STUDIES OF *rec-1* FUNCTION IN

CAENORHABDITIS ELEGANS

by

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ABSTRACT

The faithful transmission of genetic information to succeeding generations relies on the accurate segregation of chromosomes during meiosis. Central to the meiotic process is recombination. Alteration of the pattern of crossing over can lead to improper chromosome segregation. A recessive mutation in the *rec-1* gene in *Caenorhabditis elegans* eliminates the meiotic pattern of crossing over on the autosomes. Whether or not the *rec-1* mutation also affects the X chromosome was investigated. Even though the amount of variation is less on the X chromosome, *rec-1* randomized the distribution.

Crossing over initiates as a result of double strand breaks. In *C. elegans*, mutations in the *spo-11* gene, which is required for meiotic exchange, produce no viable progeny unless treated with ionizing radiation. In order to investigate the possibility that the Rec-1 mutant phenotype results from excess breaks in the DNA Rec-1; Spo-11 double mutant phenotype was determined. Rec-1 was not able to compensate for the *spo-11* defect. Thus, DNA breakage is unlikely to be the basis of the altered crossover pattern observed in Rec-1.

A recessive mutation in the *him-1* gene reduces the total number of crossovers on the X chromosome. Whether or not *rec-1* mutation can override the effect of *him-1* mutation was investigated. In *him-1 rec-1* double mutants, the total length of the X chromosome genetic map was further reduced. This indicated that wild type *him-1* function is needed for *rec-1* function on the X chromosome.

In order to understand the molecular basis of *rec-1*, candidate genes were tested for allelism. The right end of *eDf24* lies within the ribosomal gene cluster. There are 13 predicted protein encoding genes between the left breakpoint and the ribosomal gene cluster. These genes were tested using transgenic rescue, dsRNA inhibition of gene function and DNA sequencing.

Deletions, which affect genes in the region of *eDf24* where *rec-1* is known to map, were generated. None of these deletions failed to complement the Rec-1 phenotype. Thus, considerably refining potential position of the *rec-1* gene.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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QUOTATION

"In Science, when one door is opened, and a passage is revealed, ten doors are discovered in that very passage, and each one of them has to be opened in turn. Science transforms things, re-arranges them, studies their composition, re-groups their parts and releases the energy that lies latent in them. But I create the things themselves! And they are as lasting as any that is found in Nature!"

-SRI SATHYA SAI BABA

DEDICATION

I dedicate this thesis and all the remarkable learning experiences in putting it together, in memory of my father Vijayaratnam Veluppillai, with love and humility, at the Lotus Feet of my Beloved Lord, Bhagavan Sri Sathya Sai Baba, who is the real doer, and without His Divine Will this thesis would not have come into being.

CHAPTER I

GENERAL INTRODUCTION

Meiosis ensures the faithful transmission of hereditary material; it takes place in diploid cells named meiocytes. In meiosis, one round of DNA replication is followed by two successive cell divisions to produce four haploid cells. Diploid cells entering meiosis typically undergo a single round of replication followed by a lengthy prophase during which homologous chromosomes pair and recombine (reviewed in Baker *et al.* 1976b). During this stage, homologous chromosomes become intimately associated or synapsed through an elaborate structure called the synaptonemal complex (SC) (reviewed in von Wettstein *et al.* 1984). At meiosis I, homologs are aligned at the equatorial plane and undergo reductional division, which results in the separation of homologous centromeres to opposite poles (Alberts *et al.* 1994). During meiosis II, another round of chromosome segregation occurs, which is similar to a mitotic division in that sister centromeres segregate from each other (Alberts *et al.* 1994). Prophase I of meiosis can be divided into five cytological defined substages (Alberts *et al.* 1994) (Fig 1). The first stage, called leptotene, is characterized by the appearance of SC precursors, which are visible as short segments of axial elements. Each axial element represents a pair of sister chromatids associated with proteins. During the next stage, called zygotene, homologous chromosomes are aligned and synapsis begins. Pairing refers to the homologous alignment of chromosomes (perhaps at distance), whereas synapsis refers to the intimate association of chromosomes in the context of SC (Giroux, 1988; Alani *et al.* 1990). At the next stage, called pachytene, each pair of homologs is fully synapsed, and the SC appears as a ribbon-like structure along the entire length of each

Figure1: Time course of chromosome synapsis and desynapsis during meiotic prophase I (adapted from Molecular Biology of The Cell by Alberts *et al.* 1994). A single bivalent is shown. The leptotene stage represent a pair of sister chromatids associated with proteins. In zygotene stage, intimate chromosome synapsis initiates and the synaptonemal complex begins to develop. The pachytene stage is defined as the period during which a fully formed synaptonemal complex exists. In the subsequent diplotene stage the chromosomes are decondensed and very active in transcription. This ends with diakinesis-the stage of transition to metaphase- in which the chromosomes recondense and transcription halts.

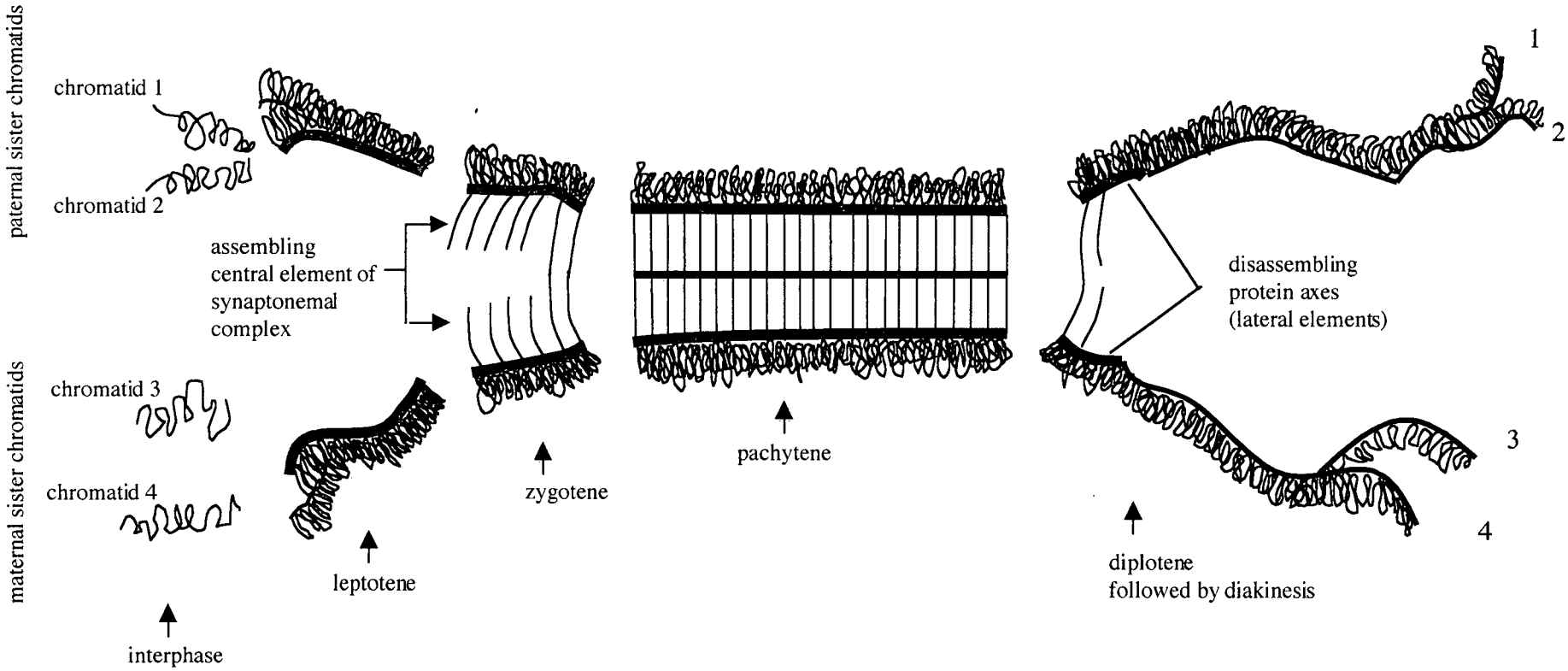

paternal sister chromatids
maternal sister chromatids
chromatid 1
chromatid 2
chromatid 3
chromatid 4
assembling
central element of
synaptonemal
complex
disassembling
protein axes
(lateral elements)
1
2
3
4
interphase
leptotene
zygotene
pachytene
diplotene
followed by diakinesis

Fig 1

chromosome pair or bivalent. At the next stage, called diplotene, desynapsis begins as the SC proteins begin to dissociate. As the homologs separate slightly, cross-shaped structures called chiasmata appear between non-sister chromatids. Each chromosome pair generally has one or more chiasmata. Chiasmata are the visible manifestations of events called crossovers that occurred earlier, probably during zygotene or pachytene. The final stage of prophase I is diakinesis, during which homologous chromosomes are attached to spindle fibers in preparation for metaphase I.

The process by which damaged DNA is repaired and the mechanism of genetic recombination are intimately related. The expanding list of human genetic diseases associated with defects in DNA metabolism makes it important in understanding how these processes occur. Recombination can be initiated by several types of DNA damage (reviewed in Paques and Haber, 1999): (1) Single-strand DNA (ssDNA) lesions may result during DNA replication or during repair, after UV-irradiation or the alkylation or cross-linking of DNA bases, or from intermediates of type I topoisomerases. (2) Double-strand breaks (DSBs) can appear as a consequence of ionizing radiation, by mechanical stress, by endonucleases, or by replication of a single-stranded nicked chromosome. Broken chromosomes pose a serious threat to cell survival. In response to this threat, cells have elaborated an impressive arsenal of DNA-repair pathways. There are two general types of repair: homologous recombination (HR) and nonhomologous end-joining (NHEJ) (reviewed in Haber, 2000). There are different types of homologous repair: gene conversion, break-induced replication and single stand annealing (reviewed in Paques and Haber, 1999). The three major types of HR all begin in the same way, as the ends of the DSB are resected by 5' to 3' exonucleases or by a helicase coupled to an endonuclease, to produce long, 3'-ended single-stranded DNA tails (Fig 2) (reviewed in Haber, 2000).

Single-strand annealing (SSA): In the simplest process (Fig 2a), resection exposes complementary regions of homologous sequences originally flanking the DSB, creating a deletion by SSA (reviewed in Haber 2000). SSA will occur with as little as 30 bp of homology, although it is much more efficient with 200-400 bp (Sugawara *et al.* 2000).

Gene conversion: SSA is in competition with gene conversion (Fig 2b), during which the two resected ends of the DSB invade and copy sequences from a homologous template located on a sister chromatid, a homologous chromosome, or at an ectopic location (Haber 2000).

Break-induced replication: Under certain circumstances only one end of a DSB might be able to engage in homologous recombination, for example in haploid or hemizygous chromosomes of G1 diploids, where there is no homologous chromosome (Haber 2000). Sequences near the centromere-proximal side of the DSB might be able to find homologous sequences elsewhere in the genome and create a nonreciprocal translocation by a process known as recombination-dependent DNA replication or break-induced replication (BIR; Fig 2c) (Bosco and Haber, 1998).

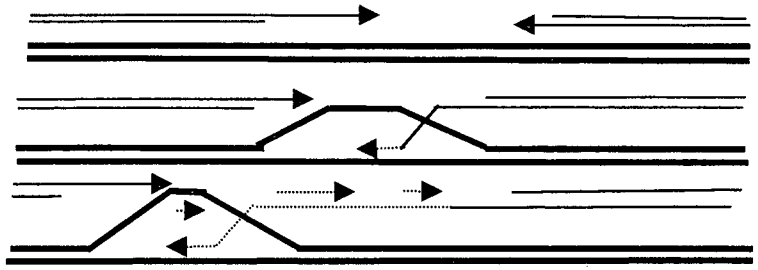
Mutations that affect different aspects of the meiotic process have been isolated from several species. In *Drosophila melanogaster*, a number of mutants have been isolated that affect recombination (Baker and Carpenter, 1985). The first class of mutant, (eg., *mei-9*) decreases the frequency of recombination but retains the normal pattern of recombination (Baker and Carpenter, 1972). The second class of mutants (eg: *mei-41, mei-218* , *mei-251* (Baker and Carpenter, 1972; Carpenter and Sandler, 1974) and *mei-217* Liu *et al.* 2000) alter both the frequency and the distribution of crossovers. In the third class of mutants, (e.g., *c*(3)G17), *mei-W68*), crossing over is nearly eliminated (Baker and Carpenter 1985). In the fourth class of mutants (e.g., *mei-38, mei-99* and *mei-160*), crossing over is altered in a nonuniform manner but the total distances are similar to control values (Baker and Carpenter 1972). The molecular basis of this phenotype is not known.

Figure 2: Alternative outcomes of the homologous recombinational repair of a double strand break (DSB) (adapted from Haber, 2000). After a double strand break is created, the ends are resected and one of the 3' single-stranded can invade an intact template. Strand invasion is believed to establish a modified replication fork, in which both leading and lagging-strand DNA synthesis occurs (Holmes and Haber, 1999). As new DNA synthesis proceeds, branch migration displaces the two newly synthesized strands.

a) If resection proceeds far enough to expose complementary strands of homologous sequences flanking a DSB (shown as boxes), repair can occur by single-strand annealing (SSA), leading to a deletion of all intervening sequences.

b) If the replication fork encounters the other end of the DSB, an intermediate containing two Holliday junctions can be formed, allowing gene conversions to be resolved both with and without crossing over.

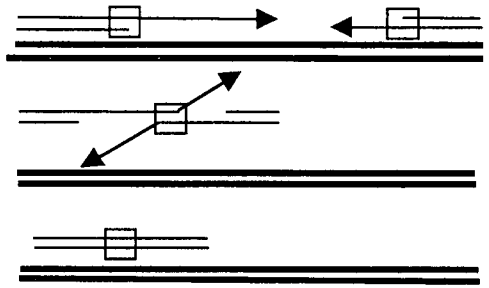
c) If the second end of the DSB fails to engage, replication can proceed all the way to the end of the chromosome (or until it encounters a converging replication fork). This process is known as break-induced replication (BIR).



5' to 3' resection

strand invasion

new DNA synthesis



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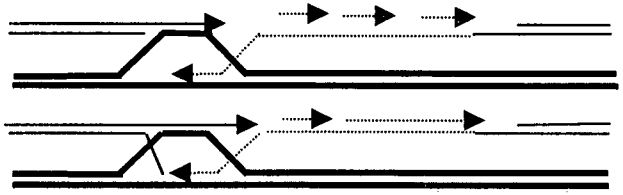
5' to 3' resection

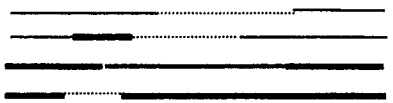
strand annealing

(a)

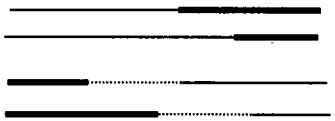


replication fork capture

Holliday junction resolution

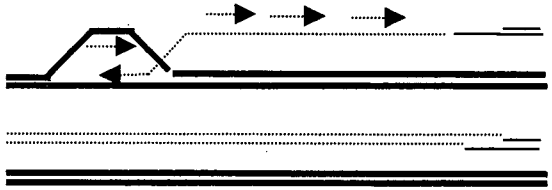


no crossing over



crossing over

(b)



continued
DNA synthesis

(c)

Fig 2

Some of these genes have been cloned and characterized. The MEI-41 protein shows sequence similarity to the ATM (ataxia telangiectasia) protein (Hari *et al.* 1995). Like the ATM protein (Savitsky *et al.* 1995), MEI-41 protein belongs to a family of kinase proteins (Hari *et al.* 1995) that includes Mec1p (Greenwell *et al.* 1995) from *S. cerevisiae,* and rad3 from *Schzosaccharomyces pombe* (Seaton *et al.* 1992). Mec1p from *S. cerevisiae* and rad3 from *S. pombe* are required for DNA damage-sensitive checkpoint controls and produce repair-deficient phenotypes when mutated (Al-Khodairi and Carr, 1992; Jimenez *et al.* 1992; Allen *et al.* 1994; Kato and Ogawa, 1994; Weinert *et al.* 1994). It is possible that *mei-41* function is dependent on kinase activity that is activated by association with double-strand breaks (McKim *et al.* 2000). Normally in *Drososphila* oocytes, meiosis arrests during metaphase I; tension on the meiotic spindle is required for metaphase arrest (McKim *et al.* 1993b; Jang *et al.* 1995). In the absence of tension, such as in a recombination-defective mutant, the metaphase arrest does not occur and meiosis proceeds precociously through the two divisions (McKim *et al.* 1993b). In some recombination-defective mutants, precocious anaphase requires *mei-41* gene product, and the effect of *mei-41* is dependent on double-strand break formation (McKim *et al.* 2000). The *mei-218* gene encodes a novel protein (McKim *et al.* 1996), which may not be required for the exchange process *per se* but instead is required to determine which recombination sites will be resolved as crossovers (Liu *et al.* 2000). MEI-217 is a novel protein (Lui *et al.* 2000). Like *mei-218*, *mei-217* is not required for the initiation of recombination but is required for recombination intermediates to be resolved as crossovers (Liu *et al.* 2000). Mutations in *mei-W68* result in a failure to initiate meiotic recombination (McKim *et al.* 1998). The *mei-w68* gene encodes a Spo11 homolog, and has vital role in generation of a double strand break (McKim and Hayashi-Hagihara, 1998). *mei-9* is required for DNA repair (Boyd *et al.* 1976). The *mei-9* gene encodes a homolog of the DNA

repair enzyme Rad1/ XPF family of endonucleases, and it is likely to have direct role in maturation of recombination intermediates (Sekelsky *et al.*, 1995).

Mutants at the *Saccharomyces cerevisiae* loci SPO11 (Klapholz *et al.* 1985), RAD50 (Game *et al.* 1980), RED1 (Rockmill and Roeder, 1990), and HOP1 (Hollingsworth and Byers, 1989) display decreased levels of meiotic recombination; strains carrying null mutations at these loci are also defective in synaptonemal complex (SC) assembly (SPO11: Dresser *et al.* 1986; RAD50: Alani *et al.* 1990; RED1: Rockmill and Roeder, 1990; HOP1: Hollingsworth and Byers, 1989). Defects in pairing and recombination in these mutants result in the production of aneuploid (and therefore usually inviable cells) meiotic products, presumably due to nondisjunction at meiosis I (Engebrecht *et al.* 1990). *zip1* mutants, in *S. cerevisiae*, make chromosomes that are homologously paired (Nag *et al*, 1995), but not intimately synapsed (Sym *et al.* 1993). *zip1* mutants exhibit two to threefold reduction in reciprocal exchange (Sym and Roeder. 1994).

Rad50 has ATP-dependent DNA binding and partial DNA unwinding activities (Raymond and Kleckner, 1993; Paull and Gellert, 1999). Spo11p is a homolog of the A subunit of a newly identified type II topoisomerase (Bergerat *et al.* 1997), which responsible for making meiotic DSBs (Keeney *et al.* 1997). The Red1 protein localizes to the cores of meiotic chromosomes before and independent of both synapsis and recombination (Smith and Roeder, 1997). The authors suggested that Red1 plays a role in meiotic sister chromatid cohesion. The establishment of cohesion between sister chromatids is presumably a necessary step in the establishment of a proteinaceous core that is shared by sisters (i.e., an axial/ lateral element) (Smith and Roeder, 1997). Red1 and Hop1 localize to the same sites on chromosomes at early stages of meiotic prophase; localization of Hop1 depends on Red1, suggesting that Red1 acts before Hop1 in SC

formation (Smith and Roeder, 1997). This observation is consistent with the observation that a *red1* null mutant fails to make any detectable axial or lateral elements (Rockmill and Roeder, 1990), while the *hop1* mutant does make axial elements (Loidl *et al.* 1994). ZIP1 encodes a component of the central region of the SC (Sym *et al.* 1993; Sym and Roeder, 1995). Zip1 localizes along the lengths of pachytene chromosomes but not is associated with unsynapsed axial elements (Sym *et al.* 1993). The *zip1* mutant displays a modest defect in sister chromatid cohesion; the only absolute defect observed in zip1 strains is a loss of crossover interference (Sym and Roeder. 1994). Thus, a primary function of Zip1, and by implication of the central region of the SC, is the regulation of crossover distribution (Smith and Roeder, 1997).

In *C. elegans*, several classes of recombination defective mutants isolated (reviewed in Zetka and Rose, 1995a). One class of meiotic mutants, represented by *him-1, him-5* and *him-8,* alters the distribution of recombination on all chromosomes with a predominant effect on the X chromosome (Hodgkin *et al.*, 1979; Broverman and Meneely, 1994). A second class of mutants, consisting of *him-6* and *him-14* , reduces crossing over on all chromosomes (Hodgkin *et al.* 1979; Kemphues *et al.* 1988). *him-1* encodes a member of the SMC (structural maintenance of chromosomes) protein family, and is implicated in homologous chromosome synapsis during meiosis and sister chromatid cohesion during both meiosis and mitosis (B. Meyer, pers. comm.). *him-5* and *him-8*, both encode novel proteins (P. Meneely, pers. comm.). *him-6* shares homology to the helicase gene responsible for Bloom syndrome in humans (C. Wicky, pers. comm.). *him-14* encodes a germline specific member of MutS family of DNA mismatch repair (MMR) proteins (Zalevsky *et al.* 1999). Although it has no apparent role in MMR, like its *Saccharomyces cerevisiae* ortholog MSH4, it has specialized role in promoting crossing over during meiosis (Zalevsky, *et al.* 1999). A deletion in the *C. elegans spo11* homologue abolishes meiotic

recombination (Dernburg *et al.* 1998). Finally one mutant (*rec-1*) was isolated (Rose and Baillie, 1979b) that shows an altered pattern of crossing over without altering the total number of recombination events (Zetka and Rose, 1995b).

Crossing over during meiosis is globally controlled in many organisms (Hawley, 1988; Carpenter, 1988). The position of crossing over is highly controlled in *Drosophila melanogaster* females, i.e. exchange only occurs in the euchromatin, and the amount of exchange is not proportional to physical distance (Lindsley *et al.* 1968). This pattern is not unique to *Drosophila* females but rather is a general feature of chiasma distribution in many organisms (Jones, 1987; Hawley, 1989). In *Drosophila melanogaster*, the proportion of spontaneous diplo-X exceptions resulting from nondisjunction, derived from single-exchange bivalents, exhibits a strong bias toward very distal crossovers (Koehler *et al.* 1996), which precisely parallels observations in human oocytes for chromosome 21 (Lamb *et al.* 1996), and chromosome 16 (Hassold *et al.* 1995). Findings in yeast also suggest that distal-exchange bivalents are often susceptible to meiotic nondisjunction at meiosis I (Ross *et al.* 1996). These observations indicate that crossover distribution is highly regulated. How crossover position is determined is not known.

Recombination distribution in *C. elegans*

The nematode *Ceanorhabditis elegans* has many advantages for the study of meiosis. The genome is completely sequenced; extensive genetic maps are available for each chromosome; there are a number of approaches available for functional analysis. These include the RNA interference (Fire *et al.* 1998), and gene knockouts can be obtained from the *C. elegans* Reverse Genetics Consortium (http://elegans.bcgsc.bc.ca/knockout.shtml). Populations consist of self-fertilizing

hermaphrodites that have a short generation time (3.5 days at 20°) and each hermaphrodite is capable of producing about 300 progeny. The frequency of males (5AA; XO) among the self progeny of wild-type hermaphrodites (5AA; XX) is about one in 500. Mutations that increase X chromosome non-disjunction will produce a *h*igh *i*ncidence of *m*ales (*him*) among the self progeny. Males can be used in mating to generate heterozygous cross progeny. One way to understand the mechanism involved in determining crossover distribution is to identify and characterize a mutation that affects crossover distribution. One mutant, *rec-1(s180)* in *Caenorhabditis elegans*, has been described to date that affects crossover distribution without affecting total frequency (Zetka and Rose, 1995b).

The distribution of crossing over has been well studied in *C. elegans*. The meiotic map of each of the chromosomes of *C. elegans* is marked by a region of gene clustering, largely the result of crossover suppression (Brenner, 1974; reviewed in Zetka and Rose, 1995a; Barnes *et al.* 1995). These observations were the first indication that the distribution of meiotic exchanges is non-random in the nematode. There are central gene clusters on the autosomes, yet, there is no central cluster region on the *X* chromosome (Brenner, 1974; Barnes *et al.* 1995), suggesting that the organization of the autosomes and the X chromosome is different. Crossover frequency is affected by a number of factors, such as age, sex, and temperature. For instance, crossover frequency decreases as the animals grow older (Rose and Baillie, 1979a), crossover frequency is lower in males than in hermaphrodites (Zetka and Rose, 1990), and an increased crossover frequency is observed at higher temperatures (Rose and Baillie, 1979a).

Crossover events preferentially occur at the regions flanking the gene clusters. When crossover is eliminated from one portion of a chromosome there is often a compensatory elevation of crossover

in other regions of *C. elegans* chromosomes (McKim *et al.* 1988b, 1993a; Zetka and Rose, 1992) suggesting that the number of crossover events is highly regulated. In *rec-1* homozygotes, crossover events are increased 3-6 fold in the cluster regions on most of the autosomes (Rose and Baillie, 1979b). On chromosome I, the crossover events increased in the cluster region and decreased in the right end of the chromosome, but the total length of chromosome I is still ~50 m.u. in *rec-1* homozygotes which is comparable to wild type (Zetka and Rose, 1995b). Since there is no change in the genetic length of the chromosome this suggests that the total number of crossovers is the same and suggests that *rec-1* mutation does not eliminate crossover interference (Zetka and Rose, 1995b). Thus, the pattern of exchange in *rec-1* homozygotes more closely represents the gene distribution on the physical map (Fig 3) (Zetka and Rose, 1995b).

Considerable progress has been made in our understanding of meiotic recombination in the past decade due to the cloning and characterization of genes involved in recombination and repair. However, it is still not clear what determines the distribution of crossing over. In this thesis following questions were investigated: if *rec-1* mutation affects the crossover distribution on the X chromosome; if Rec-1 phenotype is due to excess DSBs along the chromosomes; if *rec-1* can randomize the crossover distribution in the *him-1 (e879)* mutant background. The genes contained within *eDf24* region were characterized. Finally potential candidates for the *rec-1* coding region were examined.

Figure 3: Marey map of chromosme I in *C. elegans* (adapted from Barnes *et al.* 1995), shows the crossover distribution in wild type and in Rec-1 backgrounds. Rec-1 data is taken from Zetka and Rose (1995b).

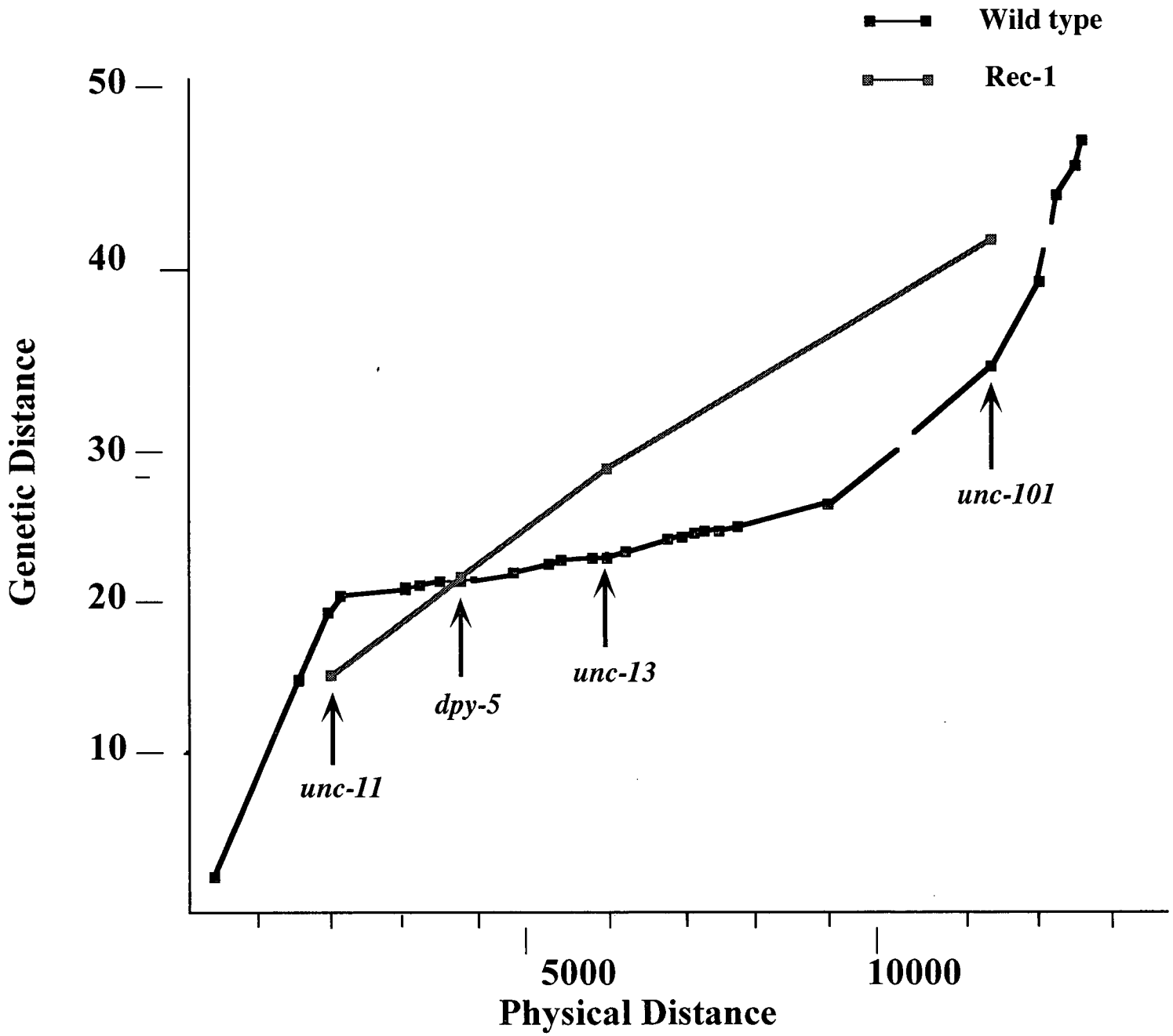

Wild type
Rec-1
50
40
30
20
10
Genetic Distance
unc-11
dpy-5
unc-13
unc-101
5000
10000
Physical Distance

Fig 3

CHAPTER II

MATERIALS AND METHODS

General methods: Wild-type and mutant strains of *C. elegans* were maintained on Petri plates containing nematode growth medium (NGM) streaked with *Escherichia coli* OP50 (Brenner, 1974). All experiments were carried out at either at 20° or room temperature (RT). The wild-type strain N2 and some mutant strains of *C. elegans* were obtained from D. L. Baillie, Simon Fraser University, Burnaby, Canada or from the Caenorhabditis Genetics Center (CGC) previously at the University of Missouri, Columbia, and now located at University of Minnesota, Minnesota. Mutations used in this study were: on chromosome I *him-1(e879)*, *dpy-5(e61), dpy-14(e188), unc-13(e51), unc-13(e450), rec-1(s180)*; on chromosome IV *unc-43 (e266) unc-22 (s8), spo-11 (ok79);* on chromosome V *dpy-11(e224), unc-42(e270); nT1[unc-x (n574) let-x]* (IV;V); and on chromosome X *lon-2(e678), dpy-6(e14), unc-1(e719), dpy-3(e27) unc-20(e112), unc-7(e5) lin-15(n309), unc-115(e2225) mab-18(bx23).*

Strain Construction

X-chromosome mutant strains: Lon-2 males were crossed to Unc-1 hermaphrodites and resulting heterozygotes plated individually. From their progeny, Lon-2 hermaphrodites were plated and in their segregants a single progeny of Unc-1 Lon-2 was isolated and used to establish KR3595, *unc-1(e719) lon-2(e678)*(X).

