5.8 S AND 2 S rDNA IS LOCATED IN THE 'TRANSCRIBED SPACER' REGION BETWEEN THE 18 S AND 26 S rRNA GENES IN *DROSOPHILA MELANOGASTER*

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Received 1 April 1977

1. Introduction

In *Drosophila melanogaster*, two small RNA species are found hydrogen bonded to 26 S rRNA:5.8 S rRNA, approximately 125 nucleotides long, and 2 S rRNA, 30 nucleotides long [1]. Evidence from pulse-chase experiments shows that they arise as the result of post transcriptional processing of 26 S RNA, and that the corresponding cleavages occur in the cytoplasm after the central cleavage of the 26 S molecule which occurs in the nucleus [1]. These results do not define completely the architecture of the 26 S molecule and a number of different models remain possible (fig.1). If it is assumed, by analogy with *Xenopus laevis* [2] that 5.8 S rRNA is coded by a DNA region located between the genes for 18 S and 26 S rRNAs, then class (c) models can be excluded. The experiments reported here examine this question directly by hybridization of ^{32}P-labelled 5.8 S and 2 S RNAs to cloned *D. melanogaster* rDNA fragments [3].

2. Methods

2.1. *Preparation of ^{32}P-labelled 5.8 S and 2 S RNAs*

These were prepared by denaturation and acrylamide urea gel electrophoresis of ^{32}P-labelled 26 S RNA obtained from tissue culture cells as previously described [1]. The specific activity of the purified material was 3.2×10^6 cpm/μg.

2.2. *Preparation of plasmid DNAs*

Plasmid DNAs were prepared as described by Wensink et al. [4] from small cultures grown under P2 conditions. For the saturation hybridization

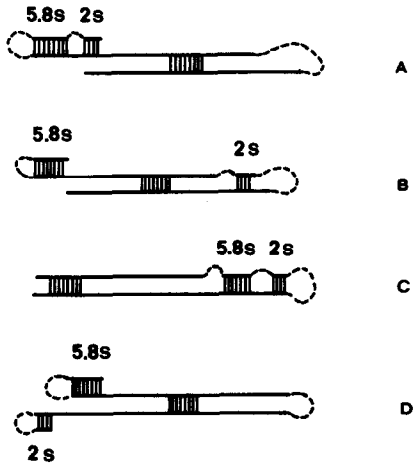

Fig.1. Possible arrangements of the four polynucleotide chains which make up mature *Drosophila* 26 S RNA. The four main classes of possible models are shown; further sub-division is possible if 3'–5' polarity is considered and also if the positions of 2 S and 5.8 S rRNAs are reversed. Dotted lines indicate loops removed during processing.

experiments ^{3}H-labelled plasmid DNAs were used to allow accurate quantitation of the amount of DNA retained on the filter; the specific activities ranged from 1–6 X 10^{4} cpm/μg.

2.3. *Hybridization conditions*

The hybridization was carried out with DNA immobilized on filters in 50% formamide, 5 X SSc as described by Glover [5]. The reaction mixtures were incubated at 37°C for 18 h, then the filters were washed extensively with 50% formamide 5 X SSc buffer but not treated with RNAase as it was found that this led to loss of the 2 S hybridization signal.

2.4. *Gel electrophoresis and transfer to nitrocellulose filter*

The restriction enzyme cleaved DNA preparations were subjected to electrophoresis on a horizontal, 0.7 cm thick, 0.7% agarose slab gel for long enough to obtain adequate resolution of the bands (which could be observed while electrophoresis was in progress). The fractionated restriction fragments were then transferred onto sheets of Schleicher and Schuell BA 85 nitrocellulose membrane as described by Southern [6]. Hybridization was performed with a concentration of either 2 S or 5.8 S RNA determined to be saturating from the experiment described in fig.2 and using the same hybridization conditions as described for this experiment. Following hybridization and extensive washing the filters were dried and autoradiographed for 24 h (5.8 S filter) or 6 days (2 S filter).

3. Results

In a first series of experiments we performed saturation hybridization measurements using ^{32}P-labelled 5.8 S or 2 S rRNAs prepared by urea–acrylamide gel electrophoresis of 26 S rRNA purified from KC cells [1] and DNA from plasmids containing the *Hind*III fragments AI, B, C or D of the 17 Kb *D. melanogaster* rDNA *Eco*RI [3] (see fig.3). Fragment AI alone showed significant hybridization to slightly less than one copy of 2 S rRNA and approximately 1.4 copies of 5.8 S rRNA (fig.2). The high saturation level observed with 5.8 S RNA could be due to non-specific hybridization since we omitted

