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GENERATING A SPARSE FLUORESCENT REPORTER FOR A

PHAGOCYTIC CELL IN C. *elegans* TO CHARACTERIZE

COMPARTMENTALIZED CELL ELIMINATION

by

IDARAROSA EKONG

Presented to the Faculty of the Honors College of

The University of Texas at Arlington in Partial Fulfillment

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for the Degree of

HONORS BACHELOR OF ARTS IN BIOLOGY

THE UNIVERSITY OF TEXAS AT ARLINGTON

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the last several years. You never let me quit even when I was on the verge of quitting, and for that I am eternally thankful. This undergraduate thesis stands as a testament to your unconditional love and encouragement.

April 1, 2022

ABSTRACT

GENERATING A SPARSE FLUORESCENT REPORTER FOR A PHAGOCYTIC CELL IN C. *elegans* TO CHARACTERIZE COMPARTMENTALIZED CELL ELIMINATION

Idararosa Ekong, B.A. Biology

The University of Texas at Arlington, 2022

Faculty Mentor: Piya Ghose

Programmed cell death is particularly important for animal development. Our lab discovered a new form of cell death called Compartmentalized Cell Elimination (CCE) in the nematode *C. elegans*. Here three segments of a complex epithelial cell called the tail-spike cell (TSC), that shapes the animal's tail, die differently. After a cell dies, it remains are taken up by a phagocyte (cell-eating cells) through a process called phagocytosis. We wish to image phagocytosis of the TSC as it dies, we have a fluorescent reporter for the phagocyte. However, in the current version of this marker expression is extremely broad, other cells are obscuring our view to see what we are interested in. The experimental strategy includes gene promoter dissection, PCR fusion of promoter fragments to GFP, microinjections, and microcopy/imaging techniques. We will use the new phagocytic reporter to look at phagocytosis in wild-type embryos through time-lapse imaging.

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CHAPTER 1

INTRODUCTION

1.1 Objective of the Ghose Lab

In Ghose lab, we study cell biology. To be able to study cell biology with ease and haste is ideal, and we achieve this using the organism *C. elegans*. This organism enables us to study our favorite cells in real time, and across several generations in a reasonable time span. The animals are easy to maintain. Multiple *C. elegans* plates can be maintained at one time for experiments. The whole genome is sequences, its cell lineage does not change, and it has a transparent body and is genetically tractable.

The term "phagocytosis" refers to an internalization process by which larger particles, such as bacteria and dead/dying cells are engulfed and processed within a membrane-bound vesicle called the phagosome. The cell that performs this process is the phagocyte.

Compartmentalized Cell Elimination (CCE) is a novel type of programmed cell death seen in *C. elegans* TSC and CEM neurons (REF). CCE involves differential elimination of soma, proximal process, and distal process. Tail-spike cell (TSC) is a morphologically complex cell in *C. elegans,* which extends a microtubule-filled process to the animal's tail tip. The TSC dies in the late 3-fold stage embryo (Ghose et. al 2018). The hyp10 cell is an epithelial cell wrapped around the tail-spike-process that forms the tip of the tail. It is also the phagocyte for the TSC process.

1.1.1 Definition and features of programmed cell death

After a cell dies, it is taken up by a phagocyte. In our lab, we have found that mutants for a kinesin and for endoplasmic reticulum stabilization genes have a part of the TSC process persisting. We want to know whether these remnants have been taken up by the phagocyte or not. The result has major implications for our work with the two mutants. I We aim to find out where the TSC process fragment of these mutants is relative to the phagocyte (hyp10 phagocyte). than five.

1.1.2 Apoptosis

Programmed cell death, for example apoptosis, is an evolutionarily conserved program of cellular self-destruction, is required for the development and survival of most multicellular animals. It is necessary to maintain tissue homeostasis and guarantee that organ architecture is functioning. Apoptosis is a gene-driven cellular self-destruction program that, in most circumstances, performs biologically vital functions. Apoptotic cells exhibit morphological changes such as chromatin condensation and DNA laddering, loss of mitochondrial membrane potential and plasma membrane phospholipid asymmetry, and dissociation from the cellular matrix. Although it is not the only sort of 'planned' cell death that occurs throughout metazoan development, apoptosis is one of them.

1.1.3 Phagocytosis

Both in the initial engulfment of pathogens and the clearance of infected or dead cells, phagocytosis plays critical functions. Phagocytic cells have two important functions: they prevent the accumulation of cell corpses, which can lead to severe inflammation and autoimmune, and they maintain host defense. In the soil, the free-living nematode C.

elegans coexists with a wide range of diseases, including bacteria, fungus, and viruses. Dying cells are engulfed by neighboring cells to maintain homeostasis.

