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Regulation of rRNA gene number in *Drosophila melanogaster*: New aspects resulting from the use of free duplications

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Summary. We have isolated a *bobbed* (*bb*) mutant on the free duplication Dp(1; f)122bb^+ and we have measured the rDNA content of the bb^+ and the *bb* loci in genetic combinations in which none of the phenomena involved in the change of the rDNA redundancy occurs. We have also measured the rDNA content of the two *bb* loci carried by the free duplications in two different genetic combinations: (1) $\hat{X}\hat{X}NO^-$/Dp122bb^+ and $\hat{X}\hat{X}NO^-$/Dp122*bb* females in which there are two attached X chromosomes completely deleted for the nucleolus organizer (NO) regions and therefore the only rDNA is contributed by the free duplication; (2) X/Dp122bb^+ and X/Dp122*bb* males, in which there are two *bb* loci, one on the X chromosome and the other on the X free duplication.

The bb^+ and the *bb* duplications produced an overall increase of the rDNA content in the two genetic conditions tested.

These results are not in favour of both a *cis* and *trans* effect of the regulator locus (cr^+ locus) hypothesised as being involved in the disproportionate replication of rRNA genes.

Introduction

In *Drosophila melanogaster* a cluster of 150–200 genes coding for 28S and 18S ribosomal RNA (rRNA) has been localized at the *bobbed* (*bb*) locus of both X and Y chromosomes (Ritossa et al. 1966). Flies with fewer rRNA genes than the wild type show a reduced rate of rRNA synthesis (Mohan and Ritossa 1970; Weimann 1972) and exhibit a mutated, hypomorphic, pleiotropic phenotype called *bb*. This phenotype is characterised by small bristles, abdominal etching and reduced growth rate, viability and fertility. The phenotype of a fly can range from extreme *bb* to bb^+ and tends to be related to the total number of rDNA cistrons (i.e. X chromosome plus X or Y chromosome contribution). However, discrepancies between rDNA content and phenotypic expression have been reported (Ritossa et al. 1971; Marrakechi and Prud'Homme 1971; Tartof 1971, 1973; Shermoen and Kiefer 1975; Gargano and Graziani 1976).

The rDNA content at the *bb* loci is remarkably unstable and several mechanisms have been proposed to explain this phenomenon: meiotic unequal crossing-over within the *bb* loci (Schalet 1969), rDNA magnification (Ritossa 1968; Boncinelli et al. 1972) and somatic gene compensation (Tartof 1971, 1973; Spear and Gall 1973; Malva et al. 1979).

When an X chromosome carrying either a wild-type or a partially deleted *bb* locus (but not a Y or an attached $\hat{X}\hat{Y}$ chromosome) is introduced in a male without a Y chromosome (X/O) or in a female with the other X chromosome carrying a complete deletion of the nucleolus organizer (NO) region (X/XNO^-), the ribosomal cistrons increase (somatic gene compensation). This increase has no phenotypic effect and is not inheritable (Tartof 1971, 1973; Hennig and Meer 1971; Spear and Gall 1973). Moreover, it has been suggested (Procunier and Tartof 1978) that rDNA compensation is regulated by the compensatory response (cr^+) locus, located in the centromeric heterochromatin of the X chromosome, which exhibits both a *cis* and a *trans* effect. It acts in *trans* to detect the presence of its partner cr^+ locus on the opposite homologue and, if only one cr^+ is present, it acts in *cis* to drive the disproportionate replication of those rRNA genes that are contiguous to it.

Free duplications can be carried as a supernumerary chromosome in addition to the normal diploid set. The use of free duplications of the X chromosome offers new possibilities to study the regulation of the rDNA level. In previous studies free duplications have been tested in combination with a partner X chromosome (Procunier and Tartof 1978) but have not been tested in $\hat{X}\hat{X}NO^-$ females in which all the rDNA present is contributed by the free duplications because the attached X chromosomes lack rRNA genes.

