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The sequence of a possible 5S RNA-equivalent in hamster mitochondria

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ABSTRACT

We have sequenced $3S_E$ RNA, an unmodified species from hamster cell mitochondria that may be a 5S rRNA-equivalent. The sequence is

60 50 40 30 20 10

pGGAGAAUGUAUGCAAGAGCUGCUAACUCCUGCUACCAUGUAUAAUAACAUGGCUUUCUUACCA$_{OH}$

The underlined stretches can form the stems of 2 hairpins whose existence is supported by S1 nuclease analysis. Residues 24 through 34 can also base-pair extensively with a sequence in the 3'-region of the small subunit ("13S") mitochondrial rRNA. These interactions resemble interactions postulated for 5S RNA.

INTRODUCTION

The ribosomes of animal and fungal mitochondria lack conventional 5S rRNA (1,2). However, we have detected in hamster cell mitochondria an unmodified low molecular weight RNA species, $3S_E$ RNA, some of whose properties suggest that it may be a 5S-RNA equivalent (3-5). We present here studies that establish the primary sequence of this RNA species, and that suggest certain secondary structural characteristics that are in accord with its functional homology to 5S RNA. This is the first complete sequence to be reported for an RNA species from animal mitochondria.

METHODS

Mitochondrial RNA was prepared from cultured hamster (BHK-21) cells, and $3S_E$ RNA was purified from the mitochondrion-associated 4S RNA fraction, by sequential electrophoresis in "warm" and "cool" gels, as previously described (5). To facilitate purification, cells were labeled lightly *in vivo* with ^{32}P. A typical batch of 6 liters of cells yielded approximately 1 μg of $3S_E$ RNA. RNA was labeled at the 5'-terminus with γ-^{32}P-ATP and T4 polynucleotide kinase (following ref. 6) or at the 3'-terminus with 5'-^{32}P-pCp and T4 RNA lig-

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ase (7). Reaction mixtures contained 0.5 to 0.6 µg of RNA.
The endlabeled product was repurified by electrophoresis in 10% acrylamide-urea gels; it yielded a discrete band running, as expected, slightly faster than mitochondrial tRNA (Fig. 1). Such RNA was subjected to mobility shift analysis (8) after partial hydrolysis with acid (9) or formamide (10). Ladder analysis was performed after partial chemical hydrolysis (11) or partial base-specific hydrolysis with ribonuclease T1, U2 and A (following refs. 10, 12). For enzymatic discrimination between C and U residues we used micrococcal nuclease in the presence of calcium (9). Structural analyses were performed with nuclease S1 (ref. 13).

RESULTS & DISCUSSION

We obtained a sequence for the entire $3S_E$ RNA molecule using 3'-terminally labeled samples. The most definitive results were obtained by base-specific partial chemical hydrolysis followed by ladder analysis on acrylamide gels, as illustrated by Fig. 2; confirmatory results were obtained by partial enzymatic analysis using ribonucleases T1, U2 and A (data not shown). 5'-Labeled samples were analyzed after partial digestion with the above enzymes, plus micrococcal nuclease in the presence of calcium to distinguish C's from

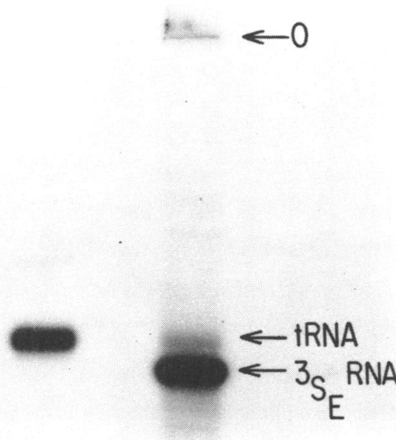

Fig. 1. Gel electrophoresis of 3'-Terminally Labeled $3S_E$ RNA. A sample of $3S_E$ RNA (0.56 µg) was labeled with ^{32}P-pCp (50 pmoles, 750 ci/mmole; Amersham) as described in Methods, and subjected to electrophoresis through a 10% acrylamide-urea gel. Mitochondrial tRNA was run as a marker (left lane). Exposure for autoradiography was seven minutes. "O" indicates the origin.

