Proc. Natl. Acad. Sci. USA
Vol. 74, No. 11, pp. 4932–4936, November 1977
Biochemistry

Cloned segment of *Drosophila melanogaster* rDNA containing new types of sequence insertion

(DNA·RNA hybridization/R-loops/heteroduplexes/restriction endonuclease sites/renaturation kinetics)

DAVID M. GLOVER

Department of Biochemistry, Imperial College of Science and Technology, London SW7 2AZ, England

Communicated by David S. Hogness, August 25, 1977

ABSTRACT **A cloned 14.3-kbase segment of *Drosophila melanogaster* rDNA (Dm207) is described in which only a 4-kbase region is homologous to a cloned 17-kbase rDNA repeating unit, Dm103; this 4-kbase region consists of part of the 28S rRNA gene and most but not all of the adjacent transcribed spacer that normally connects the 18S and 28S genes. The transcribed spacer in Dm207 is interrupted by a 2.2-kbase stretch of DNA that does not contain any 18S gene sequences. At the other end of the 4-kbase homology, the 28S gene is interrupted by an 8.1-kbase stretch of DNA at a position equivalent to the site of the 28S insertion found in the 17-kbase units. The question of whether the 2.2-kbase and 8.1-kbase interrupter segments in Dm207 derive from longer insertions into the transcribed spacer and 28S genes of a very long repeating unit (≥22 kbases) or represent a region of the chromosomal DNA into which a 4-kbase fragment of rDNA has been inserted is discussed.**

The rDNA of *Drosophila melanogaster* contains two major types of repeat units, defined by *Eco*RI endonuclease fragments 12 and 17 kilobases (kb) long. The latter class, exemplified by the cloned segment Dm103 (1), contains a 5.3-kb insertion into the 28S gene. The mature 28S rRNA consists of two "half-molecules" (α 28S and β 28S) that are hydrogen-bonded together within the ribosome. The DNA regions coding for these half-molecules are adjacent and the insertion sequence is found not at the gap that separates these regions but within the β 28S sequences (2–5). In addition, the rDNA contains other types of units as shown by a series of minor fragments intermediate in size between 12 and 17 kb together with 5.4- and 7.4-kb *Eco*RI endonuclease fragments (4, 6). In this paper, I describe a cloned segment of *D. melanogaster* rDNA (Dm207) from which a 7.5-kb fragment can be cleaved with *Eco*RI endonuclease. The transcribed spacer and β 28S sequences of Dm207 are interrupted by sequences that show no homology with the insertion of Dm103.

MATERIALS AND METHODS

Preparation of Nucleic Acids. Plasmid DNA, *D. melanogaster* embryo DNA, *D. melanogaster* tissue culture cell RNA, and cRNA from plasmids were prepared as described (1). Labeling of DNA *in vitro* was carried out as described (7).

Enzymes. The *Escherichia coli* DNA ligase used in these experiments was partially purified as far as the first phosphocellulose column step in the procedure of Modrich *et al.* (8). *E. coli* RNA polymerase (9), T4 polynucleotide kinase (10), and *Eco*RI (11) were prepared according to the indicated references and were gifts of W. Wickner, T. Barett, and A. Atkinson, respectively. *E. coli* DNA polymerase I was prepared as described by Jovin *et al.* (12) and *Xma* I, as described (13).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

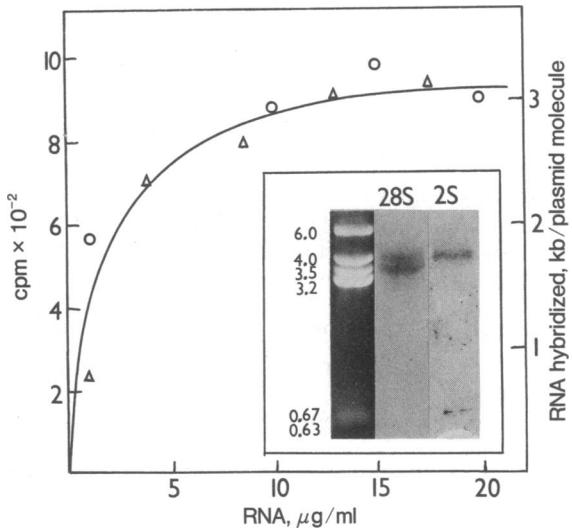

FIG. 1. Saturation hybridization of cDm207. ^{3}H-Labeled plasmid DNA (specific activity, 1.13×10^4 cpm/μg) was loaded onto filters for hybridization with [^{32}P]rRNA (specific activity, 5×10^4 cpm/μg) as described (1). The data points are means of duplicates normalized to 0.25 μg of DNA per filter. The hybridization was performed with either 28S rRNA alone (△) or a mixture of 28S and 18S rRNA (○). (*Inset*) Hybridization of 28S and 2S [^{32}P]rRNA to restriction fragments (obtained by *Xma* I and *Eco*RI cleavage of cDm207) fractionated by agarose gel electrophoresis and transferred onto a nitrocellulose filter by the method of Southern (19). (*Left*) Ethidium fluorescence of the DNA fragments and size of the fragments in kb. (*Right*) Autoradiograms of the filters after hybridization.

