Detection of a Nucleolar 7-2 Ribonucleoprotein and a Cytoplasmic 8-2 Ribonucleoprotein with Autoantibodies from Patients with Scleroderma*

(Received for publication, August 3, 1982)

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In studies on antinuclear antibodies in sera from 24 patients with scleroderma, an autoimmune disease, one serum, designated "anti-To", contained antibodies against a nucleolar 7-2 ribonucleoprotein and a novel cytoplasmic 8-2 ribonucleoprotein. The 7-2 and 8-2 RNAs are distinct RNAs with a pppG terminus. They are partially conserved between rat and human species and are present in distinct ribonucleoprotein particles. Eight sera contained antibodies that precipitated particles containing nucleolar U3 RNA; these antibodies appear to be directed against preribosomal particles containing U3 ribonucleoprotein particles, rather than the U3 ribonucleoprotein particles alone. All these ribonucleoproteins required proteins for antigenicity. These antibodies will be of use in studies on the structure and function of these novel small ribonucleoproteins.

Recently, several types of antibodies present in the sera of patients with autoimmune diseases were shown to be directed against snRNPs1 (1-7). These include anti-RNP directed against U1 RNP (1), anti-Sm directed against U1, U2, U4, U5, and U6 RNPs (1-3), anti-Ro/SS-A directed against Y RNPs (5), and anti-La/SS-B directed against a variety of cellular RNPs including Y RNPs, 4.5 S, 4.5 SI, precursor 5 S (6, 7), and viral RNPs (2, 4). In this study, antinuclear antibodies from several patients with scleroderma were characterized in part by the small RNPs which they immunoprecipitated, particularly the previously reported nucleolar 7-2 RNA and a novel cytoplasmic 8-2 RNA.

MATERIALS AND METHODS

* This study was supported by Cancer Research Grant CA-10893, P4, from the United States Department of Health, Education, and Welfare, National Cancer Institute. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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The abbreviations used are: snRNP, small nuclear ribonucleoprotein; RNP, ribonucleoprotein; NP40, Nonidet P-40; Sm, La, Ro, and To are the first two initials of patient's last names; ss-A, Sjogren's syndrome A.

RESULTS

Sera from patients with scleroderma contain antinuclear antibodies (12). All the antibodies from the 24 patients used in this study produced bright nucleolar immunofluorescence and some fluorescence in small nuclear particles. Weak cytoplasmic fluorescence was observed (Fig. 1). Fig. 2 shows the analysis of the small RNAs precipitated by different antibodies. The RNAs in the immunoprecipitates obtained using Sm, RNP, La, and Ro antibodies (lanes 2-5, respectively) were the same as reported previously (1-7). One scleroderma antibody, referred to as anti-To was found to immunoprecipitate RNPs from Novikoff hepatoma cells (lanes 6 and 12), which contained two RNA species. RNAs of similar size were found in corresponding immunoprecipitates when HeLa cells were used (results not shown). Lanes 8, 11, and 13 show the small RNAs precipitated by three different sera, each of which immunoprecipitated U3 RNA; U3 RNA was identified by fingerprinting. One of the sera (lane 8) also precipitated the Y1 and Y2 RNAs (6) precipitated by anti-Ro antibodies (lane 9). Of the 24 sera analyzed from scleroderma patients, six contained Ro-specific antibodies which immunoprecipitated the Y1 and Y2 RNPs; none contained detectable RNP-, Sm-, or La-specific antibodies.

RNAs isolated by the sodium dodecyl sulfate-phenol method or by proteinase-K treatment were not precipitated by any of the antibodies analyzed. Accordingly, the antigenicity resides in the proteins or the RNP particles because of the RNA-protein complex, the RNP protein, or the conformational state of the RNA.

Characterization of 7-2 and 8-2 RNAs—The To-RNAs of Novikoff hepatoma and of HeLa cells were characterized by the T7-RNase fingerprints (Fig. 3). The Novikoff hepatoma 7-2 RNA is nucleolar 7-2 RNA characterized previously (11), as shown by its identical fingerprint. The 8-2 RNA is a new RNA species, which differs from 8 S RNA that is hydrogen bonded to preribosomal RNA (11).