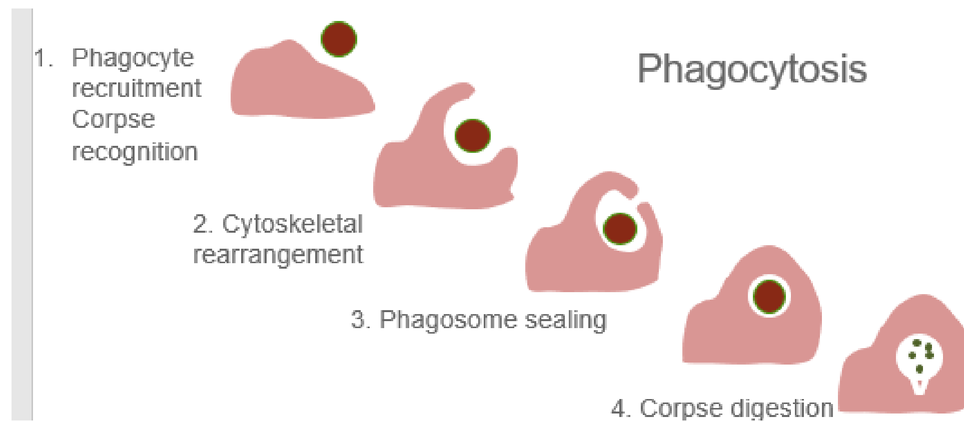

Figure 1.1: The steps of phagocytosis

1.2 *C. elegans* as a model system

The C. *elegans* genome has many genes that have functional analogues in humans, making it an excellent model for human disorders. Even though this worm is anatomically simpler than a person, it shares numerous genetic similarities with humans, making it an excellent option for a model organism. Nematodes and mammals have much in common when it comes to cell death and control. The genetic processes involved in phagocytosis of cell corpses are extremely conserved, as evidenced by worm studies that helped researchers better understand apoptotic mechanisms in humans.

1.3 Programmed cell death in *C. elegans*

Cell death is an active process that needs gene function in dying cells, according to analyses of the genes identified by these mutations. Not only are specific genes essential to cause cell death, but they are also required to safeguard cells from death. In C. *elegans*, gene interaction studies identified a genetic mechanism for the execution phase of programmed cell death. The pathway proposed by these genetic investigations is supported

by molecular and biochemical findings, which also demonstrate that the protein products of key cell-death genes interact directly. This mechanism appears to be conserved in worms, humans, and other species.

CHAPTER 2

COMPARTMENTALIZED CELL ELIMINATION

2.1 The *C. elegans* tail spike epidermal cell

During *C. elegans* embryonic development, a unique death pathway kills the morphologically complex tail-spike epithelial cell (TSC). This complex program, known as Compartmentalized Cell Elimination (CCE), is also seen in the sex-specific CEM neurons, implying that CCE is a widely employed elimination method. Differential dismantling of three cell regions—the cell soma, soma-proximal process, and soma-distal process—defines CCE. Shown in the figure is the cell undergoing it in a unique, segmented way.

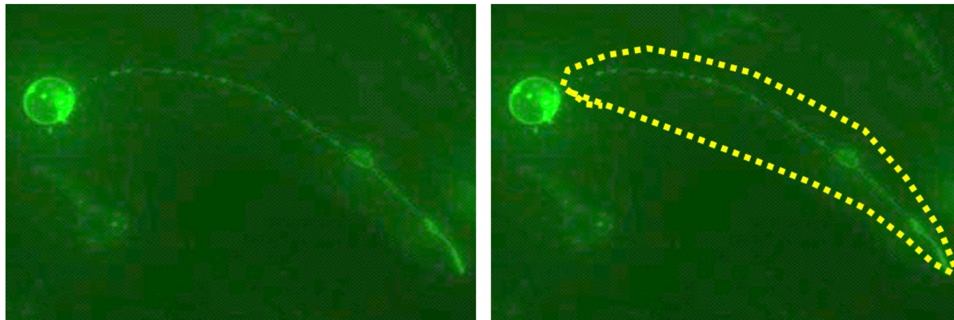

Figure 2.1: Tail-spike cell development and death

2.1.1 Tail-spike cell death

The tail-spike cell (TSC) in the animal undergoes Compartmentalized Cell Elimination as seen in figure 2.1. The yellow dotted lines show the area where there is a cell called hyp-10, which is the phagocyte that will internalize all the pieces of the dying tail-spike cell. The TSC dies in the late embryo stage. As death ensues, the cell segments into 2 compartments. The soma rounds like a simple apoptotic cell. The proximal segment of the process undergoes beading and fragmentation. The distal process segment withdraws into itself. The remains of the cell are then phagocytosed such that the TSC is eliminated by the time the animal hatches. Our objective is to understand how the cell marked in yellow interacts and internalizes all the fragments by visually witnessing it under microscopy.

2.1.2 The hyp10 epidermal cell

We aimed to create a fluorescent reporter to visualize the hyp10 phagocyte. The report that previously existed, which is based on the full promoter of *eff-1* cell fusion gene is broadly expressed, which makes it hard to study w/o obstruction from other cells. The gene *eff-1* is known be expressed in the hyp10 phagocyte, which surrounds the tail-spike cell (TSC) process. The approximately 8000 kb promoter of *eff-1* was dissected into smaller 1 kb fragments, which were fused with Green Fluorescence Protein.

2.1.3 Aims of this thesis

This thesis aims to employ PCR fusion of the *eff-1* promoter into smaller fragments with GFP to narrow down the promoter to get expression only in the desired hyp10 phagocyte.