Electron Microscopy. The spreading methods of Davis *et al.* (14) were used. Grids were examined in a Philips EM301. Length measurements of projected photographic images were made with a Hewlett-Packard digitizer and 9821 computer.

***In Vitro* Recombinants.** Strains of *E. coli* carrying *in vitro* recombinant plasmids were propagated under Category II conditions (15).

cDm207 is 1 of 140 segments of *D. melanogaster* DNA cloned in ColEl that were found to be positive in a screen by colony hybridization (16) for complementarity to [^{32}P]cRNA from pDm103. These clones were constructed from randomly sheared *D. melanogaster* DNA inserted at the *Eco*RI site of ColEl by the (dA)/(dT) connector method (17) and were constructed by G. Rubin.

pDm850 (the 7.5-kb *Eco*RI fragment of cDm207 joined to pSC101) was constructed by ligating *Eco*RI fragments of cDm207 with *Eco*RI cleaved pSC101 (mass ratio of cDm207 fragments/pSC101, 6:1) at a concentration of 95 μg/ml for 90 min at 15° and subsequently 15 μg/ml for 15 hr at 15°, under

Abbreviation: kb, kilobase.

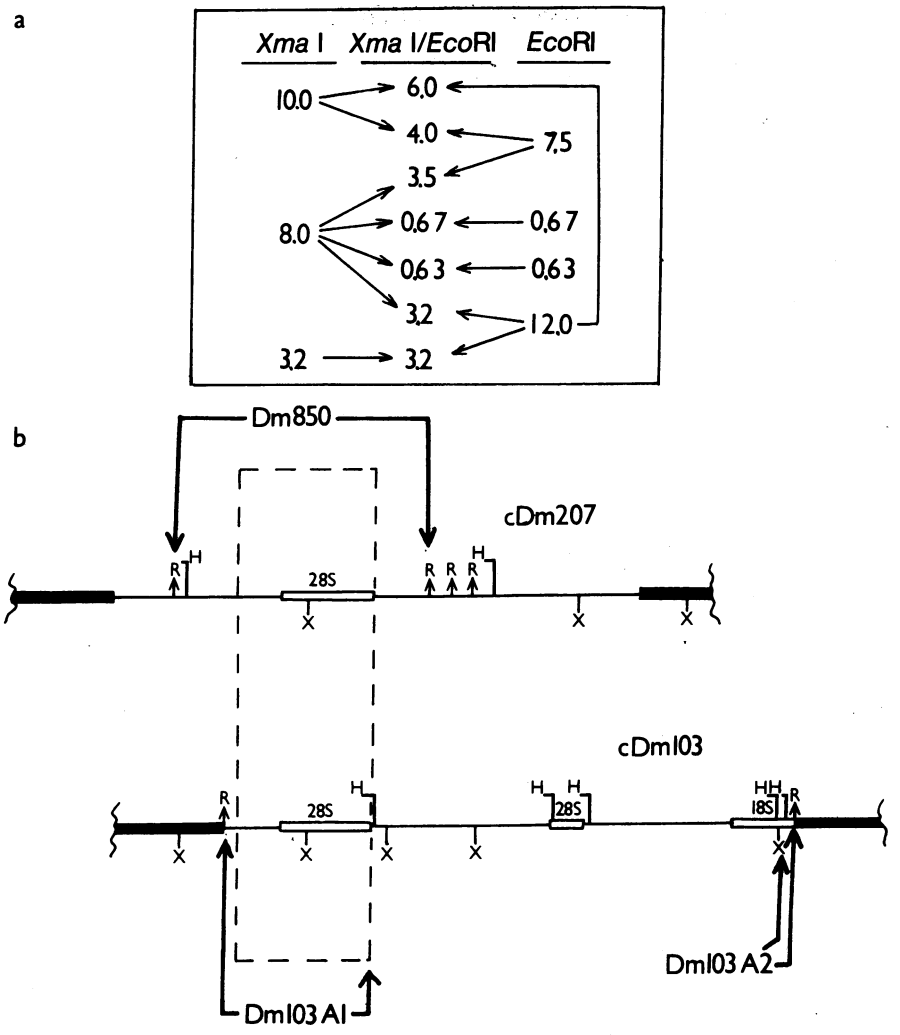

FIG. 2. Cleavage maps of cDm103 (ref. 2; B. R. Jordan and D. M. Glover, unpublished data) and cDm207 for the endonucleases *Eco*RI (R), *Hin*dIII (H), and *Xma* I (X). Regions of homology between the Dm segments (see Fig. 4) are enclosed by the dashed line. The arrows indicate sequences from within the two plasmids that have been cloned in pSC101 and to which reference is made in the text. ColE1 sequences are shown by solid bars and rRNA coding sequences, by open bars. The linear order of *Xma* I/*Eco*RI endonuclease-generated fragments on the cDm207 map is the same as the vertical order in the central column of the table at the top. *Eco*RI digestion of cDm207 yields fragments 12, 7.5, 0.67, and 0.63 kb long. All four *Eco*RI sites must be within the *D. melanogaster* segment because the *Eco*RI site of ColE1 was destroyed during construction of the plasmid. The sum of the lengths of the *Eco*RI restriction fragments (20.8 kb) is in good agreement with the length of the plasmid determined from electron micrographs (20.82 ± 0.14, n = 22). A partial *Eco*RI digest of cDm207 yielded a 1.3-kb fragment, indicating that within Dm207 the 0.67- and 0.63-kb fragments must be adjacent, although the orientation of these two fragments has not been determined. cDm207 was cleaved at three