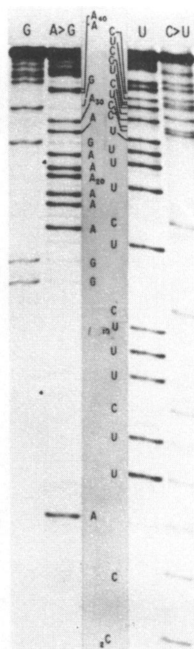

Fig. 2. Ladder Analysis of 3'-Labeled $3S_E$ RNA after Partial Chemical Digestion. The $3S_E$ RNA was recovered (14) from the gel described in Fig. 1 and aliquots were subjected to partial chemical degradation as described by Peattie (11), followed by electrophoresis through a 0.4 mm thick 20% acrylamide gel (15), 2.5h at 2,500 volts. The designations refer to the base-specificities of the reactions. We have indicated our reading for the sequence from C_2 through A_{40} (from the 3'-end). Another 10 residues could be read unambiguously on the original autoradiogram, and the rest with reasonable confidence on lighter exposures.

U's; as illustrated in Fig. 3, the identities of about 35 residues from the 5'-end were confirmed in this manner. Extreme terminal sequences were confirmed by mobility shift analyses after partial hydrolysis with acid (for 5'-labeled samples) or formamide (3'-labeled samples) (e.g., Fig. 4).

The sequence is presented in Fig. 5. It is in agreement with 5'-end group analysis of samples labeled <u>in vivo</u> with ^{32}P (5), and 3'-end group analyses of samples labeled <u>in vivo</u> with ^{3}H-adenosine and ^{14}C-uridine (R. Taylor, unpublished observations). The base ratio (23% Cp, 29.5% Ap, 18% Gp, 29.5% Up, excluding termini) is similar to that obtained from comparably purified samples labeled with ^{32}P <u>in vivo</u> and subjected to exhaustive alkaline hydrolysis (23% Cp, 31% Ap, 17% Gp, 28% Up).

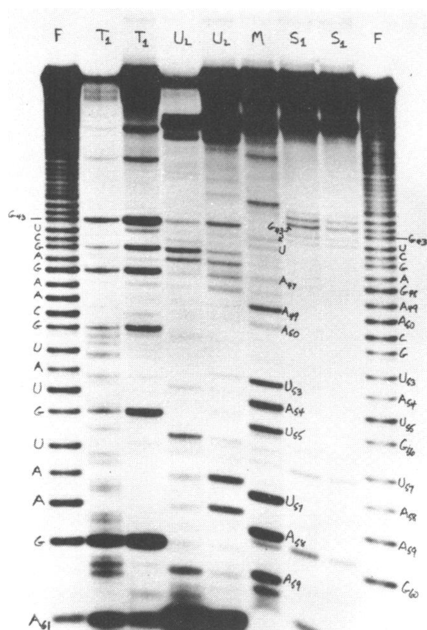

Fig. 3. Ladder Analysis of 5'-Labeled $3S_E$ RNA after Partial Enzymatic Digestion. Aliquots of a 5'-endlabeled $3S_E$ RNA sample purified as described in Fig. 1 were subjected to partial formamide or enzymatic digestion. F, formamide (100°, 60 min.); T_1, ribonuclease T_1, 0.01 or 0.002 units per µg of RNA, in 20mM sodium citrate, pH 5.0, containing 1mM EDTA and 7 M urea (50°, 5 min.); U_2, ribonuclease U_2, 0.2 or 0.04 units per µg of RNA, other conditions as for T_1; M, micrococcal nuclease, 0.01 units per µg of RNA, in 20mM Tris•HCl, pH 7.5, containing 10mM $CaCl_2$ (50°, 15 min); S_1, ribonuclease S_1, 0.1 units per µg of RNA, in 40mM sodium acetate, pH 4.5, containing 0.2 M NaCl and 10mM $ZnSO_4$ (37°, 30 or 15 min.). Electrophoresis and enumeration as for Fig. 2. We indicate our reading of the sequence from G_{43} through A_{61}. Note that by virtue of the specificities of the respective enzymes, the S_1 rungs of 5'-labeled samples run approximately one residue behind the corresponding F rungs, while the M rungs run approximately one residue ahead; thus G_{43} in the S_1 ladder is separated from G_{43} in the M ladder by two nucleotide equivalents.

