A TWO-STAGE MODEL FOR THE CONTROL OF rDNA MAGNIFICATION

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ABSTRACT

Males of the genotype *bb/Ybb*$^-$ have been shown to produce both magnified (*bb*$^{m+}$) and, less frequently, reduced (*bb*^{rl}) *X* chromosomes. An analysis of the progeny of single magnifying *bb/Ybb*$^-$ males reveals that *bb*$^{m+}$ revertants may be recovered either as rare single events or, more frequently, in large clusters. To analyze the role of the bb phenotype in the induction of rDNA magnification we have constructed a series of *bb* and *bb*$^+$ derivatives of *Ybb*$^-$. Males carrying an *X* chromosomal *bb* allele and one of these derivatives (*bb/bbYbb*$^-$ or *bb/bb*$^+$*Ybb*$^-$) produce small numbers (one to two) of *bb*$^{m+}$ progeny at a frequency similar to that observed for *bb/Ybb*$^-$ males but do not produce large clusters of *bb*$^{m+}$ revertants. In addition, *bb/bb*$^+$*Ybb*$^-$ males produce essentially equal numbers of magnified (*bb*$^{m+}$) and reduced (*bb*^{rl}) *X* chromosomes. These data, together with a consideration of the growth properties of the male germline in Drosophila, suggest that magnification/reduction may occur at two different times during development. Those events that give rise to large clusters, and, thus, necessarily arise early in germ cell development, appear to be dependent on the bb phenotype. However, those events that give rise to single *bb*$^{m+}$ chromosomes arise late in spermatogenesis, probably at meiosis, and are independent of the bb phenotype.

IN wild-type *Drosophila melanogaster* males there are two clusters of tandemly repeated rRNA genes (rDNA), each with approximately 250 copies. One of these is located in the proximal heterochromatin of the *X* chromosome and the other on the short arm of the *Y* chromosome. Partial deficiencies, known as bobbed (*bb*) mutants, exist at either cluster. Alterations in *X* chromosomal rDNA redundancy occur at very high frequency only in males and only in those carrying the aberrant *Y* chromosome known as *Ybb*$^-$. In such *bb/Ybb*$^-$ males, a phenomenon known as magnification occurs, whereby increases in rDNA redundancy are observed as reversions of *bb* to *bb*$^{m+}$ (bobbed magnified +; RITOSSA 1968). A second mutational event, in which *bb* mutates to *bb*^{rl} (bobbed reduced lethal), is also produced by *bb/Ybb*$^-$ males and is referred to as reduction (TARTOF 1974). The occurrence of both *bb*$^{m+}$ and *bb*^{rl} products in the same germline and the absence of magnification in males bearing a ring-

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X chromosome [R(1)2,*bb/Ybb*$^-$] led TARTOF (1974) to suggest that magnification and reduction are the reciprocal products of an unequal sister chromatid exchange.

In the experiments described here, we shall present evidence to indicate that magnification events may occur at two distinct times during male germline development. For purposes of simplicity, spermatogenesis may be conveniently divided into three stages (for a detailed review see LINDSLEY and TOKUYASU 1980). In the first stage (mitotic), male germ cells develop from three to seven pole cells created by the migration of nuclei into the polar cytoplasm of the egg. These cells undergo three asynchronous mitoses to give rise to an average of 37 cells which then migrate in the developing embryo to form the presumptive gonad. Approximately ten to 14 of these cells go on to form the adult stem cell population located in the apical region of the testis. In the second stage (syncytial) stem cells divide mitotically to produce a new stem cell and a primary spermatogonium. The primary spermatogonium then goes on to divide in a synchronous and syncytial fashion to produce a 16 cell cyst. The third and final stage (meiotic) is comprised of the two meiotic divisions that ultimately yield the bundle of 64 mature sperm. We will present data to indicate that there is an early (mitotic or syncytial) magnification event that is dependent on the bb phenotype. In addition, we propose that there is also a late (meiotic) event that does not require a bb phenotype but does require some aberrant aspect of the *Ybb*$^-$ chromosome, other than the deficiency for rDNA.

