Primary and Secondary Structure of U8 Small Nuclear RNA*

Ram Reddy, Dale Henning, and Harris Busch
From the Department of Pharmacology, Baylor College of Medicine, Houston, Texas 77030

U8 small nuclear RNA is a new, capped, 140-nucleotide long RNA species found in Novikoff hepatoma cells. Its sequence is: m₃GpppAmUmCGUCAGGA GGUUAAUCC UACCCUGCGA UGGCAUAGG AAAUGAUGA UUGGAGCUUG GAGUACUGC UGAUUAAAGC AUUUCGUGU AAUCAGGACC UGACAAACUC CAUGAUUGCUU CUAUCUGAUUOm. This RNA is present in approximately 25,000 copies/cell, and it is enriched in nucleolar preparations. Like U1, U2, U4/U6, and U5 RNAs, U8 RNA was also present as a ribonucleoprotein associated with the Sm antigen. The rat U8 RNA was highly homologous (>90%) to a recently characterized 5.4 S RNA from mouse cells infected with spleen focus-forming virus (Kato, N., and Harada, F. (1984) Biochim. Biophys. Acta, 782, 127-131). In addition to the U8 RNA, three other U small nuclear RNAs were found in anti-Sm antibody immunoprecipitates from labeled rat and HeLa cells. Each of these contained a m₃GpppAm cap structure; their apparent chain lengths were 60, 130, and 65 nucleotides. These U small nuclear RNAs are designated U7, U9, and U10 RNAs, respectively.

All eukaryotic cells studied thus far contain six distinct, capped small nuclear RNAs, designated U1 to U6 RNAs (1-4). These RNAs were found in human (4), rodent (1, 8), avian (5), insect (6, 7), amphibian (8), dinoflagellate (9), amoeba (10), yeast (11), and plant cells (12). These U1 to U6 RNAs, present in 2 x 10⁶ to 1 x 10⁹ copies/cell in human cells, are found in discrete ribonucleoprotein particles which are catalytically involved in nuclear processing of precursor RNAs to functional mature RNAs (2, 13-18). The present study presents the sequence of the U8 snRNA¹ and provides evidence for additional, capped small nuclear RNAs in both rodent and human cells, which extends the number of U-snRNAs from 6 to 10.

MATERIALS AND METHODS

The Novikoff hepatoma cells or HeLa cells were labeled with [³²P] phosphate as described by Maurizet et al. (19). The immunoprecipitations using anti-Sm and other antibodies were performed as described by Lerner and Steitz (2). The U8 RNA used in sequencing studies was isolated by fractionating nuclear 4-8 S RNA on 10% polyacrylamide gels (20). The fingerprinting was carried out as described by Brownlee et al. (21). The sequencing reactions were by the method of Peattie (22). The 3'-end labeling of U8 RNA was carried out as described by England and Uhlenbeck (23). The wandering spot analysis was carried out as described previously (24).

RESULTS

Fig. 1 shows the fractionation of small RNAs present in the immunoprecipitates obtained using the anti-Sm antibodies (lanes 2 and 3). Lanes 1 and 4 show the RNAs present in the starting whole cell sonicates of the HeLa cells and Novikoff hepatoma cells, respectively. In addition to the major U-snRNAs (U1, U2, U4, U5, and U6 RNAs) (2), several minor RNA bands were observed. Three bands which contain the U-snRNAs designated U8, U9, and U7/U10 were found consistently both in HeLa cells and in Novikoff hepatoma cells. RNA bands with similar mobility were observed when monoclonal anti-Sm antibodies (a kind gift from Dr. J. A. Steitz, Department of Molecular Biophysics, Yale University) were used or when sodium dodecyl sulfate/phenol-extracted total RNA was immunoprecipitated with anti-m₃G cap antibodies (a kind gift from Dr. R. Lührmann, Max-Planck-Institute, Berlin, West Germany) (results not shown). These RNAs were analyzed further.

On a 50-cm long, thin 12% polyacrylamide gel (Fig. 2), the RNA band with an apparent chain length of 60 nucleotides (designated U7/U10 RNA) was separated into several distinct bands which contained capped small RNAs. All the RNA bands were analyzed for the presence of cap structure and by fingerprinting after RNase digestion. The faster migrating bands had similar fingerprints and were uridylic acid-rich (35%). Following the designation of Strub et al. (25), these RNAs were designated U7 RNAs and these may be homologous to U7 RNA of sea urchin.

