THE NUCLEOTIDE SEQUENCE OF TURTLE 5.8 S rRNA

Ross N. NAZAR
Division of Biological Sciences, National Research Council of Canada, Ottawa K1A 0R6

and

Kenneth L. ROY
Department of Microbiology, University of Alberta, Edmonton, Alberta T6G 2E9, Canada

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1. Introduction

The 5.8 S ribosomal RNA's of human, rat and mouse cells have an essentially identical nucleotide sequence with minor differences due to terminal heterogeneity [1,2]. This sequence is 75% homologous with that of yeast 5.8 S rRNA [3,4] but appears to be considerably less homologous with the analogous RNA from flowering plants [4,5]. In order to further examine the extent of homology among other eukaryotes and to detect evolutionary drifts, we have studied the 5.8 S RNA of turtle (*Terrapene carolina*) heart cells and compared it to the presently known sequences. The results indicate that the nucleotide sequence of turtle 5.8 S rRNA differs from human cell RNA in only two respects: a single base substitution and a posttranscriptional modification.

2. Materials and methods

Turtle heart cells (CCL 50) were incubated for 48–60 h in phosphate-free Basal medium (Eagle) (with 10% dialyzed fetal bovine serum) containing 3–15 mCi of carrier free [^{32}P] orthophosphate.

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RNA was extracted from whole cells with equal volumes of liquified phenol/hydroxyquinoline (88% : 0.1%) and buffer (0.1 M NaCl, 0.02 M Tris–HCl (pH 7.8), 0.04 M Na_2EDTA, and 0.5% sodium dodecyl sulfate) [6]. 5.8 S rRNA was purified by electrophoresis on 12.5% polyacrylamide gel slabs and repurified on 20% slabs [7]. The RNA was recovered from the gel by homogenization in water followed by high-speed centrifugation and filtration through a Millipore (Millipore Corp., Bedford, Mass.) 0.45 μm filter [1].

Purified 5.8 S rRNA was digested completely by pancreatic or T_1 ribonuclease as previously described [1,8]. The complete digests were separated by two-dimensional electrophoresis on cellulose acetate, pH 3.5, and DEAE paper in 7% formic acid [8]. The larger pancreatic or T_1 oligonucleotides were further characterized by complete digestion with T_1 or pancreatic ribonuclease and subsequent analysis by one-dimensional electrophoresis on DEAE paper at pH 1.9 [8].

Oligonucleotides were analyzed for pseudouridylic acid by alkaline digestion followed by electrophoresis on Whatman 3MM paper and ascending chromatography on microcrystalline cellulose (Polygram CEL 400) thin-layers (Macherey-Nagel Co., Germany) in propan-2-ol (680 ml)/HCl (176 ml)/H_2O (to 1 litre) [1,3,8].

3. Results and discussion

Figure 1 shows autoradiographs of complete pancreatic and T_1 ribonuclease fingerprints for turtle 5.8 S rRNA. The pancreatic ribonuclease digestion map (left) contained 22 major spots; 21 were comparable in electrophoretic mobility to those of mammalian 5.8 S rRNA [1,2] with one new component (A–A–Cp). As reported for the Novikoff hepatoma [1], two spots representing methylated oligonucleotides were present; Gm–Cp, present as a satellite spot (P6′) to G–Cp (P6) and P23 corresponding to the undermethylated G–G–Um–G–G–A–Up sequence. Only 30% of the molecules contained this sequence; the unmethylated equivalents, G–G–Up and G–G–A–Up, were present as two spots, P18 and P19 (fig.1). The 5′-terminal was present as a minor spot (pCp) and no radioactive spots corresponding to the 3′-end were observed consistent with a pyrimidine nucleoside at the 3′-terminal. The T_1 ribonuclease digestion map (right) was also identical with that of Novikoff hepatoma 5.8 S rRNA except for one spot. $C_2A_2U_3G$ was absent and a new component was present $pC_3A_3U_3G$, which migrated with A_4U_3G. Um–Gp was present as a satellite spot of U–Gp and the second alkali stable dinucleotide, Gm–Cp, was present as A–A–U–U–Gm–C–A–Gp. As previously reported

