A NEW ROLE FOR THE TUMOUR SUPPRESSOR LIN-35 DURING MEIOTIC RECOMBINATION IN *CAENORHABDITIS ELEGANS*

by

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ABSTRACT

Meiosis is a fundamental biological process used by sexually reproducing species to ensure the faithful transmission of genetic material and to generate genetic diversity. In humans, failure to recombine properly during meiosis causes genetic conditions in the human conceptus such as aneuploidy and spontaneous abortion. An excellent model organism for the investigation of meiotic recombination is the nematode, *Caenorhabditis elegans,* which has many conserved meiotic processes. In this thesis, I have investigated the role of *lin-35* in meiotic crossing over. LIN-35 is the *C. elegans* ortholog of the retinoblastoma (Rb) protein, well characterized with respect to its role in gene transcription and cell proliferation. My results show that mutation in the *lin-35* gene alters recombination frequency differentially for several regions of the chromosome, causing increases in recombinationally suppressed regions and decreases in highly recombinogenic regions. In combination with Rec-1, a mutant known to alter crossover distribution, crossovers across the length of the entire chromosome, were decreased. In addition, other severely detrimental phenotypes were observed. For example, gametic viability was reduced dramatically in the double mutant, compared to either mutant alone. Thus, the Lin-35 and Rec-1 phenotypes were synergistic, indicating non-redundancy. In summary, *lin-35* function plays a role in achieving normal levels of meiotic recombination, a role that may be related to its function in chromatin modification and gene transcription.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

CO (crossover)

COI (crossover interference)

Dpy (dumpy)

DSB (double stranded break)

GFP (green fluorescence protein)

Hda (histone deacetylase locus)

Him (high incidence of males)

LG (linkage group)

Lin (cell lineage abnormal)

m.u. (map units)

p (recombination frequency)

Rec (abnormal recombination)

Rb (retinoblastoma)

SNP (single nucleotide polymorphism)

Unc (uncoordinated)

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CHAPTER 1: INTRODUCTION

1.1 The role of meiotic recombination

Fundamental to the reproductive success of most diploid organisms, meiosis is a cell division process essential for the maintenance of proper ploidy between generations. During meiosis, homologous recombination generates genomic variation and ensures genome integrity through proper chromosome segregation (Resnick 1976, Howard-Flanders and Theriot 1996, Petronezki *et al.* 2003, reviewed by Zetka 2009).

Failure to recombine properly during meiosis causes genetic conditions in the human conceptus such as aneuploidy and spontaneous abortion (Reiter *et al.* 1996, Lopes *et al*. 1998, reviewed by Handel and Schiment 2010). Reduced recombination is associated with meiosis I maternal segregation errors. Errors such as nondisjunction can occur when chromosomes do not pair and exchange genetic material properly. In humans, the direct result is aneuploid oocytes, which cause conditions such as Down Syndrome. Most pregnancies are not at a great risk of aneuploidy; however, the prevalence increases considerably with advanced maternal age (reviewed by Hassold and Hunt 2001).

1.2 Meiotic prophase and recombination in Caenorhabditis elegans

The hermaphrodite nematode *Caenorhabditis elegans* has several features that make it an excellent model organism to study meiosis including easy maintenance, a relatively compact genome, the generation of haploid gametes of both sexes, and highly conserved meiotic processes (reviewed by Muse and Boulton 2007, Zetka 2009). In *C. elegans*, meiosis begins in the germline where the distal tip cell gives rise to nuclei, which undergo pre-meiotic replication followed by prophase of meiosis I (reviewed by

Zetka and Rose 1990 and more recently Muse and Boulton 2007) (Figure 1). The spatio-temporal organization of the transparent germline facilitates visualization of meiotic prophase (Albertson *et al.* 1997).

The homologs pair and align, first at the homolog recognition region (Rosenbluth and Baillie 1981, McKim *et al.* 1993) and then along the whole chromosome (Jones *et al.* 2009). The synaptonemal complex joins homologous chromosomes to ensure that a close proximity is maintained for meiotic recombination (Zetka 2009). The axial elements promote chromosome condensation and pairing while inhibiting recombination between sister chromatids. The central element is important for synapsis and likely involved with maintaining distance between crossover events.

A highly conserved topoisomerase, SPO-11, catalyzes the double-strand break (DSB) which results in the obligate crossing over event (Figure 2). In *C. elegans*, Spo-11 mutants exhibit extensive embryonic lethality and a severe Him (high incidence of males) phenotype as a result of nondisjunction due to the absence of CO (Dernburg *et al.* 1998). The RAD-51 family of recombinases catalyzes the strand-invasion and strand-exchange reactions, resulting in products that have either exchanged flanking DNA arms (crossovers) or have not undergone exchange (non-crossovers).

The presence of one cross over event reduces the probability of a second event occurring nearby, termed crossover interference (COI) (reviewed in Zetka 2009). One advantage of studying cross over events in *C. elegans* is that there is only one cross over event between homologous chromosomes per meiosis, demonstrating complete COI (Brenner 1974, Hillers and Villeneuve 2003). The absence of double crossovers facilitates the study of the frequency and distribution of exchange events. While the

mechanism underlying COI is unclear, recent analysis by Youds *et al.* (2010) has shown that the anti-recombinase RTEL-1 promotes non-crossover events, and in the Rtel-1 mutant DSB become crossover events due to a lack of COI (Barber *et al.* 2008, Youds *et al.* 2010).

Another striking aspect of meiotic recombination in *C. elegans* is the meiotic pattern. The meiotic pattern is the cumulative effect of crossing over along the chromosome. The nematode exhibits a clear meiotic pattern as the central clusters of autosomes have fewer crossovers per unit DNA than the flanking arm regions which are highly recombinogenic (Brenner 1974). Thus, the probability of a recombination event is more likely in some genomic regions (genetic hotspots) than in others (genetic coldspots), a phenomena conserved across most species.

Many of the molecular mechanisms underlying meiotic recombination are well known, while the factors determining the distribution of CO are less clear. In *C. elegans*, a mutant known to alter CO distribution exists, Rec-1 (Rose and Baillie 1979a Zetka and Rose 1995).

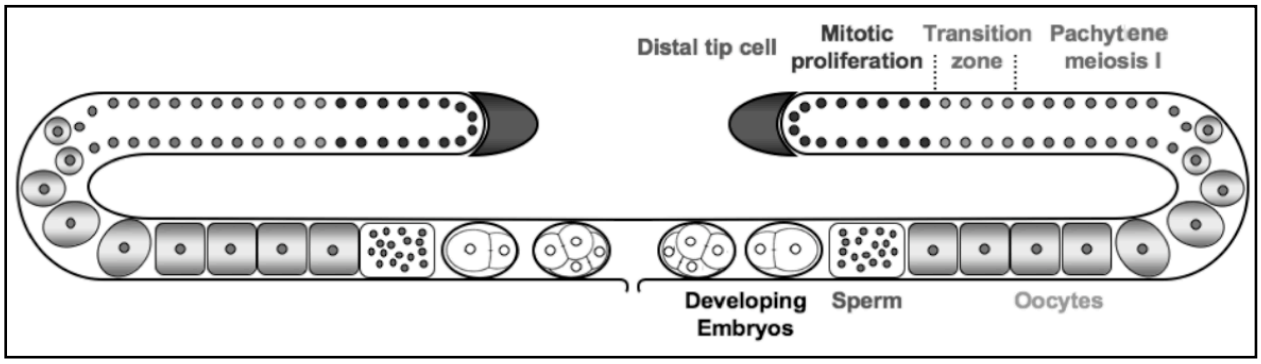

Figure 1. Schematic representation of the *C. elegans* adult hermaphrodite gonad and germ line.

