Induced Chromosomal Exchange Directs the Segregation of Recombinant Chromatids in Mitosis of Drosophila

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ABSTRACT

In meiosis, the segregation of chromosomes at the reductional division is accomplished by first linking homologs together. Genetic exchange generates the bivalents that direct regular chromosome segregation. We show that genetic exchange in mitosis also generates bivalents and that these bivalents direct mitotic chromosome segregation. After FLP-mediated homologous recombination in G2 of the cell cycle, recombinant chromatids consistently segregate away from each other (x segregation). This pattern of segregation also applies to exchange between heterologs. Most, or all, cases of non-x segregation are the result of exchange in G1. Cytological evidence is presented that confirms the existence of the bivalents that direct this pattern of segregation. Our results implicate sister chromatid cohesion in maintenance of the bivalent. The pattern of chromatid segregation can be altered by providing an additional *FRT* at a more proximal site on one chromosome. We propose that sister chromatid exchange occurs at the more proximal site, allowing the recombinant chromatids to segregate together. This also allowed the recovery of reciprocal translocations following FLP-mediated heterologous recombination. The observation that exchange can generate a bivalent in mitotic divisions provides support for a simple evolutionary relationship between mitosis and meiosis.

SEGREGATION of sister chromatids in mitosis is a vital operation: each daughter cell must receive a copy of each of the chromosomes that carry the organism's genetic heritage. Sister chromatids are reliably and efficiently segregated to the daughter cells during cell division. This is achieved by holding the replicated sister chromatids firmly together until they are aligned on the mitotic spindle and attached to opposite poles (Koshland 1994). Orientation of each pair of chromatids at the mitotic plate is generally thought to be unaffected by its homolog. However, DNA damage occasionally results in recombination between homologous nonsister chromatids. When such an exchange occurs after DNA replication, sister chromatids are no longer identical and segregation may have genetic consequences for the organism. In such a case, the alignment of the homologs that have exchanged will determine the outcome.

Stern (1936) discussed the mitotic segregation of chromosomes after an exchange between homologs in G2 of the cell cycle. He suggested that recombinants could segregate in three possible ways (Figure 1). The first, termed x segregation, results in the recombinant chromatids segregating to opposite poles. This is the segregation that makes mitotic recombination a useful tool for mosaic analysis because it produces homozygous daughter cells from a heterozygous genotype. This loss of heterozygosity may unmask recessive mutations and has been implicated in carcinogenesis in higher organisms (Stanbridge 1990; Graf *et al.* 1992; Ramel *et al.* 1996). The second, termed y segregation, would occur when sister chromatids fail to disjoin and segregate into the same daughter cell. This is equivalent to the reductional division of Meiosis I and is not normally observed. The third type of segregation, termed z segregation, results in both recombinant chromatids segregating to one daughter cell and the nonrecombinant chromatids segregating to the other daughter cell. The linkage relationships of genes flanking the site of exchange are changed in the cell with recombinant chromosomes, but both daughter cells are phenotypically indistinguishable from cells that did not experience a recombination event. Cells may also experience recombination in G1 of the cell cycle. In this event, both daughter cells will have chromosomes with altered linkage arrangements and the cells will be phenotypically and genetically indistinguishable from one of the cells that is produced by recombination in G2 followed by z segregation.

It has traditionally been assumed that when G2 mitotic recombination occurs in Drosophila, x and z segregation are equally frequent. This implies that the recombination that is measurable by the appearance of clones represents half, or less, of the total recombination. On the other hand, some experimenters have made the assumption that mitotic recombination always generates

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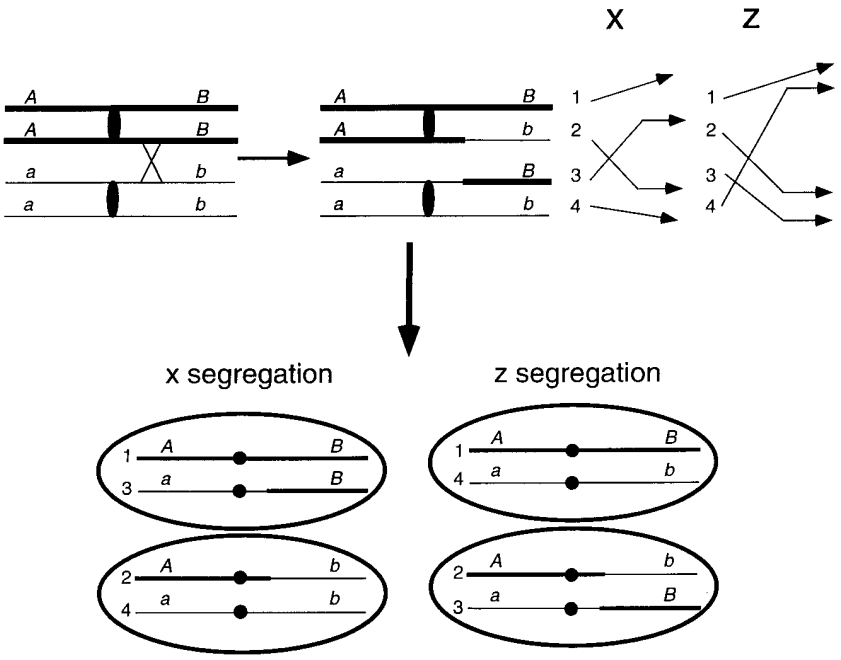

Figure 1.—Segregation of chromatids after G2 recombination. x and z segregation are diagramed. If recombination were to occur in G1 (not shown), both daughter cells would resemble the lower cell in the z segregation column. Homologous chromosomes are represented by lines of different thicknesses. X indicates a crossover. Centromeres are indicated by solid circles.

clones of cells that are homozygous for markers distal to the site of exchange. Harrison and Perrimon (1993) developed an extension of FLP-mediated mitotic recombination that marks clones by generating a functional β-*galactosidase* gene at the site of recombination. They state that β-galactosidase-positive clones also simultaneously become homozygous for more distally located mutant alleles. However, this will be true only if all recombination occurs in G2 and is followed by x segregation.

The frequencies of x and z segregation in mitotic cells are also of interest in the study of mutagens that cause mitotic recombination. Loss of heterozygosity as a result of mitotic recombination is a major causative factor in carcinogenesis (Ramel *et al.* 1996). The loss of heterozygosity caused by any given frequency of mitotic recombination will be much higher than is typically assumed if G2 recombination is always followed by x segregation. So it is clearly of interest to determine how chromatids segregate following mitotic recombination.

The mitotic segregation of recombinant chromatids has been examined in two previous reports. In Drosophila, Pimpinelli and Ripoll (1986) devised an experiment that allowed a cytological assessment of chromatid segregation after X-ray-induced exchange. They estimated that x segregation exceeded z segregation by at least two to one. Those results are complicated by use of X-ray-induced damage to induce recombination, and by the use of a large block of translocated heterochromatin on the end of one chromosome to provide a cytological marker. Heterochromatin behaves differently than euchromatin in mitotic chromosomes. Sister chromatids are observed to remain tightly adhered in regions of heterochromatin after all euchromatic sequences have separated and this extended association may have influenced chromatid segregation in their experiments.

Different results have been observed in yeast. Chua and Jinks-Robertson (1991) performed experiments that allowed the identification of cells with recombinant chromosomes, as well as the determination of their genotypes, after spontaneous mitotic recombination. Their results were interpreted as a demonstration that, in yeast, x segregation is equal to z segregation. Chua and Jinks-Robertson (1991) assert that G1 recombination must be insignificant and that their results must be wholly due to random segregation after G2 recombination because they do not see a large excess of the phenotype that is generated by G1 recombination or by G2 recombination and z segregation. However, other work indicates that approximately 70% of the mitotic recombination that occurs in yeast initiates in G1, though it may be resolved during DNA replication or be accompanied by a G2 recombination event (Wildenberg 1970; Esposito 1978). It is possible that in Chua and Jinks-Robertson's experiments, a bias for x segregation following G2 recombination was balanced by an overall G1 recombination bias. Alternatively, Drosophila and yeast may treat recombinant chromosomes differently. Dipteran chromosomes are thought to be unique in a number of structural and mechanical aspects. Most relevantly, they experience somatic chromosome pairing to a greater extent than is seen in other organisms (Stevens 1908; Metz 1916). Thus, chromatid segregation in Drosophila may be unique.