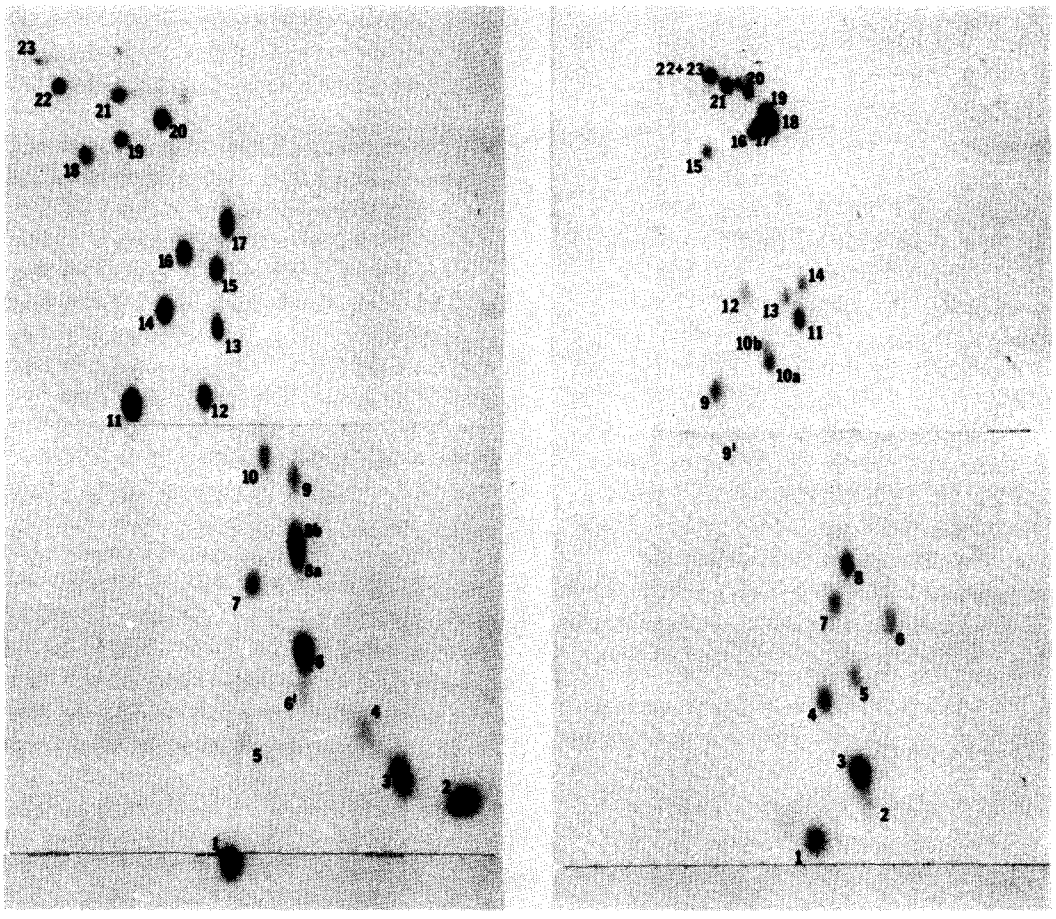

Fig.1. Autoradiographs of two-dimensional fractionations of pancreatic (left) and T_1 (right) ribonuclease digests of ^{32}P-labeled turtle heart cell 5.8 S rRNA. Electrophoresis was from right to left on cellulose-acetate, at pH 3.5, and from top to bottom on DEAE paper, in 7% formic acid. The numbers correspond to those in oligonucleotides identified in tables 1 and 2.

in human 5.8 S rRNA [2,9] the 3′-end, C–U–U, was present as a minor satellite to C–Gp.

