COMPARISON OF LARGE FRAGMENTS OBTAINED BY T_1 RNase DIGESTION OF RIBOSOMAL AND NUCLEOLAR PRERIBOSOMAL RNA OF NOVIKOFF HEPATOMA ASCITES CELLS: THE 5'-TERMINAL EICOSANUCLEOTIDE

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Summary: Large T_1 RNase fragments of 18S and 28S rRNA and their nucleolar precursors were separated by successive elution from columns of DEAE Sephadex at pH 7.6 and DEAE-cellulose at pH 3.4, respectively. The fragments that contain the methylated oligonucleotides Am-Gm-Cm-Ap, Um-Gm-Up, Gm-Up and Cm-Ap are referred to as α_2-2, α_2-4, α_2-3 and α_1-1'. Fragment α_2-2 is the eicosanucleotide Am-Gm-Cm-A-A-A-U-U-C-A-U-A-U-U-C-A-A-A-C-Gp which is the 5' end of 28S rRNA and its precursors including nucleolar 45S RNA. The dinucleotide Cm-Ap is present only in 18S rRNA and 45S nucleolar RNA.

INTRODUCTION

Studies in this and other laboratories have indicated that there are a number of methylated oligonucleotides in 18S and 28S rRNA as well as their nucleolar precursors (1-5). The sequences of large T_1 RNase fragments containing some methylated oligonucleotides have been defined for 28S rRNA as Am-Gm-Cm-A-A-A-U-U-C-A-U-A-U-U-C-A-A-A-C-Gp, Um-Gm-U-U-U-C-A-C-C-C-A-U-A-U-C-A-A-U-A-C-C-A-Gp and C-U-C-C-Gm-U-A-U-U-C-A-A-U-U-A-Gp (2). The goal of the present study was to extend these findings to the nucleolar preribosomal RNA species and to ascertain the 5' termini of these molecules. For this purpose a comparison was made of the long T_1 RNase fragments of α_1 and α_2 peaks obtained by successive column chromatography on DEAE Sephadex A-25 at pH 7.6 and DEAE cellulose at pH 3.4.

MATERIALS AND METHODS

Preparation of highly ^{32}P -labeled ribosomal RNAs and nucleolar preribosomal RNAs from Novikoff hepatoma ascites cells - The methods of preparation of ^{32}P -labeled RNA have been described previously (6). After the Novikoff hepatoma ascites cells were incubated for 18 hours for the preparation of ribosomal RNA and 6 hours for the preparation of nucleolar RNAs, the cytoplasmic ribosomes were isolated and their subunits were purified (7) and the nucleoli were isolated by sonic oscillation and purified (8). RNA was extracted by the hot sodium dodecyl sulfate-phenol method and individual RNA species were purified by repeated sucrose density gradient centrifugation (7). The specific activity of the RNAs used was approximately 100 μCi/mg RNA.

Fractionation of fragments of complete enzymic digestion products on DEAE Sephadex A-25 columns and DEAE cellulose columns - All the methods used in the present study were described previously (8-12).

Structural analyses - The conditions of enzymic digestion and the fractionation methods were similar to those employed previously (8-12).

Determination of ^{32}P-radioactivity - Both the Cerenkov method and liquid scintillation techniques (13,14) were used to determine radioactivity.

RESULTS

Sub-fractionation of peaks α_1 and α_2 - The α_1 and α_2 peaks of complete T_1 RNase digests of 18S and 28S rRNA were obtained by chromatography on DEAE Sephadex columns at pH 7.6 as described previously (9,10). These peaks were rechromatographed

