Caenorhabditis elegans: Exploring the Relationship between *srf-6* and *nsy-1*

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Abstract

Parasitic nematodes may cause biomedical and agricultural problems by avoiding host immune systems through changes in surface composition during infection, a process we call surface antigen switching. *C. elegans* is a free-living nematode serving as a model organism for antigen switching. *srf-6* mutations change the developmental surface antigen display, and previous evidence suggests that *srf-6* may be identical to the MAPKKK gene *nsy-1*. Complementation tests between *srf-6* and *nsy-1* mutants were conducted to support the hypothesis that *srf-6* and *nsy-1* affect the same genetic function.

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Introduction

C. Elegans as a Model Organism

Caenorhabditis elegans is a free-living, soil-dwelling nematode used as a model organism in many branches of biology. By using a relatively simple organism as the basis of research across the field, scientists can better understand and interpret each other's work and piece together a fairly complete understanding of the organism. The processes and patterns found within the model organism can then be applied to understand the biology of higher level organisms (Wood, 1988).

C. elegans was originally selected as a model organism for several reasons (Brenner, 1974). Its cell biology is relatively simple, as its adults have only 959 somatic cells. It also has a small genome with a haploid size of 8×10^7. Additionally, its transparency allows for easy observation under a dissecting microscope. Adults reach a size of just 1 mm, allowing for laboratory storage of large stocks. They consume bacteria, and can easily be grown on agar plates with *Escherichia coli.* The short life cycle and large brood size of *C. elegans* allows for the results of genetic crosses to be rapidly evaluated. Mutations can easily be induced by chemical exposure or radiation for genetic selection or screening. Because of the ease of cultivating *C. elegans* in a lab and these ideal anatomical and genetic characteristics, much scientific research has focused and continues on *C. elegans* (Wood, 1988).

In addition to researching *C. elegans* because of its status as a model organism, research on the species has potential to directly impact biomedical and agricultural research. *C. elegans* can be used as an in vivo model for drug therapy research because disease pathways are highly conserved between *C. elegans* and higher organisms (Kaletta & Hengartner, 2006). Additionally, other nematodes are parasitic to humans, domestic animals, and crops. Because many of these

parasitic nematodes are more difficult to culture and so much is already known about *C. elegans*, the free-living *C. elegans* can be used as a basis for learning about its parasitic cousins and the infections they cause (Kennedy & Harnett, 2001). This is a highly valuable area of research not only for the biomedical treatment potentials but also for the possibility of agricultural damage control, as nematodes can cause more than $118 billion annual loses around the world (Atkinson, Lilley, & Urwin, 2012; Wood, 1988).

The rest of this introductory section will provide a brief overview of the anatomy and life cycle of *C. elegans*, introduce the research questions addressed by this experiment, and provide more information on aspects particular to these research questions.

Anatomy and Development

The phylum Nematoda is ecologically ubiquitous and occupies a very diverse set of niches, yet all nematodes are similar in development and anatomy. Nematodes are grouped into three classes, Chromadoria, Enoplia, and Dorylaimia (De Ley, 2006). *C. elegans* can live in marine, terrestrial, or parasitic environments. Despite the differences in environment, the developmental and anatomical patterns described here for *C. elegans* are highly conserved among all members of the phylum (Wood, 1988).

Reproduction and Larval Development

The species *C. elegans* consists of two sexes, hermaphrodite and male. Hermaphrodites produce both oocytes and sperm, while males produce only sperm. Reproduction can occur via self-fertilization by hermaphrodites or fertilization between males and hermaphrodites. Fertilization cannot occur between two hermaphrodites. Once the fertilized eggs hatch, they continue through four larval development stages before reaching adulthood. These larval stages are commonly referred to as L1-L4, respectively (Wood, 1988).

Throughout the larval stages, somatic blast cells divide to form the nervous system, musculature, and gonadal structures. Between each of these larval stages, a molt occurs in which a new cuticle is synthesized underneath the old cuticle and the old cuticle is shed. The cuticles differ in structure and protein composition at the different larval stages (Cox, Kusch, & Edgar, 1981).

