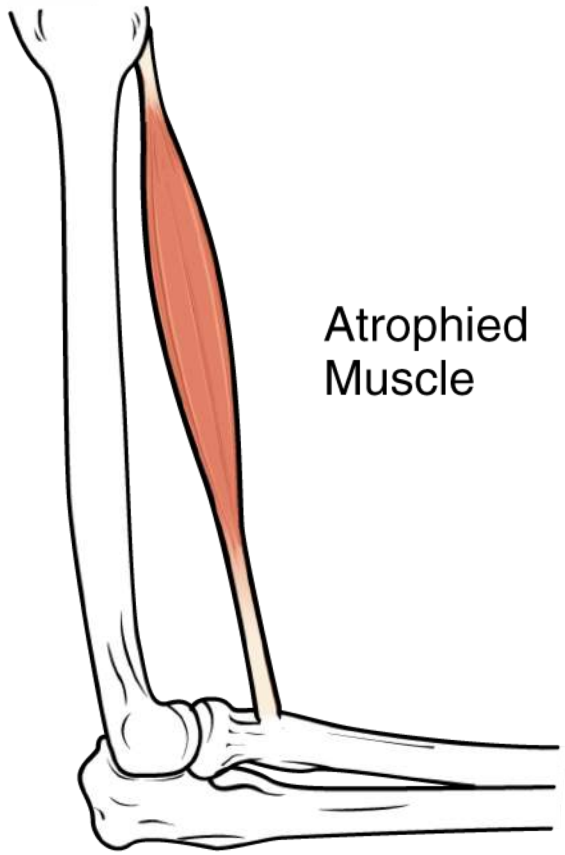
Muscular Dystrophy & *CNBP*

Paxton Paine

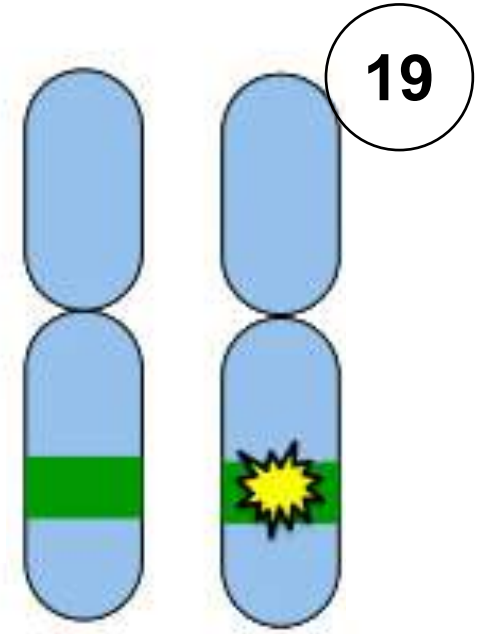
Muscular dystrophy (MD) is characterized by muscle loss and weakness



