A *Cis*-Acting Locus That Promotes Crossing Over Between *X* Chromosomes in *Caenorhabditis elegans*

Anne M. Villeneuve

Department of Developmental Biology, Stanford University School of Medicine, Stanford, California 94305

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ABSTRACT

This study reports the characterization of a *cis*-acting locus on the *Caenorhabditis elegans X* chromosome that is crucial for promoting normal levels of crossing over specifically between the *X* homologs and for ensuring their proper disjunction at meiosis I. The function of this locus is disrupted by the mutation *me8*, which maps to the extreme left end of the *X* chromosome within the region previously implicated by studies of *X;A* translocations and *X* duplications to contain a meiotic pairing site. Hermaphrodites homozygous for a deletion of the locus (*Df/Df*) or heterozygous for a deletion and the *me8* mutation (*me8/Df*) exhibit extremely high levels of *X* chromosome nondisjunction at the reductional division; this is correlated with a sharp decrease in crossing over between the *X* homologs as evidenced both by reductions in genetic map distances and by the presence of achiasmate chromosomes in cytological preparations of oocyte nuclei. Duplications of the wild-type region that are unlinked to the *X* chromosome cannot complement the recombination and disjunction defects in *trans*, indicating that this region must be present in *cis* to the *X* chromosome to ensure normal levels of crossing over and proper homolog disjunction. *me8* homozygotes exhibit an altered distribution of crossovers along the *X* chromosome that suggests a defect in processivity along the *X* chromosome of an event that initiates at the chromosome end. Models are discussed in which the *cis*-acting locus deleted by the *Df*s functions as a meiotic pairing center that recruits *trans*-acting factors onto the chromosomes to nucleate assembly of a crossover-competent complex between the *X* homologs. This pairing center might function in the process of homolog recognition, or in the initiation of homologous synapsis.

THE reductional division of meiosis is characterized by three landmark events: pairing and synapsis of homologous chromosomes, crossing over between homologs, and disjunction, the separation of homologs to opposite poles of the first meiotic spindle. Both cytological and genetic data from a wide variety of systems indicate that, in the general case, pairing and crossing over between homologs are required to ensure their proper disjunction at meiosis I (BAKER *et al.* 1976; HAWLEY 1988). Chiasmata, the cytologically visible manifestations of crossovers (JONES 1987), apparently serve as mechanical links that hold homologs together until anaphase I and ensure that homologs become oriented toward opposite spindle poles (NICKLAS 1974).

The pairing of homologous chromosomes during meiotic prophase can be broken down conceptually into two critical tasks. First, chromosomes must recognize their appropriate homologous pairing partners. Then, having identified their partners, the homologs must become physically aligned in a configuration that is productive for the formation of functional chiasmata.

The mechanistic basis of homolog recognition remains a major unsolved mystery, although many current models incorporate the idea that the DNA sequence of the chromosomes provides the information necessary for recognition (CARPENTER 1987; ROEDER 1990; KLECKNER *et al.* 1991; HAWLEY and ARBEL 1993). Identification of pairing partners, presumably through a search for DNA homology, leads to a presynaptic alignment of homologous chromosomes which has been observed in some systems as a parallel arrangement of homologs at a distance with some regions in closer association (LOIDL 1990; SCHERTHAN *et al.* 1992). Ultimately, homologs are brought into intimate contact, or synapsis, along their entire lengths by a highly ordered proteinaceous structure with a ribbon-like appearance known as the synaptonemal complex (SC) (VON WETTSTEIN *et al.* 1984).

Chromosome synapsis and recombination are intimately interrelated processes. A view that is emerging, largely from studies in the yeast *Saccharomyces cerevisiae* (HAWLEY and ARBEL 1993), is that initial pairing and prealignment of homologs is followed by the initiation of recombination by double-strand breaks in the DNA, which precedes and appears to be a prerequisite for homologous synapsis (ALANI *et al.* 1990; CAO *et al.* 1990; PADMORE *et al.* 1991). Mature recombinant molecules are first detected upon disassembly of the SC; maturation of recombination intermediates in the context of SC leads to the formation of functional chiasmata that are capable of ensuring homolog disjunction (ENGEBRECHT *et al.* 1990; ROCKMILL and ROEDER 1990; MAGUIRE 1978).

These processes of homolog recognition and chromosome synapsis clearly involve the activities of *trans*-acting protein factors that interact physically with the

chromosomes to bring about a productive pairing interaction between homologs. Are there specialized *cis*-acting chromosomal regions, so-called "pairing centers" or "pairing sites" through which these *trans*-acting factors exert their effects?

The existence of *cis*-acting homolog pairing centers has been inferred from genetic and cytological studies examining the effects of chromosome translocations and duplications on the formation of crossovers. For example, the pattern of crossover suppression in translocation heterozygotes provided evidence for the presence of four major pairing centers on the *Drosophila melanogaster X* chromosome (HAWLEY 1980). Further, cytological studies examining the frequency of chiasma formation in rearranged chromosome segments in maize suggest that maize chromosomes each have multiple specialized meiotic pairing centers, widely but unevenly dispersed along the length of the chromosomes (MAGUIRE 1986). Perhaps the most extensive studies of this type have been carried out with the nematode *Caenorhabditis elegans* (ROSENBLUTH and BAILLIE 1981; HERMAN *et al.* 1982; ROSE *et al.* 1984; MCKIM *et al.* 1988, 1993; HERMAN and KARI 1989) The accumulated data support a model proposing that each of the six *C. elegans* chromosomes has a single primary meiotic pairing center located near one end that is important for promoting homolog pairing and recombination (MCKIM *et al.* 1988)

How do such *cis*-acting pairing centers function to promote pairing and crossing over between homologous chromosomes during meiosis, thereby ensuring their disjunction? To address this question, it would be instructive to explore the consequences of loss of pairing center activity in the context of normal sequence chromosomes. The apparent concentration of pairing center activity to a single region of each chromosome makes *C. elegans* especially well suited for this purpose, since deletion or disruption of a pairing center might have much more severe consequences for chromosomes with only one such locus than for chromosomes with multiple interspersed pairing centers.

In this study, I report the characterization of a *cis*-acting locus on the *C. elegans X* chromosome that may correspond to the proposed *X* chromosome meiotic pairing center. The properties of hermaphrodites homozygous for deletions of the region indicate that the locus is crucial for promoting normal levels of crossing over along the length of the *X* chromosome, consistent with a role in homolog pairing. Further, the data suggest that an event initiates at or near the locus and propagates along the length of the chromosome in a polar fashion. The behavior of chromosomes deleted for the *cis*-acting locus argues against a class of models proposing that the information content for homolog recognition is restricted to this region of the chromosome, and suggests instead that the chromosomes may utilize the extended DNA homology shared along their lengths to identify meiotic pairing partners. The data presented here, together with previous genetic and cytological data, are best explained by models in which the meiotic pairing center at the left end of the *X* chromosome promotes the initiation of chromosome synapsis, perhaps by facilitating the process of homolog recognition.

MATERIALS AND METHODS

Strains and maintenance: General methods for culturing *C. elegans* strains were as described by BRENNER (1974). All experiments were performed at 20°. Bristol strain N2 is the wild-type strain from which all strains were derived. Nomenclature follows HORVITZ *et al.* (1979). Abbreviations are as follows: *dpy* (*dum*py), *egl* (*egg*-laying defective), *lin* (*lin*eage abnormal), *lon* (*lon*g), *unc* (*unc*oordinated), *Df* (*de*ficiency), *Dp* (*du*plication), *T* (*t*ranslocation). The following previously isolated recessive mutations and chromosome aberrations were used in the present study:

LGIII: unc-32(e189).

LGV: unc-42(e270).

LGX: egl-17(e1313), unc-1(e528 and *e1598n1201), dpy-3(e27), unc-2(e55), lon-2(e678), dpy-6(e14), unc-3(e151), lin-15(n765).*

Chromosome aberrations: szT1(X;I), mnDp66 (X;I), mnDp69(X;I), stDp2,(X;II), mnDp1(X;V), syDf1, yDf5, nDf19, mnDf1.

mnDp66 and *mnDp69* are described in HERMAN and KARI (1989), and *yDf5* is described in MILLER *et al.* (1988). *syDf1* was a gift from WENDY KATZ. The remainder of the genes and chromosome aberrations can be found in HODGKIN *et al.* (1988).

Isolation and mapping of the *me8* mutation: The *me8* mutation was isolated in a general screen for mutants with increased levels of meiotic chromosome nondisjunction. The results of the screen will be reported elsewhere. Briefly, N2 L4 hermaphrodites were treated with 40 mM ethyl methanesulfonate (EMS) (BRENNER 1974) and were plated individually onto seeded 60-mm plates and allowed to produce progeny. Ten F_1 L4 hermaphrodites from each P_0 were transferred to a fresh plate, and subsequently individual F_2 L4 hermaphrodites (10 from each F_1 plate) were picked to separate plates. These plates were screened for an increased frequency of males [the *h*igh *i*ncidence of *m*ales (Him) phenotype] in the F_3 broods. One mutation at the *me8* locus was identified in a screen of 5400 F_2 clones. In addition to the defects in recombination and meiotic chromosome segregation that are the focus of this paper, *me8* animals have a slower than wild-type growth rate and *me8* males mate less efficiently than wild-type males.

Map data for *me8* are summarized in Table 1. Mapping was carried out as in BRENNER 1974.