We have used Dp(1; f)122 (Fig. 1), which is a free duplication of the X chromosome deleted of most of the euchromatin (Lindsley and Sandler 1958) and a *bb* mutant selected on such a duplication. We measured the rDNA content of the bb^+ and *bb* duplications in $\hat{X}\hat{X}bb^+$/Dp122bb^+ and $\hat{X}\hat{X}bb^+$/Dp122*bb* females where no change in the level of rDNA occurs. We took these values as the typical rDNA content of the two duplications. We then measured the rDNA content of $\hat{X}\hat{X}NO^-$/Dp122bb^+ and $\hat{X}\hat{X}NO^-$/Dp122*bb* females or X/Dp122bb^+ and X/Dp122*bb* males.

In all these genotypes containing the free duplications we found an rDNA increase. The rDNA increase obtained with $\hat{X}\hat{X}NO^-$/Dp122 females indicates that the *bb* and bb^+

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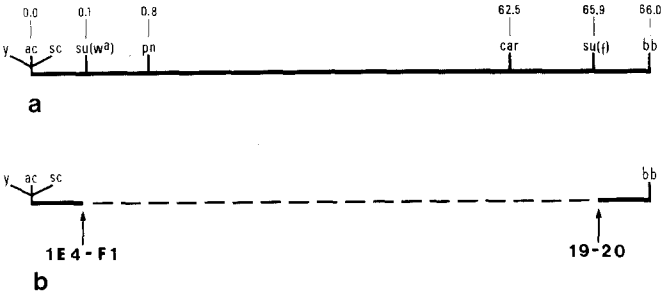

Fig. 1 a, b. Scheme of the *D. melanogaster* X chromosome (**a**) and the 122 free duplication (**b**). The Dp122 carries the wild-type alleles *y, ac, sc* and *bb* but lacks *su(w^a), pn, car* and *su(f)*. The breakpoints are 1E4-F1; 19–20. Dashed lines represent the part of the chromosome that is missing

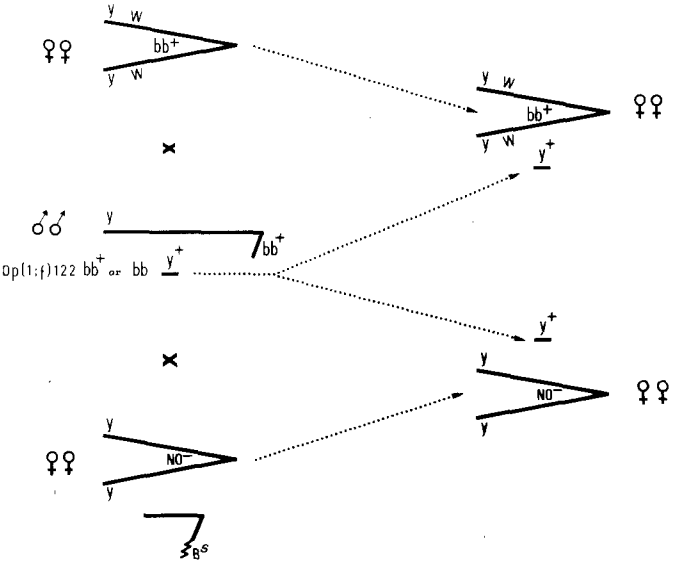

Fig. 2. Scheme of the crosses made to generate different attached $\widehat{XX}$ females with the bb^+ or the *bb* duplication. $\widehat{XY}$ attached males carrying the bb^+ or the *bb* duplication are crossed with $\widehat{XX}bb^+$ virgin females to obtain $\widehat{XX}bb^+$/Dp122 females or with $\widehat{XX}NO^-$/B^SY females to obtain $\widehat{XX}NO^-$/Dp122 females

duplications should carry a cr^+ locus adjacent to the rDNA. Nevertheless the non-additive rDNA content obtained with the different X/Dp122 males tested in which two cr^+ loci must be hypothesised, one on the X chromosome, the other on the free duplication, does not seem to support the model of the *cis-trans* effect of the cr^+ gene.