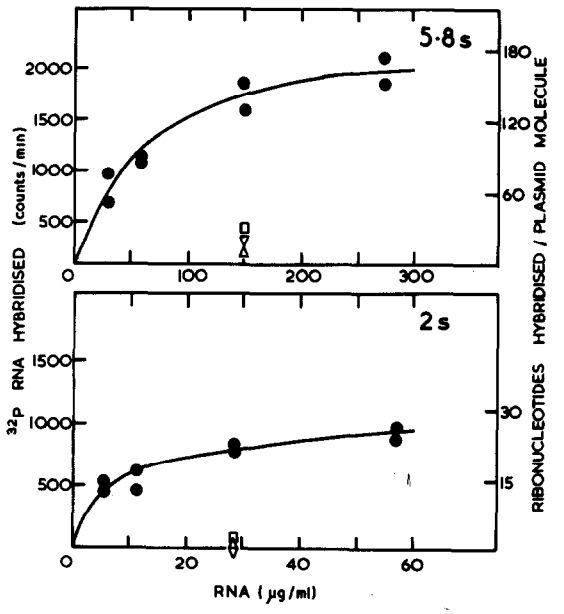

Fig.2. Saturation hybridization of 5.8 S and 2 S RNAs to plasmids containing the *Hind*III fragments AI (•), B (△), C (□), or D (▽) of the 17 kb rDNA unit Dm103. This is the unit which contains a large insertion into the 26 S gene. The upper panel shows the hybridization of 5.8 S RNA normalised to 0.1 μg plasmid DNA and the lower panel 2 S RNA normalised to 0.4 μg plasmid DNA. A physical map of Dm103 showing the nomenclature for the *Hind*III/*Eco*RI fragments is shown in fig.3.

ribonuclease treatment of the hybrids (see Methods). However we think this level reflects the fact that 5.8 S RNA is normally hydrogen-bonded to the rest of the 26 S molecule so that 5.8 S RNA would be expected to hybridize to some extent with the corresponding region in the non-coding strand. Experiments with *Sma*I digests of Dm103 segment support this interpretation (D.M.G. and B.R.J., unpublished results).

These results eliminate the class (d) models (fig.1) for the arrangement of 5.8 S and 2 S rDNA; however, since fragment AI contains more than half of the 26 S coding sequence, the possibilities remained that 5.8 S rRNA and/or 2 S rRNA could hybridize to either the transcribed spacer sequence separating the 26 S and 18 S genes or to the region of the central 26 S break. It was found that the *Hind*III/*Eco*RI fragment AI could be cleaved by the restriction endo-

