Sequence Homologies in Mammalian 5.8S Ribosomal RNA†

Ross N. Nazar,* Thomas O. Sitz, and Harris Busch

ABSTRACT: Oligonucleotide products of complete pancreatic or T_1 RNase digestion or partial T_1 RNase digestion of HeLa cell (human) and MPC-11 cell (mouse) 5.8S rRNA are identical with those obtained from Novikoff hepatoma (rat) 5.8S rRNA except for minor differences at the termini. pCp is the only major 5′ terminus of both human and mouse RNAs; both pGp and pCp 5′ termini were found in rat 5.8S RNA. Furthermore, HeLa cells contain C-U-U at the 3′ end rather than the C-U terminus of mouse and rat. The results indicate that the nucleotide sequence has been highly conserved during the evolution of mammals and suggest that, as reported for 5S rRNA, this sequence is essentially constant throughout the Mammalia.

The cytoplasmic ribosome of eukaryotic cells contains two low molecular weight RNA species, 5S and 5.8S rRNAs (Forget and Weissman, 1967a; Pene et al., 1968; Weinberg and Penman, 1968). 5S rRNAs from human, rabbit, rat, mouse, and marsupial cells have an identical sequence (Forget and Weissman, 1967b; Williamson and Brownlee, 1969; Labrie and Sanger, 1969; Averner and Pace, 1972) which differs in only eight positions from the sequence in the South African toad (Brownlee and Cartwright, 1972) and is approximately 60% homologous with yeast 5S rRNA (Hindley and Page, 1972). Although the data are limited, there appear to be similar homologies in the 5.8S rRNAs. The nucleotide sequence seems to be highly conserved during the evolution of flowering plants (Woledge et al., 1974) and 5.8S rRNA from the Novikoff ascites hepatoma is 75% homologous with the molecule in yeast (Nazar et al., 1975).

To determine the extent of homology among mammalian 5.8S rRNAs and to detect evolutionary drifts, we have analyzed 5.8S RNA from mouse and human cells and compared these to rat. The total primary sequences of myeloma MPC-11 and HeLa cell 5.8S rRNA have been derived by arranging the end products of complete pancreatic and complete or partial T_1 RNase digestion along the sequence of Novikoff hepatoma RNA (Nazar et al., 1975).

Materials and Methods

HeLa cells or mouse myeloma MPC-11 cells were incubated for 24 h in low phosphate (50 μM) modified Eagle's medium (with 5% dialyzed calf serum) or phosphate-free Dulbecco's modified Eagle's medium (with 10% horse serum), respectively, containing 5–25 mCi of [^{32}P]orthophosphate. RNA was prepared from whole cells by suspending the cell pellet in 50 ml of 0.3% (w/v) sodium dodecyl sulfate–0.14 M NaCl–0.05 M sodium acetate (pH 5.1) and extracted with 50 ml of phenol solution (Quagliarotti et al., 1970) at 65 °C. 5.8S rRNA was purified by electrophoresis on 8% polyacrylamide gel slabs (Nazar et al., 1975) and recovered from the gel by homogenization in water followed by high-speed centrifugation (30 000g, 1 h) and filtration through a Millipore (Millipore Corp., Bedford, Mass.) filter (0.45 μ).

Purified 5.8S rRNA (specific activity, 10^5 dpm/μg) was precipitated at −20 °C with 2 volumes of ethanol (2% potassium acetate) and digested completely by pancreatic or T_1 ribonuclease or partially with T_1 ribonuclease as previously described (Nazar et al., 1975a,b). The complete digests were separated by two-dimensional electrophoresis on cellulose acetate at pH 3.5 and DEAE paper in 7% formic acid (Sanger and Brownlee, 1967) and partial digests were separated by two-dimensional polyacrylamide gel electrophoresis (DeWachter and Fiers, 1972). The longer T_1 oligonucleotides were further characterized by complete digestion with pancreatic ribonuclease and subsequent analysis by one-dimensional electrophoresis on DEAE paper at pH 1.9 (Sanger and Brownlee, 1967). Fragments of partial digestion were further characterized by complete digestion with T_1 ribonuclease and subsequent analysis by one-dimensional electrophoresis on DEAE paper in 7% formic acid.

Oligonucleotides were analyzed for pseudouridylic acid by alkaline digestion followed by electrophoresis on Whatman 3 MM paper and ascending chromatography on cellulose thin layers (Eastman Kodak Co., Rochester, N.Y.) in propan-2-ol (680 ml)–HCl (176 ml)–H_2O (to 1 l.).

