CANDIDATE GENE DETERMINATION FOR *RAD-4* IN C. ELEGANS

by

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CANDIDATE GENE DETERMINATION FOR *RAD-4* IN C. ELEGANS

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<u>Introduction:</u>

Many organisms are affected by exposure to radiation and the negative side effects that follow high doses of damaging radiation, including ultraviolet light (UV). It is an important field of study because of the implications of overexposure to UV radiation that all organisms experience. Radiation generally damages the DNA, and organisms have evolved multiple ways to combat this damage (Klug 2010). When unchecked, the damage can lead to the death of the cell and if widespread enough the death of the organism. Radiation damage is also mutagenic and carcinogenic.

First studied by Brenner (1974), *Caenorhabditis elegans* are nematodes that are commonly used as a model organism. They have only six pairs of chromosomes one of which is a sex chromosome. *C. elegans* is found as both hermaphrodites and males in the wild. *C. elegans* are an ideal genetic model for investigating the effects of radiation exposure since mutations can readily be isolated and studied. In addition, the organism is popular because of its relative ease of maintenance, high homology with the human genome, and the facile and safe method of radiation exposure. Their development and other features, including sexual determination, are well documented (Riddle 1997).

Nine radiation-sensitive *C. elegan* mutants were isolated after exposure to the chemical mutagen ethyl methanesulfonate (Hartman and Herman, 1982). They all conveyed varying degrees of sensitivity to radiation and MMS (methyl methanesulfonate) at different points in development along with other observable phenotypes. This project was initiated to isolate and characterize a few of the many gene mutations that could be involved in DNA repair (Hevelone *et al.*, 1989). Some of the *rad* mutants proved to encode other things such as DNA checkpoint

proteins (Alpi, Hengartner, Gartner 2001) or proteins involved in pyrimindine synthesis (Qiao, 2014).

Rad-4, one of the isolated mutants, was shown to be sensitive to UV radiation, cold temperatures (observable at 15 °C as opposed to 20 °C and 25 °C, two other temperatures typically employed to raise *C. elegans*) and showed a decreased rate of chromosomal nondisjunction, at least of the X chromosome. Males do not contain another chromosome, such as a Y chromosome in humans, but instead are missing one of their X chromosomes (XO). This makes the detection of X-chromosomal non-disjunction relatively easy. Specifically, all self-progeny from hermaphrodites are also hermaphrodites except for rare males which arise by X-chromosome non-disjunction and are easily recognizable under a dissecting microscope. In addition to the novel phenotype of suppressing X-chromosome non-disjunction in an otherwise wild-type genetic background, the *rad-4* mutation suppresses the levels of meiotic non-disjunction in some but not all *him* mutants that are themselves characterized as possessing elevated meiotic non-disjunction (Hodgkin *et al.*, 1979). Using linkage testing and three-factor crosses *rad-4* was roughly positioned on chromosome V at the location of 9.45 +/- 3.05 map units. The goal of this study was to map *rad-4* more precisely, thus paving the way for molecular identification of the mutation. This should afford an understanding of how *rad-4* influences radiation sensitivity and, perhaps more interestingly, non-disjunction.

Radiation sensitivity was primarily measured by the survival rate of the worms after exposure to germicidal (UV) radiation. Radiation hypersensitivity is often observed in mutants lacking functional DNA repair genes (Klug 2010). Damage is induced at equal rates in wild type and mutants, but the mutants are less able to repair, and hence survive, equivalent amounts of damage. This type of mutation is likely to have some relation to the phenotypes observed in *rad-*

4. Rad-4's radiation sensitivity had not been previously tested past the early embryo stage (Hartman and Herman, 1982). This is generally the most vulnerable point in development because the likelihood of widespread DNA damage is more likely. In addition, early embryos must undergo many rounds of replication whereas many cells in larvae and adults are post-mitotic. Some of the other *rad* mutants were shown to have hypersensitivity later in development (Hartman, 1984). This indicates that UV sensitivity is temporally dependent. In addition to mapping, another goal of this study was to determine the radiation sensitivity in later stages of development including both four-hour embryos and worms in early larval stages.

One of the more unusual traits of the *rad-4* mutation is that is confers a reduced rate of chromosomal non-disjunction (Hartman and Herman, 1982). This was detected by the reduction in the number of male progeny observed in *rad-4* among the self-progeny broods of hermaphrodites. This was shown to be the result of a decreased rate of chromosomal non-disjunction and not due to a non-viability of males. In *C. elegans* sex is determined by the dosage of X chromosome compared to the ploidy of the autosomal chromosomes. Under normal conditions, hermaphrodites have 2 X chromosomes (XX), while the males have only one (XO). Thus, a non-disjunctional event in the X chromosome pair of a hermaphrodite leads to that offspring being a male. In contrast, because 50% of male sperm are nullo-X and 50% are haplo-X, the progeny of a male/hermaphrodite mating is 50% males. Interestingly, the male sperm preferentially fertilize relative to the sperm produced by the hermaphrodite (Ward 1995). The *de novo* non-disjunctional event of the X chromosome occurs in approximately 1 in 500 of wild-type *C. elegans*. This is reduced by approximately a factor of ten in *rad-4* (Hartman and Herman, 1982) Many mutations have been found to increase the rate of chromosomal non-disjunction, including those in the *him* (high incidence of males) genes in *C. elegans* (Hodgkin et al., 1979).

