TISSUE SPECIFIC DIFFERENCES IN THE 2'-*O*-METHYLATION OF EUKARYOTIC 5.8S RIBOSOMAL RNA

Ross N. NAZAR, Thomas O. SITZ and Harris BUSCH
Tumor By-Products Laboratory, Department of Pharmacology, Baylor College of Medicine, Houston, Texas 77025, USA

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1. Introduction

Although a great deal is known about protein synthesis, the means by which ribosomes carry out their multiple functions and the mechanisms for their control are not defined. The role of modified nucleotides, for example, is essentially unknown. About 3–4% of the nucleotides in eukaryotic ribosomal RNA are modified; 1–2% of the ribose residues are 2'-*O*-methylated and 10% of the uridylic acid residues are converted to pseudouridylic acid [1,2]. Some of these modifications are present in the entire RNA population while others are restricted to a fraction of the molecules [3,4]. Methylation appears to be essential in ribosome maturation [5] and ribose-methylation is largely or completely restricted to the mature RNA species [6]. It has been suggested that methylation may render crucial sequences resistant to cyclizing ribonucleases [7]. Alternately, the presence of partially methylated or modified sequences has suggested that they may exert some modulating or control activity.

Recent studies on the primary sequence of Novikoff ascites hepatoma 5.8S rRNA have revealed two sequences containing 2'-*O*-methylribose, A–A–U–U–Gm–C–A–Gp and G–G–Um–G–G–A–Up and two sequences containing pseudouridylic acid, C–ψ-Gp and ψ-Gp [8,9]. The 2'-*O*-methyl guanylic acid residue was present in molar amounts while Um was only found in about 20% of the molecules. Similarly, the ψ-Gp sequence was present in every molecule while C–ψ-Gp was only found in half molar amounts. An experimental approach to the role of modified nucleotides in ribosomal RNA requires an ability to alter the levels of modification for correlation with its biological function. As a preliminary approach towards understanding the role of these modified nucleotides, we have examined 5.8S rRNAs from various tissues of different growth rate and development in search of physiologically related changes.

2. Materials and methods

The tissues used in these studies were human, rat, mouse or chick in origin. Normal BALB/C mice and the transplanted DMBA(dimethylbenzanthracene)-induced mouse mammary tumors were kindly provided by Dr D. Medina. Mouse myeloma MPC-11 cell RNA, HeLa cells and secondary chick embryo cells were provided by Drs E. Murphy, P. N. Rao and J. Norris, respectively. Male Sprague Dawley rats were used for normal rat tissues and to maintain Novikoff hepatoma ascites cells. Cells were labeled with 5–50 mC: of [^{32}P]orthophosphate in vitro or in vivo as previously described [10]; for regenerating liver the isotope was injected interperitoneally 48 h after the partial hepatectomy and the animal was sacrificed 24 h later. Whole cell RNA was extracted with a phenol-SDS buffer at 65°C and the 5.8S rRNA was fractionated on polyacrylamide gel slabs [8,9]. The purified RNA was digested with pancreatic or T_1 ribonucleases and the resulting oligonucleotides were fractionated by two-dimensional electrophoresis [11]. The modified fragments were identified as previously reported [8].

3. Results and discussion

The autoradiographs in fig.1 compare the pancreatic or T_1 ribonuclease digestion 'fingerprints' of Novikoff hepatoma and normal rat liver 5.8S rRNAs. Both 'fingerprints' are identical except for quantitative differences in the methylation of G–G–U–G–G–A–Up to G–G–Um–G–G–A–Up. The Novikoff hepatoma contains little G–G–Um–G–G–A–Up and large amounts of the unmethylated equivalents G–G–Up and G–G–A–Up (fig.1A). In contrast, the normal rat liver contains a large amount of G–G–Um–G–G–A–Up and only traces of G–G–Up and G–G–A–Up. Table 1 shows that the hepatoma and normal liver RNAs both contained approx. 1 mol of Gm–Cp but only 0.2 and 0.7 mol of G–G–Um–G–G–A–Up, respectively. Table 2 shows that the pseudouridylic acid content was relatively constant. Each RNA which was analyzed contained approx. 1 mol of ψ-Gp and a half mol of C–ψ-Gp.