Lon-2 males were crossed to Unc-7 Lin-15 hermaphrodites and resulting heterozygous hermaphrodites plated individually and transferred 24 hours later. From their progeny, 15 - 20 Lon-2 hermaphrodites were plated one per plate. Their genotype could be one of *lon-2(e678)* + +/ *lon-2(e678)* + + , or *lon-2(e678)* + + / *lon-2(e678) unc-7(e5) lin-15(n309),* or *lon-2(e678)* + + / *lon-2(e678) unc-7(e5)* +. In the next generation, a single progeny of Lon-2 Unc-7 Lin-15 was isolated and used to establish KR3602, *lon-2(e678) unc-7(e5) lin-15 (n309)*(X).

Unc-115 hermaphrodite was segregated from *him-5(e1490); unc-115(e2225) mab-18(bx23).* Homozygotes from PB1 were crossed to N2 males, and the F1 heterozygotes plated and transferred. From their progeny, Unc-115 progeny were plated individually. Those that did not show Him-5 phenotype could be either *him-5(e1490)*/ + or +/ +. Unc-115 hermaphrodites and were progeny-tested for two generations. A single Unc-115 hermaphrodite, which did not carry the mutant *him-5* allele, was used to establish KR3530, *unc-115(e2225)*(X), which may carry *mab-18(bx23).* Unc-115 hermaphrodites from KR3530 were crossed to Lon-2 males and next generation heterozygotes plated, and transferred to new plates for three days. From their progeny, 15- 20 Lon-2 hermaphrodites were plated individually. A single Lon-2 Unc-115 hermaphrodite was isolated and used to establish the strain KR3572, *lon-2(e678) unc-115(e2225)*(X).

Rec-1 Strains: Rec-1 males were crossed to *unc-1(e719)*(X) hermaphrodites, and the resulting heterozygous hermaphrodites crossed to a male tester strain, *dpy-5 dpy-14 rec-1*/ + + *rec-1*(I); +/O (X). F2 hermaphrodites were plated one per plate, and transferred to fresh plates for three days. Those that segregated Dpy-5 Dpy-14 and Unc-1 progeny were scored. The hermaphrodites

(F2) were either *dpy-5 dpy-14 rec-1(s180)/ + + +*(I) *; unc-1(e719)/ +* (X) or *dpy-5(e61) dpy-14(e88) rec-1(s180)/ + + rec-1(s180)*(I); *unc-1(e719)/ +*(X). The standard the genetic distance between *dpy-5* and *dpy-14* in the N2 strain is 1.7 map units (m.u.). In *rec-1* homozygotes this distance increases at least three-fold (Rose and Baillie 1979b). From a plate, which had a high crossover frequency, individual Unc hermaphrodites were isolated. These were maintained for a few generations to be certain that they were not segregating Dpy progeny. From a single Unc hermaphrodite, the strain KR3082, *rec-1(s180)*(I); *unc-1(e719)*(X), was established. In a similar way, the following strains were constructed: KR3081, *rec-1(s180)*(I); *unc-20(e112)* (X), and KR3121, *rec-1 (s180)* (I); *dpy-3 (e27) (*X). In the latter case, BC378: *rec-1; unc-43 unc-22* (IV) strain was used as to test for the high crossover frequency phenotype.

Rec-1 males were crossed to KR3121 Dpy-3 hermaphrodites. The resulting Dpy males were *rec-1(s180)/rec-1(s180)*(I); *dpy-3(e27)/O*(X). These were crossed to KR3082 Unc-1 hermaphrodites. Hermaphrodites from the cross were homozygous for *rec-1*(I) and heterozygous for X-linked markers, *dpy-3(e27) +/ + unc-1(e719)*(X). These were placed one per plate. In the next generation, Unc-1 segregants were plated and their progeny examined for Dpy Unc hermaphrodites. A single Dpy Unc hermaphrodite was isolated and used to establish the strain KR3197, *rec-1(s180)*(I); *dpy-3(e27) unc-1(e719)*(X).

Rec-1 males were crossed to Dpy-6 Lon-2 hermaphrodites, and the resulting heterozygous males crossed to the tester strain, KR181: *rec-1(s180) ; dpy-11(e224) unc-42(e270)* (V). From this cross, hermaphrodites heterozygous for *lon-2 (e678)* were plated one per plate and transferred for

3 days. Those segregating Dpy-11 Unc-42 Lon-2 and Dpy-6 Lon-2 progeny were scored. Dpy is epistatic to Lon. The crossover frequency between *dpy-11* and *unc-42* was determined. There were two groups, one with a high crossover frequency and one with the normal frequency. From a single high crossover frequency hermaphrodite, all the Dpy hermaphrodites were plated individually. Those segregating Dpy Unc progeny were discarded. A single Dpy-6 hermaphrodite, failing to complement both *rec-1(s180)* and *lon-2(e678)*, was used to establish the strain KR2994, *rec-1(s180)*(I); *dpy-6(e14) lon-2(e678)*(X).

Rec-1 males were crossed to the KR181 tester strain and resulting males crossed to Unc-7 Lin-15 hermaphrodites. From a heterozgyous hermaphrodite segregating Dpy-11 Unc-42 and Unc-7 Lin-15 progeny, phenotypically wild-type hermaphrodites were plated and transferred. The progeny from those hermaphrodites segregating Dpy-11 Unc-42 and Unc-7 Lin-15 progeny were scored. Unc-7 Lin-15 progeny from a high crossover frequency hermaphrodite were plated individually. One of the Unc-7 Lin-15 hermaphrodite that gave only Unc-7 Lin-15 progeny (no Dpys, Dpy Uncs or Uncs) was tested, confirmed to be Rec-1, and used to establish strain KR3071, *rec-1(s180)*(I); *unc-7(e5) lin-15(n309)*(X).

Rec-1 Lon-2 males were crossed to Rec-1 Unc-1 hermaphrodites and resulting *rec-1(s180)/ rec-1(s180)*(I); *unc-1(e719) +/ + lon-2(e678)*(X) were plated and transferred to new plates on the next day. In the next generation, 15 - 20 Lon-2 hermaphrodites were plated one per plate. The genotype could be either *rec-1(s180)/ rec-1(s180)*(I); *+ lon(678)/ + lon-2(e678)*(X) or *rec-1(s180)/ rec-*

1(s180)(I); + *lon-2(e678)/ unc-1(e719) lon-2(e678)*(X). In the next generation, a single Rec-1 Unc-1 Lon-2 hermaphrodite was isolated and used to establish KR3596, *rec-1(s180)*(I); *unc-1(e719) lon-2 (e678)*(X).

Rec-1 Lon-2 males were crossed to Rec-1 Unc-7 Lin-15 hermaphrodites and resulting, *rec-1(s180)/ rec-1(s180); lon-2(e678)* + + / + *unc-7(e5) lin-15(n309)* hermaphrodites were plated and transferred to new plates on the next day. In the next generation, 15 - 20 Lon-2 hermaphrodites were plated one per plate. The genotype could be either *rec-1(s180)/ rec-1(s180); lon-2(e678)* + + / *lon-2(e678)* + + or *rec-1(s180)/ rec-1(s180); lon-2(e678)* + + / *lon-2(e678) unc-7(e5) lin-15(n309)*, or *rec-1(s180)/rec-1(s180); lon-2(e678)* + + / *lon-2(e678) unc-7(e5)* +. From the progeny, Rec-1; Lon-2 Unc-7 progeny were isolated and maintained for few generation to test for segregation of Lin-15 progeny. A single Rec-1 Lon-2 Unc-7 Lin-15 progeny was isolated and used to establish KR3603, *rec-1(s180)*(I); *lon-2(e678) unc-7(e5) lin-15 (n309)* (X).

Males homozygous for *rec-1* and heterozygous for *dpy-11 unc-42* were crossed to Lon-2 Unc-115 hermaphrodites. In the next generation, hermaphrodites that segregated Dpy-11 Unc-42 progeny were selected. From one of these, 20 - 27 phenotypically wild-type hermaphrodites were plated and transferred. Those that segregated Dpy-11 Unc-42 and Lon-2 Unc-115 progeny were selected. All progeny were scored and the crossover frequency between *dpy-11* and *unc-42* was measured. From an individual with a high crossover frequency, Lon-2 Unc-115 hermaphrodites

were plated one per plate. One, which did not segregate any Dpy, Unc or Dpy Unc progeny, was used to establish KR3574, *rec-1(s180)*(I); *lon-2(e678) unc-115(e2225)*(X).

Him-1 strains: Him-1 males were crossed to Dpy-5 Unc-13 hermaphrodites and F1 heterozygotes plated. From their progeny, Dpy-5 Unc-13 hermaphrodites were plated. A single Him-1 Dpy-5 Unc-13 hermaphrodite that segregated males was isolated and used to establish KR3583, *him-1(e879) dpy-5(e61) unc-13(e51)* (I). In a similar way, the following strains were constructed: KR3501: *him-1(s879)*(I); *dpy-11(e224) unc-42(e270)*(V); KR3573: *him-1(e879)*(I); *lon-2(e678) unc-115((e2225)*(X); KR3599: *him-1(e879)*(I); *unc-1(e719) lon-2(e678)*(X); KR3605: *him-1(s879)*(I); *lon-2(e678) unc-7(e5) lin-15 (n309)*(X); KR3599: *him-1(e879)*(I); *unc-1(e719) lon-2(e678)*(X); KR3580: *him-1(e879)*(I); *lon-2(e678) dpy-6(e14)*(X). In the latter case, Dpy-6 is epistatic to Lon-2. Him-1 males were crossed to Lon-2 Dpy-6 hermaphrodites and F1 heterozygotes plated. In the next generation, Dpy hermaphrodites were plated one per plate. Five individual Dpy hermaphrodites that segregated males were selected as candidates for Him-1 Dpy-6 Lon-2. The presence of *lon-2(e678)* was confirmed by complementation to Lon-2 males. A single isolate was used to establish KR3580. Broverman and Meneely (1994) reported that homozygotes for *him-1(e879); unc-1(e719)* were not recovered. KR3598: *him-1(e879)*I; *unc-1(e719) dpy-3(e27)*(X) was constructed using the double mutant KR2023: *unc-1(e719) dpy-3(e27)*.

Him-1 Rec-1 strains: Him-1 males were crossed to Rec-1; Dpy-11 Unc-42 hermaphrodites and F1 heterozygotes plated and transferred to fresh plate for three days. In their progeny, phenotypically wild-type hermaphrodites were plated one per plate and transferred for three

days. Hermaphrodites that segregated Dpy Unc progeny in the next generation were scored. Crossing over between *dpy-11* and *unc-42* was measured. From one of the plates that showed a high crossover frequency, phenotypically wild-type progeny were set up one per plate. One hermaphrodite that segregated males in the next generation was isolated as a Him-1 Rec-1 homozygote. This strain was maintained for few generations to test for segregation of Dpy, Unc or Dpy Unc progeny. A single Him-1 Rec-1 that did not segregate any Dpy, Unc or Dpy Unc progeny was isolated and used to establish KR3579, *him-1(e879) rec-1(s180)*(I).

Him-1 Rec-1 males were crossed to Rec-1 Lon-2 hermaphrodites. The resulting heterozygotes were plated. In the next generation, Lon-2 hermaphrodites were isolated. From one of these, which segregated males, KR3590 was established as *him-1(e879) rec-1(s180)*(I); *lon-2(e67)*(X). In a similar way, the following strains were established: KR3575: *him-1(e879) rec-1 (s180)*(I); *lon-2(e678) unc-115(e2225)*(X); KR3519: *him-1(e879) rec-1(s180)*(I); *unc-1(e719) dpy-3(e27)*(X); KR3581: *him-1(e879) rec-1(s180)* (I); *lon-2(e678) dpy-6(e14)*(X); KR3597: *him-1(e879) rec-1(s180)*I; *unc-1(e719) lon-2(e678)*(X); KR3604: *him-1(s879) rec-1(s180)*(I); *lon-2(e678) unc-7(e5) lin-15 (n309)*(X); KR3520: *him-1(e879) rec-1(s180)*(I); *dpy-11(e224) unc-42(e270)*(V); and KR3582: *dpy-5(e61) unc-13(e450) him-1(e879) rec-1(s180)*(I).

Other strains: *unc-101 eDf24 / eDf3* males were crossed to *hIn1*[*unc-54*] hermaphrodite which was isolated from *eDf24/ hIn1*[*unc-54*] strain. The *eDf3* deletion deletes *unc-54* gene and *eDf3 / hIn1*[*unc-54*] will give Unc-54 phenotype. In the F1 generation, single wild type hermaphrodite

was isolated to establish KR 3754: *unc-101 eDf24 / hIn1* [*unc-54*]. *unc-101 eDf24 / hIn1[unc-54]* males were crossed to *unc-101 unc-54* hermaphrodites. Resulting progeny will be either Unc-101 (*unc-101 unc-54 / unc-101 eDf24*) or Unc-54 (*unc-101 unc-54 / hIn1*[*unc-54*]). In the F1 generation, single Unc-101 was isolated to establish KR 3760.

lon-2 males were crossed to *rec-1/ rec-1; sEx 150*, Roller hermaphrodites and in the F1 generation, 20, out-crossed hermaphrodites, *lon-2 +/ + rec-1; sEx 150*, were isolated. These heterozygous Roller hermaphrodites were maintained for several generations. A Single Roller hermaphrodite was isolated and let to lay eggs. These eggs were saved in order to establish a permanent line. After a few hours, the hermaphrodite was out-crossed to the tester strain, *rec-1/ rec-1; dpy-11 unc-42* males. From this latter cross, several non-Roller hermaphrodites were plated one per plate and transferred to new plates for three days. Those hermaphrodites that segregated *dpy-11 unc-42* double mutants were selected and the recombination frequency between *dpy-11* and *unc-42* was determined for six individuals, by scoring all the progeny. If the single hermaphrodite tested is heterozygotes or homozygotes for the *rec-1* mutation, few or all six individuals tested will show a high recombination frequency. If an individual gives only the normal recombination frequency between *dpy-11* and *unc-42*, that will indicate that the individual does not carry the *rec-1* mutation. One such individual was identified to establish KR3163: *lev-11/ lev-11; sEx 150.* The extrachromosomal array, *sEx150*, carries both BO467 and ZK340 cosmids which map within *eDf24* deletion. The *rec-1* mutation maps within the *eDf24* deletion; if the extrachromosomal array carries the *rec-1* gene, it may rescue the Rec-1 phenotype and not be detected by determining the

recombination frequency. In order to avoid this possibility, non-rollers were selected to determine the recombination frequency. The same approach was taken to construct *rec-1; hIs7.*

Wild type males were out crossed to *hIn1[unc-101]* hermaphrodites and the resulting males were out-crossed to *lev-11; sEx150*, Roller hermaphrodites. From the latter cross, out crossed Roller hermaphrodites were plated one per plate. From one of the plates, a single Roller hermaphrodite was isolated to establish the line KR3638: *hIn1[unc-101]; sEx150.* Wild type males were out crossed to *unc-54* homozygous hermaphrodites and the resulting heterozygous (*unc-54*/ +) males were out crossed to *hIn1[unc-101];sEx150* hermaphrodites. From the latter cross, Roller hermaphrodites were plated one per plate. One single Roller that segregates both Unc-101 and Unc-54 in the next generation was isolated to establish KR3669: *hIn1[unc-101]/ unc-54;sEx150.* A single phenotypically wild type hermaphrodite was isolated from KR3669 strain to esatblish KR3647. The genotype of KR3647 is *hIn1[unc-101]/ unc-54* and segregates phenotypically wild type heterozygotes, Unc-101 and Unc-54.

Recombination Frequency Calculation: All the progeny of individual heterozygous hermaphrodites were scored as described by Rose and Baillie (1979a). Crossover frequency was calculated using the formula $p = 1 - (1-2R)^{1/2}$ (Brenner, 1974), R = 2 X (the number of one class of visible recombinants)/ (the total number of progeny). Genetic distance in map units was calculated by using the formula 100Xp. 95% confidence intervals were calculated using Crow and Gardner (1959).

Egg-hatching frequency: N2, Rec-1, Him-1 and Rec-1 Him-1 adult hermaphrodites, which had just molted, were plated individually, allowed to lay eggs for an 18-hr period, transferred to new plates, allowed to lay eggs for a second 18-hr period, and removed. After removing the hermaphrodites from the plates, the number of eggs was counted. After 24-hrs the number of hatched progeny was counted.

X-chromosome nondisjunction frequency: Males, which are XO, result from *X*-chromosome nondisjunction or loss. As we did not distinguish between these possibilities, we used the number of male progeny as a measure of nondisjunction frequency.

Reisolation of lethals linked to *hIn1*[*unc-101*]: Males from mutant strains, (*hIn1*[*unc-101*] Δx/ *unc-54*), were out crossed to *unc-54* homozygotes. In the F1 generation, phenotypically wild type males were isolated and crossed back to *unc-54* homozygotes. From the latter cross, a single F1, phenotypically wild type hermaphrodite (*hIn1*[*unc-101*] Δ / *unc-54)* was isolated to establish a reisolated line.

Reisolation of lethals linked to *unc-54*: Males from mutant strains, (*hIn1*[*unc-101*]/ *unc-54* Δy), were out crossed to *hIn1*[*unc-101*] homozygotes. In the F1 generation, phenotypically wild type looking males were isolated and out crossed back to *hIn1*[*unc-101*] homozygotes. From the

latter cross, a single F1, phenotypically wild type hermaphrodite (*hIn1*[*unc-101*]/ *unc-54* Δ) was isolated to establish a reisolated line.

Isolation of *eDf24* arrested embryos: Template DNA was prepared from *eDf24* arrested embryos. Embryos were isolated from the strain [CB2781: *unc-54(e1092) let-208/ eDf24*]. This strain is phenotypically wild type and segregates wild type heterozygotes, *let-208* L1 arrest and arrested embryos. Ten to 15 heterozygotes were left to lay eggs on a single plate for five to six hours (about 100 progeny); then the hermaphrodites were removed from the plate. The next day the plate was examined for un-hatched eggs, which were picked individually into PCR lysis buffer; DNA was prepared from each egg and either used in a PCR reaction or frozen for future use.

Polymerase Chain Reaction: DNA from wild-type and mutant strains was prepared and used as template as described by Barstead, *et al.* (1991). Polymerase chain reactions were carried out in a Perkin-Elmer/Cetus PCR machine for 36 cycles of denaturation (95°, 45 seconds), annealing (60°, 30 Seconds), and extension (72°, 3 minute) followed by extension at 72° for 7 minutes. Reactions were performed with *Taq* Polymerase and accompanying buffer system from Promega or with *Pfu* Polymerase and buffer system from Stratagene or with Expand™ Long Template enzyme mixture and buffer system from Boehringer Mannheim.

Agarose Gel Electrophoresis: PCR products were size separated in 0.5-1.5% w:v agarose gels in 0.5X TBE buffer [1X TBE contains 0.89 M Tris, 0.89 M Boric acid, 1mM EDTA (pH 8.0)] with

approximately 50 ng/ ml ethidium bromide. Gels were electrophoresed in 0.5X TBE running buffer at approximately 6V/ cm. One kb standard marker from Gibco-BRL was used. DNA bands were photographed using 300nm UV illumination.

Reverse Transcription Polymerase Chain Reaction (RT-PCR): Synchronized cultures of different developmental stages of wild-type were prepared by following the protocol described by (Lewis and Fleming, 1995). Total RNA was isolated from different developmental stages of wild type and mixed population of Rec-1 strains, by following the protocol described by Burdine and Stern (Pers. Comm.) using TRIZOL reagent from Gibco-BRL. Total RNA was used to synthesize cDNA by using 5'-RACE kit from Gibco-BRL. These cDNAs were used as template for Polymerase Chain reaction with gene specific primer sets as described above.

RNA interference

Preparation of double stranded RNA: Primers, specific for different genes, were used to amplify the genomic DNA or cDNA. These PCR products were cloned into Blue Script vector. This clone was used to make double stranded (DS) RNA by following the protocol described in (Fire *et al.* 1998). Some of the primers were tagged with T7 or T3 promoter sequence, and the PCR products from these primers were purified using Qiagen columns. The purified PCR products were used as template to synthesize double stranded RNA using the same protocol described in Fire *et al.* (1998). RNA was synthesized using T3 and T7 polymerase. Sense and antisense annealing was carried out in injection buffer (Mello and Fire, 1995) at 37° for 10-30 min. Formation of predominantly double-stranded material was confirmed by testing migration on a standard

(nondenaturing) agarose gel: for each RNA pair, gel mobility was shifted to that expected for dsRNA of the appropriate length.

Micro injection of double stranded (ds) RNA: The ds RNA was injected into the distal arm of one or both gonad arms in adult wild type and *dpy-11 unc-42*/ + + hermaphrodites after the method of Mello *et al.* (1991). Injected worms were transferred 10hrs and 22hrs after injection. So the first brood was 0-10 hrs, second brood was 10-22 hrs and third brood was 22 hrs after injection. The F1 generation was then examined. The purpose of injecting into the *dpy-11 unc-42* heterozygotes was to calculate the recombination frequency in the F1 and F2 generations of injected worms.

Restriction enzyme digestion: DNA was digested with restriction enzyme(s) supplied by either Pharmacia or New England Biolabs. Digests were performed with a three-fold excess of enzyme (i.e., 3 units/μg of DNA) in the appropriate buffer (supplied by the manufacturer) for at least one hour at the recommended temperature. Bovine serum albumin (BSA) was also included in restriction digest at a concentration of 100μg/ ml when recommended by the restriction enzyme manufacturer.

Mutagenesis

UV-Trimethylpsoralen: Worms were suspended in 2ug/ml TMP in acetone for one hour in the dark with occasional agitation to increase aeration; then the worms were transferred to an empty Petri plate and irradiated with ultraviolet light ,at 340/cm2, for 90 sec (D. Moerman pers. comm.).

After mutagenesis, worms were plated on to agar plates seeded with OP50 and incubated at 16° for overnight. This procedure was used for *hIn1* balanced screen. The method described by Yandell *et al.* (1994) was used for the screen using the dominant Unc-54 strain).

Screening for lethals

Using *hIn1* as a balancer: The strain used in this screen has markers on both of the chromosome I homologues (*hIn1[unc-101]/ unc-54*). Phenotypically they are wild type, and segregate wild type, Unc-101 and Unc-54 progeny in the next generation Synchronized young-adult hermaphrodites were collected and washed with M9 buffer. Mutagenesis was performed as described above. After recovery, phenotypically wild type hermaphrodites were plated three per plate. If a deletion is generated that affects a lethal site within *hIn1* balanced region in one of the homologue, homozygous for that deletion will be lethal. This deletion can be detected by identifying heterozygotes that fail to segregate either Unc-101 or Unc-54 in the F1 generation. In the F1 generation, all the phenotypically wild type hermaphrodites were plated one per plate. After two to three days the plates were examined and those that did not segregate either Unc-101 or Unc-54 progeny were selected as candidates. They were analyzed further and the arrest stage was determined.

Complementation between *rec-1* and new deletions: Males from the new deletion strain were crossed to the tester strains: *rec-1; dpy-5 unc-13* or *rec-1; dpy-11 unc-42*. The resulting F1 heterozygotes were plated, one per plate, and transferred to new plates for three days. About 50% of the F1 hermaprodites will carry the new deletion (test class); the other 50% will not (control

class). The test class will segregate deletion homozygotes, which would arrest at one of the developmental stages (it will be different for different deletions). The control class will not segregate any arrest stages. All the progeny were scored and the recombination frequency was determined for each F1 individuals. If the *rec-1* gene is deleted by a new deletion, the test scoring will show the Rec-1 phenotype and the control will be similar to wild type.

CHAPTER III

CHARACTERIZATION OF REC-1: INTERACTION WITH OTHER GENES

BACKGROUND

Meiosis ensures the faithful transmission of the genetic material to future offspring through mechanisms of pairing, recombination and disjunction. Central to the process is meiotic exchange. Both the frequency and distribution of meiotic exchange events can be altered by a number of factors, affecting the phenotype of the resulting progeny. In *Caenorhabditis elegans*, several mutants that alter recombination frequency are known, but only one has been described which alters the distribution of crossing over while retaining normal frequency (Zetka and Rose, 1995b; reviewed by Zetka and Rose, 1995a). *C. elegans* is a particularly good organism for the study of where, along a chromosome, a crossover is most likely to occur. Each autosomal pair has only one detectable crossover, and where that crossover occurred can be measured reproducibly (Rose and Baillie, 1979a), forming the basis of the meiotic recombination map. Reduction in the frequency of crossing over per unit length of DNA near the center of the autosomes results in distinctive central gene clusters on the genetic map (Brenner, 1974). Recombinationally active regions flank the central gene cluster (Zetka and Rose, 1990; 1995a; Barnes *et al.* 1995). For chromosome I, the probability of crossing over in some intervals differs as much as ten-fold. Mutation in the *rec-1* gene eliminates this meiotic preference, resulting in a crossover distribution more similar to the

physical length of DNA between markers than to the meiotic distance (Fig 3) (Zetka and Rose, 1995b).

In other genetic backgrounds, reduction or elimination of crossing over from one region of the chromosome can result in an increased probability of exchange in a different region (McKim *et al.* 1988b; McKim *et al.* 1993a; Zetka and Rose, 1992). In one extreme case, crossing over was eliminated for all but a small region of chromosome I using combinations of rearrangements that greatly reduced crossing over. A normally small genetic interval increased to nearly 50 map units (Zetka and Rose, 1992). In this case, all of the events measured occurred in a region of the chromosome normally less favored. This experiment showed that regional preference can be altered, and that the requirement for having an exchange event is stronger than the preference for where it will occur. When crossing over was eliminated completely using overlapping rearrangements, homolog pairs disjoined at random, demonstrating the importance of exchange for proper segregation in meiosis (Zetka and Rose, 1992).

The correlation between crossing over and proper disjunction is most readily observed for the X chromosome, where nondisjunction events lead to XO progeny which are male (Hodgkin *et al* . 1979). In one case, the *e879* mutation in *him-1*, a two-fold reduction in crossing over along the X chromosome is accompanied by a high incidence of male progeny. In this mutant, the distribution of crossing over was severely affected, being greatly reduced in the left half of the chromosome, and undetectable in the right portion (Broverman and Meneely, 1994).

Having a crossover is necessary for proper disjunction, and where that crossover occurs may also have an affect. Retention of the meiotic pattern may influence proper disjunction of the homologs. In the Rec-1 mutant, there was a detectable increase in X-chromosome nondisjunction (Rattray and Rose, 1988). In *Drosophila melanogaster,* alterations in the distribution of crossing over along the X chromosome resulted in elevated chromosome nondisjunction (Koehler *et al.* 1996). In humans, reductions in recombination frequency have been associated with maternal nondisjunction at meiosis I for chromosome 21 (Lamb *et al.* 1997) and meiosis II for chromosome 15 (Robinson *et al.* 1998). In addition, alteration in the distribution of crossing over has been shown to correlate with chromosome 21 nondisjunction (Lamb *et al.* 1996).