MATERIALS AND METHODS

Stocks: The flies were raised at 24.5° on standard medium (TARTOF 1973). Descriptions of most of the mutants used in this study may be found in LINDSLEY and GRELL (1968). The pertinent chromosomes used here are as follows. *In(1)sc*^{4L}*sc*^{8R}, *y sc*^{4} *sc*^{8} *cv v B*, referred to here as *sc*^{4}*sc*^{8}, is an inverted *X* chromosome deficient for rDNA; *bb*^{2}, *bb*^{6} and *bb*^{8} are *X* chromosomal *bb* mutations that arose spontaneously in this laboratory (TARTOF 1973); *bb*$^{+ORE-R}$ refers to the *bb*$^+$ allele from our Oregon-R wild-type stock; *Ybb*$^-$ is a *Y* chromosome deficient for 80% of its rDNA (TARTOF 1973); *B*^{s}*Ybb*$^-$, a derivative of *Ybb*$^-$ that carries *B*^{s} on *Y*^{L}, was constructed by D. KOMMA and obtained from S. ENDOW; *C(1)DX, y f* is an attached-*X* chromosome deficient for rDNA; *C(1)DX, y f*$^+$ is a derivative of *C(1)DX, y f* obtained from the Pasadena Drosophila Stock Center; *In(1)sc*^{8}, *sc*^{8} is an *X* chromosome inversion with a distal breakpoint near *sc* and a proximal *X* breakpoint that is proximal to *bb*$^+$; *In(1)dl-49, y Hw m*^{2} *g*^{4}, referred to here as *dl-49*, is a *bb*$^+$ inverted *X* chromosome; *B*^{s}*v*$^+$*Ybb*$^+$*y*$^+$ is a *bb*$^+$ multiply marked *Y* chromosome; the wild-type *Y* chromosome used here was obtained from our standard Oregion-R stock; *B*^{s}*Y* is an otherwise normal *bb*$^+$ *Y* chromosome whose long arm is marked with *B*^{s}; *YbbSuVar* is a *Y* chromosome that suppresses the position effect variegation of *In(1)w*^{m4} and carries a lethal to severe *bb* allele (recovered by K.D.T.); and *Ybb*^{P2} carries a spontaneous lethal to severe *bb* allele (recovered by K.D.T.).

Classification of bb *and* bb$^+$: The *bb*^{2}, *bb*^{8} and *bb*^{6} alleles used here are all strong *bb* alleles with high penetrance. They exhibit severe abdominal etching, very short thin bristles that show little or no tapering and lengthened development time. In all cases both experimental and control flies were derived from the same *bb/B*^{s}*Y* fathers.

bb$^+$ revertant females were selected on the basis of complete reversion of the bb phenotype. Moreover, each *bb*$^+$/*sc*^{4}*sc*^{8} female was testcrossed to *sc*^{4}*sc*^{8}/*B*^{s}*Y* males, and their *X*/*sc*^{4}*sc*^{8} daughters were examined with respect to bb to ensure stability and completeness of reversion. In no case was an unstable *bb*$^{m+}$ chromosome recovered. It should be noted that such a classification scheme will necessarily underestimate the frequency of magnification events.

