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Generation and Assessment of Muscular Mutations in *Caenorhabditis elegans*

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**ABSTRACT** A study of egg laying muscular mutations in *C. elegans* was conducted over a span of ten weeks. Parent (EGL-19) and wild type (N2) were exposed to mutagenesis and integration mutation techniques to generate genetic and physical different mutants. Overall, four genetic, physical and phenotypically unique worms were generated for the process of mutagenesis. The worms used in the process of integration were found to have shortened life spans, reduced size and decrease numbers of progeny.

**INTRODUCTION**

*Caenorhabditis elegans* (*C. elegans*) are free-living soil roundworms (nematodes). They commonly live in many parts of the world. Their diet primarily consists of microbes, typically *Escherichia coli* (*E. coli*). *C. elegans* are wonderful organisms to use when studying genetics and muscular mutations, reasons being that these worms have a particular fast life cycle, about three days, and worms generate large amounts of genetically identical progeny per single adult.

*C. elegans* were specifically used in this research, which looks at neurological and muscular function in the egg laying muscles because they have simple neuron and muscular network that are easy to identify with microscopic techniques. *C. elegans* also have the ability to be frozen without damaging the worms which allows for the worms to be used and kept for long periods of scientific research. *C. elegans* have two sexes, the self-fertilizing hermaphrodite (XX) and male (XO). Both these sexes were used in this analysis and are essential for working with genetic mutations. *C. elegans* exist in four larva stages and an adult stage. The four larva stages are denoted as L1, L2, L3 and L4. Worms with specific genetic mutations can be analyzed and assessed using various processes. Mutant worms were obtained through
mutagenesis and integration, which includes exposing the worms to highly toxic chemicals or radiation. More specifically, the EMS process involved exposing worms to the toxic chemical Ethyl Methanesulfate (EMS.), a highly carcinogenic compound. Integration involved exposing worms to trimethylpsoralen in DMSO (TMP) and UV radiation. Fluorescent tags and identification was used to identify specific parts of the worm in relation to neurological and muscular orientations and function.

The specific mutation assessed was egg laying muscular mutation-19 (EGL-19). EGL-19 corresponded to mutant worms with over active L-type calcium channels. These worms appeared to be phenotypically very contracted. The egg laying muscles of the worm is vital for generation of offspring.

METHODS

WORM CULTURE METHODS

Worms were obtained from previously genetically mutated (EGL-19) and wild type (N2) worms. The wild type was identified as any worm that resembled the worms in the wild. These worms were plated onto a 60 mm petri dish seeded with mutated E. coli. The E. coli used was mutated so that it did not grow at the normal rate of typical E. coli. This controlled for limited competition between the E. coli and the worms. The worm populations for each of these worms was maintained by placing 3-4 L4 worms on a seeded petri dish every 3-4 days and storing them at 20°C, an optimal temperature for growth. Worms were examined with various microscopes and transported using a pick.

