Effect of Cell Binding Domain Mutation in the *unc-52* Gene of *C. elegans*

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Abstract

- *Caenorhabditis elegans* have sequenced genome, recorded molecular pathways and simple structure
- *unc-52* gene encodes a homologue for the extracellular matrix proteoglycan perlecan
- CRISPR-Cas9 to mutate the amino acid sequence of the *unc-52* gene from RGD→RGE and successfully generated homozygous alleles
- Mutated *C. elegans* were observed and compared to N2 in order to further characterize *unc-52*
- Possible implications in development of the cytoskeleton and interaction in the Extracellular Matrix

Background

C. elegans:
- Soil nematode with quick generation time
- Easily observable phenotypes
- Easy to store specimens frozen

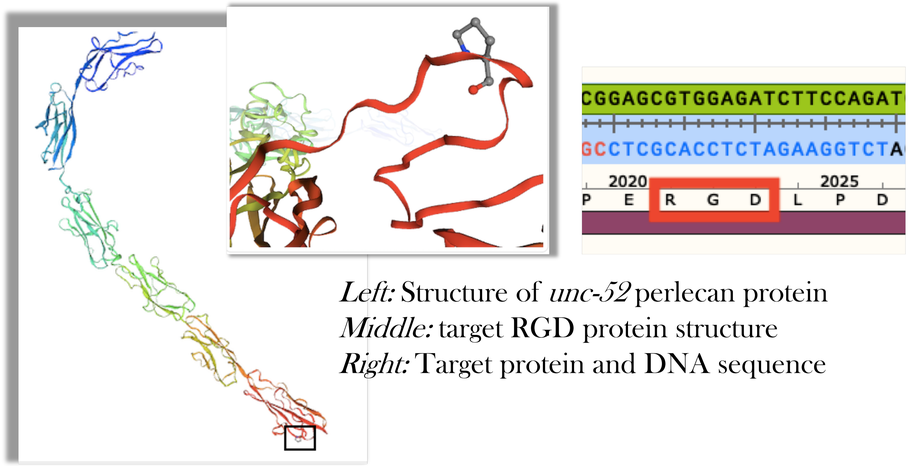
unc-52
- Encodes a proteoglycan perlecan
- Structural basement membrane protein
- Role in myofilament organization
- Regulator of growth-factor signaling in body wall muscle cells
- Known that mutations cause uncoordination and severe phenotypes are known to cause paralysis

RGD sequence
- Responsible for integrin binding in the ECM
- Changed RGD→ RGE
- Arg-gly-asp → arg-gly-glu

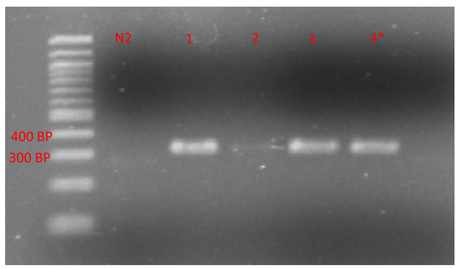
Purpose:
- Mutate RGD to further characterize the function of *unc-52* in *C.elegans*

Results

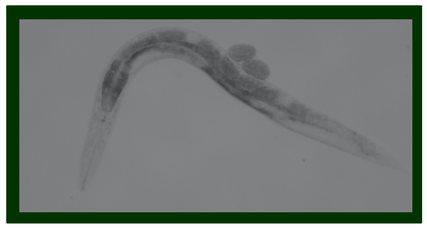
Movement Observations:	Other Observations:
- Severely uncoordinated movement - Paralysis - Lack of DTC migration led to lack of muscle cells in body wall	-Slow growing: after 3 days, Unc-52 worms are much smaller than N2 -Produce less progeny than N2 counterparts



Left: Structure of *unc-52* perlecan protein
Middle: target RGD protein structure
Right: Target protein and DNA sequence



Positive PCR results show successful transformation of RGD→RGE
*= this plate generated 100% homozygous mutants



Left: Phenotypic result of UNC-52 mutant with paralyzed uncoordinated movement, 10x
Right: N2 worms with no mutant phenotype, 10x

Methods

Treatment
- *C. elegans* treated with two CRISPRs:
  - target *unc-52*
  - indicator *dpy-10*

Selection
- 6 DUMPY or ROLLER phenotypes were selected and given individual plates
- allowed to self-fertilize

Testing
- Progeny tested with PCR for mutant RGE sequence
- Visualized with gel electrophoresis

Purification
- Positive plates selected based on gel results and treated with mutant PCR
- Testing and purification repeated two more times to isolate homozygous alleles

Analysis
- Total successful mutants counted
- Movement assays performed
- Phenotype compared to N2

Phenotype
- Observed mutants and compared to normal to observe phenotype and characterize *unc-52*

Discussion

Allows for beyond microscopic understanding regarding both intracellular and extracellular processes that may be disrupted and the resulting effects upon organisms

Implications:
- Muscle development
- Organ development
- Tissue development
- Skeletal muscle
- Myopathies and muscular dystrophies
- Corneal Epithelial development
- Basal Lamina mutations