Proc. Nat. Acad. Sci. USA
Vol. 71, No. 4, pp. 1272–1276, April 1974

Unequal Mitotic Sister Chromatid Exchange as the Mechanism of Ribosomal RNA Gene Magnification

(gene reduction/*Drosophila melanogaster*/*bobbed* mutants/gene expansion–contraction)

KENNETH D. TARTOF

The Institute for Cancer Research, Fox Chase Center for Cancer and Medical Sciences, Philadelphia, Pennsylvania 19111

Communicated by Donald D. Brown, November 28, 1973

ABSTRACT **It is hypothesized that magnification of the gene coding for ribosomal RNA occurs by unequal mitotic sister chromatid exchange on the basis of five different lines of evidence. These are: (*1*) rDNA magnification occurs in mitotically active germ cells; (*2*) decreases in rDNA redundancy can be genetically produced, a phenomenon termed *reduction;* (*3*) magnification and reduction events are reversible and reciprocal; (*4*) it is possible to generate *bb*$^+$ and *bb* somatic bristle mosaics (*bb* mutants are partially deficient for rRNA genes); and (*5*) magnification of *bb* in a ring X chromosome is reduced. Implications of these results and the unequal sister exchange (USE) hypothesis are discussed.**

In the eukaryotes thus far studied, the gene coding for 28S and 18S ribosomal RNA (rRNA) is tandemly repeated and it has been proposed that this reiterated gene sequence is clustered at the nucleolus organizer (*NO*) locus (1, 2). In *Drosophila melanogaster*, there is one *NO* on each X and Y chromosome. In addition, flies that are partially deficient for rRNA genes (rDNA) are known as *bobbed* (*bb*) mutants (2) and such a mutation can exist on either sex chromosome. The phenotype of the *bb* mutation is smaller bristles, a thinner chitinous cuticle and a reduced growth rate.

It is possible by genetic means to produce increases in the amount of rDNA per *NO* in germ line cells such that this increase can be transmitted to subsequent generations. When an X chromosome bearing a *bb* mutation is maintained for several generations with a Y*bb*$^-$ chromosome, as in *bb*/Y*bb*$^-$ males, the *bb* mutation reverts to the wild type rDNA content and *bb*$^+$ phenotype. This phenomenon has been referred to as rDNA magnification (3).

A precise understanding of the mechanism of rDNA magnification is of fundamental importance in order to elucidate those genetic principles that govern redundant genes. In 1966, in order to explain the very existence of *bobbed* mutants, Ritossa, Atwood, Lindsley, and Spiegelman (2) speculated that such *bobbed* mutants could arise as a result of unequal crossing-over. However, 2 years later, when rDNA magnification was described (3), the possibility that unequal crossing-over might explain this phenomenon was rejected. As Atwood (4) has stated, "Experiments designed to detect a correlation between crossing-over and mutation at the *bb* locus suggest that when the locus is in its normal proximal position, no such correlation exists, and that crossing-over is not the cause, or at least not the major cause, of changes in the redundancy of rDNA. Novel concepts will be required to explain the population-wide reversion called 'magnification' of *bb*." Likewise, Ritossa (5, 6) has also rejected unequal crossing-over as the explanation of rDNA magnification and has proposed a mechanism utilizing rDNA episomes: "According to this model, extra copies of rDNA are formed in all cells of *bobbed* males. After circularization, the copies can be integrated into the chromosome only in the germ line."

In spite of such statements, I know of no compelling evidence that warrants rejection of unequal crossing-over as the mechanism responsible for generating changes in rDNA redundancy during magnification. Indeed, it is my purpose here to present the results of five basic experiments which argue that unequal mitotic sister chromatid exchange is the mechanism of rDNA magnification. These experiments demonstrate: (*1*) rDNA magnification occurs in mitotically active germ cells and at a frequency several orders of magnitude higher than the rate of interchromosomal meiotic recombination; (*2*) the Y*bb*$^-$ chromosome which induces rDNA magnification of *bb* mutants can also decrease the rDNA content of the wild-type *bb*$^+$ locus (a phenomenon termed *reduction*) such that it now behaves as *bb;* (*3*) magnification and reduction are reversible and reciprocal events; (*4*) it is possible to generate *bb*$^+$ and *bb* somatic bristle mosaics; and (*5*) magnification of *bb* in a ring X chromosome is reduced. The implications of the unequal sister exchange (USE) hypothesis will be discussed.