Under harsh conditions, an L1 can alternatively molt to form a dauer larva. Dauer larvae are under developmental arrest until conditions become more favorable. Dauers are distinct from larvae that develop through the regular four larval stages. The dauer larva's cuticle protein composition differs, its pharynx is constricted, and its oral orifice is plugged internally. Most physiological processes cease or are modified to allow the organism to persist in the harsh conditions for long periods of time. The environmental conditions that typically cause the dauer larvae formation are high population density, restricted food supply, and high temperature (Hu, 2007).

Anatomy

Simplified, the body plan of nematodes can be seen as two concentric tubes with space in between. The outer tube contains the cuticle, hypodermis, musculature, and nervous system. The inner tube is the intestines, and the space in between, called the psuedocoelom, contains the gonads in adults (Wood, 1988).

Cuticle Structure and Composition

The cuticle is the extracellular structure that covers the outside of *C. elegans*. It consists of four different layers: the outermost external cortical layer, the inner cortical layer, the cortical layer, and the innermost basal layer (Cox, Kusch, et al., 1981). *C. elegans* synthesizes a new cuticle before each molt which lies below the older cuticle. Once the older cuticle is shed, the

new cuticle takes its place on the surface. During this molting period, the organism undergoes a period of lethargy with little movement or feeding. *C. elegans* is known to secrete at least four distinct cuticles: the L1, dauer, L2-L4, and adult (Cox, Staprans, & Edgar, 1981). The cuticle is shed and replaced between each larval development stage, but the cuticles produced during L2, L3, and L4 are very similar or isomorphic. Between the other life stages, changes occur in the cuticle, implying that these molts are metamorphic (Page & Johnstone, 2007).

The cuticle is an organ of particular interest because of the protein variation seen in the cuticles produced at the different molts (Proudfoot et al., 1993). In parasitic nematodes, this variation causes a serious problem because the host organism creates antibodies against the surface proteins of the cuticle. The nematode then sheds its current cuticle and the antibodies prove ineffective against the different proteins of the new cuticle (Olsen et al., 2007). The parasitic nematode *Trichinella spiralis* demonstrates this phenomenon by altering its surface proteins during the molting process (Philipp, Parkhouse, & Ogilvie, 1980). This severely challenges the immune system of the host to be able to overcome the parasitic infection.

Chemosensation

C. elegans has a chemosensory system that allows it to detect food, danger, and other individuals via olfactory and water-soluble molecules. This system is important as it drives chemotaxis towards food or away from danger and can induce the dauer larva stage. The chemosensory system is comprised of bilaterally symmetric pairs of neurons located primarily in the head (amphids) and tail (phasmids) (Bargmann, 2006). Most pairs of neurons are functionally similar, but distinct from other pairs in cilium and axon morphology and synaptic targets (White, Southgate, Thomson, & Brenner, 1986).

There are two functional classes of chemosensory neurons that transduce two distinct sensory modalities, gustation and olfaction. One pair of amphid olfactory neurons is referred to as AWC, with the left and right being AWCL and AWCR, respectively (Bargmann, 2006). AWCL and AWCR are functionally distinct in that one senses butanone and the other senses 2,3-pentanedione, but each individual organism varies in which neuron senses which chemical. Asymmetry between AWCL and AWCR is required for proper chemical detection (Wes & Bargmann, 2001).

srf and *nsy* Genes

The switching of the surface proteins is thought to be controlled at least partially by the *srf* class of genes (Grenache, Caldicott, Albert, Riddle, & Politz, 1996). These genes are separated into three subdivisions. One subdivision consists of *srf-1*, *srf-2, srf-3*, and *srf-5*, all of which exhibit mutant cuticle phenotypes but mostly wild type movement and morphology (Politz, Chin, & Herman, 1987; Politz, Philipp, Estevez, O'Brien, & Chin, 1990). The second subdivision consists of *srf-4, srf-8,* and *srf-9*. These mutants show changes in surface accessibility, uncoordinated movements, abnormal tail morphology, protruding vulva, abnormal egg-laying, and defective gonad morphology (Link, Silverman, Breen, Watt, & Dames, 1992). The final subdivision contains our gene of interest, *srf-6*. Mutations in this gene are known to affect the timing of surface antigens. *srf-6* mutants express surface epitopes recognized by monoclonal antibodies M37 and M38 at all larval stages (referred to as CLD or constitutive larval display), although wild types express this epitope only in the L1 and adult stages or at later larval stages if grown on an extract of spent culture medium (Grenache et al., 1996; Hemmer et al., 1991).