The distribution of the oligonucleotides and their sequences or compositions, as determined by secondary digestions, are tabulated and compared to mammalian 5.8 S rRNA in tables 1 and 2. Table 1 indicates that the distribution of oligonucleotides after T_1 ribonuclease digestion was identical to that of mammalian RNA with the exception of spot T23. In turtle heart, a single substitution of A for G at residue 2, increases the length of the analogous fragment in mammalian cells (A–C–U–C–U–U–A–Gp) to include the 5′-end, pC–A–A–C–U–C–U–U–A–Gp (T23). Further sequence analysis of the longer T_1 oligonucleotide fragments by digestion with pancreatic ribonuclease (table 1) showed the products to be identical to those of Novikoff hepatoma 5.8 S rRNA [1]. Both the pancreatic and U_2 ribonuclease digestion products

Table 1
Analysis of oligonucleotides obtained by complete T_1 ribonuclease digestion

Spot	Nucleotide composition	Molar yield	Pancreatic RNAase digestion products	Sequence[a]	Molar yield[b] in mammalian 5.8 S rRNA
1	Gp	10.0 (10)		Gp	10
2	CGp	6.8 (6)		C–Gp	6
3	CUU	1.2 (1)		C–U–U	1
4	AGp	2.7 (2)		A–Gp	2
5	CAGp	1.3 (1)	AG,C	C–A–Gp	1
6	C_4Gp	0.8 (1)		C–C–C–C–Gp	1
7	A_2Gp	1.3 (1)		A–A–Gp	1
8	CA_2Gp	1.6 (2)	A_2C,G	A–A–C–Gp	2
9	UGp	3.3 (2.7)		U–Gp	2.8
9′	UmGp	0.3 (0.3)		Um–Gp	0.2
10a	CUGp	2.4 (2)		U–C–Gp	2
10b	CUGp	1.1 (1)		C–U–Gp	1
11a	C_2UGp	1.0 (1)		C–U–C–Gp	1
11b	C_2UGp	1.0 (1)		C–C–U–Gp	1
12	CAUGp	1.0 (1)	AG,C,U	C–U–A–Gp	1
13	AUGp	1.3 (1)	AU,G	A–U–Gp	1
14	C_2AUGp	0.9 (1)	AC,G,C,U	C–U–A–C–Gp	1
15	CU_2Gp	1.2 (1)		U–C–U–Gp	1
16	C_2AU_2Gp	0.8 (1)	AC,G,C,$\underline{U}$	C–A–C–U–U–Gp	1
17	$C_2A_2U_2Gp$	0.7 (1)	$\underline{AU}$,G,$\underline{C}$	A–U–C–A–U–C–Gp	1
18a	$C_3A_2U_2Gp$	0.9 (1)	$\underline{AU}$,$\underline{AC}$,G,$\underline{C}$,U	A–U–C–A–C–U–C–Gp	1
18b	C_3AU_2Gp	0.9 (1)	$\underline{AC}$,G,C,$\underline{U}$	A–C–A–C–U–U–C–Gp	1
19	$C_2A_2U_3Gp$	0.7 (1)	$\underline{AU}$,AC,G,U	A–C–A–C–A–U–U–Gp	1
20	C_5U_3Gp	1.0 (1)		U–U–C–C–U–C–C–C–Gp	1
21	A_3U_2GmCGp	1.3 (1)	A_2U,AG,GmC,U	A–A–U–U–Gm–C–A–Gp	1
22	A_4U_3Gp	0.8 (1)	$\underline{A_2U}$,G,U	A–A–U–U–A–A–U–Gp	1
23	$pC_2A_3U_3Gp$	0.8 (1)	AAC,AG,pCp,C,$\underline{\underline{U}}$	pC–A–A–C–U–C–U–U–A–G^c_p	1[d]

[a] These sequences are based on the nucleotide compositions, the pancreatic ribonuclease digestion products and on analogous fragments in mammalian 5.8 S rRNA