MATERIALS AND METHODS

Drosophila melanogaster were raised at 24.5 ± 0.5° as previously described (7). The chromosomes used in these studies are: y^2bb^+/B^sY; $In(1)sc^{4L}sc^{8R}$, y sc^4sc^8cv v $B/In(1)dl\text{-}49$, y Hw m^2g^4/Ybb^-; $C(1)RM$ w^{48h}/Ybb^-; $bb^2/C(1)RM$, w^{48h}/B^sY; $In(1)sc^{4L}sc^{8R}$, y sc^4sc^8cv v $B/C(1)DX$, yf/B^sY; $R(1)2$, cv v $bb/C(1)DX$, yf/B^sY. The abbreviated designations for these chromosomes, their pertinent characteristics and their complete genetic designations (8) are given in Table 1. In all of the genetic experiments described here, adult virgin females and males 0–48 hr old (post eclosion) were utilized. The methods for nucleic acid extraction, rRNA·DNA hybridization and calculation of rRNA gene number have been previously detailed (7, 9).

RESULTS

Frequency and premeiotic origin of rDNA magnification

To determine the frequency and time at which rDNA magnification occurs, the following experiment was performed.

Abbreviations: rDNA, genes for ribosomal RNA; USE hypothesis, unequal sister [chromatid] exchange hypothesis.

TABLE 1. *Chromosome designations*

Abbreviated chromosome designation	Complete genetic designation
bb^+ (wild type)	y^2bb^+
bb^0 (deficient for *NO*)	$In(1)sc^{4L}sc^{8R}$, $y\ sc^4sc^8\ cv\ v\ B$
dl-49 (X chromosome with euchromatic inversion)	$In(1)delta\ 49$, $y\ Hw\ m^2g^4$
$\overline{XX}$ (attached X)	$C(1)RM$, w^{48h}
B^sY (dominant marker B^s to monitor presence of Y)	B^sY
Ybb^- (rDNA deficient Y chromosome)	Ybb^-
R*bb* (ring X chromosome carrying *bb*)	*R(1) 2, cv v bb*

Spontaneously occurring X chromosome *bb* mutants were used for this study (7) and each mutant stock is derived from a single X chromosome *bb* mutant by mating one bb/B^sY male to $\overline{XX}/B^sY$ females. Then bb/B^sY males from the mutant stock were mated to $\overline{XX}/Ybb^-$ females yielding bb/Ybb^- males which are in turn mated *singly* to $bb^0/dl\text{-}49$ females. The bb/bb^0 female offspring of such a mating are then examined for the presence of phenotypically bb^+ flies generated by the presence of the Ybb^- chromosome in the male parent. The control for such an experiment requires mating single bb/B^sY stock males to $bb^0/dl\text{-}49$ females and examining the bb/bb^0 female progeny for the presence of phenotypically bb^+ flies. The absence of such bb^+ flies insures that the appearance of bb^+ flies in the experimental cross is exclusively the result of a magnification response stimulated by the Ybb^- chromosome. The data for this experiment are given in Table 2, where the bb^2 mutant has been examined. Similar results have been obtained for other independently derived spontaneously *bb* mutants (10). What is immediately obvious is that some bb/Ybb^- males give rise to large numbers of bb^+ flies (vial 4) whereas others produce none or only a few bb^+ flies (vial 10). This experiment tells us three things. First, the pronounced clustering of bb^+ flies strongly suggests that

