THE 5′-TERMINI OF LOW MOLECULAR WEIGHT RNA FROM HAMSTER MITOCHONDRIA

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1. Introduction

Mammalian mitochondrial tRNA has a number of distinctive properties. It sediments slower than cytoplasmic tRNA and migrates anomalously in acrylamide gels [1–3], has a low (38%) GC content [4], and a minor nucleotide pattern that differs not only from those of bacterial and eukaryotic cytoplasmic tRNA but also from fungal mitochondrial tRNA [3–7]. We have also detected an RNA species in hamster mitochondria, '3 S_E' RNA, that migrates ahead of mitochondrial tRNA on gel electrophoresis and that may be analogous to 5 S rRNA [1,2,4]. Here we identify and quantitate the 5′-terminal nucleotides of hamster cell mitochondrial tRNA and 3 S_E RNA, and compare them with those of cytoplasmic tRNA and 5 S RNA. The results provide estimates of average chain lengths, bear on the mode of biogenesis of mitochondrial tRNA, and further underscore the unusual nature of mammalian mitochondrial tRNA.

2. Experimental

Hamster (BHK-21) cells were labeled with $^{32}P_i$ and [*methyl*-^{3}H]methionine for 18–25 h exponential growth, and mitochondrial and cytoplasmic 4 S RNA were prepared, as in [8]. Mitochondrial tRNA was purified by electrophoresis through 'warm' acrylamide gels, and 3 S_E RNA by electrophoresis sequentially through warm and then cooled gels ([3] and legend to fig.1). The apparent degeress of methylation of the mitochondrial tRNA samples were 3.0–3.1 [Me]/100 nucleotides. If pure mitochondrial tRNA is taken to contain 2.7 [Me]/100 nucleotides and cytoplasmic 8.5 [2], our mitochondrial preparations contained 5% cytoplasmic tRNA. The apparent degree of methylation of the 3 S_E RNA preparation was 0.3 [Me]/100 nucleotides, which leads to an estimate of <5% cytoplasmic tRNA contamination, taking 3 S_E RNA to be unmethylated [4].

5′-Termini were released by digesting RNA either with alkali or with T2 ribonuclease [8]. The two procedures yielded equivalent results. The termini were separated by DEAE column chromatography from the monophosphates constituting the bulk of the hydrolysates, and were fractionated using DEAE paper electrophoresis at pH 1.9 [9].

Materials were obtained from commercial sources, except for pCp and pUp markers. These were prepared by partial digestion of [^{14}C]pyrimidine-labeled cytoplasmic RNA with *Neurospora crassa* endonuclease [9,10].

3. Results

Figure 1A presents a typical DEAE column pattern for a T2 digest of mitochondrial tRNA, and fig. 1C the pattern for 3 S_E RNA. For comparison we also processed samples of cytoplasmic RNA (fig. 1B,D). The mitochondrial tRNA digest yielded a well-defined ^{32}P-peak corresponding to the -4 isostich, with an earlier-eluting shoulder. Mitochondrial 3 S_E RNA and cytoplasmic tRNA yielded symmetrical '-4' peaks, and 5 S RNA yielded a peak between '-4' and '-5', in the region of (p)ppGp. In no case was there significant ^{3}H corresponding to these presumed 5′-terminal peaks (not plotted). Electrophoretic patterns for nucleotides recovered from the peaks of fig.1 are presented in fig.2, and these results, together with those from another experiment, are summarized in

