'2 S' RNA, A NEW RIBOSOMAL RNA COMPONENT IN CULTURED *DROSOPHILA* CELLS

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1. Introduction

It has been known for some time [1] that 5.8 S RNA, an RNA molecule 150–160 nucleotides long (also called 7 S RNA or 1 RNA) is present in the large ribosomal subunit of eukaryotes. This molecule is hydrogen-bonded to 28 S (or 26 S) rRNA and can be separated from the latter by urea or heat treatment. In this article I report the existence, in a *Drosophila* cell line, of another small RNA molecule hydrogen-bonded to 26 S RNA. This molecule is approximately 30 nucleotides long and appears, like 5.8 S RNA [1–3] to be derived from 26 S RNA or its nearest precursor during one of the last steps of rRNA processing.

2. Methods

2.1. *Culture and labelling of Drosophila cells*

A cloned subline (F6) of the diploid *Drosophila* cell line KC (4) kindly provided by Drs G. Echalier and A. M. Courgeon was grown at 25°C in D22 medium [4] supplemented with 10% foetal calf serum in Falcon flasks. For labelling with ^{32}P the cells were incubated in D22 medium without phosphate, supplemented with 10% dialysed serum; 5 mCi of [^{32}P]-orthophosphoric acid (neutralized) were added to the flask. The cells, which are able to survive and grow in this medium for at least 36 hr, were usually labelled for 24 hr, then incubated in normal D22 medium (+ normal serum) for another 24 hr before harvest. For kinetic labelling experiments the cells were grown in suspension culture and labelled in the same media.

2.2. *RNA preparation*

The cells were recovered from the flasks by gentle scraping and pelleted at 600 *g* for 5 min. The cell pellet was resuspended in lysis buffer (50 mM Tris–HCl, pH 7.4: 0.5% SDS; 1% diethyl pyrocarbonate) at a concentration of approximately 10^7 cells per ml. After lysis, RNase-free DNase was added to 300 μg per ml and RNA was extracted twice with a phenol–metacresol mixture [5]. The RNA was precipitated from the aqueous phase by ethanol after addition of 0.1 volume of 20% sodium acetate pH 5.

2.3. *Acrylamide gel analysis*

All the systems used were in slab form. Large molecular weight RNAs were analysed by electrophoresis in 2.3% acrylamide, 0.5% agarose gels made up in 25 mM Tris–HCl, pH 7.8 [6]; low molecular weight RNAs were analysed on 12.5% acrylamide gels in 40 mM Tris acetate pH 8.4. Two gel systems including 8 M urea were also used: composite gels containing two layers of 10% and 20% acrylamide, both in 8 M urea and 40 mM Tris acetate, pH 8.4: also gradient gels in which the acrylamide concentration varied from 3% to 20% in 8 M urea and 40 mM Tris acetate. The latter gels were made with a Universal Scientific gradient former. In all cases a commercial acrylamide–bisacrylamide mixture ('Cyanogum 41') was used without further purification: the percentages given refer to the total acrylamide concentration. Autoradiography of the gels and elution of the bands was performed as previously described [7]. Densitometric profiles of the autoradiographs, obtained with an 'Apelab' model D.I. 4 densitometer, are presented

rather than photographs of the gel since they are more easily reproduced in the publishing process and also give semi-quantitative information. Fingerprinting was performed as described by Brownlee et al. [8].

3. Results

3.1. *Existence and homogeneity of '2 S RNA'*

When *Drosophila* ^{32}P- labelled RNA is analysed on a 12.5% polyacrylamide gel slab, the familiar pattern of fig. 1 is obtained: high molecular weight RNA's do not enter the gel, and the only bands seen are a strong, wide tRNA band and a sharp 5 S RNA band. 5.8 S RNA is not detected because under these conditions its hydrogen bonding with 26 S RNA is strong enough to keep the two together.

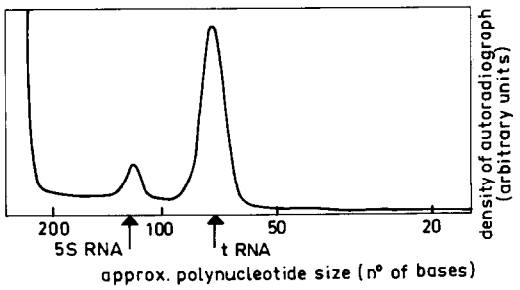

Fig. 1. Analysis of *Drosophila* ^{32}P-labelled RNA by acrylamide gel electrophoresis in the absence of urea. Electrophoresis was performed on a 12.5% acrylamide gel slab in 40 mM Tris acetate, pH 8.4. A densitometer tracing of the autoradiograph is shown. Direction of electrophoresis is from left to right on all diagrams.