How is the meiotic pattern established? A clue is provided by the *rec-1* mutant, which alters the pattern of crossing over (Rose and Baillie, 1979b; Zetka and Rose, 1995b). In this strain although the distribution is dramatically altered, the frequency is unchanged (Zetka and Rose, 1995b). Thus, the *rec-1* gene product is not required for the production of a crossover. More likely it functions at an earlier stage facilitating initiation of the exchange, or at a later stage influencing the point of resolution. One aspect of the former proposal was tested by using the Spo-11 deletion strain described in Dernburg *et al.* 1998. In yeast, the SPO11 gene product is the enzyme responsible for making meiotic DSBs (Keeney *et al.* 1997). Spo11p is a homolog of the A subunit of a newly identified type II topoisomerase (Bergerat *et al.* 1997). The *C. elegans* homolog of the yeast gene is required for meiotic exchange, and radiation-induced breaks partially alleviate the dependence (Dernburg *et al.* 1998). Meiotic map expansion results after treatment with ionizing radiation (Kim and Rose 1987), and resembles the increased crossover frequency observed in Rec-

1, for markers within the autosomal gene clusters. Thus, quite possibly Rec-1 might act in a manner similar to treatment with radiation, and cause breaks, which become substrates for crossover events. This possibility was investigated by using *rec-1; spo-11* double mutant. *him-1* encodes a member of the SMC (structural maintenance of chromosomes) (B. Meyer, pers. comm.). In *him-1* (*e879*) mutant, total length of the X chromosome is one third of the length of the wild type (Browerman and Meneely,1994). It was investigated if *rec-1* mutant can alter the crossover distribution on the X chromosome in *him-1* (*e879*) background.

RESULTS

Rec-1 alters the crossover distribution on the X-chromosome:

The differences in the amount of crossing over per physical length of DNA are not as great for different regions of the X chromosome as the autosomes. It is possible that *rec-1(s180)* may not have any effect on the X chromosome. In order to test this possibility, the crossover frequency between seven pairs of X-linked markers in a Rec-1 mutant background was determined. Crossover frequencies between markers in the left portion of the chromosome were reduced from 3.5 to 1.8 m.u., from 5.3 to 3.5 m.u., and from 14.1 to 10 m.u. for *unc-1 dpy-3, dpy-3 unc-20,* and *unc-1 lon-2*, respectively in the Rec-1 background (Table 1 and Fig 4, 5). In contrast, crossing over increased between *lon-2* and *dpy-6* (from 6.6 m.u. to 10 m.u.) and between *lon-2* and *unc-115* (from 9.6 m.u. to 11.8 m.u.). There was no change detected between *unc-7* and *lin-15* (1.9 m.u. and 1.8 m.u.). The total length of the genetic map was unchanged, 48.7 m.u. in N2, and 50.3 m.u. in Rec-1 (Table 2). Across the *unc-1* to *unc-115* interval, the number of kilobase pairs (kb) per map unit varied less between sets of markers in the Rec-1 background (411-489), than in N2 (292-602) (Table 2). This results indicates that for this region of the X chromosome, mutant Rec-1 shifts the crossover distribution in a direction that is closer to the physical distance. The results demonstrate that Rec-1 affects all chromosomes, and is not autosomal-specific.

Rec-1 does not introduce double strand breaks:

Since Rec-1 appears to alter the meiotic pattern in a manner similar to the effect of ionizing radiation, it was tested whether it would reverse the phenotype of the *C. elegans* Spo-ll mutant

Figure 4: Marey map of X chromosome in *C. elegans* (Adapted from Barnes *et al.* 1995), shows the cross over distribution in wild type and Rec-1 mutant backgrounds. The left most marker tested is *unc-1* and taken as zero map unit for the ease of plotting. Few confidence intervals (95%) in the Rec-1 background are not overlapping with the wild type and indicate that the cross over distribution is altered in the Rec-1 mutant.

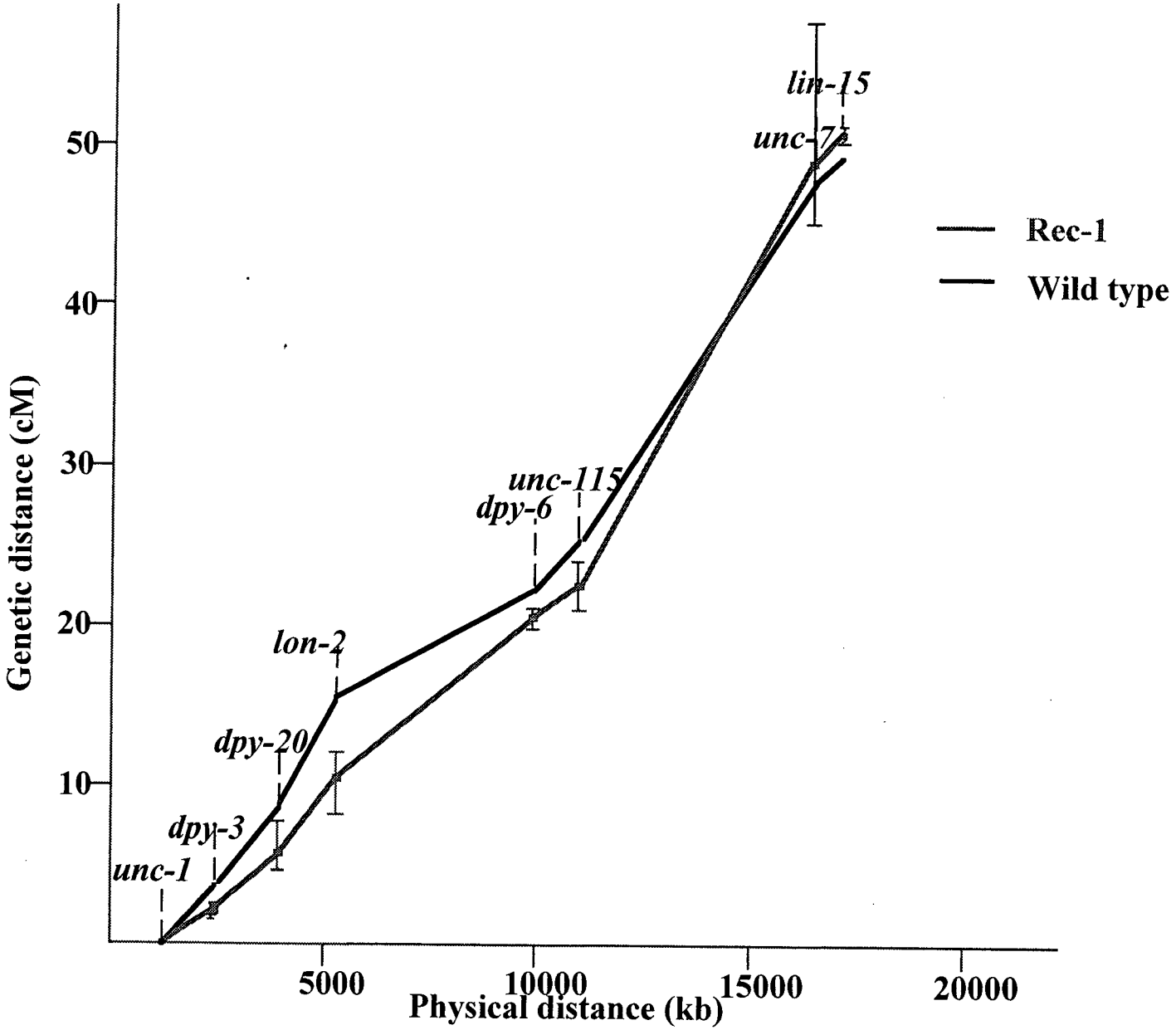

lin-15
unc-7
unc-115
dpy-6
lon-2
dpy-20
dpy-3
unc-1
50
40
30
20
10
Genetic distance (cM)
5000
10000
15000
20000
Physical distance (kb)
Rec-1
Wild type

Fig 4

Figure 5: Crossover distribution on the X chromosome in N2, Rec-1, Him-1, and Rec-1 Him-1 mutants.

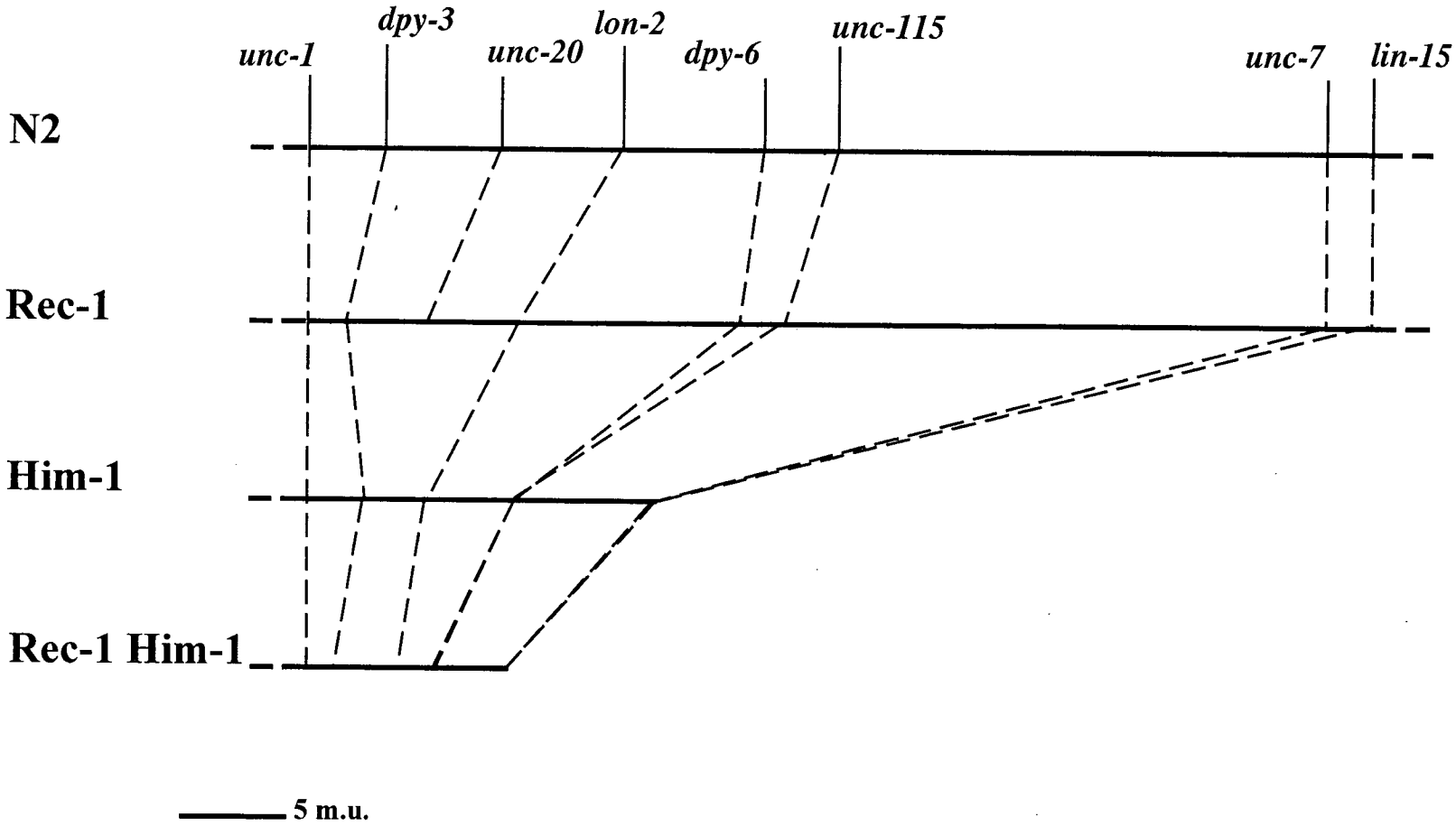

unc-1
dpy-3
unc-20
lon-2
dpy-6
unc-115
unc-7
lin-15
N2
Rec-1
Him-1
Rec-1 Him-1
5 m.u.

Fig 5

Table 1

Effect of the *rec-1* mutation on *X* chromosome crossover frequency

Parental Genotype	Total progeny	Recombinants (one class)	Distance (m.u)	C.I. 95%
unc-1 dpy-3 / + +	1308	23 Dpy-3	3.5	2.3 - 5.3
rec-1/rec-1; unc-1 dpy-3 / + +	4324	38 Dpy-3	1.8	1.2 - 2.4
dpy-3 unc-20 / + +	1286	33 Dpy-3	5.3	3.6 - 7.3
rec-1/rec-1; dpy-3 unc-20 / + +	3509	61 Dpy-3	3.5	2.7 - 4.5
unc-1 lon-2 / + +	2722	179 Lon-2	14.2	11.9 - 16.4
rec-1/ rec-1; unc-1 lon-2 / + +	2826	135 Lon-2	10.1	8.3 - 11.8
lon-2 dpy-6 / + +	4608	146 Lon-2	6.6	5.4 - 7.7
rec-1/rec-1; lon-2 dpy-6 / + +	5578	265 Lon-2	10.0	8.8 - 10.3
lon-2 unc-115 / + +	1324	61 Lon-2	9.6	7.4 - 12.4
rec-1/ rec-1; lon-2 unc-115 / + +	2611	145 Lon-2	11.8	9.7 - 13.9
lon-2 unc-7 lin-15 / + + +	1588	217 Lon-2	32.6	27.9 - 38.3
		11 Lin-15	1.4	0.6 - 2.4
rec-1/ rec-1; lon-2 unc-7 lin-15/ + + +	1572	244 Lon-2	38.4	32.3 - 45
		5 Lin-15	0.6	0.3 - 1.4
unc-7 lin-15 / + +	4809	46 Unc-7	1.9	1.4 - 2.5
rec-1/rec-1; unc-7 lin-15 / + +	5847	52 Unc-7	1.8	1.3 - 2.3

Table 2

Physical and Genetic distances

	unc-1 dpy-3[a]	*dpy-3 unc-20*[b]	[A] *unc-1 lon-2*	*lon-2 dpy-6*[c]	*lon-2 unc-115*	[B] *lon-2 unc-7*	[C] *unc-7 lin-15*	*unc-1 lin-15*
Physical	1237	1588	4144	4789	5778	11362	628	16134
Genetic m.u.	3.5	5.3	14.2	6.6	9.6	32.6	1.9	48.65
kb/ w.t m.u.	353	300	292	725	602	348	330	331
rec-1 m.u.	1.8	3.5	10.1	10	11.8	38.4	1.8	50.26
kb/ *rec-1* m.u.	687	453	411	478	489	296	348	321
him-1 m.u.	2.8	-	5.4	4.3	3.9	10.6	0	16
kb/ *him-1* m.u.	441	-	767	1113	1481	1071	-	1008
him-1 rec-1 m.u.	1	-	4.4	1.8	1.8	4.8	0.1	9.26
kb/ *him-1 rec-1* m.u.	1237	-	950	2660	3210	2367	6280	1742

[a] kb between *che-21* and *lin-32*. [b] kb between *unc-2* and *mec-18*. [c] kb between *mec-10* and *unc-115*.

Distances [A, B, C] were used to calculate the distance between Unc-1 and Lin-15.

described by Dernburg *et al.* (1998). Homozygous *rec-1* males were crossed to *rec-1*(I); *unc-43 unc-22*(IV) homozygotes. Out-cross males, *rec-1; unc-43 unc-22/ + +* were picked and crossed to *spo-11 (ok79)* IV/ *nT1 [unc-x (n574) let-x (X)]* (IV; V). From this cross, 19 phenotypically wild-type hermaphrodites were plated individually. These were transferred to fresh plates on each of four days. The Unc marking *nT1* is dominant, and thus two types of phenotypically Wild out-cross progeny were expected: *rec-1 (s180)/ +; + spo-11+/ unc-43+ unc-22;* and *rec-1 (s180)/ +; spo-11/ +*, in addition to the self-cross *spo-11/ spo-11*. Wilds, which segregate Unc-43 Unc-22 progeny were isolated. From each of two separate lines, 27 Wild hermaphrodites, were plated at the L4 stage, one per plate, and transferred to new plates for four days. Three genotypes of Wild type hermaphrodites, segregating Unc-43 Unc-22 progeny were expected: (1) *rec-1/ rec-1; + spo-11+/ unc-43+ unc-22* (the genotype of interest), (2) *rec-1/ +; + spo-11+/ unc-43+ unc-22,* and (3) *+/ +; + spo-11+/ unc-43+ unc-22*. Individuals grouped into two classes based on the crossover frequency between *unc-43* and *unc-22*, normal (0.89[0.44-1.6] for n=7 individuals) and high (5.48[3.7-7.6] for n=4 individuals). Rec-1 Spo-11 homozygotes were identified in the segregants of three of the high crossover frequency individuals (Fig 6). From each of the three lines, 36 non-Unc progeny were plated one per plate. They were of two types. Of the 108 hermaphrodites, 80 were fertile and produced Unc progeny. The remaining 28 produced mainly arrested embryos. Because of the sterility, crossover frequency in the double mutant could not be examined. The phenotype of the double mutant was indistinguishable from that of the single Spo-11 mutant (Table 3). Thus, Rec-1 was incapable of rescuing, even partially, the Spo-11 defect. Production of non-Spo-11 DNA breaks is unlikely to be the cause of the Rec-1 phenotype. It is possible that

Figure 6: Isolation of *rec-1; spo-11* homozygous by determining the distance between *unc-43* and *unc-22*. The genetic distance between *unc-43* and *unc-22*, in the Rec-1 background, is 7.1 m.u. (control) (in wild type background, the distance is less than 1 m.u. data not shown). Individuals #7, #8, #9 and #10 show Rec-1 phenotype; individuals #7, #8 and #9 were selected to test the effect of *rec-1* on Spo-11 phenotype.

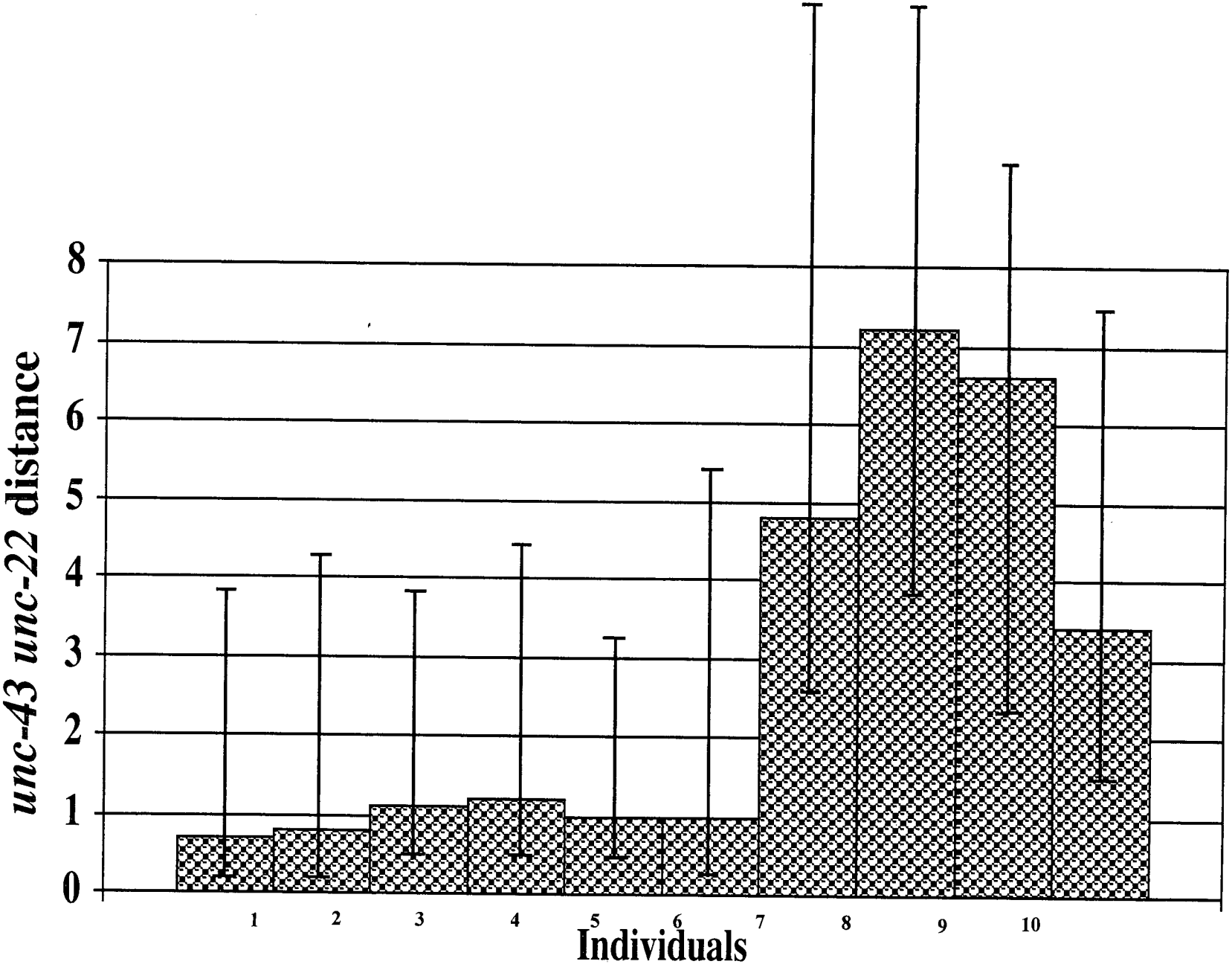


Fig 6

Table 3

Progeny analysis of Spo-11 and Rec-1 Spo-11

Genotype	Embryonic arrest	Larval arrest	adults
spo-11/ spo-11	2377	23	1
rec-1/rec-1; spo-11/ spo-11	2974	31	1

REC-1 is involved in determining the position of DSB and SPO-11 is necessary for making the breaks.

Him-1 has little effect on autosomal crossing over:

The genetic interaction between Rec-1 and Him-1, a gene believed to play a role in the maintenance of normal chromosome structure, was investigated. The Rec-1 phenotype is strongest for autosomal intervals. Him-1, however, causes only a 10% increase in autosomal crossing over, overall (Hodgkin *et al.* 1979). Crossing over between *dpy-5 unc-13* on chromosome I, and *dpy-11 unc-42* on chromosome V was measured. There was no effect of Him-1 on the chromosome I interval.

For the chromosome V interval, crossing over in the double mutant increased no more than previously observed for Rec-1 alone (Rose and Baillie, 1979b). Thus, this data (Table 4) are consistent with the interpretation that *e879* has little effect on autosomal crossing over for the intervals examined.

Despite the fact that autosomal crossing over is not dramatically affected in Him-1 mutants, hermaphrodites produce inviable zygotes (Hodgkin *et al.*1979). It was observed that approximately 10% inviable embryos in Him-1 compared to 3% in Rec-1. In the double mutant, the hatching frequency did not significantly differ from Him-1 in the first 18-hrs of egg-laying. During the second 18 hrs of egg-laying, fewer eggs hatched (Table 5). It has been shown previously that X

Table 4

Effect of *rec-1*, *him-1* and *him-1 rec-1* double mutants on autosomal crossover frequency

Parental Genotype	Total progeny	Recombinants	Distance (m.u)	C.I 95%
dpy-5 unc-13/ + + (I)	3087	25 Dpy-5	1.6	1.0 - 2.3
dpy-5 unc-13 rec-1/ + + rec-1	2627	79 Dpy-5	6.2	4.9 - 7.7
him-1 dpy-5 unc-13/ him-1 + +	2046	18 Dpy-5	1.7	1.0 - 2.7
him-1 dpy-5 unc-13 rec-1/ him-1 + + rec-1	4476	127 Dpy-5	5.8	4.7 - 6.9
dpy-11 unc-42/ + + (V)	4731	72 Dpy-5	3.0	2.3 - 3.8
rec-1; dpy-11 unc-42/ + +	1053	31 Dpy-11	6	4.1 - 8.6
him-1; dpy-11 unc-42 / + +	1802	34 Dpy-11	3.8	2.6 - 5.3
him-1 rec-1; dpy-11 unc-42/ + +	4859	197 Dpy-11	8.4	5.9 - 8

Table 5

Egg-hatching frequency in wild-type and meiotic mutants

Genotype	Hatched eggs/ total eggs first 18-hr period	Frequency	C.I. 95%	Hatched eggs/ total eggs second 18-hr period	Frequency	C. I. 95%
N2	300/ 310	96%	86% - 100%	450/ 508	88%	79% - 97%
rec-1	284/ 290	97%	86% - 100%	576/ 590	97%	92% – 100%
him-1	159/ 182	87%	74% - 100%	374/ 408	91%	82% – 99%
rec-1 him-1	360/413	87%	75% - 99%	345/417	82%	72% - 92%

chromosome nondisjunction increases with the age of the hermaphrodite (Rose and Baillie, 1979a). Thus, increasing chromosomal nondisjunction with age may have been a factor in this experiment.

X-chromosome crossing over is severely reduced in Rec-1 Him-1:

Crossing over along the X chromosome in Rec-1 Him-1 double mutants was examined. In Him-1, the genetic length of the X chromosome is much shorter than in N2. The distance from *unc-1* to *lin-15* measured 16 map units, one-third the wild-type distance. In the following intervals, Him-1 reduced the map distance: from 3.5 to 2.8 for *unc-1 dpy-3*, from 14.2 to 5.4 for *unc-1 lon-2*, from 32.6 to 10.6 for *lon-2 unc-7*, and from 1.8 to undetectable for *unc-7 lin-15*. In Him-1 Rec-1 double mutants, a total distance across the chromosome of approximately 9 map units was observed, nearly half the Him-1 distance (Fig. 5; Table 2). Crossing over in the double was less than in the Him-1 single mutant for all intervals tested (Table 6). In Rec-1 Him-1, the frequency was reduced to 1 m.u. for *unc-1 dpy-3*, 4.4 m.u. for *unc-1 lon-2*, and 1.8 m.u. for *lon-2 unc-115*. The decrease in the frequency of crossing over was greater in Rec-1 Him-1 than in Him-1. In the double mutant, the distribution of crossovers did not resemble the Rec-1 pattern, and was not proportional to physical distance (Table 2). Across the *lon-2 lin-15* region, the distribution was similar to that of Him-1 (Fig 7).

Surprisingly, fewer male progeny were observed in the Rec-1 Him-1 double than in Him-1 alone (approximately 15% compared to 24%; Table 7). With regard to this phenotype, Rec-1 appears to partially rescue the Him-1 defect.

Table 6

Effect of *him-1* and *him-1 rec-1* double mutants on *X* chromosome crossover frequency

Parental Genotype	Total progeny	Recombinants	Distance (m.u)	C . I 95%
him-1/ him-1; unc-1 dpy-3 / + +	1873	26 Dpy	2.8	1.8 – 4.1
him-1 rec-1/ him-1 rec-1; unc-1 dpy-3 / + +	4357	31 Unc 14 Dpy	1	0.7 - 1.3
him-1/ him-1; unc-1 lon-2 / + +	1770	47 Lon-2	5.4	4.0- 7.3
him-1 rec-1/ him-1rec-1; unc-1 lon-2 / + +	1405	30 Lon-2	4.4	2.8 – 6.1
him-1/him-1; lon-2 dpy-6 / + +	1026	22 Lon-2	4.3	2.7 – 6.4
him-1 rec-1/ him-1 rec-1; lon-2 dpy-6 / + +	4633	42 Lon-2	1.8	1.3 - 2.4
him-1/ him-1; lon-2 unc-115 / + +	1767	34 Lon-2	3.9	2.7 –5.5
him-1 rec-1/ him-1 rec-1; lon-2 unc-115 / + +	1499	14 Lon-2	1.8	1.0 – 3.1
him-1/ him-1; lon-2 unc-7 lin-15 / + + +	1473	74 Lon-2	10.6	8.3 – 13.3
		0 Lin-15	0	0 – 0.4
him-1 rec-1/ him-1 rec-1; lon-2 unc-7 lin-15/ + + +	1487	35 Lon-2	4.8	3.3 – 6.7
		1 Lin-15	0.1	0.0 – 0.7

Figure 7. A plot of the kilobase pairs per map unit for N2, Rec-1, Him-1, and Rec-1 Him-1. The coding region for the *dpy-6* gene not has been identified in the DNA sequence. The physical position was estimated as midway between *mec-10* and *unc-115*.

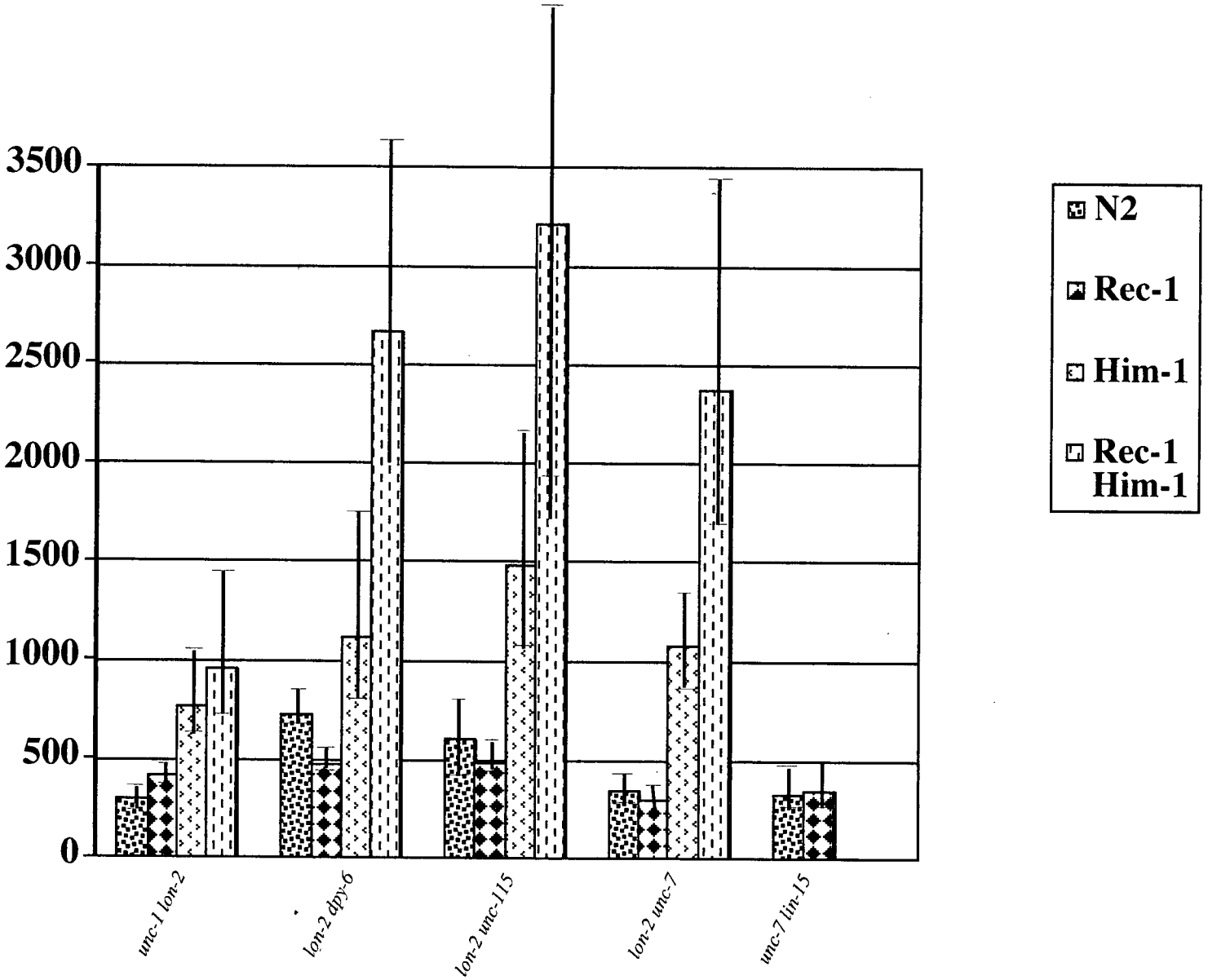

3500
3000
2500
2000
1500
1000
500
0
N2
Rec-1
Him-1
Rec-1 Him-1
unc-1 lon-2
lon-2 dpy-6
lon-2 unc-115
lon-2 unc-7
unc-7 lin-15

Fig 7

Table 7

X- chromosome non-disjunction

Genotype	Males [a]/ total progeny	Frequency	C.I. 95%
N2	3/ 2772	0.1%	0.0 – 0.3
rec-1	9/ 2800	0.3%	0.2 – 0.6
him-1	354/ 1460	24.2%	23.1 – 25.2
rec-1 him-1	269/ 1808	14.8%	13 – 16.7

[a] number of males will represent the number of X-chromosome non-disjunction.