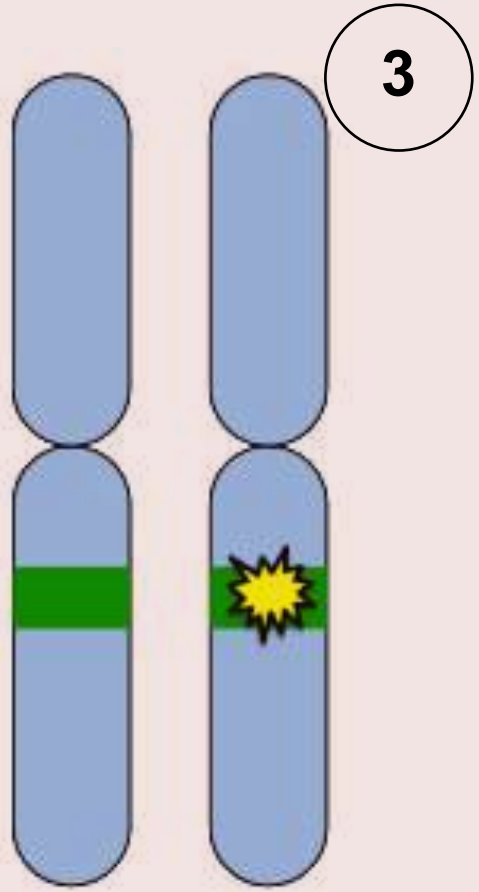


Myotonic dystrophy (DM) will be this talk's focus

Myotonic dystrophy—Most common MD found in adults

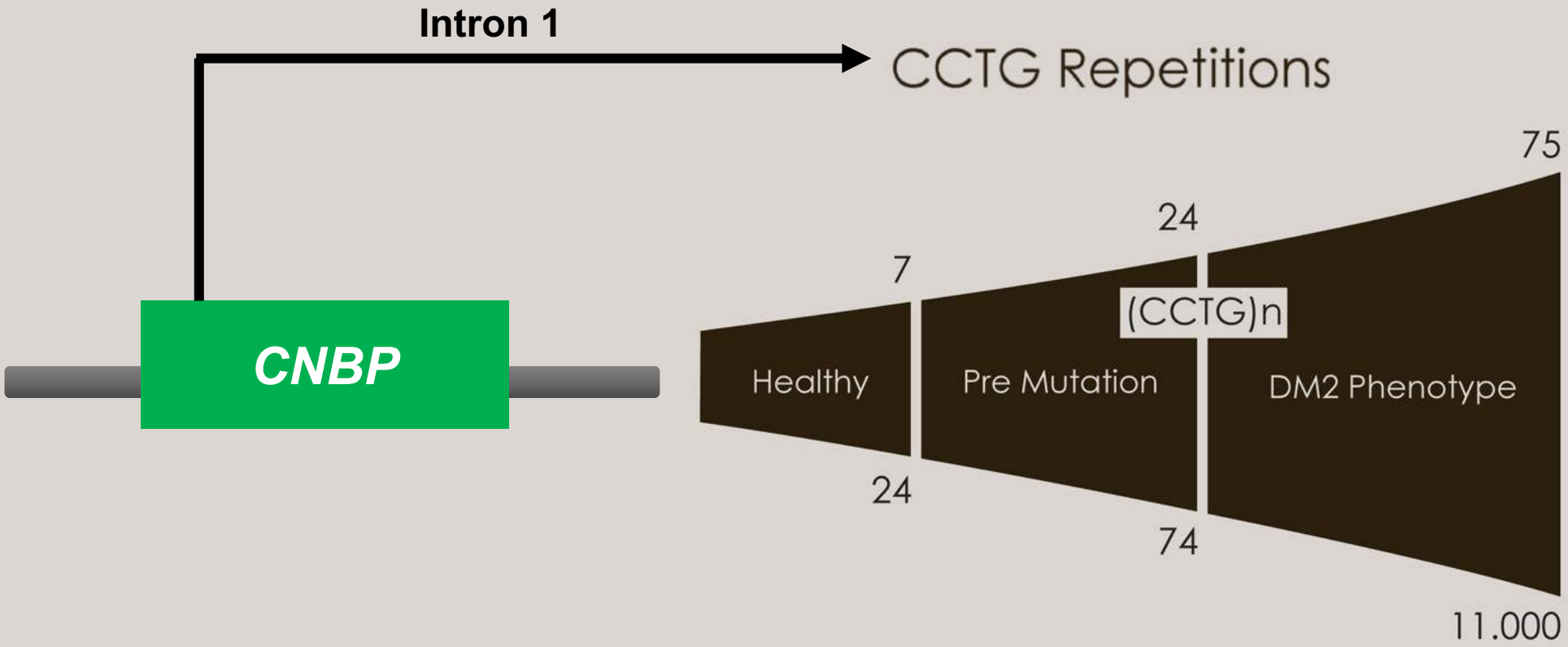


Type 1 (DM1)
***DMPK* gene mutation**



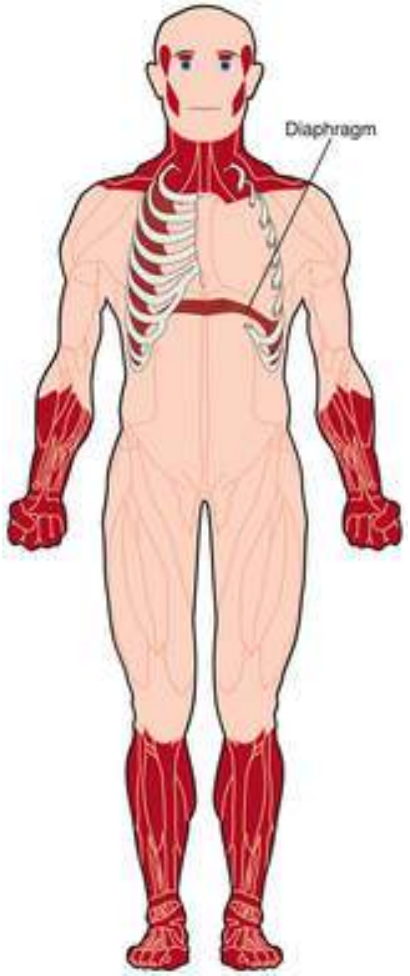
Type 2 (DM2)
***CNBP* gene mutation**

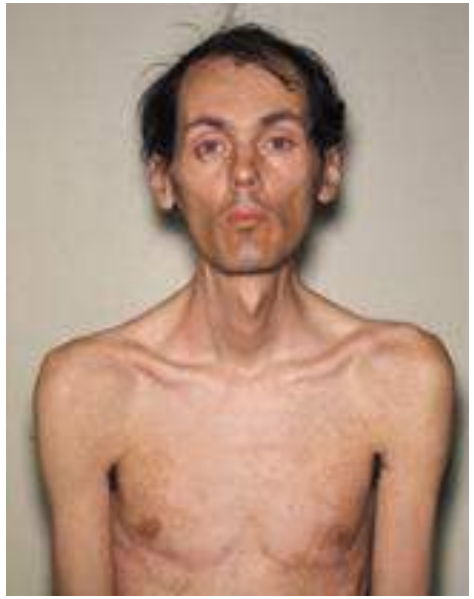
DM2 is caused by a tetranucleotide CCTG repeat in the first *CNBP* intron

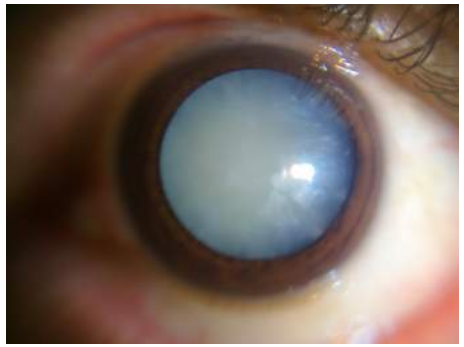


RNA-gain-of-function is a prominent mechanism in both type 1 and 2

DM2 has a wide range of symptoms







Muscle wasting

Muscle weakness

Myotonia

Early onset cataracts

Heart problems

Muscle pain

More mild than DM1

Prevalence ~1/8000

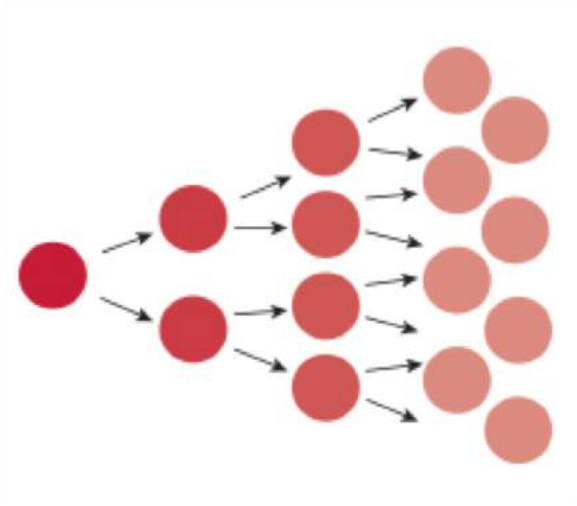
Onset at 20-30 years

What is the CNBP protein?

Homo sapiens
Humans

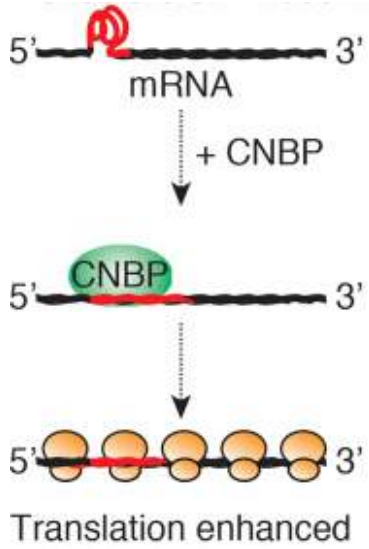
179 aa

Biological Processes



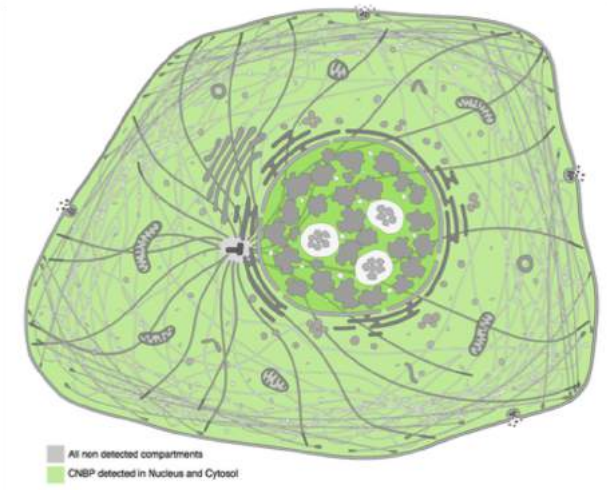
Reg. of transcription/translation
Reg. of cell proliferation
Cholesterol biosynthetic process

