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Expression and amplification of the genes for ribosomal RNA in bobbed mutants of *Drosophila melanogaster*

BY F. LEE DUTTON AND HALLIE M. KRIDER

Biological Sciences Group, Molecular Genetics and Cell Biology Section, The University of Connecticut, Storrs, CT 06268

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SUMMARY

We have employed stocks bearing clonally derived *X* chromosomes to investigate several features of the bobbed mutant syndrome, and the amplification of rDNA genes in *D. melanogaster*. We report that posterior macroscutellar bristle length correlates well with the rDNA content (i.e. dose of *ivs*−, or uninterrupted genes) in cloned *X* derivative strains. *X/O* males and *X/X* females with statistically indistinguishable rDNA contents have virtually identical bristle lengths. This indicates that (with respect to this phenotypic character) the rDNAs in these two genotypes are expressed equally, without apparent sexual dimorphism or dosage compensation. However, the severity of bobbed phenotype in terms of bristle morphology, turgite etching, and delayed eclosion is greater in the X^{bb}/X^{NO-} female than in the X^{bb}/O male genotype for the alleles examined. We estimate the minimum dose of functioning rRNA genes required for viability at 26 °C to be 70 genes per diploid genome. We have examined the capacity of several *X* chromosomes which bear bobbed mutant alleles to compensate in *X/O* males, and find that disproportionate replication of these rDNAs does not take place. In contrast, at least one of the non-compensating bobbed alleles does appear to undergo rDNA magnification.

1. INTRODUCTION

Mutations at the bobbed locus are pleiotropic, resulting in a range of effects on the development and morphology of *Drosophila* (for a review, see Ritossa, 1976). Each bobbed locus represents a certain dosage of tandemly linked ribosomal RNA genes (usually about 250) and any combination of alleles in which the total number of functional genes contributed by the two loci sums to a certain minimum will be viable. Beyond a second, higher threshold, the phenotype of the animal will be wild type. This additivity indicates that the loci on both homologues are simultaneously active in at least some tissues at some stages of development. It has been demonstrated that not all ribosomal genes have equal transcriptional potential (Long & Dawid, 1979; Long, Rebbert & Dawid, 1981*a*; Long, *et al.* 1981*b*), and that total cistron dosage does not always correlate well with the severity of the bobbed phenotype (Franz & Kunz, 1981).

Endonuclease Eco RI digestion of DNA from certain strains of *Drosophila melanogaster* produces major fragments of about 17 and 11·5 kilobases, each of

which contain sequences homologous to both the 18S and 28S rRNAs in Southern blot and R-loop analyses (White & Hogness, 1977; Glover & Hogness, 1977). The 11·5 kb fragment defines the basic repeating unit. Additional DNA in the 17 kb piece is due to the presence of an inserted DNA sequence of unknown function (Wellauer & Dawid, 1977; Glover & Hogness, 1977; White & Hogness, 1977; Pellegrini, Manning & Davidson, 1977). A large portion of the ribosomal genes of *Drosophila* contain such sequences. Renkawitz-Pohl, Glatzer & Kunz (1981) reported that ribosomal RNA genes with an intervening sequence are clustered within the *X* chromosomal rRNA genes of *D. hydei*. In contrast, initial reports based upon electron microscopic analyses indicated that *ivs*+ genes appeared to be distributed randomly in *D. melanogaster* (Pellegrini *et al.* 1977). Hawley & Tartof (1983) conducted an analysis in *D. melanogaster* similar to that of Renkawitz-Pohl *et al.* (1981), and observed large restriction fragments which they interpreted as clusters of two to three insert bearing genes. These clusters were detected at a frequency expected to result from random arrangement of *ivs*+ and *ivs*− genes. (Unfortunately, the probe used in this last analysis contained no *ivs*+ sequences, leaving the *ivs*+ nature of the large fragments unconfirmed.) Several bobbed and wild-type alleles examined by Dutton & Krider (1984*b*) showed comparable proportions of *ivs*− and Type I *ivs*+ genes, despite threefold differences in total rDNA content. This appears to be consistent with (although not proof of) a random interspersion of *ivs*+ and *ivs*− genes. The organization of rDNA in different stocks may well differ. Furthermore, cytological evidence has been presented to support the thesis that Type I inserts are located largely in the distal half of the *D. melanogaster X* chromosome rDNA locus (Hilliker & Appels, 1982). Within this distal region, *ivs*+ and *ivs*− sequences may or may not be distributed randomly.