Fig. 3 shows the analysis of cap structure of U7/U10 RNAs in the anti-Sm antibody immunoprecipitates. After each RNA was digested with P1 nuclease, the products were fractionated on DEAE-cellulose paper. U7 to U10 RNAs contained a nuclease P1-resistant structure with the mobility of m₃GpppAm of rat U2 RNA (Fig. 3, lane 1). The cap structures of U7 to U10 RNAs were also analyzed by chromatography following the method of Silberklang et al. (26), and the cap structures had the same mobility as that of m₃GpppAm obtained from U2 RNA (results not shown). The fingerprints of U7, U9, and U10 RNAs were distinct and did not match the fingerprints of other known U-snRNAs (results not shown).

Fig. 4 shows the analysis of 4-8 S RNAs isolated from nuclei (lane 1) and nuclei (lane 2) of Novikoff hepatoma. As found earlier (20), the nucleoli are enriched in 5 S RNA, U3 RNA, 7-1 RNA, 7-2 RNA, and 8 S RNA; the nucleoplasmic U1, U2, U4, U5, and U6 RNAs were present in lower concentrations. Since nucleolar RNA was enriched in U8 RNA (lane 1), U8 RNA, like U3 RNA, is probably localized to the nucleolus.

Fig. 5A shows the T; RNase fingerprints of U8 RNA of Novikoff hepatoma cells. The major oligonucleotide pattern

¹ The abbreviation used is: snRNA, small nuclear RNA.
Primary and Secondary Structure of U8 Small Nuclear RNA

FIG. 1. Analysis of RNAs in ribonucleoprotein particles recognized by anti-Sm antibodies. HeLa cells and Novikoff hepatoma cells were labeled with [32P]orthophosphate, and immunoprecipitations were carried out as described by Lerner and Steitz (2). The cell supernatant used was obtained after centrifugation at 100,000 × g for 1 h. The RNAs in the immunoprecipitates were analyzed on a 10% acrylamide, 7 M urea gel. The xylene cyanol dye marker migrated slightly slower than U7/U10 RNAs. Lane 1, HeLa cell supernatant, used as starting material; lane 2, RNAs found in anti-Sm antibody immunoprecipitate from HeLa cells; lane 3, RNAs found in anti-Sm antibody immunoprecipitate from Novikoff hepatoma cells; lane 4, Novikoff hepatoma cell supernatant used as starting material. RNA bands, designated U8, U9, and U7/U10, were observed consistently. Several other bands were observed in some experiments, especially between U4 RNA and 5 S RNA (see lane 2). These may be other minor U-snRNAs or degradation products of major U-snRNAs or RNAs precipitated nonspecifically. 7SL and 7SK RNAs are well-characterized cytoplasmic RNAs (3, 4).

did not correspond to that of any known U-snRNAs. In some experiments, oligonucleotides characteristic of U1 RNA were also found in the fingerprints of U8 RNA; these may result from co-migration of U1* RNA with U8 RNA (13). The T1 oligonucleotides of U8 RNA were analyzed after T1 RNase, RNase A, P1 nuclease, or U2 RNase digestions. Some oligonucleotide sequences such as AAAUG (spot 12, Fig. 5A) were obtained from these data. The sequences of larger oligonucleotides were obtained by mobility shift analysis of the 5'-end-labeled oligonucleotides (24). The sequences obtained for T19 and a variant of T-18 are shown in Fig. 5B; this variant suggests that there is minor heterogeneity of U8 RNA. Oligonucleotide T-20 contained the cap structure and T-3 contained the 3'-end of U8 RNA (Fig. 5A).

FIG. 2. Separation of multiple components of U7 and U10 RNAs. A, HeLa U7/U10 RNAs indicated in lane 2 of Fig. 1 were isolated and refractionated on a 50-cm long, 12% polyacrylamide, 7 M urea gel. B, rat U7/U10 RNAs indicated in lane 3 of Fig. 1 were isolated and fractionated on a 50-cm long, 12% polyacrylamide, 7 M urea gel. The xylene cyanol marker was run for a total of 45 cm, and RNAs were visualized by autoradiography. Two to three major RNA bands and several minor RNA bands were observed. All these RNAs were analyzed for cap structure and by fingerprinting. The RNA bands labeled U7 RNAs gave similar fingerprints, and RNA bands labeled U10 RNAs gave similar fingerprints but different from U7 RNAs.

