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Transcription of rDNA insertions in bobbed mutants of *Drosophila melanogaster*

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Summary

In *Drosophila melanogaster* a large number of the genes coding for 18S and 28S rRNA are interrupted in the 28S region by insertions of two types. Ribosomal insertion transcripts were compared in wild-type and bobbed strains. We found that the level of insertion transcripts increased in bobbed mutants after deletion of 50% of INS$^-$ genes, and inversely decreased in revertants when more than 50% of wild-type levels. Among type II insertion transcripts we found a predominant 3·5 kb RNA, precisely of the most frequent insertion size. No primary insertion transcript has been found, although it could be undetected if very fast splicing leads to mature 28S occurs.

1. Introduction

In *Drosophila melanogaster*, 18S and 28S rRNA genes are located in the *X* and *Y* heterochromatic regions. A wild-type locus contains from 150 to 250 tandemly arranged repeats. Partial deficiencies of rDNA lead to the bobbed phenotype, characterized by small bristles, abdominal etching and developmental delay (Ritossa, Atwood & Spiegelman, 1966). Many 28S genes are interrupted by insertions of two types, INS I and INS II, that share no homology in size or sequence (Glover & Hogness, 1977; Pellegrini, Manning & Davidson, 1977; Wellauer & Dawid, 1977; White & Hogness, 1977; Glover, 1981; Dawid *et al.* 1978; Wellauer *et al.* 1978). Ribosomal INS I occur only on the *X* chromosome, in size classes ranging from 0·5 to 6·5 kb, with a major size class of 5·5 kb. A 0·5 kb sequence homology is found at the right end of all such insertions (Wellauer & Dawid, 1978). Type I insertions are also found outside rDNA (Dawid & Botchan, 1977; Dawid *et al.* 1981; Peacock *et al.* 1981; Appels & Hilliker, 1982; De Cicco & Glover, 1983). INS II occur exclusively in rDNA of both *X* and *Y* chromosomes, in size classes ranging from 1·5 to 4 kb, with a major size class of 3·4 kb. The 8 kb precursor transcript of INS$^-$ genes is processed into 2, 5·8, 18 and 28S rRNA. Primary transcripts corresponding to INS$^+$ genes are very rare, and then these genes are at present considered as pseudogenes. In the wild-type strain Oregon R a cytoplasmic RNA about 1 kb long, hybridizing to type I insertions, exists in all developmental stages and tissues but at a very low level (Long & Dawid, 1979; Jolly & Thomas, 1980). Among INS II transcripts, the most prevalent is 3·4 kb, corresponding in length to the major INS II. 28S insertion transcripts are, however, present at 400-fold lower concentration than the primary transcripts of INS$^-$ genes in Oregon R. The level of insertion transcripts varies from one wild-type strain to another, and is higher in ovaries than in embryos, larvae or pupae (Kidd & Glover, 1981). Long *et al.* (1981) studied bobbed strains and concluded that INS$^+$ gene transcript level was characteristic for particular strains, and unrelated to bobbed phenotype. Labella *et al.* (1983) showed that during magnification (which occurs when an *X* chromosome bobbed mutation is associated with a *Ybb*$^-$ chromosome in males), INS$^+$ gene transcription increased compared to that of homozygous females. The authors inferred that their findings were due to a general increase in transcription, occurring specifically during magnification.

In this paper we compare insertion gene transcription in bobbed strains and their wild-type strains of origin to the respective copy number of the three gene types. In particular we examined the rDNA organization and transcription of a subclone issued from an extreme bobbed mutant, bb^{P2}, isolated from an M5 chromosome (Marrakechi & Prud'homme, 1971). bb^{P2} *in vivo* pulse-labelled rRNA displays an excess of 18S compared to 28S and an increased amount of 32S (Marrakechi, 1974), possibly the consequence of an

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increase in INS$^+$ gene transcription followed by incorrect splicing.

2. Materials and methods

(i) *Drosophila stocks*

The *X* chromosome from the wild-type Oregon R strain (Gif) was cloned by crossing a single male to females carrying two XM5 chromosomes. XM5 chromosome = Ins(1) $Sc^{s1L}sc^{s8R}+S$, $scS1w^aB$ (Atwood). bb^{P2} (Marrakechi & Prud'homme, 1971) and bb^{P7} were EMS-induced on the XM5 chromosome. bb^{P12} and bb^{P10} are spontaneous bobbed mutants obtained from the subcloned wild-type Oregon R strain. bb^{P12m1} is a magnified bobbed and bb^{P12R} a spontaneous wild-type revertant, both obtained from bb^{P12}. bb^{P5} was EMS-induced on wild-type Oregon R *X* chromosome (Marrakechi & Prud'homme, 1971).

