The Genetic Analysis of Achiasmate Segregation in Drosophila melanogaster. III. The Wild-Type Product of the Axs Gene Is Required for the Meiotic Segregation of Achiasmate Homologs

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ABSTRACT

The regular segregation of achiasmate chromosomes in Drosophila melanogaster females is ensured by two distinct segregational systems. The segregation of achiasmate homologs is assured by the maintenance of heterochromatic pairing; while the segregation of heterologous chromosomes is ensured by a separate mechanism that may not require physical association. Axs0 (Aberrant X segregation) is a dominant mutation that specifically impairs the segregation of achiasmate homologs; heterologous achiasmate segregations are not affected. As a result, achiasmate homologs frequently participate in heterologous segregations at meiosis I. We report the isolation of two intragenic revertants of the Axs0 mutation (Ax5c2 and Ax5c4) that exhibit a recessive meiotic phenotype identical to that observed in Axs0/Axs0 females. A third revertant (Axs+) exhibits no meiotic phenotype as a homozygote, but a meiotic defect is observed in Axs+/Axs+ females. Therefore mutations at the Axs locus define a gene necessary and specific for homologous achiasmate segregation during meiosis. We also characterize the interactions of mutations at the Axs locus with two other meiotic mutations (ald and ncd). Finally, we propose a model in which Axs+ is required for the normal separation of paired achiasmate homologs. In the absence of Axs+ function, the homologs are often unable to separate from each other and behave as a single segregational unit that is free to segregate from heterologous chromosomes.

In Drosophila melanogaster females, the proper meiotic segregation of homologous chromosomes is usually accomplished by an exchange-mediated mechanism (Hawley 1988). Recombination leads to a physical linkage, or chiasma, that orients the two homologs to opposite poles and guarantees their segregation at anaphase I (Nicklas 1974). However, nonexchange chromosomes also segregate with a high degree of fidelity. These include the obligately achiasmate fourth chromosomes, homologs which are achiasmate due to heterozygosity for aberrations or failure of exchange, and compound chromosomes.

Hawley et al. (1993) demonstrated that there are two systems that facilitate achiasmate segregation in Drosophila females. This conclusion is based on the analysis of mutations that specifically affect the segregation of achiasmate homologs without impairing the segregation of heterologs and the finding that the two systems have very different rules and prerequisites for the choice of segregational partners. Thus there is not a single “distributive system” for achiasmate segregation, as suggested by Grell (1976), but rather two separate mechanisms, here named the homologous achiasmate segregation system and the heterologous segregation system.

The homologous achiasmate segregation system requires heterochromatic homology and likely reflects the pairing of heterochromatin at pachytene. This is demonstrated by the observation that free duplications bearing specific regions of the X or fourth chromosome heterochromatin can interfere with the disjunction of the X and fourth chromosomes, respectively, without regard to their size and/or shape (Hawley et al. 1993). Because this system depends on the absence of homolog repulsion prior to prometaphase, it is likely to be unique to those meioses, such as those of Drosophila females, which bypass diploctene and diakinesis.

The heterologous segregation system may not involve any direct interaction or pairing between the chromosomes. Rather, Hawley et al. (1993) have proposed that heterologous segregation is likely to be the result of the difficulties inherent in two independent chromosomes making stable connections to the same pole on a spindle whose diameter is quite small relative to the size of meiotic chromosomes (Theurkauf and Hawley 1992). A similar model, in which heterologous segregations are mediated by kineto-
chore-to-kinetochore microtubules, without physical pairing or chromosome association, has also been proposed by Carpenter (1991). Indeed, exactly such kinetochore-to-kinetochore microtubules have recently been observed between achiasmate segregating chromosomes in Drosophila oocytes (M. Erdman, T. Arbel and R. S. Hawley, unpublished observations).

The semidominant meiotic mutation Axs$^D$ (Aberrant X segregation – Dominant) differentiates between these two achiasmate segregational systems, in that it specifically disrupts the segregation of achiasmate homologs (Zitron and Hawley 1989; Hawley et al. 1993). The frequency of nondisjunction of the obligately achiasmate fourth chromosome is also increased in the presence of Axs$^D$; but only in the presence of high levels of X chromosome nondisjunction. Greater than 50% of the Axs$^D$-induced fourth chromosome nondisjunction results from nonhomologous XX ↔ 44 disjunctions. This result suggests that in the presence of Axs$^D$, achiasmate homologs fail to segregate from each other via the homology-dependent pathway, but are still free to participate in nonhomologous segregations.

Several lines of evidence suggest that the processes that underlie heterologous achiasmate segregations are much less, if at all, affected by Axs$^D$. First, as noted by Grell (1976), the choice of partners for heterologous segregations is dependent on size. An analysis of the effects of small X chromosomal free duplications (Dp(1;f)s) on the segregation of the achiasmate fourth chromosomes reveals that in Axs$^D$/Axs$^D$ females the frequency of Dp(1;f) ↔ 44 segregations is still dependent on the size of the Dp(1;f), such that only those duplications similar in size to chromosome 4 induce high levels of fourth chromosome nondisjunction (Hawley et al. 1993). Thus, the size rule (Grell 1976) still applies in Axs$^D$-bearing females.