Isolation of *Df*s of the *me8* region: *meDf2, meDf3* and *meDf5* were identified in a screen for deficiencies (*Df*s) that failed to complement *unc-1(e1598n1201).* N2 L4 males were mutagenized with 4000 r of γ-irradiation and crossed with *unc-1 dpy-3* hermaphrodites. Candidate *Df* chromosomes were identified by screening for Unc non-Dpy hermaphrodites among F_1 cross progeny; these were picked to separate plates and allowed to produce self-progeny. If the mutagenized *X* chromosome could not be homozygosed, Unc non-Dpy hermaphrodites were crossed with *egl-17* males. If approximately half of the hermaphrodite cross progeny exhibited the Egl phenotype, it was inferred that the hermaphrodite parent was heterozygous for a *Df* that deletes both *unc-1* and *egl-17.* [Failure to complement *egl-17* was also assayed using polarized

TABLE 1

Mapping data for *me8*

Genotype of heterozygote tested	Recombinant type selected	No. of homozygous recombinants that were Him/total
+dpy-3 unc-3/me8++	Unc non-Dpy	3/3
	Dpy non-Unc	0/7
+unc-1 dpy-3/me8++	Dpy non-Unc	6/6
	Unc non-Dpy	0/12
(+egl-17) unc-1/(me8+)+[a]	Unc non-Egl	8/8
	Egl non-Unc	0/4

[a] Parentheses indicate that the relative map order of *me8* and *egl-17* is not known.

light microscopy to score for the absence of vulval muscles, a phenotype diagnostic of the *egl-17* locus (STERN and HORVITZ 1991).] Four *Df*s (*meDf2, 3, 4* and *5*) and one *Df*-translocation [*meT1(X;V)*] were identified among approximately 19,000 chromosomes screened.

meDf6 was identified in a screen for *Df*s that failed to complement *dpy-3(e27)*. N2 males were mutagenized as before, crossed with *dpy-3(e27) unc-2(e55)* hermaphrodites, and the F_1 progeny were screened for Dpy non-Unc hermaphrodites. One *Df* (*meDf6*) and one *Df*-translocation [*meT4(X;V)*] that fail to complement both *dpy-3* and *egl-17* were identified among approximately 69,000 chromosomes screened.

meT1 and *meT4* heterozygotes both produced unusually high numbers of dead embryos and self-progeny males, suggesting that the deficiencies were linked to translocations. These were shown to carry translocations by demonstrating linkage of the Him phenotype and lethality to both the *X* chromosome and chromosome *V*, and by cytological analysis of oocyte chromosomes (see below) in *meT1* and *meT4* heterozygotes. In both cases, oocytes had either: (1) four normal-sized bivalents, one large asymmetric bivalent and one univalent, or (2) four normal-sized bivalents and one large trivalent.

Progeny counts: The frequencies of *XX* (hermaphrodite), *XO* (male) and *XXX* (morphologically distinct Semi-Dpy hermaphrodite) progeny produced by hermaphrodites of a given genotype were determined by scoring the complete broods of hermaphrodites plated individually at the L4 stage. Parents were transferred daily to facilitate scoring, and progeny were scored as L4s or adults.

When the parental genotype was of the form *+/Df* or *me8/Df*, the numbers presented in Table 2 were adjusted according to the formula [4/3(no. of *XX*) + 2(no. of *XO*) + (no. of *XXX*) = total] to compensate for the inviability of *Df* homozygotes and hemizygotes. Similarly, when the parental genotype was of the form *Dp/+; +/Df* or *Dp/+; me8/Df*, the numbers were adjusted according to the formula [16/15(no. of *XX*) + 8/7(no. of *XO*) + (no. of *XXX*) = total] to compensate for the inviability of *Df* homozygotes and hemizygotes that lack the complementing *Dp*.

Only those parts of the genotypes pertaining to the *me8* mutation and *Df*s of the region are shown in Table 2; in many cases hermaphrodites were heterozygous for chromosomes that also carried additional morphological markers, as listed below. Data from congenic strains that carried different morphological markers were pooled.

+/+: Bristol strain N2, *++/dpy-3 unc-3.*

+/me8: +++/me8 unc-1 dpy-3, +++/me8 dpy-3 unc-2, +++/me8 unc-2 dpy-6, +++/me8 dpy-3 unc-3, unc-1 dpy-3/me8 dpy-3.

me8/me8: me8/me8, me8/me8 unc-1 dpy-3, me8/me8 dpy-3 unc-2, me8/me8 unc-2 dpy-6, me8/me8 dpy-3 unc-3.

+/meDf2,5: unc-1 dpy-3/Df.

+/meDf6: dpy-3 unc-2/meDf6.

mnDp66/+; +/Df: mnDp66/+; unc-1/Df, mnDp66/+; unc-2 dpy-6/Df.

mnDp69/+; +/Df: mnDp69/+; unc-1/Df.

me8/Df: me8/Df, me8 unc-1 dpy-3/Df.

Dp/+; me8/Df: Dp/+; me8 unc-1/Df, mnDp66/+; me8 dpy-3 unc-2/meDf2, mnDp66/+; me8 unc-2 dpy-6/meDf2, mnDp66/+; me8 dpy-6 unc-3/meDf2, mnDp66/+; me8 dpy-3 unc-3/meDf2, mnDp69/+; me8 dpy-3 unc-2/meDf5, mnDp69/+; me8 unc-2 dpy-6/meDf5, mnDp69/+; me8 dpy-6 unc-3/meDf5.

Dp; Df: Dp; Df, mnDp66; Df unc2 dpy-6/Df, mnDp66; Df dpy-6 unc-3/Df.

Demonstration that the *Dp*s contain a wild-type copy of the *me8* region: Although the *Dp*s used in these experiments do not recombine with wild-type *X* chromosomes in hermaphrodites at an appreciable frequency (HERMAN and KARI 1989), crossing over between the *Dp*s and a *me8* chromosome in the *unc-1 dpy-3* interval was observed in the germlines of *Dp/+; me8/Df* hermaphrodites. *X* chromosomes resulting from a recombination event between a *Dp* and a *me8* chromosome were identified in two ways.

First, hermaphrodites carrying an *X* chromosome resulting from a crossover event between the *me8 unc-1 dpy-3* chromosome and *mnDp69* in the *unc-1 dpy-3* interval were sought among the phenotypically wild-type hermaphrodite self progeny (plated individually at the L4 stage) of *mnDp69/+; me8 unc-1 dpy-3/meDf5* hermaphrodites. The genotypes of these hermaphrodites were determined by progeny testing, which revealed that most were of the expected nonrecombinant genotypes: *Dp/(Dp or +); Df/Df* (high frequency of males, all wild-type); *Dp/+; me8 unc-1 dpy-3/Df* (high frequency of males, wild-type, Dpy, and Dpy Unc); or *Dp/Dp; me8 unc-1 dpy-3/Df* (high frequency of males, wild-type and Dpy). Two hermaphrodites produced broods with exceptional compositions indicating that they carried chromosomes arising from the desired recombination event: one gave a few percent males, both wild-type and Dpy, indicating that its genotype was *Dp/(Dp or +); ++dpy-3/Df*; one gave a few percent Dpy males and no wild-type males, indicating that its genotype was *++dpy-3/Df*. Wild-type and Dpy hermaphrodite progeny were picked to verify the above inferred genotypes. Dpy hermaphrodites homozygous for these recombinant chromosomes produced large broods with <0.2% males, confirming that the recombinant chromosomes have the segregation properties of wild-type *X* chromosomes and indicating that *mnDp69* contains a wild-type copy of the region.

The frequency of recombination between the *mnDp69* and the *X* chromosome in the *unc-1 dpy-3* interval in these experiments was estimated both from the frequency of *++dpy-3* chromosomes in the above experiment as well as from the frequency of Unc non-Dpy males produced by *mnDp69/+; me8 unc-1 dpy-3/meDf5* hermaphrodites. Since Unc non-Dpy males are extremely rare among the self-progeny of *me8 unc-1 dpy-3/Df* hermaphrodites that lack the *Dp* (0 Unc non-Dpy/959 Unc Dpy males), the Unc non-Dpy male progeny of *mnDp69/+; me8 unc-1 dpy-3/meDf5* hermaphrodites are inferred to be *mnDp69[me8 unc-1]/+; meDf5/0.*, That is, they carry a *Dp* that is the reciprocal recombination product of the *++dpy-3 X* chromosome. The recombination frequency is equal to the frequency of recombinant *Dp*s, which was calculated as 3/2(Unc non-Dpy males)/total non-Dpy males. Combined data (11/283) yield a genetic map distance of 3.9 cM. This may be an overestimate, since recombinant *Dp*s may tend to segregate away from *X* chromosomes and thus be more prevalent among nullo-*X* than haplo-*X* gametes.

Second, hermaphrodites carrying an *X* chromosome resulting from a crossover event between the *me8 unc-1* chromosome and *mnDp66* in the *unc-1 dpy-3* interval were sought among the phenotypically wild-type hermaphrodite self progeny (plated individually at the L4 stage) of *mnDp66/+; me8 unc-1/meDf3* hermaphrodites. Progeny testing of these hermaphrodites revealed that most were of the expected nonrecombinant genotypes: *Dp/(Dp or +); Df/Df* or *Dp/Dp; me8 unc-1/Df* (high frequency of males, all wild-type); *Dp/+; me8 unc-1/Df* (high frequency of males, wild-type and Unc); *Dp/Dp; me8 unc-1/me8 unc-1* (intermediate male frequency, all wild-type); *Dp/+; me8 unc-1/me8 unc-1* (intermediate male frequency, wild-type and Unc). Two produced broods with exceptional constitutions indicating that they carried chromosomes arising from the desired recombination event: One gave a few percent males, all wild-type, and was inferred to be of genotype *Dp/(Dp* or +); *++/Df*; one gave only 1% males, both Unc and wild-type, and was inferred to be *(Dp or +)/+; me8 unc-1/++*. (In both cases, genotype assignments were confirmed by picking and progeny testing hermaphrodites in subsequent generations.) Hermaphrodites homozygous for these recombinant *X* chromosomes produced large broods with approximately 0.1% males, indicating that *mnDp66* had contained a wild-type copy of the region.

Measuring *X* chromosome genetic recombination frequencies: Strains carrying the *me8* mutation or a *Df* in *cis* to two *X*-linked morphological markers were constructed using standard methods. Heterozygous hermaphrodites were generated by crossing unmarked males (*e.g.*, N2, *me8*, or *Dp/Dp; Df*) with the appropriate doubly marked hermaphrodites; heterozygotes were picked individually and transferred daily to fresh plates. Complete broods were scored, and all classes of progeny were counted. Comparable numbers of the two reciprocal recombinant types were observed in each experiment. To calculate the genetic map distances presented in Table 4 and Table 7, only *XX* hermaphrodite progeny were considered. Map distances are given in centimorgans (cM); map distance = 100 × recombination frequency (p), with p calculated from the formula $p = 1 - \sqrt{(1 - 2R)}$, where R = the fraction of progeny exhibiting a recombinant phenotype (BRENNER 1974). When the heterozygote genotype was of the form *Dp/+; me8/Df*, the recombinant fraction R was calculated according to the formula: no. of phenotypically recombinant hermaphrodites/[(16/15) total no. of hermaphrodites] to compensate for the inviability of *Df* homozygotes that lack the complementing *Dp*. (Because the doubly marked chromosome in all such cases was the *me8* chromosome and not the *Df* chromosome, only those extremely rare individuals that had received two recombinant chromosomes and lacked a *Dp* would be missing from the phenotypic recombinant class; this number is negligible and may be omitted from the calculation.)