DISCUSSION

Possible mechanisms of *rec-1* gene function through analysis of genetic interactions were investigated. The gene was originally identified by means of a spontaneous mutant, which increased crossing over and apparent gene conversion between markers in autosomal gene clusters (Rose and Baillie, 1979b; Rattray and Rose, 1988). Subsequent investigation using markers covering the genetic length of autosome I, revealed that the total frequency of crossing over was not altered. The genetic length of the chromosome remained at approximately 50 map units, or one crossover per homolog pair per meiosis. However, the distribution of exchange was dramatically altered (Zetka and Rose, 1995a). Large differences in the amount of crossing over per unit length of DNA for central autosomal regions and flanking arms have been well documented (Brenner 1974; Barnes *et al.* 1995). Zetka and Rose, 1995b showed that for chromosome I, the number of kb per map unit varied more than ten-fold for different regions (96 kb/m.u. for *unc-101 unc-54* to 1291 kb/m.u. for *dpy-5 unc-13*). In Rec-1 these differences disappeared, and most intervals came close to the chromosomal average of 270 kb per map unit. With the exception of the left end of the chromosome, the intervals differed very little (254 kb/m.u. to 328 kb/m.u.). Thus, in Rec-1 the probabilility of a crossover correlated better with the physical distance between markers than it did with the chromosome I meiotic map.

Since Rec-1 effectively eliminated the autosomal meiotic pattern, and since the distribution of crossover events on the X-chromosome does not show a strong meiotic cluster, it maybe possible that Rec-1 is autosomal specific. Mutants specifically affecting the meiotic behavior of the X

chromosome have been described, and differences between the X-chromosome and the autosomes reviewed in Zetka and Rose (1995a). In the case of Rec-1, although the differences were not nearly as great as for the autosomes, changes in the distribution of crossing over for the X chromosome were observed. Between *unc-1* and *unc-115,* recombination frequency shifted in a direction closer to the physical distance between markers. In this region the number of kb per map unit varied less in Rec-1 than in N2. The amount of crossing over between the outside markers, *unc-1* and *lin-15,* was unchanged. Thus, as for the autosomes, the total frequency of crossing over is unaffected. This shows that *rec-1* affects all the chromosomes, and that factors other than the *rec-1* gene product are responsible for the differences in the meiotic organization of the autosomes versus the X chromosome.

For most of the chromosome, a Rec-1 map that correlated better with the physical distance than with the meiotic distance was observed. An exception was the left end, in the *unc-1 dpy-3* region. In this interval, the Rec-1 distance did not correlate with the physical distance. There appeared to be too few crossovers for the amount of DNA. One possibility is that the genetic and physical map correlation is inaccurate for this interval as the *dpy-3* gene has not been identified in the DNA sequence. The other possibility is that this interval responds differently than the rest of the chromosome in Rec-1. Located near here is a site involved in meiotic disjunction of the X-chromosome (Herman *et al.*, 1982; McKim *et al.*, 1988b), and shown to function in pairing, crossing over and disjunction (Villeneuve, 1994). Normally, the X chromosome in hermaphrodites experiences only a single crossover; however in cases where a second event occurs, it is near the

left end (P. Meneely, pers. comm.). Thus, this region may differ from the rest of the chromosome with regard to the distribution of crossing over.

Genetic map expansion across the central autosomal markers in the Rec-1 mutant has a number of similarities to treatment with ionizing radiation (Rose and Baillie, 1979b; Kim and Rose, 1987; Zetka and Rose, 1995a). The possibility that excess DNA breaks might be responsible for the Rec-1 phenotype was investigated. Although Rec-1 is recessive, and is unlikely to be a gain of function, a repair defect producing or accumulating breaks might randomize the position of the crossover. In yeast, the SPO11 gene product is the enzyme responsible for making meiotic DSBs (Keeney *et al.* 1997). Spo11p is a homolog of the A subunit of a newly identified type II topoisomerase (Bergerat *et al.* 1997). The *C. elegans* homolog of the yeast gene is required for meiotic exchange, and radiation-induced breaks partially alleviate the dependence (Dernburg *et al.* 1998). It was investigated whether Rec-1 could substitute for radiation-induced breaks and rescue the *C. elegans* Spo-11 phenotype. A Rec-1 Spo-11 double mutant was generated, and observed complete epistasis of Spo-11. The double mutant produced arrested embryos and larvae, but no fertile progeny. Based on the Rec-1 Spo-11 result, it seems unlikely that anomalous DNA breaks, which can become substrates for recombination, are the cause of the altered crossover distribution.

Previously, crossover distribution in Rec-1 Him-14 mutants was investigated (Zetka and Rose, 1995a). Loss of *him-14* function severely reduces crossing over, resulting in lack of chiasmata between homologs and consequent missegregation (Zalevsky *et al.* 1999). Cytological analysis showed that homologs are paired and aligned in *him-14* pachytene nuclei, indicating that *him-14* is

not needed to establish pairing or synapsis and likely has a more direct role in crossover formation. *him-14* encodes a germline-specific member of the MutS family of DNA mismatch repair (MMR) proteins. It has no apparent role in MMR, but is required to promote crossing over during meiosis, and has been implicated in branch migration during the recombination process. Rec-1 Him-14 doubles were defective in both the frequency and distribution of meiotic exchange, producing a Rec Him phenotype, suggesting that the two genes function independently (Zetka and Rose, 1995a). It is unlikely that *rec-1* is required for the exchange reaction itself, since the total number of events is normal in Rec-1.

In contrast to the Rec-1 Him-14 result, a more severe reduction was observed in crossover frequency in the Rec-1 Him-1 double mutant. The distribution of events did not resemble the Rec-1 pattern, and thus, a straightforward Rec Him phenotype was not observed. Furthermore, the data show an increased number of inviable embryos in the last half of hermaphrodite egg-laying. Seemingly paradoxically there were a decreased number of males in the Rec Him progeny. Even higher numbers of nondisjoined X chromosomes would have been expected with a decrease in crossing over. However, because exchange events may also have been redistributed in the double mutant, this may have played a compensatory role. In addition to the *e879 him-1* allele, which causes X chromosome nondisjunction, lethal alleles have been recovered (Howell and Rose, 1990). Thus, in addition to the effects on the X chromosome which have been studied (Hodgkin *et al.* 1979; Broverman and Meneely, 1994), the gene product has an essential function, which may explain some of this data. In any event, Rec-1 appears to be dependent upon the wild-type

functioning of Him-1 to redistribute crossover events in the *unc-1 unc-115* region of the X-chromosome.

At present, Rec-1 remains the sole mutant to alter the distribution of crossing over without affecting the frequency, randomizing the meiotic pattern. The mechanism of *rec-1* function is not currently known. However, this analysis of the mutant phenotype supports a hypothesis that maintenance of proper chromosome structure is important for the establishment of the meiotic pattern.

CHAPTER IV

IN SEARCH OF *rec-1*:

ELIMINATION OF CANDIDATE GENES WITHIN *eDf24*

BACKGROUND

Meiosis generates haploid germ cells in diploid eukaryotic organisms. During meiosis, a complex series of events leads to precise pairing of the homologs and genetic recombination between them. The chiasmata generated by crossing over are crucial to the fidelity of chromosomal disjunction at the first meiotic division (Baker *et al.* 1976a). Studies from a variety of model organisms suggest that many meiotic systems possess mechanisms designed to ensure the segregation of those chromosomes that, for whatever reason, fail to undergo recombination (Hawley *et al.* 1993). Despite the accuracy of chiasmate segregation and the existence of achiasmate 'back-up' systems, failed meiotic chromosome segregation, or nondisjunction, has been observed in every organism in which it has been studied. The best known consequence of meiotic nondisjunction in human is Down syndrome (trisomy 21). Recent studies of human oocytes have demonstrated an enrichment for distal exchanges among meiosis I nondisjunction events (Hassold *et al.* 1995; Lamb *et al.* 1996) and for proximal exchanges among meiosis II events (MacDonald *et al.* 1994; Lamb *et al.* 1996). These studies illuminate the need for a careful study of exchange distribution in an organism. One good candidate for this study is *C. elegans*. In *C. elegans*, several mutants that alter recombination frequency are known, but only one has been described which alters the distribution of crossing over while retaining normal frequency (Zetka and Rose 1995b; reviewed by Zetka and

Rose 1995a). *C. elegans* is a particularly good organism for the study of where, along a chromosome, a crossover is most likely to occur. Each autosomal pair has only one detectable crossover, and where that crossover occurred can be measured reproducibly (Rose and Baillie 1979a), forming the basis of the meiotic recombination map. Reduction in the frequency of crossing over per unit length of DNA near the center of the autosomes results in distinctive central gene clusters on the genetic map (Brenner 1974). Recombinationally active regions flank the central gene cluster (Zetka and Rose 1990; 1995a; Barnes *et al.* 1995). For chromosome I, the probability of crossing over in some intervals differs as much as ten-fold. Only one mutation, *rec-1* in *C. elegans*, has been described to date that alters crossover distribution without altering frequency (Zetka and Rose 1995b). The *rec-1(s180)* mutation is completely recessive (Rose and Baillie, 1979b), and shows 10-15 m.u. linkage to the gene *unc-54*, located on the right end of LGI (A. Rose, unpublished results). The gene was originally identified by means of a spontaneous mutant, which increased crossing over and apparent gene conversion between markers in autosomal gene clusters (Rose and Baillie 1979b; Rattray and Rose 1988). Subsequent investigation using markers covering the genetic length of autosome I, revealed that the total frequency of crossing over was not altered. The genetic length of the chromosome remained at approximately 50 map units, or one crossover per homolog pair per meiosis. However, the distribution of exchange was dramatically altered (Zetka and Rose 1995b). Duplication mapping indicated that *sDp1,* but not *sDp2* carries the *rec-1(+)* gene (Zetka and Rose, 1995b). *sDp1* covers the right arm of LGI, including most of the centrally located cluster (Fig 8). Deletion mapping showed that the deletion *eDf24* fails to complement *rec-1* (Fig 8) (Zetka and Rose, 1995b). *sDp1* overlaps *eDf24*, indicating that *rec-1+* is in this overlapping region.

Figure 8: Genetic map of chromosome I and extension of *sDp1* and *sDp2*.

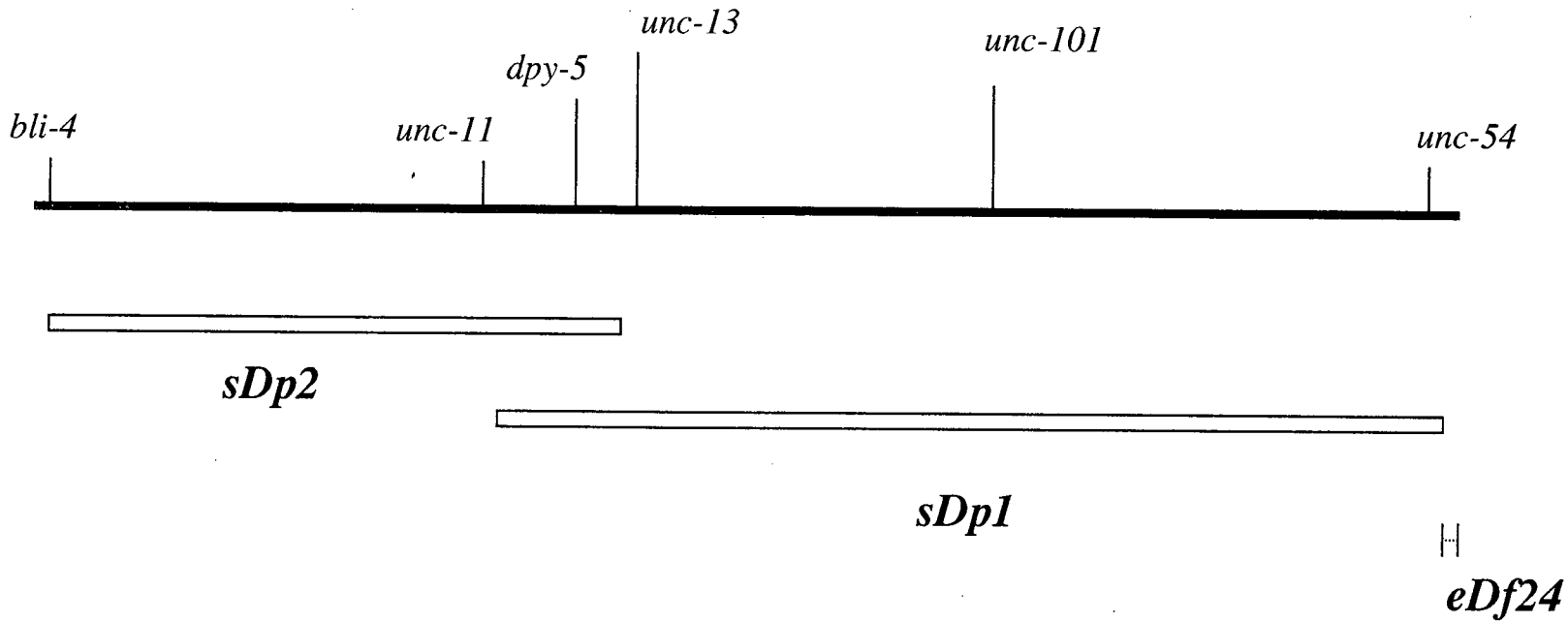


Fig 8

Understanding the function of REC-1 in *C. elegans* will shed light on the process involved in establishing crossover distribution. In order to identify the *rec-1* coding region, which maps within *eDf24*, the following approaches were taken. (1) The left breakpoint of *eDf24* was determined by PCR to identify candidate genes. (2) Rescue analysis was performed in order to test whether the cosmids that span *eDf24* (BO467 and ZK340) could rescue the lethal phenotype of *eDf24* homozygotes. Rescue would imply that the cosmids carry all the essential genes deleted by *eDf24* and that these genes are expressed at appropriate levels and at the appropriate time of development. (3) The coding regions of 12 genes within the *eDf24* region were amplified in order to determine if there is any detectable polymorphism in *rec-1* region. The *rec-1(s180)* allele was isolated as a spontaneous mutation from a strain in which Tc1 is active (Babity *et al.* 1990). It is possible that the *s180* mutation resulted from a Tc1 insertion into *rec-1* locus. (4) The temporal expression pattern of the genes within *eDf24* was determined by stage specific RT-PCR. It is possible that REC-1 has a meiosis-specific function and is expressed only in meiotic cells. Meiotic cell division occurs only in the gonad during the late developmental stages. (5) RNA interference was performed in order to determine the null phenotype of candidate genes within *eDf24* region. It has been shown that exogenous RNA can interfere with the function of its endogenous gene and create a loss of function phenotype (Fire *et al.* 1998). (6) Mutagenesis was performed in order to generate deletions with different breakpoints that overlap the essential locus or loci, and thus sub-divide the *eDf24* region; *eDf24* homozygotes arrest at embryonic development, indicating that there is at least one essential locus. If an essential locus deleted, homozygous deletion would arrest at one of the developmental stages.

EMS primarily generates single base pair lesions most commonly involving a G/C - A/T transition. EMS does produce some small deletions and chromosomal rearrangements but not at a

very high frequency (Anderson, 1995). It has been shown that UV-irradiation (Stewart *et al.* 1991), formaldehyde (Moerman and Baillie, 1981; Rogalski *et al.* 1982; Johnsen and Baillie, 1988) and UV-TMP (Trimethylpsoralen) (Yandell *et al.* 1994; Gengyo-Ando and Mitani, 2000; D. Moerman, pers. comm.) generate deletions. In this study, UV-TMP was successfully used to generate lesions within *eDf24* region. Complementation analysis was performed between *rec-1* and new deletions in order to narrow down the *rec-1* coding region.

RESULTS

Defining the left breakpoint of *eDf24* deletion

In order to identify the predicted coding regions deleted by *eDf24*, the left breakpoint of the deletion was determined. The deletion mapping results of *rec-1* of Zetka and Rose (1995b) was confirmed by using the strains CB2781 [*unc-54 (e1092) let-208/ eDf24*] and KR1953 [*unc-54 (e1092)/ eDf24*] (Table 8). *eDf24* removes part of the ribosomal gene cluster region, which maps to the right end of the chromosome I (Albertson 1984), including the ribosomal gene cluster boundary (C. Thacker, unpublished results). These results place the right break point of *eDf24* within the ribosomal gene cluster. The left breakpoint of *eDf24* deletion was determined by PCR. Three sets of primers were used in a single PCR reaction to amplify DNA from *eDf24* homozygotes and wild type individually. DNA primers (KRp12 and KRp14) designed from the Chromosome I center region should produce a PCR product of 577 bp. This primer set was used as a positive control. DNA primers (KRp30 and KRp31) designed from sequences immediately adjacent to the ribosomal genes (*rrn-1*) do not produce a PCR product with *eDf24* DNA (C. Thacker, unpublished results). In non-deleted strains (N2 or Rec-1) a 517 bp PCR product is predicted. This primer set was used as negative control to confirm *eDf24* homozygotes. A third set of primers was selected, as tester, from the sequenced cosmids at the right end of chromosome I. PCR was performed using all three sets of primers. In the wild type, all three primer sets are expected to give PCR products. In *eDf24* embryos: (1) the positive control primer set will give 577bp; (2) the negative control will not give 517bp product; (3) if the third set of primers produce the expected size PCR product, they are not deleted by *eDf24*, if there is no PCR product for the third set of primers, one or both primer sequences are deleted in *eDf24*.

Table 8

Complementation between *rec-1* and *eDf24* deletion from two different strains

Strain name	Genotype	Total progeny	Recombinants (Dpy-11)	R.F.	C.I. (95%)
CB2781	*rec-1/eDf24; dpy-11 unc-42/ + +*	1079	37	7.1	
	let-108/ rec-1; dpy-11 unc-42	829	11	2.6	
KR1953	*rec-1/ eDf24; dpy-11 unc-42/ + +*	717	23	6.7	
	unc-54/ rec-1; dpy-11 unc-42	953	14	2.9	
BC313	*rec-1/ rec-1; dpy-11 unc-42/ + +*	2047	81	8.2	
N2	*+/ +; dpy-11 unc-42/ + +*	1918	26	2.7	

BC313 (*rec-1*) males were used to generate heterozygous tester males.

KR181 (*rec-1; dpy-11 unc-42*), was used as tester strain

Testing whether or not *eDf24* deletes the 3' end of F33H2.1 gene

In order to determine if *eDf24* deletes the 3' end of F33H2.1 gene, primers KRp120 and KRp146 were selected from this region and expected to give a 400 bp PCR product. PCR was performed using this primer set along with the control primer sets. As expected, the wild type gave all three PCR products (577bp, 517bp and 400bp). *eDf24* embryos gave only the 577bp product but not the expected 400bp product indicating that the 3' end of F33H2.1 gene is deleted by *eDf24* (data not shown).

Testing whether or not *eDf24* deletes the 3' end of ZK337 cosmid

In order to determine if *eDf24* deletes the 3' end of ZK337 cosmid, primers KRp168 and KRp169 were designed from this region to generate a 409bp PCR product. PCR was performed using this primer set along with the control primer sites. As expected, wild type gave all three PCR products (577bp, 517bp and 409bp). *eDf24* embryos gave only the 577bp product but not the expected 409bp product (Fig 9) indicating that the 3' end of the cosmid ZK337 is deleted by *eDf24* deletion.

Testing whether or not *eDf24* deletes the 5' end of ZK337 cosmid

In order to test if *eDf24* deletes the 5' end of ZK337 cosmid, primers KRp184 and KRp185 were designed from this region and PCR should produce a 425bp product. PCR was performed using this primer set along with control primer sets. In wild type, all three primer sets gave PCR products (577bp, 517bp and 425bp). In *eDf24* homozygotes, the positive control primer set gave the 577 bp product and the test primer set gave the 425bp product but the negative control primer set did not give any PCR product. This shows that the primers KRp184 and KRp185 are not

Figure 9: The 3' end of the cosmid ZK337 is deleted in both *eDf24* and *hDf31*. Primers KRp 12 and KRp 14 would amplify a 577 bp product from the chromosome I gene cluster (positive control); primers KRp 30 and KRp 31 would amplify 517 bp from the ribosomal gene cluster boundary (negative control to identify the *eDf24* homozygotes); primers KRp168 and KRp 169 would amplify 409 bp from the 3' end of ZK337, if it is not deleted (test primer set).

All three bands were amplified from the wild type worm and the wild type egg. Only 577 bp product was amplified from the *eDf24* homozygotes (a), and the *hDf31* homozygotes (b) indicating that the 3' end of ZK337 is deleted in both *eDf24* and *hDf31*.

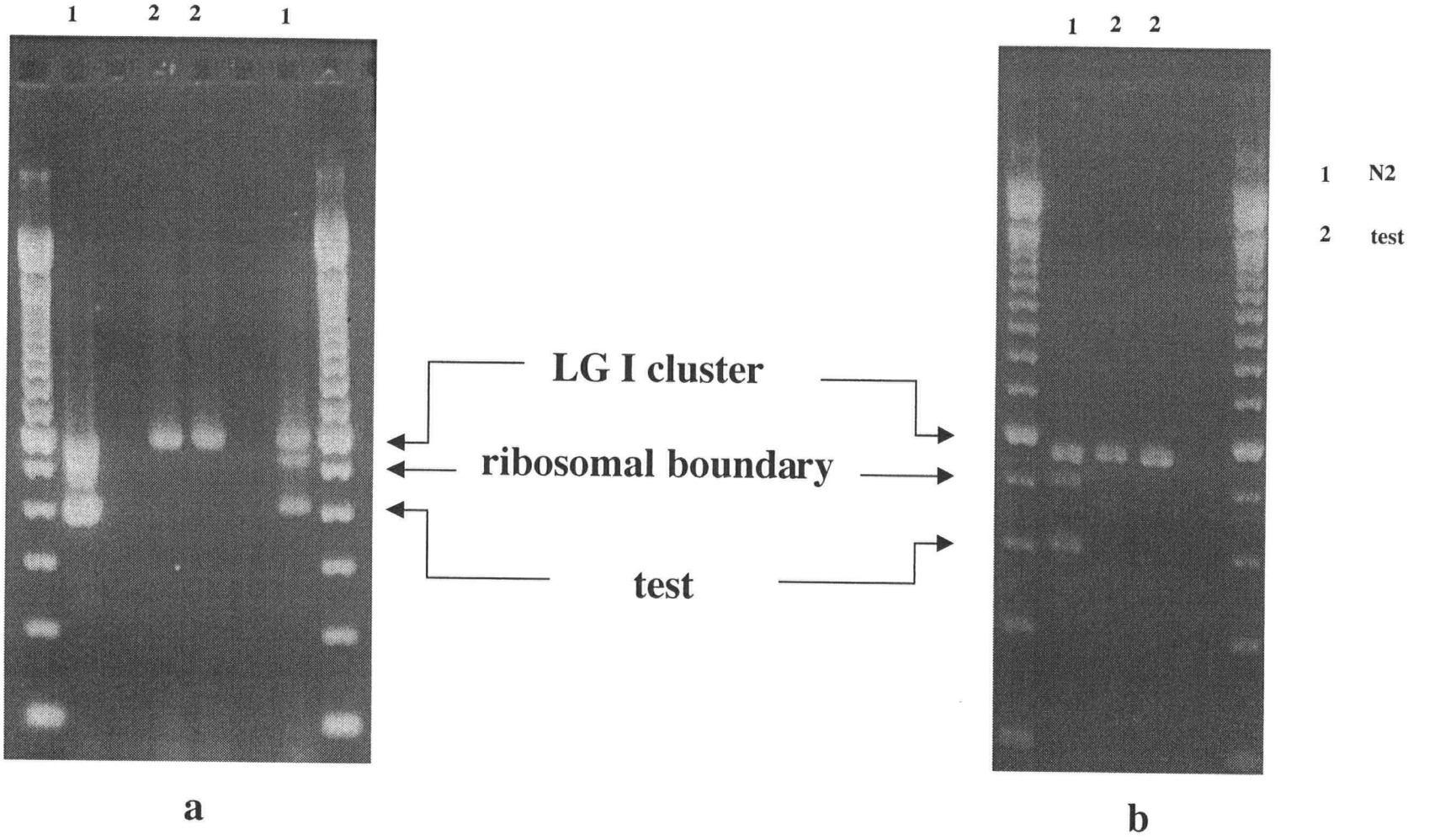


Fig 9

deleted by *eDf24*. These PCR results indicate that *eDf24* deletes the 3' end of the cosmid ZK337 but not the 5' end, so that the left breakpoint of *eDf24* is within the cosmid ZK337.

Testing whether or not *eDf24* deletes the predicted gene ZK377.2

In order to map the breakpoint more precisely, a set of primers, KRp322 and KRp323, were designed to amplify the predicted gene ZK337.2 to produce a 589 bp product. Another set of primers, KRp365 and KRp366, was designed to amplify the F33H2.8 gene and PCR should produce a 1.4 kb product in wild type but not in *eDf24*. These two primer sets were used together to perform PCR on both wild type and *eDf24* individually. In *eDf24* homozygotes, only ZK337.2 was amplified but not the F33H2.8. In wild type, both ZK337.2 and F33H2.8 were amplified. This indicates that ZK337.2 gene is not deleted by *eDf24* deletion, and that *eDf24* left breakpoint is to the right of the ZK337.2 gene.

Testing whether or not *eDf24* deletes the predicted gene ZK377.4

In order to further narrow down the *eDf24* region, a set of primers specific for the gene ZK337.4, KRp326 and KRp327, was designed to amplify 579 bp. A set of primers that amplify the F31C3.3 gene was used as a negative control for *eDf24* homozygotes. This primer set would produce a 2kb PCR product. PCR was performed using both sets of primers and *eDf24* homozygotes produced only a 579 bp, corresponding to the ZK337.4, but not the 2 kb band from F31C3.3 gene. The N2 control produced both 579 bp and 2 kb bands (Fig 10). These results indicate that *eDf24* deficiency does not delete the ZK337.4 gene. This places the left breakpoint of *eDf24* deletion between ZK337.4 and the very 3' end of the ZK337 cosmid (Fig 11).

Figure 10: The ZK337.4 gene is not deleted in either *eDf24* or in *hDf31*.

The primer set, KRp335 and KRp336, specific for the F31C3.3, gave expected 2 kb band in wild type but not in either *eDf24* (a) or in *hDf31* (b), indicating it is deleted in both *eDf24* and *hDf31*. Primers specific for the ZK337.4 gave 589 bp product in wild type, *eDf24*, and *hDf 31*. This shows that ZK337.4 is not deleted either in *eDf24* or *hDf31*.

