AN UNMETHYLATED "3 S_E" RNA IN HAMSTER MITOCHONDRIA: A 5 S RNA-EQUIVALENT?

Donald T. Dubin, Terrence H. Jones and Graham R. Cleaves

Department of Microbiology
College of Medicine and Dentistry of New Jersey
Piscataway, New Jersey 08854

Received November 29,1973

SUMMARY: A novel low molecular weight ("3 S_E") RNA associated with hamster cell mitochondria has been partially characterized. It was present at approx. 1:1 molar ratio with structural mitochondrial ribosomal RNA; it was unmethylated; and it resembled other mitochondrial RNA fractions in having a low content of G + C. These findings support the idea that 3 S_E RNA is a mitochondrial equivalent of 5 S ribosomal RNA.

We have recently described a novel low molecular weight component of hamster cell mitochondrial RNA (1). This component migrated slightly faster in acrylamide gels than the bulk of the mitochondrial 4 S* RNA, hence the designation of "3 S_E" RNA (see ref 2). In view of the absence of a conventional 5 S ribosomal RNA in mitochondria (1, 3, 4, 5), we suggested that 3 S_E RNA might be the mitochondrial equivalent of 5 S RNA (1). We now present further characterization of 3 S_E RNA, the results of which provide support for this idea.

METHODS: Procedures for growing and labeling hamster (BHK-21) cells, purifying mitochondria by differential and isopycnic centrifugation, and isolating and fractionating RNA, have been described (1, 4).

RESULTS AND DISCUSSION

Fig. 1 shows typical acrylamide gel patterns for the 4 S RNA associated with mitochondrial (A) and cytoplasmic (B) fractions of hamster cells. The experimental design was as described previously (1). Parallel

*Terminology: S-values and S_E values (ref. 2) are used as convenient designations for RNA fractions and do not necessarily reflect precise sedimentation coefficients or mobilities. "4 S" refers to the RNA fraction sedimenting in sucrose gradients between 2 S and 7 S, taking the modal S-value of cytoplasmic tRNA as 4.0.

cultures were labeled with [^{3}H]uridine in the presence of ethidium bromide [which preferentially suppresses mtRNA synthesis (6)], or with [^{14}C]uridine in its absence, and the two cultures were mixed prior to processing. The ^{14}C of resulting mtRNA preparations represents total RNA, while the ^{3}H represents ethidium-resistant (and hence contaminating cytoplasmic) RNA. After appropriate normalization, the difference between the two can be taken as ethidium-sensitive, or mitochondrion-specific, RNA (cf ref. 1).

In the pattern of total mitochondrion-associated 4 S RNA (Fig. 1A), 3 S_E RNA registers only as the faster of two leading shoulders to the main peak. However, the ethidium sensitive pattern clearly reveals 3 S_E as a peak distinct from the main ethidium sensitive peak, separated from the latter by the small amount of contaminating cytoplasmic tRNA invariably present in such preparations (1, 3). [In the gel system used, mitochondrial tRNA runs slightly slower than cytoplasmic tRNA, presumably due to conformational effects (1).] The pattern for bona fide cytoplasmic 4 S RNA (Fig. 1B) demonstrates the absence of comparable shoulders, and the presence of a substantial 5 S peak. In this experiment 3 S_E RNA constituted 4.2% of the total ethidium-sensitive 4 S radioactivity, and 3.8% of the radioactivity in 17 S RNA (the larger mitochondrial structural rRNA species), yielding a value of approx. 0.9 for the molar ratio of 3 S_E : 17 S RNA (cf ref. 1).

A distinctive feature of 5 S RNA is its absence of methylated nucleotides. To examine 3 S_E RNA in this respect, an experiment was performed in which ethidium-sensitive and -resistant RNA methylation was examined, in analogy to the uridine incorporation experiment of Fig. 1. Parallel cultures were labeled with [methyl-^{3}H]- or [methyl-^{14}C]methionine in the presence or absence of ethidium. The non-treated culture also received ^{32}P, as a general RNA label. A straightforward extension of the computational procedure used for double isotope studies (1) allows determination of the ethidium-sensitive and -resistant moieties of both the

