Comparison of nucleotide sequences of large T1 ribonuclease fragments of 18S ribosomal RNA of rat and chicken

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Received 5 September 1979

ABSTRACT

Nucleotide sequences of large T1 ribonuclease fragments of 18S ribosomal RNA of Novikoff rat ascites hepatoma cells and chicken lymphoblastoid cells were determined and compared. Among the 19 large T1 ribonuclease fragments examined of rat 18S ribosomal RNA, 12 fragments were found to be the same in chicken 18S ribosomal RNA. Three fragments of rat 18S ribosomal RNA were not found among large T1 ribonuclease fragments of chicken 18S ribosomal RNA. Four fragments of rat 18S ribosomal RNA were found to be changed in chicken 18S ribosomal RNA. All the changes were point mutations except the change in the largest T1 ribonuclease fragment 1 which is 21 nucleotides long. 2'-O-methylation at the center of the fragment was lost in chicken 18S ribosomal RNA; all the other nucleotides were the same.

INTRODUCTION

"Homochromatography" fingerprinting (1) is useful for comparison of nucleotide sequences of different RNA species, and the method has been used to compare T1 RNase digests of RNA of bacteriophages (2), tumor viruses (3), and several mammalian ribosomes (4).

Khan and Maden (5) compared rRNA of HeLa cells, Xenopus laevis, and chick embryo fibroblasts after combined T1 plus pancreatic RNase digestion. We have compared large T1 RNase fragments of 18S and 28S rRNA of rat, mouse, Chinese hamster, and man by homochromatography fingerprinting (4,6).

MATERIALS AND METHODS

Novikoff rat hepatoma cells were used as the source of rat 18S rRNA. Lymphoblastoid cells (MSB-1) (7) provided by Dr. Stubblefield were used as the source of chicken 18S rRNA.

Nucleotide sequences of the oligonucleotides were determined as described previously (4,6,8).

RESULTS

Figure 1 shows homochromatography fingerprints of large T1 RNase fragments of 18S rRNA of rat (top) and chicken (bottom). Well separated large fragments of rat 18S rRNA were numbered as previously (6).

Large T1 RNase fragments of chicken 18S rRNA which have the same nucleotide sequence as the T1 RNase fragments of rat 18S rRNA were given the same numbers. T1 RNase fragments of chicken 18S rRNA which were changed from rat 18S rRNA fragments were numbered with primes. Fragment 1' of chicken 18S rRNA moved slightly higher than fragment 1 of rat 18S rRNA in the second dimension of homochromatography. Fragment 3' migrated faster in the first dimension of electrophoresis and higher in the second dimension of homochromatography than fragment 3. Fragments 5' and 16' migrated slower in the first dimension than fragments 5 and 16, respectively. Fragments 6, 11 and 12 of rat 18S rRNA were not found among large T1 RNase fragments of chicken 18S rRNA.

The nucleotide sequences of the large T1 RNase fragments of rat and chicken 18S rRNA are summarized in Table I. The total number of nucleotides of rat 18S rRNA in Table I is 246 excluding fragment 6 which is a part of fragment 3 (9). This constitutes about 14% of the whole molecule assuming the total nucleotide number of 18S rRNA is 1,800. Between rat and chicken 18S rRNA, Table I shows three point mutations in fragments 3, 5, and 16 and a 2'-O-methylation modificational change in fragment 1 and suggests at least two point mutations in fragments 11 and 12 among the 246 nucleotides of rat 18S rRNA examined.

DISCUSSION

The present study has shown that the nucleotide sequence of 18S rRNA is highly conserved between rat and chicken. Large T1 RNase fragments of 28S rRNA of rat and chicken were also compared by homochromatography fingerprinting, but the two fingerprints were very different (Fuke, M., unpublished observation). It was