The work presented here was undertaken, in part, to assess disjunctional behavior following chromosomal exchange induced by the transgenic FLP recombinase in Drosophila (Golic and Lindquist 1989). This would seem to be of general interest owing to the widespread use of FLP-mediated mitotic recombination to generate clones for developmental studies (Golic 1991). If a

segregation preference is detectable following FLP-mediated recombination, it would be especially informative to identify the mechanism that generates that bias, in order to determine whether such a bias is specific to Dipterans or whether it applies to mitosis in general. A second motivation was our desire to extend the technique of site-specific chromosomal rearrangement (Golic and Golic 1996a) to the production of reciprocal translocations in Drosophila. If the two halves of a reciprocal translocation preferentially segregate apart at mitosis, the recovery of such translocations is considerably complicated.

MATERIALS AND METHODS

Drosophila stocks: Information about the mutations used in this work can be found in Lindsley and Zimm (1992) unless otherwise noted.

All flies used in these experiments carried the w^{1118} null mutation on the *X* chromosome except where otherwise noted.

In all experiments, FLP was produced using a heat-inducible *FLP* construct, *P[ry*$^+$*, 70FLP]* in which *FLP* is under the control of an *hsp70* promoter (Golic *et al.* 1997). An insertion of this *P* element on the *X* (*70FLP*3F) and another insertion on chromosome *3* (*70FLP*4A) were used. The *FRT*-bearing *P* elements, *RS3r* and *RS5r*, have been previously described (Golic and Golic 1996a).

***In situ* hybridization:** Chromosome squashes were prepared and hybridized according to Pardue (1986) with modifications after J. Lim (University of Wisconsin, Eau Claire; personal commumication) and hybridized with the Genius nonradioactive DNA labeling and detection kit (Boehringer-Mannheim, Indianapolis, IN).

Somatic recombination experiments: To mark the recombinant chromosomes we used the *RS3r*-2 *P* element, located at 75C-D on chromosome *3*, to supply *white* coding sequence. The promoter was supplied by an excision-remnant insertion of the *FRT*-bearing *P* element *P*[*SSINT*] (K. G. Golic, unpublished results) located at approximately the same location on the homolog. This insertion fortuitously places the *P* element adjacent to an unknown promoter that is in the proper orientation to activate the *white* gene sequences in *RS3r*-2 when FLP-mediated recombination occurs between the *FRT* in this element and the *FRT* of *RS3r*-2 on the homolog.

Recombination between heterologs: Heterologous recombination events were induced in flies that carried the transgenic *white* gene constructs designated *RS3r* and *RS5r*. A number of insertions of both *RS3* and *RS5* were used in this work (Figure 2). Each was mapped by *in situ* hybridization.

In most experiments, heterologous recombination was induced between the *X* chromosome and the autosomes, resulting in the generation of site-specific translocations. Virgin females that carried one or two *RSr* insertions on the *X* chromosome and *70FLP* 4A on the third chromosome were crossed to males that carried one or two complementary *RSr* insertions on an autosome and *70FLP* 3F on the *X* chromosome. *70FLP*4A was either homozygous or heterozygous with a *TM3, Ser e* balancer. Whenever possible, the *RS5r* and *RS3r* insertions were homozygous. In lines where homozygotes were either inviable or infertile, chromosome *2* insertions were balanced over *S*^{2}*CyO, cn bw* and chromosome *3* insertions were balanced over *TM6, Ubx e*^{s}. The *T(2;3)* was generated with an *RS5r* and a more proximal insertion of another *FRT*-bearing *P* element on chromosome *3* and an *RS3r* on chromosome *2*. Both males and females carried *70FLP* 3F.

In all cases except one, eggs from each cross were collected for 3 days in standard vials. The parents were transferred to new vials and transferred every second day thereafter. After the parents were removed, the old vials were immediately heat shocked at 38° for 1 hr in a circulating water bath. A second heat shock followed 2–3 days later, and a third was administered 4–5 days after the first. In one set of experiments with *RS5r*-2C, *RS5r*-4, and *RS3r*-19, heat shocks were done on days 3, 6, and 9, but this protocol was not as effective and was abandoned. In all cases, male and female progeny were collected within 48 hr of eclosing and brother-sister matings of three females by two or three males per vial were set up. After 17–19 days, the progeny of these crosses were scored for eye pigment. Potential heterologous recombination events were confirmed with the following tests.

Cytology: Each translocation line was confirmed by cytological examination of translocation heterozygotes. Salivary chromosomes were prepared and breakpoints were determined as described by Lefevre (1976).

Pseudolinkage: Potential translocations generated by heterologous recombination were tested for pseudolinkage of the inolved chromosomes.

PCR: DNA was prepared for PCR as previously described (Golic and Lindquist 1989). PCR reactions were performed in an Idaho Technologies RapidCycle thermocycler in 10 μl capillary tubes. Potential translocations were tested for the expected junction by amplification across the *FRT* using oligonucleotides 5′TCATCGCAGATCAGAAGCGG 3′ (w11678U) and 5′GGAGCTATTAATTCGCGGAGGCA 3′ (w7703D) for primers.

Determining the segregation pattern after exchange between the tips of *X* and *3*: Heterologous recombination was induced between *RS5r*-4, inserted at 1B1, and *RS3r*-19 at 100D. We recovered white$^+$ progeny from 5.9% of vials, an unexpectedly high rate of recovery for heterologous recombination. Since the resulting translocation, *T(1;3)*19, involves only the extreme tips of the *X* and *3R*, both halves are viable as aneuploid segregants. The autosomal hypoploids survive as both males and females; the *X* hypoploids survive as females. The *RSr* insertions are oriented so that recombination generates an entire w^+ gene on the 3^PX^D half of the translocation. The portion of the *X* chromosome that is translocated to *3R* carries the wild-type *yellow*$^+$ (*y*) gene and will be yellow$^+$ in all backgrounds. The X^P3^D half should lack the y^+ gene and give a yellow phenotype in a *y* background. Normal *X* chromosomes in these flies also carry y^+. To test for entire versus half translocations we performed a test cross. Animals carrying the w^+ gene were crossed to *y w* males or females and their progeny screened for yellow, white-eyed females, which are an indicator that the parent carried both halves of the translocation.

Metaphase chromosome cytology: To visualize mitotic bivalents, larvae of the genotype w^{1118} *70FLP*; *RS3r*-2 were heat shocked for 1 hr at 38° as previously described (Golic and Lindquist 1989) and then returned to 25°. After 18–24 hr the larvae were again heat shocked for 1 hr at 38°. Larvae were allowed to recover at 25° for 3 hr. Brains were dissected and treated as described in Ashburner (1989) using protocol 4.

RESULTS

Segregation following mitotic recombination between homologs in the soma: Nonsister chromatids that recombine in mitotic interphase may segregate apart (G2 recombination followed by x segregation), or together

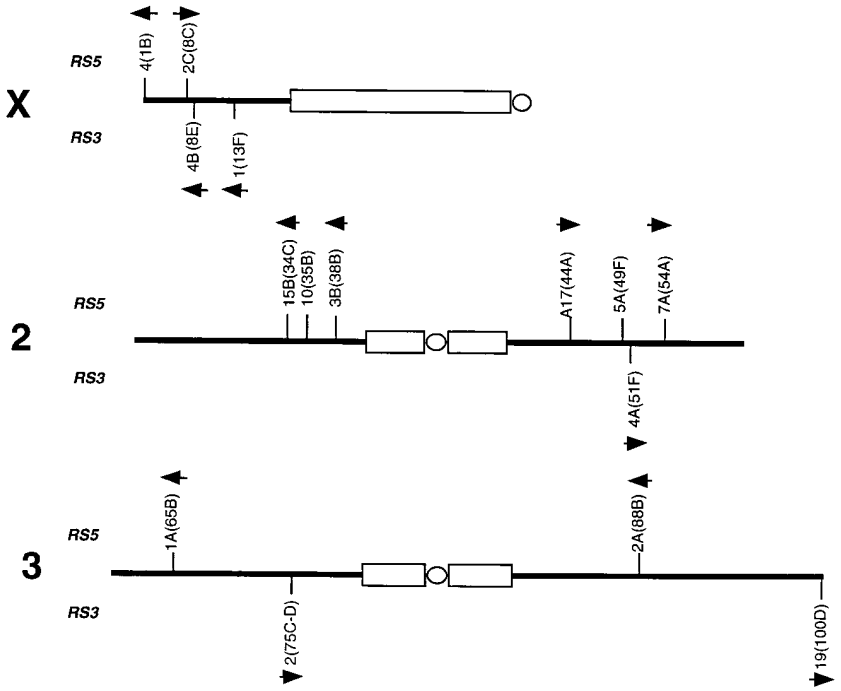

Figure 2.—Locations of *RS3* and *RS5 P* elements used in this study. *RS5* elements are located above the chromosomes; *RS3* elements are below. Insertions are designated by isolate numbers with the cytological locations given in parentheses. The arrows indicate the orientation, if known, of the *FRT*s in the *RS* elements. [See Golic and Golic (1996a) for convention.]