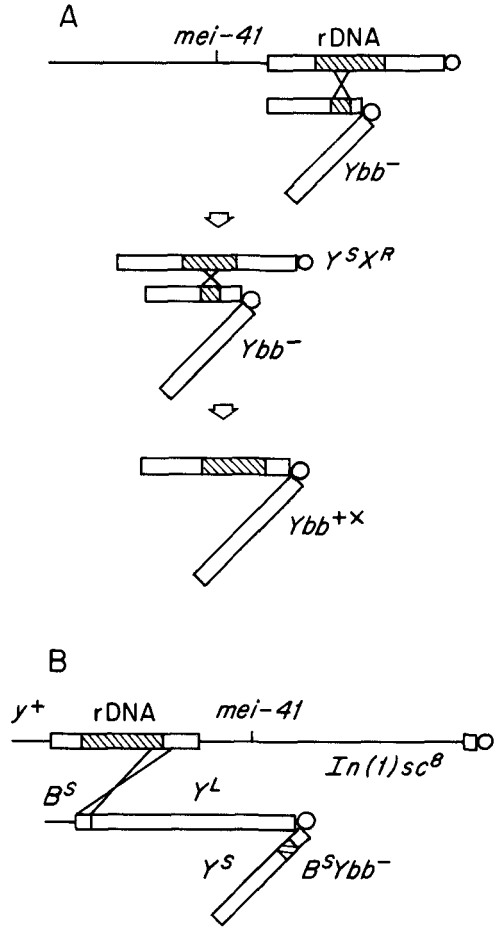

FIGURE 1.—Construction of the *bb* and bb^+ derivatives of Ybb^-. A, Diagram of the most likely origin of Ybb^{+X}. *mei-41*-induced interchange resulted in the recovery of a female carrying both Ybb^- and a Y^SX^R interchange product. In the process of recovering the interchange a bb^+ *Y* chromosome was obtained. This *Y* is presumed to have arisen by exchange between the short arm of Ybb^- and the Y^SX^R fragment. B, Diagram of the origin of y^+bbYbb^- and $y^+bb^+Ybb^-$. Interchanges were recovered from *In(1)sc*^{8}, bb^+ *mei-41*/B^SYbb^- males crossed to *C(1)DX,y f*/B^SY females. Thin lines indicate euchromatic regions of the *X*, blocks represent heterochromatin and diagonally filled blocks indicate rDNA.

Statistical analysis: All measurements of magnification frequency were obtained as averages over the progeny of a number of individual males. Statistical comparisons of frequencies of magnification were obtained by consulting the tables of KASTENBAUM and BOWMAN (1970).

RESULTS

Construction of bb^+ *and* bb *derivatives of* Ybb^-: We have previously isolated bb^+ and *bb* derivatives of Ybb^- as illustrated in Figure 1A by the following genetic crosses (HAWLEY and TARTOF 1983). In *mei-41* bb^+/Ybb^- males, interchange between the *X* chromosomal rDNA and one of a number of possible

sites on the Ybb^- chromosome occurs at a frequency of about 2×10^{-3} (HAWLEY and TARTOF 1983). Such interchanges are detected as viable and fertile *C(1)DX, y f* females that result from mating *y mei-41* bb^{+ORE-R}/Ybb^- males to *C(1)DX y f/*B^sY females. Cytogenetic analysis of the progeny of one such female revealed that it carried Ybb^- and a Y^SX^R interchange. In the process of isolating the Y^SX^R fragment, a bb^+Y chromosome was recovered that was cytologically indistinguishable from Ybb^- and fertile with a normal *X*. We infer that this chromosome arose by spontaneous exchange between Ybb^- and the Y^SX^R fragment. It is designated as Ybb^{+X} (where bb^{+X} denotes the recovery of the *X* bb^+ locus). Since *mei-41* can induce partial deficiencies of *X* chromosomal rDNA (HAWLEY and TARTOF 1983), it was possible to obtain a *bb* derivative of this chromosome by crossing *y mei-41 bb/*Ybb^{+X} males to *C(1)DX, y* f^+/B^sY females and screening for a *y* f^+ *bb* female. One such chromosome, Ybb^X, that carries a severe allele of bobbed was obtained and utilized.