The *C. elegans* gonad consists of two mirrored arms. A signal from the somatic gonadal distal tip cell keeps most distal germ line nuclei in mitosis and nuclei that migrate to the proximal region initiate meiosis, cellularlize and differentiate into oocytes. (Adapted from Minasaki *et al.* 2009)

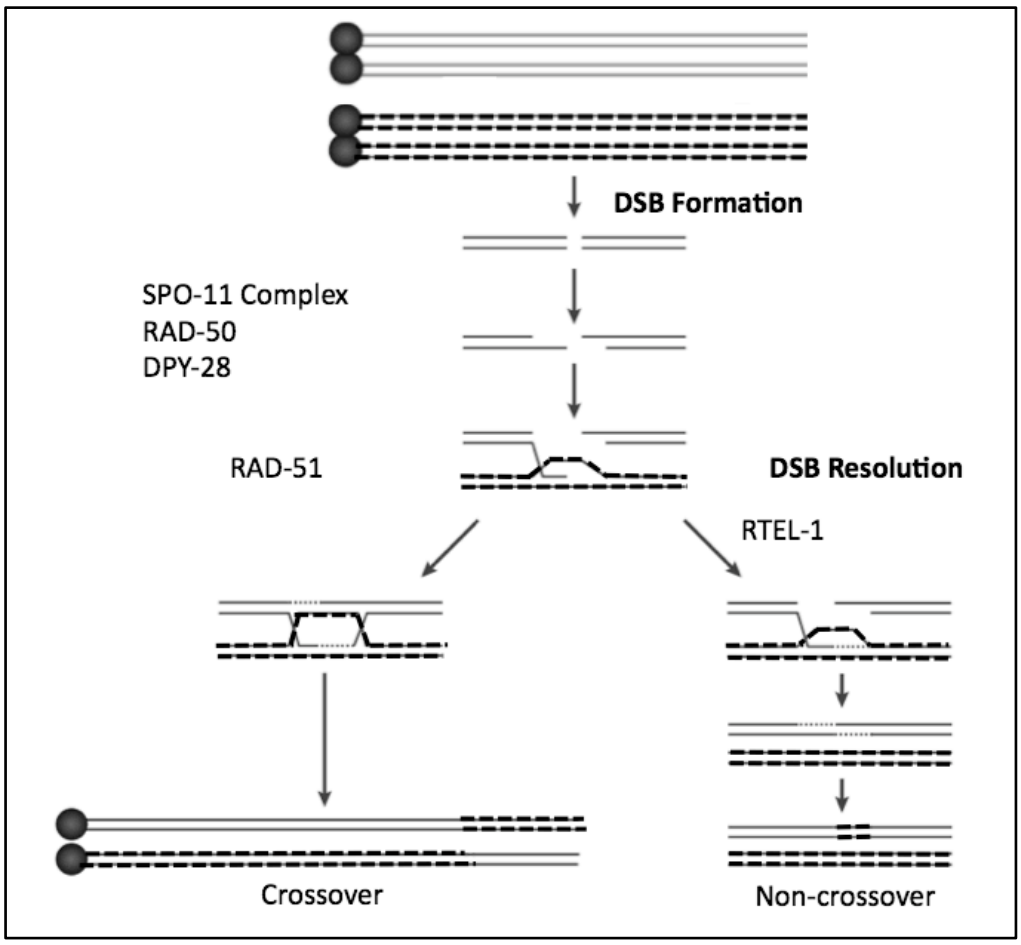

Figure 2. An overview of recombination pathways where DSB can either form a crossover product or a non-crossover product.

Two homologous unpaired chromosomes are shown at the top and the remainder of the figure focuses on segments of sister chromatids. Proteins that are known to contribute to double strand breaks (DSB) formation and DSB resolution are indicated. (Adapted from Handel and Schimenti 2010)

1.3 Rec-1 randomizes crossover distribution

First identified as a recessive mutation in a group of ethylmethane sulfonate treated strains, Rec-1 (abnormal recombination) disrupts the distribution of crossovers along the chromosome. Interestingly, Rec-1 alters the location of crossover events without affecting the total number, causing no other observable phenotypic effect (Rose and Baillie 1979a, Zetka and Rose 1995, Rose *et al.* 2010) (Figure 3). The genetic map in Rec-1 is more similar to the physical map than the wild type genetic map. While the molecular function of *rec-1* is unknown, the mutant provides a valuable opportunity to study aspects of crossover distribution.

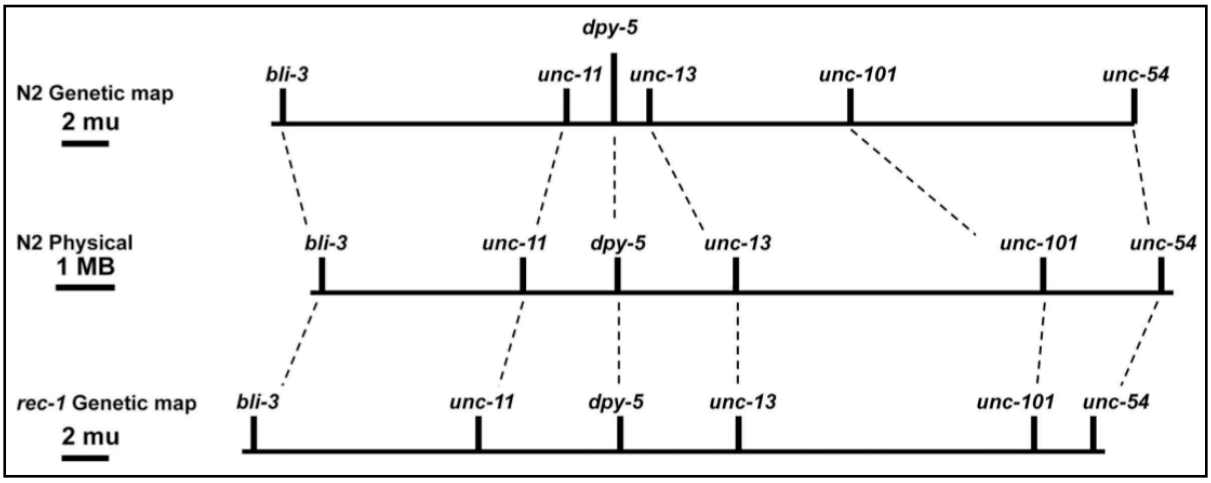

Figure 3. Comparison of the genetic map and the physical map in the *rec-1* mutant background.

The top line depicts the wild-type (N2) genetic map of autosome I as measured by Zetka and Rose (1995). The second line shows the position of the gene markers on the physical map as annotated in WormBase (http://www.wormbase.org). The bottom line is the position of gene markers in the Rec-1 mutant. The Rec-1 genetic map is more similar to the physical map than the wild-type genetic map. This indicates that crossover events are not randomly distributed along the chromosome as there are more crossover events in the chromosomal arms compared to the cluster. (Adapted from Rose *et al.* 2010)

1.4 The role of chromatin structure and chromatin remodeling during meiotic recombination

Gross chromosome structure has been linked to DSB formation and meiotic recombination. In *Drosophila melanogaster*, there are structure differences in the synaptonemal complex in euchromatin compared to heterochromatin, which lead to the absence of crossing over events in heterochromatin (Carpenter 1975). While *Drosophila* represents an extreme case, Murakami *et al.* (2003) have shown that in yeast, prior to DSB formation, chromosomes become more sensitive to micrococcal nuclease, an indicator of open chromatin. However, nuclease sensitivity is not required for recombination because deoxyribonuclease I hypersensitive sites were identified in one mouse hotspot, *Eβ1*, but not in another, *Psmb9* (Mizuno *et al.* 1996, Shenkar *et al.* 1991). Recently, Mets and Meyer (2009) showed that partial loss of the *C. elegans* dosage compensation condensin, DPY-28, results in extended chromosome axes and far more or far less RAD-51 foci than wild type worms, implicating gross chromosome structure in the formation of DSB. These pieces of data demonstrate that large-scale chromosome structure promotes recombination events in certain regions of the chromosome and suppresses these events in others; however, it is clear that additional factors are also required to facilitate recombination.

In addition to gross chromosome structure, chromatin structure influences recombination at a local scale. Chromatin structure has been documented to play a role in transcriptional control, DNA replication, repair and more recently, meiotic recombination (reviewed by Hirota *et al.* 2009, Cayrou *et al.* 2010, Szekvolgyi and

Nicolas 2010). In yeast, histone acetyltransferases open chromatin to activate transcription, and loss of this activation reduces both transcription and recombination activity at *ade-M26* and *HIS4* recombination hotspots (Yamada *et al.* 2004, Merker *et al.* 2008). In addition to acetylation, open chromatin is marked by H3K4me3, which has been associated with DSB in yeast (Kniewel and Keeney 2009). Loss of function of SET1, the only histone H3K4 methyltransferase in yeast, severely reduces DSB formation in 84% of recombination hotspots (Sollier *et al.* 2004, Borde *et al.* 2009, Kolasinska-Zwierz *et al.* 2009). In the mouse, PRDM9 was recently identified as encoding a histone methyltransferase which methylates H3K4 at recombination hotspots (Baudat *et al.* 2009, Myers *et al.* 2009). Interestingly, in spermatocytes lacking functional PRDM9, gametogenesis is disrupted at the pachytene stage (Hayashi *et al.* 2005). Taken together, this data shows that DSB formation and recombination events are influenced by open chromatin, presumably through increased access to DNA for SPO-11.

To illustrate how closed chromatin rather than open chromatin has been associated with meiotic recombination, Reddy and Villeneuve (2004) describe the case of *him-17.* In *C. elegans,* the Him-17 mutant showed reduced histone three lysine nine mono-methylation (H3K9me), less of the DNA strand exchange protein, RAD-51, and fewer DSB. H3K9me marks heterochromatin and transcriptionally inactive DNA. The authors propose that a certain degree of chromosomal compaction in one area of the chromatin may result in compensatory loosening in another area, suggesting that a variable chromatin environment facilitates meiotic recombination. In any case, it is clear that proper meiotic recombination requires both a certain chromosomal and chromatin configuration.