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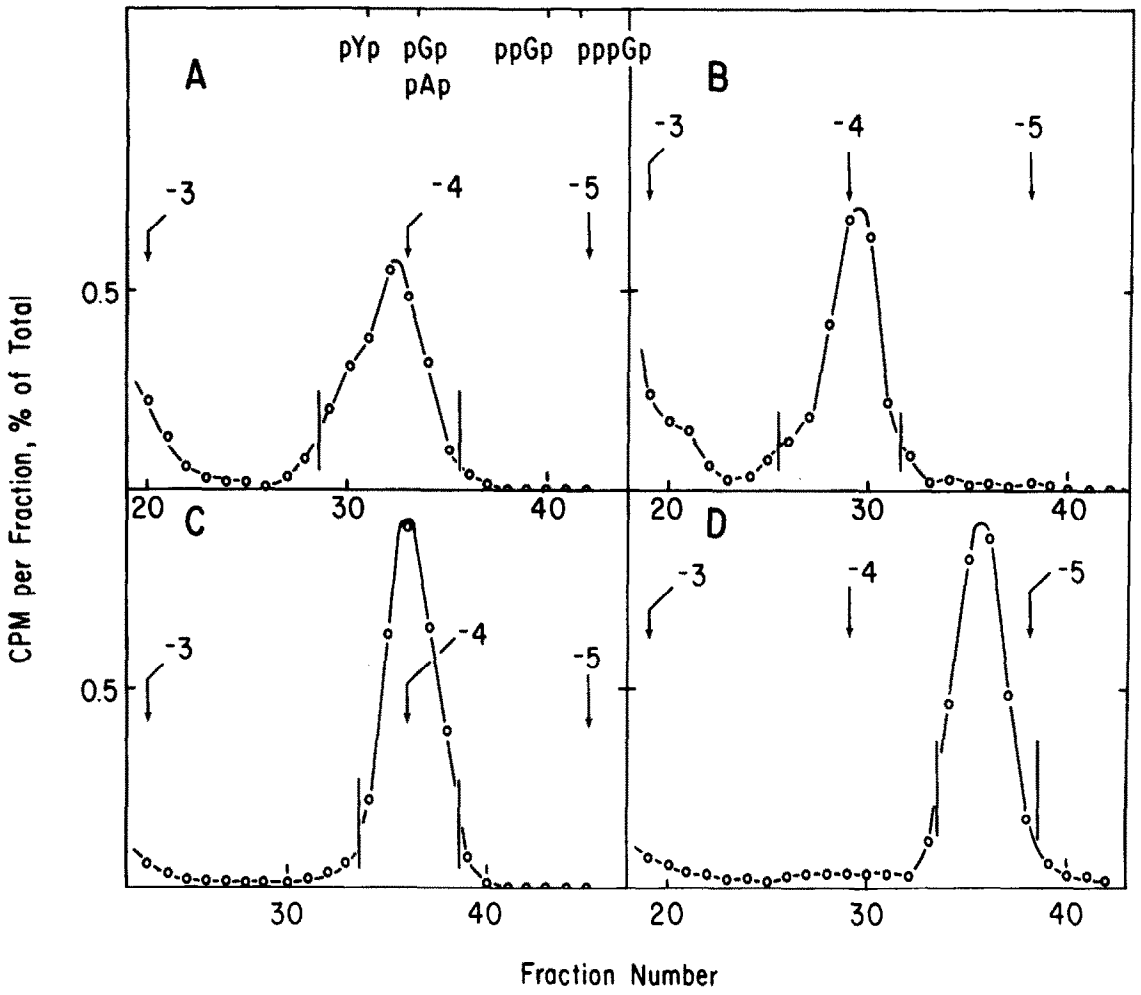

Fig.1. DEAE-cellulose column chromatography of T2 ribonuclease digests of mitochondrial and cytoplasmic low molecular weight RNA fractions. One liter of cells was labeled with ^{32}P (150 mCi, 0.5 nM) and [*methyl*-^{3}H]methionine (15 mCi, 7.5 μg/ml) for 25 h [8]. Transfer and 5 S RNA were purified by electrophoresis through 'warm' acrylamide gels [3]. The 3 S_E RNA 'shoulder' in the initial 'warm' gel [1,4] was re-run in a 'cooled' gel [3], yielding a discrete peak. Samples of mitochondrial tRNA (610 000 cpm in ^{32}P) and 3 S_E RNA (62 000 cpm), and cytoplasmic tRNA (910 000 cpm) and 5 S RNA (210 000 cpm), were subjected to hydrolysis with T2 ribonuclease followed by DEAE column chromatography together with a ribonuclease A digest of unlabeled RNA to provide markers [8]. All of each fraction was assayed for ^{32}P by Cerenkov radiation in the case of 3 S_E RNA (panel C); 0.3 or 0.5 ml portions were assayed in scintillant in the case of mitochondrial tRNA (panel A), cytoplasmic tRNA (B) and 5 S RNA (D). To facilitate comparisons, the ^{32}P has been plotted in all cases as % of total recovered. Only the regions corresponding to the '-3' through '-5' isostiches of the markers have been plotted. The positions of (pp)pNp markers were determined by running them, on separate columns, together with a sample of an alkaline hydrolysate of *methyl*-^{3}H-labeled 28 S RNA; the latter yields peaks corresponding to charges of −3, −3.6 and −5 [8]. The 'pYp' marker is the mixture of the ^{14}C-labeled pCp and pUp described in section 2.