sites by *Xma* I to yield 10-, 8-, and 3.2-kb fragments, each of which was isolated by preparative gel electrophoresis and then cleaved with *Eco*RI to establish the relationships indicated by the arrows between columns in the table. One of the three *Xma* I sites can be placed in the ColE1 DNA, 1.35 kb from the right-hand (dA)/(dT) joint, because the ColE1 plasmid contains a single *Xma* I site 1.35 kb from its *Eco*RI site (20). Because the ColE1 DNA is 6.54 kb long (2), this *Xma* I site is 5.2 kb from the left-hand (dA)/(dT) joint. This 5.2-kb segment of ColE1 DNA must be contained within the 6-kb *Xma* I/*Eco*RI fragment because all of the other fragments in the center column are too short. This places an *Eco*RI site 0.8 kb to the right of the left-hand (dA)/(dT) joint and, because the 6- and 4-kb *Xma* I/*Eco*RI fragments both derive from the 10-kb *Xma* I fragment, locates an *Xma* I site 4 kb further to the right. The 4- and 3.5-kb *Xma* I/*Eco*RI fragments comprise the 7.5-kb *Eco*RI fragment because they are cleaved from pDm850. This fact and the nature of the *Eco*RI cleavage products of the 8-kb *Xma* I fragment (one of which is the 3.5-kb *Xma* I/*Eco*RI fragment) locates the last *Xma* I site and the cluster of three *Eco*RI sites from which the 0.67- and 0.63-kb *Eco*RI fragments derive. The 3.2-kb *Xma*I fragment must then include the remaining 1.35 kb of ColE1 DNA, while the 12-kb *Eco*RI fragment must consist of this *Xma* I fragment plus the 6- and 3.2-kb *Xma* I/*Eco*RI fragments and thus include the entire ColE1 segment. This was confirmed by electron microscopic examination of heteroduplexes formed between *Eco*RI-cleaved cDm207 and randomly nicked ColE1 DNA (data not shown).

previously described conditions (18). Colonies of tetracycline-resistant *E. coli* HB101 transformed by the ligation reaction mixture were screened for recombinants with [^{32}P]cRNA from cDm207 as described (16).

***In Vitro* Labeling of 2S RNA.** A 50-μl reaction mixture containing approximately 10 μg of 2S rRNA in 5 mM Tris·HCl, pH7.5/5 mM $MgCl_2$/10 mM 2-mercaptoethanol and [γ-^{32}P]-ATP (800 μCi/ml, 2000 Ci/mmol) was incubated for 1 hr at 37° with 4 units of T4 polynucleotide kinase. The reaction mix was extracted with phenol and then with ether and then adjusted to be 50% formamide, 5 × standard saline/citrate for direct use in filter hybridization experiments.