Results and Discussion

Figures 1 and 2 show autoradiographs of complete pancreatic and T_1 ribonuclease fingerprints for mouse, rat, and human 5.8S rRNA. In each case the pancreatic ribonuclease digestion map (Figure 1) contained 21 major spots of comparable electrophoretic mobility. Gm-Cp was present as a satellite spot to G-Cp (spot 4) and a spot corresponding to the undermethylated G-G-Um-G-G-A-Up fragment (spot 21) was obtained in each digest (Nazar et al., 1974). The unmethylated equivalents, G-G-Up and G-G-A-Up, appear as spots 16 and 17, respectively. The T_1 ribonuclease digestion fingerprints (Figure 2) were also identical with respect to the 22 major spots previously reported in Novikoff hepatoma 5.8S rRNA. Um-Gp was present as a satellite spot to U-Gp and the second alkali stable dinucleotide Gm-Cp was present as A-A-U-U-Gm-C-A-Gp in each case. Both hepatoma and HeLa cell 5.8S rRNAs contained one ψ-Gp fragment and a partially modified C-(ψ)-Gp fragment and se-

† From the Tumor By-Products Laboratory, Department of Pharmacology, Baylor College of Medicine, Houston, Texas 77025. *Received July 28, 1975.* These studies were supported by the Cancer Center Research Grant CA-10893,P.1 from the National Cancer Institute.

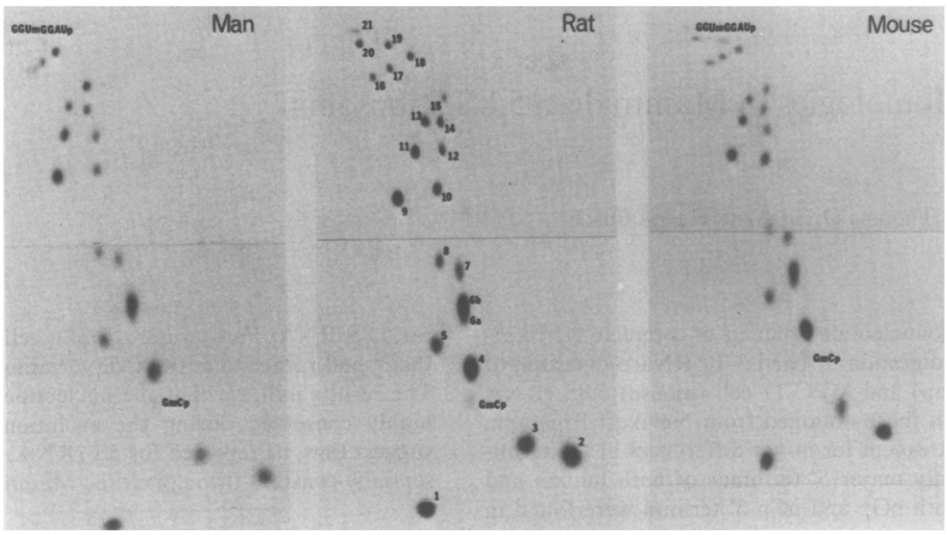

FIGURE 1: Autoradiographs for two-dimensional fractionations of pancreatic RNase digests of ^{32}P-labeled HeLa (left), Novikoff hepatoma (center), and MPC-11 (right) cell 5.8S rRNA. Electrophoresis was from right to left on cellulose acetate at pH 3.5 and from top to bottom on DEAE paper in 7% formic acid.