1 N2

2 test

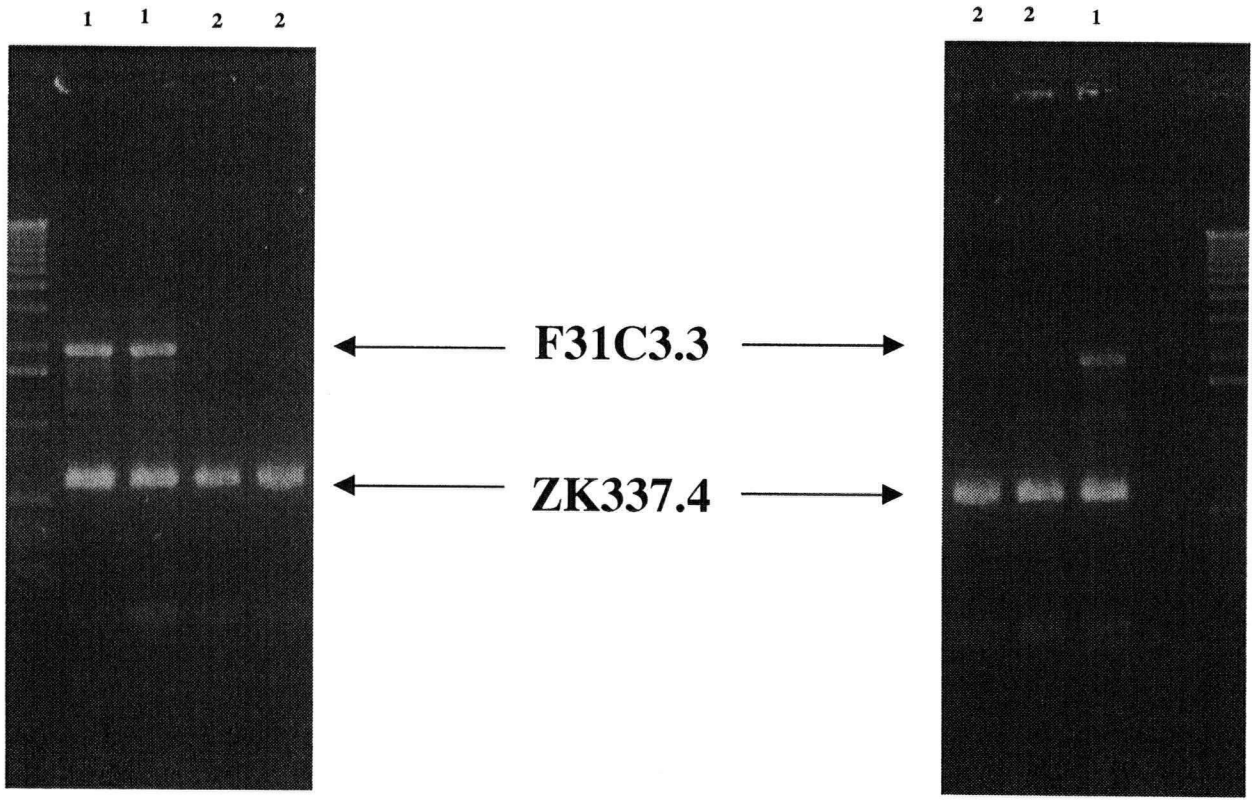
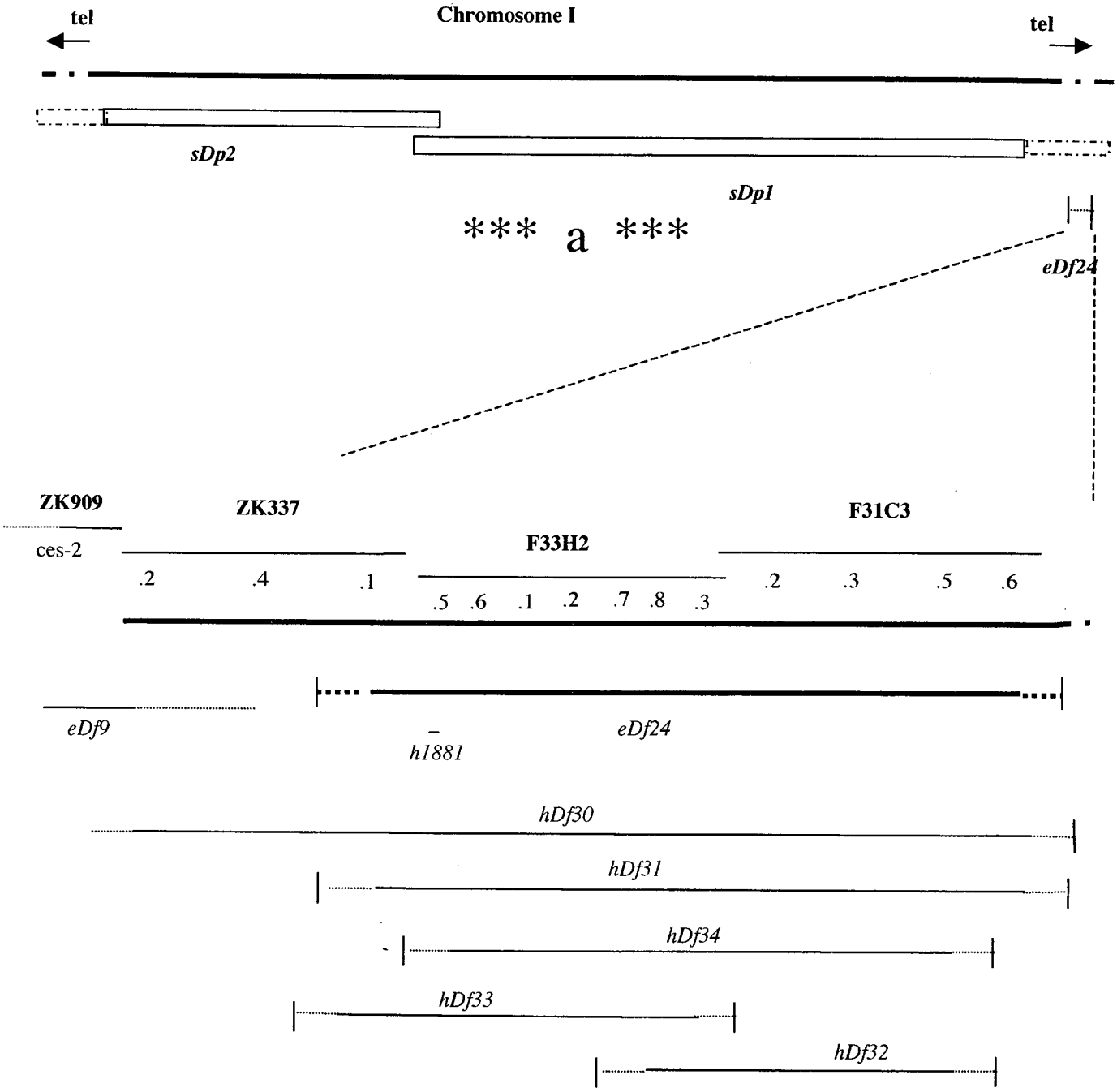


Fig 10

Figure 11: Summary of *rec-1* region.

(a) *rec-1* region in chromosome I. Duplication *sDp1* rescues the Rec-1 phenotype but not the *sDp2*.; deletion *eDf24* fails to complement Rec-1 phenotype (Zetka and Rose, 1995b). (b) Right breakpoint of *eDf24* maps within the ribosomal gene cluster (Albertson, 1984; C. Thacker, unpublished results). The left breakpoint maps between ZK337.4 gene and 3' end of ZK337 cosmid. Seven of the genes (F33H2: .1, .2, .5 and .6; ZK337: .1 .2 and .4) were analyzed for fragment length polymorphism by PCR, and there is no detectable polymorphism. Three of them were analyzed by RNAi (F33H2.1: no obvious phenotype; F33H2.5: embryonic arrest; ZK337.2: one individual produced high frequency males. Stage specific RT-PCR, for L1 and adult, was performed for 13 genes. (ZK337: .1, .2 and .4; F33H2: .1, .2, .3, .6, .7, 8; F31C3: .2, .3, .5 and .6). All the genes are transcribed in both L1 and adult stage. New deletions map in *eDf24* region. New deletions that are used for complementation analysis with *rec-1(s180): hDf30, hDf31, hDf32* and *hDf33*. All the tested deletions complement Rec-1 phenotype. The intragenic deletion *h1881*, also complement Rec-1. The *eDf9* deletion deletes the *ces-2* gene in ZK909 cosmid and complements both *rec-1* and *eDf24*.


tel
Chromosome I
tel
sDp2
sDp1
*** a ***
eDf24
ZK909
ZK337
F31C3
ces-2
F33H2
.2
.4
.1
.5
.6
.1
.2
.7
.8
.3
.2
.3
.5
.6
eDf9
h1881
eDf24
hDf30
hDf31
hDf34
hDf33
hDf32

*** b ***

Fig 11

Rescuing the genes within *eDf24* deletion

eDf24 homozygotes arrest at three-fold stage. In order to test if the cosmid BO467 and ZK340 carry all the essential genes deleted by *eDf24*, an extrachromosomal array, that carries both BO467 and ZK340, was used to test for the rescue of the *eDf24* arrest phenotype. The cosmids ZK340 and BO467 are canonical to the sequenced cosmids F33H2 and F31C3 (Fig 11). The cosmid BO467 contains part of ribosomal gene cluster and overlaps with ZK340. The cosmid ZK340 contains the 3' end of ZK337 sequence, but not the ZK337.2 gene. *hIn1*[*unc-101*]/ *eDf24* males were crossed to a transgenic strain that carries both ZK340 and BO467 as an extrachromosomal array (*lev-11; sEx150*). After 24 hrs of outcrossing, hermaphrodites were plated individually and transferred to new plates on the next day. Hermaphrodites that gave outcrossed male progeny in the F1 generation were selected as successfully outcrossed individuals. F1 Rol hermaphrodites, from these selected plates, were plated individually and transferred to new plates on the second day. On the third day, first brood plates were analyzed to select those segregating *eD24* arrest embryos. The extrachromosomal array will not segregate in Mendelian fashion and it is possible to observe *eDf24* arrested embryos even if the array rescues the arrest phenotype. These selected F1s were segregating healthy non-roller and roller progeny along with a fraction of not healthy, slow growing roller worms that are not fertile. These slow growing sterile individuals are expected to be *eDf24* homozygotes carrying the extrachromosomal array. This observation indicates that the cosmids ZK340 and BO467 can rescue *eDf24* arrest stage up to sterile adult. It could be possible that the extrachromosomal array is not expressing in the germ-line or germ-line specific gene(s) are missing in these cosmids. An alternate explanation is that *eDf24* deletes part of ribosomal gene cluster and the

extrachromosomal array is not providing enough ribosomal gene product needed in the adult development.

If the latter explanation is true, the BO467 cosmid that contains part of ribosomal gene cluster will rescue *eDf24* homozygotes similar to the rescue observed with both cosmids. On the other hand, the ZK340 cosmid will not be able to rescue *eDf24* arrest phenotype as it does not carry any ribosomal genes. In order to test this hypothesis, two different transgenic strains, one with the ZK340 cosmid (*sEx698*) and another with the BO467 cosmid (*sEx100*), were used for the rescue analysis. The crosses were made as before and the F2 progeny were analyzed. Surprisingly, both transgenics carrying single cosmids rescue *eDf24* homozygotes up to sterile adult. These observations suggest that the sterile adult phenotype observed in transgenic backgrounds is not due to insufficient ribosomal gene product.

Testing the candidate genes within *eDf24* deletion

After the completion of DNA sequencing in *eDf24* region, the following methods were employed to characterize the genes within *eDf24*. There are 13 genes predicted within the *eDf24* deletion.

Fragment length polymorphism

In order to test if there was any significant length polymorphism within *eDf24* region in Rec-1 strain, regions from wild type and Rec-1 mutant were amplified and analyzed by agarose gel electrophoresis. If there are any length polymorphisms that could be detected in an agarose gel, it would be possible to see the size differences between wild type and *rec-1* mutant strain. Almost all the coding regions within *eDf24* region were amplified, and did not identify any

polymorphisms (Table 9). If the polymorphisms were only few basepairs, it would not be possible to detect it, as the resolution in an agarose gel would not be good enough to identify small changes. This analysis did not include any regulatory regions.

Reverse transcriptase polymerase chain reaction (RT-PCR)

In order to test if any of the genes within *eDf24* region is expressed exclusively in meiotic cells, stage specific RT-PCR was performed. REC-1 is involved in determining the position of crossing over during meiosis (Zetka and Rose, 1995b), and is possible that REC-1 protein function is meiosis-specific. Any gene that expresses only in adults, would be a good candidate for the *rec-1* gene. In *C. elegans*, meiotic cell division begins during the L3 - L4 stage and continues in the adult. In order to test if any of the genes within *eDf24* is expressed only in the germline, stage-specific RT-PCR was performed. Stage-specific RNA was isolated from a synchronized population as described in Materials and Methods. For each gene, specific primers were designed to amplify two or more exons, and RT-PCR was performed on total RNA isolated from L1 and adult stages. RT-PCR was performed for 11 of the genes within *eDf24* and none of the genes was specific for adult (Table 9). This indicates that these genes are not transcribed exclusively during meiosis. It may possible that transcripts are translated only in meiotic cells. Total RNA from *rec-1* mutant was used for RT-PCR to test if any of the transcripts is missing or different in size compared to wild type. Expected size messages were amplified from all the genes tested, and indicated that transcripts were made in the *rec-1* mutant for all those genes.

RNA interference (RNAi)

In order to test the loss-of-function phenotype of each gene within *eDf24* region, RNAi was

Table 9

Analysis of genes within *eDf24* deletion

Gene designation	Primers used	RT-PCR			Fragment length polymorphisr
		L1 (wild type)	Adult (Wild type)	Mixed stage(Rec-1)	
ZK337.2	KRp322 & KRp323	np	np	np	ni
ZK337.4	KRp326 & KRp327	np	np	np	ni
ZK337.1	KRp369 & KRp370	+	+	+	ni
F33H2.5	KRp309 & KRp314	nd	nd	nd	ni
F33H2.6	KRp367 & KRp368	np	np	np	ni
F33H2.1	KRp309 & KRp 314	+	+	+	ni
F33H2.2	KRp337 & KRp338	+	+	+	ni
F33H2.7	KRp339 & KRp340	+	+	+	nd

ni - not identified, np - no PCR product, nd - not done

(continue on the next page)

Table 9 continue.....

Gene designation	Primers used	RT-PCR			Fragment length polymorphisr
		L1 (wild type)	Adult (Wild type)	Mixed stage(Rec-1)	
F33H2.8	KRp365 & KRp366	+	+	+	nd
F33H2.3	KRp359 & KRp360	+	+	+	nd
F31C3.1	KRp363 & KRp364	+	+	+	nd
F31C3.3	KRp335 & KRp336	+	+	+	nd
F31C3.5	KRp333 & KRp334	+	+	+	nd
F31C3.4	KRp361 & KRp362	np	np	np	nd
F31C3.6	KRp331 & KRp332	+	+	+	nd

ni - not identified,np - no PCR product, nd - not done

performed. It has been shown that injection of double-stranded RNA will interfere with endogenous mRNA and generate the equivalence of null phenotype (Fire *et al.* 1998). Double stranded RNA was prepared and injected into L4-early adults that were heterozygous for the markers (*dpy-11 unc-42*/ + +), as described in Materials and Methods. F1 animals were analyzed for any visible phenotype, and when this was not evident, all the progeny in the F1 and F2 generations were scored and recombination frequency between *dpy-11* and *unc-42* was determined.

F33H2.1:

The double stranded RNA was prepared from the N-terminal coding region as well as from full length cDNA. RNAi was performed as described previously. There is no visible phenotype or any change in the recombination frequency compared to wild type. There are two possibilities for this result: one possibility is that null allele of F33H2.1 does not have either visible phenotype or any effect on recombination; another possibility is that the RNAi did not work effectively. Further analysis supports the first possibility (discussed later).

F33H2.5:

For the F33H2.5 gene, a 1.6 kb genomic fragment from the initiation site was used as template for double stranded RNA preparation. RNAi was performed as described previously and an embryonic arrest phenotype was observed in the F1 generation (Table 10), as a result, the effect on crossover frequency could not be examined. This indicates that elimination of the F33H2.5 gene product will lead to a lethal phenotype i.e: it is an essential gene.

Table 10

RNA interference on F33H2.5

Genotype	worms injected	Viable adult progeny	L1 arrest	Egg arrest
dpy-11 unc-42/ + +	6	147	15	788
+ +/ + +	2	38	7	379

ZK337.2:

For ZK337.2, the whole gene, a 579 bp genomic fragment, was used as template to make ds RNA. RNAi was performed as described previously. One of the individuals gave 17.5% males with a total of 510 progeny in the F1 generation (Table 11). There might be a delay in the switch from spermatogenesis to oogenesis in hermaphrodites. There is no effect on recombination frequency indicating that this may not be *rec-1* gene.

Mutating the genes within *eDf24* deletion

In order to generate null phenotypes of the genes within *eDf24*, mutagenesis was performed. These null mutants would be used to test if they fail to complement the Rec-1 phenotype.

Using a *hIn1[unc-101]* balanced strain and UV-Trimethylpsoralen as mutagen

hIn1[unc-101] / unc-54 strain was used in this screen. The *hIn1* acts as a balancer to suppress the recombination at the right arm of chromosome I (Zetka and Rose, 1992). A new deletion can be maintained heterozygously. Some of these deletions will be within *eDf24* deletion. There were 3128 F1 individuals screened (2 X 3128 chromosomes), and 55 lethals balanced by *hIn1* were isolated. Out of these 55 candidates, 26 of them are linked to *hIn1[unc-101],* and 29 linked to *unc-54*. The lethal recovery rate is 0.9% (55/ 6256).

Complementing *eDf24* deletion with new deletions

A complementation analysis was performed between *eDf24* and the new lethals in order to identify the putative deletions that overlap with *eDf24*. The *hIn1* acts as a balancer for the region

Table 11

RNA interference on ZK337.2

Individual number	Total progeny	Males	% of males
1	158	1	0.6 %
2	132	0	-
3	313	0	-
4	270	0	-
5	510	89	17.5 %

between the visible markers Unc-75 and Unc-54. As described below, two sets of crosses were performed to select the deletions that map within *eDf24*.

Deletions linked to *hIn1[unc-101]*

To determine whether or not the new deletions linked to *hIn1[unc-101]* mapped within the *eDf24* deletion, males from the new deletion strains (*hIn1[unc-101] Δx/ unc-54*) were crossed to *unc-101 eDf24 / hIn1[unc-54]* hermaphrodites. After 24 hrs of mating, hermaphrodites were plated one per plate. In the F1 generation, wild type (either *unc-101 eDf24 / unc-54* or *hIn1[unc-54] / hIn1[unc-101]*), Unc-54 (*unc-54 / hIn1[unc-54]*) and Unc-101 (*unc-101 eDf24 / hIn1[unc-101] Δx*) males were scored as outcrossed progeny (Fig 12). *eDf24* homozygotes arrest at an embryo stage. New deletion homozygotes arrest at different developmental stages. If the *eDf24* deletion fails to complement the new deletion *x*, Unc-101 males will arrest at one of the developmental stages. In order to confirm successful out-crossing, 20 or more wild type males were scored. There were nine new deletions that fail to complement *eDf24* deletion, and these were selected for further analysis (Table 12).

Deletions linked to *unc-54*

In order to determine whether or not the new deletions linked to *unc-54* mapped within *eDf24* deletion, *hIn1[unc-101] / unc-54 Δx* males were crossed to *unc-101 eDf24 / unc-101 unc-54* hermaphrodites. After 24 hrs of mating, hermaphrodites were plated one per plate. In the F1 generation, wild type (*unc-54 Δx / unc-101 eDf24*), Unc-101 (*hIn1[unc-101 / unc-101 eDf24, hIn1 [unc-101] / unc-101 unc-54*) and Unc-54 (*unc-54 Δx / unc-101 unc-54*) males were scored as outcrossed progeny (Fig 13). Homozygotes for *eDf24* deletion arrest at the

Po *hIn1[unc-101] Δ x/ unc-54* **X** *unc-101 eDf24/ hIn1[unc-54]*

F1

(1) wild type *unc-101 eDf24 / unc-54*

hIn1[unc-54] / hIn1[unc-101]

(2)Unc-54 *unc-54 / hIn1[unc-54]*

(3) Unc-101 *unc-101 eDf24 / hIn1[unc-101] Δx*

Figure12: Complementation analysis between *eDf24* and new deletions that linked to *hIn1[unc-101]*

Table12

Complementing *eDf24* with new lethals linked to *hIn1[unc-101]*

Strain name	Allele number	Wild type males	Unc-101 males	Unc-101 hermaphrodites
KR3667	*h1887*	132	0	-
KR3668	*h1888*	10	0	-
KR3671	*h1889*	91	11	Fertile
KR3672	*h1890*	90	13	Fertile
KR3673	*h1891*	75	19	Fertile
KR3674	*h1892*	168	0	-
KR3679	*h1910*	272	0	-
KR3680	*h1896*	88	25	Fertile
KR3686	*h1898*	26	0	-
KR3687	*h1899*	68	0	-
KR3688	*h1900*	58	0	-

continue on the next page

Table 12 continues .....

Strain name	Allele number	Wild type males	Unc-101 males	Unc-101 hermaphrodites
KR3689	*h1901*	101	38	Fertile
KR3690	*h1902*	31	15	Fertile
KR3691	*h1903*	106	27	Fertile
KR3692	*h1904'*	86	0	-
KR3693	*h1905*	53	14	Fertile
KR3694	*h1906*	83	36	Fertile
KR3695	*h1907*	n/d		
KR3696	*h1908*	52	18	Fertile
KR3700	*h1914*	39	3	Sick, slow growth. Fertile
Kr3701	*h1915*	151	33	Fertile
KR3702	*h1916*	86	40	Fertile
KR3703	*h1917*	42	20	Fertile
KR3711	*h1970*	112	29	Fertile
KR3712	*h1971*	127	0	-
KR3713	*h1972*	14	5	Fertile

P0 ***hIn1[unc-101] / unc-54 Δx*** **males** **X** ***unc-101 eDf24 / unc-101 unc-54***

F1

(1) wild type ***unc-54 Δx / unc-101 eDf24***

(2) Unc-101 ***hIn1[unc-101 / unc-101 eDf24***

hIn1 [unc-101] / unc-101 unc-54

(3) Unc-54 ***unc-54 Δx / unc-101 unc-54***

Figure 13: Complementation analysis between *eDf24* and new deletions that linked to *unc-54*.

embryo stage. Homozygotes for new deletions arrest at different developmental stages. In order to confirm successful outcrossing, 15 or more Unc-101 and Unc-54 males each were scored. There is only one new deletion identified that fails to complement *eDf24* and this was selected for further analysis (Table 12). Another mutation, *h1881*, is an internal deletion in F33H2.5 gene and linked to *unc-54*. This mutation deletes 252bp, and was identified by PCR (discussed below).

Complementing new deletions

A complementation analysis between six deletions was performed, in order to identify if there are more than one essential site within *eDf24* region. For example, two of the deletions linked to *unc-101* marker were outcrossed. If they complement each other, in the F1 generation, it will be possible to isolate Unc-101 animals, which will be heterozygous for both deletions. If they fail to complement, Unc-101 progeny will not be viable in the F1 generation. The alleles *h1892, h1898, h1899, h1904, h1910,* and *h1971* were tested. This analysis shows that there are three complementation groups (Fig 14), one: *h1892, h1899 h1910* and *h1971,* two: *h1904* three: *h1998*, indicating that at least there are three essential genes. There are three more deletions linked to *hIn1*[*unc-101*] that were not tested. One has more complex rearrangement (*h1887*), and is difficult to work with; one has mutations on both of the homologues (KR3686); one has been not characterized yet.

Mapping deletions overlap *eDf24* deletion

Table 13

Complementation between *eDf24* and new mutations linked to *unc-54*

Strain name	Allele number	Wild type males	Unc-101 males	Unc-54 males	Can wild type males mate?
KR3662	*h1882*	0	3	7	-
KR3663	*h1883*	49	16	13	Yes
KR3666	*h1886*	7	5	3	Yes
KR3676	*h1894*	11	8	10	Yes
KR3677	*h1895*	11	2	1	Yes
KR3681	*h1897*	35	7	10	Yes
KR3699	*h1913*	18	2	-	Yes
KR3708	*h1969*	81	48	51	Yes
KR3709	*h2131*	29	13	11	Yes
KR3717	*h1976*	14	10	6	Yes
KR3718	*h1977*	6	1	2	Yes
KR3719	*h1978*	48	22	29	Yes
KR3720	*h1979*	48	3	-	n/t

n/t not tested

Figure 14: The new mutations fall into three complementation groups within eDf24. The *h1971* fails to complement all the alleles (*h1892, h1898, h1899, h1904*, and *h1910*). The *h1904* fails to complement *h1892, h1899* and *h1971*, but complements *h1910* and *h1898*. The *h1910* also fails to complement *h1892, h1899* and *h1971*, but complements *h1898*.

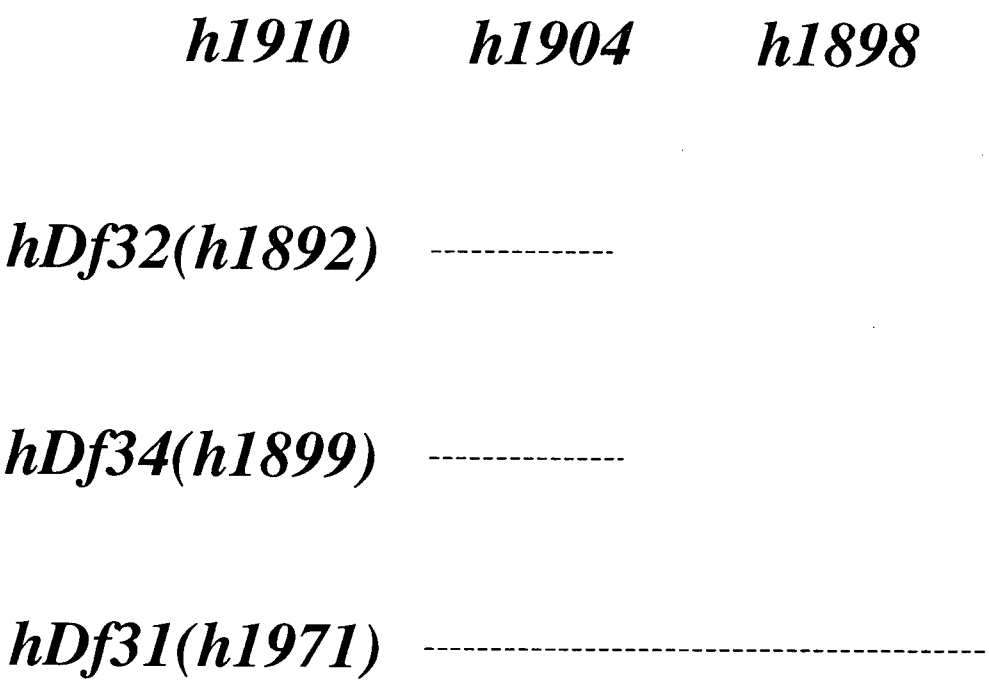


Fig 14

In order to identify the coding regions deleted by the new deletions, deletion breakpoints were mapped. These new deletions are viable as heterozygotes and arrest at different developmental stages as homozygotes. The homozygous arrest stage was determined individually and PCR was performed using primers specific for each gene within *eDf24* region. Six deletions were identified within the *eDf24* deletion. *h1881*, deletes 252bp within F33H2.5. The deletion *hDf30*, deletes more than three cosmids that cover *eDf24* deletion; the right breakpoint maps within the ribosomal gene cluster and the left breakpoint maps to the left side of ZK337 cosmid (Fig 15). This deletion is bigger than *eDf24*. Deletion, *hDf31*, is similar to *eDf24*. Both *eDf24* and *hDf31*, delete the 3' end of ZK337 and do not delete ZK337.4. This places the left breakpoint between ZK337.4 and the 3' end of the ZK337 cosmid (Fig 9, 10). The right breakpoint of each of these deletions, *eDf24* and *hDf31*, map within the ribosomal gene cluster, but the extent of the deletion within this cluster is not determined (Fig 15). There are three other deletions, *hDf32*, *hDf33* and *hDf34*, which divide *eDf24* region into four small intervals (Fig 15; Table 14).