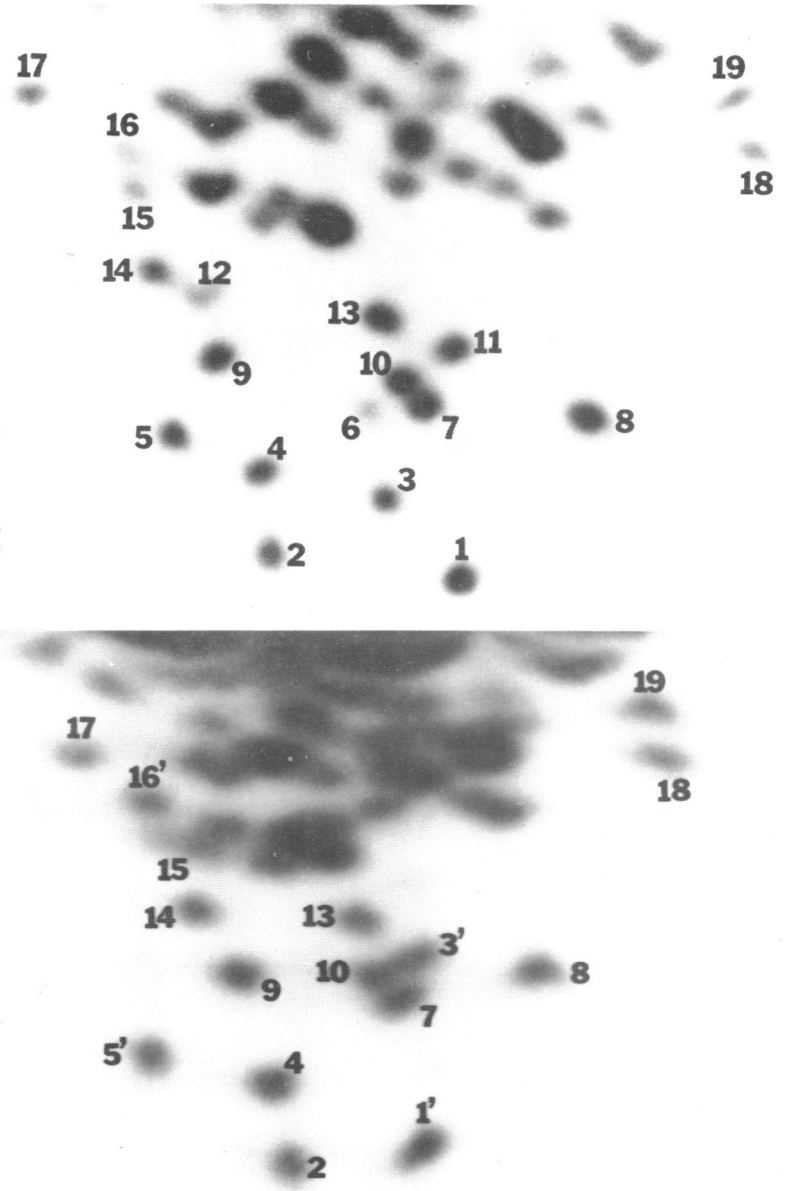

Figure 1. Two-dimensional homochromatography fingerprinting of T1 RNase fragments of 18S rRNA of rat (top) and chicken (bottom). The first dimension (electrophoresis on cellulose acetate) was run from left to right and the second dimension (homochromatography) was run from bottom to top.

Fragment	Nucleotide Sequence of Rat 18S rRNA Fragments	Change in Nucleotide Sequence of Chicken 18S rRNA Fragments
1	U-C-C-A-C-U-U-U-A-A-Am-U-C-C-U-U-U-A-A-C-Gp	Same except 2'-O-methylation is missing
2	A-A-Ā-A-A-U-A-A-C-A-A-U-A-C-A-Gp	Same
3	U-C-C-C-C-C-A-A-C-U-Um-C-U-U-A-Gm-A-Gp	Third nucleotide C ⟶ G
4	C-Am-A-A-U-U-A-C-C-C-A-C-U-C-C-C-Gp	Same
5	A-U-C-A-A-A-A-C-C-A-A-C-C-C-Gp	Second nucleotide U ⟶ C
6	U-C-C-C-C-C-A-A-C-U-Um-C-U-U-A-Gp	Missing
7	C-A-A-U-U-A-U-U-C-C-C-C-A-U-Gp	Same
8	U-U-U-U-C-A-U-U-A-A-U-C-A-A-Gp	Same
9	C-U-A-C-C-A-C-A-U-Cm-C-A-A-Gp	Same
10	C-C-C-U-A-U-C-A-A-C-U-U-U-C-Gp	Same
11	C-U-C-C-U-C-U-C-C-U-A-C-U-U-Gp	Missing
12	A-C-C-C-C-C-C-U-U-C-C-C-Gp	Missing
13	C-U-C̆Am-U-U-A-A-A-U-C̆-A-Gp	Same
14	A-A-A-C-C-U-C-A-C-C-C-Gp	Same
15	A-A-C-X-C-A-C-A-C-Gp[a]	Same
16	A-A-A-C-C-A-A-A-Gp	Sixth nucleotide A ⟶ C
17	C-A-C-C-A-C-C-A-Gp	Same
18	U-U-C-U-A-U-U-U-U-Gp	Same
19	Um-U-U-A-C-U-U-U-Gp	Same

Table I. Nucleotide sequences of large T1 RNase fragments of rat 18S rRNA and their changes in chicken 18S rRNA. Fragments were purified by homochromatography fingerprinting and numbers were given as shown in Figure 1.

a. proposed by Maden et al (12).

found that 18S rRNA sequence is conserved better than 28S rRNA sequence among mammals (4) and the same result was obtained between rat and chicken. These results are in agreement with the findings of Bendich and McCarthy (10) and Gerbi (11) by hybridization analysis.

All the detected changes between rat and chicken 18S rRNA sequences could be attributed to point mutations except the differences in fragments 1 and 1'. 2'-O-methylation found at the middle of fragment 1 of rat 18S rRNA was not found at all in fragment 1' of chicken 18S rRNA. All the other nucleotide sequences of the two fragments were the same. This raises questions about the structures that methylation enzymes recognize.

2'-O-methylation at AmU in fragment 1 of rat 18S rRNA was missing in chicken 18S rRNA but 2'-O-methylation at the same AmU in fragment 13 was conserved in rat and chicken 18S rRNA. Fragment 1 can form a tight secondary structure with 6 Watson-Crick pairs and the 2-O-methyl is located at the tip of the stem structure.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Elton Stubblefield for his kind supply of chicken lymphoblastoid cell line. This work was supported by the Cancer Research Center Grant CA 10893, P.9, awarded by the National Cancer Institute, DHEW.

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