DISTORTIONS OF THE GENETIC MAP OF CHROMOSOME *I* IN *CAENORHABDITIS ELEGANS*

by

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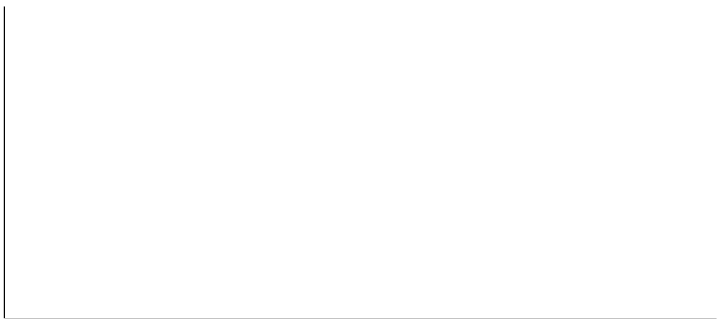

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ABSTRACT

The process of meiosis ensures heritable genetic material is passed faithfully from one generation to the next. To identify the mechanisms involved in this process, the effects of sex, mutation, and rearrangement on meiotic recombination in *Caenorhabditis elegans* were investigated. The short life cycle and existence of meiotic mutants make this organism an ideal system in which to study meiosis. To determine the effect of sex on meiotic recombination, crossing over was characterized in male gametes and then compared to the frequencies observed in hermaphrodite gametes. Male recombination across chromosome *I* was approximately one-third less than that observed in the hermaphrodite. This decrease varied with the interval being measured and in one interval, no difference was observed between the sexes. The frequency of recombination in hermaphrodite spermatocytes was two-fold higher than that observed in oocytes and male spermatocytes . Thus, recombination frequencies appear to be a function of gonad physiology rather than sexual phenotype. To test this further, recombination was measured in males sexually transformed by the *her-1* mutation. The results indicated that the sexual phenotype, rather than karyotype, determined the recombination frequency characteristic of a certain sex. Like recombination in the hermaphrodite, male recombination was also found to increase with temperature and decrease with age. Therefore, recombination frequency in *C. elegans* is influenced by physiological factors such as sexual phenotype and age, and environmental factors such as temperature.

Mutations in genes that regulate meiosis can affect the frequency of recombination and the distribution of exchange events. A recessive mutation in the gene *rec-1* was mapped, and its effects on the distribution of crossing over on LG *I* were determined. This mutation was mapped to the right end of chromosome *I* using the duplications *sDp1* (which carries a wild-type allele of the gene) and *sDp2* (which does not). A high resolution map position was determined using several deficiencies of the right end of the chromosome to map the mutation. The ribosomal deficiency *eDf24* failed to complement *rec-1*, indicating

the locus was located within its boundaries. Crossing over in five intervals on chromosome *I* was measured in *rec-1* homozygotes. The frequency of recombination in one interval located in the centre of the chromosome showed a ten-fold increase, whereas an interval located on the right end showed a three-fold decrease. Despite the changes to the frequencies of recombination in these intervals, the total genetic length of chromosome *I* remained unchanged, indicating that the *rec-1* mutation affected the distribution of a wild-type number of exchange events. This implies that the *rec-1*(+) gene product is necessary in establishing the distribution of crossovers along the chromosome.

Chromosome rearrangements can reduce or eliminate crossing over by physically disrupting the normal organization of the chromosome. In this study, a crossover suppressor for the right end of LG *I* was isolated and characterized. By inducing markers on the rearrangement and establishing the gene order in the homozygote, *hIn1(I)* was demonstrated to be the first inversion isolated in *C. elegans*. Crossing over in the heterozygote was characterized, and intrachromosomal (but not interchromosomal) effects were observed. The interaction of *hIn1(I)* with two translocations demonstrated that small homologous regions can pair and recombine efficiently, and that the formation of a chiasma between two homologues is necessary for their proper segregation. Rare recombinants bearing duplications and deficiencies were isolated from inversion heterozygotes, leading to the proposal that *hIn1(I)* is paracentric with the meiotic centromere to its left. The meiotic behaviour of the inversion was found to be consistent with the proposal that the meiotic chromosomes of *C. elegans* are monocentric.

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GENERAL INTRODUCTION

Meiosis is the process by which sexually reproducing organisms produce haploid gametes. This process consists of one round of DNA replication followed by a reductional division at meiosis I and an equational division, resembling mitosis, at meiosis II. Prophase of meiosis I is marked by two distinct processes that culminate in the segregation of replicated homologous chromosomes: pairing and recombination (reviewed by HAWLEY 1988; HAWLEY and ARBEL 1993).

Pairing between homologues is achieved by several temporally distinct events. During the first phase, called homologue recognition, homologous chromosomes are thought to find one another and align themselves at a distance in the diffuse nucleus (MAGUIRE 1984). In *C. elegans*, homologue recognition regions have been identified on every chromosome (ROSE, BAILLIE and CURRAN 1984; McKIM, HOWELL and ROSE 1988) and are absolutely required for pairing and recombination. Once aligned, the chromosomes are brought into tighter association through a homology search that may be mediated by RecA-type proteins (CONLEY and WEST 1989), which locate and homologously pair discrete sites on the chromosome (CAO, ALANI and KLECKNER 1990; KLECKNER, PADMORE and BISHOP 1991). The existence of such sites has been documented in a variety of organisms including *Drosophila melanogaster*, where pairing sites have been mapped along the *X* chromosome of the female (HAWLEY 1980) and in the ribosomal cluster of the male (McKEE and KARPEN 1990). Recently in *Saccharomyces cerevisiae*, such a site has been identified on the left arm of chromosome *III* (GOLDWAY, ARBEL and SIMCHEN 1993; GOLDWAY *et al.* 1993). During the second phase of pairing, this early alignment is locked in place by recombinational intermediates which result from the repair of double-strand breaks that appear early in meiosis (SUN *et al.* 1989; PADMORE, CAO and KLECKNER 1991). The chromosomes then begin to condense, a process thought to be

crucial to the next stage of pairing, which results in intimate synapsis and the formation of a tripartite laminar structure called the synaptonemal complex (SC), between the homologues (KLECKNER, PADMORE and BISHOP 1991). The ZIP1 locus of yeast encodes a component of the central region of the SC, indicating that specific proteins are required for the formation of the structure (SYM, ENGEBRECHT and ROEDER 1993). MAGUIRE (1978) proposed that only those recombination intermediates which occur in the context of the SC have the potential to form chiasma. This is supported by the fact that double-strand breaks, thought to be the substrate for recombination, appear before and at the same time as the first appearance of the SC (PADMORE, CAO and KLECKNER 1991). In addition, mutants in RED1, MER1, and HOP1 are defective in SC formation but still competent in meiotic exchange, indicating recombination can be initiated in the absence of the synaptonemal complex (ROCKMILL and ROEDER 1990; ENGEBRECHT, HIRSCH and ROEDER 1988; HOLLINGSWORTH, GOETSCH and BYERS 1990). In *mer1* mutants, however, the exchange events that occur do not ensure faithful disjunction of the homologues (ENGEBRECHT, HIRSCH and ROEDER 1990). This may be explained if the role of the SC during meiosis is the conversion of a number of sites of alignment and recombination into a bivalent united by a chiasmata.

An essential feature of meiosis is recombination between homologues, which serves to reassort genetic information and promote proper segregation of the chromosomes. Crossing over refers to a reciprocal event resulting in an exchange of flanking markers. The frequency and distribution of crossing over are regulated, and a number of mutations which disrupt this pattern have been identified (reviewed by BAKER *et al.* 1976).

The nematode *C. elegans* is an ideal system for the study of meiosis. Populations consist mostly of self-fertilizing hermaphrodites that are capable of producing about 300 progeny each and that have a short generation time (3.5 days at 20°). Males can be used for the introduction of genetic markers and the genetic maps of the five autosomes and the *X* chromosome are well marked with visible mutations. Recessive mutations have been

isolated that reduce crossing over (HODGKIN, HORVITZ and BRENNER 1979), confer radiation sensitivity (HARTMAN and HERMAN 1982), and increase both crossing over and conversion (ROSE and BAILLIE 1979b; RATTRAY and ROSE 1988). Thus, in *C. elegans*, gene products important in meiosis can be identified by mutations which produce phenotypes that have also been described in other systems (BAKER *et al.* 1976). In this study, meiosis in *C. elegans* has been investigated by examining the effect of sex, mutation, and rearrangement on recombination.

Chapter 1: Sex-Related Differences in Crossing Over

INTRODUCTION

The biology of *C. elegans* provides a unique opportunity to examine the effect of sex on recombination. Laboratory populations consist largely of self-fertilizing hermaphrodites (5AA;XX). Males (5AA;XO), arise spontaneously as a result of *X*-chromosome nondisjunction (HODGKIN, HORVITZ and BRENNER 1979) and are maintained by cross-fertilization with hermaphrodites. The standard genetic map of *C. elegans* (EDGLEY and RIDDLE 1990) is based upon hermaphrodite recombination frequencies that are the product of crossover events in two germlines: oocyte and hermaphrodite spermatocyte. The frequency of recombination in these two germlines has been shown to be different (ROSE and BAILLIE 1979a).

Sexual differences in crossing over are known to occur in a number of organisms. There may exist two qualitatively different situations when examining the relationship between sex and recombination frequency. The first is the absence of recombination in one sex, a characteristic of *D. melanogaster* males (MORGAN 1912) and *Bombyx mori* females (TANAKA 1913). The second, more common situation, is one where recombination exists in both sexes, but with a reduced frequency in one (reviewed by DUNN and BENNETT 1967). Recombination frequency in the female is generally higher in *D. ananassae* (MORIWAKI 1937), in mice (SLIZYNSKI 1960), and in humans (WHITE *et al.* 1985a; DONIS-KELLER *et al.* 1987). Alternatively, male recombination frequency is generally higher in maize (RHOADES 1941; ROBERTSON 1984), and in *Tribolium castaneum* (SOKOLOFF 1964). However, sex-related differences in recombination frequency are not uniform for all regions of the genome. In maize, some intervals have been reported to be longer in the female meiosis (ROBERTSON 1984). In mice, significant sex differences in recombination frequency went in opposite directions on different chromosomes (DAVISSON and RODERICK 1981) and in humans, some regions were the same genetic size in both

sexes (DONIS-KELLER *et al.* 1987). This suggests local differences in recombination between the sexes are not representative of the chromosome, nor of the genome as a whole. In this study, the effect of sex on recombination in the nematode *Caenorhabditis elegans* has been investigated. Each of the autosomes in *C. elegans* is marked by a region where genes cluster on the meiotic map as a result of less recombination per base pair than the genome average (BRENNER 1974; GREENWALD *et al.* 1987; KIM and ROSE 1987; PRASAD and BAILLIE 1989; STARR *et al.* 1989). By examining intervals spanning linkage group (LG) *I*, the effect of sex on recombination in intervals inside and outside such a region has been determined.

One approach in studying the relationship between sex and recombination frequency is measuring recombination in sexually transformed individuals. Hormone treatments have been used in the Medaka, *Oryzias latipes*, to transform XY fish, normally male, into functional females. Crossing over in these transformed males was found to occur at a higher frequency than in normal males (YAMAMOTO 1961). This suggests that differences in recombination between the sexes are not completely the result of the sex chromosome constitution, but also depend on the physiological differences associated with sex. In *C. elegans*, mutations exist which result in the complete transformation of the sexual phenotype. One such mutation, *her-1*, transforms fertile XO males into self-fertile hermaphrodites (HODGKIN 1980), and has been used in this study to examine the effect of karyotype on recombination frequency in the nematode.

Meiotic recombination frequency in higher eukaryotes is affected by several known parameters. Recombination frequency increases at temperature extremes in *D. melanogaster* (PLOUGH 1917, 1921), *Neurospora crassa* (McNELLY-INGLES, LAMB, and FROST 1966) and *Coprinus lagopus* (LU 1969, 1974). A decrease in meiotic recombination frequency with maternal age has been observed in *D. melanogaster* (BRIDGES 1927; NEEL 1941), in *C. elegans* (ROSE and BAILLIE 1979a), and on some chromosomes in the mouse, *Mus musculus* (FISHER 1949; BODMER 1961; REID and PARSONS 1963). Existing human data is not conclusive about maternal age effects although some evidence suggests a

paternal age effect may exist (LANGE, PAGE and ELSTON 1975; ELSTON, LANGE and NAMBOODIRI 1976). In *C. elegans*, hermaphrodite recombination frequency decreases with maternal age and increases with temperature (ROSE and BAILLIE 1979a) and in the presence of the *rec-1* mutation (ROSE and BAILLIE 1979b). In this study, the effect of temperature, age, and *rec-1* on recombination in *C. elegans* males has been investigated.

Chapter 1: MATERIALS AND METHODS

General Methods: *C. elegans* population consist largely of self-fertilizing hermaphrodites (5AA;XX). Males (5AA;XO) arise spontaneously as a result of *X*-chromosome nondisjunction (HODGKIN, HORVITZ and BRENNER 1979) and were maintained by mating to hermaphrodites. Wild-type and mutant strains were maintained and mated on petri plates containing nematode growth medium (NGM) and streaked with *Escherichia coli* (BRENNER 1974). All experiments were carried out at 20° unless otherwise noted. The wild-type strain N2 and most mutant strains of *C. elegans* var. Bristol used in this study were obtained from D.L. Baillie at Simon Fraser University, British Columbia or from the Caenorhabditis Genetics Centre at the University of Missouri, Columbia, Missouri. RW3072 was supplied by R.W. Waterston at Washington University School of Medicine, St. Louis, Missouri. The following genetic markers (for list of strains, see APPENDIX IV) were used in the course of this work:

LG *I*: *bli-3(e579)*; *unc-11(e47)*; *dpy-5(e61)*; *bli-4(e937)*; *dpy-14(e188)*; *unc-13(e450)*; *unc-29(e403)*; *unc-29(e193)*; *lin-11(n389)*; *unc-75(e950)*; *unc-75(h1041)*; *unc-75(h1042)*; *unc-101(m1)*; *unc-59(e261)*; *lev-11(x12)*; *let-49(st44)*; *unc-54(e190)*; *unc-54(h1040)*; *unc-54(st40)*; *let-50(st33)*; *rec-1(s180)*

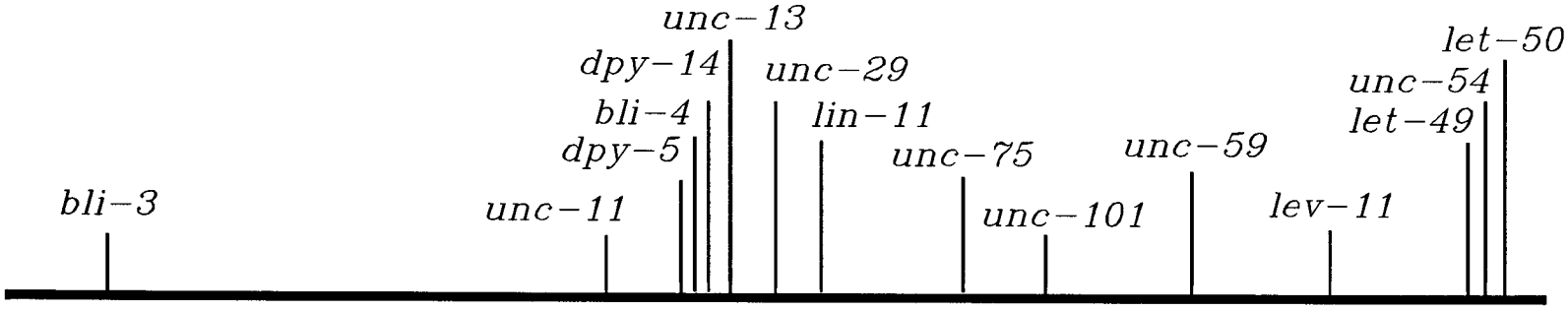
LG *III*: *dpy-18(e364)*; *unc-36(e251)*

LG *V*: *unc-42(e270)*; *her-1(e1520)*; *dpy-11(e224)*; *him-5(e1467)*

LG *X*: *lon-2(e678)*; *unc-1(e719)*; *dpy-3(e27)*

The locations of some genes on chromosome *I* are shown in Figure 1. *C. elegans* nomenclature of genes and alleles conforms to the system outlined by HORVITZ *et al.* (1979). The names of genetic loci are abbreviated using a three letter code followed by a number and then by an allele designation defining the laboratory of origin in parentheses. The most commonly used abbreviations are described in Table 1. For example, *unc-101(m1)* is a mutation in a gene which results in an uncoordinated, or *unc*, phenotype. It was the one hundred and first *unc* gene identified, and the first mutation isolated in the

FIGURE 1.-A partial genetic map of Linkage Group *I* showing the major markers used in this study.



5 m.u.

Linkage Group I

Table 1

Abbreviations

Abbreviation	Phenotype
bli	*bli*stered cuticle
dpy	*dumpy*
her	*her*maphrodization of XO animals
him	*h*igh *i*ncidence of *m*ales
let	*let*hal
lev	*lev*amisole resistant
lin	abnormal cell *lin*eage
lon	*lon*g
rec	abnormal *rec*ombination
unc	*unc*oordinated

laboratory with the *m* allele designation. The allele designation for the Rose lab is *h* and the strain designation is KR.

The following translocations were used in this study: *szT1(I;X)* (FODOR and DEAK 1985; McKIM, HOWELL and ROSE 1988), *hT2(I;III)* (McKIM, PETERS and ROSE *in press*), *hT1(I;V)* (McKIM, HOWELL and ROSE 1988), and *hT3(I;X)* (McKIM 1990). *szT1(I;X)* is inviable as a homozyote and is marked with the *lon-2* mutation on $I^R X^R$, where R denotes the right arms of chromosomes *I* and *X*. *hT2(I;III)* is viable as a homozygote and is marked with *bli-4* on $I^L III^L$, where L denotes the left arms of chromosomes *I* and *III*. Both *hT1(I;V)* and *hT3(I;X)* are inviable as homozygotes. Mutations on translocation chromosomes (T) are shown in square brackets (McKIM, HOWELL and ROSE 1988) and the formal name is used when discussing both components of the translocation (*i.e. szT1(I;X)*). When discussing the individual component chromosomes of a translocation, the nomenclature describes the segregational properties of the new chromosomes. The translocation *szT1(I;X)* is comprised of two chromosomes; *szT1(I;X)I* (of structure $I^R X^R$, where R denotes the portion of the chromosome to the right of the breakpoint), which segregates from chromosome *I*, and *szT1(I;X)X* (of structure $I^L X^L$, where L denotes the portion of the chromosome to the left of the breakpoint), which segregates from the *X* chromosome. Similarly, *hT2(I;III)* is comprised of two chromosomes; *hT2(I;III)I* (of structure $I^R III^R$), which segregates from chromosome *I*, and *hT2(I;III)III* (of structure $I^L III^L$), which segregates from chromosome *III*. *hT1(I;V)* consists of *hT1(I;V)I* (of structure $I^R V^L$) which segregates from chromosome *I*, and *hT1(I;V)V* (of structure $I^L V^R$), which segregates from chromosome *V*. *hT3(I;X)* consists of two chromosomes; *hT3(I;X)I* (of structure $I^R X^R$) which segregates from chromosome *I*, and *hT3(I;X)X* (of structure $I^L X^L$), which segregates from the *X* chromosome.

Inversions in *C.elegans* are written *In* (HORVITZ *et al.* 1979). Mutations on inversion chromosomes are shown in square brackets (*e.g. hIn1(I)[dpy-5 unc-54]*), similar to the system in use for translocations. The nomenclature does not necessarily provide

information on gene order and does not implicate the marker in the rearrangement, indicating only that the mutations are linked to the inversion.

The following duplications and deficiencies were used in this study: the free duplications *sDp1(I;f)*, *sDp2(I;f)* (ROSE, BAILLIE and CURRAN 1984), *hDp131(I;f)*, *hDp132(I;f)* (ZETKA and ROSE 1991; this study), the deficiencies *eDf4(I)*, *eDf9(I)*, *eDf10(I)*, *eDf13(I)*, *eDf24(I)* (ANDERSON and BRENNER 1984), *hDf11(I)*, and *hDf12(I)* (ZETKA and ROSE 1992). Duplications in *C. elegans* are written as *Dp* (preceeded by the laboratory designation) and followed in parentheses by their chromosome of origin and the designation *f* if they are free duplications (unlinked to an intact chromosome). *sDp1(I;f)* duplicates the right end of LG *I* and pairs and recombines with the normal homologues whereas *sDp2(I;f)* duplicates the left end of the chromosome and does not pair and recombine (ROSE, CURRAN and BAILLIE 1984). Deficiencies are abbreviated *Df* and are followed in parentheses by their chromosome of origin. *eDf24* complements *unc-54* and partially deletes the ribosomal cluster, the most distal genetic marker on LG *I*. The remaining *eDf* deficiencies fail to complement *unc-54* and were isolated using *eDf24* as a balancer (ANDERSON and BRENNER 1984). The origin and structure of *hDp131*, *hDp132*, *hDf11*, and *hDf12* are discussed in Chapter 3.

Recombination Mapping: Recombination frequency in the hermaphrodite was measured by scoring the number of recombinant progeny of a *cis*-heterozygote, under the conditions described by ROSE and BAILLIE (1979a). The recombination frequency (p) between two markers was calculated using the formula $p = 1 - (1 - 2R)^{1/2}$, where R is the number of visible recombinant individuals divided by the number of total progeny (BRENNER 1974). The total progeny number of the hermaphrodite is estimated as 4/3 X (number of Wts + one recombinant class) where Wts is the number of wild-type progeny. Map distances in the male were determined by scoring the progeny resulting from mass mating seven males heterozygous for a pair of *cis*-linked markers to five homozygous hermaphrodites (new hermaphrodites each day) every 24 hours for four days. On the fourth day the males were left on plates with the same hermaphrodites for a fifth day, after which the hermaphrodites

were transferred. Since mapping in the male involves recombination in only one germline, the recombination frequency (p) is equal to R. The total progeny number of the male is 2 X (number of Wts + one recombinant class). This differs from the total progeny number of the hermaphrodite for the following reasons. In both male and hermaphrodite recombination experiments, the double homozygote class is not scored because of its reduced viability, and the total progeny number is calculated from the wild-type class. Mapping in the hermaphrodite involves crossing two heterozygous germlines, whereas mapping in the male involves crossing one germline heterozygous for a pair of markers to one which is homozygous. For this reason, the ratio of wild-type progeny to progeny homozygous for the markers differs in hermaphrodite and male recombination experiments. Thus, the number of wild-type progeny must be multiplied by 4/3 and 2 respectively to correct for the inviable class. Both classes of recombinants were used in the calculations unless otherwise noted. In cases where only one class of recombinants was used, R = 2 X (one recombinant class) divided by the total progeny number. All hermaphrodite recombinants were progeny tested. The progeny of putative recombinants that had mated before being picked were screened for the presence of both male and hermaphrodite individuals of the recombinant phenotype. In the case of the *bli-3 unc-11* interval, *bli-3* penetrance is low and Bli-3 recombinants were scored as wild-type and later subtracted. The *unc-75 unc-101* and *unc-101 unc-54* map distances were based on the Unc-75 and Unc-101 recombinant classes respectively. 95% confidence intervals were calculated using the statistics of CROW and GARDNER (1959). In the event the number of recombinants exceeded 300, confidence intervals were approximated using the equation $1.96(nxy)^{1/2}$ where x is the number of recombinants (n), divided by the number of wild-types plus recombinants, and y is equal to 1 - x.

