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Meiotic Mutants That Cause a Polar Decrease in Recombination on the *X* Chromosome in *Caenorhabditis elegans*

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ABSTRACT

Recessive mutations in three autosomal genes, *him-1*, *him-5* and *him-8*, cause high levels of *X* chromosome nondisjunction in hermaphrodites of *Caenorhabditis elegans*, with no comparable effect on autosomal disjunction. Each of the mutants has reduced levels of *X* chromosome recombination, correlating with the increase in nondisjunction. However, normal or elevated levels of recombination occur at the end of the *X* chromosome hypothesized to contain the pairing region (the left end), with recombination levels decreasing in regions approaching the right end. Thus, both the number and the distribution of *X* chromosome exchange events are altered in these mutants. As a result, the genetic map of the *X* chromosome in the *him* mutants exhibits a clustering of genes due to reduced recombination, a feature characteristic of the genetic map of the autosomes in non-mutant animals. We hypothesize that these *him* genes are needed for some processive event that initiates near the left end of the *X* chromosome.

MEIOSIS reduces the chromosome number of gametes prior to the onset of the next generation. Despite the importance of meiosis and its near universality among eukaryotes, the mechanics of this reductional division remain poorly understood. The central process of reductional division involves each chromosome finding its homolog, pairing and then disjoining. If chromosomes fail to pair, they frequently do not disjoin correctly and chromosome loss can occur. In organisms that undergo genetic exchange, at least one exchange event per chromosome is often necessary for proper pairing and disjunction (reviewed in HAWLEY 1988). Mutations that decrease or abolish recombination lead to high levels of homolog nondisjunction (reviewed in BAKER *et al.* 1976), often a lethal event. It has been postulated (SMITHIES and POWERS 1986; CARPENTER 1987) and recently shown for one strain of yeast that initiation of recombination is one of the earliest events in the meiotic cycle (PADMORE, CAO and KLECKNER 1991).

Caenorhabditis elegans consists of two sexes: *XX* self-fertile hermaphrodites and *XO* males. Hermaphrodites are basically females that transiently make sperm, which they store and use for self-fertilization if males are absent. Hermaphrodites usually produce *XX* hermaphrodite self-progeny, but in 0.2% of all meioses spontaneous nondisjunction of the *X* chromosome generates *XO* male progeny. Since *XO* males are viable aneuploids, it is simple to isolate meiotic mutations that elevate nondisjunction or loss by screening for an enhancement of males in a brood. This was originally done by HODGKIN, HORVITZ and BRENNER (1979), who labeled this type of mutation *him*, for *h*igh *i*ncidence of *m*ales. Mutations defining nine genes were originally isolated, and since then several more *him* mutants have been identified (HODGKIN *et al.* 1988; KEMPHUES, KUSCH and WOLF 1988) bringing the total to 14 genes. The mutant phenotypes fall into two broad classes. The larger class is defined by reduced broods, a small percentage of viable males, many inviable embryos, and a general feebleness of survivors. It is likely, and indeed has been shown for some mutants, that this class of *him* mutants causes general nondisjunction of all chromosomes (HODGKIN, HORVITZ and BRENNER 1979; P. MENEELY, unpublished observations). The second class consists of recessive mutations in three autosomal genes that cause preferential nondisjunction and loss of the *X* chromosome. These mutations generate a high percentage of males with no apparent effect on autosomal transmission. The *X* chromosome specific mutants are designated *him-1*, *him-5*, and *him-8*. *him-8(e1489)* has the most dramatic phenotype, causing *X* chromosome loss in over 40% of meioses, yet unlike the other *him* mutants it has no effect on general health or brood size. [The dosage compensation mutants *dpy-26* and *dpy-28*, which affect many properties of the *X* chromosome, also apparently cause loss of the *X* (HODGKIN 1983; PLENEFISCH, DELONG and MEYER 1989).] A third class of *him* mutations has been identified that map to the *X* and have dominant effects (HERMAN, KARI and HARTMAN 1982). Many of these are translocations or other rearrangements of the *X* chromosome.

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Initial work (HODGKIN, HORVITZ and BRENNER 1979) on the *X* chromosome-specific *him* genes demonstrated that these mutations dramatically reduce recombination on the *X* chromosome but not in most of the six large autosomal regions tested. [*him-5* is the one exception, causing a decrease in recombination in one autosomal interval.] The amount of reduction in recombination roughly correlates with the degree of chromosome loss. For example, the mutation *him-1(e879)* yields 20% *XO* progeny with a decrease in recombination over a large *X*-linked interval of 32%, while *him-8(e1489)*, produces 40% *XO* males and reduces recombination in the same interval by 92% (HODGKIN, HORVITZ and BRENNER 1979). All three *him* strains also generate diplo-*X* gametes that upon self-fertilization produce morphologically distinct triplo-*X* hermaphrodites. When the two *X* chromosomes that do not disjoin are examined they are usually found to be nonrecombinant. These triplo-*X* progeny are less frequent than *XO* progeny, suggesting that nondisjoined chromosomes are often lost.

To determine how these mutations lead to nondisjunction and specific loss of the *X*, we isolated new alleles of *him-8* and undertook a more detailed analysis of recombination over the majority of the *X* chromosome in the three *X*-specific mutants. Strikingly, our results suggest that these three genes are involved in determining not only the frequency of exchange events, but also their spatial distribution along the *X* chromosome. The data suggest a biphasic mechanism for chromosome pairing.