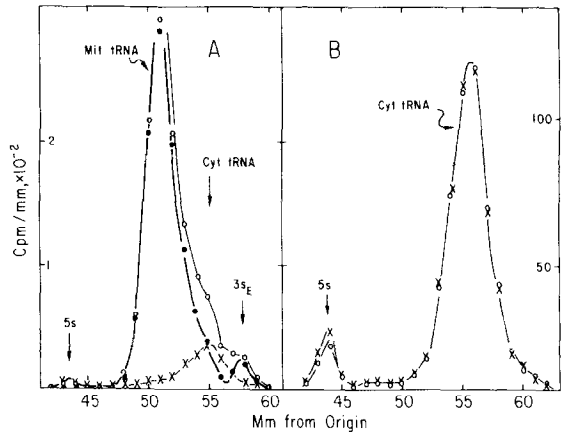

Fig. 1. Acrylamide gel electrophoresis of uridine-labeled mitochondrial and cytoplasmic 4 S RNA. Parallel 100 ml-cultures were labeled for 16 h with [^{3}H]uridine, 10^{-5}M, 10 µci/ml, in the presence of ethidium bromide (2 µg/ml) or with [^{14}C]uridine, 10^{-5}M, 0.5 µci/ml, in its absence. The cultures were mixed prior to processing, and samples of centrifugally purified mitochondrial (A) and cytoplasmic (B) 4 S RNA were subjected to acrylamide gel electrophoresis as previously described (1) at 10 mA/gel (starting) for 120 min; one-mm slices were dissolved in an "NCS" scintillation solution and counted differentially. Results were normalized on the basis of cytoplasmic isotope ratios (1). Total RNA (^{14}C):-o-o-; ethidium-resistant RNA (normalized ^{3}H);-x-x-; ethidium-sensitive RNA (difference):-●-●-.

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methyl label, and the phosphate label, of resulting RNA samples (7). As shown in Fig. 2, on acrylamide gel electrophoresis there was no ethidium-sensitive methyl peak corresponding to the 3 S_E ^{32}P peak. In a second experiment, similarly labeled 4 S RNA samples were subjected to preparative gel electrophoresis (Fig. 3A) followed by density gradient sedimentation of RNA eluted from the 3 S_E shoulder (Fig. 3B). The sedimentation served both to display the labeling patterns and to remove gel-derived impurities that interfere with nucleotide analysis (see Table 1, below). Again, there was a well-defined ethidium sensitive (3 S_E) ^{32}P peak with no corresponding ethidium-sensitive methyl peak. Residual contaminating cytoplasmic tRNA appeared as a methylated peak sedimenting, as expected (1), slightly faster than 3 S_E RNA. As a control, a "cut" from the initial cytoplasmic gel (Fig. 3A) corresponding to the mitochondrial 3 S_E shoulder was processed in parallel with the mitochondrial cut. This cytoplasmic sample was in-

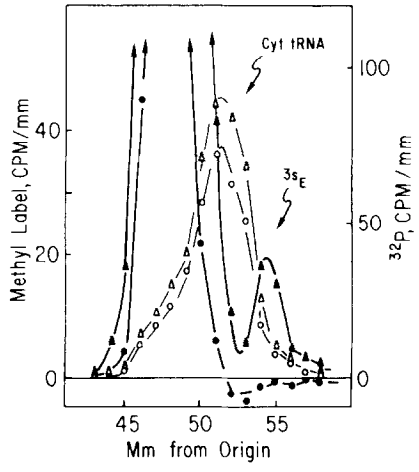

Fig. 2. Acrylamide gel analysis of methyl-,^{32}P-labeled mitochondrial 4 S RNA. Parallel cultures were labeled with [methyl-^{3}H]methionine, 50 µci/5.9 µg/ml (100 ml) in the presence of ethidium and with [methyl-^{14}C]-methionine, 2 µci/7.2 µg/ml and ^{32}P, 10 µci/0.65 µmole/ml, in its absence (500 ml). At 20 h the cultures were mixed and processed as for Fig. 1. Counting and computations were as described previously (7); for clarity, only ethidium-resistant and -sensitive data have been plotted, and the ordinate scale has been chosen to display the 3 S_E and contaminating cytoplasmic moieties rather than the main (mitochondrial tRNA) peak. Ethidium-resistant methyl label, -0-0-; ethidium-resistant ^{32}P, -Δ-Δ-; ethidium-sensitive methyl label, -●-●-; ethidium-sensitive ^{32}P, -▲-▲-.

distinguishable in sedimentation behavior from the cytoplasmic tRNA contaminating the mitochondrial cut, and its degree of methylation and nucleotide composition were indistinguishable from those of RNA from the central portion of the cytoplasmic peak.