Recombination in Hermaphrodite Germlines: Recombination frequency in oocytes was measured by scoring the male progeny of *dpy-5 unc-75/ + +* or *unc-11 dpy-5/ + +* hermaphrodites mated to a male carrying an appropriate crossover suppressor. The translocation *hT2(I;III)* was chosen because it suppresses crossing over in both these regions (McKIM, PETERS and ROSE 1993). Males of the genotype *dpy-5 unc-75; + /hT2(I;III)[+*

+;dpy-18] or *unc-11 dpy-5; + /hT2(I;III)[+ +;dpy-18]* were mated to heterozygous hermaphrodites every 24 hours and the male progeny were scored. The oocyte recombination frequency (a), is 2 X the number of recombinant individuals divided by the total progeny. The total number of progeny is 4/3 X (number of Wts + one recombinant class). Knowing the value of $\boldsymbol{R}$ for the hermaphrodite and $\boldsymbol{a}$, the recombination frequency in the oocytes, the following equation was solved for $\boldsymbol{b}$, the recombination frequency in hermaphrodite spermatocytes.

$$\boldsymbol{R} = 1/2\boldsymbol{b}(1 - \boldsymbol{a}) + 1/2\boldsymbol{a}(1 - \boldsymbol{b}) + 1/2\boldsymbol{ab}$$

Recombination in Her-1 Hermaphrodites : To measure recombination in Her-1(XO) individuals, *hT1(I;V)[unc-29; + ; +]/szT1(I;X)[+ ; + ; lon-2]* males were crossed to *her-1* homozygous hermaphrodites. Because of the segregational properties of *szT1(I;X)* (McKIM, HOWELL and ROSE 1988), all wild-type males resulting from this cross were of the genotype *+ ; her-1/hT1(I;V)[unc-29; +].* These males were then crossed to *hT3(I;X)[dpy-5 unc-29; +]* homozygotes to produce *+ + ;O/hT3(I;X)[dpy-5 unc-29; +]; her-1/ +* males. When the latter males were mated to *dpy-5 unc-75; her-1* hermaphrodites, the only wild-type hermaphrodites that resulted were of the genotype *+ + /dpy-5 unc-75; her-1/her-1; + /O.* Recombination was measured in these individuals by scoring Dpy-5 and Unc-75 recombinants.

Variation With Age: The variation of recombination with parental age was examined in two intervals; *dpy-5 unc-75* and *dpy-5 unc-13.* Young heterozygous males were individually mated to 5 new homozygous hermaphrodites every 12 hours for 4 days. Heterozygous L4 hermaphrodite controls were brooded every 12 hours for 3 days under the same conditions. The recombination frequency in every 12 hour period was calculated as described above.

Variation with Temperature: The effect of temperature on male recombination was examined in the *dpy-5 unc-75* and *dpy-5 unc-13* intervals. Seven heterozygous males were mass mated to five homozygous hermaphrodites and transferred to new hermaphrodites every 24 hours at temperatures of 15° or 25°. Hermaphrodite controls were picked from the

same plates as experimental males and were transferred every day. All progeny were permitted to develop at 20^{O} to avoid any inviablity produced by temperature extremes.

Chapter 1: RESULTS

Male recombination frequency is lower than hermaphrodite: Differences in recombination frequencies between the sexes were initially studied in two intervals; *dpy-5 unc-75* and *dpy-5 unc-13*. The latter interval is located within the chromosome *I* genetic cluster and the former includes the cluster and a genetically large interval to the right. In both intervals, the frequency of recombination was approximately two-fold lower in the male (data shown in Tables 2 and 3). To determine if the reduced recombination frequency in the male was general across the length of chromosome *I*, other intervals inside and outside the cluster were investigated. The results for six intervals spanning LG *I* is shown in Figure 2 (data shown in Table 4). In the *dpy-5 unc-29 unc-75* interval, only hermaphrodites were scored because the phenotypes of male recombinants were subtle and progeny testing was not possible. Male recombination frequency was lower in five of the intervals tested when compared to hermaphrodite controls. The differences in recombination frequencies between the hermaphrodite and the male in these intervals were not uniform; they varied from 1.3-fold in *unc-11 dpy-5* to 2-fold in *dpy-5 unc-13* and *unc-75 unc-101*. In the *unc-101 unc-54* region, the male meiotic distance was not different from that observed in the hermaphrodite. The difference for a comparably sized interval, *bli-3 unc-11* was 1.6, suggesting sex-related differences are interval-dependent and not size-dependent. Thus, the greatest differences in crossover frequency were observed near the gene cluster, and no difference was observed at the right end of the chromosome. The total genetic length of the meiotic map of LG *I* is 31.7 m.u. in the male, compared to 44.1 m.u. in the hermaphrodite (data from Table 4). As is the case with the hermaphrodite meiotic map, the male map is also marked by a centrally located cluster.

Recombination in hermaphrodite spermatocytes is higher than in oocytes: The recombination formula normally used in measuring map distances in the hermaphrodite is based on the assumption that the frequency of recombination is equal in both germlines although this has been shown not to be the case (ROSE and BAILLIE 1979a). To measure

Table 2

Male brood analysis

Genotype	Wts		Recombinants				pX100(C.I.)[a]
	♀	♂	Dpy		Unc		
			♀	♂	♀	♂	
dpy-5 unc-13/ + +							
0-12 hr	594	535	10	8	6	14	1.7(1.2-2.2)
13-24 hr	789	761	11	5	9	10	1.1(0.8-1.5)
25-36 hr	751	703	2	2	4	5	0.4(0.2-0.7)
37-48 hr	849	890	6	8	7	3	0.7(0.4-1.0)
49-60 hr	453	503	5	3	2	3	0.7(0.3-1.1)
61-72 hr	442	382	2	1	0	3	0.4(0.2-0.8)
73-84 hr	233	238	0	1	5	1	0.7(0.3-1.4)
85-96 hr	109	113	0	0	1	1	0.4(0.1-1.5)
Totals	4241	4140	36	28	34	40	0.8(0.7-1.0)
dpy-5 unc-75/ + +							
0-12 hr	667	693	46	49	58	58	7.2(6.3-8.1)
13-24 hr	556	568	28	41	29	41	6.8(4.9-6.8)
25-36 hr	712	782	48	61	44	52	6.4(5.6-7.3)
37-48 hr	879	794	45	42	34	34	4.4(3.8-5.1)
49-60 hr	378	389	21	15	18	7	3.8(3.0-4.8)
61-72 hr	611	655	27	31	27	19	3.9(3.2-4.7)
73-84 hr	352	346	16	17	16	12	4.2(3.3-5.2)
85-96 hr	76	81	3	2	0	6	3.4(1.7-5.7)
Totals	4231	4308	234	258	226	229	5.3(5.2-5.4)

[a] C.I. = 95% confidence interval (see Chapter 1: MATERIALS and METHODS).

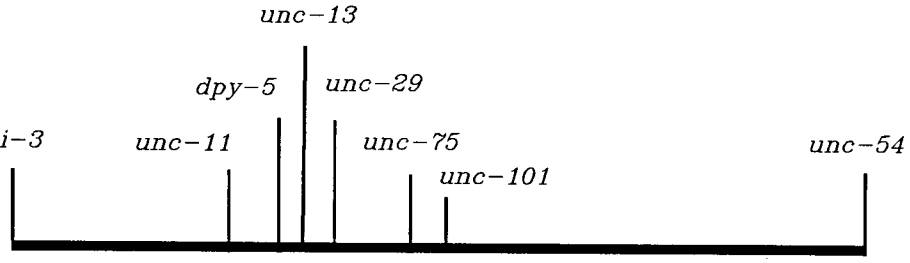
Table 3

Hermaphrodite brood analysis

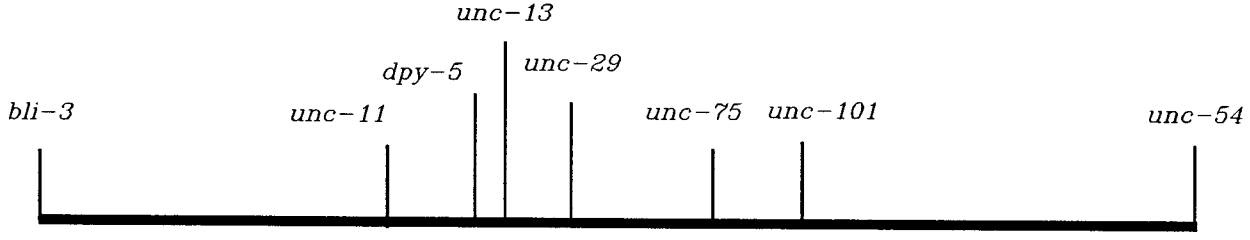
Genotype	Wts	Recombinants		pX100(C.I.)[a]
		Dpy	Unc	
dpy-5 unc-13/ + +				
0-12 hrs	334	6	7	2.8(1.5-4.6)
13-24 hrs	647	11	9	2.3(1.5-3.5)
25-36 hrs	786	5	7	1.1(0.6-1.9)
37-48 hrs	718	5	6	1.1(0.5-2.0)
49-60 hrs	383	5	1	1.2(0.5-2.5)
61-72 hrs	251	2	2	1.1(0.4-2.9)
Totals	3119	34	32	1.6(1.2-2.0)
dpy-5 unc-75/ + +				
0-12 hrs	1120	73	88	10.6(9.1-12.3)
13-24 hrs	2583	171	179	10.0(9.7-10.4)
25-36 hrs	2893	175	197	9.5(9.2-9.8)
37-48 hrs	2560	114	154	7.8(6.8-8.7)
49-60 hrs	1051	51	64	8.1(6.7-9.7)
61-72 hrs	596	39	25	8.0(6.1-9.9)
Totals	10803	623	707	9.1(8.9-9.2)

[a] C.I. = 95% confidence interval (see Chapter 1: MATERIALS and METHODS).

FIGURE 2.-Male and hermaphrodite meiotic maps of LG *I*. Three factor experiments positioned *unc-75* between *dpy-5* and *unc-101*. The LG *I* cluster extends from *unc-11* to *unc-29* (EDGLEY and RIDDLE 1990).



Male



5 m.u.

Hermaphrodite

Table 4

Male recombination on Linkage Group I

Genotype	Wts ♀	Wts ♂	Recombinants ♀	Recombinants ♂	pX100(C.I.)[a]
bli-3 unc-11/ + +					
male[b]	1392	1206	135 Unc	109 Unc	9.4[c](8.2-10.6)
hermaphrodite	1686		170 Unc		14.8[c](12.4-17.4)
unc-11 dpy-5/ + +					
male	983	962	19 Dpy	12 Dpy	1.8(1.4-2.2)
			15 Unc	25 Unc	
hermaphrodite	3786		58 Dpy		2.3(2.0-2.8)
			61 Unc		
dpy-5 unc-29/ + +					
male	2536	2451	29 Dpy	35 Dpy	1.2(1.0-1.5)
			44 Unc	61 Unc	
hermaphrodite	1822		30 Dpy		2.8(2.2-3.5)
			39 Unc		
dpy-5 unc-29 unc-75/ + +					
male	581		11 Dpy-5[d]		
			6 Unc-29 Unc-75[d]		1.4(0.8-2.2)
			17 Unc-75[e]		2.9(1.6-4.3)
hermaphrodite	1598		34 Dpy-5[d]		
			36 Unc-29 Unc-75[d]		3.4(2.6-4.2)
			2 Unc-29[d,e]		

			63 Unc-75[e]		6.0(4.7-7.6)
unc-29 unc-75/ + +					
male	3374	3568	95 Unc-75	80 Unc-75	2.7(2.6-2.8)
			126 Unc-29	90 Unc-29	
unc-75 unc-101/ + +					
male	2634	2553	45 Unc	42 Unc	1.6[c](1.3-2.0)
hermaphrodite	3192		68 Unc		3.2[c](2.7-3.8)
unc-101 unc-54/ + +					
male	392	362	71 Unc	62 Unc	15.0[c](12.7-17.2)
hermaphrodite	1187		116 Unc		14.4[c](11.8-17.1)

---

[a] C.I. = 95% confidence interval (see Chapter 1: MATERIALS and METHODS).

[b] Recombination measured in individuals of indicated sex.

[c] Calculated from one recombinant class (see Chapter 1: MATERIALS and METHODS).

[d] *dpy-5 unc-29*;

[e] *unc-29 unc-75*.

the difference in recombination frequency between the germlines, *dpy-5 unc-x; + /hT2(I;III)[+ +; dpy-18]* males were crossed to hermaphrodites *cis*-heterozygous for a pair of LG *I* markers, and the male progeny scored (see MATERIALS and METHODS). The results are shown in Table 5. In measuring the *unc-11 dpy-5* interval, an unusually small number of Dpy-5 recombinants were recovered. The most conservative approach was to use only the Unc-11 recombinants in the calculations, since this would give the minimum estimate of differences in recombination between the two germlines. In both intervals studied, the frequency of recombination in hermaphrodite spermatocytes was higher than that observed in oocytes; 2-fold in *dpy-5 unc-75* and 1.5-fold in *unc-11 dpy-5*. To further examine the effect of sexual phenotype on recombination frequency, crossing over was measured in males transformed into fertile hermaphrodites by the *her-1* mutation. The results of experiments measuring recombination in the *dpy-5 unc-75* interval in Her-1 (XO) hermaphrodites is shown in Table 6. Most of these hermaphrodites were sterile and those that were fertile produced few progeny. For this reason, recombinants that proved to be sterile upon progeny testing were also included in the calculations. The crossover frequency in these transformed males was significantly higher than that observed in normal males. An attempt was made to examine recombination in transformed hermaphrodites using the *tra-1(e1099)* mutation but these males mated poorly and rarely produced progeny.

Male recombination varies with age: ROSE and BAILLIE (1979a) found hermaphrodite recombination frequency to decrease with age. The effect of parental age on recombination in the *dpy-5 unc-75* and *dpy-5 unc-13* intervals is shown in Figure 3 (data shown in Tables 2 and 3 respectively). In both intervals, male recombination frequency shows a general decrease with age. Consistent with the previous results, the recombination frequencies of hermaphrodite controls also decreased with age. The variation in male recombination with age shows some periodicity in both intervals tested. The statistical of this fluctuation is difficult to assess due to the low recovery of recombinants in later broods. In the male, the most reproducible results were obtained in the first 36 hours. The greatest number of self-fertilization progeny were also produced in this period

Table 5

Recombination in hermaphrodite germlines

Genotype	Wts	Recombinants		pX100(C.I.)[a]
		Dpy	Unc	
dpy-5 unc-75/ + +				
oocyte[b]	4290	83	92	6.0(5.2-6.9)
sperm[c]				12.4
unc-11 dpy-5/ + +				
oocyte[b]	3707	7	24	1.9(1.2-2.8)
sperm[c]				2.7

[a] C.I. = 95% confidence interval (see Chapter 1: MATERIALS and METHODS).

[b] Only male progeny scored.

[c] Recombination frequency in hermaphrodite sperm (see Chapter 1: MATERIALS and METHODS).

Table 6

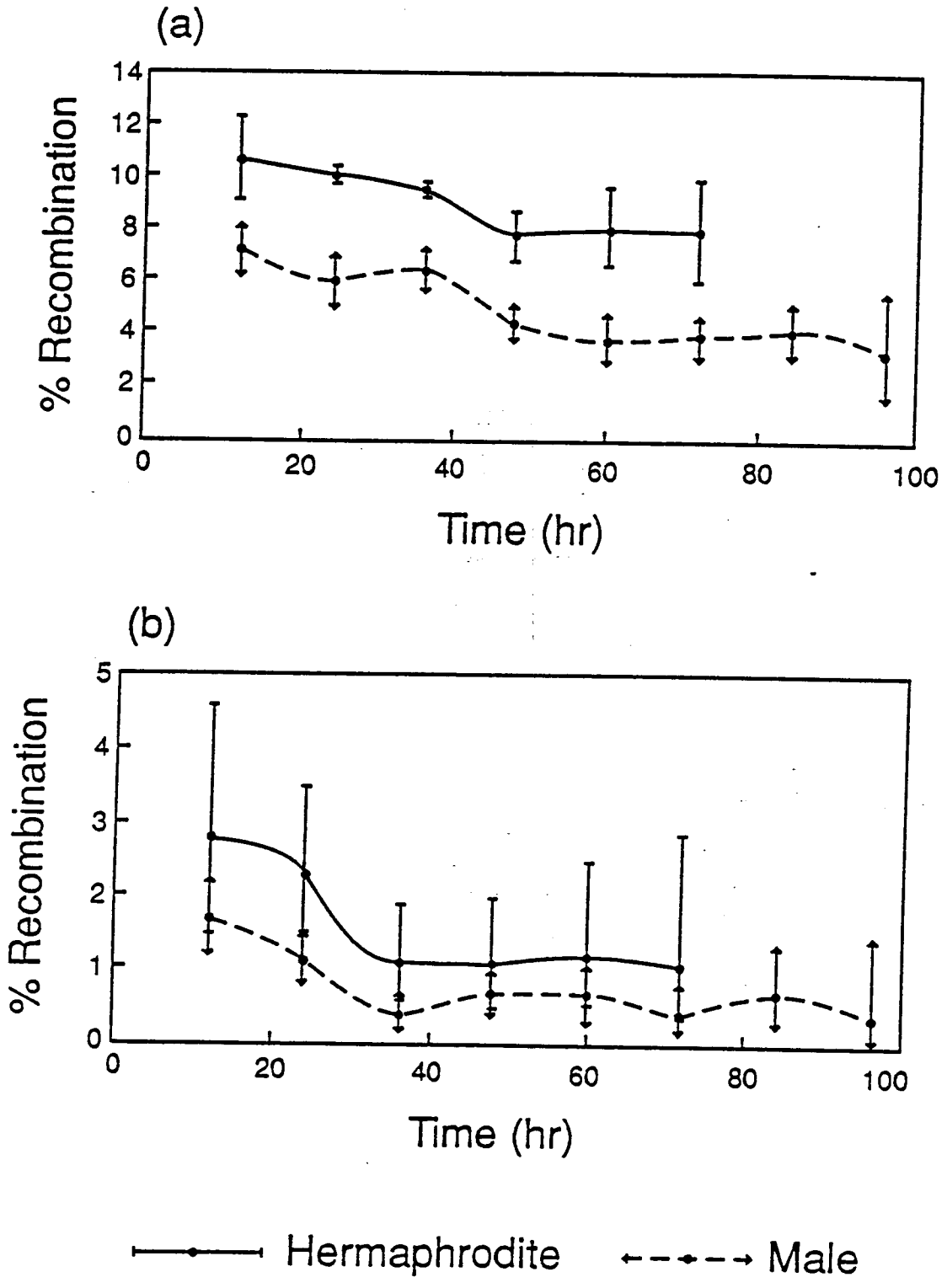
Recombination in Her-1(XO) hermaphrodites

Genotype	Wild types ♀	Wild types ♂	Recombinants Dpy ♀	Recombinants Dpy ♂	Recombinants Unc ♀	Recombinants Unc ♂	pX100(C.I.)[a]
dpy-5 unc-75/ + +; her-1/her-1(XO)	53		4				12.5(6.2-23.1)
dpy-5 unc-75/ + +[b]	10803		623		707		9.1(8.9-9.2)
male	2503	2457	148	132	122	135	5.1(5.0-5.3)

[a] C.I. = 95% confidence interval (see Chapter 1: MATERIALS and METHODS).

[b] data from Table 3.

FIGURE 3.-The variation of recombination frequency with parental age in the (a) *dpy-5 unc-75* interval and (b) *dpy-5 unc-13* interval. Brood analysis for male heterozygotes is represented by the dashed line. Hermaphrodite controls are represented by the solid line. Vertical bars represent 95% confidence intervals.


(a)
% Recombination
14
12
10
8
6
4
2
0
0
20
40
60
80
100
Time (hr)
(b)
% Recombination
5
4
3
2
1
0
0
20
40
60
80
100
Time (hr)
Hermaphrodite
Male

although the variation between individual males was high. In one experiment examining the *dpy-5 unc-75* interval in the male, a small number of progeny were recovered in the 49-60 hr period and this was likely the result of the poor physical condition of the hermaphrodites used in the matings since it was not reproduced in later experiments.

Male recombination frequency increases with temperature: Crossing over in the hermaphrodite has been found to vary with temperature (ROSE and BAILLIE 1979a). To determine if temperature has a similar effect in the male, recombination was measured in *cis*-heterozygous males at experimental temperatures of 15° and 25°. The results are shown in Table 7 with 20° controls for comparison. Recombination frequency in the male and in the hermaphrodite decreased at 15° and increased at 25° in both intervals tested. In the *dpy-5 unc-13* interval, the magnitude of the temperature effects was the same in both sexes; at 25° recombination frequency increased approximately 40% and at 15°, it decreased 40%. In the *dpy-5 unc-75* interval, however, the magnitude of the temperature effect was at least two-fold greater in males when compared to that of hermaphrodite controls. Male crossover frequency remained lower than that observed in the hermaphrodite at all temperatures and in both intervals tested.

Male recombination frequency increases with Rec-1: The *rec-1* mutation increased meiotic recombination three-fold in the hermaphrodite (ROSE and BAILLIE 1979b). This increase retained the meiotic distribution of crossover events. To determine if this mutation had the same effect in the male, recombination was measured in *unc-11 dpy-5 rec-1/ + + rec-1* and *dpy-5 unc-13 rec-1/ + + rec-1* individuals. The results of these experiments are shown in Table 8. Recombination frequency in the male increased three-fold in the *unc-11 dpy-5* interval (from 1.8 to 5.0, data in Tables 4 and 8 respectively) and five-fold in the *dpy-5 unc-13* interval (from 0.8 to 4.3, data in Tables 7 and 8 respectively). Rec-1 hermaphrodite crossover frequencies remained higher than those observed in the male.

Table 7

Effect of temperature on male recombination

Genotype	Wts		Recombinants				pX100(C.I.)[a]
	♀	♂	Dpy		Unc		
			♀	♂	♀	♂	
15°C							
dpy-5 unc-13/ + +							
male[b]	1870	1992	9	14	6	12	0.5(0.4-0.7)
hermaphrodite	1218		9		8		1.0(0.6-1.6)
dpy-5 unc-75/ + +							
male	2345	2528	93	103	111	89	3.9(3.8-4.0)
hermaphrodite	2206		121		130		8.4(7.4-9.5)
20°C							
dpy-5 unc-13/ + +[c]							
male	4242	4140	36	28	34	40	0.8(0.7-1.0)
hermaphrodite	3119		34		32		1.6(1.2-2.0)
dpy-5 unc-75/ + +[c]							
male	4231	4308	234	258	226	229	5.3(5.2-5.4)
hermaphrodite	10803		623		707		9.1(8.9-9.2)
25°C							
dpy-5 unc-13/ + +							
male	968	1077	15	10	10	11	1.1(0.8-1.5)
hermaphrodite	3024		52		42		2.3(1.9-2.8)
dpy-5 unc-75/ + +							

male	1105	1142	139	122	114	107	9.7(9.4-10.0)
hermaphrodite	1574		125		140		12.4(11.0-13.9)

[a] C.I. = 95% confidence interval (see Chapter 1: MATERIALS and METHODS).

[b] Recombination measured in individuals of indicated sex.

[c] Data from brooding experiments.