MATERIALS AND METHODS

Strains and maintenance of *C. elegans*: Nematodes (var. Bristol, wild-type designated N2) were cultivated on *Escherichia coli* strain OP50 (SULSTON and HODGKIN 1988). All crosses and growth were at 20° except for mutant screens, which were performed at 25°. Genes and alleles used were:

Linkage group (LG) I: *him-1(e879), dpy-5(e61), unc-13(e51)*;
LG II: *unc-4(e120); dpy-10(e128)*;
LG III: *unc-36(e251)*;
LG IV: *dpy-20(e1282ts), him-6(e1423);him-8(e1489), him-8 (mn253), mec-3(n578),unc-24(e138), unc-43(e408), dpy-13(e184) mDf7/nT1[let?(m435)(IV;V)]*;
LG V: *unc-42(e270), sqt-3(sc63), him-5(e1490); dpy-11(e224)*
LG X: *unc-1(e538), unc-1(e719), dpy-3(e27), unc-2(e55), lon-2(e678), unc-18(e81), dpy- 6(e14), unc-3(e151), lin-15(n309).*

Screen for new alleles that fail to complement *him-8(e1489)*: A population of N2 animals containing males was mutagenized with ethyl methanesulfonate (EMS) at room temperature as described by SULSTON and HODGKIN (1988), except that a lower concentration of EMS (30 mM) was used. Eight to ten L4 mutagenized males were mated to two *unc-24(e138) him-8(e1489) dpy-20(e1282ts)* hermaphrodites. All crosses and subsequent generations were maintained at 25° until temperature sensitivity was assayed. F_1 non-Dpy non-Unc hermaphrodites were picked to individual plates as L3 or L4 larvae and allowed to self-fertilize. F_2 progeny were screened for the presence of males. Several individuals were picked from each candidate plate and retested. New mutations were outcrossed several times, usually while mapping to a chromosome, to remove any secondary mutations.

Linkage analysis: Linkage to each chromosome was tested independently using standard techniques. Males were chosen from each *him-8* candidate mutation and mated to hermaphrodites mutant for a morphological marker, *e.g.*, *unc-13* (I). non-Unc F_1 progeny were transferred to new plates as larvae and allowed to self. Twenty-five Unc F_2 progeny were transferred to new plates and the F_3 progeny were screened for the presence of males. If unlinked, then approximately six F_2s (1/4 of the total) are expected to be Him. For new allele *ec51* the number of F_2 Hims for each linkage group was: LG I, 6; LG II, 5; LG III, 4; LG IV, 0; LG V, 7.

Analysis of recombination on the *X* chromosome in *him-1*, *him-5* and *him-8*: Recombination was initially examined in a non-Him strain to establish a wild-type standard. N2 males were mated to hermaphrodites carrying two *X*-linked markers, *e.g.*, *unc-1 dpy-3*. non-Unc non-Dpy F_1 progeny were transferred to individual plates as larvae and transferred to new plates daily once egg laying commenced. The number of recombinant and nonrecombinant F_2 progeny were determined.

To test recombination in a *him* background triple mutant strains were constructed consisting of either *him-1*, *him-5* or *him-8* and the same two *X*-linked markers used to construct the map in wild type. Each strain was then mated to unmarked *him* males to generate *him* homozygotes that were heterozygous for both X-linked markers. Since *him* strains normally produce some triplo-*X* progeny that are morphologically Dpy (HODGKIN, HORVITZ and BRENNER 1979), the Unc non-Dpy recombinant F_2 progeny were always scored whenever an *X*-linked *dpy* marker was used. F_1s were cultured until no longer fertile to ensure scoring of the entire F_2 brood, and to avoid age effects on recombination (ROSE and BAILLIE 1979). *unc-1(e719)* was used in strains containing *him-5* and *him-8*, while *unc-1(e538)* was used for *him-1*, since we found that *him-1(e879) unc-1(e719)* is not a viable strain. Deviations from the wild-type map distance in a *him* stain were expressed as a percentage of that found in N2.

Creation and analysis of double mutant strains: *him-1(e879);him-8(e1489)* was created by mating *dpy-5(e61);him-8(e1489)* males with *him-1(e879);unc-24(e138)* hermaphrodites. F_1 non-Unc hermaphrodites were transferred to new plates and allowed to self-fertilize. F_2 non-Unc non-Dpy hermaphrodites were transferred to individual plates and the F_3 screened for the absence of Unc and Dpy progeny. Those individuals producing only wild-type hermaphrodites and males were designated as being *him-1;him-8*. This was confirmed by mating *him-1;him-8* males to both *him-8(e1489)unc-24(e138)* and *him-1;(e879)unc-24(e138)*, selecting non-Unc F_1s and checking for males in the F_2 generation.

him-5(e1490);him-8(mn253) and *him-5(e1490);him-8(e1489)* were created using the above strategy and the marked strains *him-5(e1490);mec-3(n578)* or *him-5(e1490);unc-24(e138)*, and *him-8(mn253);sqt-3(sc63)*, or *him-8(e1489);sqt-3(sc63)*. *him-5;him-8* strains were confirmed by mating to *him-5(e1490);unc-24(e138)* or *him-8(mn253) unc-24(e138)* hermaphrodites, selecting non-Unc F_1s and checking for males in the F_2 generation.