FIG. 3. Analysis of cap structures of U7 to U10 RNAs. The purified U-snRNAs were digested with nuclease P1 for 16 h and fractionated on a DEAE-cellulose paper at pH 3.5. Lane 1, rat U2 RNA; lane 2, rat U8 RNA; lane 3, rat U9 RNA; lane 4, HeLa U7 RNA; lane 5, HeLa U10 RNA.

FIG. 6 shows sequencing gels obtained with 3'-end-labeled U8 RNA. The 3'-end-labeled RNA was reacted under base-specific conditions according to Peattie (22) and analyzed on sequencing gels. Fig. 6A shows the sequence of U8 RNA from nucleotides 99 to 136; Fig. 6B shows the sequence from...
nucleotide 1 to 96. Fig. 6C shows the overlapping sequencing ladders obtained with partial T1 RNase and U2 RNase cleavages using 3'-end-labeled U8 RNA. The sequences obtained by these methods were confirmatory. The sequence from nucleotides 13 to 38 was confirmed using 5'-end-labeled T1 RNase fragments; the sequences of oligonucleotides T-18 and T-19 obtained as described earlier were found to be the part of this 5'-region of U8 RNA.

Fig. 7 shows the complete nucleotide sequence of U8 RNA of Novikoff hepatoma cells. The sequence of U8 RNA is highly homologous to a 5.4 S RNA from mouse cells infected with spleen focus-forming virus (27). When the four substitutions of rat and mouse U8 RNAs are compared, all are either purine to purine or pyrimidine to pyrimidine substitutions. The 5'-97 nucleotides were identical except that nucleotides 47, 87, and 88 were absent from the mouse sequence. The substitutions were near the 3'-end of U8 RNA (Fig. 7). In addition, the rat U8 RNA was 3 nucleotides longer; it had a dinucleotide insertion at nucleotides 87-88 and one at nucleotide 47 (Fig. 7). The overall homology was 95% between the rat and mouse U8 RNAs.

Fig. 8 shows one possible secondary structure for Novikoff

![Diagram showing secondary structure of U8 Small Nuclear RNA](image_url)

**Fig. 4.** Small nuclear RNAs present in nuclei and nucleoli of Novikoff hepatoma. 100 μg of nucleolar (No.) 4–8 S RNA (lane 1) and 100 μg of nuclear (Nu.) 4–8 S RNA (lane 2) were analyzed on a 10% polyacrylamide, 7 M urea gel. The nuclei were prepared by the citric acid procedure (38), and nucleoli were prepared by sonication of nuclei prepared by the sucrose CaCl₂ procedure (39). The total RNAs were isolated by the sodium dodecyl sulfate/hot phenol procedure and fractionated on a 5–40% sucrose density gradient to obtain 4–8 S RNA (40). The numbers next to the RNA indicate the chain length of corresponding RNAs in nucleotides. The RNAs were visualized by staining with methylene blue.

**Fig. 5.** T1 RNase fingerprint of U8 RNA (A) and autoradiograph of a two-dimensional separation of a partial digest of 5'32P-labeled T1 RNase fragments T-18 and T-19 (B). A, U8 RNA of Novikoff hepatoma was digested with T1 RNase and fingerprinted by the method of Brownlee et al. (21). The first dimension was cellulose acetate at pH 5.5, and the second dimension was homochromatography on polyethyleneimine-cellulose sheets using C-15 homomixture. The sequences shown were obtained from analyzing the digestion products of these oligonucleotides with RNase A, U2 RNase, T3 RNase, P1 nuclease, or mobility shift analysis of end-labeled oligonucleotides and from sequencing gels. B, the numbers
FIG. 6. Sequencing gels for U8 RNA. Novikoff hepatoma U8 RNA was 3'-end-labeled with 5'-[32P]pCp (23) and purified on polyacrylamide gels. The base-specific chemical degradation method of Peattie (22) was used for ladders shown in A and B. The enzymatic method of Donis-Keller et al. (41) was used for ladders shown in Fig. C. A shows the sequence of U8 RNA from nucleotide 99 to 136; B shows the sequence from nucleotide 1 to 96; and C shows the sequence from nucleotide 4 to 107.

hepatoma U8 RNA which is slightly different from that reported by Kato and Harada (27) for mouse U8 RNA. In this

next to the nucleotides correspond to the nucleotide numbers in the U8 RNA sequence. The sequence of the oligonucleotide T-18 is that of a minor sequence variant, different from the T-18 shown in the fingerprint (A).