Second, the presence of Axs$^D$ does not impair the ability of a metacentric chromosome to co-orient two acrocentric heterologs, the shape rule (Grell 1976). For example, in Axs$^D$/Axs$^D$ females which also carry a metacentric compound-fourth chromosome, almost all of the observed X chromosome nondisjunction is due to the two acrocentric X chromosomes segregating from the metacentric fourth chromosomes (Zitron and Hawley 1989). In addition, in Axs$^D$/Axs$^D$/Y females, in which homologous fourth chromosome segregation is impaired or prevented, Y ↔ 44 segregations become common place. Indeed the small reduction in the frequency of XX ↔ Y segregations observed in Axs$^D$/Axs$^D$/Y females can be fully accounted for by Y ↔ 44 segregations (Zitron and Hawley 1989); suggesting that in the presence of Axs$^D$, the ability of the metacentric Y chromosome to segregate from two acrocentric X chromosomes is impaired only by its tendency to segregate from two acrocentric fourth chromosomes.

Third, one of the hallmarks of heterologous segregation is the availability rule (Grell 1976); this is to say that when there are only two nonexchange chromosomes in the cell, they will segregate from each other regardless of their identity. This rule continues to hold in the presence of Axs$^D$. For example, when the X chromosomes are involved in exchange, the two nonexchange fourth chromosomes rarely nondisjoin. Similarly, in Axs$^D$-bearing females a compound-X and a compound-fourth chromosome segregate from each other with high fidelity (A. Zitron and R. S. Hawley, unpublished data). The frequency of compound-X from Y segregations is reduced in females homozygous for Axs$^D$ (Zitron and Hawley 1989); however, most or all of this reduction is apparently due to Y ↔ 44 segregations and compound-X ↔ 44 segregations (A. Zitron and R. S. Hawley, unpublished data). Taken together these data suggest that the presence of Axs$^D$ specifically impairs (or actively prevents) the segregation of achiasmate homologs without impairing the heterologous segregation system.

It should be noted that the phenotype of the Axs$^D$ mutation is not unique. There are two other meiotic mutations with similar phenotypes; these are described below.

A class of related genes required for heterochromatin-mediated segregations: There are two other genes (ald and mei-S51) which are also specifically required for homology-dependent achiasmate segregations. ald is a female-specific meiotic mutation that primarily affects the segregation of nonexchange chromosomes (O’Toula 1982). In the presence of ald, the frequency of homologous nondisjunctions is greatly increased, primarily as a result of an increase in the frequency of nonhomologous disjunctions. As is the case for Axs, the size-dependence of heterologous disjunctions is also maintained in ald females. The ald mutation also allows some chiasmate bivalents to participate in heterologous disjunctions (O’Toula 1982). Thus, although neither chiasmate or achiasmate homologous associations are sufficient to ensure disjunction in the presence of ald, heterologous segregations are unimpaired.

mei-S51 (Robbins 1971) also affects only homology-dependent pairing and segregation. Females homozygous for mei-S51 exhibit reduced exchange and achiasmate homologs nondisjoin at high frequencies. However, mei-S51 does not impair the occurrence of heterologous segregations, as evidenced by high frequencies of XX ↔ 44 disjunction. Robbins (1971) proposed that mei-S51 disrupts chromosome pairing and alignment prior to metaphase without affecting the homology-independent pathway of achiasmate segregation.

These observations demonstrate that there are at
least three mutations that cause specific defects in the homologous associations that facilitate achiasmate segregation and which produce similar phenotypic effects when mutated.

In order to better understand this homology-dependent segregation, we set out to answer the following questions. First, does the Axs mutation define a gene whose wild-type product is required for homology-dependent segregation, or is it a neomorphic mutation that defines a locus unrelated to this process? Second, what are the functional interrelationships between these three loci and between other genes, such as nod and ned, that are also required for achiasmate segregation?

MATERIALS AND METHODS

Chromosomes and mutations: All mutations and chromosomes used in this study are described in Lindsley and Zimm (1992). Cytological breakpoints follow the polytene chromosome map of Le Févre (1976). Chromosomes used in this study are abbreviated as follows: Attached XY, v f B refers to YSX-YL. inv(1)EN, v f B, FM7 refers to inv(1)FM7a, y’16d sc’ u’4 v’3 B, a multiply inverted X chromosome that completely suppresses recombination (Hawley et al. 1993). Inversion(1) scute-8 Left scute-4 Right, y cu v is referred to as sc’ cu’. In addition, dl-49 refers to In(1)delta-49, carrying markers as indicated in the text. The symbol C(4)RM refers to an attached fourth chromosome homologous for the markers ci and ev.

In the tables, Axs (or Axs') represents the multiply marked X chromosome, y cu v Axs car; the Axs' chromosome is y cu v Axs'2; the symbol '+' or '-' represents the multiply marked X chromosome, y cu v f car. The fourth chromosome mutation spo11 refers to an allele.

The dl-49 chromosome bears a euchromatic paracentric inversion of the X chromosome. In dl-49/+ heterozygotes X chromosome exchange is suppressed to approximately 12% of normal (Sturtevant and Beadle 1936; Novitski and Braver 1954). Thus, while unrearranged X chromosomes fail to recombine in only 9% of meioses (Zitron and Hawley 1989), in a dl-49/+ heterozygote, the Xs are achiasmate in 71% of meioses.

For each Axs revertant (Axs'), recombinant dl-49, y Hu g Axs' f car chromosomes were generated by mating y cu v Axs' car/dl-49, y Hu g f Rex females to FM7/y' Y males and selecting dl-49, y Hu g f' car sons. Based on the map positions of g, Axs, and f, 85% of these recombinants are expected to carry the Axs' allele. Indeed, four out of five Axs' and three out of five Axs'3 recombinants displayed high levels of X and fourth chromosome nondisjunction in this assay. These were presumed to represent bona fide dl-49, Axs' recombinants. One recombinant for each revertant was chosen for further study.