Molecular Function



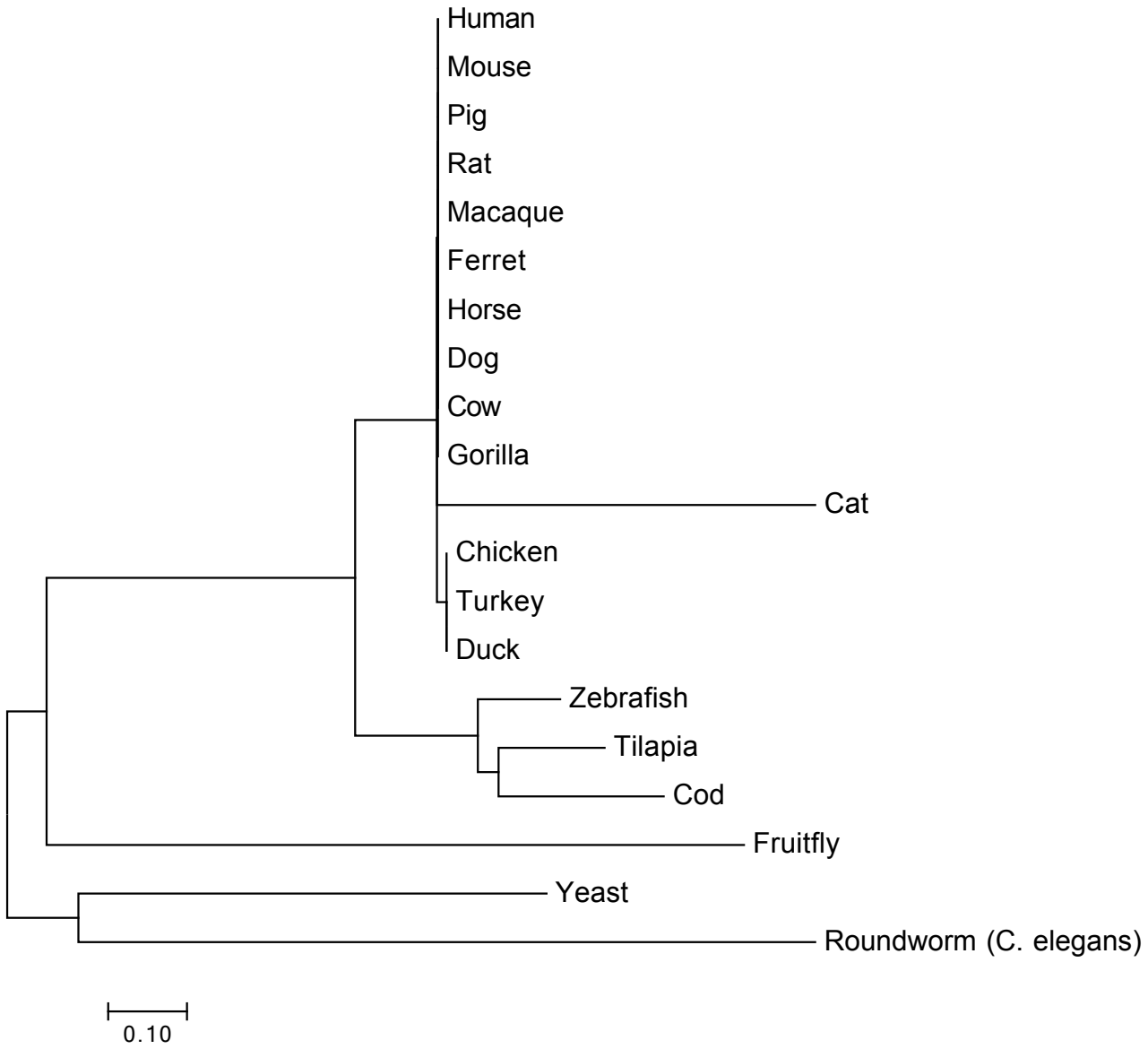
Metal ion binding
DNA & RNA binding

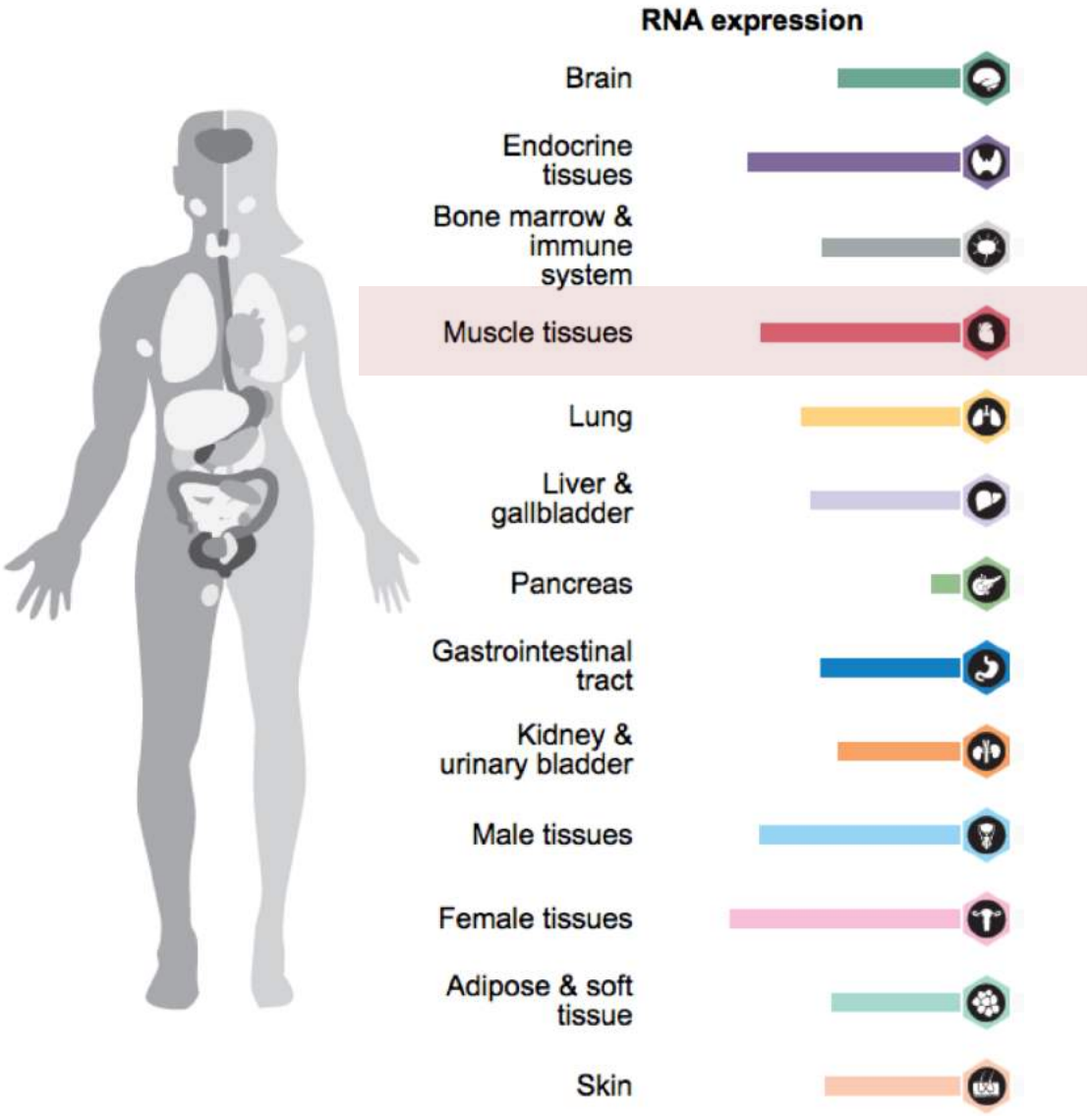
Cellular Components



Cytosol
Nucleus
ER

Phylogeny & expression of CNBP





Is CNBP conserved across species?

Humans

179 aa (100%)

Mouse

178 aa (99%)

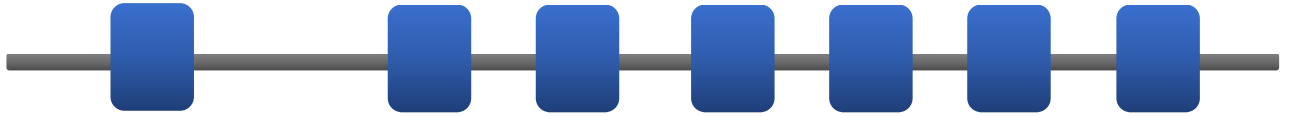
Monkey

178 aa (99%)

Rat

177 aa (99%)

Zebrafish



163 aa (64%)

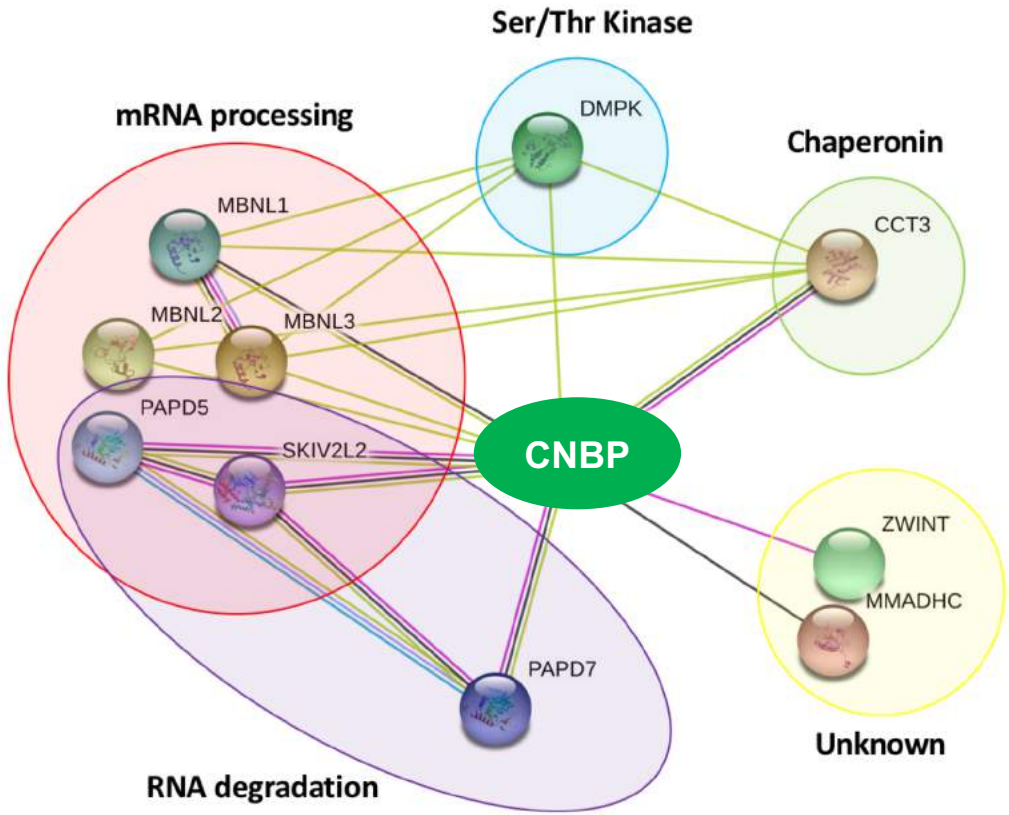
Yeast

153 aa (41%)