Essential loci within *eDf24* deletion

There are three genetic complementation groups within *eDf24* deletion, which indicates that there are at least three essential genes (Fig 15). The PCR mapping indicates that all the large deletions delete F33H2.8 gene, which could be one of the essential genes. One mutation, *h1904*, fails to complement *hDf31, hDf32* and *hDf34* but complements the *hDf33*; this means that *h1904* extends to the right of F33H2.8. Another mutation, *h1898*, complements *hDf32, hDf33, hDf34* and but fails to complement *hDf31*. Both, *hDf32* and *hDf34* delete F31C3.5, but have not been tested to see, if they delete F31C3.6. Since *hDf31* deletes part of the ribosomal gene cluster, it is

Figure 15: New deletions that overlap with the *eDf24* deletion, *eDf24* and *unc-101 eDf24*

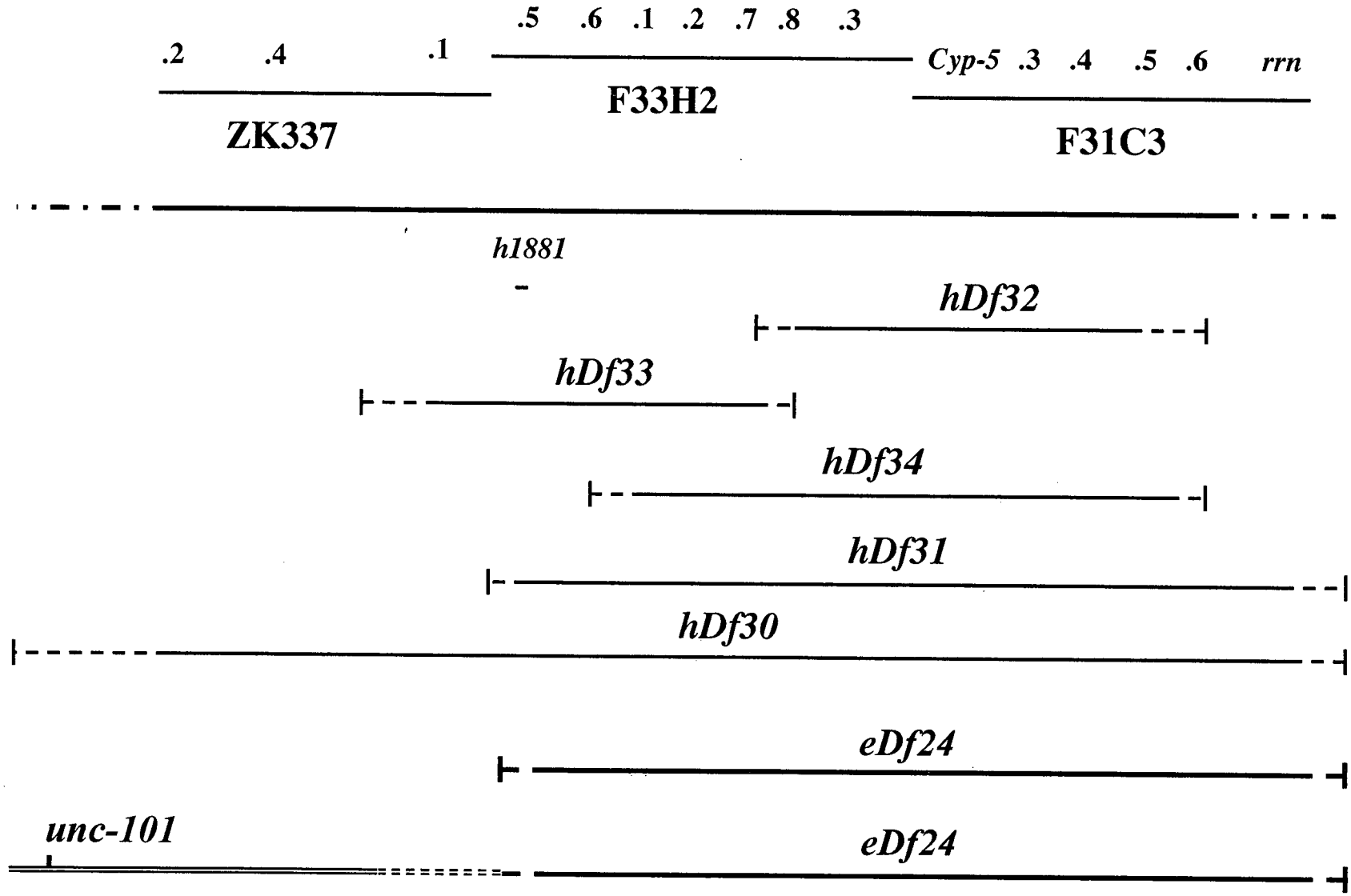

.2
.4
.1
.5
.6
.1
.2
.7
.8
.3
Cyp-5
.3
.4
.5
.6
rrn
ZK337
F33H2
F31C3
h1881
hDf32
hDf33
hDf34
hDf31
hDf30
eDf24
unc-101
eDf24

Fig 15

Table 14

New deletion mapping

Gene and/ allele	*hDf* number	Left breakpoint		Right breakpoint	
		out	in	in	out
h1882	*hDf30*	nd	ZK337.2	*rrn* boundary	nd
h1971	*hDf31*	ZK337.4	3' end of ZK337	*rrn* boundary	nd
h1892	*hDf32*	F33H2.2	F33H2.3	F31C3.5	*rrn* boundary
h1910	*hDf33*	ZK337.4	ZK337.1	F33H2.2	F33H2.3
h1899	*hDf34*	F33H2.5	F33H2.1	F33H2.5	*rrn* boundary

In- inside the deletion

Out- outside the deletion

Gene-finder predicted genes are used and the primers are covering the 5' end of the gene.

most likely that *h1898* is an allele of F31C3.6. This suggests that *hDf32* and *hDf34* do not delete F31C3.6 (Fig 14, 15). The *h1904* allele complements *h1898* and *hDf33*. This shows that *h1904* maps between F33H2.8 and F31C3.6.

Intragenic deletions

Two intragenic deletions, F33H2.1(*gk10*) and F33H2.5(*h1881*), were characterized within the *eDf24* region.

F33H2.1(*gk10*)

The predicted gene F33H2.1 contains 14 exons and has a DEAH domain characteristic of DNA helicase family of proteins. It is not known if F33H2.1 has helicase function. A deletion allele of this gene, *gk10*, was kindly provided by the Reverse Genetics Core facility at UBC. This putative knockout, deletes about 2 kb of genomic DNA extending from exon two to eight, and generates a stop codon in this latter exon (Fig 16). It maybe possible that *gk10* is a null allele. *gk10* homozygotes are viable and have no obvious phenotype.

Complementing F33H2.1(*gk10*) with *rec-1* mutation

In order to test whether or not F33H2.1 was responsible for the *rec-1* mutant phenotype, complementation analysis was done between F33H2.1(*gk10*) and *rec-1 (s180)*. The distance between *dpy-11* and *unc-42* is 3.6 m.u., 7.1 m.u. and 2.5 m.u. in F33H2.1(*gk10*)/ *rec-1*, *eDf24*/ *rec-1*, and *let-208*/ *rec-1* backgrounds respectively (Table 15). These data indicate that the distance between *dpy-11* and *unc-42* in F33H2.1(*gk10*)/ *rec-1* background is close to *rec-1*/ *let-208* control and is similar to wild type distance. If F33H2.1 is responsible for the Rec-1

Figure 16: Predicted gene structure of F33H2.5 and the position of *h1881* deletion.

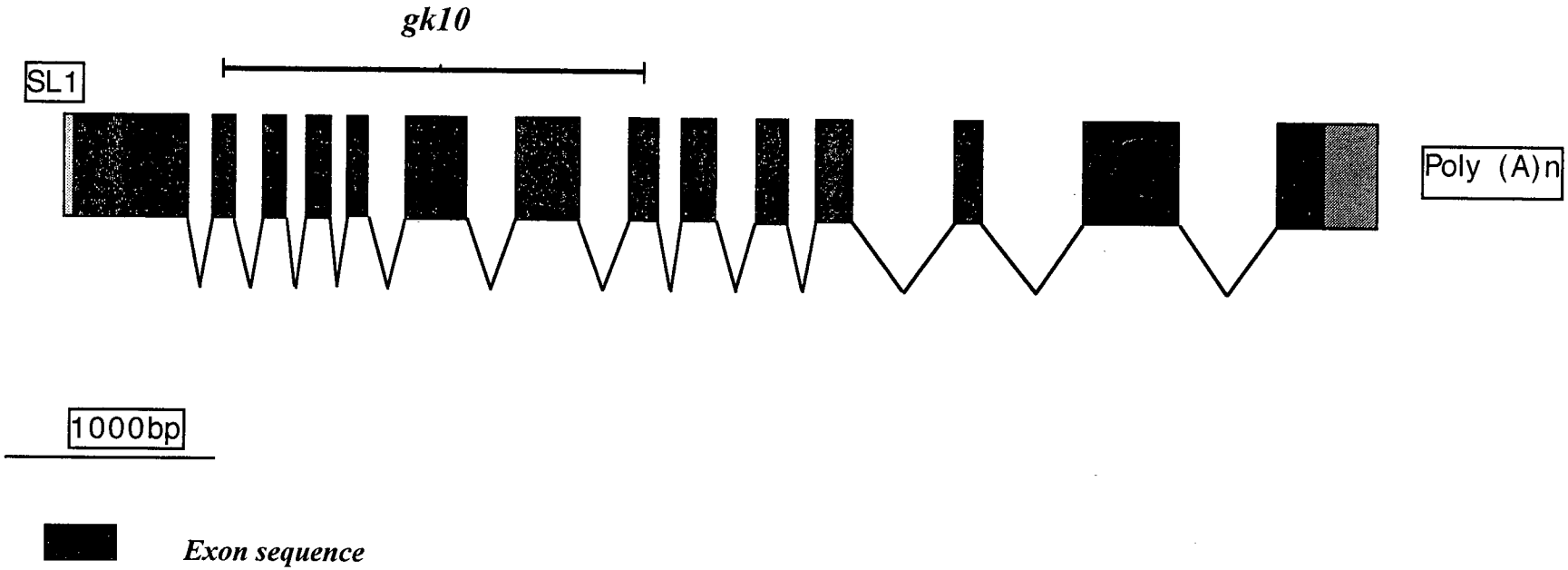


Fig 16

phenotype and *gk10* is a null allele, F33H2.1(*gk10*)/ *rec-1* will behave similar to *eDf24/ rec-1* background. At this stage there is no evidence to prove that *gk10* is a null allele. Further analysis (see below) proves that the F33H2.1 is not responsible for Rec-1 phenotype.

F33H2.5(*h1881*)

The gene-finder predicted gene F33H2.5, encodes a putative polymerase epsilon. *h1881* deletes 252 bp including part of the exons two and three, and generates a nonsense codon right after the deletion (Fig 17). *h1881* homozygotes are sterile adults. There are sixteen exons and fifteen introns in F33H2.5 gene. There is a possibility that the deletion can be excluded from the message by alternative splicing. There is no alternative splicing reported in other organisms where the polymerase epsilon is well characterized. If this mutant phenotype represents the absence of active gene product, rather than simply a reduction, then the phenotype should not change when the mutant allele is placed in *trans* to a deficiency for the locus. This assay was used to prove that the *h1881* is a null allele (discussed later).

Complementing F33H2.5(*h1881*) with *rec-1* mutation

In order to test whether or not the F33H2.5 coding region is responsible for the *rec-1* mutant phenotype, complementation analysis was performed between F33H2.5(*h1881*) and *rec-1 (s180)*, and observed distance between *dpy-11* and *unc-42* 2.2 m.u., is comparable to wild type distance (Table 15). But in a negative control, *hIn1[unc-101]* +/ + *rec-1*, the distance is 1.1 m.u., which is 50% less than the wild type distance. This difference may not be significant because 95% confident intervals for test and the control overlap. There is a possibility that there is a second site mutation which reduces the recombination frequency in both test and negative control. In

Table 15

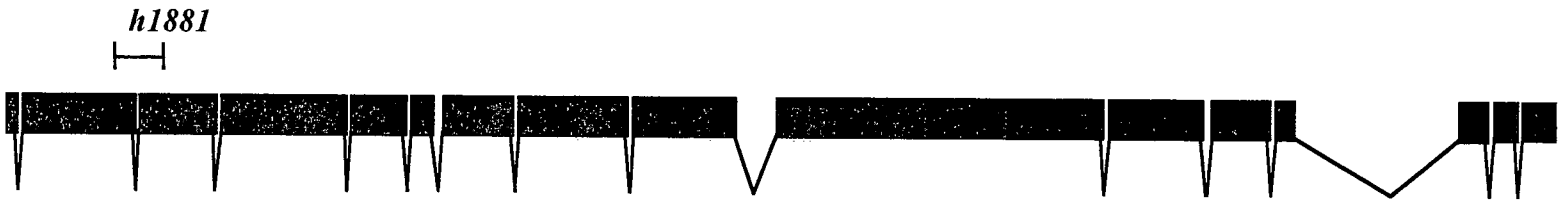
Effect of F33H2.1(*gk10*) and F33H2.1(*h1881*) on cross over distribution

Genotype	Total progeny	Recombinants (Dpy)	R.F.	C.I. (95%)
rec-1/F33H2.1(*gk10*); *dpy-11 unc-42*/ + +	1285	23	3.6	2.3-5.4
rec-1/rec-1; dpy-11 unc-42	1053	31	6.0	4.1-8.6
rec-1/eDf24; dpy-11 unc-42	1079	37	7.1	4.9-9.7
rec-1/ let-208; dpy-11 unc-42/ + +	829	11	2.6	1.2-4.6
rec-1/unc-54 F33H2.5(h1881); dpy-11 unc-42/ + +	3483✡	75*	2.2	1.6 - 2.7
rec-1/ hIn1 unc-101; dpy-11 unc-42/ + +	1475✡	8	1.1	0.5 - 2.0
rec-1/unc-54 F33H2.5(h1881); dpy-11 unc-42/ + +	1272♠	30*	2.3	1.6 – 3.3
rec-1/ hIn1 unc-101; dpy-11 unc-42/ + +	845♠	8	1.9	0.8 - 3.6

✡*h1881* original isolate

♠*h1881* reisolate

Figure 17: Predicted gene structure of F33H2.5 and the position of *h1881* deletion.



1 kb

Fig 17

order to eliminate this possibility, *h1881* was reisolated and complementation analysis was performed. The recombination frequency in *h1881* background is 2.3 m.u. and in negative control 1.9 m.u., which are similar to wild type control and indicates that F33H2.5 is not responsible for the Rec-1 phenotype (Table 15).

Rescuing with transgenic array carrying ZK340 cosmid

In order to determine whether or not or not the ZK340 cosmid would rescue F33H2.5 (*h1881*) sterility, the following cross was performed. *hIn1[unc-101] / unc-54* F33H2.5*(h1881)* males were crossed to *rec-1; sEx695* Roller hermaphrodites (kindly generated by Diana Janke in David Baillie's laboratory at Simon Fraser University, Canada). The dominant Rol-6 phenotype indicates the presence of the extrachromosomal array. After 24 hrs of outcrossing, hermaphrodites were plated one per plate. Hermaphrodites that gave males in the F1 generation were selected as successfully outcrossed individuals. Roller hermaphrodite progeny from these individuals were plated one per plate and transferred to new plates for three days. There were three different genotypes in the F1 Roller population. 1) Self crossed progeny that do not segregate either Unc-101 or Unc-54. 2) Outcrossed progeny that segregate Unc-101 in the F2 generation. 3) Outcrossed progeny that segregate Unc-54 in the F2 generation. More than 27 Unc-54 animals, from the F2 generation, were plated individually. Unc-54 is epistatic to Rol-6, therefore, Rol phenotype is masked in Unc-54 animals. If the cosmid ZK340 rescues F33H2.5(*h1881*) mutation, Unc-54 individuals that carry the extrachromosomal array will be fertile. Those that do not carry the array will not give any progeny. After three days, Unc-54 plates were examined to test if they segregate progeny. Six Unc-54 hermaphrodites were fertile and gave progeny. Five of the six fertile Unc-54 hermaphrodites did not give any progeny in the

next generation (F2); only one gave progeny in the next generation and maintained for several generations. There are two possible genotypes for the fertile Unc-54; it could be a recombinant (as a result of crossing over in F1 generation), or an extrachromosomal array carrying ZK340 rescues the sterile phenotype. In order to test if it carries the extrachromosomal array, the fertile Unc-54 hermaphrodites were outcrossed to wild type males. If these Unc-54 animals carry the extrachromosomal array, in the F1 generation, it is possible to see Rol phenotype in the outcrossed progeny. If it is a recombinant, there will not be any roller progeny in the F1 generation since there is no extrachromosomal array to give the phenotype. There were several outcrossed males and hermaphrodites in the F1 generation, but there were no roller progeny. These observations show that the stable Unc-54 hermaphrodite is a recombinant, and the other five Unc-54 hermaphrodites, which gave few progeny, should carry the extrachromosomal array. This result indicates that the cosmid ZK340 can rescue the sterile phenotype of F33H2.5(*h1881*). The fact that it could not be maintained as homozygous deletion strain in the extrachromosomal array background is maybe due to the weak or aberrant expression of the array or the array is not expressed in the tissues where it is required.

Complementing with *hDf30* deletion

In order to test if the *h1881* allele is a genetic null, *h1881* was placed over a deletion that deletes the entire F33H2.5 gene. If *h1881* is a null, *h1881* over a deletion will show the same phenotype as *h1881* homozygotes; if it is a hypomorph, *h1881* over the deficiency will be more severe than when homozygous. One of the new deletions, *hDf30*, that is linked to *unc-54*, deletes all three cosmids in *eDf24* region. *hIn1[unc-101]/ unc-54* F33H2.5(*h1881*) males were crossed to *hIn1[unc-101]/ unc-54 hDf30* hermaphrodites. *unc-54 hDf30* homozygotes arrest at the embryo

stage. If *unc-54* F33H2.5(*h1881*)/ *unc-54 hDf*30 phenotype is more severe than the *h1881* homozygotes, it will arrest at an earlier developmental stage, i.e. before adult stage. If there are any Unc-54 progeny in F1 generation, their genotype will be *unc-54* F33H2.5(*h1881*)/ *unc-54 hDf30*. Twenty-seven Unc-54 from the F1 generation were plated, one per plate. After three days, they were examined for their developmental stage. All of them grew to adult stage and did not give any progeny. This indicates that the phenotype of F33H2.5(*h1881*)/ *hDf30* is similar to F33H2.5(*h1881*) homozygotes, and conclude that *h1881* allele is a genetic null.

Complementing *rec-1* mutation with new deletions which overlap *eDf24* deletion

Complementation analysis was performed between *rec-1* mutation and new deletions within *eDf24*, in order to test if the *rec-1* gene is deleted. Markers on chromosome I, *dpy-5 unc-13*, were selected to determine recombination frequency and it was found that all the deletions tested complemented *rec-1* (Table 16). The distance between *dpy-5* and *unc-13* is 2.9 m.u., 2.5 m.u., and 3.0 m.u. in *hDf32, hDf33* or *hDf34* over *rec-1* background respectively; while in *rec-1/ rec-1* homozygotes the distance is 6.8 m.u. This indicates that observed genetic distances, in deletion backgrounds, are lower than *rec-1* homozygotes. In negative controls, +*rec-1*/ *unc-54*+, distance is 0.1 m.u., 0.9 m.u. and 1.0 m.u. for *hDf32, hDf33* and *hDf34*, respectively (Table 16). There is a slight increase in the deletion background compared to negative control. The deletions, *hDf32, hDf33* and *hDf34*, are linked to *hIn1* chromosome and it is possible that the increase is the compensatory increase for the suppression in *hIn1* region. Zetka and Rose (1992) showed that in *hIn1* heterozygotes there are compensatory increases both inside and outside the gene cluster region of chromosome I. Another explanation for this observation is that there might be a Rec-1 suppressor generated during the process of mutagenesis and interfering with the analysis. This is

Table 16

Complementation between *rec-1* and new deletion using *dpy-5 unc-13* as tester

Strain tested	genotype	Total progeny	Recombinats	R.F.	C.I. (95%)
KR3647	*dpy-5 unc-13 + rec-1/ + + hIn1[unc-101] +*	1176	15 Dpy	2.5	1.3 – 4.1
	dpy-5 unc-13 + rec-1/ + + unc-54 +	1015	2 Dpy 2 Unc	0.4	0.1 – 0.9
BC313	*dpy-5 unc-13 + rec-1/ + + rec-1*	1470	61 Dpy 40 Unc	6.8	5.7 – 8.6
KR3674	*dpy-5 unc-13 + rec-1/ + + hIn1[unc-101] hDf32*	1043	16 Dpy 15 Unc	2.9	2.0 – 4.2
	dpy-5 unc-13 + rec-1/ + + unc-54 +	862	1 Dpy	0.1	0.0 – 1.2
KR3786	*dpy-5 unc-13 + rec-1/ + + hIn1[unc-101] hDf33*	1072	15 Dpy 12 Unc	2.5	1.6 – 3.6
	dpy-5 unc-13 + rec-1/ + + unc-54 +	764	3 Dpy 4 Unc	0.9	0.4 – 1.8
KR3687	*dpy-5 unc-13 + rec-1/ + + hIn1[unc-101] hDf34*	1523	26 Dpy 21 Unc	3.0	2.3 – 4.1
	dpy-5 unc-13 + rec-1/ + + unc-54 +	860	6 Dpy 3 Unc	1.0	0.1 – 1.9

not a likely explanation, however, because it is not probable that every individual deletion is associated with a suppressor mutation. Nevertheless, four of the deletions were re-isolated and the complementation analysis was repeated. *hIn1* mutation does not affect crossover distribution on the other autosomes (Zetka and Rose, 1992), so another set of markers, *dpy-11 unc-42*, was selected on chromosome V for the analysis. The recombination frequency in all the deletion backgrounds, *hDf30*: 3.2, *hDf31*: 1.8, *hDf32*: 1.9, *hDf33*: 2.7, is similar to the control, and indicates that all the deletions tested complement Rec-1 phenotype (Table 17).

Complementing *rec-1* and *eDf24* using *unc-101 eDf24/ unc-54* strain

In order to test if the *eDf24* deletion linked to *unc-101,* which was used to select the new deletions, fails to complement *rec-1*, complementation analysis between *unc-101 eDf24* and *rec-1* was performed. The result shows that the *unc-101*-linked *eDf24*, complements *rec-1* (Table 18). However, the heterozygote segregated arrested embryos similar to *eDf24* homozygotes. It is possible that crossing over between *unc-101* and *eDf24* might have eliminated the alteration that was linked to *eDf24* and responsible for failing to complement *rec-1*. This possibility was examined as described below.

PCR analysis to test the deletion breakpoints in *unc-101 eDf24* homozygotes

In order to test if the known *eDf24* deletion is still present in *unc-101 eDf24* homozygotes, arrested embryos were isolated as described previously and PCR was performed using the same sets of primers as before, 1) chromosome I cluster region would give 577 bp, 2) ribosomal gene cluster boundary would give 517 bp, 3) 3' end of ZK337 cosmid would give 425 bp. No 517 bp product specific for the primers from ribosomal gene cluster boundary was observed (Fig 18a).

Table 17

Complementation between *rec-1* and new deletion using *dpy-11 unc-42* as tester

Genotype	Totaol progeny	Recombinants	R.F.	C.I. (95%)
+ rec-1/ hIn1[unc-101] +; dpy-11 unc-42/ + +	1030	15 Dpy 7 Unc	2.1	1.3 – 3.1
+ rec-1/ unc-54 +; dpy-11 unc-42/ + +	757	11 Dpy 13 Unc	3.1	1.9 – 4.6
rec-1/ rec-1; dpy-11 unc-42/ + +	2047	81 Dpy 78 Unc	7.7	6.7 – 9.4
dpy-11 unc-42/ + +	1918	26 Dpy 14 Unc	2	1.5 – 2.8
+ rec-1/ hIn1[unc-101] + ; dpy-11 unc-42/ + +	865	5 Dpy	1.2	0.5 – 2.6
+ rec-1/ unc-54 hDf30; dpy-11 unc-42/ + +	804	13 Dpy 13 Unc	3.2	2.1 – 4.7
+ rec-1/ hIn1[unc-101] hDf31; dpy-11 unc-42/ + +	1005	6 Dpy 13 Unc	1.8	1.1 – 2.9
+ rec-1/ unc-54 +; dpy-11 unc-42/ + +	1332	14 Dpy 13 Unc	2.0	1.3 – 2.9
+ rec-1/ hIn1[unc-101] hDf32; dpy-11 unc-42/ + +	974	10 Dpy 9 Unc	1.9	1.1 – 3.0
+ rec-1/ unc-54 +; dpy-11 unc-42/ + +	988	12 Dpy 5 Unc	1.7	0.9 – 2.6
+ rec-1/ hIn1[unc-101] hDf33; dpy-11 unc-42/ + +	1022	17 Dpy 11 Unc	2.7	1.8 – 3.9
+ rec-1/ unc-54 +; dpy-11 unc-42/ + +	865	6 Dpy 5 Unc	1.2	0.6 – 2.2

Table 18

Complementation between *rec-1* and *unc-101 eDf24* using *dpy-11 unc-42* as tester

Genotype	Total progeny	Recombinants	R.F.	C.I. (95%)
+ *rec-1/ unc-101 eDf24; dpy-11 unc-42/* + +	1420	22 Dpy 25 Unc	3.3	2.4 – 4.4
+ *rec-1/ hIn1[unc-54]; dpy-11 unc-42*	1124	13 Dpy 13 Unc	2.3	1.4 – 3.4
rec-1/ rec-1; dpy-11 unc-42/ + +	2047$	81 Dpy 78 Unc	7.7	6.7 – 9.4
dpy-11 unc-42/ + +	1918$	26 Dpy 14 Unc	2	1.5 – 2.8
rec-1/ eDf24; dpy-11 unc-42/ + +	717*	23 Dpy	6.7	4.2 9.9

* from table eight.

$ from table seventeen

However a faint ~400 bp band could be seen. This could have resulted from the primers specific for the 3' end of ZK337, or from a different combination of primers used in the reaction. In order to identify the cause for the faint band, the primer set from ribosomal gene cluster boundary or from 3' end of ZK337 was used along with the primer set from chromosome I cluster. No band from the primer set specific for ribosomal cluster boundary was observed, indicating that it is deleted (Fig 18 b). The faint band appeared in a reaction using primers from the 3' end of ZK337 cosmid (Fig 18 c). These results indicated that the *unc-101 eDf24* chromosome differs from that of the original *eDf24*. These results support the argument that the original *eDf24* chromosome contained an additional DNA alteration.

Figure 18: PCR analysis on *eDf24* breakpoints in *unc-101 eDf24* homozygous. Arrested embryos were isolated from the *unc-101 eDf24/ hIn1[unc-54]* strain and PCR was performed. Primers KRp 12 and KRp 14 would amplify 577 bp product from the chromosome I gene cluster (positive control); primers KRp 30 and KRp 31 would amplify 517 bp from the ribosomal gene cluster boundary; primers KRp168 and KRp169 would amplify 409 bp from the 3' end of ZK337.

a) All three bands were amplified from wild type egg. As expected, a 577 bp product was amplified from the *unc-101 eDf24* homozygotes and there is a faint band ~400 bp which correspond to the 3' end of ZK337.

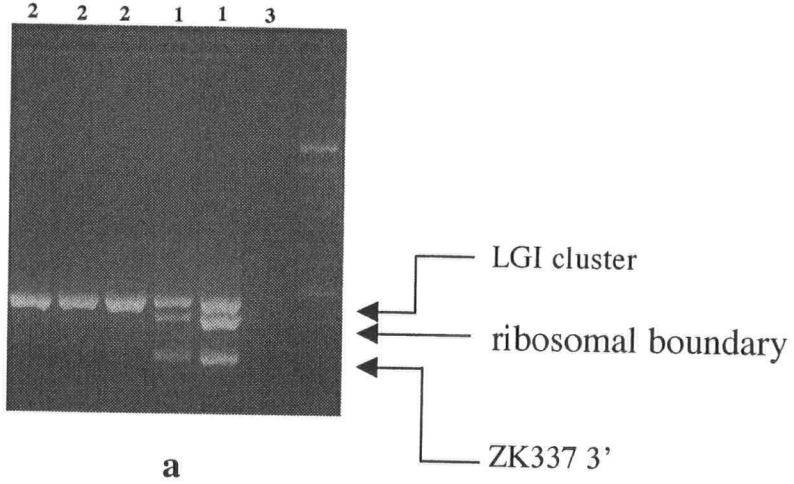
b) Only two sets of primers were used (one from chromosome I cluster and another from ribosomal gene cluster region). Both bands were amplified from wild type; only chromosome I cluster region is amplified in *unc-101 eDf24*.

c) Primers from ZK337 3' end and from the central cluster were use. There are two bright bands from the wild type; the central cluster primer set gave bright band, while the ZK337 3' end gave a very faint band.

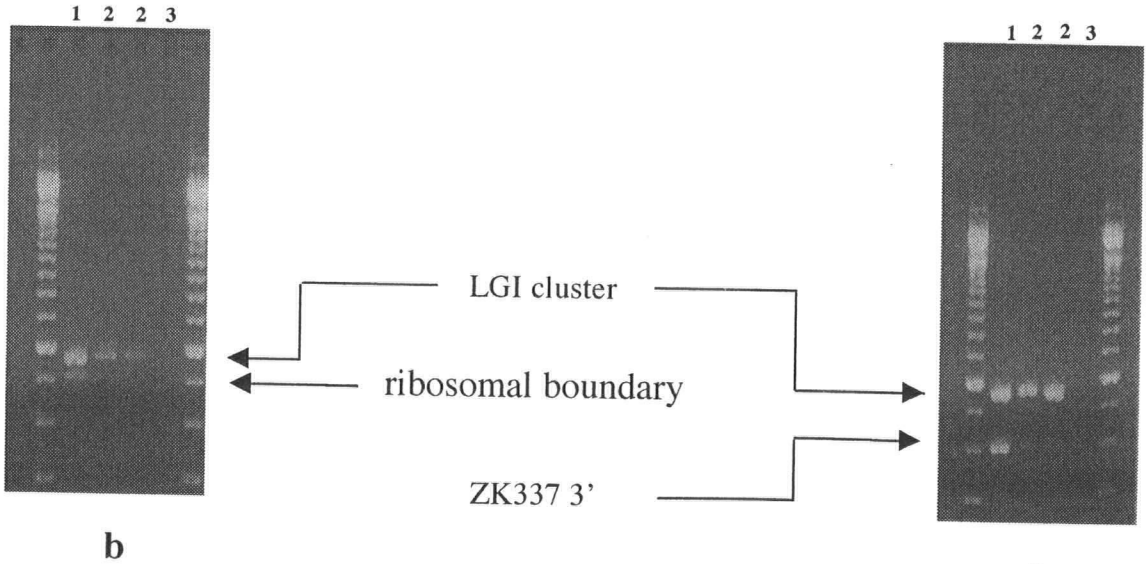
1 **N2**

2 ***unc-101 eDf24***

3 **-ve control**



a



b **c**

Fig 18

DISCUSSION

There are different classes of recombination defective mutants identified in *C. elegans* (reviewed in Zetka and Rose, 1995a). One class of meiotic mutants (eg: *him-1, him-5* and *him-8*), alters the distribution of crossing over on all chromosomes with predominant effect on the X chromosome (Hodgkin, *et al.* 1979; Broverman and Meneely, 1994). A second class of mutants (eg: *him-6* and *him-14*), reduces the crossing over on all the chromosomes (Hodgkin *et al.* 1979; Kemphues *et al.* 1988). In *spo-11* deletion strain, meiotic recombination is abolished (Dernburg *et al.* 1998). In *rec-1(s180)*, the crossover pattern is altered but the total number is not altered (Zetka and Rose, 1995b). Understanding the function(s) of *rec-1+* will help to understand the crossover distribution in meiosis and the role of recombination in general in meiosis.

