

## SHORT PAPER

### Chromosomal distribution of the major insert in *Drosophila melanogaster* 28S rRNA genes

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#### SUMMARY

The major type I insert sequence for the 28S rRNA genes of *Drosophila melanogaster* has been mapped within the chromosomes using a probe synthesized from a cloned sequence containing the entire 5.4 kb segment. The genomic distribution was shown to be complex in that the insert sequence occurred next to many different types of sequences, in addition to occurring as an insert in the 28S rRNA genes of the X chromosome. *In situ* hybridization of mitotic chromosomes showed most of the insert units not contained in the ribosomal genes to be located near the ribosomal gene cluster on the X chromosome. Additional sites were detected in polytene chromosomes in region 102C, 8-12 and in the heterochromatin of the autosomes.

#### 1. INTRODUCTION

A major proportion of the ribosomal genes of *Drosophila melanogaster* contain a DNA segment of unknown function inserted into the 28S rRNA gene (Glover & Hogness, 1977; White & Hogness, 1977). Considerable heterogeneity in the size of insert has been reported (Wellauer & Dawid, 1977; Pelligrini *et al.* 1977), the most frequent length being 5.4 kb. rDNA genes containing the 5.4 kb or smaller insert sequences have been cloned and it has been shown that the shorter sequences have homology with the right end of the 5.4 kb insert sequence (Dawid & Wellauer, 1978). This group of related sequences has been termed 'type I' inserts. In addition, a second class (type II) of insert sequence, located at the same site within the 28S gene, and having no homology with the type I insert sequences (Glover, 1977; Wellauer *et al.* 1978), has been identified. Hybridization studies (by the method of Southern, 1975) have shown that rDNA units containing the 5.4 kb type I insert are present only on the X chromosome and not on the Y chromosome (Tartof & Dawid, 1976). Wellauer *et al.* (1978) have recently shown that rDNA units with type II inserts can be cloned from flies deficient for the X chromosome nucleolus organizer, and that these rDNA units are present on both the X and Y chromosomes. Sequences homologous to the 5.4 kb type I insert are also located in the genome at places other than the repeating units of rDNA, and can be fractionated away from rDNA by centrifugation (Dawid & Botchan, 1977).

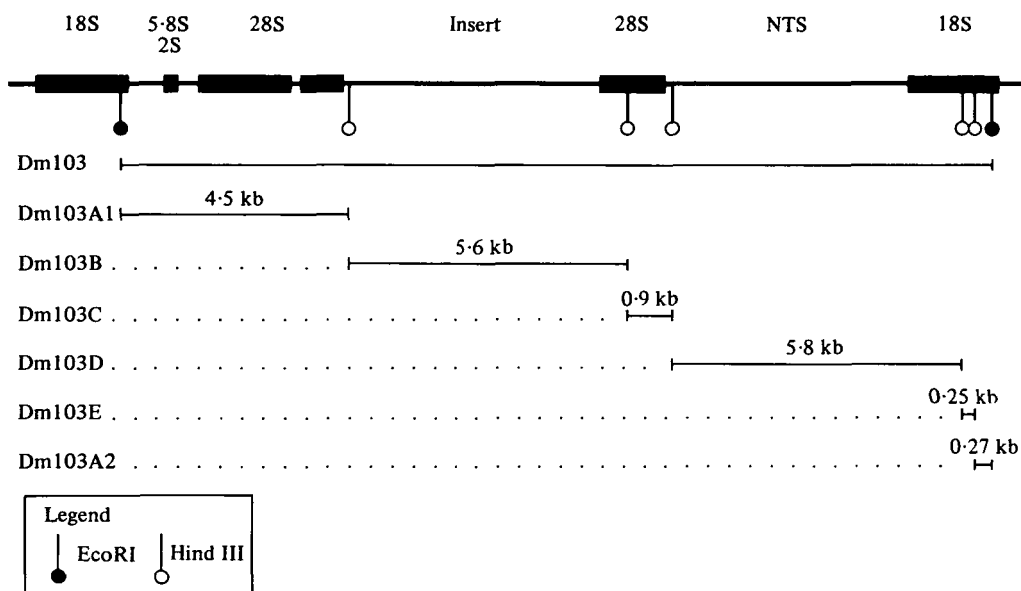
The data in this paper show the chromosomal location of these sequences. *In situ* data

are supported by molecular analyses of the distribution of type I insert sequences in Canberra wild type *D. melanogaster*. These analyses were carried out to complement those of Dawid & Botchan (1977) because a comparison of EcoRI sites in rDNA from different strains of *D. melanogaster* indicates considerable polymorphism Endow & Glover, 1979)

