THE EFFECT OF *mei-41* ON rDNA REDUNDANCY IN *DROSOPHILA MELANOGASTER*

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ABSTRACT

The recombination and repair defective mutant, *mei-41*, exhibits three rather striking effects on the genetic properties and chromosomal stability of rDNA in Drosophila. First, *mei-41* inhibits rDNA magnification. However, *mei-9*, another recombination and repair defective mutation has no similar effect. This indicates that magnification requires some, but not all, of the gene products necessary for meiotic exchange. Second, under magnifying conditions, *mei-41* induces interchanges between the X rDNA and either arm of the Ybb^- chromosome. These interchanges occur at high frequency and are independent of rDNA orientation. Third, in *mei-41* bb^+/Ybb^+ males, bobbed mutants in the X, but not the Y, also arise at high frequency. Evidence suggests that these events involve the rDNA type I insertion. The recombination and repair defective properties of *mei-41* together with our results regarding its unusual and specific effects involving rDNA are explained in a simple model that has general implications for chromosome structure.

ONE of the more curious characteristics of the eukaryotic genome is that it contains substantial amounts of tandemly redundant sequences, whereas prokaryotes have virtually none. This difference in genome structure is even more vividly illustrated by the fact that when a tandemly repeated gene cluster from a eukaryote is placed in a prokaryote by recombinant DNA techniques (BRUTLAG *et al.* 1977), or when a segment of the bacterial chromosome is tandemly duplicated by more conventional genetic methods (ANDERSON and ROTH, 1977), the redundancy is quite unstable and is eventually eliminated. In contrast, tandemly repeated sequences in eukaryotes generally exhibit remarkable quantitative and qualitative stability, although situations do exist in which certain repeated sequences are quantitatively altered (for reviews see TARTOF 1975 and LONG and DAWID 1980). These observations suggest that in eukaryotes there are genes that function both to maintain normal redundancy and to alter that redundancy when required. In this report we describe a mutational analysis of the control of rRNA gene redundancy in *Drosophila melanogaster*.

In genetically normal Drosophila males there are two clusters of tandemly repeated rRNA genes (rDNA), each with approximately 225 copies. One of these arrays is located in the proximal heterochromatin of the X chromosome and the other on the short arm of the Y chromosome. Partial deficiencies at either cluster are known as bobbed (*bb*) mutants. Although rDNA redundancy is

generally quite stable (TARTOF 1974), alterations in X chromosomal rDNA redundancy occur in males carrying the aberrant Ybb^- chromosome. These changes in rDNA redundancy may be observed as either stable reversions from *bb* to bb^+ (magnification; RITOSSA 1968) or as mutations from bb^+ to *bb* or from *bb* to $bb^{\prime}$ (reduction; TARTOF 1974). It has been suggested that both magnification and reduction are the result of unequal sister-chromatid exchanges occurring at the X chromosomal *bb* locus in the germ line of X/Ybb^- males (TARTOF 1974).

We have examined the effects of the DNA repair and recombination defective mutations *mei-9* and *mei-41* on the process of rDNA magnification. Both mutations render flies hypersensitive to a wide variety of mutagens, increase the frequency of spontaneous chromosome aberrations in somatic cells and reduce the level of meiotic exchange (BAKER *et al.* 1976; BAKER, CARPENTER and RIPOLL 1978; GATTI 1979, and BAKER and CARPENTER 1972). Although no detectable effect of *mei-9* on rDNA magnification was observed, we demonstrate here three additional and unusual effects of *mei-41* on this process and on the stability of the X chromosomal rDNA. First, *mei-41* dramatically inhibits rDNA magnification. Second, in the presence of *mei-41*, and under magnifying conditions, interchange between the X rDNA and the Ybb^- chromosome occurs at high frequency. Lastly, we note that in the presence of *mei-41*, X but not Y chromosomal bb^+ to *bb* mutations occur at high frequency in the germ lines of otherwise normal males. The implications of the repair and recombination defective properties of *mei-41*, together with our results regarding its effect on rDNA redundancy, will be explained in a simple model.

MATERIALS AND METHODS

Stocks: The flies were raised at 24.5° on standard medium (TARTOF 1973). Complete descriptions of most of the mutants used in this study, except *mei-41* and *mei-9*, may be found in LINDSLEY and GRELL (1968). The pertinent chromosomes used here are: $In(1)sc^{4L}sc^{8R}$, $y\ sc^4\ sc^8\ cv\ v\ B$, (sc^4sc^8), is an inverted X chromosome completely deficient for rDNA; $Dp(1,1)sc^{V1}$,$y\cdot y^+$ is an X chromosome carrying a y^+ duplication on the right arm and will be referred to as $y\ bb^+\cdot y^+$; bb^2 arose spontaneously in this laboratory and is an X chromosomal *bb* mutant deficient for 47% of its rDNA (TARTOF 1973); bb^{41} is a very severe allele of *bb* recovered from one of our *mei-41* stocks; $bb^{+ORE\text{-}R}$ refers to the bb^+ allele from our Oregon-R wild-type stock; $bb^+\cdot y^+$ similarly refers to the bb^+ allele carried by the $y\ bb^+\cdot y^+$ chromosome; Ybb^- is a Y chromosome deficient for 80% of its rDNA (TARTOF 1973); B^SYbb^-, which carries the Ybb^- deletion on Y^S and B^S on Y^L, was constructed by D. KOMMA and obtained from Dr. SHARON ENDOW; *C(1)DX, y f* is an attached X chromosome completely deficient for rDNA; *C(1)RM, y w* bb^+ is an attached X which is bb^+; *In(1)dl-49, y Hw* $m^2\ g^4$, (*dl-49*), is a bb^+ inversion bearing X chromosome; B^SY and y^+Y are *Y* chromosomes whose long arms are marked with B^S or y^+; $In(1)w^{m4}$, w^{m4} is an inversion of the X with one breakpoint near the white gene and the other in the distal region of the rDNA locus (APPELS and HILLIKER 1982); $In(1)rst^3$, rst^3 is also an X chromosome inversion with a distal breakpoint near white and a proximal breakpoint which is just distal to *bb* (HILLIKER, APPELS AND SCHALET 1980); $In(1)sc^8$, sc^8 is an X chromosome inversion with a distal breakpoint near *sc* and a proximal breakpoint which is proximal to bb^+; $Y^SX\cdot Y^L$ is an attached $\overline{XY}$ chromosome and is referred to as $\overline{XY}$. The meiotic mutants *mei-41* and $mei\text{-}9^b$ were obtained from L. SANDLER, and $mei\text{-}41^{195}$ was obtained from A. T. C. CARPENTER and B. S. BAKER. A number of other *mei-41* alleles were obtained from P. D. SMITH. *mei-41* and *mei-9* are X-linked mutants located at 53.3 and 6.5 cM, respectively.