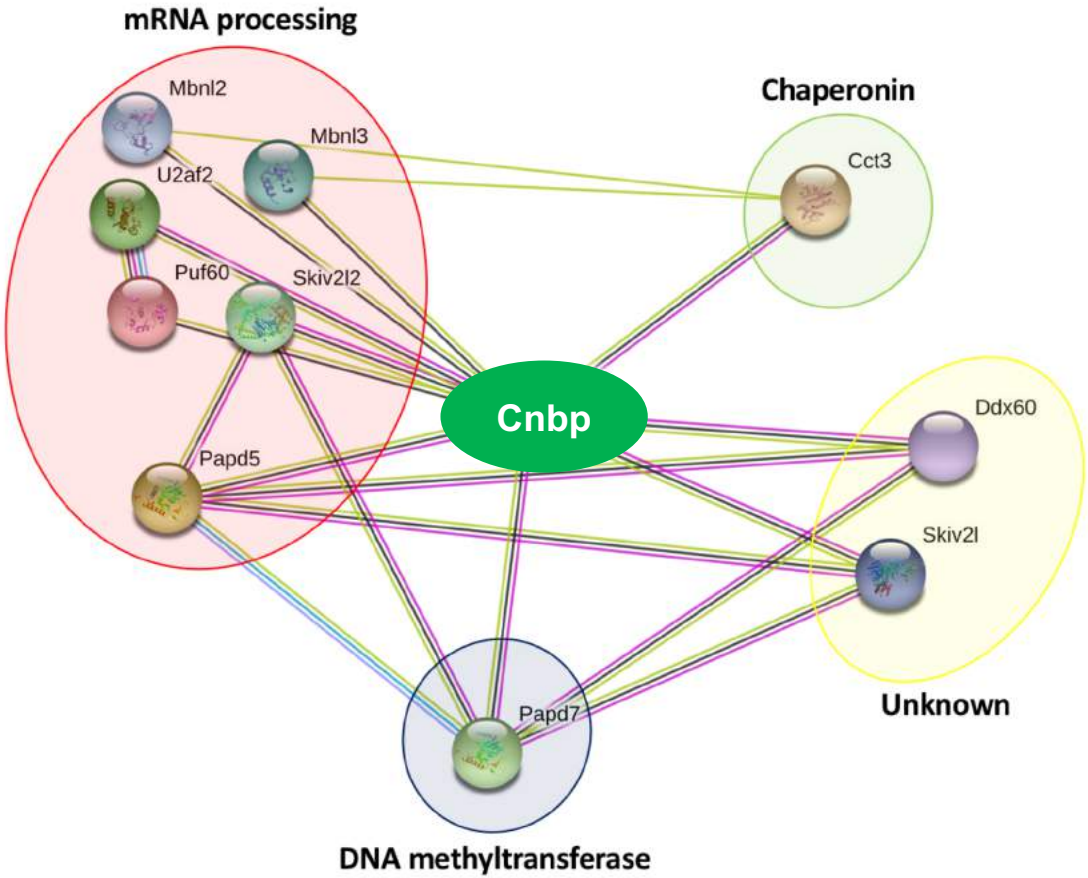
Fly

165 aa (36%)

CNBP protein-protein interactions



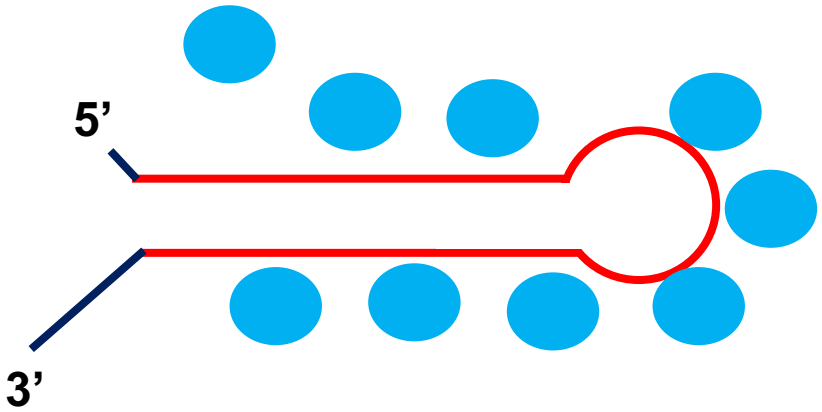
Human



Mouse

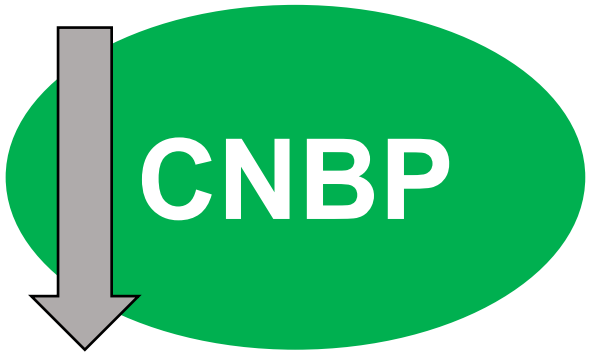
What is currently known about DM2 & CNBP?

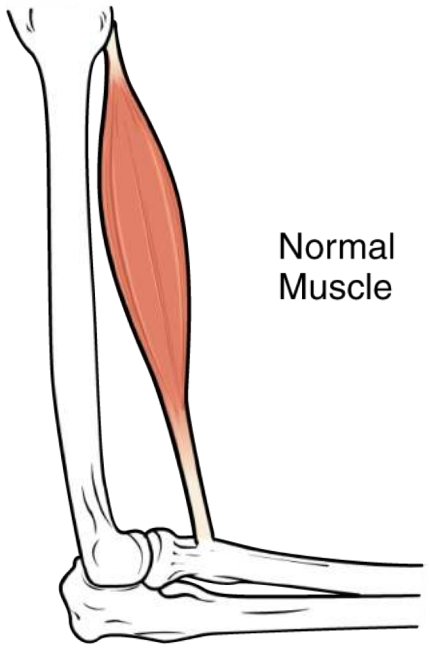
CNBP pre-mRNA is toxic, sequesters proteins, and not translated	CNBP downregulated in DM2

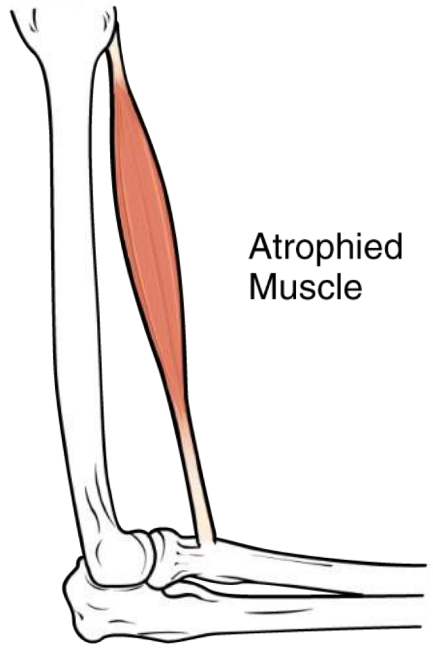


*Present in both DM1 & DM2

It is unknown how decreased CNBP contributes to muscle wasting and weakness



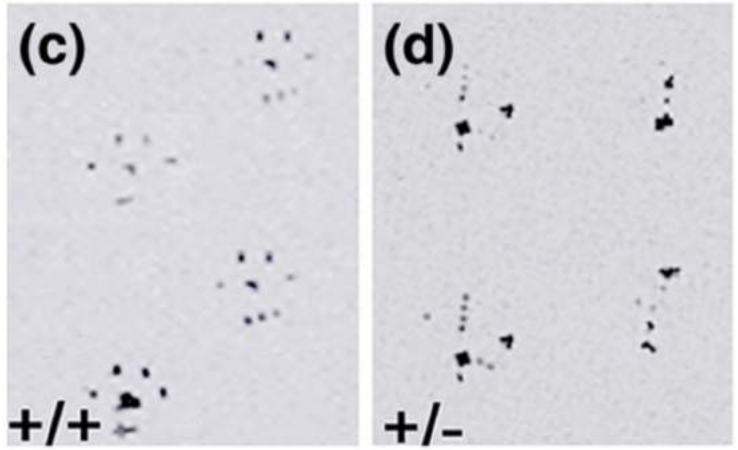




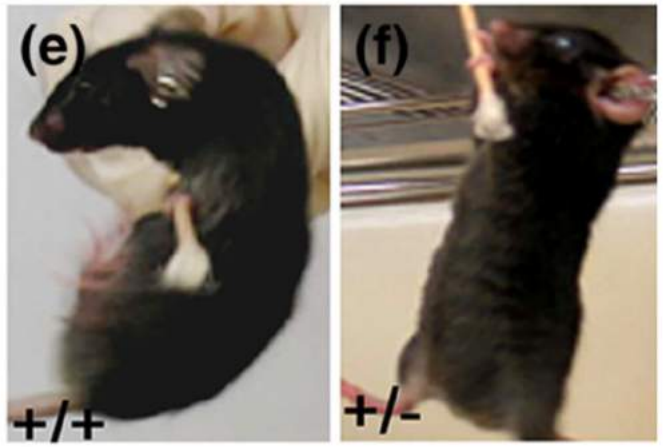
Also unclear why DM2 less severe than DM1