RESULTS

Dm207 Contains 28S rRNA Sequences. Saturation hybridization of [^{32}P]rRNA to [^{3}H]cDm207 DNA showed that cDm207 codes for 3 kb of 28S rRNA. The same saturation

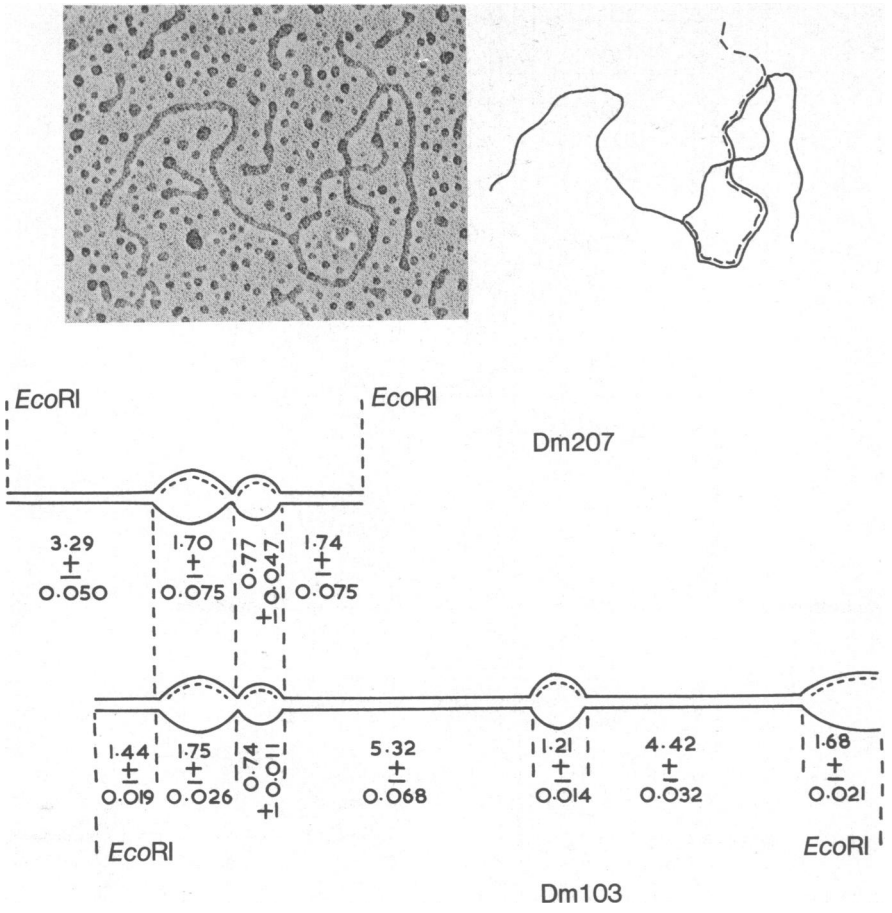

FIG. 3. R-loop map of the 7.5-kb *Eco*RI fragment of cDm207 annealed with rRNA in 70% formamide under the R-looping conditions described by White and Hogness (3) and spread for electron microscopy by their method A. Lengths were measured relative to relaxed circles of pSC101. The SEM of the measurement is indicated; $n = 18$. The previously determined R-loop map of Dm103 (3) is shown for comparison. In this map, the 1.75-kb R-loop corresponds to the α 28S half-molecule; the β 28S half-molecule forms the 0.74- and 1.21-kb loops, which are separated by the 5.32-kb insertion segment; the fork at the right end of Dm103 is formed by the 18S RNA (3). In the electron micrograph of the R-loops in the 7.5-kb *Eco*RI fragment of Dm207, the RNA tail in the upper right-hand part of the figure corresponds to that part of the β 28S half-molecule that forms the 1.21-kb R-loop in Dm103 and for which there are no homologous sequences in Dm207.

plateau was obtained whether 28S rRNA was used alone or together with 18S rRNA, indicating there are no detectable 18S sequences within Dm207 (Fig. 1). The 3-kb regions of 28S sequences in cDm207 were contained within the 3.5- and 4.0-kb *Xma* I/*Eco*RI fragments (Fig. 1 *inset*). These two fragments, which together comprise the 7.5-kb *Eco*RI fragment (Fig. 2), also were the only two that hybridized with [^{32}P]cRNA from pDm103, a plasmid that consists of the major 17-kb rDNA unit (Dm103) inserted by ligation into the pSC101 vector at its *Eco*RI site (1–3). Fig. 3 shows a representative electron micrograph of the 7.5-kb *Eco*RI fragment of cDm207 annealed with 28S rRNA to form R-loops (3), together with the R-loop maps of this *Eco*RI fragment and of Dm103. The two molecules contain what appears to be an identical complement of the 28S sequences corresponding to the entire α 28S half-molecule and about 40% of the β 28S half-molecule (see legend to Fig. 3). Both DNAs contain a segment that interrupts the 28S rRNA gene at the same position within the resolution limits of the R-loop techniques.