The fact that the X^{bb}/X^{bb} phenotype is less extreme than that of the X^{bb}/O (Lindsley & Grell, 1968), is usually taken to indicate that, unlike other *X*-linked genes, the rDNA cluster does not normally undergo transcriptional dosage compensation. However, rDNAs do appear to undergo a replicational amplification in certain contexts. In appropriate crosses, single *N.O.* progeny (X/O, X/X^{NO-}) are found to undergo a 'compensatory replication' to increase the rDNA content of individuals by 50–100% (Tartof, 1971, 1973; Yedvobnick, Krider & Dutton, 1980; Dutton & Krider, 1984*a*). This additional rDNA is evidently not heritable (Tartof, 1973). Ritossa (1968) and others have found that maintaining an X^{bb} allele in males in the presence of certain rDNA-deficient *Y* chromosomes results in a heritable elevation in the rDNA redundancy of the X^{bb} *NO*, as well as reversion of the bobbed phenotype.

In the following report we show: (1) that rDNA (*ivs*−) gene dosage is highly correlated with bristle phenotype in *D. melanogaster*, whereas some other features of the bobbed syndrome are not; (2) that rDNA cistrons are expressed equally (in terms of bristle phenotype) in X^{bb}/X^{bb} and X/O genotypes; (3) that fewer than 70 functional rDNA genes are required for survival at 26 °C; (4) that several bobbed alleles tested do not undergo rDNA compensation as X^{bb}/O males, and that (5) at least one of these non-compensating bobbed alleles can be shown to undergo rDNA magnification.

2. MATERIALS AND METHODS

Drosophila stocks and culture conditions

D. melanogaster stocks were reared on standard cornmeal, agar, and sucrose medium in half-pint milk bottles, at 26 ± 1 °C.

The Oregon R stock is described in Lindsley & Grell (1968). The attached $\overline{XX}$ and $\overline{XY}$ stock SNOC-R86 has been described previously (Dutton & Krider, 1984*a*). Two additional stocks were constructed. One contains the X^{NO-} chromosome $y\ sc^{4L}\ cv\ v\ f\ sc^{8R}$ derived from stock G21 (Oak Ridge Laboratories), $C(1)DX$ ($y\ f$), and $B^s Y$. This stock also contains uncloned autosomes which are derived from the Oregon R population. The second stock is $C(1)RM/\ Y^{bb-}$ and $X/\ Y^{bb-}$, in which the free X's and autosomes are derived from Oregon R. $sc^{4L}\ sc^{8R}$; $C(1)DX$; $B^s Y$; and Y^{bb-} are described in Lindsley & Grell (1968).

(ii) *Eclosion studies*

Vials were innoculated with 30 virgin females and 5–10 males. After 2 days these parents were transferred to fresh medium in half-pint milk bottles and maintained at 26 °C. Flies were then transferred to two additional sets of bottles at 12 h intervals. Cultures were cleared of adults 12 h after the last transfer and incubation continued at 26 °C for 9 days. Bottles were then scored for the appearance of female or male adults, and scored again every 12 h thereafter until eclosion was complete.

(iii) *Bristle measurements*

The left side posterior macroscutellar bristles of 40–100 flies of each genotype were measured using an American Optical binocular microscope equipped with an ocular micrometer calibrated in millimetres.

(iv) *Generation of* X/O *males*

Virgin females were collected within 12 h of eclosion at 18 °C, and held for several days at 21 °C. These flies were then mated to attached $\overline{XY}$ males of SNOC-R86. After egg laying, all parents were removed, and X/O males were collected from the eclosing progeny. X/O males should be sterile, and only fertility-tested X/O males were analysed.

(v) *Generation of* $\mathrm{X/X^{NO-}}$ *females*

X/X^{NO-} females were obtained by crossing females of the cloned X stocks to males of genotype X^{NO-} $(y\ sc^{4L}\ cv\ v\ f\ sc^{8R})B^s Y$. Each of the cloned X lines is a derivative of Oregon R in which the X chromosomes are identical and the autosomal population derived approximately 25% from Oregon R (Dutton & Krider, 1984*b*). SNOC-R86 and the $X^{NO-}/B^s Y$ males were both bred to contain the same autosomal population present in the female parents.

