Genet. Res., Camb. (1989), **54**, *pp.* 127–135 *With 6 text-figures* *Printed in Great Britain*

The occurrence of long ribosomal transcripts homologous to type I insertions in bobbed mutants of *Drosophila melanogaster*

MOHAMED MAKNI[1]*, MOHAMED MARRAKCHI[1] AND NICOLE PRUD'HOMME[2]

[1] *Laboratoire de Génétique, Faculté des Sciences, 1060 Tunis, Tunisie*
[2] *Centre de Génétique Moléculaire, Centre National de la Recherche Scientifique, 91190 Gif-sur-Yvette, France*

(*Received 4 October 1988 and in revised form 6 March 1989*)

Summary

In *Drosophila melanogaster* up to two thirds of the rDNA genes contain insertion sequences of two types in the 28S coding region. Comparison of the ribosomal insertion transcripts in the wild type and in two bobbed mutants reared at two temperatures showed that the level of type I transcripts is dependent on both the number of genes with type I insertions in the bobbed loci and the intensity of bobbed phenotype. Importantly, a long transcript of 8·7 kb hybridized to the ribosomal probe, the INS I probe and also to the restriction fragment of the rDNA downstream of the point of insertion was found in one bobbed mutant.This result and also those from sandwich hybridization indicate that some interrupted ribosomal genes are functional.

1. Introduction

In *Drosophila melanogaster*, the genes coding for 18S and 28S ribosomal RNA are located in the *X* and *Y* heterochromatic regions at the nucleolus organizer (NO) (Ritossa & Spiegelman, 1965; Cooper, 1959). Each NO in the wild type contains 150–250 tandemly arranged repeat units. Partial deficiencies of ribosomal DNA (rDNA) lead to the bobbed phenotype characterized by shorter bristles and slower development (Ritossa *et al.* 1966).

A large number of ribosomal genes are interrupted in the 28S coding region by non-rDNA insertions of two types, INS I and INS II, that do not cross hybridize (Glover & Hogness, 1977; Pellegrini *et al.* 1977; Wellauer & Dawid, 1977, White & Hogness, 1977; Dawid *et al.* 1978; Wellauer *et al.* 1978). These insertions occur at two different sites separated by 51 nucleotide pairs (Roiha & Glover, 1980; Roiha *et al.* 1981). Ribosomal genes with INS I occur only on the *X* chromosome (60% of ribosomal genes) with inserts ranging from 0·5 to 5 kilobases (kb). Short type I (0·5 kb) shares 3′ sequence homology with all larger insertions. INS I sequences are also found outside the rDNA (Dawid & Botchan, 1977; De Cicco & Glover, 1983) and are probably derived by transposition from rDNA. Genes with type II insertions occur exclusively in the rDNA on both the *X* and the *Y* chromosome (16% of ribosomal genes) with inserts ranging from 1·5 to 3·4 kb.

The transcription of a 38S precursor (8 kb) from the genes without insertions (INS^-) has long been established. This primary transcript undergoes a series of modifications and processing giving rise to the 2S, 5·8S, 18S and 28S mature rRNAs, while the genes with insertion (INS^+) are considered not to produce mature rRNA although they are transcribed. In wild type embryos, there is less than one copy per nucleus of insertion transcripts that hybridizes to the 5′ end fragment of INS I and about 1300 copies of the 8 kb rRNA precursor. RNA homologous to the 3′ end of this insertion occurs more frequently, the major form being 1 kb. This 1 kb RNA exists at a very low level in all developmental stages and tissues (Long & Dawid, 1979; Jolly & Thomas, 1980). Transcripts homologous to INS II are also found, the major form being 3·4 kb. There are approximately 400 fold more molecules of the 8 kb primary transcript than the 3·4 kb type II transcript in the cell nucleus. The level of insertion transcripts is strain dependent and is much higher in ovarian tissues than in embryos, larvae or pupae (Kidd & Glover, 1981). However using bobbed strains, Long *et al.* (1981) concluded that complete deletion of one NO did not affect the level of insertion transcripts in a phenotypically wild-type fly but did cause an increase in one of the two bobbed mutations studied. On the other hand, Terracol (1986) using different bobbed strains, found

* Corresponding author.

https://doi.org/10.1017/S0016672300028494 Published online by Cambridge University Press

that the amount of these transcripts depends critically on the number of INS^- genes. If this falls below 100 units transcription of INS^+ genes is activated.

In this paper we investigate the level of insertion transcripts of two bobbed mutations: bbT6 which causes a non-thermosensitive bobbed phenotype and bbP5 which causes a thermosensitive bobbed phenotype. The level of insertion transcripts is dependent both on bobbed genotypes and the intensity of bobbed phenotype. Significantly, we have identified a 8·7 kb RNA that hybridizes to the ribosomal probe, the INS I probe and also to the restriction fragment of rDNA downstream of the point of insertion.

2. Materials and methods

(i) *Fly stocks*

The wild-type Oregon R strain originated from the Centre de Génétique Moléculaire CNRS Gif/Yvette, France. The *X* chromosome was cloned by crossing a single male to females carrying two M5 chromosomes. bbT6 (Makni & Marrakchi, 1978) and bbP5 (Marrakchi & Prud'homme, 1971) were independently induced by EMS on the normal *X* chromosome. XNO^- is an inverted chromosome $In(1)sc^{4L}sc^{8R}$, *y sc cv*, devoid of all rDNA.

In homozygous females, bbT6 exhibited an intermediate bobbed phenotype (short bristles) at 25 and 18 °C while bbP5 caused a strong bobbed phenotype at 25 °C (short bristles, etched abdomen) and an intermediate one at 18 °C. In hemizygous females with XNO^-, bobbed mutations were always strong bobbed except bbP5 which was lethal at 25 °C.

