# 5S $RNA_{III}$, A NEW NUCLEUS-SPECIFIC 5S RNA

Tae Suk Ro-Choi, Ramachandra Reddy,
Dale Henning and Harris Busch

Department of Pharmacology
Baylor College of Medicine, Houston, Texas 77025

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## SUMMARY

A new species of 5S RNA, referred to as 5S $RNA_{III}$, has been found in the Novikoff hepatoma ascites cell nuclei. This RNA, which composes 28% of total nuclear 5S RNA and co-migrates with ribosomal 5S $RNA_I$ and 5S $RNA_{II}$ on 8% polyacrylamide gel electrophoresis, has been isolated by chromatography on DEAE Sephadex columns. The nucleotide composition is A 24, U 31, G 24 and C 20; its A+U/G+C ratio is 1.24. $T_1$ RNase digestion produced the following unique oligonucleotides from 5S $RNA_{III}$: $(U_2C_2)$Gp, (CUA)Gp, $(C_4A_3U_8)$Gp, [(UmU)$(C_2A_3U_5)$(AGmC)], $(CAU_2)$Gp, $(C_2AU_2)$Gp and $(C_3A_3U_2)$Gp. Similarly, unique oligonucleotides were produced by complete pancreatic RNase digestion of 5S $RNA_{III}$, including UmUp, A-A-Up, A-A-Cp, (AG)Cp, A-Gm-Cp, $(A_2G)$Up, A-A-A-Up and $(A_3G)$-Up.

## INTRODUCTION

Recent studies from this laboratory have suggested that unlike 5S rRNA (1-7), nuclear 5S RNA is heterogeneous since fractionation by DEAE Sephadex column chromatography showed that nuclear 5S RNA is separable into three components (8). Nuclear 5S RNA also differs from 5S rRNA in that it contains a number of methyl groups (9,10).

In the present study, three types of 5S RNA from Novikoff hepatoma cell nuclei are compared with nucleolar 5S RNA and 5S rRNA. Nuclear 5S $RNA_I$ and 5S $RNA_{II}$ apparently are two conformational isomers of 5S rRNA. Nuclear 5S $RNA_{III}$ is characterized by a high AMP+UMP content, i.e., these nucleotides compose 55% of the total compared to only 44% in 5S rRNA. Oligonucleotide fingerprints made after pancreatic RNase and $T_1$ RNase digestion were virtually identical for 5S $RNA_I$ and 5S $RNA_{II}$ but those for 5S $RNA_{III}$ were markedly different.

## MATERIALS AND METHODS

Labeling of Nuclear RNA by $^{32}P$ In Vitro - In each experiment, approximately 60 g of tumor cells were incubated for 10 hours in the ratio of 1:50, cell: medium with 500 mCi $^{32}P$-orthophosphate (11). Nuclear RNA was extracted by the SDS phenol procedure (12,13,14) and 4-8S RNA was separated from high molecular weight RNA by sucrose density gradient centrifugation. The 4 to 8S RNA contained 0.8 to 1.2 x $10^9$ cpm in 2-3 mg; the 5S RNA purified by preparative polyacrylamide gel electrophoresis (14) contained 0.8 to 1.4 x $10^8$ cpm.

Separation of 5S RNAs by DEAE Sephadex A-50 Columns - DEAE Sephadex A-50 was washed successively with 0.1 N HCl $H_2O$, 0.1 N HaOH and $H_2O$ (8,14), then with 1.0 M NaCl and finally with 0.6 M NaCl in 0.01 M sodium acetate, pH 5.1. The washed DEAE Sephadex was packed into a column (1.5 x 30 cm) under a pressure of 50 cm of water. A linear gradient of 0.62 to 0.72 M NaCl in 0.02 M sodium acetate, pH 5.1, was used to fractionate the RNA. Chromatography was carried out at room temperature with a flow rate of approximately 6 ml per hour; 3 ml fractions were collected. The Dowex-formate column system (15) was used for determination of nucleotide composition by absorbance after hydrolysis of RNA by 0.3 N KOH. Electrophoresis at pH 3.5 on Whatman 3 MM paper was employed for the determination of composition by $^{32}P$ content (16).