Model organism phenotypes

Mus musculus



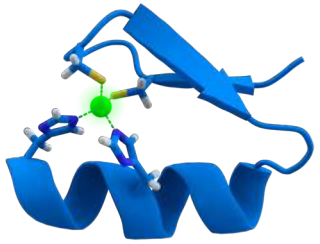
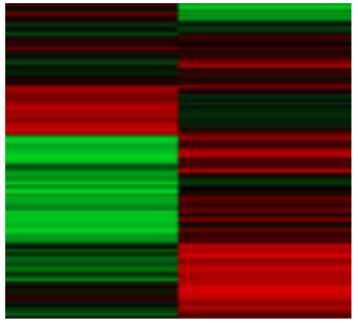
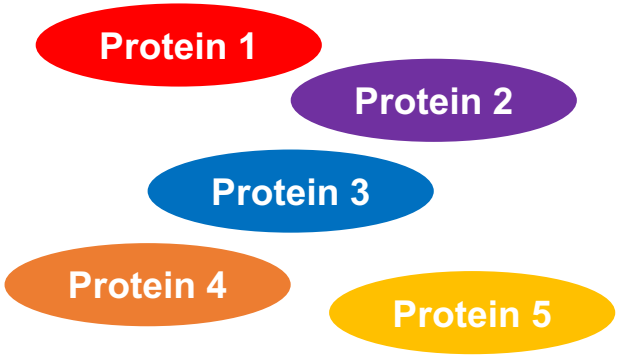
Defective walking & unclear footprints



Extremely weak & proximal/distal muscle wasting

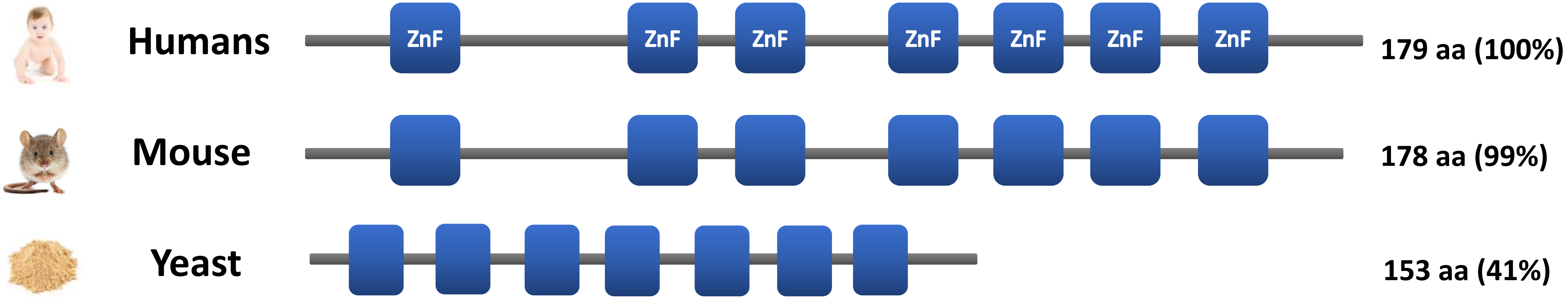
Haploinsufficient mice exhibit DM2 phenotypes

Goal: To study how low levels of CNBP contributes to muscle wasting and weakness

Aim 1	Aim 2	Aim 3
		
Identify conserved sites of CNBP important to proper muscle function	Identify genes important to proper muscle function	Quantify dysregulation of proteins involved in skeletal muscle function

Hypothesis: CNBP loss contributes to weakness and wasting by affecting pathways not associated with the RNA-gain-of-function mechanism

