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Genetics

Mismatch repair, gene conversion, and crossing-over in two recombination-defective mutants of *Drosophila melanogaster*

(meiotic mutant/*rosy* locus/postmeiotic segregation/recombination models/heteroduplex DNA)

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ABSTRACT **Recombination-defective mutants at two loci that are known to decrease drastically the frequency of meiotic crossing-over do not decrease the frequency of gene conversion at the rosy locus. *mei-9* mutant alleles produce frequent postmeiotic segregants manifested as mosaic progeny whereas controls and *mei-218* mutants produce none. It is concluded that (*i*) recombination in *Drosophila* involves a biparental DNA intermediate and (*ii*) correction of heteroduplex DNA or recognition of biparental DNA or both is necessary, but not sufficient, for this intermediate to result in crossing-over of flanking markers. It is therefore likely, at least in *Drosophila*, that the isomerization step in Meselson–Radding type molecular models of recombination is under genetic control.**

Two approaches have been used to probe the mechanism of genetic recombination in eukaryotes. The first involves deducing the properties of the wild-type recombination mechanism from the meiotic behavior of marker alleles within or between loci; this approach has been used extensively in a number of fungal systems and at several loci in *Drosophila* (for reviews and entries into the extensive literature, see refs. 1–3). In fungi, where all four products from a single meiosis can be recovered and analyzed, it has been found that biparental (heteroduplex) DNA is at least a frequent intermediate, since some marker alleles show frequent postmeiotic segregation (PMS); events are frequently nonreciprocal since marker alleles may show 3:1 or other non-Mendelian segregation (gene conversion); gene conversion may occur in either direction (+ → mutant or mutant → +) but the ratio may depend on the specific marker alleles; events detected by the occurrence of gene conversion may, or may not, simultaneously involve an exchange of flanking markers (in most systems, these two alternative outcomes are approximately equally frequent). These same properties appear to apply to recombination in *Drosophila* (3), although examination of gene conversion is of necessity indirect since at most only two chromatids can be recovered per meiosis.

The second approach involves deducing the nature of individual recombination functions by examining the properties of recombination in the presence of recombination-defective mutants. Mutant alleles of a number of loci whose wild-type alleles are necessary for normal frequencies or distributions of exchange events (or both) have been isolated and characterized in *Drosophila* (4); these loci fall into two general categories—those whose function is necessary to maintain both the nonrandom distribution of events along and between chromosomes and the normal frequency (precondition loci) and those whose function is necessary only to maintain the normal frequency of events (exchange loci). All but one of these loci also function in somatic cells in various aspects of chromosome maintenance both in the presence and in the absence of exogenous DNA-damaging agents; there is therefore considerable overlap between functions used during meiotic recombination and during repair in *Drosophila* (5, 6).

Here I examine the effects on gene conversion of mutants at two loci known to be necessary for normal frequencies of intergenic exchange. *mei-218* (precondition defective) has no detectable defect in the soma (6) but reduces meiotic exchange to 8% of wild-type levels and virtually abolishes the nonrandom distribution of exchange events (7). In *mei-218*, spherical recombination nodules (cytologically detectable structures that, in wild-type, are present at the site of exchange events and have been hypothesized to be recombination organelles; see refs. 8–10) are also reduced in frequency and approximately half of those present are morphologically abnormal, suggesting that this locus specifies a structural component of the recombination nodule (9). In the presence of mutants at the *mei-9* locus, meiotic exchange is reduced in frequency but the distribution of exchange events (7, 11) and the numbers and distributions of spherical recombination nodules (9) are normal. *mei-9*$^+$ is also necessary in the soma for the repair of both endogenous and externally induced chromosome lesions (6, 12, 13)—it is known to be involved in the excision of thymine dimers (14). Two mutant alleles at this locus have been examined here: *mei-9*^{a}, which reduces exchange to 8% of wild type, and the hypomorphic allele *mei-9*^{b}, which reduces exchange to 16% of controls. Preliminary experiments (15, 3) suggest that *mei-9* does not reduce the frequency of gene conversion but does increase the frequency of PMS; this study confirms and extends these observations and shows that these effects are due to the *mei-9* defect *per se* since homozygotes for either mutant allele and the *mei-9*^{a}/*mei-9*^{b} heterozygote all have the same phenotype.

