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Investigating the Roles of RNA Binding Protein Combinations in Neuronal Function and Organismal Behavior

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Investigating the Roles of RNA Binding Protein Combinations in Neuronal Function and Organismal Behavior

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ABSTRACT

The Norris lab recently identified two RNA binding proteins required for proper neuron-specific splicing. The lab conducted touch-response behavioral assays to assess the function of these proteins in touch-sensing neurons. After isolating *C. elegans* worms with specific phenotypes, the lab used automated computer tracking and video analysis to record the worms' behavior. The behavior of mutant worms differed from that of wild-type worms.

The Norris lab also discovered two possible RNA binding protein sites in SAD-1, a neuronal gene implicated in the neuronal development of *C. elegans*[1]. These two binding sites may control the splicing of SAD-1. The lab transferred mutated DNA into the genome of wild-type worms by injecting a mutated plasmid. The newly transformed worms fluoresced green, indicating that the two binding sites control SAD-1 splicing.

1. INTRODUCTION

The Norris lab studies splicing in neurons using *C. elegans*, a self-fertilizing worm with a simple nervous system that can be imaged in vivo. Touch-sensing neurons in *C. elegans* convert touch stimuli, such as a light poke with an eyelash, into action potentials that affect locomotion. Touch-sensing neurons in wild-type worms contain exon 15 in the SAD-1 kinase gene. A specific mutation in the RNA binding protein *Mec-8* causes exon 15 to be skipped.

The Norris lab created a reporter that fluoresces red when exon 15 in SAD-1 is included, and green when skipped[1]. In a wild-type worm with no mutations, the touch neurons are always red because exon 15 is always included. Genetic screens found that *C. elegans* with mutations in certain transcription factors like *Mec-3* have no red fluorescence. These mutated worms cannot sense a light touch such as using an eyelash as the touch stimuli. The presence of this phenotype allows these worms to be easily distinguished from wild-type worms.

It is well understood that transcription factors such as *Mec-3* and RNA binding proteins such as *Mec-8* affect SAD-1 splicing. To determine the mechanisms that control proper splicing and neuronal function in *C. elegans*, the lab sought to determine whether the RNA binding protein *Mec-8* binds directly to SAD-1 RNA.

In order to establish whether genes that regulate splicing are required for touch-sensing neurons to function, the lab also sought to investigate behavioral defects in *Mec-3*, *Mec-8*, and SAD-1 loss-of-function mutant *C.elegans* worms. Prior results with *Mec-3* and *Mec-8* mutants demonstrated that these mutant phenotypes differ from their wild-type counterparts.

2. METHODS

To determine whether *Mec-8* binds directly to SAD-1 RNA, the lab mutated plasmids, cloning vectors that are good for manipulating small DNA fragments, at specific binding sites and injected the plasmids into wild-type worms. To test possible binding sites for *Mec-8*, we used the restriction enzymes Xho-1 and Not-1 to cut a mutated SAD-1 splicing reporter in different locations. Figure 1 shows the gel results for mutated *Mec-8* binding sites showing two cuts including one that is clear and one that is faint, indicating that the two restriction enzymes made the proper cuts at the right locations.

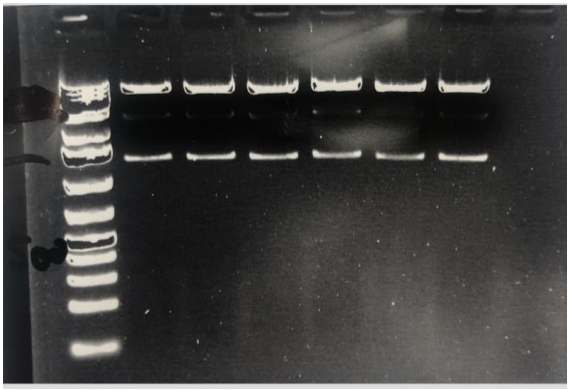

Figure 1: Gel Results for Mutated Mec-8 binding sites showing two cuts including one that is clear and one that is faint indicating that the two restriction enzymes made the proper cuts at the right locations.