(ii) *rDNA quantification, brain DNA extraction and hybridization*

These were performed as previously described (Terracol & Prud'homme, 1986).

(iii) *Total RNA extraction*

A total of 100–200 flies were ground in 5 ml of the following buffer: 10 mM tris HCl, pH 7·4, 100 mM-NaCl, 10 mM EDTA, 0·5% SDS. RNA was extracted twice with 1 vol. phenol– (saturated with 0·2 M sodium acetate pH 5) chloroform–isoamyl alcohol (100 : 96 : 4, v/v) and then with 1 vol. chloroform–isoamyl alcohol (96 : 4). Two vols. 6 M-LiCl were then added to the aqueous phase and left 24 h at 0 °C. The precipitate was centrifuged 45 min at 8000 rev/min in the HB4 sorvall rotor, dissolved in 500 μl H_2O and adjusted to 20 mM Tris HCl, pH 8, 10 mM-$CaCl_2$, 25 μg/ml DNase previously treated as follows: 1 mg/ml of DNase I was incubated 2 h at 37 °C in 20 mM Tris HCl, pH 8, 10 mM-$CaCl_2$ with 1 ng/ml of proteinase K (Tullis & Rubin, 1980). The RNA : DNase mixture was incubated 1 h at 37 °C and the reaction was stopped by adjusting the solution to 15 mM EDTA, 1% SDS followed by an incubation of 1 h at 37 °C. The RNA was then extracted with phenol–chloroform–isoamyl alcohol. The aqueous phase was adjusted to 0·3 M sodium acetate and the RNA precipitated with 2 vols. of 100% ethanol. After centrifugation, the precipitate was rinsed with 70% ethanol, oven-dried and dissolved in 30 μl of H_2O (about 10 μg/ml).

(iv) *Electrophoresis and transfer of RNA*

RNA (30–50 μg) was denatured 15 min at 55 °C in 20 μl of the following buffer: 20 mM morpholinopropane sulphonic acid, pH 7, 5 mM sodium acetate, 1 mM EDTA. The mixture was loaded on a 0·8% horizontal agarose gel (19 × 23 cm) containing this last buffer plus 2·2 M formaldehyde. After overnight migration with circulation of the buffer (70 V, 30 mA) the gel was stained with acridine orange (10 μg/ml), then rinsed and photographed under UV. The gel was treated twice for 20 min with 50 mM-NaOH, 10 mM-NaCl, and then neutralized twice for 20 min with 0·1 M Tris HCl, pH 7·5. The gel was then equilibrated for 1 h in 20 × SSC and RNA was transferred overnight to nitrocellulose filters (Scleicher and Schüll BA85) in 20 × SSC (Thomas, 1980). The blots were rinsed in 3 × SSC and dried 4 h at 80 °C in a vacuum oven. The filters were hybridized as for DNA blots.

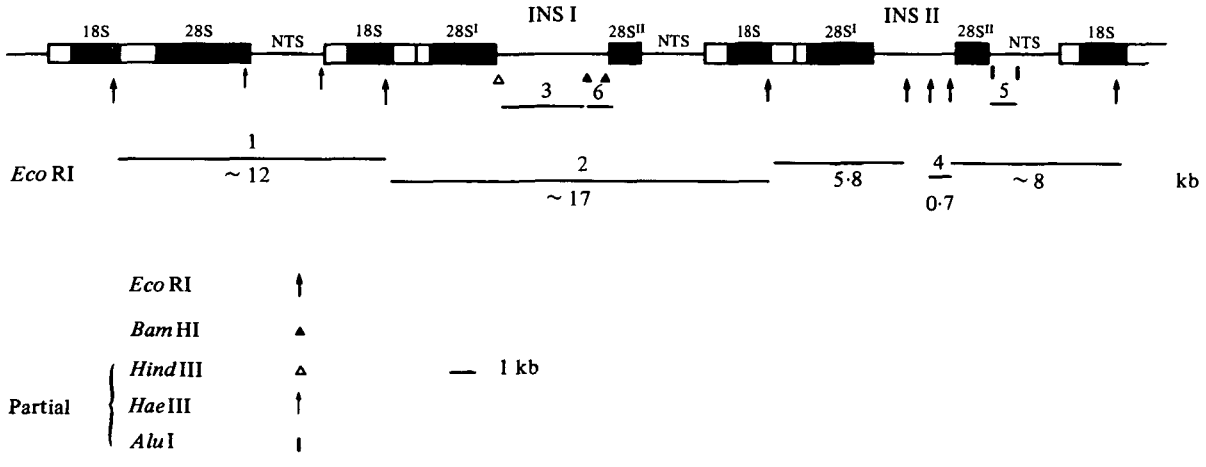

Fig. 1. Restriction map of *Drosophila melanogaster* rDNA and list of probes (adapted from the map of Long *et al.* 1981). The three gene types were arbitrarily arranged. (1) DmrY12: 12 kb *Eco* RI INS$^-$ fragment; (2) Dmr103: 17 kb *Eco* RI INS I fragment; (3) Dmr103C2: 4·5 kb *Hind* III-*Bam* HI INS I fragment; (4) Dmr205: 0·7 kb *Eco* RI INS II fragment. (5) Dmrs1150: 1150 left end *Alu* I non-transcribed spacer fragment; (6) Dmra56Bam: 1 kb *Bam* HI INS I fragment.