Male counts were done by picking larvae from the Him double mutants and transferring them to new plates daily so that entire broods could be scored.

Analysis of autosomal nondisjunction in *him+* strains: In *him+* strains the spontaneous *X* chromosome nondisjunction rate is about 3 in 1000 progeny. We looked for spontaneous autosomal nondisjunction in *him+* strains using the

procedure developed by HODGKIN, HORVITZ and BRENNER (1979). Specifically, the strains *unc-4(e120) II; dpy-11(e224) V; him-6(e1423) IV* and *dpy-10(e128) II; unc-42(e270) V; him-6(e1423) IV* were used to generate disomic and nullo-somic oocytes that were fertilized by wild-type male sperm. By screening for Unc non-Dpy and Dpy non-Unc progeny the frequency of loss of chromosomes *II* and *V* during *him+* spermatogenesis could be assesed. In more than 30,000 individuals examined no such progeny were discovered. Similar experiments, though with fewer numbers, were also conducted for chromosomes *I, III* and *IV* and again no sign was seen of spontaneous autosomal nondisjunction in a *him+* strain. The reciprocal experiments were also done using *him-6(e1423)* males and *unc-4(e120) II; dpy-11(e224) V* or *dpy-10(e128) II; unc-42(e270) V* hermaphrodites to look for spontaneous autosomal nondisjunction during *him+* oogenesis. Again, greater than 20,000 progeny were examined and no progeny indicative of autosomal loss were recovered.

RESULTS

Isolation and analysis of *him-8* alleles: When this analysis began only two alleles of *him-8* existed, *e1489* (HODGKIN, HORVITZ and BRENNER 1979) and *mn253* (HERMAN and KARI 1989), both of which were healthy and gave high frequencies of males. [A third allele of *him-8*, *him-8(g203)* (HODGKIN *et al.* 1988) has been lost (R. CASSADA, personal communication).] We wanted to examine more alleles in order to determine if the high level of non-disjunction was the only phenotype that could be obtained by mutation in *him-8*. We conducted a screen for mutations that failed to complement *him-8(e1489)*. N2 males were mutagenized with EMS and mated to *dpy-20 him-8 unc-24* hermaphrodites at 25°. non-Dpy non-Unc F_1 hermaphrodite progeny were picked to individual plates at 25° prior to reaching the adult molt in order to avoid generating males from mating. From 1728 genomes screened we recovered two mutations that failed to complement *him-8(e1489)*, neither of which was temperature sensitive. *ec51* fails to complement both *him-8* (*e1489*) and *him-8* (*mn253*), produces 44% self-progeny males when homozygous, and maps to chromosome *IV*. From 25 *ec51* +/+ *unc-24* animals, none of the Unc progeny was Him, suggesting that *ec51* maps close to *unc-24*. We thus concluded that *ec51* was a new allele of *him-8*.

The second noncomplementing mutation was homozygous lethal and failed to complement *unc-43* as well as *him-8*. We concluded that this mutation was a deficiency and named it *ecDf4*. *ecDf4* complements both *unc-24* and *dpy-20*, however, delineating the maximum size of the deficiency as less than two map units.

Excluding *ecDf4*, there now exist seven independently isolated alleles of *him-8* (this work; HODGKIN, HORVITZ and BRENNER 1979; HERMAN and KARI 1989). During the course of this work several new alleles of *him-8* were given to us by colleagues (A. VILLENEUVE and C. MELLO, personal communications). All yield similar percentages of self-progeny males, suggesting that the Him phenotype is not likely to be due to novel changes in gene function. If the 40% Him phenotype represents the absence of active gene product, rather than simply a reduction, then the phenotype should not change when the mutant allele is placed in trans to a deficiency for the locus. This was done for the canonical allele, *e1489*, using the deficiency *mDf7* and the number of self-progeny males generated was determined. No significant change in percent males produced occurred (Table 1). Similar results were seen with *him-8(e1489)/ecDf4* (data not shown). Unlike the sterility associated with *him-5* mutations (P. MENEELY, unpublished data), and the lethality (HOWELL *et al.* 1987) and semi-dominance of *him-1* mutations (HODGKIN, HORVITZ, and BRENNER 1979), *him-8(e1489)* is recessive and has no other phenotypes. Thus it is likely that the *X* chromosome nondisjunction phenotype of *e1489* and *ec51* represents the null mutant phenotype of the *him-8* gene.

TABLE 1

Percentage male progeny from different *him* strains

Genotype	No. of ♂/Total progeny	% ♂	Chi square relative to *him-8*[a]
him-1(e879)	244/1642	15	
him-5(e1490)	437/1424	31	
him-8(mn253)	851/2262	38	
him-8(e1489)	996/2459	41	
him-8(ec51)	713/1635	44	
him-8(e1489)/mDf7	327/797	41	
him-8(e1489);him-1(e879)	1283/2862	45	10 ($P < 0.005$)
him-8(e1489);him-5(e1490)	451/1172	38	1.8 ($P > 0.1$)
him-8(mn253);him-5(e1490)	717/1920	37	0.2 ($P > 0.5$)

[a] Chi square analysis was used to determine if the phenotype of the double mutant differed significantly from the phenotype of *him-8* alone. A confidence level of $P < 0.05$ was considered significant.

