Vol. 98, No. 4, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 27, 1981 Pages 1076-1083

PSEUDOURIDINE RESIDUES IN THE 5'-TERMINUS OF URIDINE-RICH NUCLEAR RNA I (U1 RNA)

Ramachandra Reddy, Dale Henning and Harris Busch

Department of Pharmacology, Baylor College of Medicine
Houston, Texas 77030

Received January 13,1981

SUMMARY

The primary nucleotide sequence was reported earlier for U1 RNA (Reddy et al, (1974) J. Biol. Chem. 249, 6486-6494), an snRNA implicated in splicing of HnRNAs. In view of the presence of homologous pseudouridine (ψ) residues in 5'-ends of several highly conserved U-snRNAs and the recent report of modified bases in the U1 RNA structure (Branlant et al, (1980) Nucleic Acids Res. 8, 4143-4154) a study was made for the presence of ψ and other modified nucleotides in the 5'-end of the U1 RNA. Identification of ψ residues at positions 6 and 7, shows the 5'-sequence of U1 RNA is: $m_3^{2,2,7}$GpppAm-Um-A-C-ψ-ψ-A-C-C-U-G-G-C-A-G-G-G-G-A-G-A-U-A-C. The ψ residues in place of U at positions 6 and 7 may affect the binding of U1 RNA at intron-exon splice junctions.

INTRODUCTION

The U-snRNAs previously described and sequenced in this laboratory (1-14) were shown to be part of snRNP particles (15-18). Interest in U-snRNAs was increased by reports that some snRNAs are hydrogen-bonded to premessenger RNA (19,20) and that some RNAs were present in the RNP particle complexes containing premessenger RNA (21-24). The U-snRNAs, particularly U1 RNA were implicated in properly aligning splice junctions (25-29). The 5'-end sequence of U1-snRNA determined in our laboratory was found to be complementary to several introns at the splice junctions (25,26,28,29).

Abbreviations used: U1 RNA, uridine rich nuclear RNA 1; U-snRNA, uridine rich small nuclear RNA; and snRNP, small nuclear ribonucleoprotein particle.

0006-291X/81/041076-08$01.00/0

In studies on homologies of snRNAs (10,11,30) sequenced in this laboratory, it was noted that ψ residues were common to the 5'-ends of U2,U3,U4 and U5 RNAs. Branlant et al (31) provided modifications to the U1 RNA sequence suggesting the U residues at positions 6 and 7 of U1 RNA were modified. Since this region of U1 RNA is implicated in recognizing and aligning splice junctions, these modifications may be important. These modified nucleotides at positions 6 and 7 were found to be pseudouridine residues. The presence of ψ in place of U residues at positions 6 and 7 of U1 RNA may affect the binding between U1 RNA and HnRNA.

MATERIALS AND METHODS

The harvested Novikoff hepatoma ascites cells or HeLa cells were incubated and labeled with [^{32}P] orthophosphate as described previously (32). Preparations of citric acid nuclei, isolation of RNA, fractionation of 4 to 8S RNA was done as reported earlier (33). Nuclear 4-8S RNA was subjected to electrophoresis on 10% acrylamide, 7 M urea, pH 8.3 gels (34) and the U1 RNA band visualized by autoradiography was excised; U1 RNA was extracted and precipitated (35). The RNA was digested with T_1-RNase and finger-printed as described by Brownlee et al (36). The kinase labelling of U1 RNA fragments was done as described by Donis-Keller et al (34) and wandering spot analysis (37) was performed to obtain nucleotide sequences of oligonucleotides. ψ residues and other nucleotides were analysed with the Wyatt system (38).

