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Characterization of two scleroderma autoimmune antigens that copurify with human ribonuclease P

PAUL S. EDER*†, RAMESH KEKUDA*‡, VIKTOR STOLC§, AND SIDNEY ALTMAN*¶

*Department of Biology, Yale University, New Haven, CT 06520; and §Department of Cell Biology, Yale University School of Medicine, New Haven, CT 06510

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ABSTRACT **Human RNase P has been purified more than 2000-fold from HeLa cells. In addition to the RNA component, H1 RNA, polypeptides of molecular masses 14, 20, 25, 30, 38, and 40 kDa copurify with the enzyme activity. Sera from two different patients with the autoimmune disease scleroderma were used to immunodeplete human RNase P activity. These same sera cross-reacted on immunoblots with two of the copurifying polypeptides, p30 and p38, whereas an autoimmune serum that does not immunodeplete RNase P activity did not react with these proteins. Peptide fragments derived from purified p30 and p38 facilitated the molecular cloning and sequencing of cDNAs coding for these two polypeptides, which are now designated as Rpp30 and Rpp38, respectively. *RPP38* cDNA encodes a polypeptide that may be identical to a previously identified antigen of ≈40 kDa, which is immunoprecipitated by Th and To autoimmune antisera, and that has been implicated as a protein subunit of human RNase P by virtue of its ability to bind to H1 RNA *in vitro*. The second autoimmune antigen, Rpp30, as such, has not been described previously.**

The ribonucleoprotein enzyme ribonuclease P removes the 5′ leader sequences from precursor transfer RNA molecules (1, 2). In eubacteria, the RNA component is the catalytic subunit (3) and the protein subunit serves as a cofactor (4, 5). Among eukaryotes and archaebacteria, however, no RNase P RNA subunit has shown catalytic capability in the absence of its protein subunits. Although the RNA subunit has been characterized from a few classes of eukaryotes, including fungi (reviewed in refs. 4 and 6), amphibians (7), zebrafish (8), and mammals (9, 10), no eukaryotic protein subunits have yet been unequivocally identified as essential participants in catalytic function in a reconstitution assay, as has been done with the protein subunit of the enzyme from *Escherichia coli* (11).

Proteins from several sources that associate with RNase P have been identified (12–16) using partial biochemical purification and genetic schemes. For example, the product of the *POP1* gene in *Saccharomyces cerevisiae* has been shown to have a function in the processing of precursor tRNA substrates (12). The protein is also found in epitope-tagged immunoprecipitates with both the precursor to and the mature form of *RPR1* RNA. The association with the precursor RNA is an indication that Pop1 could be part of a maturation complex for *RPR1* RNA rather than an essential part of the catalytic RNase P complex. Antibodies to the human orthologue of Pop1 protein efficiently immunoprecipitate RNase P activity (and the RNA component of the enzyme) from HeLa cell nuclear extracts (17). However, the human Pop1 protein has not yet been shown to copurify with human RNase P after extensive biochemical fractionation, nor has holoenzyme activity been successfully reconstituted from the H1 RNA subunit and this and/or other proteins.

Genetic data have shown that the protein product of the *RPM2* gene in *S. cerevisiae* is required for precursor tRNA maturation in the mitochondrion (13, 14), but it has not been shown directly that this protein, nor ones that are associated with partially purified RNase P from *Schizosaccharomyces pombe* (15) or *Aspergillus nidulans* (16), are essential subunits of a catalytic RNase P complex.

Nuclear ribonucleoproteins and deoxyribonucleoproteins are often the targets of autoantibodies present in patients with autoimmune disease; indeed, the presence of circulating antibodies that recognize nuclear antigens is a diagnostic marker of several autoimmune diseases (18). Patients afflicted with systemic sclerosis (SSc; scleroderma) have identifiable antibodies against nuclear antigens in greater than 95% of the cases (19). In some instances, SSc patients have sera that immunoprecipitate a ribonucleoprotein designated Th (20) or To (21). The RNA component of the Th RNP was later shown to be identical to mitochondrial RNA processing enzyme (MRP) RNA (22), first identified as 7–2 RNA (23), the RNA subunit of ribonuclease MRP (24). In *S. cerevisiae*, RNase MRP is involved in the ribosomal RNA maturation pathway (25–27).