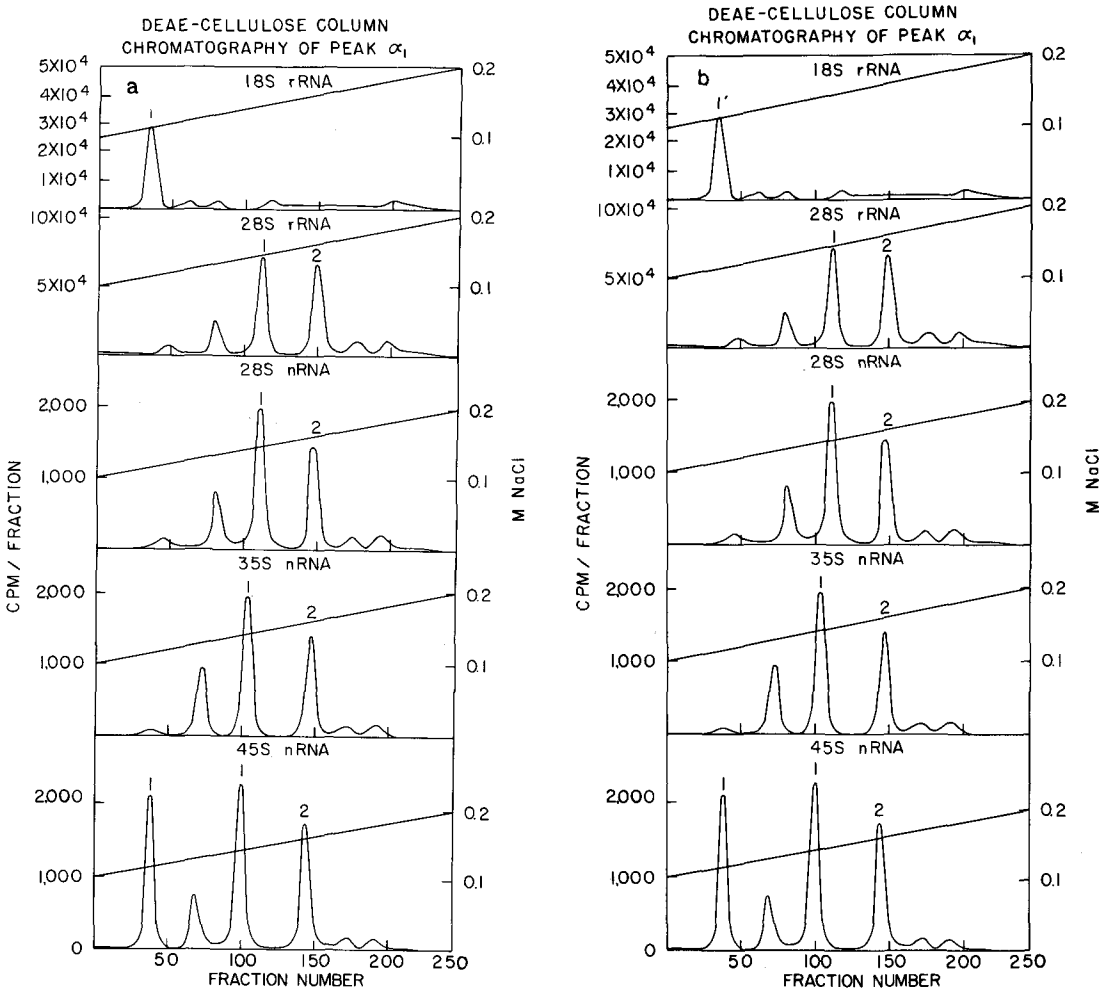
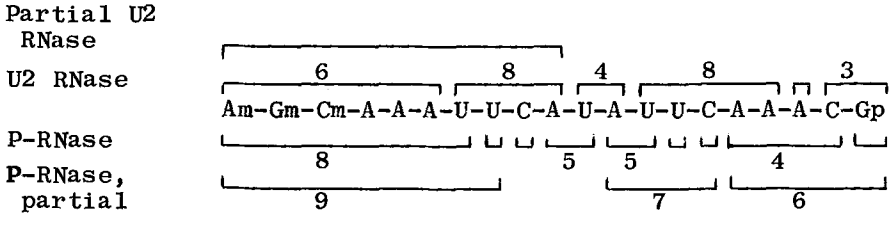


Figure 1 Fractionation of peaks α_1 and α_2 of complete T_1 RNase digests of ^{32}P-labeled RNAs. Peaks α_1 and α_2 (9,10) were eluted with an NaCl gradient from a DEAE-Sephadex column at pH 7.6 (9,10), then desalted on DEAE cellulose (carbonate form) columns (0.8 x 1.0 cm) and evaporated to dryness. The samples, dissolved in 2 ml 7 M urea at pH 3.4 (adjusted with formic acid), were loaded on DEAE cellulose columns (0.8 x 50 cm). Elution was carried out with a linear gradient from 0.10 M to 0.2 M NaCl in 7 M urea at pH 3.4 (total volume 700 ml). Each fraction was used to determine the distribution of radioactivity. (a) peak α_1, (b) peak α_2