(vi) *Induction of magnification*

Males of stock 03A ($X^{bb}/B^s Y$) were crossed to females of genotype $C(1)RM/\ Y^{bb-}$ (25 pairs of parents per bottle). The males obtained in G1 were then mated to

females of the genotype above, and the process repeated for three generations. As a control, males of the 03A stock were mated in parallel cultures to females of genotype $C(1)RM/B^s Y$.

(vii) *Molecular Biology*

DNA quantitation, liquid [^{3}H]rRNA-rDNA microhybridization, and DNA extraction were as described previously (Dutton & Krider, 1984*b*).

3. RESULTS

(i) *Correlation of rDNA content and bristle length*

When cloned X stocks with moderate, high, and low rDNA contents are chosen for comparison, a direct correlation between bristle length and rDNA content is obtained (Table 1 and Fig. 1). A correlation coefficient of 0·79 ($P < 0{\cdot}01$) can be calculated from the data in Table 1. Since the proportion of *ivs*− genes relative to total rDNA cistrons is constant for each of the chromosomes employed in this study (Dutton & Krider, 1984*b*), this result establishes a correlation similar to that reported earlier for *D. hydei* (Franz & Kunz, 1981).

The rDNA contents of X/O males in this study were calculated from corresponding female values measured previously (Dutton & Krider, 1984*b*). Bristle lengths should not be affected by possible increases in rDNA redundancy due to disproportionate replication (Tartof, 1973). It is clear that in every case X/O bristles are significantly shorter than those of females homozygous for an identical X. Notably, this is true even for chromosomes with moderate and high rDNA contents. The ability to detect slight but significant differences in bristle lengths in this study can be ascribed to the reduced component of genetic variation in cloned X strains. (Compare the S.E.M.s of cloned X strains to those of the Oregon R female and X/O populations in Table 1.)

The dramatic difference in bristle length between X^{bb}/X^{bb} females and their corresponding X^{bb}/O males is as expected (Lindsley & Grell, 1968). It is striking that flies with statistically indistinguishable rDNA contents (e.g. 03A females, 011B X/O males) have equally similar bristle lengths, regardless of sex. Thus it appears that, for the dose of rDNA genes present in these strains, the expression of an rDNA gene is equal in trichogen cells of X/X and X/O genotypes.

(ii) *Eclosion rates of bobbed mutants*

Rates of eclosion were compared for X/O males generated from seven of the cloned X strains. Six of the chosen strains are bobbed, and one provides a wild-type control. Fig. 2 illustrates the result, which shows that bobbed mutants with similar bristle lengths and rDNA contents array themselves in at least three different categories, all of which exhibit delayed eclosion relative to the wild type. It was also observed that, despite comparable bristle lengths, X^{bb}/O males displayed varying severity of turgite etching, ranging from severe to wild type. These observations merely demonstrate that, when rDNA content is limiting, factors other than the ribosomal RNA genes alone can influence certain features of the bobbed syndrome. However, as demonstrated in this report, bristle length can be a reliable reflection of rDNA content in carefully constructed stocks.

Table 1. *rDNA content and bristle length in cloned-X females and X/O males*

N	*Genotype*	rDNA content ±2 S.E.M. (%)	Bristle length ± 2 S.E.M. (mm)
100	010C Female	0·580±0·021	0·434±0·003
100	011B Female	0·383±0·008	0·425±0·007
48	010C *X/O*	0·290±0·021*	0·405±0·004
46	011B *X/O*	0·192±0·008*	0·396±0·003
100	08A Female	0·210±0·012	0·402±0·005
100	06D Female	0·214±0·007	0·369±0·006
100	03A Female	0·188±0·007	0·395±0·010
46	08A *X/O*	0·105±0·012*	0·302±0·006
43	06D *X/O*	0·107±0·007*	0·291±0·006
46	03A *X/O*	0·094±0·007*	0·293±0·005
100	Ore R Female	0·432±0·005	0·412±0·009
100	Ore R *X/O*	0·216±0·005*	0·381±0·014

N, Number of posterior macroscutellar bristles measured.
Female rDNA contents from Dutton & Krider (1984*b*).
* *X/O* Male rDNA contents = input rDNA dose (i.e. one half female content).