Hydrolysis of RNA by $T_1$ and Pancreatic RNases - After separation by DEAE Sephadex A-50, highly labeled RNAs were precipitated by two volumes of ethanol containing 2% potassium acetate. The RNA was collected on Millipore filters and dissolved in small volumes of distilled water. Carrier RNA was added to make a total of 0.2 mg and the RNA was precipitated by adding

two volumes of ethanol containing 2% potassium acetate at -20°C overnight and then centrifuged to pellet the RNA. The RNases, in a volume of 5 to 10 µl, were added directly to the pellet. The enzyme to substrate ratio was 1:20 for both the $T_1$ and pancreatic RNase digestions. Incubation was carried out at 37°C for 30 min for the $T_1$ RNase digestion and 3 to 4 hours for the pancreatic RNase digestion.

Separation of Oligonucleotides - The two-dimensional electrophoretic method of Sanger et al (16) was employed to separate the oligonucleotides.

Identification of Spots - After the DEAE-cellulose sheet was air-dried, X-ray films were exposed to the paper for a defined period of time. After development of the X-ray films, the spots were cut out and eluted with triethylamine carbonate as described by Brownlee and Sanger (4). The samples were dried, small amounts of water were added and the samples were again dried several times to remove traces of triethylamine carbonate and then the $^{32}P$-nucleotide composition was determined.

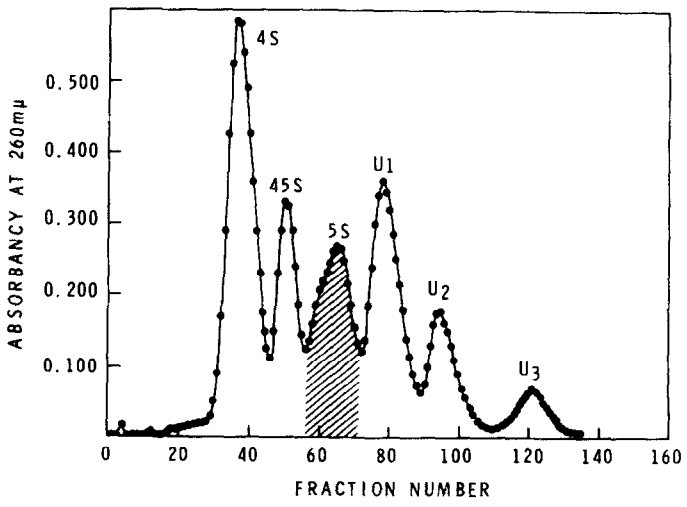


Fig. 1 - Electrophoretic pattern on a preparative polyacrylamide gel of whole nuclear 4 to 8S RNA from Novikoff hepatoma cells.

## RESULTS

Amount of 5S RNA - Nuclear 4-8S RNA was separated into six major fractions (Fig. 1), of which 5S RNA composes approximately 19.2%. Nucleolar 4-8S RNA was separated into three major fractions, of which 5S RNA comprised 47.2%. Ribosomal 4-8S RNA was separated into three fractions, including 4S RNA, 5S RNA and 7S ribosomal RNA which is hydrogen bonded to 28S ribosomal RNA. Of these, ribosomal 5S RNA composes approximately 40%.

Separation of 5S RNA by DEAE Sephadex A-50 Column Chromatography - 5S RNAs purified by preparative polyacrylamide gel

