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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Manuscript received December 28, 1992
Accepted for publication March 19, 1993

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. Axs^D (*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 Axs^D mutation (Axs^{r2} and Axs^{r3}) that exhibit a recessive meiotic phenotype identical to that observed in Axs^D/Axs^D females. A third revertant (Axs^{r1}) exhibits no meiotic phenotype as a homozygote, but a meiotic defect is observed in Axs^{r1}/Axs^{r2} females. Therefore mutations at the Axs^D 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 diplotene 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-

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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 achiasmately 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 $XX \leftrightarrow 4$ 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 $Dp(1;f) \leftrightarrow 4$ 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, $Y \leftrightarrow 4$ segregations become common place. Indeed the small reduction in the frequency of $XX \leftrightarrow Y$ segregations observed in $Axs^D/Axs^D/Y$ females can be fully accounted for by $Y \leftrightarrow 4$ 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 $Y \leftrightarrow 44$ segregations and *compound-X* $\leftrightarrow 44$ 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'TOUSA 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'TOUSA 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 $XX \leftrightarrow 44$ 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*^{D} 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 *ncd*, 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 LEFEVRE (1976). Chromosomes used in this study are abbreviated as follows: *Attached-XY,v f B* refers to *YSX•YL, In(1)EN, v f B*. *FM7* refers to *In(1)FM7a, y*^{31d} *sc*^{8} *w*^{a} *v*^{Of} *B*, a multiply inverted *X* chromosome that completely suppresses recombination (HAWLEY *et al.* 1993). *Inversion(1) scute-8 Left scute-4 Right, y cv v* is referred to as *sc*^{4} *sc*^{8}. 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 homozygous for the markers *ci* and *ey*^{R}.

In the tables, *Axs*^{D} (or *Axs*^{r}) represents the multiply marked *X* chromosome, *y cv v Axs car*; the *Axs*^{r2} chromosome is *y cv v Axs*^{r2}; the symbol " +" represents the multiply marked *X* chromosome, *y cv v f car*. The fourth chromosome mutation *spa*^{pol} is abbreviated as *pol*.

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 *X*s are achiasmate in 71% of meioses.

For each *Axs*^{D} revertant (*Axs*^{r}), recombinant *dl-49, y Hw g Axs*^{r} *f*$^{+}$ *car* chromosomes were generated by mating *y cv v Axs*^{r} *car/dl-49, y Hw g f Rex* females to *FM7/y*$^{+}$ *Y* males and selecting *dl-49, y Hw g f*$^{+}$ *car* sons. Based on the map positions of *g*, *Axs* and *f*, 85% of these recombinants are expected to carry the *Axs*^{r} allele. Indeed, four out of five *Axs*^{r2} and three out of five *Axs*^{r3} recombinants displayed high levels of *X* and fourth chromosome nondisjunction in this assay. These were presumed to represent *bona fide dl-49, Axs*^{r} 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*/+ 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 class 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*^{D}: For unambiguous nomenclature, we now rename that original allele *Axs*^{D} (for *Axs-Dominant*). The revertants reported here will be named *Axs*^{r}, for *Axs-revertant*, followed by an allele designation number in the superscript (*e.g.*, *Axs*^{r1}).

ZITRON and HAWLEY (1989) had mapped the *Axs*^{D} mutation just distal to *forked* (56.7cM, 15F1). To more precisely map *Axs*^{D}, we obtained recombinants between *rudimentary* (54.5 cM, 15A) and *forked* from *Axs*^{D} *car/r*^{39k} *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*^{D} 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*^{D} phenotype. Therefore, the *Axs*^{D} mutation is not associated with the aberration in 15D1 (WHYTE 1993).

***Axs*^{D} is only partially rescued by duplications:** Normally the morphy of *Axs*^{D} 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*^{D} mutation could be rescued by a duplication.