Table 1 summarizes the levels of methylation in twelve different tissues of human, rat, mouse or chick origin. In all cases the level of methylation for the Gm–Cp fragments was high; virtually every molecule of 5.8S rRNA appeared to contain this sequence. In contrast, the 2′-*O*-methyl uridine content varied widely; the highest was 0.72 mol in normal rat liver 5.8S rRNA and the lowest was 0.17 mol in HeLa cells. These differences were tissue specific rather than species specific; in the mouse, for example, kidney, liver and spleen had relatively high methylation levels, all greater than 0.6 mol. Pregnant mouse mammary gland and embryo RNA had intermediate levels of methylation and both of the malignant tissues contained little 2′-*O*-methyl uridine, 0.2 and 0.3 mol for the myeloma and mammary tumor, respectively.

These results indicate that while most nucleotide modifications in 5.8S rRNA are relatively constant in a large number of tissues, the level of 2′-*O*- methylation in the G–G–Um–G–G–A–Up sequence varies

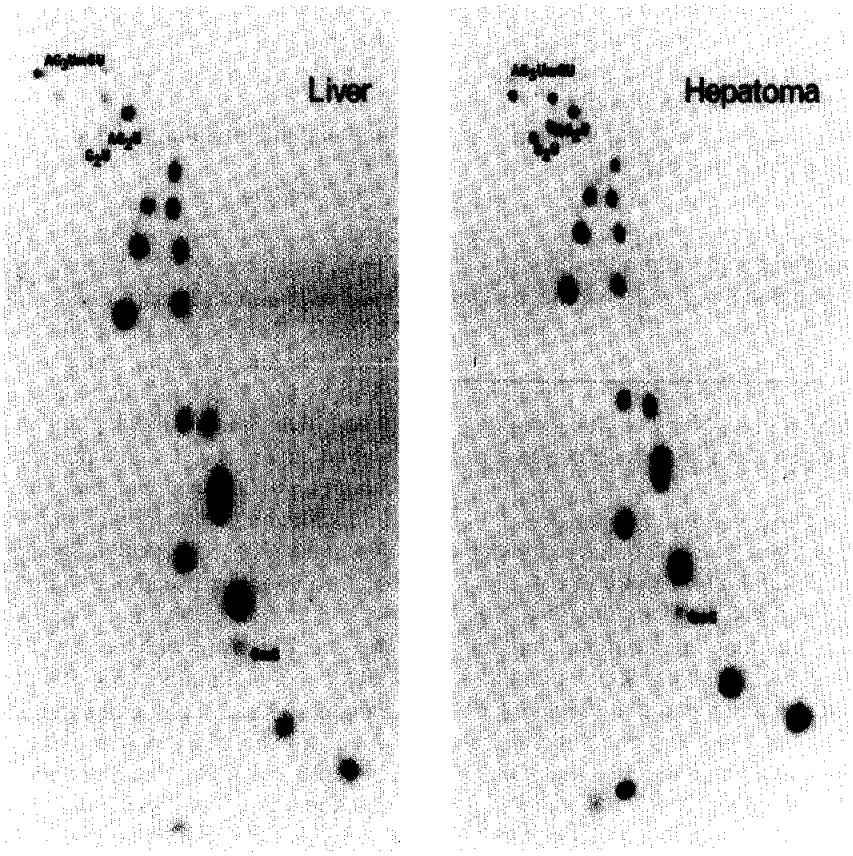

Fig.1A

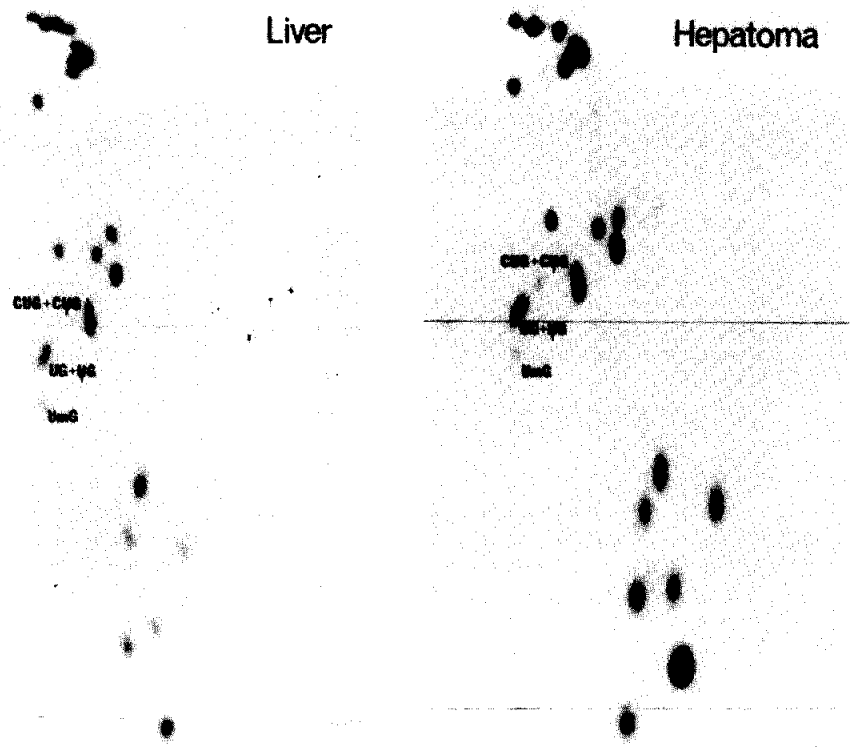

Fig.1B

Fig.1. Autoradiographs for two-dimensional fractionations of pancreatic (A) and T_1 (B) RNase digests of ^{32}P-labeled normal rat liver or Novikoff hepatoma 5.8S rRNA. Electrophoresis was from right to left on cellulose-acetate at pH 3.5, and from top to bottom on DEAE paper in 7% formic acid. The spots containing modified nucleotides are labeled to correspond to the text.

significantly. The role of this 2′-*O*- methyl uridylic acid residue and the significance of these differences, however, are unclear. Ribosomal RNA precursors in cells with low methylation such as the Novikoff hepatoma appear to be processed normally and do not require 2′-*O*-methyl uridylic acid for maturation. Tissues with high levels of methylation such as normal rat liver carry out protein synthesis efficiently and do not appear to be inhibited by methylation. Any effects, therefore, must be more subtle and not detectable by such gross measurements. Um may stabilize the secondary structure of 5.8S rRNA as has been suggested for tRNA [12] and this may alter its activity within the ribosome. Alternately, the 2′-*O*-methyl uridylic acid containing sequence may form a binding site for some class of messenger or tRNA or specific protein with the methylated nucleotide controlling this interaction.