(G1 recombination or G2 recombination followed by z segregation). To measure the frequencies of these events in the soma, we first needed a method for identifying the clones carrying a recombinant chromosome, regardless of when during the cell cycle it was produced or how it segregated. Second, we needed to distinguish the daughter cells that are produced by the two types of segregation: those with the same genotypes as the parental cells and those that are now partially homozygous. To accomplish the above, we induced recombination between *FRT*-bearing *P*-element constructs located at homologous sites that activate a *white* gene when mitotic recombination occurs. This allowed us to identify all the recombination events that occurred in cells of the eye imaginal discs by scoring white$^+$ clones in the adult eye. A distally located recessive marker that modifies the phenotypic expression of the w^+ gene allowed us to determine how the recombinant chromatids segregated.

Two *FRT*-bearing *P* elements were used in the following experiments. The *RS3r*-2 *P*-element insertion carries exons 2–6 of the Drosophila w^+ gene. A second *FRT*-bearing *P* element, recovered in a separate series of experiments, was serendipitously located at approximately the same site on another chromosome *3*. The second element carries no portion of the w^+ gene, but it can frequently recombine with *RS3r*-2 after FLP synthesis and this exchange produces a functional w^+ gene, probably as a result of transcriptional or translational fusion (Figure 3). This event uniformly marks one recombinant chromosome with a functional w^+ gene regardless of segregation events. We recombined the *scarlet* (*st*) mutation onto the *RS3r*-2 chromosome (as diagrammed in Figure 3). If the recombination event that generates a w^+ gene occurs in G2 and is followed by x segregation, the w^+ clone will also be homozygous for *st*. If recombination occurs in G1 or in G2 and is followed by z segregation, the w^+ clone will be st/st^+ (phenotypically scarlet$^+$). Thus, the ratio of [white$^+$ scarlet] : [white$^+$ scarlet$^+$] clones is the ratio of [G2 recombination followed by x segregation] : [G1 recombination plus G2 recombination followed by z segregation]. The scarlet phenotype cannot be reliably scored in the w^+ clones, but cells that are homozygous for both *brown (bw)* and *st* do not make pigment regardless of whether they are w^- or w^+. In brown flies, w^+ clones will be observed only if they are st/st^+. By comparing the frequency of pigmented clones produced in a brown$^+$ background, where all recombination events are visible, to their frequency in a brown background, where only clones produced by G1 recombination or G2 recombination followed by z segregation are visible, we can calculate the frequency of G2 recombination followed by x segregation.

A heat-inducible FLP gene (*70FLP*) was used to supply FLP protein to flies with the genotype diagrammed in Figure 3. Because the *70FLP* gene produces some FLP protein without heat shock, a substantial number of white$^+$ clones were observed in brown$^+$ flies without heat shock (−HS). This number was reduced by two thirds in brown flies, indicating that two thirds of the clones derive from G2 recombination followed by x segregation (Table 1). After heat shock induction of *70FLP*, the number of white$^+$ clones was much greater, and again that number was reduced by two thirds in brown flies (Table 1). We conclude that, when FLP-

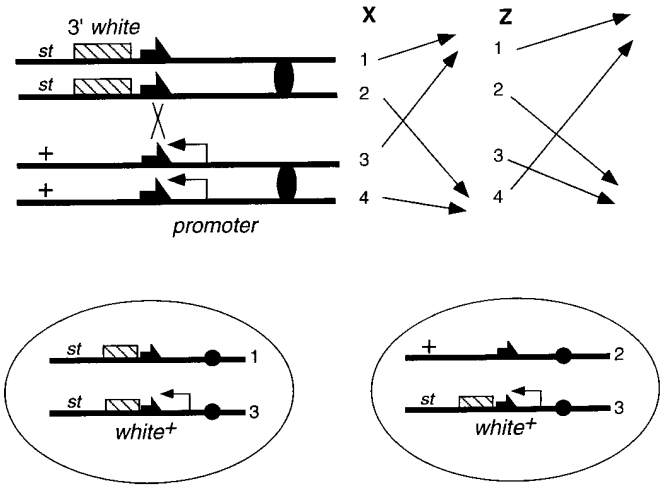

Figure 3.—Measuring x and z segregation. FLP mediates recombination between *FRT*s on homologous chromosomes, joining the *white* coding sequence to an unidentified promoter and generating a white$^+$ gene. The white$^+$-marked daughter cells produced by x or z segregation are diagrammed, and the clone phenotypes in *bw*$^+$ or *bw* backgrounds are indicated below each cell. The four chromatids are identified by numbers 1–4. Half arrows indicate *FRT*s; the hash-marked box indicates the *white* gene coding sequence; the exchange is indicated by X.

	clone phenotypes	
	x segregation	z segregation
bw$^+$ background	white$^+$	white$^+$
bw background	white	white$^+$

mediated mitotic recombination occurs, at least two thirds of the time the recombinant chromatids segregate to opposite daughter cells.

An alternative explanation might be that recombinant chromatids segregate randomly, but that multiple rounds of recombination occur. If a *st*/*st*$^+$ daughter cell (produced by z segregation) undergoes a second round of recombination, then at the next mitosis there exists a second opportunity for the *w*$^+$ cell to become homozygous *st*/*st*. Thus, it is conceivable that additional rounds of recombination in succeeding cell cycles could generate the observed excess of *st*/*st* homozygotes. We think this is very unlikely, at least for the −HS experiments, because the frequency of multiple events per fly is low. In the −HS bw$^+$ experiment, where 38% of the flies exhibited w$^+$ clones, the number of individual clones was also scored and the average frequency of w$^+$ clones per fly was 0.6. The clones were distributed randomly throughout the eyes, and their size suggested that they were generated mainly in first instar, when there are, in total, 20–200 cells in the eye imaginal discs (Postlethwait 1978). Therefore, noninduced expression of *70FLP* appears to be limited to a short period of development, and its level of expression is most likely insufficient to produce multiple rounds of recombination in any given cell lineage. For the same reason, double exchanges in a single cell cycle are unlikely to account for a significant fraction of recombination and need not be considered here.

TABLE 1

x and z segregation in somatic cells

Genotype	HS[a]	*N*[b]	Frequency of mosaics[c] (%)
bw/+	−	255	38
bw/*bw*	−	291	12
bw/+	+	485	91
bw/*bw*	+	478	28

This experiment is diagrammed in Figure 3.

[a] HS, heat shock. For the +HS experiments flies were given a 1-hr 38° heat shock at 24–48 hr of development.

[b] *N*, number of males scored.

[c] Calculated as 100 × (males with white$^+$ clones)/*N*.

Frequency of G1 recombination in somatic cells: The previous experiments allowed us to determine the fraction of recombination that occurred in G2 and was followed by x segregation, but did not allow us to distinguish between G1 recombination and G2 recombination followed by z segregation. This was done by recombining a second *FRT* onto one of the chromosomes used in these experiments (Figure 4). Because FLP-mediated unequal sister chromatid exchange occurs very frequently in Drosophila (approaching 100%; Golic 1994), this additional *FRT* should allow frequent sister chromatid exchange at the site where it is inserted. When G2 recombination between homologs generates an intact *white* gene, and sister chromatid recombination occurs at the more proximal *FRT*, we expect the linkage between the site of homologous recombination and the centromere to be changed.

When centromeres segregate in an x-like fashion, the distal portions of the recombinant chromatids should segregate together, to produce a white$^+$ cell with a *st*/*st*$^+$ genotype. Z-like segregation of the centromeres will produce a white$^+$ cell with a *st*/*st* genotype. G1 recombination will still result in *st*/*st*$^+$ cells. Therefore, in a

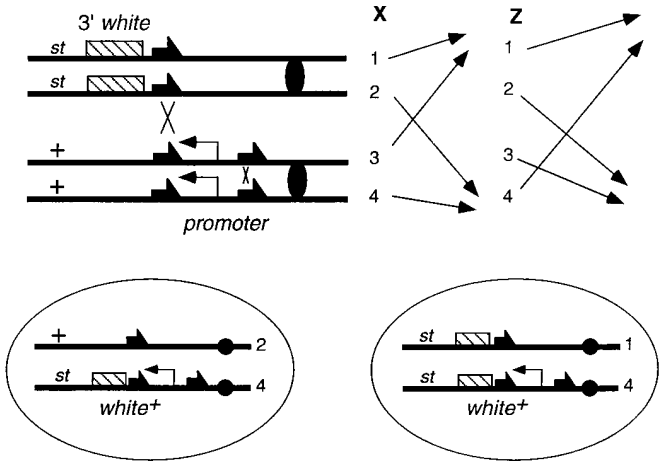

Figure 4.—Measuring x and z segregation with sister chromatid exchange. This diagram indicates the daughter cells of x and z segregation of centromeres when a sister chromatid exchange also occurs at a more proximal site on one homolog. The expected clone phenotypes are indicated at the bottom. Symbols are as in Figure 3.

	clone phenotypes	
	x segregation	z segregation
bw+ background	white+	white+
bw background	white+	white

brown background, only clones that have undergone G1 recombination or G2 recombination followed by sister chromatid exchange and x segregation will be visible (Figure 4). Thus, with sister chromatid exchange, the ratio of [white$^+$ scarlet] : [white$^+$ scarlet$^+$] is the ratio of [G2 recombination followed by z segregation] : [G1 recombination plus G2 recombination followed by x segregation].