Table 8

The effect of Rec-1 on male recombination

Genotype	Wts		Recombinants				pX100(C.I.)[a]
	♀	♂	Dpy		Unc		
			♀	♂	♀	♂	
unc-11 dpy-5 rec-1/ + + rec-1							
male[b]	866	755	40	43	57	30	5.0(4.3-5.7)
hermaphrodite	2033		91		91		6.7(5.7-7.6)
unc-11 dpy-5/ + +							
male[c]	983	962	19	12	15	25	1.8(1.4-2.2)
hermaphrodite[c]	3786		58		61		2.3(2.0-2.8)
dpy-5 unc-13 rec-1/ + + rec-1							
male	922	908	46	41	36	43	4.3(3.7-5.0)
hermaphrodite	2111		103		86		6.6(5.7-7.7)
dpy-5 unc-13/ + +							
male[d]	4241	4140	36	28	34	40	0.8(0.7-1.0)
hermaphrodite[e]	3119		34		32		1.6(1.2-2.0)

[a] C.I. = 95% confidence interval (see Chapter 1: MATERIALS and METHODS).

[b] Recombination measured in individuals of indicated sex.

[c] Data from Table 4.

[d] Data from Table 2.

[e] Data from Table 3.

Chapter 1: DISCUSSION

BRENNER (1974) first observed that each *C. elegans* autosome is marked by a cluster of genes and proposed that this clustering was a result of recombination suppression. This has been supported by studies which have compared the genetic and physical maps (GREENWALD *et al.* 1987; PRASAD and BAILLIE 1989; STARR *et al.* 1989) and by the enhancement observed in the clusters when treated with gamma radiation (KIM and ROSE 1987) and elevated temperatures (ROSE and BAILLIE 1979a; this study). The results presented in this thesis show that the frequency of recombination is generally higher in the *C. elegans* hermaphrodite than in the male, although the increases are not uniform along the length of the chromosome and one interval showed no sex-related difference. In addition, the gene cluster of LG *I* appeared to be larger in the male than in the hermaphrodite. That the genetic map of the male is smaller than that of the hermaphrodite, while the gene cluster is larger, may be explained if the recombination suppression observed in the hermaphrodite is more pronounced in the male, or if interference values in the male (leading to double-crossing over) are low. HODGKIN, HORVITZ, and BRENNER (1979) found complete interference on the *X* chromosome of the hermaphrodite but measured a moderate C value (coefficient of coincidence) on an autosome in the male. This may be explained if high interference is limited either to the hermaphrodite or to the *X* chromosome but neither possibility has been confirmed. It is unlikely that low interference in the male is the basis of sex-related differences in recombination frequency for several reasons. Firstly, large decreases in the male meiotic map were observed in small intervals in the cluster, a region in which double-crossing over would be extremely rare. Secondly, in a large interval like *bli-3 unc-11*, the male meiotic map showed a 36% decrease in recombination when compared to the hermaphrodite. The number of double-crossovers one would expect in this interval (approximately 2), cannot account for the magnitude of this decrease. Thus, while it is possible that interference values differ between the

hermaphrodite and the male, it is unlikely to be the sole explanation of differential rates of crossing over between the sexes.

Elevated temperatures produce increases in recombination values in a number of organisms including Drosophila (PLOUGH 1917), Coprinus (LU 1969, 1974), and Neurospora (McNELLY-INGLES, LAMB and FROST 1966). In Drosophila, the greatest temperature related changes in crossover frequency occur in centromeric regions, where recombination is normally suppressed (PLOUGH 1917; BRIDGES 1915, 1927; STERN 1926; MATHER 1939). ROSE and BAILLIE (1979a) examined two intervals in the LG *I* cluster of the hermaphrodite and found 2-3 fold increases in recombination frequency at elevated temperatures. In this study, similar increases of recombination values have been observed in the male in two intervals. The *dpy-5 unc-13* interval has been well characterized and includes a portion of the chromosome which is the most recombinationally suppressed (KIM and ROSE 1988; PRASAD *et al.* 1993). The adjacent *unc-13 unc-75* interval showed a two-fold map expansion in the male compared to the hermaphrodite. This suggests that the recombination suppression responsible for the appearance of the gene cluster on the genetic map extends further in the male than in the hermaphrodite and can be expanded by temperature over a larger interval.

The results of experiments using the *rec-1* mutation can also be interpreted in light of sex-related differences in cluster size. This mutation increased the frequency of male recombination in both intervals tested. In both sexes, a greater enhancement effect (6-8-fold increase) was observed in the *dpy-5 unc-13* interval, located within the cluster, when compared to the *unc-11 dpy-5* interval (3-fold increase), a larger region at the left end of the cluster. The *dpy-5 unc-13* region is more recombinationally suppressed (discussed above) than flanking regions, suggesting that the most suppressed regions may be more sensitive to the effects of *rec-1*.

BRENNER (1974) measured recombination frequency in oocytes on the *X* chromosome and found this frequency to be the same as the hermaphrodite frequency. In this study, oocyte recombination frequency was measured in two intervals on LG *I* and

found to be lower than both the total hermaphrodite frequency and the crossover frequency in hermaphrodite spermatocytes. These results may be explained if differences in recombination frequency between the hermaphrodite germlines are genetic interval-dependent or limited to the autosomes. Although recombination was found to vary with age in male spermatocytes, it is unlikely hermaphrodite spermatocytes contribute to the variation of recombination frequency with age in the hermaphrodite since spermatogenesis in hermaphrodites is restricted to the fourth larval stage, at which time about 300 sperm are produced (HIRSH, OPPENHEIM and KLASS 1976; WARD and CARREL 1979). This has previously been pointed out in studies examining the variation of recombination with hermaphrodite age (ROSE and BAILLIE 1979a). If the recombination frequency in hermaphrodite spermatocytes (b) is constant, it follows that as the oocyte recombination frequency approaches zero with increasing age, the value of R in the hermaphrodite should never fall below $1/2b$. For example, in the *dpy-5 unc-75* interval the value of R in the final brood (0.08) is still higher than $1/2b$ (0.06). Of further interest is the possibility that the variation of recombination frequency with age is a continuum of the two germlines. Since the first brood measures the earliest oocyte recombination frequency (those events occurring right after the switch from spermatogenesis), one would expect the two germlines to have similar frequencies in this brood. In the *dpy-5 unc-75* interval for example, knowing the value of R in the first brood (0.10) and the value of b (0.12), the value of a (0.09), the frequency of recombination in the oocyte, can be calculated. As predicted, the oocyte recombination frequency in this brood is close to, but not higher, than the spermatocyte frequency.

YAMAMOTO (1961) measured recombination in hormonally transformed XY males of the Medaka and found the recombination frequency to be much higher than that observed in normal males. In this study, recombination was measured in males sexually transformed by the *her-1* mutation. Similar to the previous results, the recombination frequency was significantly higher in the transformed males when compared to normal

males. This result can be interpreted as evidence that it is the sexual phenotype and not genotype that determines the frequency of recombination during gametogenesis.

Meiotic recombination frequency in both sexes of *C. elegans* is affected by age and temperature. Recombination frequency decreases with maternal age in Drosophila (BRIDGES 1927; NEEL 1941), in mice (FISHER 1949), and in *C. elegans* (ROSE and BAILLIE 1979a). A fall in crossover frequency with paternal age was observed in two intervals. This variation of recombination frequency with parental age does not affect the results of other experiments. As described in MATERIALS and METHODS, only L4 hermaphrodites, which are easily identifiable at that stage, and young males were used in later experiments further characterizing recombination. The population of males used in these experiments was considered to be synchronous since all male recombination experiments were replicated and reproducible results were obtained. For example, the curves derived from four separate experiments examining the variation of recombination frequency with paternal age in the *dpy-5 unc-13* interval could be superimposed.

In conclusion, male recombination across the length of LG *I* was found to be approximately one-third less than that observed in the hermaphrodite. This decrease, however, was not uniform and one interval showed no sex-related difference in crossover frequency. By measuring recombination in the two germlines of the hermaphrodite and in transformed males, it has been concluded it is the physiology of the gonad, rather than the sexual karyotype of the germline, that determines the recombination frequency characteristic of a specific sex. It was also observed that male recombination in *C. elegans* varies with age and temperature, suggesting recombination is quantitatively rather than qualitatively different between the sexes. For this reason, it is recommended that the standard practices suggested by ROSE and BAILLIE (1979a) for hermaphrodite recombination experiments be also applied to male recombination studies (*i.e.* that studies measuring recombination frequency be carried out at 20° and all progeny from the male should counted).

Chapter 2: Characterization and Mapping of *rec-1*

INTRODUCTION

Mutations which disrupt the normal frequency and distribution of crossing over can identify genes important in the control of meiosis. Study of these mutations has revealed that in organisms in which recombination normally occurs, one crossover between the homologues is necessary for their proper disjunction (reviewed by JONES 1984, 1987). One class of genes is defined by mutants which are defective in the recombinational machinery and another is defined by mutants that alter the normal patterns of exchange during meiosis (reviewed by BAKER *et al.* 1976). The majority of mutations identified are recombination defective and result in reduced levels of recombination. In *D. melanogaster*, recombination defective mutants have been divided into three groups based on the distribution of their exchange events (reviewed by BAKER *et al.* 1976). One group is represented by the gene *mei-9*, whose mutations decrease the frequency of crossing over but maintain the wild-type pattern of events (BAKER and CARPENTER 1972; CARPENTER and SANDLER 1974). The second group includes the genes *mei-218*, *mei-41*, and *mei-251*, whose mutations reduce the frequency of crossing over by differing amounts in different intervals, thereby disrupting both the frequency and pattern of exchange events (BAKER and CARPENTER 1972; CARPENTER and SANDLER 1974; SANDLER and SZAUTER 1978). The third group is represented by mutations in *mei-352* which alter the distribution, but not the overall frequency of crossing over (BAKER and CARPENTER 1972).

In *C. elegans*, the majority of meiotic mutants have been isolated as recessive mutations that increased the nondisjunction frequency of the *X*, resulting in a *Him* (*h*igh *i*ncidence of *m*ales) phenotype (HODGKIN, HORVITZ and BRENNER 1979). Mutations in the genes *him-6* and *him-14* are recombination defective and produce nondisjunction of the autosomes as well as of the *X* chromosome, presumably as a result of the reduction in crossing over (HODGKIN, HORVITZ and BRENNER 1979; KEMPHUES, KUSCH and WOLF 1988). A class of meiotic mutant not previously described is represented by the

recessive *rec-1* mutation which increases both crossing over (ROSE and BAILLIE 1979b) and conversion (RATTRAY and ROSE 1988) on all chromosomes. Since *rec-1* mutants are not radiation sensitive (HARTMAN and HERMAN 1982), the function of this gene appears to be specific to meiosis rather than to general DNA metabolism. In this thesis, the effect of *rec-1* on the distribution of crossing over has been characterized and a map position has been determined.

Chapter 2: MATERIALS AND METHODS

The source of mutations and chromosomal rearrangements is given in Chapter 1: MATERIALS and METHODS.

Duplication mapping of *rec-1*: The possibility that *sDp1(I;f)* included the *rec-1* locus was examined by measuring recombination in the *dpy-5 dpy-14* interval in the presence of the duplication (*sDp1* carries wild-type alleles of both of these markers). Rec-1 or N2 males were mated to *dpy-5 dpy-14 rec-1/dpy-5 dpy-14 rec-1/sDp1(I;f)* hermaphrodites and *dpy-5 dpy-14/dpy-5 dpy-14/sDp1(I;f)* controls respectively. Wild-type hermaphrodite progeny resulting from this cross were individually plated and their progeny scored. *sDp1*-bearing hermaphrodites have lower brood sizes (duplication homozygotes are inviable) and increased nondisjunction of the *X* resulting in male progeny (ROSE, BAILLIE and CURRAN 1984). To identify the individuals that carried the duplication, broods of the size characteristic for *sDp1(I;f)* were examined for the presence of males, and the frequency of the double homozygote class was determined. This class was expected to approach a frequency of 0.125 in the presence of the duplication and and a frequency of 0.25 in its absence. The recombination frequency in individuals lacking the duplication was calculated as described in Chapter 1: MATERIALS and METHODS. A gametic frequency of 0.43 for *sDp1(I;f)*(ROSE, BAILLIE and CURRAN 1984) was used to calculate the frequency of crossing over in individuals determined to be of the genotype *dpy-5 dpy-14 rec-1/ + + rec-1/sDp1(I;f)* and *dpy-5 dpy-14 + / + + rec-1/sDp1(I;f)* using the formula:

$$p = 1 - [\, 1 - 148\mathbf{D}/17(\mathbf{D} + \mathbf{W})]^{1/2}$$

where **D** is the number of Dpy-5 recombinants and **W** is the number of wild-type progeny. This formula is based upon the assumptions that the *sDp1* homozygote is inviable and that recombination between *sDp1* and LG *I* does not occur in the *dpy-5 dpy-14* interval. Similarly, *sDp2* was used to map *rec-1* by measuring recombination in *dpy-5 dpy-14 rec-1/*

+ + rec-1/sDp2(I;f) hermaphrodites and in *dpy-5 dpy-14 rec-1/ + + + /sDp2* controls. This duplication covers both markers and *sDp2*-bearing worms were identified by the frequency of segregation of the double mutant as described for *sDp1*. A gametic frequency of 0.38 for *sDp2(I;f)* (ROSE, BAILLIE and CURRAN 1984) was used to calculate the frequency of crossing over in the presence of the duplication using the formula:

$$p = 1 - [\, 1 - 75\mathbf{D}/19(\mathbf{D} + \mathbf{W})]^{1/2}$$

where **D** is the number of Dpy-5 progeny and **W** is the number of wild types. This formula assumes that the *sDp2* homozygote is not viable. Recombination was also measured in *dpy-5 unc-13 rec-1/ + + rec-1/sDp2* hermaphrodites and in *dpy-5 unc-13 rec-1/ + + + /sDp2* controls. In this case, however, the duplication does not extend to *unc-13* and as a result, *sDp2*-bearing hermaphrodites were identified by the presence of a large number of Unc-13 segregants amongst their progeny. Recombination in the *dpy-5 unc-13* interval was calculated using the formula:

$$p = 1 - [\, 1 - 19\mathbf{D}/(\mathbf{D} + \mathbf{W})]^{1/2}$$

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where **D** is the number of Dpy-5 recombinants and **W** is the number of wild-type progeny.

Deficiency mapping of *rec-1*: To test if the ribosomal deficiency *eDf24(I;f)* deleted the *rec-1* locus, *dpy-11 unc-42/ + + ; rec-1/rec-1* or *dpy-11 unc-42/ + +* males were crossed to *unc-54/eDf24* hermaphrodites and the resulting wild-type progeny individually plated. Since *eDf24* does not include *unc-54*, only plates that segregated Dpy-11 Unc-42 progeny and failed to segregate Unc-54 individuals (indicating the presence of the deficiency) were scored. Recombination was measured in the *dpy-11 unc-42* interval using the general recombination formula discussed in Chapter 1: MATERIALS and METHODS. The

deficiencies *eDf4*, *eDf9*, *eDf10*, and *eDf13* were isolated using *eDf24* as a balancer and all complement *eDf24* and fail to complement *unc-54* (ANDERSON and BRENNER 1984). To test if any of these deficiencies included *rec-1*, *eDfX/eDf24* hermaphrodites were mated to *unc-54/ +* males and the resulting Unc-54 hermaphrodites were then mated to males of the genotype *dpy-11 unc-42/ + + ; rec-1/rec-1* or *dpy-11 unc-42/ + +* . Wild-type hermaphrodite progeny were individually plated and their progeny screened for the presence of Dpy-11 Unc-42 segregants and the absence of Unc-54 segregants. The recombination frequency in the *dpy-11 unc-42* interval was then measured and calculated as described above for *eDf24*.

Chapter 2: RESULTS

***rec-1* alters the distribution of crossing over:** ROSE and BAILLIE (1979b) showed that *rec-1* greatly enhanced the frequency of crossing over in small intervals. To determine the effect of *rec-1* on recombination along the whole chromosome, four intervals spanning LG *I* were examined and the results are shown in Figure 4 (Data shown in Table 9). The *bli-3 unc-11* interval, located on the left arm of LG *I*, was 12.8 in *rec-1* homozygotes and 14.8 m.u. in controls, showing no recombination enhancement in the presence of *rec-1*. Recombination in the *unc-11 dpy-5* interval, however, showed a 3-fold enhancement in *rec-1* homozygotes (6.7 m.u. compared to 2.3 m.u. in controls). The *dpy-5 unc-101* interval, normally 12.0 m.u., was 21.2 m.u. in *rec-1* homozygotes, demonstrating extensive enhancement in the presence of *rec-1*. The *unc-101 unc-54* interval, located on the right arm of the chromosome, was severely reduced from 14.4 m.u. in controls to 4.6 m.u. in *rec-1* homozygotes. The *dpy-5 unc-54* interval, however, was 31.6 m.u. in controls and 30.6 m.u. in *rec-1* homozygotes, indicating that total recombination on the right arm of LG *I* in *rec-1* homozygotes did not change when compared to controls. The total genetic length of LG *I* was 45.3 m.u. in *rec-1* homozygotes and 43.5 m.u. in controls.

***sDp1(I;f)* suppresses the Rec-1 phenotype:** ROSE and BAILLIE (1979b) found no linkage between *rec-1* and any markers located in the gene clusters of the autosomes. When markers located at the ends of the chromosomes were tested, *rec-1* showed loose linkage to the gene *unc-54*, located on the right end of LG *I* (ROSE *unpublished results*). Since *rec-1* is completely recessive (ROSE and BAILLIE 1979b), a strategy using two large duplications of LG *I*, *sDp1* and *sDp2*, to map the gene was developed (data shown in Table 10; extent of duplications shown in Figure 4). Although *sDp1* does pair and recombine with LG *I*, it does so rarely in the *dpy-5 dpy-14* region (ROSE, BAILLIE and CURRAN 1984; McKIM, PETERS and ROSE 1993) and in conjunction with the small size of this interval, it is unlikely that any recombinants recovered were the result of a recombination event with the duplication. In the absence of the duplication, the frequency of crossing over in *dpy-5*

FIGURE 4.-Meiotic maps of LG *I* in the presence of the *rec-1* mutation and in controls.

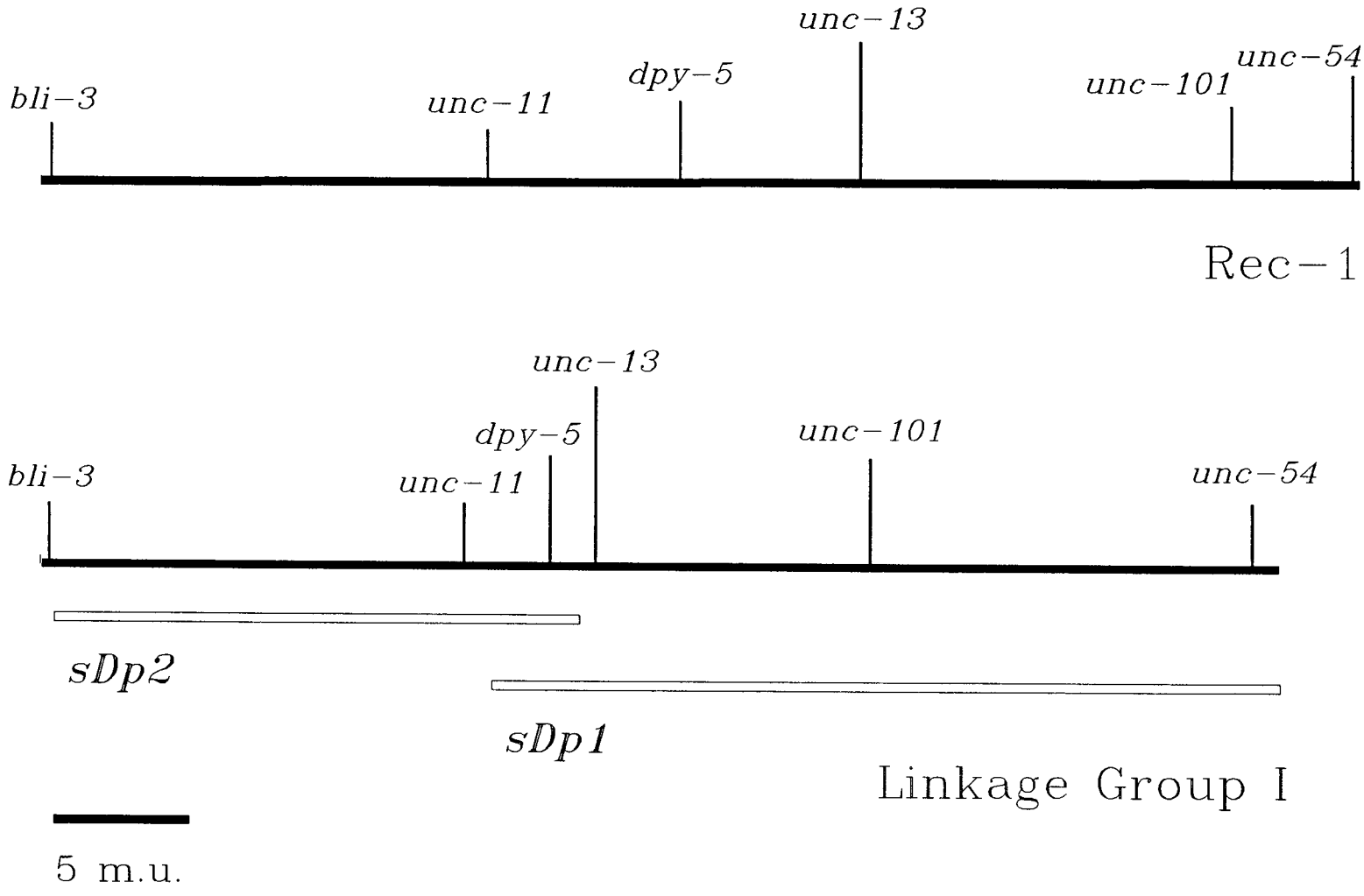

unc-13
unc-54
dpy-5
bli-3
unc-11
unc-101
Rec-1
unc-13
dpy-5
unc-101
bli-3
unc-11
unc-54
sDp2
sDp1
Linkage Group I
5 m.u.

Table 9

The effect of *rec-1* on crossing over on LG *I*

Genotype	Wts	Recombinants	pX100(C.I.)[a]
bli-3 unc-11/ + + [b]	1686	170 Unc	14.8(12.4-17.4)
bli-3 unc-11 rec-1/ + + rec-1	990	79 Unc	12.8(10.0-16.1)
unc-11 dpy-5/ + + [b]	3786	58 Dpy	2.3(2.0-2.8)
unc-11 dpy-5 rec-1/ + + rec-1	2033	91 Dpy 91 Unc	6.7(5.7-7.6)
dpy-5 unc-13/ + + [c]	3119	34 Dpy 32 Unc	1.6(1.2-2.0)
dpy-5 unc-13 rec-1/ + + rec-1 [d]	3706	156 Dpy	6.3(5.3-7.3)
dpy-5 unc-101/ + + [e]	889	79 Dpy 66 Unc	12.0(10.0-14.0)
dpy-5 unc-101 rec-1/ + + rec-1	1369	183 Dpy 213 Unc	21.2(20.1-22.2)
unc-101 unc-54/ + + [b]	1187	116 Unc-101	14.4(11.8-17.1)
unc-101 unc-54 rec-1/ + + rec-1	1973	61 Unc-101	4.6(3.6-5.8)
dpy-5 unc-54/ + +	1620	349 Dpy	31.6(30.3-32.9)
dpy-5 unc-54 rec-1/ + + rec-1	1698	355 Dpy 267 Unc	30.6(29.2-32.0)

[a] C.I. = 95% confidence interval (see Chapter 1: MATERIALS and METHODS).