In Dm103, the DNA lying to the left of this common 28S complement consists of the transcribed spacer which contains the 5.8S and 2S rDNA sequences (21) plus a small part (about 0.2 kb) of the 18S gene at the extreme left end of Dm103 (2, 3). The lack of any detectable 18S sequences in Dm207 and the longer length of the DNA segment lying to the left of the 28S sequences (Figs. 1 and 3) suggest that the transcribed spacer in Dm207 is also interrupted by a DNA segment. That this segment contains some part of the transcribed spacer is suggested by the fact that the 4-kb *Xma* I/*Eco*RI fragment of Dm207 (Fig. 2) hybridizes with the 2S rRNA (Fig. 1).

Transcribed Spacer and 28S Gene Are Interrupted in Dm207 by Sequences Not Homologous to Any in Dm103. The presence and nature of the interrupter sequences in Dm207 were determined by analyzing heteroduplex molecules formed between its *Eco*RI fragments and those of Dm103. A typical heteroduplex (Fig. 4) shows a region of homology measuring 4.06 ± 0.25 kb with single-stranded forked ends. The branches of the fork at one end of the structure were 12.46 ± 0.46 and 1.61 ± 0.12 kb, corresponding to lengths from the position of the interruption of the 28S gene to the *Eco*RI endonuclease site measured from the R-loop maps of Dm103 and Dm207, respectively, in which these distances are 12.6 and 1.7 kb. The DNA segments that interrupt the 28S gene in the two clones are therefore not sufficiently related that they will form stable duplex regions under the heteroduplex annealing conditions. The 0.45- and 1.4-kb single-stranded prongs of the fork at the other end of the heteroduplex can be assigned to Dm103 and the 7.5-kb *Eco*RI fragment of Dm207, respectively. The 0.45-kb Dm103 prong must contain some of the transcribed spacer sequences because it is longer than the 0.2-kb of 18S sequences located at the left end of Dm103 (2, 3). The transcribed spacer in Dm207 is therefore interrupted near its left end by the

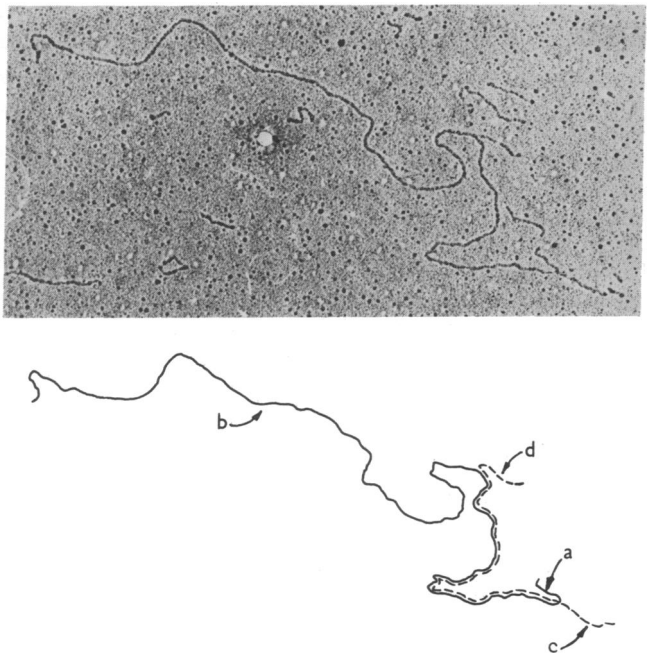

FIG. 4. Heteroduplex formed between Dm103 and the 7.5-kb *Eco*RI fragment of Dm207. cDm103 and pDm207 were cleaved with *Eco*RI, extracted with phenol, passed over a Sephadex G-50 column, and finally denatured and annealed to form heteroduplexes (14). The mean lengths were determined relative to previously characterized single- and double-strand heteroduplex structures formed between Dm103 and *Eco*RI-cleaved ckDm103D (2) included in the annealing mixture. The length (mean ± SEM) of the indicated single-strand regions was determined to be: a, 0.45 ± 0.04 kb; b, 12.46 ± 0.46 kb; c, 1.40 ± 0.11 kb; d, 1.61 ± 0.12 kb (n = 20).

nonhomologous sequences that form the 1.4-kb prong of the fork.