TABLE 2. *Frequency of magnification determined by the phenotype of bb^2/bb^0 females derived from mating a single bb^2/Ybb^- male × $bb^0/dl\text{-}49$ females*

Vial no.	*bb* Phenotype of bb^2/bb^0 females: *bb*	*bb*$^+$	% Magnification
Control (1–10)*	409	0	0
1	75	3	3.8
2	40	4	9.1
3	62	3	4.6
4	12	55	82.1
5	10	0	0
6	38	6	13.6
7	34	20	37.0
8	29	4	12.1
9	22	14	38.9
10	52	0	0
Total (excluding control)	374	109	average = 22.6

* The control for this experiment requires mating bb^2/B^sY males × $bb^0/dl\text{-}49$ females and examining the *bobbed* phenotype of the bb^2/bb^0 female progeny.

TABLE 3. *Progeny of mating bb^+/B^sY males × $bb^0/dl\text{-}49$ females*

Bottle no.*	bb^+/bb^0 ♀ Phenotype: *bb*	*bb*$^+$	*bb*$^+$/*dl-49*	bb^0/B^sY	*dl-49*/B^sY
1–10	0	1710	1859	1446	566

* Five male and 5 female parent flies mated in each bottle.

the magnification process is premeiotic, that is, it occurs in germ cells when they are still mitotically active. Secondly, the frequency of magnification can be as high as 80% in a single fly. This high frequency of magnification cannot be reconciled with interchromosomal meiotic recombination since magnification occurs in males where the frequency of meiotic recombination is at least three orders of magnitude below that sufficient to account for the rate of rDNA magnification observed here. Finally, the stability of the phenotypically wild type magnified *bb* (bb^{m+}) locus has been studied as follows: 89 bb^{2m+}/bb^0 phenotypically bb^+ virgin females from above were randomly selected and singly mated to bb^0/B^sY males. In every case, the next generation progeny of bb^{2m+}/bb^0 females remained a stable bb^+.

Genetically directed reduction of rDNA redundancy

Under appropriate genetic conditions it is possible to reduce the number of rRNA genes in a wild type bb^+ locus. The construction of genotypes to detect such a genetically directed mutation from bb^+ to *bb* is as follows: bb^+/B^sY males are mated to $\overline{XX}/Ybb^-$ females and the bb^+/Ybb^- male progeny are then mated to $bb^0/dl\text{-}49$ females. Of the four different progeny genotypes from this second cross, the bb^+/bb^0 female class will reveal any changes that have occurred at the *bb* locus. In this way, the specific mutation of bb^+ to *bb* as a result of bb^+ coexisting with the Ybb^- chromosome in the immediately preceding generation can be detected. Critically important controls for this experiment are required. It is possible that some *bb* flies might arise because of the presence of a *bb* mutant in the bb^+ stock before the experiment began. In order to eliminate this possibility, bb^+/B^sY males and bb^+/bb^+ females are mated to $bb^0/dl\text{-}49$ females and bb^0/B^sY males, respectively. The resulting bb^+/bb^0 female progeny are then examined for a *bb* phenotype. These controls also permit an unequivocal demonstration that any bb^+ to *bb* transition is due strictly to the presence of the Ybb^-, neither the B^sY nor the bb^+ chromosomes being similarly effective.

The data of Tables 3 and 4 summarize the results of the control matings. When bb^+/B^sY males are crossed to $bb^0/$

TABLE 4. *Progeny of mating bb^+/bb^+ females × bb^0/B^sY males*

Bottle no.*	bb^+/bb^0 ♀ Phenotype: *bb*	*bb*$^+$	*bb*$^+$/B^sY ♂	*bb*$^+$/bb^0/B^sY ♀ †	*bb*$^+$/0 ♂ †
1–10	0	2718	2138	316	102

* Five female and 5 male parent flies were mated in each bottle.

† These progeny classes are the result of nondisjunction in the bb^0/B^sY male parent.