Statistical analysis: Statistical comparisons of mutation rates were obtained by consulting the tables of KASTENBAUM and BOWMAN (1971). Chi-square tests were conducted, using the 2 × 3 contingency tables.

Chromosome preparation: Metaphase preparations of neuroblast chromosome were obtained by dissecting third instar larvae into 45% acetic acid with 2% orcein. The tissue was fixed for 4 to 5 min and then squashed.

rDNA Restriction enzyme analysis: DNA was extracted from the appropriate flies (PROCUNIER and TARTOF 1975), digested with EcoRI and run on a 0.7% agarose gel. The DNA fragments were then transferred to a nitrocellulose filter (SOUTHERN 1975) and hybridized (TARTOF 1975) to 10^6 cpm of a DNA probe labeled with ^{32}P by nick translation to about 10^8 cpm/μg (RIGBY *et al.* 1977). The probe was a recombinant DNA known as Y22 that contains a single 11-kb Drosophila rDNA gene inserted into pMB9 (DAWID, WELLAUER and LONG 1978). After hybridization the filter was washed several times in 1 mM Tris, pH 7.4, and 0.1% SDS at room temperature, dried and exposed to Kodak AR-5 X-ray film at −70°C.

RESULTS

mei-41 inhibits rDNA magnification: rDNA magnification is defined experimentally by crossing single bb/Ybb^- males to five to ten $sc^4sc^8/dl\text{-}49$ females and scoring the X/sc^4sc^8 female progeny for *bb*. X/sc^4sc^8 progeny females that are phenotypically bb^+ are then retested by crossing to sc^4sc^8/B^SY males to insure that a stable reversion to bb^+ has occurred. As indicated in Table 1, in the presence of Ybb^- both bb^2 and bb^{41} revert to bb^+ at a high frequency (0.16 to 0.19). However, in the presence of *mei-41* the frequency of magnification for both bb^2 and bb^{41} was decreased ten- and fivefold, respectively. This effect is due to *mei-41* because another allele, $mei\text{-}41^{195}$, similarly suppresses magnification of bb^2. Thus *mei-41* defines a locus whose wild-type product is necessary for magnification. However, not all repair defective mutations define such loci because $mei\text{-}9^b$, a mutation that affects both recombination and repair, fails to have any effect on magnification. Although POLITO *et al.* (1982) have reported some impairment of magnification in males carrying $mei\text{-}9^a$, we have been unable to confirm their result.

Each magnifying (paternal) genotype was also scored according to how many bb^+ progeny it produced (0, 1 or $\geqq$ 2) as shown in Table 1. Approximately 50 to 70% of the $y\ bb^2/Ybb^-$ and ybb^{41}/Ybb^- males produce two or more bb^+ progeny. However, in the presence of *mei-41* this is reduced seven- and tenfold, respectively. A χ^2 analysis using the 2×3 contingency tables shows that the distribution of bb^+ progeny from magnifying males is significantly different compared to when *mei-41* is present ($P < 0.01$). This difference is due to the conspicuous reduction of clusters of two or more bb^+ progeny that derive from fathers carrying *mei-41*. Note that the number of males producing single bb^+ revertants remains the same, whether *mei-41* is present or absent. This indicates then, that *mei-41* exerts its effect by interfering with the initial magnifying event rather than by reducing the size of the clusters.

mei-41 promotes interchange between the X *and* Ybb^- *chromosome:* In the cross involving $mei\text{-}41^{195}\ bb^2/Ybb^-$ males, a *bobbed* sc^4sc^8 male was recovered that might be accounted for by interchange between the X and Ybb^- chromosomes (see Table 1, line 6). Because this male was sterile, and thus unavailable for further study, experiments were performed to recover such putative interchanges in females. $y\ mei\text{-}41\ bb^{+ORE\text{-}R}/Ybb^-$ males were crossed to *C(1)DX, y f/* B^SY females (Table 2A, lines 1–4), where *bb* or bb^+ exceptions could be

TABLE 1

Magnification as measured by matings of single bb/Ybb$^-$ *males to* sc^{4}sc^8/dl-49 *females and scoring of the* X/sc^{4}sc^8 *female progeny with respect to* bb

Paternal genotype	bb Phenotype of X/sc^{4}sc^8 females		Frequency of magnification[a]	No. of tested males producing 0, 1 or ≥2 bb$^+$ progeny		
	bb	bb$^+$		0	1	>2
y bb^2/Y	499	0	0.0	25	0	0
y bb^{41}/Y	216	0	0.0	20	0	0
y bb^2/Ybb$^-$	433	100	0.19	4	4	14
y bb^{41}/Ybb$^-$	107	20	0.16	5	1	6
y *mei-41* bb^2/Ybb$^-$	798	20	0.02	15	3	2
y *mei-41*^{195} bb^2/Ybb$^-$	663	23[b]	0.03	12	5	5
y *mei-41* bb^{41}/Ybb$^-$	185	5	0.03	17	3	1
y *mei-9*^{b} bb^2/Ybb$^-$	315	69	0.18	6	2	11

[a] Calculated on the number of bb$^+$ flies divided by the total number of flies examined.
[b] One bb sc^{4}sc^8 male was also recovered, see text.

recovered as y *f* non-B^S females. Similarly, y *mei-41* bb$^+$·y$^+$/Ybb$^-$ males were crossed to either *C(1)DX*, y *f*/B^SY females where bb$^+$ or bb exceptions could be recovered as y$^+$ *f* non-B^S females, (Table 2A, lines 5–10) or to *C(1)RM*, y *w* bb$^+$/Y females, where the putative interchanges could be recovered as y$^+$ *w* females regardless of their bb allele (Table 2B). Such exceptional females were recovered from all crosses involving *mei-41* bb$^+$/Ybb$^-$ males at a frequency of greater than 1.7×10^{-3}, whereas the frequency in bb$^+$/Y, bb$^+$/Ybb$^-$ or *mei-41* bb$^+$/Y controls was less than 5×10^{-4}. Frequent production of these exceptional chromosomes requires, therefore, the presence of both the *mei-41* mutation and the Ybb$^-$ chromosome.

Cytological analyses of neuroblast metaphases from females carrying *C(1)DX* and one of these exceptions reveals the presence of small acrocentric chromosomes with a nucleolus organizer flanked by two larger blocks of heterochromatin (Figure 1). The following experiments allow the identification of the centromere, rDNA and the distal heterochromatin of these aberrations.