Table 9 presents genetic map distances calculated from scoring male progeny; in this case, $p = R$.

Staining oocyte chromosomes: Meiotic chromosomes were fixed and stained with DAPI (diamidinophenolindole) using a modification of the method of FIXSEN (1985). Worms were picked to a minimal volume of M9 buffer (SULSTON and HODGKIN 1988) on a microscope slide and excess liquid was wicked away. Whole worms were fixed by dropping 2–3 drops of Carnoy's fixative (6 ethanol:3 chloroform:1 glacial acetic acid) onto the slide from a height of 2 inches; slides were allowed to air dry. Seven to ten microliters of staining solution (2 μg/ml DAPI, 2 μg/ml phenoxypropanol in M9) were added to the sample and covered with a coverslip. Alternatively, in a few cases chromosomes in unfixed tissues were stained with Hoechst 33342 as follows. Anterior and posterior gonad arms were extruded from adult hermaphrodites by cutting just posterior to the posterior bulb of the pharynx and just anterior to the anus; dissection was carried out on slides directly in the staining solution (100 μg/ml Hoechst 33342 in sperm medium salts) (NELSON *et al.* 1982). For unfixed preparations, coverslips were supported by two thin stripes of petroleum jelly to prevent compression of the tissue. Stained chromosomes were visualized using a Zeiss Axioplan microscope equipped for fluorescence microscopy; Kodak TMY400 film was used for photography.

Determining the frequencies of nullo-*X* and diplo-*X* ova: The frequencies of nullo-*X* and diplo-*X* ova were determined by crossing N2 males with hermaphrodites homozygous or hemizygous for recessive *X* chromosome markers. Cross progeny that had received a paternally derived *X* chromosome were identified by their wild-type (non-marker) phenotype. The frequencies of *X*, O and *XX* ova produced are equal to the frequencies of *XX* hermaphrodites, *XO* males and *XXX* hermaphrodites among wild-type cross progeny. Hermaphrodites from the marked strains *unc-1 dpy-3/meDf5*, *me8 unc-1 dpy-3*, *me8 unc-1 dpy-3/meDf2,5*, and *mnDp66; meDf2,3,5 dpy-6 unc-3* were used for these experiments.

Determining the genotypes of diplo-*X* ova: The genotypes of ova carrying two nondisjoined *X* chromosomes were determined by progeny testing *XXX* individuals resulting from crosses between N2 males and marked *XX* hermaphrodites.

First, N2 males were crossed with *me8 unc-1 dpy-3/Df* hermaphrodites; *XXX* cross progeny were picked to separate plates and progeny tested to determine which *X* chromosome markers were present in the diplo-*X* ova from which they had arisen. Unc Dpy progeny were diagnostic of the *unc-1* and *dpy-3* markers, and Unc non-Dpy progeny suggested that the *Df* was also present. These Unc non-Dpy progeny were picked to separate plates to verify their suspected genotype (*me8 unc-1 dpy-3/Df*) by progeny testing: animals of this genotype will give >1/4 dead embryos and a high frequency of Unc Dpy males but no Unc non-Dpy males. 124/127 *XX* ova tested in this way proved to be heterozygous for the *Df* and *me8 unc-1 dpy-3*; 2/127 were homozygous for the *Df* and 1/127 was homozygous for *me8 unc-1 dpy-3*.

The *lin-15* marker, which maps approximately 40–45 cM from *dpy-3*, was included on the *me8 unc-1 dpy-3* chromosome in the parent for 42 of the 127 ova tested. All ova that were heterozygous for the *Df* and *me8 unc-1 dpy-3* (41/42) were also heterozygous for *lin-15*, consistent with the chromosomes being nonrecombinant. The 1/42 that was homozygous for the *Df* was heterozygous for *lin-15*, indicating that the *X* chromosomes had recombined.

Second, N2 males were crossed with *Dp/Dp; Df unc-2 dpy-6/Df unc-2* hermaphrodites; *XXX* cross progeny were picked to separate plates and progeny tested to determine whether the diplo-*X* ova were heterozygous or homozygous at the *dpy-6* locus. The presence of both Unc non-Dpy and Unc Dpy progeny, with Unc non-Dpy > Unc Dpy, is indicative of ova that were *dpy-6/+*. The presence of Unc but no Dpy progeny is indicative of ova that were *+/+*. The presence of both Unc non-Dpy and Unc Dpy progeny, with Unc non-Dpy ≪ Unc Dpy, is indicative of ova that were *dpy-6/dpy-6* (Unc non-Dpys in this case result from recombination with a wild-type chromosome in the *XXX* germline). 44/49 ova tested were heterozygous at the *dpy-6* locus (*dpy-6/+*); 2/49 were *dpy-6/dpy-6*, and 3/49 were *+/+*.

RESULTS

C. elegans mutants defective in meiotic crossing over and/or homolog pairing were identified in systematic genetic screens for mutants exhibiting high levels of

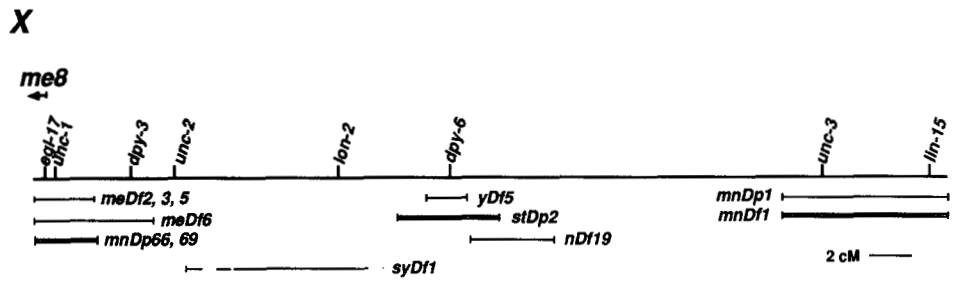

FIGURE 1.—Genetic map of the *X* chromosome. Genetic map positions of markers used are indicated on the main line; the map position of *me8* is shown above the line using an arrow to indicate that *me8* maps left of *unc-1* and near and/or to the left of *egl-17*. The extents of the various chromosomal deficiencies (*Df*s, single line) and duplications (*Dp*s, double line) are indicated below the main line.

meiotic chromosome nondisjunction (see MATERIALS AND METHODS; A. VILLENEUVE, unpublished results). These screens exploit the fact that *C. elegans* exists as two morphologically distinct sexes, a self-fertilizing hermaphrodite, which has two sex chromosomes (*XX*), and a male, which has a single sex chromosome (*XO*). Because males arise spontaneously among the self progeny of hermaphrodites at a frequency of 0.2% due to nondisjunction of the *X* chromosome in the hermaphrodite germline (HODGKIN *et al.* 1979), *C. elegans* mutants with increased nondisjunction can be readily identified as self-fertilizing hermaphrodites that produce increased frequencies of self-progeny males. A number of such mutants were identified previously and were termed *him* (*h*igh *i*ncidence of *m*ales) mutants (HODGKIN *et al.* 1979; HERMAN *et al.* 1982; HERMAN and KARI 1989; KEMPHUES *et al.* 1988).

Whereas most of the new *him* mutations identified in the current screens define *trans*-acting loci (A. VILLENEUVE, unpublished results), the data presented below provide evidence that one mutation, *me8*, disrupts the function of a *cis*-acting locus that is important for normal *X* chromosome recombination and segregation. Hermaphrodites homozygous for the *me8* mutation produce 14% male self progeny, reflecting an increase of two orders of magnitude over the wild-type level of *X* chromosome nondisjunction.

The *me8* mutation maps to the left end of the *X* chromosome: The *me8* mutation maps to the extreme left end of the *X* chromosome, to the left of *unc-1* and near or to the left of *egl-17*, the leftmost known genetic marker (Figure 1, Table 1). This map position is within the 4–5-cM region to the left of *dpy-3* that was previously implicated to contain one or more sites involved in *X* chromosome pairing by studies examining the recombination and segregation properties of *X;A* translocations and duplications (HERMAN *et al.* 1982; MCKIM *et al.* 1988; HERMAN and KARI 1989).

Deficiencies of the left end of *X* cause defects in *X* chromosome segregation: The *me8* mutation has a semidominant effect on *X* chromosome disjunction. While hermaphrodites with two wild-type *X* chromosomes produced 0.1% male self progeny and *me8/me8* hermaphrodites produced 14.0% male self progeny, *+/me8* hermaphrodites produced 1.2% male self progeny (Table 2). Further, deficiencies (*Df*s) that delete the *me8* region of the chromosome were generated (see Figure 1; MATERIALS AND METHODS) and were also found to have a semidominant effect on *X* chromosome disjunction. Heterozygotes for these deficiencies produced 5.3–7.7% male self progeny (Table 2).

+/me8 hermaphrodites do not exhibit as strong a defect as *+/Df* hermaphrodites, suggesting that the *me8* mutation may interfere with but not completely eliminate function of the locus. Alternatively, the heterogeneity in the length of the two *X* homologs in *+/Df* heterozygotes may account in part for this difference in meiotic stability.

Hermaphrodites that carry two deletion chromosomes (*Df/Df*), or one *me8* chromosome and one deletion chromosome (*me8/Df*), exhibit extremely high levels of nondisjunction, producing broods composed of approximately one-third or more males (Table 2). These hermaphrodites also produce a substantial proportion of progeny with three *X* chromosomes (*XXX*), which are identifiable as slower growing hermaphrodites with a dumpy (Dpy) body morphology (HODGKIN *et al.* 1979). The production of triplo-*X* progeny indicates that diplo-*X* as well as nullo-*X* gametes are being produced; thus nondisjunction of homologous chromosomes and not only chromosome loss is occurring. As described below, this nondisjunction occurs at meiosis I, the reductional division. The frequency of *XO* male self progeny is invariably higher than the frequency of *XXX* self progeny despite the fact that *XXX* and *XO* animals do not differ markedly in viability (HODGKIN *et al.* 1979). This indicates that when two *X* chromosomes fail to disjoin properly, an excess of nullo-*X* over diplo-*X* gametes is produced.

The high level of *X* chromosome nondisjunction caused by deletion of the *me8* region of the chromosome is clearly not a common feature of *X* chromosome deficiencies. Deficiencies of several other regions of the *X* chromosome were tested (Figure 1, Table 3), and none were found to cause increased levels of *X* chromosome nondisjunction either as heterozygotes or homozygotes.