[b] Data from Table 4.

[c] Data from Table 3.

[d] Data from Table 10.

[e] Data from Table 12.

Table 10

Duplication mapping of *rec-1*

Genotype	Wts	Recombinants	pX100(C.I.)[a]
dpy-5 dpy-14 + / + + rec-1	3238	28 Dpy-5	1.3(0.88-1.8)
dpy-5 dpy-14 + / + + rec-1/sDp1	1614	6 Dpy-5	1.6(0.7-3.5)
dpy-5 dpy-14 rec-1/ + + rec-1	8659	321 Dpy-5	5.5(5.4-5.6)
dpy-5 dpy-14 rec-1/ + + rec-1/sDp1	1201	5 Dpy-5	1.8(0.7-4.1)
dpy-5 dpy-14 rec-1/ + + +	2213	25 Dpy-5	1.7(1.1-2.4)
dpy-5 dpy-14 rec-1/ + + +/sDp2	1976	7 Dpy-5	0.7(0.3-1.4)
dpy-5 dpy-14 rec-1/ + + rec-1	1729	67 Dpy-5	5.8(4.4-7.2)
dpy-5 dpy-14 rec-1/ + + rec-1/sDp2	1002	13 Dpy-5	2.6(1.3-4.2)
dpy-5 unc-13/ + + [b]	3119	34 Dpy 32 Unc	1.6(1.2-2.0)
dpy-5 unc-13 + / + + rec-1	2133	23 Dpy	1.6(1.0-2.4)
dpy-5 unc-13 rec-1/ + + rec-1	3706	156 Dpy	6.25(5.3-7.3)
dpy-5 unc-13 + / + + rec-1/sDp2	1057	4 Dpy	0.2(0.08-0.6)
dpy-5 unc-13 rec-1/ + + rec-1/sDp2	1977	41 Dpy	1.4(0.9-1.9)

[a] C.I. = 95% confidence interval (see Chapter 1: MATERIALS and METHODS).

[b] Data from Table 3.

dpy-14 rec-1/ + + + heterozygotes was 0.013. In *rec-1* homozygotes, the frequency of recombination increased to 0.055 in this interval. In the presence of *sDp1*, the frequency of recombination in the *dpy-5 dpy-14* interval was reduced to 0.018 in *sDp1/dpy-5 dpy-14 rec-1/ + + rec-1* heterozygotes, similar to the value of 0.016 observed in *sDp1/dpy-5 dpy-14 + / + + rec-1* heterozygotes, indicating that *sDp1* carried a wild-type allele of *rec-1*. These results are also consistent with the finding that *rec-1* is completely recessive to its wild-type allele (ROSE and BAILLIE 1979b). To ensure that the suppression observed was not a general feature of LG *I* duplications, recombination was also measured in the presence of *sDp2*, a large duplication of the left half of the chromosome. The frequency of crossing over between *dpy-5* and *dpy-14* in the presence of *sDp2* and Rec-1 (0.026) was 3-fold higher than in the absence of Rec-1 (0.007), indicating that the Rec-1 phenotype was expressed despite the presence of the duplication. Although the frequency of crossing over in the presence of *sDp2* and Rec-1 was significantly higher than in the absence of Rec-1, the frequencies were much lower than those obtained in the absence of the duplication (0.06 in *rec-1* homozygotes and 0.02 in heterozygotes). To confirm the possibility that the overall decrease in recombination frequencies could be attributed to a reduced recovery of recombinants in the presence of *sDp2*, recombination was examined in another interval. The frequency of crossing over was examined in *sDp2/dpy-5 unc-13 rec-1/ + + rec-1* and *sDp2/dpy-5 unc-13/ + + rec-1* heterozygotes. The frequency of recombination between *dpy-5* and *unc-13* in the presence of *sDp2* and Rec-1 (0.014) was 7-fold higher than that observed in the absence of Rec-1 (0.002). Since the recombination formula used to calculate the frequencies assumes that both *sDp1* and *sDp2* are inviable as a homozygotes, these results may be explained if *sDp2* homozygotes can be recovered and are affecting the recovery of recombinants. An alternative explanation may be that *sDp2* suppresses recombination between the two homologues, however, recombination between the duplication and the chromosomes *I* has not been observed (ROSE, BAILLIE and CURRAN 1984).

***eDf24(I)* fails to complement *rec-1*:** The duplication *sDp1* covers the right arm of LG *I*, including most of the centrally located cluster. Since *rec-1* was suppressed by *sDp1*,

deficiencies of the right end were tested for failure to complement the mutation (data shown in Table 11). The deficiencies used in this study and their known breakpoints are shown in Figure 5. The *dpy-11 unc-42* interval, normally 2.7 m.u., increases to 6.4 in *rec-1* homozygotes. In *eDf24/rec-1; dpy-11 unc-42/ + +* heterozygotes, this interval showed a 2-fold enhancement in recombination (5.6 m.u.) when compared to *eDf24/ + ; dpy-11 unc-42* controls (3.0 m.u.), indicating that the deletion failed to complement the *rec-1* mutation. *eDf24* had previously been used as a balancer to isolate a number of deletions of the *unc-54* locus (including *eDf4*, *eDf9*, *eDf10*, and *eDf13*) (ANDERSON and BRENNER 1984) which had undefined right breakpoints. Although these deficiencies genetically complemented *eDf24*, the possibility remained they physically overlapped *eDf24* in a region that did not include any essential genes. All of the deficiencies tested complemented *rec-1*, indicating that if the deletions did overlap with *eDf24*, *rec-1* was not included in the region of overlap.

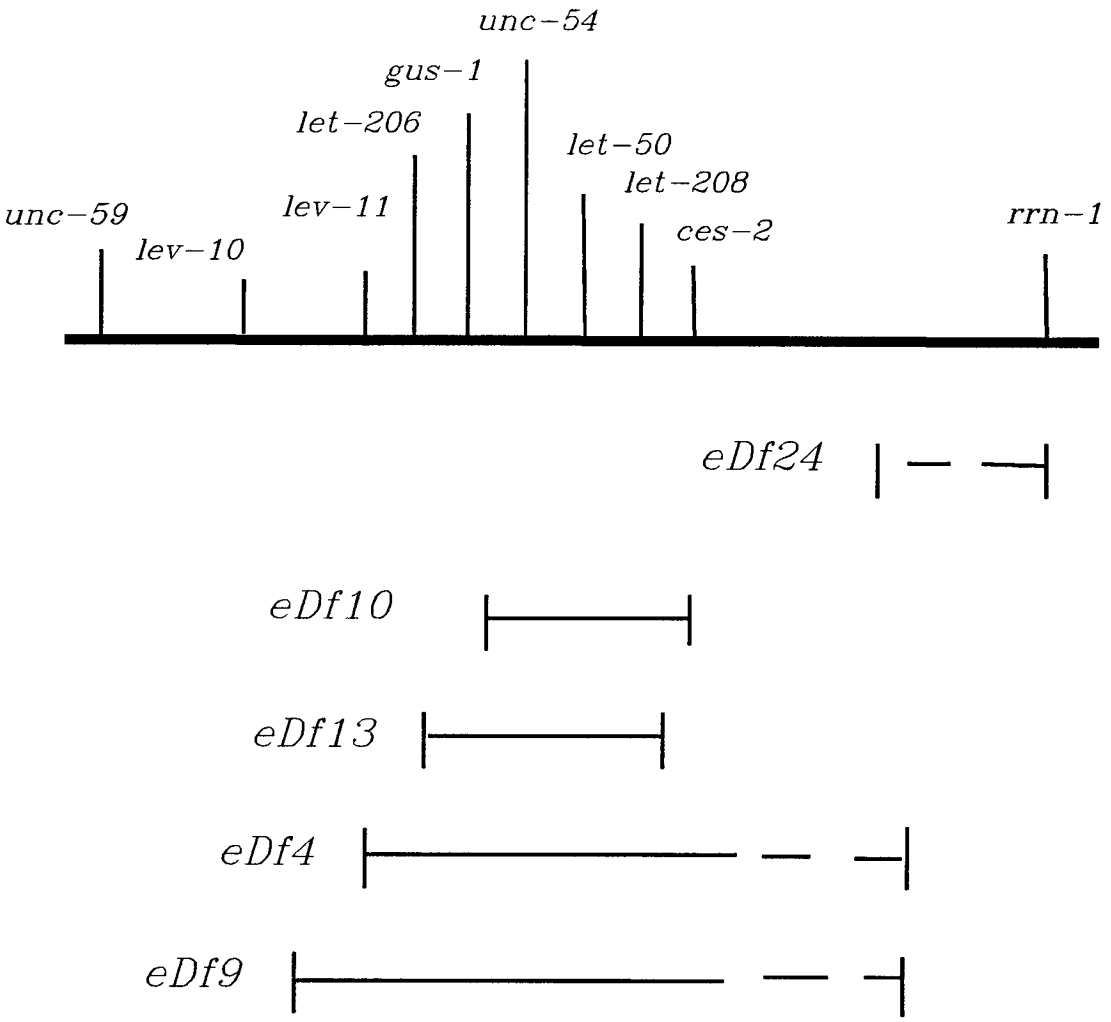
Table 11

Deficiency mapping of *rec-1*

Genotype	Wts	Recombinants	pX100(C.I.)[a]
unc-42 dpy-11/ + +	1250	26 Dpy 20 Unc	2.7(2.0-3.6)
unc-42 dpy-11/ + + ; rec-1/rec-1	1219	66 Dpy 59 Unc	7.6(6.4-9.0)
unc-42 dpy-11/ + + ; rec-1/eDf4	999	19 Dpy 23 Unc	3.1(2.2-4.1)
unc-42 dpy-11/ + + ; rec-1/eDf9	693	8 Dpy 11 Unc	2.0(1.2-3.1)
unc-42 dpy-11/ + + ; rec-1/eDf10	1127	29 Dpy	3.8(2.5-5.4)
unc-42 dpy-11/ + + ; rec-1/eDf13	1558	46 Dpy 49 Unc	4.5(3.6-5.5)
unc-42 dpy-11/ + + ; + /eDf24	1668	35 Dpy 33 Unc	3.0(2.3-3.8)
unc-42 dpy-11/ + + ; rec-1/eDf24	2119	88 Dpy 72 Unc	5.6(4.8-6.5)

[a] C.I. = 95% confidence interval (see Chapter 1: MATERIALS and METHODS).

FIGURE 5.-Deficiency map of the *unc-54* region of LG *I*. The right breakpoint of *eDf24* is known to map within the ribosomal cluster whose genetic locus is *rrn-1* (see APPENDIX III). The left breakpoint maps within nonribosomal sequences to the right of *unc-54* (see Chapter 2: RESULTS). It is not known how far *eDf9* and *eDf4* extend to the right, only that they complement *eDf24*. Both deficiencies may delete common sequences with *eDf24*, if no essential genes are included.


unc-54
gus-1
let-206
let-50
let-208
unc-59
lev-11
ces-2
rrn-1
lev-10
eDf24
eDf10
eDf13
eDf4
eDf9

LG I (right)

Chapter 2: DISCUSSION

The *rec-1* mutation, initially described as a general recombination enhancer, increases meiotic crossing over (ROSE and BAILLIE 1979b) and conversion (RATTRAY and ROSE 1988), without disrupting the normal pattern of meiotic exchange. The intervals tested in these studies, however, were located in the central gene clusters, or at the distal tips of the chromosome arms, where a small clustering effect also exists. BRENNER (1974) first proposed that the gene clusters observed on the meiotic map were the result of recombination suppression in the region. This has been supported by several studies that have shown that the cluster is a result of 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). In this thesis, crossing over in the gene cluster of LG *I* was enhanced in *rec-1* homozygotes, consistent with previous results (ROSE and BAILLIE 1979b). The level of enhancement, however, was dependent on the interval tested within the cluster; the frequency of recombination increased 3-fold in the *unc-11 dpy-5* interval and 5-fold in the *dpy-5 unc-13* interval. The differential level of enhancement may be explained if *rec-1* alleviates the recombination suppression normally present in the cluster and if this suppression is more extreme in some regions. This interpretation is supported by the facts that the *dpy-5 unc-13* interval contains the most recombinationally suppressed region of the cluster (STARR *et al.* 1989), and shows the most enhancement in the presence of Rec-1. This enhancement, however, is not a general feature of the *rec-1* phenotype since in two large intervals flanking the cluster the frequency of crossing over was unaffected or reduced. Recombination in the *bli-3 unc-11* interval was not different in controls, whereas *unc-101 unc-54*, a comparably-sized interval on the right end, showed a 4-fold reduction. The apparent suppression of crossing over in this interval may be explained if *rec-1* reduced interference values on the right arm, or if the recombination frequency normally observed in that region is enhanced per base pair, compared to the genome average, however, neither possibility has been confirmed.

Darlington (1937) proposed that the formation of a chiasmata between homologues was necessary for their proper disjunction. In *C. elegans*, the suppression of crossing over between homologues results in their random segregation (ZETKA and ROSE 1992; this study). Consistent with this result, the genetic length of chromosome *I* approaches 50 m.u. (corresponding to an average of one crossover per meiosis) in wild-type hermaphrodites, males (ZETKA and ROSE 1990; this study), translocation heterozygotes (McKIM, HOWELL and ROSE 1988; McKIM, PETERS and ROSE 1993) and inversion heterozygotes (ZETKA and ROSE 1992; this study). The conservation of a 50 m.u. genetic length can be explained by some cytological evidence suggesting that the meiotic chromosomes are held together by a terminalized chiasma at metaphase of meiosis I (ALBERTSON and THOMSON 1993). The genetic size of chromosome *I* in *rec-1* homozygotes also approached 50 m.u., compatible with an average of one crossover every meiosis. Although *rec-1* does not affect the total number of crossovers, its effect of expanding some regions and contracting others, disrupted the normal distribution of exchanges. This suggests the mechanism responsible for ensuring that one crossover occurs between the homologues every meiosis is epistatic to *rec-1*(+), the role of which appears to be in determining preferred sites of exchange. The existence of such sites is supported by studies in *S. cerivisiae* where a secondary pairing site on chromosome *III* (GOLDWAY, ARBEL and SIMCHEN 1993) was found to be a recombination hotspot (GOLDWAY *et al.* 1993). The role of *rec-1(+)* may be in the identification of such sites during pachytene and in its absence, the distribution of crossover events on the chromosome becomes related to physical size. On chromosome *I*, a preferential site of exchange may be located on the right arm and would explain the high frequency of recombination in the *unc-101 unc-54* region in spite of its small physical size (COULSON *et al.* 1986, 1988).

In Drosophila, mutations that alter the distribution of exchanges have been isolated (reviewed by Baker *et al.* 1976). Of these, however, mutations in all but one gene also reduce the frequency of crossing over. Mutations in the exceptional gene, *mei-352*, disrupt the distribution of exchanges along the chromosome, but do not alter their frequency

(BAKER and CARPENTER 1972), similar to *rec-1*. In both mutants, the frequency of recombination is increased in regions which are normally recombinationally suppressed. Recombination in *mei-352* mutants is enhanced in the centric heterochromatin and on the fourth chromosome of *mei-352* mutants (BAKER and CARPENTER 1972; SANDLER and SZAUTER 1978). In *rec-1* mutants, crossing over is enhanced in the gene clusters, and in the case of chromosome *I*, also in a region adjacent to the cluster (the *unc-29 unc-101* interval). This region has not been cloned (COULSON *et al.* 1986, 1988), presumably because of the presence of repetitive sequences. The meiotic centromere of chromosome *I* has been mapped to the *dpy-5 unc-75* interval and is thought to be tightly linked to the left breakpoint of the inversion *hIn1(I)*, which lies between *unc-29* and *unc-75* (ZETKA and ROSE 1992; this study). This is supported by the structure of two recombinant chromosomes, derived from inversion heterozygotes, which proved to be deficiencies with indistinguishable left breakpoints. These deficiencies are thought to have arisen as a result of the breakage of a dicentric bridge at anaphase II. That two independent events could result in identical breakpoints can be explained by the presence of a fragile site in the region, or if the meiotic centromere is tightly linked to the breakpoint of *hIn1(I)* (mapping to the *unc-29 unc-75* interval) and as a result, the region pulled apart at anaphase is small, and the probability of two independent breaks in the region is high. In addition to the presence of potential centromeric sequences, a family of repetitive sequences (La VOLPE, CIARAMELLA, BAZZICALUPO 1988) have also been mapped to this region. For these reasons, it is possible that *rec-1* disrupts two distinct forms of crossover suppression; one form responsible for establishing the gene cluster and the other responsible for the crossover suppression associated with repetitive sequences. *mei-352* mutants also exhibit a decreased frequency of single-exchange tetrads and an increased frequency of double-exchange tetrads, suggesting the defect lies in the establishment of spatial restrictions on chiasma formation (BAKER and CARPENTER 1972). While it is possible that the crossover suppression observed on the right arm of LG *I* in *rec-1* mutants can be explained by lower interference values, the number of double crossovers expected in the *unc-101 unc-54* interval

(approximately two) cannot explain the 3-fold reduction in genetic length. Furthermore, the genetic length of chromosome *I* in *rec-1* mutants approached the 50 m.u. observed in controls, suggesting that if double-crossing over occurred, it did so at a low frequency.

While *rec-1* and *mei-352* may be defective in genes that specify a precondition for exchange necessary for determining its probability or distribution on the chromosome, they differ in two important respects. Firstly, *mei-352* specifically increases recombination in recombinationally-suppressed regions and does not decrease recombination in any interval, whereas *rec-1* increased recombination in the cluster and also decreased the probability of exchange in the *unc-101 unc-54* region. Secondly, *mei-352* females are partially sterile and this phenotype cannot be explained by nondisjunction of the autosomes as a result of reduced recombination, since mutants have normal levels of exchange (BAKER and CARPENTER 1972). Paradoxically, *mei-352* mutants exhibit increased nondisjunction and chromosome loss which occurs at a frequency too low to explain the sterile phenotype (BAKER and CARPENTER 1972). Recent evidence suggests that the *mei-352* gene product is actually involved in the sex-determination pathway and that its effect on meiosis is indirect (K. McKIM *pers. comm.*); the mutation may disrupt sex-determination genes which regulate meiosis-specific genes. These observations suggest that while *mei-352* and *rec-1* clearly disrupt a meiotic process necessary for establishing spatial limits on the distribution of exchange, *rec-1* specifically disrupts this process and as a result, remains the lone representative of an unusual class of meiotic mutants.

Chapter 3: The Meiotic Behaviour of an Inversion

INTRODUCTION

Chromosome rearrangements that result in crossover suppression are useful for a wide range of genetic experiments, including the dissection of chromosomal features responsible for meiotic behaviour. An understanding of the mechanisms responsible for the elimination of meiotic events in the presence of the rearrangement can lead to the discovery and description of sites necessary for the recognition and synapsis of homologues, meiotic exchange, and subsequent disjunction. For example, studies of translocations in *C. elegans* have led to the proposal that each chromosome contains a single region necessary for homologue recognition and pairing (ROSENBLUTH and BAILLIE 1981; McKIM, HOWELL and ROSE 1988; reviewed by ROSE and McKIM 1992). Translocations are the major class of dominant crossover suppressor in *C. elegans* (HERMAN 1978; ROSENBLUTH and BAILLIE 1981; HERMAN, KARI and HARTMAN 1982; FERGUSON and HORVITZ 1985; CLARK *et al.* 1988; McKIM, HOWELL and ROSE 1988), although intrachromosomal crossover suppressors have also been described (HERMAN 1978; ANDERSON and BRENNER 1984; ROSENBLUTH, JOHNSEN and BAILLIE 1990). For example, deletions of the chromosome *V* end that does not contain the region necessary for homologue recognition were found to suppress recombination for several map units beyond the breakpoint of the deletion (ROSENBLUTH, JOHNSEN and BAILLIE 1990). The authors proposed that the deletions eliminated sites required for meiotic synapsis which occurs after homologue recognition has taken place. Insertional duplications have a polar effect on recombination (HERMAN, ALBERTSON and BRENNER 1976; McKIM 1990; reviewed by ROSE and McKIM 1992). The intrachromosomal suppressor *mnC1(II)* has been used to balance a large region of chromosome *II* (HERMAN 1978) and although *mnC1(II)* is widely believed to be an inversion, no reversal of gene order has been demonstrated.

In this thesis, the first genetic inversion in *C. elegans* is described. Since this inversion, *hIn1(I)*, suppresses crossing over in a region not previously balanced by translocations, it is representative of a new class of balancers for the genome. Furthermore, the meiotic behavior of *hIn1(I)* with respect to homologue recognition and the centromeric behavior of chromosome *I* has been characterized.

Chapter 3: MATERIALS AND METHODS

The source of mutations and chromosomal rearrangements is given in Chapter 1: MATERIALS and METHODS.

Isolation of *hIn1(I)*: N2 males were treated with 1500 rads of *gamma* radiation (ROSENBLUTH, CUDDEFORD and BAILLIE 1985) and mated to *unc-101(m1) unc-54(e190)* homozygotes. *unc-101 unc-54/ + +* hermaphrodites resulting from this mating were individually plated and their progeny screened for the absence of Unc-101 recombinants. In total, 900 chromosomes were screened and one isolate recovered that suppressed crossing over.

Egg-hatching frequency: Hermaphrodites of the genotype *unc-101 lev-11/hIn1(I)[+ +], hIn1(I)/hIn1(I), hDp131/unc-101 lev-11* or *hDf11/unc-101 unc-54* were individually plated and allowed to lay eggs for two 10-12 hour periods. The hermaphrodites were then transferred and the eggs remaining on the plate counted. All resulting progeny were counted three days later.

Induction of genetic markers on *hIn1(I)*: *hIn1(I)* homozygous males were treated with 25 mM EMS (ROSENBLUTH, CUDDEFORD and BAILLIE 1983) using the procedure described by BRENNER (1974). The mutagenized males were then mated to *unc-75(e950) unc-101(m1)* homozygotes for 24 hours. These hermaphrodites were individually plated and their progeny screened for the presence of Unc-75 individuals. Since the *unc-75 unc-101* interval is located in the crossover suppressed region of *hIn1(I)* heterozygotes, any Unc-75 individuals recovered were expected to be the result of an induction of a new mutation on the *hIn1(I)* chromosome. 18,700 chromosomes were screened and two mutations were recovered; *unc-75(h1041)*, and *unc-75(h1042)*. Both new *unc-75* alleles were lethal as homozygotes and were maintained as heterozygotes. Both new alleles produced the Unc-75 visible phenotype when crossed to males heterozygous for *unc-75(e950)*. To induce an *unc-54* mutation on the *hIn1(I)* chromosome, *hIn1(I)* males mutagenized in the procedure described above were mated to *lev-11 let-49 + + / + + unc-*

54 let-50 (RW3072) hermaphrodites for 24 hours. These hermaphrodites were then individually plated, and their progeny screened for Unc-54 individuals. A total of 5200 chromosomes were screened, of which one-half are heterozygous with the *unc-54 let-50* chromosome. Some Unc-54 isolates could arise from recombination between *unc-54* and *let-50* in the parental strain. To test if the new *unc-54* mutations were linked to *hIn1(I)*, the progeny of putative *hIn1(I)[+ + unc-54]/unc-101 lev-11 +* hermaphrodites were screened for the presence of Unc-101 and Lev-11 recombinants. One strain, KR2151, exhibited complete recombination suppression in heterozygotes indicating the new mutation, *unc-54(h1040)*, was linked to *hIn1(I)*.