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(v) *Dot blot hybridization*

RNA (2·5 μg/ml) in 2 × SSC was denatured 10 min at 100 °C and quickly chilled on ice. Fractions of 2 μl were loaded on nitrocellulose filters previously soaked in 20 × SSC and dried. When the fraction was absorbed on paper the operation was repeated until the desired quantity of RNA had been applied. The filter was then dried at room temperature (30 min) and oven-dried for 2 h at 80 °C.

(vi) *Sandwich hybridization*

Sandwich hybridizations were adapted from Dunn & Hassel (1977) and Wahl *et al.* (1979). Nitrocellulose filters with plasmid DNA were prehybridized at 42 °C for 2 h in 50% formamide, 5 × SSC, 5 × Denhardt's in 50 mM sodium phosphate buffer (pH 6·5) 50 μg of yeast tRNA per ml and 0·1% SDS. Filters were hybridized overnight in 50% formamide, 5 × SSC, 1 × Denhardt's, 20 mM sodium phosphate buffer (pH 6·5), 10% dextran sulphate 500, 0·1% SDS and 25 μg per ml of cold Drosophila RNA. They were rinsed at 42 °C three times for 20 min each in 2 × SSC 0·1% SDS and three times for 20 min each in 0·5 × SSC 0·1% SDS. Filters were then prehybridized and hybridized overnight with ^{32}P probe in the same buffers (without SDS) as with RNA and rinsed as previously described. Control filters were treated, after the first rinse in 2 × SSC 0·1% SDS, for 2 h at 42 °C with RNAse A (20 μg/ml) in 2 × SSC, then rinsed in 0·5 SSC 0·1% SDS. They were then hybridized as previously described.

(vii) *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 *Col* EI (Wellauer *et al.* 1978). pDmr103, used as size marker, contains a 17 kb *Eco* RI ribosomal fragment with 5·5 kb INS I inserted at the *Eco* RI site of *Col* EI (Glover *et al.* 1975; Glover & Hogness, 1977). pDmr103C2 contains the 4·5 kb *Hind* III-*Bam* HI INS I fragment of pDmr103 inserted in pBR322 (Kidd & Glover, 1980). pDmr205 contains the 0·7 kb *Eco* RI INS II fragment cloned into the *Eco* RI sites of pBR322 (Long *et al.* 1980). pDmra56Bam contains the 1 kb *Bam* HI INS I fragment in the *Bam* HI sites of pBR322 (Long & Dawid, 1979). pDmr1150 contains a 1150 bp *Alu* I non-transcribed spacer left end fragment.

3. Results

(i) *Pattern and distribution of rDNA*

For all strains, namely Oregon R wild type, M5, bb^{P2}, bb^{P5}, bb^{P7}, bb^{P10}, bb^{P12}, bb^{P12m1}, bb^{P12R}, we determined the number of rDNA genes by hybridization of homozygous female DNA with labelled rRNA. For these same strains the distribution of the three types of genes, INS$^-$, INS I, INS II, was achieved by scanning blots of genomic DNA digested with *Eco* RI and *Eco* RI/*Bam* HI and hybridized to a ribosomal probe (pDmrY12). *Eco* RI cuts once in the 18S region of each gene and at least once in INS II. *Bam* HI cuts at least once in INS I (Map, Fig. 1). Non-transcribed spacer organization was determined by probing Hae III digested genomic DNA transfers with a spacer probe (pDmrs 1150). All spacers were found to be between 3·5 and 7 kb, with the exception of some long spacers of 10–20 kb in bb^{P5}. Since the structural gene is 8 kb long and the major size class of non-transcribed spacer is near 5 kb (very few spacers are longer than 7 kb, except in bb^{P5}), *Eco* RI fragments longer than 10 kb correspond to INS$^-$ and INS I genes. Fragments shorter than 10 kb correspond to the left and right ends of INS I genes. *Eco* RI–*Bam* HI fragments longer than 10 kb correspond to INS$^-$ genes alone. Fragments shorter than 10 kb correspond to the two parts of INS I and INS II genes.