Materials and methods

Drosophila strains. (1) C(1)DX, *y f*/B^SY and In(1)sc^8, bb^L *cv*/B^SY from the Pasadena collection. In this strain the females have attached X chromosomes which have lost the NO regions. In the text these attached chromosomes are indicated as $\widehat{XX}NO^-$.

(2) C(1)RM, *y w* and XY^LY^S, *y w f*, from the University of Chicago. In this strain the females have attached X chromosomes carrying bb^+ loci; these chromosomes are indicated in the text as $\widehat{XX}bb^+$.

(3) C(1)RM, *y v f*/Dp(1; f)122 and XY^L, Y^S lJ^{259}, *y w*/Dp122 from the Bowling Green collection. This is the strain carrying the original free duplication in combination with attached X females and attached $\widehat{XY}$ males.

Isolation of bb mutation on Dp(1;f)122. The bb^+ duplication mentioned as Dp122bb^+ carries, besides the *bb* locus, *y ac sc* wild-type loci (Fig. 1). To isolate a *bb* mutant on this free duplication, $\widehat{XY}$/Dp122bb^+ X-ray treated males (about 2,000 r) were crossed with $\widehat{XX}NO^-$/B^SY virgin females with a complete deletion of the NO region on the $\widehat{XX}$ chromosomes. Therefore, in such a cross, survival and *bb* phenotype of female offspring depends, respectively, on the presence and number of rRNA genes carries by the free duplication. Hence, a strong *bb* mutant could be isolated and introduced in a suitable strain ($\widehat{XX}ybb^+$/Dp122*bb*; $\widehat{XY}ybb^+$/Dp122*bb*).

To generate $\widehat{XX}bb^+$/Dp122bb^+ and $\widehat{XX}bb^+$/Dp122*bb* females, the following procedure was used: $\widehat{XY}$/Dp122bb^+ or $\widehat{XY}$/Dp122*bb* males were crossed with $\widehat{XX}bb^+$ virgin females: the y^+ females were collected for 10 days and stored for DNA (Fig. 2).

To generate $\widehat{XX}NO^-$/Dp122bb^+ and $\widehat{XX}NO^-$/Dp122*bb* females, $\widehat{XY}$/Dp122bb^+ and $\widehat{XY}$/Dp122*bb* males were crossed with $\widehat{XX}NO^-$/B^SY virgin females; the y^+ females were collected for 10 days and stored for DNA extraction (Fig. 2).

rRNA extraction. [^{3}H]-labelled ribosomal RNA was extracted from wild-type larvae according to Ritossa et al. (1971) and purified on sucrose density gradients (Scherrer and Darnell 1962). The specific activity was not lower than 100,000 cpm/μg.

DNA extraction. DNA from adult flies was extracted as described by Ritossa et al. (1966).

rRNA/DNA hybridisation. rRNA/DNA hybridisations were performed after sticking alkali-denatured DNA to nitrocellulose filters according to Gillespie and Spiegelman (1965). For each determination the percentage of DNA hybridised was averaged from at least ten values at saturation, the standard error was approximately ±0.005%. For each genotype, the rDNA content was calculated from three to four separate determinations; the standard error was approximately ±0.010%. In X/O males the values obtained from rRNA/DNA hybridisation experiments have been multiplied by 0.9 to account for the 10% reduction in the total nuclear DNA content (Henderson and Ritossa 1970).

Results

Dp122bb^+ is a free duplication in which most of the X euchromatin has been deleted (Lindsley and Sandler 1958) (Fig. 1).

A strong *bb* mutant was obtained with the use of X-ray mutagenesis. This new *bb* free duplication remained unchanged for the other genetic markers carried by the original Dp122bb^+. We then calculated the amount of rDNA carried by the bb^+ and by the *bb* duplication, according to the rRNA/DNA hybridisation technique, in different genetic combinations.