In crosses of y *mei-41* bb$^+$·y$^+$/Ybb$^-$ males to *C(1)DX*, y *f*/B^SY females (Table 2A) all but one (see footnote *e*) of the exceptional progeny were y$^+$, so these aberrations carry at least the centromere of the paternal X chromosome. The rDNA of interchanges is also derived from the X chromosome as shown by restriction enzyme analysis of interchanges arising from y *mei-41* bb^{+ORE-R}/Ybb$^-$ males. X and Y chromosomal rDNA differ in that *Eco*RI digestion of Y rDNA yields primarily 11-kb fragments whereas X rDNA results in both 11- and 17-kb fragments (TARTOF and DAWID 1976). 17-kb fragments arise from the presence of a 5-kb insertion known as type I that occurs in the 28S coding region of the 11-kb repeat (WHITE and HOGNESS 1977). The Ybb$^-$ chromosome like the wild-type Y lacks the type I insertion (ENDOW 1982). *Eco*RI digests of DNA from *C(1)DX*/bb$^+$ females were run on a 0.7% agarose gel, transferred to a nitrocellulose filter that was then hybridized to a ^{32}P-labeled plasmid carrying the 11-kb rDNA repeat. Data for three interchanges and the parental Oregon-R strain

TABLE 2

X-Ybb$^-$ *interchange in the presence of* mei-41 *and* Ybb$^-$

A. Results of crossing males of the indicated genotype to *C(1)DX, y f/B*^{S}Y females

Paternal Genotype	Progeny			Frequency of X-Y Interchange[b] × 10^3
	B^S♂♂	*y f* ♀♀	y^+f ♀♀[a]	
bb^{+ORE-R}/y^+Y	2059	0	1683	≤0.5
bb^{+ORE-R}/Ybb^-	2320	0	0	≤0.4
y mei-41 bb^{+ORE-R}/y^+Y	4424	0	5977	≤0.2
y mei-41 bb^{+ORE-R}/Ybb^-	2875[c]	5[d]	0	1.7
y $bb^+ \cdot y^+/Y$	2825	2298	1	0.3
y $bb^+ \cdot y^+/Ybb^-$	3568	0	1	0.3
y mei-41 $bb^+ \cdot y^+/Y$	2606	2182	1	0.4
y mei-41 $bb^+ \cdot y^+/Ybb^-$	3375	1[e]	5 (2)	1.8
y mei-41^{195} $bb^+ \cdot y^+/Ybb^-$	1656	0	3[f]	1.8
y mei-41 $bb^+ \cdot y^+/B^SYbb^-$	1285	0	6 (2)[g]	4.7

B. Results of crossing males of the indicated genotype to *C(1)RM, y w* bb^+/Y females

Paternal genotype	Progeny				Frequency of X-Y[h] Interchange × 10^3
	y^+ ♂♂	*y* ♂♂	y^+ *w* ♀♀	*y w* ♀♀	
y $bb^+ \cdot y^+/Y$	2,422	0	0	2,692	≤0.4
y $bb^+ \cdot y^+/Ybb^-$	3,772	0	1	4,988	≤0.2
y mei-41 $bb^+ \cdot y^+/Y$	2,830	0	1	2,703	≤0.4
y mei-41 $bb^+ \cdot y^+/Ybb^-$	13,499	2[i]	28[j]	12,653	2.2

[a] The numbers in parentheses are the numbers of individuals that were also *bb*.

[b] Measured as the number of interchange bearing females (*y f* or y^+ *f*) divided by the number of B^s males.

[c] One *y* B^S male was also recovered. Cytological analysis revealed that this male carried a normal X chromosome that appears to have simply lost the y^+. The mechanism of this event is not understood. However, similar events have been observed in the presence of meiotic mutants in females by SANDLER and SZAUTER (1978).

[d] One of these females also carried Ybb$^-$.

[e] This female appears to be a double recombinant and is the object of further study.

[f] One female also carried B^SY in addition to the y^+ bearing interchange.

[g] Two of the bb^+ interchanges also carried B^S.

[h] Measured as the number of interchange bearing females (y^+ *w*) divided by the number of total females (*y w*).

[i] These two males were shown to carry XL.Y recombinant chromosomes that are presumed to be the reciprocal product of those exchange events that generate the YX recombinant. The low frequency of recovery of XL.Y chromosomes, when compared to that for YX chromosomes, is not understood. Zimmering (1976) has demonstrated nonrandom disjunction of heteromorphic dyads in male meiosis, so perhaps this represents an explanation.

[j] One female was a (y^+/y) mosaic some of whose progeny also carried the interchange.

are presented in Figure 2. It may be seen that these recombinants possess a restriction pattern identical to that of the paternal X chromosome having both 17 and 11-kb major repeats. Thus the interchanges are caused by breakage of the X chromosome within or distal to the rDNA.

The distal heterochromatin of these interchanges may be derived from either arm of the Y chromosome. Two of the five interchanges recovered from *y mei-41* bb^{+ORE-R}/Ybb^- males were shown to be capped by Y^S by constructing males

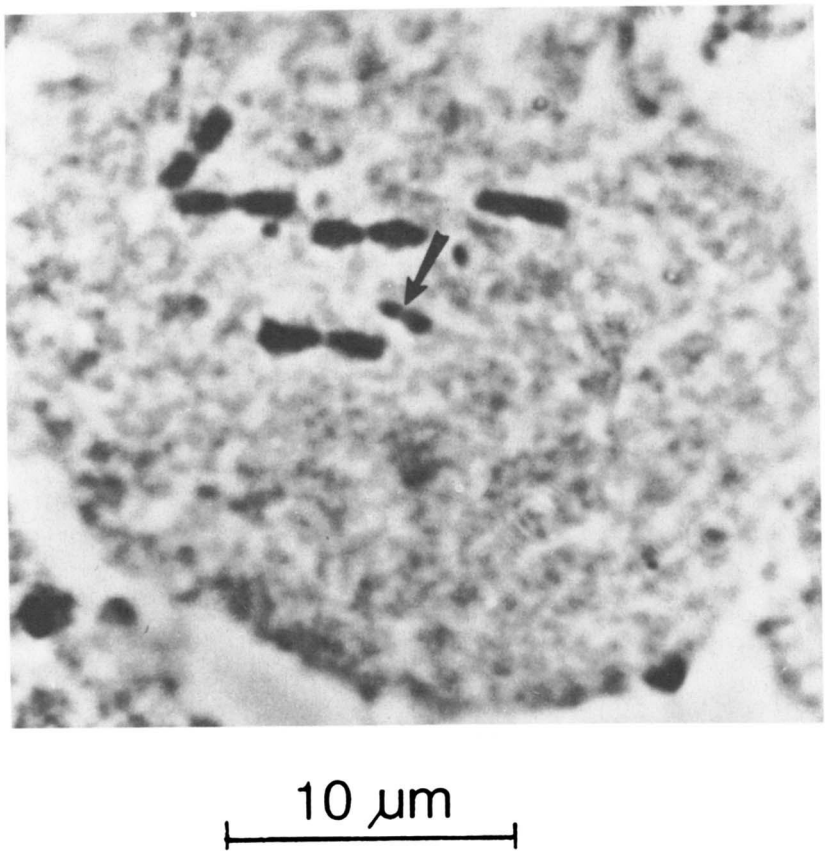

FIGURE 1.—Neuroblast metaphase from a *C(1)DX, y f*/Y^SX^R feamle. The arrow indicates the nucleolus organizer on Y^SX^R. Similar figures were observed for all of the interchanges examined.