As an example, we show in Fig. 2 comparison of bb^{P2} and bb^{+} strains. The pattern of the original M5 wild-type strain differs from that of the Oregon R wild-type (OR) by the presence of a single band corresponding to 12·5 kb INS$^-$ genes, and by a higher proportion of INS II genes (Fig. 2 I). Fig. 2 II shows that INS$^-$ genes (greater than 10 kb *Eco* RI/*Bam* HI fragments) are less abundant in M5.bb^{P2}; E.M.S. induced on the M5 chromosome, differs from it by a clear decrease in 12·5 kb INS$^-$ fragments. Other fragments disappear entirely, especially those longer than 10 kb in double *Eco* RI/*Bam* HI digests (INS$^-$ genes). The relative proportions of the different gene types were deduced from densitometric analysis (Fig. 3). The number of genes in each class can be easily calculated from the total. M5, when compared with OR, contains 27 fewer INS$^-$ genes, an equal number of INS I genes, and 5 more INS II genes per locus. bb^{P2} lost 62% of the INS$^-$ genes, 30% of the INS I genes and 25% of the INS II genes compared to M5, its strain of origin. To summarized, bobbed strains have fewer genes and show modified distributions of insertion and spacer size classes when compared with the original wild-type strain (Table 1, columns 4–6).

(ii) *Insertion transcripts*

bb^{P2} and bb^{P7} insertion transcripts were compared with those of the original M5 strain. bb^{P7} contains the same number of ribosomal genes as bb^{P2}, about 100, with 29% INS$^-$, 41% INS I and 30% INS II units. Total RNA was hybridized either to an INS$^-$ ribosomal probe (pDmrY12: Fig. 4 I), to the *Bam* HI INS I fragment common to each INS I (pDmra56Bam, Fig. 4 II) or to the 0·7 kb INS II *Eco* RI fragment (pDmr205, Fig. 4 III). The hybridization intensities

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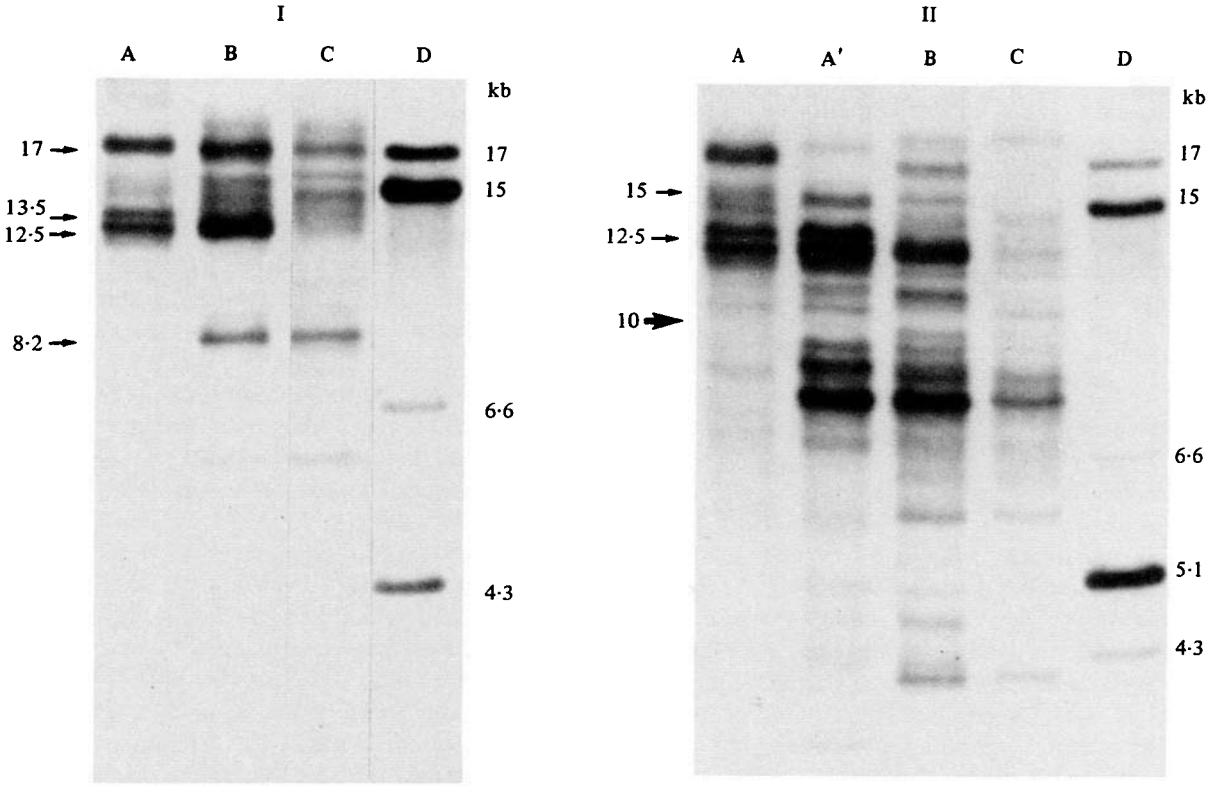

