Comparison of RNase T, Fingerprints of U1, U2, and U3 Small Nuclear RNA's of HeLa Cells, Human Normal Fibroblasts, and Novikoff Hepatoma Cells

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ABSTRACT

To determine whether there are differences between the U1, U2, and U3 small nuclear RNA's of human cancer cells (HeLa cells) and human normal fibroblasts (IMR-90 cells), and between these uridine-rich small nuclear RNA's of human and Novikoff hepatoma cells, the cells were first incubated in Eagle's medium with [32P]P, to label these RNA's uniformly.

No differences were found between the RNase T, fingerprints of the purified U1, U2, and U3 RNA's of HeLa cells and IMR-90 cells. The RNase T, fingerprints of U1 RNA's from human tissues were very similar to that of the U1 RNA of Novikoff hepatoma cells. The RNase T, fingerprints of U2 and U3 RNA's from human tissues had many similarities to those of Novikoff hepatoma cells, but a few differences were found, such as a point mutation of the U-U-Gp in the rat U2 RNA to A-U-Gp (U → A) in human U2 RNA. Unlike the three U3 RNA's of Novikoff hepatoma cells, U3 RNA from human tissues appears to be only one species.

These results indicate that U1, U2, and U3 RNA's of human cancer cells are essentially the same as those of human normal cells. In addition, the uridine-rich small nuclear RNA's appear to be conserved through evolution.

INTRODUCTION

The snRNA's, which have been purified and sequenced in this laboratory (21), have been reported recently to be implicated in processing of precursor RNA's (10, 13, 23). Lerner et al. (10) suggested that a U1 ribonucleoprotein particle is involved in splicing hnRNA to mature RNA. The possibility has been suggested that U2, U4, U5, and U6 snRNA's may be also involved in processing of hnRNA, since they are associated with hnRNA (6, 7, 9, 14) and are precipitated by the same antibodies (11). U3 RNA is localized to the nucleolus and was shown to be hydrogen bonded to precursor RNA's (15, 28). Accordingly, U3 RNA might be involved in cleaving precursor rRNA's as suggested previously (18).

The present study was carried out in order to determine whether there are differences between U1, U2, and U3 RNA's of human cancer cells and human normal fibroblasts using RNase T, fingerprinting. In this study, U1, U2, and U3 RNA's of Novikoff hepatoma cells were also compared with those from human cells.

MATERIALS AND METHODS

Log-phase cultures of HeLa cells and IMR-90 cells were grown in Eagle's minimal essential medium supplemented with 5% fetal calf serum and 5% calf serum and in McCoy's medium 5A supplemented with 10% fetal calf serum and 10% calf serum, respectively.

Preparation of 32P-labeled 4 to 8S RNA's from HeLa Cells, IMR-90 Cells, and Novikoff Hepatoma Cells. The harvested Novikoff hepatoma cells were incubated in Eagle's medium with 32P as described previously (12). For HeLa cells and IMR-90 cells, the procedure used was similar to that described for Novikoff hepatoma cells, except that the Eagle's medium was supplemented with 20%, rather than 10%, fetal calf serum. The precipitation of citric acid nuclei and isolation of RNA with sodium dodecyl sulfate-phenol were carried out as described previously (22). The 4 to 8S RNA fraction was separated by sucrose gradient centrifugation (22).

Purification of U1, U2, and U3 RNA's. Nuclear 4 to 8S RNA obtained by sucrose gradient centrifugation was subjected to electrophoresis on 10% polyacrylamide gels (40 x 20 cm) containing 7 M urea (5). To separate the U1, U2, and U3 RNA's from other snRNA species, electrophoresis was carried out until the 4S RNA migrated to the bottom of the gel. The RNA's were visualized by methylene blue staining and/or autoradiography. The U1, U2, and U3 RNA bands were excised, and RNA was extracted as described by Winter and Brownlee (25). The purity of these RNA's was sufficient to carry out the RNase T, fingerprinting. If further purification was required, the RNA's were rerun on 12% acrylamide-7 M urea gels.

Chemicals. RNase T1, RNase U2, and pancreatic RNase were obtained from Calbiochem, La Jolla, Calif. RNase T2 was obtained from Sankyo, Tokyo. Nuclease P1 was purchased from Sigma Chemical Co., St. Louis, Mo. The PEI-cellulose sheets were obtained from Brinkman Instruments, Westbury, N. Y.