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In order to create a recombinant plasmid, the lab used ligase to connect the purified minigene with the purified cut plasmid. We then used *E.coli* to produce large quantities of the plasmid. To isolate *E. coli* containing the plasmid, we treated the *E. coli* with ampicillin, as the plasmid also carries an ampicillin resistance gene. The ligated plasmid was then used to transform bacteria. Lastly, we added a promoter to the plasmid to help initiate transcription. We successfully cut the resulting DNA and injected the mutant plasmid into wild-type worms.

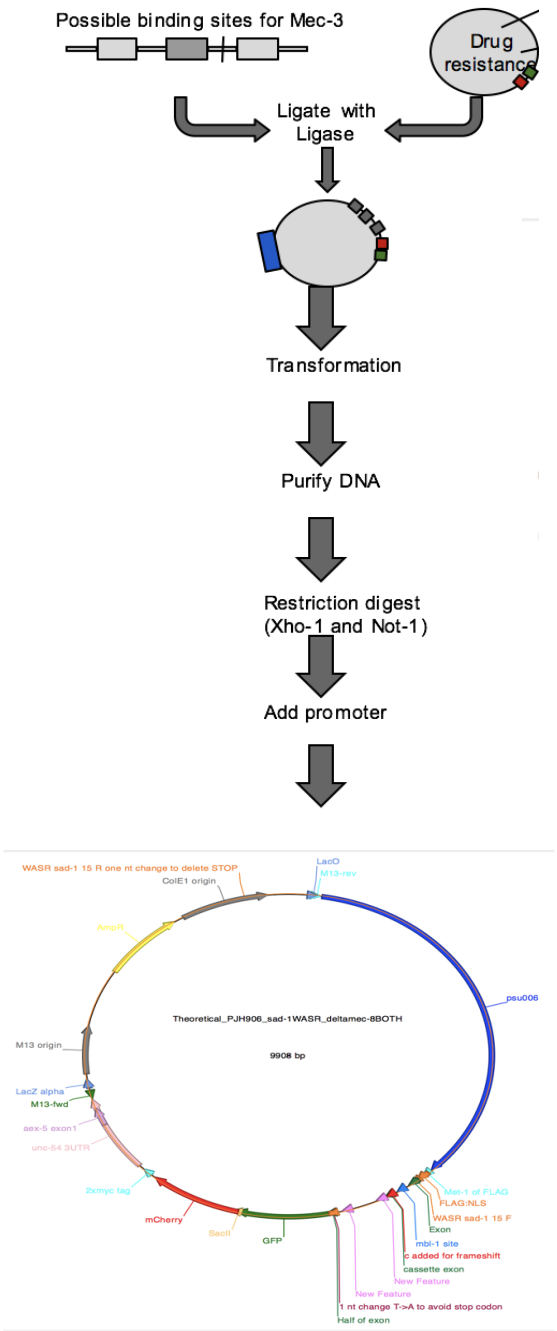

Figure 2**:** Diagram showing the steps used to create the plasmid containing the mutated sites

We conducted behavioral video assays to assess behavioral defects with *Mec-3* mutant, wild-type, and SAD-1 mutant worms. To continuously perform these assays, we replated the worms frequently to ensure that there were young larvae readily available. The lifespan of *C. elegans* worms is short, and it is important to use worms in the L4 larval stage, which occurs just before the worms reach adulthood. L4 worms are easy to detect by their crescent-moon-shaped spot on their body. Since they have yet to reach adulthood, using L4 worms helps keep the experiment consistent and prevents self-fertilization from occurring. If the worms self-fertilize, the experiment is no longer controlled, as young larvae behave differently than adult worms.