In contrast, the number of mutations leading to a reduction in non-disjunction events is not as common, and in fact several searches of Web of Science have failed to find another case in which a mutation actually decreases non-disjunction. In addition, *rad-4* was shown to partially or completely reduce the effect of some *him* mutants including up to an eighty percent reduction in number of males when combined with *him-1* and *him-3* (Hartman and Herman, 1982)

Rad-4 also appears to be unique in that the mutant is both radiation hypersensitive and has reduced X-chromosome non-disjunction. It is at least formally possible that these two mutant phenotypes were caused by two mutations in different genes. However, *rad-4* was repeatedly outcrossed using wild-type animals to reduce or eliminate multiple mutations being responsible for the different phenotypes. This eliminates or at least greatly reduces the possibility of instances in which the two mutations are on different chromosomes or are widely separated on chromosome V. There is a small probability that another mutation could be closely linked to *rad-4* mutation but that is unlikely. This suggests that the protein for which *rad-4* codes for has at least two functions in the organism.

A common approach to elucidating such matters is to molecularly identify the gene, which is currently only genetically identified as being at an approximate position on a chromosome. Therefore, the primary focus of this project was to map more precisely the *rad-4* mutation using observable phenotypes and to search the more narrowly defined area for candidate genes. Such mapping was successfully accomplished over the course of the 2014-2015 academic year and preceding summer. As well, candidate genes were identified by running protein BLAST analyses of candidate genes in the region defined by the above-mentioned mapping experiment. This should allow the molecular identity of and function of *rad-4* to be discovered by sequencing the *rad-4* strain to show a mutation is present in a candidate gene.

Materials and Methods

Strains:

The following strains were obtained from the Caenorhabditis Genetics Center: N_2 (wild type), SP497 [*rad-4(mn158)*], MH1914 [*lin-40(ku285)*], DR181 [*unc-60(m35) dpy-11(e224)*] and CB644 [*unc-62(e644)*]. For each strain, the capital letter and number (*e.g.,* SP497) indicate the strain name, the letters and number in bracket (*e.g.*, *rad-4*) indicate the gene mutated and the letters and numbers in parentheses (*e.g.*, *mn158*) indicates the specific allele.

Stock Maintenance:

Stocks were maintained in 60x15mm Petri dishes on Nematode Growth Medium with an OP50 *Escherichia coli* bacterial lawn prepared in the manner described by Sidney Brenner (1974) and by the use of a "dot" preparation, which led to the bacterial lawn being smaller than the entire surface of the plate. They were checked regularly to ensure the survival of the worms. If there appeared to be shortage of bacteria or the presence of a contaminant, the stocks were transferred by one of two methods. The first was the transfer of several gravid hermaphrodites via a sterile platinum wire to a newly seeded plate. The second was the mass transfer of a "chunk" of the agarose plate to a new Petri dish by cutting it with a sterile surgical scalpel. The length of time between each transfer varied between strains.

Preparing Petri dishes with Nematode Growth Medium:

A 2 L Erlenmeyer flask was filled with 1 L of deionized (DI) water and 17 g agar, 3 g NaCl, and 2.5 g peptone were added to the water and autoclaved for 30 minutes. Also autoclaved were 3 solutions: 1 M $CaCl_2$ (solution B), 1 M $MgSO_4$ (solution C), and 1 M $KHPO_4$ (solution D). A 5 mg/ml solution of cholesterol (solution A) dissolved in ethanol was also used but not autoclaved. After autoclaving, the flask was placed in a water bath at 50 °C to prevent premature

solidification. After the solution had cooled, 1 ml of solutions A, B, and C along with 25 ml of solution D and 2.5 ml of a fungicide (Niacin) (5 mg/ml in ethanol) were added and swirled into the flask. The agar solution was then poured in to the Petri dishes and allowed to harden overnight. Plates were stored at $4^{o}C$.

Three-factor cross:

OP50 "dot" plates were used throughout these particular experiments. First, 5-7 male N_2 animals were placed on a transfer plate and then allowed to locomote for 15 minutes. The plates were then scanned to insure there were no larvae or adult hermaphrodites. Eight to ten late L4 or young mature double homozygous hermaphroditic mutants (for example *lin-40 unc-60*) in which each of the two mutations conferred a visible phenotype (unlike the *rad-4* mutation that does not confer a visible phenotype) were then placed on a separate plate. After ensuring only male N_2s were present on the transfer plate, the males were added to the cross plate with the *lin40 unc60* hermaphrodites. The plates were checked daily after this to obtain the progeny at the appropriate time. There were two types of progeny: 1. Self-progeny from the *unc60 lin40* hermaphrodites, which have a distinctive phenotype, and 2. Cross progeny, which could be symbolized as *++/unc60 lin40* (both males and hermaphrodites) and have an obviously different phenotype. Due to the low probability of a *de novo* male arising from the hermaphrodites, all male progeny were assumed to be cross progeny. These males were picked from the cross plate while still young and placed on another transfer plate. On a new dot plate were placed 8-10 *rad-4*