TABLE 5. *The genetic production of bb mutants in the X chromosome observed by mating bb^+/Ybb^- males × $bb^0/dl\text{-}49$ females*

Bottle no.*	bb^+/bb^0 ♀ Phenotype bb	bb^+	$bb^+/dl\text{-}49$ ♀	$dl\text{-}49/Ybb^-$ ♂	$bb^+/0$ ♂†
1	0	155	168	158	1
2	3	182	193	174	1
3	2	137	169	123	0
4	1	137	132	123	1
5	1	222	209	186	1
6	5	209	229	191	4
7	(1)‡	175	179	151	2
8	3	193	224	201	0
9	(1)‡	221	191	160	1
10	0	161	198	177	0
Total	15§	1792	1892	1644	11

* Five male and 5 female parent flies were mated in each bottle.

† This progeny class is the result of nondisjunction in the $bb^0/dl\text{-}49$ female.

‡ Phenotypically *bb* flies that were sterile.

§ Confirmed *bb* mutants (see *text*).

dl-49 females (Table 3), of the 1710 bb^+/bb^0 female progeny, no *bb* flies were observed. The under-representation of *dl-49*/B^sY male progeny is probably due to the poor viability or late hatching of the genotype since a similar experiment utilizing a wild-type Y chromosome rather than the B^sY yielded the expected proportion of *dl-49*/Y males. In Table 4, the results of crossing bb^+/bb^+ females to bb^0/B^sY males are given. Of the 2718 bb^+/bb^0 class of females inspected, no *bb* flies were observed. Thus, in these controls 4428 bb^+/bb^0 females were examined for the presence of a *bb* mutation in the bb^+ stock, and none was found.

However, if the X chromosome bb^+ locus is placed opposite Ybb^- chromosome to produce bb^+/Ybb^- males and these males are then mated to $bb^0/dl\text{-}49$ females, a large number of phenotypically *bobbed* flies appear among the bb^+/bb^0 female class (Table 5). The class of *bobbed* mutants produced in this manner will henceforth be denoted as bb^r, each individual mutant receiving a number of (bb^{r1}, bb^{r2}, etc.). Among 1802 bb^+/bb^0 females, 17 phenotypically *bb* flies were found, 15 of which were confirmed by the following genetical tests to be stable *bb* mutants. Each of the 15 bb^r mutants are phenotypically *bb* when maintained with the bb^0 chromosome and all are allelic to three separate X chromosome *bb* mutants that are maintained in this laboratory. All 15 bb^r mutants are phenotypically *bobbed* as $bb^r/0$ or bb^r/Ybb^- males but are phenotypically bb^+ when maintained with a wild-type Y chromosome.

TABLE 6. *The rDNA content of genetically induced bb^r mutants*

Genotype	% DNA hybridized	% DNA contributed from bb^r chromosome*	No. of rRNA genes per X chromosome *NO*
bb^0/B^sY	0.223	0	0
bb^+/B^sY	0.426	0.203	232
bb^{r61}/B^sY	0.303	0.080	91
bb^{r78}/B^sY	0.315	0.092	105
bb^{r80}/B^sY	0.288	0.065	74
bb^{r78m+}/B^sY	0.402	0.179	205

* Determined by subtracting the rDNA contribution of B^sY.

TABLE 7. *The status of the bobbed locus in $bb^{2?}/B^s$Y males assayed by mating these males to $bb^0/dl\text{-}49$ females and observing the phenotype of the $bb^{2?}/bb^0$ female offspring*

bb^2/Ybb^- male parent no.	Phenotype of $bb^{2?}/bb^0$ females bb^{m+}	bb	bb^{r1}
1	2	57	1
2	12	38	2
3	22	15	2
4	6	38	2
5	30	12	0
6	0	57	0
7	7	52	1
8	15	28	4
9	21	34	2
10	8	41	2
Total	123	372	16
Percent of Total	24	72.9	3.1

The amount of rDNA in the bb^+ and bb^r chromosomes was determined by means of the rRNA·DNA filter hybridization technique (7). In order to avoid rDNA compensation effects (7, 11), the amount of rDNA in bb^+ and bb^r chromosomes was measured when maintained opposite the B^sY chromosome. The data (Table 6) demonstrate a considerable reduction (about 50%) in the amount of rDNA in the bb^r chromosomes (each derived from a separate bottle, Table 5) as compared to the parental bb^+ chromosome. I shall refer to this phenomenon as *rRNA gene reduction*.