Recombination in *hIn1(I)* homozygotes: Recombination was examined in *hIn1(I)* homozygotes in three intervals; *dpy-5 unc-75*, *dpy-5 unc-29*, and *dpy-5 unc-54*. To examine crossing over in *dpy-5 unc-75*, Dpy-5 recombinants were picked from amongst the progeny of *hIn1(I)[+ unc-75 +]/hIn1(I)[dpy-5 + unc-54]* hermaphrodites and mated to *unc-75(e950)/+* males to confirm the presence of *unc-75(h1041 or h1042)*. Unc-75 progeny resulting from this cross were then mated to *dpy-5 unc-75(e950)/+ +* males to ensure the *dpy-5* mutation was still present. The resulting *hIn1(I)[dpy-5 unc-75]/dpy-5 unc-75(e950)* progeny were crossed to *hIn1(I)[unc-54]/ +* males and a fraction of the wild-type individuals resulting from this cross were of the desired genotype *hIn1(I)[dpy-5 + unc-75]/hIn1(I)[+ unc-54 +]*. This experiment also confirmed the gene order in *hIn1(I)* was *dpy-5 unc-54 unc-75* (see Chapter 3: RESULTS). Knowing the map distance between *dpy-5* and *unc-54* in *hIn1(I)* homozygotes (see Table 13), recombination was measured in the *unc-54 unc-75* interval in the same heterozygotes used in the three-factor experiment using the formula:

$$p = 9 - \frac{[81 - 20(2\mathbf{D} + \mathbf{W})(9\mathbf{D} - \mathbf{W})/(\mathbf{D} + \mathbf{W})^2]^{1/2}}{10(2\mathbf{D} + \mathbf{W})/(\mathbf{D} + \mathbf{W})}$$

where **D** is the number of Dpy-5 recombinants and **W** is the number of wild-type progeny. Since *dpy-5* and *unc-29* are outside the boundary of *hIn1(I)* crossover suppression, the mutations can be crossed onto the inversion chromosome. Since *hIn1(I)* is viable as a homozygote, recombination was measured in *hIn1(I)[+ +]/hIn1(I)[dpy-5 unc-29]* and *hIn1(I)[+ +]/hIn1(I)[dpy-5 unc-54]* heterozygotes using the general mapping methods described in Chapter 1: MATERIALS and METHODS.

Analysis of recombinants from *hIn1(I)* heterozygotes: Four rare recombinants falling into two classes, duplications and deficiencies, were recovered from *hIn1(I)* heterozygotes. The deficiencies *hDf11* and *hDf12* were recovered from *hIn1(I)[+ +]/unc-101 unc-54* and *hIn1(I)[+ +]/unc-75 unc-101* heterozygotes respectively. The duplications *hDp131* and *hDp132* were both recovered from *hIn1(I)[+ +]/unc-101 lev-11* heterozygotes, based on the Lev-11 visible phenotype. These duplications were mapped with respect to visible markers. For example, markers inside the region of *hIn1(I)* crossover suppression were tested by mating males of the genotype *unc-75 unc-101/hIn1(I)[+ +]* to *hDp(I)/unc-101 lev-11* hermaphrodites. A fraction of the wild-type progeny from the cross were of the desired genotype *hDp(I)/unc-75 unc-101 + / + unc-101 lev-11.* Upon examining the progeny of such individuals, Unc-75 individuals were observed if the duplication did not carry *unc-75(+).* In the event the duplication did carry *unc-75(+)*, no Unc-75 individuals were observed. A similar procedure was followed for markers outside the region of crossover suppression with the exception that males heterozygous for a wild-type, rather than a *hIn1(I)* chromosome, were used. To determine if the duplications also carried *unc-54(+)*, *hIn1(I)[+ +]/unc-75 unc-54* males were mated to *hDp(I)/unc-75 unc-101* hermaphrodites. A number of the progeny resulting from this mating were of the genotype *hDp(I)/unc-75 unc-101 +/unc-75 + unc-54.* Since Unc-75 Unc-54 individuals are similar in phenotype to Unc-54 individuals, several wild type progeny from the latter heterozygote were plated and their progeny examined. If the duplication carried *unc-54(+)*, a fraction of these individuals would be of the genotype *hDp(I)/unc-75 unc-54.*

The deficiencies *hDf11* and *hDf12* were complementation tested with several visible markers by mating *hDf11/unc-101 unc-54* and *hDf12/unc-75 unc-101* hermaphrodites to either *unc-x/ +* or *lev-11/ +* males. The F_1 progeny resulting from this mating were screened for both males and hermaphrodites Unc-x or Lev-11 in phenotype, the presence of which indicated the deficiency did not carry either *unc-x(+)* or *lev-11(+)*.

DAPI staining of *hDp132*: To determine if *hDp132* was a free duplication, the meiotic chromosomes of an *hDp132/unc-29 unc-75* hermaphrodite were stained with DAPI as described by MOENS and PEARLMAN (1991). Hermaphrodites were placed in a solution of 0.03% TWEEN and the gonads were removed. The tissue and cells were then fixed in 4% formaldehyde and allowed to dry at room temperature overnight. The slides were then treated with 5-10 ul/ml of DAPI solution (0.1 mg/ml DAPI in PBS) in 1 ml of mounting solution and examined under a fluorescence microscope (330-380 nm, reflector 420 nm, barrier 420 nm).

Interaction of *hIn1(I)* with *szT1(I;X)*: Recombination between the boundary of *hIn1(I)* crossover suppression and the *szT1(I;X)I* breakpoint was measured by scoring the Unc-101 progeny from hermaphrodites of the genotype *hIn(I)[+]; + /szT1(I;X)[unc-101; lon-2]*. To measure crossing over between *szT1(I;X)I* and chromosome *I*, Unc-101 hermaphrodite progeny were scored from *+ + ; + /szT1(I;X)[unc-101; lon-2]* hermaphrodites. In both cases, the crossover frequency *(p)* between the *szT1(I;X)* breakpoint and *unc-101* (or the *hIn1(I)* boundary of crossover suppression) is defined by the following formula:

$$p = 4 - [16 - 60\ \mathbf{U}/(\mathbf{U} + \mathbf{W})]^{1/2}$$

$$6$$

where **U** is the number of Unc-101 recombinants and **W** is the number of wild-type progeny.

Interaction of *hIn1(I)* with *hT2(I;III)*: To examine the interaction of *hIn1(I)* with the translocation *hT2(I;III)*, recombination was measured in *+ + + /hT2(I;III)[bli-4 dpy-5 unc-54]* and *hIn1(I)[+ + +]/hT2(I;III)[bli-4 dpy-5 unc-54]* heterozygotes using the formula:

$$p = 1 - [1 - 20\mathrm{D}(3\mathbf{D} + \mathbf{W})/(4\mathbf{D} + 2\mathbf{W})^2]^{1/2}$$

$$\text{----------}$$

$$(3\mathbf{D} + \mathbf{W})/(2\mathbf{D} + \mathbf{W})$$

where **D** is the number of Dpy-5 recombinants and **W** the number of wild types. The segregation of *hIn1(I)* and *hT2(I;III)I* was examined by scoring the Dpy-5 Unc-29 progeny of a *hIn1(I)[+ + +]/hT2(I;III)[bli-4 dpy-5 unc-29]* hermaphrodite and *+ + + /hT2(I;III)[bli-4 dpy-5 unc-29]* control.

Lethal screen using *hIn1(I)* as a balancer: Hermaphrodites of the genotype *hIn1(I)[unc-54]/unc-101 lev-11 +* were treated with 17 mM EMS (ROSENBLUTH, CUDDEFORD and BAILLIE 1983) using the procedure described by BRENNER (1974). Wild-type F_1 progeny from these hermaphrodites were individually plated and their progeny screened for the absence of Unc-101 Lev-11 individuals.

Chapter 3: RESULTS

Isolation of a crossover suppressor for the right end of LG *I*: *hIn1(I)* was identified in a screen for gamma mutations that suppressed crossing over between *unc-101* and *unc-54*, a 14 m.u. interval located at the right end of LG *I*. This map distance was reduced to 0.04 m.u. in *hIn1(I)[+ +]/unc-101 unc-54* heterozygotes (see Table 12). Since recombination in this interval was measured using the Unc-101 recombinant class (Unc-54 recombinants are indistinguishable from the double mutant), the possibility that *hIn1(I)* was a suppressor of the Unc-101 phenotype remained. For this reason, crossing over was examined in *unc-101 lev-11* heterozygotes from which both recombinant classes were recovered. This interval was 9.0 m.u. in *unc-101 lev-ll/ + +* heterozygotes and 0.07 m.u. in *hIn1(I)[+ +]/unc-101 lev-11* heterozygotes, demonstrating extensive crossover suppression of the right arm of LG *I* in *hIn1(I)* heterozygotes. Individuals homozygous for *hIn1(I)* were fertile and wild type in appearance. Since most crossover suppressors identified in *C. elegans* are tranlocations, the segregation of *hIn1(I)* from a normal homologue marked with an *unc-101* mutation was examined. The predicted segregation pattern of wild-type and Unc progeny for a translocation heterozygote is 5:1 (HERMAN 1978; ROSENBLUTH and BAILLIE 1981). *hIn1(I)* heterozygotes segregated wild-type and Unc progeny in a 3:1 ratio (2060 wild types: 672 Unc-101 individuals); a segregation pattern characteristic of an intrachromosomal rearrangement.

***hIn1(I)* heterozygotes have wild-type zygote viability:** To further confirm that *hIn1(I)* was an intrachromosomal rearrangement, the egg-hatching frequency of individuals homozygous and heterozygous for the mutation was determined and is shown in Table 13. The egg-hatching frequencies for heterozygotes is not statistically different than for homozygotes, both of which are high, suggesting that few or no aneuploid gametes are being produced in the former. The egg-hatching frequencies of two recombinants derived from *hIn1(I)* heterozygotes is also shown for comparison. The egg-hatching frequency of

Table 12

Effects of *hIn1(I)* on crossing over on Linkage Group *I*

Genotype	Wild types	Recombinants	pX100(C.I.)[a]
unc-101 unc-54/ + + [b]	1187	116 Unc-101	14.4(11.8-17.1)
unc-101 unc-54/hIn1	1584	1 Unc-101	0.04(0.002-0.25)
unc-101 lev-11/ + +	1492	99 Unc 82 Lev	9.0(7.8-10.3)
unc-101 lev-11/hIn1	2062	1 Lev	0.07(0.004-0.39)
unc-75 unc-101/ + + [b]	3192	68 Unc-101	3.2(2.7-3.8)
unc-75 unc-101/hIn1	2211	1 Unc-75	0.07(0.003-0.30)
dpy-5 unc-101/ + +	889	79 Dpy 66 Unc	12.0(10.1-14.0)
dpy-5 unc-101/hIn1	1975	165 Dpy 148 Unc	11.7(11.2-12.2)
unc-29 lin-11/ + + ; him-5/+	1514	14 Unc-29	1.4(0.8-2.3)
unc-29 lin-11/hIn1; him-5/+	1381	44 Unc-29	4.7(3.4-6.3)
dpy-5 unc-29 unc-75/ + + +[b]	1598	34 Dpy[c]	
		36 Unc-29 Unc-75[c]	3.4(2.6-4.2)
		2 Unc-29[c,d]	
		63 Unc-75[d]	6.0(4.7-7.6)
dpy-5 unc-29 unc-75/hIn1	1669	68 Dpy[c]	
		52 Unc-29 Unc-75[c]	5.5(4.6-6.5)
		4 Unc-29[c,d]	
		81 Unc-75[d]	7.5(5.9-9.5)
unc-11 dpy-5/ + + [b]	3786	58 Dpy 61 Unc	2.3(2.0-2.8)
unc-11 dpy-5/hIn1	1345	46 Dpy 41 Unc	4.8(3.9-5.9)
bli-3 unc-11/ + +[b]	1686	170 Unc	14.8(12.4-17.4)
bli-3 unc-11/hIn1	1232	191 Unc	22.7(19.4-26.0)

[a] C.I. = 95% confidence interval (see Chapter 1: MATERIALS and METHODS).

[b] Data from Table 4.

[c] *dpy-5 unc-29* interval.

[d] *unc-29 unc-75* interval.

Table 13

Egg-hatching frequencies of *hIn1(I)* heterozygotes and recombinants

Genotype	Egg-hatching Frequency
hIn1/hIn1	0.985(465/472)
unc-101 lev-11/hIn1[+ +]	0.983(567/577)
unc-101 unc-54/hDf11	0.356(73/205)
unc-101 lev-11/hDp131	0.982(567/577)

hDf11/unc-101 unc-54 and *hDp131/unc-101 lev-11* heterozygotes was 36% and 98% respectively.

Gene order is inverted in *hIn1(I)*: STURTEVANT (1921) established that three loci in *D. melanogaster* and *D. simulans* were not in the same sequence on the genetic maps of the two species, thus defining the first inversion. To determine if *hIn1(I)* was an inversion, the order of genes was examined by the induction of three mutations on the rearranged chromosome: *unc-54(h1040)*, *unc-75(h1041)*, and *unc-75(h1042)*. The *unc-75* mutations were recessive lethals that produced an Unc-75 phenotype when heterozygous with *unc-75(e950)*, and both were used in the following experiments. Dpy-5 and Unc-54 recombinant progeny from a *hIn1(I)[dpy-5 unc-54 +]/hIn1(I)[+ + unc-75]* hermaphrodite were individually mated to *unc-75(e950)/ +* males to determine if the recombinant chromosome carried one of the lethal *unc-75* mutations. The normal order of these genes is *dpy-5 unc-75 unc-54* (EDGLEY and RIDDLE 1990). If the order of *unc-75* and *unc-54* were reversed, all Dpy-5 recombinants should fail to complement *unc-75(e950)*, whereas the Unc-54 recombinants should complement *unc-75*. Of 17 Dpy progeny examined with *h1042* and 10 with *h1041*, all 27 failed to complement *unc-75*. Of 12 Unc-54 progeny examined with *h1042* and 6 with *h1041*, all 18 complemented *unc-75*. This demonstrated that either the gene order in *hIn1(I)* is *dpy-5 unc-54 unc-75*, or that the order is unchanged but *unc-54* is now tightly linked to *unc-75*. To distinguish between these two possibilities, Dpy-5 progeny from a *hIn1(I)[dpy-5 + unc-75(h1042)]/hIn1(I)[+ unc-54 +]* hermaphrodite were individually plated and their progeny examined for Dpy-5 Unc-54 segregants. If the gene order in *hIn1(I)* were *dpy-5 unc-54 unc-75*, only some of the Dpy-5 recombinants were expected to segregate Dpy Uncs. Of 243 Dpy progeny examined, 120 segregated the double mutant and 123 did not, indicating that gene order of *unc-75* and *unc-54* is reversed in *hIn1(I)* with respect to wild type and establishing that *hIn1(I)* is an inversion.

Recombination frequency in *hIn1(I)* homozygotes is normal: Inversion homozygotes do not experience the pairing problems inherent in heterozygotes. To determine if crossing over occurred in *hIn1(I)* homozygotes, and at what frequency,

recombination was measured in three intervals: one interval outside the boundary of crossover suppression in heterozygotes and two spanning the boundary. The results are shown in Table 14. The *dpy-5 unc-75(h1042)* distance in *hIn1(I)* homozygotes was obtained from the same experiment as the gene order. The *dpy-5 unc-54* and *dpy-5 unc-75* distances in *hIn1(I)* homozygotes were 9.8 and 18.8 respectively. The *dpy-5 unc-75* distance is probably an underestimate since it was measured in *trans* and relied upon the recovery of a less viable double homozygote class. The map distances between *dpy-5* and *unc-54* and *dpy-5* and *unc-75* in controls were 26.4 and 9.4 (data from Table 12) respectively. This confirms the gene order indicated by the three-factor experiment and suggests that recombination frequency in homozygotes is wild type. Recombination was also measured in the *dpy-5 unc-29* interval, a region located outside the inversion in the LG *I* cluster. The map distance in this interval in homozygotes was found to be 3.7 m.u., not significantly different than observed in wild types (data in Table 12).

***hIn1(I)* crossover suppression is associated with recombination enhancement on LG *I*:** To determine the extent of *hIn1(I)* mediated crossover suppression, intervals to the left of *unc-101* were examined and the results are shown in Figure 6 (data shown in Table 12). The *unc-75 unc-101* interval, normally 3.2 m.u., was reduced to 0.07 m.u. in *hIn1(I)* heterozygotes. The *dpy-5 unc-101* interval, however, was not significantly different in *hIn1(I)* heterozygotes when compared to the control (11.7 and 12.0 m.u. respectively), thereby raising two possibilities; recombination to the left of *unc-75* was normal or the interval contained a region of recombination enhancement with an associated region of recombination suppression. To distinguish between these alternatives, recombination was examined in *dpy-5 unc-29 unc-75* heterozygotes. Recombination in the *dpy-5 unc-75* interval was 13.0 m.u. in *hIn1(I)* heterozygotes and 9.4 m.u. in the control. Crossing over in the *unc-29 unc-75* region was not significantly affected by the presence of *hIn1(I)*, whereas the *dpy-5 unc-29* interval showed a 1.6-fold increase in recombination in *hIn1(I)[+ +]/dpy-5 unc-29* heterozygotes. To further map the boundary of crossover suppression, recombination was measured between *unc-29* and *lin-11*. This interval was 4.7 m.u. in heterozygotes and

Table 14

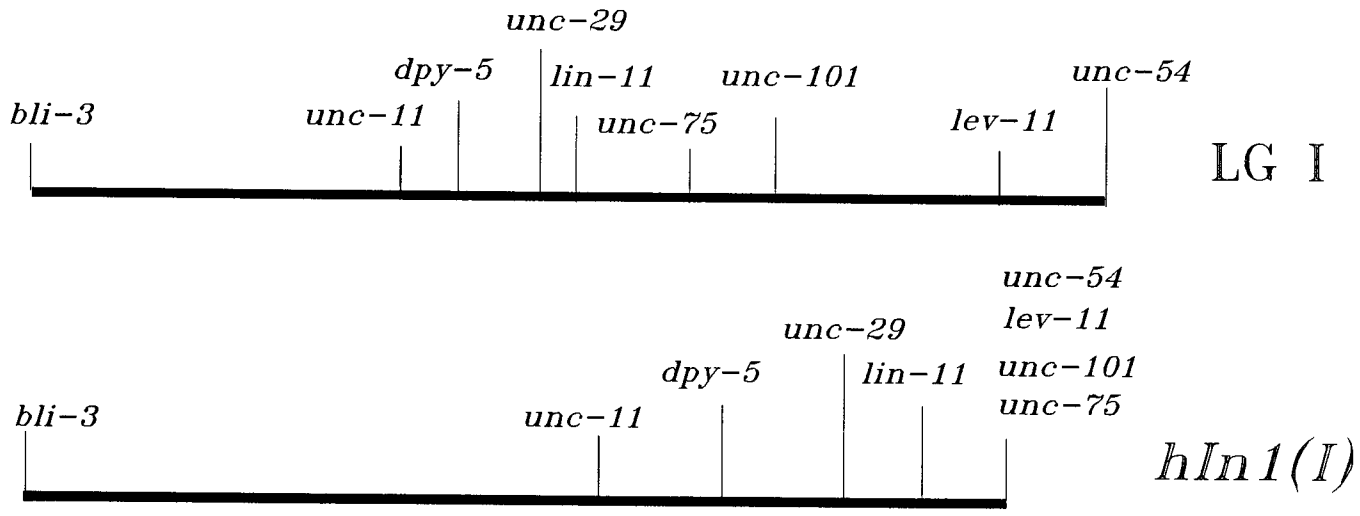
Recombination in *hIn1(I)* homozygotes

Genotype	Wild types	Recombinants	pX100(C.I.)[a]
hIn1[dpy-5 unc-29]/hIn1[+ +]	1500	35 Dpy 46 Unc	3.7(2.9-4.6)
dpy-5 unc-54/hIn1[+ +]	1464	151 Dpy	15.2(12.7-17.7)
hIn1[dpy-5 unc-54]/ + +	1285	136 Dpy	15.6(13.2-18.2)
hIn1[dpy-5 unc-54]/hIn1[+ +]	1312	87 Dpy	9.8(7.9-11.9)
hIn1[dpy-5 + unc-75(h1042)]/hIn1[+ unc-54 +]	1135	243 Dpy[b]	9.0(6.6-11.4)

[a] C.I. = 95% confidence interval (see Chapter 1: MATERIALS and METHODS).

[b] *unc-54 unc-75* distance measured in *trans* (see Chapter 3: MATERIALS and METHODS).

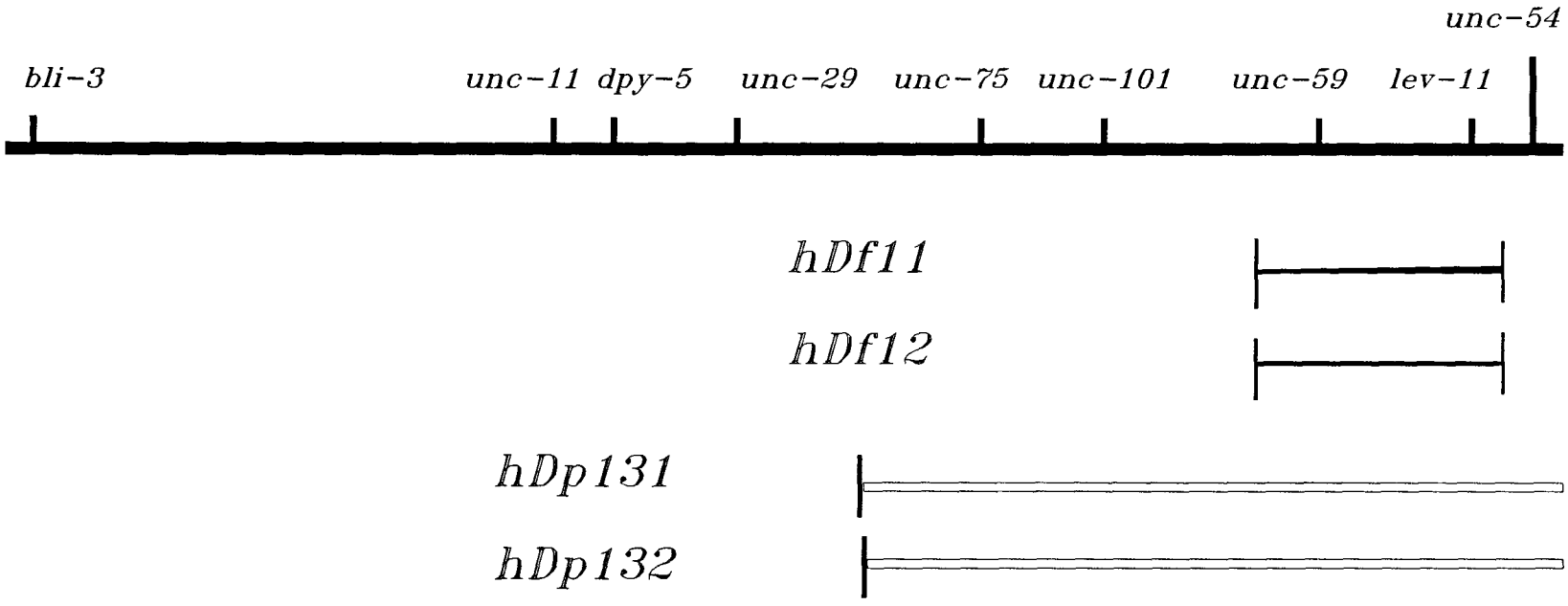
FIGURE 6.-Meiotic maps of LG *I* in *hIn1(I)* heterozygotes and controls. The LG *I* cluster extends from *unc-11* to *unc-29* (EDGLEY and RIDDLE 1990).


unc-29
dpy-5
lin-11
unc-101
unc-54
bli-3
unc-11
unc-75
lev-11
LG I
unc-54
lev-11
unc-29
dpy-5
lin-11
unc-101
unc-75
bli-3
unc-11
hIn1(I)
2 m.u.