This reagent will be used in a variety of lab projects. We obtained CCE-defective mutants with incorrectly persistent TSC pieces and mutations in certain genes through forward genetic screens. We will employ a novel phagocytic reporter to look at phagocytosis in wild-type embryos using time-lapse imaging, as well as see if our CCE-defective mutants' fragments are within or outside the phagocyte. These experiments will reveal how the linked genes work and will aid in the diagnosis of CCE.

CHAPTER 3

METHODOLOGY

The following will describe the main laboratory techniques employed: PCR fusion and microinjection which were both performed under the use of microscopy.

3.1 Isolating *eff-1* the fragments

3.1.1 Dividing eff-1 promoter into smaller fragments

We dissected the large 8kb *eff-1* promoter into small 1kb (kilobase) fragments that were predicted to have fewer enhancer elements therefore driving gene expression in fewer cells.

3.1.2 Amplifying eff-1 promoter fragments

Next, we fused each individual fragment with GFP (Green Fluorescent Protein, a fluorescent tag) by PCR fusion and transformed into the *C. elegans* germline. PCR is a method of replicating a single fragment of DNA to produce millions of identical copies of that DNA fragment. PCR fusion is a simple and easy method that produces fusion DNA fragments without the need for restriction enzyme digestion and DNA ligation. A major bonus to this approach is its rapid generation of reporter constructs more quickly (Evans, 2006). The reagents used include DNA templates, forward primers, reverse primers, Taq Polymerase, dNTP, buffer and distilled water. Primers serve the starting point for DNA synthesis as a short nucleic acid sequence. As seen in the figure, the 5' ends of both primers attach to the 3' of the DNA strand. dNTP (deoxynucleotide triphosphate) are the building blocks for sequencing new DNA strands. The four types are dATP, dTTP, dCTP and dGTP,

which fill the blanks for complimentary base pairing. Adenine pairs with thymine, guanine with cytosine. Buffers optimize the conditions for Taq polymerase by maintaining a pH. Distilled water is rid of all impurities and balances the stoichiometry of the final solution. PCRs are performed in thermocyclers. The polymerase used was NEB Phusion Taq polymerase. The accuracy of the chosen temperatures determines the failure or success of PCR. It will repeat cycles as you specify in the program upon setup. Once the thermocycler is run, PCR product is extracted to be visualized on a gel.

This table illustrates what a program looks like in terms of temperature and number of cycles.

95C	Denaturation, separation of DNA strands
55C	Annealing, Taq polymerase amplifies primers attachment to DNA strands
72C	Extension, building blocks continue filling the blanks in the sequence
4C	Hold, sample cools down for future storage

Table 3.1: PCR cycle parameters

3.1.3 PCR fusion of eff-1 promoter fragments with GFP

We employed the PCR fusion technique (citation). For each promoter fragment, two distinct fusions are required. First is the A/B fusion PCR, which amplifies the desired fragment and includes an overhang that has homology (matched) the 5' end of GFP. Second is fusion PCR with C/D fragment, which amplifies GFP. Once these have been amplified, the fragments are fused together using special primers and a special PCR reaction. The final fused product was microinjected into the worm. Primers were designed using the software called Ape DNA Plasmid Editor and NEB Tm Calculator®.

Table 3.2: Promoter fusions generated

Fusion 2 KB PCR Thermocycler Run

Temp	Time	Cycle
98C	30 sec	
98C	10 sec	
54C	30 sec	35
72C	60 sec	
72C	10 mins	
10C	Hold	

Fusion 1 PCR Thermocycler Run

Temp	Time	Cycle
98C	30 sec	
98C	15 sec	
54C	35 sec	9
72C	6 mins	
10C	10 mins	
10C	Hold	

Fusion 2 PCR Thermocycler Run

Temp	Time	Cycle
98C	30 sec	
98C	15 sec	
54C	35 sec	23
72C	6 mins	
72C	10 mins	
10C	Hold	