that carried an $X \cdot Y^L$ or an $X \cdot Y^S$ chromosome and an interchange. Although none of the five interchanges restored fertility to males carrying $X \cdot Y^S$, two did complement $X \cdot Y^L$ and are designated as Y^SX^R to indicate that they carry the fertility factors of Y^S. Since the Y^S fertility factors are distal to the *bb* locus, these chromosomes are the result of an exchange or translocation event that involves the X chromosome at or distal to the *bb* locus and the Y chromosome at or proximal to *ks-1*. The remaining three interchanges did not complement either $X \cdot Y^S$ or $X \cdot Y^L$ and so their breakpoints cannot be determined unambiguously. It is likely that their breakpoints are distal to the proximal-most fertility factor on either Y^S or Y^L. That interchanges can involve Y^L is clearly demonstrated by the cross involving *y mei-41* $bb^+ \cdot y^+/B^SYbb^-$ males. In this cross two of the six interchanges recovered showed tight linkage of the y^+ duplication and the B^S marker located on Y^L. In these cases the X chromosomal material is capped by Y^L and these chromosomes are designated as Y^LX^R. The four *non-*B^S interchanges recovered in this cross complement $X \cdot Y^L$ and are therefore the result of interchange involving Y^S at a site proximal to both fertility factors. Therefore, the interchanges observed among the progeny of *mei-41/*Ybb^- males

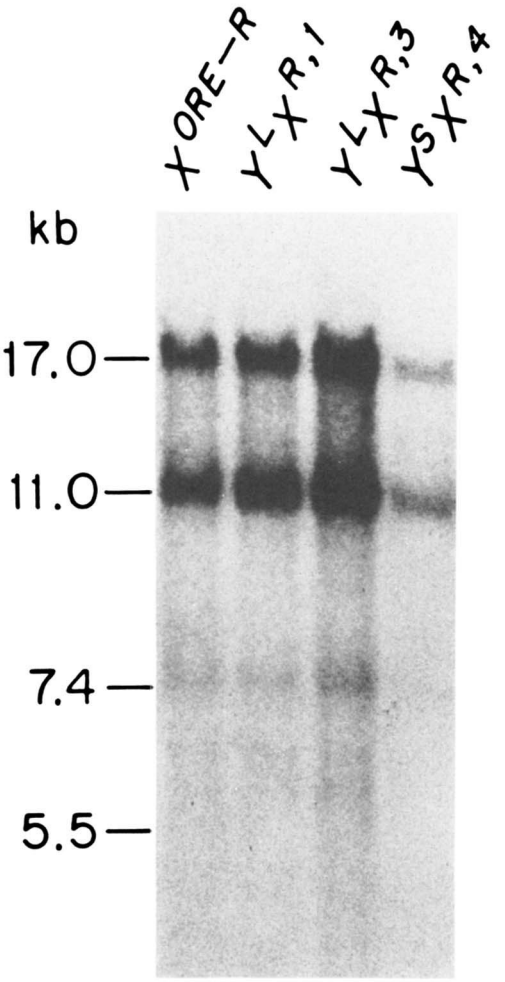

FIGURE 2.—rDNA restriction patterns from wild type and interchange bearing females. DNA from each female was digested with *Eco*RI, submitted to electrophoresis on a 0.7% agarose gel and then hybridized to a ^{32}P-labeled plasmid carrying the 11 kb rDNA repeat.

may be thought of as resulting from exchanges between the X at a site within or just distal to its rDNA and either a site(s) on Y^S that may be within or proximal to the rDNA or one or more sites on Y^L.

Finally, it should be noted that nearly all interchanges were recovered as single exceptions in a bottle and in no case were 4 or more exceptions found in the same bottle. This lack of clustering suggests, although does not prove, that these interchanges are meiotic in origin.

The X-chromosome breakpoint occurs within the rDNA: Since 4 out of 20 interchanges recovered in crosses to *C(1)DX, y f/B*^{S}Y females were *bb*, these at least have breakpoints within the X rDNA. Three other lines of evidence also indicate that most if not all of such interchanges have breakpoints within the X chromosomal rDNA.

First, to examine the effect of inverting the rDNA to a more distal position, X-Ybb^- interchange was measured in males carrying *In(1)sc*^{8} (Table 3, A and B). If interchanges involve the X rDNA regardless of its position, then such males should still produce exceptional chromosomes, although in this case one arm of the Y chromosome would be capped by some or all of the X rDNA rather than *vice versa*. It may be seen that in both experiments, *In(1)sc*^{8}, *sc*^{8} *mei-41*/Ybb^- males produced X-Ybb^- interchanges at frequencies similar to those observed for normal sequence X chromosomes. Moreover, a genetic and cytological analysis of exceptions recovered in crosses to *C(1)DX, y f*/B^SY females confirms that such interchanges carry a bb^+ or *bb* allele capping either arm of the Y chromosome (see footnotes to Table 3). Hence, even in a case in which the *bb* locus has been inverted, the site of interchange still corresponds to the region containing the rDNA.

Second, to determine whether sites distal to the *bb* locus, which would also have been inverted by *In(1)sc*^{8}, are necessary for interchange, X-Ybb^- interchange was examined in males carrying either of two inversions, *In(1)w*^{m4} or *In(1)rst*^{3} (Table 3B). Both *In(1)w*^{m4} and *In(1)rst*^{3} invert the material normally just distal to the rDNA to the tip of the X chromosome. In fact, the proximal breakpoint of *In(1)w*^{m4} lies near or within the distal region of the X rDNA (APPELS and HILLIKER 1982). As demonstrated by the data in Table 3B, X-Y interchanges were recovered from *In(1)w*^{m4}, *mei-41*/Ybb^- and *In(1)rst*^{3}, *mei-41*/Ybb^- males at frequencies similar to or greater than those observed for normal sequence X chromosomes (compare Table 2 with Table 3B). Therefore, sites normally distal to the rDNA are not necessary for interchange. It appears then, that most if not all of the X-Y interchanges resulting in bb^+ or *bb* exceptions occur very close to, and probably within, the X chromosomal rDNA.