1.4 m.u. in controls (3-fold enhancement), thus localizing the *hIn1(I)* boundary of crossover suppression between *lin-11* and *unc-75*. To determine if the recombination enhancement observed in the *dpy-5 unc-29* interval extended to the left arm of LG *I*, recombination was examined in the *bli-3 unc-11* and *unc-11 dpy-5* regions. In *hIn1(I)* heterozygotes, a 1.5-fold increase in recombination was observed in the *bli-3 unc-11*, and a 2-fold increase was observed in the *unc-11 dpy-5* interval when compared to controls. The total genetic length of chromosome *I* was 44 m.u. in controls and 41 m.u. in *hIn1(I)* heterozygotes (see Figure 6).

Rare recombinants from *hIn1(I)* heterozygotes contain duplications and deficiencies: Single crossovers within a classical inversion heterozygote produce chromosomes that contain duplications and deficiencies. Four rare (~ 1/2500) recombinants were recovered from *hIn1(I)* heterozygotes. Three of these originated from mapping experiments (see Table 12), while the fourth was isolated independently from a *hIn1(I)[+ +]/unc-101 lev-11* hermaphrodite on the basis of its visible Lev-11 phenotype. To determine if the individuals homozygous for the chromosome of interest were viable, all four recombinants were crossed to N2 males. The progeny of wild-type hermaphrodites resulting from this cross were screened for the presence of individuals with the original recombinant phenotype. Two of the four recombinants proved to be homozygous lethal, and both failed to complement *unc-59* and *lev-11* establishing them as deficiencies, later designated as *hDf11* and *hDf12*. *hDf11* was known to complement *unc-54* because of the original phenotype of the recombinant (Unc-101 when heterozygous with an *unc-101 unc-54* chromosome). *hDf11* complemented *unc-75*, indicating the left deficiency breakpoint is to the right of this gene. PCR analysis of the left breakpoint of *hDf11* indicates the deficiency does not include *unc-101*, suggesting the deficiency bearing chromosome carries the original *unc-101* mutation (J.-Y. HO *unpublished results*). *hDf12* also complemented *unc-54* and was known to complement *unc-101* based on the original recombinant phenotype (Unc-75 when heterozygous with an *unc-75 unc-101* chromosome). Thus the left breakpoint of *hDf12* is to the right of *unc-101*. The extent of these deficiencies is shown diagramatically in Figure 7. The remaining two recombinants, *hDp131* and *hDp132*, were both Lev-11 in phenotype

FIGURE 7.-Position of breakpoints of recombinant chromosomes derived from *hIn1(I)* heterozygotes. *hDf11* complements *unc-75* and *unc-101*. The right breakpoints of *hDp131* and *hDp132* are not known but both duplications cover *unc-54*.


unc-54
bli-3
unc-11
dpy-5
unc-29
unc-75
unc-101
unc-59
lev-11
hDf11
hDf12
hDp131
hDp132

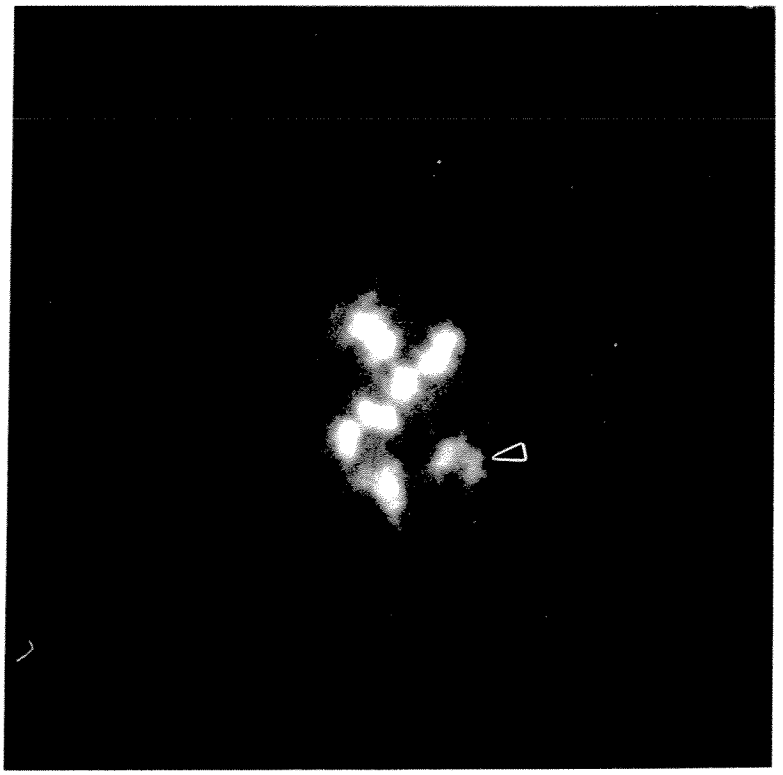
when heterozygous with an *unc-101 lev-11* chromosome. When crossed to N2 males, however, all resulting wild-type progeny segregated Unc-101 Lev-11 individuals, suggesting the recombinants were diploid for the *unc-101 lev-11* chromosome and carried a duplication of *unc-101*. These two duplications were mapped to visible markers and segregated from chromosome *I* as though unlinked. The meiotic chromosomes of an *hDp132/unc-29 unc-75* hermaphrodite were stained with DAPI to determine if the duplication was unlinked. Figure 8 shows a cell carrying a seventh chromosome, indicating that *hDp132* is a free duplication. Both *hDp131* and *hDp132* have breakpoints between *unc-29* and *unc-75*, and carry *unc-75(+)*, *unc-101(+)*, *unc-59(+)*, and *unc-54(+)*. That the duplications are Lev-11 in phenotype when heterozygous with *unc-101 lev-11* chromosomes suggests that they are linked to the original *lev-11* mutation. The extent of the duplications and their known breakpoints is shown in Figure 7.

***hIn1(I)* has no effect on crossing over on other chromosomes:** In *D. melanogaster*, inversion heterozygosity produces interchromosomal effects; an increase in crossing over in regions surrounding the centric heterochromatin and the distal tips of chromosome arms on the other pairs of chromosomes (SCHULTZ and REDFIELD 1951; RAMEL 1962; reviewed by LUCCHESI 1976). To determine if *hIn1(I)* produces a similar effect in *C. elegans*, recombination was measured on other chromosomes in *hIn1(I)* heterozygotes. The results are shown in Table 15. Two regions located on autosomes and one located on the *X* chromosome were examined. In all three cases, the presence of *hIn1(I)* did not significantly affect recombination in heterozygotes.

***hIn1(I)* recombines with *szT1(I;X)*:** The meiotic behaviour of the translocation *szT1(I;X)* has been extensively characterized (FODOR and DEAK 1985; McKIM, HOWELL and ROSE 1988). The breakpoint of the translocation on LG *I* is close to the left of *unc-29*, and translocation homozygotes are inviable. The extent of crossover suppression was determined; recombination was suppressed to the left of the breakpoint and enhanced to the right (McKIM, HOWELL and ROSE 1988). Since crossing over is suppressed in the *unc-75 unc-54* interval in *hIn1(I)* heterozygotes, it was of interest to

FIGURE 8.-DAPI staining of chromosomes from the meiotic cells of an *hDp132/unc-29 unc-75* hermaphrodite showing a) an oocyte bearing a seventh chromosome, indicating the duplication is unlinked and b) an oocyte lacking the duplication.

a)



b)

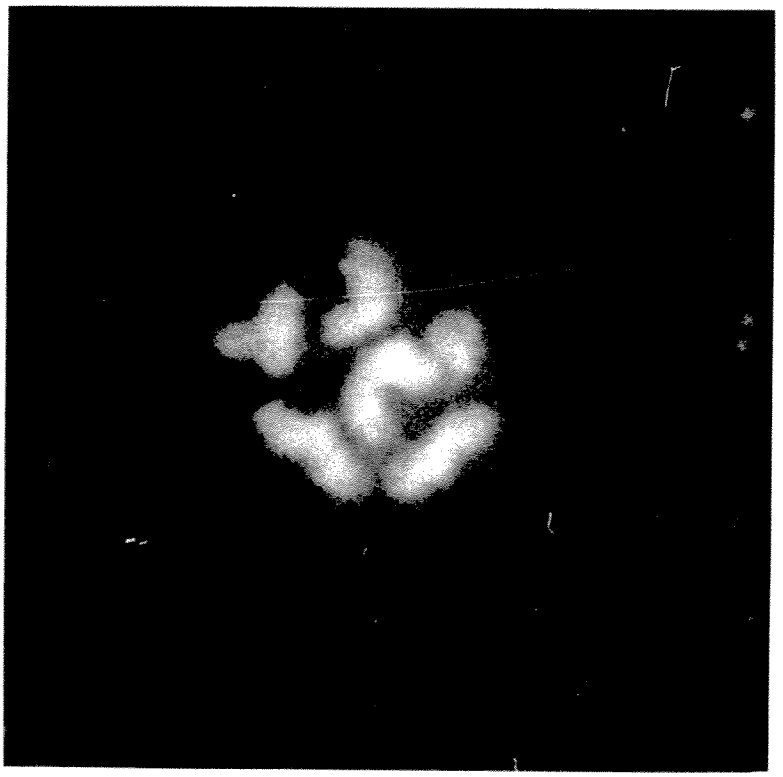


Table 15

Effect of *hIn1(I)* on crossing over on other chromosomes

Genotype	Wild types	Recombinants	pX100(C.I.)[a]
dpy-18 unc-36/ + +	1561	77 Dpy 94 Unc	8.1(6.9-9.3)
dpy-18 unc-36/ + +;hIn1/+	1881	98 Dpy 96 Unc	7.6(6.6-8.7)
unc-1 dpy-3/ + +	1373	23 Dpy 27 Unc	2.7(2.1-3.5)
unc-1 dpy-3/ + +;hIn1/+	2147	32 Dpy 36 Unc	2.4(1.8-2.9)
unc-42 dpy-11/ + +	1357	22 Dpy 20 Unc	2.3(1.8-3.2)
unc-42 dpy-11/ + +;hIn1/+	1324	19 Dpy 14 Unc	1.9(1.3-2.6)

[a] C.I. = 95% confidence interval (see Chapter 1: MATERIALS and METHODS).

determine if pairing was possible between the two rearrangements. Crossing over between the *szT1(I;X)* breakpoint and the *hIn1(I)* boundary of crossover suppression was measured in hermaphrodites of the genotype *hIn1(I)[+]; + /szT1(I;X)[unc-101;lon-2].* The map distance between the breakpoint of *szT1(I;X)* and the boundary of *hIn1(I)* crossover suppression (between *lin-11* and *unc-75*) was 45 m.u. The recombination frequency between the *szT1* breakpoint and *unc-101* was measured in *+ ; + /szT1(I;X)[unc-101;lon-2]* controls and was 25 m.u., approximately 2-fold lower. The data for these experiments are shown in Table 16 and the Punnett square diagramming recombination between the two rearrangements is shown in Figure 9.

***hIn1(I)/hT2(I;III)* heterozygotes suppress crossing over on LG *I*:** The translocation *hT2(I;III)* is comprised of two chromosomes; *hT2(I;III)I* segregates from chromosome *I* and, *hT2(I;III)III* segregates from chromosome *III*. In heterozygotes, recombination on LG *I* is suppressed to the left of *unc-101* and enhanced to the right of this marker. Since *hIn1(I)* suppresses recombination from *unc-75* to *unc-54*, it was of interest to determine whether recombination could be completely suppressed on LG *I*. Crossing over between *dpy-5* and *unc-54* was measured in *hIn1(I)[+ + +]/hT2(I;III)[dpy-5 bli-4 unc-54]* heterozygotes and *+ + + /hT2(I;III)[dpy-5 bli-4 unc-54]* controls (results shown in Table 16). The map distance of chromosome *I* was reduced to 0.8 m.u. in *hIn1(I)* heterozygotes, compared to 32.1 m.u. in controls, thus demonstrating recombination could be effectively suppressed along the entire length of the chromosome.

***hIn1(I)* and *hT2(I;III)I* segregate randomly:** While examining recombination in *hIn1(I)[+ + +]/hT2(I;III)[dpy-5 bli-4 unc-54]* heterozygotes, an unusually small number of Dpy-5 Unc-54 progeny, representing the viable translocation homozygote, were recovered. To investigate the possibility that *hIn1(I)* and *hT2(I;III)I* were segregating abnormally, segregation was examined in *hIn1(I)[+ + +]/hT2(I;III)[dpy-5 bli-4 unc-29]* heterozygotes and *+ + + /hT2(I;III)[dpy-5 bli-4 unc-29]* controls. Since both *dpy-5* and *unc-29* map in the crossover-suppressed arm of *hT2(I;III)III*, the recovery of the double mutant, representing the viable translocation homozygote class, is dependent upon the proper

Table 16

Effect of *hIn1(I)* on crossing over with LG *I* translocations

Genotype	Wild types	Recombinants	pX100(C.I.)[a]
+ ; + /szT1(I;X)[unc-101;lon-2]	1110	200 Unc	25.0(24.0-26.0)
hIn1(I)[+]; + /szT1(I;X)[unc-101;lon-2]	1775	563 Unc	45.5(45.4-48.7)
+ + + /hT2(I;III)[dpy-5 bli-4 unc-54]	441	59 Dpy	32.1(24.1-$\geq$50)
hIn1(I)[+ + +]/hT2(I;III)[dpy-5 bli-4 unc-54]	591	2 Dpy[b]	..0.8(0.15-2.8)

[a] C.I. = 95% confidence interval (see Chapter 1: MATERIALS and METHODS).

[b] Both Dpy individuals were fertile and gave Dpy progeny.

FIGURE 9.-Punnett square diagramming the predicted segregation of a *hIn1(I)[+]; + /szT1(I;X)[unc-101;lon-2]* heterozygote (see RESULTS). Empty boxes represent presumed lethal zygotes resulting from severe aneuploidy. Viable classes are indicated by phenotype and any aneuploidy they may carry. *szDp1* progeny are duplicated for $I^L X^L$ and are viable (McKIM, HOWELL and ROSE 1988).

	$I^{R}X^{R}$ $[u^{101}l^{2}]$; $I^{L}X^{L}$	$I^{R}X^{R}$ $[u^{101}l^{2}]$; X^{N}	I^{N}; $I^{L}X^{L}$	I^{N}; X^{N}	$u^{101}I^{N}$; $I^{L}X^{L}$	$u^{101}I^{N}$; X^{N}	$I^{R}X^{R}$ $[+l^{2}]$; $I^{L}X^{L}$	$I^{R}X^{R}$ $[+l^{2}]$; X^{N}
$I^{R}X^{R}$ $[u^{101}l^{2}]$; $I^{L}X^{L}$				WT		Unc-101		
$I^{R}X^{R}$ $[u^{101}l^{2}]$; X^{N}			WT		Unc-101			
I^{N}; $I^{L}X^{L}$		WT						WT
I^{N}; X^{N}	WT					WT	WT	
$u^{101}I^{N}$; $I^{L}X^{L}$		Unc-101						WT
$u^{101}I^{N}$; X^{N}	Unc-101					Unc-101	WT	
$I^{R}X^{R}$ $[+l^{2}]$; $I^{L}X^{L}$				WT		WT		
$I^{R}X^{R}$ $[+l^{2}]$; X^{N}			WT		WT			

segregation of the translocation from the normal homologues. In the control, the predicted ratio (5:1) of wild types to Dpy-5 Unc-29 progeny was observed (771 Wild types: 164 Dpy Unc). The frequency observed in *hIn1(I)* heterozygotes, however, was 13.7:1 (411 Wild types: 30 Dpy Unc), close to the predicted ratio of 11:1 if *hT2(I;III)I* and *hIn1(I)* were segregating randomly, resulting in aneuploid gametes (shown in Figure 10). Both the recovery of rare recombinants in the previous experiment and the difference between the predicted and observed segregation ratios may be explained by a low frequency of pairing between the two rearrangements.

***hIn1(I)* effectively balances lethal mutations:** One objective in isolating a crossover suppressor for a region associated with the homologue recognition region was the demonstration that such rearrangements, presumably intrachromosomal, would be effective balancers. The efficiency of *hIn1(I)* was tested by screening for recessive lethal mutations in the region of crossover suppression. In total, 1412 mutagenized chromosomes were screened and 54 mutations, including those resulting in adult sterility, were recovered. Strains representing the recovered mutations were effectively balanced in *hIn1(I)* heterozygotes for at least 20 generations (before being frozen) without breakdown of the balancer being observed.

FIGURE 10.-Punnett square diagramming the predicted segregation of a *hIn1(I)[+ + +]/hT2(I;III)[dpy-5 bli-4 unc-29]* heterozygote (see RESULTS). Empty boxes represent presumed lethal zygotes resulting from severe aneuploidy. Viable classes are indicated by phenotype and any aneuploidy they may carry. *hDp134* progeny are duplicated for $I^{R}III^{R}$ and are viable (McKIM, PETERS and ROSE *in press*). Progeny duplicated for $I^{L}III^{L}$ are viable (K. McKIM *pers. comm.*).

	$I^{L}III^{L}$ $[d^{5}u^{29}]$; *hIn1*	$I^{L}III^{L}$ $[d^{5}u^{29}]$; $I^{R}III^{R}$	$I^{L}III^{L}$ $[d^{5}u^{29}]$	$I^{L}III^{L}$ $[d^{5}u^{29}]$; $I^{R}III^{R}$; *hIn1*	III^{N}; *hIn1*	III^{N}; $I^{R}III^{R}$	III^{N}	III^{N}; $I^{R}III^{R}$; *hIn1*
$I^{L}III^{L}$ $[d^{5}u^{29}]$; *hIn1*						WT		
$I^{L}III^{L}$ $[d^{5}u^{29}]$; $I^{R}III^{R}$		Dpy-5 Unc-29			WT			
$I^{L}III^{L}$ $[d^{5}u^{29}]$								WT
$I^{L}III^{L}$ $[d^{5}u^{29}]$; $I^{R}III^{R}$; *hIn1*							WT	
III^{N}; *hIn1*		WT			WT			WT *hDp134*
III^{N}; $I^{R}III^{R}$	WT							
III^{N}				WT				
III^{N}; $I^{R}III^{R}$; *hIn1*			WT		WT *hDp134*			

Chapter 3: DISCUSSION

In this thesis, evidence has been presented for an inversion in *C. elegans*, *hIn1(I)*, that inverts a region of chromosome *I*, including the genes *unc-75* and *unc-54*. The meiotic properties of *hInI(I)* were similar to those observed for inversions in Drosophila, including crossover suppression within the inverted region and intrachromosomal effects. *hIn1(I)* is capable of recombining efficiently with the translocation *szT1(I;X)I*, indicating that the two rearrangements also synapse efficiently, a prerequisite to chaisma formation. For this reason, it has been concluded that *hIn1(I)* and chromosome *I* are capable of homologue recognition and synapsis, but that physical constraints inside the inversion loop limit chiasma formation, resulting in the suppression of crossing over in the region.

That exchange events are rare seems likely for three reasons. Firstly, in Drosophila, crossing over inside *In(1)dl-49*, an inversion located at the end of the *X* chromosome, has been well characterized. NOVITSKI and BRAVER (1954) designed a system to recover the products of single exchanges inside *In(1)dl-49* using a compound chromosome. They observed a 75% reduction in crossing over in heterozygotes despite cytological evidence that this inversion was capable of pairing by forming loops in mitotic cells (PAINTER 1933). This suggests that topological constraints exist that reduce the frequency of chiasmata formation inside such inversions when heterozygous. By analogy, *hIn1(I)*, which is also located at the end of a chromosome and is even smaller than *In(1)dl-49*, should experience constraints in pairing for recombination in heterozygotes. Secondly, compensatory increases in recombination are large in *hIn1(I)* heterozygotes, as would be expected if exchanges within the inversion were rare (*i.e.* in *hIn1(I)* heterozygotes, the map distance from *bli-3* to *lin-11*, normally 22 m.u., approaches 50 m.u.). The fact that *hIn1(I)* heterozygotes efficiently recombine in other regions of the chromosome indicates that the ability of the homologues to recognize one another is intact, and that recombination suppression on the right arm is limited to the inverted segment. Thirdly, reciprocal recombination events were isolated from *hIn1(I)* heterozygotes, suggesting that all meiotic products can be recovered,

but that their frequency is low. Figure 11 shows the possible pairing conformations between the inversion and the normal chromosome. The first shows a pairing loop resulting from homologous pairing of all sequences and the second shows the inversion remaining unpaired. It is not possible to distinguish between these two configurations genetically.

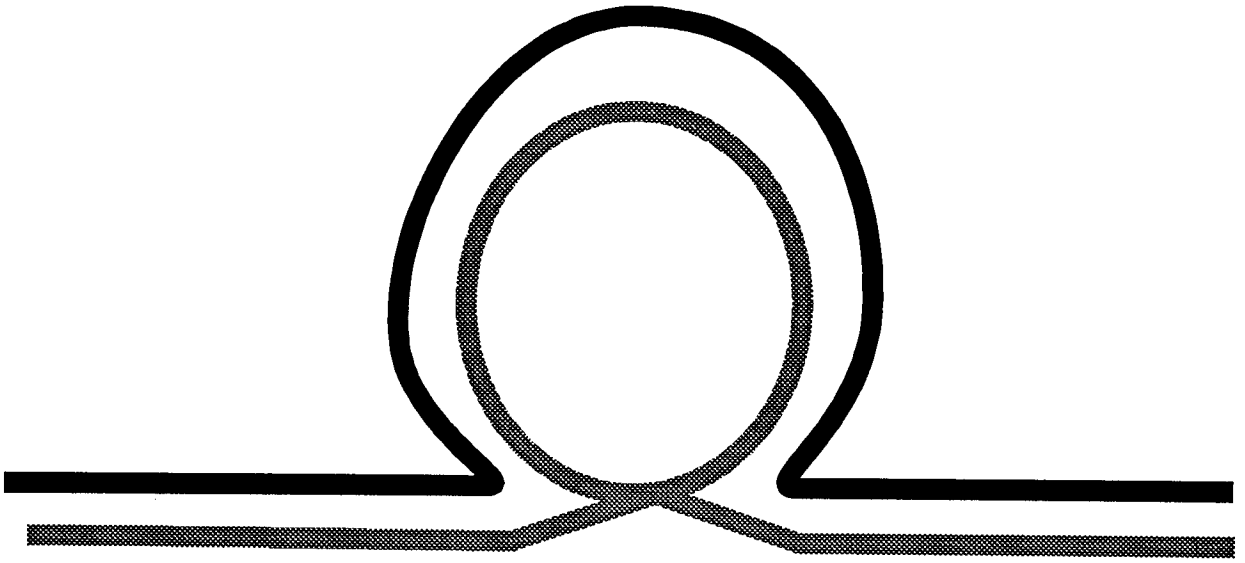
Recombination was examined between *hIn1(I)* and two translocations; *szT1(I;X)* and *hT2(I;III)*. The pairing portion of *szT1(I;X)I* and *hIn1(I)* share sequences not included in either rearrangement, whereas the pairing portion of *hT2(I;III)I* and *hIn1(I)* have no common unrearranged sequences. The crossover frequency between *hIn1(I)* and *szT1(I;X)I* was 0.45, demonstrating that synapsis and recombination were efficiently conducted between the two in spite of the genetically small size of the homologously paired region. In contrast, the frequency of recombination between *hIn1(I)* and *hT2(I;III)I* was less than 0.01. These results agree with the conclusion that exchanges within the inversion are rare, since the only DNA available for pairing is within the inverted segment.