ZITRON and HAWLEY (1989) demonstrated that *Axs*^{D} was at least partially rescued by *Dp(1;4)r*$^{+}$ *f*$^{+}$ (13F10;16A1–2). However, the analysis of this exper-

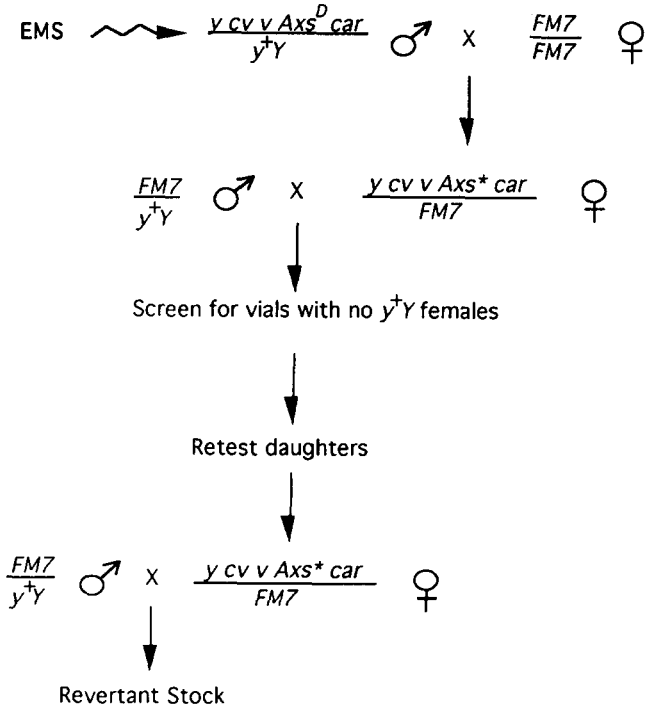

FIGURE 1.—Scheme for reversion mutagenesis of Axs^D. About 5% of the progeny of $Axs^D/FM7$ females will be *XXY* daughters, while $Axs^+/FM7$ females produce only 0.2% *XXY* daughters (ZITRON and HAWLEY 1989). Thus, by individually mating *yellow* females carrying a mutated Axs^D chromosome over *FM7*,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

iment was complicated by high background levels of fourth chromosome nondisjunction induced by the duplication itself. We repeated this experiment using a duplication of the Axs^+ region transposed to chromosome *2*. This much smaller duplication ($Dp(1;2)r^{+75c}$, 14B13;15A9) does not by itself induce *X* or fourth chromosome nondisjunction in Axs^+ females. In $Axs^D/FM7$, Axs^+; $Dp(1;2)r^{+75c}/+$ females the frequency of *X* nondisjunction is reduced by more than three fold (from 36% in $Axs^D/FM7$ females to 10% in $Axs^D/FM7$ females carrying the duplication) and fourth chromosome nondisjunction was reduced to background levels (WHYTE 1993). Thus, this duplication also appears to only partially suppress the effect of Axs^D on *X* nondisjunction.

These experiments suggested that Axs^D is a semidominant antimorph that is only partially rescued by the addition of an additional wild-type copy of Axs^+. If this is the case then Axs^D should be easily revertible by a second inactivating lesion within the gene.

Isolating intragenic revertants of Axs^D: The scheme for isolating EMS-induced revertants of Axs^D is diagrammed in Figure 1. Axs^D-bearing males were treated with ethyl methanesulfonate (EMS), as described by LEWIS and BACHER (1968), and mated to *FM7/FM7* females, as described in Figure 1. The

TABLE 1

X and fourth chromosomal nondisjunction in females bearing revertants of Axs^D

Female genotype	Percent nondisjunction				Adjusted total progeny
	X	*4*	*X, 4*	*XX ↔ 44*	
+/FM7	0.4	0.2	0.1	0.0	7459
$Axs^D/FM7$	17.0	8.0	3.8	2.6	3906
$Axs^{r1}/FM7$	0.7	0.6	0.4	0.0	2182
$Axs^{r2}/FM7$	1.7	0.4	0.4	0.0	475
$Axs^{r3}/FM7$	1.6	0.0	0.0	0.0	372

Females of the indicated genotype were mated to *attached-XY,v f B; C(4)RM,ci* ey^R males.

resulting $Axs^*/FM7$ 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 *XXY* females) that are characteristic of Axs^D 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 Axs^D chromosomes. These revertants are here named Axs^{r1}, Axs^{r2} and Axs^{r3}. When heterozygous with *FM7*, Axs^+, 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 Axs^D when present in trans (*i.e.*, in Axs^r/Axs^D females). Thus they cannot simply be dominant suppressors of Axs^D. Moreover, all three of the revertants are inseparable from the Axs^D mutation by direct recombinational mapping (WHYTE 1933). Thus, the revertants are tightly linked to, if not inseparable from, the Axs^D lesion.

