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Constitutive magnification by the Y^{bb^-} chromosome of *Drosophila melanogaster*

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Summary

Y^{bb^-} is an rDNA-deficient chromosome of *Drosophila* that has often been used in magnification experiments to induce high-frequency reversion of *bobbed* (*bb*) chromosomes. We observed previously that Y^{bb^-} causes ring chromosome loss even when the rings are bb^+, suggesting that Y^{bb^-} induces magnifying sister chromatid exchanges in bb^+ rings. Here we show that the Y^{bb^-} chromosome causes low levels of *bb* magnification in bb^+ flies. We refer to the ability of Y^{bb^-} to bypass the rDNA deficiency requirement for magnification as 'constitutive' magnification. We have magnified the ribosomal genes on the Y^{bb^-} chromosome and analysed the revertant chromosomes using genetic and molecular methods. We find that magnified Y^{bb^-} chromosomes also exhibit constitutive magnifier activity. Molecular analysis shows that both type 1 and type 2 intron$^+$ ribosomal gene repeats are associated with magnified Y^{bb^-} chromosomes. Type 2 introns have been described previously in the rDNA of both *X* and *Y* chromosomes. However, type 1 intervening sequences are thought to be present only in *X*, but not *Y*, ribosomal genes. Some of the Y^{bb^-} type 1 insertions differ from those present in the rDNA of *X* chromosomes in that they contain an *Eco*R I site, and some may be present in tandem arrays. The constitutive magnifier activity of Y^{bb^-} may reside either in the structurally unusual ribosomal gene intervening sequences associated with the chromosome, or in the locus on Y^L that is required for magnification to occur.

1. Introduction

The Y^{bb^-} chromosome was used by Ritossa (1968) in his initial demonstration of magnification in *Drosophila*. Ritossa observed frequent reversion of rDNA-deficient or *bobbed* (*bb*) chromosomes in male *Drosophila* that had subnormal levels of ribosomal genes. He named this phenomenon magnification. Magnification differs from other systems of gene amplification in that it occurs in the germline of rDNA-deficient flies and results in offspring with a heritable increase in ribosomal gene number. Present evidence supports a mechanism of unequal sister chromatid exchange (USCE) to account for the rDNA increase observed in *bobbed magnified* (bb^m) revertants (Tartof, 1974; Endow, Komma & Atwood, 1984; Endow & Atwood, 1988). Genetic studies of magnification in female *Drosophila* have defined two requirements for the induction of magnification (Komma & Endow, 1986; Komma & Endow, 1987). Magnification requires both rDNA deficiency and a locus on the long arm of the *Y* chromosome (Y^L). In the absence of either of these requirements, magnification does not normally occur.

Although the Y^{bb^-} chromosome can be used to create magnifying conditions, it is not a necessary component of the system: magnification is also observed when Y^{bb^-} is replaced by other Y^{bb} chromosomes. However, Y^{bb^-} causes ring chromosome loss even when the rings are bb^+ (Endow, Komma & Atwood, 1984), suggesting that the sister chromatid exchanges of magnification occur in bb^+ chromosomes in the presence of Y^{bb^-}. In addition, derivatives of Y^{bb^-} that carry ribosomal genes from an *X* chromosome cause low levels of magnification in bb$^+$ flies (Hawley & Tartof, 1985). These observations suggest that Y^{bb^-} can bypass the rDNA deficiency requirement for magnification. We refer to the ability to bypass one of the genetic requirements for magnification as 'constitutive' magnification (Komma & Endow, 1987).

Here we demonstrate that unaltered Y^{bb^-} causes low frequencies of magnification in phenotypically bb$^+$ flies. The Y^{bb^-} chromosome therefore shows consti-

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tutive magnifier activity. Further, we have magnified the ribosomal genes on the Y^{bb^-} chromosome and analysed the revertant ($Y^{bb^{-m}}$) chromosomes using genetic and molecular methods. $Y^{bb^{-m}}$ chromosomes cause constitutive magnification of *bb* alleles in bb$^+$ flies. $Y^{bb^{-m}}$ chromosomes also possess many intron$^+$ ribosomal genes, both type 1 and 2. The presence of type 2 repeats in the Y^{bb^-} chromosome was inferred in previous studies (Endow, 1982*a*). Type 1 repeats are thought to be absent from *Y* chromosomes, with the exception of *Y* derivatives such as *Y mal*$^+$ (de Cicco & Glover, 1983) which carry markers and presumably rDNA from the *X* chromosome. The type 1 insertions associated with $Y^{bb^{-m}}$ chromosomes are unusual in that some of them contain an *Eco*R I site, unlike the type 1 introns that have been described previously (Wellauer & Dawid, 1978), and some may be present in tandem arrays. The constitutive magnifier activity of the Y^{bb^-} chromosome may reside in its unusual ribosomal gene intervening sequences. Alternatively, the Y^L locus which is required for magnification in females (Komma & Endow, 1987) and probably also in males, may be altered in the Y^{bb^-} chromosome.