Further data distinguishing the roles of these genes in meiosis comes from HARTMAN and HERMAN (1982), who demonstrated that *him-1* is sensitive to UV and ionizing radiation, while *him-5* and *him-8* are not. *him-1* mutations can also be suppressed by a mutation in the UV radiation-sensitive gene *rad-4*, while *him-5* and *him-8* mutations can not (HARTMAN and HERMAN 1982). HODGKIN, HORVITZ and BRENNER (1979) reported that the effects of mutation in *him-1(e879)* and *him-5(e1490)* are additive. We find that a *him-1(e879);him-8(e1489)* double mutant strain produces 45% males, a slight but statistically significant increase in production of nullo *X* gametes above what *him-8* alone produces (Table 1). In contrast, the *him-5(e1490);him-8(e1489)* strain gave 38% males and *him-5(e1490);him-8(mn253)* gave 37% males, not significantly different from either *him-8* allele alone (Table 1). Thus, *him-1(e879)* is additive with both *him-8(e1489)* and *him-5(e1490)*.

TABLE 2

Frequency of recombination on the X chromosome in *him+*

Interval	Frequency of recombinants	Map units[a]
unc-1 dpy-3	53/3120	3.4
dpy-3 unc-2	31/1619	3.8
unc-2 lon-2	47/1587	6.0
lon-2 unc-18	79/2858	5.6
unc-18 dpy-6	11/1448	1.6
dpy-6 unc-9	142/2903	9.8
unc-9 unc-3	83/2652	6.2
unc-3 lin-15	73/2659	5.4

[a] Since only one recombinant class was scored, only half of all recombination events were counted. Thus the number of recombinant progeny was doubled before being divided by the total number of progeny and then multiplied by 100 to be expressed as map units.

***him-1*, *him-5* and *him-8* cause a reduction and redistribution of recombination frequencies along the X chromosome:** HODGKIN, HORVITZ and BRENNER (1979) examined recombination in *him-1*, *him-5* and *him-8* for two large intervals on the *X* chromosome and concluded that all three *him* mutants had decreased recombination on the *X*. In fact, the allele *him-8(mn253)* was originally isolated in a screen for mutations that reduce recombination on the *X* chromosome (HERMAN and KARI 1989). Only *him-5* has been seen to affect recombination dramatically in any one of six autosomal regions tested (HODGKIN, HORVITZ and BRENNER 1979). In order to determine if these reductions in *X* chromosome recombination reflect local phenomena specific to one site or a more global effect on *X* chromosome recombination, we measured exchange rates in small intervals from *unc-1* to *lin-15*. These totaled a distance of 41.8 map units (m.u.), representing 84% of the genetically defined *X* chromosome. This was initially done in *him+* hermaphrodites in order to develop an internal map for comparison (Table 2; Figure 1). We also examined recombination in several overlapping intervals and found results comparable to those presented (data not shown).

As previously reported by HODGKIN, HORVITZ and BRENNER (1979) we also saw a decrease in *X* chromosome recombination when examining hermaphrodite progeny of *him-8(e1489)*. By summing the amount of recombination in small intervals we find that the genetic map in *him-8* consists of only 12.2 m.u. from *unc-1* to *lin-15*, a threefold reduction in exchange from the wild type (Figure 1). However, the pattern of reduction is neither uniform nor random, but highly skewed from one end of the chromosome to the other. In a region near the right end of the chromosome (*unc-3 lin-15*), only 2% of the wild-type level of crossing over occurred (Figure 2). Strikingly, reduction in the frequency of crossing over becomes less severe in intervals approaching the opposite end

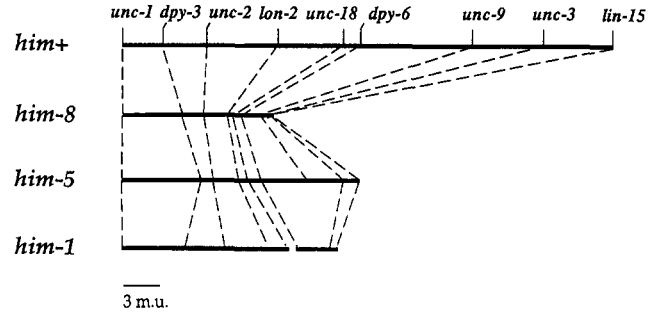

FIGURE 1.—Genetic map of the *X* chromosome in *him* and *him+* strains. Genetic maps were constructed by summing the map distances of each interval measured in each strain. Numbers of progeny counted for each interval are given in the figure legends of the corresponding graphs for each mutant. *him* alleles used were: *him-8(e1489)*, *him-5(e1490)* and *him-1(e879)*.

