PARTIAL METHYLATION OF 18 S RIBOSOMAL RNA DETECTED BY T1 RIBONUCLEASE DIGESTION AND HOMOCHROMATOGRAPHY FINGERPRINTING

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1. Introduction

Studies on methylation of oligonucleotides of ribosomal RNA have shown quantitative [1,2], species [1] and growth related variations [2].

In previous studies on the oligonucleotides produced by digestion of ^{32}P-labeled 18 S rRNA from four mammalian species with T1 RNAase and mapped by homochromatography fingerprinting, ^{32}P-content in the spots of the large oligonucleotides was determined and the molar yield was calculated [3,4]. The molar yield was approximately one for each large oligonucleotide except oligonucleotides 3 and 6 [4].

Oligonucleotide 3 was found in less than one molar yield in the 18 S rRNA of rat, mouse, hamster and man. Oligonucleotide 6 was found at approximately 10% molar yield in the four 18 S rRNAs. The nucleotide composition of oligonucleotide 3 was $A_3C_6U_4$,GmA,UmC,G and of oligonucleotide 6 it was $A_3C_6U_4$,UmC,G [4]. Both the oligonucleotides contained the same 2′-*O*-methylated dinucleotide, UmC, and $A_3C_6U_4G$. Oligonucleotide 3 contained the extra 2′-*O*-methylated dinucleotide, GmA. The present study shows that a partial 2′-*O*-methylation at the GmA position is responsible for the appearance of the two spots and that this methylation is quantitatively similar in several species.

2. Materials and methods

All procedures for isolation of the RNA, digestion of the RNA and nucleotide sequencing were described previously [3–6].

3. Results and discussion

Oligonucleotides 3 and 6, which were obtained by T1 RNAase digestion of ^{32}P-labeled 18 S rRNA of Novikoff rat ascites hepatoma cells, were completely digested with pancreatic RNAase and analyzed by electrophoresis on DEAE-cellulose paper at pH 1.9. The results showed that oligonucleotide 3 contains (A,GmA)G, AAC, UmC, 4U and 5C (fig.1). Oligonucleotide 6 contained AG, AAC, UmC, 4U and 5C. These oligonucleotides have the same components except that oligonucleotide 3 contains (A,GmA)G and oligonucleotide 6 contains AG.

It was found after U2 RNAase digestion that the oligonucleotides 3 and 6 contain two large polypurines, C_5UA and CU_3,UmC,A. Oligonucleotide 3 had an extra fragment, GmA.

Oligonucleotides 3 and 6 labeled with ^{32}P at the 5′-ends [6] were completely digested with T2 RNAase and analyzed by Whatman 3 MM paper electrophoresis, at pH 3.5. Most of the radioactivity was in the p*Up position for both oligonucleotides. Therefore, both oligonucleotides 3 and 6 have the nucleotide U at their 5′-ends.

Oligonucleotides 3 and 6 labeled in vitro at the 5′-ends were partially digested with U2 RNAase and analyzed by one-dimensional homochromatography (fig.2) to determine the positions of A in the T1 RNAase fragments [6]. Figure 2 shows AA which was found as AAC as noted earlier (fig.1) is in the same position from the 5′-ends of the oligonucleotides. The third A from the 5′-end was also in the same position in both oligonucleotides (fig.2). The 3′-terminus of oligonucleotide 3 is (A,GmA)G