Another type of bb^+ derivative of Ybb^- was constructed by crossing *In(1)*sc^8, sc^8 bb^+ *mei-41/*B^sYbb^- males to *C(1)DX, y f/*B^sY females (Figure 1B). Among the rare y^+ B^+ *f* female progeny, both bb^+ and *bb* flies, which also showed slight "hairy wing" effects, were recovered. Two of these exceptions, one of which was *bb* and the other bb^+, were chosen for further study. In both cases the B^s marker on Y^L of Ybb^- was replaced by y^+ bb^+ or y^+ *bb* from the *In(1)*sc^8 chromosome. This indicates that the site of exchange on the *X* occurred within or proximal to the rDNA of *In(1)*sc^8. Since both chromosomes are male fertile with a normal *X*, the exchange sites on the *Y* were distal to the distal-most fertility factor on the long arm of Ybb^-. These chromosomes are designated y^+bbYbb^- and $y^+bb^+Ybb^-$. A bb^{rl} derivative of y^+bbYbb^-, designated $y^+bb^{rl}Ybb^-$, was also recovered during the course of single pair matings of bb^8/y^+bbYbb^- males to sc^4sc^8*/dl-49 females.*

bb$^+$ *and* bb *derivatives of* Ybb$^-$ *induce magnification*: To examine the ability of these chromosomes to induce rDNA magnification, males were constructed that carried a *bb* allele on the *X* and a bb^+ or *bb* derivative of Ybb^-. These males were then singly mated to sc^4sc^8*/dl-49* females and the *X*/sc^4sc^8 progeny scored with respect to bb phenotype. All bb^+ exceptions were crossed to sc^4sc^8/ B^sY males, both to ensure that the putative reversions were not a consequence of nondisjunction of the *X* and *Y* chromosomes and to test the stability of those reversions that had occurred. As shown in Table 1, magnification of bb^2 does not occur in the presence of a wild-type *Y* or B^sY chromosome but does occur at high frequency (0.198 to 0.398) in males carrying Ybb^-. bb^+ derivatives of Ybb^-, Ybb^{+X} and $y^+bb^+Ybb^-$ also induced magnification of bb^2 but at a much lower frequency (0.013 to 0.095) than was observed for Ybb^-. Magnification is also observed in bb^2 males carrying *bb* derivatives of Ybb^- (Ybb^X, y^+bbYbb^- and $y^+bb^{rl}Ybb^-$) but at frequencies (0.107 to 0.018) that are intermediate between those observed for Ybb^- and its bb^+ derivatives. Similarly, these bb^+ and *bb* derivatives of Ybb^- also induce magnification of two other bobbed alleles, bb^6 and bb^8, although again at frequencies much lower than were obtained with Ybb^- (Table 2).

These data demonstrate that bb^+ or *bb* derivatives of Ybb^- are still capable of inducing magnification of bb^2, bb^6 and bb^8, albeit at frequencies that are at

TABLE 1

bb *and* bb$^+$ *derivatives of* Ybb$^-$ *induce magnification*

Paternal genotype	Paternal phenotype	bb phenotype of X/sc^4sc^8 progeny		No. of males producing 0, 1, or ≥2 bb^+ progeny[a]			Frequency of magnification	Level of significance[b]
		bb	bb^+	0	1	≥2		
bb^2/Y	bb$^+$	2710	0	(mass mating)			≤0.001	
bb^2/B^sY	bb$^+$	1010	0	45	0	0	≤0.001	
$y\ bb^2/Ybb^-$	bb	433	100	4	4	14 (6.8)	0.188	0.01
bb^2/B^sYbb^-	bb	171	113	2	3	8 (13.7)	0.398	0.01
bb^2/Ybb^X	bb$^+$	318	8	16	3	2 (2.5)	0.024	0.01
bb^2/Ybb^{+X}	bb$^+$	750	10	26	8	1 (2)	0.013	0.01
$bb^2/y^+bb^{r1}Ybb^-$	bb	649	78	20	5	10 (7.3)	0.107	0.01
bb^2/y^+bbYbb^-	bb$^+$	433	8	15	3	2 (2.5)	0.018	0.01
$bb^2/y^+bb^+Ybb^-$	bb$^+$	I. 499	3	18	1	1 (2)	0.006	0.05
		II. 1428	28	(mass-mating)			0.019	0.01

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

[a] The numbers in parentheses indicate the mean numbers of bb^+ progeny produced by males giving more than one revertant. In cases in which only one cluster of ≥two revertants was observed, the numbers in parentheses indicate only the size of that cluster.