hermaphrodites. The F_1 cross progeny males (++/*lin40 unc60*) were then added to the new dot
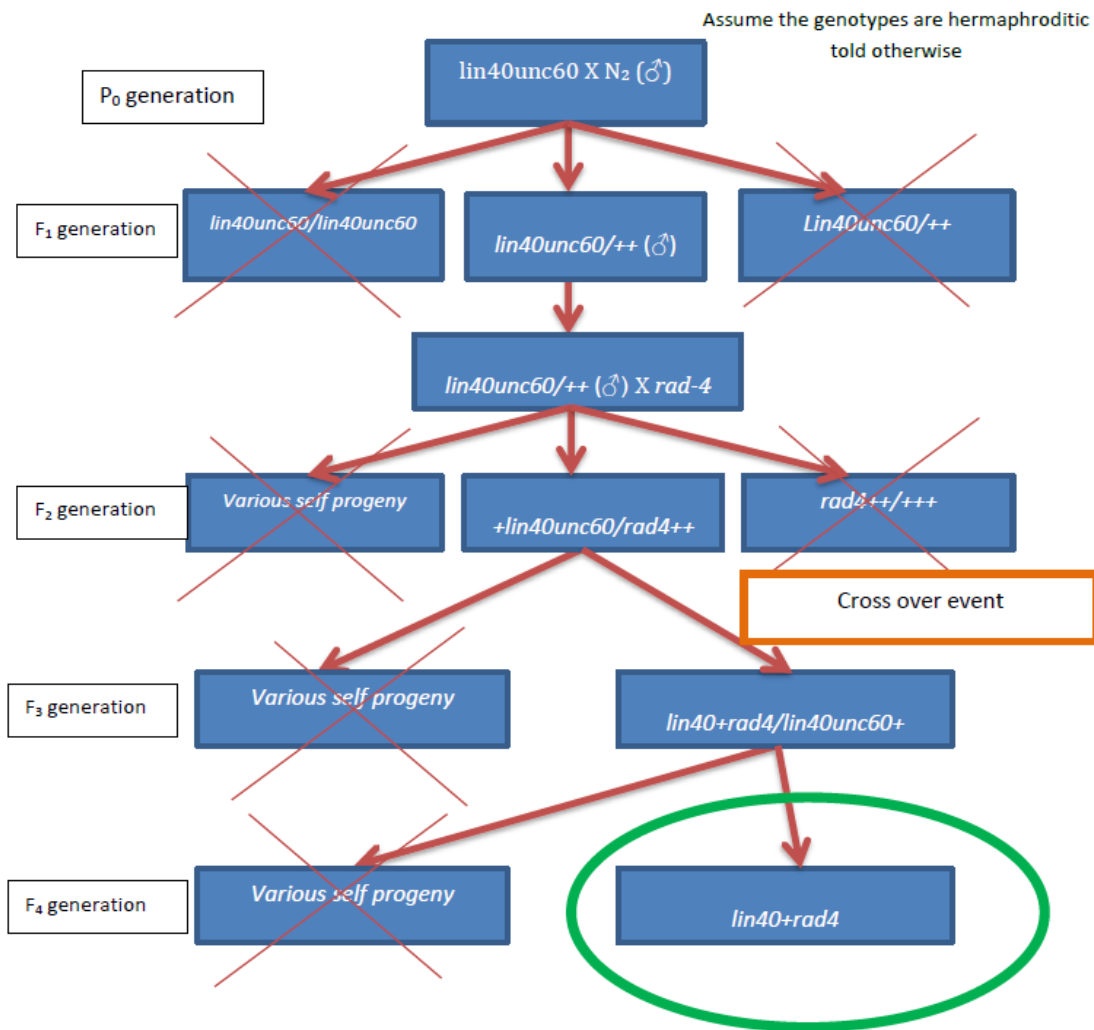

Figure 1: Three factor cross between *lin-40 unc60* x *rad-4*.

plate for the second cross. The plate was checked daily after this to obtain the progeny at the

appropriate time. There would be three types of progeny from this cross: 1. Self-progeny from

the *rad-4* hermaphrodites, 2. Cross progeny (+/ *rad-4*), and 3. Cross progeny rad-*4*/ double

mutant (for example *rad-4/ lin-40 unc-60*). This third group of progeny is the desired genotype and can readily be recognized by the presence of phenotypically Lin Unc progeny. For it to be considered an efficient cross there needed to be a plethora of males in the F_2 generation to increase the likelihood of many cross progeny. However, there is no way to distinguish between them and *rad-4* self-progeny. Because of this, many of the phenotypically wild-type hermaphrodites were plated individually. In the F_3 generation it was apparent which genotype was obtained by the double mutant phenotype being observed in one-fourth of the progeny of the needed genotype. Any sample that did not contain these observable phenotypes was disposed of at this point. On the plates where the original hermaphrodite was *rad-4*/double mutant, a daily scoring was performed to locate F_3 progeny that appeared to be only single mutants. This signified that a crossover event had happened in between the two visible mutations thus unlinking them. These F_2 genotypes were either + single mutant/double mutant (for example: + *lin-40/lin-40 unc-60*) or *rad-4* single mutant/ double mutation (for example: *rad-4 lin-40/lin-40 unc-60*).

Generating Homozygotes from the Recombinants:

Progeny from the various recombinants were then individually plated. They would be one of three genotypes; 1. Visible double mutants (for example *lin-40 unc-60/lin-40 unc-60)*, 2. Heterozygotes (+ single mutant/double mutant or *rad-4* single mutant/ double mutations) (for example *rad4+lin40/+unc60lin40*), or 3. Homozygous single mutants (for example: *rad4+lin40/rad4+lin40*). Observing only single mutants (*e.g.*, no Unc Lin progeny) meant that the original animal plated was a single mutant homozygote. At least 5 progeny of the crossover event were used to maximize the chance of obtaining the desired homozygote. Throughout all experiments described, it was imperative to obtain the correct generation to ensure there were no

multiple crossover events and to guarantee knowledge of the genotypes. These homozygotes

obtained were then stable and could be treated in the same way any other stock was maintained.

