A RAPIDLY-LABELED RNA SPECIES FROM THE SILKGLAND OF THE WAX MOTH, GALLERIA MELLONELLA (L.)

H. Ishikawa and R. W. Newburgh, Dept. of Biochemistry and Biophysics, Oregon State University, Corvallis, Oregon 97331

Received June 23, 1970

Summary: Extraction of the Galleria 28-S rRNA from silkgland in a SDS-phenol solution at 45° results in the conversion of this rRNA to a 18S rRNA component. If the silkgland RNA is labeled with ^{3}H-uridine for 2 hours, a new highly radioactive RNA species is found that separates by sucrose gradient centrifugation at the same place as the 28S-rRNA. This radioactive RNA is not converted by heat-treatment to the 18-S rRNA component.

The messenger RNA for silk fibroin of Bombyx mori is believed to be unusually stable. (1,2) For this reason a rapidly-labeled RNA species is usually not detected in the posterior silkgland of spinning larvae. In comparing the extraction of RNA by a hot sodium dodecyl sulfate (SDS) phenol procedure (3) to a cold extraction a rapidly-labeled RNA species was detected in the posterior silkgland of spinning larvae of Galleria.

Materials and Methods

A stock culture of the greater wax moth, Galleria mellonella (L.) was maintained basically by the methods of Pipa (4). Silkglands of last-instar larvae which had just started spinning were used throughout the study. Each larva was injected with 5 µl sterile distilled water containing 25 µC of [5-^{3}H] uridine (28 Ci/mmole) or 10 µC of [methyl-^{3}H]-L-methionine (194 mCi/mmole). After injection the larvae were left for 2 or 8 hours at 30°C prior to removal of the posterior part of each silkgland. After dissection the posterior silkglands were washed once with a balanced salt

solution (5) and then homogenized with a glass homogenizer in equal volumes of acetate-sodium dodecyl sulfate (SDS) buffer (sodium acetate (pH 5.0) 0.01M, potassium polyvinyl sulfate 2 μg/ml, SDS 0.5%) and water-saturated redistilled phenol (6.) For the "hot"

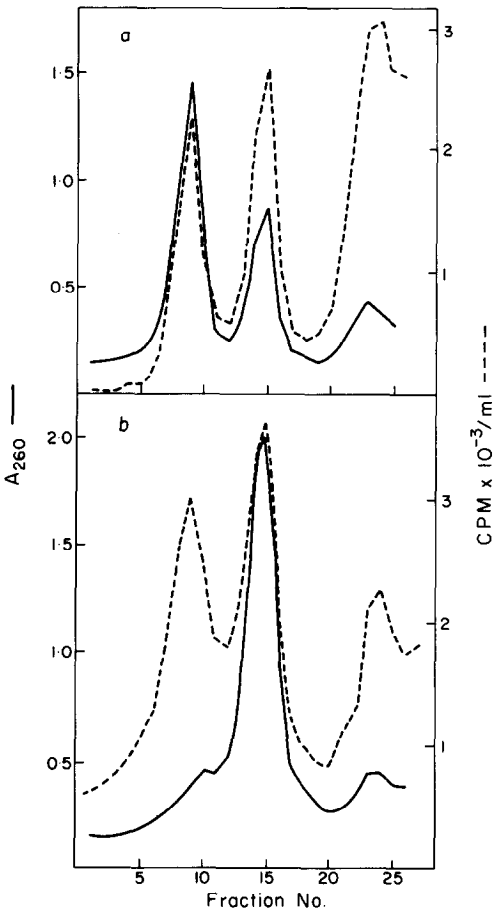

Fig. 1. Effect of extraction at 45° on the sedimentation profile of silkgland RNA.

Groups of last instar larvae were injected with [5-^{3}H]-uridine and the posterior silkglands isolated 2 hours later. The RNA was extracted according to the methods given in the text. The final RNA solution was layered over a linear [15 to 30% (W/V)] sucrose gradient containing 0.01 M Tris (pH 7.4), 0.1 M NaCl, 1 mM EDTA and 0.5% SDS and centrifuged at 23,000 rev./min. in a Spinco 25.1 rotor for 16 hours at 20°C. Fractions were collected after puncturing the bottom of the centrifuge tube and read for A_{260}. An aliquot of each fraction was counted for radioactivity in a Packard liquid-scintillation counter using a solution consisting of toluene-PPO-POPOP (6 parts) and 2-methoxyethanol (4 parts). a = control; b = extracted at 45°.