Base-pairing interactions between 5S RNA and regions near the 3'-end of 16S or 18S RNA have been proposed to play roles in ribosome function (16). Although we detected little direct homology between the $3S_E$ RNA sequence and those for eukaryotic and prokaryotic 5S RNA's (17), an impressive stretch of complementarity occurs between $3S_E$ RNA and the small ribosomal subunit ("13S") RNA of hamster mitochondria, also as illustrated in Fig. 5. We summarize in Table 1 the positions involved in the putative interactions. The complementary stretches of 13S, 16S and 18S RNA begin in all cases in the 5'-portion of

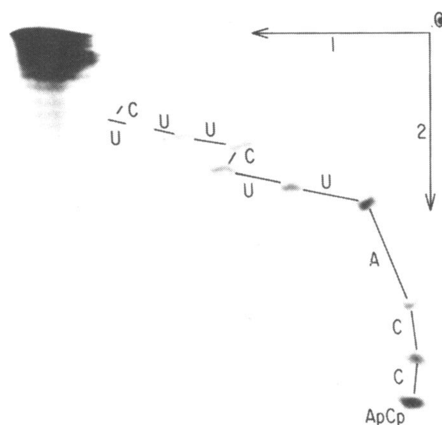

Fig. 4. Mobility Shift Analysis of 3'-Labeled $3S_E$ RNA. A sample was subjected to partial formamide hydrolysis followed by fingerprinting (8). Dimension 1, cellulose acetate electrophoresis at pH 3.5; dimension 2, homochromatography on a PEI plate. The pCp moiety of the fastest moving spot in the second dimension derives from the 5'-^{32}P pCp used for endlabeling.

the "m_2^6A" hairpin that each of these RNA species appears to have (18; also Baer & Dubin, in preparation). Equilibria between such intermolecular interactions and intramolecular base-pairing figure in ideas on the possible function of 5S RNA (16). To evaluate intramolecular secondary structure in $3S_E$ RNA we performed structural analyses using nuclease S1. As shown in Fig. 6 for a 3'-endlabeled sample, the palindromic sequence AUAAUA (positions 18-23) was strikingly sensitive to S1. A region with lesser, but definite, enhanced sensitivity to nuclease S1 was also detected around G_{43}. This can be seen as a lighter series of bands in the first S1 channel of Fig. 6, and can also be

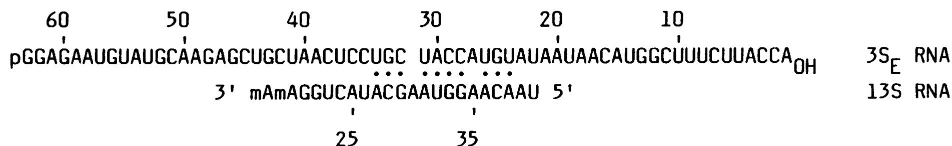

Fig. 5. Sequence of $3S_E$ RNA and Proposed Interaction with 13S RNA. The numbers refer to residues counted from 3'-termini. "mA" represents $m_2{}^6Ap$. The gap between U_{31} and C_{32} of $3S_E$ RNA indicates simply the absence of base-pairing with A_{30} of 13S RNA. The 5'-portion of the $m_2{}^6A$ hairpin of 13S RNA runs from U_{23} through U_{32} (Baer & Dubin, in preparation).

Table 1. Positions of sequences involved in putative interactions between $3S_E$ or 5S RNA, and Small Ribosomal Subunit RNA's.