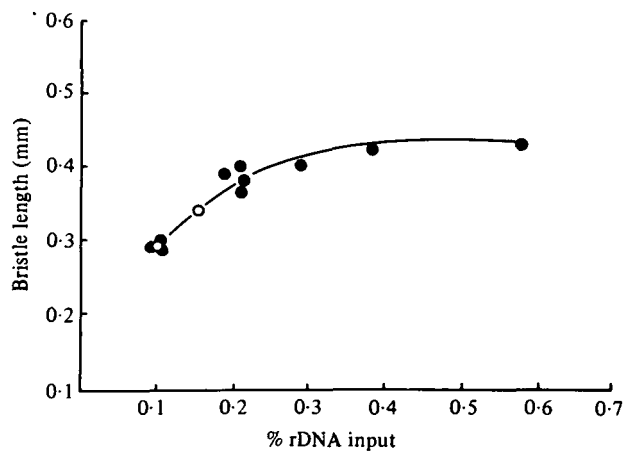

Fig. 1. Correlation of rDNA content and bristle length. The rDNA contents of the ten cloned *X* genotypes reported in Table 1 are plotted against bristle lengths measured in the appropriate adults (●). ○, 03A X/Y^{bb-} genotypes before and after magnification (see text).

(iii) *Comparison of* X^{bb}/O *and* X^{bb}/X^{NO-} *phenotype*

Females from each of several bobbed lines were mated to males of the genotype $X^{NO-}/B^{s}Y$. Non-disjunction of these two modified sex chromosomes is approximately 39% when spermatogenesis occurs at 25 °C (Peacock, Miklos & Goodchild, 1975). As a result, X^{bb}/O and X^{bb}/X^{NO-} genotypes can be generated with comparable frequency from the same cross. When this was done, the resulting X^{bb}/O males were viable, and obtained in large numbers. In contrast, the majority of X^{bb}/X^{NO-} females were unable to complete eclosion. Those which did emerge were more severely bobbed than their X^{bb}/O male counterparts in terms of bristle and turgite morphology, and eclosed later. Thus among the bobbed alleles tested, the expression of the bobbed syndrome is most severe in X^{bb}/X^{NO-} females, least

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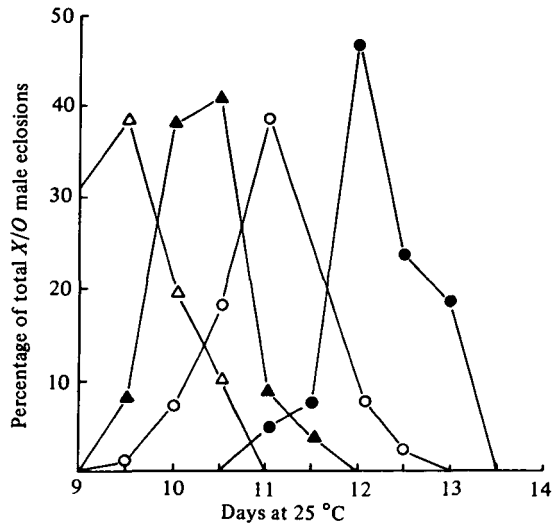

Fig. 2. Eclosion of X/O males at 26 °C. The rDNA contents of the cloned X stocks employed in this study are found in Dutton & Krider (1984*b*). △, 011B (bb^+, rDNA % 0·19); ▲, 08A (*bb*, rDNA % 0·10); ○, 03A (*bb*, rDNA % 0·09); ●, 012D (*bb*, rDNA % 0·11). For simplicity, only eclosions of 011B, 08A, 03A, and 012D are shown. Eclosions of 011D (*bb*, rDNA % 0·09) and 02D (*bb*, rDNA % 0·11) are indistinguishable from that of 03A. Eclosion of 06D (*bb*, rDNA % 0·11) is indistinguishable from that of 08A. Results are the average of two experiments.

severe in X^{bb}/X^{bb} females, and intermediate in X^{bb}/O males. A greater severity of X^{bb}/X^{bb-1} relative to X^{bb}/O and X^{bb}/X^{bb} is characteristic of bobbed alleles studied previously (Lindsley & Grell, 1968).