MATERIALS AND METHODS

Intragenic events were studied at the rosy locus since the wild-type products of intragenic events can be selectively recovered and close flanking markers and a large variety of well-characterized alleles are available (16). rosy is the structural locus of xanthine dehydrogenase (XanDHase); mutants affect eye color.

The rosy alleles used here, *ry*^{5} and *ry*^{41} (rosy, 3—52.0), map near the left and right ends, respectively, of the rosy locus. Map distances to the flanking markers *cu* (curled), *kar* (karmoisen), *Ace*^{l26} (lethal allele of the acetylcholinesterase structural locus, herewith abbreviated *l26*), and *e* (ebony: the *e*^{4} allele is reliably classifiable in the heterozygote) are given in Fig. 2. Other markers used include *y* (yellow, 1—0.0), *Ubx* (Ultrabithorax, 3—59), and *Sb* (Stubble, 3—58). All combinations of markers are phenotypically distinct. The *mei-9* (1—6) and *mei-218* (1—57)

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Abbreviations: PMS, postmeiotic segregation; XanDHase, xanthine dehydrogenase.

alleles used are recessive in females and do not affect male meiosis. For further descriptions of markers, see refs. 17 and 18.

The following format was used for all crosses. *y (mei)/y (mei); ry^5 e^4/cu kar ry^{41} l26* virgins from *y (mei)/FM7* mothers, 0–72 hr post-eclosion and uniformly distributed across this age span, were mated *en masse* to *+/Y; In(3LR)P18, kar ry^{41} Ubx e/ Tp(3LR)MKRS, M(3)S34* [invisible] *kar ry^2 Sb*. For control (*y*) cultures, 15 females and 12 males were used per quarter-pint bottle (1 pint = 473 ml); since the meiotic mutants have reduced fertility, 30 females and 25 males were used per bottle. Parents were transferred to fresh (yeasted) food every 3 days for a total of five cultures; immediately after parent transfer, 0.8 ml of 0.111 (wt/vol) aqueous purine (or 0.8 ml of distilled water) was dispensed onto each culture. Under the culture conditions used, this dose of purine does not kill ry^+ individuals but results in the pre-eclosion death of all but a very few *ry* individuals; such escapers were more frequent in cultures having low larval density. Bottles were numbered through all five transfers to detect potential "clusters" of events (=premeiotic). Two clusters of two were observed, both in mei^+ controls. Since the two genotypes were dissimilar in both cases, they probably represent independent meiotic events.

All purine-treated bottles were checked daily for eclosed flies through days 9–17 of culture. The phenotype of each ry^+ fly for the flanking markers *kar* and *e* was recorded and the fly was then mated to *P18/MKRS* flies of the opposite sex to test for gonadal mosaicism for rosy, confirm the *kar-e* genotype, and recover the ry^+ chromatid for further testing. F_1 ry^+ (and/or *ry* if the original fly was mosaic)/*P18* and */MKRS* males were mated to *l26/T(3;4) + In(3LR)Ubx^A, cu kar Ubx^A spa^A* females to determine the ry^+ chromatid's genotype at the *cu* and *l26* loci. The maternally derived X was also tested for the appropriate meiotic mutant as a control for contaminants; in all cases, the presence of the expected meiotic mutant was confirmed.

Bottles to receive water rather than purine were chosen randomly (each transfer independent) at the frequency of 2 per tray of 32 bottles. Progeny were scored for sex and *y* daily through days 9–17; in addition, one randomly chosen water-control bottle per set was also scored for *Sb*, *Ubx*, and crossing-over between *kar* and *e*. These data give an estimate of the number of total progeny (average progeny per water-control bottle times total bottles per set) and the frequencies of X nondisjunction and crossing-over. ry^+ flies occurring in the water controls were treated like those from purine-treated cultures.