## 2. MATERIALS AND METHODS

### (i) DNA isolation and fractionation

DNA from *D. melanogaster* embryos was prepared and fractionated in actinomycin D/CsCl gradients as described by Brutlag *et al.* (1977). DNA from larval brains was prepared as described by Endow & Glover (1979).



Text-fig. 1. The diagram shows a map of pDm103 and the parts which were subcloned (Glover & Hogness, 1977) to give the Dm103A1, Dm103B and Dm103D plasmids referred to in the text.

### (ii) Hybridization procedures

To determine the genomic arrangement of sequences by hybridization, DNA was cleaved by an endonuclease, fractionated by gel electrophoresis in an agarose gel and transferred to nitrocellulose filters as described by Southern (1975). Radioactive probes for hybridization were prepared using either RNA polymerase or DNA polymerase.  $^3\text{H}$ -cRNA was used for some hybridization under the following conditions:  $3 \times \text{SSC}$  plus 50% formamide at  $45^\circ\text{C}$  for 5 h, followed by a washing procedure described by Birnstiel *et al.* (1972).  $^{32}\text{P}$ -cDNA as prepared by nick-translation was hybridized following the protocol of Denhardt (1966) but in  $3 \times \text{SSC}$  at  $65^\circ\text{C}$  with washing at  $65^\circ\text{C}$  in progressively lower salt concentrations, the final wash being in  $0.2 \times \text{SSC}$ .

The *in situ* hybridization procedure used here is described by Peacock *et al.* (1978). Selected chromosome preparations were pre-photographed prior to hybridization.

(iii) *Probes utilized*

The radioactive hybridization probes used were synthesized from the sub-clones of Dm103 (Glover & Hogness, 1977) shown in Text-fig. 1. The 4.5 kb fragment (A1) of Dm103 contains the transcribed spacer sequences and 3 kb of 28S sequences; fragment B contains virtually the entire 5.4 kb type I insert sequence and only 200 base pairs of 28S sequences; fragment D contains non-transcribed spacer sequences and 1.1 kb of the 18S gene.

In some experiments a radioactive probe synthesized from a plasmid pXlr101 was used. This plasmid was constructed by Dr R. Reeder and contains a complete repeating unit of *Xenopus laevis* rDNA (provided by Dr A. Bird).

### 3. RESULTS AND DISCUSSION

(i) *Genomic distribution of rDNA sequences*

When Canberra wild type embryonic DNA (approximately 5 kb long) was fractionated in actinomycin-D/CsCl gradients, Dm103AI sequences were found in the region expected for ribosomal genes whereas Dm103B sequences were detected in part as a satellite banding as a sharp peak of lower density and in part overlapping with Dm103AI sequences (Plate 1, Fig. 2). This is qualitatively similar to the result obtained by Dawid & Botchan (1977) using DNA fragments from the right and left halves of an independent clone of the 5.4 kb type I insert from Oregon R (namely the Sma D and Bam HI fragments, respectively, of their plasmid Dmra 56). This suggests that as much as 50% of the Dm103 insert sequences are *not linked* to ribosomal genes and so are free to move away from these genes in the buoyant density gradient. The Dm103B sequences banding in the ribosomal gene region of the buoyant density gradient (plate 1, Fig. 2) were shown to be linked to ribosomal genes by rebanding this DNA after denaturation, reannealing with rRNA and treating the reannealed molecules with RNase. Sequences homologous to both Dm103AI and Dm103B were shifted to the density of partial DNA-RNA duplexes, and a proportion of sequences homologous to Dm103AI shifted to the density expected for complete rRNA-DNA duplexes.