[b] These are predicted molar yields for mammalian 5.8 S rRNA [1,2]

[c] The products of further U_2 digestion were C_2U_3Ap, pCAp, Gp and Ap

[d] This is the molar yield for the analogous fragment, A–C–U–C–U–U–A–Gp

The T_1 ribonuclease digests were fractionated by two-dimensional electrophoresis (fig.1), the radioactivity was determined and each spot was eluted and identified by its nucleotide composition and by further digestion with pancreatic ribonuclease. The experimental molar yields are averages of two determinations based on a 158 nucleotide chain length. The predicted yields are noted in parentheses. The notation for the relative molar yields of the products is: no underline, one underline and two underlines for molar yields of 1, 2 and 3, respectively.

(table 1) of spot T23 (fig.1) were consistent with a simple elongation in the mammalian A–C–U–C–U–U–A–Gp sequence by a single substitution of A for G. Further analysis for pseudouridylic acid in U–Gp (spot T8) and C–U–Gp (spot T9a) showed 1 mol of pseudouridylic acid in U–Gp (fig.2) as found in mammalian 5.8 S rRNA [1,2] but not the partially modified C–(Ψ)–Gp sequence observed in mammalian cells [1,2].

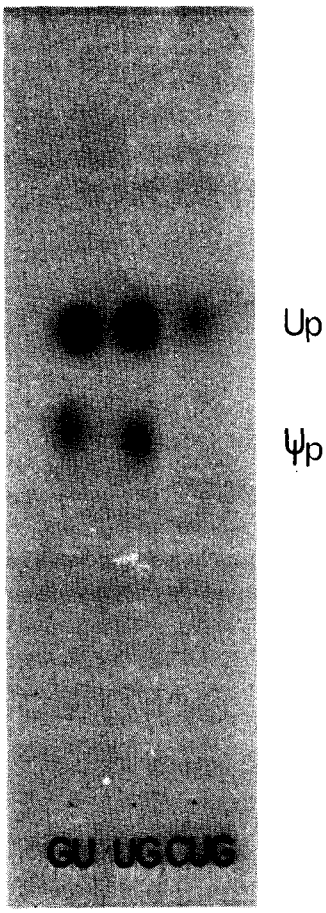

Fig.2. Analysis of oligonucleotides containing pseudouridylic acid. ^{32}P-labeled turtle heart cell 5.8 S rRNA was digested with pancreatic or T_1 ribonuclease and fractionated as described in fig.1. The oligonucleotides were eluted with 30% TEA–carbonate, digested with 0.3 N NaOH for 18 h at 37°C and the nucleotides were fractionated by electrophoresis on Whatman 3 MM paper at pH 3.5. Spots corresponding to uridylic acid were eluted with water and further fractionated by chromatography on cellulose thin-layers with propan-2-ol–HCl–water [8].

Table 2 indicates that the distribution of oligonucleotides after pancreatic ribonuclease digestion was also identical to mammalian RNA with the exception of one new spot, A–A–Cp, and one less G–A–Cp sequence. Again, in turtle heart, the new spot is consistent with a single substitution of A for G at residue 2 which gives a pC–A–A–Cp 5′-terminal end and results in the loss of a G–A–Cp oligonucleotide in the mammalian sequence. Further analysis for pseudouridylic acid in G–Up (spot P9) indicated one mole of pseudouridylic acid (fig.2) as found in mammalian 5.8 S rRNA [2].

Since, with the exception of the single substitution at residue 2, the observed molar yields and the products of complete, and secondary digestion by pancreatic or T_1 ribonuclease of turtle 5.8 S rRNA are identical to those of mammalian RNA, the primary nucleotide sequences of these molecules must be identical, assuming no minor sequence displacements. Such displacements must be highly specific and are, therefore, improbable. With the single substitution at residue 2 the sequence homology between yeast and turtle 5.8 S rRNA is slightly higher than between yeast and man; the 5-terminal sequence of yeast is pA–A–A–C [3] rather than pC–G–A–C in man [2]. The substitution also reduces the stability of the 3′- to 5′-terminal interaction which has been proposed in the 'burp gun' model [1]. This would be consistent with an increased RNA stability during the evolution of higher eukaryotes [1]. Because of the very limited difference this RNA sequence is not useful in further consideration of a generalized model for 5.8 S rRNA and other more diverse species are being examined.

