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Preliminary Study on the Metabolic Characteristics of Supernatant 5SRNA of the Rat Liver

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Summary. **The 5'- and 3'-terminal sequences of the rat liver supernatant 5SRNA were shown to be the same as the corresponding sequences of ribosomal 5SRNA. The time course of the specific activity of the supernatant 5SRNA in the livers of rats receiving an intraperitoneal injection of [^{3}H] orotic acid showed that there was no lag in the labeling of this RNA, and that the specific activity increased up to 3 h and then decreased. The specific activity of ribosomal 5SRNA increased gradually after a lag of 90 min. The specific activity of supernatant 5SRNA was markedly higher than that of ribosomal 5SRNA at each time of labeling. These findings indicate a high metabolic rate for supernatant 5SRNA of rat liver.**

INTRODUCTION

Our previous reports[1,2] indicated that 5SRNA in the rat liver supernatant was present in the macromolecular complexes. One of them containing 9 aminoacyl-tRNA synthetases was highly purified. It was found that 5SRNA and ribosomal protein L5, which was shown to be bound to 5SRNA in the 60S ribosomal subunit in our previous report,[3,4] were present in this macromolecular aminoacyl-tRNA synthetase complex. On the other hand, there have been few reports concerning the metabolism of supernatant 5SRNA. From the time course of the labeling of rabbit reticulocytes labeled with [^{32}P]phosphate *in vivo*, it was reported that the rate of labeling of supernatant 5SRNA was much lower than that of ribosomal 5SRNA.[5] In the case of HeLa cells, the existence of rapidly labeled 5SRNA in the supernatant was reported, which was assumed to be transferred to the nucleus.[6] Recently, using anti-La autoantibody, Rinke and Steiz[7] showed the presence of 5SRNA* in a HeLa cell sonicate, which migrated slightly slower than ribosomal 5SRNA on the gel electrohoresis and had additional sequences of uridine at the 3'end as compared with ribosomal 5SRNA. They further showed that 5SRNA* was the precursor of ribosomal 5SRNA.

Since the sequence homology had not been confirmed between supernatant and ribosomal 5SrRNAs, we compared 5'- and 3' terminal sequences of the supernatant 5SRNA with those of ribosomal 5SRNA. Then we made preliminary experiments on the metabolic characteristics of supernatant 5SRNA of the rat liver.

*precursor of ribosomal 5SRNA

MATERIALS AND METHODS

Materials: [5-^{3}H] orotic acid (20 or 15.6 Ci/m mole), 5'-[γ-^{32}P] ATP (6000 Ci/m mole), [^{32}P] CpC (3000 Ci/m mole) were obtained from Amersham International, UK. NENSORB™20 was acquired from DU Pont, USA and a RNA sequencing kit, glycogen and RNA ligase were from Pharmacia, Sweden, with RNase CL3 and calf intestine alkali phosphatase from Boehringer Mannheim, Germany. The T4 ligase was from Takara Shuzo Co., Japan.

Methods: *(1) Purification of ribosomal and supernatant 5SRNA from rat liver.* Ribosomal 5SRNA was prepared from 5SRNA-L5 protein particles by sodium dodecyl sulfate phenol-cresol extraction followed by ethanol precipitation.[1] The RNA was further purified by proteinase K treatment, phenol-chloroform extraction, followed by ethanol precipitation.[8] Supernatant 5SRNA was prepared from the pH 5 fraction[1] by the same methods described above.

These RNAs were then subjected to acrylamide gel electrophoresis described in reference.[1] The 5SRNA bands on the gel were extracted as described in reference[8] and RNA was purified by phenol-chloroform extraction followed by ethanol precipitation. When necessary, glycogen (20 μg/ml) was added to the water phase as a carrier before ethanol precipitation. RNA was then applied to the NENSORBTH 20 column and eluted with methanol, and then methanol was evaporated in a vacuum. RNA was dissolved in H_2O. The ratio of A_{260nm}/A_{280nm} of the RNA was usually higher than 1.8. The concentration of RNA was determined by measuring A_{260nm} with a Beckman DU-62 spectrophotometer.

