EVIDENCE THAT SPECIES OF RNA FOUND IN RAT LIVER MITOCHONDRIA PREPARATIONS ARE ASSOCIATED WITH PURIFIED CYTOPLASMIC RIBOSOMES

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Received March 29, 1971

Summary

Species of RNA sedimenting at 20S and 22S in sucrose-SDS* gradients, originally observed in RNA preparations from rat liver mitochondria contaminated with microsomes, are shown to be present in cytoplasmic ribosome preparations free of mitochondria, microsomal membranes, nuclei, lysosomes and peroxisomes. It is concluded that these RNA's are non-mitochondrial and arise from contamination of mitochondria by microsomal membrane-bound ribosomes.

Introduction

In RNA preparations from HeLa cell mitochondria, Attardi and Attardi (1,2) have observed RNA species sedimenting at approximately 21S and 23S in sucrose-SDS gradients. These RNA's do not hybridize with mitochondrial DNA (1). Dubin and Czaplicki (3) have observed a 21S RNA in preparations from BHK cell mitochondria. The labelling of this RNA is not inhibited by ethidium bromide, indicating a non-mitochondrial origin. In our studies of RNA from rat liver mitochondria (4), we consistently observe two species of RNA sedimenting at approximately 20S and 22S in addition to the 18S and 28S cytoplasmic ribosomal RNA's which arise from contaminating microsomes. We now show that the 20S and 22S RNA's can be demonstrated in preparations from purified cytoplasmic ribosomes.

Methods

Mitochondria were prepared as described (5) with some modifications (4) The isolation medium contained 0.001M EDTA and nuclei and cell debris were

*Abbreviation: SDS, sodium dodecyl sulfate.

removed with two centrifugations at 1400 x $g_{max.}$ for 6 min. The mitochondria were pelleted and washed by centrifuging twice at 4300 x $g_{max.}$ for 10 min. and once at 10,800 x $g_{max.}$ for 10 min. For ribosome fractions, rat liver was homogenized with a motor driven teflon-glass homogenizer running at 500 rpm in two volumes of buffer AS (0.1M Bicine, pH 7.4, 0.025M KCl, 0.005M $MgCl_2$, 0.25M sucrose). The homogenate was centrifuged at 15,000 x $g_{max.}$ for 10 min. and the supernatant centrifuged at 50,000 rpm in the Spinco 50 Ti rotor for 20 min. to obtain the "crude microsome" pellet. "Purified microsomes" and "free ribosomes" were prepared by homogenizing liver from rats starved for two days in two volumes of buffer AS and centrifuging at 10,000 x $g_{max.}$ for 10 min., and then at 20,000 x $g_{max.}$ for 20 min. Seven ml of the supernatant was layered over 2 ml of 1.15M sucrose in buffer A (buffer AS minus sucrose) which had been layered over 3 ml of 2M sucrose in buffer A in Spinco SW41 tubes. After centrifuging at 40,000 rpm for 18 hours at 4°C, "purified microsomes" were collected at the 2M sucrose interface and "free ribosomes" from the pellet (6,7). The preparation and analysis of RNA is described in the figure legends. Enzymes were assayed as described previously: the microsomal membrane-specific glucose-6-phosphatase (9), the lysosome-specific β-N-acetylglucosaminidase (10), the mitochondria-specific cytochrome oxidase (10) and the peroxisome-specific urate oxidase (11). Protein was estimated by the method of Lowry et al. (12).

Results

Figure 1(A) shows RNA from mitochondria preparations. The 13S mitochondrial RNA is apparent along with a shoulder at 16S in the region of the larger species of mitochondrial RNA (4,13). The 18S and 28S RNA's from contaminating microsomes are clearly seen. The mole ratio of 28S to 18S RNA is always greater than one in these preparations since the mitochondria are washed in EDTA-containing buffers and EDTA preferentially removes the smaller ribosomal subunit from microsomal membranes (14). Peaks

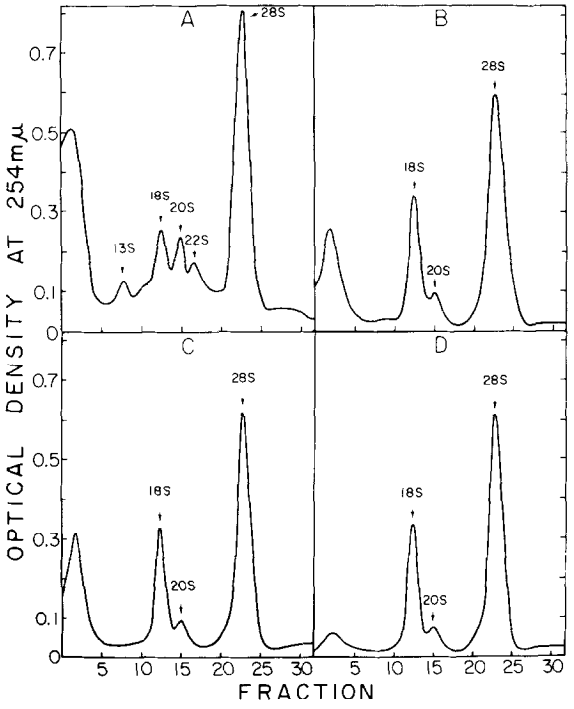

Figure 1: Sucrose gradient analyses of RNA. RNA was prepared from mitochondria by phenol-cresol extractions (8) with the additional step of incubating the RNA in buffer BN (0.01M Bicine, pH 7.4, 0.15M NaCl) containing 0.01M $MgCl_2$ and 20 μg/ml ribonuclease-free deoxyribonuclease for 15 min. at room temperature. RNA was prepared from ribosome fractions by dissolving the samples in buffer BN containing 1% SDS just prior to layering on the gradients. 0.2 ml samples were layered over 17 ml linear sucrose gradients (10-25% [w/v] sucrose in buffer BN containing 0.1% SDS) in Spinco SW27 tubes and centrifuged at 26,000 rpm for 15 hours at 15°C. Optical density at 254 mμ was monitored with an ISCO UV analyzer. All S values are approximate and are based on assigning values of 18S and 28S to cytoplasmic ribosomal RNA's.