2. Materials and methods

Drosophila stocks. Most of the stocks used in these studies are described in Lindsley & Grell (1968) or Lindsley & Zimm (1992). The *Muller-5* (*M5*) chromosome is $In(1)sc^{S1L}sc^{8R}+S$, w^a *B* sc^{S1} sc^8. The *C(1)DX*, *y* w^a and $In(1)sc^{4L}sc^{8R}$, *y cv* sc^4 sc^8 [also referred to as $In(1)sc^4sc^8$] chromosomes carry bb^o deficiencies of the nucleolus organizer region. They are denoted $\widehat{XX}_{-NO}$ and X_{-NO}, respectively. *C(1)RM*, *y v bb*, denoted $\widehat{XX}$, is an attached *X* chromosome that carries sufficient rDNA to result in viable, but bb, $\widehat{XX}/O$ flies. *Dp(1;f)1209* was obtained from K. W. Cooper in 1967 and was found to carry a *bb* allele soon after receipt. bb^2 (Lindsley & Zimm, 1992) was originally recovered by Schultz from an Oregon R background (Tartof, 1973), and was obtained from R. S. Hawley in 1984. Y^{bb^-} was in our stock collection. The $B^S Y^{bb^-}$ chromosome was constructed by D.J.K. from the original Y^{bb^-} of Schultz as described in Endow (1982*a*).

Recovery of magnified revertants of Y^{bb^-}. Magnified Y^{bb^-} chromosomes were recovered by mating a $bb^2/B^S Y$ male to a *C(1)RM*, *y v bb*/Y^{bb^-} female to produce bb^2/Y^{bb^-} magnifying males. Single F_1 magnifying males were crossed to $X_{-NO}/X_{-NO}/B^S Yy^+$ females to test for magnification of the bb^2 chromosome and to $M5/bb^2$ females to continue the magnification. F_2 $bb^2/Y^{bb^{-m}}$ males were mated in single pairs both to *C(1)RM*, *y v bb*/$B^S Y^{bb^-}$ females to test for bb^2 magnification and to $M5/bb^2$ females to continue the magnification. In subsequent generations, $bb^2/Y^{bb^{-m}}$ males were taken only from vials in which bb^{2m^+} revertants were observed, and were mated in single pairs both to *C(1)RM*, *y v bb*/$B^S Y^{bb^-}$ tester females and to $M5/bb^2$ females to continue the magnification. The Y^{bb^-} chromosome is almost completely deficient for rDNA (Endow, 1982*a*) and is lethal in combination with *C(1)DX* or $In(1)sc^4sc^8$. Thus, in addition to improvement in phenotype with an X^{bb} chromosome, an independent measure of magnification is viability with *C(1)DX* or $In(1)sc^4sc^8$.

Tests for constitutive magnification. Tests of the Y^{bb^-} chromosome for constitutive magnification were carried out by mating phenotypically bb$^+$ males carrying *y* bb^2 and Y^{bb^-}, and either *Dp1209* or *Dp1185*, to tester y^2 w^{bf}/X_{-NO} females and monitoring the yellow X^{bb}/X_{-NO} and $X^{bb}/X_{-NO}/Y^{bb^-}$ female offspring for *bb* reversion. *Dp1209* and *Dp1185* are free duplications of the *X* chromosome that carry y^+ and *bb* or bb^+, respectively. Both free duplications give a bb$^+$ phenotype in combination with *y* bb^2. *bobbed* magnification was monitored by measurement of posterior scutellar bristles and by noting the presence or absence of abdominal etching. bobbed magnified (bb^m) refers to any improvement in phenotype relative to bobbed controls while bb^{m+} is reversion to wildtype.

Magnified revertants of Y^{bb^-} were tested for constitutive magnification by mating phenotypically bb$^+$ males carrying an X^{bb} chromosome and $Y^{bb^{-m}}$ to y^2 w^{bf}/X_{-NO} or $X_{-NO}/X_{-NO}/B^S Y$ tester females. X^{bb}/X_{-NO} female offspring were monitored phenotypically for reversion at *bb* as described above.

Tests for stability of bb^m revertant chromosomes were carried out by mating magnified females to males carrying $In(1)sc^4sc^8$ and examining the offspring for their phenotype with respect to *bb*.

Cytological analysis. Larval neuroblast squashes were prepared as described previously (Endow & Komma, 1986) and scanned under fluorescence after staining with DAPI (4′,6-diamidino-2-phenylindole).