Creation of *lin-40 unc-60* double mutant:

The creation of the double homozygote required a similar crossover event that was used
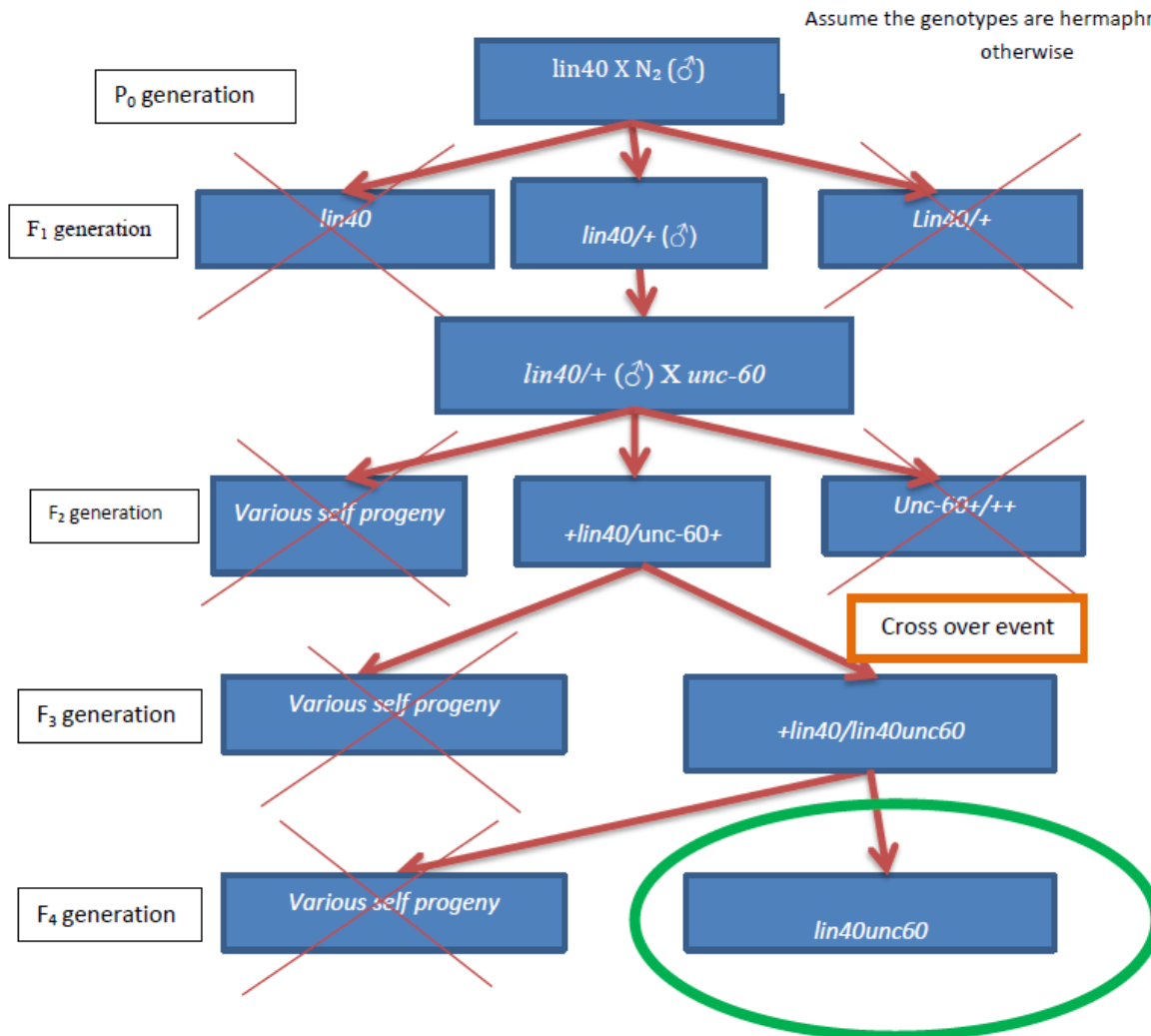

Figure 2: Generating a double homozygote mutant of *lin40* and *unc60*

in the three-factor cross later in the experiment. *Lin-40* L4s were combined with wild-type males

on an "OP50" dot plate. Their male progeny were then transferred to another dot plate that

contained *unc-60* young hermaphrodites. Their progeny could contain both the *lin-40* and *unc-60*

genes, and this would allow for a crossover event that would cause the two mutations to become linked. Homozygotes could be generated using the techniques described above. They could then be used in the three-factor crosses also described above.

Preparing plates for radiation experiments:

Young gravid hermaphrodites, 8-10, were plated and placed on a recently seeded plate and allowed to lay eggs for 2 hours. At the end of this period the hermaphrodites were removed with a flame sterilized platinum wire. The number of eggs on each plate was counted and noted on each Petri dish.

Radiation experiments:

In order to determine if embryos or early larva were sensitive to radiation, they were exposed to a dose of ultraviolet radiation. A box of 15 inches in height was used along with a radiation dosage of 1 joule/m^2 of germicidal (254 nm) radiation emitted from a low pressure mercury fluorescent bulb. There were several experiments done at different times in the developmental life cycle of *C. elegans*. Time periods of 2 hours, 4 hours, and 24 hours from the removal of the hermaphrodites were tested for multiple strains. A kill curve was determined for N$_2$ and *Rad-4* in order to test for the presence of *Rad-4*.

Scoring the results:

There were two different strategies used for scoring radiation sensitivity. For the 2-hour embryos, the results were either death or normal development. This makes determining radiation sensitivity in these early embryos relatively easy. The later embryos and early larva had to be scored in a less quantifiable way. They were judged against the virility of the control group in comparison to their continued development.