Table 3.3: Primers used to amplify *eff-1* fragment

Oligo Name	Primer Name	Fragment Name	Sequence
oGC1	GFP_primer C	D	AGCTTGCATGCCTGCAGGTCG
oGC2	GFP_primer D		AAGGGCCCGTACGGCCGACTA
oGC3	GFP_Primer D*		GGAAACAGTTATGTTTGGTATA
oGC4	eff1p 0--1000 F_A	1 A/B	TCCAAAAATGTCAGAAGATAGTTG
oGC5	eff1p 0-1000_F_A*		AATGTCAGAAGATAGTTGGGAA
oGC6	eff1p 0-1000_R_B		CTCTAGAGTCGACCTGCAGGCATGCAAGCTGCTTCTGAAAATAAAATGATACG
oGC7	eff1p 800--1800 F_A	2 A/B	ACATTGTTTCTCCCAACAACTTC
oGC8	eff1p 800-1800_F_A*		ttgtttctcccaacaacttctc
oGC9	eff1p 800-1800_R_B		CTCTAGAGTCGACCTGCAGGCATGCAAGCTAGATACCCGAAAAAAACGGGAG
oGC10	eff1p 1600--2600 F_A	3 A/B	TTTAAGAATCCGTTATGCAGATTTC
oGC11	eff1p 1600-2600_F_A*		agaatccgttatgcagatttcc
oGC12	eff1p 1600-2600_R_B		CTCTAGAGTCGACCTGCAGGCATGCAAGCTCAGGTCACATAGTATGAGAGGTG
oGC13	eff1p 2400--3400 F_A	4 A/B	TGAAAACTCTTTTCTTGTTATCC
oGC14	eff1p 2400-3400_F_A*		aactcttttcttgttatccaaa
oGC15	eff1p 2400-3400_R_B		CTCTAGAGTCGACCTGCAGGCATGCAAGCTAAAACAAATGAAAAACTTTGAATTT
oGC16	eff1p 3200-4200 F_A	5 A/B	TAATGAAAAATTACATATAAATGTGTAA
oGC17	eff1p 3200-4200_F_A*		gaaaaattacatataaatgtgtaattacag
oGC18	eff1p 3200-4200_R_B		CTCTAGAGTCGACCTGCAGGCATGCAAGCTGGATTCAAGCGAAAAAAGAGAG
oGC19	eff1p 4000--5000 F_A	6 A/B	GTATTGGCTGTCCAAAAATAGT
oGC20	eff1p 4000-5000_F_A*		ggctgtccaaaaatagttaaaac
oGC21	eff1p 4000-5000_R_B		CTCTAGAGTCGACCTGCAGGCATGCAAGCTATAGGGAAAAAAGAGAGAAGCA
oGC22	eff1p 4800-5800 F_A	7 A/B	TTTCGACTGTGACTCATTGTAG
oGC23	eff1p 4800-5800_F_A*		TGACTCATTGTAGTCGGATATT
oGC24	eff1p 4800-5800_R_B		CTCTAGAGTCGACCTGCAGGCATGCAAGCTAGATTTTAGAGCTCGTTTTAGTG
oGC25	eff1p 5600--6600 F_A	8 A/B	TCTATCTGAGTATCATTCTCTTA
oGC26	eff1p 5600-6600_F_A*		tctgagtatcattctcttaatttc
oGC27	eff1p 5600-6600_R_B		CTCTAGAGTCGACCTGCAGGCATGCAAGCTTTCAGAAAGTGTTTTTCATTTG
oGC28	eff1p 6400--7495 F_A	9 A/B	CGAGAAATGGGATATGCAGGCA
oGC29	eff1p 6400--7495_F_A*		AACGATGAATTGATGGATCACC
oGC30	eff1p 6400--7495_R_B		CTCTAGAGTCGACCTGCAGGCATGCAAGCTCATGGATTGACATCTAAGTGATGATGG
oGC33	minus 4000 to minus 4250_A	6A	tcaacgttctgttagtttccaatagg
oGC34*	minus 4000 to minus 4250_A*		gttctgttagtttccaataggaaggc
oGC35	minus 4000 to minus 4250_B		CTCTAGAGTCGACCTGCAGGCATGCAAGCTatagggaaaaaagagagaagcaaaaaaa
oGC36	minus 4200 to minus 4450_A	6B	ttttgatactttttaatcaaaaagtttacc
oGC37*	minus 4200 to minus 4450_A*		ctttttaatcaaaaagtttaccgccttgc
oGC38	minus 4200 to minus 4450_B		CTCTAGAGTCGACCTGCAGGCATGCAAGCTttttaaaacaaaaaaagatgccttcctattg
oGC39	minus 4400 to minus 4650_A	6C	ctccttctcatctacccatcatttttc
oGC40*	minus 4400 to minus 4650_A*		ctcatctacccatcatttttcttgtttg
oGC41	minus 4400 to minus 4650_B		CTCTAGAGTCGACCTGCAGGCATGCAAGCTactatttggttatgcaaggcggtaaac
oGC42	minus 4600 to minus 4850_A	6D	ttccgaaatatttgaaaaaatcggaagcac
oGC43*	minus 4600 to minus 4850_A*		gaaatatttgaaaaaatcggaagcactaaaa
oGC44	minus 4600 to minus 4850_B		CTCTAGAGTCGACCTGCAGGCATGCAAGCTtagagaaaaggagggcaaacaagaaaaaa
oGC45	minus 4800 to minus 5000_A	6E	gtattggctgtccaaaaatagttaaaac
oGC46*	minus 4800 to minus 5000_A*		ggctgtccaaaaatagttaaaacacatt
oGC47	minus 4800 to minus 5000_B		CTCTAGAGTCGACCTGCAGGCATGCAAGCTagattttagagctcgttttagtgcttc
oGC48	(1)0tominus75_A	hyp10-1	ACTCATTCTTCCTCTTCTTTTC
oGC49	(1)0tominus75_A*		cttcctcttcttttcactcaac
oGC50	(1)0tominus75_B		CTCTAGAGTCGACCTGCAGGCATGCAAGCTcttcctcttcttttcactcaac
oGC51	(2)minus50tominus125_A	hyp10-2	cttcctcttcttttcactcaac
oGC52	(2)minus50tominus125_A*		TTTACGACGTCCGTGAAATCG
oGC53	(2)minus50tominus125_B		CTCTAGAGTCGACCTGCAGGCATGCAAGCTGAGTGAAAAGAAGAGGAAGAATG
oGC54	(3)minus100tominus175_A	hyp10-3	TACCTTTTTTAAGGAATTGTCTTGTC
oGC55	(3)minus100tominus175_A*		CTTTTTTAAGGAATTGTCTTGTCTG
oGC56	(3)minus100tominus175_B		CTCTAGAGTCGACCTGCAGGCATGCAAGCTTCGATTTCACGGACGTCGTAA
oGC57	(4)minus150tominus225_A	hyp10-4	CTTTTTTAAGGAATTGTCTTGTCTG
oGC58	(4)minus150tominus225_A*		CCTTGCATAACCAAATAGTTC
oGC59	(4)minus150tominus225_B		CTCTAGAGTCGACCTGCAGGCATGCAAGCTACAAGACAATTCCTTAAAAAAGG
oGC60	(5)minus200tominus250_A	hyp10-5	TTTTGATACTTTTTAATCAAAAAGTTTAC
oGC61	(5)minus200tominus250_A*		GATACTTTTTAATCAAAAAGTTTACC
oGC62	(5)minus200tominus250_B		CTCTAGAGTCGACCTGCAGGCATGCAAGCTAACTATTTGGTTATGCAAGGC