extraction procedure, the above homogenate was placed in a 45°C bath for 10 min with occasional shakings. After centrifugation the lower phenolic layer was re-extracted as before with an equal volume of buffer. The pooled aqueous layers were again treated with fresh phenol. For the control the homogenate was placed in an ice-bath instead of the 45° bath. The RNA obtained by either method was precipitated by the addition of 0.5 vol. of 10% KCl and 2.5 vol. of cold 95% ethanol. The resulting precipitate was resuspended in 0.05M Tris-HCl buffer (pH 7.6) containing 1mM $MgCl_2$, treated with 20 μg of DNase/ml at 4°C for 20 min. and made to 0.5% SDS by the addition of 5% SDS to the solution (7). The RNA was precipitated with cold ethanol, and resuspended in 0.01M Tris-Cl (pH 7.4) containing 0.1M NaCl, 1 mM EDTA and 0.5% SDS.

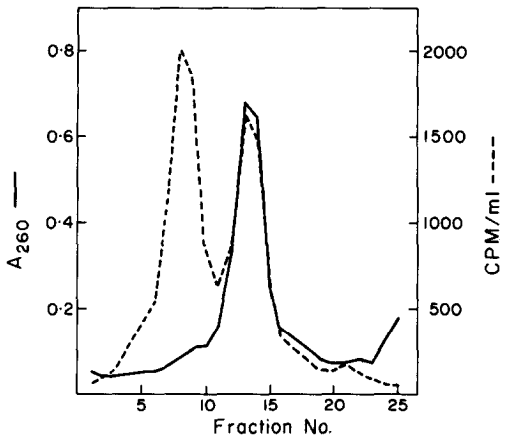

Fig. 2. Effect of heating on the "28-S rRNA".

The RNA occurring between fractions 8-10 (Fig. 1a) comprising "28S rRNA" were combined and the RNA precipitated with 2.5 vol. of ethanol. The precipitate was dissolved in a small volume of acetate-SDS buffer (0.01M sodium acetate, 2 μg/ml potassium polyvinyl sulfate, 0.5% SDS, pH 5.0, and incubated at 45°C for 30 min. After precipitating with KCl and ethanol the RNA then was dissolved in 0.01M Tris-Cl (pH 7.4) containing 0.1M NaCl, 1mM EDTA and 0.5% SDS, and analyzed on the sucrose density gradient under the conditions used in Fig. 1.

Results

The effect of the hot extraction procedure on the RNA from the posterior silkglands is shown in Fig. 1. In the control (Fig. 1a) three major components are evident. These are a 28-S, 18-S and 4-S fraction. When the extraction is carried out at 45° the bulk of the 28-S component as shown by plotting the optical density at 280Mμ is converted to a 18-S component. (Fig. 1b) In contrast to this the radioactive component of the 28-S peak is not converted to the 18-S component.

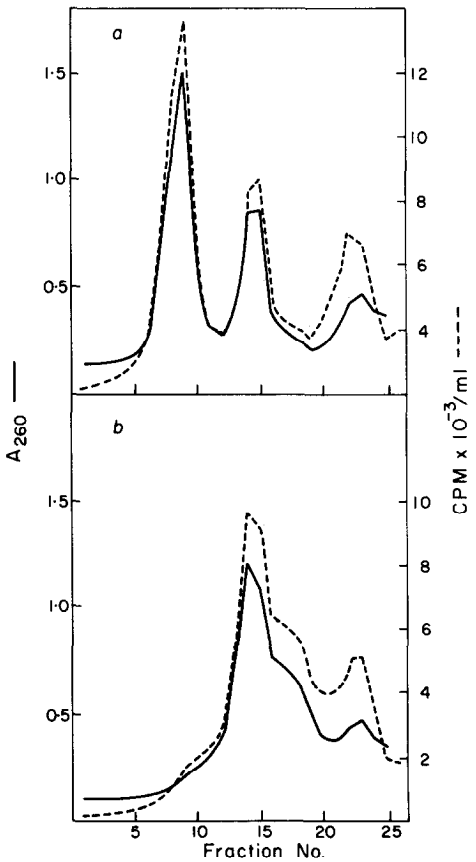

Fig. 3. Effect of extraction at 45° on the sedimentation profile of silkgland RNA.

The posterior silkgland was labeled with ^{3}H-uridine for 8 hours, and the RNA extracted and centrifuged as for Fig. 1. a = control; b = extracted at 45°.