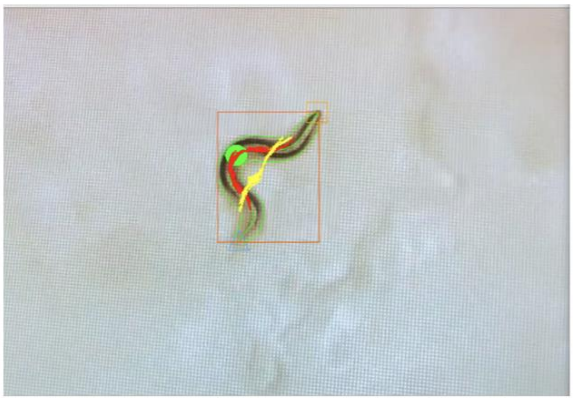

Figure 3: Video Analysis software showing the outline of a worm and the track line of its movements

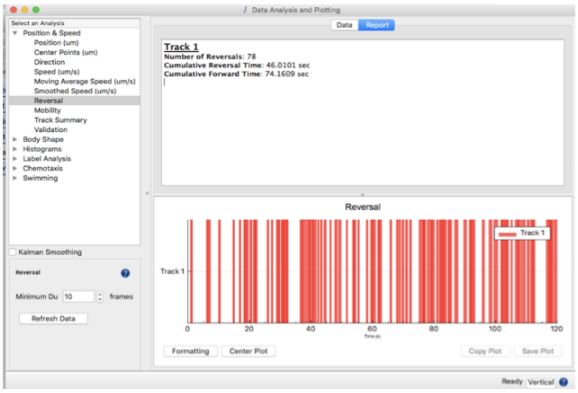

Figure 4: Video Analysis Software showing the reverse movements in the course of the worms recorded track

To conduct the behavioral assay, we isolated individual worms of each phenotype and placed each worm on its own plate. To avoid disrupting the worm's natural movement, it was crucial to wait an hour to allow each worm to adjust to its new environment. Next, we recorded the movement of each worm for 2 minutes by measuring time moving forward, time in reverse, and time without movement. We used an automated computer tracking program to confirm the results obtained manually. The automated system recognizes worm movement based on a predefined threshold. However, the automated system

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appeared to work properly only in ideal conditions, so we decided to use the results of the manual movement analysis.

3. RESULTS

The Norris lab successfully transformed and injected the splicing reporter plasmid containing the mutated *Mec-8* binding sites. We found that both mutations alter SAD-1 splicing. The splicing reporter also showed a change in the fluorescence of touch neurons. We found that mutating either binding site caused the fluorescence of touch neurons to shift from 100% red to approximately 50% red and 50% green. This pattern indicates that exon 15 is partially skipped in the mutants. We concluded that (a) mec-8 binds to both sites and (b) both Mec-8 mutations alter SAD-1 splicing and are necessary for proper neuronal function in *C. elegans*.

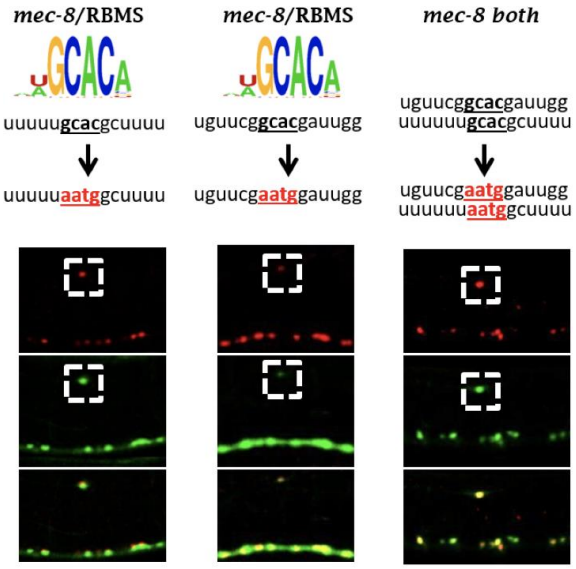

Figure 5: Fluorescence imaging of Injected Mutants showing the location of the mutation along and imaging of mechanosensory neurons

After pooling the lab's data, we found minor behavioral defects in SAD-1 worms compared to wild-type worms. SAD-1 was associated with slightly decreased time spent in reverse compared to the wild-type gene.