Aim 1: Identify conserved sites of CNBP critical to muscle function

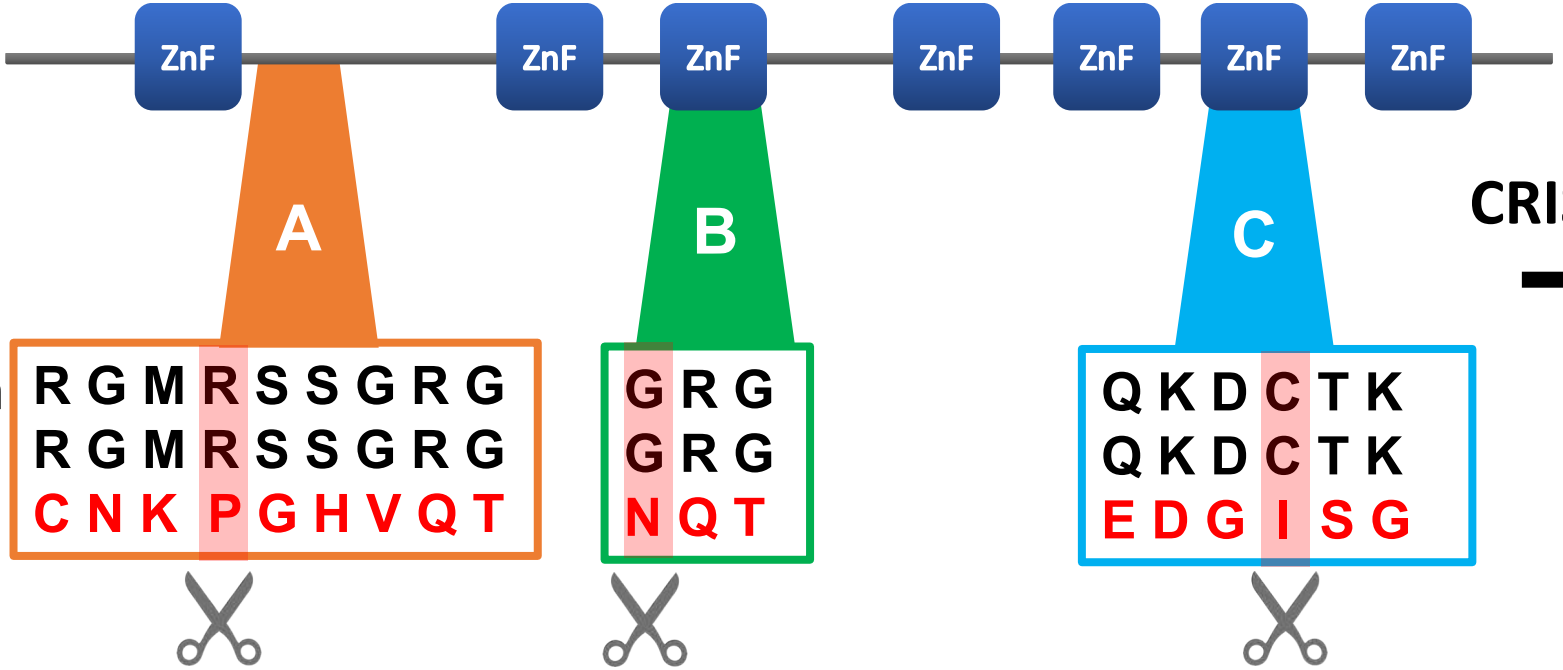


Species/Abbrv	Group Name									*																												*															
1. Human		R	G	R	G	M	R	S	R	G	R	G	G	F	T	S	D	R	G	F	Q	F	V	S	S	S	L	P	D	I	C	Y	R	C	G	E	S	G	H	L	A	K	D	C	D	L	Q	E	D	V	E	A	C	Y
2. Mouse		R	G	R	G	M	R	S	R	G	R	G	G	F	T	S	D	R	G	F	Q	F	V	S	S	S	L	P	D	I	C	Y	R	C	G	E	S	G	H	L	A	K	D	C	D	L	Q	E	D	E	-	A	C	Y
3. Fruitfly		G	G	P	G	G	V	G	G	G	G	G	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	G	G	G	M	R	G	N	D	G	G	G	M	R	R	N	R	E	-	-	-	-	-	-	K	C	Y
4. Tilapia		R	G	R	G	K	-	G	R	G	R	G	K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D	L	F	C	Y	R	C	G	E	L	G	H	V	A	R	D	C	E	R	T	E	D	-	-	A	C	Y
5. Cow		R	G	R	G	M	R	S	R	G	R	G	-	-	-	-	-	-	-	F	Q	F	V	S	S	S	L	P	D	I	C	Y	R	C	G	E	S	G	H	L	A	K	D	C	D	L	Q	E	D	-	-	A	C	Y
6. Yeast		L	C	Y	N	C	N	K	P	G	H	V	Q	-	-	-	-	T	D	C	T	M	P	R	T	V	E	F	K	Q	C	Y	N	C	G	E	T	G	-	-	H	V	R	S	E	C	T	V	Q	-	-	R	C	F
7. Dog		R	G	R	G	M	R	S	R	G	R	G	G	F	T	S	D	R	G	F	Q	F	V	S	S	S	L	P	D	I	C	Y	R	C	G	E	S	G	H	L	A	K	D	C	D	L	Q	E	D	V	E	A	C	Y
8. Horse		R	G	R	G	M	R	S	R	G	R	G	-	-	-	-	-	-	-	F	Q	F	V	S	S	S	L	P	D	I	C	Y	R	C	G	E	S	G	H	L	A	K	D	C	D	L	Q	E	D	-	-	A	C	Y

1. Identify protein domains using SMART and align FASTA sequences with ClustalW

Aim 1: Identify conserved sites of CNBP critical to muscle function

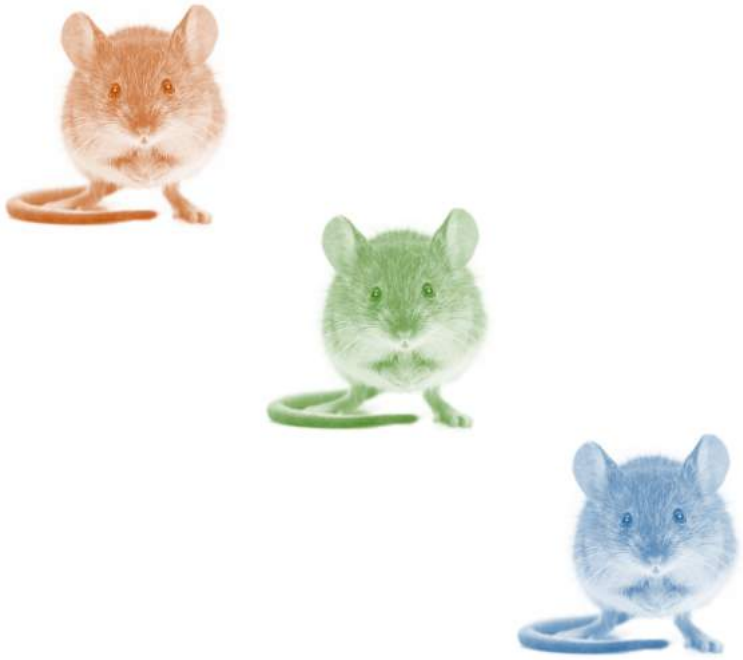
179 aa



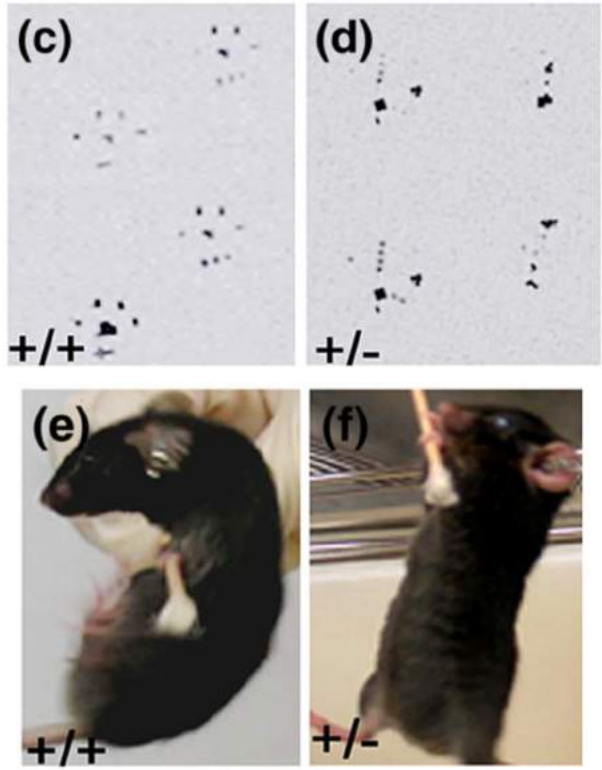
CRISPR/Cas9

2. Create mutants in conserved regions with CRISPR/Cas9

Aim 1: Identify conserved sites of CNBP critical to muscle function



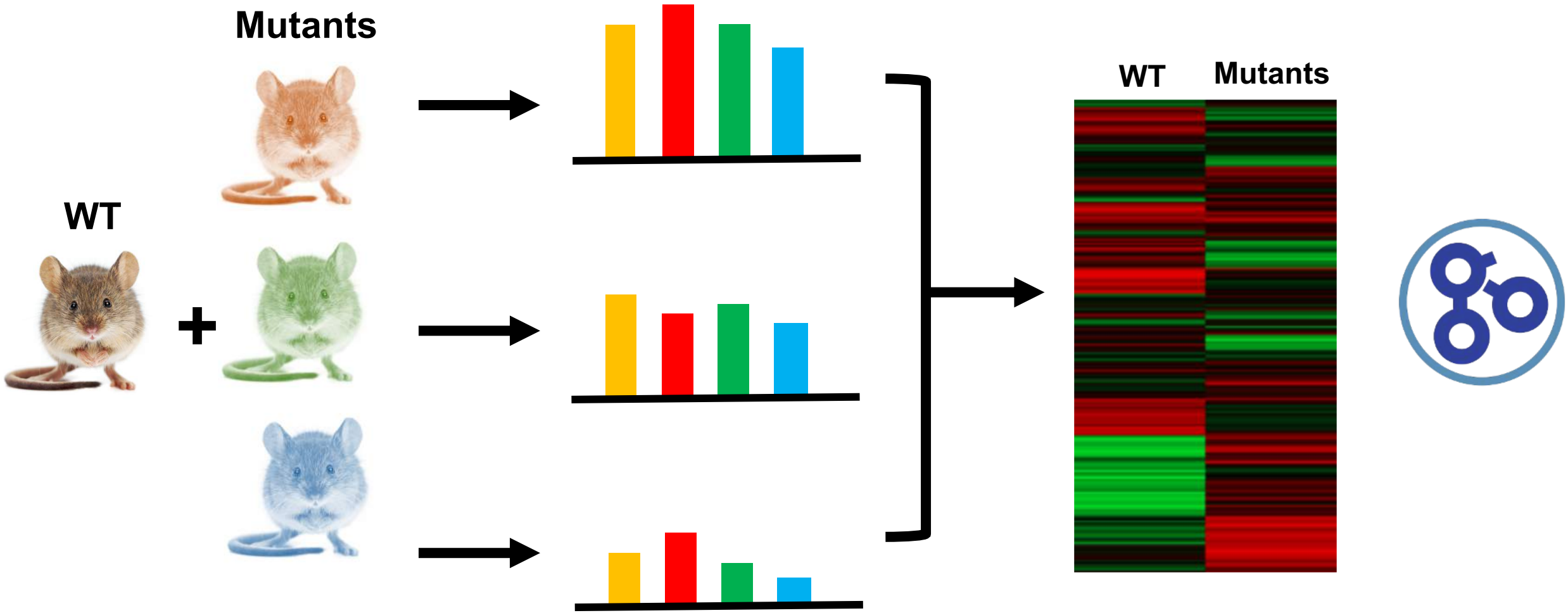
Phenotype screen



<u>Hypothesis</u>: Specific sites conserved only within muscular organisms will be critical in regulating muscle function