A - RNA from mitochondria preparations
B - RNA from "crude microsomes"
C - RNA from "purified microsomes"
D - RNA from "free ribosomes"

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at 20S and 22S are also apparent. Figure 1(B,C,D) shows RNA from "crude microsomes," "purified microsomes" and "free ribosomes"; the patterns are not different, each showing a minor peak at 20S corresponding in position to the 20S RNA from mitochondria preparations. When RNA is prepared from "free ribosomes" using the same method employed for mitochondria, a small 22S peak is seen along with the 20S material (Figure 2). The 20S and 22S peaks are

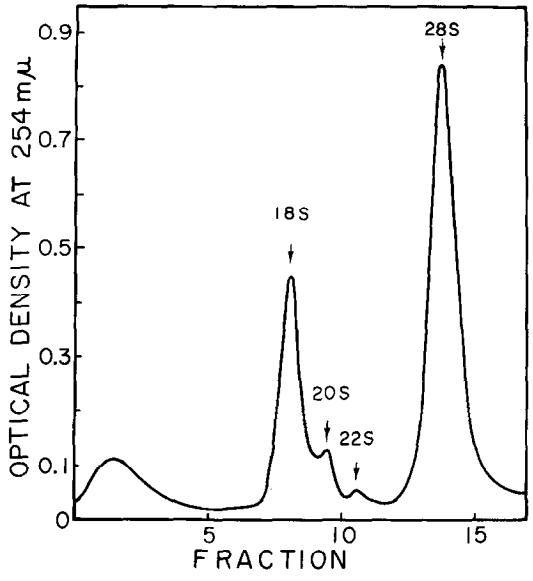

Figure 2: Sucrose gradient of RNA prepared from "free ribosomes" by exactly the same method used for mitochondrial RNA. RNA in 0.2 ml of buffer BN containing 1% SDS was layered over an 11.75 ml linear sucrose gradient (5-20% [w/v] sucrose in buffer BN containing 0.1% SDS) in a Spinco SW41 tube and centrifuged at 41,000 rpm for 5 hours at 15°C. Optical density at 254 mμ was monitored with an ISCO UV analyzer.

ribonuclease-sensitive (100 μg/ml, 15 min., room temperature) and deoxyribonuclease-insensitive (20 μg/ml, 15 min., room temperature).

The "free ribosome" preparation should be free of nuclei, microsomal membranes, mitochondria, lysosomes and peroxisomes both because of the preliminary high speed centrifugations and the high density of 2M sucrose. This is borne out by the fact that no activity was detected with any of the marker enzymes described in Methods using an amount of ribosomes containing 0.1 mg of protein per assay.

Discussion

A minor 22S peak has been observed previously in analytical ultracentrifuge patterns of RNA prepared from rat liver ribosomes. Its detection was dependent on the conditions of buffer composition and RNA concentration (15). In our hands, the detection of the 20S and 22S RNA's is highly dependent on the use of high resolution techniques, i.e., SDS-containing

gradients [Attardi and Attardi (1) also could not resolve the HeLa cell 21S and 23S RNA's in gradients lacking SDS], long tubes (Spinco SW41 or the 17 ml SW27 tubes) and continuous UV monitoring with a flow-through cell. Even with these precautions, resolution of the peaks is impaired at RNA concentrations much above 100 μg per gradient.

The observance of the 20S and 22S RNA's in preparations from purified ribosomes supports earlier observations that RNA prepared from HeLa and BHK cell mitochondria and found in the same region of sucrose gradients is non-mitochondrial in origin (1,3). However, this earlier work indicated only that these RNA's are not transcribed on mitochondrial DNA. The results presented here indicate that these RNA's are truly non-mitochondrial and arise from contamination of mitochondria by cytoplasmic ribosomes. The nature of the 20S and 22S RNA's is not known at the present time. The presence of the 20S RNA as a constant % component (compared to 18S and 28S RNA) in both highly contaminated and purified ribosomes indicates that it does not arise from non-ribosomal sources and furthermore suggests that it is not a degradation product of 28S RNA since the preparation time, and thus possible exposure to ribonuclease activity, varies greatly for the different ribosome preparations. In addition, preparations relatively rich in lysosomes (and thus lysosomal ribonuclease), i.e. both "crude" and "purified microsomes," do not show an increased 20S/28S ratio. Attardi and Attardi (2) reported that the HeLa cell 21S and 23S RNA's are somewhat less methylated than 28S ribosomal RNA. That the 22S RNA may be a degradation product of 28S RNA is suggested by the fact that it is observed only when RNA is prepared by a rather lengthy procedure involving an incubation at room temperature under non-protein denaturing conditions.

Supported by grant #P-540 from the American Cancer Society. I would like to thank Miss Mary Abell for expert technical assistance.

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