These data demonstrate that the dominant phenotype of the Axs^D mutation is indeed revertible. Taken together with the inability of Axs^+ duplications to fully rescue the Axs^D mutation, these data suggest that the Axs^D 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 Axs^D, and which can be rescued by duplications that carry Axs^+, suggesting that Axs^D is an antimorphic allele.

The revertants display a recessive meiotic phenotype similar to that of Axs^D: The revertants were tested for a meiotic phenotype in exchange-suppressed (*dl-49/+*) and in exchange competent (+/+) females. The Axs^D allele induces 20–25% *X* nondisjunction in $Axs^D/dl\text{-}49,Axs^+$ females in contrast to only 3% *X* nondisjunction in the $Axs^D/+$ females (ZITRON and HAWLEY 1989). None of these recombinants induced significant levels of nondisjunction in $+/dl\text{-}49,Axs^r$ females, confirming their lack of a dominant phenotype (*cf.* Table 2). However, as shown in Table 2, $Axs^{r2}/dl\text{-}49,Axs^{r2}$ females displayed the same high

TABLE 2

***Axs*^{r} alleles induce nonexchange *X* chromosomes to nondisjoin at high frequencies**

Gamete types		Maternal genotype									
		+/revertant				Homozygous revertant			Revertant/*Axs*^{r2}		
Maternal	Paternal	+/+	+/*Axs*^{r1}	+/*Axs*^{r2}	+/*Axs*^{r3}	*Axs*^{r1}/*Axs*^{r1}	*Axs*^{r2}/*Axs*^{r2}	*Axs*^{r3}/*Axs*^{r3}	*Axs*^{r1}/*Axs*^{r2}	*Axs*^{r2}/*Axs*^{r2}	*Axs*^{r3}/*Axs*^{r2}
Regular											
X4	$\widehat{XY44}$	868	395	584	348	306	423	674	855	392	689
X4	$O\widehat{44}$	637	421	697	340	380	632	760	661	334	726
X Nondisjunctional											
O4	$\widehat{XY}\,\widehat{44}$	10	0	6	0	0	73	56	54	38	81
XX 4	$O\,\widehat{44}$	4	1	5	0	1	57	81	62	54	92
4 Nondisjunctional											
X44	$\widehat{XY}O$	0	0	1	2	0	8	7	7	7	18
XO	$O\widehat{44}$	0	0	0	0	0	3	5	6	6	10
X44	OO	0	1	0	0	0	10	9	10	3	18
XO	$\widehat{XY}\widehat{44}$	0	0	0	0	0	11	5	9	3	10
X,4 Nondisjunctional											
XX44	OO	0	0	0	0	0	2	1	0	0	0
OO	$\widehat{XY44}$	0	0	0	0	0	1	1	0	0	0
XXO	$O\widehat{44}$	0	0	0	0	0	5	6	8	7	20
O44	$\widehat{XY}O$	2	0	0	0	0	14	11	12	11	25
Total progeny											
Adjusted total		1521	818	1293	690	687	1239	1616	1684	855	1689
		2174	819	1304	690	688	1391	1772	1820	965	1907
% Nondisjunction											
X		1.47	0.24	1.69	0.00	0.29	21.86	17.61	14.95	22.80	22.86
4		0.18	0.12	0.08	0.29	0.00	5.46	3.61	3.96	5.70	7.66
% Simultaneous *X,4* nondisjunction		0.18	0.00	0.00	0.00	0.00	3.16	2.15	2.20	3.73	4.72
% Nonhomologous disjunction		0.18	NA	NA	NA	NA	2.30	1.69	2.20	3.73	4.72

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

levels of *X* and fourth chromosomal nondisjunction as do *Axs*^{D}/*dl-49,Axs*^{D} females (compare Tables 2 and 3). Moreover, in both genotypes a large fraction of the fourth chromosome nondisjunction was the consequence of simultaneous *X,4* nondisjunction.

A similar, but weaker, phenotype is observed in *Axs*^{r3}/*dl-49,Axs*^{r3} females. Thus both *Axs*^{r2} and *Axs*^{r3} behave as recessive meiotic mutations whose phenotypes are similar to that of the *Axs*^{D} mutation. The observation of high levels of achiasmate nondisjunction in *Axs*^{r3}/*dl-49,Axs*^{r2} females also demonstrates that *Axs*^{r2} and *Axs*^{r3} are allelic.