DARLINGTON (1937) suggested that the formation of a chiasma between homologues during meiosis facilitates their proper disjunction (reviewed by HAWLEY 1988). One consequence of the crossover suppression observed in *hIn1(I)/hT2(I;III)* heterozygotes was the random segregation of *hIn1(I)* and *hT2(I;III)I*. This suggests that in *C. elegans*, the formation of a chiasma between two homologues is necessary to ensure their proper disjunction at meiosis I. This interpretation is supported by cytogenetic studies which have documented that at metaphase I the bivalents orient axially and may be held together at the metaphase plate by a terminalized chiasma (ALBERTSON and THOMSON 1993).

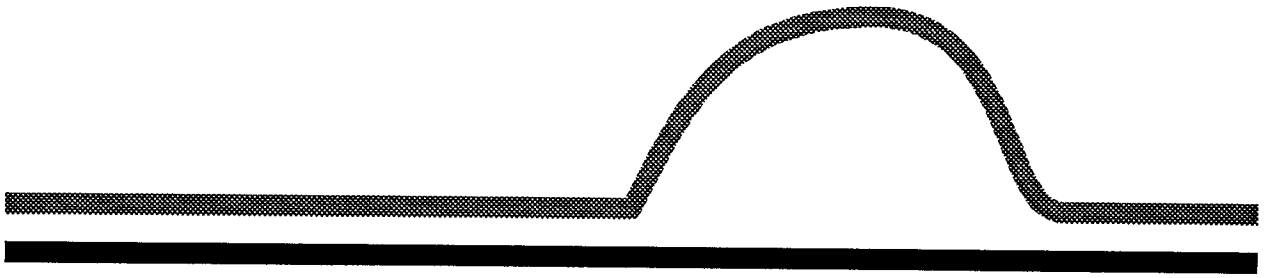
In Drosophila, it has been observed that inversions can effect increases in recombination frequency on the rearranged chromosome and on the other major chromosomes (STURTEVANT 1919; STURTEVANT 1931; DOBZHANSKY 1933; reviewed by LUCHESSI 1976). The analysis presented here showed that the total genetic length of chromosome *I* was 41 m.u. in *hIn1(I)* heterozygotes and 44 m.u. in controls. These values are similar to the recombination frequency reported for the pairing portion of

FIGURE 11.-Possible pairing conformations within a chromosome arm heterozygous for *hIn1(I)*. The *hIn1(I)* chromosome is represented by gray lines and the normal homologue by black. a) Synapsis is shown for both the inversion and the normal homologue, resulting in a conventional pairing loop. b) Synapsis is shown only for the uninverted regions. The inversion does not pair with the normal homologue.

a)



b)



chromosome *I* in individuals heterozygous for four translocations involving chromosome *I*: *hT1(I;V)*, *szT1(I;X)* (McKIM, HOWELL and ROSE 1988), *hT3(I;X)*, and *hT2(I;III)* (McKIM 1990). These results indicated that while compensatory increases can occur on both arms of LG *I*, the amount of exchange is limited to approximately one crossover event per meiosis. Unlike inversions in Drosophila, *hIn1(I)*-mediated recombination enhancement in heterozygotes did not extend to other linkage groups. No increase in crossing over was observed in the three intervals examined in the presence of *hIn1(I)*, regardless of their location on the autosomes (small interval inside the cluster or large interval spanning the cluster) or on the *X* chromosome. Interchromosomal effects have been observed in *C. elegans* with mutations that result in *X*-chromosome nondisjunction (HODGKIN, HORVITZ and BRENNER 1979; HERMAN and KARI 1989). Thus in *C. elegans*, as in *D. melanogaster*, the mechanism that regulates the number of crossovers per meiosis may involve compensatory increases of events on other chromosomes in the event crossing over is suppressed or reduced along an entire chromosome. The failure to observe interchromosomal effects in *hIn1(I)* heterozygotes may have been expected since recombination was not reduced on chromosome *I* as a whole.

Exchange events resulting from an intrachromosomal effect are not distributed randomly along the chromosome. In Drosophila for example, such increases occur in regions sufficiently removed from the inversion breakpoint (GRELL 1962), and near the centric heterochromatin and distal tips of other chromosomes, regions of low intrinsic exchange (SCHULTZ and REDFIELD 1951; RAMEL 1962). Each of the autosomes in *C. elegans* are marked by a region where genes cluster on the meiotic map resulting from a reduction in recombination (BRENNER 1974) per base pair compared to the genomic average (GREENWALD *et al.* 1987; KIM and ROSE 1987; PRASAD and BAILLIE 1989; PRASAD *et al.* 1993). In *hIn1(I)* heterozygotes, recombination frequency was enhanced in intervals both inside (1.5 fold in *dpy-5 unc-29*) and outside (1.5 fold in *bli-3 unc-11*) the chromosome *I* gene cluster. This suggests that the regulatory mechanism responsible for establishing the distribution of crossing over is independent of the mechanism determining

the number of exchanges. The meiotic pattern specific to chromosome *I* is retained; the enhancement observed is not greater in the cluster than it is at the left end. The frequency and distribution of exchange events was found to be normal in *hIn1(I)* inversion homozygotes. This suggested that when the pairing difficulties experienced in heterozygotes were removed in homozygotes, exchange within the inversion maintained the distribution observed in wild types. Chromosomal sites that are necessary for normal levels of meiotic exchange have been mapped in Drosophila (HAWLEY 1980; SZAUTER 1984). A similar mechanism may exist in *C. elegans* since recombination frequency is enhanced in the region adjacent to the *szT1(I;X)* breakpoint on LG *I*, suggesting the break may have disrupted the mechanism responsible for the regional distribution of exchange (McKIM, HOWELL and ROSE 1988). If this mechanism is mediated by chromosomal elements, the level of crossing over in *hIn1(I)* homozygotes suggests that such elements can operate normally in either orientation.

Inversions are classically defined by their exclusion (paracentric) or inclusion (pericentric) of the centromere (MULLER 1938; reviewed by ROBERTS 1976). Cytogenetic analysis of the salivary glands of inversion heterozygotes demonstrated that inverted homologous segments were capable of pairing by forming a loop (PAINTER 1933). A single exchange in a paracentric inversion loop led to the formation of acentric and dicentric fragments. The formation of these structures had been observed cytogenetically during meiosis in *Zea mays* (McCLINTOCK 1933). Single exchanges within paracentric inversions were not observed in Drosophila until single crossover products were recovered from individuals heterozygous for a long paracentric inversion on the *X* using an attached chromosome (SIDOROV *et al.* 1935). These results demonstrated that single crossovers do occur but that single crossover recombinants are not recovered. Nevertheless, information transfer in the form of gene conversion occurred in undiminished frequency in inversion heterozygotes, except near the breakpoints where effective homologous pairing may not be possible (CHOVNICK 1973). Unexpectedly, no concomitant loss of zygote viability was observed in heterozygotes despite the formation of aberrant chromosomes (STURTEVANT

and BEADLE 1936; NOVITSKI 1952). To explain this, STURTEVANT and BEADLE (1939) proposed that chromatids involved in single exchanges were excluded from a functional nucleus, a theory later corroborated by genetic and cytological evidence (STURTEVANT and BEADLE 1939; CARSON 1946; HINTON and LUCHESSI 1960). In contrast, single crossovers in pericentric inversion heterozygotes produced chromosomes with terminal duplications and deficiencies that were segregated into gametes and resulted in reduced fertility (ROBERTS 1967). The frequency at which single exchanges occurred was dependent on the size and location of the inversion; a reduced frequency of such events was observed with both small inversions, and inversions located at the ends of chromosome arms (STURTEVANT and BEADLE 1936; NOVITSKI and BRAVER 1954). Individuals heterozygous for *hIn1(I)* showed no reduction in egg-hatching frequencies, compatible with the behaviour of a paracentric inversion for which the products of single exchanges are either excluded from functional nucleii or for which single exchanges in the inverted segment are rare.

The recovery of a chromosomal rearrangement in *C. elegans* that behaves as a paracentric inversion may seem suprising given that the mitotic chromosomes are holokinetic (ALBERTSON and THOMSON 1982) and evidence for holocentric meiotic chromosomes has been reviewed (HERMAN 1988). Recent cytological studies, however, suggest that the ends of *C. elegans* chromosomes adopt centromeric functions for meiotic disjunction; one end holds the bivalent together and the other probably provides a site for the attachment of microtubules. These roles do not appear to be specific to one end of the chromosome and either end can be the inner or outer end of the bivalent (ALBERTSON and THOMSON 1993). This meiotic behaviour is similar to that observed in other mitotically holokinetic species; the nematode *Parascaris univalens* (GODAY, CIOFI LUZZATTO and PIMPINELLI 1985; PIMPINELLI and GODAY 1989; GODAY and PIMPINELLI 1989), the insects *Euchistus servus* (HUGHES-SCHRADER and SCHRADER 1961) and *Myrmus miriformis* (NOKKALA 1985), where the mitotic chromosomes are holocentric but during

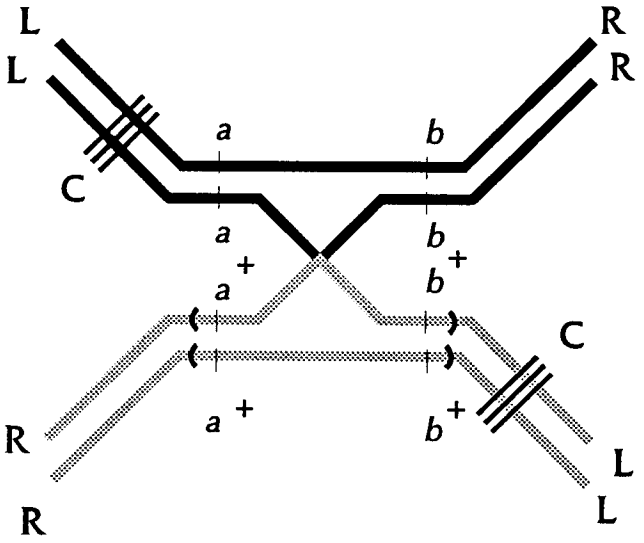
meiosis centromeric activity is restricted to a limited chromosome region, often at chromosome ends.

The results of genetic analyses in *C. elegans* have consistently been compatible with the predicted behaviour of monocentric chromosomes. For example, the segregation ratios of aneuploid and viable progeny observed from translocation heterozyotes were compatible with the presence of a single centromere (HERMAN 1978; ROSENBLUTH and BAILLIE 1981; McKIM, HOWELL and ROSE 1988; this study). In the case of the translocation *eT1(III;V)*, recombination is suppressed to one side of the translocation breakpoint while the other recombines and segregates from the chromosome with which it had paired (ROSENBLUTH and BAILLIE 1981). Thus, in any one meiosis, only one meiotic segregator (centromere) was functional. The data presented in this thesis strongly suggest that the meiotic chromosomes are monocentric, a suggestion compatible with both genetic and cytogenetic observations.

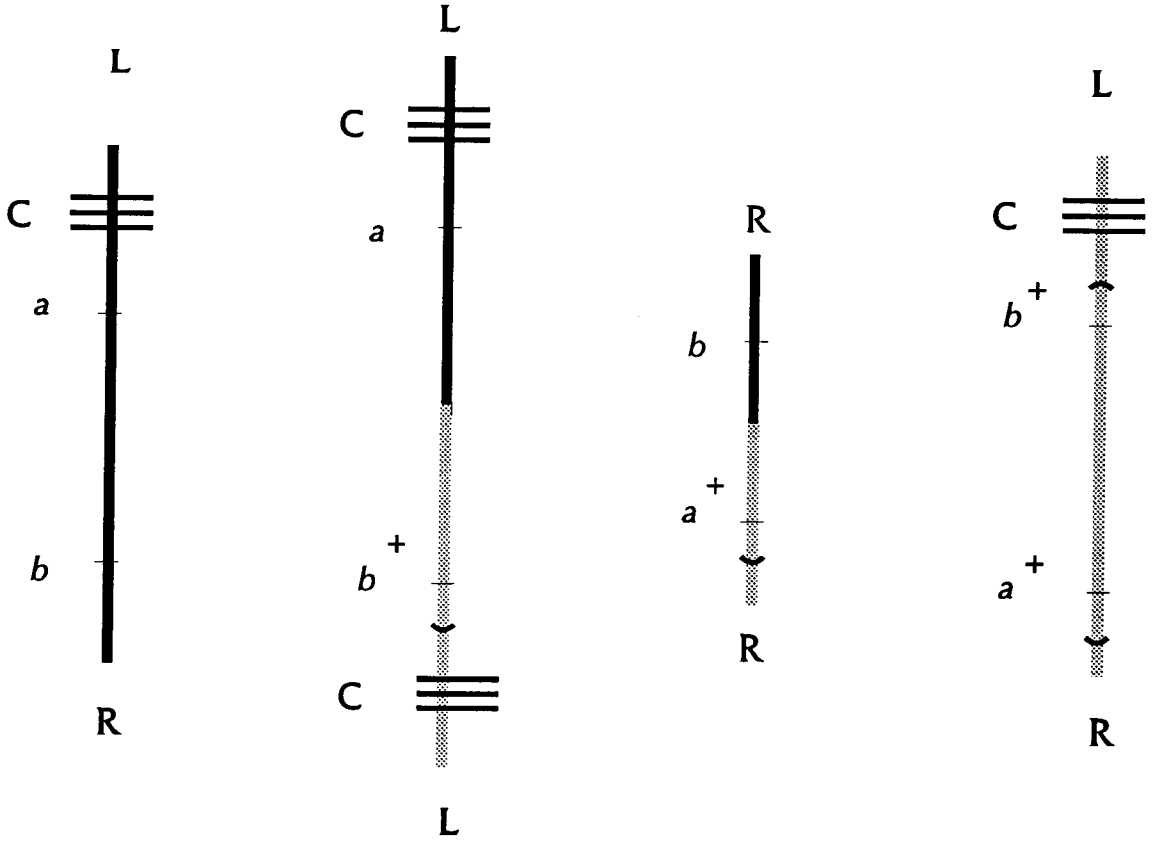
The isolation of four recombinants from *hIn1(I)* heterozygotes raised the possibility that their genotypes would provide information on the location of the meiotic centromere. Two free duplications, *hDp131* and *hDp132* were recovered following a single exchange event inside the inversion loop. The structure of these duplications is consistent with the products formed by the events illustrated in in Figure 12. In this model, *hDp131* and *hDp132* are represented by the acentric fragment that results from a single exchange within a paracentric inversion where the centromere is to the left of *unc-75*. The facts that the duplications were isolated independently, and that the left endpoints were nonrandom and coincided with the boundary of *hIn1(I)* crossover suppression (between *unc-29* and *unc-75*) support this interpretation. In Drosophila, the acentric fragment generated by a single exchange within a paracentric inversion loop is not recovered under ordinary circumstances. In *C. elegans*, however, free duplications are readily recovered (HERMAN, ALBERTSON and BRENNER 1976). According to the model shown in Figure 12, the reciprocal product is a duplication of the sequences to the left of the inversion including the centromeric sequences. This structure is analogous to the dicentric chromosomes generated by single

FIGURE 12.-Effects of single crossing over within a chromosome arm heterozygous for *hIn1(I)*. a) Synapsis is shown only for the inversion. *hIn1(I)* is represented by gray lines (inversion boundaries shown by parentheses) and the normal homologue by black lines. **L** denotes the left end and **R** denotes the right end of LG *I*. **C** represents the gene cluster. **a** and **b** represent markers on the normal chromosome; in the case of *hDp131* and *hDp132*, *unc-101* and *lev-11* respectively. In the case of *hDf11*, **a** = *unc-101* and **b** = *unc-54*. In the case of *hDf12*, **a** = *unc-75* and **b** = *unc-101*. Wild-type alleles of these markers are shown on the inverted chromosome. b) Meiotic products resulting from the exchange event (see Chapter 3: DISCUSSION). The chromosome duplicated for the cluster is proposed to be dicentric and the origin of *hDf11* and *hDf12*. The small chromosome duplicated for the right ends has the same structure as *hDp131* and *hDp132*.

a)



b)



exchanges within paracentric inversions in Drosophila. Although this reciprocal product was not recovered intact in our experiments, *hDf11* and *hDf12* may have resulted from its breakage. Dicentric chromosomes in other organisms have been observed to form chromatid bridges at anaphase I, and as a result are meiotically unstable and subject to chromosome breakage (McCLINTOCK 1933, 1941; CARSON 1946; HABER, THORBURN and ROGERS 1984). The two deficiencies recovered, *hDf11* and *hDf12*, could have resulted from a similar event followed by the broken end of one product being capped by sequences on the right end (including *unc-54(+)*), presumably derived from its normal homologue to which it is still attached. To stabilize broken ends of chromosomes, double-stranded breaks can be repaired by fusing with other chromosomes (McCLINTOCK 1941; 1942) or by recombining with homologous sequences (HABER and THORBURN 1984). KADYK and HARTWELL (1992) have shown that sister chromatids are preferred over homologues as substrates for recombinational repair. Both deficiencies recovered have breakpoints independent of the site of the original exchange event. Thus, the structures of the recombinant chromosomes recovered are compatible with the interpretation that *hIn1(I)* is a paracentric inversion with the centromere to its left. That cytogenetic analysis has demonstrated that both ends of LG *I* can adopt centromeric function (ALBERTSON and THOMSON 1993) is not inconsistent with the genetic data. Firstly, in any one meiosis only one end of the chromosome adopts centromeric function (*i.e.* spindles do not attach to both ends). Secondly, the right end of chromosome *I* was observed to take on centromeric function in the majority of meioses (ALBERTSON and THOMSON 1993), thereby explaining the preferential recovery of recombinant products whose derivation is most easily explained by centromeric activity of the right end of LG *I* rather than the left.

The recombinant products recovered are not consistent with the predicted behaviour of a paracentric inversion with centromeric sequences to the right of the inversion. The recovery of *hDp131* and *hDp132* is not compatible with dicentric products (see below), and no acentric fragment of the predicted structure was recovered. The possibility that *hIn1(I)* is a pericentric inversion cannot formally be ruled out, however, it is unlikely for the

following reasons. No significant reduction in egg-hatching frequency was observed in *hIn1(I)* heterozygotes as would have been expected if the inversion were pericentric, however, it is possible that the frequency of recombination within the inversion was so low that no reduction was observed. Most importantly, however, if *hIn1(I)* included the centromere, *hDp131* and *hDp132* would be centric and would have segregated from their homologue at meiosis II. To recover these duplications, a nondisjunction event would be needed to generate a viable zygote, and the probability of recovering two such rare events is very low. The reciprocal recombinant product would also have possessed one centromere and would have been meiotically stable. Size is unlikely to be a consideration since large, rearranged chromosomes exist in *C. elegans* which are meiotically stable (HERMAN, KARI and HARTMAN 1982; SIGURDSON *et al.* 1986; McKIM 1990; ALBERTSON *unpublished results*).

In conclusion, the meiotic behaviour of a *C. elegans* inversion *hIn1(I)*, that suppresses crossing over in a previously unbalanced region, has been characterized. The simplest interpretation of the data presented is that *hIn1(I)* is a paracentric inversion with the meiotic centromere to its left. *hIn1(I)* was used to successfully balance lethal mutations in a region previously impenetrable to extensive essential gene analysis, demonstrating its value as a new class of balancer for the genome of *C. elegans*.

CONCLUSIONS

The intent of this thesis has been the description of conventional meiotic phenomena in *C. elegans*. Meiosis in *C. elegans* is marked by the classical features that distinguish it from mitosis: pairing, recombination, and segregation of homologous chromosomes. Some of the functional elements responsible for these processes have now been described in the nematode. The term "pairing" has been used to describe several chromosomal behaviours that are now understood to be temporally and functionally distinct. Homologue pairing is the alignment of chromosomes at a distance, perhaps as early as interphase, and is thought to be mediated by discrete sites whose number may be related to chromosome size (MAGUIRE 1984). These homologue pairing sites may be the attachment sites for fibrullar proteins that anchor the two homologues together during interphase and early prophase. In *C. elegans*, the characterization of two free chromosome *I* duplications demonstrated that one covering the right arm of the chromosome was capable of pairing for recombination with the normal chromosomes, while the duplication covering the left arm was not. This led to the proposal that the right end of chromosome *I* contained sequences necessary for recombination and pairing between the homologues (ROSE, BAILLIE and CURRAN 1984; McKIM, HOWELL and ROSE 1988). The characterization of translocations and duplications has led to the identification of a single site on each chromosome that is necessary for pairing and recombination, discussed by McKIM, HOWELL and ROSE (1988). This site, called the homologue recognition region, may correspond to the region discussed by MAGUIRE (1984), described as the site of first contact between homologues during meiosis. The genetic behaviour of rearrangements lacking this region supports this interpretation since such rearrangements fail to pair for recombination with their homologues. This demonstrates that the function associated with this site temporally precedes any later meiotic event in the pathway, consistent with the predicted behaviour of a specialized site required for initial homologue recognition early in meiosis. Such behaviour, however, would also be predicted for rearrangements that delete the site of telomere

attachment to the nuclear membrane. The failure of the chromosomes to pair and recombine could be attributed to the failure of the homologue lacking the telomere attachment site to become properly oriented and anchored in the spatial organization of the nucleus. As a result, it would float free in the nucleus, unable to participate in later meiotic events. In *C. elegans*, electron microscopy of pachtene nucleii has demonstrated that while only one telomere of each chromosome is attached to the nuclear membrane, both telomeres have the ability to do so (GOLDSTEIN 1982, 1984a, 1984b, 1985, 1986; GOLDSTEIN and SLATON 1982), indicating the telomere attachment site does not correspond to the homologue recognition region.

The second form of meiotic pairing is thought to bring the homologues into a tighter association as a result of numerous recombination events which occur at certain sites at a higher frequency. Such pairing sites have been mapped on the *X* chromosome of Drosophila (HAWLEY 1980) and in yeast a pairing site identified on chromosome *III* has been shown to be a recombination hotspot (GOLDWAY, ARBEL and SIMCHEN 1993; GOLDWAY *et al.* 1993). In *C. elegans*, these secondary pairing sites are not sufficient to ensure recombination between homologues in the absence of the homologue recognition region (ROSENBLUTH, JOHNSEN and BAILLIE 1990; McKIM, PETERS and ROSE 1993). In the presence of the HRR, however, a small set of these sites may be preferentially used to ensure secondary pairing between rearranged chromosomes. Intrachromosomal effects in translocation (McKIM, HOWELL and ROSE 1988; McKIM, PETERS and ROSE 1993) and inversion heterozygotes (ZETKA and ROSE 1992) enhance crossing over in the regions capable of homologous pairing to levels approaching 50 map units. In *hIn1(I)/szT1(I;X)* heterozygotes, the genetic size of chromosome *I* was 50 map units even though all crossing over had to occur in a small region flanked on one side by nonhomologous translocated sequences, and on the other by the inversion. This supports the interpretation that pairing between such sites is independent of the pairing of neighbouring sites, and does not support a model whereby pairing for recombination is initiated only at the end(s) of a chromosome

and progresses from this site. Rather, it suggests that recombination events can be initiated internally.

