Molecular Analysis of Recombination Events in Drosophila

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ABSTRACT

The locations of crossover junctions and gene conversion tracts, isolated in the *rosy* gene of *Drosophila melanogaster,* were determined using DNA sequencing and denaturing gradient gel electrophoresis. Frequent DNA sequence polymorphisms between the parental genes served as unselected genetic markers. All conversion tracts were continuous, and half of the reciprocal crossover events had conversion tracts at the crossover junction. These experiments have also identified the sequence polymorphisms responsible for altered gene expression in two naturally occurring *rosy* variants.

THE mechanisms of recombination have been inferred, for the most part, from examination of the products of recombination events (for reviews, see FOGEL, MORTIMER and LUSNAK 1981; WHITEHOUSE 1982; ORR-WEAVER and SZOSTAK 1985; HASTINGS 1987). The models for the process have become more sophisticated as the resolution of genetic mapping has increased. The more closely spaced the markers used in a cross, the more apparent are gene conversions, and the use of multiple markers has permitted a measurement of the length of DNA involved in a recombination event. Most of our knowledge of recombination in eukaryotes has come from studies in yeast and other fungi, primarily because of selective systems that permit the recovery of recombinants, and because all products of a single meiosis are packaged together.

Drosophila is unique among higher eukaryotes in the resolution available for the study of recombination. There are selective systems, for the products of genes such as *maroon-like* and *rosy,* that allow recovery of recombinants between very close intragenic markers (FINNERTY, DUCK and CHOVNICK 1970; CHOVNICK *et al.* 1970). It is possible to recover two of the four meiotic products, that is, a half-tetrad, by using "compound" chromosomes, in which the two copies of one chromosome arm are attached to the same centromere. An additional feature has emerged as the techniques of nucleic acid molecular biology have been applied to the mapping: Drosophila strains are quite polymorphic. At loci such as *rosy* and *Adh* there is typically from 0.5 to 1% sequence mismatch between strains (BENDER *et al.* 1983; BENDER, SPIERER and HOGNESS 1983; KREITMAN 1983; LEE *et al.* 1987). All of these sequence polymorphisms are potential markers in a cross, so that it should be possible to describe recombinant chromosomes with greater resolution than has yet been possible in other systems.

In earlier experiments, designed to map genetic sites known to affect *rosy* gene expression, we recovered pairs of chromosomes from half-tetrads that represented meiotic recombinants within the *rosy* locus (CLARK *et al.* 1984). We have now used DNA sequencing and denaturing gradient gel electrophoresis to define the variant sites, and to study the regions of recombination.

MATERIALS AND METHODS

Genetic procedures: The isolation of the recombinant chromosomes and their genetic characterization has been previously described (CLARK *et al.* 1984).

Molecular cloning: Genomic DNA was isolated from stocks carrying a recombinant chromosome over a chromosome with a deficiency for the *rosy* gene, *Df(3R)ry*^{36}. DNA was prepared by a rapid "miniprep" method (BENDER, SPIERER and HOGNESS 1983) with the addition of a phenol/chloroform extraction step, and was digested to completion with *Hin*dIII. The digested DNA was size fractionated by velocity centrifugation through 5–30% sucrose gradients, ligated into *Hin*dIII cut, phosphatase treated, pEMBL 9 plasmid vector DNA (DENTE, CESARANI and CORTESE 1983), and transformed into competent bacteria (HANAHAN 1983) of strain KH802. Colony lifts were probed with gel purified ^{32}P-labeled *rosy Hin*dIII fragment. To verify that no errors had been made in cloning, we compared denaturing gradient gel patterns of the genomic and cloned DNA for each *rosy* gene cloned.

DNA sequencing: Plasmids carrying *rosy* inserts were transformed into bacterial strain JM105 and single stranded template DNA was prepared by superinfection with helper phage strain M13K07 (VIEIRA and MESSING 1987). Sequencing was carried out by the dideoxy chain termination method of SANGER, NICKLEN and COULSEN (1977), using the Sequenase protocol (U.S. Biochemical Corp.), and T7 DNA polymerase kindly provided by Dr. Stan Tabor. The genes were sequenced on one strand using gene-specific oligonucleotide primers spaced at 200–400-bp intervals.

Denaturing gradient gel electrophoresis: The procedures were based on those of FISCHER and LERMAN (1983), and will be presented in detail elsewhere (M. GRAY and W. BENDER, unpublished results). The gels used were approxi-

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mately 15 × 17 cm in size and 0.75 mm thick, and consisted of 6.5% acrylamide in 1 × TAE buffer (40 mM Tris, 20 mM NaOAc, 1 mM EDTA, pH 7.4), with a gradient of urea and formamide concentration increasing from top to bottom. Two solutions were prepared, an "80% denaturant" solution (6.5% acrylamide, 1 × TAE, 32% (v/v) formamide, 5.6 M urea), and a "20% denaturant" solution, (6.5% acrylamide, 1 × TAE, 8% (v/v) formamide, 1.4 M urea). The gels were poured using these solutions and a gradient maker to produce a linear gradient from 20% to 80% denaturant concentration. Genomic DNA samples were digested to completion with the restriction enzymes *Hae*III, *Alu*I, *Hha*I, *Msp*I or *Rsa*I. The samples (3 μg/lane) were electrophoresed at 75 V for 17.3 h, with the gels submerged in a buffer tank maintained at 60° with buffer circulation. After electrophoresis, the gels were treated with 0.5 M NaOH for 5 min, followed by 0.5 M Tris (pH 8.0) for 5 min, and then allowed to equilibrate for 10 min in transfer buffer (20 mM Tris (pH 8.0), 1 mM EDTA). The DNA was then electrotransferred to nylon membrane (Nytran, Schleicher & Schuell), and the blots were hybridized with radiolabeled *rosy* DNA probes.

RESULTS AND DISCUSSION

Genetic mapping experiment: Many ry^+ isoalleles have been isolated from laboratory strains of Drosophila, and among these, two show a quantitative difference in the production of the enzyme xanthine dehydrogenase (XDH), the product of the *rosy* gene. ry^{+4} produces twofold more, and ry^{+10} twofold less XDH protein and poly(A)$^+$ mRNA than other wild-type strains such as ry^{+0}, ry^{+2}, and ry^{+5} (CHOVNICK *et al.* 1976; MCCARRON *et al.* 1979; CLARK *et al.* 1984). The ry^{+4} overproduction is due primarily to increased synthesis of XDH in one tissue, the fat body, while in ry^{+10} the underproduction is common to at least two tissues, the fat body and the Malpighian tubules (CLARK *et al.* 1984). Sites responsible for these differences have been defined by genetic mapping; the site in ry^{+4} is called *i409H* (High), and the site in ry^{+10} is called *i1005L* (Low). The corresponding alleles in strains producing normal levels of XDH are designated *N*.