The expression patterns of the surface protein recognized by antibody M37 for wild type and *srf-6* mutants are shown in Figure 1 below.

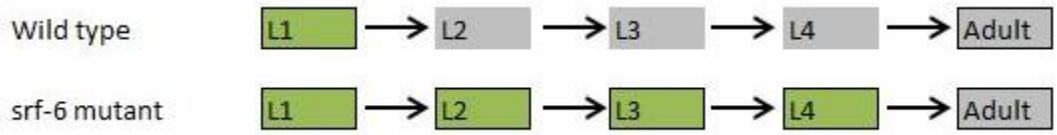

Figure 1: Expression Patterns of the Protein Recognized by Antibody M37 in Wild Type and *srf-6* Mutants
The M37 recognized antigen is displayed only in the L1 in wild type *C. elegans*. *srf-6* mutants display this antigen throughout all of the larval stages. In the figure, stages where the antigen is displayed are represented by green boxes, while the stages lacking this antigen display are represented by gray boxes.

srf-6 is a gene of high interest because of the potential difficulties surface protein switching creates in parasitic infections. In learning more about the genes that control protein switching, perhaps therapies could be developed that disrupt this switching in parasitic nematodes.

In working to learn more about *srf-6*, a previous WPI project by Caitlin Rush and Nicholas Van Sciver, advised by Professor Samuel Politz and Professor Robert Dempski, identified a likely location for the *srf-6* mutation. Genome sequencing and additional traits suggested that *srf-6* maps to the gene *nsy-1*(Caitlin Rush and Nicolas Van Sciver, unpublished results). In sequencing the genomes of three different *srf-6* mutants, mutations were found in several genes. *nsy-1* stood out as a prospective location because all three *srf-6* sequences had homozygous mutations in *nsy-1*. *nsy-1* encodes a MAPKKK that has roles in many processes, including chemosensation and immune responses to infection, and *srf-6* is known to have a chemotaxis defect (Olsen et al 2007).

A known phenotype of *nsy-1* mutants occurs in the bilateral olfactory AWC neurons. In wild-type organisms, only one of these neurons expresses *str-2*, an olfactory receptor. When a mutation occurs in *nsy-1*, this asymmetry is lost and both receptors express *str-2* (Sagasti et al., 2001, Figure 2). *nsy-1* is also expressed throughout the organism in the intestine, hypodermis, rectal glands, and neurons, suggesting that it controls additional functions (Sagasti et al 2001).

nsy-1 is therefore considered a likely candidate for *srf-6* because of its location, homozygous mutations, and role in chemosensation.

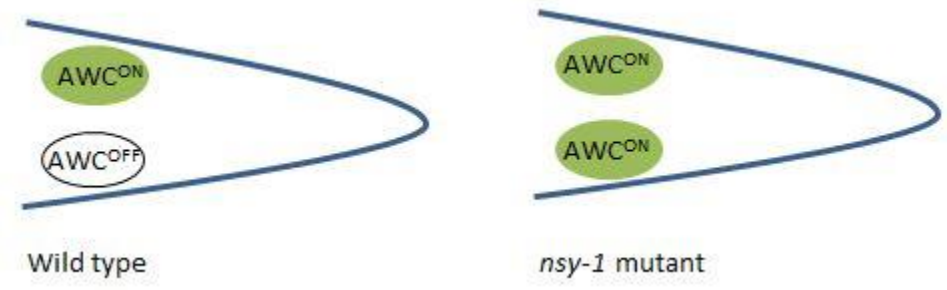

Figure 2: ON/OFF Expression Patterns of the AWC Neurons in Wild Type and *nsy-1* Mutants
In wild type *C. elegans*, the neurons are stochastically assigned to ON or OFF status, meaning either the left or right is ON and the other is OFF. In *nsy-1* mutants, both AWC neurons are in the ON status. In the figure, the green ovals represent the neurons that are ON and the white ovals represent the neurons that are OFF.

Experimental Design/Research Course of Action

The purpose of this project is to gather additional evidence to support the hypothesis that *srf-6* and *nsy-1* are the same gene. In order to do this, I will use complementation testing. In a complementation test, a double heterozygote is created containing mutations in two genes that separately result in the same recessive phenotype. If the double heterozygote displays the mutant phenotype, it is said that the mutations fail to complement, and the simplest explanation is that both mutations affect the same genetic function. If the double heterozygote displays the wild

type phenotype, it is said that the mutations complement. The explanation in this case is that the heterozygote is simultaneously heterozygous in two different genes.