Since 130 rRNA genes were lost from the bb^+ chromosome during reduction, had they integrated with the Ybb^- chromosome, which contains no more than 40 rRNA genes (7), then 130–170 rRNA genes would be present and this would be detected as a bb^0/Ybb^- male that was phenotypically *bb* or bb^+ (7). However, no such males were found. Therefore, it appears that there is no exchange of rDNA between the Ybb^- and bb^+ chromosomes during rDNA reduction.

The frequency of rDNA reduction in this experiment is approximately 0.8%. In similar experiments utilizing two other bb^+ X chromosomes of independent origin, the frequency of rDNA reduction was 0.1% and 0.03%. These frequencies probably underestimate the true rDNA reduction frequency for two reasons: (*1*) there would be severe selection against bb^r/Ybb^- cells which would grow slower than bb^+/Ybb^- cells; and (*2*) bb^r/Ybb^- might revert to bb^+/Ybb^- by magnification.

To determine if the bb^r mutants can magnify and return to the bb^+ condition, five bb^r/B^sY males from each of the 15 genetically derived bb^r mutants were mated to five $\overline{XX}/Ybb^-$ females and the resulting bb^r/Ybb^- male progeny were then mated to $bb^0/dl\text{-}49$ females. The bb^r/bb^0 female progeny from this cross were then examined for the presence of phenotypically bb^+ flies and for each of the 15 bb^r mutants, magnified bb^+ flies were obtained. A bb^{r78} mutant that magnified in this manner to the bb^+ phenotype (bb^{r78m+}) was assayed for its rDNA content and found to contain nearly the wild-type parental number of rRNA genes (Table 6). This experiment

demonstrates that it is possible to obtain a 2-fold decrease or increase in the rDNA content in a single step and that bb^r mutants generated by the presence of a Ybb^- chromosome can also revert rapidly to bb^+ when again placed opposite a Ybb^- chromosome. The ability of Ybb^- to generate reciprocal reduction–magnification events suggests recombination as the mechanism for altering rDNA redundancy.

That reduction is indeed the reciprocal event of magnification can be demonstrated as follows. Single bb/Ybb^- males are mated to $\overline{XX}/B^sY$ females and all of the $bb^?/B^sY$ male progeny derived from this single bb/Ybb^- male parent are collected. The $bb^?$ locus now present in the $bb^?/B^sY$ male may be magnified (bb^{m+}), unmagnified (bb) or reduced lethal (bb^{rl}) as a result of coexistence with the Ybb^- in the preceding generation. The status of the $bb^?$ locus in each $bb^?/B^sY$ male is assayed by mating them to $bb^0/dl\text{-}49$ females and observing the phenotype of the $bb^?/bb^0$ female progeny. Ideally, each bb/Ybb^- male parent should produce equal numbers of bb^{m+} and bb^{rl} chromosomes if rDNA magnification and reduction are reciprocal events. This experiment has been repeated 4 times and the results of one such typical experiment are given in Table 7. There are three important facts that derive from these data. First, note the occurrence of bb^1 is dependent on the occurrence of bb^{m+} in the germ line of the same single bb/Ybb^- male parent. In all 4 repeats of this experiment, bb^{rl} has never been observed to arise independently of bb^{m+}. Second, the overall frequency of bb^{m+} is approximately 24%. In Table 2, the average frequency of bb^{m+} was 22.5%. Thus, magnified *bobbed* loci behave in a perfectly stable manner whether or not they are initially paired with a bb^0 or bb^+ locus. This striking stability of the magnified *bobbed* locus conflicts with previously reported observations claiming instability of magnified bb^+ loci (3). Finally, the average frequency of bb^{rl} was about 3% compared to 24% for bb^{m+}. That bb^1 are under-represented probably results from: (*1*) bb^{rl}/Ybb^- sperm would be cell lethals and grow considerably slower than bb or bb^{m+} sperm, and (*2*) bb^{rl} might revert by magnification to bb or bb^{m+}. It is worth noting that the numbers of bb^{rl} are vastly reduced when large numbers of bb^{m+} are produced (e.g., parent nos. 3 and 5). In these cases the magnification-reduction event may have occurred early with bb^{m+} overpopulating the small number of bb^{rl} initially produced. The rDNA content of a bb^{2rl} and a bb^{2m+} have been measured and found to contain 20 and 218 rRNA genes respectively, compared to the 110 rRNA genes present in the parent bb^2 chromosome. Thus, the 90 rRNA genes lost by reduction is roughly equal to the number of rRNA genes gained by magnification.