Nondisjunction assays and calculations: The total progeny class is adjusted in each table to correct for the lethality of certain progeny classes. X nondisjunction is always doubled, as triplo-X and nullo-X exceptions are lethal. In Table 5, the mothers carrying the duplications are heterozygous pol+/pol rather than homozygous pol/pol; therefore, fourth chromosome nondisjunction can be measured only in progeny receiving no fourth chromosomes from their mother. In each case, the number observed in the nullo-4 exceptional is substituted into the diplo-4 class and subtracted from the normal disjunction class. Corrections specific to each cross are described in the table legends.

Nondisjunction frequencies were calculated as described by Hawley et al. (1993). The proportion of the simultaneous X, 4 nondisjunctions which resulted from the segregation of the two X chromosomes from the fourth chromosomes is calculated as: 2 × (XX;O + O;44) − (XX;44 + O;O)/(adjusted total progeny).

When no progeny resulting from simultaneous X, 4 nondisjunction were recovered, the frequency of nonhomologous disjunction is designated as NA (not applicable).

Comparing frequencies of nondisjunction: Although there is some variance in the absolute frequency of Axs-induced X chromosome nondisjunction between experiments, that variance is considerably less within a given series of experiments. Hence the Axs phenotype is presumably very sensitive to differences in genetic background. Therefore considerable caution must be exercised in comparisons of raw frequencies of nondisjunction between different experiments. In all experiments, controls were run in parallel, using sisters with non-mutant X chromosomes. In repetitions, the order of severity and interactions among alleles was invariant.

RESULTS

Nomenclature and mapping of Axs: For unambiguous nomenclature, we now rename that original allele Axs (for Axs-Dominant). The revertants reported here will be named Axs', for Axs-revertant, followed by an allele designation number in the superscript (e.g., Axs'1).

Zitron and Hawley (1989) had mapped the Axs mutation just distal to forked (56.7 cM, 15F1). To more precisely map Axs, we obtained recombinants between rudimentary (54.5 cM, 15A) and forked from Axs car/f380 f B mothers. Eighteen of these recombinants were assayed for X and fourth chromosomal nondisjunction in X/FM7 females. Three of 18 tested recombinants resulted from exchanges that occurred between r and Axs, while 15 occurred between Axs and f. This places the gene nearer to r than to f, at 54.9 cM, presumably in polytene region 15A-B, a position concordant with the cytogenetic studies reported below.

We previously noted the presence of an extra polytene chromosome band in 15D1 which was tightly linked to the original Axs mutation and was absent from its parent chromosome (Zitron and Hawley 1989). However, the cytological aberration was absent in one of the recombinants carrying the Axs phenotype. Therefore, the Axs mutation is not associated with the aberration in 15D1 (Whyte 1993).

Axs is only partially rescued by duplications: Normally the morph of Axs could simply have been tested by asking whether or not it could be mimicked by a deficiency. Unfortunately, due to the high density of Minutes in this region, deficiencies are not available. The alternative approach was to inquire whether or not the Axs' mutation could be rescued by a duplication.

Zitron and Hawley (1989) demonstrated that Axs was at least partially rescued by Dp(1;4)A1+ f* (13F10;16A1–2). However, the analysis of this exper-
X and fourth chromosomal nondisjunction in females bearing revertants of Ax$^{d}$

<table>
<thead>
<tr>
<th>Female genotype</th>
<th>Percent nondisjunction</th>
<th>Adjusted total progeny</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/FM7</td>
<td>0.4 0.2 0.1 0.0</td>
<td>7459</td>
</tr>
<tr>
<td>Ax$^{d}$/FM7</td>
<td>17.0 8.0 3.8 2.6</td>
<td>3906</td>
</tr>
<tr>
<td>Ax$^{s}$/FM7</td>
<td>0.7 0.6 0.4 0.0</td>
<td>2182</td>
</tr>
<tr>
<td>Ax$^{s}$/FM7</td>
<td>1.7 0.4 0.4 0.0</td>
<td>475</td>
</tr>
<tr>
<td>Ax$^{s}$/FM7</td>
<td>1.6 0.0 0.0 0.0</td>
<td>372</td>
</tr>
</tbody>
</table>

Females of the indicated genotype were mated to asex-XY; vJ B; C(4)RM, M ey females.

resulting Ax$^{s}$/FM7 daughters were then individually mated to appropriate males and their progeny scored to identify those females which did not produce the high levels of X nondisjunctional offspring (specifically XXX females) that are characteristic of Ax$^{s}$/FM7 females. Only those chromosomes which caused no more than 2% X chromosome nondisjunction were retained.

Three revertant alleles were isolated from a total of 1998 EMS-treated Ax$^{d}$/FM7 chromosomes. These revertants are here named Ax$^{s}$/FM7, Ax$^{s}$/FM7, and Ax$^{s}$/FM7. When heterozygous with FM7, Ax$^{s}$/FM7, all three revertants yield only background levels of nondisjunction for either X or fourth chromosomes, indicating complete loss of the dominant phenotype (Table 1). As shown below, none of these three revertant chromosomes can suppress Ax$^{d}$ when present in trans (i.e., in Ax$^{s}$/Ax$^{d}$/FM7 females). Thus they cannot simply be dominant suppressors of Ax$^{d}$. Moreover, all three of the revertants are inseparable from the Ax$^{d}$/FM7 mutation by direct recombinational mapping (WHYTE 1933). Thus, the revertants are tightly linked to, if not inseparable from, the Ax$^{d}$/FM7 lesion.

These data demonstrate that the dominant phenotype of the Ax$^{d}$/FM7 mutation is indeed revertible. Taken together with the inability of Ax$^{s}$/FM7 duplications to fully rescue the Ax$^{d}$/FM7 mutation, these data suggest that the Ax$^{d}$/FM7 mutation is a dominant gain-of-function mutation. In the following section we show that these revertants possess a recessive meiotic phenotype identical to that exhibited by Ax$^{d}$/FM7, and which can be rescued by duplications that carry Ax$^{s}$/FM7, suggesting that Ax$^{d}$/FM7 is an antimorphic allele.