As soon as there were sufficient F_1 progeny (usually after about 7 days), each surviving ry^+ fly was frozen and immediately sectioned serially (12-μm sections) on a Slee cryostat; the sections were picked up on clean slides and stained for XanDHase activity. The reaction mixture (sufficient for one experimental ry^+ fly as well as stock control *ry* and ry^+ flies all sectioned in one block) contained 0.6 ml of hypoxanthine (25 mg/5 ml of 0.1 mM HCl), 1.35 ml of nitro blue tetrazolium (20 mg/4.5 ml of Tris), 1.35 ml of phenazine methosulfate (6 mg/ 4.5 ml of Tris), and 0.6 g of gelatine in 2.7 ml of Tris [0.2 M Tris·HCl (pH 8.1)] and was held at 60°C. All chemicals were from Sigma. The mixture was pipetted over the sections immediately after they were cut and was solidified by chilling the slide to 0°C. Coated slides were held for 4–12 hr at room temperature in the dark, then the reaction mixture was dissolved in warm tap water, the sections were dehydrated through ethanol to propanol, and a coverslip was mounted with Euperal. XanDHase activity results in a dark blue stain/precipitate in the tissue.

Consistent specific XanDHase activity was found in eyes, anterior and posterior malphigian tubules, and peripheral fat (in head, thorax, and anterior and posterior abdomen); in addition, anterior testis sheath stains specifically but not consistently. There are therefore 14 scorable regions per fly (left and right sides scored independently). Of these regions, anterior malphigian tubules proved least reliable with 14% staining failure in ry^+ controls; all other regions had less than 5% failure.

RESULTS

Frequencies of Inter- and Intragenic Recombination. None of the *mei-9* or *mei-218* mutant combinations examined reduce the frequency of intragenic events (Table 1)—in fact, the frequencies of ry^+ flies are equal to or higher than controls. Nevertheless, *mei-218*, *mei-9^a*, and *mei-9^b* reduce crossing-over by factors of 1/12, 1/12, and 1/6, respectively (Table 2), in excellent agreement with previous determinations (7, 11). The *mei-9^a/mei-9^b* combination reduces crossing-over by a factor of 1/12 (Table 2), in agreement with previous (unpublished) tests. Together, these data suggest that mutants at both the *mei-218* and *mei-9* loci have differential effects on gene conversion and reciprocal recombination.

Although reconstruction experiments indicated that the level of purine used here had no effect on the survival of *ry/ry^+* heterozygotes, the best tests are to compare the frequencies of ry^+ progeny recovered from the water-control bottles with those from bottles treated with purine (Table 1). In neither control nor *mei-218* crosses is there much, if any, difference in recovery of ry^+ progeny from the two sources, so the total data provide the best estimates of ry^+ progeny. However, for all allelic combinations of *mei-9*, ry^+ progeny are 2 to 3 times more likely to be recovered in the absence of purine than in its presence; this suggests that the selective agent is selecting against some ry^+ progeny which in turn suggests that the "ry^+" progeny from *mei-9* mothers are qualitatively different from those from control or *mei-218* mothers. Indeed, as shown below, many of these "ry^+" progeny are *ry^+/ry—ry/ry* mosaics. These experiments were begun using a 1.6-fold higher concentration of purine; at this dose, recovery of ry^+ progeny from control and *mei-218* was unaffected (these data are included in Table 1) but no ry^+ progeny were recovered from *mei-9^a* or *mei-9^b* out of 244,000 progeny (purine data excluded from Table 1). For *mei-9* alleles, therefore, the frequencies of ry^+ progeny from the total data are underestimates.

Types of Events at the *rosy* Locus. ry^+ strands that retain the parental configuration of the two closest flanking markers

Table 1. Frequencies of ry^+ progeny

	Progeny, no. × 10^{-5}			ry^+, no.			ry^+, frequency × 10^5		
Mutant	Water	Purine	Total	Water	Purine	Total	Water	Purine	Total
y (control)	1.61	23.1	24.8	4	66	70	2.5	2.9	2.8
mei-218	0.27	3.7	4.0	0	20	20	—	5.4	5.0
mei-9^a	0.21	1.1	1.3	1	2	3	4.7	1.8	2.2
mei-9^b	0.23	2.0	2.2	1	3	4	4.3	1.5	1.8
mei-9^a/9^b	0.39	5.3	5.7	4	15	19	10.3	2.8	3.3
Σ*mei-9*	0.83	8.5	9.3	6	20	26	7.2	2.4	2.8

Table 2. Recombination and disjunction data from water-control counts

Mutant	*kar-e* recombination: Total progeny	Map	% control	X nondisjunction: Total progeny	Gametic frequency, %
y (control)	15,959	18.0	100.0	160,586	0.1
mei-218	2,911	1.6	8.9	27,467	28.6
mei-9^a	3,041	1.2	6.7	21,253	25.8
mei-9^b	3,978	3.0	16.7	23,277	19.9
mei-9^a/9^b	5,899	1.4	7.8	38,762	26.5

(*kar* and *l26*) are assumed for simplicity to have arisen by nonreciprocal events and are referred to as (gene) convertants; those that are accompanied by the crossover configuration of these flanking markers are referred to as crossover events without prejudice regarding the exact nature of the events at, or between, the rosy sites monitored. These conventions are necessary to avoid proliferating terminology needlessly.