Locker and Prud'homme (12) have reported the occurrence of bb^{rl} arising from bb/Ybb^- males in a mass mating experiment, but concluded that magnified and lethal *bobbed* loci were not reciprocal products of the same events. They drew this conclusion because bb^{rl} were recovered less frequently than bb^{m+}. However, for the reasons given above (slower growth and reversion of bb^{rl}) this result is expected and, therefore, their conclusion is not necessitated by their results.

Somatic mosaics of *bobbed*

It is possible under a variety of genetic conditions to generate somatic mosaics for the magnified bb^+ phenotype. The details of such experiments have been published elsewhere (10) and I shall only attempt to summarize them here. Several independent spontaneous bb mutants were mated to flies of the appropriate sex and genotype to produce bb/Ybb^- males, $bb/0$ males, and bb/bb^0 females. Twenty-five head and thorax bristles on each side of the fly were examined for the presence of one or a patch of several wild-type bristles. For each genotype, such mosaics were found in agreement with the observations of Atwood (4) who described *bobbed* bristle mosaics in bb/Ybb^- flies. Many flies contain patches of several bb^+ bristles clustered in one region, suggesting that they are clonally derived from a single event. In addition, the bb^+ chromosome used in the rDNA reduction study was placed opposite a Ybb^- chromosome to produce bb^+/Ybb^- males and the soma of these flies were examined for the presence of bb bristles. Of 2350 flies examined, 12 mosaics were found to contain at least one bb bristle, the rest being wild type. Experiments to detect bb bristles in $bb^+/0$ males and bb^+/bb^0 females give similar results (10). Photographs of such mosaic flies have appeared elsewhere (10). It is possible that the mosaic patches of wild-type bristles in bb/Ybb^- flies are the result of X chromosome nondisjunction leading to $bb/bb/Ybb^-$ (phenotypically bb^+) cells. The fact that bb^+/Ybb^- flies can produce bb bristles suggests that this explanation is not likely. In addition, bb/Ybb^- flies that bear several bb^+ bristles yield a four to five times higher frequency of magnification than do bb/Ybb^- flies that contain no bb^+ bristles (10). These results are similar to the behavior of mosaics that are obtained after chemical mutagenesis.