Fingerprinting of RNA Fragments. Complete digestion of RNA with RNase T1 and separation of the resulting oligonucleotides on cellulose acetate were carried out as described by Brownlee et al. (2). The second dimension was carried out by an improved homochromatography on PEI-cellulose sheets using the C-15 homomixture (3).

RESULTS

Fig. 1 shows an autoradiograph of the gel obtained with 32P-labeled snRNA's from HeLa cells and IMR-90 cells. It has been reported already that snRNA's of Novikoff hepatoma cells separate well under the conditions used (16, 17). snRNA's of HeLa cells and IMR-90 cells also separated well under the
same conditions. Although U3 RNA of Novikoff hepatoma cells separated into 3 distinct bands as shown previously (17), the U3 RNA of HeLa cells and IMR-90 cells migrated as a single band as shown in Fig. 1.

RNase T1 Catalog of U1 RNA. Complete digestion of Novikoff hepatoma U1 RNA with RNase T1 and separation of these fragments by electrophoresis and homochromatography produced 22 fragments (Fig. 2) as described previously (19). Complete RNase T1 digestion of the U1 RNA from HeLa cells and IMR-90 cells also yielded 22 fragments as shown in Fig. 2. The RNase T1 fingerprint of U1 RNA from HeLa cells was the same as that of IMR-90 cells. The mobility of 3′-terminal Fragment 17 of HeLa cells was more variable than other oligonucleotides. The RNase T1 fingerprints of U1 RNA’s from HeLa cells and IMR-90 cells were very similar to that of Novikoff hepatoma cells except that the mobility of Fragment 17 was clearly different.

RNase T1 Catalog of U2 RNA. Fig. 3 shows the autoradiograph of uniformly labeled U2 RNA digested with RNase T1 and fractionated by electrophoresis and homochromatography. The RNase T1 fingerprint of U2 RNA from HeLa cells was the same as that of IMR-90 cells. When compared to the RNase T1 fingerprint of U2 RNA of Novikoff hepatoma cells, the RNase T1 fingerprints of U2 RNA’s from human cells had many similarities. However, Fragment 7 of the Novikoff U2 RNA was absent from U2 RNA’s of the human cells. Fragment 6′ was found in the human cells but not in Novikoff hepatoma cells. When digested further with RNase T2 and separated by the Wyatt system (27), Fragment 6′ yielded Ap, Up, Gp; it yielded Ap, Up-Gp after digestion with RNase U2. These data show the sequence of Fragment 6′ in human U2 RNA is A-U-Gp. Thus, there is a point mutation of the U-U-Gp (T-7) in the rat (24) to A-U-Gp (T-6′) (U → A) in the human.

RNase T1 Catalog of U3 RNA. As shown previously (17, 18), complete digestion of U3A and U3B RNA’s of Novikoff hepatoma cells with RNase T1 produced 27 fragments and 25 fragments, respectively, that were separated by electrophoresis and homochromatography (Fig. 4). The RNase T1 fingerprints of U3 RNA of HeLa cells and IMR-90 cells yielded 26 fragments (Fig. 4).

There was no difference between RNase T1 fingerprints of U3 RNA’s from HeLa cells and IMR-90 cells. The RNase T1 fingerprint for U3 RNA from humans was similar to those of U3A and U3B RNA’s of Novikoff hepatoma cells, but somewhat different. Fragments B, C, E, F, G, and H in U3A RNA and Fragments 17, 18, 22, and 25 in U3B RNA of Novikoff hepatoma cells were absent from human U3 RNA. Fragments 17, 24′, C, and H′ in human U3 RNA were not found in Novikoff hepatoma cells. There was only one detectable U3 RNA species in human cells which differs in some respects from both rat U3A and U3B RNA’s. Chart 1 shows the sequence of U3A RNA of Novikoff hepatoma cells (18); the underlined areas show the few oligonucleotides which differ from those of human U3 RNA.