Aberrant nucleic acid methylation has previously been reported in tumor tissues and has been suggested as a factor in malignant transformation [13]. The apparently excessive tRNA methyltransferase activities in tumors [14] have led to the assumption that overmethylation may occur in oncogenesis. In contrast, the present studies suggest some site specific undermethylation in the ribosomal RNAs of malignant cells. In each series of related tissues, the lowest levels of methylation were found in the tumor. However, there was some correlation with growth rate and it is not clear whether this change is required in malig-

Table 1
2′-*O*-Methylated nucleotides in 5.8S ribosomal RNA from different tissues

Source of 5.8S rRNA	GGUmGGAUp (moles)	GmCp (moles)
Normal rat liver	0.72	1.03
Normal mouse liver	0.69	–
Normal mouse spleen	0.62	–
Normal mouse kidney	0.61	1.16
Pregnant mouse mammary gland	0.51	0.98
Secondary chick embryo	0.49	1.04
Mouse embryo	0.40	0.99
Transplanted DMBA induced mouse mammary tumor	0.34	–
Regenerating rat liver	0.31	0.90
Novikoff Ascites hepatoma cells	0.23	0.90
Mouse myeloma MPC-11	0.18	1.02
HeLa cells	0.17	0.96

[32] P-labeled 5.8S rRNA was digested with T_1 RNase and the oligonucleotide fragments were separated as described in fig.1. Fragments containing 2′-*O*-methylated nucleotides were identified as previously described [8,9]. The molar yields were calculated in assuming 1 GGUGGAU and 8 GC oligonucleotide fragments per molecule [9]. Mouse embryo and mammary gland RNA were prepared from 12–15 day pregnant mice.

nant transformation. Undermethylation has also been described in specific sequences of ribosomal high mol. wt RNAs [4,7,15] but no tissue comparison was made. Therefore, it is also unclear whether the differences in methylation reported here are unique or represent a coordinated series of changes in ribosomal RNA in general. Nevertheless, the observation remains an attractive experimental system for examining the enzymology, function and control of rRNA methylation and possible relationships to cancer.

Table 2
Pseudouridylic acid residues in 5.8S ribosomal RNA from different tissues

Source of 5.8S rRNA	C–ψ-Gp (moles)	ψ-Gp (moles)
HeLa cells	0.59	0.98
Mouse myeloma MPC-11	0.64	0.82
Novikoff Ascites hepatoma cells	0.54	1.01
Secondary chick embryo	0.39	1.20
Normal mouse liver	0.62	0.94

[32] P-labeled 5.8S rRNA was digested with T_1 RNase and the oligonucleotide fragments were separated as described in fig.1. Fragments containing pseudouridylic acid were analyzed as previously described [8,9]. The molar yields were calculated assuming 1 CUG and 3 UG oligonucleotide fragments per molecule [9].

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