In our original experiment, one third of recombination events were not followed by x segregation. If these were all cases of G2 exchange followed by z segregation, then in this experiment the frequency of white$^+$ clones observed in a brown$^+$ background should be reduced by one third in a brown background. If all recombination events that are not followed by x segregation are the result of G1 recombination, then we should observe no reduction in the number of white$^+$ clones when measured in a brown background. When the site of sister chromatid exchange was proximal to the site of homologous recombination, the number of white$^+$ clones observed in the brown background was essentially equal to that observed in the brown$^+$ background (Table 2). There was no measurable reduction in clone frequency in a brown background. Thus, within the limits of resolution of this experiment, all recombination that occurs in G2 is followed by x segregation, and G1 recombination is responsible for non-x segregation.

As a control, a chromosome carrying a second *FRT* distal to the site of homologous recombination was also constructed. We expect that sister chromatid exchange at this site will not affect observed segregation ratios. In this experiment, we observed no change in the segregation ratio from that observed in the experiment without sister chromatid exchange (Table 2). The number of white$^+$ clones observed in a brown$^+$ background was reduced by more than two thirds in a brown background, indicating that at least two thirds of recombination occurred in G2 and was followed by x segregation. The results of these and the previous experiments lead us to conclude that approximately two thirds of FLP-mediated mitotic recombination occurs in G2 and that this is always, or nearly always, followed by x segregation. The remaining one-third fraction of recombination occurs in G1. It is conceivable that the proportions of G1 and G2 recombination may vary from tissue to tissue, possibly in accord with the length of time that a cell spends in each part of the cell cycle.

The results of this experiment provide further evidence that the observed excess of x segregation cannot simply be a result of random segregation with multiple

TABLE 2

x and z segregation in somatic cells with an additional FRT

Genotype	Heat shock	Additional *FRT*[a]	*N*[b]	Frequency of mosaics[c] (%)
bw/+	−	P	430	48
bw/*bw*	−	P	354	51
bw/+	−	D	405	47
bw/*bw*	−	D	461	12

This experiment is diagrammed in Figure 4. Flies were not heat shocked.

[a] P indicates that the added *FRT* is proximal to the site of homologous exchange; a *P* element with a single *FRT* inserted at 79D was used. D indicates that the added *FRT* is distal to the site of homologous exchange; an *FRT*-bearing *P* element inserted at 67A was used.

[b] *N*, number of males scored.

[c] Calculated as 100 × (males with white$^+$ clones)/*N*.

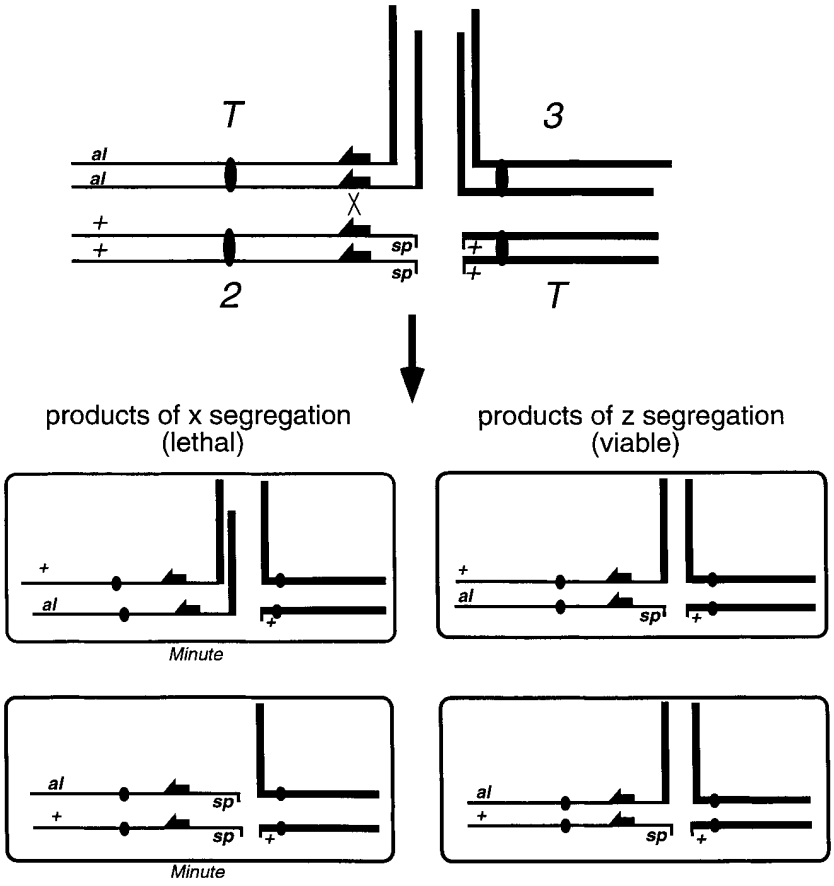

Figure 5.—Measuring x and z segregation in the germline. G2 recombination between a translocation and a normal chromosome in germline stem cells results in aneuploidy if x segregation ensues. When G_2 recombination followed by z segregation occurs (or G_1 recombination), both daughter cells are euploid. The positions of the marker genes are indicated. Chromosome *2* is represented by a thin line. Chromosome *3* is thick.

rounds of recombination. As discussed previously, such a circumstance would tend to produce an excess of *st*/*st* homozygous cells at the expense of *st*/*st*$^+$ heterozygotes. However, in this experiment, the *st*/*st*$^+$ cells are in excess. This result is easily explained by preferential segregation.

We also note that, in this experiment, each cell appears to have undergone only a single sister chromatid exchange event after homologous recombination. Previous work in our lab has indicated that unequal sister chromatid exchange between *FRT*s, leading to the formation of dicentric chromosomes, can occur in 90% or more of larval neuroblast cells after heat shock induction of FLP synthesis (Golic 1994; Ahmad and Golic, 1998). In light of this, we considered it possible that multiple equal sister chromatid exchanges might occur in each cell and randomize the pattern of segregation. This would drive the ratio of *st*/*st*$^+$:*st*/*st* clones derived from G2 recombination to approach 1:1 and predicts that the frequency of clones observed in a *bw* background would be approximately two thirds of the total observed in a *bw*$^+$ background. Because this is not what we observed, we repeated this experiment twice, with the same results. To ensure that the stocks were correct, we collected experimental males and performed backcrosses to confirm their genotype. When the putative *bw*/*bw* males were crossed to *w*$^+$/*w*$^+$; *bw*/*bw* virgins, all progeny were brown. When crossed to *w*$^+$/*w*$^+$; *st*/*st* virgins, half the progeny were scarlet, as expected. PCR was used to confirm the presence of the proximal *FRT*. All stocks showed the expected band. It may be that the noninduced level of FLP expression does not generally produce multiple sister chromatid exchanges. Alternative models to explain this result will be presented in the discussion.

Segregation of recombinant chromatids in the germline: To determine segregation ratios following mitotic recombination in germline cells, it was necessary to devise a system that allowed us to measure mitotic segregation after the recombinant chromosomes had also undergone meiosis. When recombination occurs between a large reciprocal translocation and a normal chromosome, it creates a situation in which the products of x segregation have a greatly reduced viability. An *FRT*-bearing *P* element located at 54A on the polytene chromosome map was recombined onto *T(2;3)bw*^{v5}. This translocation has the entire euchromatic left arm of chromosome *3* translocated to the tip of *2R*. The normal homolog carried the same *FRT*-bearing *P* element. The chromosomes were marked as indicated. (Figure 5).

In this experiment we examined FLP-mediated recombination in the male germline to avoid the complication of normal meiotic recombination observed in females. The FLP construct used in this work, *70FLP*,

TABLE 3

x and z segregation in the germline

Genotype	HS[a]	*N*[b]	Nonrecombinant		Recombinant		Frequency (%)
			al+	+*sp*	++	*al sp*	
T/+	−	58	792	1399	0	2	0.1
+/+	+	40	748	1351	723	349	33.8
T/+	+	75	798	1358	78	132	8.9

This experiment is diagrammed in Figure 5.