The revertants display a recessive meiotic phenotype similar to that of Ax$^{d}$/FM7: The revertants were tested for a meiotic phenotype in exchange-suppressed (dl-49/+) and in exchange competent (+/+ ) females. The Ax$^{d}$/FM7 allele induces 20–25% X nondisjunction in Ax$^{d}$/dl-49,Axs$^{s}$/FM7 females in contrast to only 3% X nondisjunction in the Ax$^{d}$/+ females (ZITRON and HAWLEY 1989). None of these recombinants induced significant levels of nondisjunction in +/dl-49,Axs$^{s}$/FM7 females, confirming their lack of a dominant phenotype (cf. Table 2). However, as shown in Table 2, Axs$^{s}$/dl-49,Axs$^{s}$/FM7 females displayed the same high

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**FIGURE 1.**—Scheme for reversion mutagenesis of Ax$^{d}$. About 5% of the progeny of Ax$^{s}$/FM7 females will be XXX daughters, while Ax$^{s}$/FM7 females produce only 0.2% XXX daughters (ZITRON and HAWLEY 1989). Thus, by individually mating yellow females carrying a mutated Ax$^{d}$/FM7 chromosome over FM7, Ax$^{s}$/ females to males with a y$^{+}$ Y chromosome, we could recognize revertants as those females who bore no yellow$^{+}$ daughters. y Ax$^{s}$/FM7, Ax$^{s}$/ daughters of the putative revertants were selected and rescreened in the same manner.

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**TABLE 1**

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Females of the indicated genotype were mated to asex-XY; vJ B; C(4)RM, M ey females.
levels of X and fourth chromosomal nondisjunction as do Axs<sup>r</sup>/dl-49,Axs<sup>r</sup> females (compare Tables 2 and 3). Moreover, in both genotypes a large fraction of the fourth chromosome nondisjunction was the consequence of simultaneous X<sub>4</sub> nondisjunction.

A similar, but weaker, phenotype is observed in Axs<sup>r</sup>/dl-49,Axs<sup>r</sup> females. Thus both Axs<sup>r</sup><sub>2</sub> and Axs<sup>r</sup><sub>3</sub> behave as recessive meiotic mutations whose phenotypes are similar to that of the Axs<sup>r</sup> mutant. The observation of high levels of achiasmate nondisjunction in Axs<sup>r</sup><sub>2</sub>/dl-49,Axs<sup>r</sup><sub>3</sub> females also demonstrates that Axs<sup>r</sup><sub>2</sub> and Axs<sup>r</sup><sub>3</sub> are allelic.

Surprisingly, no meiotic phenotype was observed in putative y<sup>cy</sup> v<sup>Axs<sup>r</sup></sup> car<sup>/dl-49</sup>,Axs<sup>r</sup><sup>+</sup> females for any of the ten dl-49,Axs<sup>r</sup> constructions tested. However, 9 out of 10 of these constructions did display a strong meiotic phenotype in y<sup>cy</sup> v<sup>Axs<sup>r</sup></sup><sub>2</sub>/dl-49,Axs<sup>r</sup><sub>1</sub> females. (As shown in Table 2, the same strong meiotic phenotype is also observed when the coupling relationships of Axs<sup>r</sup><sub>1</sub> and Axs<sup>r</sup><sub>2</sub> are reversed in Axs<sup>r</sup><sub>1</sub>/dl-49,Axs<sup>r</sup><sub>2</sub> females). These observations demonstrate that the Axs<sup>r</sup><sub>1</sub> mutation is allelic to Axs<sup>r</sup><sub>2</sub> and Axs<sup>r</sup><sub>3</sub>, and suggests that the Axs<sup>r</sup><sub>1</sub> mutation may be a hypomorph that can confer wild-type function only when present in two doses.

Thus the three revertants define a single complementation group and all have a phenotype identical to that of Axs<sup>r</sup>.

**Loss-of-function alleles of Axs specifically impair homologous achiasmate disjunctions:** For all allelic combinations of Axs-revertants, greater than 70% of the simultaneous X and 4 nondisjunction events are nonhomologous disjunctions (XX<sup>c.*</sup>44). Thus, as is the case for Axs<sup>r</sup>-induced nondisjunction, the effect of the Axs<sup>r</sup> alleles is restricted to homologous achiasmate segregation, while heterologous segregations still occur with relatively high fidelity. In this section we also demonstrate that the effects of the Axs<sup>r</sup> mutations, like those of the Axs<sup>r</sup> allele, are restricted to homologous achiasmate segregation and do not extend to other meiotic or mitotic processes.