(ii) *rDNA quantification*

Adult female flies were ground in 50 mM Tris HCl pH 8·4, 150 mM-NaCl, 100 mM-EDTA and 1 % SDS. After incubation for 10 min at 60 °C, the mixture was adjusted to 1 mM-$NaClO_4$ and incubated for 5 min at room temperature. DNA was extracted with phenol–chloroform–isoamyl alcohol (100–96–4) and then with chloroform–isoamyl alcohol (96–4). After precipitation with ethanol, the DNA was treated for 2 h at 37 °C with RNase A (15 μg/ml), and extracted with chloroform–isoamyl alcohol. It was purified by elution from a hydroxyapatite column with a phosphate buffer gradient and then dialysed for 24 h against 0·1 × SSC (1 × SSC is 0·15 M-NaCl plus 0·015 M sodium citrate). The DNA was denatured with NaOH, neutralized with HCl and fixed on nitrocellulose filters. These filters dried for 2 h at 80 °C, were incubated overnight at 65 °C in a vial containing 2 × SSC, 0·1 % SDS, 0·1 mg/ml yeast tRNA and 5 μg/ml of *in vivo*-labelled ^3H rRNA extracted from wild-type third instar larvae. The filters were then rinsed three times for 20 min at 42 °C in 2 × SSC, 0·1 % SDS and incubated for 2 h at 42 °C in 2 × SSC containing 20 μg/ml of RNase A. They were rinsed three times for 20 min at 42 °C in 0·5 × SSC, 0·1 % SDS. The amount of rDNA hybridized was measured by scintillation counting.

(iii) *Brain DNA preparation, restriction and transfer*

Brains from 50 third instar larvae were homogenized in 250 μl of 50 mM Tris HCl pH 7·8, 100 mM-EDTA, 0·5 % SDS, 50 μg pronase. After adding sodium acetate to a final concentration of 1 M, DNA was extracted with chloroform–isoamyl alcohol. Lambda DNA (1 μg) was added and the DNA was precipitated for 15 min at −70 °C with ethanol. The precipitate was dried and dissolved in 10 mM Tris HCl pH 8·0, 1 mM-EDTA. All restriction endonuclease digestions were performed according to the supplier's specifications (Boehringer, Mannheim). For double digestions, DNA was precipitated with ethanol after completion of the first digest. The DNA was fractionated by eletrophoresis through 0·6 % agarose gels, and then Southern blotted (Southern, 1975) to nitrocellulose filters (Schleicher and Schull BA 85) after HCl treatment to facilitate transfer of large fragments (Wahl *et al.* 1979).

(iv) *Total RNA extraction*

RNA was extracted from adult flies of the appropriate genotype. Flies were homogenized in 10 mM Tris HCl pH 7·4, 100 mM-NaCl, 10 mM-EDTA, 0·5 % SDS. The mixture was treated twice with one volume of phenol–chloroform–isoamyl alcohol, then with one volume of chloroform–isoamyl alcohol. Two volumes of 6 M-LiCl were added to the aqueous phase and the mixture left 24 h at 0 °C. After centrifugation, the pellet was dissolved in 20 mM Tris HCl pH 8·0, 10 mM-$CaCl_2$ and 25 μg/ml of RNase-free DNase predigested according to the method of Tullis & Rubin (1980) to remove traces of RNase. After incubation at 37 °C for 1 h, the RNA was extracted with phenol–chloroform–isoamyl alcohol and precipitated with 2 volumes of 100 % ethanol. The pellet was then dried and dissolved in sterile distilled water.

(v) *Electrophoresis and transfer of RNA*

RNA was denatured 15 min at 55 °C in 20 mM morpholinopropane sulphonic acid (MOPS) pH 7·0, 5 mM sodium acetate, 1 mM-EDTA, 50 % formamide and 2·2 % formaldehyde. The mixture was loaded onto 0·8 % horizontal agarose gel containing 20 mM-MOPS, 5 mM sodium acetate, 1 mM-EDTA and 2·2 % formaldehyde. After overnight migration (70 V, 30 mA) the gel was treated twice with 50 mM-NaOH, 10 mM-NaCl and then washed twice with 0·1 M Tris HCl pH 7·5 to gain neutralization. RNA was transferred after equilibration in 20 × SSC onto nitrocellulose filters (Thomas, 1980).

https://doi.org/10.1017/S0016672300028494 Published online by Cambridge University Press

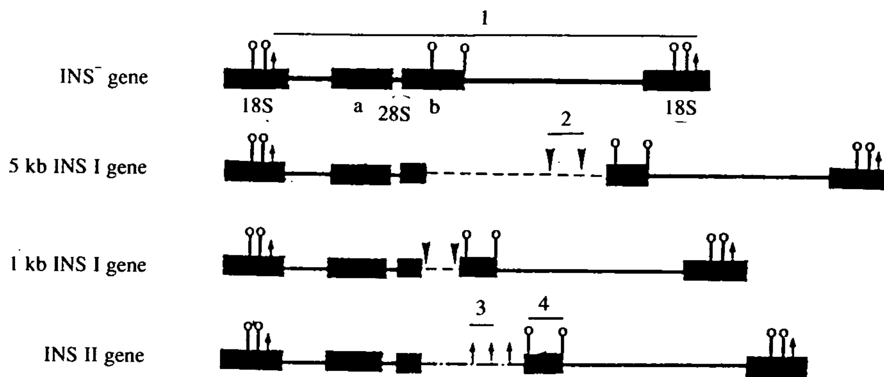

Fig. 1. Restriction maps of rRNA genes. An uninterrupted gene; a gene with a 5 kb type I insertion; a gene with a short type I insertion and a gene with type II insertion are shown. The sites for restriction enzymes: (↑) *Eco* RI, (Ɏ) *Bam* HI, (⚲) *Hind* III. Hybridization probes are illustrated above the repeats: (1) DmrY12, *Eco* RI INS⁻ of 12 kb; (2) Dmra56Bam, *Bam* HI INS I of 1 kb; (3) Dmr205, *Eco* RI INS II of 0·7 kb and (4) a gel-purified *Hind* III fragment.