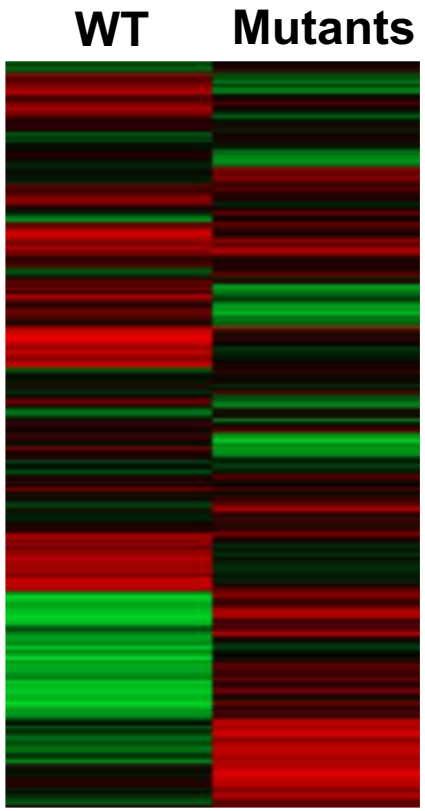
3. Screen for mutant phenotypes

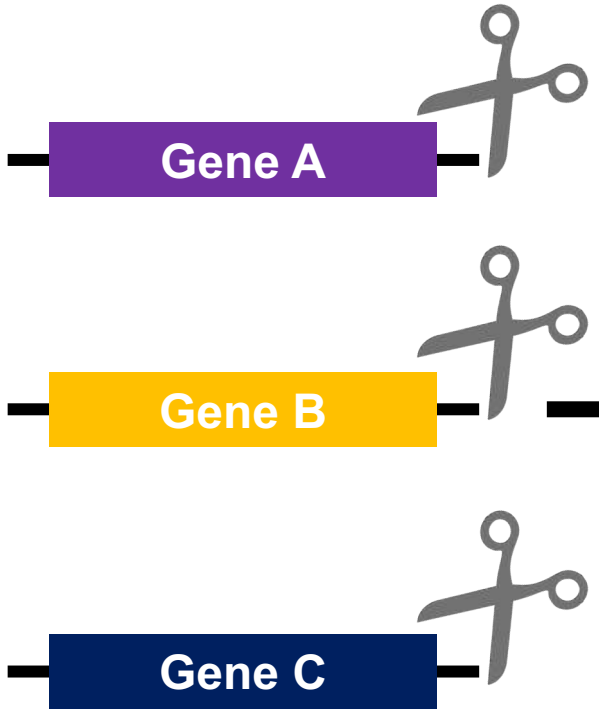
Aim 2: Identify genes that are important in regulating muscle function

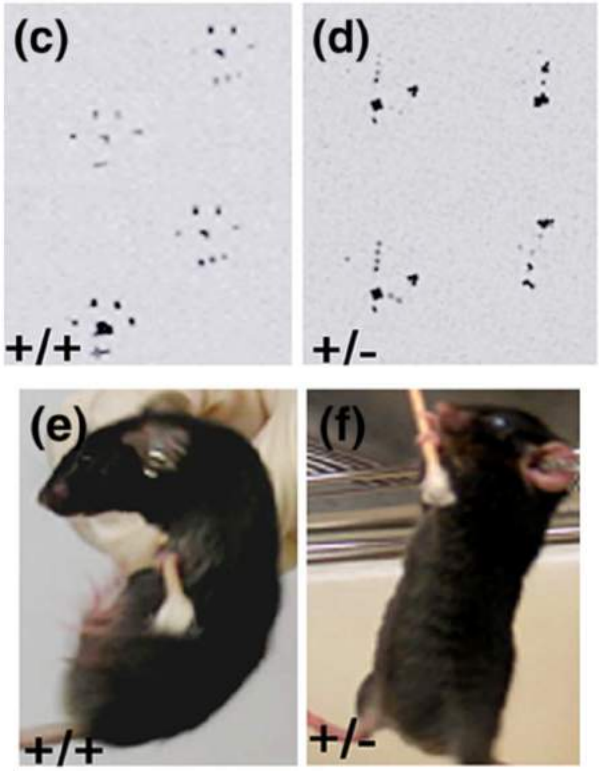


1. Perform RNA-seq in mouse muscle tissue and sort with GO

Aim 2: Identify genes that are important in regulating muscle function



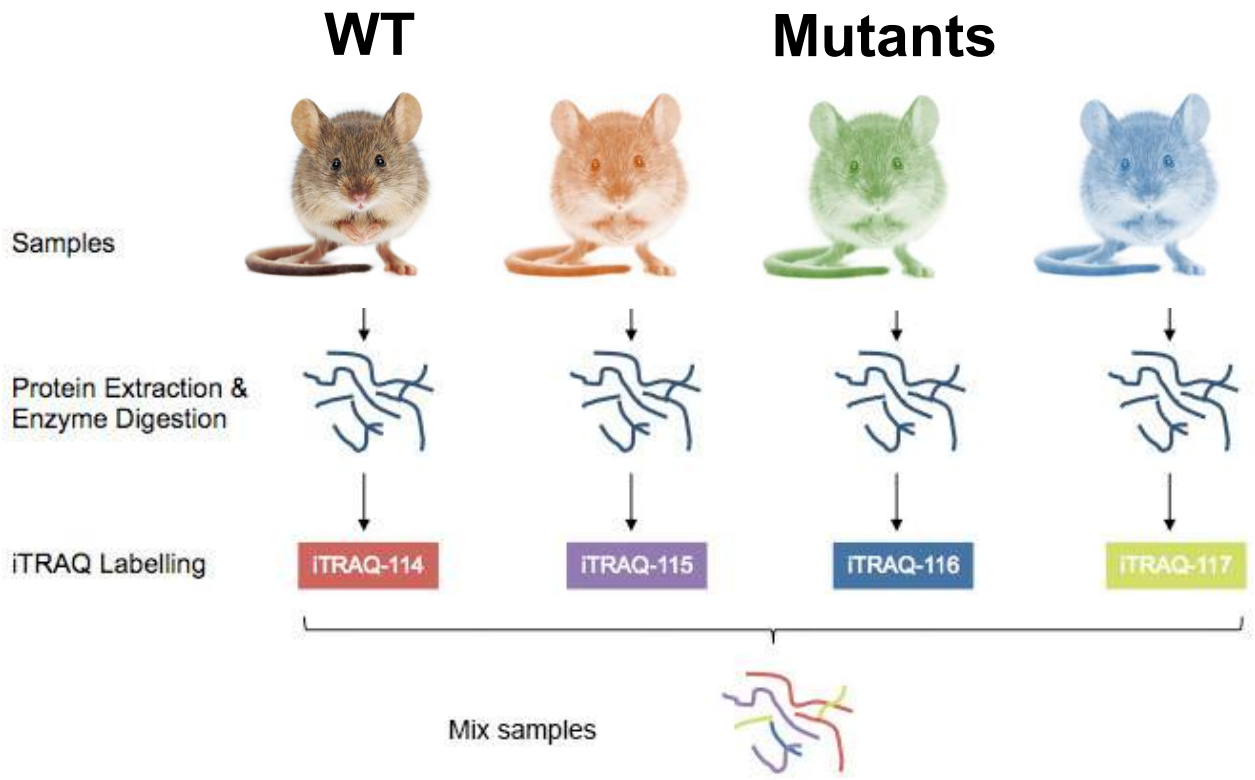


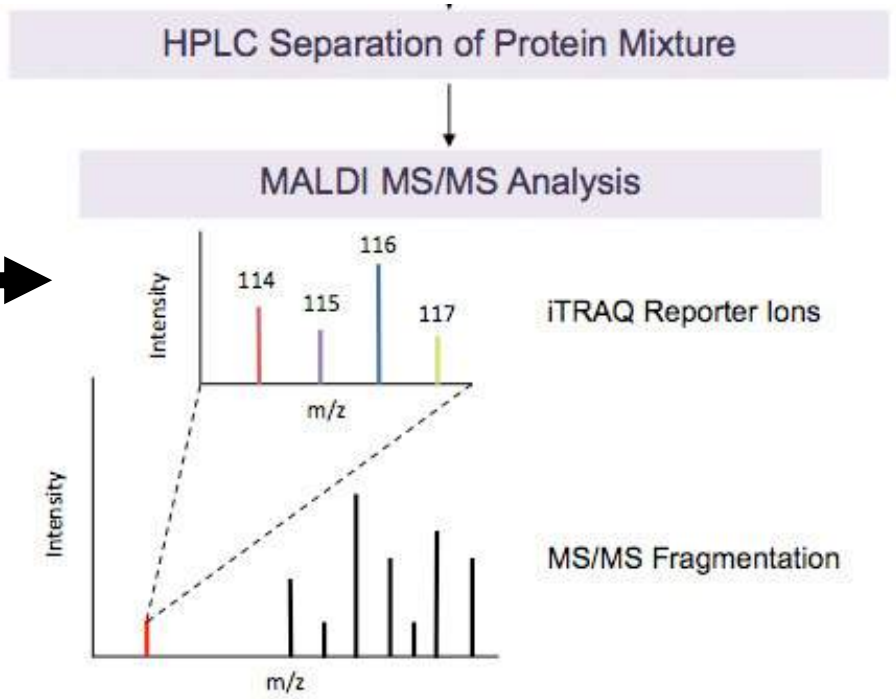


<u>Hypothesis</u>: CNBP mutants will have differentially expressed genes due to changes in CNBP-DNA binding and these genes are critical to regulating muscle function