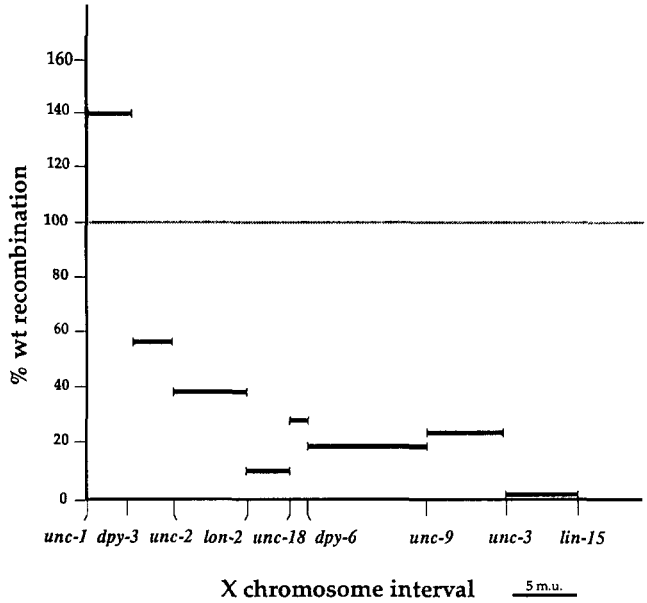

FIGURE 2.—Recombination on the *X* chromosome in *him-8(e1489)*; hermaphrodite progeny scored. In this and in Figures 3 and 4, the genes are placed along the *x* axis as determined by the recombination distance between them in non-Him strains. Horizontal lines indicate the amount of recombination in that interval as a percentage of the recombination in wild type. Thus, 100% (the dotted line) is the amount of recombination in each interval in non-mutant strains. The number of individuals scored (no. of one recombinant class/no. total progeny) for each interval is: *unc-1 dpy-3* (39/1647); *dpy-3 unc-2* (24/2295); *unc-2 lon-2* (18/1620); *lon-2 unc-18* (3/1416); *unc-18 dpy-6* (4/1602); *dpy-6 unc-9* (13/1374); *unc-9 unc-3* (3/369); *unc-3 lin-15* (1/2039).

of the chromosome, and the frequency in the most leftward interval examined, *unc-1 dpy-3*, was actually elevated above wild type. These data suggest that *him-8* mutants confer a polarity in the ability of the *X* chromosome to undergo recombination, and that the *unc-1 dpy-3* region is capable of undergoing normal or elevated rates of crossing over in the absence of *him-8* gene product.

We performed the same type of analysis with *him-5(e1490)*. The reduction in exchange was not as severe as was seen in *him-8*: the *unc-1* to *lin-15* interval was

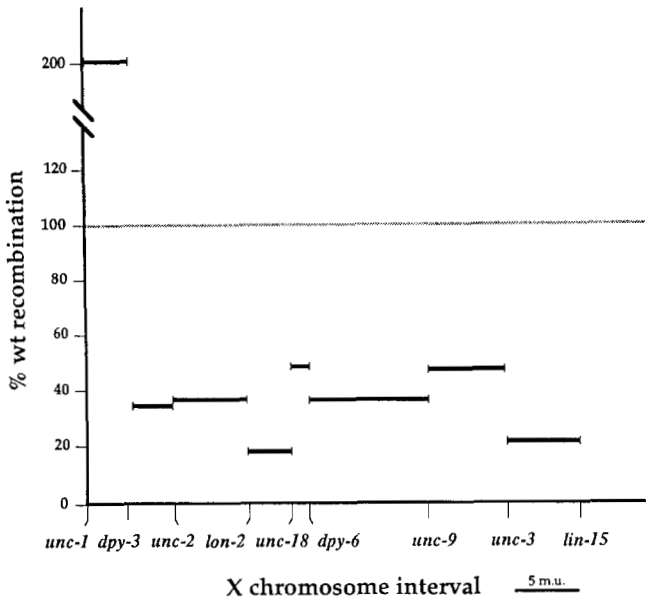

FIGURE 3.—Recombination on the *X* chromosome in *him-5(e1490)*; hermaphrodite progeny scored. The graph is explained in more detail in the legend to Figure 1. The number of individuals scored (no. of one recombinant class/no. total progeny) for each interval is: *unc-1 dpy-3* (42/1224); *dpy-3 unc-2* (4/623); *unc-2 lon-2* (13/1193); *lon-2 unc-18* (3/579); *unc-18 dpy-6* (3/759); *dpy-6 unc-9* (14/774); *unc-9 unc-3* (9/598); *unc-3 lin-15* (4/558).

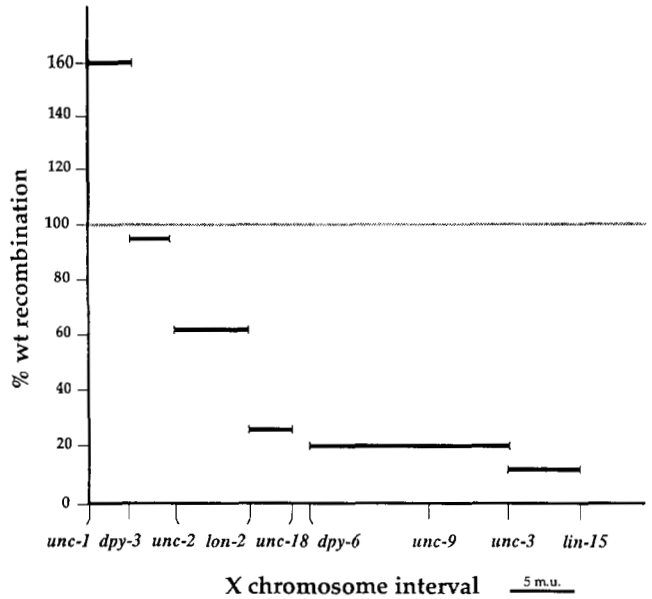

FIGURE 4.—Recombination on the *X* chromosome in *him-1(e879)*; hermaphrodite progeny scored. The graph is explained in more detail in the legend to Figure 1. The number of individuals scored (no. of one recombinant class/no. total progeny) for each interval is: *unc-1 dpy-3* (38/1371); *dpy-3 unc-2* (31/1745); *unc-2 lon-2* (31/1649); *lon-2 unc-18* (11/1609); *dpy-6 unc-3* (24/1743);*unc-3 lin-15* (8/2235).