Once a *srf-6 / nsy-1* double heterozygote is created, it can be tested for the surface antigen that binds antibody M37 via a florescent antibody staining experiment. In this process, *C. elegans* L2-L4 worms are treated with the M37 antibody which will bind to the surface if the complementary antigen is present. The worms are then treated with a secondary antibody that binds M37, if present on the surface, and fluoresces. Examination under a fluorescent microscope allows us to discern whether each strain displays the surface marker, our mutant phenotype.

In a separate experiment, a *srf-6 / nsy-1* double heterozygote containing a p*str-2:: GFP I* marker can be examined directly under a fluorescent microscope. If one AWC neuron fluoresces, it would indicate that the two mutations do not affect the same function. If both AWC neurons fluoresce, it would indicate that both mutations affect the same function.

Methodology

Nematode Culture

Nematode stocks were grown on nematode growth agar plates with *Escherichia coli* OP50 as a food source and stored at 16^{o} C. Strains were perpetuated by moving a few gravid individuals to a fresh plate (Grenache et al., 1996).

Immunofluorescent Staining

Immunofluorescent staining has been previously described and the methodology here varies only slightly (Hemmer et al., 1991). Live nematodes in the L3 and L4 life stages were removed from the stock plates described above and placed on a nematode growth agar plate with no *E. coli*. Fifty individual worms in these stages were selected and moved for each strain to be stained. The worms were then rinsed off of the agar plates with PBS (phosphate buffered saline, 0.15 M NaCl, 0.01 M Na phosphate pH 7.0) into conical glass centrifuge tubes. They were then washed by three cycles of centrifugation and resuspension in PBS and transferred to 2 mL glass vials. Two microliters of M38 was added to the vials and they were incubated at 0^{o} C for 2.5 hours. Samples were transferred back to conical glass centrifuge tubes and rinsed three times via centrifugation and resuspension. Samples were transferred into fresh 2 mL glass vials and 20 microliters of FITC-conjugated goat anti-mouse IgM were added. These were then incubated for 1.5 hours at 0^{o} C followed by six more rinses as before. Samples were moved one at a time to a pre-chilled glass well plate for examination under a fluorescent microscope.

Male Formation

In order to complete genetic crosses between strains, males were needed in addition to the hermaphrodites. To encourage X nondisjunction, a number of L4 worms were placed on a plate and incubated at 28^{o} C for 24 hours. After this incubation period, these worms were stored at 16^{o} C. The offspring of these worms were visually examined for individuals with the

distinguishable male tail. Multiple males were plated with a few hermaphrodites in the L4 stage to encourage mating. If mating occurred, half of the cross progeny offspring would be male and half hermaphrodite. Male cultures were maintained by continually replating males and hermaphrodites together to allow mating.

Genetic Crosses

Crosses between different strains of *C. elegans* were conducted by plating multiple males of one strain with a few hermaphrodites of another strain. If one of the males successfully mated with a hermaphrodite, half of the offspring would be hermaphrodite and half male. Several males and hermaphrodites could then be plated again on new plates to continue to propagate progeny male-producing stock.

Strains

Various *C. elegans* strains were used throughout this experiment. The strains are detailed here.

N2 is the standard laboratory wild type strain. It is the parent that consists of the genetic background of *nsy-1* and *srf-6* mutants.

AT18 is the strain name for a strain containing *srf-6(yj13).*

AU3 contains a mutation in *nsy-1*. The genotype for this strain is *nsy-1(ag3)* II. It is known to have an enhanced susceptibility to pathogens. It was created using the mutagen EMS.

VC390 contains a mutation in *nsy-1*. The genotype for this strain is *nsy-1(ok593)* II. It was created from UV/TMP mutagenesis.

CX4998 contains a mutation in *nsy-1*. The genotype for this strain is *kyIs140* I; *nsy-1(ky397)* II. *kyIs140* is an integrated transgene containing *pstr*-2: *GFP*. The GFP marker allows determination that *str-2* is expressed in one or both AWC neurons.