Surprisingly, no meiotic phenotype was observed in putative *y cv v Axs*^{r1} *car*/*dl-49, Axs*^{r1} females for any of the ten *dl-49,Axs*^{r1} constructions tested. However, 9 out of 10 of these constructions did display a strong meiotic phenotype in *y cv v Axs*^{r2}/*dl-49,Axs*^{r1} females. (As shown in Table 2, the same strong meiotic phenotype is also observed when the coupling relationships of *Axs*^{r1} and *Axs*^{r2} are reversed in *Axs*^{r1}/*dl-49,Axs*^{r2} females). These observations demonstrate that the *Axs*^{r1} mutation is allelic to *Axs*^{r2} and *Axs*^{r3}, and suggests that the *Axs*^{r1} 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*^{D}.

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* ↔ *44*). Thus, as is the case for *Axs*^{D}-induced nondisjunction, the effect of the *Axs*^{r} alleles is restricted to homologous achiasmate segregations, while heterologous segregations still occur with relatively high fidelity. In this section we also demonstrate that the effects of the *Axs*^{r} mutations, like those of the *Axs*^{D} allele, are restricted to homologous achiasmate segregation and do not extend to other meiotic or mitotic processes.

The original *Axs*^{D} 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*^{r2}, there was little, if any, effect on the segregation of

TABLE 3

Interaction of Axs^D with revertant alleles

Gamete types		Maternal genotype					
Maternal	Paternal	+/+	$Axs^D/+$	Axs^D/Axs^D	Axs^{r1}/Axs^D	Axs^{r2}/Axs^D	Axs^{r3}/Axs^D
Regular							
X4	$\widehat{XY}\,\widehat{44}$	868	522	445	352	457	552
X4	O$\widehat{44}$	637	272	222	242	236	335
X Nondisjunctional							
O*4*	$\widehat{XY}\,\widehat{44}$	10	46	64	57	72	57
XX4	O$\widehat{44}$	4	36	65	51	66	74
4 Nondisjunctional							
X44	$\widehat{XY}$O	0	6	9	8	21	14
*X*O	O$\widehat{44}$	0	3	7	3	1	2
X44	OO	0	4	6	2	9	4
*X*O	$\widehat{XY}\widehat{44}$	0	4	6	3	15	14
X,4 Nondisjunctional							
XX 44	OO	0	0	1	1	0	1
OO	$\widehat{XY}\widehat{44}$	0	0	2	0	1	0
*XX*O	O$\widehat{44}$	0	4	13	11	12	8
O *44*	$\widehat{XY}$ O	2	6	12	13	27	15
Total progeny[a]		1521	903	852	743	917	1076
Adjusted total		2174	1274	1244	1123	1341	1572
% Nondisjunction							
X		1.47	14.44	25.24	23.69	26.55	19.72
4		0.18	3.45	7.80	6.32	10.14	5.60
% Simultaneous *X,4* non disjunction		0.18	1.57	4.50	4.45	5.97	3.05
% Nonhomologous dis		0.18	1.57	3.54	4.10	5.67	2.80

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

[a] The *In(1)dl-49, y Hw g* Axs^D and *In(1)dl-49, y Hw m g* Axs^+ chromosomes are hemizygous lethal. Therefore the *X*O progeny classes are doubled in the adjusted total progeny.

TABLE 4

Axs^{r2} does not induce recombinant *X* chromosomes to nondisjoin

Female genotype	Percent nondisjunction				Adjusted total progeny
	X	*4*	*X, 4*	*XX* ↔ *44*	
+/+	0.39	0.11	0.06	0.00	7111
Axs^{r2}/Axs^{r2} [a]	2.33	0.93	0.00	NA	430
$Axs^{r2}/dl\text{-}49,Axs^{r2}$	22.80	5.70	3.73	3.73	965

Females of the indicated genotype were mated to *attached-XY,v f B; C(4)RM,ci ey*^{R} males.

[a] These females are *y cv v* $Axs^{r2}/y\ w^a ct^6\ m\ Axs^{r2}$.

exchange *X* chromosomes. As shown in Table 4, the frequency of *X* chromosome nondisjunction in Axs^{r2}/Axs^{r2} 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^r 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 Axs^D 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^D, nondisjunction is not accompanied by a significant level of chromosomal loss.