The original Axs<sup>r</sup> allele had no effect either on the frequency or distribution of exchange or on the segregation of exchange bivalents (Zitron and Hawley 1989). Similarly, in females homozygous for Axs<sup>r</sup><sub>2</sub>, there was little, if any, effect on the segregation of
TABLE 3
Interaction of Axs with revertant alleles

<table>
<thead>
<tr>
<th>Gamete types</th>
<th>Maternal genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+/+</td>
</tr>
<tr>
<td>Regular</td>
<td></td>
</tr>
<tr>
<td>X4</td>
<td>866</td>
</tr>
<tr>
<td>X4</td>
<td>637</td>
</tr>
<tr>
<td>X Nondisjunct.</td>
<td></td>
</tr>
<tr>
<td>O4</td>
<td>10</td>
</tr>
<tr>
<td>X4</td>
<td>4</td>
</tr>
<tr>
<td>4 Nondisjunct.</td>
<td></td>
</tr>
<tr>
<td>X44</td>
<td>0</td>
</tr>
<tr>
<td>XXO</td>
<td>0</td>
</tr>
<tr>
<td>XO</td>
<td>0</td>
</tr>
<tr>
<td>X44</td>
<td>0</td>
</tr>
<tr>
<td>Total progeny</td>
<td>1521</td>
</tr>
</tbody>
</table>

TABLE 4
Axs² does not induce recombinant X chromosomes to nondisjoin

<table>
<thead>
<tr>
<th>Female genotype</th>
<th>Percent nondisjunction</th>
<th>Adjusted total progeny</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td>X, 4</td>
</tr>
<tr>
<td>+/-</td>
<td>0.39</td>
<td>0.11</td>
</tr>
<tr>
<td>Axs²²/Axs² a</td>
<td>2.33</td>
<td>0.93</td>
</tr>
<tr>
<td>Axs²²/dl-49/Axs²</td>
<td>22.80</td>
<td>5.70</td>
</tr>
</tbody>
</table>

TABLE 3 continued

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<tr>
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</tr>
</tbody>
</table>

No combination of the revertants, nondisjunction of the fourth and X chromosomes produces equal frequencies of nullo- and diplo-exceptions. Thus, as is the case for Axs, nondisjunction is not accompanied by a significant level of chromosomal loss.

We isolated no mosaics or gynandromorphs in any nondisjunction assays for Axs and the revertants, indicating these alleles do not cause levels of postmeiotic chromosome loss which exceed those due to background effects. Nor were the frequencies of X and fourth chromosome nondisjunction elevated above background in Axs²/Y; pol pol males, so the Axs locus has no measurable function in male meiosis (data not shown). No combination of homozygotes or heterozygotes produced sterility or inviability. Therefore, there are no obvious phenotypes for any Axs mutation other than the nondisjunction of achiasmate homologs.

dl-49/+ females of the indicated genotype were mated to attached-XY;v f B; C(4)RM,ci ef⁴ males.

The In(I)dl-49, y Hu g Axs² and In(I)dl-49, y Hu m Axs² chromosomes are hemizygous lethal. Therefore the X0 progeny classes are doubled in the adjusted total progeny.

exchange X chromosomes. As shown in Table 4, the frequency of X chromosome nondisjunction in Axs²²/ Axs²² females was 2.33% or approximately one-third of the expected frequency of nonexchange chromosomes (6–10%, HAWLEY et al. 1993). Similar data were also obtained for the remaining two revertants (WHYTE 1993). In females that carry two normal sequence X chromosomes, no combination of Axs alleles induces levels of X nondisjunction that are above 50% of the frequency of achiasmate X chromosomes (data not shown). These data argue that the meiotic defect caused by homozygosity for Axs-revertants, like that caused by the AxsD mutation, uniquely affects nonexchange segregation. For all combinations of the revertants, nondisjunction of the fourth and X chromosomes produces equal frequencies of nullo- and diplo-exceptions. Thus, as is the case for Axs, nondisjunction is not accompanied by a significant level of chromosomal loss.

We isolated no mosaics or gynandromorphs in any nondisjunction assays for Axs and the revertants, indicating these alleles do not cause levels of postmeiotic chromosome loss which exceed those due to background effects. Nor were the frequencies of X and fourth chromosome nondisjunction elevated above background in Axs²/Y; pol pol males, so the Axs locus has no measurable function in male meiosis (data not shown). No combination of homozygotes or heterozygotes produced sterility or inviability. Therefore, there are no obvious phenotypes for any Axs mutation other than the nondisjunction of achiasmate homologs.
Genetic studies of the revertant alleles: In terms of their effects on X and fourth chromosome nondisjunction, the dominant mutation and its three revertants form an allelic series: such that the phenotype of AxS²/AxS⁰ is similar to that of AxS²/AxS² while AxS³/AxS³ females exhibit an intermediate phenotype and AxS¹/AxS⁷ females are phenotypically normal (Table 2, columns 5–7, and Table 3, column 3). More detailed characterizations of each of the three revertants are presented below.

The AxS² allele: As a homozygote, AxS² is as severe as the dominant AxS⁰ mutation. That AxS² is a loss of function allele is demonstrated both by the fact that it is fully recessive and by the observation that a duplication of AxS+ can completely rescue the mutant phenotype in AxS²/dl-49,AxS²; Dp(AxS+) females (see below). AxS² provides no rescue of the AxS function in AxS²/dl-49,AxS⁰ females (Table 3, columns 5 and 6). Thus AxS² has completely lost the antimorphic phenotype of AxS², while failing to gain any AxS+ function, suggesting that it might be an amorphic allele of AxS. However, without the ability to compare the meiotic effects of AxS² to those of a homoygous viable and fertile deficiency of the locus, we cannot rule out the possibility that AxS² retains some wild-type function.

The AxS³ allele: Homozygosis for AxS³ induces only about 80% as much nondisjunction as does AxS² (see Table 2). Moreover AxS³ exhibits a reduced level of nondisjunction in AxS³/dl-49,AxS³; Dp(AxS+) females as compared with AxS⁰/dl-49,AxS⁰ females (see Table 3). Both of these results are consistent with only a partial loss of function. The fact that in flies heterozygous for AxS³/dl-49,AxS², the phenotype is as strong as that of the AxS² homozygote (see Table 2) may indicate that the residual level of AxS+ function falls below some essential threshold in AxS³/dl-49,AxS² females.