structure, all the S1 nuclease-sensitive sites observed are in single-stranded regions (marked by arrows). A putative Sm antigen-binding site found to be uridylic acid-rich and single-stranded in U1, U2, U4, and U5 RNAs (28-31) is also single-stranded in this proposed secondary structure for U8 RNA (nucleotides 85-98) and may be the Sm-binding site (Fig. 8). Fig. 9 shows the T1 RNase fingerprints of two human U10
Primary and Secondary Structure of U8 Small Nuclear RNA

**FIG. 8.** One possible secondary structure of U8 RNA. The secondary structure was constructed to have maximal base pairing and to fit the partial digestion products of this RNA molecule from partial digestion with T1 RNase, S1 nuclease, and P1 nuclease. The stability numbers were calculated according to Tinoco et al. (42). The overall structure has a Tinoco value of \( G = -25 \text{ K cal/mol} \). A putative Sm antigen-binding site is indicated by a *line under* the sequence corresponding to nucleotides 85 to 97.

RNAS and a rat U7 RNA. The T1 RNase fingerprint of U10B RNA was obtained from the U10 RNA species with most radioactivity (see Fig. 2A). The fingerprints of other U10 RNAs in this size range were identical to the fingerprint of U10B RNA. The fingerprints showed 11 oligonucleotides, 1 to 11, in molar yield and 2 oligonucleotides, 12 and 13, in less than molar yield. These oligonucleotides were digested with U2 RNase or with RNase A, and the results are as follows: 1, Gp; 2, AAp; 3, UGap; 4, CAGp; 5, AACpG; 6, CUGp; 7, AUCCpG and CACUGp; 8, CUUGp; 9, UAUGp; 10, CAGp; 11, CAUGp. In addition to the three U10 RNA species with apparent chain length of 60 nucleotides, an RNA species with apparent chain length of 120 nucleotides was also found in anti-Sm antibody immunoprecipitates. This RNA was designated U10D, since it contained all the oligonucleotides, 1 to 13, found in U10B RNA and at least 4 additional oligonucleotides, numbered 14 to 17 (Fig. 9). Both of these RNAs contained the cap structure, indicating that the 5’-end may be the same and that the U10D species may be longer at the 3’-end when compared to U10B RNA. The analysis of RNase A or U2 RNA digests of 1 to 13 oligonucleotides from U10B and U10D RNA yielded the same products. The U10D RNA contained about 15–20% of the total radioactivity in U10 RNAs.

The U7 RNA from the Novikoff hepatoma cells was fingerprinted and shown in Fig. 9. The RNA species still contained some U10 RNA as a contaminant, and the oligonucleotides consistently observed were designated 1 to 11 and shown in Fig. 9. Since all the U-snRNAs previously characterized in higher eukaryotes contained modified nucleotides (1, 3), the U7 RNA was analyzed for modified nucleotides. The human U7 RNA contained 2-O-methylated cytidine and pseudouridine in 0.6–0.8 molar yield, in addition to the cap structure (Fig. 9). Other modified nucleotides were observed in less than 0.5 molar yield.

**FIG. 9.** Characterization of U7 and U10 RNAs. **H.U10B, T1** RNase fingerprint of the most abundant U10 RNA species (see Fig. 2A); **H.U10D, T1** RNase fingerprints of a minor U10 RNA species with an apparent chain length of 120 nucleotides; **RU7, T1** RNase fingerprint of the most abundant U7 RNA species found in Novikoff hepatoma cells (see Fig. 2B); **H.U7,** autoradiograph of a nuclease P1 digest of human U7 RNA fractionated on thin layer cellulose plates. The spots were quantitated by counting in a Packard scintillation counter. The fingerprinting was carried out as described by Brownlee et al. (21); the first dimension was electrophoresis on cellulose acetate strips, and the second dimension was homochromatography using C-15 homomixture. The analysis of modified nucleotides was by the method of Silberklang et al. (26). The molar yield of pm6A was between 0.6 and 0.8. The molar yield of pm6A was 0.3.

**DISCUSSION**

In this study, four minor U-snRNAs were analyzed. The number of U-snRNAs identified in higher eukaryotes is therefore extended from 6 to 10. The U7 RNA identified in this study probably corresponds to the U7 RNA shown in the 12 S ribonucleoprotein particle involved in the accurate processing of sea urchin histone mRNAs (18, 25, 32, 33). Birnstiel and colleagues (25) showed this U7 RNA was immunoprecipitable with anti-Sm antibodies. Our results confirm this observation and the indication that U7 RNA contains a trimethylguanosine cap structure (18); therefore, U7 RNA is a member of the U-snRNA series by the criteria that it has a cap structure and is present in Sm-containing ribonucleoprotein particle.