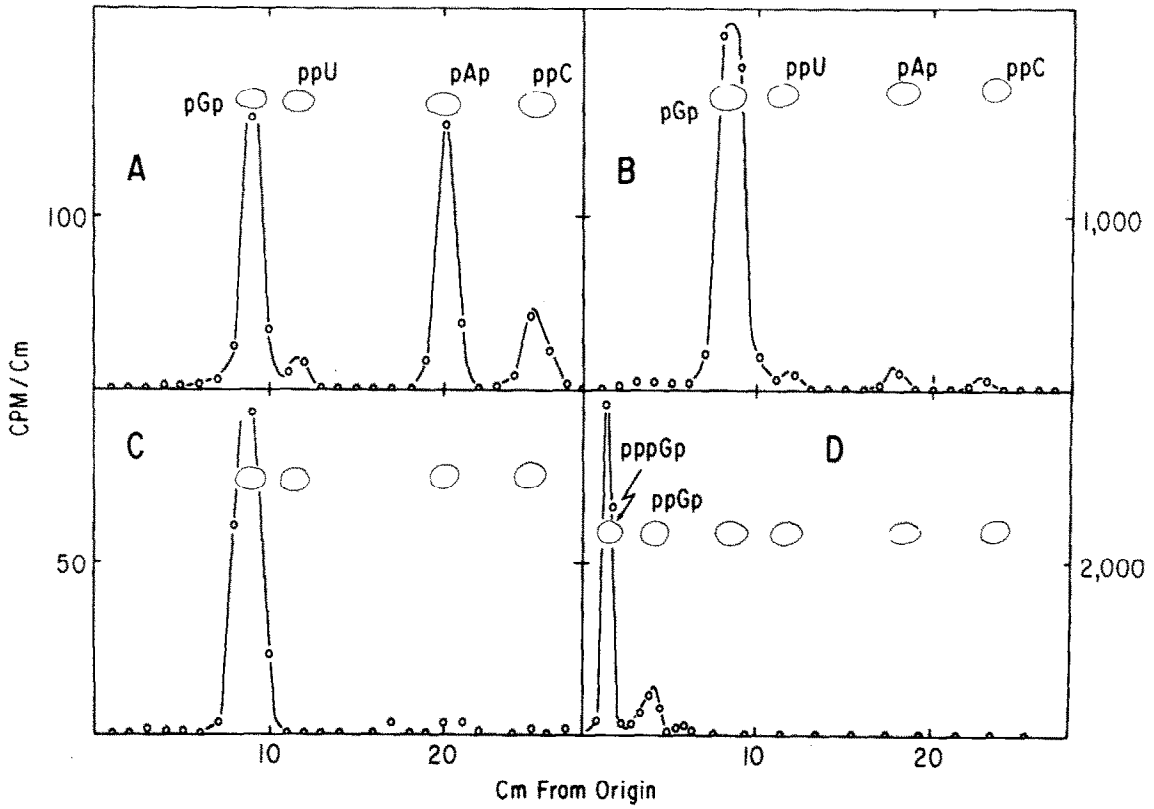

Fig.2. Separation of termini of mitochondrial and cytoplasmic low molecular weight RNA fractions by DEAE ionophoresis at pH 1.9. The nucleotides in the fractions indicated by the bars in fig.1 were recovered, portions were subjected to DEAE ionophoresis (pH 1.9; 5 h; 1000 V/37 cm) [2/3 of mitochondrial tRNA pool (600 cpm in ^{32}P); 2/3 of mitochondrial 3 S_E RNA pool (160 cpm); 1/3 of the cytoplasmic tRNA pool (4700 cpm); and 1/2 of the 5 S RNA pool (3100 cpm)], and segments were assayed in a scintillation counter. The ovals represent 260 nm absorbing spots from added marker compounds; in other runs, ^{14}C-labeled pUp and pCp (of section 2) were found to run with ppU and ppC, respectively, except for minor ^{14}C-labeled peaks running slightly faster in each case; these presumably are 2′-phosphate isomers. The panel designations correspond to fig.1.

table 1. Cytoplasmic tRNA yielded predominantly (86%) pGp termini, and 5 S RNA yielded peaks migrating with ppGp plus pppGp; these latter identities were confirmed as described in [11]. Mitochondrial tRNA in contrast yielded ~40% each of pGp and pAp, 13% pCp and 7% pUp; the relative enrichment for pCp and pUp explains the asymmetry of the DEAE column peak. Mitochondrial 3 S_E RNA yielded only pGp as its terminal nucleotide.

Apparent chain lengths as estimated from fig.1,2 and table 1 are summarized in table 2. The mean length of mitochondrial tRNA, averaged from 4 experiments, was 75 nucleotides, compared to 83 for cytoplasmic tRNA. Correcting the mitochondrial value for 5% cytoplasmic tRNA lowers it to 74. The 5 S RNA value is close to that of other mammalian 5 S RNA, 121 [12], lending confidence to the accuracy of our procedure. The 3 S_E RNA result, 66 nucleotides, is in general accord with its gel behavior [1,2].