[b] Statistical significance is computed using the tables of KASTENBAUM and BOWMAN (1970) by comparing the number of magnificants observed in the given experiment with that seen in the bb^2/B^sY control.

TABLE 2

bb *and* bb$^+$ *derivatives of* Ybb$^-$ *also induce magnification of* bb^6 *and* bb^8

Paternal genotype	Paternal phenotype	bb phenotype of X/sc^4sc^8 progeny		No. of males producing 0, 1, or ≥2 bb^+ progeny[a]			Frequency of magnification	Level of significance[b]
		bb	bb^+	0	1	≥2		
bb^6/B^sY	bb$^+$	605	0	20	0	0	≤0.002	
bb^6/Ybb^-	bb	722	105	13	7	10 (9.8)	0.127	0.01
bb^6/Ybb^{+X}	bb$^+$	748	6	21	3	1 (3)	0.008	0.05
bb^6/y^+bbYbb^-	bb$^+$	1914	3	46	3	0	0.002	NS
bb^8/B^sY	bb$^+$	417	0	20	0	0	≤0.002	
bb^8/Ybb^-	bb	382	57	4	3	9 (6.0)	0.130	0.01
bb^8/Ybb^{+X}	bb$^+$	755	10	5	4	1 (6)	0.013	0.05
bb^8/y^+bbYbb^-	bb$^+$	1626	5	35	5	0	0.003	NS

[a] The numbers in parentheses indicate the mean numbers of bb^+ progeny produced by males giving more than one revertant. In cases in which only one cluster of ≥two revertants was observed, the numbers in parentheses indicate only the size of that cluster.

[b] Statistical significance is computed using the tables of KASTENBAUM and BOWMAN (1970) by comparing the number of magnificants observed in the given experiment with that seen in the bb/B^sY control. NS, not significant.

least an order of magnitude less than those observed with the rDNA-deficient Ybb^- chromosome. Moreover, it is important to note that, as shown in Tables 1 and 2, this is primarily the result of the failure of males carrying *bb* or bb^+ derivatives of Ybb^- to produce the clusters of two or more bb^+ revertants that

TABLE 3

bb *and* bb$^+$ *derivatives of* Ybb$^-$ *induce magnification and reduction at similar frequencies*

Paternal genotype	No. of X/B^sY sons tested	Genotype of sons with respect to *bb*			Frequency of		Level of significance	
		bb^{+a}	*bb*	bb^1	Magnification	Reduction	Magnification	Reduction
bb^2/B^sY	2450	0	2540	0				
bb^2/Ybb^-	1027	360	653	14	0.350	0.014	0.01	0.01
bb^2/B^sYbb^-	780	89	683[b]	8	0.114	0.010	0.01	0.01
bb^2/y^+bbYbb^-	1313	9 (2)	1288	14	0.007	0.011	0.01	0.01
$bb^2/y^+bb^+Ybb^-$	800	5 (1)	791	3	0.006	0.004	0.01	0.05
bb^2/Ybb^{+X}	1013	0	1003	10		0.010		0.01

Males of the indicated genotype were crossed to *C(1)DX, y f/B^{s}Y* females, and the *X/B^{s}Y* sons were individually tested for bb^+, *bb* or bb^1 by crossing to four to five $sc^4sc^8/dl\text{-}49$ females. All putative bb^+ or bb^1 mutations were retested for at least two generations. In addition cytological examinations were performed to confirm that these exceptions are not the consequence of *X-Y* interchange.

[a] Numbers in parentheses indicate other males scored that produced both bb^+ and *bb* progeny. Testcrosses revealed that these males carried weak alleles of *bb* with low penetrance.

[b] One *X-Y*($y^+bb^+ \cdot B^s$) interchange was also recovered.

are so common among the progeny of Ybb^- bearing males. The frequencies with which males bearing Ybb^- or one of its *bb* and bb^+ derivatives produce single magnified bb^{m+} offspring are nearly the same.