A half-tetrad recombination experiment was designed in order to map the sites responsible for differential expression of these two alleles. The construction of the stocks, the selection protocol, and the recovery of the recombinants has been described (CLARK *et al.* 1984). Figure 1a diagrams the right arm of the compound third chromosome used in the selection experiment. Flies with this genotype produce a low amount of XDH (25% of wild type) and die on purine-supplemented medium. However, rare recombination events within the *rosy* gene will generate flies that produce more XDH, and consequently survive the selection. One such recombinant, a reciprocal crossover between *i1005L* and *i409H*, is illustrated in Figure 1b. Other possibilities include crossovers between *i409H* and ry^{406}, conversions of *i1005L* to *i1005N*, conversions of *i409N* to *i409H*, and conversions of ry^{406} to ry^+.

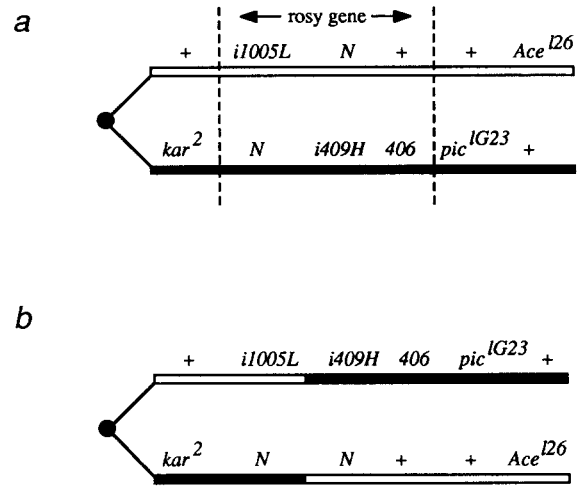

FIGURE 1.—**a,** Schematic representation of the compound 3R chromosome used in the genetic recombination experiment. Both right arms of the third chromosome are attached to the same centromere. The arm deriving from the ry^{+10} strain is colored white, and the arm deriving from ry^{+4} is black; ry^{406} is a null *rosy* point mutation on the ry^{+4} background. Relative positions of the markers are not drawn to scale; the rosy gene spans about 0.005 cM, while the genetic distance between *kar* and *Ace* is 0.5 cM. **b,** An example of a recombinant between *i1005L* and *i409H*. A fly carrying this recombinant half-tetrad will survive the purine selection.

Crossover and conversion events were operationally distinguished by the behavior of the closely linked flanking markers. ry^+ recombinants were classified as crossovers when accompanied by the exchange of flanking markers, while those without associated flanking marker exchange were classified as gene convertants. Gene conversion is a nonreciprocal process, by which information on one chromatid is lost and replaced by the corresponding information on another chromatid. To demonstrate this phenomenon unequivocally requires tetrad analysis, where conversion is observed as nonmendelian segregation during meiosis: a heterozygote for a marker gene (+/−) gives rise to progeny with the marker ratio 3+:1− or 1+:3−, rather than the expected 2+:2−. We believe, from the extensive free strand and half-tetrad experimental data (reviewed in HILLIKER and CHOVNICK 1981), that the noncrossover exchange events observed in Drosophila correspond to the nonreciprocal conversion events seen in fungi. In the gene conversion half-tetrads recovered in this experiment we cannot determine if we have recovered the donor chromatid as well as the recipient. However, in the case of crossovers, the heterozygous lethal mutations distal to the *rosy* locus (Figure 1) ensured that only half-tetrads carrying both products of the exchange would be recovered (CLARK *et al.* 1984).

From a total of 1.75×10^6 tested progeny, 20 recombinant survivors were recovered, including examples of each of the expected classes (CLARK *et al.* 1984). Nine of the 20 were reciprocal crossovers, while the other 11 were conversion events retaining the parental flanking marker configuration. The com-

pound chromosomes were detached, and balanced stocks of the individual members of each pair were established. Some recombinant chromosomes carry a ry^+ gene, and for these the quantity of XDH protein produced was determined directly. For the chromosomes still carrying the ry^{406} mutation, the amount of XDH synthesized by the recombinant transcriptional control region could not be assayed. A ry^+ protein coding region was therefore reintroduced into some of these through a second round of recombination against ry^{606} (CLARK *et al.* 1984). Two chromosomes derived from this second experiment, labeled #2 and #6, were included in our sample. We describe here the molecular analysis of 18 of the 20 original recombinant half-tetrads.

Molecular characterization of recombinant chromosomes: In order to define molecular positions for the genetic sites affecting XDH expression, we needed to sequence a subset of the recombinant *rosy* alleles. We cloned a 7.3-kb *Hin*dIII fragment containing the *rosy* gene from each of 23 individual recombinant chromosomes. All nine crossover half-tetrads were included. For eight of these we cloned both members of the half-tetrad (chromosomes #10–#25, Figure 2), and the ninth was represented by the chromosome #1 and two derivatives of its reciprocal arm, #2 and #6 (described above). For three gene conversion half-tetrads we cloned only the member of the compound pair which had been converted (#3–#5). We also included a recombinant recovered from a previous free stand recombination experiment. This recombinant (*#26*) is a conversion of *i1005L* to *i1005N* against a *ry* mutant chromosome derived from the ry^{+11} background (MCCARRON *et al.* 1979). We also isolated the *Hin*dIII fragment from the three parental chromosomes: kar^2 ry^{606} Ace^{126}, ry^{+10}, and ry^{+4}. The latter two alleles had previously been recovered from bacteriophage lambda libraries (LEE *et al.* 1987, and our unpublished results). Using a set of oligonucleotide primers specific to the *rosy* gene, we sequenced from 2 to 5 kb from each of the 26 genes. Figure 2 illustrates the extents of the genes sequenced and the patterns of polymorphisms found in each. The sequence of the ry^{+5} allele (LEE *et al.* 1987, and this work) serves as the baseline, and only the nucleotide positions at which the alleles differ from each other or from the baseline sequence are indicated.