Leger (2007) examined chromatin modifier mutants, including Him-17, and their effect on meiotic recombination in *C. elegans*. Mutation in *him-17* caused an increase in recombination in a chromosomal hybrid region while *rec-1* mutation caused a decrease. Thus, mutation in Him-17 failed to recapitulate the Rec-1 pattern.

Another gene analyzed by Leger (2007) encodes the tumour suppressor LIN-35 (abnormal cell lineage). Mutation in *lin-35* caused an increase in recombination in a chromosome cluster and a decrease in an arm, a similar effect to *rec-1* mutation. Since HIM-17 shares structural properties with LIN-35, Reddy and Villeneuve (2004) constructed the double mutant and analyzed RAD-51 foci staining along the germline axis. Although the role of *lin-35* in meiotic recombination was not investigated, the Him-17 Lin-35 double mutant showed a reduction in foci compared to wild-type and the Him-17 single mutant, suggesting fewer crossing over events in the double mutant. These are the first pieces of evidence suggesting that *lin-35* plays a role during meiotic recombination.

1.5 The tumour suppressor and chromatin modifier lin-35

Mutation in the Rb gene was identified in malignant tumours of the retina. Rb mutation is now recognized as one of the most common events preceding the onset of tumourigenesis in humans (Dunn *et al.* 1988, reviewed by Sherr and McCormick 2002, Giacinti and Giordano 2006). In *C. elegans,* LIN-35 is an ortholog of the Rb tumour suppressor family. LIN-35 has been well-studied with respect to several cellular processes including gene transcription, mRNA stability, cellular proliferation, cell cycle regulation, soma germline transformation and apoptosis (reviewed by Kirienko *et al.* 2010).

pRb family members not only prevent tumorigenesis but also regulate the general organization of chromosomes. Loss of pRb in mouse adult fibroblasts increased mobility of heterochromatin protein 1, and the authors suggested that pRb plays a role in formation of compact chromatin (Siddiqui et al. 2007). In Drosophila neuroblasts, loss of the pRB homolog exhibits fused and broken chromosomes. These pieces of evidence suggest a role for Rb in maintaining gross chromosome structure (Longworth and Dyson 2010).

While pRb family members have been shown to influence chromosome structure, LIN-35 has been shown to be a chromatin modifier in *C. elegans*. As a member of the DRM (Db, Rb and Muv genes) complex, LIN-35 binds to the transactivation domain of E2F transcription factors that bind to gene promoters to regulate gene transcription (Harrison *et al.* 2006). The DRM complex recruits other complexes to gene promoters such as SWI/SNF (switch/sucrose nonfermentable) and NuRD (nucleosome remodeling and deacetylase complex), which are involved with chromatin remodeling and histone deacetylation respectively (Sawa 2000, Cui *et al.* 2004). Affecting chromatin structure causes an indirect effect on gene transcription. When *lin-35* is non-functional, 535 genes are up-regulated and 175 are down-regulated, including several meiotic genes (such as *syp-1, htp-1, him-3, rad-51)* (Grishock *et al.* 2008). Altered transcription of indirect targets may provide a means for *lin-35* mutation to influence recombination. Thus, *lin-35* may influence recombination in two ways: directly through chromatin structure or indirectly through gene transcription.

1.6 lin-35 (n745)

Since first identified in a screen for genes involved with vulval development, the *n745* allele of *lin-35* has been used in numerous experiments (Ferguson and Horvitz 1989). The *n745* allele causes a single nucleotide change, "TGG" in wild type to "TGA" in the mutant, causing an opal stop in the fourth codon of the gene (Lu and Horvitz 1998). For this reason, *n745* is considered a null allele. However, Ouellet and Roy (2007) showed mRNA levels were similar to wild type in the allele, *rr33*, which introduces an amber stop codon in the same position as *n745*. The authors hypothesized that readthrough resulted in this mRNA stabilization. Previous investigators have interpreted the *n745* allele as a null allele, although definitive proof has not been obtained.

1.7 Thesis objectives

Chromatin structure has been long proposed to play a role during meiotic recombination. However, the role of chromatin structure in establishment of the meiotic pattern is unclear in *C. elegans*. To initiate this investigation, this thesis aims to determine whether or not the chromatin modifier, Lin-35, affects meiotic recombination. Using pairs of linked markers (Dpy and Unc mutants), the frequency and distribution of crossing over will be measured along the entire length of a chromosome. In this way, information will be gained regarding whether or not mutation in the *lin-35* influences recombination, providing a starting point for understanding its functional role in the recombination process.

CHAPTER 2: MATERIALS AND METHODS

2.1 General methods

Strains were obtained from the *C. elegans* Genetics Center at the University of Minnesota. The following alleles were used (shown left to right): LGI, *rec-1(s180), dpy-5(e61), lin-35(n745), unc-13(e51);* LGIII*, unc-45(e286), dpy-1(e1), dpy-17(e164), unc-32(e189), dpy-18(e364), unc-64(e246);* LGV*, dpy-11(e224), unc-42(e270);* LGX, *hda-4(ok518)*. The chromosomal rearrangement, *hT2 [bli-4]*, was employed in this study as a genetic balancer. *hT2* is a reciprocal translocation that balances left LGI through *unc-101* and right LG III through *unc-59* (McKim *et al.* 1993). Refer to Appendix I for a complete listing of the strains used (Table 10, Table 11).

All strains were maintained and mated at 20 Degrees Celsius (°C) on petri dishes containing nematode growth medium (NGM) streaked with *Escherichia coli* strain OP50 (Brenner 1974). The CB286 strain, *unc-45 (e286)*, is temperature sensitive and was maintained at 15 °C. Worms were visualized on a M38 dissecting microscope.

Males were generated by incubating L4 hermaphrodites at 30°C for 5 to 6 hours. At this temperature, hermaphrodites produce male progeny more frequently than at 20°C because there is an increase in X-chromosome nondisjunction with an increase in temperature. Hda-4 and Lin-35 Rec-1 animals produced males at 20°C so the aforementioned step was unnecessary for these strains.

2.2 Strain construction

In order to measure the recombination frequency between various markers in mutant backgrounds, several strains were constructed (Table 11). Dpy and Unc mutants were followed with their visible phenotypes while Lin-35, Rec-1 and Hda-4 required further analysis.

lin-35 has been mapped to +0.46 cM on LGI (WormBase). The *n745 lin-35* allele causes a base pair change that does not introduce a restriction enzyme digestion site, so it cannot be followed with PCR. Rather, quantitative brood size analysis and *hT2::GFP* were used to follow the non-green homozygous Lin-35 mutant. Since *hT2* balances the genomic region on Chromosome I where *lin-35* has been mapped, it served as an effective means to assure a homozygous Lin-35 mutant. In some cases when *hT2* could not be used, brood size analysis distinguished strains because homozygous Lin-35 mutant animals have significantly fewer progeny than wild-type or heterozygous Lin-35 mutants.

A molecular marker was used to follow Rec-1. PCR products were tested by running 5 μl of PCR product on a 1% agarose gel stained with ethidium bromide to distinguish between wild-type, Rec-1 homozygous and heterozygous mutants. Scoring recombination confirmed the Rec-1 phenotype.

hda-4 has been mapped to +24.06 on the X Chromosome (WormBase). The *hda-4* (*ok518)* allele is a 1090 bp deletion mutant, which was tracked through PCR products (Table 1). The same PCR method was applied to *hda-4* mutants as the *rec-1* mutants.

Table 1. Primers used to amplify mutant strains.

Primer Name	Annealing Temperature	Primer Sequence (5' to 3')
hda-4(ok518)		
ZL7	60°C	cgt tag cat ggg atc tca cc
ZL8	60°C	tgc taa ggg atc agc aaa cc
ZL9	60°C	ttg att tag gtt gcc gaa gg

2.3 Crosses

Hermaphrodites have not yet produced oocytes at the L4 larval stage and the vulva has not yet developed (Schedl 1997, Greenwald 1997). Thus, L4 hermaphrodites are selected at this stage because they have neither mated nor self-fertilized. Matings were set up with 8-10 males and 2-3 hermaphrodites. To note, crosses homozygous for the double mutant Lin-35 Rec-1 included 8-10 males and 8-10 hermaphrodites due to the reduced viability of this double mutant. Large numbers of males in the first filial generation (F1) indicates a successful mating. F1 hermaphrodites were picked to individual plates and allowed to self-fertilize to produce the F2 generation. The F2 generation was scored for most crosses.