Finally, it is possible that interchange frequently occurs proximal to the *bb* locus resulting in bb^ℓ (bobbed lethal) interchanges. To test this, 20 interchanges recovered as y^+ *w* females from crosses to *C(1)RM, y w* bb^+/*Y* females (see Table 2B) were examined for their *bb* phenotype by crossing y^+ *w* females to *sc*^{4}*sc*^{8}/B^SY males. Eleven gave *sc*^{4}*sc*^{8} sons that were bb^+, 1 gave *bb sc*^{4}*sc*^{8} male offspring, 5 produced bb^ℓ interchanges because no *sc*^{4}*sc*^{8} males were observed, and 3 were sterile. Since five of 17 bb^ℓ interchanges that do occur can be accounted for by the fact that 30% of interchanges occurring within the *bb* locus would be expected to recover fewer than 80 genes and thereby be bb^ℓ, there is no evidence to suggest interchange frequently occurs proximal to the *bb* locus.

mei-41 *induces* X *chromosomal* bb *mutants:* It had been noticed by A. T. C. CARPENTER and B. S. BAKER (personal communication) that *mei-41* stocks tended to accumulate X chromosomal *bb* mutations despite repeated replacement of mutant *bb* alleles with bb^+. In fact, among 21 separate alleles of *mei-41* that had been induced on a bb^+ X chromosome and provided to us by P. D. SMITH, 14 were *bb*. We have tested *mei-41* for its ability to mutate bb^+ in both *y mei-41* bb^+/B^SY and *y mei-41* bb^{+ORE-R}/Y males. In order to guard against pre-existing *bb* mutations, single males of these genotypes were mated to *sc*^{4}*sc*^{8}/*dl-49* females. bb^+/*sc*^{4}*sc*^{8} daughters were selected, crossed to *sc*^{4}*sc*^{8}/B^SY males and their sons used to establish stocks. Stock males were then crossed to *sc*^{4}*sc*^{8}/*dl-*

TABLE 3

X-Ybb$^-$ *interchange in males bearing* X *chromosomal inversions*

A. Results of crossing males of the indicated genotype to *C(1)RM, y w bb*$^+$/Y females

Paternal genotype	Progeny				Frequency of interchange $\times 10^{-3a}$
	y^+ ♂♂	y ♂♂	y^+ w ♀♀	y w ♀♀	
In(1)sc^8,*sc*^{8} *bb*$^+$/*B*^{S}Y	1747	—	2[b]	2246	<0.9
In(1)sc^8,*sc*^{8} *bb*/Ybb$^-$	1425	—	0	2260	<0.4
In(1)sc^8,*sc*^{8} *mei-41 bb*$^+$/*B*^{S}Y	711	—	0	1366	<0.7
In(1)sc^8,*sc*^{8} *mei-41 bb*$^+$/Ybb$^-$	4206	—	11	6640	1.7

B. Results of crossing males of the indicated genotype to *C(1)DX, y f*/*B*^{S}Y females

	Progeny[c]					Frequency of interchange $\times 10^{-3d}$
	y^+ B^S ♂♂	y f ♂♂	y f B^S ♀♀	y^+ f ♀♀	y^+ f B^S ♀♀	
In(1)sc^8,*sc*^{8} *bb*$^+$/*B*^{S}Y	6160	0	4752	0	3	0.5
In(1)sc^8,*sc*^{8} *bb*$^+$/*B*^{S}Ybb$^-$	1742	0	0	0	1	≤0.6
In(1)sc^8,*sc*^{8} *mei-41 bb*$^+$/*B*^{S}Y	3225	4[e]	3667	2[f]	3	1.5
In(1)sc^8,*sc*^{8} *mei-41 bb*$^+$/*B*^{S}Ybb$^-$	2027	0	0	4 (2)[g]	1[h]	2.5
In(1)w^{m4},*w*^{m4} *mei-41 bb*$^+$/Ybb$^-$	2513	3 (2)	—	0	—	1.2
In(1)rst^3,*rst*^{3} *mei-41 bb*$^+$/Ybb$^-$	4007	14 (2)	—	0	—	3.5

[a] Measured as the number of y^+ *w* females divided by the number of y *w* females.

[b] Both of these females also carried B^S, and test crosses revealed tight linkage between y^+ and B^S. These interchanges also were shown to restore fertility to X·YS males.

[c] The number of parentheses is the number of individuals that were also *bb*.

[d] Measured as the number of y^+ *f* and y^+ *f* B^S females divided by the number of y^+ B^S males.

[e] These females were shown to carry Y chromosomes that appear to have lost the B^S element. This phenomenon is not understood and is being investigated further.

[f] Both of these interchanges were recovered in one of the 30 bottles examined and were shown to restore fertility to X·Y^S males.

[g] All of these exceptions were shown to complement X·Y^S with respect to male fertility and thus are the result of interchange between the X and Y^L.

[h] This interchange was shown to carry both y^+ and B^S and restored fertility to X Y^S males. Thus it appears to be the result of interchange between the X and Y^S.

49 females (each bottle contained 5 males and 10 females) and the X/*sc*^{4}*sc*^{8} progeny scored for *bb*. As shown in Table 4A, y *mei-41* *bb*$^+$·y^+/B^SY males produce X chromosomal *bb* mutants at the rate of approximately 2×10^{-3}, although no such mutants were obtained in the controls ($P < 0.01$). However, only two mutations were observed among the 5693 X/*sc*^{4}*sc*^{8} female progeny of y *mei-41* *bb*$^{+ORE\text{-}R}$/Y males. This mutation rate is significantly less ($P < 0.01$) than that observed in y *mei-41* *bb*$^+$·y^+/B^SY males and is, in fact, so low as not to be significantly different from the control cross. Thus, the frequency at which *mei-41* induces bb$^+$ to *bb* mutations appears to be a characteristic of the particular *bb*$^+$ allele used. We shall return to this observation later.

To show that these induced *bb* mutants do not depend on the continued presence of *mei-41* for their *bb* phenotype, *mei-41* was removed from three independently induced *bb* mutants. In all cases the *bb* phenotype remained unchanged.