TABLE 2

Frequency of *X* chromosome nondisjunction: production of *XO* male and *XXX* self progeny

Genotype[a]	Percent *XO* males[b]	Percent *XXX*[c]	No. of progeny scored (no. of broods)[b]
+/+	0.1	0	4,075 (13)
+/*me8*	1.2	ND	9,478 (34)
me8/me8	14.0	3.2	12,375 (63)
+/*meDf2*	5.3	ND	938 (6)
+/*meDf5*	5.7	ND	1,658 (8)
+/*meDf6*	7.7	ND	2,675 (15)
mnDp66/+; +/*meDf2*	6.5	(1.9)	2,833 (12)
mnDp66/+; +/*meDf3*	5.7	(1.2)	2,574 (11)
mnDp66/+; +/*meDf5*	7.6	(2.0)	2,659 (11)
mnDp69/+; +/*meDf2*	7.0	ND	769 (3)
mnDp69/+; +/*meDf3*	6.4	ND	281 (1)
mnDp69/+; +/*meDf5*	6.6	ND	274 (1)
me8/meDf2	38.8	(4.4)	2,812 (21)
me8/meDf3	39.0	ND	1,005 (6)
me8/meDf5	39.1	(4.6)	3,865 (24)
mnDp66/+; *me8/meDf2*	37.6	5.4	7,276 (30)
mnDp66/+; *me8/meDf3*	42.1	5.3	1,347 (8)
mnDp66/+; *me8/meDf5*	37.0	5.5	1,544 (8)
mnDp69/+; *me8/meDf2*	35.7	5.7	1,644 (8)
mnDp69/+; *me8/meDf3*	37.4	5.3	1,517 (8)
mnDp69/+; *me8/meDf5*	37.4	(6.0)	5,606 (25)
mnDp66; *meDf2*	31.2	6.2	6,629 (26)
mnDp66; *meDf3*	32.4	6.0	8,083 (28)
mnDp66; *meDf5*	31.7	5.3	7,289 (26)
mnDp69; *meDf3*	34.4	5.6	2,371 (10)

[a] Only those parts of the genotypes pertaining to the *cis*-acting locus defined by the *me8* mutation and *Df*s of the region are shown; in many cases hermaphrodites were heterozygous for chromosomes that also carried additional morphological markers (see MATERIALS AND METHODS for complete genotypes).

[b] When the parental genotype was of the form +/*Df* or *me8/Df*, the "No. of progeny scored" was adjusted according to the formula [4/3(no. of *XX*) + 2(no. of *XO*) + (no. of *XXX*) = total number of progeny scored] to compensate for the inviability of *Df* homozygotes and hemizygotes. The "Percent *XO* males" was then calculated as 100 × 2(no. of *XO*)/total. Similarly, when the parental genotype was of the form *Dp*/+; +/*Df* or *Dp*/+; *me8/Df*, "No. of progeny scored" was adjusted according to the formula [16/15(no. of *XX*) + 8/7(no. of *XO*) + (no. of *XXX*) = total number of progeny scored] to compensate for the inviability of *Df* homozygotes and hemizygotes that lack the complementing *Dp*; "Percent *XO* males" was then calculated as 100 × [8/7(no. of *XO*)]/total.

[c] Parentheses indicate that *XXX* progeny were scored in a subset of the broods counted for a given genotype. ND indicates that the frequency of *XXX* progeny was not determined.

TABLE 3

***Df*s of other regions of the *X* chromosome do not cause increased nondisjunction**

Genotype	Percent *XO* males	No. of progeny scored
+/+	0.1	1444
syDf1/+[a]	0.2	1011
yDf5/+[a]	<0.1	3093
stDp2/+; *yDf5/yDf5*	0.4	1062
nDf19/+[a]	<0.2	1044
mnDp1/+; *mnDf1/mnDf1*	0.2	1397

[a] For *Df*/+ hermaphrodites, "No. of progeny scored" and "Percent *XO* males were adjusted according to the formulae given in the Table 2 footnotes to compensate for the inviability of *Df* homozygotes and hemizygotes. Complete genotypes for *Df*/+ hermaphrodites were: *syDf1/unc-2 lon-2*, *unc-42*/+; *yDf5*/+, *unc-32*/+; *nDf19*/+.

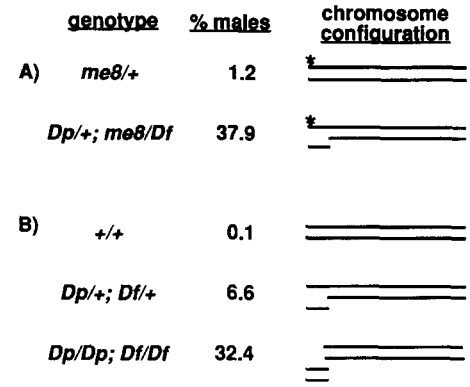

FIGURE 2.—The locus is *cis*-acting. These diagrams depict differences in the configuration of the *X* chromosomes in hermaphrodites of various genotypes that differ in their ability to faithfully segregate their *X* chromosomes. A long line represents a wild-type *X* chromosome; a long line with an asterisk indicates an *X* chromosome carrying the *me8* mutation; a shorter line represents an *X* chromosome that is deleted for the left end region; a very short line indicates a chromosomal duplication of the left end region that is unlinked to the *X* chromosome. The duplications used in these experiments are translocated duplications attached to the right end of chromosome *I* (HERMAN and KARI 1989). Each entry in the "% males" column is the average of the values presented in Table 2 for members of the indicated genotypic class.

The *me8(+)* chromosomal region must be present in *cis* to the *X* chromosome to function in ensuring proper disjunction of the *X* homologs: Chromosomal duplications (*Dp*s) of the *me8(+)* region that are unlinked to the *X* chromosome cannot provide in *trans* the disjunction function deleted by the *Df*s. In contrast, these same *Dp*s do fully complement in *trans* for lack of all essential genes and visible markers that are deleted. The duplications used in these experiments are translocated duplications attached to the right end of chromosome *I* (HERMAN and KARI 1989).

The requirement for this chromosomal region to be present in *cis* to the *X* chromosome is illustrated in Figure 2, which compares the *X* chromosome segregation properties in animals that presumably carry the same dose of the region, but in different chromosome configurations. For example, the key genetic difference between hermaphrodites of the genotypes *me8*/+ and *Dp*/+; *me8/Df* is in the configuration of their chromosomes (Figure 2A). Specifically, in one case the left end region of the *X* chromosome is attached in *cis* to the rest of *X* (*me8*/+), while in the other case it is not (*Dp*/+; *me8/Df*). The phenotypic consequences of this difference are substantial: *me8*/+ hermaphrodites exhibit a relatively low level of nondisjunction, producing only 1.2% self-progeny males, while *Dp*/+; *me8/Df* hermaphrodites exhibit an extremely high level of nondisjunction, producing 38% self-progeny males. A similar phenomenon is seen when comparing hermaphrodites of the genotypes +/+, *Dp*/+; +/*Df*, and *Dp/Dp; Df/Df*,

which likewise differ in the configuration of their *X* chromosomes (Figure 2B). Once again, the ability of the two *X* chromosomes to disjoin properly is highly dependent on whether at least one of the two homologs has the left end region present in *cis* to the *X* chromosome. Thus having a wild-type copy of the *me8* chromosomal region elsewhere in the nucleus is not the same as having it attached to the *X* chromosome. These findings argue that the locus affected by *me8* and deleted by the *Df*s is *cis*-acting: it must be present in *cis* to the *X* chromosome to function in ensuring proper *X* homolog disjunction.

To validate the conclusion that the locus deleted by the *Df*s is *cis*-acting, it was necessary to demonstrate that the *Dp*s used in these experiments do in fact contain a wild-type copy of the region. This was accomplished by identifying *X* chromosomes that had resulted from a crossover event between the *Dp* and a *me8* chromosome in the *unc-1 dpy-3* interval (see MATERIALS AND METHODS). In these recombinant *X* chromosomes, the interval extending from the left end of the *X* chromosome to the right of *unc-1* was derived from the *Dp*, replacing the homologous region of the chromosome containing the *me8* and *unc-1* mutations. Several such recombinant chromosomes were recovered and were found to have segregation properties identical to wild-type chromosomes, indicating that a functional wild-type copy of the left end of the *X* chromosome was indeed present on both of the *Dps* used in these experiments.

The data presented in Table 2 further argue against the possibility that *me8* might be a hypomorphic or null mutation in a dose-sensitive *trans*-acting locus. If this were the case, then a *Dp/+; me8/Df* hermaphrodite should exhibit a phenotype no more severe, and perhaps less severe, than that seen in a *+/Df* hermaphrodite, since each carries one copy of *me8(+)*. On the contrary, *Dp/+; me8/Df* hermaphrodites exhibit a much more severely defective phenotype, indicating that the *me8(+)* copy on the duplication does not complement the *me8* mutation on the chromosome.

High *X* chromosome nondisjunction is correlated with reduced crossing over between the *X* homologs: The high nondisjunction seen in *me8/Df* and *Df/Df* hermaphrodites is correlated with a sharp decrease in crossing over between the *X* chromosomes. The reduction in crossover frequency has been documented in two ways: by measurement of genetic map distances and by cytological examination of meiotic chromosomes in oocytes.

Recombination frequencies in *me8/Df* and *Df/Df* hermaphrodites were significantly decreased for several different genetic map intervals along the *X* chromosome (Table 4, Figure 3). Taking into account both the measured map distances presented here and the fact that the *Df*s delete approximately 5–8% of the genetic map length, it is estimated that the *X* chromosomes in *me8/Df* and *Df/Df* hermaphrodites undergo crossovers only 10–25% as frequently as normal *X* chromosomes. Since wild-type *C. elegans X* chromosomes typically have one crossover, and thus one chiasma, per homolog pair (BRENNER 1974; HODGKIN *et al.* 1979), this amount of reduction in crossing over means that 75–90% of the *X* chromosomes will have no chiasmata.