2.4 Calculating recombination frequency

Recombination frequency in the hermaphrodite was measured at 20°C by scoring the number of recombinant progeny from hermaphrodites that were cis-heterozygous for selected visible markers (Rose and Baillie 1979b). Recombination frequency (p) was calculated according to the following formula where $p = 1 - (1 - 2R)^{1/2}$ (Brenner 1974). R is the total number of recombinant progeny divided by the total number of progeny. Recombinants were segregated on individual plates to ensure that the phenotype had been correctly identified. Both classes of recombinants were used in all calculations. For strains constructed during this thesis, multiple isolates were scored in some cases to further validate the data. Refer to appendix I for the scoring results of individual isolate strains (Table 12). Data represented in results is pooled from the isolates.

Confidence intervals (95% CI) were calculated using the statistics of Crow and Gardner (1959) where fewer than 300 recombinants were observed. When more than 300 recombinants were scored, the CI was calculated with the following formula where $n \pm 1.96\ (nxy)^{1/2}$ where *n* is the number of recombinants, x is the number of recombinants divided by the number of recombinants plus wild-type progeny, and y is 1-x.

2.5 Brood analysis

To characterize distinguishing features, brood analysis was performed. Hermaphrodites were picked at the L4 stage to individual plates and allowed to self-fertilize. The F1 progeny were counted to determine brood size and examined for distinguishing features such as the protruding vulva phenotype (Pvl). Data represents mean values from ten broods of each strain and error represents one standard deviation from the mean.

2.6 RNAi

To phenocopy the results of the brood analysis, RNA-mediated interference (RNAi) was employed. RNAi constructs were obtained by the RNAi feeding library of Kamath *et al.* (2003). Bacteria expressing double stranded RNA from *lin-35* was administered by feeding as described by Fraser *et al.* (2000). Worms were transferred to fresh RNAi plates each day for three consecutive days and scored for features after five days. Ten wild-type hermaphrodites and ten Rec-1 mutants were subjected to *lin-35* (RNAi) by feeding. Data includes mean values from ten broods of the strain.

CHAPTER 3: RESULTS

3.1 Mutant LIN-35 affects recombination frequency in a chromosome cluster and a chromosome arm

The first question addressed was whether or not mutation in *lin-35* would alter the frequency of crossing over in recombinantly suppressed or enhanced regions. To do this, the recombination frequency (*p*) within two genomic intervals was analyzed: *dpy-11 unc-42* in the cluster region of Chromosome V and *dpy-18 unc-64* in the arm region of Chromosome III (Table 2).

The strain mT10430 *lin-35(n745)* was used and compared to the VC2010 N2 wild-type strain. The genetic distance between *dpy-11* and *unc-42* (LGV) in wild type was 1.7±0.5 map units (m.u.) and 5.4±1.1 m.u. in Lin-35. The genetic distance between *dpy-18* and *unc-64* (LGIII) in wild type was 9.9±0.3 m.u. and 6.5±1.4 m.u. in Lin-35. Lin-35 caused an increase in crossing over in the cluster of one chromosome and a decrease in the arm of another.

Table 2. Crossing over in a Lin-35 mutant strain in the cluster region of Chromosome V and flanking arm region of Chromosome III.

		Recombinants				
Genotype	**wild-type progeny[a]**	**Dpy**	**Unc**	**Total**	***p* x 100**	**95% C.I.[b]**
dpy-11 unc-42/++	2320	26	26	52	1.69	1.24-2.18
dpy-11 unc-42/++; lin-35/lin-35	1554	65	44	109	5.38	4.51-6.67
dpy-18 unc-64/+ +	2972	212	194	406	9.91	9.56-10.10
dpy-18 unc-64/+ +; lin-35/lin-35	1079	32	61	93	6.50	5.42-8.23

[a] Male progeny included.
[b] C.I. = 95% confidence interval; See Materials and Methods.

3.2 *Like Rec-1, Lin-35 increases chances of CO in the central cluster of Chromosome III*

The initial result for Lin-35 is similar to previous analysis of the Rec-1 phenotype. To investigate this further, crossing over within three genomic intervals was analyzed in the Rec-1 mutant: *dpy-5 unc-13* in the cluster region of Chromosome I, *dpy-11 unc-42* in the cluster region of Chromosome V, and *dpy-18 unc-64* in the arm region of Chromosome III (Table 3). In both cluster intervals, Rec-1 increased crossing over more than three fold in; *dpy-5 unc-13:* 1.8±0.4 m.u. (*wild type)*, 6.6±1.0 m.u. (Rec-1) and *dpy-11 unc-42:* 1.7±0.5 m.u. (*wild type*), 7.1±1.1 m.u. (Rec-1). Within the arm, recombination was reduced in the Rec-1 mutant compared to wild type; *dpy-18 unc-64:* 9.9±0.3 m.u. (wild type), 6.5±0.8 m.u. (Rec-1). I observed the published result for the LGV cluster (7.6 m.u. in Rec-1 compared to 2.7 m.u. in wild type) (Zetka and Rose 1995) and observed the expected decrease in the arm of LGIII, a region not previously investigated.

Table 3. Crossing over in a Rec-1 mutant strain in the cluster region of Chromosome I and Chromosome V and flanking arm region of Chromosome III.

		Recombinants				
Genotype	**wild-type progeny[a]**	**Dpy**	**Unc**	**Total**	***p* x 100**	**95% C.I.[b]**
dpy-5 unc-13/++	4142	51	48	99	1.79	1.46-2.20
dpy-5 unc-13/++; rec-1/rec-1	2086	84	95	179	6.64	5.65-7.66
dpy-11 unc-42/++	2320	26	26	52	1.69	1.24-2.18
dpy-11 unc-42/++; rec-1/rec1	2085	97	100	197	7.11	6.08-8.19
dpy-18 unc-64/+ +	2972	212	194	406	9.91	9.56-10.10
dpy-18 unc-64/+ +; rec-1/rec-1	3215	119	170	289	6.53	5.98-7.58

[a] Male progeny included.
[b] C.I. = 95% confidence interval; See Materials and Methods.

3.3 Lin-35 and Rec-1 have similar meiotic phenotypes, which complement in trans

Results thus far have shown that mutations in Lin-35 and Rec-1 display similar meiotic phenotypes compared to wild type, that is, there is an increase in crossing over in the chromosome cluster and a decrease in crossing over in the chromosome arm. Both *lin-35* and *rec-1* map to LG I. Lu and Horvitz (1998) mapped *lin-35* to the cluster of LGI between *dpy-5* and *unc-13*. Although previous strain constructions eliminated the *dpy-5 unc-13* interval as a position for the *rec-1* gene, I constructed the trans-heterozygote and examined the phenotype (Baillie and Rose 1979, Zetka and Rose 1995). *lin-35(n745)* complements *rec-1(sI80)* (Table 4), indicating that *lin-35* and *rec-1* are not allelic and that there is no dominant interaction.

Table 4. Complementation test data for Lin-35 and Rec-1 mutant strains in the cluster of Chromosome I.

		Recombinants				
Genotype	**wild-type progeny[a]**	**Dpy**	**Unc**	**Total**	***p* x 100**	**95% C.I.[b]**
dpy-5 unc-13/++	4142	51	48	99	1.79	1.46-2.20
dpy-5 unc-13 rec-1/++rec-1	1792	74	85	159	7.01	5.89-8.15
dpy-5 unc-13 rec-1+/+++lin-35	1918	20	26	46	1.86	1.37-2.43

[a] Male progeny included.
[b] C.I. = 95% confidence interval; See Materials and Methods.

3.4 Crossing over analysis in the double mutant, **Lin-35 Rec-1,** *indicates that* **LIN-35** *and* **REC-1** *function non redundantly*

To analyze the relationship between *lin-35* and *rec-1*, I constructed a Lin-35 Rec-1 double mutant.

Between *dpy-11* and *unc-42* of LGV, the genetic map distance in the double mutant was 2.1±0.4 m.u. compared to 1.7±0.5 m.u. in wild type, 7.1±1.0 m.u. in the Rec-1 mutant, and 5.4±1.1 m.u. in the Lin-35 mutant (Table 5, Figure 5). The double mutant recombination frequency was less than the single mutants and similar to wild type.

To observe an arm interval, the *dpy-18 unc-64* arm interval on Chromosome III was scored. The recombination frequency of the double mutant was lower than both wild type and the single mutants (wild type: 9.9±0.3 m.u., Lin-35: 6.5±0.9 m.u., Rec-1: 6.5±0.8 m.u., Lin-35 Rec-1: 4.6±1.5 m.u.) (Table 5, Figure 5). Clearly, in this case the wild-type distance was not restored. Nor was the distance the same as the single mutants, as it was reduced. Within both the cluster and arm intervals, the recombination frequency of the double mutant was less than the single mutants.

Table 5. Crossing over in Lin-35 and Rec-1 mutant strains in the cluster of Chromosome V and the arm of Chromosome III.