3.2 Germline transformation and microinjection

Microinjection refers to taking a glass micropipette to inject the fusion DNA product into the living animal. Microinjection-based germline transformation was done were used to introduce PCR fusion DNA into the gonad of worms at P0 stage to generate transgenic animals (Evans, 2006). In our case, the *eff-1* gene promoter fragments fused to GFP that we generated were introduced into worms through the technique of microinjection. DNA transformation is effectively achieved by injecting DNA into the core of cytoplasm to target germ cell nuclei. This microinjection injects experimental DNA into the gonad of a young adult stage worm. A successful injection will result in the P0 animal generating transgenic F1 progeny. These transgenics are then isolated and propagated. If F2 progeny are also transgenic, this is referred to as a stable line.

3.2.1 Microinjection method & selecting transgenics

1. Fill a needle-loading pipette by capillary action with DNA injection mix.
2. Watch to see that the injection solution has diffused to the needle tip using microscopy. To save time, prepare several injections needles the day before. Store the needles in clay or wax ridges in a closed box.
3. Position the needle into the needle holder and mount on the manipulator.
4. Turn on the microscope and align the needle tip in the center of the microscope's field of view.

3.2.2 Equipment for imaging

1. Needle puller. (Sutter Instrument Co, Model P-2000) A machine that is excellent for creating precise needles with preferred shapes/sizes.

2. Microinjections microscope. (Zeiss Axiovert) will allow for simultaneous visualization and manipulation of the worms. It should come with a flat, free-sliding glide stage with centered rotation.

3. Micromanipulator. Zeiss makes a sufficient manipulator to hold and position the needle holder. A practical option will be a device that has mobility on all 4 directions (up, down, left, right).

3.3. Microscopy

Two types of microscopes were used for viewing transgenic animals. The first was a Zeiss Stereodiscovery fluorescent dissecting microscope, which was used for isolating transgenics. For imaging, a Nikon Spinning Disc Confocal microscope was used. Nikon NS elements and Fiji/ImageJ were used to process images obtained.