We have also compared the pattern of hybridization of Dm103B and rRNA coding sequences in total genomic DNA isolated from diploid tissue (Oregon R wild type), cleaved with EcoRI and fractionated by gel electrophoresis (Plate 1, Fig. 3). Dm103B can be seen to hybridize to at least ten more bands than the probe for the coding sequences, suggesting that the Dm103B sequences not inserted into the rDNA are located adjacent to a number of different genomic sequences. Parallel results were obtained with DNA of Canberra wild type origin.

The data in Plate 1, Fig. 3 show that Dm103B sequences are linked to a complex set of EcoRI restriction fragments. We have recently characterized a cloned segment of *D. melanogaster* DNA which consists of tandemly linked sequences homologous to Dm103B but are not linked to rDNA (Glover *et al.* 1978; unpublished experiments of S. Kidd). EcoRI fragments greater than 17 kb to which Dm103B hybridizes (Plate 1, Fig. 3) may thus represent blocks of tandemly arranged sequences of type I inserts, since the Dm103B segment contains no EcoRI cleavage sites.

(ii) *The chromosomal distribution of rDNA sequences*

The chromosomal location of sequences homologous to Dm103B were determined directly by *in situ* hybridization to salivary gland chromosomes. Grain counts show 95% of the Dm103B sequences to be in the Giemsa positive strands of the nucleolus. There is also hybridization of Dm103B to a band in the euchromatin of chromosome 4, and to the

pericentromeric heterochromatin of all chromosome arms, the heterochromatin of chromosome 3 being more prominently labelled than that of chromosome 2 (Plate 1, fig. 4*a*). Dm103A1 and Dm103D on the other hand, hybridize virtually exclusively to the nucleolus but with a significant number of grains over the heterochromatin at the base of the X chromosome, the origin of the nucleolus. The Dm103B sequences present in the euchromatin of chromosome 4 were located to 102C 8–12. In some preparations (e.g. insert Plate 1, fig. 4*a*) the sequence appears to be present in two bands close together. The translocation T(3; 4) A12 which breaks chromosome 3 at 73C 1–2 and chromosome 4 at 102C 15–102D1 (see Lindsley & Grell, 1968) leaves the Dm103B sequences on the portion of chromosome 4 attached to the chromocentre. We have been unable to repeat an observation made by Dr David Finnegan (cited in White & Hogness, 1977), in which, following a 350-day autoradiographic exposure, grains are seen over a number of euchromatic bands. We presume that in his experiments weak sequence homologies were detected under hybridization conditions not as stringent as those used by ourselves.

The studies of Tartof & Dawid (1976) indicated that DNA extracted from male flies carrying the *sc<sup>4</sup>sc<sup>8</sup>* X chromosome, which is deficient for the X chromosome nucleolus organizer region, contained no detectable rDNA units with 5.4 kb type I insert sequences suggesting these sequences are absent from the Y chromosome. We examined the hybridization of Dm103B sequences to mitotic chromosomes *in situ* (prepared from male larval brain tissue) in order to determine whether this distribution of the Dm103B sequences applied in Canberra wild type *D. melanogaster*. The 200 bp of 28S coding sequences present in the <sup>3</sup>H-cRNA probe are largely competed by unlabelled rRNA on the slides as judged from the virtual absence of hybridization of Dm103A1 <sup>3</sup>H-cRNA probe unless slides are RNase treated before hybridization. The Dm103B sequences occupy a single, well defined site in the middle of the heterochromatin of the X chromosome (Plate 1, Fig. 5). We estimate from grain counts of longer autoradiographic exposures than that shown in Plate 1, fig. 5, that at least 80–95% of the Dm103B sequences are at this site. In long exposures some hybridization was observed at the nucleolus organizer site of the Y chromosome (less than 10% relative to that seen on the X chromosome) but we have not been able to detect significant hybridization over the remaining heterochromatin of the X chromosome or in any of the autosomes. The result is in accord with the conclusion of Tartof & Dawid (1976) that rDNA units containing type I inserts are largely restricted to the X chromosome.