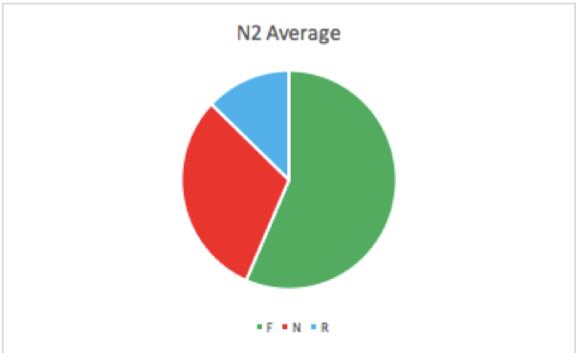

Figure 6: Average Proportions of movements for wild-type worms showing Forward, Pause, and Reversals

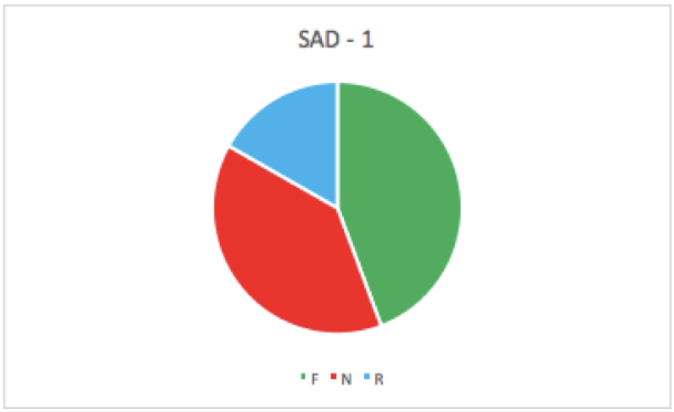

Figure 7: Average proportion of movements for SAD-1 worms showing Forward, Pause, and Reversal time in seconds

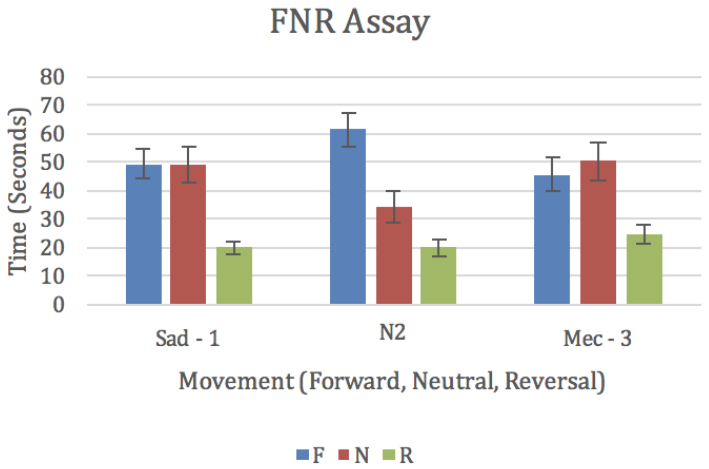

Figure 8: Average Movements for wildtype, SAD-1, and Mec-3 worms showing Forward, Pause, and Reversal time in seconds

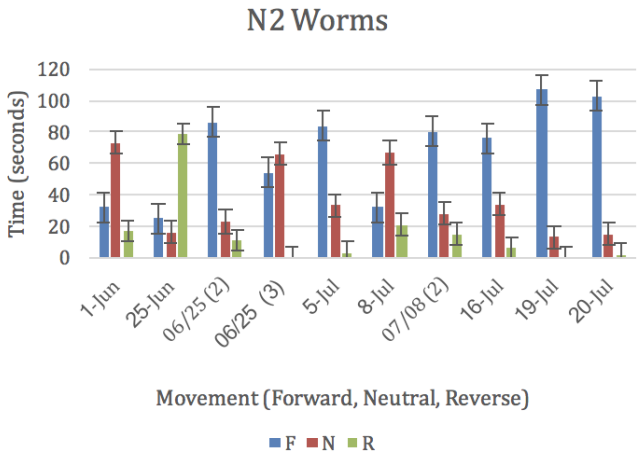

Figure 9: Cumulative Individual data for wildtype worms for Forward, Pause, and Reversal time in seconds

The second part of this project reflected on the effect of the RNA binding proteins and transcription factors in relation to RNA splicing and mutations that can alter the phenotype of the worm. We confirmed that Mec-3, Mec-8, and possibly SAD-1 are functionally important for worm behavior. Thus, these genes contribute to normal functioning and locomotion in *C. elegans*, and mutations in these genes can significantly impact normal functioning.

4. REFERENCES

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