<p style="text-align:center;">Results:</p>

1. **Kill curve confirmation for N$_2$ and *rad-4* two hour embryos:**
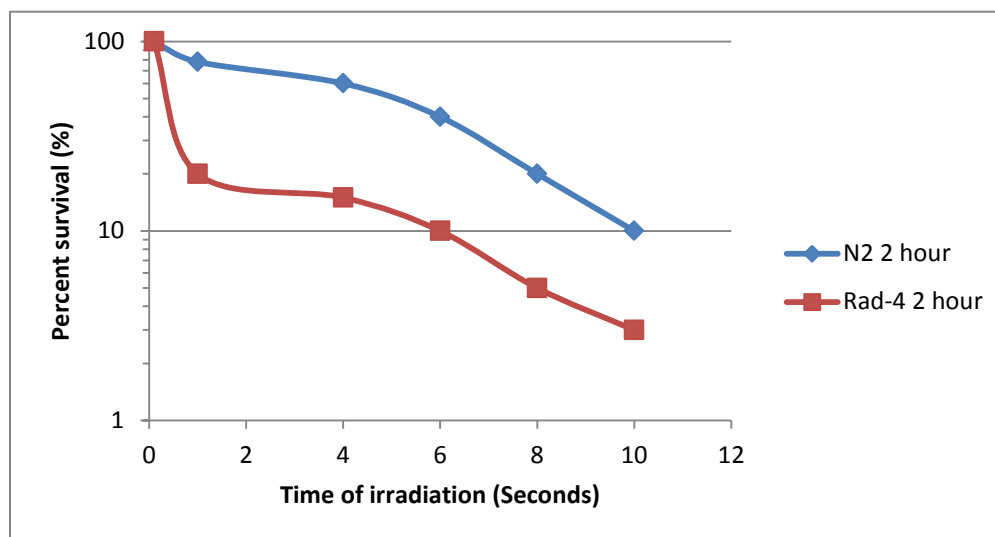

Figure 3: Initial determination of a kill curve of N$_2$ vs. *rad-4*. This figure shows the Percent Survival vs. the time of irradiation. The number of eggs irradiated per trial was anywhere from 8 to 132 depending on the number of eggs laid in the two-hour period.

It was first necessary to confirm that *rad-4* was in fact still sensitive to radiation since it has been decades since this mutant was last studied (Hartman and Herman, 1982). In order to do so, gravid hermaphrodites were allowed to lay eggs for two hours and the plates were exposed to various doses of germicidal radiation. The survivors were then scored. *Rad-4* was clearly more sensitive than the wild-type strain N2 (Figure 3). These experiments were repeated several times, with *rad-4* clearly more sensitive in all cases.

Survival rate was determined by the number of embryos to reach adulthood after 3-4 days. The survival rates even at only a second of irradiation were clearly different but approached similar values by 10 seconds. This arises from the presence of older embryos present in the experiments. Two reasons for this difference in age of the eggs could be the eggs were actually laid earlier in the two hour egg laying process or because older hermaphrodites tend to hold their eggs longer. The fact that survival rates approach similar values supports the claim later made that *rad-4's* susceptibility is limited to early embryogenesis. *Lin-40's* kill curve was determined to be similar to N_2's by the same method (data not shown). This was important to determine because *lin-40* was employed in mapping experiments (see below). The irradiation time period of one second was selected for future trials to determine whether a strain was *rad-4* or *rad-4$^+$*.

2. ***Rad-4's* susceptibility to radiation later in development (2 hour embryos and 24 hour larva)-**
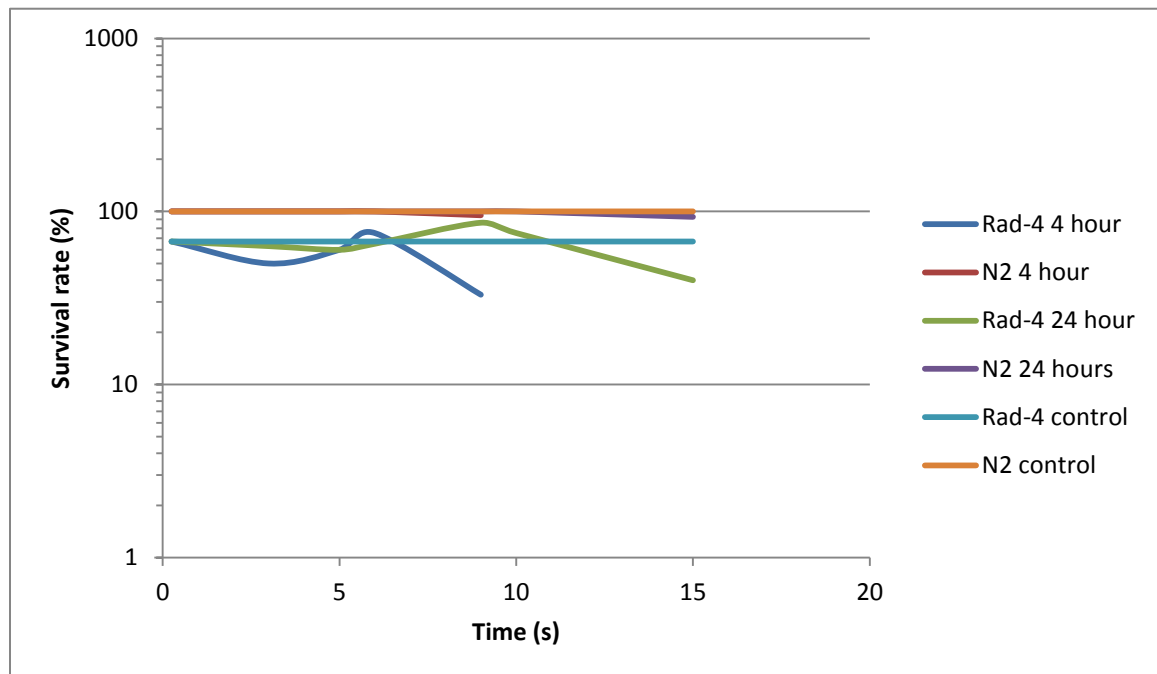

Figure 4: Graph of irradiation tests of later embryos and early larva.