Genotype	wild-type progeny[a]	Recombinants			*p* x 100	95% C.I.[b]
		Dpy	Unc	Total		
dpy-11 unc-42/++	2320	26	26	52	1.69	1.24-2.18
dpy-11 unc-42/++; rec-1/rec1	1867	82	89	171	6.86	5.78-7.85
dpy-11 unc-42/++; lin-35/lin-35	1554	65	44	109	5.38	4.51-6.67
dpy-11 unc-42/++; lin-35 rec-1/lin-35 rec-1	3321	44	47	91	2.07	1.67-2.54
dpy-18 unc-64/+ +	2972	212	194	406	9.91	9.56-10.10
dpy-18 unc-64/+ +; rec-1/rec-1	3215	119	170	289	6.53	5.98-7.58
dpy-18 unc-64/+ +; lin-35/lin-35	1079	32	61	93	6.50	5.42-8.23
dpy-18 unc-64/+ +; lin-35 rec-1/lin-35 rec-1	634	19	23	42	4.58	3.33-6.24

[a] Male progeny included.
[b] C.I. = 95% confidence interval; See Materials and Methods.

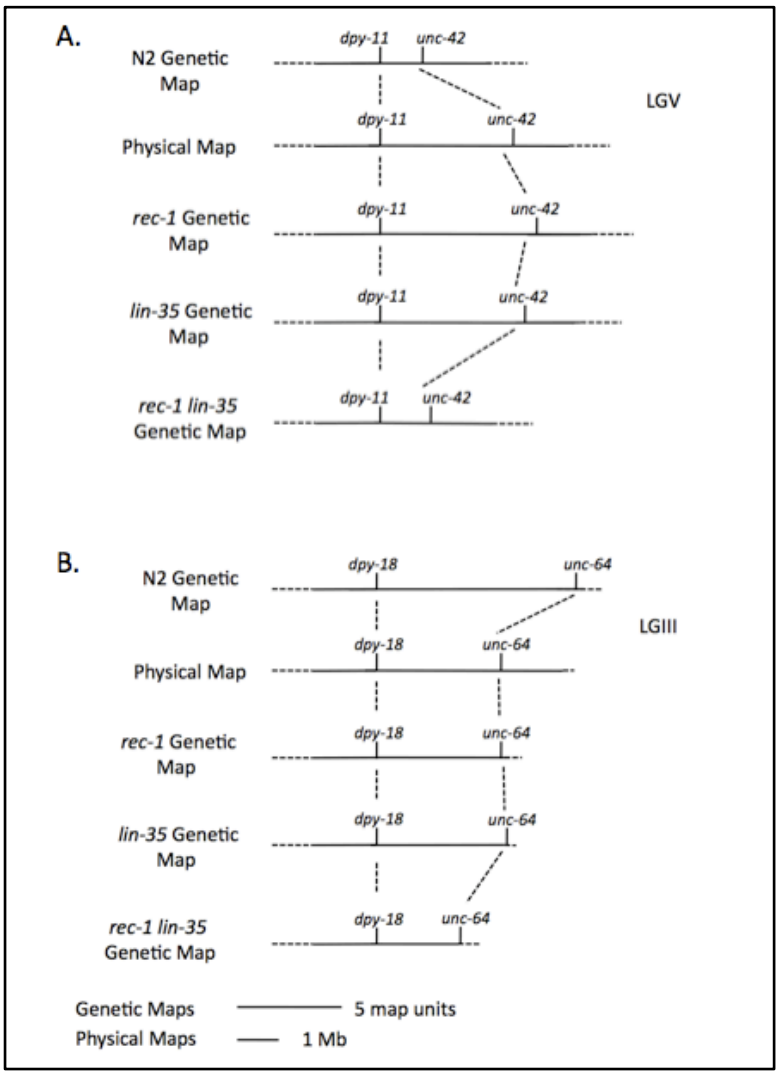

Figure 4. Genetic maps and physical maps for the cluster region of Chromosome V and the arm region of Chromosome III.

A. The top portion of the figure depicts the cluster or Chromosome V between the markers *dpy-11* and *unc-42* **B**. The bottom portion depicts the right arm of Chromosome 3 between *dpy-18* and *unc-64*.

The position of the gene markers on the physical map is shown for each interval for wild-type (N2) worms as annotated by WormBase (http://www.wormbase.org). The genetic map of Lin-35 and Rec-1 mutants is more similar to the physical map than the genetic map.

3.5 Mutation in lin-35 reduces recombination across a whole chromosome

To further analyze the relationship between Lin-35 mutation and meiotic recombination, the entire length of Chromosome III (LGIII) was analyzed. The chromosome was divided into five sections comprising of the chromosome arms (*dpy-1 unc-45, dpy-18 unc-64)*, cluster (*dpy-17 unc-32)* and two halves (*dpy-17 unc-45, dpy-17 unc-64)* (Figure 6, Table 6, Figure 7). Determining the genetic length of these segments serves to test previous findings, in addition to determining effects along an entire chromosome.

The genetic distance within the cluster and arm intervals in the Lin-35 mutant was similar to intervals previously analyzed. Within the cluster of LGIII between *dpy-17* and *unc-32*, the genetic distance in Lin-35 was 3.4±0.8 m.u., higher than wild type worms (1.9±0.6 m.u.). Rec-1 exhibited a more striking effect in this interval (5.1±0.94 m.u.). Within the left arm of LGIII between *dpy-1* and *unc-45*, wild type worms displayed a genetic distance of 11.1±0.4 m.u. while in Lin-35 mutants the distance was 6.4±1.2 m.u. and in Rec-1 mutants the distance was 5.6±0.9 m.u.. The right arm interval, *dpy-18 unc-64*, was described in a previous section (Table 5). These results confirmed previous observations that Lin-35 increased crossing over in chromosomal clusters and reduced it in the flanking arms.

To determine the genetic length of the whole chromosome, the two halves of Chromosome III were added together. In wild type, left half of LG III between *dpy-17* and *unc-45* is 22.3±0.9 m.u.. In Rec-1, this distance was unchanged (22.9±0.9 m.u.); since this interval included both a cluster which increases and an arm with reduces, this

result was expected (Zetka and Rose 1995). The Lin-35 mutant measured gave 21.0±1.0 m.u.. For the right half of Chromosome III, between *dpy-17* and *unc-64,* wild type and Rec-1 worms showed similar genetic distances (p = 26.3±1.0 m.u. and 24.8±0.9 m.u. respectively) while the Lin-35 mutant was 21.3±3.3 m.u.. Adding together the two halves gives a genetic length for the whole chromosome of 48.6 m.u. in wild type, 47.7 m.u. in Rec-1 and 42.3 m.u. in Lin-35. Thus, wild type and Rec-1 worms were close to the theoretical value of 50 m.u., whereas the Lin-35 mutant chromosome was shorter at 42.3 m.u.

The left and right halves of LGIII was investigated in the Lin-35 Rec-1 double mutant. Between *dpy-17* and *unc-64*, the double mutant displayed a genetic distance that was less (14.7±0.7 m.u.) than wild type (26.3±1.0 m.u.) and the single mutants (Rec-1: 24.8±0.95 m.u., Lin-35: 21.3±3.3 m.u.) (Table 6, Figure 7). Similarly, between *dpy-17* and *unc-45*, the double mutant displayed a genetic distance that was less (13.3±1.7 m.u.) than wild type (22.3±0.9 m.u.) and the single mutants (Rec-1: 22.9±0.9 m.u., Lin-35: 21.0±1.0 m.u.). Thus, the length of Chromosome III in the Lin-35 Rec-1 double mutant is severely truncated at 28.0 m.u.. These results show that the genetic length of Chromosome III is shorter than wild type in both Lin-35 single mutants and Lin-35 Rec-1 double mutants.

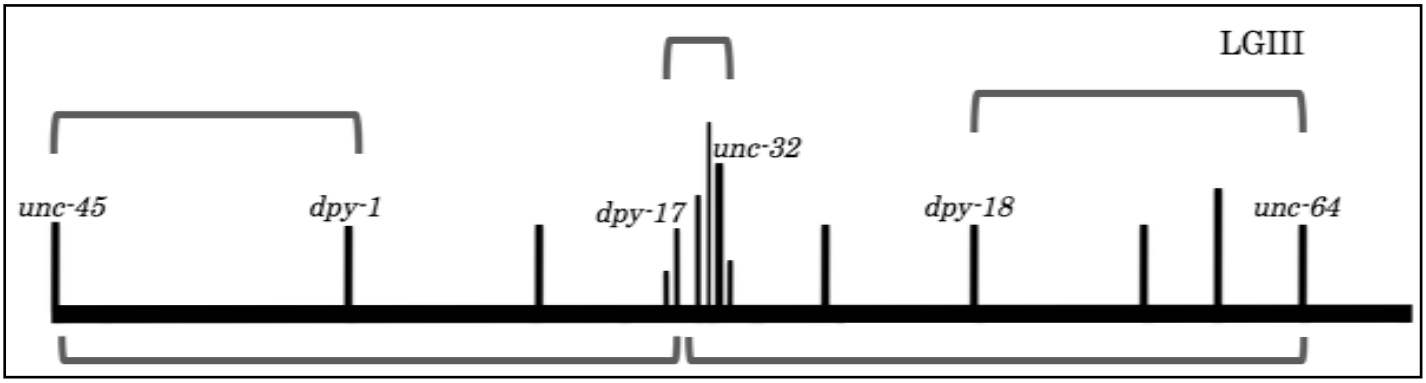

Figure 5. The standard genetic map of Chromosome III highlighting the five intervals examined in this study.