CHAPTER 4

RESULTS

4.1 Promoter fusions generated (gels)

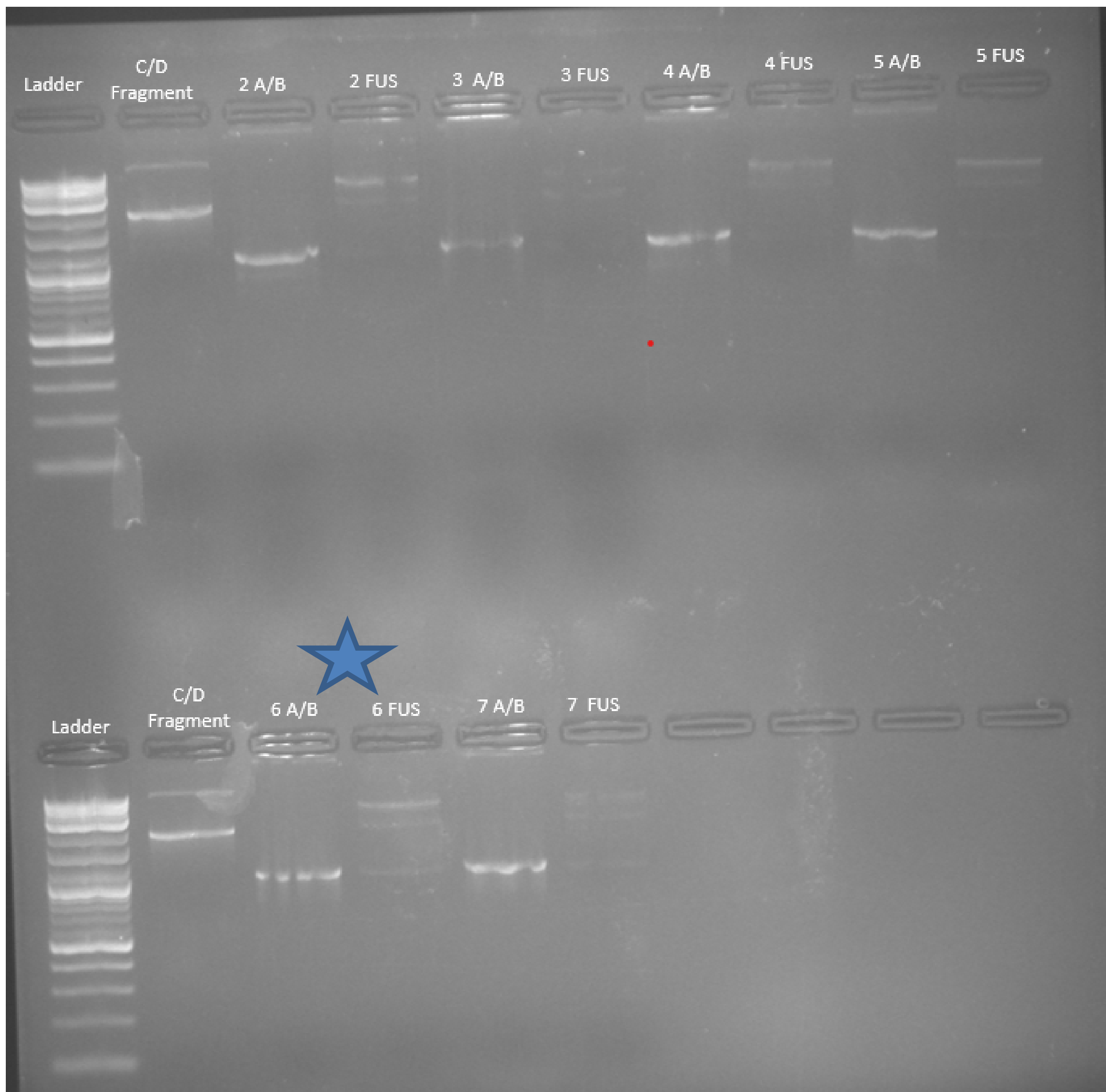

Figure 4.1: Promoter fragment generation and PCR fusion strategy; 7 out of the 9 fused fragments produced stable lines for observation

The gene eff-1 is known to be expressed in the hyp10 phagocyte, which surrounds the tail-spike cell (TSC) process. We dissected the 7495 kb promoter of *eff-1* into ~1kb fragments, which were fused with Green Fluorescence Protein (GFP) as below.

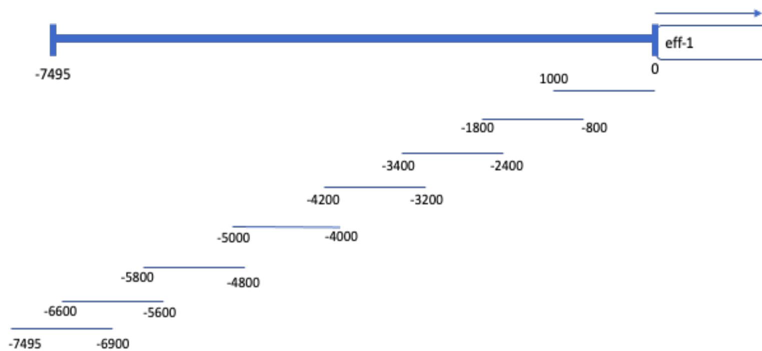

Figure 4.2: Promoter fragments and GFP fusions generated; All nine one-kilobase eff-1 promoter fragments were successfully amplified and fused to GFP

We divided the *eff-1* promoter into 8 1kb fragments and amplified them (Figure 4.1). We then fused these fragments (A-B) with GFP (C-D). All fragments were introduced into worms through microinjection (see Methods) The 1kb DNA fragments were fused to GFP via the polymerase chain reaction (PCR) and injected into the C. elegans gonad in a linear form (Hobert, 2002). This approach is referred to as PCR fusion. GFP was fused to the 3′ end of a DNA fragment (e.g., promoter). Gene-specific primers were used to amplify the *eff-1* promoter fragments primers A, A*, B; A* is nested relative to A. Primers were also used to amplify GFP and 3′ UTR (primers C, D, D*; D* is nested relative to D).