The numbers of events of the various types at the *ry* locus are presented in Table 3; ry^+ chromatids that had a crossover in the adjacent *l26-e* interval are presented separately from those that did not (no crossovers were observed in the other adjacent interval, *cu-kar*).

In the control, 40% of the events at the rosy locus are associated with crossing-over of flanking markers; the remaining 60% are parental for flanking markers and hence are gene convertants. Of the gene convertants, 3/4 are convertants of the ry^{41} allele, 1/4 of the ry^5 allele. All crossovers have the flanking marker combination *kar l26*$^+$, confirming the order *kar* ry^5 ry^{41} *l26*. These observations are in good agreement with the more extensive observations of Chovnick, Ballantyne, and Holm (20) on these two alleles.

The proportion of events at the *rosy* locus that are associated with recombination of flanking markers is reduced in *mei-218* as well as all *mei-9* combinations relative to the control. This is consistent with the observation that they reduce crossover events throughout the genome. Although a few more such crossovers were recovered than expected from the general effects of the mutants on crossing-over, the number of cases is as yet too small to deem significant.

The proportion of conversion events that affect ry^5 vs. ry^{41} is more nearly equal both for *mei-218* and for *mei-9* combinations than for the control. Although in no case do the relative numbers of the two types of convertants differ significantly from the control (2 × 2 contingency tests, $P > 0.05$), the trend is suggestive.

Completeness of Events at the *rosy* Locus. PMS is a characteristic attribute of intragenic recombination events in fungi and it is generally accepted that PMS results from failure to correct recombinationally generated biparental heteroduplex DNA prior to the first round of postmeiotic DNA synthesis. In flies, PMS will produce mosaic embryos that will, in turn, produce mosaic adults. Since only a fraction of the nuclei at blastoderm are progenitors of adult structures, the composition of individual mosaic flies is expected to range from nearly all of one component to nearly all of the other with a mean of 50%.

Mosaic progeny that involve at least one ry^+ component can be detected in three ways in these experiments: (*i*) by transmitting two distinct types of maternally derived chromatids to their progeny (this will detect only a small fraction of mosaic flies); (*ii*) by transmitting only a rosy maternal chromatid with unambiguous evidence of rosy$^+$ somatic tissue (this should detect nearly one-half of mosaic flies); and (*iii*) by having, after sectioning and staining, gross amounts of both stained (ry^+) and unstained (*ry*) tissue (this will detect the majority of mosaics).

All of the 67 ry^+ flies from control that were successfully progeny tested transmitted ry^+ as the sole maternal chromosome, and all of the 50 that were successfully sectioned and stained were apparently wholly ry^+; consequently, in agreement with the more extensive data of Chovnick and co-workers (see ref. 20), postmeiotic segregation is at best a very rare occurrence in wild-type *D. melanogaster*.

None of the ry^+ progeny from *mei-218* mothers appeared to be mosaic: all 19 that yielded progeny transmitted only ry^+ and all 15 that were successfully sectioned and stained were apparently wholly ry^+.

Strikingly, however, many of the ry^+ progeny from *mei-9* mothers are unambiguously *ry*//+ mosaics and must therefore have developed from a maternally contributed *ry*/+ heteroduplex. All three combinations of *mei-9* alleles tested gave mosaics; for simplicity, the data in Table 4 are summed over *mei-9* genotype.

One of the 18 fertile ry^+ progeny transmitted two maternal rosy alleles; the rosy and the rosy$^+$ chromatids were alike at all other sites, so the presumptive heteroduplex was restricted to (a part of) the rosy locus. The abdomen of this fly contained both XanDHase$^+$ and XanDHase$^-$ tissue. Six of the fertile ry^+ progeny transmitted only a rosy maternal chromosome; all of these had XanDHase$^-$ malphigian tubules and XanDHase$^+$ tissue in the head, thorax, or both. The remaining 11 fertile progeny transmitted only a ry^+ maternal chromosome and 8 progeny were sterile. Nine of these 19 flies are also judged to be mosaic since they had extensive staining and nonstaining internal tissue.