We examined both arms of an additional six half-tetrads (pairs #27–#32) carrying conversions of ry^{406} to ry^+ (CLARK *et al.* 1984) using both southern blotting, as described (CLARK, HILLIKER and CHOVNICK 1988), and denaturing gradient gel electrophoresis (FISCHER and LERMAN 1983). The latter technique has recently been adapted for the purpose of detecting single nucleotide point mutations in genomic restriction digests of Drosophila DNA, and will be described in detail elsewhere (M. GRAY and W. BENDER, unpublished results). Because the melting point of a fragment depends on its nucleotide sequence, as little as a single base pair change in the sequence can shift the position of the fragment on the gradient gel. This technique allowed us to detect many more of the polymorphisms between the parental strains than was possible by scoring restriction fragment length polymorphisms on nondenaturing gels.

Figure 3a shows an example of a denaturing gradient gel of *Hha*I digested genomic DNA from five of these half-tetrad pairs and the two parental strains, electroblotted to nylon membrane, and probed with the 7.3-kb *rosy* *Hin*dIII fragment. Nearly every fragment shows a melting difference between the ry^{406} and ry^{+10} parental strains. By comparing the fragment patterns of the recombinants to those of the parents, the lengths of conversion intervals were determined to within a few hundred nucleotides (Figure 3b). The analysis was repeated using four other enzymes: *Hae*III, *Alu*I, *Rsa*I and *Msp*I (not shown), and the extents of the six conversion tracts, based on the combined data, are shown (Figure 3d). The precision in measurement of conversion tract lengths by gradient gels approached that obtained by sequencing.

We are confident that we have analyzed the entire interval of each of the recombination events in the sample, and that each represents a single isolated event. This is based on the following: first, the genetically determined flanking marker configurations are consistent with the directions of exchanges that we see at the molecular level. Second, we see no evidence on denaturing gradient gels for additional exchange events within the 7.3-kb *Hin*dIII fragment for any of the recombinants. And third, the low overall frequency of recombination at *rosy*, coupled with the established phenomenon in Drosophila of positive interference, in which a crossover event inhibits additional crossovers in adjacent intervals on the same chromosome arm (HILLIKER and CHOVNICK 1981), argues against the possibility of additional, incidental recombination in the interval.

Variant strain regulatory sites: The recombinant chromosomes that proved useful in locating the sites of *i1005L* and *i409H* are diagrammed in Figure 2b. Chromosome pairs #1/#2 and #24/#25 were isolated as crossover half-tetrads. #2 also carries an independent conversion of ry^{406} to ry^+, recovered in the second round of recombination against ry^{606}. #3, #4, #5, #6 and #26 are simple conversion events. The conversion tract in #4 extends beyond the end of the cloned *Hin*dIII fragment, but we determined by denaturing gradient gels that the conversion tract ends less than 1 kb to the left of the *Hin*dIII site (not shown).

Based on their XDH overproduction phenotypes (Figure 2d) (CLARK *et al.* 1984), #2, #25, #3 and #6 each carry *i409H*. The only ry^{+4}-specific polymorphism common to all four of these recombinants is