[a] HS, + indicates males were heat shocked; − indicates no heat shock control. Males were heat shocked for 1 hr at 38°, at 6–30 hr of development.

[b] *N*, number of males crossed.

is inducible in only the earliest stages of spermatogenesis, so after recombination the recombinant chromosomes undergo several mitotic divisions before meiosis (Bonner *et al.* 1984; Golic and Golic 1996b). When germline recombination is induced in normal males, the frequency of recombinant gametes is a measure of the frequency of recombination (Golic 1991; Golic and Golic 1996b). The translocation genotype allows x segregation to be measured as a reduction in the frequency of recombinant gametes relative to control males with a normal karyotype. If recombination occurs in G2 and the recombinant chromatids undergo x segregation, both daughter cells will be aneuploid for portions of the translocation (Figure 5). One cell has only one copy of *3L* and the other cell is aneuploid for the tip of *2R*. Both cells are Minute and, therefore, slow growing. In addition, aneuploidy for an entire chromosome arm is expected to be cell lethal (Ripoll 1980; Ashburner 1989). We expect that cysts consisting of these cells will die or divide so poorly that they will contribute very few sperm. When recombination occurs during G1 of the cell cycle or during G2 and is followed by z segregation, the daughter cells are euploid and capable of making viable sperm. Z segregation results in one cell with the parental genotype and one cell containing both recombinant chromosomes. G1 recombination results in two euploid daughter cells, each carrying both recombinant chromosomes.Thus, the frequency of recombination that is measured by a test cross will be reduced relative to the control in proportion to the fraction of recombination that occurred in G_2 and was followed by x segregation. Therefore, essentially all recombinant progeny recovered from this cross should derive from either G1 recombination or G2 recombination followed by z segregation.

In the control, recombination induced between two normal chromosomes *2* resulted in 33.8% germline recombination (Table 3). Recombination between the translocation and a normal *2* yielded only 8.9% of progeny carrying recombinant chromosomes. These ratios are consistent with G2 recombination followed by x segregation accounting for approximately three quarters of recombination in germline mitotic cells (Table 3). If G1 recombination accounts for the remaining recombinants, then the frequency of G2 recombination followed by x segregation is underestimated by these results, because a single G1 exchange produces twice as many recombinant chromosomes as does a single G2 exchange.

One alternative explanation for the reduction in the observed frequency of recombination could be that the translocation interferes with pairing of the homologs and reduces recombination by this mechanism. However, when Lewis (1954) examined chromosome rearrangments that disrupted pairing-dependent complementation (transvection) at *BX-C*, he showed that only those rearrangements with one breakpoint proximal to the locus were capable of disrupting transvection. Rearrangements with breakpoints solely distal to *BX-C* do not affect transvection, leading to the conclusion that they do not affect pairing. In two other cases of transvection it was also observed that pairing was disrupted only by rearrangements that had one breakpoint proximal to the transvecting locus (Gelbart 1982; Leiserson *et al.* 1994). The effects of rearrangements on FLP-mediated mitotic recombination in the male germline are very similar to the effects of rearrangements on transvection at *BX-C* (Golic and Golic 1996b). For these reasons we do not expect that the translocation interferes with the frequency of recombination at the proximal *FRT* in our experiment. In our experiment, the reduction in the number of progeny with recombinant chromatids is most likely caused by segregation. Therefore we conclude that, in the male germline, approximately three fourths of FLP-mediated mitotic recombination occurs in G2 and is followed by x segregation. Thus, whether FLP-mediated homologous recombination is induced in mitotically dividing cells of the germline or of the soma, most recombination occurs in G2 of the cell cycle and the recombinant chromatids segregate apart at mitosis.

Segregation following recombination between heterologs: We wished to determine whether site-specific recombination between heterologous chromosomes was

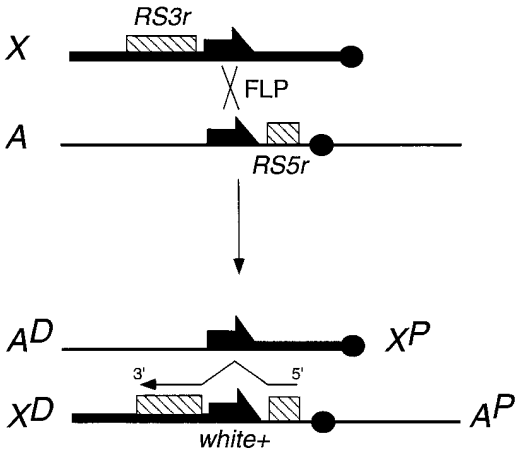

Figure 6.—Chromosome rearrangement scheme. *RS5r* carries the first exon of the *white* gene and an *FRT* within the first intron; *RS3r* carries the remainder of the *white* gene, with an *FRT* located at the identical position in the first intron. When recombination occurs between complementary *RSr* elements located on heterologous chromatids, a translocation is generated that is marked by a reconstituted *white*$^+$ gene on one chromatid. In this example, the *X* chromosome is indicated by a thick line; an autosome (*A*) is indicated by a thin line. The proximal and distal parts are indicated by superscripted *P* and *D*.

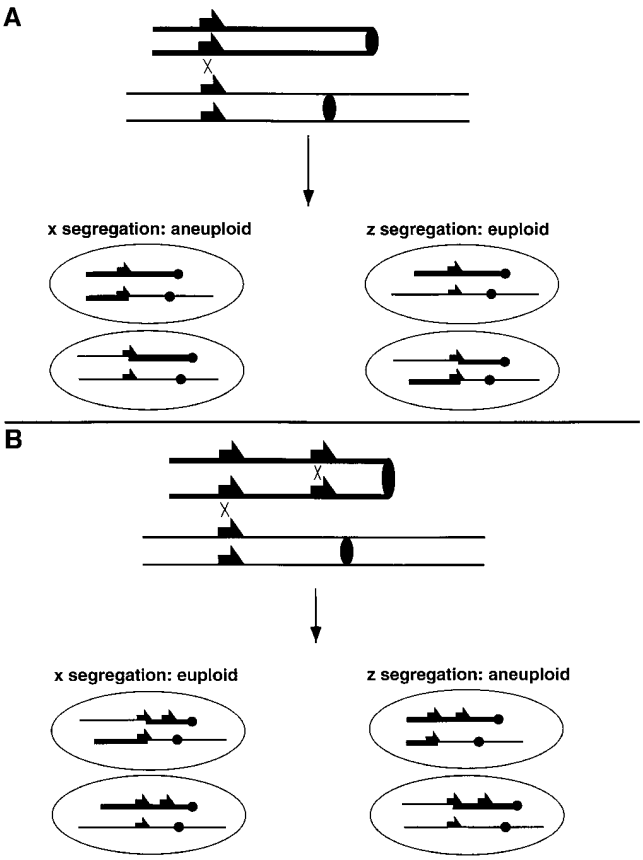

Figure 7.—Segregation of recombinant chromosomes following heterologous recombination. (A) The consequences of x and z segregation after recombination in G2 are diagrammed. G1 recombination will produce the lower cell diagrammed under z segregation. (B) The expected outcomes of segregation after heterologous exchange with the addition of a proximal *FRT* on one chromosome. Sister chromatid exchange is expected to alter the outcome of x segregation, so that the translocated portions segregate in a z-like pattern, allowing recovery of the translocation.

also followed by preferential segregation of recombinant chromatids. To identify recombination events between heterologous chromosomes, we again used a system that marks recombinants with the generation of an intact w^+ gene. The *P* element *RS5r* carries the first exon of the *white* gene followed by an *FRT* in the first intron. The *P* element *RS3r* carries the remainder of the *white* gene with an *FRT* at an identical position in the first intron. Collectively, we refer to *RS3r* and *RS5r* as *RSr* elements (*RS* stands for rearrangement screen). When FLP is used to catalyze recombination between the *FRT*s of these elements, chromosomal rearrangements, marked by the generation of a functional w^+ gene at the site of recombination, are produced at a low frequency (Figure 6; Golic and Golic 1996a).

For recovery of translocations by FLP-mediated recombination between *FRT*s on heterologous chromosomes, the complementary *RSr* elements must be in the same orientation with respect to their centromeres. If they are in reverse orientation, recombination will generate a dicentric chromosome and an acentric fragment. Equally critical, the two recombinant chromatids, which are the two halves of a reciprocal translocation, must segregate together. This can occur by G2 recombination and z segregation, or by G1 recombination (Figure 7A). If the recombinant chromatids segregate apart, the daughter cells will be aneuploid. In most cases it is not possible to recover viable progeny after this type of segregation.