Molecular analysis. DNA was prepared from larval neuroblasts and imaginal disks of individuals carrying magnified Y^{bb^-} chromosomes together with the X_{-NO} or $\widehat{XX}_{-NO}$ chromosome. Southern blots were carried out as described previously (Endow, 1982*b*) to visualize rDNA patterns in genomic DNA. Ribosomal genes from the $Y^{bb^{-m}}$ chromosomes were cloned by ligating *Hind* III-digested, gel-fractionated DNA to lambda 1149 (Murray, 1983). *In vitro* packaging, and plaque screening and purification were carried out as described (Maniatis, Fritsch & Sambrook, 1982). Recombinant phage carrying $Y^{bb^{-m}}$ ribosomal genes were analysed by digestion with restriction enzymes and by Southern blot hybridization with ribosomal gene, type 1 and type 2 intervening sequence probes labelled with ^{32}P by oligonucleotide primed synthesis (Feinberg & Vogelstein, 1984). In some experiments gel-purified restriction fragments from $Y^{bb^{-m}}$ ribosomal gene recombinants were used as probes for Southern blots of cloned ribosomal gene or type 1 intervening sequences. The cloned ribosomal genes used in these studies were pDmr·a51 #1 (Dawid, Wellauer & Long, 1978; Endow, 1982*b*), an intron$^-$ ribosomal gene repeat; pDm103 (Glover & Hogness, 1977), a

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Table 1. *Constitutive magnification by* Y^{bb^-}

Male parent	Offspring					Frequency of magnification
	+ or y^2 ♀	y bb ♀	y bb^m ♀	y bb^{m+} ♀	♂	
1. *y* bb^2/B^SY	450	311	0	0	643	< 0·003
2. *y* $bb^2/Dp1209/Y^{bb^-}$	752	273	8	0	633	0·028
3. *y* $bb^2/Dp1185/Y^{bb^-}$	1100	495	8	0	937	0·016

Males of the indicated genotypes were mated to y^2 $w^{bf}/In(1)sc^4sc^8$, *y cv* females. y female offspring of these matings were scored for their phenotype with respect to *bb*. *Dp1209* and *Dp1185* both carry y^+.

type 1 intron$^+$ repeat; and ckDm103A1 (Glover & Hogness, 1977), a subclone of pDm103 that includes the sites of type 1 and type 2 insertion in the 28S coding region (Dawid & Rebbert, 1981; Roiha *et al.* 1981). ckDm103A1 also contains 231 bp of type 1 intervening sequence (Roiha *et al.* 1981) and is missing 28S rDNA sequences 3′ to the type 1 insertion site. The type 1 and 2 intervening sequence recombinants were ckDm103B (Glover & Hogness, 1977) and the 0·7 kb *Eco*R I fragment from the 3·5 kb type 2 insertion (Long, Rebbert & Dawid, 1980), respectively. The 0·7 kb fragment is homologous to all size classes of type 2 insertion.

Statistical methods. Chi-square tests were carried out on samples in which expected values were $\geqslant 5$. The null hypothesis was that the test and control samples were from the same population. The expected frequency was calculated as the average obtained for the test + control samples. In samples in which an expected value was < 5, statistical tests were carried out assuming a Poisson probability distribution. The probability of observing i events, p(i), is $(e^{-m}\ m^i)/(i!)$ for a standard Poisson distribution, where m and i are the numbers expected and observed, respectively. m is calculated as (sample size) × (expected frequency).

3. Results

(i) *Unaltered* Y^{bb^-} *chromosomes show constitutive magnifier activity*

Reversion of *bb* in the presence of the Y^{bb^-} chromosome was tested by mating $X^{bb}/Dp/Y^{bb^-}$ males carrying a free duplication of the *X*, either *Dp1209* or *Dp1185*, to X/X_{-NO} tester females. *Dp1209* and *Dp1185* are y^+ and *bb* or bb^+, respectively. Males carrying either free duplication together with an X^{bb} chromosome and Y^{bb^-} are bb^+. X^{bb}/X_{-NO} and $X^{bb}/X_{-NO}/Y^{bb^-}$ female offspring of these matings were examined for magnification of the X^{bb} chromosome. Results of these crosses are shown in Table 1. *y* bb^2/B^SY control males (cross 1) produced 0 bb^m among 311 y female offspring. Males carrying *Dp1209* and Y^{bb^-} (cross 2) produced 8 bb^m among 281 y female offspring, a reversion frequency for the *y* bb^2 chromosome of 0·028. The probability of recovering both 0 bb^m among 311 offspring and 8 bb^m among 281 offspring is very low ($P = 5{\cdot}45 \times 10^{-4}$), indicating that the reversion frequency of the *y* bb^2 chromosome in $X^{bb}/Dp1209/Y^{bb^-}$ males differs significantly from that in X^{bb}/B^SY males. $X^{bb}/Dp1185/Y^{bb^-}$ males (cross 3) produced 8 y bb^{2^m} among 503 y female offspring, a reversion frequency which is not significantly different from that in $X^{bb}/Dp1209/Y^{bb^-}$ males. Tests of *y* bb^2 magnification in $X^{bb}/Dp/B^SY$ males carrying the bb^+ B^SY and either *Dp1209* or *Dp1185* resulted in no bb^m (not shown), thus the bb^m produced by $X^{bb}/Dp/Y^{bb^-}$ males can be attributed to Y^{bb^-} rather than to the *y* bb^2 chromosome or the free duplication of the *X*. The recovery of bb^m from bb^+ flies carrying Y^{bb^-} indicates that Y^{bb^-} causes *bb* reversion even in the absence of an rDNA-deficient phenotype. The frequency of *y* bb^2 magnification induced by Y^{bb^-} in bb^+ flies is low compared with the 0·490 $bb^m + bb^{m+}$ produced by *y* bb^2/Y^{bb^-} (Komma, Graves & Endow, 1989). We conclude that the Y^{bb^-} chromosome induces a low frequency of magnification in rDNA-non-deficient flies.