TABLE 4

Reduced *X* chromosome recombination in *me8/Df* and *Df/Df* hermaphrodites

Genotype and interval tested	Map distance (cM)	Percent wild-type map distance	No. of hermaphrodite progeny scored
+/+			
dpy-3 unc-2	2.8	100	2531
unc-2 dpy-6	16.2	100	1907
dpy-6 unc-3	19.1	100	2357
dpy-3 unc-3	39.4	100	2631
mnDp66/+; me8/meDf2			
dpy-3 unc-2	0.8	29	742
unc-2 dpy-6	0.9	6	604
dpy-6 unc-3	0.9	5	697
dpy-3 unc-3	4.1	10	1063
mnDp69/+; me8/meDf5			
dpy-3 unc-2	0.7	25	818
unc-2 dpy-6	0.3	2	552
dpy-6 unc-3	1.6	8	948
mnDp66; meDf2			
unc-2 dpy-6	3.7	23	1080
dpy-6 unc-3	6.2	32	1385
mnDp66; meDf3			
unc-2 dpy-6	2.8	17	1073
dpy-6 unc-3	5.0	26	1574
mnDp66; meDf5			
unc-2 dpy-6	4.1	25	1143
dpy-6 unc-3	3.4	18	1512

Map distances were calculated as described in MATERIALS AND METHODS. The duplications used in these experiments (*mnDp66* and *mnDp69*) do not readily recombine with or prevent recombination between normal *X* chromosomes (HERMAN and KARI 1989).

A reduction in chiasma frequency has been visualized directly by cytological examination of meiotic chromosomes in oocytes using fluorescent DNA stains. *C. elegans* oocytes arrest at a late stage of meiotic prophase (diakinesis) prior to fertilization. By this stage, homologous chromosomes have desynapsed but are highly condensed and remain held together by chiasmata, the cytological manifestations of crossovers that occurred at an earlier stage (JONES 1987). Chromosomes remain in this configuration until after fertilization occurs, at which time the spindle assembles and homologs disjoin (ALBERTSON and THOMSON 1993). In oocytes from wild-type hermaphrodites, six sets of attached homolog pairs, or bivalents, are seen (Figure 4a), indicating that all six chromosome pairs are recombinant. In *me8/Df* or *Df/Df* hermaphrodites, oocytes typically have five bivalents and two univalents, presumably corresponding to two noncrossover *X* chromosomes (Figure 4, c and d). The fraction of oocytes in *me8/Df* and *Df/Df* hermaphrodites that exhibit this 5 + 2 meiotic karyotype (88% and 72%,

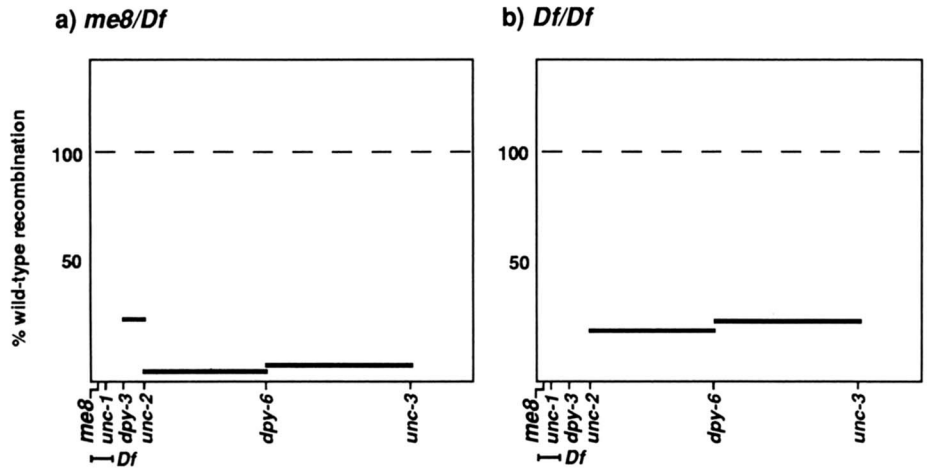

FIGURE 3.—Reduced crossing over along the *X* chromosome in *me8/Df* and *Df/Df* hermaphrodites. Graphical representation of the marked reduction in genetic map distances on the *X* chromosome in (a) *me8/Df* and (b) *Df/Df* hermaphrodites. The *x* axis indicates the position of markers along the *X* chromosome based on the control wild-type map distances presented in Tables 4 and 7; the *y* axis indicates the percent of the wild-type level of recombination observed; the horizontal bars indicate the genetic interval tested. The percent of wild-type recombination shown for each genetic interval is the average of the values presented in Table 4 for members of the indicated genotypic class.

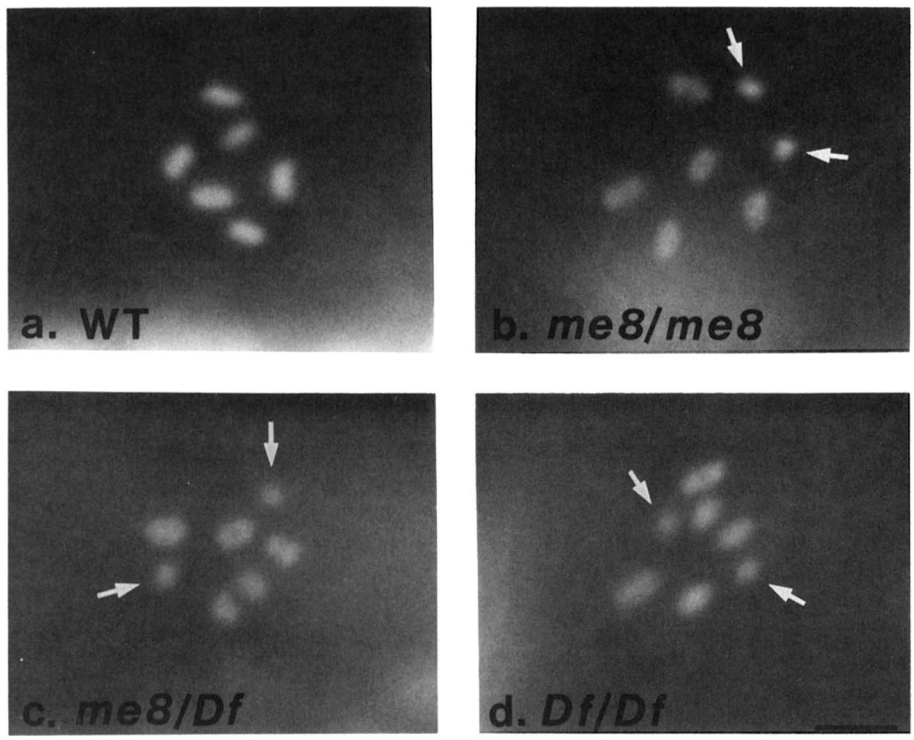

FIGURE 4.—Cytological visualization of achiasmate *X* chromosomes. Photomicrographs of oocyte nuclei at the diakinesis stage of meiotic prophase that have been fixed and stained with DAPI to allow visualization of meiotic chromosomes. By this stage, homologous chromosomes have become desynapsed but remain held together by chiasmata, the physical links between homologs created by crossovers that occurred at an earlier stage. The condensation state of the chromosomes is maximal at this stage. (a) Oocyte nucleus from a wild-type hermaphrodite; the six oblong stained bodies correspond to the six sets of attached homolog pairs, or bivalents. In the micrographs of oocyte nuclei from (b) *me8/me8*, (c) *me8 unc-1 dpy-3/meDf2* and (d) *mnDp66; meDf5* hermaphrodites, five bivalents and two smaller stained bodies corresponding to a pair of achiasmate chromosomes (indicated by arrows) are seen. Scale bar = 5 μm. See Table 5 for frequencies of normal and 5 + 2 meiotic karyotypes in oocytes from hermaphrodites of various genotypes.

respectively; see Table 5) agrees well with the fraction of noncrossover *X* chromosomes predicted on the basis of genetic recombination frequency data, suggesting that the presence of achiasmate chromosomes most likely reflects a defect in chiasma formation rather than in chiasma maintenance.

A positive correlation was observed between the frequency of achiasmate chromosomes in oocytes and the frequency of nullo-*X* ova produced by hermaphrodites of several different genotypes (Table 5). The frequency of nullo-*X* ova was determined by crossing wild-type males with appropriately marked hermaphrodites and scoring the frequency of patroclinous *XO* males among those cross progeny that had received a paternally derived *X* chromosomes (see MATERIALS AND METHODS). This correlation between an increase in the frequency of achiasmate chromosomes and the failure to segregate *X* chromosomes properly suggests that the majority of *X* chromosome nondisjunction observed in *me8/Df* and *Df/Df* hermaphrodites may be a consequence of failure in crossing over.

As is required for a *cis*-acting locus, the observed reductions in chiasma frequency and the defects in chromosome segregation in *me8/Df* and *Df/Df* are apparently *X* chromosome specific. *X* specificity was initially suggested by the fact that *me8/me8*, *me8/Df* and *Df/Df* hermaphrodites produce large broods (Table 2) with few inviable embryos (after accounting for the inviable *Df* homozygotes and hemizygotes produced by *me8/Df* hermaphrodites) (Table 6); mutants in which the au-

TABLE 5

The presence of achiasmate chromosomes in oocytes correlates with the production of nullo-*X* ova

Genotype	Oocyte karyotype			Ovum genotype	
	6 bivalents	5 bivalents + 2 univalents	Percent 5 + 2	Percent nullo-*X* ova	No. of ova scored
+/+	199	0	0	0.1	2417[a]
+/*meDf5*	186	6	3	2.4	1415
me8/me8	233	39	14	4.2	1079
mnDp66; meDf2/meDf2	44	113	72	28.5	1545
mnDp66; meDf5/meDf5	116	286	71	28.4	1196
me8/meDf2	16	112	88	40.2	1665
me8/meDf5	29	195	87	40.6	2334

[a] Taken from HODGKIN, *et al.* (1979).

TABLE 6

Embryonic viability

Genotype of parent	No. of eggs laid	Percent embryos hatching	Corrected percent embryos hatching[a]
+/+	1451	99.8	NA
me8/me8	1916	97.0	NA
mnDp66; meDf2	1732	95.8	NA
mnDp66; meDf5	1709	93.5	NA
me8/meDf2	1029	63.3	95.3
me8/meDf5	1043	62.1	93.7

[a] Approximately 1/4 of *XX* zygotes and 1/2 of *XO* zygotes produced by *me8/Df* hermaphrodites will die as embryos due to homozygosity (*XX*) or hemizygosity (*XO*) for the *Df* chromosome. Based on the proportions of *XX* and *XO* progeny they produce (Table 2), *Df* homozygosity or hemizygosity is therefore expected to account for the embryonic lethality of 33.6% of the progeny of *me8/meDf2* hermaphrodites and 33.7% of the progeny of *me8/meDf5* hermaphrodites. "Corrected percent embryos hatching" was calculated as: percent embryos hatching/(100 − expected percent *Df/Df* and *Df/0* dead embryos). NA = not applicable.