Magnification and reduction events occur at similar frequencies in bb$^+$/bb$^+$Ybb$^-$ *males*: As noted bb^{m+} progeny produced by $bb/bbYbb^-$ or bb/bb^+Ybb^- males are not recovered in large clusters but rather as rare single events. This suggests that bb^{m+} loci recovered from bb/bb^+Ybb^- males may be arising very late in spermatogenesis, perhaps at meiosis. If this is the case then one might expect to recover the reciprocal event, namely, reduced (bb^{rl}) *X* chromosomes, at a frequency similar to that observed for bb^{m+} chromosomes.

To measure magnification and reduction simultaneously, males bearing a bb^2 *X* chromosome and the indicated *Y* chromosome were crossed to *C(1)DX,y f/B^{s}Y* females, and the bb^2/B^sY sons were then individually crossed to four or five $sc^4sc^8/dl\text{-}49$ females. Those males that yielded only bb^+/sc^4sc^8 daughters carry bb^{m+} alleles, and those that produce no X/sc^4sc^8 daughters but substantial numbers of *X/dl-49* progeny carry bb^{rl} alleles. For each putative bb^{m+} or bb^{rl} exception a number of *X/dl-49* sisters were testcrossed to confirm the phenotype.

As shown in Table 3, y^+bbYbb^-, $y^+bb^+Ybb^-$ and Ybb^{+X} derivatives of Ybb^- induce reduction at frequencies similar to those observed for Ybb^-. The failure to Ybb^{+X} to produce bb^{m+} loci in this particular experiment is not understood since this chromosome is capable of inducing magnification at a frequency similar to the frequency of reduction observed in this experiment (see Table 1). However, in the case of y^+bbYbb^- and $y^+bb^+Ybb^-$ in which magnification and reduction events are both observed, bb^{m+} and bb^{rl} alleles are recovered at virtually equal frequencies. This observation is consistent with the hypothesis that those bb^{m+} progeny arising in the germlines of bb/bb^+Ybb^- males are the

TABLE 4

Other Y *chromosomes also induce magnification*

Y chromosome	bb/Y phenotype	bb phenotype of bb^2/sc^4sc^8 progeny		Frequency of magnification	Level of significance[a]
		bb	*bb*$^+$		
$Ybb^{+ORE\text{-}R}$	Wild type	2151	0	≤0.001	
Ybb^-	Severe bb	653	360	0.355	0.01
Ybb^{P2}	Moderate-severe bb	2309	203	0.080	0.01
YbbSuVar	Slight bb	533	7	0.011	0.01
$B^sv^+Ybb^+y^+$	Wild type	1287	4	0.003	0.05

Males bearing a bb^2 *X* chromosome and the indicated *Y* were crossed to $sc^4sc^8/dl\text{-}49$ females and the X/sc^4sc^8 female progeny were scored with respect to *bb*.

[a] Level of significance is computed using the tables of KASTENBAUM and BOWMAN (1970) by comparing the number of magnificants observed in the given experiment with that seen in the control.

consequence of a reciprocal meiotic event, presumably unequal sister chromatid exchange.

Y *chromosomal variants unrelated to* Ybb$^-$ *also induce rDNA magnification*: In the preceding sections, it has been demonstrated that *bb* and *bb*$^+$ derivatives of Ybb^- are capable of inducing magnification, albeit at a reduced frequency. This demonstrates that the ability of Ybb^- to induce at least some magnification is not merely due to the deficiency for rDNA, or of the bb phenotype, but, rather, is a consequence of some other aberrant feature of this chromosome. Accordingly, one might predict that other *Y* chromosomes, whose origins are unrelated to Ybb^-, should also be capable of inducing rDNA magnification.