In addition to immunoprecipitating MRP RNA, some Th-positive antisera also immunoprecipitate H1 RNA, previously identified as 8–2 RNA (21), the RNA component of human RNase P (10, 20–22, 28). Although it can be isolated from cytosolic extracts (10, 29), this enzyme is known to function in the nucleus (30). Evidence for the localization of the RNA subunit suggests that it is both nucleolar (31, 32), as is RNase MRP (33), and cytoplasmic, but the latter subpopulation may represent an assembly intermediate or be destined for mitochondria. An antigen of about 38–40 kDa that is sometimes referred to as the Th or To antigen is found in both RNase P and RNase MRP complexes that have been immunoprecipitated with Th antisera (34, 35). Pop1 has similar antigenic properties. It has been suggested that these antigens are subunits common to both RNase MRP and RNase P enzyme complexes, or at least that they have a transient association with both enzymes in the cell.

Although RNase P from HeLa cells has been partially purified previously (10), the lack of sufficient pure protein has precluded rigorous characterization of its protein subunits. In this report, we describe a modified purification scheme for

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Abbreviations: SSc, systemic sclerosis (scleroderma); MRP, mitochondrial RNA processing enzyme; FPLC, fast protein liquid chromatography.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. U77664 and U77665).

†Present address: Howard Hughes Medical Institute, Department of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.
‡Present address: Department of Biochemistry and Molecular Biology, Medical College of Georgia, Augusta, GA 30912-2100.
¶To whom reprint requests should be addressed. e-mail: sidney.altman@qm.yale.edu.

RNase P from HeLa cells and describe the isolation and characterization of some of the proteins that copurify with RNase P activity in these preparations. Two of these proteins, p30 and p38, cross-react with sera from two different SSc patients. The two sera also immunodeplete RNase P activity from highly purified preparations of the holoenzyme, an indication that both antigenic polypeptides play a role in some aspect of RNase P function. The two antigens are now named Rpp30 (RNase P Protein) and Rpp 38, respectively.

MATERIALS AND METHODS

Materials. *Taq* polymerase and deoxyribonucleoside triphosphates for PCR, Pefabloc protease inhibitor, and calf intestinal alkaline phosphatase were purchased from Boehringer Mannheim. Avian myeloblastosis virus reverse transcriptase, ribonucleoside triphosphates, and SP6 RNA polymerase were purchased from Promega. All other enzymes were from New England Biolabs.

Autoimmune antisera were a kind gift of J. Craft (Yale University School of Medicine). Polyclonal rabbit antisera against C5 protein were prepared by Pocono Rabbit Farms (Canadensis, PA). Enzyme-linked secondary antibodies were purchased from ICN. Oligodeoxyribonucleotides were synthesized by the W. M. Keck Biotechnology Resource Center at Yale University.

Assays for RNase P Activity. Each RNase P reaction of 10 μl contained 10 mM Tris·HCl (pH 7.8), 10 mM $MgCl_2$, 200 mM KCl, 1 mM DTT, and 1 pmol precursor tRNA substrate, radiolabeled to a specific activity of 1000 cpm/pmol. The substrate, the precursor to the suppressor $tRNA^{Ser}$, *supS1*, from *Sc. pombe* (36), was transcribed in the presence of [α-^{32}P]GTP (37). The reaction mixture for the assay of RNase P activity was incubated for 1–15 min at 37°C, and the reaction was terminated by the addition of an equal volume of 40 mM EDTA/8 M urea. To facilitate migration of products into 8% denaturing polyacrylamide gels, 1 μl of phenol was added to each reaction tube, and the samples were electrophoresed at 15 W for 2 h. Reaction products were detected by autoradiography of the polyacrylamide gels.

Purification of RNase P Holoenzyme. The enzyme has been partially purified previously from HeLa cells (10). Modifications of that protocol are described here. Suspension cultures of HeLa cells, strain S3 (Cellex, Minneapolis), were grown to a density of 5×10^5/ml. Sixty liters of suspension culture were centrifuged to pellet the cells, which were stored at −70°C until use. Five volumes of buffer A (10 mM Tris·HCl, pH 7.5/2.5 mM $MgCl_2$/2 mM DTT/0.2 mM Pefabloc protease inhibitor/5 mM KCl) were added to the thawed cell pellets, and the suspensions were incubated for 30 min on ice, followed by further disruption with 10 strokes by a Dounce homogenizer. The mixture was centrifuged at 100,000 × *g* to remove cellular debris and organelles. The 100,000 × *g* supernatant (S100) was applied to a column of DEAE–Sepharose Fast-flow (Sigma), 2.5 × 56 cm, equilibrated with buffer A that contained 100 mM KCl. The enzyme was eluted with buffer A in a gradient of 100–500 mM KCl. Peak RNase P activity was pooled and concentrated in Centriprep-10 concentrators (Amicon).