(iv) *Minimum dose of rDNA*

The rDNA contents of the bobbed stocks employed in this study have been carefully determined, and the relative stability of these contents has been demonstrated (Dutton & Krider, 1984*b*). Stability is further confirmed by the failure to observe any wild-type flies among several thousand putative X^{bb}/O males generated during the course of these studies. Likewise, no bobbed flies were observed among the X/Os generated from cloned wild-type alleles. Since an input of 0·1 % rDNA will allow only a few X^{bb}/X^{NO-} progeny to eclose at 26 °C, while permitting X^{bb}/O progeny to develop fully, this can be considered to represent a reasonable estimate of the minimum amount of rDNA required for viability. Since we further estimate that about one half of the X chromosomal rDNA genes in each of these stocks contain a Type I intervening sequence, and are therefore transcriptionally inactive (Long & Dawid, 1979), the actual minimum may be taken as 0·05 %.

(v) *Compensatory response of* X^{bb}/O *males*

Tartof (1973) reported that the compensatory response among bobbed chromosomes in X/X^{NO-} females was roughly proportional to input dose of rDNA. In

contrast, we have found that for wild-type X chromosomes, the amount of disroportionate replicaton in X/O males is not a simple function of starting dose (Dutton & Krider, 1984*a*). It was of interest to examine the disproportionate replication of several bobbed isolates from Oregon R. Four such chromosomes were studied,representing at least two different rDNA contents. The results, displayed in Table 2, reveal that no compensatory replication was observed in any of these crosses.

Table 2. *rDNA content in X/X and X/O gentoypes of bobbed mutants*

Genotype	rDNA % ± S.E.M.	rDNA %/X	Change (%)
011D X/X	0·176 ± 0·008 (8)	0·088	—
011D X/O*	0·061 ± 0·009 (8)	0·061	−30·7
03A X/X	0·187 ± 0·011 (18)	0·094	—
03A X/O*	0·086 ± 0·012 (18)	0·086	−8·5
06D X/X	0·208 ± 0·004 (5)	0·104	—
06D X/O*	0·097 ± 0·007 (5)	0·097	−6·7
08A X/X	0·219 ± 0·018 (8)	0·110	—
08A X/O*	0·113 ± 0·011 (8)	0·113	+2·7

Numbers in parentheses indicate number of tests.
* X/O values were corrected by a factor of 0·9 (Tartof, 1971).

Table 3. *Bristle lengths during magnification in cloned-X derivative 03A (bobbed)*

Generation	$X/B^s Y$ ± 2 S.E.M.	X/Y^{bb-} ± 2 S.E.M.	$Y^{bb-}/B^s Y \times 100$
*G*1	0·421 ± 0·006	0·303 ± 0·006	71·9
*G*2	0·420 ± 0·005	0·346 ± 0·008	82·4
*G*3	0·426 ± 0·010	0·353 ± 0·013	82·9

Table 4. *Magnification of rDNA in 03A/ Y^{bb-} males*

Genotype	rDNA content ± 2 S.E.M.	rDNA/X	Increase (%)
X^{bb}/X^{bb}*	0·188 ± 0·024 (5)	0·094	—
$X^{bb}/B^s Y$†	0·390 ± 0·022 (5)	0·094	—
X^{bb}/Y^{bb-}†	0·150 ± 0·027 (5)	0·150	60 %

(Numbers in parentheses indicate number of hybridization.)
* Female of 03A cloned X line. This value has been determined previously (Dutton & Krider, 1984*b*), and was employed here as internal standard.
† Males of the third generation.