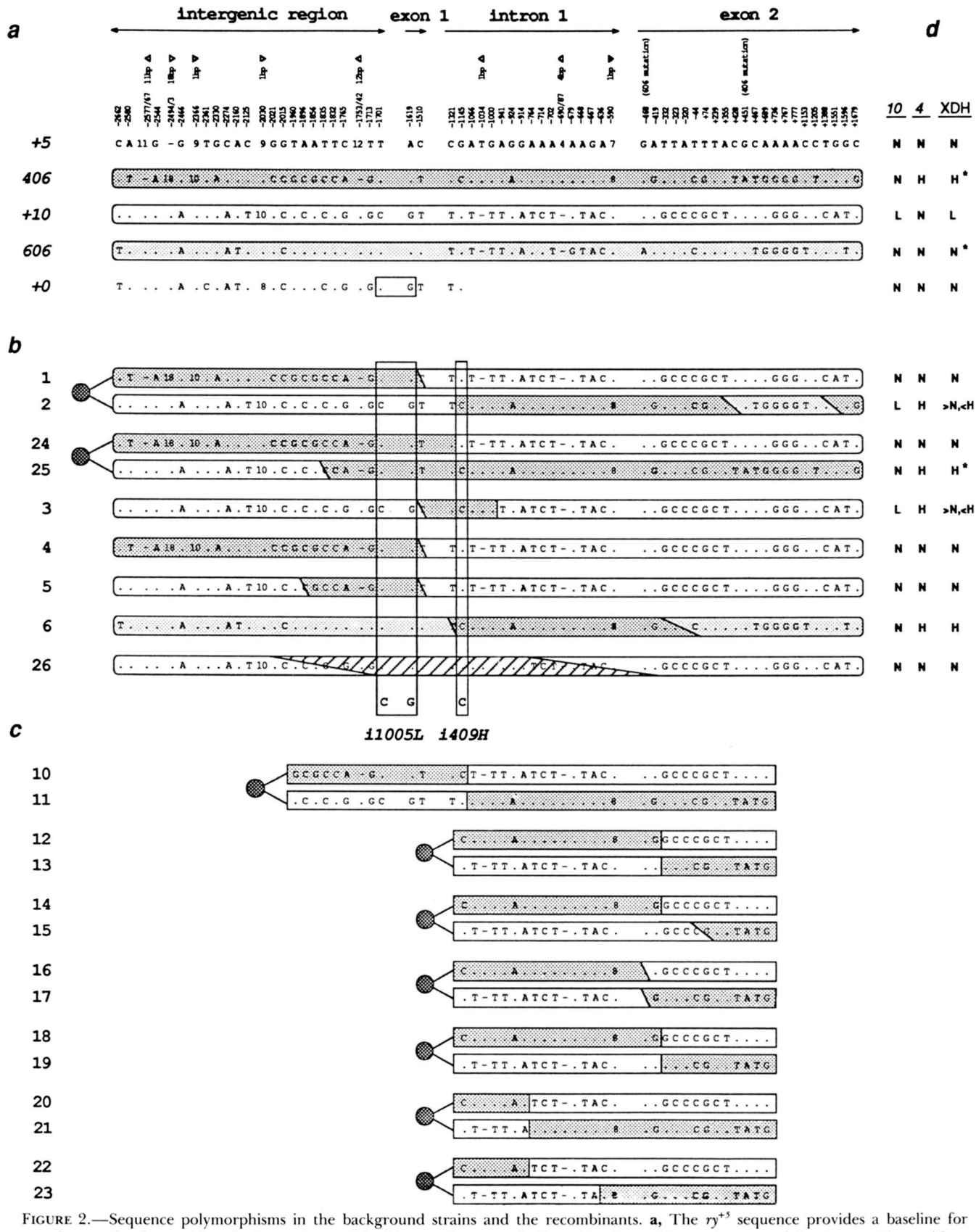

FIGURE 2.—Sequence polymorphisms in the background strains and the recombinants. **a,** The ry^{+5} sequence provides a baseline for comparison, and polymorphisms in the strains used in this experiment are indicated by the position and nature of the change. The positions of the polymorphisms with respect to the intron/exon structure of *rosy* are indicated along the top of the figure. "Intergenic region" refers to the space between *rosy* and the next gene to the left (centromere-proximal), *l(3)S12.* A dot indicates that a sequence is identical to the baseline sequence at a position, a letter indicates a nucleotide substitution, and a number or a dash indicates an insertion (▽) or deletion (△) of the indicated number of base pairs. Polymorphisms indicated by numbers at positions −2366, −2030 and −590 are length differences

the single guanine to cytosine transversion at −1145, located in the first intron of the gene (Figure 2). We conclude that this single nucleotide change is *i409H*, and that it is sufficient to bring about the increase in XDH production found in the fat body of ry^{+4} animals. This polymorphism is located within the limits of the 0.5-kb deletion of intron sequences in a *rosy* mutant $sd^{[ry2216-547]}$ (DANIELS *et al.* 1985), which reduces fat body expression of XDH (DUTTON and CHOVNICK 1988).

Two recombinants, #2 and #3, express XDH at a level greater than normal, but less than the overproducer ry^{+4}, and are genotypically *i1005L i409H* (CLARK *et al.* 1984). Four other recombinants, the #24/#25 pair, #4, #5, and #26 are convertant for *i1005L* to *i1005N*. Correlating the ry^{+10} specific polymorphisms with the phenotypes of these recombinants, we can restrict the sites for *i1005L* to only two possibilities, nucleotides −1701 and −1619 (Figure 2). The start site for *rosy* transcription is at approximately −1645, based on primer extension data and cDNA 5′ endpoints (our unpublished results). This places the −1701 polymorphism just 5′ of the transcription start site, and the −1619 polymorphism just within the transcribed region.

We sequenced this region from several other wild-type alleles which had been characterized (CHOVNICK *et al.* 1976) as normal level XDH producers. The sequence polymorphisms for 1.6 kb of one of these, ry^{+0}, are given in Figure 2a. ry^{+0} (and also two other normal level XDH producing strains, ry^{+2} and *Okanogon #31*, data not shown), shares the −1619 polymorphism with ry^{+10}, but it does not share the −1701 polymorphism. This suggests that *i1005L* consists of the single polymorphism at −1701.

In summary, at most two nucleotides at the 5′ end of the gene are responsible for the general underexpression found in the ry^{+10} strain, and probably only one of them, the thymidine to cytosine transition at −1701, corresponds to *i1005L*. Although the *rosy* gene does not have any easily recognized promoter sequence motifs, such as the TATA box (BUCHER and TRIFONOV 1986), which would be altered by this polymorphism, we expect that *i1005L* might influence the rate of transcriptional initiation at the *rosy* promoter. Further *in vitro* mutagenesis experiments will investigate the full sizes and functions of the *cis*-acting sequences identified by the *i1005L* and *i409H* polymorphisms.

Product of recombination: The large number of polymorphisms serving as unselected markers in these experiments allows us to observe the nature and size of conversion tracts, and to see conversion tracts associated with crossover junctions. In fungi, complex recombination events are sometimes observed. These include independent or partial mismatch repair in a single heteroduplex tract, crossover points separated from converted markers by unconverted markers, and events involving three or four chromatids (FOGEL *et al.* 1979; KALOGEROPOULOS and ROSSIGNOL 1980; BORTS and HABER 1987; SYMINGTON and PETES 1988). However, none of the events in this Drosophila sample were complex. We have examined ten gene conversion events at the sequence level, and an additional six using denaturing gradient gels. Fourteen of these conversion tracts include three or more markers, and all 14 are completely continuous. If conversion results from the repair of heteroduplex DNA mismatches, we do not see "patchy" repair.

We examined both arms of the half-tetrads for eight simple conversions using denaturing gradient gels (half-tetrads #27–#32, #4 and #5; Figure 3, and not shown). Although in these events the reciprocal arm was only one of two potential nonsister chromatid donors, we saw no changes in the reciprocal arms. We thus see no evidence for symmetric heteroduplex in generating these events, since symmetric heteroduplex formation, followed by independent repair of the

of one nucleotide in homonucleotide runs. The sequence of the 18-bp insertion in ry^{+4} at −2494/3 is: AATGAGCAGTAGAAATCT. The sequences of the mutant genes ry^{406} and ry^{606} are given; these each differ from their respective background gene sequences, ry^{+4} and ry^{+6}, by a single nucleotide substitution (M. GRAY, unpublished results, and this work). The positions of the two mutations, +451 and −468, respectively, are indicated; both mutations cause glycine to glutamic acid amino acid substitutions in the XDH polypeptide. The polymorphisms of a portion of the ry^{+0} gene are presented, and the box encloses the region of the *i1005L* site as delimited by the recombination mapping. The full sequence of the *rosy* gene has been published (LEE *et al.* 1987; KEITH *et al.* 1987); nucleotides −2920 to 0 in that sequence were derived from a ry^{+5} gene, and nucleotides 0 to +4624 from a Canton-S gene. The ry^{+5} and Canton-S sequences from 0 to +1679 differ in some positions; Canton-S is identical to ry^{+10} in this interval. Part of the sequence of the ry^{+10} gene was previously published (LEE *et al.* 1987). Four errors in that sequence are corrected here: differences at positions −2030 and −702 were not previously noted, while positions −1620 and −440 do not differ, as previously reported, from ry^{+5}. **b,** Recombinant chromosomes which define the molecular sites of *i1005L* and *i409H*. The shadings inside the horizontal boxes indicates parental identity of the polymorphisms: ry^{406} is darkly shaded, ry^{+10} is unshaded, and ry^{606} is intermediate, as in part **a** above. A diagonal line through a polymorphism indicates that that position is uninformative for the particular genes involved. Members of original half-tetrads, for example #1 and #2, are joined by the schematic centromere linkage at the left. #3, #4, #5, #6 and #26 are simple gene conversion events. The limits of the conversion segment of #26 were determined by comparison to the genomic sequence of the ry^{+11} allele (not shown). **c,** The sequences of the half-tetrad pairs in which a reciprocal crossover occurred between *i409H* and ry^{406}. **d,** XDH expression phenotype of the genes and the deduced genotypes. The column headed XDH gives the level of expression of XDH protein (from CLARK *et al.* 1984) as H(igh), N(ormal), or L(ow). (*) indicates that the expression level was determined for the corresponding wild-type genes, or, in the case of #25, for a derivative in which the ry^{406} site was converted to ry^{+} against a ry^{606} chromosome. Under the columns labeled *10* and *4* are genotypes for the sites *i1005L* and *i409H*, respectively, as deduced from the expression phenotypes.