Two major classes of mutations that disrupt meiotic exchange exist in *C. elegans*. The largest is represented by several *him* mutants which are recombination-defective on both the *X* chromosome and the autosome (HODGKIN, HORVITZ and BRENNER 1979). The second class is represented by one mutation, *rec-1*, which disrupts the normal distribution of crossing over and does not decrease the viability and brood sizes of mutants. The genetic size of chromosome *I* in *rec-1* homozygotes approaches the 50 map units observed in rearrangement heterozygotes (McKIM, HOWELL and ROSE 1988; ZETKA and ROSE 1992; McKIM, PETERS and ROSE 1993) and wild-type individuals (ZETKA and ROSE 1990), suggesting a flexible mechanism exists to ensure the formation of one crossover between the homologues. In this study, crossing over was eliminated in *hIn1(I)/hT2(I;III)* heterozygotes resulting in the random segregation of the two chromosomes. This demonstrates that in *C. elegans*, one chiasma per bivalent is necessary for the proper disjunction of homologous chromosomes.

Recombination in *C. elegans* is also regulated by a mechanism based on the sexual phenotype of the individual. Male recombination frequency on chromosome *I* is reduced by one third when compared to the hermaphrodite frequency, suggesting the formation of a chiasma is not guaranteed in every male meiosis. Three possibilities may explain the orderly disjunction of chromosomes in male gametes in the event a chiasma does not form. Firstly, substantial levels of recombination may occur at the distal tips of the chromosome where crossing over is difficult to measure. Secondly, male meiosis may make more use of secondary pairing sites to ensure proper segregation in the absence of chiasma formation. In Drosophila, deletion mapping of the ribosomal cluster has demonstrated that sequences between the rRNA genes function as *X-Y* pairing sites in male meioses (McKEE, HABERA and VRANA 1992). Thirdly, studies have documented that free duplications segregate from the *X* chromosome in the male and it is possible that nonrecombinant chromosomes may pair distributively (McKIM, PETERS and ROSE 1993; this study APPENDIX I).

Recombination-independent segregation systems have been documented in Drosophila (GRELL 1962) and in yeast (DAWSON, MURRAY and SZOSTAK 1986).

The third form of meiotic pairing culminates in the intimate association of the homologues that is mediated by the formation of the synaptonemal complex. Normal synaptonemal complexes, consisting of two lateral elements and a central element form in both males and hermaphrodites (GOLDSTEIN and SLATON 1982).

Although *C. elegans* chromosomes behave holokinetically during mitosis (ALBERTSON and THOMSON 1982), evidence has been presented in this thesis that this is not true for meiosis. Analysis of recombinants derived from inversion heterozygotes has suggested that *hIn1(I)* is a paracentric inversion with the meiotic centromere to its left (ZETKA and ROSE 1992). This is supported by recent cytological studies which have concluded that while centromeric activity is localized to one end of the chromosome during meiosis, either end can perform this function (ALBERTSON and THOMSON 1993). Thus, in any one meiosis, one end of the chromosome adopts centromeric activity by attaching to spindles (monocentric), rather than both ends (dicentric), or the whole chromosome (holocentric). On chromosome *I*, the right end of LG *I* adopts centromere function in the majority of meioses (ALBERTSON and THOMSON 1993). This is consistent with the structure of *hIn1(I)*-derived recombinants since each recombinant chromosome is representitive of one meiosis, and their derivation has been explained by the presence of a meiotic centromere on the right arm (ZETKA and ROSE 1992). This proposal was based upon the genetic definition of the centromere, defined as the last point of attachment between sister chromatids at anaphase II. By this definition, *C. elegans* chromosomes have two potential spindle attachment sites at Meiosis I (of which only one is used) and at Meiosis II they possess one genetic (and cytological) centromere. As a result, it can be concluded that while *C. elegans* chromosomes behave holocentrically during mitosis, centromeric activity during meiosis is restricted to one site and the chromosomes are functionally monocentric.

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APPENDIX

I. Distributive pairing of the *X* and *sDp1(I;f)* in males: To test if the free duplication *sDp1* distributively paired with the single *X* chromosome in males, *dpy-5 unc-13; 0 /szT1(I;X)[+ + ;lon-2]* males were mated to *dpy-5 dpy-14/sDp1* hermaphrodites. All wild-type males resulting from this cross were of the genotype *dpy-5 unc-13/dpy-5 dpy-14/sDp1* and were mated to *dpy-5; unc-36* homozygous hermaphrodites and the wild-type and Dpy-5 progeny were scored. If the duplication and the *X* chromosome segregated from one another, the resulting male progeny were predicted be wild-type as consequence of inheriting *sDp1* and the hermaphrodite progeny were predicted to Dpy-5 as a consequence of inheriting the paternal *X*. If the duplication and the *X* segregated randomly, one half of the wild-type and Dpy-5 progeny were expected to be male. 269/293 wild-type progeny were male and 275/298 Dpy-5 progeny were hermaphrodite indicating that in approximately 87% of the male meioses, *sDp1* and the single *X* segregated from one another, consistent with the proposal that distributive pairing occurs in males.

II. Cosmid mapping of *rec-1*: A Rec-1 transgenic strain (KR2357), constructed by coinjecting *rol-6* cosmid pRF4 and three overlapping cosmids (ZK219, BO195, CO1F5) (mapping to the right of *unc-54*), was obtained from J. McDowall. To determine if these cosmids carried a wild-type allele of *rec-1*, Rol-6 transformants (*rol-6* is dominant and establishes the presence of the extrachromasomal array containing the cosmids discussed above) were mated to *dpy-11 unc-42/ + + ; rec-1/rec-1* or *dpy-11 unc-42/ + +* males. Rol-6 progeny resulting from this cross were individually plated and their progeny screened for the presence of Dpy-11 Unc-42 segregants. Recombination in these individuals was calculated using the formula:

$$p = 1 - [\ 1 - 3(\mathbf{D} + \mathbf{U})/2(\mathbf{W} + \mathbf{R} + \mathbf{D})$$

where **D** is the number of Dpy recombinants, **U** is the number of Unc recombinants, **W** is the number of wild types and **R** is the number of roller progeny. Rol-6 animals will not roll in an Unc-42, Dpy-11 or Dpy-11 Unc-42 background.

The region of chromosome *I* located between *unc-54* and the ribosomal cluster (*rrn-1*) at the right distal tip is spanned by six overlapping cosmids shown in Figure 13. In order to map *rec-1* to one of these cosmids, the six cosmids were coinjected as sets of three (one group containing the cosmids ZK219, BO195, CO1F5 and the other ZK340, BO467, ZC556) into *rec-1* homozygotes (J. McDOWALL *unpublished results*) using the *rol-6* transformation system (MELLO *et al.* 1991). Attempts to construct a transgenic line containing the second group of cosmids (ZK340, BO467, ZC556) were unsuccessful and no stable lines were isolated (J. McDowall *pers. comm.*). Transgenic Rol-6 hermaphrodites bearing an extrachromosomal array containing the cosmids ZK219, BO195 and CO1F5 were tested for the presence of Rec-1 by measuring recombination in *dpy-11 unc-42/ + + ; rec-1/rec-1* individuals (data shown in Table 17). Since the cosmids were injected into a Rec-1 individual, the presence of a wild-type allele of *rec-1* on one of the cosmids would result in a normal map distance for the *dpy-11 unc-42* interval. The *dpy-11 unc-42* interval was 8.8

FIGURE 13.-Physical map of cosmids in the region of *unc-54*.

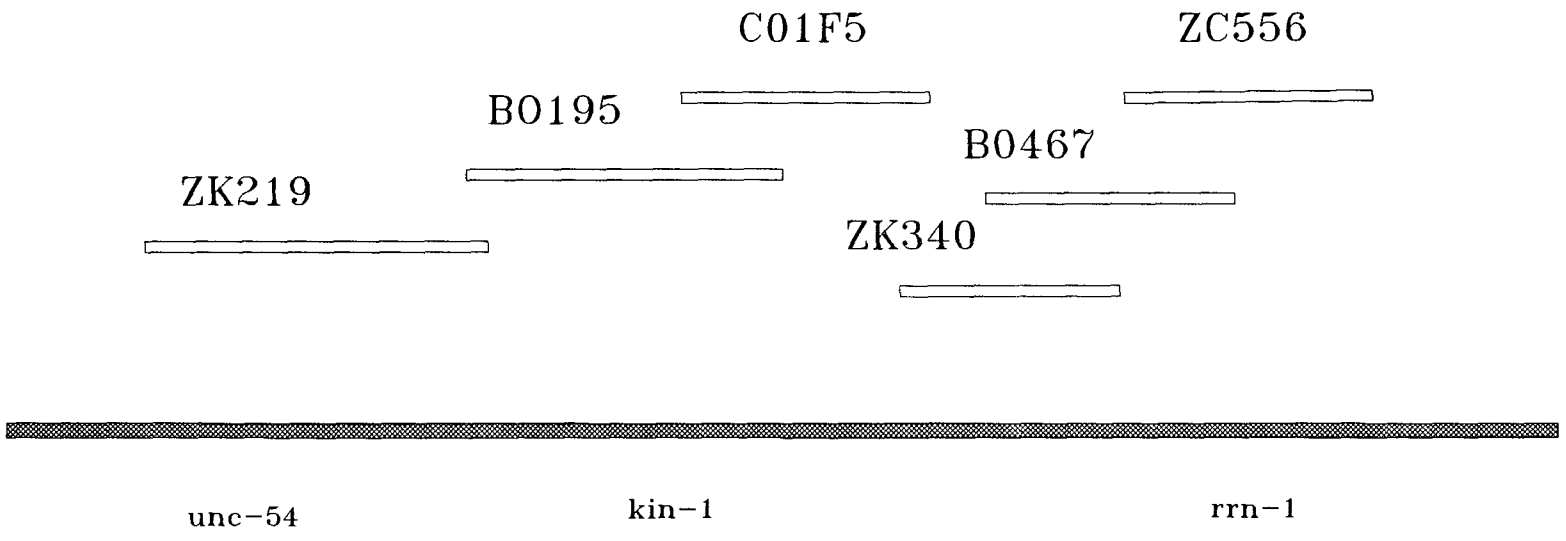

C01F5
ZC556
B0195
B0467
ZK219
ZK340
unc-54
kin-1
rrn-1

Table 17

Cosmid mapping of *rec-1*

Genotype	Wts	Rol-6	Dpy	Unc	pX100(C.I.)[a]
dpy-11 unc-42/ + + ;rec-1/rec-1[b]	1219		66	59	7.6(6.4-9.0)
dpy-11 unc-42/ + +[b]	1250		17	12	2.7(2.0-3.6)
dpy-11 unc-42/ + + ;rec-1/rec-1;hEx12[c]	637	271	60	49	8.9(7.3-10.6)
dpy-11 unc-42/ + + ;rec-1/ + ;hEx12[c]	494	403	19	11	2.5(1.7-3.5)

[a] C.I. = 95% confidence interval (see Chapter 1: MATERIALS and METHODS).

[b] data from Table 10.

[c] The extrachromosomal array *hEx12* includes the *rol-6* dominant gene, and the cosmids ZK219, B0195 and C01F5 in unknown copy number (J. McDOWALL *unpublished results*).

m.u. in transgenic individuals homozygous for *rec-1* and 6.4 m.u. in *dpy-11 unc-42/ + + ; rec-1/rec-1* controls, indicating that the cosmids did not carry a wild-type allele of *rec-1*. In the event normal meiotic recombination was affected by the presence of the cosmids, recombination was also measured in *dpy-11 unc-42/ + + ; rec-1/ +* individuals carrying the extrachromasomal array. The frequency of recombination was 2.5 m.u. in transgenic individuals heterozygous for *rec-1* and 2.7 m.u. in *dpy-11 unc-42/ + +* controls, indicating the presence of the array does not affect recombination in this interval. Because the presence of the array was not confirmed by PCR, however, the negative results of these experiments are not conclusive.

III. PCR mapping of *eDf24(I)*: Young *eDf24/hIn1[unc-101]* or *hIn1(I)/hIn1(I)* hermaphrodites were plated and allowed to lay eggs for 12 hours and then removed. Eggs which remained unhatched after 20 hours were removed to agar plates lacking OP50 and treated according to the protocol of BARSTEAD, KLEIMAN and WATERSTON (1991) with the exception that the eggs were transferred using cut fishing line which then remained in the PCR tubes. The cosmids ZK340, BO467, and ZC556 were provided by J. McDowall.

The reaction volume of 22.5 *ul* included 2.5 *ul* of DNA preparation, 0.125 units of *Taq* polymerase (Sigma), 4 *ul* each of 2.5 mM dNTP (dATP, dCTP, dGTP, and dTTP) and 1 *ul* of each oligonucleotide in a PCR buffer (Sigma) containing 1.5 *ul* of 25 mM $MgCl_2$. The reaction mixtures were overlaid with mineral oil (Fisher), incubated for 3 min at 95°C, 30 sec at 50°C, taken through 30 cycles of 1 min at 72°C, 45 sec at 94°C, and 20 sec at 55°C. After these cycles, the samples were incubated for 7 min at 72°C and cooled to 4°C using a brand name thermal cycler. After cycling, 5 *ul* of 5 X DNA sample buffer (1 X = 0.25% bromophenol blue, 0.25% xylene cyanole, 15% Ficoll) was added the sample. 20 *ul* of each sample was removed and analyzed on a 1.2% agarose gel containing ethidium bromide. After electrophoresis at 140 V for about 1 hour, the gels were removed and photographed.

ALBERTSON (1984b) observed that *eDf24* deletes a portion of the ribosomal cluster located at the right end of LG *I*, but could not determine whether the deficiency contained sequences to the left of the cluster or lay completely within it. To distinguish between these two possibilities, primers flanking the left ribosomal junction (one specific for nonribosomal sequences adjacent to the cluster and the other specific for 26S ribosomal RNA) were used to determine if the junction was present in *eDf24* homozygotes. The sequences of these primers and control primers derived from the adenosyl homocysteine hydrolase gene (AHH)(PRASAD, STARR and ROSE 1993) are shown in Figure 14 and the PCR products are shown in Figure 15. The primer set specific for the ribosomal junction produces a 517 b.p. product and the AHH primers a 577 b.p. product. Both products are present in *hIn1(I)* homozygote controls, however, only the 577 b.p. product is present in *eDf24* homozygotes,

FIGURE 14.-Sequences of PCR primers used in this study. a) RL30 anneals to nonribosomal DNA adjacent to the ribosomal cluster and RL31 anneals to 26S ribosomal genes. b) RL12 and RL14 anneal to sequences within the AHH locus, located in the central cluster of chromosome *I*.

a)

RL30 5′ – TGG GAA TTT TCT GTT CAG GT – 3′

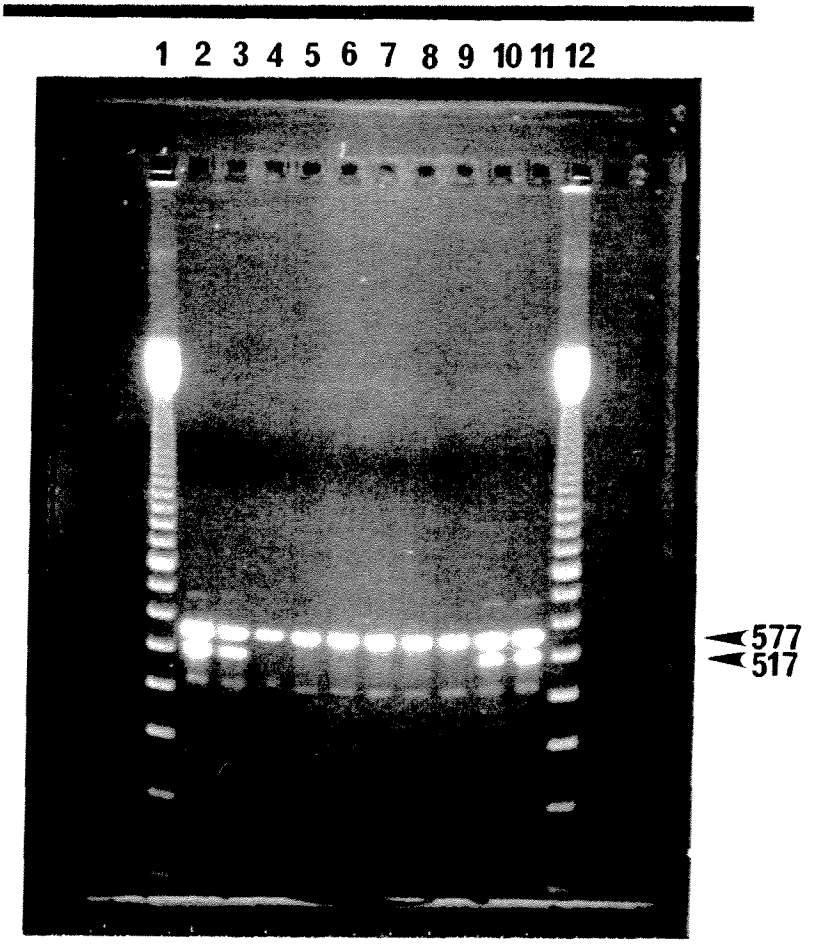
RL31 5′ – CGC AAT AAC AAG TCA ACA GT – 3′

b)

RL12 5′ – CGT CCG TTC TTG AGG GTG – 3′

RL14 5′ – CTA AGA TGC TCG CCA AGG – 3′

FIGURE 15.-PCR analysis of *eDf24*(I). PCR products obtained from lane 2) N2 L4 hermaphrodite, lane 3) N2 egg, lanes 4-9) *eDf24(I)/eDf24(I)* egg, lane 10) *eDf24(I)/hIn1(I)[unc-101]* L4 hermaphrodite, and lane 11) *hIn1(I)/hIn1(I)* L4 hermaphrodite using the left ribosomal junction primers (517 b.p. predicted product size) and AHH control primers (577 b.p. predicted product size). *eDf24(I)* homozygotes lack the 517 b.p. product, indicating that the deficiency spans the junction (THACKER and ZETKA *unpublished results*).


1 2 3 4 5 6 7 8 9 10 11 12
577
517

indicating the deficiency spans the ribosomal junction and includes nonribosomal DNA adjacent to the cluster. Identical primer sets were also used to determine which of the three remaining cosmids contained the ribosomal junction. The cosmids ZC556 and BO467 (not ZK340) produced a 517 b.p. product, indicating the ribosomal junction maps to these cosmids (data not shown).

III. Table 18

Strains used in this study

Strain	Genotype	Strain	Genotype
BC62	*dpy-5(e61)unc-75(e950)*	CB719	*unc-1(e719)*
BC64	*unc-35(e259)dpy-5(e61)*	CB1479	*him-6(e1423)*
BC89	*dpy-5(e61)unc-54(e190)*	DR1	*unc-101(m1)*
BC196	*dpy-5(e61)dpy-14(e88)rec-1(s180)*	KK596	*him-14(i144ts)*
BC207	*dpy-5(e61)unc-29(e403)*	KR181	*unc-42(e270)dpy-11(e224)rec-1(s180)*
BC251	*unc-42(e270)dpy-11(e224)*	KR236	*dpy-5(e61)unc-13(e450)/sDp2*
BC260	*unc-11(e47)dpy-5(e61)rec-1(s180)*	KR307	*dpy-5(e61)unc-101(m1)*
BC415	*dpy-5(e61)unc-13(e450)*	KR309	*bli-3(e579)unc-11(e47)rec-1(s180)*
BC563	*dpy-18(e364)unc-36(e261)*	KR387	*unc-13(e450)rec-1(s180)*
BC1195	*sDp3/dpy-17(e251)unc-36(e261)*	KR642	*dpy-5(e61)rec-1(s180)*
CB51	*dpy-5(e51)*	KR900	*dpy-5(e61)unc-13(e450)/szT1(I;X)[+ +;lon-2]*
CB88	*dpy-7(e88)*	KR1004	*dpy-5(e61)dpy-14(e88)*
CB151	*unc-3(e151)*	KR1005	*sDp2/dpy-5(e61)dpy-14(e88)rec-1(s180)*
CB190	*unc-54(e190)*	KR1012	*dpy-5(e61)unc-29(e403)unc-75(e950)*
CB261	*unc-59(e261)*	KR1064	*bli-3(e579)unc-11(e47)*
CB450	*unc-13(e450)*	KR1071	*dpy-5(e61)unc-11(e47)*

Strain	Genotype
KR1301	*rec-1(s180)* males
KR1473	*unc-101(m1)unc-54(e190)*
KR1546	*sDp1/dpy-5(e61)dpy-14(e88)rec-1(s180)*
KR1701	*dpy-7(e88)unc-3(e151)*
KR1714	*unc-29(e403)unc-75(e950)*
KR1734	*unc-75(e950) unc-101(m1)*
KR1735	*sDp1/dpy-5(e61)dpy-14(e88)*
KR1898	*unc-101(m1)lev-11(x12)*
KR1906	*unc-11(e47)rec-1(s180)*
KR1949	*hIn1(I)* males
KR1953	*unc-54(e190)/eDf24*
KR1954	*dpy-5(e61) unc-75(e950); her-1(e1520)*
KR1955	*dpy-5(e61)unc-101/szT1[unc-101;lon-2]*
KR1956	*dpy-5(e61) unc-13(e450) rec-1(s180)*
KR2017	*unc-75(h1041)/unc-75(e950)unc-101(m1)*
KR2019	*hDp132/unc-75(e950)unc-101*
KR2020	*hDf12/unc-75(e950)unc-101(m1)*
KR2025	*hDp131/unc-101(m1)lev-11(x12)*
KR2151	*hIn1[unc-54(h1040)]*
KR2152	*hDp131/unc-75(e950)unc-101(m1)*
KR2153	*hIn1[dpy-5(e61)unc-54(h1040)]*
KR2156	*unc-29(e403)lin-11(n389)*
KR2158	*unc-75(e950)unc-59(e261)*
KR2159	*unc-75(h1042)/unc-75(e950)unc-101(m1)*
KR2226	*hIn1[unc-54(h1040)]/unc-101(m1)lev-11(x12)*
KR2228	*lev-11(x12)* males
KR2387	*unc-1(e719)dpy-7(e88)*
KR2390	*hDp132/unc-75(e950)unc-54(e190)*
KR2391	*hDp131/unc-75(e950)unc-59(e261)*
KR2392	*hDf11/unc-101(m1)unc-54(e190)*
KR2394	*hDp2/dpy-5(e61)*
KR2423	*dpy-5(e61)unc-36(e261)*
MT633	*lin-11(n389);him-5(e1467)*
RW3072	*lev-11(x12)let-49(st44)/unc-54(st60)let-50(st33)*
SP580	*mn164(I;X)*
ZZ3003	*lev-11(x12)*