It should also be noted that the *mei-41* effect on the X *bb*$^+$ locus is unidirec-

TABLE 4

mei-41 Permits the generation of bb *mutations in* bb$^+$ *X chromosomes but not* Ybb$^+$ *chromosomes*

A. Males of the indicated genotype were crossed to $sc^4sc^8/dl\text{-}49$ females and the sc^4sc^8-bearing progeny scored with respect to *bb*

Paternal genotype	Progeny: sc^4sc^8/X females *bb*	Progeny: sc^4sc^8/X females *bb*$^+$	Progeny: sc^4sc^8/Y males *bb*	Progeny: sc^4sc^8/Y males *bb*$^+$	*bb*$^+$ to *bb* mutation frequency $\times 10^3$	Significance[a]
y bb$^+ \cdot y^+/B^S$Y	0	3660	0	1664	—	—
y mei-41 bb$^+ \cdot y^+/B^S$Y[b]	19	8455	0	2063	2.2	$P < 0.01$
y bb$^{+ORE\text{-}R}/$Y	0	3465	0	3119	—	—
y mei-41 bb$^{+ORE\text{-}R}/$Y	2	5693	0	2098	0.3	NS
R(1)2, cv v f/B^{S}Y	0	4193	0	1074	—	—
R(1)2, cv mei-41/B^{S}Y	2	4739	0	733	0.4	NS

B. Other crosses in which Y chromosomal *bb* mutations could have been detected

Male parent	Female parent	Y chromosomes screened: *bb*$^+$	Y chromosomes screened: *bb*	X chromosomes screened: *bb*$^+$	X chromosomes screened: *bb*
y mei-41 bb$^{+ORE\text{-}R}/y^+$Y	*C(1)DX, y f/B*^{S}Y	5977	0	—	—
y mei-41 bb$^+ \cdot y^+/$Y	*C(1)DX, y f/B*^{S}Y	2182	0	—	—
y mei-41 bb$^{41}/y^+$Y	$sc^4sc^8/dl\text{-}49$	2507	0	0	3159
In(1)sc^8, *sc*^{8} *mei-41/B*^{S}Y	*C(1)DX, y f/B*^{S}Y	3667	0	—	—

C. Effect of *mei-41* on a Y chromosome-bearing X chromosomal rDNA[c]

Paternal genotype	Progeny: y^+B^S ♂♂	Progeny: *y* B^S ♂♂	Progeny: *y f bb*$^+$ ♂♂	Progeny: *y f bb* ♀♀	Progeny: y^+ *f bb*$^+$ ♀♀
y bb$^+ \cdot y^+/$Y*bb*$^{+X}$	4920	0	4216	0	1
y mei-41 bb$^+ \cdot y^+/$Y*bb*$^{+X}$	7518	14[d]	6323	2	4[d]

[a] Statistical comparisons with *bb*$^+$/Y males were obtained by using the tables of KASTENBAUM and BOWMAN (1971).

[b] These numbers are the sum of those obtained in seven separate experiments. No significant differences in the mutation frequency were observed among the individual experiments.

[c] Males of the indicated genotype were crossed to *C(1)DX, y f/BSY* females.

[d] These exceptions were shown to be the result of interchanges between the X and Y*bb*$^{+X}$.

tional in that *mei-41* does not induce reversions of *bb* to *bb*$^+$. This conclusion is supported by three lines of evidence. First, although our *bb*$^+$ strains of *mei-41* routinely accumulate *bb* mutants, our *bb* stocks of *mei-41* are stable over a large number of generations and show no evidence of reversion. Second, in an experiment designed to screen for increases in rDNA redundancy, *y mei-41 bb*$^{41}/y^+$Y males were mated to $sc^4sc^8/dl\text{-}49$ females. No *bb*$^+$ revertants were recovered among 3159 females examined, although the *bb*^{41} allele can revert as was demonstrated in Table 1. Third, in an experiment designed to find both up and down mutations, *y mei-41 bb*$^{41}/y^+$Y males were crossed to *C(1)DX, y f/y*$^+$Y females and the sons were mated singly to $sc^4sc^8/dl\text{-}49$ females. Of the 935 males tested, 150 were sterile despite replacement of the females or produced fewer than 10 progeny, 783 gave X/sc^4sc^8 female progeny that were *bb*, whereas

2 gave no X/sc^4sc^8 progeny but large numbers of X/*dl-49* progeny. Further test crosses showed that these two X chromosomes were bb^6. No males gave X/sc^4sc^8 progeny that were bb^+. Thus, in a situation where both rDNA increases and decreases could have been detected only deficiencies were obtained.

These experiments indicate that the *bb* mutations induced by *mei-41* result from the intrachromatid excision of rDNA, an event that can produce only deficiencies and not duplications. Further support for this hypothesis comes from an experiment that measures the frequency at which *mei-41* induces *bb* mutants in a ring chromosome. Interchromatid exchanges in rings, such as unequal sister chromatid exchange or X-Y interchange, would result in the formation of dicentrics, and hence *R(1)2, mei-41* bb^+ might be expected to fail to produce *bb* mutants. The only way to recover unequal sister chromatid exchanges in ring chromosomes is to have two exchange events, only one of which need occur at the *bb* locus. If the frequency of a single event at the *bb* locus is 2×10^{-3} (the higher of the two values observed for normal sequence X chromosomes), then using 0.036 as the estimated frequency of sister chromatid exchange in *R(1)2* (WELSHONS and HINTON 1955), production of *bb* mutants would be expected at a maximum frequency of 7×10^{-5}. However, as shown in Table 4A, *bb* mutants are produced in the ring chromosome at 4×10^{-4}, a rate intermediate between that observed for the two bb^+ loci located on normal sequence X chromosomes and sixfold higher than expected for sister chromatid exchange. These data, together with the results described above, indicate that the production of *bb* mutants by mei-41 is not the result of sister chromatid events, but rather, is a consequence of intrachromatid excision of rDNA.

Although 23 X chromosomal *bb* mutants were recovered in the experiments listed in Table 4A, no Y chromosomal *bb* mutants were found. Table 4B summarizes several other experiments (previously discussed) in which Y chromosomal *bb* mutants could also have been detected. It may be seen that no such mutations were observed among more than 24,000 Y chromosomes examined. This might be the result of some effect of the Y chromosome *per se* on its rDNA, or it might reflect an intrinsic difference between the X and Y rDNA clusters. In an attempt to resolve this issue a Y chromosome was constructed that carries X chromosomal rDNA.

The effect of mei-41 *on a* Y *chromosome carrying* X *chromosomal rDNA:* The ability of *mei-41* to promote interchange between X rDNA and Ybb^- has allowed us to construct a derivative of the Ybb^- chromosome that carries the bb^+ locus from the $y\ bb^{+ORE\text{-}R}$ chromosome. Although details of the construction and behavior of this chromosome will be published elsewhere (R. S. HAWLEY and K. D. TARTOF, in preparation), suffice it to say that it arose from recombination between a Y^SX which contains $bb^{+ORE\text{-}R}$ and both Y^S fertility factors (Table 2A, line 4) and a Ybb^- chromosome in a y *mei-41* $bb^{+ORE\text{-}R}/Ybb^-/Y^SX^R$ male. It carries X chromosomal rDNA as shown by analysis of *Eco*RI digests. Cytologically, it is indistinguishable from Ybb^- or Ybb^+ This chromosome is designated as Ybb^{+X}.