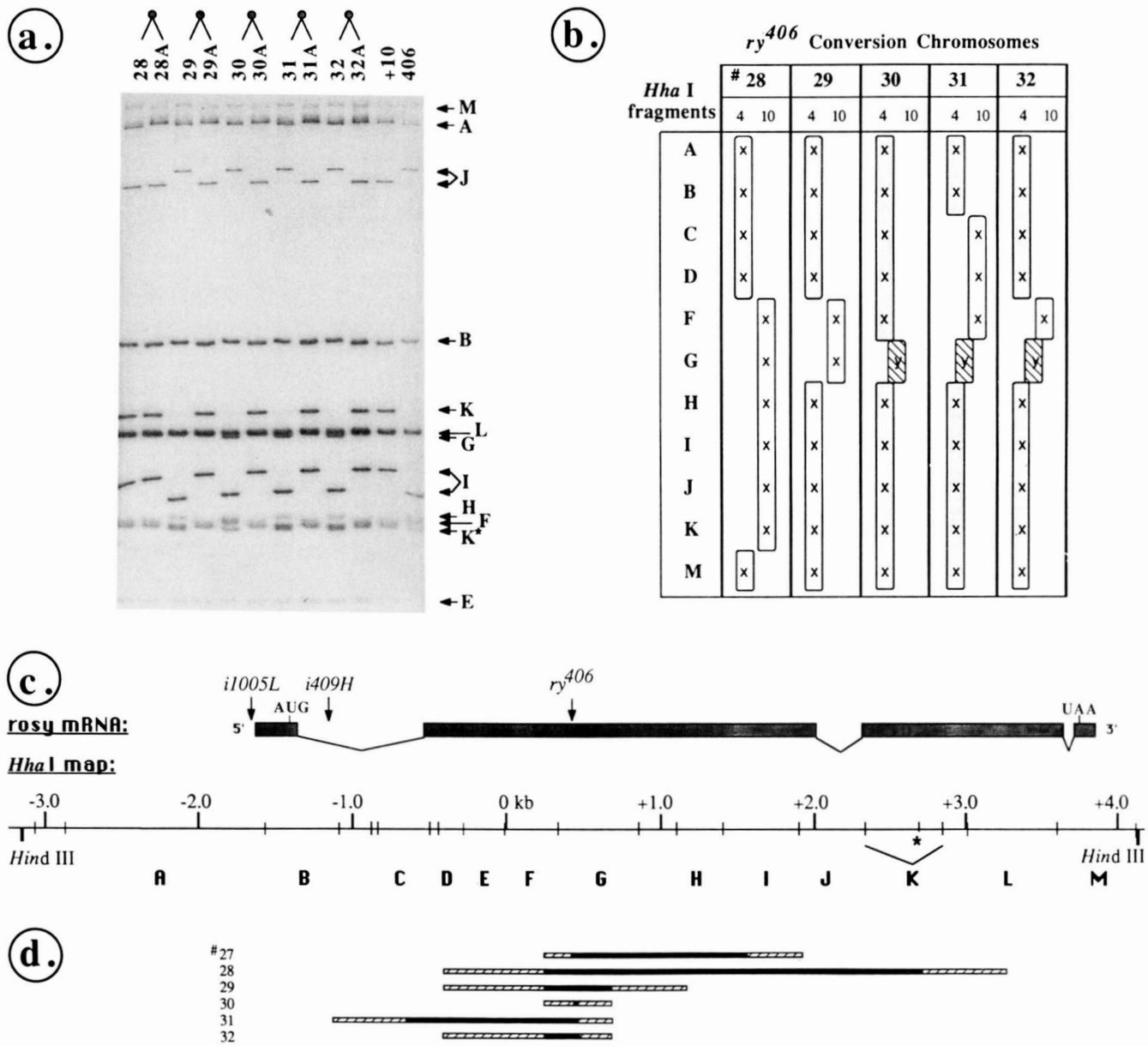

FIGURE 3.—Mapping of conversion intervals using denaturing gradient gel electrophoresis. **a,** A denaturing gradient gel of genomic DNA digested with *Hha*I, blotted to nylon membrane, and probed with the 7.3-kb *Hin*dIII fragment. Each pair, for example #28 and #28A, are the members of an original recombinant half-tetrad characterized as a conversion of ry^{406} to ry^{+}. The A denotes the unconverted, $ry^{+10}Ace^{126}$ members of the half-tetrads. Bands are labeled by the letter designations indicated on the restriction map in part **c.** All of the bands on the blot except E, G and L (G and L ran together here) show melting point differences between the two parental strains, ry^{406} and ry^{+10}. The band labeled K* is present in ry^{406}, instead of K, due to the gain of the restriction site indicated by * in part **c. b,** Schematic representation of the results of the blot shown in **a.** An "x" under the 4 or 10 column indicates that a chromosome exhibits melting behavior of the ry^{406} or ry^{+10} parental chromosome, respectively, for that fragment. A "y" shows that the conversion chromosome exhibits a non-parental melting type, indicating that the endpoint of the conversion tract lies between polymorphisms within the fragment. Shaded boxes represent the ry^{406} parental chromosome strand, and white boxes ry^{+10}. Only one member of each half-tetrad is diagrammed, since none of the other members (labeled A in part **a**) show evidence of being changed in the event. **c,** The *rosy* transcriptional unit is shown relative to the restriction map. The locations of the *i1005L, i409H,* and ry^{406} sites (Figure 2) are indicated. *Hha*I sites are indicated by unlabeled vertical bars, and the *Hha*I fragments visible on the gel in **a** are lettered. Fragments C and D are visible upon longer exposure of the same blot. **d,** Extents of the conversion tracts in the six events in which ry^{406} was converted to ry^{+}. Endpoints were determined from denaturing gradient gel analysis repeated with a total of five enzymes. Black bars indicate the minimum conversion tract length, and the hatched regions extend to the maximum length.

two tracts, could have led to reciprocal conversions.