If the majority of Dm103B-like sequences not linked to rDNA are tandemly arranged, we could expect to detect them by *in situ* hybridization of mitotic as well as polytene chromosomes. Since in both these cases, most hybridization is to the region of the nucleolus or nucleolus organizer these sequences could still be in some way associated with rDNA regions of the genome although *not inserted into rDNA genes*. The nucleolus may in fact contain a variety of other DNA sequences: the satellites of buoyant density of 1.686 and 1.688 g/ml, for example, are drawn out into the nucleolus (Peacock *et al.* 1978). Other workers have isolated cloned segments of DNA which hybridize *in situ* to the nucleolus but apparently do not contain rDNA (Gehring, personal communication). The significance of these observations is not clear, but they serve to emphasize the complex nature of DNA sequences in the nucleolus.

The 'non-rDNA' sites of the type I insert sequences may be associated with other coding sequences of importance in nucleolus structure and/or function. Similarly the site(s) in chromosome 4 and those in the heterochromatic regions of chromosome 2 and 3, may be associated with functional genes. Essential gene functions have, for example, been mapped to the heterochromatin of chromosome 2 (Hilliker, 1976) and the region of chromosome 4 where type I insert sequences have been found (Hochman, 1976). It should be possible to determine if the chromosomal distribution of the chromosome 2

type I insert sequences parallels the distribution of the heterochromatic genes when they are manipulated by chromosomal rearrangement. It will be of some interest to determine if other 'insert sequences' occur in a number of specific sites in the genome and whether in at least some locations they occur in tandem arrays.

## REFERENCES

- BIRNSTIEL, M. L., SELLS, B. H. & PURDOM, I. F. (1972). Kinetic complexity of RNA molecules *Journal of Molecular Biology* **63**, 21-39.
- BRUTLAG, D. L., APPELS, R., DENNIS, E. S. & PEACOCK, W. J. (1977). Highly repeated DNA in *Drosophila melanogaster*. *Journal of Molecular Biology*, **112**, 31-47.
- 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 Science (Wash.)* **74**, 4233-4237.
- DAWID, I. B. & WELLAUER, P. K. (1978). Ribosomal DNA and related sequences in *Drosophila melanogaster*. *Cold Spring Harbor Symposium of Quantitative Biology*. **42**, 1185-1194.
- ENDOW, S. A. & GLOVER, D. M. (1979). Differential replication of ribosomal gene repeats in polytene nuclei of *Drosophila*. *Cell* **17**, 597-605.
- DENHARDT, D. (1966). A membrane-filter technique for the detection of complementary DNA. *Biochemical and Biophysical Research Communications* **23**, 641-646.
- GLOVER, D. M. (1977). Cloned segment of *Drosophila melanogaster* rDNA containing new types of sequence insertion. *Proceedings of the National Academy of Science (Wash.)* **74**, 4932-4936.
- GLOVER, D. M. & HOGNESS, D. S. (1977). A novel arrangement of the 18S and 28S sequences in a repeating unit of *Drosophila melanogaster* rDNA. *Cell* **10**, 167-176.
- GLOVER, D. M., KIDD, S. J., ROIHA, H. T., JORDAN, B. R., ENDOW, S. & APPELS, R. (1978). Interrupter sequences that are widely distributed in the *Drosophila* genome. *Biochemical Society Transactions* **6**, 732-736.
- HILLIKER, A. (1976). Genetic analysis of the centromeric heterochromatin of chromosome 2 of *Drosophila melanogaster*: Deficiency mapping of EMS-induced lethal complementation groups. *Genetics* **83**, 765-782.
- HOCHMAN, B. (1976). The fourth chromosome of *Drosophila melanogaster*. In *Genetics and Biology of Drosophila*, vol. 1b (ed. M. Ashburner and E. Novitski), pp. 903-928. New York: Academic Press.
- LINDSLEY, D. L. & GRELL, E. G. (1968). Genetic variations in *Drosophila melanogaster* *Carnegie Institute of Washington Publications* 627.
- PEACOCK, W. J., BRUTLAG, D., GOLDRING, E., APPELS, R., HINTON, C. W. & LINDSLEY, D. L. (1973). The organization of highly repeated DNA sequences in *Drosophila melanogaster* chromosomes. *Cold Spring Harbor Symposium of Quantitative Biology* **38**, 405-416.
- PEACOCK, W. J., LOHE, A., GERLACH, W. L., DUNSMUIR, P., DENNIS, E. S. & APPELS, R. (1978). Fine structure and evolution of DNA in heterochromatin. *Cold Spring Harbor Symposium of Quantitative Biology* **42**, 1121-1135.
- PELLEGRINI, M., MANNING, J. & DAVIDSON, N. (1977). Sequence arrangement of the rDNA of *Drosophila melanogaster*. *Cell* **10**, 213-224.
- RUDKIN, G. T. (1972). Replication in polytene chromosomes. In *Results and Problems in Cell Differentiation*, vol. 4 (ed. W. Beermann), pp. 59-85. New York: Springer-Verlag.
- SOUTHERN, E. (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. *Journal of Molecular Biology* **98**, 503-517.
- TARTOF, K. D. & DAWID, I. B. (1976). Similarities and differences in the structure of X and Y chromosome rDNA Genes of *Drosophila*. *Nature* **263**, 27-30.
- WELLAUER, P. K. & DAWID, I. B. (1977). The structural organization of ribosomal DNA in *Drosophila melanogaster*. *Cell* **10**, 193-212 (1977).
- WELLAUER, P. K., DAWID, I. B. & TARTOF, K. D. (1978). X and Y chromosome ribosomal DNA of *Drosophila*: comparison of spacers and insertions. *Cell* **14**, 269-278.
- WHITE, R. L. & HOGNESS, D. S. (1977). R loop mapping of the 18S and 28S sequences in the long and short repeating units of *Drosophila melanogaster* DNA. *Cell* **10**, 177-192.