(vi) *Hybridization of DNA and RNA*

After baking for 2 h at 80 °C, filters (DNA or RNA) were prehybridized for 2 h at 42 °C in the following buffer: 50% formamide, 5 × SSC, 5 × Denhardt's solution, 50 mM sodium phosphate buffer pH 6·5, 50 μm/ml yeast tRNA. Hybridizations were performed overnight in the same mixture but with 10% dextran sulphate added using the ^{32}P nick-translation-labelled probe after denaturation for 10 min at 100 °C. The filters were rinsed at 42 °C three times in 2 × SSC, 0·1% SDS and three times in 0·5 × SSC, 0·1% SDS. Filters were then autoradiographed using Kodak XAR-5 film with intensifying screens.

(vii) *Dot-blot hybridization*

After denaturation, RNA in 2 × SSC was spotted on nitrocellulose filters that had been equilibrated with 20 × SSC and dried. Prehybridization, hybridization and washing were as described above.

(viii) *Plasmids*

Plasmid DNA was isolated according to the method of Birnboim & Doly (1979). pDmrY12 contains a 12 kb *Eco* RI INS⁻ ribosomal fragment in the *Eco* RI site of ColEI (Wellauer *et al.* 1978). pDmr103, used as size marker, contains a 17 kb *Eco* RI ribosomal fragment with a 5·5 kb INS I inserted at the *Eco* RI site of ColEI (Glover *et al.* 1975; Glover & Hogness, 1977). pDmra56Bam contains the 1 kb *Bam* HI INS I fragment inserted at the *Bam* HI site of pBR322 (Long & Dawid, 1979). pDmr205 contains the 0·7 kb *Eco* RI INS II fragment cloned into the *Eco* RI site of pBR322 (Long *et al.* 1980). Fig. 1 illustrates the different kinds of rDNA genetic units and probes that were used.

3. Results

(i) *rDNA content and restriction pattern*

Measurements of rDNA content for Oregon R wild-type, bbP5 and bbT6 mutants were done by hybridization of DNA from homozygous females to labelled rRNA. To evaluate the number of INS⁻, INS I and INS II genes, the brain DNA from third instar larvae was digested with *Eco* RI or *Eco* RI–*Bam* HI (Fig. 2) and hybridized to plasmid pDmrY12. *Eco* RI has one cleavage site in the 18S coding region of each repeat unit and at least one in the INS II sequences, while *Bam* HI has at least one cleavage site in the INS I sequences. Since the structural gene is 8 kb long, the major size class of nontranscribed spacer is about 4 kb and the major size of insertion sequence is 5 kb. Thus, *Eco* RI fragments greater than 17 kb and *Eco* RI–*Bam*

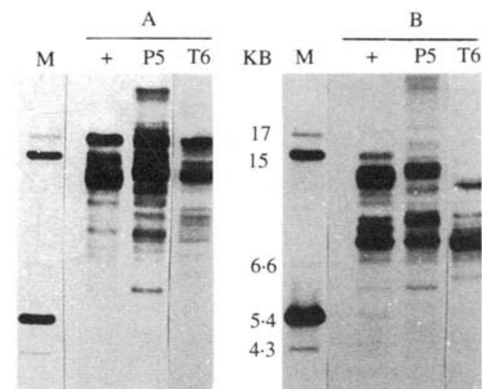

Fig. 2. Southern blot analysis of rRNA genes in wild type and the two bobbed stocks analysed. Brain DNA from third instar larvae of Oregon R (wt), bbT6/bbT6 and bbP5/bbP5 were digested either with *Eco* RI or *Eco* RI–*Bam* HI, electrophoresed through 0·6% horizontal agarose gels, transferred onto nitrocellulose filters and hybridized to ^{32}P-labelled pDmrY12 probe. Sizes of relevant bands are given in kb. A, *Eco* RI digests and B, *Eco* RI–*Bam* HI digests.

https://doi.org/10.1017/S0016672300028494 Published online by Cambridge University Press

Table 1. *Number and distribution of ribosomal genes in the wild-type Oregon R and bobbed mutants*

Ribosomal locus	% ± S.E.M. rDNA (homozygous females)[a]	No. ribosomal genes per locus[b]	No. INS⁻ (%)[c]	No. INS I (%)[c]	No. INS II (%)[c]
bb+	0·35 ± 0·02	200	98 (49)	70 (35)	32 (16)
bbP5	0·16 ± 0·01	91	42 (46)	29 (32)	20 (22)
bbT6	0·21 ± 0·01	124	45 (36)	55 (44)	24 (19)

[a] Percentages of rDNA were obtained by hybridization of genomic DNA to ^{3}H rRNA. The values are mean values obtained from at least three independent DNA extractions and with hybridization of each to 4 or 5 filters. Standard error of mean (S.E.M.) = $\sqrt{[\Sigma(Xi-M)^2/n(n-1)]}$.

[b] The number of ribosomal genes was calculated from the two following values:
Molecular mass of *D. melanogaster* haploid genome = $1{\cdot}2 \times 10^{11}$ D.
Molecular mass of *D. melanogaster* rRNA = $2{\cdot}1 \times 10^6$ D.
The number of ribosomal genes = $1{\cdot}2 \times 10^{11} \times$ %rDNA/$2{\cdot}1 \times 10^6$.

[c] Values were obtained from densitometric analyses of autoradiographs by calculating the percentages of areas defined by the scannings.