(2) 5'- and 3'-terminal sequencing of supernatant and ribosomal 5SRNAs. 5SRNA was labeled either at its 5'-terminus by the 5' [γ-^{32}P] ATP/T4 polynucleotide reaction after alkali phosphatase digestion or at its 3'-terminus by [^{32}P] CpC/T4 ligase. 5'- or 3'-terminal labeled 5SRNA was then subjected to urea-acrylamide gel electrophoresis[8] and 5SRNA was extracted and purified as described in reference.[1] The RNA was then digested with various kinds of RNase as described in the legend to Fig. 1, and then subjected to urea-acrylamide gel electrophoresis. Radioautographs were then established by exposing the gel to X-ray film at −80°C. These procedures were carried out as described in reference.[9]

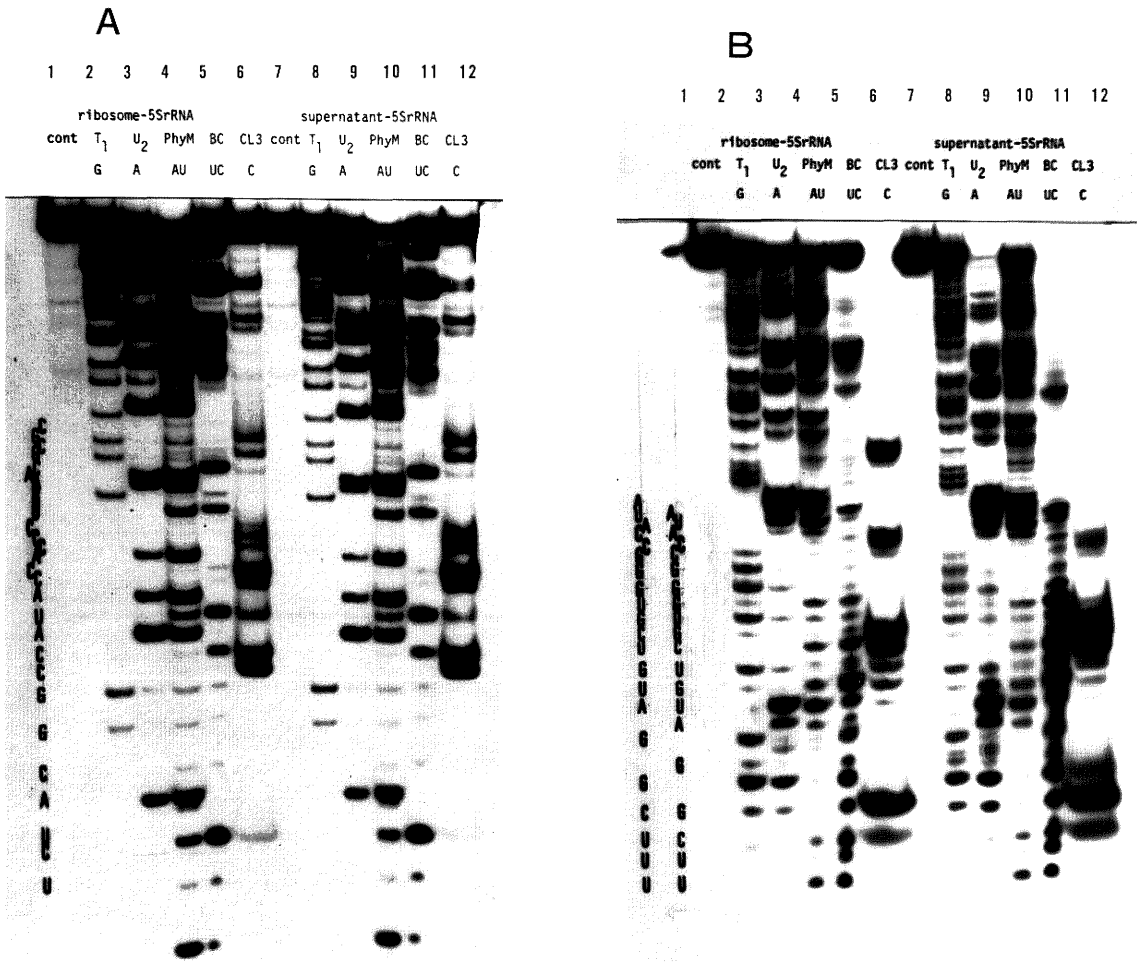

Fig. 1. 5'- and 3'-terminal nucleotide sequences of the supernatant and ribosomal 5SRNAs of rat liver. Ribosomal and supernatant 5SRNA were made radioactive at either the 5'end (A) or 3' end (B) with ^{32}P as described in reference.[7] Labeled 5SRNA was subjected to 7M urea-10% acrylamide gel electrophoresis. The 5S bands were extracted and 5SRNA was purified as described in MATERIALS AND METHODS. The nucleotide sequences of the ^{32}P-labeled 5SRNA was determined by enzymatic digestion methods.[9] The^{32}P-labeled 5SRNA was digested by RNase T1 (lane 2 and 8), RNase U2 (lane 3 and 9), RNase Phy M (lane 4 and 10), RNase B cereus (lane 5 and 11) and RNase CL3 (lane 6 and 12). Lane 1 or 7 are the controls without RNase digestion.