Nine crossover half-tetrads were sequenced; in the four cases in which a conversion tract was associated with the crossover, the tracts were not separated from the crossover. In one experiment in Drosophila, putative convertant classes that might have resulted from complex events at *rosy* have been reported (CHOVNICK *et al.* 1970; BALLANTYNE and CHOVNICK 1971). This stands in contrast to the large number of experiments which have monitored several markers within the *rosy* gene, as well as flanking markers, and which have provided no evidence for multiple events (MC-

TABLE 1

Conversion tract lengths

Chromosome #	Lengths (bp) Min – Max	Midpoint
a. Conversions without crossover, unrestricted:		
27	1100 – 1684	1392
28	2468 – 3679	3074
29	450 – 1622	1036
30	1 – 448	224
31	1119 – 1832	1476
32	213 – 1106	660
		Mean: 1310
b. Conversions without crossover:		
2	778 – 1521	1150
3	322 – 677	500
4	1512 – 2782	2147
5	238 – 638	438
6	727 – 1583	1155
26	788 – 1697	1243
		Mean: 1106
c. Conversions associated with crossovers:		
1/2	1 – 473	237
14/15	12 – 657	335
22/23	97 – 278	188
24/25	512 – 710	611
		Mean: 343
d. Crossover without associated conversion:		
10/11	0 – 78	
12/13	0 – 86	
16/17	0 – 170	
18/19	0 – 86	
20/21	0 – 149	

The lengths of the conversion tracts illustrated in Figures 2 and 3 are listed. Part a gives the lengths of tracts determined by denaturing gradient gels, while all other lengths, excepting the left end of the interval in #4, were determined by sequencing. Part d of the table lists the spacing between polymorphisms flanking the recombination points in the crossovers which did not show conversion.

CARRON, GELBART and CHOVNICK 1974; GELBART *et al.* 1974; GELBART, MCCARRON and CHOVNICK 1976; HILLIKER, CLARK and CHOVNICK 1988). From the previous results and from those reported here, we conclude that such complex events are very rare in Drosophila.

Conversion tract length: The lengths of all 16 conversion tracts examined are given in Table 1. The six events in Table 1a, conversions of the *406* site, were unselected in length. In the six events in Table 1b, conversions of *i1005L* or *606,* the conversion tracts could not have extended so far as to cover the *406* site (as far as the *606* site in the case of #2). The lengths are thus not a completely unbiased sampling of conversion events, since some long tracts might have been lost. Nevertheless, the average lengths of the events in Table 1, a and b, are not significantly different. When grouped, the average length of these 12 noncrossover associated intervals is 1208 bp (standard deviation ± 790 bp). Another bias which probably affected this sample is that the length of a conversion tract would influence the likelihood of its recovery in the selection. Long tracts were more likely to cover the selected site, and be recovered in the selection, than short ones. We can adjust the lengths of the noncrossover associated conversion tracts to represent unweighted values; this adjustment gives an average tract length of 752 bp.[2] The previous estimate of an average conversion tract length at *rosy* of 400 bp was based on co-conversion events (HILLIKER, CLARK and CHOVNICK 1988). The present results provide a more accurate figure because of uncertainty in the molecular locations of markers used to derive the earlier estimate. Other factors such as strong initiator sites or polarity of recombination events could also bias the lengths of conversion events recovered, but previous results (HILLIKER and CHOVNICK 1981; CLARK, HILLIKER and CHOVNICK 1988), and examination of the endpoints of the events in this study (Figures 2 and 3) provide no evidence for such phenomena at *rosy*. Measures of average meiotic conversion tract lengths in yeast are similar to this figure, and, depending on the genetic interval monitored, range from a minimum of 0.7 kb to over 2 kb (BORTS and HABER 1987; JUDD and PETES 1988, SYMINGTON and PETES 1988).

The average length of the crossover-associated conversion tracts is 343 bp (standard deviation ± 189 bp, Table 1c). There is little or no experimental bias on these tract lengths, since these crossover events were recovered regardless of presence or length of the associated conversion. When we compare the lengths of conversion tracts associated with crossing over (343 bp), to those not associated with crossing over (1208 bp), the difference is significant. However, it is more appropriate to use the unweighted length figure in making the comparison between the two classes. We cannot use simple statistical tests to compare the crossover associated average (343 bp) to the unweighted average (752 bp), and given the small sample number, we can only say that the data are suggestive of a size difference. This contrasts with recent data from experiments in yeast which indicate an equivalence in conversion tract lengths with or without associated crossing over (BORTS and HABER 1989).

Association of gene conversion and crossing over: Gene conversion and crossing over are associated (HURST, FOGEL and MORTIMER 1972), and are envisioned in many recombination models as alternate outcomes of a single underlying process. Most models of recombination mechanisms involve formation of a Holliday junction (MESELSON and RADDING 1975; SZOSTAK *et al.* 1983). This structure can be resolved as either a crossover or non-crossover, depending on the choice of strands which are cleaved and religated. An implication of the models is that, since heteroduplex and/or gap (SZOSTAK *et al.* 1983) formation precedes the resolution step, the lengths of conversion

[2] The unweighted mean (harmonic mean) X was calculated from the actual experimental lengths w_i, with N = number of events in the sample, by the following formula: $X = N/\sum 1/w_i$.

tracts resulting from repair of the heteroduplex, or gapped, DNA should be independent of how the Holliday junction is resolved. A length difference such as suggested by our data could imply a mechanistic distinction between recombination events resolved as crossovers versus those resolved as conversions, as has been suggested in other contexts (CARPENTER 1984, 1987; SMITHIES and POWERS 1986). It will be important to confirm this suggestion, and a larger sample size, as well as a variety of parental allele combinations will be necessary to determine if tract lengths in Drosophila are consistently shorter when associated with crossing over. Our results clearly do not support the hypothesis that only longer heteroduplex tracts can be substrates for crossing over.