Fig. 2. *Eco* RI and *Bam* HI restriction patterns of bb^+ and bb^{P2} strains. A total of 1/10 of *Eco* RI (I and IIA) or *Eco* RI/*Bam* HI (IIA'BCD) DNA digests from 50 homozygous female larvae brains were fractionated on a 0·6% agarose gel, blotted on a nitrocellulose filter and hybridized to the ^{32}P-labelled pDmrY12 probe (12 kb *Eco* RI INS I fragment). (A) (A') bb^+ Oregon R; (B) bb^+ M5; (C) bb^{P2}; (D) size marker pDmr103 *Eco* RI+pDmr103 *Pst* I.

cannot be directly compared because the probes had different specific activities.

Hybridization with rDNA (pDmrY12) shows transcripts of 8, 4·6 and 3·7 kb and a group of near 1·8 kb. The 8 kb corresponds to the 38S primary transcript (Dawid *et al.* 1978), the 4·6 kb to the 32S precursor of 38S, and the 3·7 kb to the mature 28S. Under the denaturing conditions used, a large proportion of 28S rRNA central cleavage is seen as 28Sa and 28Sb molecules of respectively 1·6 and 1·9 kb. Mature 18S is 1·8 kb (Long & Dawid, 1979). For the three strains, the intensities of hybridization are quite comparable. In bb^{P2} and bb^{P7} we found a new 4·3 kb band not well separated from that of the 4·6 kb (32S), but together these represent a higher transcription level than the M5 4·6 kb band alone. The two insertion probes reveal the presence of INS I (Fig. 4 II) and INS II (Fig. 4 III) transcripts in bb^{P2} and bb^{P7}, while in the M5 strain no hybridization is detectable under identical conditions. bb^{P2} (B) mutant displays stronger hybridization with both types of insertion probes than bb^{P7} (C). Using INS I as probe, we found 3·9, 3, 1·45, 1·25, 0·8 and 0·6 kb transcripts in these two strains. bb^{P2} also shows some longer transcripts though in lower proportions. INS I gene primary transcripts, i.e. longer than 8 kb, are not detectable. The 0·8 kb fragment, hardly visible in the M5 strain, is predominant in both bobbed strains. It could be of the same origin as the cytoplasmic 1 kb transcript complementary to short INS I and to ribosomal sequences flanking the insertion (Long & Dawid, 1979). It is noteworthy that the same filter hybridized to the *Hind* III–*Bam* HI long INS I fragment (pDmr103C2, specific to long insertions) shows no signal either in M5, bb^{P2} or bb^{P7} (data not shown). This is in good agreement with the results of Long & Dawid (1979) and Jolly & Thomas (1980), indicating that transcripts from long insertion genes are very rare. In bb^{P2}, hybridization with the INS II probe demonstrates transcripts of 8·4, 7·5, 6·2, 5, 4·2, 3·5 and 1·7 kb. The 3·5 kb RNA is as predominant in total RNA as it was found to be in the nuclei of wild-type strains by Kidd & Glover (1981) and corresponds to the major INS II size class usually found in these strains. The other RNAs are not the same sizes as those found by Kidd and Glover, and could correspond to precursors with insertions in the process of maturation or degradation. In particular, the 8·4 kb RNA could correspond to a 28S precursor, equivalent to 32S, but additionally containing the INS II 3·5 kb sequence. However, no 3·5 kb INS II gene primary transcripts (whose size would thus be 11·5 kb) are seen. The 4·2 kb RNA could be the same as the 4·3 kb previously described for the ribosomal probe. One possible hypothesis is that it is a mature 28S RNA (3·7 kb) with a non-spliced 0·5 or 0·6 kb INS II.

