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ISOLATION OF NUCLEOLAR METHYLASE PRODUCING ONLY 5-METHYLCYTIDINE IN RIBOSOMAL RNA

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SUMMARY: Methylation of rRNA mostly occurs in the nucleolus of mammalian cells. We have isolated nucleoli from Ehrlich ascites tumor cells of mice and purified RNA methylase taken from them. This highly purified nucleolar methylase produces only 5-methylcytidine in hypomethylated 18S and 28S rRNAs prepared from the mouse tumor cells after treatment with cycloleucine. This enzyme, however, did not transfer the methyl-group to normally methylated rRNA from the same mouse tumor cells. This high substrate specificity and enrichment of this enzyme in the nucleoli strongly suggest that we have isolated one of the enzymes which physiologically methylate rRNA precursor in the nucleoli.

Most of methylation of rRNA occurs in the nucleoli of mammalian cells and these methylations of rRNA are carried out in a highly specific manner. The methylations are accomplished during a short period after the transcription of rDNA, that is, before the complete processing of ribosomal precursor RNA to mature RNA. Some physiological roles of methylated nucleotides in precursor RNA might be supposed for accurate processing of nucleolar 45S RNA (1), because the methylations occur in the limited regions of nucleotide sequences, which are conserved during the cleavage of precursor RNA. Furthermore, it has been revealed recently that the secondary structures of rRNA are extensively conserved during the evolution and many of the

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modified nucleotides are located in the well conserved regions (2). These facts suggest that the methylations of rRNA might have an important role in ribosomal functions.

After considering the existence of various methylated nucleotides in both 18S and 28S rRNA (3), we think there might be heterogeneous enzymes for the methylations of rRNA in the nucleoli. However, none of methylases for rRNA have been purified so far, as compared with tRNA methylases and DNA methylases (4). Recently we have purified RNA methylase taken from the isolated nucleoli of mouse tumor cells (5), in which the biosynthesis of rRNA was significantly enhanced. The present study revealed that the product, which was specifically methylated by this purified nucleolar enzyme, was 5-methylcytidine.

MATERIALS AND METHODS

Preparation of hypomethylated RNA from mouse tumor cells Ehrlich ascites tumor cells were treated with cycloleucine according to the procedure described by Amalric et al. (6). The cytoplasmic RNAs were extracted and fractionated into tRNA, 18S and 28S rRNA by sucrose density gradient and used as substrate in the standard methyltransferase assay.

DEAE-Sephadex A-25 column chromatography of the product by the nucleolar methylase RNA from E.coli was incubated with both nucleolar methylase and [^{3}H-methyl]-S-adenosyl-L-methionine. The reaction products were extracted with phenol, collected by ethanol precipitation and digested in 0.3 N KOH at 37°C for 18h. After neutralization with perchloric acid, the digests were chromatographed with DEAE-Sephadex A-25 column in the presence of 7 M urea at pH 7.8.

Two dimensional thin-layer chromatography RNAs methylated in vitro by the nucleolar enzyme were hydrolyzed with RNase T_2. Aliquots of the hydrolysates were spotted onto Avicel SF cellulose thin-layer plates. Development of the chromatography and identification of the modified nucleotides after autoradiography were carried out as described previously (7).

RESULTS AND DISCUSSION

During the early steps of purification, the specific activity of RNA methylase was increase about 10 fold by merely isolating nucleoli from the purified nuclei of Ehrlich ascites tumor cells. The purification and characterization of the nucleolar methylase will be described elsewhere (5). The purified enzyme, which is

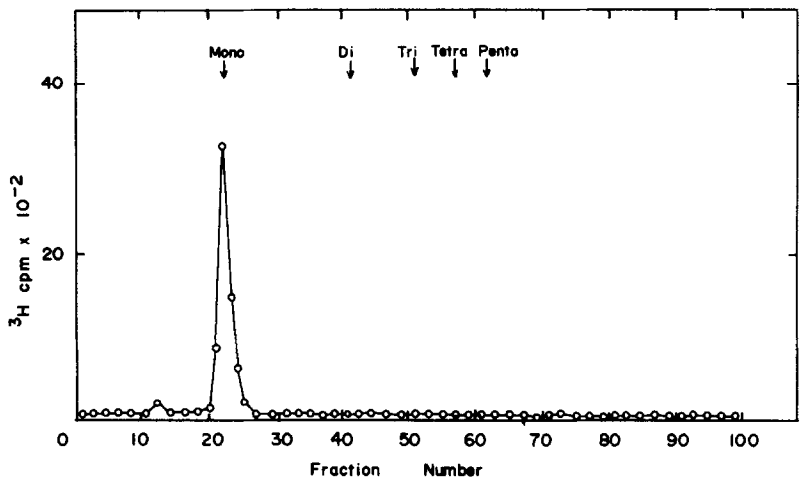

Fig. 1 DEAE-Sephadex A-25 column chromatography of the products by nucleolar RNA methylase after alkaline digestion [^{3}H-methyl]-labeled RNA from E.coli was digested in alkaline solution and chromatographed on a DEAE-Sephadex A-25 column as described under MATERIALS AND METHODS. Elution was performed by using 100 ml of a linear gradient from 0.1 to 0.5 M NaCl in 7 M urea at pH 7.8.

free from DNA methylase-activity, does not methylate homologous RNA from the same tumor cells of mice. Therefore, heterologous RNA from E.coli was employed as methyl acceptors during the purification of the present enzyme (5). In the present study we analyzed the methylation-product by this nucleolar enzyme.