Sample radiated	Time of irradiation	Wild type % survival	*Rad-4* % survival
24 hour larva	5 seconds	100 % survival	60% survival rate
	10 seconds	100 % survival	75% survival rate
	15 seconds	93% survival rate	40% survival rate
4 hour embryos	3 seconds	100% survival rate	50% survival rate
	6 seconds	100% survival rate	50% survival rate
	9 seconds	95% survival rate	33% survival rate
Control	NA	100% survival rate	67% survival rate

Table 1: This chart demonstrates the survival rate of N_2 compared to *rad-4* at later stages in development. An un-irradiated *rad-4* control was also included in this table. These animals were scored in the same manner as described in the kill curve confirmation. However an extra variable of healthy appearance was also noted in this experiment. This experiment was performed because others of the original mutants were shown to be sensitive later in development (Hartman, 1984). It was important to know if *rad-4's* sensitivity was limited to early embryogenesis or not.

Rad-4 at first glance appears to be affected by the exposure but when compared to the control there was no significant difference in the survival rates. The appearance of

both N$_2$ and *rad-4* seemed slightly less healthy at the extremes of exposure but equally so.

3. **Three factor cross:**

Chromosome 5 (1 to -19 map units)

Dpy-11 (.03)   *Lin-40 (-8.19)*   *rad-4 (-9.45 +/- 3.05)*   *Unc-60 (-18.88)*

-11.75

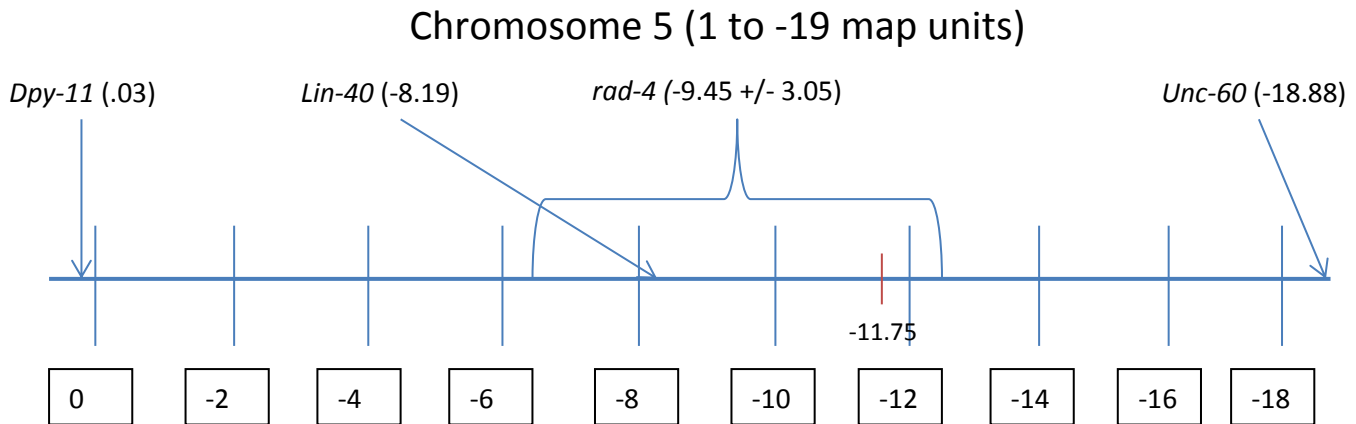| 0 | | -2 | | -4 | | -6 | | -8 | | -10 | | -12 | | -14 | | -16 | | -18 |

Figure 5: This shows the estimated location of *rad-4* on chromosome V (Hartman and
Herman, 1982) and the more specific estimation determined by this study (-11.75 map
units). It also includes all the pertinent genes used in the three-factor crosses from both
experiments.

As previously stated, *rad-4* was located to the 5th chromosome by several three-
factor crosses. Specifically the following cross: *unc-60 rad-4 dpy-11* (Hartman and
Herman, 1982), using the nomenclature of Horvitz *et al.* (1979). This narrowed the field
of search to what is shown in the figure above (Figure 5). In this study a similar three
factor cross was employed: *lin-40 rad-4 unc60.* The mutations chosen for the final three-
factor cross were based on their locations (Figure 4). This narrowed window was more
useful because it provided a smaller range to study and allowed for a more accurate

location determination. They are also easily differentiated phenotypes which allowed for

easy scoring. The method for strain construction is stated in the materials and methods.

Approximately one third of the *lin-40* recombinants included *rad-4.* This allowed the

location determined by Hartman and Herman (1982) to be refined to the location of -

11.75 map units on chromosome V.