RESULTS

Fig. 1A shows the T_1-RNase fingerprint of Novikoff hepatoma U-1 RNA; electrophoresis was on cellulose acetate in the first dimension and with the 7% formic acid system on DEAE-cellulose paper in the second dimension. All the oligonucleotides were separated by this method except T-18 to T-21, which did not move in the second dimension. Using homochromatography in the second dimension (Fig. 1B) these four oligonucleotides

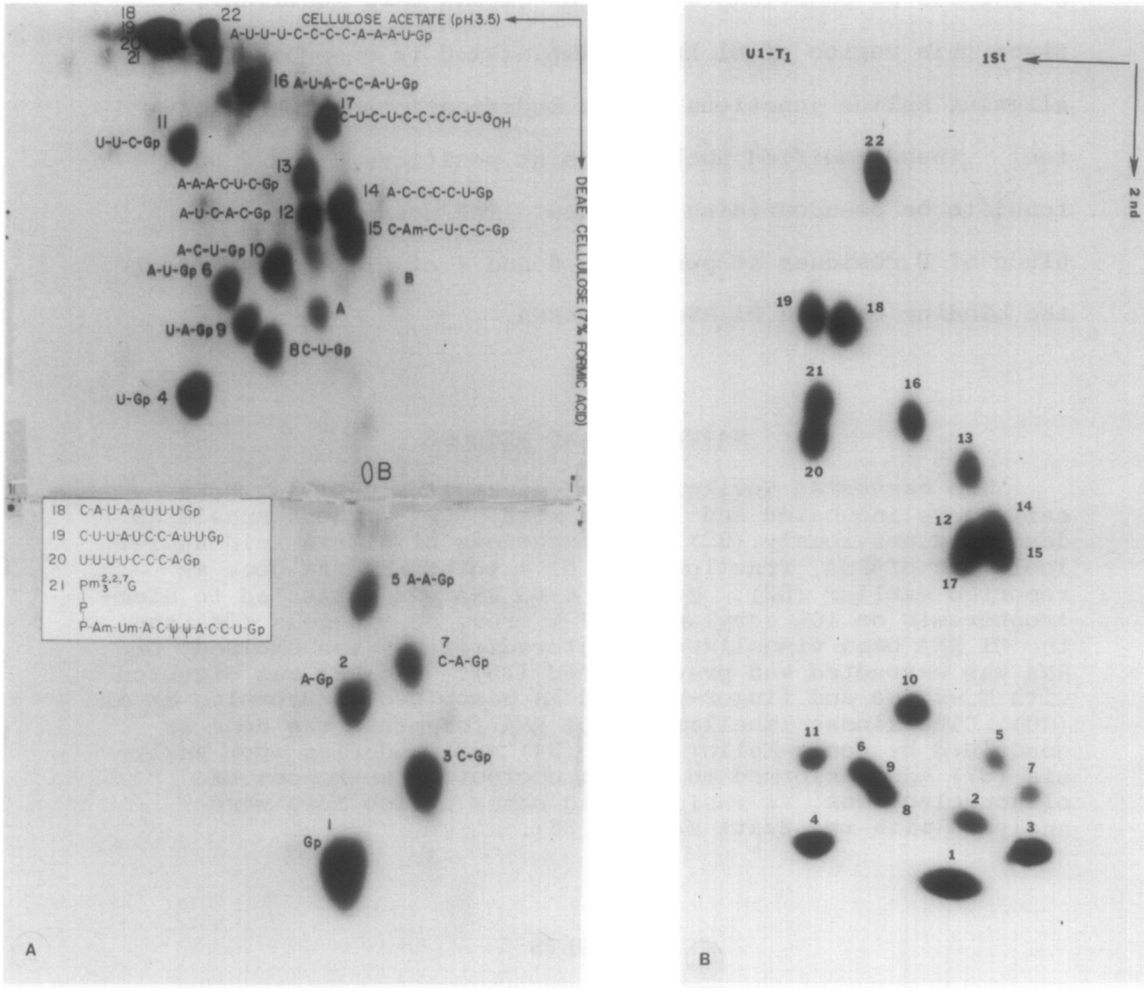
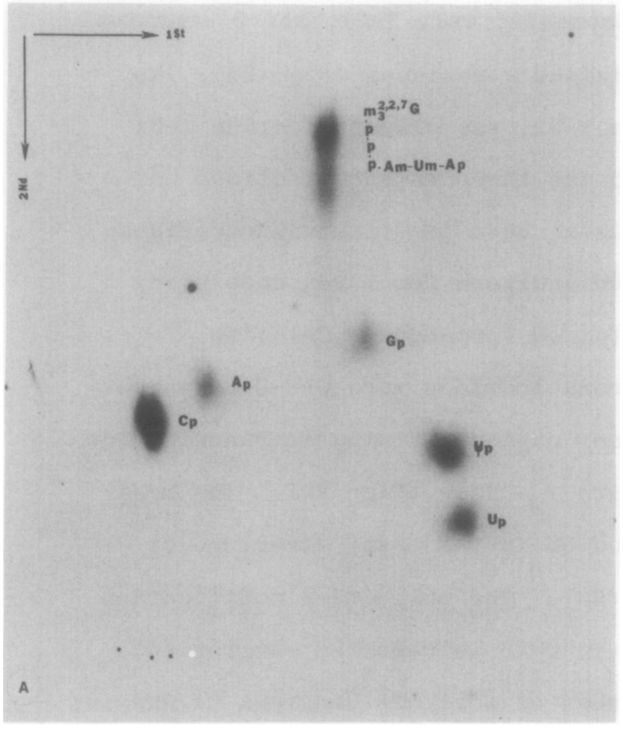