Table 1: Strains of *C. elegans* used in Crosses and Experiments

Strain	Genes Mutated	Genotype	Details
N2	None		Wild type
AT18	*srf-6*	*srf-6 (yj13)* II	Altered expression of the surface antigen recognized by mAb M37
AU3	*nsy-1*	*nsy-1(ag3)* II	More susceptible to pathogens
VC390	*nsy-1*	*nsy-1(ok593)* II	Chemotaxis defect
CX4998	*nsy-1*	*kyIs140 I; nsy-1(ky397)* II	Both AWC receptors express *str-2* tagged with GFP

Complementation Testing

Complementation testing is the procedure of crossing two strains that are each homozygous mutants for a recessive gene that result in the same phenotype. If the offspring of this cross demonstrate the mutant phenotype, the mutations fail to complement and affect the same genetic function. If the offspring do not demonstrate the mutant phenotype, the mutations complement and affect different genes.

Because neither *nsy-1 n*or *srf-6* mutant phenotypes have phenotypes visible in the dissecting microscope, cross progeny are not distinguishable from self-progeny without the presence of an additional visible mutation. By crossing *unc-4 dpy-10* hermaphrodites with *srf-6* males and selecting the F2 progeny that displayed only the *unc-4* mutant phenotype, a *srf-6 unc-4* mutant was created. These genes are linked because both are on chromosome II, and this now allows *srf-6* to be tracked by the visible Unc-4 phenotype.

Construction of *srf-6nsy-1* heterozygotes

In order to cross *srf-6* and *nsy-1* mutants, males first had to be obtained from one strain. Males and hermaphrodites are then placed on the same plate to reproduce. A difficulty in this process comes in distinguishing the cross-progeny from the self-progeny, since neither mutant phenotype is visible under the dissecting microscope. To overcome this obstacle and allow for

visible distinction of cross progeny, a strain was created that links the *srf-6* mutation with the visible *unc-4* mutation.

To create the *srf-6 unc-4* mutant strain, *dpy-10 unc-4* hermaphrodites were mated with the *srf-6 (yj13)* males. All three of these genes are located on chromosome II. The strategy for creating the double mutant depended on the fact that *srf-6* is located to the left of the pair *dpy-10 unc*-4, in the map order *srf-6 dpy-10 unc-4*. The recombinant outcross offspring were then separated from the self-progeny by selecting the non-Dpy non-Unc hermaphrodite progeny. Ten of these F1 outcross were picked onto separate plates. These F1 worms were then allowed to self-fertilize for another generation. In the F2 offspring, plates were searched for individuals showing an Unc non-Dpy phenotype. These contained at least one recombinant chromosome. A large number of Unc non-Dpy F2 were picked individually to separate plates. Their offspring were observed. Individuals that segregated both Unc and Dpy Unc offspring were discarded. Individuals that produced only Unc progeny were the desired *srf-6 unc-4* double mutant, and were maintained for the complementation crosses. These crosses are shown in Figure 3.