Rose and Baillie (1979b) first reported that the *rec-1(s180)* allele increases crossover frequency in the autosomal gene cluster regions. Further studies showed that *rec-1* alters the crossover distribution along chromosome I without altering the total number of recombinant events (Zetka and Rose, 1995b). There is 10-15 m.u. linkage between *rec-1* and *unc-54* gene on the right end of the chromosome I (A. Rose, unpublished results). Zetka and Rose (1995b) showed that *rec-1* is covered by duplication *sDp1* and uncovered by deletion *eDf24*. *eDf24* deletion maps to the right end of the chromosome I and removes part of ribosomal gene cluster (Albertson, 1984). PCR analysis on *eDf24* homozygotes showed that the ribosomal gene cluster boundary is deleted in *eDf24* (C. Thacker, unpublished results). This places the right breakpoint of the *eDf24* deletion within the ribosomal gene cluster. In order to identify the *rec-1* coding sequence, *eDf24* region was extensively analyzed (summarized in Fig 11).

PCR mapping defined the left breakpoint of the *eDf24* deletion to right side of ZK337.4 gene. The deletion extends into the ribosomal gene cluster on chromosome I. The cosmid BO467 contains a few repeats of the ribosomal genes on the right and overlaps with the cosmid ZK340 on the left. The left end of ZK340 cosmid contains the 3' end of sequenced cosmid ZK337, but does not carry the ZK337.4 gene (Fig 12). This places the left end of ZK340 cosmid between ZK337.4 and the 3' end of ZK337 cosmid, which is similar to the left breakpoint of *eDf24* deletion. However, the ZK340 cosmid need not necessarily contain all ZK337 sequence deleted by *eDf24*.

If both cosmids, BO467 and ZK340, contained all the sequence that is deleted by the *eDf24*, they would rescue *eDf24* homozygotes phenotype. The rescue results show that these cosmids can rescue the embryonic arrest phenotype of *eDf24* homozygotes up to adult stage, which are sterile. The straightforward explanation is that the cosmids do not have all the sequences deleted by *eDf24* deletion. This could be at the left end or at the right end. The possibility being the right end was favored because the copy number of the ribosomal gene may result in sterile phenotype, i.e. the BO467 does not have same number of ribosomal genes that was deleted by *eDf24*. So that BO467 could not compensate the loss of ribosomal gene product in *eDf24* homozygotes. On the other hand, the extrachromosomal array contains multiple copies of BO467 cosmid, and the total number of ribosomal genes in the array might be more than the number of genes deleted by *eDf24*. The effect of ribosomal gene copy number on the rescue was investigated by using a single cosmid for the rescue experiment. It was expected that BO467 cosmid would rescue *eDf24* homozygotes, similar to double cosmid rescue, but not with ZK340. Surprisingly, both cosmids

singly gave the same rescue phenotype. This eliminates the possibility that the sterile phenotype is due to low copy number for ribosomal genes.

A second possibility is that there might be different gene(s) within the ribosomal gene cluster and that *eDf24* deletes the gene(s). The sequencing project did not identify any other genes within the ribosomal gene cluster, i.e. the gene cluster has not been sequenced through. However, until it is proven that there are no other genes within the ribosomal gene cluster, this possibility cannot be eliminated.

The third explanation for the sterile phenotype of *eDf24* homozygotes in the extrachromosomal array background is that the gene(s) in the array is not expressing in the germline. It has been shown that multicopy transgenes can get shut off in the germline but not in the soma; while same constructs are expressed in the germline in a less repetitive environment (Kelly *et al.* 1997).
A fourth explanation is that *eDf24* might be a discontinuous deletion, part of which is not covered by the cosmids (BO467 and ZK340).

There was no detectable polymorphism, between wild type and Rec-1, within the regions tested. One explanation for the result is that these genes are not responsible for Rec-1 phenotype. Alternatively, since the regulatory elements were not analyzed, a sequence alteration responsible for the Rec-1 phenotype could lie within a regulatory element. However, further analyses supported the former possibility.

RT-PCR did not identify any genes that were expressed specifically in the adult, in *rec-1* region of chromosome I. One possibility is that *rec-1* gene is not a meiosis specific gene, it may have other function(s). A second explanation is that the transcript might be made at a different time than when it functions. A third explanation is that the *s180* alteration is in a regulatory element, leading to an altered spatial expression pattern not detectable by RT-PCR. For example increased or decreased amount of product. Fourth possibility is that the *rec-1* gene does not map to any of the tested genes.

rec-1(s180) is a recessive mutation (Rose and Baillie, 1979b). The expression of most genes can be suppressed by injection of corresponding double-stranded RNA to generate the equivalent of a null allele, a process known as RNA-mediated interference or RNAi. This mechanism has been shown to work in *C.elegans* (Fire *et al.* 1998), *Drosophila* (Kennerdell and Carthew, 1998), trypanosomes (Ngo *et al.* 1998), plants (Waterhouse *et al.* 1998), and mouse (Wianny and Zernicka-Goetz, 2000). Only a few molecules of injected dsRNA are required per affected cell, arguing against stochiometric interference with endogenous mRNA in *C. elegans* and suggesting that there could be a catalytic or amplification component in the interference process (Fire *et al.* 1998). It could be possible that RNAi of *rec-1* gene could result in Rec-1 phenotype. RNAi phenotype was examined for three of the candidates within *eDf24* region and could not phenocopy Rec-1 phenotype. One possibility is that these genes are not responsible for Rec-1 phenotype. A second possibility is that null allele of *rec-1* could be lethal. There is no evidence to prove that *rec-1(s180)* is null allele. It is difficult to test if the recombination frequency is significantly different in *rec-1/ eDf24* compared to *rec-1* homozygotes.

RNAi on F33H2.1 did not result in any visible phenotype or have any effect on crossover frequency. This observation indicates that F33H2.1 is not an essential gene. It is possible that other genes, i.e. redundancy, compensate loss of function of F33H2.1. There is no obvious phenotype for a putative knockout strain, F33H2.1(*gk10)* (isolated by the Reverse Genetics Core Facility at UBC).

One of the ZK337.2 RNAi animals produced high frequency males. It maybe possible that RNAi with this gene affects the X chromosome disjunction, but not the autosomes. If there is an effect of autosome disjunction, there will be embryonic arrest due to aneuploidy. But there is no embryonic lethality, instead large brood size was observed, about 500 progeny; average brood size is about 300 progeny.

One possibility is that the hermaphrodite was outcrossed with male before ds RNA injection. If this was true, it is most likely that the male progeny would be in the early broods not in the late broods, because the male sperm will be used preferentially to the sperms produced in the hermaphrodites (Ward and Carrel, 1979; LaMunyon and Ward, 1995). There is a possibility that the hermaphrodite had enough male sperm to be used for all four broods. If this was the case, there would be difference in the segregation pattern of the marked chromosomes in the F1 generation (P_o is *dpy-11 unc-42/* + +); i.e. in the normal situation, a heterozygous animal will segregate 75% wild type and 25% mutants in the F1 generation; if it is outcrossed with a heterozygous male, the segregation pattern will not be 3:1. In this situation the segregation pattern is 3:1 wild type to mutant (data not shown). This eliminates the possibility of the hermaphrodites being outcrossed before injection.

Rose and Baillie (1979a) showed that X chromosome non-disjunction increased at high temperature. It is possible that high frequency males are produced due to temperature effect. This explanation is not likely because all the injected animals were kept at the same temperature, 20°, and only one showed high frequency males. If temperature is the cause, all the injected animals should have produced high frequency males. This observation indicates that the temperature is not the cause for the high male frequency observed.

In *C. elegans* hermaphrodites, approximately the first, 40 germ cells that enter meiotic prophase in each gonad arm develop as male, producing about 160 sperm. Thereafter, a switch in sexual fate occurs so that all additional germ cells differentiate as oocytes (Schedl, 1997). The brood size of a self-fertilized hermaphrodite depends on the number of sperm made. The results indicate that there are more sperm made than average. This is only possible if the spermatogenesis to oogenesis switch is delayed. ZK337.2 maybe involved directly or indirectly in the spermatogenesis to oogenesis switch.

RNAi with F33H2.5 gene gave an embryonic arrest phenotype. A mutation of this gene, F33H2.5(*h1881*), results in a sterile adult phenotype. One explanation for the sterile adult phenotype of *h1881* is that *h1881* is a hypomorph and is not producing enough gene product in the adults to be fertile. Another explanation is that *h1881* is a null allele and the maternal contribution, of F33H2.5 gene product, to the embryo is enough to develop up to adult stage. RNAi eliminate the maternal contribution and results in embryonic arrest.

Yandell *et al.* (1994) examined the nature of 23 TMP-induced alleles of either *unc-22* or *pal-1*. More than one-half (13) were deletions and eight of these deletions ranged in size from 0.1 to 15 kb, and the forward mutation rate is 1X 10^{-4}. Gengyo-Ando and Mitani (2000), identified 22 deletions out of 91 alleles of *ben-1* gene. The deletion range from 50 bp to more than 10 kb, and the forward mutation rate is 1X 10^{-3}. In the experiments reported in this thesis, deletions ranging from 252 bp to more than 90 kb were isolated from the UV-TMP mutagenesis using an *hIn1* balanced strain. The region targeted is left to the *unc-75* marker on chromosome I. The lethal recovery rate is 1x10^{-2} (0.9%). This is a higher frequency than previously reported. However on a gene basis, one internal deletion was observed making that frequency 1x10^{-4}, in agreement with Gengyo-Ando and Mitani (2000).

hIn1 chromosome was used to isolate new alleles of *unc-75* and *unc-54* using EMS; two new alleles of *unc-75* were isolated from 18,700 chromosomes screened (1X 10^{-2}) and one new allele for *unc-54* from 2,600 chromosomes screened (3X 10^{-2}) (Zetka and Rose, 1992). In this thesis, *hIn1* was used as a balancer to isolate lethals, using UV-TMP, letahls were recovered at a rate of 1x10-2 higher than the frequency reported by Zetka and Rose (1992) for EMS. These differences would explain the different recovery rate observed between this screen and the screens reported by Zetka and Rose (1992). Forward mutation rate for different mutagens have been well documented. Forward mutation rate for EMS, LGV(left): 6.7 X 10-5 (Johnsen and Baillie, 1991); *hDf6* region: 4.3 X 10-5 (Howell and Rose, 1990); *sDp2* region: 5 X 10 -5 (Johnsen *et al.* 2000); for an average gene: 5 X 10-4 (Anderson, 1995). The frequency of formaldehyde-induced *unc-22* mutations is 2 X 10^{-4} (Moerman and Baillie, 1981). Forward mutation rate for UV irradiation

(120J/m2), *eT1(III;V)* balanced region, is about half that observed with EMS (Stewart *et al.* 1991; Johnsen and Baillie, 1991).

There are three complementation groups identified within *eDf24* region, indicating that there are three essential loci. The complementation analysis and PCR mapping of deletions indicate that F33H2.8 is one of the essential loci.

Complementation analysis between the new deletions and *rec-1(s180)* shows that all the new deletions complement *rec-1* mutation. Four of the five large deletions are linked to *hIn1[unc-101]* chromosome; another linked to *unc-54*, where there is no *hIn1*. The markers from chromosome I, *dpy-5* and *unc-13*, were used as tester markers to identify Rec-1 phenotype. The crossover frequency between the markers, in new deletion over *rec-1* background is slightly higher than the internal control, which does not carry the deletion (~3 in the tester and ~1.5 in the control). This difference may reflect Rec-1 phenotype. Zetka and Rose (1992), showed that in *hIn1* background, the crossover frequency was enhanced in intervals both inside and outside the gene cluster. It is also possible that the slight increase in the deletion background is the compensatory elevation of the crossover suppression in *hIn1* region. This increase is not comparable to crossover frequency observed in *rec-1* homozygotes . This indicates that the new deletions do not delete *rec-1* gene.

The new deletions are isolated from mutagenesis and it is possible that there are more than one alterations in the genome. A possibility for not observing Rec-1 phenotype in the deletion background, is that the deletions are linked to a crossover suppressor mutation. It is not likely

that all the new deletions are linked to the crossover suppressor. However, in order to eliminate this possibility, new deletions were reisolated as described in materials and methods. This would eliminate the possibility of a crossover suppressor as a second site mutation in the strain, but may not eliminate any mutations that are closely linked to the deletions. In order to avoid *hIn1* effect on recombination, another set of markers, *dpy-11* and *unc-42*, was selected from chromosome V as tester markers. *hIn1* does not have any effect on crossover frequency on other chromosomes (Zetka and Rose, 1992). Again all the deletions tested complement *rec-1*. This indicates that *rec-1* gene is not in the defined deletions.

There are three deletions (*hDf32, hDf33* and *hDf34*) that map within *eDf24* deletion. Another deletion, *hDf30*, bigger than *eDf24*, deletes more than three cosmids in *eDf24* region. The breakpoints of one of the deletions, *hDf31*, are more or less similar to known *eDf24* breakpoints. Both *eDf24* and *hDf31* delete the ribosomal gene cluster boundary and the right breakpoints map within the ribosomal gene cluster, although the extent within the cluster is not known (Fig 10); they also delete the 3' end of the ZK337 cosmid, but not the ZK337.4 gene. These results place the left breakpoint between the ZK337.4 gene and the 3' end of ZK337 (Fig 15). The only difference is that *eDf24* fails to complement *rec-1* mutation, while *hDf31* complements *rec-1*. This indicates that the genes within the known deletion breakpoints are not responsible for Rec-1 phenotype. One possibility is that both of these deletions have different breakpoints within ribosomal gene cluster, and there might be additional genes deleted by these two deletions. As discussed above, this explanation is based on the assumption that there are non-rDNA genes within the ribosomal cluster.

A possible approach is to map the left breakpoints precisely by PCR, then amplify the fragment spanning the breakpoint, using one primer close to the left breakpoint and another primer specific for the ribosomal gene. The PCR product can then be sequenced and the breakpoint can be determined. In order to determine if there are any other functional genes within the ribosomal gene cluster, the whole cluster has to be sequenced and annotated, eventually by the sequencing consortium.

Since the exact breakpoint on the left has not been determined, it is also possible that *eDf24* deletes ZK337.1 but *hDf31* does not and that might be the reason for the difference in the complementation with *rec-1*. This possibility was eliminated by the fact that *hDf30* deletes the whole ZK337 cosmid (Fig 16), and still complements Rec-1 phenotype indicating that ZK337.1 is not responsible for Rec-1 phenotype.

Another possibility is that there might be a discontinuous deletion or alteration, associated with *eDf24*, which maps to the left side of the breakpoint. If this alteration is another deletion, it should be possible to identify it by PCR; but if the alteration is small, then different deletions have to be tested for failure to complement Rec-1 phenotype. *unc-101* maps to the left of *eDf24*. An *unc-101 eDf24* double was constructed as a result of recombination between *unc-101* and *eDf24*, and the genetic material to the left of *eDf24* was exchanged with that on the *unc-101* chromosome. The homozygous deletion arrest stage was unaffected. PCR indicated that there might be a sequence alteration at the 3' end of ZK337. If there is a discontinuous alteration on the left side of *eDf24*, that alteration could be lost due to crossing over between *unc-101* and *eDf24*. This could be the reason that *unc-101 eDf24* complement *rec-1*, but *eDf24* fails to complement.

The fact the band is faint indicates that one or both of the primers are not annealing properly. Sequencing the faint band would help to identify the exact change at the 3' end of ZK337 cosmid in *unc-101 eDf24* chromosome. Since the faint band will not give enough PCR product for sequencing, a set of primers that span the boundary can be used amplify the region and the sequence can be determined. An alternative approach would be testing the progenitors of *unc-101 eDf24* strain if they fail to complement *rec-1*. If they fail to complement *rec-1*, then it will support that the alteration happened only in *unc-101 eDf24* strain. Another explanation would be that the ribosomal gene cluster might have undergone recombination and the number of ribosomal genes linked to *eDf24* is altered. This can be tested by *in situ* hybridization using probes specific for the ribosomal genes. This will not identify any small differences. Alternatively, all the spacer sequences in wild type can be sequenced and determine if there are any coding regions there. If coding regions are identified, they can be sequenced from *rec-1* mutant to test if there are any alterations.

There are several deletions available at the right end of the chromosome I and that complement Rec-1 phenotype. A few deletions delete *unc-54* gene and extent towards the center of the chromosome I. One of the deletions is *eDf9*, which deletes *ces-2* gene that maps between *unc-54* and *eDf24* breakpoint (Fig 11). *ces-2* locus is mapped to the cosmid CO6A3, which overlaps with sequenced cosmids ZK337 and ZK909. ZK909 is the next cosmid sequenced to the left of ZK337. *eDf9* deletion complements both *eDf24* and *rec-1* mutation. Both deficiencies *eDf24* and *eDf9* may delete common sequences that are not essential. Determining the right breakpoint of *eDf9* would give the left boundary to the *rec-1* coding region. *hDf30* deletes ZK337 and extends to the left. Determining the left breakpoint of *hDf30* gives the right boundary for *rec-1* region.

Since *sDp1* maps to the right end of the chromosome I, and complements *rec-1 (s180)* (Zetka and Rose, 1995b), *rec-1* gene is somewhere within that region. Overlapping deletions to the left of ZK337 could be tested for failure to complement *rec-1*.

In this study, 55 mutations balanced by *hIn1* chromosome were generated. Eleven deletions fail to complement *eDf24*. Six of the 11 candidates are proven deletions, with breakpoints mapped to cosmids. There are three complementation groups identified within the *eDf24* region. Complementation analysis between *rec-1* mutation and the new deletion shows that *rec-1* gene is not within the known breakpoints of *eDf24* deletion. This raises the possibility that *eDf24* might have an additional sequence alteration. Alternatively, *rec-1* could be within the ribosomal gene cluster.

CHAPTER V

GENERAL DISCUSSION

In this thesis the following questions were addressed. First, does the *rec-1* mutation affect the crossover distribution on the X chromosome. Second, is the *rec-1* mutant phenotype due to random double strand break along the chromosomes. Third, can the *rec-1* mutation randomize the crossover distribution in the *him-1(e879)* mutant background. Finally, potential candidates for the *rec-1* coding region were investigated.

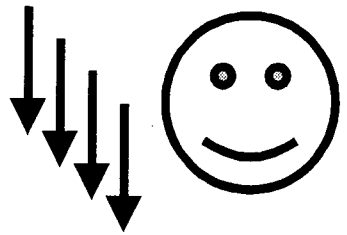
Even though there is no central gene cluster, on the X chromosome the *rec-1* mutant flattens the meiotic pattern of the X chromosome. These data demonstrate that the crossover distribution is regulated in all six chromosomes. In *C. elegans*, some gene products are required for both autosome and X chromosome functions. Mutations in the genes *him-6* and *him-14* show general reduction in crossing over on all chromosomes (Hodgkin *et al*, 1979; Kemphues *et al.* 1988; reviewed in Zetka and Rose, 1995a). In contrast, mutation in *him-1 him-5* and *him-8* reduce crossover only on the X chromosome (Hodgkin *et al*, 1979; Broverman and Meneely, 1994). The *rec-1* mutation dramatically affects crossing over in the central gene cluster and flanking regions along chromosome I (Zetka and Rose, 1995b), and the other autosomes (Rose and Baillie, 1979b; Rose, A. M. Pers. comm.). Each *C. elegans* autosome is marked by a cluster of genes and this clustering is a result of recombination suppression (Brenner, 1974; Barnes *et al.* 1995). This has been supported by several studies, which showed that the gene cluster results, in part, from less recombination per base pair than the genomic average (Greenwald *et al.* 1987; Kim and Rose,

1987; Prasad and Baillie, 1989; Starr *et al.* 1989; Barnes *et al.* 1995). There is no pronounced gene cluster on the X chromosome and the organization differs from autosomes (Brenner, 1974; Barnes *et al.* 1995). Despite this organizational difference, crossover distribution is regulated on the X chromosome and the pattern changes in the absence of the *rec-1* gene product.

Even though the Rec-1 phenotype is similar to the effect of radiation on crossing over, the Spo-11 phenotype was not suppressed in the *rec-1 spo-11* double mutant. The results show that the Rec-1 phenotype is not due to excess DSBs in the DNA. Functional SPO-11 is necessary to randomize the crossover distribution in the *rec-1* background. In *rec-1* homozygotes, crossover frequency is enhanced at least two-four fold within the autosomal cluster regions (Rose and Baillie, 1979b). However, in the large intervals flanking the cluster the frequency is reduced (Zetka and Rose, 1995b). Thus the total length of the chromosome I genetic map in Rec-1 is comparable to wild type; the total number of crossovers is not altered but the meiotic distribution is eliminated and crossing over distances made more proportional to the physical distance (Zetka and Rose, 1995b). In *C. elegans*, treatment with ionizing radiation increased recombination frequency in the region of the gene cluster (centrally located in the autosomes), but not outside the cluster (Kim and Rose, 1987; Rattray and Rose, 1988). These observations indicate that the Rec-1 phenotype is similar to the radiation effect on crossover frequency. The disruption of crossover distribution observed in Rec-1 could be the result of random double strand breaks in the DNA (Fig 19). The *spo-11* null mutant is defective in making double strand breaks (DSB) in *C. elegans* and can be partially suppressed by inducing DSB by ionizing radiation treatment (Dernberg *et al.* 1998).

Figure 19: Double strand breaks (DSB) initiation in wild type and Rec-1. DSB occur preferentially at the chromosomal arm region in the wild type. DSB initiate randomly in Rec-1.

wild-type



Rec-1

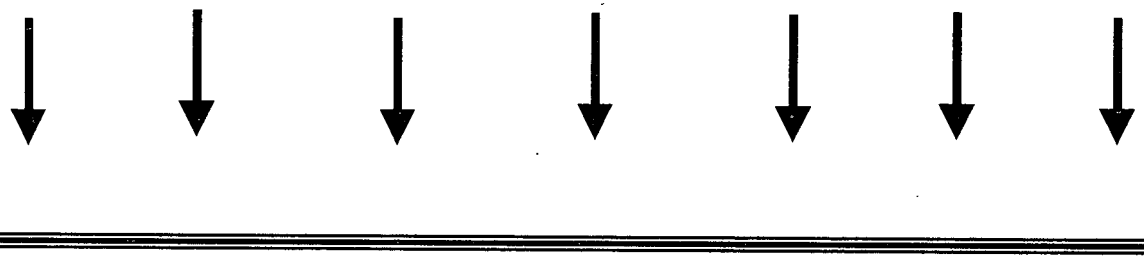


Fig 19

The creation of double-strand breaks in yeast meiosis represents a distinct ability of the Mre11 complex. Meiotic DSB formation requires Spo-11, Mre11/Rad50/Xrs2, and at least five other proteins, two of which may associated with Mre11 complex (Usui *et al.* 1998). Although *rec-1* could not suppress the Spo-11 phenotype, it does not eliminate the possibility that REC-1 has sequence specificity and recruit SPO-11 or a complex similar to Mre11 complex, in yeast, for making DSB. In *rec-1* mutant, a possibility is that, the specificity is altered and the crossover distribution is random. This proposal is supported by the fact that in wild type *E. coli,* most conjugational recombination proceeds via the RecBCD pathway (Smith, 1991), which is stimulated by the presence of *lambda* (x), a sequence [5' -GCTGGTGG- 3'] (Smith *et al.* 1981a, 1981b) that creates a recombination hotspot (Lam *et al.* 1974). The m26 hotspot of the fission yeast *Schizosaccharomyces pombe* has been well characterized (reviewed in Fox and Smith, 1998). The hotspot activity of M26 depends upon a specific nucleotide sequence, the heptamer ATGACGT (the site of the M26 mutation is underlined; Schuchert et al. 1991). Fox *et al.* (2000) identified a new family of sequences that can act as meiotic recombination hotspot (C/T/G)TGACGT(A/T) in *S. pombe*. Little is known about the nature of recombination hotspots in the human genome. Comparisons of linkage maps with physical maps or sequences of human chromosomes have revealed evidence for long domains showing elevated recombinational activity (Janson *et al.* 1991; Oudet *et al.* 1992; Hubert *et al.* 1994; Robinson and Lalande, 1995; Malfroy *et al.* 1997; Dunham *et al.* 1999). No primary sequence similarities could be found between any of the very few well defined crossover hotspots in the human genome, all of which show recombinationally active domains 1-2 kb long (Jeffreys *et al*, 2000).

Sen and Gilbert (1992) showed that DNA oligomers containing three or more contiguous guanines form tetrastranded parallel complexes and suggest that naturally occurring chromosomal telomeres, which all have guanines at their 3' termini, maybe able to form these superstructures. In *C. elegans* the GC content (36%) is essentially unchanged across all the chromosomes (The *C.elegans* sequencing consortium, 1998), unlike the GC content in vertebrate genomes, such as human or yeast (Dujon *et al*, 1994; Bernardi, 1995). It maybe possible that there is difference in guanine or similar complex formation between wild type and Rec-1 which could be responsible for the random distribution of crossing over in Rec-1.

Mre11p, Rad50p and Xrsp proteins are also involved in non-homologous recombination and play a key role in telomere maintenance. Deleting any one of the Mre11/Rad50/Xre2 proteins creates a yeast strain with shortened telomeres and, in some strain backgrounds, cells become senescent as they do without telomerase (Kironmai and Muniyappa, 1997; Boulton and Jackson, 1998; Nugent *et al.* 1998). Normal telomere maintenance requires both the Mre11 complex and Ku (Ku70 and KU80) proteins (reviewed in Haber, 1998). Recently it has been reported that the Ku70/80 heterodimer is recruited rapidly to double-strand cuts (Martin *et al.* 1999). It is possible that Ku proteins are involved in homologous recombination as well.

It has been demonstrated that in *Drosophila* females the centromere can suppress the occurrence of meiotic exchange over long distances in a polar fashion (Dobzhansky, 1930; Hawley, 1980). In *Saccharomyces cerevisiae,* meiotic crossover frequency was increased near centromere when the centromere structure was altered (Lambie and Roeder, 1986). On the other hand, centromere (CEN3) exerts a bidirectional repression of crossing over when it is moved to adjacent sequence

(Lambie and Roeder, 1988). One explanation for the central clusters in *C. elegans* autosomes is that it correlates with the location of the meiotic centromere. If the central gene cluster in the autosomes is due to the location of meiotic centromere, one possible explanation for the Rec-1 phenotype is that the centromere structure might have been altered in *rec-1* mutant. In Rec-1, there is a slight increase in the spontaneous male frequency compared to wild type (Rattray and Rose, 1988; Zetka and Rose, 1995b; this study). There is no effect in the brood size or in the egg hatching frequency (Zetka and Rose, 1995b and this study). If the autosomes were to missegregate, with a similar frequency as the X chromosome, the effect on brood size, due to aneuploidy, would not be significant. However, there is currently no defined centromeric sequence in *C. elegans*. The sequencing consortium reports (1998) that the extensive, highly repetitive sequences that are characteristic of centromeres in other organisms maybe represented by some of the many tandem repeats found scattered among the genes, particularly on chromosome arms. Identifying the functional centromere would help to support or to reject this possibility.

It has been postulated that the presence of repetitive DNA may influence the rate of recombination (Brutlag, 1980). The *C. elegans* sequencing consortium identified both inverted and tandem repetitive sequences that are more frequent on the autosomal arms than in the central regions of the chromosomes or on the X chromosome (The *C. elegans* sequencing consortium, 1998). It was also observed that the conserved set of eukaryotic genes shared by yeast and *C. elegans* are excluded from the arm region with sharp boundaries; these are close to the boundaries in the genetic map that separate regions of high and low rates of recombination (Barnes *et al.* 1995). These features suggest that DNA on the arms might be evolving more rapidly than in the central regions of the autosomes. This would explain the fact that the genes in the cluster regions are less

diverged in evolution (Jones, 1999). These observations could be interpreted to suggest that the Rec-1 phenotype, which alters the crossover distribution throughout the genome, may not have selective advantage. This makes the Rec-1 phenotype interesting not only to study crossover distribution but also to study evolution and selection. Rattray and Rose (1988) showed that there was no substantial difference in the survival of Rec-1 and N2 strains when they were plated together, under laboratory conditions, for sixteen generations. One possible explanation is that the laboratory conditions are not providing enough selective pressure to observe evolution and selection. Another possibility is that a difference could be observed after a long period of time. Alternatively, Rec-1 phenotype may not have any effect on evolution or selection at all.

Even though the *rec-1* mutation alters the crossover distribution throughout the entire genome, a more severe Him-1 phenotype was observed in *him-1 rec-1* double mutant animals and suggests that functional HIM-1 is necessary for the redistribution of crossing over on the X chromosome in *rec-1* background. Crossing over during meiosis is globally controlled in many organisms (Hawley 1988; Carpenter 1988). This is evident in *C. elegans* by the reduction of crossing over at the central region of each autosome, but not the X chromosome, leading to a tight genetic cluster (Brenner 1974; Greenwald *et al.* 1987; Starr *et al.* 1989; Edgley and Riddle 1990; Barnes *et al.* 1995). *him-1* encodes a member of SMC (Structural Maintenance of Chromosomes) protein family (B. Meyer, pers. comm.). Members of the highly conserved SMC protein family are central for diverse chromosome dynamics, including mitotic chromosome condensation and sister-chromatid cohesion. The *him-1(e879)* allele causes abnormal X-chromosome nondisjunction, producing 16% males (Hodgkin *et al.* 1979), and crossover distribution is severely affected on the X chromosome (Broverman and Meneely, 1994). The *him-3* gene in *C. elegans* is necessary for

synapsis and chiasma formation; hypomorphs are defective in crossing over but proficient in synapsis (Zetka *et al.* 1999). These observations indicate that integrity of the chromosomal structure is necessary for the distribution of crossing over in the *rec-1* background.