Figure 1 Autoradiographs of a two-dimensional separation of a complete T_1-RNase digest of ^{32}P-labeled U1 RNA Novikoff hepatoma. Electrophoresis was carried out in the first dimension on cellulose acetate pH 3.5. The second dimension was electrophoresis on DEAE-cellulose paper (Fig. 1A) or homochromatography (36, 37) on DEAE-cellulose plates using C-15 homomixture (Fig. 1B).

(T-18 to T-21) were separated. The sequences of oligonucleotides were derived from earlier data (3) and from wandering spot analysis (37) of 5'-end labeled oligonucleotides. These sequences are in agreement with the sequences confirmed by Branlant et al (31).

The T_1-RNase oligonucleotides of U1 RNA containing U residues were analysed for modified nucleotides using the Wyatt system (38) and electrophoresis; only T-21, the 5'-terminal oligonucleotide of U1-RNA contained Ψ residues (Fig. 2A). To localize the Ψ residues within T-21, the oligonucleotide T-21 was digested with U2 RNase and the three tetranucleotides found earlier (3) were separated. When these tetranucleotides were digested with T_2-RNase and analysed for Ψ residues using the Wyatt system (38) one contained Ψ residues, C-Ψ-Ψ-Ap (Fig. 2B). The other two tetranucleotides were C-C-U-Gp which contained only unmodified U, and 'cap' containing tetranucleotide which could not be digested with T_2 RNase (Fig. 2B). The modified nucleotides $m_3^{2,2,7}$G, Am and Um in the 'cap' structure of U1 RNA were reported earlier (39). The only other modification observed in U1 RNA was the Am in T-15 as reported earlier (3). With these results, the sequences of Novikoff hepatoma U1 RNA is:

$m_3^{2,2,7}$G-ppp-Am-Um-A-C-Ψ-Ψ-A-C-C-U-G-G-C-A-G-G-G-G-A-G-A-U-A-C-
C-A-U-G-A-U-C-A-C-G-A-A-G-G-U-G-G-U-U-U-U-C-C-C-A-G-G-G-C-G-A-
G-G-C-U-U-A-U-C-C-A-U-U-G-C-Am-C-U-C-C-G-G-A-U-G-U-G-C-U-G-A-C-
C-C-C-U-G-C-G-A-U-U-U-C-C-C-C-A-A-A-U-G-C-G-G-G-A-A-A-C-U-C-G-
A-C-U-G-C-A-U-A-A-U-U-U-G-U-G-G-U-A-G-U-G-G-G-G-G-A-C-U-G-C-G-
U-U-C-G-C-G-C-U-C-U-C-C-C-C-U-G$_{OH}$