We performed experiments to recover reciprocal translocations by FLP-mediated recombination. The same method has been successfully used to recover intrachromosomal rearrangements (Golic and Golic 1996a). Three combinations of *RS5r* and *RS3r* elements were tested. In all three cases, the *FRT*s of the two elements were known to be oriented in the same direction with respect to the centromere. We were unable to recover translocations from two combinations of single *RS5r* and *RS3r* insertions despite extensive crosses and the occurrence of w^+ clones in the eyes as evidence of translocation generation in somatic cells (Table 4). Three translocations were recovered from a third combination of single insertions, at a rate lower than that achieved in the experiments described below.

There are two possible explanations for the failure to recover progeny carrying reciprocal translocations in these experiments. One possibility is that the two translocation halves preferentially segregate from each other during mitosis. The resulting aneuploid daughter cells may survive and produce white$^+$ clones in the soma, but, in the germline, the aneuploidy leads to either a

TABLE 4

Effect of proximal *FRT* insertions on translocation recovery

	RS3r insertions (*X*-linked or autosomal)	*RS5r* insertions (autosomal)	N^a	T^b	$\%T^c$
		A. Single *FRT*s on each chromosome			
T(X;A)	4B•	•7A	688	0	0
		1A•	722	0	0
T(2;3)	•4A	1A•	2072	3	0.14
		B. Proximal *FRT*s on at least one chromosome			
T(X;A)	4B(1)•	•7A	443	4	0.90
		•(5A)7A	918	6	0.65
		1A•(2A)	403	2	0.50
T(2;3)	•4A	1A([>]2A)•[d]	603	2	0.33

The large dots indicate the position of the centromere on each chromosome with respect to the *RS* element insertions. Insertions are indicated by arbitrary isolate numbers. Locations are shown in Figure 2. The insertions indicated in parentheses are not involved in the translocation. This experiment is diagrammed in Figures 6 and 7.

[a] *N*, total number of vials scored. Each vial produced ~100 progeny.

[b] *T*, number of independently recovered translocations. Occasionally a single vial produced two white$^+$ progeny. This was scored as one event.

[c] $100 \times T/N$.

[d] [>]2A is the excision product of P[>w^{hs}>]2A located at 79D-F. It consists of an *FRT*, *P*-element sequences, and no portion of the *white* gene.

failure to produce gametes or the death of aneuploid progeny. Alternatively, in the germline, recombination between *FRT*s on heterologous chromosomes may be too rare to recover easily. We can think of no way to easily demonstrate the latter possibility, so we concentrated on devising a test of the first theory by attempting to alter the segregation in the germline, thereby allowing the recovery of reciprocal translocations that were being lost due to aneuploidy.

We reasoned that it might be possible to alter the segregation pattern of exchange chromatids by providing a more proximal site at which sister chromatid exchange could occur, as in our previous experiments. If FLP-mediated heterologous exchange were to occur in G2, it is very likely that sister chromatid exchange would occur in the same cell and change the linkage between the centromere and the site of heterologous exchange. This might allow the two translocation halves to segregate together so that the entire translocation could be recovered (Figure 7B). When we repeated our attempts to recover translocations with the same combinations of *RSr* elements and the addition of a second, proximal *FRT* on at least one chromosome, reciprocal translocations were recovered in all combinations (Table 4). These results demonstrate that the failure to recover translocations in the first experiments was not simply a result of the rarity of their formation. Instead, we conclude that reciprocal translocations were not recovered because the two halves tended to segregate apart in the subsequent mitosis. The addition of a second *FRT* on one chromosome allows a segregation event that is mechanically x-like to give a result that is genetically z-like, and the two halves of the translocation segregate together.

However, we also discovered a second method for recovering reciprocal translocations. In two cases we recovered translocations when the additional *FRT* insertion was not proximal to the translocation breakpoint, but distal. In another instance there was a third *FRT*-bearing *P* element on the chromosome arm opposite the translocation breakpoint. In an additional experiment, translocations were recovered when the third element was located on an entirely different chromosome (Table 5). Without invalidating the ability of sister chromatid exchange to alter segregation, this indicates that segregation of recombinant heterologs may be affected by mechanisms additional to the one that appears to affect segregation of recombinant homologs.

Segregation after exchange near chromosome termini: Meiotic exchanges that occur near the ends of chromosomes are less effective in directing segregation at the reductional division than are exchanges that occur in the middle of arms (Carpenter 1973; Koehler *et al.* 1996; Lamb *et al.* 1996; Ross *et al.* 1996). We investigated whether recombination near the ends of chromosomes could direct segregation in mitosis. For this experiment we induced heterologous recombination between *RSr* elements located at the tips of the *X* and *3R*. Recombination between these two elements generates a translocation in which both aneuploid segregants are viable. The portion of the translocation with the chromosome *3* centromere will carry the reconstructed *white*$^+$

TABLE 5

Effect of additional *FRT* insertions on translocation recovery

RS3r insertions (*X*-linked)	*RS5r* insertions (autosomal)	N^a	T^b	$\%T^c$
A. Additional *FRT*s on at least one chromosome				
4B•	(10)•7A	1290	6	0.47
(4B)1•	3B•	327	2	0.61
(4B)1•	•A17	411	1	0.97
B. Single *FRT* on each chromosome				
4B•	•7A;1A•	342	6	1.75[d]

The large dots indicate the position of the centromere on each chromosome with respect to the *RS* insertion elements. Insertions indicated in parentheses are not involved in the translocation.

[a] *N*, total number of vials scored. Each vial produced ~100 progeny.

[b] *T*, number of independent occurrences of each heterologous recombination event. Occasionally a single vial produced two white$^+$ progeny. This was scored as one event.

[c] $100 \times T/N$.

[d] This number indicates the total rate of recovery of translocations. Two were T(1;3)s; one was a T(1;2). The remainder were sterile males and we were unable to determine pseudolinkage.

gene. We induced germline recombination with *70FLP*, recovered the progeny with the *white*$^+$ half of the translocation, and then performed a test cross to determine whether they also carried the other half of the translocation. If the *white*$^+$ offspring carried both halves, then the translocation must have arisen by recombination in G1 or have undergone z segregation following recombination in G2. In half of such cases, the reciprocal halves of the translocation will segregate apart in meiosis (Ashburner 1989). Thus, the corrected frequency of x segregation is given by subtracting the number of complete translocations recovered from the number of white$^+$ half-translocations recovered. We find that the frequencies of the two types of segregation are approximately equal (Table 6). This is not changed by the presence of a second *FRT* insertion (Table 6). We conclude that when recombination occurs at the tips of heterologous chromosomes, preferential x segregation does not occur.

Cytological analysis of mitotic exchange: The results of our experiments imply that, in most cases, a chromosome does influence the segregation of the chromatids of its homolog following mitotic recombination. It seems most likely that any mechanism that achieves this would require that the exchange homologs remain in contact until they attach to the mitotic spindle. Because FLP-mediated recombination occurs with a high efficiency (Golic 1991), it seemed that it might be possible to unambigously detect such persistent associations by cytological examination of metaphase chromosomes. Accordingly, we induced FLP synthesis in flies that were homozygous for an *FRT* insertion at 75C-D on chromosome *3*. Metaphase chromosomes from larval brains were examined after DAPI staining. In these spreads cruciform structures that involved specifically the chromosome *3* homologs were observed in ~10–20% of metaphase nuclei (Figure 8; a diagrammatic interpretation of these structures is presented in Figure 10). (Chromosome *3* can be identified in most spreads by its two brightly staining pericentric bands.) These structures are not observed in normal metaphase spreads. Thus, we conclude that homologs do remain physically associated after mitotic exchange.

TABLE 6

x *vs.* z segregation after exchange at chromosome tips

	Half *T*'s	Full *T*'s	% *X*
One *FRT*/chromosome	23	9	44
Two *FRT*/chromosome	22	7	51

% *X* segregation = 100 × (half *T* − full *T*)/total.