4. **Candidate gene determination:**

Gene	Gene Sequence Name	Gene Gene Name	Gene Location: Start (base pair)	Gene Location: End (base pair)
WBGene00006126	C17B7.1	str-63	3351566	3352773
WBGene00015886	C17B7.12		3353263	3353921
WBGene00015436	C04E12.6		3354669	3355513
WBGene00015437	C04E12.7	scrm-3	3355980	3357193
WBGene00015435	C04E12.5		3358599	3361093
WBGene00015434	C04E12.4		3362784	3368321
WBGene00015433	C04E12.2		3369081	3369877
WBGene00006012	C04E12.8	srx-121	3370942	3372773
WBGene00015438	C04E12.9	srbc-2	3374362	3377073
WBGene00015439	C04E12.10		3377536	3382850
WBGene00015440	C04E12.11	arrd-20	3386491	3388156
WBGene00015441	C04E12.12	arrd-21	3389330	3390952
WBGene00020619	T20D4.13		3391683	3393294
WBGene00020620	T20D4.15		3393478	3394688
WBGene00020622	T20D4.17		3395275	3395966
WBGene00020618	T20D4.12		3397065	3397789
WBGene00020617	T20D4.11		3398693	3399389
WBGene00020616	T20D4.10		3400255	3401045
WBGene00044718	T20D4.20		3401645	3402356
WBGene00020615	T20D4.9		3402631	3405161
WBGene00020614	T20D4.8		3405709	3407893
WBGene00020613	T20D4.7		3408870	3409519
WBGene00020612	T20D4.6	arrd-22	3410520	3412962
WBGene00020611	T20D4.5		3413344	3415816

WBGene00020610	T20D4.4		3415993	3417770
WBGene00020609	T20D4.3		3418263	3422733
WBGene00020623	T20D4.18	srab-21	3422949	3424441
WBGene00020608	T20D4.2	srab-22	3426376	3427733
WBGene00020607	T20D4.1	srab-20	3428999	3430408
WBGene00020624	T20D4.19		3430669	3431446
WBGene00005597	T20C4.1	srj-9	3432259	3433658
WBGene00000478	F27E11.3	cfz-2	3441501	3444877
WBGene00174026	F27E11.5	21ur-15093	3448742	3448762
WBGene00195825	F27E11.6		3455131	3455293
WBGene00017868	F27E11.2		3459719	3463001
WBGene00167632	F27E11.4	21ur-8722	3463423	3463443
WBGene00017867	F27E11.1		3463814	3466150
WBGene00001781	C02A12.1	gst-33	3467453	3468501
WBGene00015315	C02A12.2	srbc-29	3469445	3470589
WBGene00044088	C02A12.8	oac-2	3474277	3475121
WBGene00044089	C02A12.9	srbc-31	3476405	3477596
WBGene00015316	C02A12.3	srbc-30	3478031	3480446
WBGene00003096	C02A12.4	lys-7	3481419	3482600
WBGene00044090	C02A12.10	srbc-33	3483091	3484781
WBGene00015317	C02A12.5	srbc-32	3485549	3486957
WBGene00015318	C02A12.6	srbc-36	3489228	3490535

Table 2: This displays the list of genes centered on the suspected location of *rad-4*. These genes were searched for a function that could cause the phenotype observed in *rad-4*.

Using an online database (WormBase), a list of genes centered around -11.75 map units on the genetic map (which corresponds to 3.35-3.50 Megabase pairs on the physical map) was obtained (Table 2). The function of these genes was then determined either by information from WormBase or by conducting P-BLAST analyses. An example of a non-candidate gene would be the srab-21 gene, a 7TM GPCR, serpentine receptor, whose primary role is in olfaction and presumably has little to do with radiation sensitivity. Using this information, a short list of candidate genes was determined by the likelihood that they could have some sort of link to a function of the *rad-4* mutation. The criterion was data published

on the gene having an effect on radiation resistance or having a function in DNA repair. Some of the genes were of an unknown function, so a BLAST analysis was necessary to determine possible functions.

5. BLAST analysis:

Candidate Genes	Function
C04E12.5	Nucleotide excision repair (Domain homology)
C04E12.4	Nucleotide excision repair (Domain homology)
C04E12.10	Nucleotide excision repair (Domain homology)
T20D4.5	Nucleotide excision repair (Domain homology)
T20D4.4 (3)	Nucleotide excision repair (Domain homology)
F27E11.1	Solute carrier protein when over expressed leads to radiation resistance

Table 3: This shows the final list of candidate genes (narrowed from table 2) after the BLAST analysis. They are all within the determined range and have function that makes them strong candidates for having a role in a similar phenotype as observed in *rad-4*.

A BLAST analysis is a common way in which to elicit similarities between both nucleotide and protein sequences (Madden, 2002). For this study a protein BLAST (pBLAST) was used. After running this analysis, the sequences were scoured for similarities for any gene that showed a function for anything related to the phenotypes observed in *rad-4*, specifically radiation sensitivity and decreased chromosomal non-disjunction.

Discussion:

In this study we employed a number of techniques to map *rad-4* more precisely and narrow the number of candidate genes for the *rad-4* gene of the nematode *C. elegans*. Both previously obtained data and data collected in this study were used in this search. We narrowed the list of candidate genes by the use of three-factor crosses, which two mutations that confer visible phenotypes are employed. We used this to determine the relative location of the *mn158* mutation in *rad-4*, which does not confer a visible phenotype. Hypersensitivity to UV radiation was used to assess the presence of *mn158*. Using the ratio of the recombinational events, the location of *rad-4* was narrowed. After this range was obtained, a candidate gene approach was used to limit further the search window. However, this strategy can be shortsighted due to unknown interactions between certain genes and radiation sensitivity.