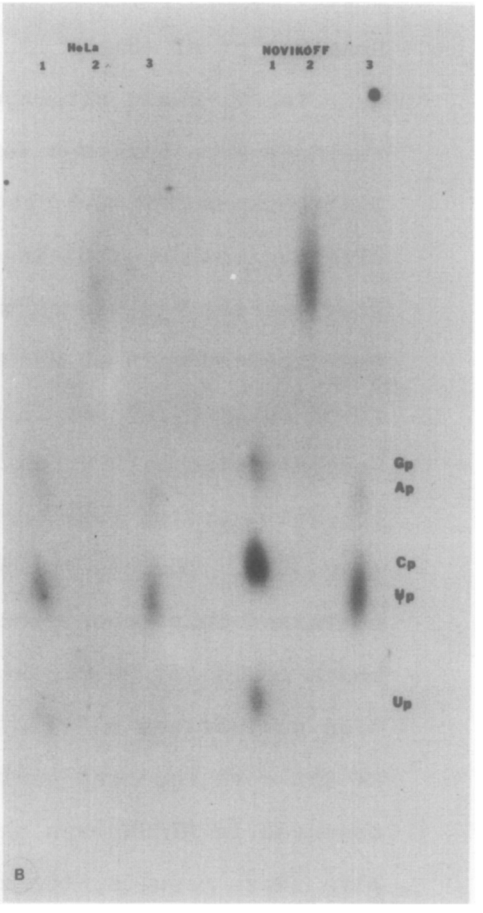


Figure 2A Autoradiograph of a complete T_2-RNase digest of ^{32}P-labeled oligonucleotide T-21 of U1 RNA. Electrophoresis was carried out on Whattman 3 MM paper at pH 3.5 in the first dimension and second dimension was descending chromatography in isopropyl alcohol/HCl/H_2O (680:176:144,v/v).

Figure 2B Analysis of oligonucleotides for Ψ residues. U_2-RNase digestion products of T-21 were digested with T_2-RNase and fractionated on 3 MM paper by Wyatt system (28). The numbers 1,2, and 3 correspond to the U_2 RNase digestion products C-C-U-Gp, $m_3^{2,2,7}$Gppp Am-Um-Ap and C-Ψ-Ψ-Ap, respectively of HeLa (left) and Novikoff hepatoma (right).

DISCUSSION

Following initial studies from this laboratory (3) on Novikoff hepatoma U1 RNA, a highly conserved nuclear RNA, Branlant et al (31) showed that there were only minor differences in this structure in human, chicken and rat molecules. The modification Am at 71, the "cap" structure at the 5'-end, and the two ψ residues at position 6 and 7 also appear to be conserved. Our present data shows that positions 6 and 7 contain ψ residues in Novikoff hepatoma and HeLa cells. The U residues at position 6 and 7 of chicken U1 RNA are also modified (31) and are probably ψ residues. The significance of these modifications is not understood at present, but the presence of these modifications in U1 RNA sequence implicated in recognizing splice junctions (25-29) makes the identification of these modifications important. G-ψ or A-ψ complementary bonds are marginally weaker than G-U or A-U bonds, and the modification of U to ψ in these positions may affect the binding of U1 RNA to HnRNA. One interesting possibility is that ψ residues instead of U residues in the 5'-end of U1 RNA may allow U1 RNP to turn over faster during splicing.

ACKNOWLEDGEMENTS

These studies were supported by the Research program Project Grant CA-10893-P4, awarded by the National Cancer Institute, DHEW. We also thank Dr. K. Nohga for HeLa U1 RNA, and Mrs. Rose K. Busch for supplying tumor-bearing rats.

REFERENCES

1. Muramatsu, M., Hodnett, J. L. and Busch, H. (1966) J. Biol. Chem. 241: 1544-1550.
2. Nakamura, T., Prestayko, A. W. and Busch, H. (1968) J. Biol. Chem. 243: 1368-1375.
3. Reddy, R., Ro-Choi, T. S., Henning, D. and Busch, H. (1974) J. Biol. Chem. 249: 6486-6494.
4. Shibata, H., Reddy, R., Henning, D., Ro-Choi, T. S., and