Males carrying a bb^2 *X* chromosome and either $Ybb^{+ORE\text{-}R}$, Ybb^{P2}, *YbbSuVar* or $B^sv^+Ybb^+y^+$ were crossed to $sc^4sc^8/dl\text{-}49$ females, and the results are presented in Table 4. Although no magnificants were observed among the progeny of males bearing a wild-type *Y* chromosome, they are obtained at modest frequencies (0.003 to 0.080) among progeny of males bearing the variant *Y* chromosomes. It may also be noted that, although all three variant *Ys* produce some bb^{m+} progeny, a high frequency of magnification is only observed in the presence of Ybb^{P2} which, like Ybb^-, produces a strong bb phenotype in combination with bb^2. These experiments demonstrate both that *Y* chromosomes unrelated to Ybb^- can in fact induce magnification and that the severity of the bb phenotype is correlated with frequency of magnification.

Magnification of bb Ybb$^-$ *chromosomes*: In the crosses presented magnification was always assayed with respect to the *X* chromosomal *bb* locus. However, it is also possible to demonstrate that *X* chromosomal rDNA is capable of being magnified when present on a *Y* chromosome. Table 5 presents the results of a series of crosses in which males bearing the sc^4sc^8 chromosome and either y^+bbYbb^- or Ybb^X were crossed to *C(1)DX*, $y\ f^+/B^sY$ females and the *C(1)DX*, $y\ f^+/Y$ daughters examined with respect to the bb phenotype. All *bb*$^+$ exceptional progeny were retested by crossing these females to sc^4sc^8/B^sY males and then mating the sc^4sc^8/Y sons back to *C(1)DX*, $y\ f^+/B^s$Y females. (The bb$^+$ phenotype

TABLE 5

Y chromosomal magnification as measured by matings of single sc^4sc^8/Ybb *males to* C(1)DX, y f^+/B^sY *females and scoring the* C(1)DX, y f^+/Y *female progeny with respect to* bb

Paternal genotype	bb phenotype of *C(1)DX y f⁺/Y* females		Frequency of magnification
	bb	*bb⁺*	
sc^4sc^8/Ybb^X	819	37	0.043
sc^4sc^8/y^+bbYbb^-	431	18	0.040

cannot be accurately assessed in sc^4sc^8/Y males due to the presence of scute.) In each case a modest frequency of magnification (about 0.040) was observed. This demonstrates that the ability of *X* chromosomal rDNA to undergo magnification is not solely a consequence of its location on the *X* chromosome.

DISCUSSION

The construction of *bb* and bb^+ derivatives of Ybb^- allows us to distinguish between events that produce large clusters of bb^{m+} revertants and those that produce rare single bb^{m+} and bb^{rl} chromosomes. To explain our data we will argue that there are two times at which magnification events can occur: an early event induced by the bb phenotype that gives rise to the large clusters of magnified loci and a late (meiotic) event, induced by Ybb^-, that is independent of the bb phenotype and gives rise to small numbers (one or two per male) of bb^{m+} and bb^{rl} loci.

First, we consider the class of rare magnification events that appear with similar frequencies in bb/bb^+Ybb^- and bb/Ybb^- males. These events necessarily occur late in spermatogenesis, well after the mitotic and syncytial stages, since they arise at low frequency and show no evidence of clustering. Therefore, they probably take place sometime during the meiotic process. The occurrence of bb^{m+} and bb^{rl} products in a phenotypically bb^+ germline demonstrates that these late magnification events are neither a consequence of the bb phenotype nor of the deficiency for rDNA carried by Ybb^-. Thus, the induction of meiotic magnification and reduction events is unrelated to rDNA dosage and appears to be the consequence of some other property of this chromosome, perhaps a deficiency for some site at or near the ribosomal RNA gene cluster. That is, the late class of events seem to represent a form of *X* rDNA mutability induced by an aberrant homologue. Moreover, the occurrence of bb^{m+} and bb^{rl} products at equal frequencies from late magnification events supports the suggestion of TARTOF (1974) that magnification and reduction are the reciprocal products that result from an unequal sister chromatid exchange. These data for late magnification events cannot be easily explained by an alernative mechanism for rDNA magnification proposed by RITOSSA (1968) in which rDNA genes are excised from the chromosome, replicated episomally and then reintegrated in an unstable fashion.