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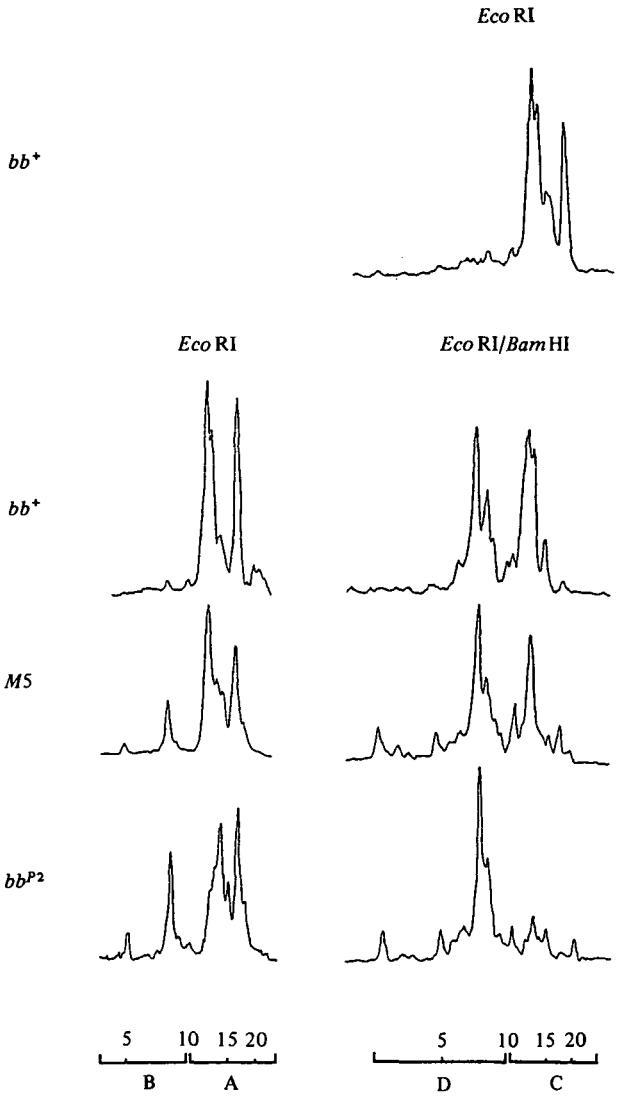

Fig. 3. Scannings of Fig. 2 autoradiographs. Autoradiographs were scanned on a Joyce Loebel microdensitometer. The areas were determined within the linear response range of the film. (A) *Eco* RI fragments longer than 10 kb corresponding to INS$^-$ and INS I genes. (B) *Eco* RI fragments shorter than 10 kb corresponding to INS II genes. (C) *Eco* RI/*Bam* HI fragments longer than 10 kb corresponding to INS$^-$ genes. (D) *Eco* RI/*Bam* HI fragments shorter than 10 kb that correspond to INS I and INS II genes.

(iii) *Dot blot analysis of insertion transcripts*

Increasing amounts to total RNA from females of various strains were hybridized with the *Bam* HI INS I probe pDmra56Bam, then washed and rehybridized to the 0·7 kb *Eco* RI INS II fragment. In order to permit the best photographic representation for all strains, and in view of large signal variations from one strain to another, some dot blots were over-exposed on the autoradiographs presented in Fig. 5. Relative transcript amounts were measured by densitometric analyses at concentrations giving a linear response range of the film. Under these conditions, the error was estimated to be about 10%. The results presented in Table 1 give the ratios of INS I or INS II transcripts in different strains compared to OR. For any given strain, increases are the same for type I and II insertions. M5 contains about 3·5-fold more insertion transcripts than OR. In bb^{P2}/bb^{P2} insertion transcripts are present at 80-fold higher concentration than in OR, and in bb^{P7}/bb^{P7} about 50-fold higher. bb^{P5}/bb^{P5} is slightly bobbed and shows 30-fold more insertion transcripts. bb^{P12}/bb^{P12} is wild type, and has 15-fold more insertion transcripts than OR. bb^{P12m1}, a magnification product of bb^{P12}, and bb^{P12R} are wild type when homozygous or when associated with a deficiency. These two strains have about 2-fold more insertion transcripts than OR. bb^{P10}/bb^{P10} is wild type and contains 3-fold more insertion transcripts. The three strains that show the largest increase in transcript levels are those that are bobbed when homozygous (bb^{P2}, bb^{P7} and bb^{P5}).

The transcript levels relative to the different gene types in each strain are in inverse proportion to the INS$^-$ genes. The variation is not associated with a greater or lesser number of INS$^+$ genes nor with a particular chromosome structure: bb^{P2} and bb^{P7} were induced on a double inverted M5 structure, while the other mutant strains derive from the wild-type Oregon R strain. However, the decrease of transcripts in revertants arising from bb^{P12} clearly shows that this phenomenon is associated with the bobbed locus itself. Finally, the level of insertion transcripts in bb^{P2}/M5 is lower than expected when compared to bb^{P2} and M5 homozygous strains. Thus, the strong increase in INS$^+$ transcripts observed in bb^{P2} is inhibited by the presence of a bb^+ homologue chromosome: the active regulating factor then comes from the locus containing many INS$^-$. However, this result could possibly be the consequence of the activation of a single rDNA locus, bb^+ in this context.