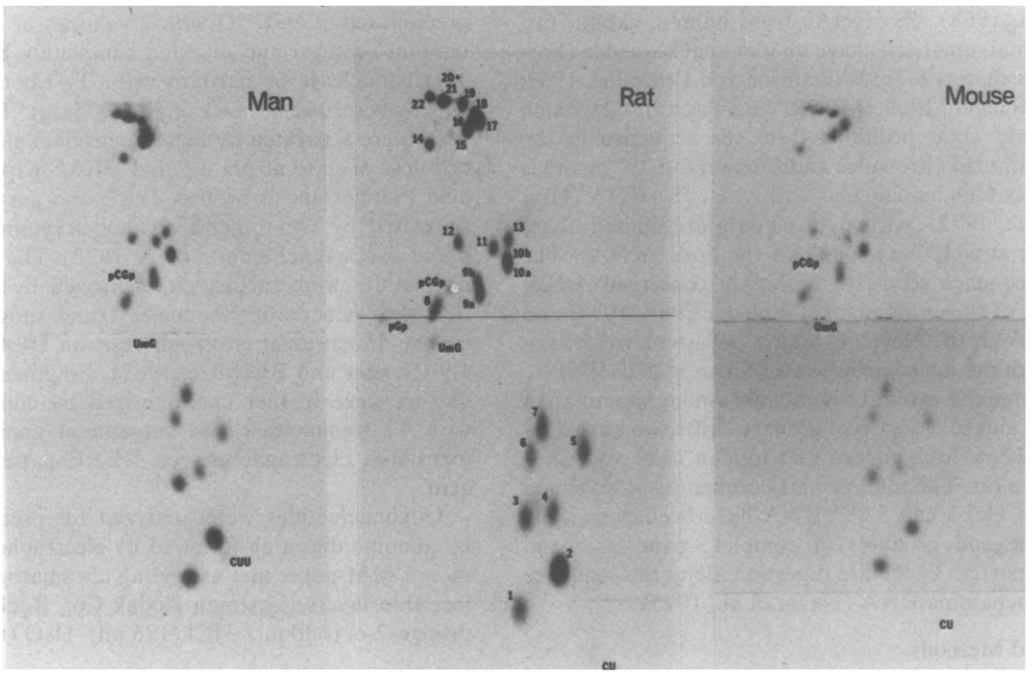

FIGURE 2: Autoradiographs for two-dimensional fractionations of T_1 RNase digests of ^{32}P-labeled HeLa (left), Novikoff hepatoma (center), and MPC-11 (right) cell 5.8S rRNA. Electrophoresis was from right to left on cellulose acetate at pH 3.5 and from top to bottom on DEAE paper in 7% formic acid.

quence analysis of the longer T_1 oligonucleotides showed them to be identical (Nazar et al., 1975a,b; Maden and Robertson, 1974). Further analysis of mouse 5.8S rRNA indicated that the modified nucleotides were also present in this RNA. Figure 3 shows 1 mol of pseudouridylic acid in U-Gp (spot 8) and about 0.5 mol in C-(ψ)-Gp (spot 9a), but none was found in the adjacent spot 9a (U-C-Gp). The longer T_1 oligonucleotides were further digested with pancreatic ribonuclease and their products (Table I) were found to be identical with those observed in the hepatoma and HeLa cell 5.8S rRNAs.

Some differences were found in the minor spots after T_1 RNase digestion corresponding to differences in the termini. In Figure 2, Novikoff hepatoma 5.8S rRNA contains two major termini, pGp and pC-Gp (Nazar et al., 1974). In contrast, both mouse and human cell RNA contain pC-Gp as the major 5′ termini and no significant amounts of pGp were detected. Furthermore, the 3′ terminus of HeLa cell 5.8S rRNA is C-U-U (Maden and Robertson, 1974) rather than C-U which was found in the Novikoff hepatoma and myeloma MPC-11 cells. C-U has also been reported to be the 3′ terminus of rabbit reticulocyte and BHK (hamster) cell 5.8S rRNA (Shine et al., 1974).

Figure 4 shows autoradiographs for two-dimensional polyacrylamide gel fractionations of limited T_1 RNase digest of human, rat, and mouse 5.8S rRNAs. The major

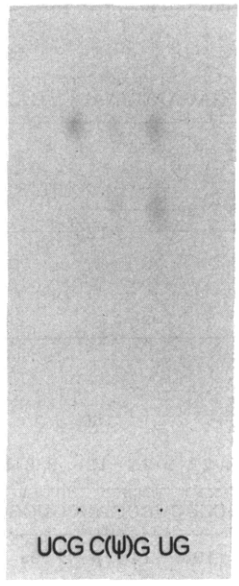

FIGURE 3: Analysis of oligonucleotides containing pseudouridylic acid. ^{32}P-labeled MPC-11 5.8S rRNA was digested with T_1 RNase and fractionated as described in Figure 2. The approximate positions of the origins and the solvent fronts are given in the margin. The positions of the nucleotides were identified with markers under uv light.