(ii) *Magnification of the* Y^{bb^-} *chromosome*

Magnified revertants of the Y^{bb^-} chromosome were recovered using the scheme shown in Fig. 1. In the F_0 generation, a single bb^2/B^SY male was mated to a *C(1)RM*, *y v* bb/Y^{bb^-} female to produce bb^2/Y^{bb^-} magnifying males. An F_1 magnifying male was crossed to a female carrying the X_{-NO} chromosome to test for reversion of the bb^2 chromosome, and to a $M5/bb^2$ female to continue the magnification. In subsequent generations single males were mated both to $\widehat{XX}/B^SY^{bb^-}$ females to test for reversion of the bb^2 chromosome and to $M5/bb^2$ females to continue the magnification. Males to continue the magnification were taken only from vials that produced $bb^{2^{m+}}$ chromosomes, with the rationale that males that produced X^{bb^m} were more likely to produce $Y^{bb^{-m}}$. No visible improvement in phenotype of $bb^2/Y^{bb^{-m}}$ males was apparent during the first three magnifying generations. $bb^2/Y^{bb^{-m}}$ males first showed an improvement in phenotype in the F_4 generation. $Y^{bb^{-m}}$ chromosomes were tested with the $\widehat{XX}_{-NO}$ chromosome and maintained with the y^2 w^{bf} *X* chromosome.

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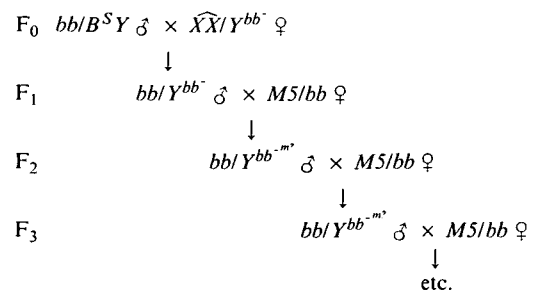

Fig. 1. Magnification of the Y^{bb^-} chromosome. A bb/B^SY male was mated to a $\widehat{XX}/Y^{bb^-}$ female to produce bb/Y^{bb^-} magnifying males. Magnifying males were mated in single pairs to $M5/bb$ females in each successive generation to continue the magnification. The same males were mated to females carrying an X_{-NO} (F_1) or $B^SY^{bb^-}$ (F_2-F_5) chromosome to test for magnification of the X^{bb} chromosome. $\widehat{XX} = C(1)RM$, *y v bb*.

Using this scheme, seven $Y^{bb^{-m}}$ revertant chromosomes representing three independent lineages were recovered. Chromosomes in three of the lines subsequently reverted to *bb*, leaving four revertant chromosomes derived from a single line. All of the $Y^{bb^{-m}}$ chromosomes showed a bb^+ phenotype with bb^2, however, two lines did not produce viable female offspring with $\widehat{XX}_{-NO}$. The other two lines, 7132-1 and 7132-2, produced viable, but bb, female offspring with $\widehat{XX}_{-NO}$ and bb male offspring with X_{-NO}. Magnification of 7132-2 for one further generation resulted in two lines which were bb^+ in combination with $\widehat{XX}_{-NO}$. The two lines were maintained with $\widehat{XX}_{-NO}$ and X_{-NO}.