MUTAGENESIS

Mutagenesis was used to mutate worms. The original worms used in this process resembled the EGL-19 strain. 5-10 plates of with mutated worms were first washed with M9, a simple buffer for this experiment. The worms were then collected with a pipette and placed into a conical tube, which is then centrifuged for one minute. The supernatant was removed and the plates were washed for the second time with M9 and centrifuged again. The supernatant was then removed and 3 mL of M9 was added to a tube. In a separate glass tube 1 mL of M9 and 20 µL of EMS was added and then the solution was mixed with a vortex. It is important that this reaction is completed inside the hood because EMS is hazardous. Worms were then added to the solution, about 3 mL. The solution was then quickly capped and sealed with parafilm. The tube was then rotated in a tube for 4 hours. After this the worms were then spun using a centrifuge and the supernatant was removed. M9 was then added and repeated 3 times. After the washes were complete the worms were then transferred into a plated 60 mm plate. The solution was then allowed to dry. After the solution was dried worms were then picked (8 worms/plate, total of 10 plates). Three days after, L4 worms were picked (10 worms/plate, total of 80 plates). After the picking was complete 50 plates were placed in 15°C fridge and 3 plates were placed in 20°C. The various temperatures helped correct for the growth rates of the worms, number of worms picked and the time allotted for mutagenesis. 2-4 days after the plates were assessed for mutant worms. The assessment of mutants consisted of scanning the plated for two days visibly looking for mutants worms. In the case of EGL-19 the mutant worms picked were either non-motile or resembled N2 worms. These worms when then mated with fluorescent GFP worms to deliver fluorescent tags on the trans gene of EGL-19 expressed in the neuron to the offspring, this represents the parent generation (P0). The next generation of the fluorescent worms (F1) was then picked into four individuals plates. The next generation (F2) was later selected from these plates. The worms picked were either worms that resembled the original mutant, EGL-19, worms that resembled the mutants after the mutagenesis process and worms that looked like neither of the mutants or N2. N2-like worms were present in these plates but these worms were not picked. The progeny of these worms were later checked to have 100% fluorescent worms, if so the worm was maintained and used for further analysis. Due to the fact that GFP is a sex, X-linked trait, once the homozygous fluorescent mutant is found all progeny had fluorescence. Mutagenesis was completed twice
and four different mutant worms were maintained.

INTEGRATION

The second process used to mutate worms was TMP/UV Integration. The original worms used in this were strain Ex[myo-2,3-EGL-19] M. Cherry, a fluorescent worm with a marker on a specific neuron similar to EGL-19. M. Cherry is not integrated; therefore, mutation of these worms must follow the integration process in order to maintain the fluorescent characteristics of the worms. Six plates contained adult worms were washed with M9 buffer. The worms were then collected with a pipette and placed into a conical tube, which is then centrifuged for one minute and the supernatant removed. In a separate microcentrifuge tube 20 \( \mu L \) of TMP was added to 380 \( \mu L \) of M9, a final concentration of 50 \( \mu g/mL \). TMP is a light sensitive solution, therefore the tubes were wrapped in tin foil and transfers were performed in a dark hood to protect the solution from light. The solution was then transferred to an unseeded 60mm plate and then exposed to 350 \( \mu J \) of long wave UV light. The worms on this plate were maintained for five hours. Later, worms were picked (10 worms/plate, total of 15 plates); this represented the parent generation (\( P_0/g2868/g4667 \). The \( P_0 \) worms were transferred to new plates after 24 hours. 100-120 \( F_1/g2870 \) worms were then individually picked and placed on individual plates from the parent generation. Later, 200 \( F_2 \) worms were selected from the \( F_1 \) plates and placed on individual plates. In this part of the experiment worm fertility was accessed and many worms were found to be sterile with zero progeny. The worms were then analyzed under a microscope for fluorescents, plates that did not contain 100% fluorescent worms were discarded and plates with 100% fluorescents were maintained and used in further study. The process of integration was completed twice.

GENERATION OF FLUORESCENT WORMS

Furthermore, the fluorescent worms used were originally produced using a cloning process via plasmid delivery of the florescence. First the plasmid was isolated from the original bacteria strain. Plasmids are individual forms of DNA that are separate from chromosomal DNA and can replicate independently. This is the vehicle used to transport the fluorescent code into the worms existing DNA sequence. The DNA is sliced using a preemtor with a corresponding enzyme sequence. This performed with the desired worm DNA containing the fluorescent sequence using a cloning process. This is how the worms used in integration were fluorescent. The process of cloning takes about one day. In this mutagenesis part of this experiment fluorescent worms were used to deliver fluorescent tags though the mating process where 8-15 male fluorescent worms were places in an unseeded tray with 1-3 hermaphrodite mutants. After successful mating and three generations the fluorescent mutant was identified and maintained.