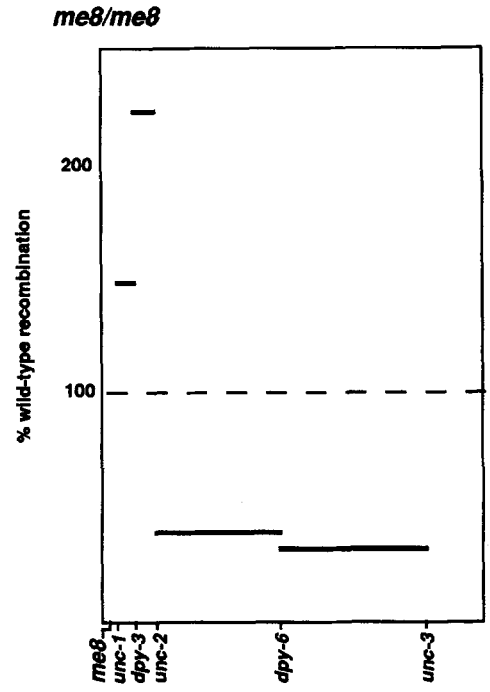

FIGURE 5.—Altered distribution of crossovers in *me8/me8* hermaphrodites. In *me8/me8* hermaphrodites, the frequency of crossovers is elevated above wild-type levels in genetic map intervals proximal to the *me8* locus and is reduced below wild-type levels in intervals more distal to the locus. Axes and symbols as in Figure 3; data from Table 7.

tosomes undergo nondisjunction or loss at frequencies comparable to the *X* chromosomes in these hermaphrodites produce broods consisting mainly of dead aneuploid embryos (HODGKIN *et al.* 1979; KEMPHUES *et al.* 1988; A. M. VILLENEUVE, unpublished results). Further, *X* chromosome specificity is strongly indicated by the cytological examination of oocytes just described: the 5 + 2 meiotic karyotype characteristic of *me8/Df* and *Df/Df* oocytes indicates that one and only one pair of homologs lacks a chiasma.

Altered distribution of *X* chromosome crossovers in *me8/me8* hermaphrodites: Chiasma frequencies and *X* chromosome recombination frequencies were also examined in *me8* homozygotes, which exhibit a more moderate increase in *X*-chromosome nondisjunction. Cytological examination of meiotic chromosomes in *me8/me8* oocytes indicates that 14% contain one pair of achiasmate chromosomes (Figure 4, Table 5).

Measurement of genetic map distances in *me8* homozygotes uncovered a hidden defect in *X*-chromosome recombination not revealed by the cytological assay. The striking result of these studies is that *me8* homozygotes exhibit an altered distribution of crossovers along the *X* chromosome. Specifically, the frequency of crossing over is elevated above wild-type levels in genetic map intervals immediately proximal to the *me8* locus, and then decreases to below wild-type levels in intervals more distal to the *me8* locus (Figure 5, Table 7). This altered crossover profile suggests that the *me8* mutation causes a defect in propagation along the *X* chromosome of an event that initiates at the left end.

Nondisjunction occurs at the reductional division: The presence of chiasmata is thought to play an important role in orienting homologous chromosomes toward opposite poles of the meiosis I spindle (HAWLEY 1988; NICKLAS 1974). Because chiasma frequency is strongly reduced in *me8/Df* and *Df/Df* hermaphrodites, it was

TABLE 7

Altered distribution of crossovers along the *X* chromosome in *me8/me8* hermaphrodites

Genotype and interval tested	Map distance (cM)	Percent wild-type map distance	No. of hermaphrodite progeny scored
+/+			
unc-1 dpy-3	2.3	100	2638
dpy-3 unc-2	2.8	100	2531
unc-2 dpy-6	16.2	100	1907
dpy-6 unc-3	19.1	100	2357
dpy-3 unc-3	39.4	100	2631
me8/me8			
unc-1 dpy-3	3.4	148	1796
dpy-3 unc-2	6.2	221	2250
unc-2 dpy-6	6.3	39	1470
dpy-6 unc-3	6.1	32	1142
dpy-3 unc-3	23.5	60	772

expected that the nondisjunction in *me8/Df* and *Df/Df* oocytes occurs mainly, if not exclusively, at meiosis I, the reductional division. This expectation was verified by determining the genetic constitution of gametes that had two nondisjoined *X* chromosomes. Specifically, *XXX* cross progeny were picked from crosses between wild-type males and *me8/Df* or *Df/Df* hermaphrodites carrying two differently marked *X* chromosomes (see MATERIALS AND METHODS). These *XXX* animals were then progeny tested to determine which markers were present on the two nondisjoined oocyte *X* chromosomes. In the absence of recombination, nondisjunction at meiosis I (reductional division) will result in *XX* gametes that are all heterozygous for diagnostic *X*-linked markers.In contrast, nondisjunction at meiosis II (equational division) in the absence of recombination will result in *XX* gametes that are all homozygous for *X*-linked markers.

In these experiments, 124/127 *XX* ova from *me8/Df* hermaphrodites and 44/49 *XX* ova from *Df/Df* hermaphrodites were heterozygous for the diagnostic markers. Since previous experiments showed that the overwhelming majority of oocyte chromosomes are nonrecombinant in these hermaphrodites, these results indicate that most, if not all, of the nondisjunction occurs at meiosis I. The few observed cases (3/127 and 5/49) of marker homozygosis in *XX* ova could have arisen as a result of an infrequent recombination event between the two *X* chromosomes in the oocyte and thus do not necessarily constitute evidence of any equational nondisjunction. Inclusion of an additional *X*-linked marker in some of the *me8/Df* experiments verified that the *X* chromosomes in *XX* ova that were heterozygous for the diagnostic markers were nonrecombinant as expected, and that in the one *XX* ovum that was homozygous for the markers, one of the two *X* chromosomes was indeed recombinant (see MATERIALS AND METHODS).

Nondisjunction in oocytes *vs.* spermatocytes: The frequency of *X* chromosome nondisjunction during spermatocyte meiosis appears to be significantly lower than during oocyte meiosis in *me8/Df* and *Df/Df* hermaphrodites. Although nondisjunction frequencies in hermaphrodite spermatocytes cannot be measured directly as was done for oocytes, the percentages of *XX*, *XO* and *XXX* self progeny produced reflect the combined contributions of nondisjunction in both gamete lines. Thus the measured frequencies of *X*, O and *XX* gametes produced during oocyte meioses were used to calculate the expected percentages of *XX*, *XO* and *XXX* self-progeny that would be produced if *X* chromosome nondisjunction in spermatocytes occurred at the same frequency as in oocytes. As shown in Table 8, the actual percentage of *XO* self-progeny produced by *me8/Df* and *Df/Df* hermaphrodites is substantially lower and the percentage of *XX* self-progeny is substantially higher than predicted if the frequency of nondisjunction in spermatocytes equaled that in oocytes.

Despite the fact that meiotic disjunction of the *X* chromosomes appears to be less severely affected in spermatocytes than in oocytes in *me8/Df* and *Df/Df* hermaphrodites, the severely reduced genetic map distances reported in Table 4 can only be accounted for by a reduction in recombination in both oocytes and spermatocytes in the genetic intervals tested. Moreover, since nondisjunction in oocytes appears to account for most of the nullo-*X* gametes produced by *me8/Df* or *Df/Df* hermaphrodites, map distances that largely reflect spermatocyte recombination can be calculated from scoring male self progeny. Map distances calculated in this way also suggest a reduced level of spermatocyte recombination in these genetic intervals (Table 9). Because technical limitations preclude measurement of genetic recombination frequencies in the leftmost region of the chromosome, and since the compact structure of the spermatocyte nucleus hinders the visualization of individual chromosomes, we cannot rule out the possibility that spermatocyte chromosomes in these hermaphrodites are in fact more frequently recombinant than oocyte chromosomes and that these recombination events are concentrated in the untested intervals. If most of these spermatocyte *X* chromosomes do prove to be nonrecombinant, however, then it follows that hermaphrodite spermatocytes must possess a mechanism for disjoining achiasmate *X* chromosomes.

Nondisjunction apparently occurs in both spermatocytes and oocytes in +/*Df* and *me8/me8* hermaphrodites (Tables 2 and 5). Since +/*Df* hermaphrodites retain one wild-type copy of the *cis*-acting locus and since *me8/me8* hermaphrodites appear to retain partial function of the locus, this spermatocyte nondisjunction might reflect a difference between failed and incomplete homologous pairing.

DISCUSSION

This study describes the characterization of a *cis*-acting locus on the left end of the *C. elegans X* chro-

TABLE 8

X chromosome nondisjunction occurs more frequently in ocytes than in spermatocytes in *me8/Df* and *Df/Df* hermaphrodites

	Ova		Self progeny		
				Fraction of progeny	
Genotype	Ovum type	Fraction of ova	Progeny type	Expected[a]	Observed
me8/Df	*X*	0.56	*XX*	0.41	0.56
	O	0.40	*XO*	0.54	0.39
	XX	0.04	*XXX*	0.04	0.05
Dp; Df	*X*	0.66	*XX*	0.51	0.62
	O	0.28	*XO*	0.41	0.32
	XX	0.06	*XXX*	0.08	0.06

Numbers presented are the averages of the values determined for *me8/meDf2* and *me8/meDf5*, and the averages of the values determined for *mnDp66; meDf2* and *mnDp66; meDf5*, respectively. The fractions of *X*, O and *XX* ova produced were determined as described in MATERIALS AND METHODS; primary data for nullo-*X* ova are presented in Table 5. The fractions of *XX*, *XO* and *XXX* self progeny are taken from Table 2. The "Expected" values were calculated using a Punnet square, assuming that the frequencies of *X*, O and *XX* gametes among sperm equaled their frequencies among ova; inviable aneuploid zygote classes (*XXXX* and OO) were excluded from the totals.

[a] If nondisjunction in spermatocytes = nondisjunction in oocytes.

TABLE 9

Genetic map distances calculated from scoring the frequency of recombinants among *XO* male self progeny

Genotype and interval tested	Map distance (cM)	Percent wild-type map distance[a]	No. of *male* self progeny scored
mnDp66/+; me8/meDf2			
dpy-3 unc-2	<0.2	<7	502
unc-2 dpy-6	<0.3	<2	375
dpy-6 unc-3	0.5	3	506
dpy-3 unc-3	0.8	2	530
mnDp69/+; me8/meDf5			
dpy-3 unc-2	0.2	7	499
unc-2 dpy-6	<0.4	<2	263
dpy-6 unc-3	0.5	3	576
mnDp66; meDf2			
unc-2 dpy-6	1.8	11	563
dpy-6 unc-3	1.7	9	637
mnDp66; meDf3			
unc-2 dpy-6	0.8	5	637
dpy-6 unc-3	2.3	12	795
mnDp66; meDf5			
unc-2 dpy-6	1.9	12	529
dpy-6 unc-3	1.6	8	802

Map distances were calculated as described in MATERIALS AND METHODS.