HI fragments greater than 12 kb, which are easily detectable only in bbP5 mutation, indicate the presence of some long spacers. If very large spacers are not considered, *Eco* RI fragments shorter than 10 kb correspond to the 5′ and 3′ ends of the INS II genes, while *Eco* RI–*Bam* HI fragments longer than 10 kb correspond to INS⁻ genes alone. The relative proportions of the different gene types were estimated by scanning the autoradiographs. Table 1 summarizes the distribution of ribosomal genes. In the wild-type strain, rDNA comprises 49% INS⁻ genes, 35% INS I genes and 16% INS II genes. The two bobbed strains were deleted for about 109 genes in bbP5 and 76 genes in bbT6 with preferential deletions of INS⁻ and INS I genes in bbP5 and INS⁻ in bbT6. Since they have roughly the same number of INS⁻ and INS II genes, those genes with type I insertions discriminate the bbP5 and bbT6 mutations.

(ii) *Insertion transcripts*

Total RNA from homozygous or heterozygous *X/NO⁻* females reared at 25 and 18 °C was transferred from denaturing agarose gels onto nitrocellulose filters and hybridized either to an INS⁻ ribosomal probe (pDmrY12), to the *Bam* HI INS I fragment common to all INS I (pDmra56Bam) or to the *Eco* RI INS II fragment (pDmr205). Since the total amount of RNA loaded on the gel for each sample is not equal, the intensities of the hybridizations are not quantitatively comparable.

Hybridization to the ribosomal probe shows the usual transcripts such as the 38S primary transcript of 8 kb, the intermediates in the processing of ribosomal RNA of 7·2, 4·6 and 3·3 kb, and finally the mature rRNA 28S of 3·7 kb, 28Sb of 1·9 kb, 28Sa of 1·6 kb and 18S of 1·8 kb (Fig. 3) (Dawid *et al.* 1978; Long & Dawid, 1979, 1980*a*, *b*). In addition, three other bands of 8·7, 4·4 and 4·1 kb of slight intensity appear when RNA was extracted from bbT6 heterozygous females (indicated by arrows in Fig. 3). The 4·4 kb is probably the intermediate (c) of 4·46 kb described by Long & Dawid (1980*b*), while the two others, specific to the bbT6 mutation, could correspond to intermediates flanked by an insertion sequence. The same results are obtained when RNA was extracted from *X/O* males. The observation of the 8·7 and 4·1 kb transcripts detected by hybridization to the ribosomal probe suggests that interrupted genes can be transcribed at a very high level.

For the two bobbed mutants studied, hybridization to the INS I probe reveals the presence of several INS I transcripts not detectable when RNA was extracted from wild-type homozygous or heterozygous females (Fig. 4). For bbP5 mutant, we detect fewer bands ranging between 4·8 and 1·3 kb. The most abundant RNA species is 4·8 kb in homozygous bbP5 females, while in hemizygous bbP5 females the pattern of type I transcripts is slightly different and the most intense band is 1·5 kb. At the four experimental conditions, bbT6 mutant displays stronger hybridization than bbP5 and shows principally transcripts of 8·7, 7·4, 5·4, 4·3, 4·1, 3·2, 1·9 and 1·5 kb. These results are reproducible and suggest that the level of INS I transcripts is higher in the bbT6 than in the bbP5 mutant. To our knowledge, this is the first report of a transcript longer than the primary precursor of 8 kb when using hybridization either to the uninterrupted ribosomal probe or to the short INS I probe. This RNA could be a full length rRNA precursor derived from a gene with a 0·7 kb type I insertion. It could be a primary transcript that is synthesized from the rDNA promoter and terminates at the right hand end of a 2·3 kb type I insertion. An alternative hypothesis is that it could correspond to an intermediate in the processing comprising the 32S precursor of mature 28S rRNA sequences containing a 4 kb type I insertion. However, the latter two hypotheses seem unlikely since primary transcripts longer than 8·7 kb are not detectable, even with long autoradiographic exposure, and no pre-

https://doi.org/10.1017/S0016672300028494 Published online by Cambridge University Press

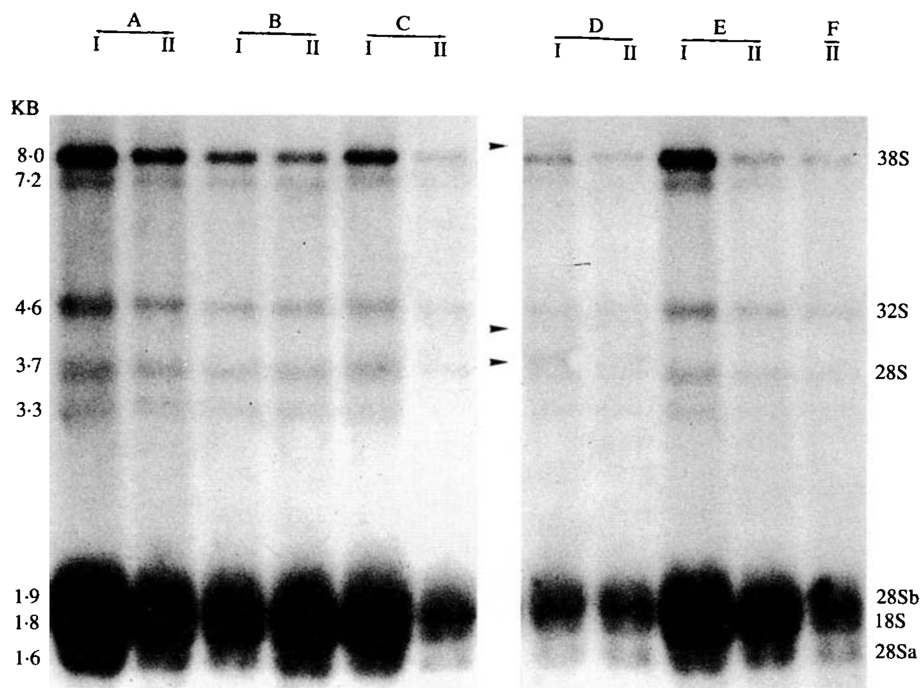

Fig. 3. RNA homologous to ribosomal gene sequences. Total RNA was electrophoresed in a horizontal 0·8% agarose gel and transferred onto nitrocellulose paper. The immobilized RNA was hybridized to labelled pDmrY12 (probe 1 of Fig. 1). Sizes of some of the transcripts are indicated. A, bbP5/bbP5; B, +/+; C, bbT6/bbT6; D, bbT6/XNO⁻; E, +/XNO⁻ and F, bbP5/XNO⁻. I, 25 °C and II, 18 °C.