Table 1. The percentage of rDNA carried by the Dp122bb^+ and Dp122bb measured in attached $\widehat{XX}bb^+$ females

Genotype	% rDNA hybridization	$XXbb^+$ contribution	Dp122 contribution
$\widehat{XX}bb^+/O$	0.215	0.215	–
$\widehat{XX}bb^+/Dp122bb^+$	0.338	0.215	0.123
$\widehat{XX}bb^+/Dp122bb$	0.285	0.215	0.070

a) rDNA content of the free duplications in combination with $\widehat{XX}bb^+$ chromosomes

To avoid possible changes in the percentage of rDNA due to somatic gene compensation or magnification, we measured the percentage of rDNA carried by the duplications in phenotypically bb^+ females with attached $\widehat{XX}$ chromosomes ($\widehat{XX}bb^+/Dp122$) where these phenomena do not occur. The compound X chromosomes have an rDNA percentage of 0.215 (Table 1) and therefore the rDNA contributed by the bb^+ and bb duplications in combination with these X chromosomes is 0.123% and 0.070%, respectively (Table 1). We consider these values as the basic rDNA content of the two duplications.

b) rDNA content of the free duplications in combination with $\widehat{XX}NO^-$ chromosomes

We then measured the rDNA content of the two free duplications in combination with the $\widehat{XX}NO^-$ chromosomes, which have completely lost the NO regions. In these flies, the bb phenotype depends exclusively on the rDNA carried by the free duplications: $\widehat{XX}NO^-/Dp122bb^+$ females are phenotypically bb^+ and $\widehat{XX}NO^-/Dp122bb$ are strongly bb flies. We obtained an rDNA percentage of 0.203 for the bb^+ duplication and 0.129 for the deleted bb (Table 2, lines a, b). Our results indicate that: (1) the rDNA contributed by the two duplications differs depending on whether they are combined with $\widehat{XX}NO^-$ or $\widehat{XX}bb^+$ chromosomes; (2) there is an rDNA increase with the bb^+ as well as with the bb duplication in $\widehat{XX}NO^-/Dp122$ females although their phenotype is in one case bb^+ and strongly bb in the other; (3) the increase in the bb^+ females is lower than in the bb ones. In fact, the bb^+ locus shows an increase of 0.080 (a 65% increase with respect to the original level), while the bb locus has an increase of 0.059 (an 84% increase).

c) rDNA content of males carrying the free duplications in combination with different X chromosomes

The rDNA content has also been tested in males carrying bb^+ and bb free duplications with a $y\ bb^+$ X′ chromosome. The rDNA content of this X′ chromosome, measured under conditions in which the rDNA level does not change (homozygous X′/X′ females) is 0.276% (Table 2, line c).

This X′ chromosome has also been tested for its ability to undergo somatic gene compensation in X′/O males; as we can see from Table 2, line d, the rDNA content is 0.344% as compared to the original value of 0.276%. These values are in good agreement with similar measurements previously reported by Malva et al. (1979).

When we measured the rDNA content of males with the bb^+ or the bb duplication in combination with this X′ chromosome, we obtained higher values than those expected from the sum of the rDNAs carried by the two NOs present: 0.488% in X′/Dp122bb^+ (Table 2, line e) and 0.410% in X′/Dp122bb (Table 2, line f). This occurs with the bb^+ duplication as well as with the bb one.

To confirm these results we performed the same experiments with a second $y\ bb^+$ X″ stock and obtained very similar results (see Table 2, lines g, h, i, j).

Discussion

It has been reported (Spear and Gall 1973) that in polytene cells the amount of rDNA is not dependent on the number of NOs and, more recently, that genes from only one NO replicate in X/Y (Endow and Glover 1979) and X/X (Endow 1980) polytene cells. It has been proposed that in such cells replication of rDNA genes occurs at the same level as replication of genes from the single NO present in X/O polytene cells and that this could account for the somatic compensation of rDNA (Endow 1980). In $\widehat{XX}NO^-/Dp122$ females the only NO is carried by the free duplication. Therefore, the rDNA increase may be due to disproportionate replication of the rDNA carried by the free duplications in non-diploid (polytene) tissues of adult females. Moreover, it has been suggested (Procunier and Tartof 1978)