[a] Numbers reported in this column compare map distances calculated from scoring *XO* male self-progeny with control map distances from Table 4. The map distances reported here largely reflect recombination in spermatocytes, however, whereas the control map distances reflect recombination in both gamete lines. Since previous studies have suggested that recombination frequencies in hermaphrodite spermatocytes and oocytes may be unequal in some genetic intervals (ZETKA and ROSE 1990), these numbers represent a rough estimate of the extent of recombination reduction in spermatocytes.

mosome that plays a key role in promoting normal levels of meiotic crossing over specifically between *X* homologs. Deletion of this locus on both *X* chromosomes leads to high levels of *X* chromosome nondisjunction during the reductional division of meiosis, presumably as a consequence of the failure to form chiasmata. The fact that homozygosity for a deletion of a *cis*-acting locus at one end of the chromosome suppresses recombination along the entire length of the chromosome suggests a role for the locus in homolog pairing. Mechanistically, how might such a *cis*-acting locus function in the pairing process?

Models for pairing center function: The properties of deletions of the *cis*-acting locus demonstrated here argue against one particular type of model for the role of this chromosomal region in homolog pairing. This model proposed that chromosomes do not utilize the information contained in the extended DNA homology shared along their lengths to identify meiotic pairing partners, but rather that the information content for homolog recognition is restricted to a specialized "homolog recognition site" located near one end of the chromosome (ROSE and MCKIM 1992). According to this model, homolog recognition would be achieved by the matching of recognition sites on two chromosomes. The homolog recognition site model makes the strong prediction that deletion of the locus from only one of the two homologs would be just as deleterious as deleting it from both homologs, in either case rendering the homologs unable to find each other. Clearly this prediction is not borne out by the behavior of the *cis*-acting locus characterized in the present study. Quite the contrary, a catastrophic failure in crossing over and chromosome segregation is observed only when the *cis*-acting locus is deleted from both homologs. In deletion heterozygotes, the vast majority of chromosomes are able to form chiasmata with and segregate normally from their homologs. This holds true even for the larger deletion *meDf6* which appears to remove the entire 4–5-cM region to which pairing activity had been localized by previous studies of translocation chromosomes (although we cannot at present rule out the possibility that the *Df* chromosomes might retain some material from the extreme tip of the chromosome). Thus while it is possible that the *cis*-acting locus might function in the homolog recognition process

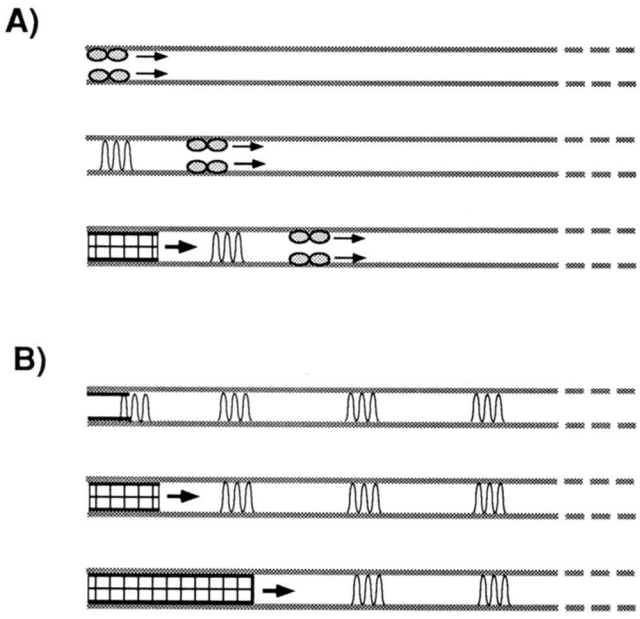

FIGURE 6.—Models for pairing center function. See text for a discussion of models in which the *cis*-acting "pairing center" functions as a binding site that loads onto the chromosome *trans*-acting factors involved either in (A) a homology search that leads to the identification and prealignment of homologs, or (B) the initiation of chromosome synapsis. In this diagram, thick gray lines represent the *X* chromosomes, ovals represent *trans*-acting factors that carry out the DNA homology search, wavy lines represent regions of presynaptic alignment resulting from a successful identification of homology, plain black lines represent synaptonemal complex precursors, and black parallel lines with cross hatching represent the synaptonemal complex.

(see below), models in which the information content required for homolog recognition is restricted to this chromosomal region seem less compelling.

I propose instead a model in which the *cis*-acting locus affected by the *me8* mutation and the *Df*s acts as a meiotic "pairing center" involved in nucleating or facilitating the assembly of a crossover-competent complex between the two *X* homologs. This pairing center could function in the early phase of homolog pairing, by promoting a DNA homology search that leads to identification and prealignment of homologous pairing partners (Figure 6A). Alternatively, the pairing center could function after homolog recognition, in the initiation of chromosome synapsis (Figure 6B). In either case, the locus would act as a binding site that recruits or loads *trans*-acting factors onto the chromosome, thereby initiating assembly of a complex which would then proceed to translocate (A) or elongate (B) in a polar fashion along the length of the homologs. According to this model, failure to form chiasmata when both homologs lack the pairing center results from a failure to initiate a key event in homolog pairing.

When the pairing center is deleted from only one of the two *X* homologs most *X* chromosomes are able to form chiasmata, suggesting that productive pairing can occur although somewhat less efficiently than with two wild-type homologs. Clearly in this case the pairing center on one homolog is providing function for both homologs. How might this be accomplished? If the pairing center functions by promoting a search for DNA homology, normally both chromosomes would load the homology-search machinery and actively search for each other (Figure 6A); initiation of the homology search on only one of the two homologs will usually result in homolog recognition and productive pairing, albeit with reduced efficiency. Alternatively, according to models in which the pairing center functions by promoting chromosome synapsis (Figure 6B), a prior DNA homology search would have brought the homologs into close proximity at sites of presynaptic association which could then allow factors that were loaded onto one homolog to interact with and initiate synapsis between the two homologs.

If the pairing center functions in homolog recognition *per se*, it is necessary to propose that the successful identification of homology and the initiation of synapsis are temporally coupled processes in order to account for the pairing and recombination behavior observed for translocation chromosomes (see below). Accordingly, a directional homology search that proceeds from a pairing center at one end of the chromosome will first identify homology proximal to the pairing center, thereby triggering initiation of synapsis in this proximal region (Figure 6A). Thus the activity of the pairing center, either directly or indirectly, may dictate the site of initiation of chromosome synapsis. Independent cytological evidence is consistent with a single site of initiation of synapsis for *C. elegans X* chromosomes. Reconstruction from serial section electron micrographs of pachytene nuclei from *XXX* hermaphrodites has shown that the *X* chromosomes are present as one completely synapsed bivalent and one separate univalent (GOLDSTEIN 1984); in organisms with multiple interstitial sites of synaptic initiation, trivalents arising from pairing partner switching are often observed at the pachytene stage in aneuploids or triploids (*e.g.*, MOENS 1969; RASMUSSEN 1977; MOENS and ASHTON 1985; LOIDL and JONES 1986)).

A variation on the class of models proposed is that the pairing center serves as a "molecular address," targeting the chromosomes to a specific location in the nucleus; this localization would then serve to facilitate homolog pairing. The nuclear envelope might appear to be a reasonable target location since the pairing center maps to the end of the *X* chromosome, and chromosome ends have been shown to be attached to the nuclear envelope during meiotic prophase (VON WETTSTEIN *et al.* 1984), although the functional significance of this attachment is not known. Further, nematode chromosomes are attached to the nuclear envelope at only one end (GOLDSTEIN 1982). However GOLDSTEIN (1982) found that either end of the *C. elegans* chromosome *I*

bivalent could be attached to the nuclear envelope. The possibility of attachment at either chromosome end argues against the nuclear envelope as a target since the pairing center maps at only one end. Are there other possible target locations to which a pairing center might be directed? (MAGUIRE 1984) has proposed the existence of intranuclear specialized structures that interact with specific chromosomal regions to facilitate homolog pairing, but concrete experimental evidence for such structures is lacking.

Disruption of pairing center function by the *me8* mutation: In animals homozygous for the *me8* mutation, reduction in the total amount of recombination on the *X* chromosome is relatively moderate but there is a dramatic alteration in the distribution of crossover events along the length of the chromosome. The crossover frequency is elevated above wild-type levels in genetic intervals proximal to *me8*, and is reduced below wild-type levels in more distal intervals. This concentration of crossover events toward the *me8* end of the chromosome makes recombination frequency less proportional to physical distance than in +/+ hermaphrodites (ALBERTSON 1993; T. BARNES, personal communication; A. VILLENEUVE, unpublished results), arguing that the *me8* mutation does not affect the process of recombination itself but instead affects a precondition for reciprocal exchange. It further suggests that the *me8* mutation causes a defect in processivity along the *X* chromosome of an event that initiates at the chromosome end. The most compelling and straightforward conclusion is that *me8* is a mutation in the *X* chromosome meiotic pairing center itself that disrupts but does not completely eliminate pairing center function. However we cannot rigorously exclude the possibility that *me8* might be a gain-of-function mutation in a *trans*-acting locus that both interacts with and fortuitously maps to the same location as the pairing center. In either case, it is clear from the interactions between *me8* and the *Df* chromosomes that the *me8* mutation interferes with the function of the *X* chromosome pairing center defined by the *Df*s. One interpretation of the change in crossover distribution in *me8/me8* animals is that the *X* chromosomes are at least partially capable of carrying out an initial nucleation event, perhaps the loading of factors onto the chromosomes, but are defective in a function required for these factors to translocate or propagate assembly of a complex along the chromosome. Alternatively, a reduction in affinity or cooperativity of binding of factors at the pairing center could result in a population of chromosome pairs in which productive homologous pairing extends only part of the length of the chromosomes, from the left end to random internal positions. Restriction of most crossover events to these properly paired regions would then yield the observed crossover distribution. Similar altered distributions of *X* chromosome crossovers have been observed in strains carrying mutations in the *trans*-acting genes *him-8*, *him-5* and *him-1* that cause high nondisjunction specifically or preferentially of the *X* chromosome (BROVERMAN and MENEELY 1994; HODGKIN *et al.* 1979; HERMAN and KARI 1989). Independent evidence has further implicated the *him-8* gene product in the process of *X* chromosome pairing (S. BURGESS and W. WOOD, personal communication; A. M. VILLENEUVE unpublished).