4.2 Expression patterns of transgenics generated

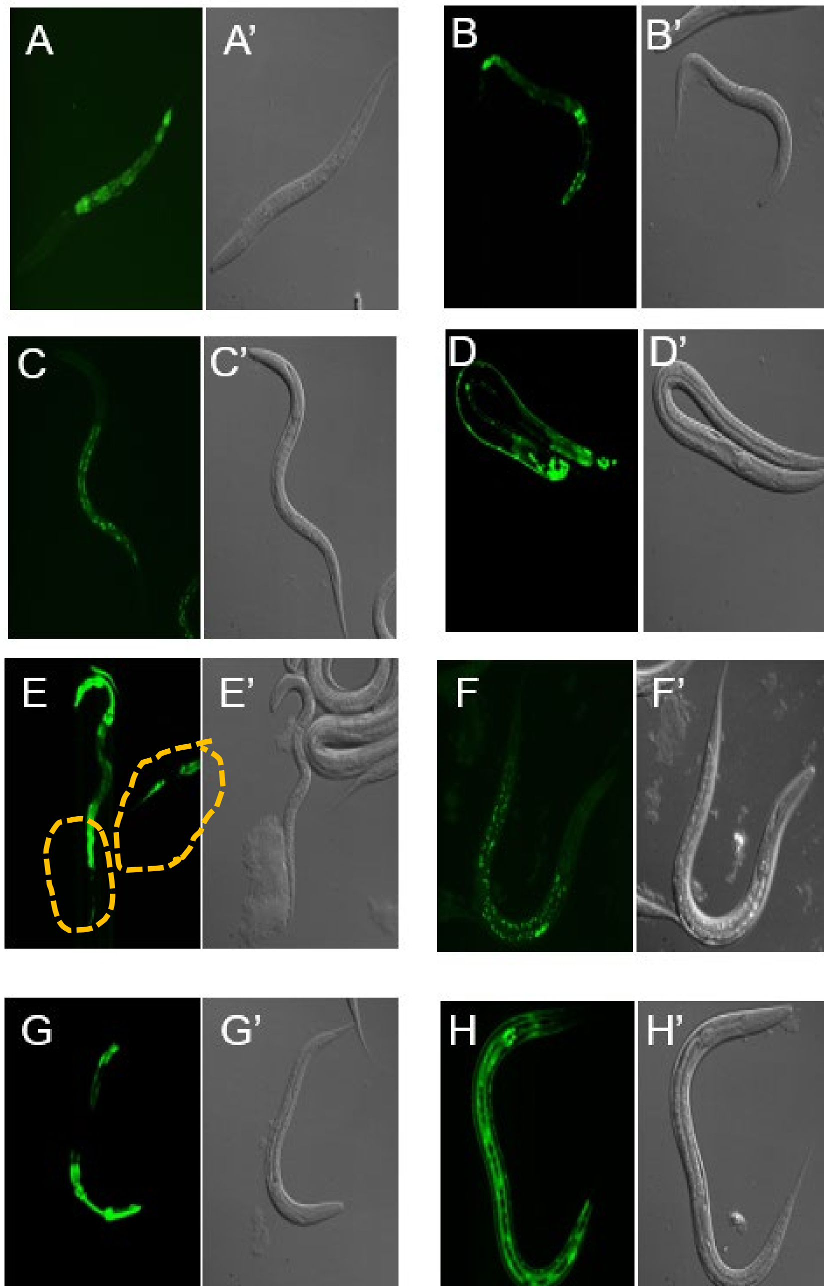

Figure 4.3: Promoter fragment expression patterns of transgenic animals

Each 1kb GFP fragment was injected into wildtype *C. elegans*. F2 animals were examined by imaging on the confocal microscope; GFP expression was imaged and only one fragment showed the appropriate expression in *hyp 10* as seen in figure 4.2.1 in

fragment 6. The yellow dotted lines highlight the areas where there is a cell called hyp-10, which is the phagocyte that will internalize all the pieces of the dying tail-spike cell. The objective is to understand how the cells marked in yellow interact and internalize all the fragments through visualization. Each image captured from A to H displayed some form of GFP expression, but the cells in E, E' (fragment 6) had expression in the tip of the tail or hyp10 region, which is the desired result. This is the correct fusion as it corresponds to the band in gel as seen in figure 4.1.

Key

A. Fragment 2 *eff-1* (-800 to -1800)

B. Fragment 4 *eff-1* (-2400 to -3400)

C. Fragment 2 (-800 -> -1800) (negative control)

D. Fragment 5 *eff-1* (-3200 to -4200)

E. Fragment 6 *eff-1* (-4000 to -5000)

F. Fragment 7 *eff-1* (-4800 to -5800)

G. Fragment 8 *eff-1* (-5600 to -6600)

H. Fragment 9 *eff-1* (-6400 to -7495)

CHAPTER 5

DISCUSSION

5.1 A sparse hyp10 marker was successfully made

We have obtained a reporter that is more specific for hyp10. However, there is still expression in some undesired cells, so in the future the lab may narrow down the promoter further. To do this, we will take the fragment we isolated and divide into 100 bp fragments and fuse to GFP as described above.

We have already applied our reporter to a project in the lab. We have found that the remaining part of the TSC seen in our kinesin (a microtubule motor protein) mutant is internalized by hyp10, but the hyp10 is not fully closed around it. This suggests that the remnant is recognized by the phagocyte, but the phagocyte arms cannot close around it. It will be interesting to decipher what is occurring here.

Our reporter also revealed something interesting in wild-type animals. The hyp10 cell only encompasses the proximal part of the TSC process—the distal extends long passed the tip of the hyp10. This may explain why the proximal and distal process die differently—the proximal may receive a juxtracrine signal from hyp10 to die and the distal gets retracted towards the hyp10.

CHAPTER 6

CONCLUSION

We were able to narrow down a kilobase fragment that shows expresses ion more discretely in hyp10 as well as some other cells. We have applied this tool and have made two interesting observations. We have been able to generate a sparse reporter for the hyp10 phagocyte and this tool has revealed interesting aspects of Compartmentalized Cell Elimination.