The increased transcription of insertions could result from copies of the sequences found outside rDNA. Although type II insertions have never been found outside rDNA, type I insertions are known to exist in the 102C region of chromosome 4 and on the *X* chromosome in the heterochromatin distal to rDNA. In order to demonstrate a link between insertion transcription of both types and rDNA, we carried out sandwich hybridizations. We first hybridized total cold RNA from each strain to the insertion I fragment from pDmra56Bam and insertion II fragment from pDmr205 fixed to filters, and then examined the resulting DNA/RNA hybrid for free RNA tails by hybridizing with labelled pDmrY12 rDNA probe. Hybridization occurred in each case, indicating that INS$^+$ RNA molecules were linked to rDNA (data not shown).

4. Discussion

Insertion transcripts have been detected at higher levels in bobbed mutants in comparison to the original bb^+ strains. The amount of these transcripts

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Table 1. *Comparison of the quantities of insertion transcripts with the distribution of the different types of genes per diploid genome*

	RNA/RNA$^+$					
	INS I	INS II	Phenotype*	INS$^-$	INS I	INS II
+/+	1	1	+	196	140	64
M5/M5	3·3	3·5	+	138	138	78
bb^{P2}/bb^{P2}	76	88	bb^s	52	98	56
bb^{P2}/M5	1·2	0·8	+	95	117	68
bb^{P7}/bb^{P7}	46	52	bb^s	56	80	58
bb^{P12}/bb^{P12}	16	15	+	94	110	58
bb^{P12m1}/bb^{P12m1}	2	2·2	+	156	124	56
bb^{P12R}/bb^{P12R}	2·2	1·6	+	138	118	68
bb^{P10}/P^{P10}	3·5	3·1	+	102	114	64
bb^{P5}/bb^{P5}	26	35	bb^l	84	58	40

The quantities of insertion transcripts were obtained by scanning dot blot autoradiographs in the linear response range of the film. The distribution of the three types of genes was obtained by scanning auotoradiographs of *Eco* RI and *Eco* RI/*Bam* HI blots, in the linear response range of the film.
* s = strong, l = light.

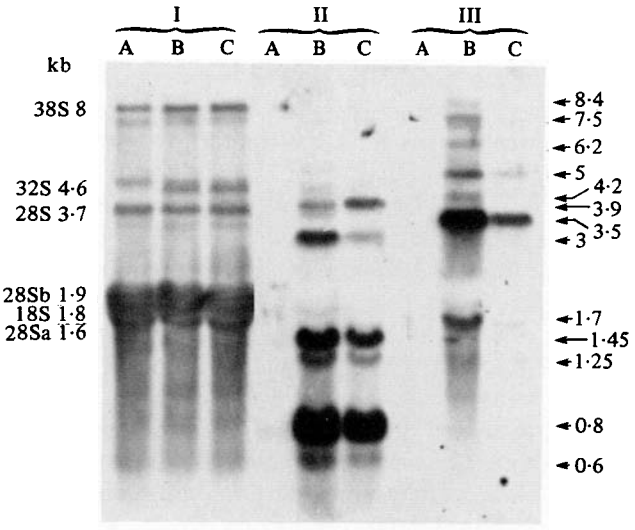

Fig. 4. Total RNA hybridization of bb^+ M5, bb^{P2}, and bb^{P7}. 45 μg of total RNA were fractionated on 0·8% denaturing agarose gels (formaldehyde) blotted on to nitrocellulose and hybridized to ^{32}P-labelled probes (I) pDmrY12 (12 kb *Eco* RI INS$^-$ fragment), (II) pDmra56Bam (1 kb *Bam* HI short INS I fragment), (III) pDmr205 (0·7 kb *Eco* RI INS II fragment). (A) bb^+ M5; (B) bb^{P2}; (C) bb^{P7}.