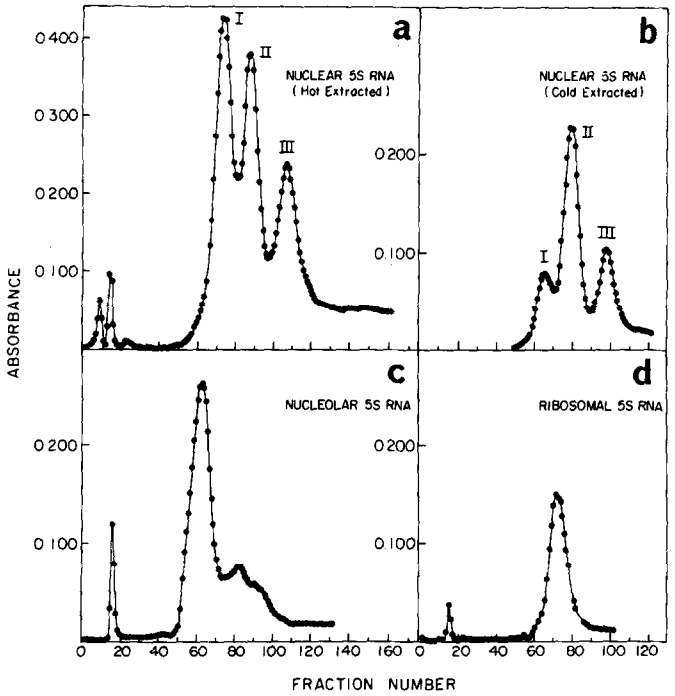


Fig. 2 - Chromatographic pattern of 5S RNAs from various fractions on DEAE Sephadex A-50. A linear gradient of 0.6 to 0.8 M NaCl in 0.02 M sodium acetate, pH 5.1, was used for the fractionation.

a) Nuclear 5S RNA extracted with SDS phenol at 65°C (10-15 min).

b) Nuclear 5S RNA extracted with SDS phenol at 23°C.

c) Nucleolar 5S RNA extracted with SDS phenol at 65°C.

d) Ribosomal 5S RNA extracted with SDS phenol at 65°C.

electrophoresis were separated by chromatography on DEAE Sephadex A-50 columns. When the RNA was extracted by the hot SDS phenol procedure, 38%, 34% and 28% of the nuclear 5S RNA were found in the 5S $RNA_I$, 5S $RNA_{II}$ and 5S $RNA_{III}$, respectively (Fig. 2a). The proportion of 5S $RNA_I$ was markedly decreased when the RNA was extracted at 23°C (Fig. 2b). When nucleolar or ribosomal 5S RNA was chromatographed in the same way, only one main peak was found (Fig. 2c, 2d).

Nucleotide Compositions of 5S RNAs - Table I shows the nucleotide compositions of various 5S RNAs. Nuclear 5S $RNA_I$ and 5S $RNA_{II}$, as well as 5S rRNA and nucleolar 5S RNA, have virtually identical nucleotide compositions. The composition of

TABLE I

NUCLEOTIDE COMPOSITIONS OF 5S RNA

FROM NOVIKOFF HEPATOMA CELL

| Fractions | AMP | UMP | GMP | CMP | $\frac{AMP+UMP}{GMP+CMP}$ | Ref. |
|---|---|---|---|---|---|---|
| Nuclear 5S RNAs | | | | | | |
| 5S RNA I | 19.2 | 25.2 | 30.6 | 25.0 | 0.80 | |
| 5S RNA II | 20.7 | 23.4 | 30.0 | 25.9 | 0.79 | |
| 5S RNA III | 24.0 | 31.3 | 24.5 | 20.1 | 1.24 | |
| Whole 5S RNA | 18.8 | 23.2 | 31.9 | 26.0 | 0.72 | 19 |
| Nucleolar 5S RNA | 19.0 | 24.8 | 28.9 | 26.6 | 0.79 | |
| Ribosomal 5S RNA | 19.0 | 23.5 | 31.6 | 25.9 | 0.74 | |

Nucleotide compositions of 5S RNAs from various fractions. Compositions were determined by UV absorbance after separation of the nucleotides on Dowex formate columns (see Materials and Methods).