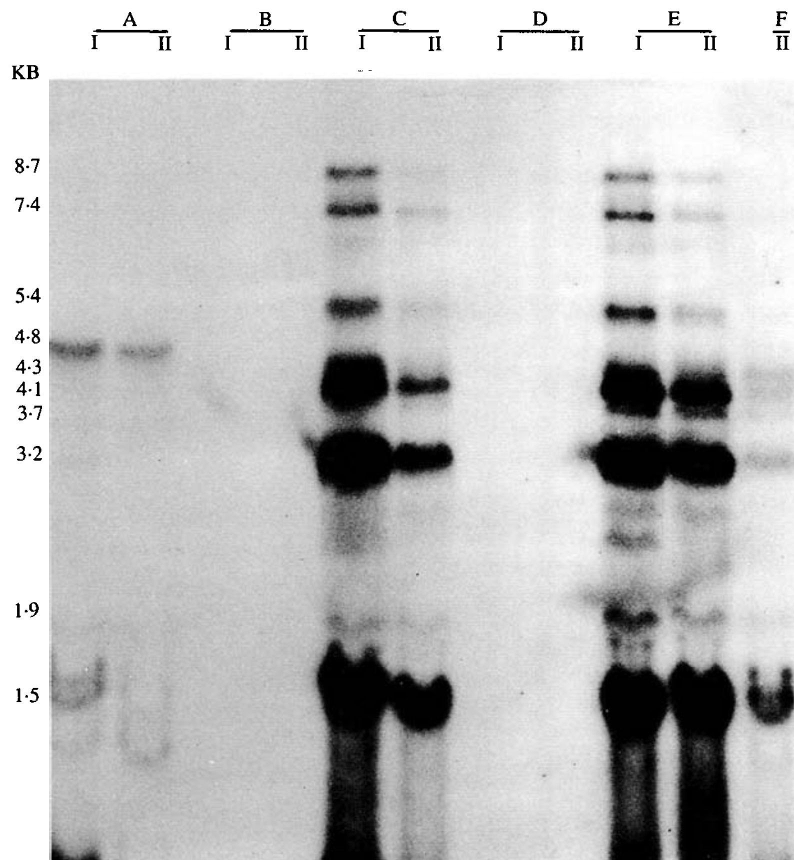

Fig. 4. RNA homologous to short type I insertion. Total RNA was separated on horizontal 0·8% agarose gel, transferred onto nitrocellulose paper and hybridized to labelled pDmra56Bam (probe 2 of Fig. 1). A, bbP5/bbP5; B, +/+; C, bbT6/bbT6; D, +/XNO⁻; E, bbT6/XNO⁻ and F, bbP5/XNO⁻. I, 25 °C and II, 18 °C.

https://doi.org/10.1017/S0016672300028494 Published online by Cambridge University Press

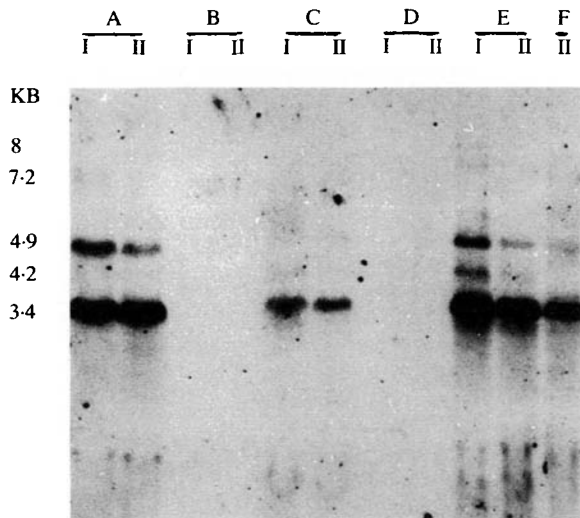

Fig. 5. RNA homologous to type II insertions. Filter shown in Fig. 4 was rehybridized, after washing, to labelled pDmr205 (probe 3 in Fig. 1).

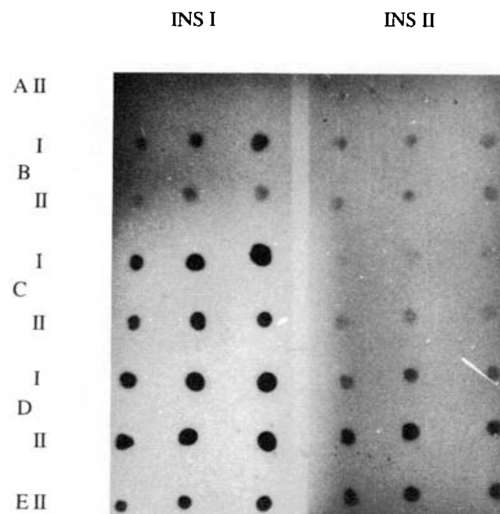

Fig. 6. Dot–blot assay. Increasing amounts of RNA were spotted on nitrocellulose filters and hybridized either to INS I-labelled pDmra56Bam (probe 2 in Fig. 1) or to INS II-labelled pDmr205 (probe 3 in Fig. 1). From left to right 5, 10 and 15 μg respectively. A, +/+; B, bbP5/bbP5; C, bbT6/bbT6; D, bbT6/NO⁻ and E, bbP5/NO⁻. I, 25 °C and II, 18 °C.

dominant 2·3 or 4 kb RNAs homologous to type I insertions are seen.