4. Discussion

Earlier studies on 'conventional' (bacterial or cytoplasmic) mixed tRNA [13,14] revealed a preponderance of 5′-terminal G, as have sequence analyses of many individual species [15]. Thus our cytoplasmic results are as expected for a conventional tRNA population. The majority of fungal mitochondrial elongator tRNA for which 5′-termini are known also terminate in G (yeast tRNA corresponding to serine, leucine, histidine, phenylalanine, and cystine [16–18], and *N. crassa* alanine and valine [U. L.

Table 1
5′-Termini of mitochondrial and cytoplasmic low molecular weight RNA fractions

	Transfer RNA			
	Mitochondrial	Cytoplasmic	3 S_E RNA	5 S RNA
pGp	40.8 ± 3.5[a]	86.0 ± 0.8[a]	88.6, 89.0	–
pUp	7.2 ± 2.2	5.5 ± 1.3	–	–
pAp	37.0 ± 1.1	4.2 ± 0.9	–	–
pCp	12.9 ± 1.1	1.9 ± 0.4	–	–
ppGp	–	–	–	14
pppGp	–	–	–	85

[a] Standard deviation

The results are expressed as % of total ^{32}P counts recovered in DEAE paper runs such as those of fig.2. The tRNA values represent 6 (mitochondrial) or 4 (cytoplasmic) separate determinations for the preparations described in fig.1, plus those from a second similar experiment. Values from alkaline and T2 ribonuclease digests were not significantly different (cf. table 2 and text) and have been pooled. The 5 S RNA values are from the pattern of fig.2D, and the 3 S_E RNA values from that of fig.2B plus a duplicate analysis from the same experiment

RajBhandry, personal communication]). The heterogeneous nature of our mitochondrial pattern thus may constitute a distinctive feature of animal mitochondrial tRNA. Appreciation of this may help in interpreting animal mitochondrial tRNA-containing DNA sequence that may be generated (cf. [17]).

Our finding of 20% 5′-terminal pyrimidines in mitochondrial tRNA suggests the occurrence of post-transcriptional processing. RNA polymerases in general (e.g., [19–21]) and a mitochondrial polymerase in particular [22] highly prefer purine triphosphates for initiation of transcription. Although pulse labeling studies failed to demonstrate discrete precursors to mitochondrial tRNA [1], there is in fact evidence that all mitochondrial RNA species arise via processing of very large transcripts [23].

Finding a single species of 5′-terminal nucleotide for mitochondrial 3 S_E RNA is in accord with our earlier impression [1,4] that the RNA is a single species, and the fact that the terminus is G is in general accord with the idea that 3 S_E RNA is a mitochondrial equivalent to eukaryotic 5 S rRNA [12].

The value for average chain length of mitochondrial tRNA, 74, is the shortest that can be accomodated by a standard version of generalized tRNA structure [15]. Thus, insofar as our figure is applicable, animal mitochondrial tRNA would be just long enough to fold into a standard cloverleaf but would be deficient in extra nucleotides in their D-loops and would lack variable arms. *N. crassa* mitochondrial $tRNA^{Tyr}$ is 90 nucleotides long [24], and yeast mitochondrial $tRNA^{Leu}$ migrates in urea gels as relatively long tRNA would [16]. On the other hand, gel analysis of rodent mitochondrial tRNA suggest that $tRNA^{Leu}$ species from this source are relatively short (71–74 nucleotides) [25,26]. We propose that the uniform absence of variable arms is peculiar to animal mitochondrial tRNA. If this is confirmed by sequence analysis it will contribute to the evidence (see, e.g., [27]) that fungal and animal mitochondria, and their tRNA, have arisen via separate evolutionary events, or have diverged remarkably since their evolutionary origin.

Table 2
Chain lengths of mitochondrial and cytoplasmic low molecular weight RNA fractions

Expt.	Transfer RNA				3 S_E RNA		5 S RNA	
	Mitochondrial		Cytoplasmic		T2	alkali	T2	alkali
	T2	alkali	T2	alkali				
1	75	–	81	–	69	–	119	–
2	75	79	84	83	–	–	124	–
3	73	–	85	86	–	–	120	–
4	–	74	–	81	–	63	–	138
Av.	75.2 ± 2.0[a]		83.3 ± 1.9[a]		66		125 ± 8[a]	

[a] Standard deviation

Values are expressed as total nucleotides per 'average' chain in each preparation. Expt. 1,2 were those described in table 1; expt. 3,4 were similar. The last line indicates the overall averages of all results, combining T2 and alkaline digest values

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