The genetic distance between various markers is shown (adapted from WormBase). The regions indicated are genetic intervals that were analyzed in this study: *unc-45 dpy-1* (left arm), *dpy-17 unc-32* (cluster), *dpy-18 unc-64* (right arm), *dpy-17 unc-45* (left half), *dpy-17 unc-64* (right half).

Table 6. Crossing over in Lin-35 and Rec-1 mutant strains along Chromosome III.

Genotype	wild-type progeny[a]	Recombinants			*p* x 100	95% C.I.[b]
		Dpy	Unc	Total		
dpy-18 unc-64/+ +	2972	212	194	406	9.9	9.56-10.1
dpy-18 unc-64/+ +; rec-1/rec-1	3215	119	170	289	6.5	5.98-7.58
dpy-18 unc-64/+ +; lin-35/lin-35	2112	71	90	161	5.93	5.01-5.92
dpy-18 unc-64/+ +; lin-35 rec-1/lin-35 rec-1	634	19	23	42	4.58	3.33-6.24
dpy-17 unc-32/+ +	2008	21	27	48	1.88	1.36-2.47
dpy-17 unc-32/+ +; rec-1/rec-1	2033	64	74	138	5.11	4.35-6.22
dpy-17 unc-32/+ +; lin-35/lin-35	2011	40	45	85	3.35	2.62-4.12
dpy-1 unc-45/+ +	2021	146	157	303	11.1	10.7-11.52
dpy-1 unc-45/+ +; rec-1/rec-1	2021	79	80	159	5.79	4.88-6.74
dpy-1 unc-45/+ +; lin-35/lin-35	1414	63	57	120	6.38	5.25-7.65
dpy-17 unc-45/+ +	2052	276	320	596	22.3	21.4-23.1
dpy-17 unc-45/+ +; rec-1/rec-1	2037	325	268	593	22.9	22.0-23.8
dpy-17 unc-45/+ +; lin-35/lin-35	1450	197	162	359	21.0	20.0-22.0
dpy-17 unc-45/+ +; lin-35 rec-1/lin-35 rec-1	1688	137	133	270	13.3	11.6-15.0
dpy-17 unc-64/+ +	2035	379	338	717	26.3	25.3-27.3
dpy-17 unc-64/+ +; rec-1/rec-1	2004	290	373	663	24.8	23.9-25.8
dpy-17 unc-64/+ +; lin-35/lin-35	702	109	101	210	21.3	18.5-25.1
dpy-17 unc-64/+ +; lin-35 rec-1/lin-35 rec-1	2083	186	192	378	14.7	14.0-15.3

[a] Male progeny included.
[b] C.I. = 95% confidence interval; See Materials and Methods.

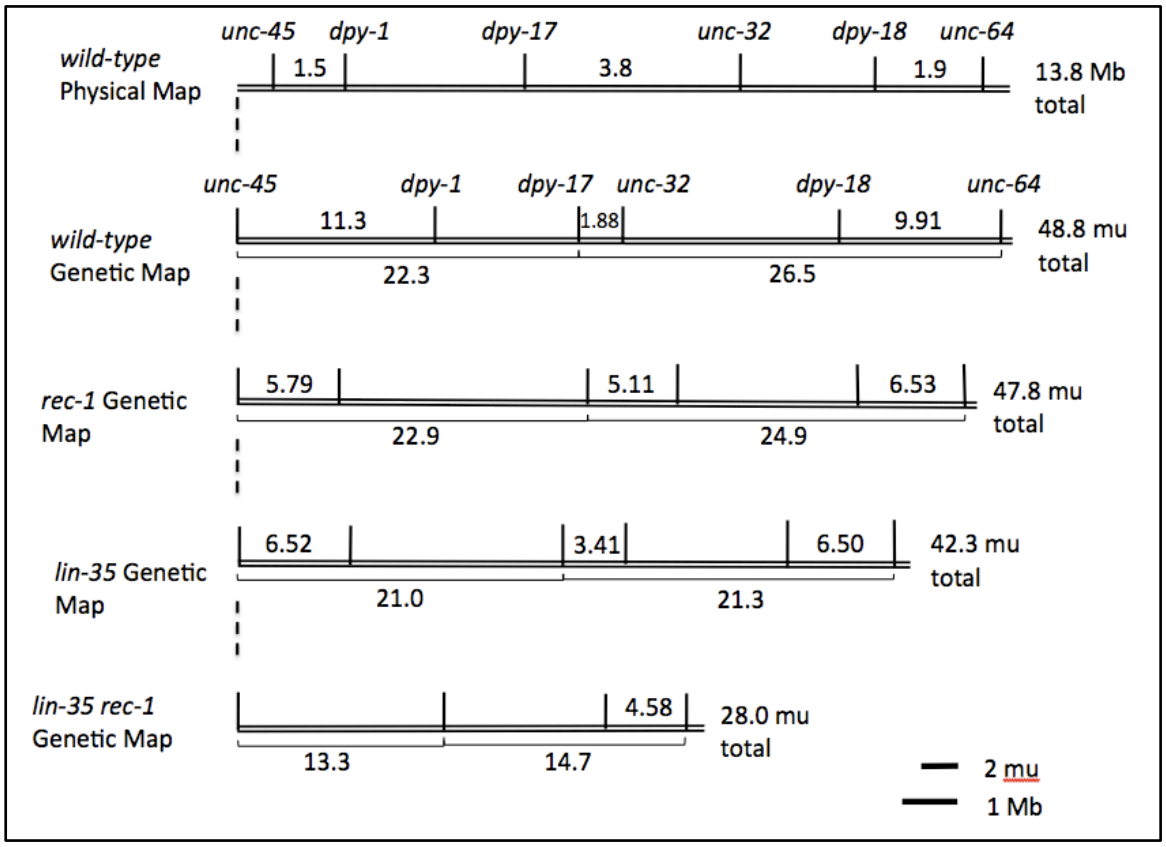

Figure 6. The genetic map of Chromosome III in Rec-1 and Lin-35 mutant backgrounds.

The five markers (*unc-45, dpy-1, dpy-17, unc-32, dpy-18, unc-64*) are labeled on the wild-type chromosome in both the physical and genetic map (top). Genetic distances between markers (map units, m.u.) are shown where measured in the genetic maps. The total genetic length of the chromosome was determined by adding the two halves together.

3.6 The double mutant, **Lin-35 Rec-1***, has a more severe morphological phenotype than the single mutants*

In constructing the double mutant, Lin-35 Rec-1, somatic and meiotic characteristics were scored. The progeny of ten hermaphrodite worms were counted and analyzed for morphological changes, viability and fertility. The double mutant progeny displayed protruding vulvae (Pvl), everted vulvae (Evl), reduced fertility and arrested embryonic progeny (Table 7). These features were phenocopied when RNAi was directed against *lin-35* (Table 7). To note, the standard deviation included for each piece of data is higher for some values compared to others, indicating phenotypic variation. Embryonic viability was reduced in the double mutant. Clearly, the Lin-35 Rec-1 double mutant exhibits a more severe phenotype than the single mutants.

Table 7. Mutant Lin-35 and Rec-1 progeny analysis.

Genotype	Brood Size[1]	%Unhatched Eggs	% Vulval Defects (Pvl, Evl)[2]	% Male
+[3]	253±25	0.1±0.2%	0	0.2±0.4%
rec-1[3]	234±48	0.2±0.2%	0	0.1±0.2%
lin-35[3]	115±29	0.8±3.3%	0	0.4±0.5%
lin-35 rec-1[3]	101±28	3.4±9%	14.3±1.5%	1.5±0.6%
+ ; lin-35 (RNAi)[4]	194±39	0	0	0
rec-1; lin-35 (RNAi)[4]	184±42	0	7.6±3.8%	0

[1]Brood size excludes unfertilized oocytes.
[2] Pvl, protruding vulva phenotype; Evl, everted vulva phenotype.
[3] Mutant alleles were employed to observe *rec-1* and *lin-35* mutants. Data includes mean values from ten broods of each strain.
[4] Lin-35 and Rec-1 mutants were observed through interference RNA (RNAi). Ten wild-type hermaphrodites and ten Rec-1 mutants were subjected to *lin-35(RNAi)* by feeding. Data includes mean values from ten broods of the strain.
Error represents one standard deviation from the mean.