Table I: Analysis of Unique T_1 RNase Oligonucleotide Products.[a]

Spot	Nucleotide Composition	Pancreatic RNase Digestion Products: Novikoff Hepatoma	Pancreatic RNase Digestion Products: MPC-11
T13	C_2AUG	AC,C,U,G	AC,C,U,G
T15	C_2AU_2G	AC,U,C,G	AC,U,C,G
T16	$C_2A_2U_2G$	AU,C,G	AU,C,G
T17a	$C_3A_2U_2G$	AU,G,AC,C,U	AU,G,AC,C,U
T17b	$C_3A_2U_2G$	G,AC,C,U	G,AC,C,U
T18	$C_2A_3U_2G$	G,AU,AC,U	G,AU,AC,U
T19	C_5U_3G	$G,C_{(5)},U$	$G,C_{(5)},U$
T20	A_3U_2GmCG	A_2U,AG,GmC,U	A_2U,AG,GmC,U
T21	$C_2A_2U_3G$	AG, AC,C,U	AG,AC,C,U
T22	A_4U_3G	A_2U,G,U	A_2U,G,U

[a] The T_1 RNase digests were fractionated by two-dimensional electrophoresis (Figure 2) and further analyzed by complete pancreatic digestion (Nazar et al., 1975a,b). The notation for the relative molar yields of the products is: no underline, one underline, two underlines, and the subscript 5 for molar yields of 1, 2, 3, and 5, respectively.

fragments which had previously been reported for Novikoff hepatoma RNA (Nazar et al., 1975a,b) were also present in both HeLa and MPC-11 cell 5.8S RNA. Particularly obvious is the upper ridge of spots beginning with PT 25, PT 29, PT 31, etc., which are characteristic of the very stable GC-rich hairpin loop in 5.8S rRNA (Nazar et al., 1975a,b). Furthermore, subsequent analysis by complete digestion with T_1 RNase indicated that each fragment contained the same oligonucleotides as had been found in rat.

Based upon the pancreatic and T_1 RNase digestion "fingerprints" and upon the products of limited T_1 RNase digestion, the primary nucleotide sequences of human, rat, and mouse ribosomal 5.8S RNAs are highly conserved and probably identical with minor exceptions at the termini (Figure 5). Apparently the structures of both low molecular weight rRNAs of the Mammalia are essentially conserved.

The differences at the termini are interesting but not understood. Two 3′ termini, C-U-U and C-U-U-U, have also been reported in 5S rRNAs and the 3′ end of yeast 5.8S rRNA is U-C-A-U-U-U (Rubin, 1973). It appears that both 5S and 5.8S rRNAs may terminate in a poly(U) sequence within their precursor molecules which may act as a specific site for the processing enzyme. Since the termini are homogeneous within each species, the cleavage is stringent and cell specific. Processing at the 5′ end, however, was somewhat variable in the Novikoff hepatoma but is quite specific in mouse and HeLa cells. Therefore, although there is little variation in the rRNAs, there may be differences in processing mechanisms that give rise to the mature RNA species.