RESULTS

Once mutagenesis worms was completed four mutant worms were obtained and analyzed. These worms were selected for the physical mutations present when compared to parent worms, EGL-19 (Figure 3) and wild type (N2) worms (Figure 1). All worms picked throughout the mutagenesis were hermaphrodite worms; therefore, male worms (Figure 2) were not selected. Mutant 3-1 and 5-1 (Figure 4 & 6) had the most severe physical mutations with no movement in the adult worms and slower development. Mutants 2-1 and 12-1 (Figure 5 & 7) all exhibited similar mutations with limited mobility in the adult worms reduced development and decreased progeny but not as severe as the 1-3 and 5-1 mutants.

FLUORESCENT MICROSCOPY

The Inverted Magnification Microscope Zeiss X-EO Observer z1 was used to take detailed pictures of specific parts of the parent worm, EGL-19 with GFP marker. The GFP, or Green Fluorescent Protein, illuminates certain proteins related to egg laying motion inside of the worm. This illumination accounts for the lighter areas in the picture below. Figure 8 shows the nucleus of a neuron cell is \textit{C. elegans} EGL-19. The size of this cell is considerably smaller when
comparing it to the size of the muscle nucleus in Figure 9. Figure 10 shows the end of the worm, including the anus. Defecation by the worm is control by smooth muscles along the sides of the anal opening, which when contracted release waste. This type of contraction is identical to the muscle contraction for the release of eggs from the uterus of the worm, thus similar mutations can be seen between these areas of the worm. Figure 12 shows the muscles along the opening to the uterus, which when contracted releases eggs. Eggs can be seen to the left and right of this opening. Lastly, Figure 12 shows the distinctive neuronal pattern seen in EGL-19 and the most common identifier of this strain. The pattern is a stagger formation of neuronal cell nuclei along the synaptonemal complex of the worm. No analysis was completed on the resulting worms from integration due to the reduced viability of the worms. The M. Cherry fluorescent tag would have also eliminated myosin heavy chain protein of the egg laying muscle complex.

**DISCUSSION**

Of the two techniques, mutagenesis was the most successful. The process of mutagenesis produced four significantly different worms from the parent generation, EGL-19, and wild type, N2. These worms show the wide variety in egg laying genetic mutations that exist in *C. elegans* and the various characteristics associated with these mutants. Overall, each of these mutants expresses its own significance in the overall study of egg-laying muscular mutations.

**REFERENCES**


Kage-Nakadai, E., et al. 2012. Single/low-copy integration of transgenes in *Caenorhabitis elegans* using ultraviolet trimethylpsoralen method. *Methods*. 12:1 Integration was very unsuccessful throughout the course of this experiment. This process was performed twice and each time the worms were proven to have reduced viability. Each of the offspring from parent generation contained a large amount of sterile worms, identified by there slim bodies. This proves that the worms struggled to withstand the effects of the TMS/UV light and still produce progeny. The reduced progeny and viability of the mutants was a result of small deletion in the DNA code of the mutants.

In conclusion, mutagenesis was found to be the most significant for creating mutant worms from the original parent strain, EGL-19.

**Further Information**

This research was conducted over a span of ten weeks. Due to the time constraint and fast paced timeline, many interesting and supplemental investigations of the research could not be completed. Therefore, many questions that arose have been left unanswered. For future investigation additional research can be conducted on the genetic code of the mutants that were insolated and maintained in this research. This would include techniques such as Polymerase Chain Reaction (PCR) and the process of lysing. In addition, further identification and greater analysis of the fluorescent patterns of the mutants would also be something worthy of investigation.
Figure 1. Wild Type (N2) Hermaphrodite Adult
Figure 2. Wild Type (N2) Male Adult
Figure 3. EGL-19 Adult

Figure 4. 1-3 Mutant Adult
Figure 5. 2-1 Mutant Adult
Figure 6. 5-1 Mutant Adult
Figure 7. 12-1 Mutant Adult