We isolated no mosaics or gynandromorphs in any nondisjunction assays for Axs^D 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 $Axs^r/y^+\ Y;\ pol/pol$ 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 Axs^D/Axs^D is similar to that of Axs^{r2}/Axs^{r2} while Axs^{r3}/Axs^{r3} females exhibit an intermediate phenotype and Axs^{r1}/Axs^{r1} 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 Axs^{r2} allele: As a homozygote, Axs^{r2} is as severe as the dominant Axs^D mutation. That Axs^{r2} 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 $Axs^{r2}/dl\text{-}49,Axs^{r2}$; $Dp(Axs^+)$ females (see below). Axs^{r2} provides no rescue of the Axs^+ function in $Axs^{r2}/dl\text{-}49,Axs^D$ females (Table 3, columns 5 and 6). Thus Axs^{r2} has completely lost the antimorphic phenotype of Axs^D, 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 Axs^{r2} to those of a homozygous viable and fertile deficiency of the locus, we cannot rule out the possibility that Axs^{r2} retains some wild-type function.

The Axs^{r3} allele: Homozygosis for Axs^{r3} induces only about 80% as much nondisjunction as does Axs^{r2} (see Table 2). Moreover Axs^{r3} exhibits a reduced the level of nondisjunction in $Axs^{r3}/dl\text{-}49,Axs^D$ females as compared with $Axs^D/dl\text{-}49,Axs^D$ females (see Table 3). Both of these results are consistent with only a partial loss of function. The fact that in flies heterozygous for $Axs^{r3}/dl\text{-}49,Axs^{r2}$, the phenotype is as strong as that of the Axs^{r2} homozygote (see Table 2) may indicate that the residual level of Axs^+ function falls below some essential threshold in $Axs^{r3}/dl\text{-}49,Axs^{r2}$ females.

The Axs^{r1} allele: Axs^{r1} appears completely wild type in homozygous females, and in a *trans*-heterozygote with Axs^{r2} it causes less nondisjunction of both the *X* and fourth chromosomes than does the Axs^{r2} homozygote (see Table 2). This is exactly the phenotype expected of a weak hypomorph in the presence of a stronger allele.

Surprisingly, the combination $Axs^{r1}/dl\text{-}49,Axs^D$ produces a more severe meiotic phenotype than does the genotype $Axs^{r1}/dl\text{-}49,Axs^{r2}$ (Table 3). One could imagine that when alone, or in the presence of Axs^+ protein, the Axs^{r1} protein assumes an active conformation, but in combination with Axs^D, it reverts to a dysfunctional or nonfunctional state.

Cytogenetic mapping of *Axs*: As shown above, recombinational mapping placed Axs^D 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, Axs^{r2}. 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 $Axs^{r2}/dl\text{-}49,Axs^{r2}$ females. For each duplication, sisters of the genotype $Axs^{r2}/dl\text{-}49,Axs^{r2}$; *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 Axs^{r2} (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 $XX \leftrightarrow 44$ 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 Axs^{r2} 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 Axs^D and *ald/ald* on *X* chromosome nondisjunction appear to be additive, two lines of evidence suggest that Axs^D may be epistatic to *ald* with respect to its effect on fourth chromosomal disjunction. First, the level of fourth chromosome nondisjunction observed in $Axs^D/FM7$; *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 $Axs^D/FM7$; +/+ 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 Axs^D-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 Axs^{r2} by derivatives of the $Dp(1;4)r^+f^+$ chromosome

Gamete types		Maternal genotype							
		$Axs^{r2}/+$		Axs^{r2}/Axs^{r2}					
Maternal	Paternal	No *Dp*	$Dp(1;4)r^+f^+$	No *Dp*	$Dp(1;4)r^+f^+$	*Dp81j6c*	*Dp81j23a*	*Dp81g1i*	*Dp80g12a*
Regular									
X4	$\widehat{XY}\,\widehat{44}$	560	508	694	854	751	323	804	783
X4	$O\widehat{44}$	382	280	1031	830	881	439	1044	913
X Nondisjunctional									
O4	$\widehat{XY}\,\widehat{44}$	0	2	42	4	8	45	61	103
XX4	$O\widehat{44}$	2	3	57	19	5	26	31	47
4 Nondisjunctional									
X 44	$\widehat{XY}O$	0		12					
X O	$O\,\widehat{44}$	0	0	7	4	0	1	6	1
X 44	*OO*	0		14					
X O	$\widehat{XY}\,\widehat{44}$	1	0	7	6	1	0	5	3
X,4 Nondisjunctional									
XX 44	*OO*	0		2					
OO	$\widehat{XY}\,\widehat{44}$	0	0	0	4	0	0	0	0
XXO	$O\,\widehat{44}$	0	0	12	3	0	1	2	6
O 44	$\widehat{XY}O$	1		10					
Total progeny		946	793	1888	1724	1646	835	1953	1856
Ajusted total		1328	1032	2011	1754	1659	907	2047	2012
% Nondisjunction									
X		0.5	1.0	12.2	3.4	1.6	15.9	9.2	15.5
4		0.2	0.0	4.4	2.7	0.1	0.5	1.4	2.0
% Simultaneous *X,4* nondisjunction		0.2	0.0	2.4	1.6	0	0.4	0.4	1.2