The results of this heteroduplex analysis were confirmed by hybridizing ^{32}P-labeled pDm850 DNA to the *Hin*dIII/*Eco*RI restriction fragments of cDm103 (2) by the method of Southern (19) (Fig. 5). This experiment demonstrated that only the 4.5-kb *Hin*dIII/*Eco*RI restriction fragment A1 (see Fig. 2) of cDm103 (2) shows homology with Dm207. This fragment contained the same 28S sequence found in Dm207 plus all of the transcribed spacer including the 2S and 5.8S sequences (2, 3, 21). The fact that pDm850 did not hybridize to the 5.6-kb *Hin*dIII fragment B of Dm103, which contains the 5.3-kb insertion found in 17-kb units, confirms the conclusion that the sequences that interrupt the 28S gene in Dm207 and Dm103 are nonhomologous.

Hybridization of Dm207 with Total *D. melanogaster* DNA. The hybridization of ^{32}P-labeled pDm850 to total *D. melanogaster* DNA digested by *Eco*RI or *Hin*dIII endonucleases is also shown in Fig. 5. The plasmid hybridizes predominantly to *Eco*RI fragments 17, 12, and 5.4 kb long. Additional bands, including one at 7.5 kb, could be detected at a much longer exposure time. The predominant hybridization of pDm850 to the *Hin*dIII digest of total *D. melanogaster* DNA was to a 4.7-kb band. This is equivalent to the fragments A1 and A2 cut from Dm103 by a combined digestion with *Hin*dIII and *Eco*RI endonucleases (2). These fragments would be adjacent within the tandem array of chromosomal rDNA as a 4.7-kb *Hin*dIII fragment. A *Hin*dIII fragment of this size also was excised from a cloned segment of rDNA that contains the major 12-kb *Eco*RI unit (cDm238). The 4.7-kb fragment therefore represents the major *Hin*dIII fragment from total *D. melanogaster* DNA which contains the 28S sequences and transcribed spacer sequences found in Dm207.

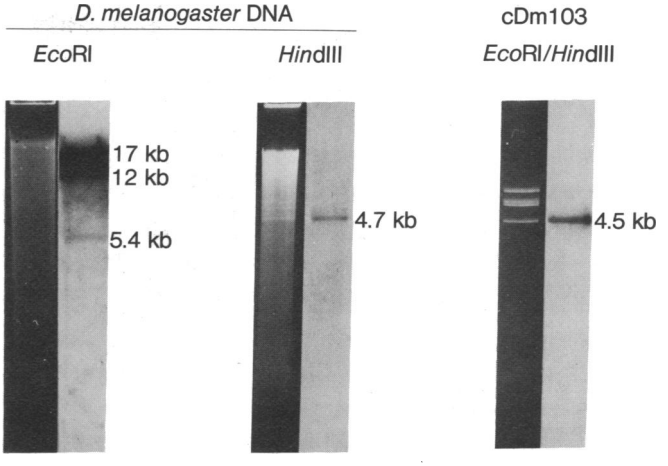

FIG. 5. Southern hybridization (19) of restriction digests of cDm103 and total *D. melanogaster* DNA with nick-translated pDm850 (7). The hybridization protocol of Denhardt (22) was used. The sizes of the fragments that show hybridization in the autoradiogram are indicated.

The reassociation kinetics of Dm207 driven by total *D. melanogaster* DNA is shown in Fig. 6. If these data are treated as one kinetic component, then a value of 180 is obtained for the repetition frequency. However, the curve is a composite; 4 kb of Dm207 should anneal with the rate constant of the sequences in the cloned fragments A1 and A2 of Dm103 (see Fig. 2) and the interrupter sequences will also have their own rate constants. If the contribution of the former is subtracted from each data point, one obtains the dotted curve shown in Fig. 6 *inset*, which corresponds to an average repetition frequency for the interrupter sequences of 80.

DISCUSSION

If it is assumed that Dm207 was cut out from a complete rDNA unit containing the entire 18S and 28S genes, then the 2.2- and 8.1-kb segments must be considered as parts of insertions into the transcribed spacer and 28S gene, respectively. Although interruption of the transcribed spacer has not been observed previously, interruptions of the 28S gene, at the same site as in Dm207, have been characterized (2–5). However, the interrupter sequences of Dm207 show no homology to the insertion sequences in the 28S gene of a 17-kb unit, Dm103. We have now detected the 0.67- and 0.63-kb *Eco*RI fragments characteristic of the 8.1-kb interrupter segment in four other rDNA plasmids—two (cDm249 and cDm264) of 64 from our collection and two (14C9 and 4G3) of 18 rDNA plasmids from the collection of P. Schedl, S. Artavanis-Tsakonas, and W. J. Gehring. Because both collections were formed from randomly sheared Dm segments that, in the cloned plasmids, exhibit an average length between 10 and 15 kb, this suggests that sequences related to the 8.1-kb segment are linked to sequences of the rRNA genes at a frequency somewhat greater than 0.06. Previous characterizations of cloned *Eco*RI fragments of rDNA or of *Eco*RI fragments from gradient purified rDNA have biased most of the existing data because insertions containing *Eco*RI sites have been excluded from the analysis (2–4). Pellegrini *et al.* (5), however, examined rDNA that had not been cleaved by *Eco*RI and described three broad size classes of insertion: 1.42 ± 0.47, 3.97 ± 0.55, and 6.59 ± 0.62 kb. It is conceivable that insertions into the 28S gene of Dm103 and of Dm207 both could fall within this third size class.