When however the RNA sample is treated with urea (e.g. dissolved in 8 M urea) and/or analysed in a acrylamide gel containing 8 M urea, two additional bands become visible. This is best seen on gradient gels (fig. 2): 5.8 S RNA, whose mobility is slightly lower than that of 5 S RNA and a fast moving band, '2 S RNA', are apparent. The size of the molecules which give rise to this '2 S' band can be estimated to be approximately 30 nucleotides from their migration in standard 10 or 20% acrylamide, 8 M urea gels.

Evidence that the material contained in this band does correspond to a defined molecular species has been obtained by finger-printing of the eluted product (fig. 3). The fingerprints are quite simple and are consistent with a unique sequence 28–30 nucleotides long.

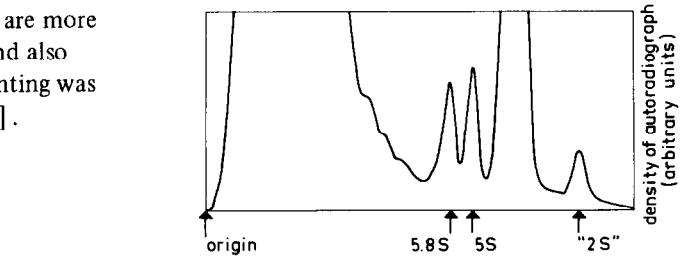

Fig. 2. Analysis of *Drosophila* ^{32}P-labelled RNA by gradient acrylamide gel electrophoresis in the presence of 8 M urea. The RNA sample was dissolved in 8 M urea and applied to a 3% to 20% linear acrylamide gel gradient (in slab form) made up in 8 M urea, 40 mM Tris acetate, pH 8.4. A densitometer tracing of the autoradiograph is shown in which the 26 and 18 S regions are grossly overexposed: some degradation products are also present (shoulders after the first peak).

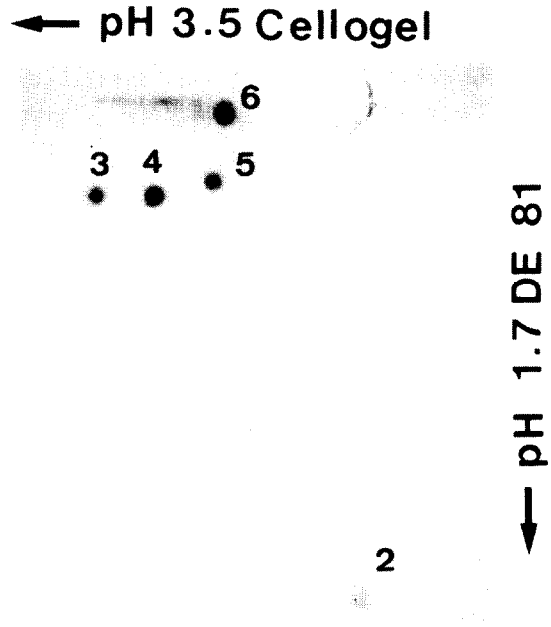

Fig. 3. Fingerprint after RNase T1 digestion of *Drosophila* 2 S RNA. Spot 1 (Gp) has run out of the paper.

3.2. *Where does '2 S RNA' originate from?*

Since 2 S RNA only appears when the RNA sample is analysed after denaturation or under denaturing conditions, it follows that it must be bound to another RNA molecule before urea treatment. A sample of whole cell RNA was therefore analysed on a 2.3% acrylamide, 0.5% agarose gel slab (fig. 4) and the different molecular species (26 S, 18 S, 5 S and tRNA) were separately analysed under denaturing conditions as follows: the corresponding gel slices were cut out,

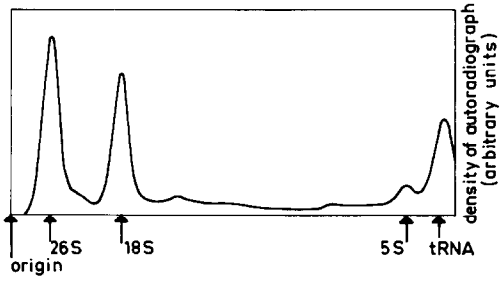

Fig. 4. Acrylamide-agarose gel electrophoresis of *Drosophila* ^{32}P-labelled RNA. Electrophoresis was performed on a 2.3% acrylamide 0.5% agarose gel slab in 25 mM Tris–HCl, pH 7.8. The autoradiograph was overexposed in order to display 5 S RNA, thus 18 and 26 S RNA are recorded close to the maximum density of the film and the peak heights in the densitometer tracing do not accurately represent the amount of the radioactivity present.