20 m.u. in length, a twofold overall reduction (Figure 1). The distribution of crossovers, however, was qualitatively similar to that seen with *him-8(e1489)*, with a large increase in exchange in the *unc-1* to *dpy-3* interval and a reduction over the remainder of the regions tested (Figure 3).

A similar analysis of exchange in *him-1(e879)* produced a pattern strikingly similar to that seen in *him-8* and *him-5*. There is a twofold decrease in overall recombination in the region of the *X* chromosome examined (Figure 1), but the distribution of exchange in different regions is again highly skewed. The most dramatic difference between *him-1* and either *him-5* or *him-8* is the presence of nearly normal levels of exchange between *dpy-3* and *unc-2* (Figure 4). This suggests that a greater physical length of the chromosome is proficient for wild-type levels of exchange in *him-1* mutants than in the other two *him* mutants. This might account for the greater stability of the X chromosome in *him-1*, as assayed by the smaller number of males generated by non-disjunction or loss.

In the analyses above, the data were collected from hermaphrodite self-progeny, which receive an *X* chromosome from both hermaphrodite germlines. It is not possible to directly assay nondisjunction during hermaphrodite spermatogenesis. However, HODGKIN, HORVITZ and BRENNER (1979) measured *X* chromosome nondisjunction rates during spermatogenesis in sexually transformed *him-8;tra-1 XX* males. They found 11% nullo-*X* sperm produced, as compared to the 38% nullo-*X* ova formed in *him-8* hermaphrodites, and suggested that disjunction during spermatogenesis may be less affected than during oogenesis. The origin of the single *X* present in male progeny of hermaphrodites will therefore reflect the frequencies of nondisjunction or loss in each germline: higher levels of loss during oogenesis means that the majority of the *X* chromosomes found in *XO* male progeny come from spermatogenesis. When the *X* chromosomes in male *him-8* progeny were examined, the frequency of recombination was reduced to a summed map distance of 18.6 m.u., and the distribution of the remaining crossovers was similar to that seen when hermaphrodite progeny were examined (data not shown). Thus recombination is reduced to similar degrees in both germlines, but this may lead to higher levels of loss during oogenesis.

A recombinational analysis of the *X* chromosome inherited by *him-5* male progeny was also performed. Again, the reduction and pattern of exchange on the *X* chromosome in *him-5* males was very similar to that in *him-5* hermaphrodites (data not shown). Analysis by HODGKIN, HORVITZ and BRENNER (1979) had indicated that both germlines of a hermaphrodite are also likely to be affected in *him-5*. Thus in *him-5* hermaphrodites both oogenesis and spermatogenesis are similarly affected for disjunction and exchange.

In all three mutants the interval *unc-1* to *dpy-3* undergoes elevated levels of recombination. This elevation could be due in part to a suppression of exchange in the remainder of the chromosome. However, there is an overall decrease in exchange on the

X chromosome, as well as an alteration in distribution. Since this region of the chromosome is proficient for at least wild-type levels of recombination in the absence of the wild-type *him* gene products, it suggests that the products of *him-1*, *him-5* and *him-8* may not normally function in this region, but may perhaps be necessary for extension of the ability to undergo recombination to the remainder of the *X* chromosome (discussed below).

The severe reduction in total map distance seen in the *him* mutants suggests frequent recovery of nonrecombinant chromosomes: To generate the extreme compression of the genetic map observed in the *him* mutants, particularly in *him-8*, one of two things must be occurring. Either nonrecombinant chromosomes must be recovered the majority of the time, or significant increases in recombination must be occurring in regions not examined at the ends of *X* chromosome. Unfortunately, at this time it is not possible to distinguish between these two possibilities as few useful genetic markers map to the ends of the *X* chromosome. Efficient recovery of nonrecombinant chromosomes may suggest the presence of a secondary disjunction system, such as the distributive disjunction system first reported by GRELL (1976) that routinely segregates the nonrecombinant fourth chromosome in Drosophila. Evidence from other studies (discussed below) suggest that a similar system may also function in *C. elegans*.

DISCUSSION

Many mutations that alter exchange during meiosis have been isolated and examined in a variety of organisms [for reviews see BAKER *et al.* (1976) and HAWLEY 1988). We find that mutations in the genes *him-1*, *him-5* and *him-8* in *C. elegans* reduce recombination on the *X* chromosome in a way that is neither uniform nor random, but polar with respect to the chromosome. In all three *him* mutants, recombination occurs at normal or elevated levels in the region of the *X* chromosome thought to be involved in pairing, but at reduced levels elsewhere on the *X* chromosome. Some meiotic mutants in Drosophila alter the probability of exchange along a chromosomal arm (BAKER and CARPENTER 1972; reviewed in BAKER *et al.* 1976). Such mutants were called "precondition mutants" by SANDLER *et al.* (1968) and were hypothesized to establish the ability of chromosomal regions to undergo exchange.