Previous genetic results in Drosophila (SMITH, FINNERTY and CHOVNICK 1970; CHOVNICK, BALLANTYNE and HOLM 1971; CLARK *et al.* 1984) led us to anticipate conversion at crossover sites, and we did see conversion of polymorphisms adjacent to the crossover in four of nine pairs sequenced. There are at least two possible reasons for the lack of conversion in the other five cases. One is that there was no heteroduplex tract, or gap generated during the event which could have resulted in conversion, or there was such a tract but it was too short to detect. The distances between informative polymorphisms flanking these five crossovers range from 78 to 170 bp (Table 1d), and conversion tracts shorter than this would be invisible. Alternatively, a region of heteroduplex was generated, but it was restored back to the parental configuration. If all crossovers are in fact associated with the generation of heteroduplex DNA, and if there is no bias in the direction of repair of mismatches, then 50% of the heteroduplexes should be repaired back to the parental arrangement. This is consistent with the frequency that we find in our sample, and with results from studies on yeast (BORTS and HABER 1987, SYMINGTON and PETES 1988). Alternative models have been proposed. CARPENTER (1987) postulates separate pathways and separate roles in meiosis for gene conversion and crossing over, based on electron microscope studies of recombination nodules, and on mutations which affect the spectrum of recombination events observed at *rosy*. ROSSIGNOL *et al.* (1984), in their genetic studies of recombination in Ascobolus, note that crossing over may arise by two pathways. Crossover events with certain allele combinations apparently occur in the absence of conversion or heteroduplex DNA formation.

The principal evidence requiring the inclusion of heteroduplex DNA formation in all models of recombination is the phenomenon of postmeiotic segregation (PMS). Although common for certain alleles and at certain genetic loci in the fungi (WHITE, LUSNAK and FOGEL 1985; reviewed in ORR-WEAVER and SZOSTAK 1985; HASTINGS 1987), PMS is normally very rare in Drosophila (CHOVNICK, BALLANTYNE and HOLM 1971). However, in the presence of recombination-defective mutations at the *mei-9* locus, PMS is frequently observed at *rosy* in conjunction with both conversions and crossovers (CARPENTER 1982). This result suggests that a heteroduplex DNA intermediate is normally involved in recombination in Drosophila, and the *mei-9* mutants are defective in the recognition and repair of mismatched bases (CARPENTER 1982). Our data are consistent with models in which heteroduplex DNA is generated during the process of recombination, and mismatches in a heteroduplex segment are efficiently corrected as a continuous block.

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LITERATURE CITED

BALLANTYNE, G. H., and A. CHOVNICK, 1971 Gene conversion in higher organisms: non-reciprocal recombination events at the rosy cistron in *Drosophila melanogaster*. Genet. Res. **17:** 139–149.

BENDER, W., M. AKAM, F. KARCH, P. A. BEACHY, M. PEIFER, P. SPIERER, E. B. LEWIS and D. S. HOGNESS, 1983 Molecular genetics of the bithorax complex in *Drosophila melanogaster*. Science **221:** 23–29.

BENDER, W., P. SPIERER and D. S. HOGNESS, 1983 Chromosomal walking and jumping to isolate DNA from the Ace and rosy loci and the Bithorax complex in *Drosophila melanogaster*. J. Mol. Biol. **168:** 17–33.

BORTS, R. H., and J. E. HABER, 1987 Meiotic recombination in yeast: alteration by multiple heterozygosities. Science **237:** 1459–1465.

BORTS, R. H., and J. E. HABER, 1989 Length and distribution of meiotic gene conversion tracts and crossovers in *Saccharomyces cerevisiae*. Genetics (in press).

BUCHER, P., and E. N. TRIFONOV, 1986 Compilation and analysis of eukaryotic POL II promoter sequences. Nucleic Acids Res. **14:** 10009–10026.

CARPENTER, A. T. C., 1982 Mismatch repair, gene conversion, and crossing-over in two recombination-defective mutants of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **79:** 5961–5965.

CARPENTER, A. T. C., 1984 Meiotic roles of crossing-over and of gene conversion. Cold Spring Harbor Symp. Quant. Biol. **49:** 23–29.

CARPENTER, A. T. C., 1987 Gene conversion, recombination nodules, and the initiation of meiotic synapsis. BioEssays **6:** 232–236.

CLARK, S. H., S. DANIELS, C. A. RUSHLOW, A. J. HILLIKER and A. CHOVNICK, 1984 Tissue-specific and pretranslational character of variants of the rosy locus control element in *Drosophila melanogaster*. Genetics **108:** 953–968.

CLARK, S. H., A. J. HILLIKER and A. CHOVNICK, 1988 Recombination can initiate and terminate at a large number of sites within the *rosy* locus of *Drosophila melanogaster*. Genetics **118:** 261–268.

CHOVNICK, A., G. H. BALLANTYNE and D. G. HOLM, 1971 Studies on gene conversion and its relationship to linked exchange in *Drosophila melanogaster*. Genetics **69:** 179–209.

CHOVNICK, A., G. H. BALLANTYNE, D. L. BAILLIE and D. G. HOLM, 1970 Gene conversion in higher organisms: Half-tetrad analy-

sis of recombination within the rosy cistron of *Drosophila melanogaster*. Genetics **66:** 315–329.

CHOVNICK, A., W. GELBART, M. MCCARRON, B. OSMOND, E. P. M. CANDIDO and D. L. BAILLIE, 1976 Organization of the rosy locus in *Drosophila melanogaster*: evidence for a control element adjacent to the xanthine dehydrogenase structural element. Genetics **84:** 233–255.

DANIELS, S. B., M. MCCARRON, C. LOVE and A. CHOVNICK, 1985 Dysgenesis-induced instability of rosy locus transformation in *Drosophila melanogaster*: analysis of excision events and the selective recovery of control element deletions. Genetics **109:** 95–117.

DENTE, L., G. CESARANI and R. CORTESE, 1983 pEMBL: a new family of single stranded plasmids. Nucleic Acids Res. **11:** 1645–1655.