Preparative 15–30% glycerol density gradients were prepared in buffer A that contained 200 mM KCl. RNase P from the DEAE step was layered onto 11 ml of the gradient solution. Centrifugation was at 2°C for 26 h at 200,000 × *g* in a Beckman SW 41 rotor. Fractions of 300 μl were collected from the top and assayed for RNase P activity. The pooled glycerol gradient fractions containing peak RNase P activity were concentrated in a Centriprep-10 concentrator, and the sample was loaded onto a Mono Q HR-10/10 fast protein liquid chromatography (FPLC) column (Pharmacia). The column was washed with 10 bed volumes of buffer B (10 mM Tris·HCl, pH 7.5/10 mM $MgCl_2$/2 mM DTT/0.2 mM Pefabloc/10% glycerol/300 mM KCl) and the enzyme was eluted with a linear gradient of 300–700 mM KCl in buffer B. Fractions that contained the peak of activity were pooled and concentrated and then loaded onto an S-12 size exclusion FPLC column (1.0 × 10 cm) in buffer B with 500 mM KCl. Fractions (0.5 ml each), which contained the peak of RNase P activity, were analyzed for purity of protein content by SDS/PAGE, followed by staining with reduced silver. The appropriate factions were then pooled and concentrated to facilitate isolation of individual polypeptides by preparative SDS/PAGE.

Protein Sequence Analysis. The purified sample (100–150 μg) from an S-12 column was denatured and electrophoresed through a discontinuous gradient gel (38), and the proteins were visualized by Coomassie brilliant blue staining. The desired proteins (1–3 μg; 30–100 pmol) were excised from the gel, and submitted to the W. M. Keck Biotechnology Resource Center for determination of amino acid composition, tryptic digestion, HPLC purification, mass spectrometry, and peptide sequencing.

Immunoblotting. After electrophoretic separation in polyacrylamide/12% SDS gels, proteins were transferred to nitrocellulose in 25 mM CAPS (pH 10.4), 20% (vol/vol) methanol at 4°C. The nitrocellulose was blocked with TNT buffer (10 mM Tris·HCl, pH 7.5/150 mM NaCl/0.05% Tween 20) and 2.5% nonfat dry milk for 30 min at room temperature. Autoimmune serum was used at a dilution of 1:200 in TNT plus 1.25% nonfat dry milk. Horseradish peroxidase-linked goat anti-human IgG secondary antibody was used at a dilution of 1:1500. Blots were then incubated with LumiGLO chemiluminescent substrate (Kirkegaard & Perry Laboratories) according to the manufacturer's protocol.

Immunodepletion. Antiserum (40 μl) was incubated for 1 h with 5 mg (dry weight) protein A-Sepharose beads (Pharmacia) in 1 ml TMKT buffer (10 mM Tris·HCl, pH 7.5/10 mM $MgCl_2$/100 mM KCl/0.02% Tween 20), washed four times in TMKT, followed by three washings in TMK buffer (TMKT minus Tween 20). The beads were then incubated for 1 h at room temperature in 100 μl of purified RNase P. The suspension was centrifuged, and the supernatant was assayed for depletion of enzyme activity.

Identification and Cloning of *RPP30* cDNA. The peptides obtained from the Rpp30 protein, LVETAAHLGY, PPINVAIDR, and VIISIAAERPLEIR, were used to search the DBEST (expressed sequence tag) database for a matching cDNA clone. The TBLASTN (39) program compared each peptide individually against the DBEST nucleotide sequence database (40, 41) translated in all reading fames. Several partial clones (I.M.A.G.E. consortium cDNA clones) were identified and obtained from Research Genetics (Huntsville, AL). The two clones used to obtain the full-length coding sequence were numbered 220430 and 190158. These clones were sequenced in their entirety to demonstrate overlap of the coding sequence for Rpp30 in the two clones. Clone 220430 was digested with *Eco*RI and *Hin*dIII to release a fragment of 356 bp that contained the 5′ end (N terminal) of Rpp30. Clone 190158 was digested with *Hin*dIII and *Not*I (made blunt with Klenow polymerase) to release a fragment about 700 bp long. These two fragments were cloned into pUC19 that had been digested with *Eco*RI and *Sma*I. The clone that was used for additional analysis was sequenced to ascertain that the *RPP30* sequence was correct and in the appropriate orientation.