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

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 $Axs^{r2}/FM7$; *ald/+* females; nor does heterozygosity for *ald* enhance the phenotype of Axs^D.

***Axs^{r2}* 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 Soler-Niedziela 1990). As shown in Table 6, $FM7/Axs^{r2}$; *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;

TABLE 6

Interaction of *Axs* with other meiotic mutants

Mother's genotype	Percent nondisjunction				Total progeny
	X	*4*	*X, 4*	$XX \leftrightarrow 44$	
A. *ncd* enhances Axs^{r2}					
$FM7/+$; $+/+$	0.2	0.1	0.0	NA	14246
$FM7/Axs^{r2}$; $+/+$	1.3	0.2	0.0	NA	946
$FM7/+$; $+/ncd$	0.6	0.4	0.0	NA	7198
$FM7/Axs^{r2}$; $+/ncd$	3.4	3.6	0.5	0.5	414[a]
$+/Axs^{r2}$; $+/ncd$	0.0	1.6	0.0	NA	815
B. *ald* and Axs^{r2} fully complement					
dl-49,$Axs^{r2}/+$; $+/+$	0.5	0.0	0.0	NA	413
dl-49,$+/+$; $+/ald$	0.5	0.4	0.1	0.1	2062
dl-49,$Axs^{r2}/+$; $+/ald$	0.3	0.4	0.1	0.1	2019
C. Axs^D is epistatic to *ald*					
$FM7/Axs^D$; $+/+$	23.5	6.2	4.3	3.5	1380
$FM7/+$; *ald/ald*	10.3	17.3	5.4	5.4	1550
$FM7/Axs^D$; *ald/+*	24.8	4.1	3.0	2.3	1120
$FM7/Axs^D$; *ald/ald*	35.7	8.1	5.8	4.9	902

Females of the indicated genotype were mated to *attached-XY; C(4)RM,ci* ey^R males.

[a] These females displayed a significantly reduced fertility.

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 Axs^D (ZITRON and HAWLEY 1989). Moreover, disjunction appears normal in *FM7,nod/* Axs^{r2} 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 *ncd*, do not disrupt proper spindle formation (THEURKAUF and HAWLEY 1992).

DISCUSSION

We previously described a semidominant mutation, Axs^D, which impairs the segregation of achiasmate homologs at meiosis I (ZITRON and HAWLEY 1989; HAWLEY *et al.* 1993). Three lines of evidence argue that the original Axs^D mutation represents a poisonous (antimorphic) allele. First, it is revertible. Second, it is only partially rescued by an additional wild-type copy of the gene: that is, in terms of phenotypic severity, $Axs^D/Axs^D > Axs^D/+ > Axs^D/+/+ > +/+$. Third, loss of function alleles of *Axs* have a meiotic phenotype that is similar to that of Axs^D, thus ruling out the possibility that Axs^D 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 $X \leftrightarrow 4$ 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)]:

$sc^4\ sc^8,Axs^D/dl\text{-}49,Axs^D;\ 4/4$
$sc^4\ sc^8,Axs^D/dl\text{-}49,Axs^D/Dp(1;f);\ 4/4$
$sc^4\ sc^8,Axs^D/dl\text{-}49,Axs^D/Y;\ 4/4$
$sc^4\ sc^8,Axs^D/dl\text{-}49,Axs^D;\ C(4)RM.$

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 *X*s 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 segregate from the *compound-fourth* chromosome, exactly as was observed by ZITRON and HAWLEY (1989). Similarly, $XX \leftrightarrow 44$ segregations in chromosomally normal females would result from the alignment of an interlocked pairs of *X*s 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 *X*s 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 $Axs^D/FM7$ and $Axs^{r2}/dl\text{-}49$, Axs^{r2} 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^D- or Axs^{r2}-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 centrioles, 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 1936; 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 combined haplo-insufficiency with *ncd* has also been demonstrated for mutations at the *nod* locus (KNOWLES and HAWLEY 1991). Like the putative wild-type function for *Axs*, the wild-type product of *nod* is required to position achiasmate chromosomes on the developing spindle (THEURKAUF and HAWLEY 1992).