on DEAE cellulose columns at pH 3.4 as shown in Figs. 1a and 1b. Peak α_1-1' was found only in 18S rRNA and 45S nRNA. On the other hand, peaks α_1-1 and α_1-2 were found in 28S rRNA, 28S nRNA, 35S

nRNA and 45S nRNA. Fragment α_1-1' contained the methylated dinucleotide Cm-Ap. The compositions of α_1-1', α_1-1 and α_1-2 were $A_3U_4C_9$(Cm-Ap)Gp, U_6C_9Gp and $A_{10}U_5C_2\psi$Gp, respectively. These compositions differed markedly from other fragments found in peak α_2; the compositions of α_2-1', α_2-1, α_2-2, α_2-3 and α_2-4 were respectively, $A_{13}U_3C_3$Gp, U_5C_{11}Gp, Am-Gm-Cm-A-($A_7U_5C_3$)Gp, $A_4U_5C_4$(Gm-Up)Gp and $A_7U_5C_7$(Um-Gm-Up)Gp. Like α_1-1', α_2-1' is only found in 18S rRNA and 45S nRNA. All of the other fragments are found in 28S rRNA and its nucleolar precursors.

Analyses of subfractionated fragments - The complete pancreatic RNase digestion products of fragments α_1-1' and α_1-2 are shown in Figs. 2a and 2b. Fragments α_1-1' of 18S rRNA and 45S nRNA were found to contain identical digestion products (Fig. 2a). Similarly the complete pancreatic RNase digestion products of fragment α_1-2 were the same for 28S rRNA and 28S, 35S and 45S nucleolar RNA (see Fig. 2b). These fragments were found to be essentially identical to one another and to those of 28S rRNA. Fragment α_2-2 was sequenced as follows:



The other sequences are:

α_1-1': (Cm-A-A-Up)(A-A-Cp)$(Cp)_8(Up)_3$Gp

α_1-2: (A-A-A-A-Cp)(A-A-Up)(A-A-ψp)(Cp)$(Up)_4$A-A-Gp

α_2-1': (A-A-A-A-Up)(A-A-Up)(A-A-Up)(A-A-Cp)$(A-Cp)_2$A-Gp

α_2-3: C-U-C-C-Gm-U-A-U-U-C-A-A-U-U-A-Gp

α_2-4: Um-Gm-U-U-U-C-A-C-C-C-A-U-A-U-C-A-A-U-A-C-C-A-Gp

The 5' terminus of 28S rRNA and its nucleolar precursors -

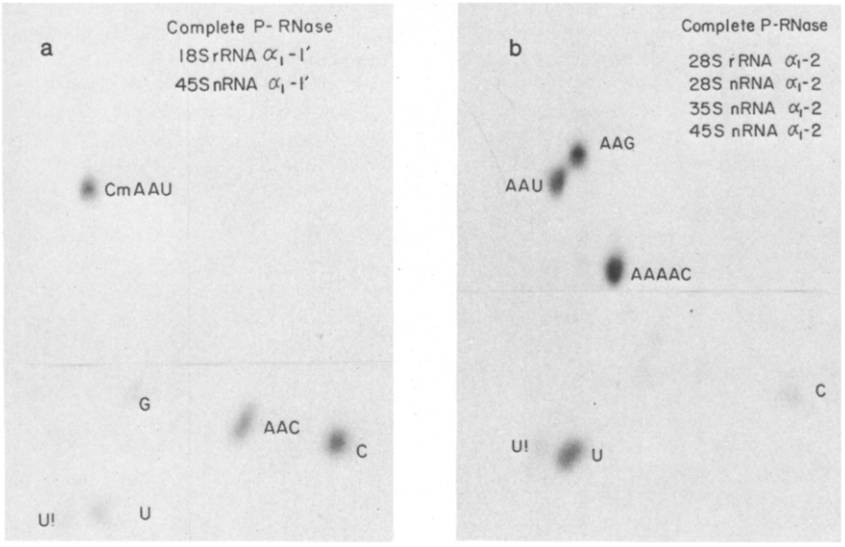


Figure 2 Two-dimensional high voltage electrophoresis of complete pancreatic RNase digests. The first dimension (direction, right to left) was on cellulose acetate in 7 M urea - 5% acetic acid at pH 3.5 (adjusted with pyridine) with 45 volts/cm for 5 hours. The second dimension (direction, from top to bottom) was on DEAE paper in 7% formic acid with 15 volts/cm for 16 hours. The sample was digested completely with pancreatic RNase (enzyme: substrate, 1:20) for 30 minutes.
(a) α_1-1', (b) α_1-2