When the same filter was hybridized to the INS II probe (Fig. 5), we detected transcripts of heterogeneous size in RNA extracted only from bobbed mutants. In particular, the most abundant RNA species are the 3·4 kb corresponding to the length of the major INS II and the 4·9 kb as found in the nuclei of the wild type strains (Kidd & Glover, 1981).

In order to determine whether genes with INS I sequences can be transcribed productively, total RNA from homozygous and hemizygous bbT6 females was transferred from agarose gels to nitrocellulose paper and hybridized to a labelled 1 kb *Hin*d III (gel purified) fragment of pDmrY12 corresponding to the 3′ end of the primary transcript, downstream of the insertion site (probe 4 in Fig. 1). In such an experiment we detected among many signals a 8·7 kb transcript as shown in Fig. 3. Additionally, we performed a sandwich hybridization experiment according to Dunn & Hassel (1977). We first transferred the 1 kb restriction *Hin*d III fragment (probe 4 in Fig. 1) onto nitrocellulose. This filter was hybridized to unlabelled total RNA extracted from homozygous bbT6 females and then the resulting DNA/RNA hybrids observed for free RNA tails that would hybridize to labelled INS I DNA probe (probe 2 in Fig. 1). Hybridization occurred in this case, indicating that some INS I genes can be completely transcribed (data not shown).

(iii) *Dot blot assay*

In order to estimate the proportion of insertion transcripts in mutants relative to the wild-type, a dot–blot assay was used (Thomas, 1980). Increasing amounts of total RNA, extracted from wild-type Oregon R homozygous females, homozygous Xbb/Xbb and heterozygous Xbb/XNO⁻ females reared at 25 and 18 °C, were hybridized either to the INS I probe pDmra56Bam or to the INS II probe pDmr205. The relative amounts of transcripts were measured by densitometric analysis at concentrations giving a linear response range on the film. Since ribosomal genes interrupted either with INS I or INS II sequences are transcribed at a very low level in wild-type flies (Long & Dawid, 1979; Long *et al.* 1980; Kidd & Glover, 1981), the hybridization of the Oregon R samples, though positive, was difficult to represent photographically (Fig. 6). Results are summarized in Table 2.

Hybridization to the INS I probe (Fig. 6, on the left) showed a high level of transcripts in bobbed

Table 2. *Comparison of the quantities of insertion transcripts in homozygous and hemizygous bobbed females reared at two temperatures.*

		RNA/RNA⁺	
		INS I	INS II
+/+	18 °C	1	1
bbP5/bbP5	25 °C	15·1	4·5
bbP5/bbP5	18 °C	7·5	4·3
bbT6/bbT6	25 °C	35·4	5·0
bbT6/bbT6	18 °C	36·5	6·3
bbT6/XNO⁻	25 °C	94·4	7·7
bbT6/XNO⁻	18 °C	98·5	10·9
bbP5/XNO⁻	18 °C	36·5	12·5

The proportions of insertion transcripts in bobbed mutants compared to the wild type were obtained by scanning dot–blot autoradiographs. The error was estimated to be about 10%.

https://doi.org/10.1017/S0016672300028494 Published online by Cambridge University Press

mutants compared to the Oregon R females. In the bbT6 mutant, insertion transcripts at 18 °C as well as 25 °C were 35-fold higher in homozygous and about 95-fold higher in heterozygous bbT6/XNO$^-$ than in Oregon R homozygous females reared at 18 °C. While for the bbP5 mutant, transcripts in homozygotes were 7-fold higher at 18 °C and 15-fold higher at 25 °C, and about 35-fold higher in heterozygotes bbP5/XNO$^-$ females at 18 °C.

Hybridization to the INS II probe (Fig. 6, on the right) indicated only a small increase of insertion transcripts in bobbed females. As observed for the INS I transcripts, in heterozygous bobbed females INS II transcripts are more abundant than those in homozygous bobbed females.

These experiments reveal a clear correlation between the high level of insertion transcripts and the intensity of bobbed phenotype for the two bobbed mutations (homozygous–hemizygous); moreover the amount of insertion transcripts seems to be related to the proportion of INS$^+$ genes (bbT6–bbP5), since these two mutants have approximately an equal number of INS$^-$ genes.

4. Discussion

It has been demonstrated that in wild-type flies, rRNA genes interrupted by type I or type II insertion do not contribute significantly to produce mature rRNA (Long & Dawid, 1979; Long *et al.* 1980; Kidd & Glover, 1981). In bobbed mutants, transcripts of short type I insertions are more abundant while type II insertions are transcribed at a very low level (Long *et al.* 1981). Compared to homozygous bobbed females, INS$^+$ transcripts increased also during reversion by a magnification phenomenon (Labella *et al.* 1983). We investigated the transcription of rRNA in two bobbed mutants: bbP5 which showed a thermosensitive bobbed phenotype (strong bobbed phenotype at 18 °C and lethal at 25 °C in the heterozygous bbP5/NO$^-$ females) and bbT6 which showed non-thermosensitive bobbed phenotype (strong bobbed phenotype at 18 and 25 °C in the heterozygous bbT6/NO$^-$ females).

By rRNA/DNA hybridization, it was demonstrated that the two bobbed mutations are deficiencies. Southern analysis using *Eco* RI and *Eco* RI–*Bam* HI digestions showed that the organization of the rDNA locus is slightly modified in the mutants. INS$^-$ genes decreased nearly equally in bbP5 and bbT6 (about 50%), INS II genes decreased slightly and also equally in the two bobbed mutants, while INS I genes decreased preferentially in bbP5. These results suggest the occurrence of differential clustering of these genes in the rDNA of the mutants as has already been demonstrated for INS II genes in the nucleolus organizer of the *Y* chromosome by Wellauer *et al.* (1978), for INS I genes by Appels & Hilliker (1982), Sharp *et al.* (1983) and England *et al.* (1988) and for the three classes of genes by Terracol & Prud'homme (1986).