In vitro [^{3}H-methyl]-labeled RNA was digested in an alkaline solution and analyzed by DEAE-Sephadex A-25 column chromatography in 7 M urea (8). All of the [^{3}H-methyl]-labeled materials after alkaline-digestion were eluted extensively in the mononucleotide fraction (Fig.1). This chromatographic behavior of alkaline-digests suggests that the products of nucleolar methylase do not contain 2'-O-[^{3}H-methyl]-labeled ribose, but accepted [^{3}H-methyl]-groups in their bases. Furthermore, [^{3}H-methyl]-labeled RNA products in vitro were digested completely with RNase T_2 and analyzed by two dimensional thin-layer chromatography. The autoradiograms shown in Fig.2 indicate only one spot of radioactive product. Only in the fifth position of cytidine residue(s) in

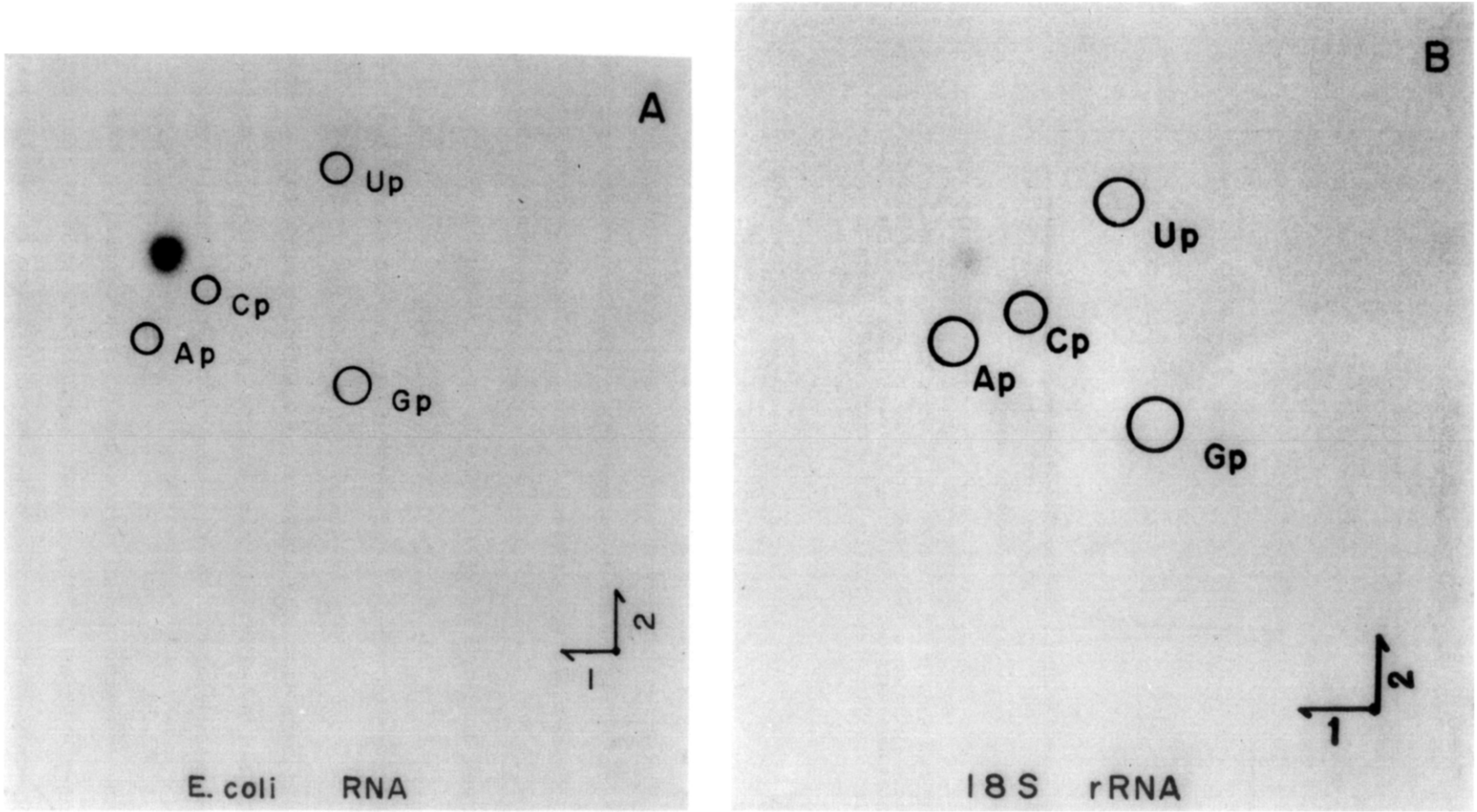

Fig. 2 Two dimensional thin-layer chromatography of the products in vitro by nucleolar methylase Several RNAs including E.coli RNA (A), 18S rRNA (B) and 28S rRNA (C) prepared from mouse tumor cells treated with cycloleucine were methylated by purified enzyme, digested with RNase T_2 and autoradiographed after the two dimensional thin-layer chromatography (7).

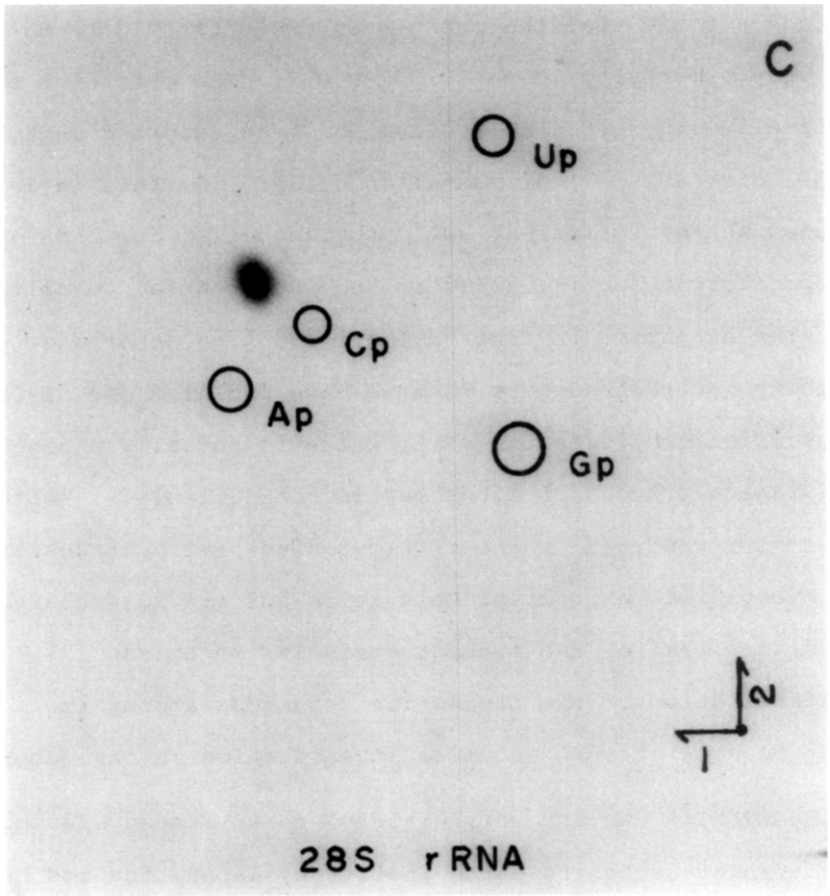

Fig. 2--Continued.

E.coli RNA does the purified nucleolar enzyme specifically transfer the methyl group.