The numbers of nonstaining regions per ry^+ fly are most conveniently displayed graphically (Fig. 1). ry^+ flies from controls show a unimodal distribution with the mode at zero to one neg-

Table 3. Numbers of ry^+ flies classified by type of generating event as determined by flanking markers

Mutant	*l26-e* interval	rosy interval: Convertant ry^5	Convertant ry^{41}	Crossover (*kar* +)	Crossover (+ *l26*)	Total	Frequency,* no. × 10^5: rosy convertants	rosy crossovers
y (control)	NCO	9(+1)	25(+4)	25	0	72†		
	CO	0	5	3	0		1.3–**1.8**–2.4	0.7–**1.0**–1.5
mei-218	NCO	6	10(+2)	3	0	21†	2.4–**4.3**–6.6	0.2–**0.7**–2.0
mei-9^a	NCO	1	2	0	0	3		
mei-9^b	NCO	0	2	(+1)	1	4		
mei-9^a/9^b	NCO	5(+3)	4(+4)	2	1	19		
Σ*mei-9*: Total	NCO	9	12	3	2	26	1.4–**2.3**–3.4	0.1–**0.3**–0.9
: Water	NCO	3	3	0	0	6	3.1–**7.2**–15.4	**0** –4.0

Values in parentheses indicate genotypes deduced only from the phenotype of the original fly.
* Results represent means and Poisson 95% confidence intervals (from ref. 19).
† Totals include two (for control) and one (for *mei-218*) ry^+ progeny that came from a nonstandard experiment not included in Table 1.

Table 4. Flanking-marker–mosaic distributions of ry^+ flies from *mei-9*

Status	Transmitted	Conversion ry^5	Conversion ry^{41}	Crossover (*kar* +)	Crossover (+ *l26*)
Nonmosaic	ry^+	2	4	1	
	Sterile	2	1		
Mosaic	ry^+	0	2	1	1
	ry	3	2	0	1
	ry/ry^+	1			
	Sterile	1	3	1	

ative regions per fly (mean = 0.2 regions per fly). ry^+ flies from *mei-9* show a bimodal distribution, with one mode at zero to one regions and the second at six to seven. (One sterile fly with no peripheral fat is excluded from this figure; it had XanDHase$^-$ eyes and XanDHase$^+$ malphigian tubules.) Both comparison with control and calculations of Poisson distributions indicate that flies with two or more negative regions are mosaic whereas those with one or fewer are probably not mosaic. There are therefore 16 mosaics among the 26 ry^+ flies from *mei-9* (i.e., 61%).

Mosaics are distributed across flanking marker combinations (Table 4) except that both flies with the reverse crossover configuration of flanking marker (*kar*$^+$ *l26*, discussed below) were mosaic.

DISCUSSION

The majority of the gene conversion events occurring in the presence of *mei-9* demonstrably give rise to PMS and must therefore have involved unrepaired heteroduplex at the rosy locus whereas no events in control meioses generate PMS. This must be due to the *mei-9* defect itself since two independently isolated mutant alleles and their heterozygote all behave alike. Since *mei-9* is known also to be repair defective in somatic tissues (14), the *mei-9*$^+$ function is involved in both somatic and meiotic repair systems. The high frequency of persistent heteroduplexes is, however, remarkable, since some 6–8 *days* elapse between the time of meiotic recombination and the time of fertilization and zygotic replication. Either there are no other efficient heteroduplex repair systems expressed in the oocyte or else these heteroduplexes are inaccessible to them.

That these heteroduplexes persist in the presence of the repair-defective *mei-9* mutants strongly suggests that they were present, but repaired, in wild type (15, 3). This in turn implies that meiotic gene conversion in *Drosophila* occurs primarily, if not exclusively, by a process that involves extensive biparental DNA—e.g., Meselson–Radding heteroduplex-type models (21) rather than Stahl sex-circle type models (22).