(iii) *Cytological examination of* $Y^{bb^{-m}}$ *chromosomes*

Metaphase chromosomes of the $Y^{bb^{-m}}$ revertant lines 7132-1 and 7132-2 were examined after staining larval neuroblast squash preparations with the fluorescent dye, DAPI. The $Y^{bb^{-m}}$ chromosomes resemble wildtype *Y* chromosomes with respect to the number of DAPI-bright regions on Y^L and the presence of a nucleolar organizer constriction. The Y^{bb^-} chromosome has also a nucleolar organizer constriction (Komma & Endow, 1986) although it is not associated with a nucleolus [K. W. Cooper, cited in (Ritossa, 1976)]. The $Y^{bb^{-m}}$ revertant chromosomes are similar in appearance to Y^{bb^-} and wildtype *Y* chromosomes, and are not $\widehat{XY}$ chromosomes.

(iv) $Y^{bb^{-m}}$ *chromosomes exhibit constitutive magnifier activity*

$Y^{bb^{-m}}$ chromosomes were tested for constitutive magnifier activity by mating phenotypically bb^+ males carrying *y* bb^2 and $Y^{bb^{-m}}$ to $X_{-NO}/X_{-NO}/B^SY$ females. All of the $Y^{bb^{-m}}$ chromosomes that were tested caused low frequencies of *bobbed* magnification. Table 2 shows results of constitutive magnification tests for $Y^{bb^{-m}}$ chromosomes 7132-1 and 7132-2. Control males carrying *y* bb^2 with a normal bb^+ *Y* (cross 1) produced 0 bb^m among 295 y female offspring. $X^{bb}/Y^{bb^{-m}}$ 7132-1 males (cross 2) produced 3 bb^m and 8 bb^{m^+} among 150 y females, while $X^{bb}/Y^{bb^{-m}}$ 7132-2 males (cross 3) produced 6 bb^m among 183 y females. The probability of recovering both 0 bb^m among 295 offspring and 11 $bb^m + bb^{m^+}$ among 150 is very low ($P = 1{\cdot}75 \times 10^{-6}$). Similarly, the probability of recovering both 0 bb^m among 295 offspring and 6 bb^m among 183 is low ($P = 1{\cdot}33 \times 10^{-4}$). These results indicate that the frequency of bb^m observed in progenies of males carrying $Y^{bb^{-m}}$ chromosomes differs significantly from that observed for males carrying the same X^{bb} chromosome with a normal *Y* chromosome. Four of the $Y^{bb^{-m}}$ chromosomes were also tested for constitutive magnification of the *y* uco_3 *bb X* chromosome, resulting in frequencies of 1–6% bb^m. The $Y^{bb^{-m}}$ chromosomes therefore induce reversion of two different X^{bb} chromosomes in phenotypically bb^+ flies.

(v) *rDNA of* Y^{bb^-} *revertants contains type 1 and 2 introns*

DNA from $\widehat{XX}_{-NO}/Y^{bb^{-m}}$ larval neuroblasts and imaginal disks was analyzed on Southern blots using rDNA probes. Diploid larval tissue was used as the source of DNA to avoid changes in rDNA copy number due to polytenization or compensation (Spear, 1974). DNA was digested with restriction enzyme *Eco*RI before gel fractionation. Intron$^-$ ribosomal

Table 2. *Constitutive magnification by* Y^{bb^-} *magnified chromosomes*

Male parent	Offspring					Frequency of magnification
	y B^s ♀	y bb ♀	y bb^m ♀	y bb^{m^+} ♀	♂	
1. *y* bb^2/Y	266	295	0	0	408	< 0·003
2. *y* bb^2/Y^{bb^-} 7132-1	259	139	3	8	338	0·073
3. *y* bb^2/Y^{bb^-} 7132-2	367	177	6	0	422	0·033

Males of the indicated genotype were mated to $In(1)sc^4sc^8$, *y cv*/$In(1)sc^4sc^8$, *y cv*/B^SY females. y female offspring of these matings were scored for their phenotype with respect to *bb*.

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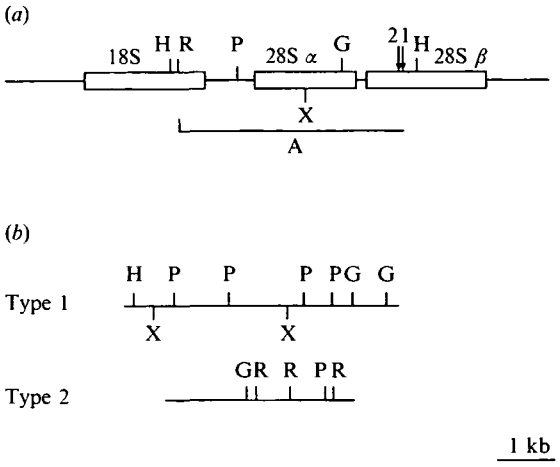

Fig. 2. Structure of ribosomal gene repeat and intervening sequences. (*a*) An 18S + 28S ribosomal gene repeat with adjacent spacer regions. The 28S gene consists of the 28Sα and 28Sβ RNA regions. The positions of type 1 and 2 insertion (1, 2) and the *Eco*R I-*Hind*III A fragment are indicated. The *Hind*III site at the righthand end of the A fragment lies in a type 1 insertion (see b). (*b*) Restriction enzyme maps of a 5 kb type 1 and a 3·5 kb type 2 insertion (Roiha & Glover 1980). R = *Eco*R I, P = *Pst* I, X = *Xma* I, G = *Bgl* II, H = *Hind*III.