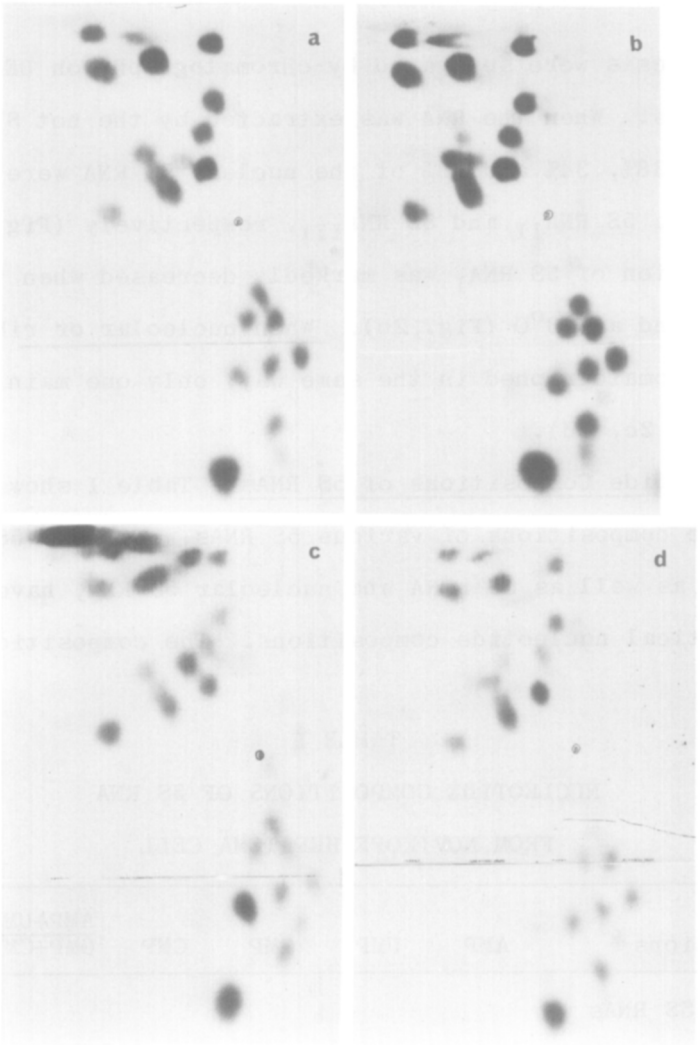


Fig. 3 - Autoradiograph of two-dimensional electrophoretic pattern of $T_1$ RNase digestion products of 5S RNAs (enzyme substrate ratio 1:20) at 37°C for 30 minutes. Electrophoresis in the first dimension was done on cellulose acetate strips (3 x 100 cm) in a 7 M urea, 5% acetic acid and 0.1% pyridine at pH 3.5 with a constant voltage of 2.5 KV; second dimension was on DEAE-cellulose paper (42 x 80 cm) in 7% formic acid system with a constant voltage of 1 KV. In both cases, Savant high voltage power supplies (Model HV-5000-3TC) and Lucite electrophoresis tanks (Model LT-48A) were used.

a) Nuclear 5S $RNA_I$.

b) Nuclear 5S $RNA_{II}$.

c) Nuclear 5S $RNA_{III}$.

d) Nucleolar 5S RNA.

nuclear 5S $RNA_{III}$ differs in its higher AMP and UMP content and lower CMP content. The AMP+UMP/GMP+CMP ratio for the ribosomal type 5S RNAs are 0.74-0.80, but that of 5S $RNA_{III}$ is 1.24.

Results of Enzymatic Digestion with $T_1$ RNase - Figure 3 shows the fingerprints of the $T_1$ RNase digestion products of the 5S RNAs. Two-dimensional electrophoresis was performed by the method of Brownlee and Sanger (4,16). The fingerprints of the $T_1$ RNase digest of nuclear 5S $RNA_I$ and 5S $RNA_{II}$ were identical to those of nucleolar 5S RNA and ribosomal 5S RNA. The fingerprint of 5S $RNA_{III}$ contains 27 spots as compared to the 22 spots found for 5S $RNA_I$ and 5S $RNA_{II}$.

The unique oligonucleotides found in 5S $RNA_{III}$ were $(U_2C_2)G$, (CUA)G, $(C_4A_3U_8)G$, [(UmU)$(C_2A_3U_5)$(AGmC)], $(CAU_2)G$, $(C_2AU_2)G$ and $(C_3A_3U_2)G$ which are not present in 5S rRNA.

Products Obtained by Hydrolysis with Pancreatic RNase - Figure 4 shows fingerprints obtained after complete pancreatic RNase digestion of the 5S RNAs. 5S $RNA_I$ and 5S $RNA_{II}$ each contained 20 spots. The fingerprints of 5S $RNA_{III}$ differed from those of the other 5S RNAs studied, i.e., the unique oligonucleotides in 5S $RNA_{III}$, UmUp, A-A-Up, A-A-Cp, (AG)Cp, A-Gm-Cp, $(A_2G)$Up, A-A-A-Up and $(A_3G)$Up were found, they are not found in 5S $RNA_I$ and 5S $RNA_{II}$.

## DISCUSSION

Nuclear 5S RNA composes 19.2% of the total nuclear 4-8S RNA and 5S $RNA_{III}$ composes 28% of the total 5S RNA. Since approximately 10 mg of 4-8S RNA can be obtained by the hot SDS phenol procedure from 100 gm of packed tumor cells, 0.7 mg, 0.6 mg and 0.5 mg of 5S $RNA_I$, 5S $RNA_{II}$ and 5S $RNA_{III}$, respectively, can be obtained from isolated nuclei from 100 gm of packed tumor cells.