The radioactive 28-S peak from a 2 hour pulse labeling with [^{3}-H] uridine likely is not totally "mRNA". (Fig. 2) If the 28-S peak obtained in the control (Fig. 1a) is isolated and heated at 45^o some of the radioactive 28-S peak is converted to the 18-S RNA peak.

When the larvae are exposed to [5-^{3}H]-uridine for 8 hours and the silkgland isolated the results shown in Fig. 3 are obtained. After this length of time most of the radioactive peak found in the 28-S position also is converted to the 18-S RNA. (Fig. 3b)

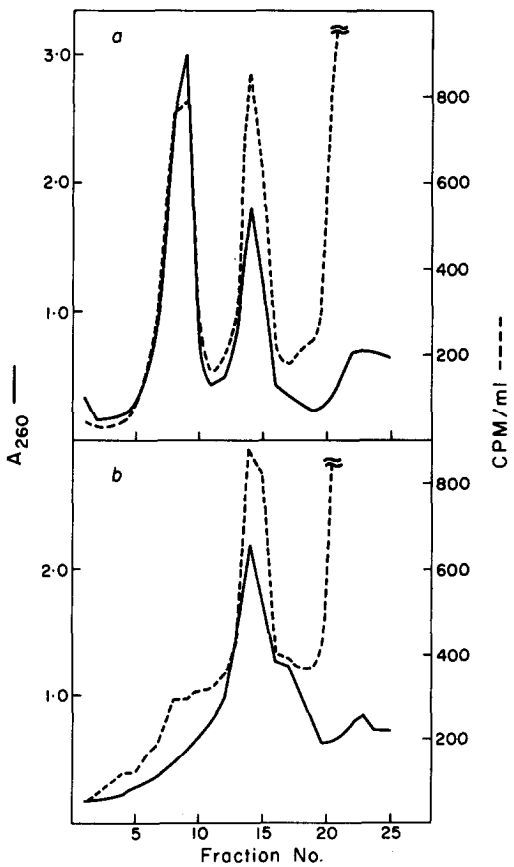

Fig. 4. Methylation of silkgland RNA.

The RNA of the posterior silkgland was labeled with ^{3}H-methionine for 2 hours and then treated as in Fig. 1. a = control; b = extracted at 45^o.

Using [methyl-^{3}H] methionine it was found that within 2 hours both the 28S and 18S rRNA underwent considerable methylation (Fig. 4a). The bulk of this labelled RNA was converted to the 18-S RNA (Fig. 4b) by heat treatment in contrast to the results with [^{3}H]-uridine.

Discussion

The possibility exists that the rapidly labeled RNA species reported in this paper may be either a rRNA precursor or messenger RNA. The methylation experiments (Fig. 4) suggest that this component is not a rRNA. Although rRNA was methylated to a considerable extent, this did not occur to the rapidly-labeled RNA which is not converted to the 18-S component. Since the methylation of rRNA in insect tissues occurs at the level of the 38-S precursor (8), the results reported here would also tend to indicate that this new rRNA species is not a non-methylated rRNA precursor.

The heat-sensitivity of insect 28-S rRNA has been reported by others (3,6,8,9). In these reports there was no evidence for the RNA species reported in this paper.

Acknowledgment: This work was supported by Research Grant NS-09161 from the National Institutes of Health.

References

1. Hosoda, J., Shigematsu, H., Takeshita, H., Mizuno, S., Takahashi, H., and Maruo, B., Biochim. Biophys. Acta, 72, 544 (1963).
2. Bensam, A., Kitasume, Y., and Ycas, M., Exptl. Cell Res., 31. 329 (1963).
3. Applebaum, S. W., Ebstein, R. P., and Wyatt, G. R., J. Mol. Biol., 21, 29 (1966).

4. Pipa, R. L., Biol. Bull., 124, 293 (1963).
5. Martignoni, M. E., and Scallion, R. J., Biol. Bull., 121, 507 (1961).
6. Howells, A. J., and Wyatt, G. R., Biochim. Biophys. Acta, 174, 86 (1969).
7. Kedes, L. H., and Gross, P. R., J. Mol. Biol., 42, 559 (1969).
8. Greenberg, J. R., J. Mol. Biol., 46, 85 (1969).
9. Hastings, J. R. B., and Kirby, K. S., Biochem. J., 100, 532 (1966).