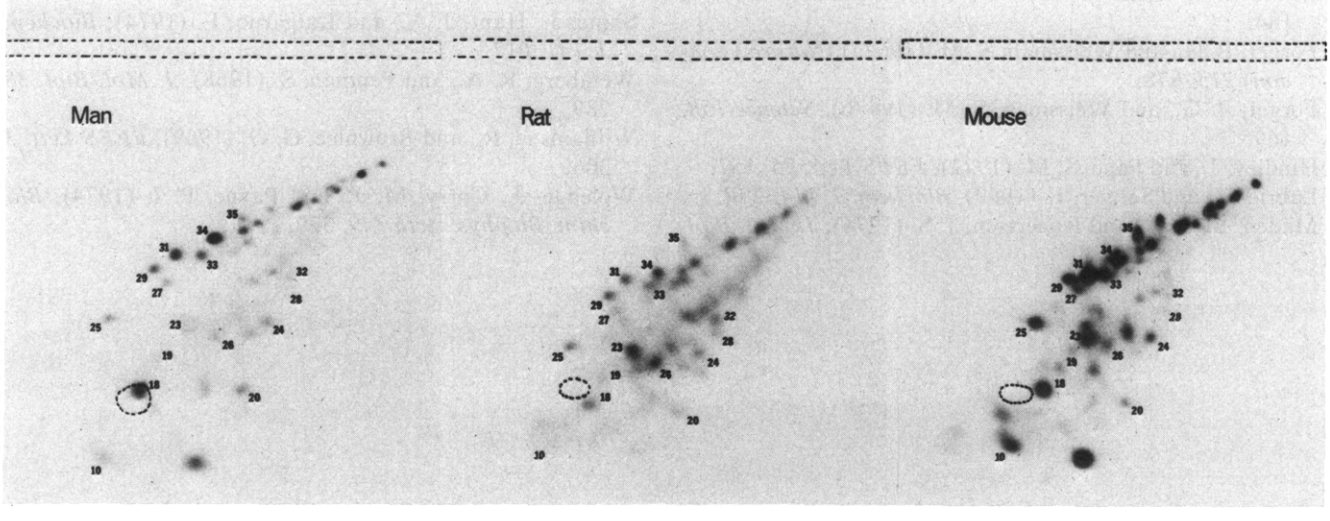

FIGURE 4: Autoradiographs for two-dimensional polyacrylamide gel fractionations of limited T_1 RNase digests of ^{32}P-labeled HeLa (left), Novikoff hepatoma (center), and MPC-11 (right) cell 5.8S rRNA. Electrophoresis was from right to left in a 16% gel slab at pH 3.3 and from top to bottom in a 20% gel slab at pH 7.2. The positions of the first dimension gel strip and the bromophenol blue dye marker are identified by the broken rectangle and circle, respectively.

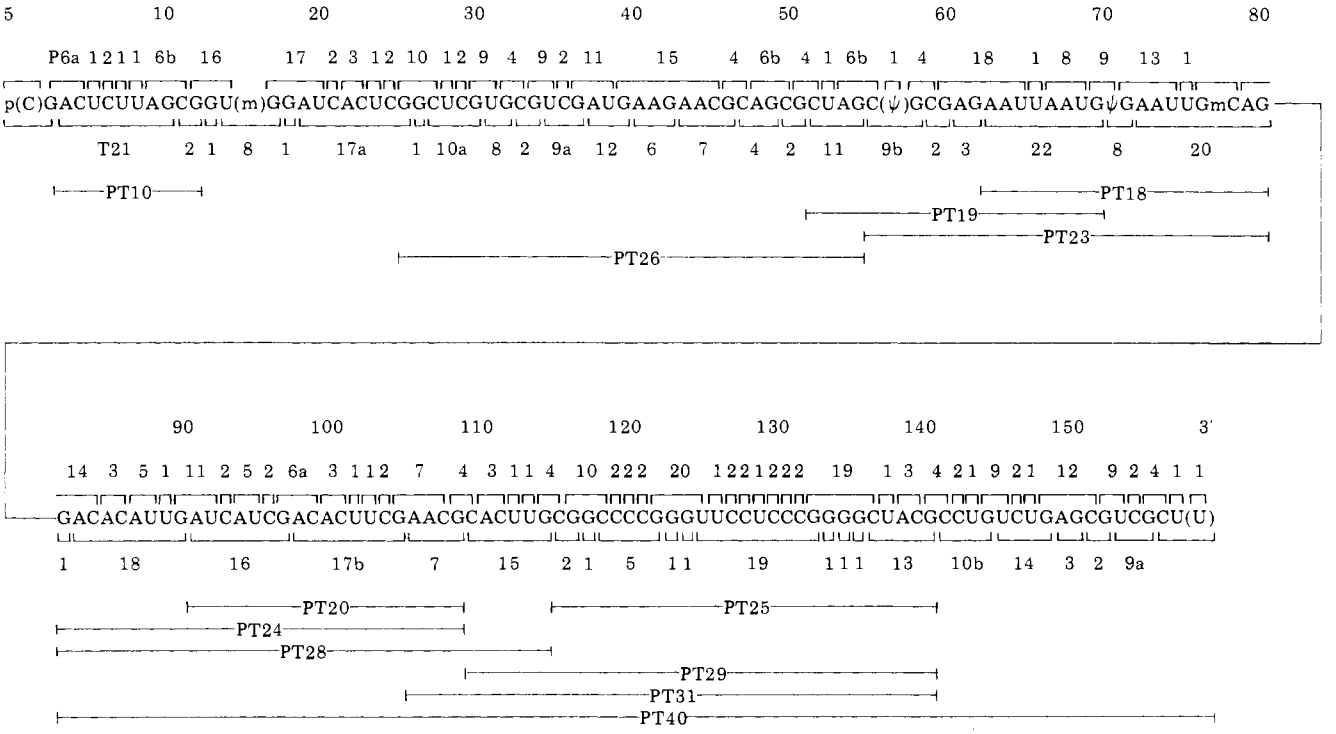

FIGURE 5: The primary nucleotide sequence of HeLa, Novikoff hepatoma, and MPC-11 cell 5.8S rRNA. Products of complete pancreatic RNase digestion are designated by square brackets above the sequence and numbered to correspond with Figure 1; products of complete T_1 RNase digestion are designated by square brackets below the sequence and numbered to correspond with Figure 2. Fragments of limited T_1 RNase digestion are numbered to correspond with Figure 4 with the prefix PT.

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