To determine whether X chromosomal rDNA placed in the Y chromosome was mutable in the prsence of *mei-41*, y *mei-41* $bb^+ \cdot y^+/Ybb^{+X}$ males were

mated to *C(1)DX, y f/B*^{S}·Y females and their B^+ female progeny examined with respect to *bb* (Table 4C). Of the 6323 *Ybb*$^{+X}$ chromosomes scored, two proved to be *bb*. This mutation rate (3.2×10^{-4}) is very close to that observed for *bb*$^{+ORE-R}$ X chromosomes (3.5×10^{-4}, Table 4). Although the reciprocal experiment of placing Y rDNA in the X and observing the effect of *mei-41* on it has not been possible, these results, nevertheless, indicate that the production of *bb* mutants from X rDNA and not from Y rDNA is most likely the result of an intrinsic difference between X and Y rDNA clusters and not their chromosomal location.

The mutabilities of bb$^+$ *alleles and their restriction patterns:* As noted in Table 5A, the *bb*$^+$ to *bb* mutation rates of *y bb*$^+$·*y*$^+$ and *y bb*$^{+ORE-R}$ differ by more than sevenfold in the presence of the same allele of *mei-41*. This suggests that the difference in the mutation rates of the two *bb*$^+$ alleles might be reflected in the structure of their rDNAs. As shown in Figure 3, substantial differences exist in their *Eco*RI restriction patterns. *y bb*$^+$·*y*$^+$ possesses a number of fragments not seen in *y bb*$^{+ORE-R}$. The bands between 11.5 and 16.5 kb correspond to rDNA repeats containing size variants of the type I insertion whereas those less than 11.5 kb may be caused by type II insertions or to spacer heterogeneity (DAWID, WELLAUER and LONG 1978). These results confirm and extend the idea that the ability of *mei-41* to induce *bb* mutations depends on intrinsic structural features of the rDNA cluster.

mei-41 *does not cause deletions of random breakage elsewhere in the genome:* Since GATTI (1979) has shown that *mei-41* increases the frequency of spontaneous chromosome breakage in neuroblasts by more than tenfold, it is possible that the observed X-*Ybb*$^-$ interchanges are simply a local manifestation of a large number of breaks and interchanges occurring throughout the genome. The

TABLE 5

Attempt to recover Minutes induced by mei-41

Cross	No. of offspring recovered		Confirmed Minutes[b]
	Females	Males	
y mei-41 bb$^+$·*y*$^+$/Y × *C(1)DX, y f/B*^{S}Y[a]	—	2,606	0
y mei-41 bb$^+$·*y*$^+$/Y × *C(1)RM, y w/Y*	2,703	2,830	0
y mei-41 bb$^+$·*y*$^+$/Ybb$^-$ × *C(1)DX, y f/B*^{S}Y	—	3,375	0
y mei-41 bb$^+$·*y*$^+$/Ybb$^-$ × *C(1)RM, y w/Y*	12,653	13,495	0
In(1)sc^8, *mei-41/Y* × *C(1)DX, y f/B*^{S}Y	—	3,225	0
In(1)sc^8, *mei-41/Y* × *C(1)DX, y f/B*^{S}Y	1,366	711	0
In(1)sc^8, *mei-41/Ybb*$^-$ × *C(1)RM, y w/Y*	—	2,027	0
In(1)sc^8, *mei-41/Ybb*$^-$ × *C(1)RM, y w/Y*	6,640	4,206	0
y mei-41 bb^{+ORE}/Y × X/X	3,521	3,500	0
y mei-41 bb^{+ORE}/Ybb$^-$ × X/X	3,510	4,068	0
y mei-41 bb^{+ORE}/Ybb$^-$ × *C(1)DX, y f/B*^{S}Y	—	2,875	0
Total	30,393	42,918	0

[a] Although no Minutes were observed among the *C(1)DX, y f* bearing progeny, the numbers of such females examined are not listed since *f* might have obscured the Minute phenotype.

[b] Five Haplo-4 Minute males and one female were eliminated from consideration after test cross to *y/y; pol/pol* female or *y/Y; pol/pol* males.

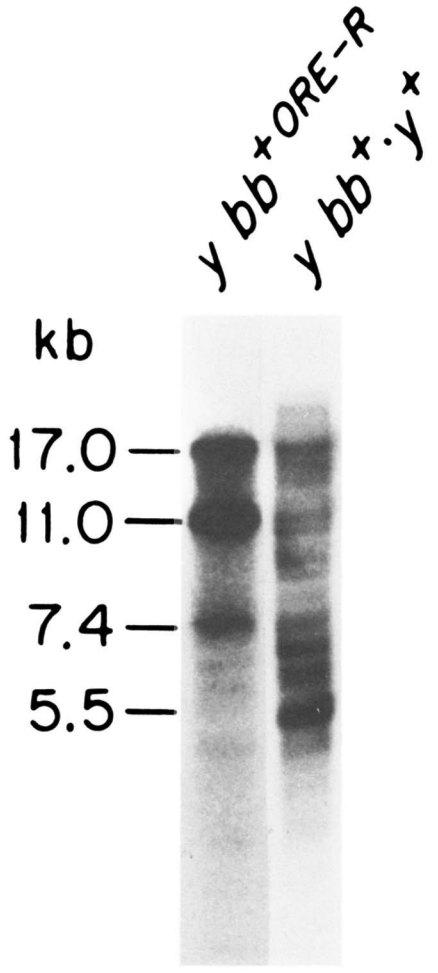

FIGURE 3.—Comparison of the *Eco*RI restriction patterns of *y bb*$^{+ORE}$ and *y bb*$^{+}$·*y*$^{+}$ females.

following experiments were designed to look for deletions as reflected by the recovery of Minutes on all chromosomes or to detect recombination (interchange) on the second chromosome. Table 5 lists those crosses in which Minutes might have been observed. Examination of more than 73,000 offspring from *mei-41* males revealed no confirmed Minute mutations. If interchanges were occurring randomly throughout the genome, then *mei-41* should also increase the frequency of autosomal exchange. To test this possibility, males heterozygous for the second chromosomal markers *al dp b pr cn* were mated to females homozygous for these genes. Since the region of chromosome 2 analyzed is about nine times larger than the *bb* locus, the frequency of homologous interchange should be quite high. As shown in Table 6, in the absence of *mei-41* a single (mitotic) event was observed. In the presence of *mei-41* eight separate interchanges were obtained. The frequency of second chromosomal interchange in both of the crosses involving *mei-41* is less than that observed for the *bb*

TABLE 6

Effect of mei-41 *and* Ybb$^-$ *on second chromosomal exchange in males*

Paternal genotype	NCO[a]	SCO				DCO		
		al-dp	*dp-b*	*b-pr*	*pr-cn*	*dp*	*b*	*pr*
bb$^+$/Y	7139	0	1[b]	0	0	0	0	0
bb$^+$/Y*bb*$^-$	5269	0	0	0	0	0	0	0
y mei-41 bb$^+$/Y	7021[c]	0	0	0	2	0	0	0
y mei-41 bb$^+$/Y*bb*$^-$	7608[d]	0	0	1	4	1[e]	0	0

Males of the indicated X/Y genotype and heterozygous for *al dp b pr* and *cn* were crossed to *al dp b pr cn* females.