DUTTON, F. L., JR., and A. CHOVNICK, 1988 Chap. 10: Developmental regulation of the *rosy* locus in *Drosophila melanogaster*, pp. 267–316 in *Developmental Biology*, Vol. 5, edited by L. W. BROWDER. Plenum, New York.

FINNERTY, V. G., P. DUCK and A. CHOVNICK, 1970 Studies on genetic organization in higher organisms. II. Complementation and fine structure of the maroon-like locus of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **65:** 939–946.

FISCHER, S., and L. LERMAN, 1983 DNA fragments differing by single base-pair substitutions are separated in denaturing gradient gels: correspondence with melting theory. Proc. Natl. Acad. Sci. USA **80:** 1579–1583.

FOGEL, S., R. MORTIMER and K. LUSNAK, 1981 Mechanisms of meiotic gene conversion, or "wanderings on a foreign strand," pp. 289–339 in *The Molecular Biology of the Yeast Saccharomyces*, Vol. 1, edited by J. STRATHERN, E. JONES and J. BROACH. Cold Spring Harbor Labororatory, Cold Spring Harbor, N.Y.

FOGEL, S., R. MORTIMER, K. LUSNAK and F. TAVARES, 1979 Meiotic gene conversion: a signal of the basic recombination event in yeast. Cold Spring Harbor Symp. Quant. Biol. **43:** 1325–1341.

GELBART, W., M. MCCARRON and A. CHOVNICK, 1976 Extension of the limits of the XDH structural element in *Drosophila melanogaster*. Genetics **84:** 211–232.

GELBART, W., M. MCCARRON, J. PANDEY and A. CHOVNICK, 1974 Genetic limits of the xanthine dehydrogenase structural element within the rosy locus in *Drosophila melanogaster*. Genetics **78:** 869–886.

HANAHAN, D., 1983 Studies on transformation of *Escherichia coli* with plasmids. J. Mol. Biol. **166:** 557–580.

HASTINGS, P. J., 1987 Chap. 4: Meiotic recombination interpreted as heteroduplex correction, and Chap. 5: Models of heteroduplex formation, pp. 107–156 in *Meiosis*, edited by P. B. MOENS. Academic Press, Orlando, Fla.

HILLIKER, A. J., and A. CHOVNICK, 1981 Further observations on intragenic recombination in *Drosophila melanogaster*. Genet. Res. **38:** 281–296.

HILLIKER, A. J., S. H. CLARK and A. CHOVNICK, 1988 Genetic analysis of intragenic recombination in *Drosophila*, pp. 73–90 in *The Recombination of Genetic Material*, edited by K. B. LOW. Academic Press, San Diego, Calif.

HURST, D. D., S. FOGEL and R. K. MORTIMER, 1972 Conversion-associated recombination in yeast. Proc. Natl. Acad. Sci. USA **69:** 101–105.

JUDD, S. R., and T. D. PETES, 1988 Physical lengths of meiotic and mitotic gene conversion tracts in *Saccharomyces cerevisiae*. Genetics **118:** 401–410.

KEITH, T. P., M. A. RILEY, M. KREITMAN, R. C. LEWONTIN, D. CURTIS and G. CHAMBERS, 1987 Sequence of the structural gene for xanthine dehydrogenase (*rosy* locus) in *Drosophila melanogaster*). Genetics **116:** 67–73.

KALOGEROPOULOS, A., and J.-L. ROSSIGNOL, 1980 Evidence for independent mismatch corrections along the same hybrid DNA tract during meiotic recombination in *Ascobolus*. Heredity **45:** 263–270.

KREITMAN, M., 1983 Nucleotide polymorphism at the alcohol dehydrogenase locus of *Drosophila melanogaster*. Nature **304,** 412–417.

LEE, C. S., D. CURTIS, M. MCCARRON, C. LOVE, M. GRAY, W. BENDER and A. CHOVNICK, 1987 Mutations affecting expression of the *rosy* locus in *Drosophila melanogaster*. Genetics **116:** 55–66.

MCCARRON, M., W. GELBART and A. CHOVNICK, 1974 Intracistonic mapping of electrophoretic sites in *Drosophila melanogaster*: fidelity of information transfer by gene conversion. Genetics **76:** 289–299.

MCCARRON, M., J. O'DONNELL, A. CHOVNICK, B. S. BHULLAR, J. HEWITT and E. P. M. CANDIDO, 1979 Organization of the rosy locus in *Drosophila melanogaster*: further evidence in support of a cis-acting control element adjacent to the xanthine dehydrogenase structural element. Genetics **91:** 275–293.

MESELSON, M. S., and C. M. RADDING, 1975 A general model for genetic recombination. Proc. Natl. Acad. Sci. USA **72:** 358–361.

ORR-WEAVER, T. L., and J. W. SZOSTAK, 1985 Fungal recombination. Microbiol. Rev. **49:** 33–58.

ROSSIGNOL, J.-L., A. NICOLAS, H. HAMZA and T. LANGIN, 1984 Origins of gene conversion and reciprocal exchange in *Ascobolus*. Cold Spring Harbor Symp. Quant. Biol. **49:** 13–21.

SANGER, F. S., S. NICKLEN and A. R. COULSON, 1977 DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74,** 5463–5467.

SMITH, P. D., V. G. FINNERTY and A. CHOVNICK, 1970 Gene conversion in *Drosophila*: non-reciprocal events at the maroon-like cistron. Nature **228:** 442–444.

SMITHIES, O., and P. A. POWERS, 1986 Gene conversions and their relation to homologous chromosome pairing. Phil. Trans. R. Soc. Lond. B **312:** 291–302.

SYMINGTON, L. S., and T. D. PETES, 1988 Expansions and contractions of the genetic map relative to the physical map of yeast chromosome III. Mol. Cell. Biol. **8:** 595–604.

SZOSTAK, J. W., T. L. ORR-WEAVER, R. J. ROTHSTEIN and F. W. STAHL, 1983 The double-strand-break repair model for recombination. Cell **33:** 25–35.

VIEIRA, J., and J. MESSING, 1987 Production of single-stranded plasmid DNA. Methods Enzymol. **153:** 3.

WHITE, J. H., K. LUSNAK and S. FOGEL, 1985 Mismatch-specific post-meiotic segregation frequency in yeast suggests a heteroduplex recombination intermediate. Nature **315:** 350–352.

WHITEHOUSE, H., 1982 *Genetic Recombination: Understanding the Mechanisms*. John Wiley & Sons, New York.

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