In addition to chromatographic evidence that the new 5S

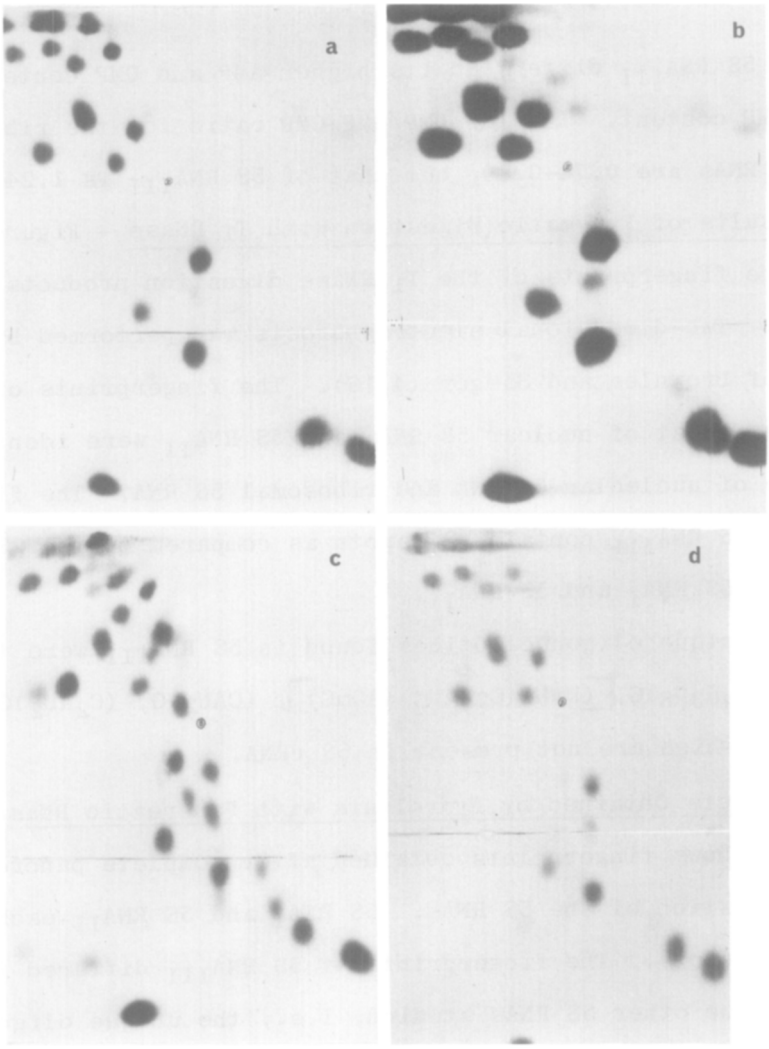


Fig. 4 - Autoradiograph of two-dimensional electrophoretic patterns of P-RNase digestion products of 5S RNAs (enzyme:substrate ratio 1:20 at 37°C for 2.5-3 hr). The conditions for electrophoresis are the same as those in Fig. 3.

a) Nuclear 5S $RNA_I$.

b) Nuclear 5S $RNA_{II}$.

c) Nuclear 5S $RNA_{III}$.

d) Nucleolar 5S RNA.

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$RNA_{III}$ is separated from the two conformationally different types of 5S rRNA, the evidence that it is a hitherto undescribed species of RNA is its content of AMP and UMP is high and its

AMP+UMP/GMP+CMP ratio is 1.24 compared to 0.80 for 5S $RNA_I$ and 5S $RNA_{II}$, and its oligonucleotides produced by $T_1$ RNase digestion differ markedly from that of 5S $RNA_I$ and 5S $RNA_{II}$.

Like those of most other recently discovered low molecular weight nuclear RNAs, the function of the 5S $RNA_{III}$ is unknown at present. The structural findings preclude the possibility that nuclear 5S $RNA_{III}$ is a precursor of 5S rRNA. Moreover, it is also unlikely that this AU-rich RNA is derived from nuclear heterogeneous RNA because its rate of labeling is much lower than that of the heterogeneous nuclear RNA. Since 5S $RNA_{III}$ is rich in both adenylic and uridylic acids, it seems possible that it may be a template for synthesis of a subunit of a nuclear protein (18,19).

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