Busch, H. (1974) Mol. Cell. Biochem. 4: 3-19.
5. Prestayko, A., Tonato, M. and Busch, H. (1970) Mol. Biol. 47: 505-515.
6. Shibata, H., Ro-Choi, T. S., Reddy, R., Choi, Y. C., Henning, D. and Busch, H. (1975) J. Biol. Chem. 250: 3909-3920.
7. Ro-Choi, T. S., Choi, Y. C., Henning, D., McCloskey, J., Busch, H. (1975) J. Biol. Chem. 250: 3921-3928.
8. Reddy, R., Henning, D. and Busch, H. (1979) J. Biol. Chem. 254: 11097-11105.
9. Reddy, R., Henning, D. and Busch, H. (1980) J. Biol. Chem. 255: 7029-7033.
10. Reddy, R., Henning, D. and Busch, H. (1981) J. Biol. Chem. (in Press).
11. Reddy, R. and Busch, H. (1980) in Cell Nucleus Vol. VIII, Academic Press, in press.
12. Ro-Choi, T. S. and Busch, H. (1974) in The Cell Nucleus (H. Busch, Ed.) Vol. VIII, pp. 151-208.
13. Weinberg, R. A. and Penman, S. (1968) J. Mol. Biol. 38: 289-304.
14. Hellung-Larsen, P., Frederiksen, S. and Plesner, P. (1971) Biochim. Biophys. Acta 254: 78-90.
15. Enger, M. D. and Walters, R. A. (1970) Biochemistry 9: 3551-3562.
16. Rein, A. (1971) Biochim. Biophys. Acta 232: 306-313.
17. Raj, N. B., Ro-Choi, T. S. and Busch, H. (1975) Biochemistry 14: 4380-4385.
18. Lerner, M. and Steitz (1979) Proc. Natl. Acad. Sci. (U.S.A.) 76: 5495-5499.
19. Flytzanis, C., Alonso, A., Louis, C., Krieg L. & Sekeris, C. E. (1978) FEBS Lett., 96: 201-206.
20. Jelinek, W. & Leinwand, L. (1978) Cell 15: 205-214.
21. Deimel, B., Louis, C. & Sekeris, C. E. (1977) FEBS Lett. 73: 80-84.
22. Northemann, W., Scheurlen, M., Gross, V. & Heinrich, P. C. (1977) Biochem. Biophys. Res. Commun. 76: 1130-1137.
23. Guimont-Ducamp, C., Sri-Widada, J. & Jeanteur, P. (1977) Biochimie 59: 755-758.
24. Gallinaro, H. & Jacob, M. (1979) FEBS Lett. 104: 176-182.
25. Lerner, M. R., Boyle, J. A., Mount, S. M., Wolin, S. L. & Steitz, J. A. (1980) Nature 283: 220-224.
26. Rogers, J. & Wall, R. (1980) Proc. Nat. Acad. Sci. U.S.A. 77: 1877-1879.
27. Murray, V. & Holliday, R. (1979) FEBS Lett. 106: 5-7.
28. Avvedimento, V., Vogeli, G., Yamada, Y., Maizel, J., Pastan, I. and Crombrugghe, B. (1980) Cell 21: 689-696.
29. Ting, A. C., Tsai, M. and O'Malley, B. W. (1980) Proceedings of 33rd Annual Symposium on Fundamental Cancer Research, "Genes, Chromosomes and Neoplasia". Raven Press, New York, in Press.
30. Busch, H., Reddy, R., Henning, D. and Epstein, P. (1981) Cell Biology, Schweiger pp. 47-52 Springer, Heidelberg.
31. Branlant, C., Krol, A., Ebel, J., Lazar, E., Gallinaro, H., Jacob, M., Sri-Widada, J. and Jeanteur, P. (1980) Nucleic Acids Res. 8: 4143-4154.
32. Mauritzen, C. M., Choi, Y. C. and Busch, H. (1970) Methods Cancer Res. 6: 253-282.
33. Ro-Choi, T. S., Moriyama, Y., Choi, Y. C. and Busch, H. (1970) J. Biol. Chem. 245: 1970-1977.

34. Donis-Keller, H., Maxam, A. M. and Gilbert, W. (1977) Nucleic Acids Res. 4: 2527-2538.
35. Winter, G. and Brownlee, G. G. (1978) Nucleic Acids Res. 5: 3129-3138.
36. Brownlee, G. G., Sanger, F. and Barrell, B. G. (1968) J. Mol. Biol. 34: 379-412.
37. Fuke, M. and Busch, H. (1977) Nucleic Acids Res. 4: 339-352.
38. Wyatt, G. R. (1951) Biochem. J. 48: 584-590.
39. Ro-Choi, T. S. and Henning, D. (1977) J. Biol. Chem. 252: 3814-3820.