(3) In vivo labeling of supernatant and ribosomal 5SRNA of rat liver and the determination of specific activity. Three or four male rats of the Wistar strain weighing about 150 g were used in each experiment after fasting overnight. They received an intraperitoneal injection of 500 μCi of [^{3}H] orotic acid per 150 g of body weight. At the time indicated, rats were sacrificed, and ribosomal and supernatant 5SRNA were prepared from the livers and their amounts were determined as described in reference.[1] The ^{3}H radioactivity was measured by a Beckman LS 3801 liquid scintillation system.

RESULTS AND DISCUSSION

Comparison of the nucleotide sequence of supernatant 5SRNA with that of ribosomal 5SRNA

As shown in Fig. 1, the 5'- and 3'-terminal sequences, of supernatant 5SRNA were the same as the corresponding ones of ribosomal 5SRNA. It must be mentioned that the 3'-terminal sequence of the supernatant 5SRNA did not show $CUUUU_{OH}$ or $CUUUUU_{OH}$ which are the 3'-terminal sequences of the precursor of ribosomal 5SRNA,[7] although CUU_{OH} and $CUUU_{OH}$ were present at the 3'-terminal of both kinds of 5SRNA examined. These findings indicate that supernatant 5SRNA of the rat liver is mature 5SRNA, having the same sequence as that of ribosomal 5SRNA.

Time courses of *in vivo* labeling of supernatant and ribosomal 5SRNAs

The results are shown in Fig. 2. The lag in labeling of up to 90 min after an administration of [^{3}H] orotic acid was observed in the case of ribosomal 5SRNA, and the extent of labeling gradually increased up to 14h. On the other hand, there was no lag in the labeling of supernatant 5SRNA; it increased almost linearly up to 3 h and then sharply decreased. It must be emphasized that the specific activity of supernatant 5SRNA was markedly higher than ribosomal 5SRNA at each time period, indicating the high metabolic rate of supernatant 5SRNA in the rat liver. From these time courses of labeling, supernatant 5SRNA does not appear to be the precursor of ribosomal 5SRNA, and has a peculiar metabolism. Concerning the metabolism of ribosomal 5SRNA in mammalian cells, it is generally accepted that it is synthesized in the nucleoplasm by RNA polymerase III and then transferred to the nucleolus where it is incorporated into ribosomal 60S precursor. The precursor is then released from the nucleus to cytoplasm. Detailed studies of the time courses of the labeling of ribosomal 5SRNAs in these cellular components after an administration of labeled orotic acid will be necessary before elucidating the metabolic characteristics of supernatant 5SRNA. Such experiments are now in progress in our laboratories.

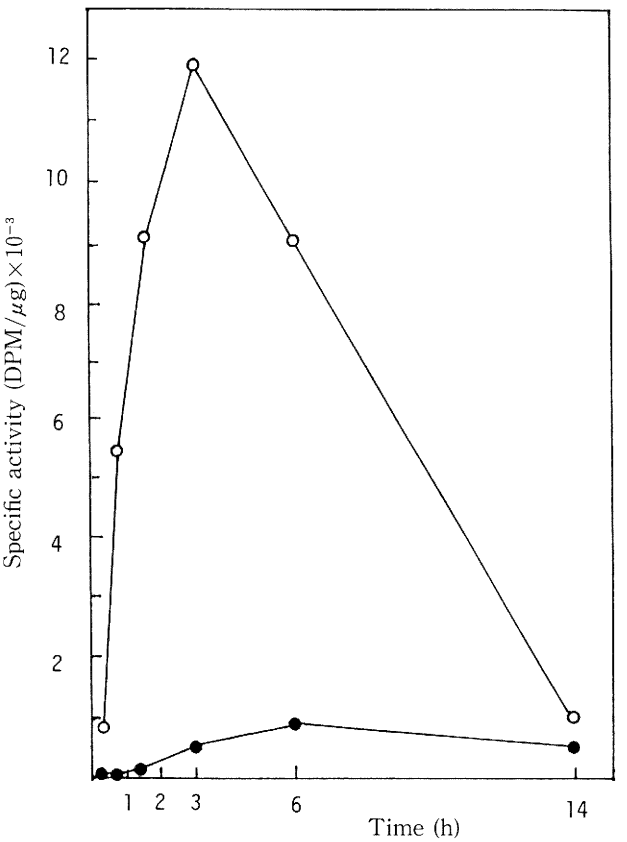

Fig. 2. Time courses of labeling supernatant and ribosomal 5SRNA with [^{3}H] orotic acid. The procedures are given in MATERIALS AND METHODS. supernatant 5SRNA (○—○), ribosomal 5SRNA (●—●).

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