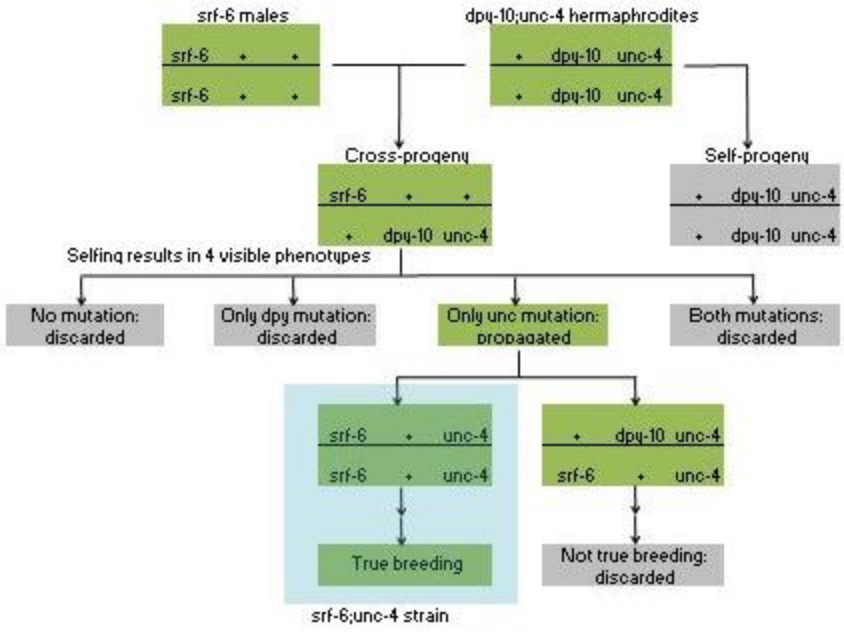

Figure 3: Cross and Selection Process for Developing a True Breeding *srf-6 unc-4* Strain
srf-6 males were crossed with *dpy-10 unc-4* hermaphrodites. Cross progeny were distinguished from self-progeny based on the lack of *dpy-10* and *unc-4* visible mutations. Of the F2 phenotypes, those showing the Unc non-Dpy phenotype were propagated. *srf-6 unc-4* individuals were distinguished by the lack of Dpy phenotypes appearing in subsequent generations.

Once the *srf-6 unc-4* strain was constructed, *srf-6 unc-4* hermaphrodites were mated with *nsy-1* males. The *unc-4* phenotype was used to distinguish the F1 cross progeny from the *srf-6 unc-4* self-progeny, as only the self-progeny would demonstrate the Unc-4 phenotype. The offspring not displaying the Unc-4 phenotype were tested for complementation. This selection process is shown in Figure 4.

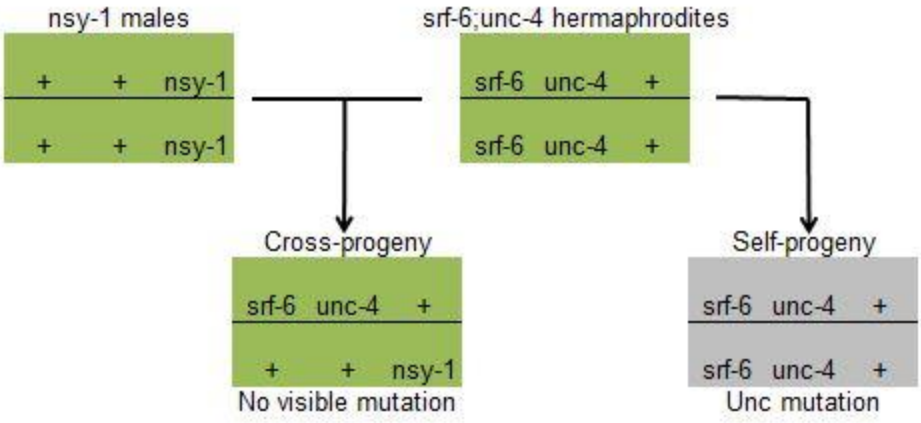

Figure 4: Selection of *srf-6;nsy-1 Double Heterozygotes*

Hermaphrodites from the *srf-6 unc-4* true breeding strain were crossed with *nsy-1* males. The cross progeny were distinguished from the self-progeny by their non-Unc-4 phenotype. The cross progeny represent the complementation cross offspring, i.e., double heterozygotes for the two genes being examined. These offspring can be tested for each mutant phenotype to determine if the genes affect the same or different genetic functions.

Results

The goal of these experiments was to determine if *nsy-1* and *srf-6* affect the same genetic function. To accomplish this, complementation tests between *srf-6* and *nsy-1* mutants were conducted. The two different mutants were crossed, and the offspring were tested for the mutant phenotype. If the offspring show the mutant phenotype, the mutations fail to complement and the genes affect the same genetic function. If the offspring display the wild type phenotype, the mutations complement and affect different genetic functions.

The visible phenotype of a *srf-6* mutation is the surface display of the M37 recognizing surface epitope in the L2-L4 larval stages. The visible phenotype of a *nsy-1* mutation is the expression of *str-2* in both AWC neurons, as opposed to just one. The recombinant offspring were tested for these phenotypes.

Complementation Tests for the *srf-6* phenotype

The *srf-6 (yj13) unc-4* and *nsy-1* (*ag3)* cross progeny at the L2-L4 stage were examined by fluorescent antibody staining with M37 and its fluorescent second antibody for the *srf-6* mutant phenotype. *srf-6* (*yj13*), two different *nsy-1* mutants, and two strains of *srf-6 unc-4* were also examined for the mutant phenotype. The results of these staining experiments are shown in Figure 5.