Table 2. rDNA content in different genotypes in which a bb^+ or bb duplication is present

Lane	Sex	Genotype	% rDNA hybridized	% rDNA originally present on the X	% rDNA originally present on the Dp	rDNA increase
a	♀	$\widehat{XX}NO^-/Dpbb^+$	0.203	/	0.123	0.080
b	♀	$\widehat{XX}NO^-/Dpbb$	0.129	/	0.070	0.059
c	♀	X′y/X′y	0.552	0.276	/	/
d	♂	X′y/O	0.344	0.276	/	0.068
e	♂	X′y/Dpbb^+	0.488	0.276	0.123	0.089
f	♂	X′y/Dpbb	0.410	0.276	0.070	0.064
g	♀	X″y/X″y	0.286	0.143	/	/
h	♂	X″y/O	0.190	0.143	/	0.047
i	♂	X″y/Dbbb^+	0.330	0.143	0.123	0.064
j	♂	X″y/Dpbb	0.270	0.143	0.070	0.057

that rDNA compensation is regulated by a genetic locus, the cr^+ locus, located in the distal centromeric heterochromatin of the X chromosome which displays both a *cis* and *trans* effect. According to Procunier and Tartof, this gene acts in *trans* to sense the presence or the absence of its partner cr^+ locus on the opposite chromosome. If only one cr^+ is present, then it acts in *cis* leading to disproportionate replication of the rRNA genes contiguous to it on the X chromosome. This implies that if a given chromosome can disproportionately replicate the rDNA it carries, it will not induce an rDNA increase on its partner chromosome because it contains a cr^+ gene.

Our results indicate that the bb^+ and *bb* duplications, in combination with $\widehat{XX}NO^-$, increase their rDNA content and therefore should have the cr^+ gene. However, when the free duplications were combined with two different X chromosomes, we obtained a non-additive rDNA content which cannot simply be attributed to over-replication of one or the other chromosome. On the basis of the *cis-trans* model, the free duplications capable of increasing the rDNA they carry ($\widehat{XX}NO^-$/Dp122bb^+ and $\widehat{XX}NO^-$/Dp122*bb* females) should not induce an rDNA increase on their partner chromosomes. Also, the two X chromosomes, which in X/O males can increase the rDNA they carry, should not induce an rDNA increase on the free duplications.

From our results we cannot discern whether the increased rDNA is due to the NO of the free duplications or of the X′ chromosome; in fact, the resulting values from X′/Dp122bb^+ (0.488) and X′/Dp122*bb* (0.410) males are very close to those expected regardless of whether we add the increased X′ value obtained from the X′/O males (Table 2, line d) to the original values of the two duplications (Table 1): $0.344+0.123=0.467$ and $0.344+0.070=0.414$) or the original value of the X′ (Table 2, line c) to the increased values of the free duplications (Table 2, lines a, b), obtained in $\widehat{XX}NO^-$/Dp122 females: $0.276+0.203=0.479$; $0.276+0.129=0.405$. Therefore, we cannot state unequivocally which of the two NOs present is involved in this rDNA increase. This ambiguity remained unresolved even though the other X″ was used in the second set of experiments.

The non-additive rDNA content in the different X/Dp122 males tested, if due to disproportionate replication, could be explained by assuming that the cr^+ locus is necessary only in *cis* to ensure the ability of disproportionate replication. The role of triggering the phenomenon would then have to be played by different genes. Alternatively, given the unknown behaviour of these duplications during polytenisation, the rDNA increase can be explained by assuming that the X/Dp122 males mimic the X/O males and therefore the rDNA from the X or from the duplication replicates disproportionately.

The *bb* duplication behaves like bb^+ in most of the genetic combinations we tested. $\widehat{XX}NO^-$/Dp122*bb* females are an exception. The greater increase in the rDNA shown by the *bb* duplication in these phenotypically *bb* females could be explained by the simultaneous occurrence of somatic compensation and a premagnification of the type described by Gargano and Graziani (1976) in $\widehat{XX}NO^-$/Y*bb* females.

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