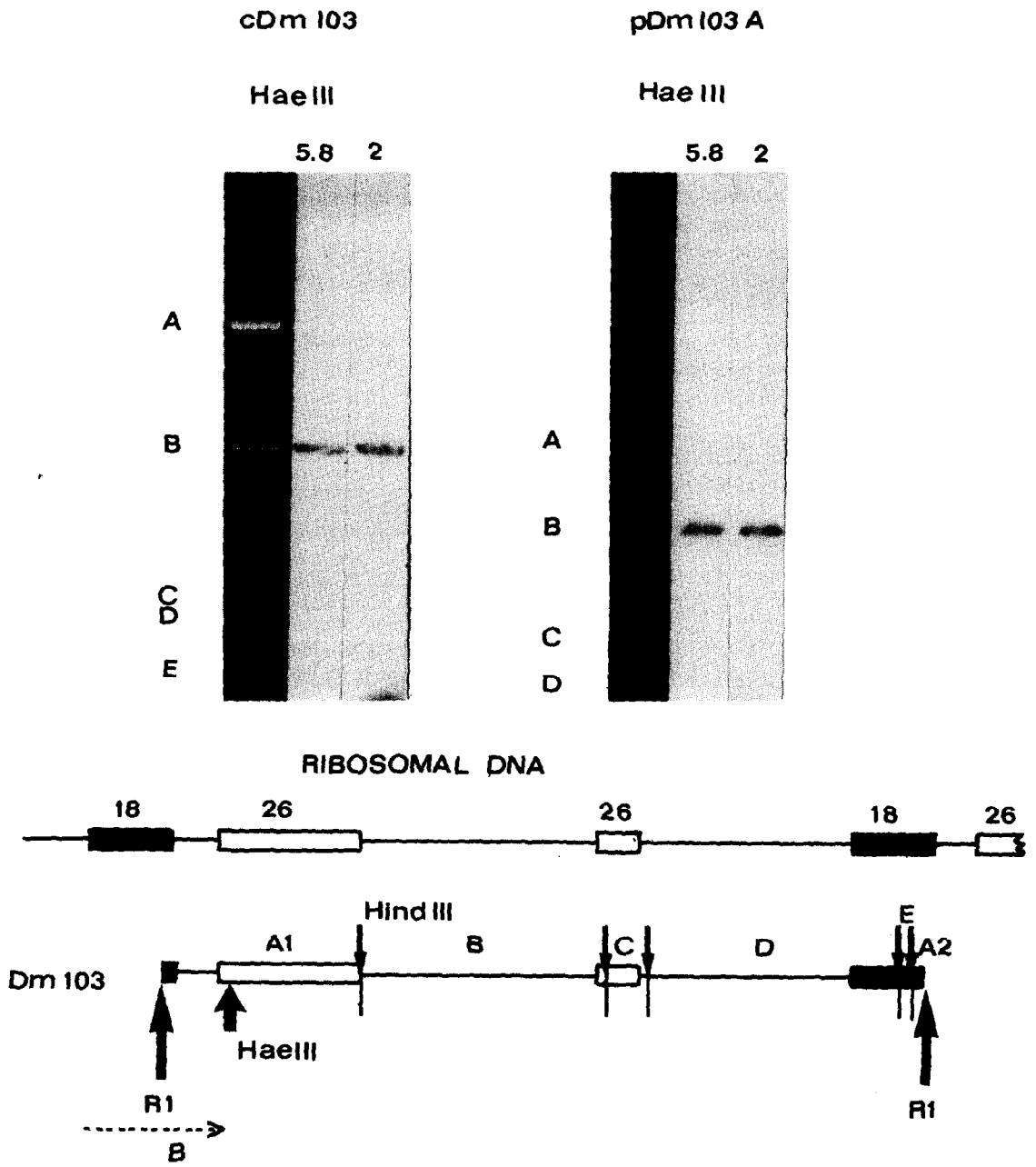

Fig.3. Top. Gel-transfer hybridization of 5.8 S and 2 S rRNAs to restriction fragments from Dm103. The following DNA preparations were subject to electrophoresis: slot 1, cDm103 digested with *Hae*III; slot 2, pDm103A digested with *Hae*III. In each case about 1 μg DNA was digested and this divided between two gel slots for electrophoresis. The fractionated restriction fragments were then transferred onto two sheets of Schleicher and Schuell BA85 filter paper and hybridization was performed with either 5.8 S or 2 S ^{32}P-labelled RNAs.

Bottom. map of the 17 kb *Drosophila* rDNA unit and of the corresponding *Eco*RI Dm103 segment. The fragments obtained after *Hind*III digestion of this segment (these which were used in the saturation hybridization experiments) are shown above the Dm103 map. *Hae*III fragment B (left panel on top) is shown below the Dm103 map.

nuclease *Hae*III so as to permit the resolution of these possibilities by the gel-transfer hybridization technique of Southern [6].

A *Hae*III digestion of the plasmids pDm103A (pSC101 carrying fragments AI and A2) or cDm103/2 (Dm103 inserted via *Eco*RI cuts in Col E vector [7]) generates approximately 30 fragments, the majority of which are shorter than Ikb and are therefore not particularly suitable for gel-transfer hybridization [6]. The second largest *Hae*III fragments of cDm103 and pDm103A are, however, 2.4 kb and 1.6 kb, respectively, and each of these breaks down to 1.55 kb with *Eco*RI, establishing the position of a *Hae*III site 1.55 kb from the *Eco*RI site within fragment AI.

A gel-transfer hybridization experiment with the restriction fragments from a digest of cDm103/2 with *Hae*III shows that 5.8 S and 2 S rRNA both hybridize to fragment B. Similarly, both 5.8 S and 2 S rRNA show hybridization to the *Hae*III fragment B from pDm103A (fig.3). This experiment therefore localises sequences complementary to 5.8 S and 2 S rRNA to within the transcribed spacer region which separates the 18 S and 26 S rRNA genes, at some point within 1.55 kb from the *Eco*RI site (fig.3).

Thus we conclude that model (a) (fig.1) correctly depicts the arrangement of polynucleotide chains in the 26 S molecule although the order of 5.8 S and 2 S rDNA within the transcribed spacer is still unknown. Our interpretation is in agreement with the recent observation by electron microscopy of a small region of DNA–RNA duplex of the approximate length of 5.8 S RNA within the short spacer separating the 18 S and 26 S genes on the coding strand of *D. melanogaster* rDNA hybridized with rRNAs [8].

Acknowledgements

This work was supported by a Medical Research Council grant to D.M.G. and by grants from the Commissariat à l'Energie Atomique and the Centre National de la Recherche Scientifique to B.R.J. B.R.J. is also grateful for a short term EMBO fellowship which enabled him to work at Imperial College, London.

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