To ascertain the substrate specificity under more physiological conditions, we decided to prepare the hypomethylated RNAs from mouse tumor cells according to the procedure by Amalric et al. (6). In the presence of cycloleucine both tRNA and rRNA were reported to be extensively undermethylated (more than 90%) (6,9). In contrast to the normally methylated homologous rRNA, both 18S and 28S rRNAs from cycloleucine-treated tumor cells were efficiently methylated by the nucleolar methylase. More methyl groups were transferred to hypomethylated rRNA than to tRNA (5). The methylated products of hypomethylated 18S and 28S rRNA were

analyzed by the same procedure employed for that of E.coli RNA. It was again found that the methylated products of both RNAs were exclusively 5-methylcytidine (Fig.2-B,C). The existence of the nucleoside 5-methylcytidine residue had been reported in both rRNA (10) and tRNA (11) of mammalian cells. So far, it is not well-known whether different RNA methylase exist for tRNA and rRNA separately or not. However, the preferential localization of the present enzyme in the nucleoli and high substrate specificity for rRNA suggest that we have isolated one of the base-specific methylases for rRNA, which is actually engaged in the maturation process of ribosomes in the nucleoli. Keith et al. (12) have reported on the tRNA(cytosine-5)methyltransferase from a cytoplasmic extract of Hela cells but its molecular weight differed from that of the present nucleolar methylase (5).

Determination of the nucleotide sequences around the methylation site of rRNA is under investigation in our laboratory.

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