U8 RNA was first identified in nucleolar preparations and was designated 5.4 S RNA because of its electrophoretic migration between 5 S RNA and 5.8 S RNA (20). Although the function of this RNA is not known, the characterization of U8 RNA (5.4 S RNA) in mouse cells (27) and in Novikoff hepatoma cells in this investigation establishes this RNA to be a distinct member of the U-snRNA series. These results also show that U8 RNA is present in a ribonucleoprotein particle in association with Sm antigen and may be localized to nucleoli because it, like U3 RNA, was enriched in nucleolar preparations. However, its association with high molecular weight, nucleolar ribosomal RNAs was weak because no detectable U8 RNA was associated with high molecular weight nucleolar RNAs extracted at room temperature (20).

Although U3 RNA was conclusively shown to be present in
nucleoli (1, 3, 4) and U8 RNA is found in nucleoli, no detectable nucleolar localization was observed with mG cap-specific antibodies (34) or anti-Sm antibodies (35, 36). The reason(s) for these apparently inconsistent results are not clear. One possibility is that the U8 RNA is a minor RNA and may not be contributing significant nucleolar immunofluorescence compared to nucleoplasm using anti-Sm antibodies. The other possibility is that nucleolar snRNAs may be tightly bound to proteins and are not available for binding to antibodies. Yet another, although unlikely, possibility is that both U3 and U8 RNA are only co-purifying with nucleoli and are really not inside the nucleolus. More studies will be needed to distinguish between these possibilities. The subcellular localization of U7 and U10 RNAs is not known since the U7 and U10 RNAs were run off this gel. Further experiments are needed to clarify this question. The amounts of U9 RNA were not sufficient to be visualized by methylene blue staining in both total nuclear and nucleolar 4-8 S RNA preparations.

All U-snRNAs associated with Sm antigen were shown to contain a uridylic acid-rich, smear-shaped tail that appears to be the Sm antigen-binding site (29-31). The second-variety of similar features.

Although this nomenclature is imperfect, these symbols reflect molecular species with a stem regions. These features are found in other Sm antigen-bound snRNAs including U1, U2, U4/U6, and U5 RNAs (28-31). Therefore, this region of US RNA may be the Sm antigen-binding site.

The fingerprints of U9 and U10 RNAs were not similar to those of the other known U-snRNAs although they contained cap structures. Adams et al. (7) found a distinct trimethylguanosine-containing small RNA unrelated to other known U-snRNAs in silk worms. The RNA described by Adams et al. (7) was slightly larger than U4 RNA and smaller than U43. Therefore, this region of US RNA may be the Sm antigen-binding site.

The designation of U-snRNAs U7 to U10 was done using the following criteria. U-snRNAs U1 to U3 were first designated in the ascending order by Hodnett and Busch (37), and these were extended by Lerner and Steitz (2) to include U4 to U6 RNAs in descending order. A 60-nucleotide long Sm antigen-associated RNA was added by Strub et al. (25) to this series as U7 RNA. The designation of U7 RNA is supported by its cap structure. The other three distinct, capped, Sm antigen-associated RNAs are designated U8 to U10 RNAs, again in descending order. Although this nomenclature is imperfect, these symbols reflect molecular species with a variety of similar features.

Acknowledgments—We would like to thank Professor Eng Tan for providing anti-Sm antibodies, Professor Joan A. Steitz for providing monoclonal anti-Sm antibodies, and Professor R. Lührmann for providing mG cap-specific antibodies. We would also like to thank Rose K. Busch for supplying tumor-bearing rats and William Spohn for providing HeLa cells.