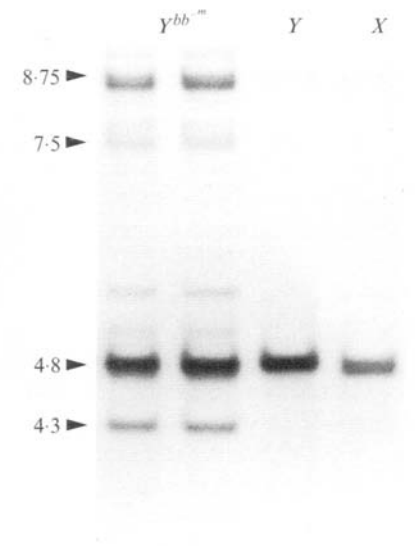

Fig. 3. Ribosomal genes in $Y^{bb^{-m}}$ chromosomes. Autoradiograph of a Southern blot after digestion of DNAs with *Hind*III and hybridization with ^{32}P-A fragment. DNA from $\widehat{XX}_{-NO}/Y^{bb^{-m}}$ 7132-1 or 7132-2 ($Y^{bb^{-m}}$), $\widehat{XX}_{-NO}/Y^{OR}$ (*Y*) and X^{OR}/X^{OR} (*X*) larval brains and imaginal disks. OR = Oregon R.

genes of *D. melanogaster* have a single *Eco*R I site per repeat (Fig. 2). The type 1 intervening sequences that have been described have no *Eco*R I sites, while type 2 introns have 1–3 *Eco*R I sites. Digestion with *Eco*R I can therefore reveal type 1 repeats as those larger than intron$^-$ repeats, and type 2 repeats as those smaller than intron$^-$ repeats.

Southern blots of *Eco*R I-digested DNA from $\widehat{XX}_{-NO}/Y^{bb^{-m}}$ flies carrying the 7132-1 and 7132-2 $Y^{bb^{-m}}$ chromosomes, hybridized with an intron$^-$ rDNA probe, showed a major repeat of $\sim$ 10·5 kb with minor rDNA bands of $\sim$ 16–17 kb (not shown). The *Eco*R I hybridization patterns were compared with the pattern of the Oregon R *Y* chromosome (Endow & Glover, 1979) and found to be similar, although the major rDNA repeat associated with the $Y^{bb^{-m}}$ chromosomes was somewhat smaller than the 12 kb major repeat of the Oregon R *Y*. Digestion with *Eco*R I did not reveal any unusual intron content of the $Y^{bb^{-m}}$ chromosomes.

Southern blots of *Hind*III-digested DNA from revertants 7132-1 and 7132-2 were hybridized with the A fragment from the cloned rDNA repeat, ckDm103A1 (Glover & Hogness, 1977) (Fig. 2). The A fragment contains 18S and 28S rDNA sequences, including the sites of type 1 and 2 insertion in the 28S gene. *Hind*III cuts in the major type 1 rDNA repeat to produce an A-homologous fragment of $\sim$ 4·8 kb that is slightly smaller than for intron$^-$ repeats. The Oregon R *X* rDNA pattern (Fig. 3) shows a doublet at $\sim$ 4·8 kb that arises from intron$^-$ and type 1 intron$^+$ repeats. The Oregon R *Y* chromosome lacks such type 1 repeats and shows a band of 4·8 kb that, in shorter exposures, can be seen to correspond to the upper band of the doublet in the *X* (Fig. 3). The 4·8 kb band of the $Y^{bb^{-m}}$ chromosomes corresponds to the lower band of the *X* doublet and probably arises from a shorter transcribed spacer rather than a difference in the 28S gene, based on further mapping experiments. The $Y^{bb^{-m}}$ chromosomes also exhibit prominent bands of 8·75 kb and 4·3 kb that are present in much lower amount (8·75 kb), or missing (4·3 kb) from the Oregon R *X* and *Y* rDNA patterns.

The unusual rDNA fragments in the $Y^{bb^{-m}}$ chromosomes were cloned from *Hind*III-digested 7132-1 DNA. Four cloned fragments, one of 8·75 kb, one of 7·5 kb and two of 4·3 kb, were analyzed. The structures of the four fragments are shown in Fig. 4. The 8·75 kb fragment is a type 2 repeat bearing two 0·7 kb *Eco*R I fragments at one end. The 2·4 kb *Bgl*II-*Hind*III fragment containing these *Eco*R I fragments hybridizes with a type 2 intervening sequence probe. The other end of the 8·75 kb fragment hybridizes with the A fragment and has a similar, but non-identical, restriction enzyme pattern: the *Pst* I site in the transcribed spacer is missing in the 8·75 kb fragment and a *Bgl*II site is present near the lefthand *Hind*III site that is not found in previously described ribosomal gene repeats. A similar *Bgl*II site is present in the 7·5 kb fragment.