The T7-RNase oligonucleotides of Novikoff hepatoma and HeLa 7-2 RNA were analyzed after U2 RNase or RNase A digestion and electrophoresis on DEAE-cellulose paper at pH 3.5 (9). Although some of the oligonucleotides analyzed were not structurally related, two large oligonucleotides (marked by arrows, Fig. 3, A- and C-) gave identical products when digested by U2 RNA or RNase A. Therefore, 7-2 RNA appears to be partially conserved between rat and human tissues. The 7-2 RNA differs from other small RNAs like U1 RNA (13, 14).
or 7 S RNA (11, 15, 16) where the conservation between rat and human species is over 98%. Earlier, 7-2 RNA was shown to lack modified nucleotides (11), and in this study Novikoff hepatoma 8-2 RNA was also found to lack modified nucleotides.

The 5'-ends of HeLa and Novikoff hepatoma 7-2 and 8-2 RNAs obtained by immunoprecipitation were analyzed after digestion with nuclease P1 and chromatography on polyethyleneimine-cellulose sheets using 0.375 M phosphate buffer, pH 3.4, as the solvent (6). Both HeLa and Novikoff hepatoma 7-2 and 8-2 RNAs contained pppG in 0.6 to 0.9 M yield. These results showed that 7-2 and 8-2 RNAs are primary transcription products. The 7-2 RNAs of both HeLa and Novikoff hepatoma obtained from total nuclear 4-8 S RNA contained 3'-hydroxyl groups which permitted efficient ligation with 32P Cp and RNA ligase; U was the terminal nucleotide (70%) and A was a minor terminal (30%). The 3'-end of Novikoff hepatoma 8-2 RNA is indicated by an arrowhead (Fig. 3B); the nucleotide composition of this oligonucleotide was C:U. The pppG 5'-terminus, and the defined 3'-termini indicate that 7-2 and 8-2 RNAs are unique RNA species.

7-2 and 8-2 RNP Are Distinct RNP Particles—To test whether 7-2 and 8-2 RNAs were in different particles, experiments were carried out on immunoprecipitation from cytoplasmic fractions and nuclear fractions (Fig. 4). Anti-To antibodies precipitated only 8-2 RNP from cytoplasm (Fig. 4A, lane 7) but not 7-2 RNP. Similarly, an RNP containing RNA identical in fingerprint to 7-2 RNA but with slower electrophoretic mobility on polyacrylamide gels was precipitated from nuclear extracts with very little 8-2 RNP (Fig. 4A, lane 2), indicating that 7-2 and 8-2 RNPs are distinct RNP particles. These results also indicate that 8-2 RNP is in the cytoplasm obtained by the NP-40 fractionation method.

7-2 RNP and 7-2* RNP Contain Common Antigens—As described above (Fig. 2), 7-2 and 8-2 RNPs were immunoprecipitated by anti-To antibodies. When these sonicates were centrifuged at 120,000 x g for 2 h and the supernatant was used as a source of antigen, a 7-2* RNP (Fig. 4A) was immunoprecipitated; the 7-2* RNA had a slightly slower electrophoretic mobility on polyacrylamide gels. This result...
suggests 7-2 and 7-2* RNPs contain common antigen(s). In low speed sonicates, the predominant 7-2 RNP competed out the available antibody. Since 7-2 RNP appears to be associated with some larger particles which sediment at 120,000 x g for 2 h, the high speed supernatant is essentially free of 7-2 RNP, permitting 7-2* RNP to immunoprecipitate.

Comparative Sedimentation of 7-2, 7-2*, and 8-2 RNPs—Most of the 7-2 RNP is associated with large (>20 S) particles. Only 7-2* RNP could be immunoprecipitated (and not 7-2 RNP) from the 120,000 x g supernatant of nuclear sonicates (Fig. 4A, lane 2). Similar results were obtained for 8-2 RNP starting from whole cell sonicates (Fig. 4B, lane 8). These results indicate that 7-2* and 8-2 RNPs are approximately the same size as U snRNPs i.e. 10 S size. However, 7-2 RNP was present in 10,000 x g supernatants, but was found in the pellet after high speed centrifugation, indicating that 7-2 RNP is part of a larger complex (>20 S).