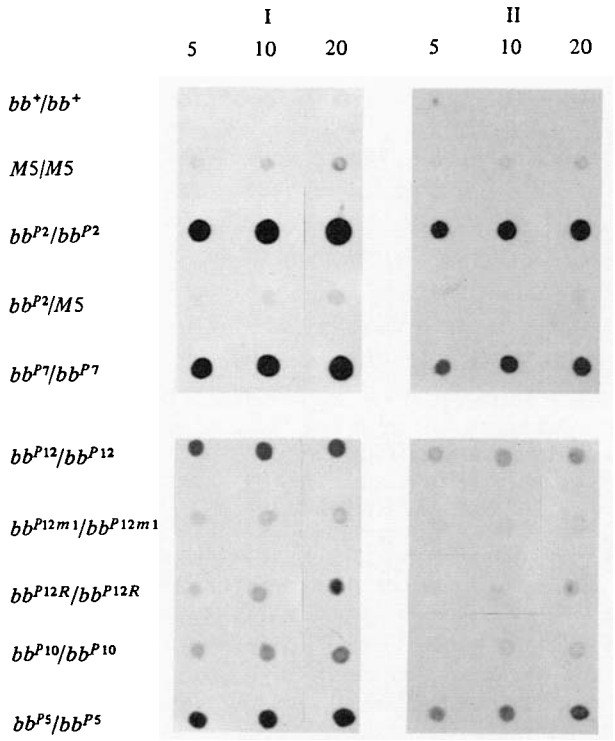

Fig. 5. Comparison of insertion transcripts of various genotypes by dot blot analyses. Increasing quantities of total RNA (5–20 μg) were loaded on a nitrocellulose filter and hybridized to ^{32}P-labelled (I) pDmra56Bam probe (1 kb INS I fragment). (II) The filter was dehybridized and hybridized to pDmr205 probe (0·7 kb *Eco* RI fragment of INS II). Some dot blots were over-exposed to facilitate photographic reproduction.

does not seem related to the number of INS$^+$ genes in each strain but depends on the INS$^-$ gene number: a threshold of about 100 INS$^-$ per diploid genome might exist, below which the transcription of INS$^+$ genes would be activated, yet without reaching the transcriptional level of INS$^-$ genes. The increase observed in mutants derived from two bb^+ strains (bb^{P5}, bb^{P10} and bb^{P12} arose from Oregon R; bb^{P2} and bb^{P7} issued from M5) and the decrease observed in the revertants directly arising from one of these mutants (bb^{P12R} and bb^{P12m} arose from bb^{P12}) clearly indicate that the phenomenon is linked to the bobbed locus itself and is a consequence of mutational events at the locus. That transcription of both insertion types is linked is suggested by proportionality in the increase and decrease in the transcript levels. We have ob-

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served, however, only the result of transcription followed by maturation processes, and not primary transcription. The maturation process may be differential, according to the size classes of insertions contained in a given strain. Indeed, at least at the level of INS I genes in embryo nuclei, transcripts of 5 kb insertions are rarer than those of shorter insertions (Long & Dawid, 1979), probably because these molecules are rapidly degraded (Jamrich & Miller, 1984). In the same way, with total RNA, we found molecules complementary only to short insertions. Our sandwich hybridizations, and the fact that some RNA molecules revealed by the insertion probes are longer than the insertions themselves, suggest that in bobbed mutants INS^+ genes are effectively transcribed. But our results do not demonstrate that transcription and splicing are accurate. Some previous observations might be interpretable if we hypothesize inaccurate splicing of INS^+ genes, activated in bobbed strains, leading to non-functional products. Among these is the fact that the rDNA transcription level in strong bobbed premagnified males (Ritossa *et al.* 1971; Shermoen & Kiefer, 1975; Graziani & Gargano, 1976; Locker & Marrakechi, 1977) and also in strong bobbed females (Terracol & Prud'homme, 1981) was found to be higher than in the wild-type control. Moreover, 28S maturation defects were described in bb^{P2} rRNA (Marrakechi, 1974).

The lack of INS^- genes could be one of several factors leading to induction of INS^+ gene transcription. In fact, the level of insertion transcripts is not the same in several wild-type strains: we found 3·5-fold more in bb^+M5 than in Oregon R. Kidd & Glover (1981) showed that the concentration of type II transcripts in Canton S females was 10-fold lower than in Oregon R females. They also found that in flies carrying a translocation of *X* heterochromatin on the *Y* chromosome ($y^+Y/sc^{4L}sc^{8R}/sc^{4L}sc^{8R}$) the concentration of type II transcripts is 2 orders of magnitude greater than in wild-type Oregon R females. In this genotype the *X* chromosomes do not contain any rDNA and the number of the different gene types on the *Y* chromosome was not determined. The number of INS^- genes is probably reduced even if the flies do not express a bobbed phenotype. If we examine our results we note, for instance, a 16-fold increase in the amount of insertion transcripts in bb^{P12} homozygous females, which are wild-type in phenotype, as opposed to a 100 × increase. We can infer that the level of transcripts depends on other factors – the heterochromatic structure around the rDNA locus possibly one of them. One way to interpret all the results is to suppose that INS^+ genes are transcribed when they are sufficiently accessible to RNA polymerase, perhaps achieved by a modification of the heterochromatin-structure-surrounding region or by deletion of INS^- genes in the locus intself. This hypothesis is supported by the results of Wayne *et al.* (1985), indicating that INS^+ repeats are less sensitive than INS^- genes to DNase I digestion, implying that they are folded into a higher-order chromatin configuration. Futhermore, Dawid & Rebbert (1986) have shown that intercalating drugs increase up to 60-fold the 0·8 kb type I insertion transcript level. They conclude that type I insertion genes are assembled into chromatin configuration lacking torsional stress. The presence of two different chromatin structures further supports the previously hypothesized clustering of genes according to type (Terracol & Prud'homme, 1986).

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