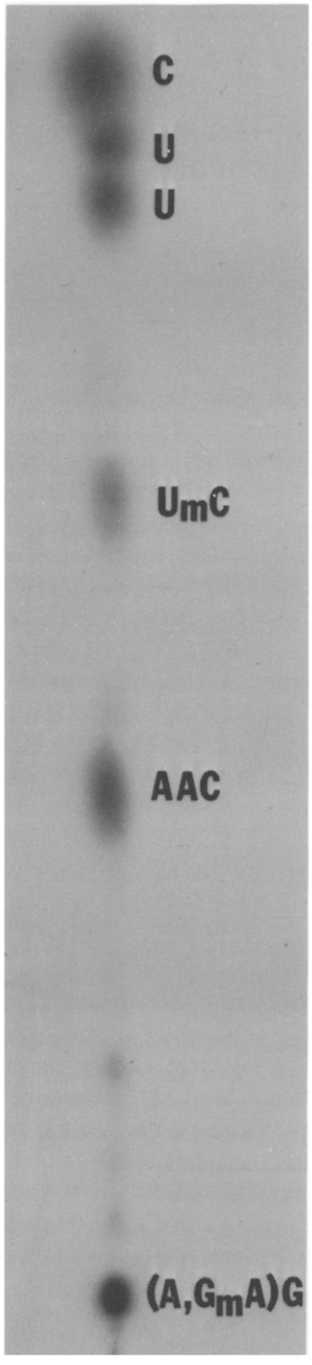

Fig.1. DEAE-Cellulose paper electrophoresis analysis, at pH 1.9, of complete pancreatic RNAase digestion products of oligonucleotide 3. The slowest migrated spot was determined to be (A,GmA)G after nucleotide compositional analysis. U appeared as a doublet in this system.

(fig.1). Of the two possible sequences, –PyAGmAG and –PyGmAAG, fig.2 shows that –PyAGmAG is in this oligonucleotide. This result agrees with the finding that homogeneously labeled oligonucleotide 3 yields fragment GmA after complete U2 RNAase digestion.

Oligonucleotides 3 and 6 labeled at the 5′-ends were completely digested with U2 RNAase. The fragments were purified, partially digested with pan-

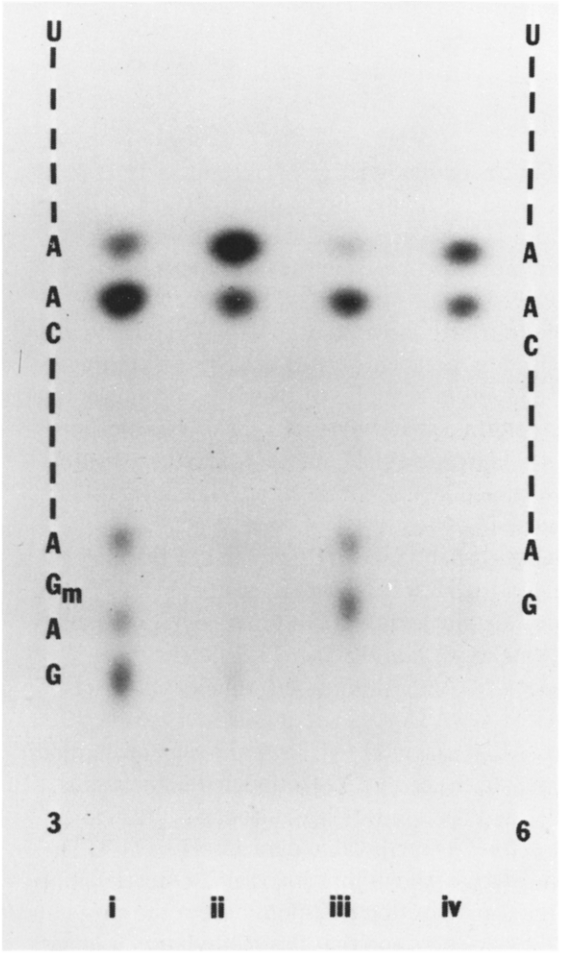

Fig.2. One-dimensional homochromatography analysis of oligonucleotides 3 and 6 labeled at the 5′-ends and partially digested with U2 RNAase. (i) Oligonucleotide 3, U2 RNAase 0.1 unit/10 μl; (ii) oligonucleotide 3, U2 RNAase 0.2 units/10 μl; (iii) oligonucleotide 6, U2 RNAase 0.1 unit/10 μl; (iv) oligonucleotide 6, U2 RNAase 0.2 units/10 μl. Digestion was performed at 37°C for 1 h.

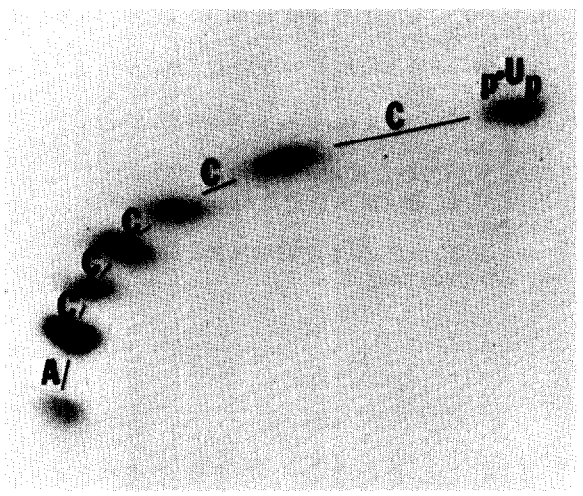

Fig.3. Nucleotide sequence determination of UCCCCCA at the 5′-end of oligonucleotide 3.