In contrast to the rare reciprocal events described before, individual bb/Ybb^- males also frequently give rise to large clusters of bb^{m+} progeny that appear in vast excess to their bb^{rl} sibs. Because of the size of these clusters and their distribution among bb/Ybb^- individuals, they necessarily reflect an early magnification event that probably arises during the mitotic or syncytial stage of germline development. To explain these observations it has been proposed (TARTOF 1974) that both magnification and reduction are reciprocal products of rare premeiotic unequal sister chromatid exchanges, and the excess of magnified loci compared to reductants is the result of a selective advantage that would accrue to a bb^+ revertant cell occurring in an otherwise bb germline. However, in bb/bb^+Ybb^- germlines the expected clusters of bb^{m+} are eliminated. Furthermore, we do not observe large clusters of bb^{rl} alleles that would be expected from phenotypically bb^+ cells that are genotypically bb^{rl}/bb^+Ybb^-. The fact that these clusters are eliminated in phenotypically bb^+ germlines and that the ability of various Y chromosomes to induce magnification is strictly correlated with the severity of the bobbed phenotype lead us to propose that the early magnification event is dependent on the bobbed phenotype. Although we propose that the bb phenotype is necessary for magnification, we do not know whether it is sufficient. It may be that the structural aberration associated with Ybb^- is also required. In any case, according to this model, bb/Ybb^- flies give rise to large clusters of bb^{m+} alleles as a consequence of early magnification events that are amplified by the further proliferation of the germline during the mitotic or syncytial stage. bb^{rl}/Ybb^- cells arising from early events would presumably die or otherwise fail to proliferate at this time, so that those bb^{rl} chromosomes that are obtained from bb/Ybb^- flies probably arise from late events. Thus, large clusters of bb^{m+} and bb^{rl} chromosomes would not be observed among the progeny of bb/bb^+Ybb^- males because the early germline events require a bb phenotype for induction.

This model also explains two observations regarding the behavior of bb^+/Ybb^- males. First, the rDNA content of a bb^+ X chromosome does not increase beyond the amount required for a bb^+ phenotype even when that X is maintained over Ybb^- for as many as ten generations (TARTOF 1973). This is explained by the hypothesis that the early high-frequency magnification event requires a bobbed phenotype and, therefore, does not occur in bb^+/Ybb^- germlines. Second, bb^+/Ybb^- flies will produce rare (presumably meiotic) bb progeny by reduction (TARTOF 1974). This is explained by the occurrence of late reciprocal events that require the Ybb^- but are independent of the bb phenotype. We would predict that reciprocal supermagnified bb^+ offspring also occur at low frequency but are not phenotypically distinct from their bb^+ sibs and so do not accumulate in the population.

Thus, according to our model, magnification would appear to be under two levels of control. At one level, the germline can adjust its abundancy of rDNA in response to the bb phenotype early in development at the mitotic or syncytial stages. Perhaps the induction of early magnification events is a consequence of the bb phenotype similar to the way in which Minutes enhance mitotic recombination in the somatic cells (KAPLAN 1953). A second level of

control occurs late in spermatogenesis, probably about the time of meiosis, and is independent of the bobbed phenotype. In at least this case the Ybb^- chromosome is required, and as we have suggested previously (HAWLEY and TARTOF 1983), the observed rDNA mutability may reflect aberrant homologue-homologue interactions, such as failed pairing. Although our hypothesis proposes two distinct times for the induction of magnification events, we are not suggesting that the magnification events themselves arise by different chromosomal processes. The failure of $R(1),bb/Ybb^-$ males to produce any bb^{m+} progeny (TARTOF 1974) suggests that the stable genetic reversion of bb to bb^+ is a consequence of unequal sister chromatid exchange whenever it occurs.

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