The tetranucleotide Am-Gm-Cm-Ap is the 5'-end of 28S rRNA and its nucleolar precursors because: 1) the pancreatic RNase fragment Am-Gm-Cm-A-A-A-Up which was obtained from both whole 28S rRNA and its nucleolar RNA precursors (Fig. 3,4) was not cleaved further by spleen phosphodiesterase, indicating there are no nucleotides on the 5' end of Am-Gm-Cm-Ap, 2) in the sequence of fragment α_2-2 there are no Ap, Up or Cp residues on the 5'-end of Am-Gm-Cm-Ap and 3) partial U2-RNase digestion of fragment α_2-2 produced the fragment Am-Gm-Cm-A-A-Ap which contained no pyrimidine residues and was not cleaved further by spleen phosphodiesterase. Each of the other methylated fragments studied was found to contain additional nucleotides on the 5'-end of the

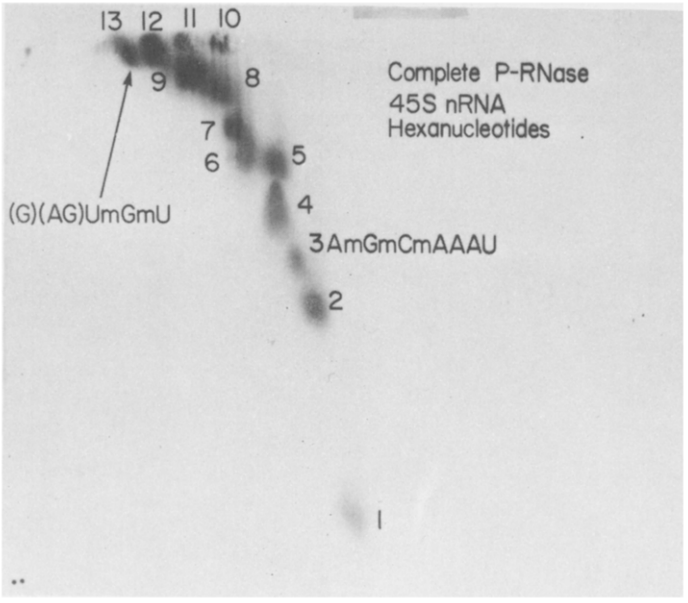


Figure 3 Two-dimensional high voltage electrophoresis of the -7 to -8 charge peak obtained by DEAE-Sephadex chromatography of a complete pancreatic RNase digest of 45S nRNA. The conditions for electrophoresis and digestion were the same as in Figure 2.

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methylated oligonucleotide, i.e., the 5'-end of Um-Gm-Up of α_2-4 contains (Gp)(A-Gp) as shown in Figure 3 and in α_2-3 C-U-C-Cp is at the 5'-end.

DISCUSSION

The present study shows that the examination of the subcomponents of peaks α_1 and α_2 provides some important structural features of 45S nRNA. In addition, it was found that 45S nRNA contains unique fragments which are present only in either 18S rRNA or 28S rRNA. Such fragments contained modified nucleotides such as pseudouridylate or alkali resistant oligonucleotides including Cm-Ap, Gm-Up, Um-Gm-Up and Am-Gm-Cm-Ap. The previous evidence that the 5'-end of 45S nRNA is the 5'-end of 28S rRNA