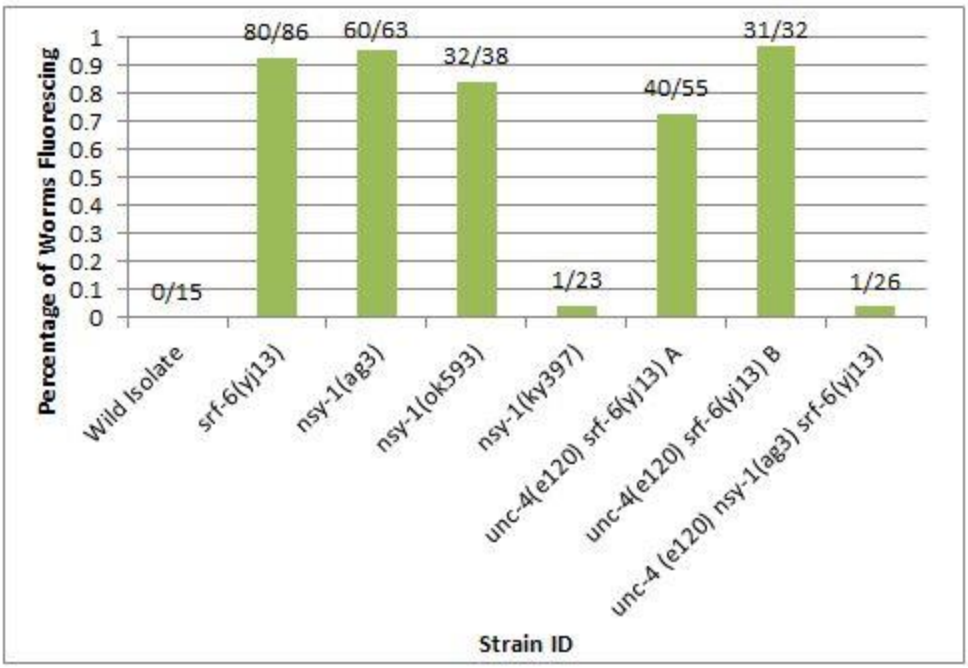

Figure 5: Antibody Staining of *C. elegans* Strains for *srf-6* Phenotype
The percent displayed was calculated as the total number of worms fluorescing/total scored. The fractions above each bar represent these total numbers. Construction of the *unc-4 srf-6* mutants are described in Materials and Methods. The *nsy-1(ky397)* mutant also contained *ks140* I.

Each strain tested for the *srf-6* phenotype fell into one of two categories: 70-97% fluorescent or less than 4% fluorescent. This allowed for a clear distinction between the *srf-6* mutant phenotype and the wild type phenotype. As expected based on published results, none of the wild type negative control worms stained positively, and 93% of positive control *srf-6 (yj13)* worms stained positively. Of the three *nsy-1* strains tested, two of the three displayed the mutant phenotype. Both of the two *srf-6 unc-4* strains constructed showed a percentage of stained worms comparable to *srf-6 (yj13).* Only 4% of the *srf-6;nsy-1* double heterozygotes showed staining.

Complementation Tests for the *nsy-1* phenotype

The cross progeny of *srf-6 unc-4* hermaphrodites and *ksy140* I; *nsy-1 (ky397)* males were also examined for the *nsy-1* phenotype. *nsy-1(ky397)* mutants show two fluorescent AWC neurons as opposed to just one (Sagasti et al 2001). Double heterozygote adult hermaphrodites were examined under a GFP filter microscope for the Nsy-1 phenotype. Of the 20 worms examined, 18 showed two fluorescent AWC neurons, one showed one fluorescent neuron, and one showed no fluorescent neurons.

The results of the two complementation tests are not strictly comparable, because *nsy-1 (ky397)* was used for the Nsy-1 phenotype, whereas *nsy-1(ag3)* was used for the Srf-6 phenotype.

Discussion

The objective of this project was to determine if *nsy-1* and *srf-6* affect the same or different gene functions in *C. elegans.* In order to do this, double heterozygotes for those genes were created and tested for each mutant phenotype. Additionally, *nsy-1* mutants were tested for the *srf-6* phenotype.