The AxS¹ allele: AxS¹ appears completely wild type in homozygous females, and in a trans-heterozygote with AxS² it causes less nondisjunction of both the X and fourth chromosomes than does the AxS² homozygote (see Table 2). This is exactly the phenotype expected of a weak hypomorph in the presence of a stronger allele.

Surprisingly, the combination AxS¹/dl-49,AxS⁰ produces a more severe meiotic phenotype than does the genotype AxS²/dl-49,AxS² (Table 3). One could imagine that when alone, or in the presence of AxS+ protein, the AxS¹ protein assumes an active conformation, but in combination with AxS⁰, it reverts to a dysfunctional or nonfunctional state.

Cytogenetic mapping of AxS: As shown above, recombinational mapping placed AxS⁰ proximal to rudimentary in 15A-B. This allowed us to test a group of eighteen duplications derived from Dp(1;4)r+f+ whose distal breakpoints were in 14B-C and whose proximal breakpoints were in the 15A region (Falk et al. 1984; Falk and Halladay 1986) for their ability to rescue the recessive loss-of-function mutation, AxS². Although all 18 duplications delete varying amounts of the 15A interval, ambiguities in our genetic and cytological characterization of this region preclude the more precise ordering of breakpoints within this region at this time.

These derivatives of Dp(1;4)r+f+ were tested for their ability to enable regular disjunction in AxS²/dl-49,AxS² females. For each duplication, sisters of the genotype AxS²/dl-49,AxS²;pol/pol were tested simultaneously for the induction of nondisjunction (data not shown). This allowed us to control for the nondisjunction caused by the duplication-carrying fourth chromosome, especially in the presence of AxS² (Table 5, columns 1–4). Only one of the eighteen duplications tested was able to rescue the meiotic defect (81j6c, Table 5, column 5). Complete progeny counts for only three of the noncomplementing duplications are presented (Table 5, columns 6–8).

While there is a significant level of residual nondisjunction in flies carrying the full duplication, it is not characteristic of AxS-induced nondisjunction (note the absence of XX ↔ 44 segregations). The Dp(1;4)81j6c chromosome, which removes about half of the X euchromatin from the parent duplication, essentially completely rescues the AxS phenotype (compare columns 3 and 5, Table 5). These results clearly place AxS² somewhere in 15A.

The interaction of AxS with ald: As a means of attempting to elucidate the functional interrelationships between AxS and other genes involved in homologous achiasmate segregation, we have searched for genetic interactions between dominant and recessive alleles of AxS and the sole existing allele of ald.

Although the effects of AxS⁰ and ald/ald on X chromosome nondisjunction appear to be additive, two lines of evidence suggest that AxS⁰ may be epistatic to ald with respect to its effect on fourth chromosomal disjunction. First, the level of fourth chromosome nondisjunction observed in AxS⁰/FM7; ald/ald females is not additive, but rather is substantially lower than that observed in +/FM7; ald/ald females. Indeed, it quite close to that observed in AxS⁰/FM7; +/- females (8.1% and 6.2%, respectively). Second, the observed ratio of X and fourth chromosome nondisjunction (~4:1), and the fact that greater than 70% of the fourth chromosome nondisjunction results from simultaneous X,4 nondisjunction, is more typical of AxS²-induced fourth chromosome nondisjunction than of ald-induced 4th chromosome nondisjunction.

We propose the following interpretation for these results. The ald mutation allows a larger fraction of X chromosomes (both exchange and nonexchange) to enter the achiasmate disjunctional system (O'Tousa 1982). We propose that these additional X chromo-
TABLE 5
Mapping of AmrZ by derivatives of the Dp(1;4)r+f chromosome

<table>
<thead>
<tr>
<th>Gamete types</th>
<th>Maternal genotype</th>
<th>Table 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regular</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X4</td>
<td>X4 44</td>
<td>560</td>
</tr>
<tr>
<td>X4</td>
<td>O44</td>
<td>382</td>
</tr>
<tr>
<td>X Nondisjunctial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O4</td>
<td>X4 44</td>
<td>0</td>
</tr>
<tr>
<td>XX4</td>
<td>O44</td>
<td>2</td>
</tr>
<tr>
<td>4 Nondisjunctial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X 44</td>
<td>X4 O4</td>
<td>0</td>
</tr>
<tr>
<td>X4</td>
<td>O0</td>
<td>0</td>
</tr>
<tr>
<td>X 44</td>
<td>X4 O4</td>
<td>1</td>
</tr>
<tr>
<td>X,4 Nondisjunctial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>XX 44</td>
<td>O0</td>
<td>0</td>
</tr>
<tr>
<td>X0</td>
<td>X4 O4</td>
<td>0</td>
</tr>
<tr>
<td>O 44</td>
<td>X4 O0</td>
<td>1</td>
</tr>
<tr>
<td>Total progeny</td>
<td>946</td>
<td>793</td>
</tr>
<tr>
<td>Adjusted total</td>
<td>1328</td>
<td>1052</td>
</tr>
<tr>
<td>% Nondisjunction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>4</td>
<td>0.2</td>
<td>0.0</td>
</tr>
<tr>
<td>% Simultaneous X,4 nondisjunction</td>
<td>0.2</td>
<td>0.0</td>
</tr>
</tbody>
</table>

dl-49/+ females of the indicated genotype were mated to attached-XY,vfB; C(4)RM,cf eyR males.