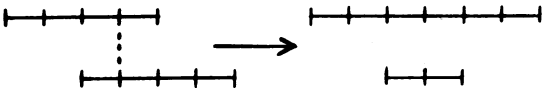

FIG. 1. This diagram shows how a USE mechanism can generate reciprocal magnification and reduction events. Each *segmented line* represents the tandemly repeated rRNA gene of one chromatid.

rDNA magnification in a ring chromosome

The preceding data support the hypothesis that the mechanism of rDNA magnification involves unequal mitotic sister chromatid recombination. If this is the case, then it should be possible to reduce the frequency of magnification of a bb mutation when in a ring X chromosome configuration since only even number (2, 4, 6, etc.) sister chromatid crossovers permit intact ring chromosomes to be recovered. A ring X chromosome carrying a bb mutation (Rbb) has been maintained opposite a Ybb^- chromosome by mating each successive generation of Rbb/Ybb^- males to $\overline{XX}/Ybb^-$ females. Although this experiment is still in progress, over 1500 Rbb/Ybb^- flies have been examined and after four generations of maintaining Rbb opposite Ybb^-, not a single magnified Rbb chromosome has been obtained.

DISCUSSION

On the basis of all the evidence presented here, I propose that rDNA magnification-reduction results from unequal mitotic sister chromatid exchange. Such a recombination event would lead to the production of two new sister chromatid strands, one containing a greater number and the other a lesser number of rDNA tandem repeats than originally contained in either parental chromatid (Fig. 1). I shall refer to this proposed mechanism as the USE (unequal sister exchange) hypothesis.

Specifically, the USE hypothesis is supported by the observations presented here: (1) magnification occurs in mitotically active germ cells and at a frequency not compatible with meiotic recombination; (2) rDNA reduction, like magnification, requires the presence of the Ybb^- chromosome in males but this chromosome does not gain rDNA lost from the bb^+ locus during its reduction; (3) the reduction–magnification reactions are reciprocal and reversible events; (4) somatic mosaics for the bb^+ and bb phenotype under genetic conditions that produce rDNA magnification and reduction have been observed and are consistent with the mitotic origin of rDNA magnification and reduction; and finally, (5) magnification does not occur or is severely inhibited when the bb locus is in a ring X chromosome, a condition which would reduce the number of recoverable single sister chromatid exchanges. The virtue of this hypothesis rests on the fact that it is consistent with all observations regarding the *bobbed* locus, does not require special *ad hoc* assumptions, is readily testable and relies on fundamental orthodox genetic principles.

Since the Ybb^- chromosome can induce rDNA reduction as well as magnification, it appears that disproportionate rDNA replication (7, 11) is not the mechanism of magnification. Since there is no detectable exchange of rDNA between the Ybb^- and bb^+ chromosomes during reduction, it appears that rDNA episomes (5) may not be involved in the magnification–reduction reactions. Furthermore, a mechanism of magnification involving disproportionate replication or rDNA episomes would not predict hindrance to magnification in ring chromosomes, or that magnification and reduction be reciprocal events.

Stern (13) was the first to document mitotic recombination in *Drosophila*. He demonstrated that mitotic exchange occurs most frequently in the heterochromatic centromeric portion of the X, a region now known to be rich in reiterated polynucleotide sequences (14). Although Stern's work only considered mitotic exchanges between homologous chromosomes, sister chromatid exchanges would also be expected in view of Taylor's direct physical evidence for mitotic sister chromatid exchange for a wide variety of organisms (15, 16), though some of these exchanges may be radiation-induced (17). However, there is also considerable cytogenetic evidence for spontaneous sister chromatid exchange in maize (18–20).

The USE hypothesis has a rather intriguing evolutionary implication. Consider the problem of maintaining all copies within a redundant gene cluster among a given species the same, and yet, permitting in the course of evolution that homologous redundant gene to rapidly diverge with respect to its nucleotide sequence. This has been shown to be the case for the spacer region in the rDNA of two closely related amphibians, *Xenopus laevis* and *Xenopus mulleri* (21). The master-slave concept could be used to solve this problem (22). Here, the master copy at some time in the life cycle pairs with its retinue of slave copies. If a mutation occurs in the slave, it is corrected when pairing with the master. On the other hand, a mutation in the master would be immediately transmitted throughout the tandem repeat. Alternatively, the continual expansion–contraction of a tandemly repetitious gene through unequal exchange would tend to maintain homogeneity provided that the frequency of exchange is greater than the mutation rate. At the same time, the occurrence of a single spontaneous mutation in the tandem repeat could be vastly increased by a USE mechanism and thereby account for the divergence of a redundant gene cluster in the course of evolution. This concept has already been discussed in some detail (10, 23).