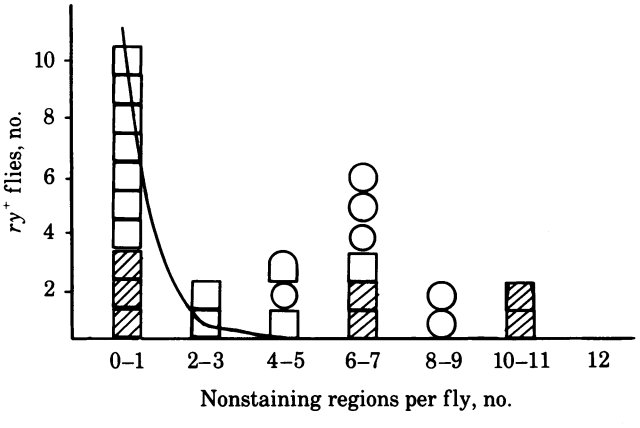

FIG. 1. Number of regions that did not show XanDHase activity per ry^+ fly (discounting anterior malphigian tubules). Symbols = progeny from *mei-9* (□, transmitted ry^+; ○, transmitted ry; ▨, sterile); curve = progeny from control normalized to 10 progeny with zero to one nonstaining segments.

If the only recombinational difference between *mei-9* and wild type is the frequency with which heteroduplex is converted to homoduplex, then conversion events should be more frequent in *mei-9* than in wild type because those events that would, in wild type, have been repaired to homoduplex rosy mutant (and therefore be undetectable) will remain heteroduplex and detectable in *mei-9*; if heteroduplexes involving either of the two rosy alleles examined are equally likely to be repaired to + or to ry, then the increase should be 60%. The data are consistent with an increase of at least this much among convertants not subject to purine selection (Table 3: observed, 6; expected, 1.5). This is a minimum estimate because recognition and testing of potential convertants depend on phenotypically ry^+ eye color. Although the eyes do not have to be ry^+ themselves to be phenotypically ry^+ (rosy is nonautonomous), nevertheless the genotypic ry^+ tissue in a mosaic must be a part of the body that expresses XanDHase in order to result in ry^+ eyes. Consequently, although there is at least a twofold increase in the frequency of detectable conversion events in *mei-9* relative to control, this value is likely to be an underestimate.

The recombinational phenotype of *mei-9* involves two differences from wild type—decrease in correction of heteroduplex and decrease in crossing-over (reciprocal events). These two attributes either represent two different types of function for the *mei-9*$^+$ product or else they are causally related. The most straightforward causal relationship is the hypothesis that the functioning of the *mei-9*$^+$ product in the correction of mismatch is integral to the transition of an event from one that can resolve only as gene conversion (i.e., without recombination of flanking markers) to one that can resolve as a crossover (isomerization step on the Meselson–Radding model). Analogous evidence for the involvement of repair in the isomerization decision has been found in fungi (23) in that the frequency of flanking marker exchange is much lower among asci with asymmetrical PMS (5:3) than it is in 6:2 asci.

Important support for this hypothesis is provided by the two *kar*$^+$ *l26* reverse crossovers detected here from *mei-9*. The only way to generate a rosy$^+$ strand with this combination of flanking markers by a single recombination event is to have had a heteroduplex that included both rosy mutant sites (Fig. 2) followed by correction of only one of the mismatches (generating 5 +/+ + or + +/+ 41) or of both mismatches but off opposite strands (generating + +/+ +). Such ry^+ reverse crossovers have never been observed in wild type, implying either that such extensive heteroduplex is very rare or that correction rarely occurs off opposite strands (correction of both sites off the same strand will not generate ry^+) or both. Both reverse crossovers were recovered as mosaics, implying that only one of the two mismatches was repaired; this repair was, however, associated with a crossover, strengthening the correlation be-

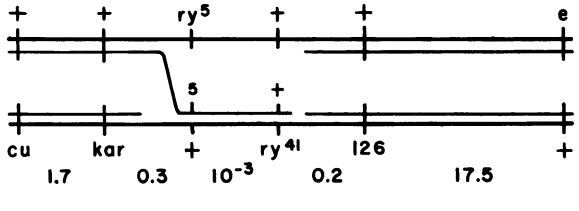

FIG. 2. One of several specific heteroduplex configurations that can generate reverse crossovers. Map distances (not to scale) are indicated below.

tween repair and crossing-over (isomerization).