3.7 Mutation in the histone deacetylase, Had-4, does not influence crossing over

Histone acetyltransferases (HAT) modify chromatin to activate transcription (Struhl 1998,Yamada *et al.* 2004). In yeast, histones near recombination hotspots are often hyperacetylated by HAT, and suppression of HAT reduces recombination activity (Krebs *et al.* 1999, Vogeleuer *et al.* 2000). Recently, Petes *et al.* (2007) showed that mutation in the histone deacetylase, SIR-2, affected the distribution of Spo-11 induced DSB in yeast. In *C. elegans*, there are at least four genes encoding histone deaceytlases. One of these, *hda-4,* results in a viable null mutant, facilitating the scoring of recombination. HDA-4 regulates chemoreceptor gene expression and deacetylates histones. It is proposed to function independently of the LIN-35 pathway (Choi *et al.* 2002).

The recombination frequency resulting from mutation in *hda-4(ok518)* was analyzed for three genomic intervals: *dpy-5 unc-13* in the cluster of Chromosome I, *dpy-11 unc-42* in the cluster of Chromosome V, and *dpy-18 unc-64* in the arm of Chromosome III (Table 9). Within all three intervals examined, the recombination frequency of Hda-4 mutants did not deviate significantly from wild type; *dpy-5 unc-13*: 1.8±0.4 (wild type) and 1.5±0.3 (*hda-4*); *dpy-11 unc-42*: 1.7±0.5 (wild type) and 2.0±0.4 (*hda-4)*; *dpy-18 unc-64*: 9.9±0.3 (wild type) and 9.9±1.3 (*hda-4*). These results indicate that *hda-4* does not play a role in meiotic recombination.

Table 8. Crossing over in a Hda-4 mutant strain in the *dpy-5 unc-13* region of Chromosome I, the *dpy-11 unc-42* region of Chromosome V, and the *dpy-18 unc-64* region of Chromosome III.

		Recombinants				
Genotype	**wild-type progeny[a]**	**Dpy**	**Unc**	**Total**	**p x 100**	**95% C.I.[b]**
dpy-5 unc-13/+ +	4142	51	48	99	1.79	1.46-2.20
dpy-5 unc-13/+ +; hda-4/hda-4	5949	60	54	114	1.50	1.24-1.79
dpy-11 unc-42/+ +	2320	26	26	52	1.69	1.24-2.18
dpy-11 unc-42/+ +; hda-4/hda-4	4712	61	64	125	2.01	1.67-2.38
dpy-18 unc-64/+ +	2972	212	194	406	9.91	9.56-10.1
dpy-18 unc-64/+ +; hda-4/hda-4	2086	125	140	265	9.88	8.69-11.2

[a] Male progeny included.
[b] C.I. = 95% confidence interval; See Materials and Methods.

CHAPTER 4: DISCUSSION

In this thesis, I have shown a meiotic phenotype for Lin-35, and a synthetic interaction with Rec-1, a well-characterized meiotic mutant in *C. elegans.*

4.1 Lin-35 affects meiotic crossing over in C. elegans

A chromosome cluster (*dpy-11 unc-42*) and a chromosome arm (*dpy-18 unc-64*) were first analyzed because these intervals have been well studied previously and represent both recombinantly suppressed and enhanced regions. Within the cluster interval, Lin-35 mutation caused a three-fold increase in recombination (wild type: 1.7±0.5 m.u., *lin-35*: 5.4±1.1 m.u.) while the arm demonstrated an approximate 30% decrease (wild type: 9.9±0.3 m.u., Lin-35: 6.5±1.4 m.u.) (Table 2). Analysis of Rec-1 mutation yielded similar results to Lin-35 mutation (*dpy-11 unc-42*: 7.1±1.1 m.u., *dpy-18 unc-64:* 6.5±0.8 m.u.) (Table 3).

This initial data indicated that LIN-35 functions to alter the distribution of the crossover events along the chromosome. Similar findings were observed previously in the Rose Lab by M. Leger (Leger 2007). I have furthered this analysis by investigating the effect of Lin-35 on the frequency of crossing over for an entire chromosome.

4.2 The Lin-35 mutant alters both the distribution and frequency of crossing over

To determine the genetic length of a whole chromosome, recombination was scored across five intervals on Chromosome III: the two halves, the two arms, and the

cluster (Figure 6). Since crossing over interference is high in *C. elegans* autosomes, only one crossover per chromosome pair is expected, producing a genetic map of 50 m.u. per linkage group. The genetic length of Chromosome III in Lin-35 was 42.3 m.u., shorter than wild-type worms and Rec-1 (48.8 m.u., 47.8 m.u. respectively) (Table 6, Figure 7). This is the first piece of evidence showing that the phenotype of Lin-35 differs from that of Rec-1.

Zetka and Rose (1995) scored recombination across LGI and found that the genetic length of Chromosome I was similar in Rec-1 mutants and wild-type worms (45.3 m.u., 43.5 m.u. respectively), indicating that Rec-1 mutation alters the distribution of crossing over but not the frequency; moreover, Rec-1 affects the location of crossovers while maintaining the normal number. Here, it can be concluded that Lin-35 reduced the frequency of crossing over and consequently the distribution, as crossovers were located in different regions compared to wild-type.

To determine whether or not there are in fact fewer crossover events in Lin-35 and Lin-35 Rec-1 mutants, RAD-51 foci staining could be employed. In *C. elegans,* RAD-51 is a strand exchange protein that can be used to quantify the number of DSB (Alpi *et al.* 2003, Martin *et al.* 2005). This technique has been applied to Him-17 Lin-35 double mutants where a reduction in DSB was observed compared to wild type and Him-17 single mutants (Reddy and Villeneuve 2004). Since RAD-51 foci staining was not shown for Lin-35 single mutants, this would be a valuable avenue for additional research.

In addition to lending insight into the number of crossover events, the Chromosome III genetic map can be compared to the physical map. Zetka and Rose (1995) determined that the Rec-1 genetic map for Chromosome I is more similar to the

physical map than the wild type genetic map in terms of the spacing of genes (Zetka and Rose 1995 Figure 3). Similarly, results here show that the Rec-1 and Lin-35 genetic maps for Chromosome III are more similar to the physical map than the wild-type genetic map, reflecting the effects these mutants have on the frequency and distribution of crossing over. These results indicate that mutation in *lin-35* reduces the ability to crossover in *C. elegans*.

4.3 Lin-35 in a Rec-1 background exhibits even fewer crossover events

The four intervals analyzed in the Lin-35 Rec-1 double mutant shed light on the relationship between Lin-35 and Rec-1; however, when examined independently, these intervals point towards different types of genetic relationships. Within the cluster of chromosome V (*dpy-11 unc-42*), the recombination frequency of the double mutant was similar to wild type and less than the single mutants (Table 5). The phenotype of Lin-35 was suppressed in a Rec-1 mutant background (or vice versa). In contrast, the arm of chromosome III (*dpy-18 unc-64*) exhibited a partially additive relationship between Lin-35 and Rec-1 because the recombination frequency of the double mutant was less than wild type and each single mutant (Table 5). Finally, the left and right halves of chromosome III demonstrated a synergistic relationship as the recombination frequency of the double mutant was even less than the reductions of each single mutant (Table 6). While these relationships appear to be contradictory, it is more informative to draw conclusions from the whole chromosome as chromosome arms and clusters behave differently in terms of meiotic recombination.

The genetic length of the chromosome in the Lin-35 Rec-1 mutant was 28.0 m.u., considerably shorter than wild-type worms (48.8 m.u.), Rec-1 mutants (47.8 m.u.) or Lin-

35 mutants alone (42.3 m.u.) (Table 6, Figure 7). This data represents a synergistic relationship between *lin-35* and *rec-1* because the recombination frequency of the double mutant is less than the single mutants across the chromosome. This non-redundancy may result from *lin-35* and *rec-1* functioning in distinct pathways. Thus, not only do Lin-35 and Rec-1 have different phenotypes, but genetic interaction analysis indicates independence of function.

The severe reduction in crossing over in the Lin-35 Rec-1 double mutant is supported by the percentage of males observed in the Lin-35 Rec-1 double mutant brood analysis. There were more than seven times more males in Lin-35 Rec-1 double mutants compared to wild-type worms (0.2% in wild-type and 1.5% in Lin-35 Rec-1) (Table 7). Wild-type hermaphrodites (5A XX) are self-fertilizing by producing sperm, and males (5A XO) arise spontaneously through X-chromosome loss or nondisjuction at a rate of 1.07 per 1000 wild-type progeny at 20°C (Rose and Baillie 1979). A reduction in crossing over, as observed in the double mutant, is associated with increased chromosomal nondisjunction, manifesting as an increase in male worms in *C. elegans* (Lamb *et al.* 2005).