To explain the difference in the expression of the bobbed phenotype between these two mutants, the identification of RNA molecules containing ribosomal sequences was determined. A classical pattern resulted when hybridization was performed to the ribosomal probe pY12; however, the bbT6 mutant exhibits 3 other bands of 8·7, 4·4 and 4·1 kb. The number of bands that were detected with INS I probe is greater in bbT6 than in bbP5 mutants. This differs from the pattern of INS II transcripts for which a 3·4 kb predominant RNA is seen in both the bbT6 and bbP5 mutants (as found by Kidd & Glover, 1981). Moreover, the bbP5 mutant displays a slightly different pattern in the homozygous females as compared to hemizygous bbP5/NO$^-$ females. In addition, we obtained RNA of 4·1 and 8·7 kb homologous to the type I insertion from bbT6 mutant as shown by hybridization to the ribosomal probe, indicating a strong tendency to transcribe genes with INS I. In particular, the 8·7 kb RNA could be an INS I gene primary transcript. The autoradiographic signal obtained, especially at 8·7 kb, after hybridization to ribosomal probe from downstream of the insertion site, and when sandwich hybridization was performed, suggests that in the bbT6 mutant some INS I genes are entirely transcribed. This result may explain the significant proportion of long transcription units noted by Chooi (1979). Ribosomal RNA longer than the 38S primary transcript hybridizing to type II insertion sequences but not to type I sequences, have been reported in wild-type stocks by Kidd & Glover (1981), in one bobbed mutation EMS induced on the inverted M5 chromosome by Terracol (1986) and in premagnified bobbed males by Labella *et al.* (1983). In all cases, no primary insertion transcript was found. Dot-blot analysis showed that the concentration of type I transcripts is greater in bbT6 mutant than in bbP5 mutant indicating a probable correlation of INS I transcripts with the number of type I units. High temperature (25 °C) induced an increase of INS I transcripts in the thermosensitive bbP5 mutant but not bbT6. This concentration increases in the two hemizygous bb mutants but remains much lower in bbP5 suggesting that for each bobbed mutation the level of INS I transcripts is dependent on the severity of bobbed phenotype as demonstrated for the car bb mutation by Long *et al.* (1981). The concentration of type II transcripts increased slightly in the two bobbed mutants studied. Differences in the concentration of INS I and INS II transcripts have been reported in bobbed premagnified males and in homozygous bobbed females (Labella *et al.* 1983). Thus, we think that, among other factors, each bobbed mutation behaves differently according to the rDNA content and the distribution of the different types of genes. The bbP5 mutant was studied for the quantities of insertion transcripts by Terracol (1986) who found a

https://doi.org/10.1017/S0016672300028494 Published online by Cambridge University Press

higher increase of insertion transcripts. The differences with our results could be mainly due to a difference in the original wild-type strain used as control. Variable levels of type II transcripts in different wild-type strains were described by Kidd & Glover (1981). One explanation of the temperature effect on the level of INS I transcripts in bbP5 mutant is to suppose that a specific structural change at the promoter sites for INS I genes occurs at the high temperature, resulting in an increased rate of INS I transcripts following a greater accessibility to RNA polymerase. Our hypothesis is supported by the finding of some long spacers of 10–20 kb in bbP5 (Terracol, 1986). The promoter site for rRNA genes is located in the non-transcribed spacer that contains many *Alu* I repeats perfectly homologous with the pol I transcription initiation site (Coen & Dover, 1982). Thus, differences in the lengths of the non-transcribed spacers, mainly due to the *Alu* I repeated sequences, could modulate the transcription rate of ribosomal genes.

We thank Hamouda Hemissi (Faculté des Sciences de Tunis), Jean Claude Mounolou and Monique Fukuhara (Université Paris XI) and Douglas E. Eveleigh (Rutgers University, New Brunswick) for their critical review of the manuscript. This work was supported by a grant from the Ministère de l'Enseignement Supérieur et de la Recherche Scientifique de Tunisie (Contract project) and a grant from the Centre National de la Recherche Scientifique de France (ATP Biologie Moléculaire du Gène).

References

Appels, R. & Hilliker, A. J. (1982). The cytogenetic boundaries of the rDNA region within heterochromatin of the *X* chromosome of *Drosophila melanogaster* and their relation to male meiotic pairing site. *Genetical Research* **39**, 149–156.

Birnboim, H. C. & Doly, J. (1979). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Research* **7**, 1513–1523.

Chooi, W. Y. (1979). The occurrence of long transcription units among the X and Y ribosomal genes of *Drosophila melanogaster*: transcription of insertion sequences. *Chromosoma* **74**, 57–74.

Coen, E. S. & Dover, G. A. (1982). Multiple Pol I initiation sequences in rDNA spacers of *Drosophila melanogaster*. *Nucleic Acids Research* **10**, 7017–7026.

Cooper, K. W. (1959). Cytogenic analysis of major heterochromatic elements (especially Xh and Y) in *Drosophila melanogaster* and the theory of 'heterochromatin'. *Chromosoma* **10**, 535–588.

Dawid, I. B. & Botchan, P. (1977). Sequences homologous to ribosomal insertions occur in the *Drosophila* genome outside the nucleolus organizer. *Proceedings of the National Academy of Sciences* (*USA*) **74**, 4233–4237.

Dawid, I. B., Wellauer, P. K. & Long, E. O. (1978). Ribosomal DNA in *Drosophila melanogaster*: I. Isolation and characterization of cloned fragments. *Journal of Molecular Biology* **126**, 749–768.

De Cicco, D. V. & Glover, D. M. (1983). Amplification of rDNA and type I sequences in *Drosophila* males deficient in rDNA. *Cell* **32**, 1217–1225.

Dunn, A. R. & Hassel, J. A. (1977). A novel method to map transcripts: evidence for homology between an Adenovirus mRNA and discrete multiple regions of the viral genome. *Cell*, **12**, 23–36.