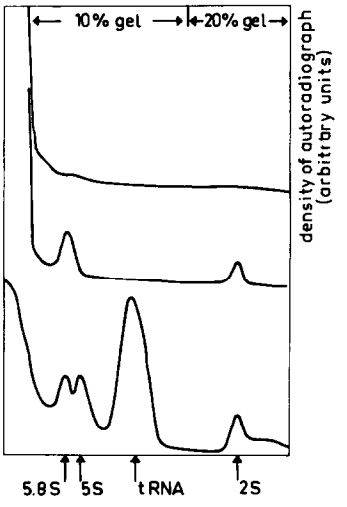

Fig. 5. Analysis of 18 S RNA (top), 26 S RNA (middle) and whole cell RNA (bottom) on a composite 10%–20% acrylamide gel slab in 8 M urea. 5 S RNA and tRNA (not shown) were also analysed in the same way and gave rise to no band in the 2 S RNA region.

Table 1
Molar yields of 5.8 S and 2 S RNAs on acrylamide gel electrophoresis in 8 M urea of isolated 26 S RNA

RNA species	26 S	5.8 S	2 S
Experiment (a)			
dpm	10.3×10^6	41×10^4	7.4×10^4
Molar ratio	1	1.17	1.08
Experiment (b)			
dpm	1.46×10^6	0.5×10^4	0.3×10^4
Molar ratio	1	0.10	0.31
Experiment (c)			
dpm	2.0×10^6	6.8×10^4	1.5×10^4
Molar ratio	1	1	1.12

Experiment (a): cells labelled for 24 hr and 'chased' for 24 hr; Experiment (b): cells labelled for 2 hr; Experiment (c): cells labelled for 2 hr and 'chased' for 6 hr. The gel slices corresponding to 26 S, 5.8 S and 2 S RNA were cut out and their radioactivity determined by Čerenkov counting. The radioactivity in corresponding background slices was subtracted. Molar yields respective to 26 S RNA are indicated assuming molecular weights of 1.5×10^6, 5.1×10^4 (150 nucleotides) and 10^4 (30 nucleotides) for 26 S, 5.8 S and 2 S RNAs, respectively.

soaked for 15 min in 10 M urea at room temperature and incorporated into a two layer (10%, 20%) acrylamide gel in 8 M urea, 40 mM Tris acetate, pH 8.4. Only 26 S RNA gave 2 S RNA (and 5.8 S RNA) (fig. 5). Moreover, the amount of 2 S RNA obtained was approximately stoichiometric with respect to 26 S and 5.8 S, i.e. one molecule of each was present (table 1, expt. a).

3.3. *'2 S RNA' is produced during one of the last steps in rRNA processing*

To investigate the kinetics of labelling of 2 S RNA, *Drosophila* cells in suspension culture were labelled with ^{32}P for 2 hr; half of the culture was harvested and the rest was resuspended in normal medium and allowed to grow for a 6 hr-'chase' period. RNA was extracted and analysed on a 2.3% acrylamide 0.5% Agarose gel and the amount of 2 S RNA present in the 26 S RNA bands was determined as in the preceding paragraph. 5.8 S and 2 S RNAs are present in a low yield in the first sample while they are found in normal yield in the second sample (table 1). 2 S RNA was not detectable after shorter labelling periods or in 30 S precursor rRNA (results not shown).

These results indicate that 2 S RNA is *not* produced by rRNA degradation during the extraction procedure (since then it should be present in both samples, which were extracted in exactly the same way) and also that 2 S RNA is generated (approximately at the same time as 5.8 S RNA) by a late processing event which occurs either on 26 S RNA or on a precursor of very similar molecular weight.

4. Conclusion

The results reported above show the existence in insect rRNA of a new molecular species, 2 S RNA, which is non-covalently bound to 26 S RNA. 2 S RNA may well be present in the RNA of other eukaryotes: it could have been overlooked because of its high mobility on acrylamide gel electrophoresis.

A number of questions can be raised with regard to this molecule: since the endonucleolytic cleavage which gives rise to 2 S RNA seems to take place during a terminal step in 26 S rRNA processing, one may wonder whether this occurs at the same time as the cleavage which gives rise to 5.8 S RNA. The relation of this processing event to the introduction of the central nick generally found in mature insect 26 S RNA's [9] is open to study. It is also possible to ask whether the 2 S and 5.8 S sequences are distal or proximal in the pre-26 S molecule. Finally, it would be important to determine whether the existence of 2 S (and 5.8 S) RNA is necessary for activity of the eukaryotic ribosome.

Acknowledgements

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