6.1 Results reveal new cell-cell associations

An intact TSC (magenta) with its distal process segment extending beyond the hyp10 phagocyte. Arrows also indicate that hyp10 ensheathed the TSC process at specific points. A dying TSC with a shorter distal process extending beyond hyp10. Specific points of the TSC process are unsheathed by hyp10.

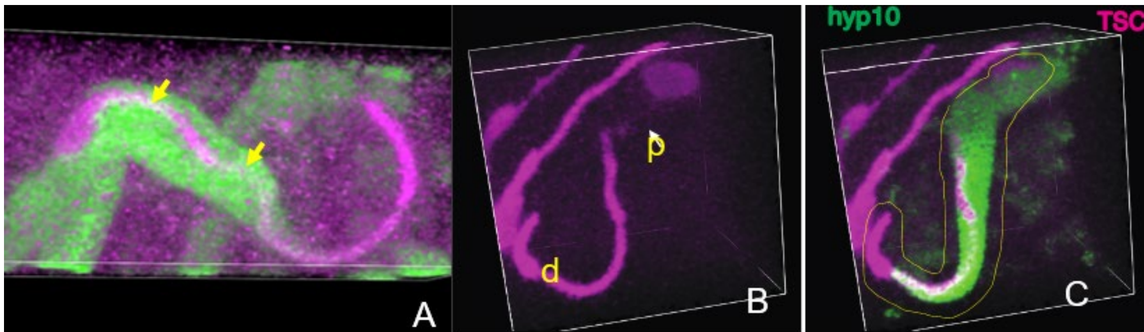

Figure 6.1: Hyp10 selectively ensheaths the intact tail-spike cell

6.2 Kinesin loss leads to phagocytosis defects

Our lab has found that loss of a kinesin (a microtubule motor) results in the persistence of a fragment of the TSC process (magenta). Our new hyp10 reporter reveals

that this fragment cannot be properly phagocytosed: the fragment is not fully internalized by the hyp10 phagocyte.

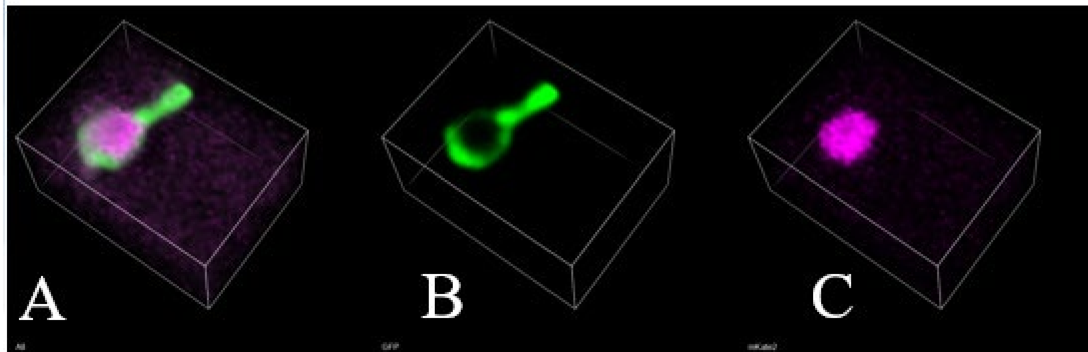

Figure 6.2: A kinesis motor mutant showing failed phagocytosis and an open hyp10 phagosome

6.3 Future directions

We will employ this new tool for several projects in the lab. We will use it to determine the relationship between the TSC and the hyp10 phagocytes in different mutants. We have several mutants from forward genetic screens and this tool will help us characterize these. We will also perform dual color time-lapse imaging.

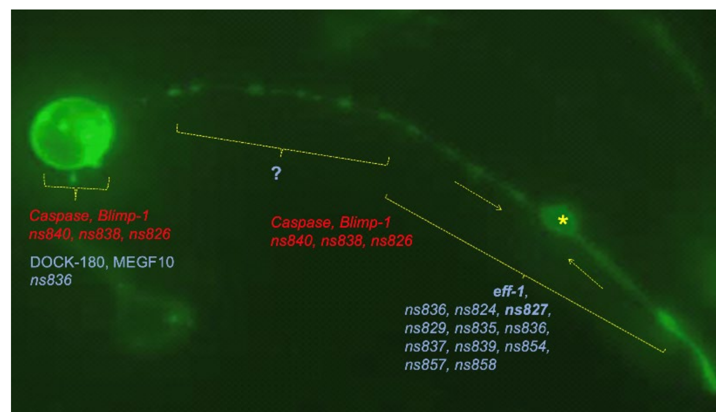

Figure 6.3: Future applications with the TSC

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BIOGRAPHICAL INFORMATION

Idararosa Ekong is earning an Honors Bachelor of Arts in Biology with another major with Public Health. Idara has had the privilege to be member of Dr. Piya Ghose's lab and conducted research regarding programmed cell death in *C. elegans*. Idara has also been involved with various on-campus organization including Peer Academic Leader, Maverick Terry Scholars, Minority Association of Premedical Students and Honors College Council. After graduating UTA, she hopes to apply to medical school for a career in Obstetrics/Gynecology.