TABLE 6
Interaction of Axs with other meiotic mutants

<table>
<thead>
<tr>
<th>Mother's genotype</th>
<th>Percent nondisjunction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>

A. ncd enhances Axs

FM7/+/+: /+ | 0.2 | 0.1 | 0.0 | NA | 14246 |
FM7/Axs'/: /+ | 1.3 | 0.2 | 0.0 | NA | 946 |
FM7/+; /+ncd | 0.6 | 0.4 | 0.0 | NA | 7198 |
FM7/Axs'/: /+ncd | 3.4 | 3.6 | 0.5 | 0.5 | 414* |
FM7/Axs'/: /+ncd | 0.0 | 1.6 | 0.0 | NA | 815 |
B. ald and Axs' fully complement

dl-49,Axs'/: /+; /+ | 0.5 | 0.0 | 0.0 | NA | 413 |
dl-49,+/+; /+ald | 0.5 | 0.4 | 0.1 | 0.1 | 2062 |
dl-49,Axs'/: /+ald | 0.3 | 0.4 | 0.1 | 0.1 | 2019 |
C. Axs' is epistatic to ald

FM7/Axs'/: /+ | 23.5 | 6.2 | 4.3 | 3.5 | 1380 |
FM7/+; ald/ald | 10.3 | 17.3 | 5.4 | 5.4 | 1550 |
FM7/Axs'/: ald/+ | 24.8 | 4.1 | 3.0 | 2.3 | 1120 |
FM7/Axs'/: ald/ald | 35.7 | 8.1 | 5.8 | 4.9 | 902 |

Females of the indicated genotype were mated to attached-XY; C(4)RM,cf cf eyR males.
* These females displayed a significantly reduced fertility.

Axs' is enhanced by the ncd mutation: The ncd gene encodes a kinesin-like protein and is specifically required for proper spindle assembly (McDonald and Goldstein 1990; Endow, Henikoff and Solor-Niedziela 1990). As shown in Table 6B, ncd/+ females show much higher levels of both X and fourth chromosomal nondisjunction than do either of the single heterozygotes. Increased X nondisjunction is only observed when X chromosome exchange is suppressed and high levels of X nondisjunction are accompanied by high levels of fourth chromosome nondisjunction. This demonstrates that the Axs-ncd interaction is due to an enhancement of the Axs phenotype by a single dose of ncd". This interaction with ncd is not unexpected in that mutations at both Axs and ncd disrupt proper spindle formation (Kimble and Church 1983; M. Erdman, T. Arbel, W. Whyte and R. S. Hawley, unpublished observations).

We have also tested the interaction of Axs alleles

somes are then acted upon to produce an Axs-like defect in X,4 segregation.

As shown in Table 6B, there is no evidence for second site noncomplementation in Axs"/FM7; ald/+ females; nor does heterozygosity for ald enhance the phenotype of AmrZ.

AmrZ is enhanced by the ncd mutation: The ncd gene encodes a kinesin-like protein and is specifically required for proper spindle assembly (McDonald and Goldstein 1990; Endow, Henikoff and Solor-Niedziela 1990). As shown in Table 6, FM7/Axs"; ncd/+ females show much higher levels of both X and fourth chromosomal nondisjunction than do either of the single heterozygotes. Increased X nondisjunction is only observed when X chromosome exchange is suppressed and high levels of X nondisjunction are accompanied by high levels of fourth chromosome nondisjunction. This demonstrates that the Axs-ncd interaction is due to an enhancement of the Axs phenotype by a single dose of ncd". This interaction with ncd is not unexpected in that mutations at both Axs and ncd disrupt proper spindle formation (Kimble and Church 1983; M. Erdman, T. Arbel, W. Whyte and R. S. Hawley, unpublished observations).

We have also tested the interaction of Axs alleles

with loss of function alleles of the nod locus. The nod gene encodes a kinesin like protein which is required to position and/or maintain achiasmate chromosomes during spindle development (Zhang et al. 1990;
Theurkauf and Hawley 1992). In the case of nod, there was no evidence for genetic interactions with Axs, in that heterozygosity for nod did not enhance the phenotype of AxsD (Zitron and Hawley 1989). Moreover, disjunction appears normal in FM7,nod/Axsp females (R. S. Hawley, unpublished data). The failure to find an interaction with nod may not be surprising, in that nod mutations, unlike mutations in Axs and nod, do not disrupt proper spindle formation (Theurkauf and Hawley 1992).

DISCUSSION

We previously described a semidominant mutation, AxsD, which impairs the segregation of achiasmate homologs at meiosis I (Zitron and Hawley 1989; Hawley et al. 1995). Three lines of evidence argue that the original AxsD mutation represents a poisonous (antimorphic) allele. First, it is reversible. Second, it is only partially rescued by an additional wild-type copy of the gene: that is, in terms of phenotypic severity, AxsD/AxsD > AxsD/+ > AxsD/+ > +/+. Third, loss of function alleles of Axs have a meiotic phenotype that is similar to that of AxsD, thus ruling out the possibility that AxsD is a neomorphic allele.

Both dominant and recessive alleles of Axs impair homologous achiasmate segregations while not impairing heterologous segregations. Moreover, none of the three Axs revertants is homozygous lethal or sterile in either sex, even though such mutations would have been recovered by this screen. These observations suggest that the function of Axs+ is limited to female meiosis I and is specific for homologous achiasmate segregations. As demonstrated by Hawley et al. (1993), such segregations require heterochromatic homology. It is thus reasonable to think of Axs+ as a gene required for the facilitation, maintenance or proper release of heterochromatic pairing.

The Axs function may be required for the separation of achiasmate homologs: The genetic analysis of Axs reveals that Axs mutations exhibit a defect in achiasmate segregation. Other data reveal that the heterologous size-dependent system functions normally in the presence of Axs mutations (Hawley et al. 1993). These two observations raise a serious paradox. Why then does not a competent size-dependent disjunctional system prevent X ↔ 4 segregations even in the absence of heterochromatic pairings?