The patterns of recombination seen in *him-1*, *him-5* and *him-8* mutants suggest that in *C. elegans* the *X* chromosome can be divided into two general domains. One region, defined by the region *unc-1* to *dpy-3* [hereafter called the "*unc-1* region"], can undergo at least normal levels of exchange in the absence of normal levels of *him* wild-type gene products. The remainder of the *X* chromosome, however, requires all of these gene products for correct level and spatial distribution of crossover events. In *him-1* and *him-8* mutants recombination decreases progressively in intervals farther away from the *unc-1* region. This skewed pattern of recombination might help to reveal the mechanism of chromosome pairing and exchange for the *X* chromosome in *C. elegans*. The data suggest an inability to propagate some process out of the *unc-1* region into the remainder of the chromosome.

The *unc-1* region of the chromosome has elevated levels of crossing over in the *him* mutant backgrounds we tested, and may contain a pairing site (HERMAN, KARI and HARTMAN 1982; HERMAN and KARI 1989) or homolog recognition region (ROSE and MCKIM 1992). These studies demonstrated that it is necessary to have the *unc-1* region in *cis* for correct disjunction and correct exchange anywhere along the *X* chromosome, and that when a third unlinked copy of this region is present *X* chromosome disjunction is disrupted. For example, a small duplication of the region in a hermaphrodite competes with the two normal *X* chromosomes for a pairing partner, recombines at near wild-type rates, and excludes one normal homolog from the pair, causing *X* chromosome loss (HERMAN and KARI 1989). Duplications of other regions of the *X* chromosome do not recombine at high levels with the chromosome and do not cause nondisjunction, prompting HERMAN and KARI (1989) to suggest that the *unc-1* region contains one or more sites necessary for *X* chromosome pairing. It should be emphasized that while the region around *unc-1* resides near one end of the chromosome as defined by the genetic map, the physical end of the chromosome has not been located. Indeed several genes map to the left of *unc-1*, suggesting that the physical end may not be immediate.

The phenomenon of one chromosomal region being necessary for exchange over the remainder of the chromosome is not unique to the *X* chromosome of *C. elegans*. While studies of the autosomes have not yet defined as small a region, it is clear that homolog recognition regions are asymmetrically localized (reviewed by ROSE and MCKIM 1992; MCKIM, PETERS and ROSE 1993). Thus the overall mechanism of pairing might be similar for the *X* chromosome and the autosomes. However, the *X* chromosome pairing and recombination process may require the additional wild-type gene products of *him-1*, *him-5* and *him-8*. It has also been demonstrated in other species that certain regions of the genome are specifically involved in initiating genetic exchange. In yeast a 7.5-kb chromosomal fragment increases nondisjunction of its chromosomal cognates, presumably by competing for pairing (GOLDWAY *et al.* 1993). This fragment contains a "hot spot" for meiotic recombination and a

strong double strand break (DSB) site. GOLDWAY *et al.* (1993) suggest that DSB sites might serve as pairing sites for homologs during meiosis. Studies in Drosophila suggest that a chromosome might contain multiple pairing sites along its length (HAWLEY 1980). Whether these sites are functionally homologous to those defined in yeast and implicated in worms is as yet unknown.

The work of HERMAN and ROSE indicates that recombination along the majority of the *X* chromosome depends upon a small region near *unc-1*. Our work suggests that when *him-1*, *him-5* or *him-8* are mutant, this region can still pair and recombine, while the remainder of the chromosome is affected. Taken together, the data lead us to suggest that these *him* gene products are not necessary for the process that initiates in the *unc-1* region, but are essential for processivity or extension of this process into the majority of the X chromosome. Several testable predictions for *X*-specific *him* function can be constructed based on this hypothesis.

Models for *X*-specific him function

***him* mutations might affect synaptonemal complex extension or function:** In many organisms formation of a synaptonemal complex (SC) correlates with meiotic recombination (reviewed in VON WETTSTEIN, RASMUSSEN and HOLM 1984), and many meiotic mutants cause both recombination and the SC to be aberrant (SMITH and KING 1968; ALANI, PADMORE and KLECKNER 1990; ENGEBRECHT, HIRSCH and ROEDER 1990). ENGEBRECHT, HIRSCH and ROEDER (1990) have postulated that only those exchange events that occur in the context of a synaptonemal complex lead to efficient disjunction. In *C. elegans* the synaptonemal complex may initiate in a region localized near the left end of the *X* chromosome independent of the *him* genes described here, but the products of these genes may be necessary for extension into the remainder of the *X* chromosome. If a functional SC very rarely extended into the right end of the chromosome, then few exchange events involving the right end would be recovered, giving the impression that recombination occurred only at low levels in that region. This model suggests that *him-8* is involved in formation or extension of the SC, and that the SC, for the *X* chromosome at least, should be aberrant or absent in the mutant.

GOLDSTEIN (1982) investigated the SCs in *him-8* mutants by serial sectioning the gonad and forming three-dimensional reconstructions of four nuclei. In *C. elegans* the *X* chromosome is not cytologically distinct and so a numerical test must be applied. He found six apparently normal SCs, even though *him-8* has dramatic effects on recombination. It is possible that GOLDSTEIN examined the SC during a phase of pachytene when the defect was not apparent. It is also possible that *him-1*, *him-5* and *him-8* are not structural components of the SC, but might instead regulate its function.