One end of the 7·5 kb fragment hybridizes with the A fragment, and 1·5 kb of the righthand, non-

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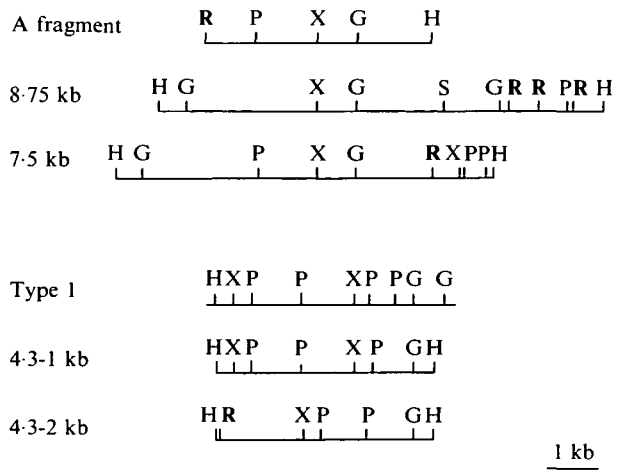

Fig. 4. Structure of $Y^{bb^{-m}}$ cloned rDNA fragments. Structures of the A fragment and a 5 kb type 1 intervening sequence are shown for comparison. The *Eco*R I sites are shown in bold. R = *Eco*R I, P = *Pst* I, X = *Xma* I, G = *Bgl* II, H = *Hind* III, S = *Sal* I.

hybridizing end is homologous to a type 1 insertion probe. The restriction map of the A-homologous region of the 7·5 kb recombinant is similar to that of the A fragment. The 7·5 kb fragment may therefore correspond to an rDNA repeat with a type 1 intron in its normal insertion site. However, the presence of an *Eco*R I site in the insertion makes it different from previously described type 1 intervening sequences. The restriction map of the type 1 sequences in the 7·5 kb fragment resembles the internal region of a 5 kb type 1 intron. This is unlike previously characterized 1 kb and 0·5 kb type 1 insertions, which correspond to the right-most sequences of a 5 kb intron (Dawid & Wellauer, 1977).

There are at least two classes of 4·3 kb fragments that are distinguished by differing *Eco*R I, *Xma* I and *Pst* I sites. The two characterized in this study are designated 4·3-1 and 4·3-2. Only 0·4 kb at one end of each fragment (*Bgl* II-*Hind* III fragment) hybridizes with the 1·4 kb *Bgl* II-*Hind* III fragment of A. The hybridization is due to type 1 sequences in the A fragment, since neither 4·3-1 nor 4·3-2 hybridizes with an intron$^-$ rDNA repeat. Both 4·3 kb fragments hybridize with a type 1 insertion, but show no hybridization with the 0·7 kb *Eco*R I fragment from a type 2 insertion. The 4·3 kb fragments therefore correspond to type 1 intervening sequences. However, the restriction maps of the 4·3 kb fragments differ from those of *X* chromosome type 1 insertions (Dawid, Wellauer & Long, 1978; Roiha & Glover, 1980). 4·3-1 is missing a *Pst* I site, while 4·3-2 contains an *Eco*R I site which is not present in previously described type 1 insertions, and differs from cloned type 1 insertions in position and number of *Pst* I and *Xma* I sites. Finally, both 4·3 kb fragments have *Hind* III sites at either end, but neither fragment contains ribosomal gene sequences. The 4·3 kb type 1 sequences may be present in tandem arrays in the rDNA of the $Y^{bb^{-m}}$ chromosomes, or present outside of the rDNA. Alternatively, the $Y^{bb^{-m}}$ type 1 intervening sequences may have more than one *Hind* III site, differing from previously characterized type 1 insertions on the *X* chromosome.

The 7·5 kb and 4·3 kb fragments thus represent type 1 insertions that are associated with the $Y^{bb^{-m}}$ chromosome. The restriction patterns of the three type 1 fragments are atypical compared with previously described type 1 intervening sequences. Type 1 repeats are thought to be absent from *Y* chromosome rDNA. The probable reason that type 1 repeats are not observed as a major 17 kb band on Southern blots of *Eco*R I-digested $Y^{bb^{-m}}$ DNA is that some of the insertions contain *Eco*R I sites, in contrast to the type 1 insertions described for the *X* chromosome.