Studies such as those of Figs. 2 and 3 would have detected as low a degree of methylation as 0.3 methyl groups/100 nucleotides [compared to approx. 3 and 8 for mitochondrial and cytoplasmic tRNA (4, 7)]. Since 3 S_E RNA is less than 100 nucleotides long (1), we infer that it is unmethylated.

RNA from the gradient of Fig. 3 was analyzed for major nucleotide composition, and the results are presented in Table 1 together with those for 5 S RNA and other mitochondrial and cytoplasmic RNA fractions. Both 5 S RNA, and 3 S_E RNA, resembled their homologous transfer and structural ribosomal RNA's in this regard.

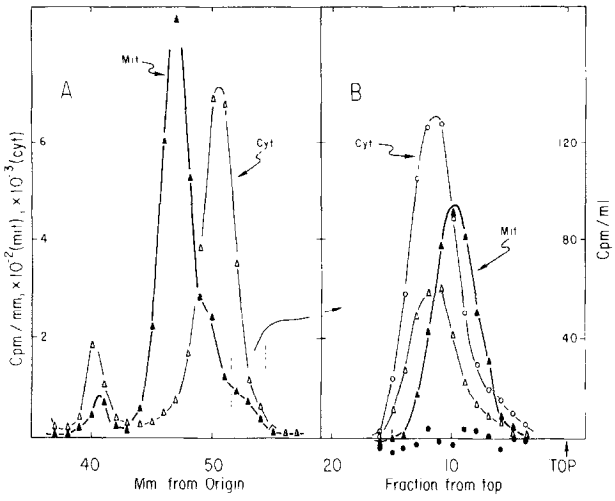

Fig. 3. Density gradient analysis of gel-purified, methyl-,^{32}P-labeled 3 S_E RNA. Cultures were labeled and processed essentially as for Fig. 2. Panel A represents parallel gels of 4 S RNA from the mitochondrial and cytoplasmic fractions, run as for Fig. 1 except that slices were assayed non-destructively for ^{32}P (only), using an end-window counter. RNA was eluted from the slices corresponding to the 3 S_E shoulder of the mitochondrial pattern (vertical bars), and was subjected to density gradient analysis, as previously described (ref. 7; "standard" salt, 38,000 RPM, 18 h, 32 fractions). Panel B represents the normalized patterns of the "cut" from the mitochondrial gel; a corresponding cytoplasmic cut (not shown; see text) was sedimented in parallel. Panel A: cytoplasmic ^{32}P, -Δ-Δ-; mitochondrion-associated ^{32}P, -▲-▲-. Panel B: Symbols are as for Fig. 2B.

We have thus far been unsuccessful in demonstrating specific association between 3 S_E RNA and the large ribosomal ("33 S") subunit released from hamster cell mitochondria by Triton X-100 (ref. 8). However, little is known about the stability of such a putative association in mitochondrial systems, and further studies on mitochondrial ribonucleoprotein particles are in progress.

In summary, 3 S_E RNA appears to be a well defined, unmethylated component that resembles the structural RNA of mitochondrial ribosomes in base ratio and in molar abundance. These properties, while not definitive, favor the idea that 3 S_E RNA is, in fact, a "mini-5 S" RNA, and that the "mini-ribosomes" of mammalian mitochondria resemble conventional ribosomes in having at least a functional equivalent of 5 S rRNA.

Table 1. Base Ratios of 3 S_E and Other RNA Fractions from BHK Cells

(Mole %)

	Mitochondrial RNA			Cytoplasmic RNA		
	3 S_E	tRNA	17 S	5 S	tRNA	28 S
Cp	24.0	20.2	22.7	27.0	26.2	29.1
Ap	33.2	31.0	31.2	16.2	18.7	16.0
Gp	15.8	18.3	20.2	33.2	32.7	35.7
Up	27.1	29.8	26.0	23.6	22.4	19.2

Results were obtained by high voltage electrophoresis of alkaline hydrolysates (4). The 3 S_E RNA was obtained from the gradient peak of Fig. 3B, and the raw results were corrected for residual contaminating cytoplasmic tRNA (approx. 30% of total ^{32}P). Other RNA samples were obtained from the same experiment. Low molecular weight RNA was purified by gel electrophoresis, and 17 S and 28 S rRNA (representative of structural rRNA) were purified by density gradient sedimentation.

This work was supported by Research Grant No. GM 14957 of the National Institute of General Medical Sciences, U.S.P.H.S. G.R.C. is a trainee of the National Cancer Institute (Grant No. CA-05234). The expert technical assistance of Miss Kathleen Daye is gratefully acknowledged.

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