Because not all of the X nondisjunction in Axs can be accounted for by heterologous disjunctions, we argue that Axs mutations do not impair homologous pairings per se, but rather they prevent paired homologs from separating. Note that the level of X nondisjunction is virtually the same (~35%) in the following classes of females [data from Zitron and Hawley (1989) and Hawley et al. (1993)]:

\[sc^I\cdot AxsD^p/dl-49,AxsD^p; 4/4\]

Clearly, the frequency of X chromosome nondisjunction is independent of the number or identity of the heterologous chromosomes that are present. Rather, the number and type of available heterologs appears to influence only the fraction of X chromosome nondisjunction which is due to heterologous segregations. Thus, although nondisjoining Xs are free to undergo heterologous disjunctions in Axs oocytes, it is not the heterologous associations that cause nondisjunction.

Rather we suppose that Axs mutations cause nonexchange chromosomes to become interlocked (or intertwined) preventing their separation at prometaphase. We also propose that this interlocked pair of chromosomes then behaves as a single segregation unit that is free to use the heterologous system.

To explain the large direct effect of Axs mutations on the segregation of the X chromosomes and major autosomes, and their minimal direct effect on the fourth chromosome, we also propose that the probability of Axs-induced interlocking increases with increasing chromosome size. For example, one could easily imagine that the cases of individual X or fourth chromosome nondisjunction are due to the movement of an interlocked pair of chromosomes to a single pole. In the case of exchange suppressed XX females carrying a compound-fourth chromosome (C(4)RM), the two interlocked X chromosomes would separate from the compound-fourth chromosome, exactly as was observed by Zitron and Hawley (1989). Similarly, XX ↔ 4 segregations in chromosomally normal females would result from the alignment of an interlocked pair of Xs with one or both fourth chromosomes.

That such interlockings can occur is suggested by Carpenter's (1973) analysis of secondary nondisjunction in the presence of the nod mutation. She showed that, in the presence of nod, the two achiasmate Xs still co-segregate as a single unit despite segregating at random from the Y. This demonstrates that two chromosomes can interact in some fashion as to commit them to move to the same pole.

The ability of chiasmata to rescue the Axs defect is explained by the fact that in Drosophila female meiosis, achiasmate segregations precede chiasmate segregations (Theurkauf and Hawley 1992), such that chiasmate bivalents would be precluded from moving to the poles at the time heterologous segregations occur. Thus either the chiasmata themselves resolve the interlocking or it is resolved while the chiasmate chromosomes remain at the metaphase plate during metaphase arrest.

Two lines of evidence support such a segregational defect for Axs. First, confocal studies of meiotic spin-
dle development in Axs\(^{0}/\)FM7 and Axs\(^{2}/\)dl-49, Axs\(^{2}\) females suggest that mutations at Axs cause serious malformations in meiotic spindle development (M. Erdman, T. Arbel, W. Whyte and R. S. Hawley, manuscript in preparation). These defects are not observed in Axs\(^{2}\) - or Axs\(^{2}\)-bearing females carrying normal sequence X chromosomes, but rather are observed only in oocytes with exchange-suppressed X, second or third chromosomes. Given that the chromosomes, and not centrosomes, organize the meiotic spindle in Drosophila females, a defect in chromosome alignment such as intertwined achiasmate homologs might be expected to cause serious difficulties in establishing a normal spindle and thus produce such a phenotype.

Second, we have shown here that the ncd mutation behaves as a dominant enhancer of Axs. Mutations at the ncd locus have long been known to produce abnormally wide or multipolar spindles (W. Theurkauf, personal communication; Kimble and Church 1983; Wald 1956; Hatsumi and Endow 1992). We propose that a twofold reduction in ncd\(^{+}\) function (due to heterozygosity for a loss-of-function mutation) creates a slightly less stable spindle on which the Axs-induced defect becomes more pronounced. A similar case of a slightly less stable spindle on which the Am-induced (nodDTW) demonstration for mutations at the Axs locus have long been known to produce abnormalities at the ncd locus; but is simply a weakly dominant loss-of function mutation. The amino acid sequence of ncd\(^{+}\) differs from the canonical wild-type sequence by two residues (Komma, Horne and Endow 1991). One of these changes occurs in the putative microtubule binding domain; the second occurs in an unconserved region outside the motor domain. Although it is tempting to ascribe the phenotypic effects of the ncd\(^{+}\) allele to the defect in the motor domain, it should be noted that three null alleles of ncd are due to changes in the unconserved carboxy-terminal domain of the protein (R. S. Rasooly and R. S. Hawley, unpublished observations).

By comparison with the cases of dominant alleles of nod and ncd, we propose that the Axs\(^{2}\) mutation also results from a mutational alteration that both prevents normal function and poisons the wild-type allele. As to the exact biochemical function of this protein we can only say that the mapping studies reported in this paper have placed the Axs locus within an existing chromosomal walk and provide the landmark to delimit its boundaries. Efforts to identify and sequence the Axs-coding region are well underway in the laboratory.

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Note added in proof: Since the date of this submission, Wendy Hurley, Cindy Ramirez and R. S. Hawley have validated the hypothesis that the AxsD mutation can induce high levels of X chromosomal nondisjunction in the absence of any achiasmate heterolog (see above). Specifically, they have observed high levels of X chromosome nondisjunction in females of the genotype AxsD/dl-49,AxsD/T(3;4)S6D/T(3;4)ll6D in which the normal achiasmate fourth chromosomes have been appended to the virtually always achiasmate third chromosomes. This result demonstrates that the Axs defect reflects a failure of the ability of achiasmate X chromosomes to separate and not a consequence of heterologous pairings or alignments.

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