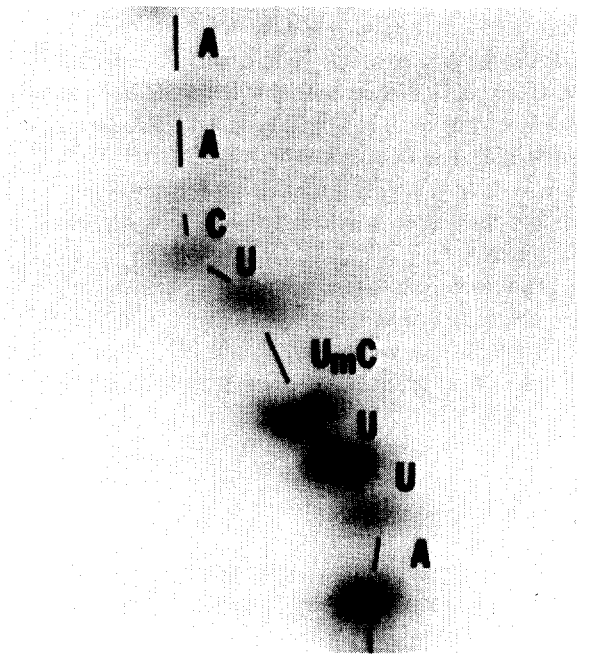

Fig.4. Nucleotide sequence determination of (UmC,U_3) in oligonucleotide 3.

creatic RNAase and analyzed by homochromatography fingerprinting. Both 5′-terminal fragments from the two oligonucleotides had the same pattern with the sequence of UCCCCCA (fig.3). This polypyrimidine fragment, UCCCCCA, in rat 18 S rRNA was previously reported from our laboratory [7]. Oligonucleotide 3 has the nucleotide sequence UCCCCCAAC-(UmC,U_3)AGmAG and oligonucleotide 6 has the nucleotide sequence UCCCCCAAC-(UmC,U_3)AG.

The sequences of (UmC,U_3) in oligonucleotides 3 and 6 remained to be determined. Oligonucleotide 3 labeled at the 5′-end was partially digested with pancreatic and U2 RNAases and analyzed by homochromatography fingerprinting (fig.4) [6]. The small spaces in fig.4 were assigned to U residues. Since only one large space could be associated with a 2′-*O*-methylated dinucleotide, it was assigned to UmC. The nucleotide sequence of oligonucleotide 3 is UCCCCCAACUUmCUUAGmAG. Oligonucleotide 6 had the same sequence at (UmC,U_3). Its nucleotide sequence is UCCCCCAACUUmCUUAG. Accordingly, oligonucleotide 6 is a part of oligonucleotide 3 and GmA in this position is partially methylated. Table 1 shows the percentage of GmA methylation in four 18 S rRNAs calculated from the molar yields of oligonucleotides 3 and 6 (table 1 of ref. [4]).

To determine whether UmC in oligonucleotide 3 is partially methylated, polypyrimidine CUUmCUUA was purified after U2 RNAase digestion of homogeneously labeled oligonucleotide 3 and nucleotide composition was determined. The ratio between the radioactivity in UmC and A was 1.6 : 1. Therefore, UmC is also partially methylated to the extent of about 80%.

Khan and Maden [1] examined methylated nucleotides in the combined digestion products with T1 RNAase and pancreatic RNAase of 18 S rRNA.

Table 1
Percent methylation at GmA

Rat	Mouse	Hamster	Man
88	85	84	92

They reported a half molar yield for AGmAG and suggested a half molar yield for AAAmU of HeLa 18 AS rRNA. The molar yield observed for AGmAG by them was less than the value observed in this study. AAAmU was found in the largest T1 RNAase oligonucleotide 1, UCCACUUUAAAmUCCUUUAACG, but AmU in this position has been found to be almost completely methylated in current studies on 18 S rRNA.

The reasons for these partial 2′-*O*-methylations are obscure at present. Although in some instances a relationship to growth was found [2], other factors may be involved in the partial methylation at GmA of 18 S rRNA.

Acknowledgement

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