REFERENCES

1. Busch, H., Reddy R., Rothblum, L., and Choi, Y. C. (1982) Annu. Rev. Biochem. 51, 617-654
2. Lerner, M. R., and Steitz, J. A. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 5495-5499
3. Reddy, R., and Busch, H. (1983) Prog. Nucleic Acids Res. Mol. Biol. 30, 127-162
4. Zieve, G., and Penniman, S. (1976) Cell 8, 19-31
5. Roop, D. R., Kristo, P., Stumph, W. E., Tsai, M. J., and O’Malley, B. W. (1981) Cell 25, 671-680
6. Mount, S., and Steitz, J. A. (1981) Nucleic Acids Res. 9, 6351-6368
7. Adams, D. S., Herrera, R. J., Lührmann, R., and Lizardi, P. M. (1986) Biochemistry 25, 117-125
8. Zieve, G., Carri, M., Mattaj, I. W., and DeRobertis, E. M. (1984) EMBO J. 3, 1075-1081
9. Reddy, R., Spector, D., Harneing, D., Liu, M.-H., and Busch, H. (1983) J. Biol. Chem. 258, 13965-13969
10. Wise, J. A., and Weiner, A. M. (1981) J. Biol. Chem. 256, 965-968
11. Wise, J. A., Tellervay, D., Maloney, D., Swerdlov, H., Dunn, E. J., and Guthrie, C. (1983) Cell 35, 743-751
12. Krol, A., Ebel, J., Rinke, J., and Lührmann, R. (1983) Nucleic Acids Res. 11, 8863-8894
13. Lerner, M. R., Boyle, J. A., Mount, S. M., Wolin, S. L., and Steitz, J. A. (1980) Nature 283, 220-224
14. Padgett, R. A., Mount, S. M., Steitz, J. A., and Sharp, P. A. (1983) Cell 35, 101-107
15. Yang, V. W., Lerner, M. R., Steitz, J. A., and Flint, S. J. (1981) Proc. Natl. Acad. Sci. U.S.A. 78, 1371-1375
16. Rogers, J., and Wall, R. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 1877-1879
17. Krämer, A., Keller, W., Appel, B., and Lührmann, R. (1984) Cell 38, 299-307
18. Birnstiel, M. L., Busslinger, M., and Strub, K. (1985) Cell, 41, 349-359
19. Mauritzen, C. M., Choi, Y. C., and Busch, H. (1971) Methods Cancer Res. 6, 253-292
20. Reddy, R., Li, W.-Y., Henning, D., Choi, Y. C., Nohga, K., and Busch, H. (1981) J. Biol. Chem. 256, 8452-8457
21. Brownlee, G. G., Sanger, F., and Barrell, B. G. (1968) J. Mol. Biol. 34, 379-412
22. Fritzie, D. A. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 1760-1764
23. England, T. E., and Uhlenbeck, O. C. (1978) Nature 275, 560-561
24. Fuke, M., and Busch, H. (1977) Nucleic Acids Res. 4, 339-352
25. Strub, K., Galli, G., Busslinger, M., and Birnstiel, M. L. (1984) J. Mol. Biol. 162, 623-643
26. Siöberklang, M., Gillum, A. M., and Raj Bhanderi, U. L. (1979) Methods Enzymol. 59, 58-109
27. Kato, N., and Harada, F. (1984) Biochim. Biophys. Acta 782, 127-131
28. Branlant, C., Krol, A., Ebel, J. P., Lazar, E., Haendler, B., and Jacob, M. (1982) EMBO J. 1, 1295-1296
29. Jacob, M., Lazar, E., Haendler, B., Gallinaro, H., Krol, A., and Branlant, C. (1984) Biol. Cell. 51, 1-10
30. Liutard, J. P., Sri-Widada, J., Brunel, C., and Jeanteur, P. (1982) J. Mol. Biol. 162, 623-643
31. Mattaj, I. W., and DeRobertis, E. M. (1985) Cell 40, 111-118
32. Galli, G., Hofstetter, H., Stannenberg, H. G., and Birnstiel, M. L. (1983) Cell 34, 823-828
33. Georgiev, O., and Birnstiel, M. L. (1985) EMBO J. 4, 481-489
34. Reuter, A., Appel, B., Bringmann, B., Rinke, J., and Lührmann, R. (1984) Exp. Cell Res. 154, 549-560
35. Lerner, E. A., Lerner, M. R., Janeway, C. R., and Steitz, J. A. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 2737-2741
36. Spector, D. (1984) Biol. Cell. 51, 109-112
37. Hodnett, J. L., and Busch, H. (1988) J. Biol. Chem. 243, 6334-6342
38. Higashi, K., Adams, H. R., and Busch, H. (1966) Cancer Res. 26, 2196-2201
39. Prestayo, A. W., Tonato, M., and Busch, H. (1971) J. Mol. Biol. 57, 505-515
40. Reddy, T. S., Moriymaya, Y., Choi, Y. C., and Busch, H. (1970) J. Biol. Chem. 245, 1970-1977
41. Donis-Keller, H., Maxam, A., and Gilbert, W. (1977) Nucleic Acids Res. 4, 2527-2538
42. Tinoco, I., Jr., Borer, P. N., Dengler, B., Levine, M. D., Uhlenbeck, O. C., Brothers, D. M., and Gralla, J. (1973) Nature New Biol. 246, 40-41