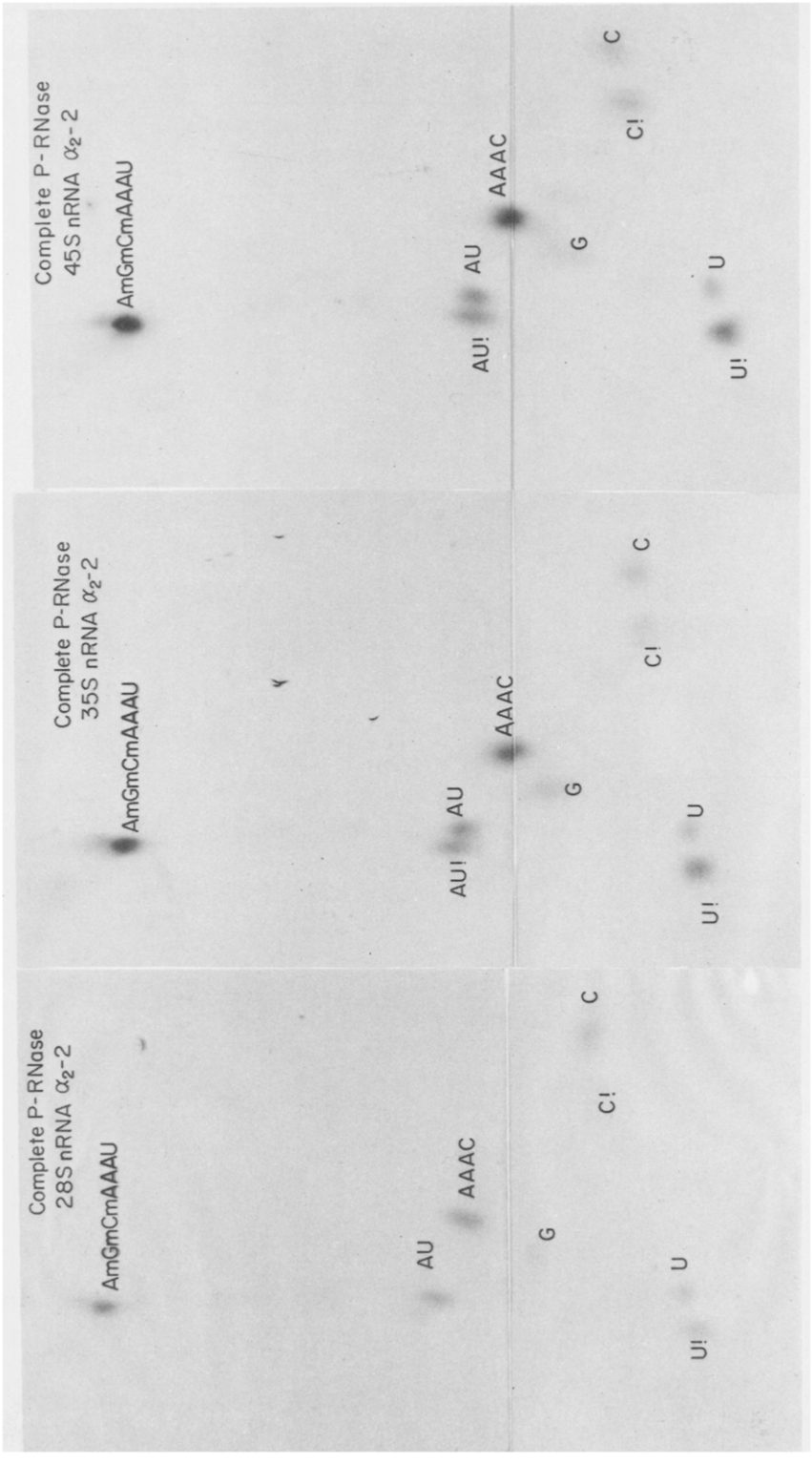

Figure 4 Two-dimensional high voltage electrophoresis of complete pancreatic RNase digest of peak α_2-2 of 45S, 35S and 28S nRNA. The conditions for digestion and electrophoresis were the same as in Figure 2.

(3,4,15) was confirmed by the presence of eicosanucleotide fragment α_2-2 both in 28S rRNA and 45S nRNA. This fragment contained the 5' terminal alkali resistant tetranucleotide Am-Gm-Cm-Ap in the pancreatic RNase fragment Am-Gm-Cm-A-A-A-Up.

The presence of the chain of methylated nucleotides and absence of a terminal triphosphoryl group in 45S nRNA must reflect a specific functional role for this portion of the molecule. The post-transcriptional methylation of three consecutive nucleotides is so specific that either more than one kind of 2'-O-methylase is involved or that some other factors must direct the activity of the methylases. Highly methylated alkali resistant 5'-terminal oligonucleotides have been found in a number of nuclear low molecular RNA species including U1, U2 and U3 RNA (16). Since the highly methylated tetranucleotide is resistant to various endonucleases and alkali and has greater hydrophobicity than adjacent oligonucleotides, it would seem that this oligonucleotide may play an important role in protein binding.

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