The changes in rDNA redundancy as revealed by the magnification and reduction reactions demonstrate gene directed mutational events occurring at high frequency. Precisely how the Ybb^- chromosome is able to induce these changes in X chromosome rDNA redundancy is a question of some considerable interest. It should be noted that *Drosophila* is not the only organism in which such events have been described. Strikingly similar observations in maize have been referred to as paramutation (24). Both paramutation and rDNA magnification–reduction share certain common features. Both arise mitotically, both occur at high frequency, both can be triggered by deficiencies in the opposite homologue and both are readily reversible. The possibility that such similar phenomena have been maintained in the course of evolution from maize to *Drosophila* suggests that we are dealing with a basic and fundamental genetic principle that we are only just beginning to understand.

Marilyn Jones, Dana Tartof, and Joseph Gibbs provided excellent technical assistance. Our work was supported by NSF Grant GB-32487, NIH Grants GM-19194, CA-06927, and RR-05539, and an appropriation from the Commonwealth of Pennsylvania.

1. Birnstiel, M. L., Wallace, H., Sirlin, J. L. & Fischberg, M. (1966) *Nat. Cancer Inst. Monogr.* **23**, 431–447.
2. Ritossa, F. M., Atwood, K. C., Lindsley, D. L. & Spiegelman, S. (1966) *Nat. Cancer Inst. Monogr.* **23**, 449–472.
3. Ritossa, F. M. (1968) *Proc. Nat. Acad. Sci. USA* **60**, 509–516.
4. Atwood, K. C. (1969) *Genetics* **61**, 319–327.
5. Ritossa, F. (1972) *Nature New Biol.* **240**, 109–111.
6. Ritossa, F. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 1950–1954.
7. Tartof, K. D. (1973) *Genetics* **73**, 57–71.
8. Lindsley, D. L. & Grell, E. H. (1968) *Carnegie Inst. Wash. Publ.* **627**.
9. Tartof, K. D. & Perry, R. P. (1970) *J. Mol. Biol.* **51**, 171–183.
10. Tartof, K. D. (1973) *Cold Spring Harbor Symp. Quant. Biol.*, in press.
11. Tartof, K. D. (1971) *Science* **171**, 294–297.
12. Locker, D. & Prud'homme, N. (1973) *Mol. Gen. Genet.* **124**, 11–19.
13. Stern, C. (1936) *Genetics* **21**, 625–730.
14. Gall, J. G., Cohen, E. H. & Polan, M. L. (1971) *Chromosoma* **33**, 319–344.
15. Taylor, J. H., Woods, P. S. & Hughes, W. L. (1957) *Proc. Nat. Acad. Sci. USA* **43**, 122–128.
16. Taylor, J. H. (1963) in *Molecular Genetics*, ed. Taylor, J. H. (Academic Press, New York), Part I.
17. Gibson, D. A. & Prescott, D. M. (1972) *Exp. Cell Res.* **74**, 397–402.
18. McClintock, B. (1938) *Genetics* **23**, 315–376.
19. McClintock, B. (1941) *Cold Spring Harbor Symp. Quant. Biol.* **9**, 72–80.
20. Schwartz, D. (1952) *Genetics* **38**, 251–260.
21. Brown, D. D., Wensink, P. C. & Jordan, E. (1972) *J. Mol. Biol.* **63**, 57–73.
22. Callan, H. G. & Lloyd, L. (1960) *Phil. Trans. Roy. Soc. B.* **243**, 135–219.
23. Smith, G. P. (1973) *Cold Spring Harbor Symp. Quant. Biol.*, in press.
24. Brink, R. A., Styles, E. D. & Axtell, J. D. (1968) *Science* **159**, 161–170.