*Eco*RI endonuclease cleavage at minor sites within the nontranscribed spacer and insertion sequences results in two

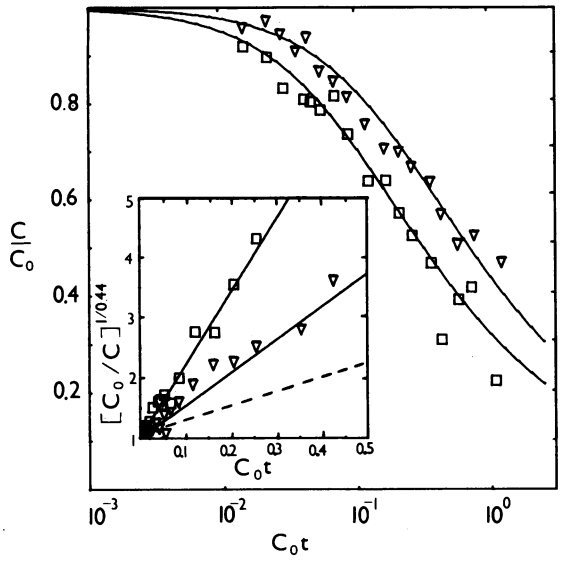

FIG. 6. Reassociation of cDm207 and pDm103A (segments A1 and A2 cloned in pSC101) driven by *D. melanogaster* DNA. Plasmid DNA was labeled to a specific activity of approximately 1.5×10^8 cpm/μg by nick translation. A mixture of ^{32}P-labeled plasmid DNA (~0.5 ng) and *D. melanogaster* DNA (78.5 μg) was denatured and the reassociation of the labeled DNA in a volume of 1 ml was followed by the S1 assay as described (1). The pSC101 and ColE1 sequences in the plasmids make no significant contribution to the annealing within the values of C_0t to which the reaction was carried and so the contribution of these sequences to the concentration of single-stranded DNA at each time point has been subtracted. The curves are a linear regression best fit of the equation $C/C_0 = (1 + K'C_0t)^{-0.44}$ to the data. Values of K' for pDm103A of 5.34 M^{-1}·sec^{-1} and for cDm207 of 2.4 M^{-1}·sec^{-1} were obtained. The dashed line represents the theoretical curve obtained if the contribution of 4 kb of sequences with the rate constant of Dm103A is subtracted from the cDm207 data. In this case, K' is calculated as 1.06. The repetition frequencies, α, have been calculated from the equation $\alpha = (K'/K\text{pSC101})(\text{LD/LpSC101})$ in which LD and LpSC101 are the haploid genome content of *D. melanogaster* DNA and the length of pSC101 DNA, respectively, and KpSC101 is the rate constant for the self-driven reassociation of pSC101. Previous reports (1, 17) have given values of K that are, in fact, $K/0.44$. This has no effect on the value of α finally calculated from these data. ▽, cDm207; □, pDm103A.

prominent minor rDNA fragments 5.4 and 7.4 kb long (4, 6). The 7.5-kb *Eco*RI fragment of Dm207 hybridized strongly with total *Eco*RI cleaved DNA to a 5.4-kb band and very weakly to a 7.5-kb band (Fig. 5). It is possible that these 5.4-kb fragments are similar to the 7.5-kb fragment from Dm207 but do not contain an insertion into the transcribed spacer. The weak hybridization of Dm207 to the 7.5-kb band probably reflects heterogeneities between one inbred population of flies and another, because rRNA is also found to hybridize only very weakly to 7.5-kb *Eco*RI fragments in this DNA preparation.