2. Knock-out differentially expressed transcripts with CRISPR & screen

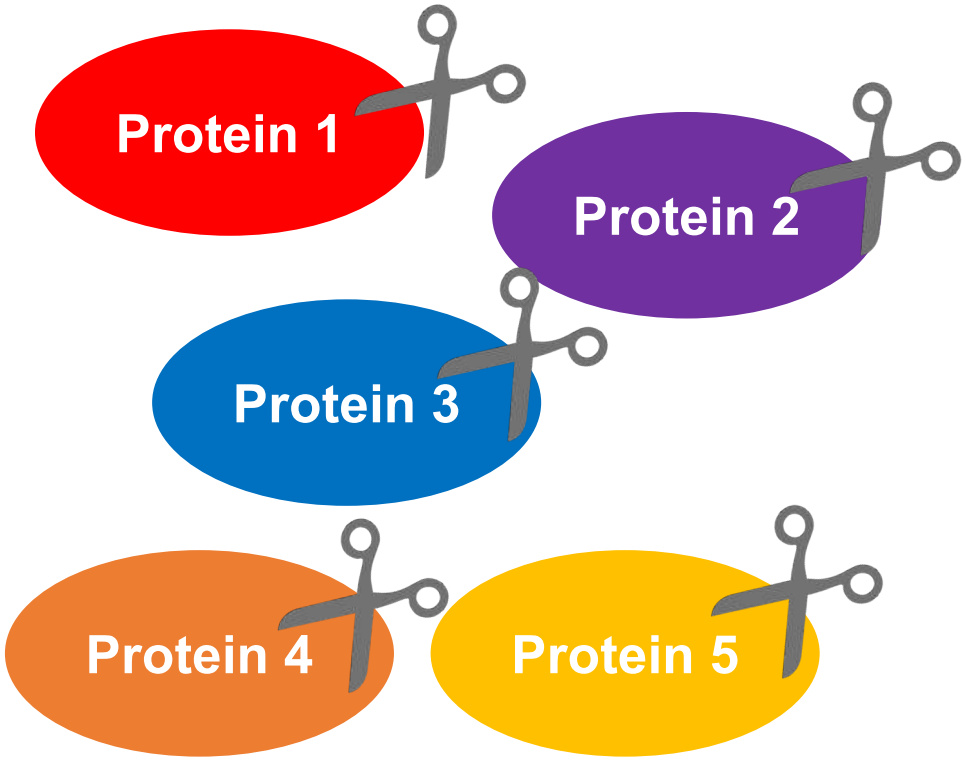
Aim 3: Quantify dysregulation of proteins involved in skeletal muscle function





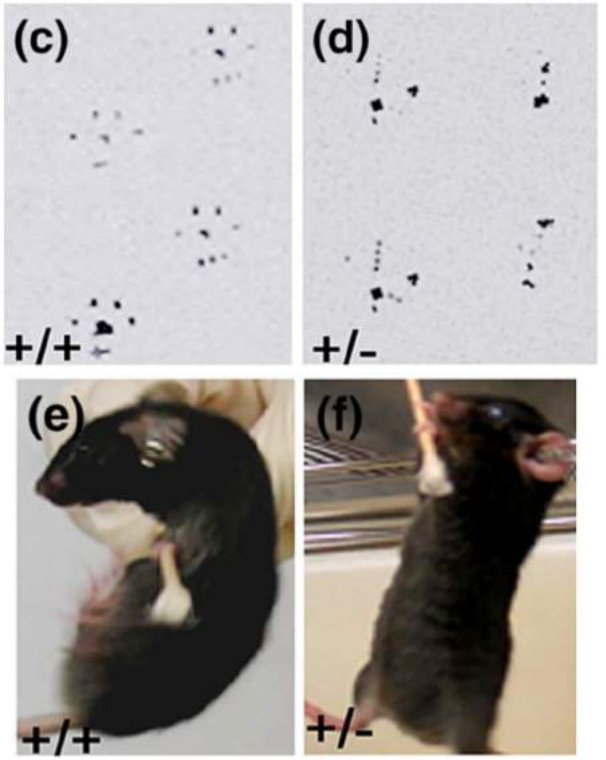
1. Isolate proteins from skeletal muscle of WT and CNBP mutants

Aim 3: Quantify dysregulation of proteins involved in skeletal muscle function



CRISPR/Cas9

Knock-out



<u>Hypothesis</u>: CNBP mutants will have dysregulated proteins levels and these proteins will be critical to skeletal muscle function

2. Quantify proteins, KO dysregulated proteins and screen for mutant phenotypes

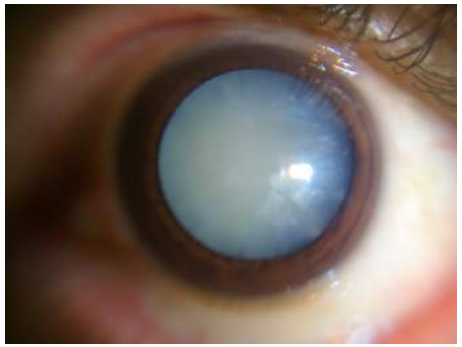
Summary of specific aims

Aim 1 Identifies regions of CNBP critical to regulating muscle function

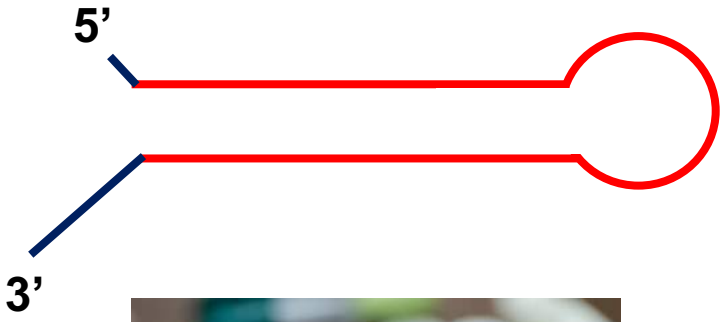
Aim 2 Identifies mRNAs dysregulated by loss of CNBP

Aim 3 Identifies proteins and protein classes dysregulated by CNBP

Future directions



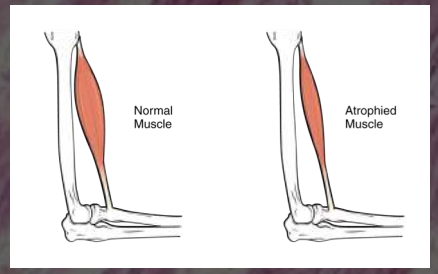
Characterize CNBP interactions in other tissues, such as eye and endocrine tissues



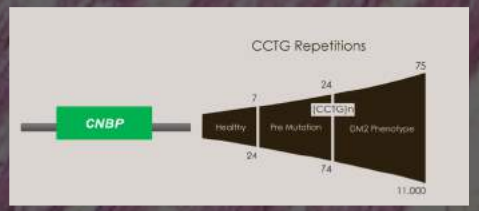
Chemical screens to remove *CNBP* pre-mRNA

Continue developing drugs for management of symptoms, such as pain and myotonia

Conclusions



DM2 is a disease characterized by many symptoms



Nucleotide repeats in the first intron of *CNBP* causes DM2

The effects of lower CNBP levels are unclear

Learning more about each disease mechanism will aid in treatment of disease


Questions?

References

https://www.ncbi.nlm.nih.gov/pubmed/28329689

https://www.ncbi.nlm.nih.gov/pubmed/28078562

https://www.ncbi.nlm.nih.gov/pubmed/17335846

https://ghr.nlm.nih.gov/gene/CNBP

https://ghr.nlm.nih.gov/condition/myotonic-dystrophy