4. Discussion

The Y^{bb^-} chromosome arose spontaneously in a stock of *equational producer* (*eq*) flies (Bridges & Brehme, 1944). It carries a strong deletion of *bb*, although some ribosomal genes can be detected in backgrounds of *X* chromosomes with different patterns of length and intron heterogeneity (Endow, 1982*a*). The ribosomal genes that were detected previously were inferred to be high in type 2 intron content from digestion patterns with *Eco*R I. The present analysis substantiates this finding and indicates the presence of several classes of type 1 intervening sequences associated with the $Y^{bb^{-m}}$ chromosomes.

The Y^{bb^-} chromosome was used as a helper chromosome by Ritossa (Ritossa, 1968) in his original demonstration of magnification, and has been widely used to induce magnifying conditions. It is known, however, that other Y^{bb} chromosomes can also serve as helper chromosomes in magnification experiments. The finding that the Y^{bb^-} chromosome can induce magnification even in rDNA-non-deficient flies indicates that it possesses genetic activity that can bypass the normal requirement of rDNA deficiency for the induction of magnification. We refer to this as constitutive magnifier activity. Evidence from ring chromosome experiments indicated previously that the Y^{bb^-} chromosome causes loss even of bb^+ rings (Endow, Komma & Atwood, 1984). This suggests that magnifying sister chromatid exchanges are induced by Y^{bb^-} in bb$^+$, as well as bb, flies. Y^{bb^-} chromosomes carrying *X* ribosomal genes have been demonstrated to cause low frequencies of *bb* reversion in bb$^+$ flies (Hawley & Tartof, 1985). With the use of these *bb* or bb^+ Y^{bb^-} chromosomes, however, it was not certain whether the constitutive magnifier activity was a property of the Y^{bb^-} chromosome or the *X* ribosomal genes associated with the chromosome. Here we demonstrate that unaltered Y^{bb^-} induces *bb* reversion in rDNA non-deficient flies, and that magnified Y^{bb^-} chromosomes also show this ability.

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The basis of the constitutive magnifier activity of the Y^{bb^-} chromosome and its magnified derivatives is not certain. It may represent an alteration of one of the normal components of the magnification system, or an alteration of an unrelated function, resulting in neomorphic activity. Meiotic loss of ring X^{bb} chromosomes in the presence of Y^{bb^-} can be attributed to increased sister chromatid recombination that in rod X^{bb} chromosomes results in magnification (Endow, Komma & Atwood, 1984). The observation that bb^+ ring chromosomes undergo loss in the presence of Y^{bb^-} (Endow, Komma & Atwood, 1984) therefore suggests that the constitutive magnifier activity of Y^{bb^-} affects a component of the normal system of magnification that is induced by rDNA-deficient conditions.

The Y^{bb^-} magnified chromosomes that we recovered and analysed contain ribosomal gene repeats with type 1 intervening sequences, as well as type 1 sequences that may be present in tandem arrays. Except for *Y* chromosomes that are known to carry *X* chromosome sequences, type 1 insertions are thought to be absent from *Y* chromosomes. The type 1 sequences of the Y^{bb^-} magnified chromosomes may have been acquired by recombination or other interactions with an *X* chromosome. However, some of the type 1 sequences on the $Y^{bb^{-m}}$ chromosomes are structurally unusual in that they contain an *Eco*R I site. Such type 1 insertions have not been described previously and are not known to exist in *X* chromosomes.

The constitutive magnifier activity of Y^{bb^-} may represent the low level activation of the locus on Y^L (Komma & Endow, 1987) that is needed for magnification in females, and presumably also in males. A second possibility is that the constitutive magnifier activity of Y^{bb^-} is related to the presence of the structurally unusual type 1 ribosomal gene insertion sequences associated with the magnified Y^{bb^-} chromosomes. Hawley & Tartof (1983) have suggested that gaps or nicks made in or near type 1 insertions may be recombinogenic, and that type 1 sequences may be part of the system that regulates magnification. The mere existence of type 1 sequences does not lead to magnification, however, since X^{bb} chromosomes that contain type 1 sequences do not magnify in *X*/*Y* males unless the *Y* chromosome is *bb*. Type 1 variants could explain the ability of Y^{bb^-} to facilitate the process, if they encode active type 1 elements. Active elements may show low-level activity, which is increased under rDNA-deficient conditions. Evidence that ribosomal gene elements are potentially capable of transposing is provided by the identification of putative integrase-encoding regions in the type 1 and 2 insertions of *D. melanogaster* (Jakubczak, Xiong & Eickbush, 1990) and the observation that the integrase-analogous gene of the R2 insertion in *Bombyx mori* is functional *in vitro* (Xiong & Eickbush, 1988). The nicks or breaks made in the rDNA during the transposition process may serve as the initiation sites for the sister chromatid exchanges of magnification.

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