An alternative explanation of the structure of Dm207 would be that the 4-kb stretch of transcribed spacer and 28S sequences represents an insertion of rDNA into a region of the chromosome consisting of the repeated sequences of the 2.2- and 8.1-kb interrupter segments. The average frequencies for the interrupter sequences of Dm207 and for the sequences in the 4.7-kb segment corresponding to Dm103A1 and Dm103A2 (Fig. 2; this segment contains the entire 4 kb of 28S and transcribed spacer sequences present in Dm207 and is free of interrupter sequences) are 80 and 400, respectively (Fig. 6). A comparison of the ratio of these two values, or 0.20, with the linkage frequency of approximately 0.06 given above, suggests that a significant fraction of the Dm207 interrupter sequences may not be linked to the rRNA genes. The weak hybridization of Dm850 (Fig. 2) to several *Eco*RI and *Hin*dIII fragments of total *D. melanogaster* DNA (Fig. 5) is consistent with this interpretation. In a similar vein, the observation that Dm103 sequences are found at chromosomal sites other than the nucleolus organizer (D. Finnegan and W. J. Peacock, data cited in ref. 3; S. Kidd, S. Endow, R. Appels, and D. M. Glover, unpublished data) suggests that these sites may represent regions containing the Dm103 insertion sequences free of rRNA gene sequences. This suggestion has encouraged speculation that the 28S gene insertions result from some sort of exchange between uninterrupted 12-kb rDNA units and the insertion sequences located in such regions. If this is the case, then the reciprocal of such an exchange may occasionally result in the insertion of rDNA segments into regions consisting of insertion sequences.

The question of the origin of Dm207 is therefore one of considerable interest. It can be attacked by mapping the Dm207 interrupter sequences within the genome, by using *in situ* hybridization techniques, and by the examination of additional cloned plasmids that contain both these interrupter sequences and the rRNA gene sequences.

I thank Pete Burrows for his excellent technical assistance in some of the experiments described. This work was supported by a project grant from the Medical Research Council.

1. Glover, D. M. White, R. L., Finnegan, D. J. & Hogness, D. S. (1975) *Cell* **5**, 149–157.
2. Glover, D. M. & Hogness, D. S. (1977) *Cell* **10**, 167–176.
3. White, R. L. & Hogness, D. S. (1977) *Cell* **10**, 177–192.
4. Wellauer, P. K. & Dawid, I. B. (1977) *Cell* **10**, 193–212.
5. Pellegrini, M., Manning, J. & Davidson, N. (1977) *Cell* **10**, 213–224.
6. Tartof, K. D. & Dawid, I. B. (1976) *Nature* **263**, 27–30.
7. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113**, 237–251.
8. Modrich, P., Anraku, Y. & Lehman, I. R. (1973) *J. Biol. Chem.* **248**, 7495–7501.
9. Berg, D., Barrett, K. & Chamberlin, M. (1971) in *Methods in Enzymology*, eds. Grossman, L. & Moldave, K. (Academic Press, New York), Vol. 21D, pp. 506–519.
10. Richardson, C. (1971) in *Procedures in Nucleic Acid Research*, eds. Cantoni, G. L. & Davies, D. R. (Harper and Row, New York), pp. 815–828.
11. Greene, P. J., Betlach, M. C., Goodman, H. M. & Boyer, H. W. (1974) in *Methods in Molecular Biology*, ed. Wickner, R. (Marcel Dekker, New York), Vol. 6, pp. 87–111.
12. Jovin, T. M., Englund, P. T. & Bertsch, L. (1969) *J. Biol. Chem.* **244**, 2996–3008.
13. Endow, S. & Roberts, R. (1977) *J. Mol. Biol.*, **112**, 521–530.
14. Davis, R. W., Simon, M. & Davidson, N. (1971) in *Methods in Enzymology*, eds. Grossman, L. & Moldave, K. (Academic Press, New York), Vol. 21D, pp. 413–428.
15. Her Majesty's Stationery Office (1976) *Report of the Working Party on the Practice of Genetic Manipulation* (Her Majesty's Stationery Office, London).
16. Grunstein, M. & Hogness, D. S. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 3961–3965.
17. Wensink, P. C., Finnegan, D. J., Donelson, J. E. & Hogness, D. S. (1974) *Cell* **3**, 315–325.
18. Glover, D. M. (1976) in *New Techniques in Biophysics and Cell Biology*, eds. Pain, R. & Smith, B. (John Wiley & Sons, New York), Vol. 3, pp. 125–145.
19. Southern, E. (1975) *J. Mol. Biol.* **98**, 503–517.
20. Collins, J. (1976) *Hoechst Workshop Conference* (North Holland Press), Vol. 5.
21. Jordan, B. R. & Glover, D. M. (1977) *FEBS Lett.* **78**, 271–274.
22. Denhardt, D. (1966) *Biochem. Biophys. Res. Commun.* **23**, 641–646.