4.4 The phenotype of the Lin-35 Rec-1 double mutant is more severe than the single mutants or wild-type

The brood analysis of the Lin-35 Rec-1 double mutant showed morphological changes, and a reduction in viability and fertility compared to the single mutants and wild type (Table 7). Discussion here will draw links between my data, additional Lin-35 phenotypes, and meiotic recombination where possible to substantiate results and raise further questions.

Lin-35 mutants are synthetic multi-vulva class B (synMuv B) mutants such that synthetic vulval defects arise when combined with a synMuv A or synMuv C mutant (Lu and Horvitz 1998). The *C. elegans* vulva is an excellent model system to study development because the vulva phenotype responds to inducers, which create visible changes (reviewed by Fay and Yochem 2007). Since the vulva is susceptible to these changes, it is not surprising to observe defective vulval phenotypes (Pvl and Evl) in the Lin-35 Rec-1 double mutant. However, a link between these phenotypes and recombination is not clear.

Lin-35 also exhibits increased penetrance and strength for germline, embryonic, and post-embryonic RNAi phenotypes (Lehner *et al.* 2006). Thus, mutation in *lin-35* has wide affects on both transcription and mRNA stability. This complicates analysis because several genes affected by *lin-35* mutation may have caused the meiotic, viability and fertility phenotypes observed.

To shed light on this issue, Grishok *et al.* (2008) examined how mutant LIN-35 influences gene transcription. Loss of *lin-35* function results in the up-regulation of 535 genes and the down-regulation of 175 genes. Since several of these genes have a meiotic function, this provides an indirect means for meiotic control. The meiotic genes that are up-regulated in Lin-35 mutants include several synaptonemal complex genes (*syp-1, syp-3, htp-1, him-3*) and the strand exchange protein, *rad-51*. While the loss of function of these genes results in aberrant recombination events, it is unclear how an enhancement of gene transcription would affect meiotic recombination. Analyzing the consequences of up-regulating these secondary transcripts would be an intriguing avenue of future study.

In addition to transcriptional effects, Lin-35 may affect meiosis by modifying chromatin. While it is clear that Lin-35 interacts with several chromatin modifying complexes as part of the DRM complex, the precise histone modifications influenced by *lin-35* have not been determined (Sawa 2000, Cui *et al.* 2004, Harrison *et al.* 2006). Immuno-staining *C. elegans* germline with molecular antibodies for specific histone modifications may shed light on this question.

It is unclear whether the effects *lin-35* has on recombination are direct and local through chromatin structure, or indirect and global through gene transcription. In fact, it is likely a combination of these because a change in chromatin structure alters the transcription environment. Further experimental support is required to elucidate the relationship between chromatin modifications, transcriptional regulation and meiotic recombination. A unified model may include a mechanistic link between distinct DNA loci and chromatin structure to encompass the factors known to influences meiotic recombination (reviewed by Wahls and Davidson 2010).

4.5 Conclusion

The findings presented in this thesis expand upon the knowledge surrounding meiotic recombination, establishing a new role for the Rb ortholog, LIN-35, during meiosis. LIN-35 maintains wild type levels of meiotic recombination, possibly through its role in modifying chromatin and regulating gene transcription.

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APPENDIX 1

Table A1. Strains that can be found at the *C. elegans* Genetics Center

Strain Name	Genotype
N2	Wild-type, variety Bristol
BC313	*rec-1(s180)*
MT10430	*lin-35(n745)*
BC26	*dpy-5(e61) unc-13(e51)*
KR177	*dpy-5(e61) unc-13(e51) rec-1(s180)*
BC502	*dpy-18(e364) unc-64(e246)*
KR4479	*rec-1(s180); dpy-18(e364) unc-64(e246)*
BC251	*dpy-11(e224) unc-42(e270)*
CB164	*dpy-17(e164)*
KR1234	*hT2/[bli-4(e937)]*
BC503	*unc-64(e246)*
CB286	*unc-45(e286)*
CB1	*dpy-1(e1)*
RB758	*hda-4(ok518)*
C32F10.2	*lin-35(RNAi)*

dpy, dumpy; *unc*, uncoordinated; *lin*, abnormal cell lineage; *rec*, abnormal recombination; *hda*, histone deacetylase.

Table A2. Strains constructed by Z. Lohn

Strain Name	Genotype
KR4841	*lin-35(n745) rec-1(s180)*
KR4918	*lin-35(n745); dpy-18(e364) unc-64(e246)*
KR4919	*rec-1(s180) lin-35(n745); dpy-18(e364) unc-64(e246)*
KR4830	*lin-35(n745); dpy-11(e224) unc-42(e270)*
KR4868	*rec-1(s180) lin-35(n745); dpy-11(e224) unc-42(e270)*
KR4821	*dpy-17(e164) unc-64(e246)*
KR4839	*lin-35(n745); dpy-17(e164) unc-64(e246)*
KR4829	*rec-1(s180); dpy-17(e164) unc-64(e246)*
KR4825	*dpy-17(e164) unc-45(e286)*
KR4870	*lin-35(n745); dpy-17(e164) unc-45(e286)*
KR4867	*rec-1(s180); dpy-17(e164) unc-45(e286)*
KR4917	*rec-1(s180) lin-35(n745); dpy-17(e164) unc-45(e286)*
KR4820	*dpy-1(e1) unc-45(e286)*
KR4823	*rec-1 (s180); dpy-1(e1) unc-45(e286)*
KR4869	*lin-35 (n745); dpy-1(e1) unc-45(e286)*
KR4912	*lin-35 (n745); dpy-17(e164) unc-32 (e189)*
KR4822	*hda-4 (ok518); dpy-18 (e364) unc-64 (e246)*
KR4819	*hda-4 (ok518); dpy-11 (e224) unc-42 (e270)*
KR4840	*hda-4 (ok518); dpy-5 (e61) unc-13 (e51)*
KR4824	*dpy-17(e164) unc-32 (e189)*

dpy, dumpy; *unc*, uncoordinated; *lin*, abnormal cell lineage; *rec*, abnormal recombination; *hda*, histone deacetylase.

Table A3. Crossing over in Lin-35 and Rec-1 mutant strains along Chromosome III. Data includes multiple isolate labeled with strain numbers.

		Recombinants				
Genotype	**wild-type progeny[a]**	**Dpy**	**Unc**	**Total**	***p* x 100**	**95% C.I.[b]**
dpy-17 unc-32/+ +	2008	21	27	48	1.88	1.36-2.47
dpy-17 unc-32/+ +; rec-1/rec-1	2033	64	74	138	5.11	4.35-6.22
dpy-17 unc-32/+ +; lin-35/lin-35 (Isolate 1)	1127	22	24	46	3.45	2.55-4.56
dpy-17 unc-32/+ +; lin-35/lin-35 (Isolate 2)	884	18	21	39	3.22	2.28-4.34
dpy-17 unc-32/+ +; lin-35/lin-35 (Combined)	2011	40	45	85	3.35	2.62-4.12
dpy-1 unc-45/+ +	1884	141	148	289	11.3	9.9-12.7
dpy-1 unc-45/+ +; rec-1/rec-1 (Isolate 1)	1269	55	51	106	6.12	4.92-7.33
dpy-1 unc-45/+ +; rec-1/rec-1 (Isolate 2)	752	24	29	53	5.23	3.92-6.81
dpy-1 unc-45/+ +; rec-1/rec-1 (Combined)	2021	79	80	159	5.79	4.88-6.74
dpy-1 unc-45/+ +; lin-35/lin-35	1414	63	57	120	6.38	5.25-7.65
dpy-17 unc-45/+ +	2052	276	320	596	22.3	21.4-23.1
dpy-17 unc-45/++; rec-1/rec-1 (Isolate 1)	1477	201	191	392	21.2	20.1-22.0
dpy-17 unc-45/+ +; rec-1/rec-1 (Isolate 2)	560	124	77	201	27.6	23.2-32.2
dpy-17 unc-45/+ +; rec-1/rec-1 (Combined)	2037	325	268	593	22.9	22.0-23.8
dpy-17 unc-45/+ +; lin-35/lin-35	1450	197	162	359	21.0	20.0-22.0
dpy-17 unc-64/+ +	1812	343	302	645	26.5	25.3-27.4
dpy-17 unc-64/+ +; rec-1/rec-1 (Isolate 1)	943	137	179	316	24.9	23.6-26.4
dpy-17 unc-64/+ +; rec-1/rec-1 (Isolate 2)	920	138	182	321	25.9	24.6-27.4
dpy-17 unc-64/+ +; rec-1/rec-1 (Combined)	2004	290	373	663	24.8	23.9-25.8
dpy-17 unc-64/+ +; lin-35/lin-35	702	109	101	210	21.3	18.5-25.1
dpy-17 unc-64/+ +; lin-35 rec-1/lin-35 rec-1	2083	186	192	378	14.7	14.0-15.3

[a] Male progeny included.
[b] C.I. = 95% confidence interval; See Materials and Methods.