DISCUSSION

x segregation predominates in Drosophila: We measured the segregation of recombinant chromatids in mitosis after FLP-mediated recombination. Our results show that, in both somatic and germline cells, x segregation outweighs z segregation and G1 recombination by at least two to one. This is in complete accord with the results of Pimpinelli and Ripoll (1986) on segregation following X-ray-induced mitotic recombination and indicates that the directed segregation observed by Pimpinelli and Ripoll is not solely due to associations mediated by the heterochromatic blocks used to mark the chromosomes. Instead, the preference for x segregation is a general property of mitotic recombination in Drosophila. Formally, the recombinant chromosomes that did not undergo x segregation may have been produced by recombination in G1 or may have experienced z segregation after recombination in G2. To the limits that our experiments can measure, the recombinants that did not go through x segregation were a result of recombination that occurred in G1. Thus, when mitotic recombination occurs in G2, it is almost inevitably followed by x segregation. If homologous chromosomes align independently on the metaphase plate, then it is unexpected that the segregation of one pair of sister chromatids should exert any influence on the segregation of homologous sisters. It seems that the only way that a chromosome could influence the segregation of its homolog is if the homologs remain attached to each other until their kinetochores attach to the spindle. Our results can be easily interpreted if, when mitotic recombination occurs in G2, a mitotic bivalent is formed. This mitotic bivalent may then be responsible

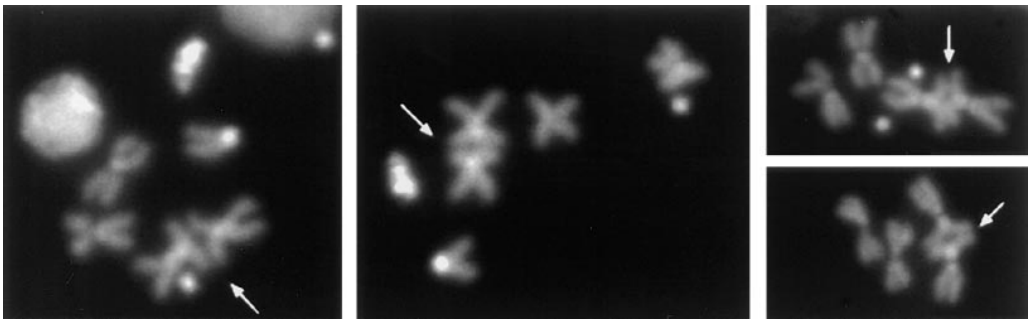

Figure 8.—Mitotic bivalent cytology. Mitotic figures were prepared as described in materials and methods. Several examples of the observed chromosome *3* bivalents are shown; in each case the chromosome *3* pair is indicated with an arrow. Figure 10A gives a diagrammatic interpretation of these bivalents.

for the directed segregation of recombinant chromatids.

Mitotic bivalent model: We imagine two ways that a bivalent could be maintained and lead to x segregation. First, mitotic pairing may maintain the association of homologs until they attach to the mitotic spindle. Although this pairing is most fully developed in interphase, it can also be clearly observed cytologically in prophase and metaphase cells (Metz 1916; Kopczynski and Muskavitch 1992; Hiraoka *et al.* 1993). If the exchange chromatids are oriented to the center of this bivalent, then x segregation is expected (Figure 9). If this model is correct, the tendency to x segregation may be specific to organisms with strong mitotic pairing.

A second model supposes that a G2 exchange persists as a physical crossover until metaphase, and orients chromosomes in a fashion similar to a chiasma in meiosis (Nicklas 1967, 1977; Hawley 1988). The force that maintains this crossover might be attributable to the components that maintain the association of sister chromatids. This force, termed sister chromatid cohesion (SCC), holds together the sister chromatids that make up each homolog from S phase through metaphase (Maguire 1982; Murray and Szostak 1985; Holm 1994; Miyazaki and Orr-Weaver 1994). SCC is strong enough to hold an exchange bivalent intact during meiosis even when enough mechanical force is applied to break the microtubule attachments (Nicklas 1967). Therefore, the mechanical linkage created by an exchange event in concert with SCC may be capable of maintaining the association of homologs during spindle attachment and alignment on the metaphase plate (Figure 10). Such a bivalent may well be capable of directing segregation. If the mitotic spindle first attaches to a recombinant chromatid in the bivalent, it is probable that the chromatid of the homolog that is in proper alignment to attach to the same pole will be the nonrecombinant chromatid. Their sister chromatids must each attach to the opposite pole in order for anaphase to proceed (McIntosh 1991; Li and Nicklas 1995; Nicklas *et al.* 1995; Reider *et al.* 1995; Skibbens 1995; Chen *et al.* 1996). Thus, the recombinant chromatids will ultimately be attached to opposite poles and will segregate away from each other owing to mechanistic constraints of the cell's mitotic apparatus. This model predicts that the preference for x segregation after an exchange in G2 would not be limited to organisms with strong mitotic pairing, but would be universal, because the maintenance of sister chromatids in close apposition is an elemental feature of mitosis in eukaryotes.

In order to decide between these two models we consider the following data. In meiosis, chiasmata maintain the physical linkage of homologs until anaphase I. Current opinion favors the view that these chiasmata are maintained by the cohesion of sister chromatids (Maguire 1982; Bickel and Orr-Weaver 1996). This coupling of homologs allows the meiotic bivalent to establish a stable linkage to both poles for the reductional division. Anaphase segregation of homologous chromosomes is accomplished by the simultaneous release of SCC on all chromosomes, except in the vicinity of kinetochores.

Meiotic chiasmata that result from exchange between nonhomologous chromosomes can also direct the anaphase I disjunction of the two chromosomes involved

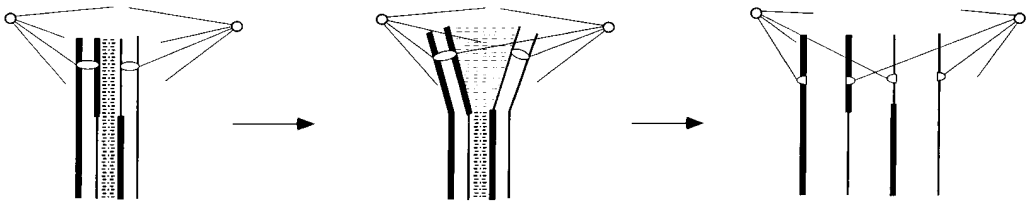

Figure 9.—Orientation of a mitotic bivalent by mitotic pairing forces. Homologs are distinguished by thick or thin lines. The force of mitotic pairing is indicated by dashed lines between homologs. Recombination in G2 occurs between chromatids oriented to the interior of the homolog pair, because they are most closely associated. The kinetochores of the recombinant chromatids are also oriented inward, obscuring them from the spindle microtubules until the outer kinetochores have attached to the spindle pole. The force generated by the spindle attachment pulls the homologs apart, allowing access to the interior kinetochores, which, of necessity, must attach to opposite poles, resulting in x segregation.

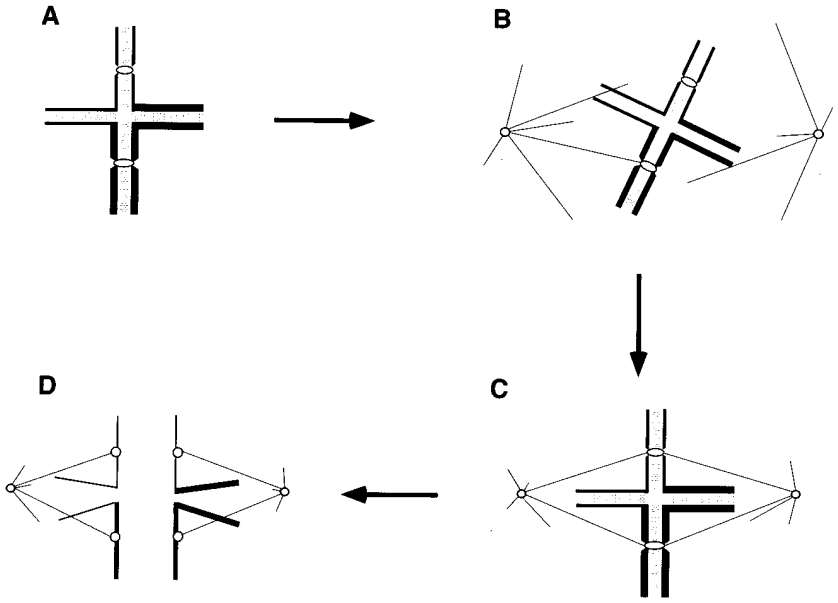

Figure 10.—Orientation of a mitotic bivalent mediated by sister chromatid cohesion. Homologs are distinguished by thick or thin lines. The force of sister chromatid cohesion is indicated by dashed lines between sister chromatids. (A) The recombinant homologs associate in a mitotic bivalent, in which replicated sister chromatids are held together. (B) Bivalents tend to be oriented so that, if the first attachment is to a recombinant chromatid, the nonrecombinant chromatid adjacent to it will attach to the same spindle pole. (C) The sister of each attached chromatid must attach to the opposite spindle pole. Mitosis cannot proceed until each centromere is under tension as illustrated. (D) The mechanical constraints of the bivalent result in x segregation.

in the exchange (Bateman 1968; Parker 1969; Parker and Williamson 1970; Traut 1970; Busby 1971; Golden and Zimmering 1972; Parker and Williamson 1976; Hawley 1988; Jinks-Robertson *et al.* 1997). Our results suggest that heterologous exchanges are also able to dictate the pattern of segregation in mitosis, just as an exchange between homologs does. In this case, we expect minimal pairing of the heterologous chromosomes because there is only a small region of matching homology (within the two *P* elements) between the two chromosomes. However, cohesion of the sister chromatids distal to the exchange should still apply and could produce a bivalent just as easily after heterologous exchange as after homologous exchange. Thus, our observation that heterologous exchanges drive x segregation favors the SCC model.