$3S_E$ RNA	24-34	13S RNA	27-38
Prokaryotic 5S	20-30	16S RNA	26-34
Eukaryotic 5S	16-31	18S RNA	22-33

Numbers are counted from 3'-termini. Prokaryotic results are from *E. coli* RNA's and eukaryotic from yeast; other sources of conventional RNA yielded similar correlations (16).

seen in S1 channels in Fig. 3. We indicate in this latter figure an anomolous enhancement of sensitivity of the $U_{44}pG_{43}$ band (the M channel) which we also attribute to secondary structure effects. Assuming a correlation between enhanced S1 sensitivity and localization in loops of hairpin structures (13), we infer the existence of two hairpins in $3S_E$ RNA, designated I and II in Fig. 7. Both are reasonably stable according to the model of Salser (19),

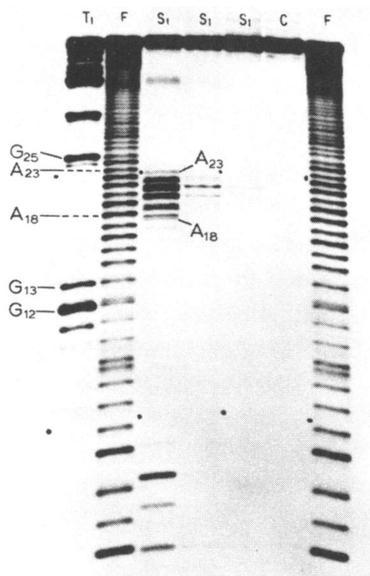

Fig. 6. Ladder Analysis of Secondary Structure of $3S_E$ RNA. Aliquots of the 3'-labeled $3S_E$ RNA preparation of Fig. 1 were subjected to partial hydrolysis with formamide or ribonuclease T1 to provide marker bands, as for Fig. 3. In addition, aliquots were digested with nuclease S1 under the conditions described for Fig. 3, 0.1, 0.02 or 0.004 units per µg of RNA, 37° for 15 min. C refers to a control (undigested) sample. Electrophoresis and enumeration as for Fig. 2.

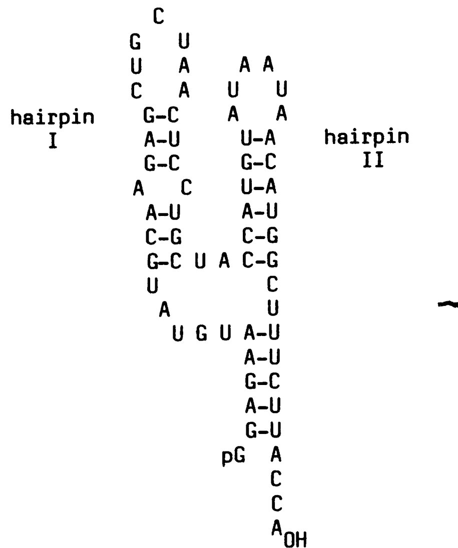

Fig. 7. A Proposed Secondary Structure for $3S_E$ RNA.

having free energies of formation of -5.1 and -6.5 kcal per mole respectively. There is in addition a modest stretch of complementarity between regions near the 5' and the 3' termini of $3S_E$ RNA, also shown in Fig. 7. These general features - two hairpins and a terminal stem - also occur, albeit in more elaborate form, in the proposed universal structure of prokaryotic 5S RNA (20). Of course, the putative participation of $3S_E$ RNA in the ribosome dissociation-association reaction (16) would require substantial unwinding of helices I and II. Presumably the resulting gain in free energy would be balanced by compensating losses related to formation of the $3S_E$-13S RNA helix (cf. Fig. 5).

We believe that the present results support, on balance, the idea that $3S_E$ RNA is a 5S rRNA-equivalent. However, the presence of 3'-terminal CCA_{OH} suggests as an alternative possibility that $3S_E$ RNA may be a transfer RNA, albeit a very bizarre one. Clearly, definitive assignment of function will require, at the least, systematic studies on ribosome association and on chargeability, and such studies are planned.

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