In addition to a substitution in the primary sequence, turtle 5.8 S rRNA contains one less modified nucleotide. As has been found in many 5.8 S RNA species [1,5,10], this molecule contains one mole of 2′-*O*-methyl guanosine in the T_1 ribonuclease derived octanucleotide A–A–U–U–Gm–C–A–Gp and a partially methylated Um–Gp dinucleotide. About half the molecules contain 2′-*O*-methyl uridine, similar to that found in normal chick or mouse cells [11]. However, while this 5.8 S rRNA species also contains one mole of Ψ–Gp, unlike the mammalian cells [2], turtle cell 5.8 S rRNA does not contain a partially modified C-(Ψ)-Gp sequence. As the role of these modifications

Table 2
Analysis of oligonucleotides obtained by complete pancreatic ribonuclease digestion

Spot	Nucleotide composition	Molar yield	Sequence[a]	Molar yield[b] in mammalian 5.8 S rRNA
1	Up	15.5 (16)	Up	16
2	Cp	17.4 (18)	Cp	18
3	ACp	4.8 (5)	A–Cp	5
4	A_2Cp	0.9 (1)	A–A–Cp	0
5	pCp	0.7 (1)	pCp	0.4–1
6	GCp	6.6 (7)	G–Cp	7
6	GmCp	1.0 (1)	Gm–Cp	1
7	AUp	2.4 (2)	A–Up	2
8a	AGCp	1.1 (1)	G–A–Cp	2
8b	AGCp	3.2 (3)	A–G–Cp	3
9	A_2GCp	1.1 (1)	G–A–A–Cp	1
10	A_2Up	1.4 (1)	A–A–Up	1
11	GUp	5.3 (5)	G–Up	5
12	G_2Cp	1.8 (2)	G–G–Cp	2
13	AG_2Cp	1.2 (1)	G–A–G–Cp	1
14	AGUp	2.4 (2)	G–A–Up	2
15	A_2G_2Cp	1.1 (1)	A–G–G–A–Cp[c]	1
16	A_2GUp	1.3 (1)	G–A–A–Up	1
17	A_4G_2Cp	0.9 (1)	G–A–A–G–A–A–Cp[c]	1
18	G_2Up	0.7 (0.7)	G–G–Up	0.3–0.8
19	AG_2Up	0.7 (0.7)	G–G–A–Up	0.3–0.8
20	A_3G_2Up	1.2 (1)	G–A–G–A–A–Up[c]	1
21	G_4Cp	0.8 (1)	G–G–G–Cp	1
22	G_3Up	0.8 (1)	G–G–G–Up	1
23	AG_3UmGUp	0.3 (0.3)	G–G–Um–G–G–A–Up	0.8–0.3

[a] These sequences are based on the nucleotide compositions, electrophoretic mobilities, and on analogous fragments in mammalian 5.8 S rRNA.

[b] These are predicted molar yields for mammalian 5.8 S rRNA [1,2].

[c] These sequences were confirmed by further digestion with T_1 ribonuclease.

The pancreatic digests were fractionated by two-dimensional electrophoresis (fig.1), the radioactivity was determined and each spot was eulted and identified by its nucleotide composition. The experimental molar yields are averages of two determinations based on a 158 nucleotide chain length. The predicted yields are noted in parentheses.

is presently unknown the absence of this modification is not understood. Nevertheless, this molecule could provide a useful unmodified substrate for studying the conversion of C–U–Gp to C–Ψ–Gp in mammalian cells.

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