***X*-specific *him* genes might be involved in chromatin structure:** Many organisms globally control recombination during meiosis to coordinate exchange throughout the genome (HAWLEY 1988; CARPENTER 1988). This is evident by the nonrandom distribution of exchange over chromosomes, and the non-uniform relationship between map units and kilobases in different regions of the genome (SZAUTER 1984; LEFEVRE 1971; SYMINGTON and PETES 1988; MORTIMER and SCHILD 1985) In *C. elegans* chromosome wide control of recombination is evident by an exclusion of exchange from the central region of each autosome, but not the *X* chromosome, leading to a tight genetic cluster of 3–5 m.u. that encompasses the majority of the genes identified (BRENNER 1974; GREENWALD *et al.* 1987; STARR *et al.* 1989; EDGLEY and RIDDLE 1990).

An alternative possibility for the role of *him-1*, *him-5* and *him-8* gene products is that they are involved in generating domains of chromatin that are differentially receptive to recombination. This model is analogous to the way some transcription factors alter transcriptional rates by modulating chromatin structure (WINSTON and CARLSON 1992). Unlike the autosomes the *X* chromosome in *C. elegans* is distinguished by the absence of a genetically defined cluster. The coordination of recombination events to generate the more uniform genetic map characteristic of the *X* chromosome might be due to the gene products of the *X*-specific *him* genes, *him-1*, *him-5* and *him-8*. Indeed, when *him-1*, *him-5* or *him-8* is mutant, the *X* chromosome assumes the tight cluster of genes typical of an autosome, the cluster occurring at the end opposite the hypothesized initiation site near the *unc-1* region (Figure 1). On the autosomes, however, the cluster is more centrally located, perhaps because processive events affecting recombination initiate at both ends of an autosome instead of only one, as on the *X* chromosome. This may also explain the observation that an autosome can undergo double crossovers while the *X* chromosome in a hermaphrodite apparently undergoes only single events (HODGKIN, HORVITZ and BRENNER 1979). The presence of a cluster distinguishes the *X*-specific *him* mutants from Drosophila mutants that alter the distribution of exchange by creating a more uniform map such as is seen after irradiation (BAKER and CARPENTER 1972). Thus the Drosophila mutants seem to relax the constraints on exchange, while *him-1*, *him-5* and *him-8* alter them, or reveal underlying constraints.

The asymmetry of effect in the absence of *him* function suggests a polar localization of *him* gene product. Elucidating the specific process affected will

require molecular analysis of the genes and localization of the gene products.

Evidence for distributive disjunction in *C. elegans*: Our analysis of recombination on the *X* chromosome in *C. elegans* suggests many nonrecombinant chromosomes are faithfully transmitted. This may occur in both germlines of a hermaphrodite and perhaps more frequently during spermatogenesis. We speculate that meiosis in *C. elegans* might employ a mechanism for disjoining nonrecombinant *X* chromosomes, such as a distributive disjunction system (GRELL 1976). None of the *X*-specific *him* mutants affects transmission of the *X* during male spermatogenesis, suggesting that the *X* chromosome in males uses some other mechanism for disjunction (HODGKIN, HORVITZ and BRENNER 1979). HERMAN and KARI (1989) showed that *him-8* functions during male spermatogenesis by examining recombination between duplications and the *X* chromosome. *him-8* again appears to decrease and redistribute recombination between the *X* chromosome and a homologous duplication, but as during hermaphrodite spermatogenesis, this does not lead to increased nondisjunction or loss of nonrecombinants. Finally, HERMAN, MADL and KARI (1979) and ROSE, BAILLIE and CURRAN (1984) showed that in males free autosomal duplications tend to segregate from the single *X* chromosome, demonstrating that homology is not necessary for pairing as is typical of the distributive disjunction system in Drosophila (reviewed by GRELL 1976).

Evolutionary implications of *X* chromosome specific meiotic functions: The meiotic control of the *X* chromosome in *C. elegans* appears to be at least partially independent of the autosomes, as evidenced by greater frequency of loss of the *X* chromosome in wild-type strains (see MATERIALS AND METHODS), the existence of *X* chromosome-specific meiotic mutations, the lack of a genetic cluster, and the presence of complete interference over most of its length in hermaphrodites. *C. elegans* is a facultative hermaphrodite, and when male sperm are present they are preferentially used, yielding 50% male outcross progeny. However, in the absence of male sperm oocytes are self-fertilized and generate almost 100% hermaphrodite progeny. Thus even in the best conditions males are not maintained at high levels in a wild-type population. Outcrossing generates new allelic combinations and distributes alleles more rapidly through a population than self-crossing. Thus, *X* chromosome-specific meiotic processes that are less efficient than autosomal processes might have evolved in hermaphroditic species due to the benefit accrued from specifically generating male progeny via *X*-specific loss during self-fertilization. *X* chromosome-specific components of a meiotic system might be absent or have assumed different functions (*i.e.*, no longer be *X* chromosome specific) in related nematode species that are dioecious and have a more typical 1:1 sex ratio. With the molecular probes being generated in our laboratory, this hypothesis should now be testable.

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