(vi) *Magnification in 03A/ Y^{bb-}*

Especially in light of the fact that the X^{bb} mutants analysed above did not compensate as X^{bb}/O males, it was of interest to examine the ability of these alleles to undergo magnification. Stock 03A was selected, and magnification of the X^{bb} locus induced by carrying out the crosses described in Methods. At each generation, the posterior macroscutellar bristles of at least 25 males were measured, and the results of these measurements are presented in Table 3. Clearly, the mean bristle length of X^{bb}/Y^{bb-} males increased in G2, with no significant further increase in G3. We found that in both G2 and G3 virtually all bristles were longer than the G1 mean, but shorter than the chosen control ($X^{bb}/B^s Y$). At each generation, after

measuring their bristles and allowing them to mate $C(1)RM/Y^{bb-}$ females, the males were sacrificed and their DNA extracted. The rDNA contents of 03A females, males, and G3 X^{bb}/Y^{bb-} males were determined by saturation rRNA-rDNA hybridization. The results of this experiment are recorded in Table 4. In G3, the 03A X^{bb} chromosome appars to have undergone a 60 % elevation in rDNA content.

4. DISCUSSION

The results presented in Fig. 1 illustrate that bristle morphology correlates well with total rDNA dose for the *D. melanogaster* strains examined in this report. The proportion of rDNA genes which bears an intervening sequence is approximately 50 % in each of these strains (Dutton & Krider, 1984*b*). Since *ivs*+ sequences are known to be transcriptionally inactive (Long & Dawid, 1979; Long *et al.* 1981*a*, *b*) we can infer from the above that bristle phenotype is actually dependant upon the dose of *ivs*− genes, over the range of rDNA contents reported here. Franz & Kunz (1981) have reported that a similar correlation of bristle morphology and *ivs*− gene redundancy can be detected in *D. hydei*.

When females and X/O males with identical rDNA contents are compared, bristle lengths do not exhibit detectable sexual dimorphism (Table 1 and Fig. 1). For example, 03A females, homozygous for a bobbed X, have an rDNA content of 0·19 %. These females have bristles which are indistinguishable from those of 011B X/O males, in which the same dose of rDNA (0·19 %) is present in a single chromosome. In this case, males and females each have only X chromosomal rDNAs, and the two genotypes each have the same total rDNA dose and proportion of *ivs*+ genes. Weinmann (1972), Shermoen & Kiefer (1975), and Clark, Strausbaugh & Kiefer (1977) have established that bristle length is a direct reflection of transcriptional activity, both in whole flies and in certain isolated tissues. As described above, we have observed that bristle length in X/X and X/O genotypes of our derivative strains correlates well with rDNA dose. We therefore infer that, in these two genetic contexts, transcription of rDNA is dependant only upon the availability of template. In other words, here we observe neither transcriptional regulation of the type observed by Shermoen & Kiefer (1975), nor dosage compensation of the kind which typifies other X-linked loci. Nevertheless, it is clear that transcriptional regulation of the rDNAs does occur in certain situations. Krider & Plaut (1972) obtained evidence of increased transcription per nucleolus organizer in salivary gland preparations of X/Os relative to X/Xs, while Shermoen & Kiefer (1975), Clark & Kiefer (1977), and Clark *et al.* (1977), have established that some component or components of the chromosome Y^{bb} SuVar elevates transcription in general, including that of rDNAs.

We have noted that all aspects of the X^{bb}/X^{NO-} phenotype are more severe than that of X^{bb}/O males. If the phenotype (bristle length) correlates well with transcriptional activity for a given dose of rDNA, as we have concluded above, then we could infer that transcription of rDNA genes differ between X^{bb}/O and X^{bb}/X^{NO-} genotypes. Tartof (1973) has reported compensatory replication of rDNAs in the X^{bb}/X^{NO-} genotype, and finds that disproportionately replicated rDNA apparently is not functional in bristle forming cells. Assuming that a

compensatory response does occur in X^{bb}/X^{NO-} females, the process could conceivably interfere with transcription, resulting in the greater severity of X^{bb}/X^{NO-} phenotype. Alternatively, regulation of transcription may differ between X^{bb}/X^{NO-} and X^{bb}/O.

Study of X^{bb}/O eclosions demonstrates that not all features of the bobbed syndrome correlate equally well with rDNA dose. Despite similar rDNA contents and autosomal backgrounds, X^{bb}/O males with virtually indistinguishable bristle lengths exhibit eclosion rates which vary greatly (although all X^{bb}/O males eclose later than X^{bb+}/O controls, Fig. 2). Clearly, factors in addition to rDNA (*ivs*−) dose are also influencing phenocritical events in X/O development.