[a] NCO, SCO and DCO indicate the noncrossover, single crossover and double crossover classes, respectively.

[b] This represents a single cluster of four such exchanges occurring in 1 of 30 bottles examined.

[c] Two white-eyed males were recovered. Such white-eyed males have also been observed to occur spontaneously in the *al dp b pr cn* stock and thus may indicate the presence of an unstable allele of w^+.

[d] One white-eyed male was also recovered (see footnote *c*).

[e] This female was *al dp*$^+$ *b pr cn* and thus likely represents a true double exchange.

locus alone. Moreover, those interchanges that were recovered occurred primarily in the heterochromatin (6/8) indicating that breakage may occur preferentially in heterochromatic domains. In sum, these results demonstrate that *mei-41* does not generate extensive chromosome breakage in the male germ line leading to deletions or interchange. Rather, *mei-41* appears to induce such aberrations at the bobbed locus and perhaps a small number of other sites such as in the heterochromatin of chromosome 2.

DISCUSSION

We have shown that *mei-41* has three distinct effects on X chromosomal rDNA in the germ line cells of Drosophila males. First, *mei-41* suppresses magnification before or at the initial magnifying event. Second, *mei-41* enhances the frequency of chromatid interchange involving the X chromosomal rDNA and two or more sites on the *Ybb*$^-$ chromosome. Third, *mei-41* causes the production of rDNA deletions at high frequency in otherwise normal males. These deletions arise from an intrachromatid excision event that is intrinsic to the X chromosomal rDNA itself. Such frequent and specific interaction of *mei-41* with the *bb* locus is surprising in view of the other phenotypes of this gene. *mei-41* is known to suppress recombination (BAKER and CARPENTER 1972) and to be defective in post-replication repair (BOYD 1978), resulting in flies being sensitive to a wide variety of mutagens (BAKER *et al.* 1976), as well as increasing the frequency of random spontaneous chromosome aberrations throughout the genome of somatic cells (GATTI 1979). The question than arises: How does a mutation like *mei-41*, with general and widespread effects, result in such apparently specific and frequent events at the *bb* locus?

From the evidence presented here, it is clear that the *mei-41*$^+$ gene product, necessary for meiotic recombination, is also required for rDNA magnification. However, the *mei-9*$^+$ gene product is not. Two conclusions can be drawn from

our results. First, magnification requires some, but not all, of the gene products required for meiotic exchange. Second, although both *mei-9* and *mei-41* apparently increase the rate of random spontaneous chromosome breakage and rearrangement in somatic cells (GATTI 1979; BAKER, CARPENTER and RIPOLL 1978), we do not observe comparable random events in the male germ line. In fact, germ line deletions and interchanges induced by *mei-41* appear to be limited to specific heterochromatic domains. These data suggest there is a difference in the way somatic and germ line cells cope with spontaneous DNA damage.

The observation that *mei-41* generates intrachromatid deletions only in X rDNA, and not Y, indicates that *mei-41* responds to an intrinsic difference between the two rDNA clusters. One such difference is that about half of the X rDNA contains an insertion in the 28S coding segment, known as type I, that is not present in theY (TARTOF and DAWID 1976). These inserts are similar to transposable elements in that they are flanked by target site duplications (DAWID and REBBERT 1981) and are distributed at several, primarily heterochromatic, locations in the genome (PEACOCK *et al.* 1981). We propose that recombinogenic lesions, such as gaps or nicks, are periodically made in or near type I insertions. These may be the result of a system that creates them in order to promote their exchange or transposition. We suggest that in the presence of *mei-41* these recombinogenic lesions are not properly repaired, and as a result, are allowed to interact so as to generate intrachromatid deletions. This hypothesis is also supported by the fact that *mei-41* induces *bb* mutations more frequently in $y\ bb^{+} \cdot y^{+}$ than in $bb^{+ORE\text{-}R}$ chromosomes. The former contains a number of classes of insertion bearing rRNA genes not possessed by the latter.

In the presence of *mei-41*, interchange between the X and Ybb^{-} (but not Ybb^{+}) occurs at high frequency. The Ybb^{-} chromosome is also responsible for driving the unequal sister chromatid exchanges that generate magnified and reduced *bb* loci (TARTOF 1974). Ybb^{-} may achieve both effects by being deficient not only for rDNA, but perhaps for some other site as well, thereby inducing anomolous pairing in the rDNA of sister chromatids of the X. The X-Y interchanges produced in *mei-41* bb^{+}/Ybb^{-} males may be viewed as a consequence of such a special pairing relationship between the X and Ybb^{-} chromosomes in the presence of recombinogenic lesions that remain unrepaired. In this regard, the observation that either arm of the Y may be involved in *mei-41* induced interchanges is of interest. Although COOPER (1964) noted that the X could pair with either arm of the Y, with one curious exception (MULLER 1948), all previously reported cases (143/143) of interchange between the X and Ybb^{+} or Ybb^{-} have involved only Y^{S} (NEUHAUS 1937; SIDEROV 1940; LINDSLEY 1955; RITOSSA *et al.* 1974; MADDERN 1981). The fact that *mei-41* permits $Y^{L}X^{R}$ interchanges to occur at high frequency indicates that *mei-41* may relax those conditions that normally lead to rearrangements only with Y^{S} so that interchanges involving Y^{L} now arise.

In the control crosses reported here Y^{S} is able to undergo interchange with the rRNA genes in both wild-type and inverted X chromosomes. Likewise, STERN and DOAN (1936), NEUHAUS (1937), SIDEROV (1940) and MADDERN (1981)

have demonstrated Y^S interchanges with the rDNA of a normal sequence X. All of these experiments fail to confirm the hypothesis of PALUMBO, CAIZZI and RITOSSA (1973) that the rDNA clusters of X and Y chromosomes are oriented with opposite polarities relative to the centromere. Rather, as initially suggested by MADDERN (1981), the data indicate that the rDNA clusters of either (or both) the X and Y chromosome are composed of genes whose polarity is not uniform.

Our data also fail to confirm the suggestion of PALUMBO, CAIZZI and RITOSSA (1973) and that of RITOSSA *et al.* (1974) that Ybb^- induces X-Y interchange as part of the magnification process. In no case did Ybb^- increase the frequency of interchange in bb^+/Ybb^- males when compared to that observed for bb^+/Y males.

If our hypothesis that type I inserts are sites at which recombinogenic lesions are constantly being made (but not efficiently repaired by *mei-41*) is correct, then this has an interesting implication for chromosome structure. It is possible that type I (and similar) sequences bring to heterochromatic regions, otherwise devoid of exchange, a specialized recombination system that may be part of the mechanism regulating events such as rDNA magnification-reduction.

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