Although repair of heteroduplex or an equivalent metabolic event may be necessary for isomerization, it is not sufficient. In wild type, conversion events do not show PMS; they have therefore undergone repair of heteroduplex without isomerization. Moreover, *mei-218*, which is repair competent both in the soma (5, 6) and in meiosis (no PMS), does not decrease the frequency of recombination events as measured by the frequency of gene conversion but nevertheless drastically decreases the frequency of crossovers.

All current molecular models for recombination have been designed not only to generate gene conversion events with and without exchange as alternative outcomes of one series of steps but also to account for the relative probability of the two outcomes; since the two outcomes are on average approximately equally likely in many systems, the differentiating step (isomerization) has (not unreasonably) been assumed to be a nonenzymatic reaction that randomly occurs at sites where recombination is in progress. That this view is too simple for meiotic recombination in *Drosophila* (and therefore probably for other eukaryotes as well) was suggested by the observation that *mei-9* does not alter the frequency, distribution, or morphology of spherical recombination nodules although it drastically decreases the frequency of exchange (9). Since spherical recombination nodules correspond exactly with exchange events in wild type, the most reasonable interpretation of this observation in *mei-9* is that spherical recombination nodules are present at recombinational sites *before* the molecular event(s) that differentiates exchange from nonexchange outcomes has occurred. In wild type, their presence predetermines the outcomes to be exchange events; in *mei-9*, this outcome is prevented. This requires that the differentiating step (presence or absence of isomerization) be predetermined (9).

What, then, is the outcome of an event that has been predetermined to exchange (has a spherical nodule) but is prevented from executing that predetermination by the *mei-9* defect? It is likely that these events as well as those predetermined not to undergo exchange are recovered as convertants, but a numerical argument is confounded by two variables. First, some *ry*/+ heteroduplex is presumably corrected to *ry*/*ry* homoduplex in wild type. The probability of this event is not known for the *ry* alleles monitored, so the contribution to (mosaic) convertants in *mei-9* from this source cannot be calculated. Second, the effect of *mei-9* on the average length of biparental (heteroduplex) DNA is not known; since the probability that the monitored sites are included in heteroduplex is a function of heteroduplex length, the possibility that this may be affecting conversion frequencies in *mei-9* cannot yet be eliminated. However, it is clear that most events that would have resulted in exchange tetrads in wild type result in nonexchange tetrads in *mei-9* rather than in incomplete recombination events. Incomplete events should produce either damaged chromosomes or incomplete exchanges. Recombinationally damaged chromosomes should be zygotic lethals; although *mei-9* reduces female fertility, this is at least primarily due to the increased frequencies of nondisjunction that result from the increased frequency of nonexchange tetrads (7). Incomplete exchanges should produce anaphase I bridges; however, distributive disjunction of nonexchange chromosomes is frequent in *mei-9* (7).

Although the recombinational defect in *mei-9* appears to occur relatively late in the process (being a reduced ability to execute the command to exchange), the defective step in *mei-218* appears to be earlier (reduced ability to make the command) because *mei-218* does drastically affect spherical recombination nodules. The most straightforward interpretation of the observation that *mei-218* reduces only the frequency of ry^+ crossovers and not the overall frequency of ry^+ events is that the proportion of conversion events that would have been chosen for exchange in wild type yield conversion without exchange in *mei-218*; this assumes no effect of *mei-218* on heteroduplex length, an issue for which there are as yet no data. *mei-218* is known not to make "faulty" exchanges for the same reasons adduced for *mei-9*.

The various aspects of the phenotypes of these mutants suggest the following model for the recombination process in *Drosophila* females. Recombination events begin at a number of sites along the euchromatic portions of the bivalent arms; both the observations on ellipsoidal recombination nodules (24) and the residual exchange in precondition-defective mutants (7) suggest that these events are randomly distributed both along and between bivalents. These events by themselves can resolve only as gene conversion without exchange. The generation of exchange capability at such sites involves at least $mei\text{-}218^+$ and presumably also the wild-type alleles of all other known precondition loci. This (assembling a sperical recombination nodule?) must be an involved process both because a large number of loci are involved and because the resulting distribution of exchange events is highly *non*random both between and along bivalents. In wild type, attainment of exchange capability at a site is followed by exchange with a probability of 1; however, this is an active process requiring at least $mei\text{-}9^+$.

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