Females of the strains 03A and 08A have an rDNA content equal to about 0·19 % of total adult DNA (Dutton & Krider, 1984*b*). X/O males and X/X^{NO-} females derived from these strains therefore receive parental inputs of approximately 0·09 %. We have found that X/O males of these strains do not compensate rDNAs (see below). Furthermore, about one half of the X chromosomal rDNA cistrons in the strains we have investigated contain Type I intervening sequences, and are presumed to be transcriptionally inactive. This lowers the effective dose to about 0·047 % of the total weight of adult DNA. A diploid female genome contains 0·40 pg of DNA (Mulligan & Rasch, 1980). 0·047 % of this value equals $1{\cdot}88 \times 10^{-4}$ pg. The weight of combined 18S and 28S transcripts which we have used as probe is taken to be $2{\cdot}1 \times 10^{6}$ AMU (Tartof & Perry, 1970), or $3{\cdot}486 \times 10^{-6}$ pg. Based upon these figures, there will be about $1{\cdot}88 \times 10^{-4}/3{\cdot}486 \times 10^{-6} = 54$ functional rDNA cistrons in an 03A X/O male or X/X^{NO-} female. An additional correction to this estimate is necessary. In Oregon R (from which 03A is derived), 0·56 % of diploid DNA hybridizes to rDNA (Dutton, 1982), as compared to 0·43 % in adult females (Dutton & Krider, 1984*b*). Under-replication of rDNAs in polytene tissue is presumably the reason for the apparently 30 % lower adult rDNA contents. (Note that there may be a bias for *ivs*− gene replication in polytene tissue.) The actual input of functional rDNA cistrons in 03A X/O males may therefore be corrected to $1{\cdot}3 \times 54 = 70$ genes. Since an input of about 70 genes will allow X/O males to develop, while this same number will allow some X/X^{NO-} females to develop, this can be taken as a reasonable estimate of the minimum required dose of functional rDNA genes for *D. melanogaster* reared at 26 °C.

As reported previously, we have not found that the magnitude of the compensatory response among wild type derivatives of Oregon R is correlated with the initial redundancy of the rDNAs (Dutton & Krider, 1984*a*). Tartof (1973) reported that a rough correlation exists between magnitude of the compensatory response and the input dose of rDNA in bobbed alleles, when measured in X^{bb}/X^{NO-} females. Due to the poor viability of the alleles in this study when reared at 26 °C, we were unable to confirm this result. However, our X^{bb} chromosomes survived well as X^{bb}/O males, and all failed to compensate in this genotype. We do not know if this failure is a general feature of bobbed X/O's, or is unique to the chromosomes which we have analysed. Furthermore, autosomal loci are known to affect the compensatory response of Canton S (Dutton & Krider, 1984*a*). Although no such effects were detected in previous analyses of Oregon R, they cannot be ruled out in the present study.

Although failing to compensate in several repeats of the experiment, bobbed allele 03A does undergo magnification in the standard cross first described by Ritossa (1968). Thus, it is clear that magnification can occur independently of the compensatory response. Since 03A does not compensate (in X^{bb}/O males), it was possible for the first time to analyse magnification in the absence of compensatory disproportionate replication. In this analysis, the rDNA content of Y^{bb^-} was presumed to be virtually zero (Spear, 1974; Hilliker & Appels, 1982; Yedvobnick, 1980). This estimate is further supported by the fact that G1 X^{bb}/Y^{bb^-} bristle lengths (0·303 mm) are not significantly different from those of X^{bb}/O (0·293 mm). Virtually all of the increase in bristle length during magnification occurred in G2. No non-magnified bristles were detected in G2, although non-magnified individuals may have been selected against by the mass mating protocol employed. Since we have established that bristle length can accurately reflect rDNA content, we infer that magnification has occurred to roughly the same extent in all of the individuals scored. We do not know if the rDNA content of G3 would be stable upon outcrossing from $03A/Y^{bb^-}$. The rDNA content and bristle lengths of G1 and G3 X^{bb}/Y^{bb^-} males can be plotted on the curve of Fig. 1. The locations of the resulting points provide support for the estimate of rDNA amplification obtained in the hybridization experiment. If the estimate is accepted, the curve can be taken to demonstrate that magnified and non-magnified rDNAs are expressed equally.

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