## PLATE 1

Fig. 2. Distribution of Dm103A1 and Dm103B sequences in DNA fractionated in actinomycin-D/CsCl. *D. melanogaster* DNA was extracted from embryos and fractionated in actinomycin-D/CsCl gradients as described by Brutlag *et al.* (1977). To assay the distribution of sequences within the gradients 10  $\mu$ l aliquots of each fraction were loaded on nitrocellulose filters (Birnstiel *et al.* 1972) and hybridized with either Dm102A1 (○ ○) or Dm103B (■ ■)  $^3$ H-cRNA in 3xSSC plus 50% formamide at 45 °C for 5 h. Filters were washed as described by Birnstiel *et al.* (1972). The  $T_m$  of  $^3$ H-cRNA hybrids to DNA immobilized on nitrocellulose filters was 62 °C.

Fig. 3. Southern hybridization of Dm103B and pXlr101 to *D. melanogaster* DNA and Dm103. *D. melanogaster* DNA was extracted from hand dissected brains and imaginal discs of ten male or female larvae. Following cleavage with EcoR1, the DNAs were fractionated by gel electrophoresis, transferred to nitrocellulose and hybridized with either pXlr101 or Dm103B labelled by nick-translation to specific activities of  $9.8 \times 10^7$  and  $1.5 \times 10^8$  respectively. The plasmid pXlr101 contains a complete repeating unit of *Xenopus laevis* rDNA and gives an identical pattern of hybridization as *D. melanogaster* rRNA. The tracks are as follows: (1) male *D. melanogaster* DNA hybridized with pXlr101, (2) female *D. melanogaster* DNA hybridized with pXlr101 (3) male *D. melanogaster* DNA hybridized with Dm103B, (4) female *D. melanogaster* DNA hybridized with Dm103B, (5) Dm103 DNA hybridized with Dm103B. The differences in the pattern of hybridization between male and female *D. melanogaster* DNA with the pXlr101 probe has been discussed in a separate publication (Endow & Glover, 1979).

Fig. 4. *In situ* location of Dm103B sequences in salivary gland chromosomes. *In situ* hybridization of Dm103B  $^3$ H-cRNA to salivary gland chromosomes was carried out as described by Peacock *et al.* (1978). The arrows indicate the autoradiography grains on chromosome 4.

Fig. 5. *In situ* location of Dm103B sequences in mitotic chromosomes. *In situ* hybridization was carried out as described in the legend to Fig. 4. The arrow indicates the autoradiography grains near the middle of the X heterochromatin.