Altered crossover distributions have also been reported for hermaphrodites heterozygous for a variety of chromosome aberrations including translocations, inversions, insertions and deletions (MCKIM *et al.* 1988; ROSENBLUTH *et al.* 1990; ZETKA and ROSE 1992; MCKIM *et al.* 1993). An important distinction exists between these instances and the altered crossover distribution in *me8* mutants, however. Notably, in *me8* homozygotes the distribution of crossovers is altered despite the fact that the chromosomes are completely collinear along their entire lengths. In contrast, the altered crossover distributions observed in aberration heterozygotes appear to result from the lack of parity in length and/or lack of collinearity between the chromosome aberration and the normal sequence chromosome.

Mechanism of crossover suppression in translocation heterozygotes: The initial proposal that each of the six *C. elegans* chromosomes has a unique pairing region located near one end was based largely on studies examining the recombination and segregation properties of reciprocal translocations (MCKIM *et al.* 1988). The basic observation was that reciprocal translocations can act as efficient crossover suppressors in *C. elegans*. In heterozygotes for certain reciprocal translocations, crossing over occurs readily between the normal sequence chromosomes and translocated segments from one end of the chromosome to the translocation breakpoint, but is strongly suppressed from the breakpoint to the other end of the chromosome (ROSENBLUTH and BAILLIE 1981; HERMAN *et al.* 1982; ROSE *et al.* 1984; FERGUSON and HORVITZ 1985; FODOR and DEAK 1985; MCKIM *et al.* 1988, 1993). For several *X*;autosome (*X;A*) translocation heterozygotes studied, the crossover suppression along the *X* chromosome was accompanied by high *X* chromosome nondisjunction (HERMAN *et al.* 1982; FODOR and DEAK 1985; A. VILLENEUVE, unpublished results).

Any model for the mechanism of crossover suppression in translocation heterozygotes must explain why heterozygosity for certain *X;A* translocations and deficiency-translocations causes strong crossover suppression and concomitant high nondisjunction (HERMAN *et al.* 1982; A. VILLENEUVE, unpublished), while heterozygosity for the deletions with similar breakpoints described in the present study allows most *X* chromosomes to recombine and to disjoin properly. Since the deletion heterozygotes suggest that separation of a large chromosomal segment from its endogenous pairing center on one of the two homologs is not in itself sufficient to cause strong crossover suppression

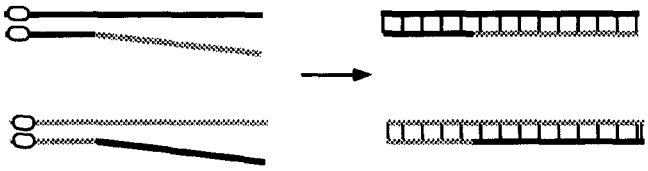

FIGURE 7.—Mechanism of crossover suppression in translocation heterozygotes. See text for discussion of a model proposing that efficient crossover suppression in translocation heterozygotes is achieved in part by sequestering translocated chromosome segments away from their homologous segments on normal sequence chromosomes in a nonproductive pairing configuration. In this diagram, the thick black and gray lines represent two different chromosomes involved in a reciprocal translocation, and the ovals indicate the chromosome ends at which the pairing centers are located. The right half of the figure indicates the predicted configuration of the chromosomes at the pachytene stage of meiotic prophase, when chromosomes are fully synapsed; cross-hatching represents the synaptonemal complex.

along the entire chromosome segment, an additional factor must contribute to the crossover suppression caused by translocations.

I suggest that attachment of chromosome segments to new chromosomal locations plays a major role in recombination suppression in translocation heterozygotes. In reciprocal translocations that act as efficient crossover suppressors, each half-translocation has one pairing center (Figure 7). Models in which the pairing center directs initiation of synapsis predict that in such translocation heterozygotes, synapsis will initiate formation of two separate bivalents in homologous segments and will eventually reach the translocation breakpoint where the chromosomes become nonhomologous. Since the SC structure itself is apparently indifferent to homology (CARPENTER 1987), synapsis may then continue along the same two chromosomes on which it started, despite the fact that beyond the breakpoint this synapsis will juxtapose nonhomologous chromosome segments. The occurrence of nonhomologous synapsis during meiotic prophase is a well-established and widespread phenomenon. In Bombyx and Allium triploids, for example, nonhomologous bivalents or fold-backs are frequently observed during the late pachytene stage (RASMUSSEN 1977; LOIDL and JONES 1986). Likewise, in haploid plants and yeast (*e.g.*, SEN 1970; TING 1973; GILLIES 1974; LOIDL *et al.* 1991) most chromosomes show extensive synapsis with other chromosomes despite the fact that no homologs are present. Moreover, localized regions of nonhomologous synapsis have been observed in mice and maize heterozygous for chromosome aberrations (MOSES and POORMAN 1981; ASHLEY 1983; MAGUIRE 1972; GILLIES 1983). Thus upon completion of synapsis in these translocation heterozygotes, the model predicts that six normal-looking synapsed bivalents will be present in the nucleus. This is in fact exactly what was observed in a small sample of pachytene nuclei reconstructed from serial section electron micrographs for one translocation heterozygote of this type (GOLDSTEIN 1986). Hence, I argue that efficient crossover suppression is achieved because translocated chromosome segments are sequestered away from their homologous segments on normal sequence chromosomes in a nonproductive synapsed configuration.

The relationship between recombination and disjunction: The data presented in this report reveal a positive correlation between the frequency of oocytes with a pair of achiasmate chromosomes and the frequency of *X* chromosome nondisjunction during oocyte meiosis. This observation is consistent with other instances of correlation between crossing over and chromosome segregation in *C. elegans* (HODGKIN *et al.* 1979; ROSENBLUTH and BAILLIE 1981; HERMAN *et al.* 1982; ROSE *et al.* 1984; MCKIM *et al.* 1988; HERMAN and KARI 1989; ZETKA and ROSE 1992; K. KEMPHUES, personal communication; A. M. VILLENEUVE, unpublished results). Thus it appears that in *C. elegans* oocytes, as in most other systems studied (reviewed in HAWLEY 1988), crossing over between homologous chromosomes is required to ensure their proper segregation at meiosis I.

Although *X* chromosome recombination is strongly reduced when both *X* chromosomes carry deletions affecting the pairing center, it is not completely eliminated. Residual *X* chromosome recombination in the apparent absence of a pairing center is reminiscent of the residual recombination observed in several *S. cerevisiae* mutants that lack *trans*-acting factors involved in chromosome synapsis. Although yeast strains carrying null mutations in the *MER1*, *RED1* or *HOP1* genes exhibit strongly reduced recombination, they nevertheless retain approximately 10–25% of the wild-type levels of crossing over (HOLLINGSWORTH and BYERS 1989; ENGEBRECHT *et al.* 1990; ROCKMILL and ROEDER 1990). Moreover, the residual crossover events in *mer1* and *red1* null mutants are less efficient at ensuring proper disjunction of homologs than crossover events occurring during wild-type meioses, suggesting that crossovers must mature in the context of properly paired chromosomes in order to serve reliably as functional chiasmata in meiosis I segregation (ENGEBRECHT *et al.* 1990; ROCKMILL and ROEDER 1990). Likewise, the residual recombination events in *me8/Df* or *Df/Df* hermaphrodites do not always guarantee disjunction of homologs, as evidenced by the production of some diplo-*X* ova that carry recombinant *X* chromosomes.

Observations from several systems (triploid and haploid plants, haploid yeast; see above) argue that there is a strong propensity for chromosomes to synapse even when no homolog is available. This suggests that the two *X* chromosomes in *Df/Df* or *me8/Df* germ cell nuclei may be present in some type of synapsed configuration at the pachytene stage even though the initial homologous phase of synapsis may have failed. The juxtaposition of the *X* homologs via nonhomologous synapsis could account for the somewhat higher frequency of

nonrecombinant *X* chromosomes and nullo-*X* gametes in *me8/Df* than in *Df/Df* hermaphrodites: the unequal length of the two *X* chromosomes in *me8/Df* hermaphrodites may result in an out-of-register alignment that interferes with the production of residual crossover events.

Whereas oocytes are dependent on the formation of chiasmata to ensure proper disjunction of their *X* chromosomes, spermatocytes in *C. elegans* hermaphrodites may be capable of segregating achiasmate *X* chromosomes. This possibility is suggested by the fact that the level of *X* chromosome nondisjunction appears to be substantially higher in oocytes than in spermatocytes in *me8/Df* and *Df/Df* hermaphrodites despite a strong reduction in recombination in both gamete lines in all genetic intervals tested. Similarly, in mutants defective in the *C. elegans* gene *him-8*, *X* chromosome nondisjunction appears to be more frequent in oocytes that in spermatocytes (HODGKIN *et al.* 1979) despite an apparent reduction in recombination in both gamete types (HERMAN and KARI 1989; BROVERMAN and MENEELY 1994). The conclusion that *C. elegans* spermatocytes have the capacity to segregate *X* chromosomes that lack chiasmata must remain tentative at present, since in any of these studies an increased frequency of *X* chromosome recombination in untested intervals in spermatocytes would have gone undetected. Mechanisms for segregating achiasmate chromosomes have been well documented in a few other systems, however, most notably in the *D. melanogaster* female where the phenomenon was first described (reviewed in HAWLEY 1989).

Two obvious differences between spermatocytes and oocytes that might account for a difference in the ability to segregate achiasmate *X* chromosomes are their physical size and the timing of their progression through meiosis. Oocytes proceed more slowly through the late stages of meiotic prophase and arrest prior to fertilization at the diakinesis stage, with homologs desynapsed but highly condensed and held together by chiasmata; the nuclear volume is quite large by this stage and bivalents are substantially separated in space. In oocytes in which the *X* chromosomes have failed to recombine, the two noncrossover *X*s bear no obvious spatial relationship to each other (Figure 4) and may be far apart when the meiotic spindle begins to assemble. Spermatocytes proceed more rapidly from the end of the pachytene stage, during which chromosomes are fully synapsed, through the meiosis I and meiosis II divisions, with the chromosomes crowded into a relatively compact nuclear volume. If when the normal pairing process fails the two *X* chromosomes are nevertheless juxtaposed in a nonproductive synapsed configuration as discussed above, the timing and spacing of desynapsis and spindle assembly in spermatocytes may allow attachment of a pair of achiasmate *X* chromosomes to spindle fibers from opposite poles.

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