Kill curves were first determined for N2, *rad-4*, and *lin-40*. This was necessary to test for the presence of *rad-4*. This was done because it was necessary to determine a time period of radiation in which the survival rates for N2 and *rad-4* were observably different. This will allow confirmation of the presence of *rad-4* in the crosses mentioned later. This is imperative because the *rad-4* does not have any other readily observable phenotypes. The kill curve for *lin-40* being similar to N2's is imperative. Some mutants, including *lin* mutants hold their eggs for a variable amount of time in comparison to N2 (Johnsen *et. al.*, 1989). If the kill curve had been different for *lin-40* it would have suggested they held their eggs for a prolonged period of time. This would have required further studies using Nomarski scope techniques to confirm the findings.

Once the kill curve was determined, the three-factor crosses could begin. The results from the *lin-40* and the *unc-60* positioned *rad-4* in a smaller range than determined by Hartman

and Herman (1982). These two mutations were chosen for several reasons. They were easily distinguishable from each other and the location of *rad-4* in proximity to *lin-40* made it an attractive selection. From this cross four of twelve recombinants were *rad-4lin-40⁺*. These recombinants were all the result of a crossover between *lin-40* and *unc-60*. This suggests that *rad-4* is located one third of the way in between *lin-40* and *unc-60*. Specifically, it was calculated to the location of -11.75 map units on chromosome V. There are two complicating factors worth noting. First, *lin-40* also has a reduced fecundity that, when combined with *rad-4*'s reduced brood size, made it difficult to obtain enough embryos to determine radiation sensitivity. Second, the combination of *lin-40* and *rad-4* appeared to inconsistently appear "Dumpy." This could have allowed for a higher rate of recognition of the *lin-40 rad-4* recombinants, thereby altering the actual ratio. While possible, this is unlikely because the recombination events were detected one generation before the *lin-40 rad-4* recombinants were made homozygous. These data with *lin-40* and *unc-60* are generally consistent with mapping data obtained when *rad-4* was first isolated (Hartman and Herman, 1982) and successfully narrow the possible location of the gene.

Another aspect studied was to determine whether *rad-4*'s susceptibility to radiation continued later in development. This could help with the narrowing the pool of candidate genes by determining if the gene conveyed sensitivity beyond the early development. Some of the other radiation sensitive genes discovered in the original study were sensitive past this early stage (Hartman, 1984). *Rad-4* was similar to *rad-1* and *rad-7* in that its hypersensitivity is limited to embryogenesis while *rad-2* and *rad-3*'s persisted into the larval stages of development.

The multi-faceted effects of the *rad-4* mutation make this study even more interesting. *Rad-4* was somewhat difficult to work with due to its reduced fecundity (Hartman and Herman,

1982) and the lack of males that derive from hermaphrodites owing to the fact that meiotic non-disjunction is reduced by the *rad-4* mutation. In addition, despite extensive efforts early in the project to propagate a *rad-4* male stock, this proved extremely difficult. Not only were fewer males obtained, but they were largely infertile.   This necessitated that the three-factor crosses were initiated differently than the more direct method of crossing *rad-4* males times the double mutant.

Another interesting point about *rad-4* is the lack of other mutations in *C. elegans* and other organisms that reduce chromosomal non-disjunction relative to wild type.   There are extensive reports that mutations can elevate meiotic non-disjunction, including in *C. elegans* (Hodgkin et al., 1979). In contrast, an extensive literature search did not yield a single example of a mutation that actually reduced meiotic non-disjunction. In all the searches of multiple databases and journals, there were no sources concerning a gene that limited this event's probability. However, there were countless articles and studies about the increase of the rate of chromosomal non-disjunction. This is not to say that it is a possibility there could be other agents of similar function but that through our research nothing was found.

In the future, these candidate genes will be sent to a colleague of Dr. Hartman's who will sequence the candidate gene or genes in *rad-4*-bearing strain to see if the evidence gathered by this study is correct. As stated previously, we are limited in the further studies before the sequencing of the genes.

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ABSTRACT

Radiation sensitivity can be caused by many factors. A mutant, *rad-4*, of the nematode *Caenorhabditis elegans* was isolated in 1982 that possesses interesting properties in addition to conferring sensitivity to UV radiation. The purpose of this study was to use observable recombinational events to more precisely locate the mutation on the genetic map. Once the mutation is located candidate genes can then be sequenced in order to molecularly identify *rad-4*. First, kill curves were performed (comparing *rad-4* to the wild-type strain N2) to confirm the radiation hypersensitivity of *rad-4*. Most experiments employed two-hour embryos; however, the sensitivity of *rad-4* was also tested at later stages in development, four and twenty-four hours. There was no increased sensitivity observed in *rad-4* in later stages in development. Once the dosage of a one second was shown to be sufficient to distinguish *rad-4* from *rad-4*$^+$ in two hour embryos, the mapping experiments began. Three factor crosses were employed in which *rad-4* was position relative to two other mutations that conferred visible phenotypes. The results from the original study were first confirmed by the *dpy-11 unc-60/ rad-4* cross. The next cross performed necessitated a creation of a new double homozygote, *lin-40 unc-60*. A triple heterozygote (*rad-4/lin-40 unc-60*) was then generated and Lin, Non-Unc recombinants were selected. One third of these recombinants observed included *rad-4*. This allowed the possible genes to be narrowed to a much smaller range centered around -11.75 map units on the fifth chromosome. Finally, WormBase was used to locate the possible genes and NIH pBLAST analysis was employed to determine possible functions for genes on the list with unknown functions. The list was then limited to seven sequences, six of them having high homology suggesting that they were the same gene with different splice sites. Several mutant strains of each

gene were ordered and will be tested for radiation sensitivity. After this confirmation *rad-4* will

be able to be sequenced and studied further.