In meiosis, insufficient SCC causes increased nondisjunction. This is true whether SCC is reduced genetically (Miyazaki and Orr-Weaver 1992) or because an exchange has occurred too near the end of the chromosome, so that SCC distal to the exchange is insufficient to maintain the bivalent (Carpenter 1973; Rasooley *et al.* 1991; Koehler *et al.* 1996; Lamb *et al.* 1996; Ross *et al.* 1996). Very small chromosomes also suffer increased nondisjunction, possibly due to a lack of sufficient SCC (Dani and Zakian 1983; Murray and Szostak 1983; Koshland *et al.* 1985). In our experiments we obtained one case in which no preference for x segregation was observed. In this instance, exchange occurred very near the tips of the involved chromosomes. If mitotic pairing is the force that drives x segregation, then x segregation should predominate, just as it does when exchange occurs at the more proximal sites. However, if SCC maintains the association of exchange chromosomes, then it might be reasonably expected that when the sites of exchange were near chromosome termini, the exchange chromosomes could fall apart. In such a circumstance no preference for x segregation would be observed, as previously proposed by Pimpinelli and Ripoll (1986). This result, therefore, also favors the SCC model.

Finally, SCC is sufficient to account for the mitotic bivalents that we observed cytologically (compare Figures 8 and 10). Cytological figures that appear to be such bivalents have been previously observed in mitotic metaphase after X-irradiation (Gatti *et al.* 1974; Pimpinelli and Ripoll 1986). Our present results confirm that these bivalents are produced at a high rate after FLP-mediated mitotic recombination. The bivalents that we observed showed no sign of being paired in homologous regions. Colchicine treatment was used to obtain these figures, and it is possible that a mitotic pairing that existed at metaphase was lost because of the treatment. But, if such pairing does normally exist in metaphase cells, it is apparently not necessary to maintain the mitotic bivalents that are produced by an exchange between chromosomes in G2. The evidence of our experiments supports the model that the association of exchange chromosomes is maintained by SCC. Thus, we believe that our results demonstrating a preponderance of x segregation after mitotic recombination will pertain to a broad range of organisms.

Altering segregation by sister chromatid exchange: We showed that the tendency of exchange chromatids to segregate from each other in mitosis could be altered by providing one of the exchange chromosomes with an additional more proximal *FRT*. We presume that sister chromatid exchange at this proximal site can alter the segregation pattern of the distal arms. Normally, such a sister chromatid exchange would have no effect on the genetic constitution of the daughter cells. However, when that chromosome has also undergone a nonsister exchange at a more distal site, then the al-

tered pattern of chromatid segregation may be of consequence.

The results obtained from the experiment diagrammed in Figure 4 necessitate that there be one and only one effective sister chromatid exchange prior to segregation. We propose that, for a sister chromatid exchange to alter the linkage of the more distal portions of chromatids, the sister chromatids must be rigidly oriented with respect to one another and unable to swivel about the site of sister chromatid exchange. As chromosomes condense for division, such rigidity may result. There is good reason to believe that chromosome condensation may also limit FLP activity. *FRT*s inserted near constitutive heterochromatin are recombined much less efficiently by FLP than are *FRT*s inserted at euchromatic sites (Ahmad and Golic 1996). Thus, chromosome condensation may have two consequences: the limitation of FLP activity and the prevention of any further change in the arrangement of chromatids about their axis. Perhaps only an exchange that occurs after the chromosomes begin to condense and become rigid is effective in changing their linkage. The time available between reaching this level of condensation and becoming too condensed for further sister chromatid exchange may be short, limiting the number of effective exchanges to one. Sister chromatid exchange events that occur before the onset of chromosome condensation would have no effect on segregation.

Alternatively, sister chromatids may be prevented from swivelling about their axis by the force of homologous pairing, and there may be but one FLP-mediated sister chromatid exchange in a given cell cycle. After recombination, the FLP protein complex must dissociate and reform before another round of recombination can occur at any *FRT*, and this event is slow *in vitro* (Waite and Cox 1995). Our observations could also accommodate a model that supposes that there is, in general, time for only a single sister chromatid exchange to occur within a given G2 period.

Other factors affecting segregation after recombination between heterologs: When recombination was induced between heterologs, in most cases we were unable to recover translocations by relying on the recombinant chromatids to segregate together. An additional *FRT* proximal to the site of recombination allowed the cosegregation of recombinant chromatids resulting from heterologous recombination. We postulate that heterologous recombinants segregate by a mechanism that is equivalent to the one that drives segregation of homologous recombinants.

However, other factors may also influence the segregation of recombinant heterologs. Unexpectedly, we discovered that the extra *FRT* did not need to be proximal to the site of heterologous recombination, or even on the same chromosome, to facilitate cosegregation of the recombinant chromatids. We recovered translocations with the additional *FRT* distal to the site of heterologous recombination, on the opposite chromosome arm and on the uninvolved chromosome. We propose two possible explanations for this result. First, additional *FRT*s present in the germline may simply facilitate rapid pairing of the sites so that G1 recombination is more frequent. Recombination that occurs during G2 would continue to result primarily in x segregation, but a greater percentage of recombination events would occur in G1. A second possible model is that the orientation of the centromeres is influenced by both SCC and mitotic pairing. When FLP catalyzes exchanges between a single pair of *FRT*s located on heterologous chromosomes, the resulting exchange causes the involved chromatids to behave as recombinant homologs and preferentially segregate away from each other. However, when there are multiple *FRT*s in the genome, ectopic pairing and multiple homolog associations may produce a mechanical strain on the chromosomes that can disrupt the orientation of the bivalent. At this point we are unable to discern between these two models, but, practically, we are able to utilize this phenomenon in attempting to recover heterologous recombination events in Drosophila.

Recently, generation of translocations with the Cre-*lox* transgenic recombinase has been reported in mouse and in tobacco. No allowances were made for segregation in either of these systems. However, translocations were recovered at very low frequencies. In mouse cells, the rate of recovery was reported as 5×10^{-8}; this low rate of recovery might easily be accounted for by G1 recombination (Smith *et al.* 1995). In tobacco, Cre expression was constitutive in every cell until a translocation was generated, and 50% of plants experienced at least one recombination event in several weeks of growth (Qin *et al.* 1994). This may correspond to a similarly low rate of recovery. Also, three of the four tobacco translocations were viable as half-translocation plants. Because the applied selection required the maintenance of only one of the two breakpoints, many of the selected cells may have carried only half the translocation. It is possible that both of these systems could be made more efficient by allowing for alteration of segregation ratios as discussed above.

The relationship between meiosis and mitosis: It has been proposed by a number of workers that the meiotic cell cycle is a modified mitotic cycle and that much of the meiotic apparatus and biochemical mechanisms have been co-opted or modified from mitotic machinery (Murray and Szostak 1985; Kleckner and Weiner 1993; Miyazaki and Orr-Weaver 1994; Kleckner 1996; Nicklas 1997). In support of this view, many proteins and enzymes are necessary for both mitotic and meiotic cell cycle progression (Baker *et al.* 1978). Frequently these gene products are responsible for DNA repair in mitotic cells and are involved in recombination

in meiotic cells (Baker *et al.* 1978; Gatti *et al.* 1980; Carpenter and Baker 1982; Wagstaff *et al.* 1985).

We propose that recombination, whether in mitosis or meiosis, is capable of mechanically directing the attachments that the involved chromosomes make to the spindle. Our data provide genetic and cytological evidence for the existence of a mitotic bivalent that is produced by exchange in G2 of the cell cycle, and for its ability to drive segregation in a manner analogous to the meiotic bivalent. However, because homologs do not undergo a reductional division in mitosis, the segregation that results is that of recombinant nonsister chromatids.

Kleckner (1996; see also Kleckner *et al.* 1991) suggests that the first step in progressing from a mitotic cell cycle to a meiotic cycle was the co-opting of the double-strand break repair mechanisms in somatic cells to create the chiasmata that link homologs for meiosis I. Because the capability to generate a bivalent via exchange exists in the mitotic cycle, the steps needed to produce the meiotic cycle through evolution are simplified.

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