Analysis of small RNAs from immunoprecipitates obtained using anti-La antibodies showed the presence of a 7-2* RNA by electrophoretic mobility (Fig. 5) which was identified by fingerprinting. The fingerprint did not show any differences when compared to 7-2 RNA (Ref. 11 and Fig. 3A). This result is analogous to precipitation of 5 S RNA by anti-La antibodies (7). 7-2* RNA contained pppG on its 5'-terminus and the slower electrophoretic mobility may reflect the presence of a few additional nucleotides on the 3'-end. It is not yet known whether 7-2* RNA precipitated by anti-La antibodies and anti-To antibodies is the same or a different population.

FIG. 5. Precipitation of 7-2* RNA with anti-La antibodies. Whole cell sonicates (10,000 x g supernatants) were precipitated with normal human serum (lane 2), anti-RNP (lane 3), anti-Sm (lane 4), anti-Ro (lane 5), anti-La (lane 6), and anti-To (lane 7). Lane 1 shows the 4-8 S RNAs as standards. 7-2* indicated by an arrow was identified by fingerprinting and was similar to that shown in Fig. 2A.

Discussion

Evidence is presented for the presence of a new cytoplasmic RNA, 8-2 RNA; this RNA and a previously described 7-2 RNA are shown to be present in the cell as RNP particles. Thus, these two RNAs fall into the category of other small RNAs which are present in RNPs. 7-2* RNA differs from 7-2 RNA in its electrophoretic mobility and probably contains extra nucleotides on its 3'-end, since both 7-2 and 7-2* RNA contain pppG as 5'-terminus.

Using the NP40 method, the 8-2 RNA was found to be in the cytoplasmic fraction and 7-2 RNA and 7-2* RNA in the nuclear fraction. Since most of 8-2 RNP was present in the 120,000 x g supernatant, most of 8-2 RNP appears to be present as a small RNP particle not associated with larger particles. The 7-2 RNP was mostly associated with larger (>20 S) particles; the 7-2* RNP was small like 8-2 RNP but it is nuclear. It is not known if 7-2 and 8-2 RNPs contain a common antigen. Experiments carried out by competing nuclear sonicates which contain only 7-2 RNP with varying amounts of cytoplasmic sonicates, which contain mostly 8-2 RNP, did not yield conclusive results. The reasons for this may be the presence of free antigenic protein. Free antigenic protein recognized by La-antibodies is known to be present in cell sonicates (15). Dr. J. Steitz and her colleagues independently identified a serum from another patient that precipitates 8-2 and 7-2 RNPs. The fingerprinting of 7-2 and 7-2* RNPs indicated by fingerprinting and was similar to that shown in Fig. 2A.

2 J. Seitz, personal communication.
common antigen has been demonstrated in the case of Sm, La, and Ro RNPs (1-7).

None of the 24 sera tested contained anti-U3 RNP antibodies. Eight of the 24 sera analyzed reacted with large particles, presumably preribosomal particles containing U3 RNP. Most of U3 RNP in the cell was associated with preribosomal RNPs but 5-10% of the U3 RNP was not sedimented at 120,000 x g for 2 h. This portion of U3 RNP was not recognized by these antibodies.

It was previously estimated that 50,000 copies of 7-2 RNA are present in Novikoff cells (11). Assuming that the specific activity of 7-2 RNA and 7-2* RNA is the same, in this study the percentage of 7-2* RNA was estimated to be less than 1% of the 7-2 RNA. Since a stainable RNA band corresponding to 8-2 RNA was not observed, 8-2 RNA appears to be present in less than 10,000 copies/cell.

Isolation and characterization of small RNP particles is important for understanding their function (16-18). The availability of these antibodies should be helpful in defining the cellular functions of the cytoplasmic 8-2 RNP and nucleolar 7-2 RNPs.

Acknowledgments—We would like to thank Rose Busch for supplying tumor-bearing rats, Dr. Morris Reinich for providing some of the sera used in this study, and Dr. Paul Epstein for very useful discussions.

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R Reddy, E M Tan, D Henning, K Nohga and H Busch

J. Biol. Chem. 1983, 258:1383-1386.

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