Another possible model to explain the Rec-1 phenotype is that chromatin structure is altered in Rec-1 becoming more accessible to the recombination machinery (Fig 20). In *S. cerevisiae*, DSBs occur not only at hotspots, but also in regions that display more normal frequencies of meiotic recombination (Lichten and Goldman, 1995); DSB distributions mirror those of meiotic crossovers, both over entire chromosomes (Game, 1992; Zenvirth *et al* 1992) and within smaller regions (Wu and Lichten, 1994). DSBs are coincident with upstream of initiation site of gene transcription in *S. cerevisiae* (reviewed in Lichten and Goldman, 1995). In *C. elegans*, more genes are mapped in autosomal center regions than in autosomal arm regions or in the X chromosome (The *C. elegans* sequencing consortium, 1998), If the crossover distribution is correlated with promoter sequence in *C. elegans*, the crossover frequency would be high in the centers of the autosomes, than the autosome arms. In the genetic map of autosomes, genes are clustered in the center (Brenner, 1974; Barnes *et al.* 1995). These observations indicate that, in *C. elegans*, the DSB sites do not coincide with the promoter sequences in wild type.

If the crossover pattern is affected by chromatin structure, it is possible that alteration in the expression pattern will also alter the crossover pattern. For example, the *tam-1* (*t*andem-*a*rray *m*odifier) mutation in *C. elegans* reduces the expression of highly repeated transgenes, in somatic tissues, and shows high a incidence of males with small brood size (Hsieh *et al.* 1999). The high

Figure 20: Alteration in the chromatin structure affects the DSB formation. Central region of the autosomes are highly condensed compared to the arm region and the recombination machinery could not access the whole chromosome equally and favor the arm region. While in Rec-1, chromatin structure is altered and the recombination machinery can access equally along the chromosome.

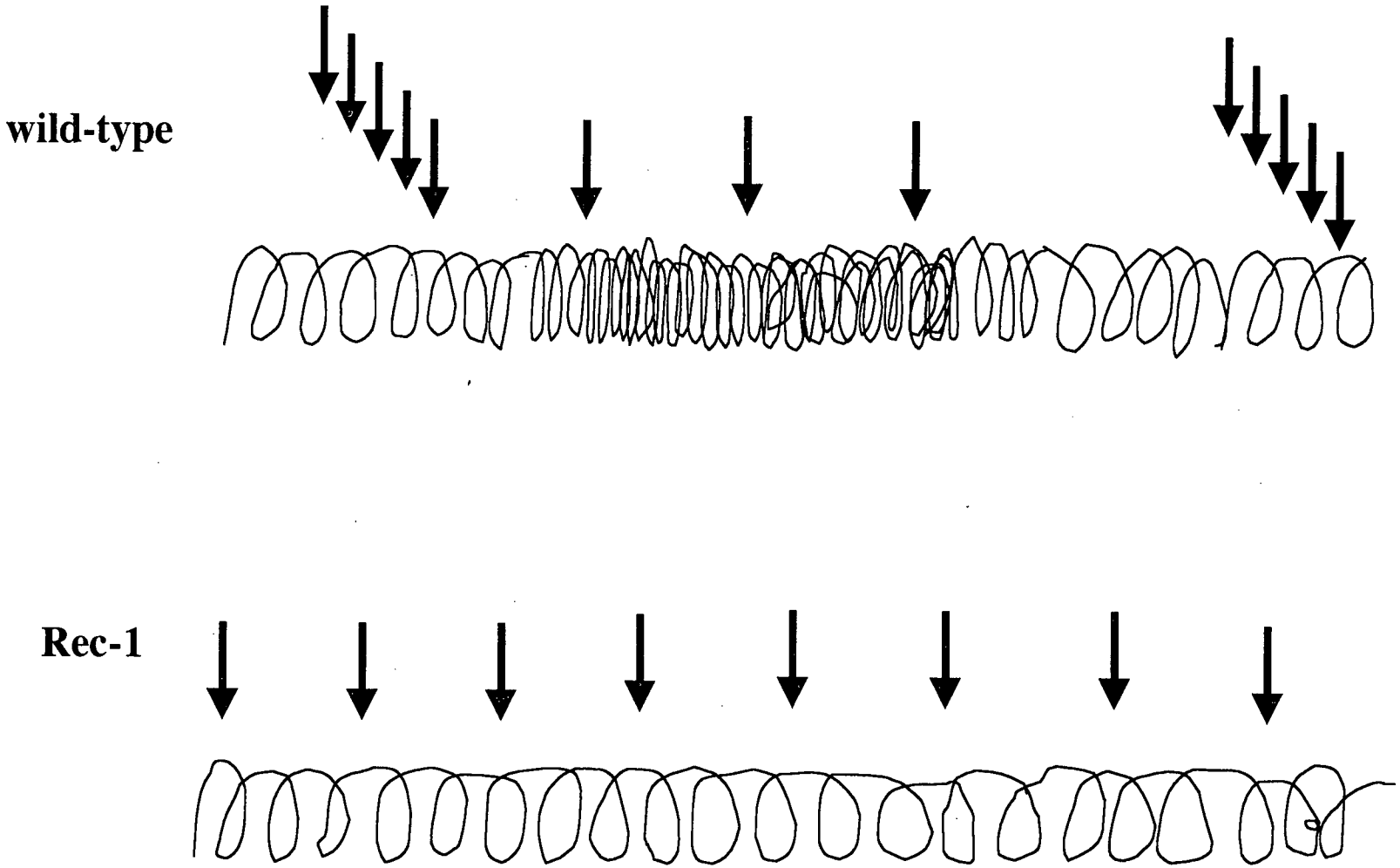


Fig 20

frequency of males indicates a defect in X chromosome disjunction and the small brood size maybe due to autosomal non-disjunction. It is possible that *tam-1* might affect the crossover distribution or frequency, or both, along chromosomes. The predicted TAM-1 protein contains two cysteine-rich regions, a C3HC4 (RING finger) motif and a B-box motif (Hsieh *et al.* 1999). Although both domains may act as metal (zinc)-binding domains, there is not yet any basis to predict a specific biochemical function (Saurin *et al.* 1996). Five additional mutations, *lin-9(n112), lin-15B(n744), lin-35(n745), lin-51(n770*) and *lin-52(n771),* were found to produce a transgene-silencing phenotype similar to *tam-1* (Hsieh *et al.* 1999). It will be informative to test if these mutations, that affect the chromatin structure, would affect the crossover distribution along the chromosomes.

In addition to transgene-silencing, *tam-1* also affects several endogenous genes (Hsieh *et al.* 1999). For example, a partial loss-of-function allele of *lin-2, let-2* and *lin-3* gave a more severe phenotype in a *tam-1* mutant background. There was an approximately two-fold effect. No effect was seen for *unc-22* mutation. Since *tam-1* has effect on some of the endogenous genes, it would be possible to investigate if the conversion rate is affected in a *tam-1* mutant background. Gene conversion and crossing over are associated events in yeast (Fogel and Hurst, 1967) and other fungi (reviewed in Whitehouse, 1982). One interpretation to explain this association is that these events are mechanically connected, and such a connection is explicit in most models of recombination (Holliday 1964; Meselson and Radding 1975; Szostak *et al.* 1983). In these models, gene conversion events reflect an intermediate in the formation of crossovers. An alternative possibility is that conversion and crossing over represent mechanistically distinct events that require a common condition (e.g., accessible DNA), a model which has not been ruled out

(reviewed in Fink and Petes 1984; Hastings 1987; Ray *et al.* 1988). In *Drosophila,* analysis of intragenic recombination using the rosy locus has demonstrated a series of parallels between crossing over and gene conversion (Hilliker and Chovnick, 1981; Hilliker, Clark and Chovnick, 1987). Two mutants, *mei-9* (Hilliker and Chovnick 1981; Carpenter 1982, 1984) and *mei-218* (Carpenter 1982, 1984) have increased conversion frequencies with an accompanying decrease in crossing over. Conversion events were also observed in *C. elegans*. For example, *unc-22* (Moerman and Baillie, 1979), *unc-15* (Rose and Baillie, 1980), *unc-54* region (Waterson *et al.* 1982), *unc-60* region (McKim *et al.* 1988a), *cha-1-unc-17* (Rand, 1989), *unc-26* (Charest *et al.* 1990). In *rec-1* strain, not like *mei-9* and *mei-218* in *Drosophila*, the frequency of both crossing over and conversion increases two to three-fold (Rattray and Rose, 1988).

The basic unit of chromatin is the nucleosome, which consists of ~146 bp of DNA wrapped around the four core histones arranged in an octamer. The amino-terminal tails of the histones, in particular H3 and H4, are highly conserved and contain four lysine (K) residues that can be reversibly acetylated (Felsenfeld, 1996; Workman and Kingston, 1998). A frequently suggested function of histone acetylation is that it serves as a control for transcription, since active sections of chromatin appear to contain hyperacytylated histones (Davie and Candido, 1978; Levy-Wilson *et al.* 1979). Histone hypoacetylation is generally correlated with transcriptional inactivity, telomeric and centromeric heterochromatin, and silenced areas of the genome, such as the donor mating-type loci in yeast (Turner, 1998; Workman and Kingston, 1998).

It maybe possible that the mutations that alter the chromatin structure and expression affect crossover distribution as well. Position-effect variegation (PEV) is the mosaic inactivation of a

euchromatic gene and most often occurs when a chromosomal rearrangement abuts a normally euchromatic region of a chromosome to a breakpoint in centromeric heterochromatin (Grigliatti, 1991; Henikoff, 1992; Reuter and Spierer, 1992). An analogous situation is thought to occur in the phenomenon of telomeric position effects (TPEV). This occurs when a reporter gene is inserted in or near the heterochromatin of the telomeres of *S. cerevisiae* chromosomes (De Rubertis *et al.* 1996; Grewal *et al.* 1998; Grunstein 1998). Many dominant mutations that either suppress (*Su(var)s*) or enhance (*E(var)s*) euchromatic PEV have been isolated and characterized (Reuter and Wolff, 1981; Sinclair *et al.* 1983; Reuter *et al.* 1986). Molecular cloning of several of these modifier genes indicates that they have the potential to encode regulatory or structural components of chromatin (Tschiersch *et al.* 1994; Cleard *et al.* 1997; reviews by: Eissenberg, 1989; Grigliatti, 1991; Reuter and Spierer, 1992; Lohe and Hilliker, 1995; Elgin, 1996).

Specific missense mutations in the structural gene of HDAC1 (histone deacetylase) suppress silencing and increase the expression of a *w+* gene subject to PEV (Mottus *et al.* 2000). Heterochromatic regions never undergo spontaneous meiotic crossing over in *Drosophila* females regardless of their position in the genome; even mutations that greatly alter the distribution of meiotic exchange in euchromatin do not release the heterochromatic suppression of exchange (reviewed in Hawley *et al.* 1993). It is possible that heterochromatin may undergo crossing over in Su(var) or E(var) mutant background.

In *S. cerevisiae*, all the natural and artificial DSB sites examined are located in regions of chromatin hypersensitive to DNAse I and micrococcal nuclease (Mnase) (Ohta *et al*, 1994; Wu and Lichten, 1994, 1995). Thus, DSBs appear to occur where nucleosomes are absent or disrupted

and the underlying DNA is exposed. Although chromatin structure plays a role in determining DSB sites, it is also clear that other factors are important. For example, adjacent sites with similar degrees of nuclease hypersensitivity can display markedly different DSB frequencies (Wu and Lichten, 1994). Wu and Lichten (1995) suggest that the DSB sites compete for regionally limited factors necessary for DSB formation, and that different amounts of factors are available in different parts of the genome. If this is true, it is conceivable that REC-1 functions to control the distribution of the limiting factors, such that in *rec-1* mutant these elements become evenly distributed leading to the more random initiation of crossovers along each chromosomes.

In summary, it is demonstrated that the crossover distribution is altered on the X chromosome in *rec-1* mutant background; random distribution of crossing over in *rec-1* mutant background is not due to excess DSB in the genome; functional HIM-1 protein is necessary for the random distribution of crossing over on the X chromosome. In addition, several candidates for the *rec-1* gene has been tested and eliminated.

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Appendix I

Genes mapped within the *eDf24* deletion

Genes	Similarity
ZK337.1	Alpha-2-Macroglobulin family
ZK337.2	Zinc-finger c2H2 type
ZK337.4	Rad11
F33H2.1	Rad3
F33H2.2	Zn-15 transcription factor
	Dachahund variant – Droso
	Zinc-finger protein
F33H2.3	Leucine rich repeat
F33H2.5	DNA Polymerase epsilon family B
F33H2.6	Secretory pathway protein
	Surface protein
F33H2.7	Skm-BOP2
	Skm-BOP1
F33H2.8	Nucleoside diphosphate kinaseI
Cyp-5	Cyclophillin
F31C3.3	Probable memrane protein
	DNA polymerase III, beta chain
F31C3.4	Growth arrest inducible gene product
	Putative zinc transporter
F31C3.5	Hypothetical protein
	Type III restriction enzyme
F31C3.6	Localized in the nucleoli
	Fms-related tyrosine kinase1
	Similar to Zinc-finger C2H2 type

Appendix II

Studies of F33H2.1

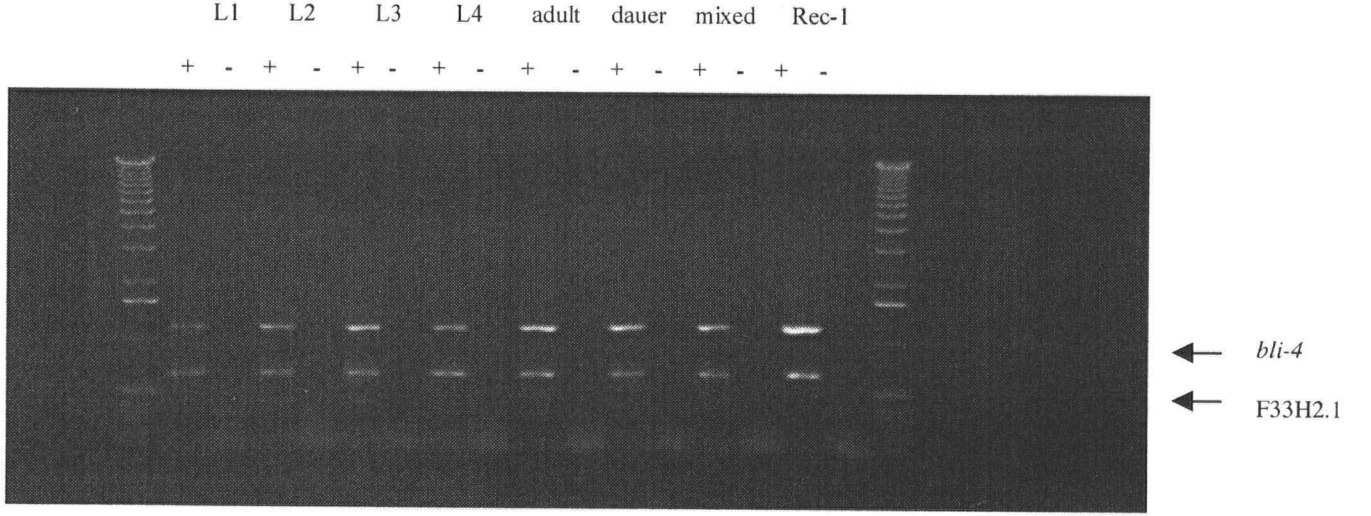
A. Temporal expression pattern of F33H2.1

Total RNA was used to perform RT-PCR (+); as negative control, RNA sample was treated with RnaseI and used as template (-). The stages used are indicated above (L1, L2, L3, L4, adult, dauer, mixed stage, and Rec-1).

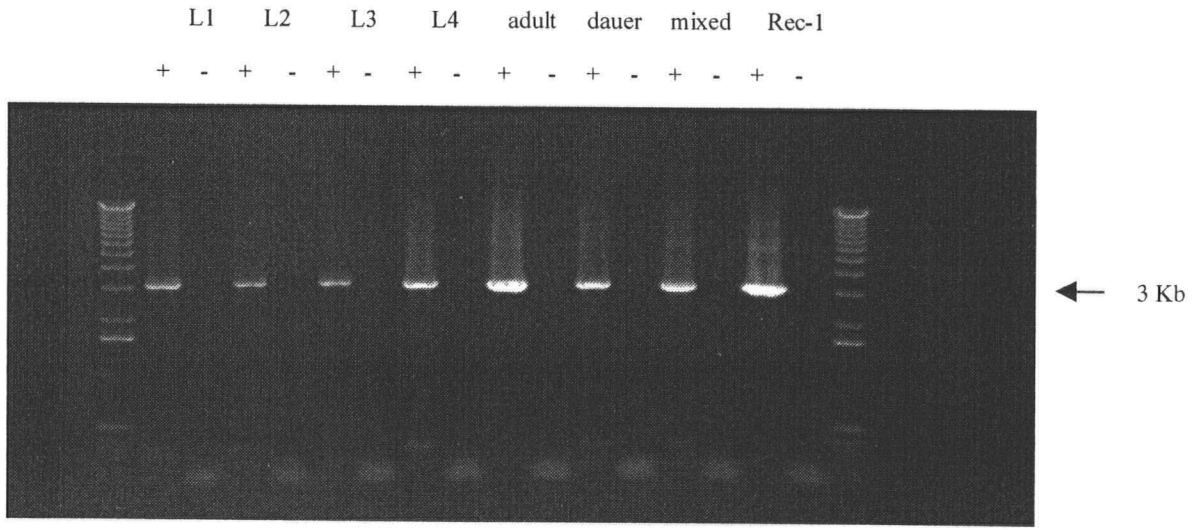
(a) RT-PCR using primers specific for F33H2.1 gene and *bli-4* gene.

As expected, F33H2.1 primers are producing 650 bp and *bli-4* primers are producing 1.2 kb.

(b) RT-PCR to amplify the full-length cDNA from F33H2.1using primers specific for F33H2.1 cDNA. These primer will produce a 3.2 kb product. This shows that 3.2 kb transcript is produced in all developmental stages.



(a)



(b)

Appendix II.A

Appendix II

Recombination frequency using cosmid arrays

B.1. Recombination frequency using the cosmid BO467

Strain name	Genotype	Total progeny	Recombinants (Dpy-11 and Unc-42)	R.F	C.I. (95%)
BC4772	*rec-1/ rec-1; dpy-11 unc-42/ + +; sEx100*	1627	71	4.4	3.4 - 5.6
	rec-1/ rec-1; dpy-11 unc-42	1837	136	7.6	6.4 - 9.0

Appendix II-B.2

Recombination frequency using both BO467 and ZK340

Strain name	Genotype	Total progeny	Recombinant (Dpy-11)	R.F.	C.I. (95%)
BC4934	*rec-1/rec-1; dpy-11 unc-42/ + +; sEx150*	2407	33	2.9	1.9-3.8
	rec-1/rec-1; dpy-11 unc-42/ + +	948	32	6.2	4.5-9.8

Data from Cheng and Rose (unpublished results)

Appendix II. B. 3

Recombination frequency using new transgenic satains

Strain # & *sEx#*	cosmid(s)	Total progeny	Recombinants (Dpy)	% of Rollers	R.F.	C.I. (95%)
BC5675 *sEx688*	BO467 &ZK340	1629	54	21%	6.8	5.1 – 8.9
BC5677 *sEx690*	BO467 & ZK340	2822	71	30%	5.1	3.9 – 6.5
BC5683 *sEx696*	BO467 & ZK340	2988	101	19%	7.1	5.6 – 8.4
BC5674 *sEx687*	ZK340	1241	38	42%	6.3	4.3 – 8.6
BC5676 *sEx689*	ZK340	3129	141	31%	6.3	7.8 – 11.1
BC5679 *sEx692*	ZK340	2214	77	27%	7.0	5.6 – 8.9
BC5680 *sEx693*	ZK340	1738	54	33%	6.4	4.8 – 8.3
BC5681 *sEx694*	ZK340	1405	47	11%	6.9	5.0 – 9.2

All the parents scred here are Rollers and carry the extrachromosomal array.

Appendix II-C

Recombination frequency using plasmids that can constitute full length F33H2.1 gene

Strain name	Genotype	Total progeny	Recombinants (Dpy-11)	R.F.	C.I. (95%)
KR3227	*rec-1/ rec-1; dpy-11 unc-42/ + +; hEx142*	2443	78	6	5.2-8.2
	rec-1/ rec-1; dpy-11 unc-42/ + +	2290	91	8.3	6.6-10.1

Appendix II.D

Recombination frequency with an integrated array (cosmids BO467 and ZK340)

Strain name	Genotype	Total progeny	Recombinants (Dpy-11)	R.F.	C.I. (95%)
KR3388	*rec-1/ rec-1; dpy-11 unc-42; hIs7*	1128	44	8.1	6.9 – 11.0
BC4934	*rec-1/ rec-1; dpy-11 unc-42; sEx150*	3858	113	6	4.9 – 7.2
	rec-1/ rec-1; dpy-11 unc-42;	574	22	6	4.8 – 7.7

The BC4934 was freshly thawed and tested.

APPENDIX II. E

MSSSDAFWRMFANKNKGKSNTRSAFQVVKEEQPSTST

EPDDKKPLHHEIAGEMIKNPAKGKRKRVQIKNDEYE

QLMMLGVPVRVPRGLSLYSTQKLMIVRILTALKNSQN

VLGESPTGSGKTMALLASTCAWLK

a) Amio acids in the GFP::F33H2.1 promoter construct.

5' CTTGAGCCATGCACACGTAG 3'

5' CTTCCACTGATGTCTGAGCC 3'

b) Primers used for cloning 5' end of F33H2.1 with GFP vector pPD95.75 using BamHI site.

One primer has BamHI site and the other end is blunt end ligation.

APPENDIX II. F

**MRGSHHHHHHGMASMTGGQQMGRDLYDDDDKDR
WGSELEIFSAITKHNEPEMQYFDAFKPSSTAIVCIEKW
LYFQSYFGNQQYQSTYRLNISIEPINQNGRFNHTFDAD
VSMSTSFGNPRPTTTRSSAGPRNMQYKEENAWLADA
AADGDDWKDPSMSETGHKPISEGCKTTISLWCMSPAL
SFFDAGIRSLIRLLTKPERKLSWLLPPLSNNZ**

Antigen used for antibody production

Appendix III

Additional new mutations linked to *unc-54*

Strain name	Allele number
KR3664	*h1884*
KR3665	*h1885*
KR3675	*h1893*
KR3678	*h1909*
KR3697	*h1911*
KR3698	*h1912*
KR3704	*h1965*
KR3705	*h1966*
KR3706	*h1967*
KR3707	*h1968*
KR3714	*h1973*
KR3715	*h1974*
KR3716	*h1975*
KR3721	*h1980*
KR3722	*h1981*
KR3723	*h1982*

Appendix IV

New him mutation isolated

Mutagen	Strain used	Allele number	Strain name	Brood size	% of males	Chromosome #
UV	*lev-11; sEx150*	*h1552*	KR3199	37	16 %	nd
EMS	*lev-11; sEx150*	*h1541*	KR3200	184	41%	IV
EMS	*lev-11; sEx150*	*h1548*	KR3176	12	19 %	nd

Appendix V

Strains used in this study

Strain name	Genotype
BC313	*rec-1(s180)*I
	*him-1(e879)*I
	*dpy-5(e61) unc-13(e51)*I
	*dpy-5(e61) unc-13(e450) rec-1(s180)*I
	*dpy-5(e61) unc-14(e88) rec-1(s180)*I
	*dpy-11(e224) unc-42(e270)*V
	*rec-1(s180)*I *unc-43(e266)unc-22(s8)*IV
	*spo-11(ok79)*IV/ *nT1[unc-x(n574) let-x]*(IV;V)
	*lon-2(e678) dpy-6(e14)*X
	*unc-1(e719)*X
	*dpy-3(e27)*X
	*lon-1(e678)*X
	*rec-1(s180)*I; *lon-2(e678)*X
	*unc-7(e5) lin-15(n309)*X
	*him-5(e1490)*V *unc-115(e2225) mab-18(bx23)*X
	*unc-20(e112)*X
	*unc-1(e719) dpy-3(e27)*X

Appendix V continues....

Strain name	Genotype
	*dpy-3(e27) unc-20(e112)*X
KR2994	*rec-1(s180)*(I); *dpy-6 lon-2*(*e678*)(X)
KR3071	*rec-1(s180)*I; *unc-7(e5) 7 lin-15(n309)*X
KR3081	*rec-1 (s180)* I; *unc-20 (e112)* X
KR3082	*rec-1(s180)*I; *unc-1(e719)*X
KR3098	*rec-1(s180)*I; *dpy-3(e27) unc-20 (e112)*X
KR3121	*rec-1 (s180)* I; *dpy-3 (e27)* X
KR3163	*lev-11; sEx150*
KR3197	*rec-1(s180)*I; *unc-1(e719) dpy-3 (e27)*X
KR3501	*him-1(s879)*I; *dpy-11(e224) unc-42(e270)*V
KR3519	*him-1(e879) rec-1(s180)*I; *unc-1(e719) dpy-3(e27)*X
KR3520	*him-1(e879) rec-1(s180)*I; *dpy-11(e224) unc-42(e270)*V
KR3530	*unc-115(e2225)*
KR3572	*lon-2(e678) unc-115(e2225)*X
KR3573	*him-1(e879)*I; *lon-2(e67) unc-115(e2225)*X
KR3574	*rec-1(s180)*I; *lon-2(e678) unc-115(e2225)*X
KR3575	*him-1(e879) rec-1 (s180)*I; *lon-2(e67) unc-115(e2225)*X
KR3579	*him-1(e879) rec-1(s180)*I
KR3580	*him-1(e879)*I; *lon-2(e678) dpy-6(e14)*X
KR3581	*him-1(e879) rec-1(s180)*I; *lon-2(e678) dpy-6(e14)*X
KR3582	*dpy-5(e61) unc-13(e450) him-1(e879) rec-1(s180)*I

Appendix V continues ....

Strain name	Genotype
KR3583	*dpy-5(e61) unc-13(e51) him-1(e879)*I
KR3595	*unc-1(e719) lon-2(e678)*X
KR3596	*rec-1(s180)*I; *unc-1(e719) lon-2 (e678)*X
KR3597	*him-1(e879) rec-1(s180)*I; *unc-1(e719) lon-2(e678)*X
KR3598	*him-1(e879)*I; *unc-1(e719) dpy-3(e27)*X
KR3599	*him-1(e879)*I; *unc-1(e719) lon-2(e678)*X
KR 3602	*lon-2(e678) unc-7(e5) lin-15(n309)*X
KR3603	*rec-1(s180)*I; *lon-2(e678) unc-7(e5) lin-15 (n309)* X
KR3604	*him-1(s879) rec-1(s180)*I; *lon-2(e678) unc-7(e5) lin-15 (n309)*X
KR3505	*him-1(s879)*I; *lon-2(e678) unc-7(e5) lin-15 (n309)*X
KR3638	*hIn1[unc-101(sy241)]*I; *sEx150*
KR3647	*hIn1[unc-101(sy241)]/ unc-54(e190)*I
KR3754	*unc-101 eDf24/ hIn1[unc-54]*I
KR3669	*hIn1[unc-101(sy241) / unc-54(e190)*I; *sEx150*
KR3760	*unc-101 eDf24/ unc-101(m1) unc-54(e190)*I

Appendix VI

List of Primers used for the analysis of genes within *eDf24*

Gene designation	Primers used	Primer sequence
ZK337.2	KRp322	GTAATACGACTCACTATAGGGCGATCTGAACATACTTACCGCTG
	KRp323	AATTAACCCTCACTAAAGGGTTTGCCATGCAGAATGCAACC
ZK337.4	KRp326	AATTAACCCTCACTAAAGGGATGTACTTGAACTTTCAGCGAG
	KRp327	GTAATACGACTCACTATAGGGCTTGTTCGAGGTCTTAATTCGAG
ZK337.1	KRp369	GTAGCTCTAACGGCATTTGTG
	KRp370	TCATGCAATAAGGAGCACAGC
F33H2.5	KRp309	TATGGAATGCTTCAACCATC
	KRp314	ATGAGCTCCAAGGATGACATTC
	KRp372	TATGATTGTGTGCGTCCATCC
	*KRp353	ATCAAACTCTCATTCACCAGC
	*KRp354	ATCAGCCGACACGATTTCTC
F33H2.6	KRp367	ATTTCGAATTTCTCTCGCCGC
	KRp368	AATCGTACCATTTATGTGCTCC
F33H2.1	KRp87	GCCGTTACACCATGAAATTG
	KRp144	TGGAAGACGGGAGAACTC
	†EL	TCGTTCGGTAATTCTCAGGG
	†ER	CGCGGTGAAAATTTGTTTTT
	πIL	TGTCCATTGGGCACAGAGTA
	πIR	GTTGTGAAAGAGGAGCAGCC

Gene designation	Primers used	Primer sequence
F33H2.2	KRp337	GCGATAATATTCCCTGGGCG
	KRp338	GTTCCATAAGAAATCAGCTGAC
F33H2.7	KRp339	TAATGCTTGATTCGATGGCAAG
	KRp340	AGTTGCTCGTGAACAACCGC
F33H2.8	KRp365	GCGCCTCTTCTCTGTGAAAC
	KRp366	CACCCAGAACTCACATAATCG
F33H2.3	KRp359	TGCCGTCTATGGCCGAAATC
	KRp360	GCTCTTCTCCGTTATCAGAAG
F31C3.1	KRp363	ATGAAGTCGCTTCTTGTTGTGG
	KRp364	ACGACGGCCTCGCGTTCAACAG
F31C3.3	KRp335	AGAGGGTACAGTGTAACTGAG
	KRp336	CTGCTTCATAAACTGTTGTACG
	KRp371	CGAATCGCTCAGTACTGTGG
F31C3.5	KRp333	AATGCAGAACGTTGCGAGTTC
	KRp334	GTTTATTACGGACGACAGCTC
F31C3.4	KRp361	CGTATGGGAAGAGCATGGAG
	KRp362	AGCGACAACGATTGCAAATCC
F31C3.6	KRp331	CAGTGCCAATTGAGACGAAAC
	KRp332	GCAATGACTTGTTGAACGTTCC

* primers flanking *h1881* deletion in F33H2.5
† external primer set flanking *gk10*
π internal primer set flanking *gk10*