The nature of dominant meiotic mutations: Although dominant mutations are common in Drosophila, dominant meiotic mutations are indeed quite rare. Of the over 60 female meiotic loci identified to date, dominant alleles exist for only three (*Axs*, *nod* and *ncd*). Because the *nod* and *ncd* genes have been studied in detail at both the genetic and molecular levels, it is worth summarizing those observations in light of their similarities and differences with respect to *Axs*.

Like the *Axs* gene, the *nod* locus, which encodes a kinesin-like protein specifically required for distributive segregation, is also defined by both dominant (nod^{DTW}) and recessive (*e.g.*, nod^{b27}) alleles (ZHANG and HAWLEY 1990; ZHANG *et al.* 1990; RASOOLY *et al.* 1991). Recessive loss-of-function alleles of *nod* either prevent protein synthesis or disrupt conserved regions of the protein (such as microtubule binding sites) while the dominant antimorphic allele of the *nod* gene results from a change in the ATP-binding domain of the protein and may cause a rigor binding phenotype (RASOOLY *et al.* 1991).

$nod^{DTW}/+$ heterozygotes are phenotypically identical to homozygotes for loss-of-function *nod* alleles (RASOOLY *et al.* 1991 and J. JANG and R. S.HAWLEY, unpublished data). However, the phenotype exhibited by nod^{DTW} homozygotes is considerably more severe, including the meiotic nondisjunction of chiasmate and achiasmate chromosomes, anomalies in both meiotic and mitotic spindle formation (J. JANG and R. S. HAWLEY, unpublished data), and mitotic chromosome bridging and breaking.

The *ncd* locus, described above, is also defined by recessive loss-of-function alleles and by a weak dominant allele, ncd^D, all of which cause high levels of meiotic nondisjunction and loss when homozygous (O'TOUSA and SZAUTER 1980; LEWIS and GENCARELLA 1952; LINDSLEY and ZIMM 1992). Null alleles of *ncd* also cause substantial amounts of mitotic loss (SEQUIERA, NELSON and SZAUTER 1989). Although KOMMA, HORNE and ENDOW (1991) have claimed that the dominant allele ncd^D retains wild-type mitotic function, in that it prevents mitotic loss, ncd^D/ncd females show very high levels of mitotic loss (KOMMA, HORNE and ENDOW 1991). Thus this mutation does not separate the meiotic and mitotic phenotypes of lesions at the *ncd* locus; but is simply a weakly dominant loss-of function mutation. The amino acid sequence of ncd^D 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^D allele to the defect in the motor domain, it should be noted that three null alleles of *nod* 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^D 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 landmarks to delimit its boundaries. Efforts to identify and sequence the *Axs*-coding region are well underway in the laboratory.

We wish to thank MICHELLE D. WHITLEY and CHRISTINE NEW for technical assistance, BILL THEURKAUF, PAUL SZAUTER, CATHERINE NELSON, KIM MCKIM, MARIA ERDMAN, ANNE ZITRON, KENNETH BURTIS and BRENDA KNOWLES for helpful discussions, and BARR E. BAUER for computer help. We especially thank ARTHUR CHOVNICK, WILLIAM GELBART, PAUL SZAUTER and an anonymous reviewer for helpful comments on the manuscript. This work has been supported by a grant to R.S.H. from the Eukaryotic Genetics Program of the National Science Foundation (DCB-8815749). W.L.W. was supported by National Institutes of Health Training Grant (5T32-GM07491). G.Y. was supported by a grant from the National Science Foundation (DCB-9104087). Parts of this paper are derived from a thesis submitted by W.L.W. in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Sue Golding Graduate Division of Medical Sciences, Albert Einstein College of Medicine, Yeshiva University.

Note added in proof: Since the date of this submission, WENDY HURLEY, CINDY RAMIREZ and R. S. HAWLEY have validated the hypothesis that the Axs^D 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 $Axs^D/dl\text{-}49,Axs^D$; *T(3;4)86D/T(3;4)86D* in which the normal achiasmate fourth chromosomes have been appended to the virtually always chiasmate 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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Communicating editor: A. CHOVNICK