DISCUSSION

The RNase T1 fingerprints of U1, U2, and U3 RNA’s from HeLa cells were identical with those of human fibroblasts (IMR-90 cells). This result indicates that the nucleotide sequences of U1, U2, and U3 RNA’s in human cancer cells are essentially the same as those of normal human tissue. The RNase T1 fingerprint of U1 RNA from humans was very similar to U1 RNA from Novikoff hepatoma cells except that the mobilities of the 3′-terminal Fragment 17 were different. Brantlant et al. (1) have shown recently that only 2 base substitutions were observed from the rat to the human in nucleotide sequences of U1A RNA’s. One base substitution C→U was observed at position 159 near the 3′-terminus. The difference in the mobilities of Fragment 17 in U1 RNA’s may result from the base substitution at position 159. Earlier studies of Lerner and Steitz (11) showed that the RNase T1 fingerprint of mouse U1 RNA was similar to that of the rat (19).
The RNase T1 fingerprints of U2 RNA's from HeLa cells and the IMR-90 cells were also similar to that of Novikoff hepatoma cells. Lerner and Steitz (11) have shown also that U2 RNA of the mouse has a fingerprint similar to that of the rat (24).

However, Fragment 6' A-U-Gp was found in U2 RNA from human cells instead of Fragment 7 U-U-Gp. Fragment 16 (A-A-C-U-A-Gp) was found in lower yield in HeLa cell and IMR-90 U2 RNA compared to Novikoff hepatoma. The reason for this is not clear. One possibility is that there is heterogeneity in U2 RNA resulting in submolar yields of Spot 16; however, no other evidence is available to suggest heterogeneity of U2 RNA's.

The RNase T1 fingerprints of U3 RNA from HeLa cells and IMR-90 cells were somewhat different from those of U3A and U3B RNA's for Novikoff hepatoma cells. It has been shown (17) that there are 3 U3 RNA species in Novikoff hepatoma cell nuclei. However, the results obtained from RNase T1 fingerprints of U3 RNA's of HeLa cells and IMR-90 cells indicate that human U3 RNA is mainly one species. In addition to studies on the mammalian U3 RNA, Wise and Weiner (25) noted recently that there are large regions of homology of the D2 RNA of Dictyostelium and the Novikoff hepatoma U3B RNA (17) in which over 40% of the sequences were 75% or more homologous. These results strongly support earlier suggestions that U1, U2, and U3 RNA's are conserved through evolution (8). However, there appears to be less conservation of the U3 RNA sequence inasmuch as there were more differences between the oligonucleotides of the U3 RNA of the HeLa and Novikoff species.

Ringuette et al. (20) have found that one snRNA purified from the chromatin of SV40-transformed WI-38 human fibroblasts stimulated the transcription of chromatin in homologous isolated nuclei as well as in nuclei of untransformed human and monkey cells. It seems possible that some RNA's may play some role in differentiation or development (4, 20), but their precise function requires more definitive studies.

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REFERENCES

Fig. 2. Autoradiograph of a 2-dimensional separation of a complete RNase T1 digest of 32P-labeled U1 RNA's from HeLa cells (A), IMR-90 cells (B), and Novikoff hepatoma cells (C). Electrophoresis was carried out in the first dimension on cellulose acetate (C. A.), pH 3.5 (5% acetic acid-0.01% pyridine-7 M urea). The second dimension was homochromatography (H. C.) on PEI-cellulose sheets.

Fig. 3. Autoradiograph of a 2-dimensional separation of a complete RNase T1 digest of 32P-labeled U2 RNA's from HeLa cells (A), IMR-90 cells (B), and Novikoff hepatoma cells (C). Electrophoresis and homochromatography were performed as described in the legend to Fig. 2. Fragments 10 and 11, and Fragments 12 to 14 comigrated on PEI-cellulose sheets. The identity of these fragments was determined by digestion of these fragments with RNase U2 or pancreatic RNase. C. A., cellulose acetate; H. C., homochromatography.
Comparison of RNase T1 Fingerprints
Fig. 4. Autoradiograph of a 2-dimensional separation of a complete RNase T1 digest of $^{32}$P-labeled U3 RNA's from HeLa cells (A), IMR-90 cells (B), and Novikoff hepatoma cells (C, D). Electrophoresis and homochromatography (H.C.) were performed as described in the legend to Fig. 2. C.A., cellulose acetate.
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