Initially, we had to construct the necessary strains for these experiments. After several weeks of attempting to develop males in the starting strains and preparing to do the genetic crosses, we tested the stock strains for the *srf-6* phenotype. None of the *srf-6* or *nsy-1* stocks showed the Srf-6 phenotype, including the *srf-6* and *nsy-1* mutants that previously had displayed the phenotype. The L1 worms on these plates still stained with the antibody, providing an internal positive control for the antibody staining.

The differences between these and previous results could be due to reversion of the mutant phenotypes in the stocks. Particularly when picking worms for stock maintenance from starved plates, non-mutant revertants may have a selective advantage in growth and reproduction, and eventually prevail in the stock population. This problem is exacerbated by the fact that neither *srf-6* nor *nsy-1* mutants display visible phenotypes, so that reversion would be undetected. This problem was solved by starting with stocks from unstarved plates.

Another possible problem was that worms grown on plates with bacterial or fungal contaminants do not stain well with M37. After obtaining these negative results, care was taken to use worms from uncontaminated plates for M37 staining experiments.

Once fresh stocks were obtained, we began the previously described crosses to obtain the *srf-6 / nsy-1* double heterozygotes. To do this, we first had to obtain a strain of *srf-6 unc-4*

mutants to allow for distinction between self- and cross-progeny in the offspring of the complementation cross. Once the *srf-6 / nsy-1* double heterozygotes were created, they were tested for both the Srf-6 and Nsy-1 phenotypes. The two *srf-6 unc-4* putative double mutants showed the *srf-6* phenotype as expected. This indicated that the *srf-6* mutation was not lost in the construction of the double mutants.

In testing for the Srf-6 phenotype, as expected, the wild type did not show the Srf-6 mutant phenotype. *srf-6(yj13), nsy-1(ag3),* and *nsy-1(ok593)* mutants all showed the Srf-6 phenotype. However, *nsy-1 (ky397)* did not show the Srf-6 phenotype. The fact that some of the *nsy-1* mutants showed the *srf-6* mutation is crucial because it indicates that *nsy-1* mutation can cause the Srf-6 phenotype. Finally, the *srf-6/nsy-1* double heterozygotes did not display the Srf-6 phenotype.

It would appear that the *srf-6/nsy-1* double heterozygotes complemented, meaning that the genes do not affect the same gene function. Since this data was based on one experiment, however, it is possible that this could be artifactual. However, it should be noted that the *nsy-1* allele used for the Srf-6 phenotypic complementation test was *ag3*, whereas *ky397* was used for the Nsy-1 AWC complementation test. The Srf-6 complementation test should be repeated using *nsy-1 (ky397).*

In contrast to the Srf-6 complementation results, when the Nsy-1 AWC phenotype was evaluated in *srf-6 / nsy-1* heterozygotes, the 2 AWC on phenotype was obtained.

The NSY-1 protein sequence is similar to the sequence of the human ASK-1 MAPKKK. ASK-1 contains three distinct domains, a regulatory N terminal domain, a kinase catalytic domain, and a C terminal dimerization domain. In future complementation tests, attention needs

to be paid to the locations and types of mutations used. For example, *nsy-1(ky397)* is a nonsense mutation in the C terminal domain that would be expected to encode a NSY-1 protein truncated at its C terminus. However, this mutation would not affect function of the N terminal or catalytic domains per se. Only one study of NSY-1 domain function has been carried out. Sagasti et al (2001) noted that an ASK-1 gain of function was achieved in a mutant with a deletion of the N terminal domain. Building on this information, Sagasti et al created an N terminal deletion of *nsy-1*, and showed that this mutant did display the gain of function as well. Studies of this nature, together with the consideration that the surface antigen phenotype and AWC phenotype may require differential activity of the three NSY-1 protein domains, suggests that careful attention needs to be paid to which alleles are used in complementation tests. An early indication that this may be important is that *nsy-1 (ky397)* does not show the Srf-6 phenotype in antibody staining experiments.

Conclusions

The goal of this project was to determine if *srf-6* and *nsy-1* genes affect the same genetic function. In complementation testing double heterozygotes for the Srf-6 phenotype, the genes appeared to complement, however in complementation testing for the Nsy-1 phenotype, the genes appeared to fail to complement. Because of this discrepancy, further studies should be conducted to determine whether or not the genes affect the same genetic function.

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