England, P. R., Stokes, H. W. & Frankham, R. (1988). Clustering of rDNA containing type I insertion sequence in the distal nucleolus organizer of *Drosophila melanogaster*: implications for the evolution of X and Y rDNA arrays. *Genetical Research* **51**, 209–216.

Glover, D. M. & Hogness, D. S. (1977). A novel arrangement of the 18S and 28S sequences in a repeating unit of *Drosophila melanogaster*. *Cell* **10**, 167–176,

Glover, D. M., White, R. L., Finnegan, D. J. & Hogness, D. S. (1975). Characterization of six cloned DNAs from *Drosophila melanogaster*, including one that contains the genes for rRNA. *Cell* **5**, 149–157.

Jolly, D. J. & Thomas, C. A. (1980). Nuclear RNA transcripts from *Drosophila melanogaster* ribosomal RNA genes containing introns. *Nucleic Acids Research* **8**, 67–84.

Kidd, S. J. & Glover, D. M. (1981). *Drosophila melanogaster* ribosomal DNA containing type II insertions is variably transcribed in different strains and tissues. *Journal of Molecular Biology* **151**, 645–662.

Labella, T., Vicari, L., Manzi, A. & Graziani, F. (1983). Expression of rDNA insertions during rDNA magnification in *Drosophila melanogaster*. *Molecular and General Genetics* **190**, 486–493.

Long, E. O., Collins, M., Kiefer, B. I. & Dawid, I. B. (1981). Expression of the ribosomal DNA insertions in bobbed mutants of *Drosophila melanogaster*. *Molecular and General Genetics* **182**, 377–384.

Long, E. O., & Dawid, I. B. (1979). Expression of ribosomal DNA insertions in *Drosophila melanogaster*. *Cell* **18**, 1185–1196.

Long, E. O. & Dawid, I. B. (1980*a*). Repeated genes in Eucaryotes. *Annual Review of Biochemistry* **49**, 727–764.

Long, E. O. & Dawid, I. B. (1980*b*). Alternative pathways in the processing of ribosomal RNA precursor in *Drosophila melanogaster*. *Journal of Molecular Biology* **138**, 873–878.

Long, E. O., Rebbert, M. L. & Dawid, I. B. (1980). Structure and expression of ribosomal RNA genes of *Drosophila melanogaster* interrupted by type 2 insertions. *Cold Spring Harbor Symposium on Quantitative Biology* **45**, 667–672.

Makni, M. & Marrakchi, M. (1978). Mise en evidence d'une mutation bobbed à effet cryosensible chez *Drosophila melanogaster*. *Biologie Cellulaire* **33**, 39a.

Marrakchi, M. & Prud'homme, N. (1971). A study of bobbed mutants induced by ethyl methane sulfonate in *Drosophila melanogaster*. *Biochemical and Biophysical Research Communications* **43**, 273–277.

Pellegrini, M., Manning, J. & Davidson, N. (1977). Sequence arrangement of the rDNA of *Drosophila melanogaster*. *Cell* **10**, 213–224.

Ritossa, F. M., Atwood, K. C. & Spiegelman, S. (1966). A molecular explanation of bobbed mutants of *Drosophila* as partial deficiencies of ribosomal DNA. *Genetics* **54**, 819–834.

Ritossa, F. M. & Spiegelman, S. (1965). Localization of DNA complementary to ribosomal RNA in the nucleolus organizer region of *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences* (*USA*) **53**, 737–745.

Roiha, H. & Glover, D. M. (1980). Characterization of complete type II insertions in cloned segments of ribosomal DNA from *Drosophila melanogaster*. *Journal of Molecular Biology* **140**, 341–355.

Roiha, H., Miller, J. R., Woods, L. C. & Glover, D. M. (1981). Arrangements and rearrangements of sequences flanking the two types of rDNA insertions in *Drosophila melanogaster*. *Nature* **290**, 749–753.

Sharp, Z. D., Gandhi, V. V. & Procunier, J. D. (1983). X chromosome nucleolus organizer mutants which alter

https://doi.org/10.1017/S0016672300028494 Published online by Cambridge University Press

major type I repeat multiplicity in *Drosophila melanogaster*. *Molecular and General Genetics* **190**, 438–443.

Southern, E. M. (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. *Journal of Molecular Biology* **98**, 503–517.

Terracol, R. (1986). Transcription of rDNA insertions in bobbed mutants of *Drosophila melanogaster*. *Genetical Research* **48**, 167–174.

Terracol, R. & Prud'homme, N. (1986). Differential elimination of rDNA genes in bobbed mutants of *Drosophila melanogaster*. *Molecular and Cellular Biology* **6**, 1023–1031.

Thomas, P. S. (1980). Hybridization of denatured RNA and small DNA fragment transferred to nitrocellulose. *Proceedings of the National Academy of Sciences* (*USA*) **77**, 5201–5205.

Tullis, R. H. & Rubin, H. (1980). Calcium protects DNase I from proteinase K: a new method for the removal of contaminating RNase from DNase I. *Analytical Biochemistry* **107**, 260–264.

Wahl, G. M., Stern, M. & Stark, G. R. (1979). Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl paper and rapid hybridization using dextran sulfate. *Proceedings of the National Academy of Sciences* (*USA*). **76**, 3683–3687.

Wellauer, P. K. & Dawid, I. B. (1977). The structural organization of ribosomal DNA in *Drosophila melanogaster*. *Cell* **10**, 193–212.

Wellauer, P. K., Dawid, I. B. & Tartof, K. D. (1978). X and Y chromosomal ribosomal DNA of *Drosophila*: comparison of spacers and insertions. *Cell* **14**, 269–278.

White, R. L. & Hogness, D. S.(1977). R loop mapping of 18S and 28S sequences in the long and short repeating units of *Drosophila melanogaster* rDNA. *Cell* **10**, 177–192.

https://doi.org/10.1017/S0016672300028494 Published online by Cambridge University Press