Sequence of Drosophila 5S RNA Synthesized by Cultured Cells and by the Insect at Different Developmental Stages.

Homogeneity of the Product and Homologies with Other 5S RNAs at the Level of Primary and Secondary Structure

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Summary. The nucleotide sequence of Drosophila melanogaster 5S RNA has been determined and appears to be homogeneous both in the KC cell line and in the insect at different developmental stages. Experimental evidence on the conformation of this molecule is in agreement with a general class of 5S RNA models.

Key words: 5S RNA — Drosophila — Evolution — Secondary structure — Development

Introduction

Sequence studies have now been performed on 5S RNA from a large number of organisms, although so far no insect had been included. The determination of the sequence of Drosophila 5S RNA (Benhamou and Jordan, 1976) provides new data for comparative analysis both at the level of primary structure and at the possibly more significant level of secondary structure. Moreover the very specific advantages of Drosophila melanogaster as a developmental system accessible to genetic analysis should make this sequence particularly useful. In this article, we briefly present the evidence for the sequence and show that a homogeneous group of 5S RNA genes is expressed both in the cell line and in the organism at all developmental stages. We also show by partial hydrolysis studies that the secondary structure of Drosophila 5S RNA is very similar to that proposed as a general 5S RNA model, thus giving additional experimental support to the idea (Fox and Woese, 1975) that the sequence of 5S RNA molecules has evolved in such a way as to conserve a definite and — presumably — biologically significant shape.

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Methods

a. Cell Culture and Labelling. Subline F 6 (A.M. Courgeon, 1975) of the KC Drosophila cell line (Echalier and Ohanessian, 1969) was grown in suspension culture and labelled for 36–48 h in low phosphate medium as previously described (Jordan et al., 1976).

b. Extraction and Purification of RNA. The cell pellet was lysed in 0.05 M Tris − 0.5 % SDS buffer in the presence of 1 % diethylpyrocarbonate as previously described (Jordan et al., 1976). After DNAase treatment (5 min at 4 °C with 20 μg/ml of RNAase-free DNAase) RNA was extracted twice with a phenol / m-cresol / hydroxyquinolene mixture (Loening, 1969) and loaded on a 12.5 % acrylamide gel slab in 0.04 M tris-acetate buffer pH 8.3. The 5S RNA band (Fig. 1) was either eluted directly (Jordan, 1971) for partial digestion studies or incorporated, after dialysis against 8 M urea, into a second 10 % acrylamide gel slab in 8 M urea for repurification (Fig. 1). Typical yields were 20 x 10^6 dpm of purified 5S RNA (5 to 10 x 10^6 dpm per μg) when the labelling was performed with 10 mCi of 32P-orthophosphate.

For the preparation of 5S RNA from ribosomes a cytoplasmic fraction was first obtained as previously described (Jordan et al. 1976); this was centrifuged for 15 min at 30,000 x g, then the supernatant was sedimented for 4 h at 100,000 x g to yield a crude ribosome pellet.

c. Labelling of Drosophila Adults, Larvae and Embryos. This was based on the use of 32P-labelled yeast (5 to 50 x 10^6 dpm/mg) obtained by growing brewer’s yeast in a low-phosphate medium in the presence of 32P-orthophosphate (Bellemare et al., 1973).

Adults and larvae were labelled by transfer to bottles containing agar-NaCl medium (9 g/l NaCl in 0.5 % agar) on the surface of which a small amount of 32P-labelled yeast (approx. 1 mg per adult or larva) was added. After one to three days, the adults or larvae were transferred to a similar bottle containing unlabelled yeast for one day before harvest. To obtain 32P-labelled embryos young adult females were kept in those conditions for 2–3 days at 22 °C, then transferred to perspex cages containing a small amount of unlabelled yeast. After 12 h the eggs were collected and dechorionated by treatment with sodium hypochlorite for 2 min.

Fig. 1. Purification of 5S RNA. Left: Fractionation of whole cell RNA by electrophoresis in a 12.5 % acrylamide gel slab. Right: Repurification of 5S RNA by dialysis and embedding of the 5S RNA gel slice in a 10 % acrylamide, 8 M urea gel slab (autoradiographs of the gels)