Identification and Cloning of *RPP38* cDNA. Human cDNA that was amplified by PCR from a human fetal liver cDNA library in λgt10 (CLONTECH) was used as target DNA in a two-step, nested amplification reaction to identify a cDNA fragment that encodes two of the peptide fragments from the Rpp38 protein. The peptides used to design degenerate oligonucleotide primers were ALNNPYIIR and IEDSGENLETEPLESQDR. The sense-strand degenerate primer in the first and second PCR reactions was 5′-GCN CTN AA(C/T)

AA(C/T) CCN TA(C/T) AT(A/C/T) AT-3′, corresponding to the peptide ALNNPYIIR. The antisense-strand primers for the first reaction corresponded to the anticodons of amino acids 15–9 of the second peptide: 5′-GA (C/T)TC NAG NGG (C/T)TC NGT (C/T) TC-3′ and 5′-GA (C/T)TC (C/T)AA NGG (C/T)TC NGT (C/T)TC-3′. In the second reaction, the antisense primers were nested to the same second peptide, corresponding to the anticodons of amino acids 12–5: 5′-GG (C/T)TC NGT (C/T)TC NAG (A/G)TT (C/T)TC NCC-3′ and 5′-GG (C/T)TC NGT (C/T)TC (C/T)AA (A/G)TT (C/T)TC NCC-3′. The first PCR reaction included 35 cycles of denaturation at 94°C for 1 min, annealing at 42°C for 1 min, and extension at 72°C for 1 min. An aliquot was removed for the second reaction, which was performed for 35 cycles that included denaturation at 94°C for 1 min, annealing at 42°C for 1 min, and extension at 72°C for 1 min. A 650-bp fragment was isolated after the second, nested PCR reaction, and this was subcloned into pBluescript II SK+ (Stratagene) and sequenced using the Sequenase kit (Amersham).

A variation of 3′ RACE (rapid amplificaton of cDNA ends) was used to identify the 3′ coding sequence and untranslated region (42). Briefly, poly(A)$^+$ RNA from HeLa cells was used in a reverse transcription reaction with a T_{17}-adapter primer, 5′-CGACTCGAGTCGACATCGA$(T)_{17}$-3′. The second step was a p38-specific linear amplification of the antisense cDNA, using only a sense-strand primer, 5′-GGA CGA AGT AAG AGC CAT CAT CC-3′. The PCR included 35 cycles with denaturation at 94°C for 1 min, annealing at 63°C for 30 s, and extension at 72°C for 1 min, using a nested sense primer, 5′-GAG TCC CCA GTT TAA GTG TAC C-3′ and the adapter primer, 5′-CGA CTC GAG TCG ACA TCG-3′. A 400-bp product was obtained, of which 74 bp were an overlapping coding sequence from the first PCR product. This fragment was aligned and extended the coding sequence for 258 nt.

To obtain the remaining 5′ coding sequence of the *RPP38* cDNA, 5′-RACE was performed essentially as described (41), with the following modifications. In the first reaction, using the same target DNA described above; the 5′ coding sequence of *RPP38* was amplified using the *RPP38*-specific antisense primer, 5′-CAA TCT TCT GAA GTC CAA TAG C-3′, and a λgt10-specific sense-strand primer, 5′-GAG GTG GCT TAT GAG TAT TTC TTC CAG GGT A-3′. In the second reaction, the nested, *RPP38*-specific antisense primer was 5′-GCG TCT GTA GGA TGA AGT GC-3′, with the sense-strand primer the same as in the first reaction. A 250-bp product was obtained, which was cloned and sequenced as described above. This product gave 120 bp of new 5′ sequence. The new sequence was used to search the EST database, which identified a matching cDNA clone from a human fetal brain cDNA library in λgt22 (F3053). A PCR product that contained the insert in F3053 was subcloned into the PCR–Script-Amp vector (Stratagene) by David Hwang in the laboratory of C. C. Liew (University of Toronto), and this clone was provided courtesy of C. C. Liew. The DNA insert in the PCR–Script-Amp vector was sequenced and shown to contain full-length cDNA for *RPP38*.

RESULTS

Purification and Stability. To purify active HeLa RNase P sufficiently for identification and isolation of adequate quantities of its protein subunits, we modified the purification scheme described in ref. 10. The new protocol includes two FPLC purification steps. Optimal resolution of enzymatic activity was obtained using the anion exchanger, Mono Q (peak of enzymatic activity eluted between 450 and 500 mM KCl), and the size exclusion column, S-12 (Fig. 1). Other schemes typically used for the purification of nucleic acid-modifying enzymes were unsuccessful in enhancing significantly the purification of this ribonucleoprotein enzyme. Furthermore, the enzyme was not stable in the presence of even low concentrations of ammonium sulfate.

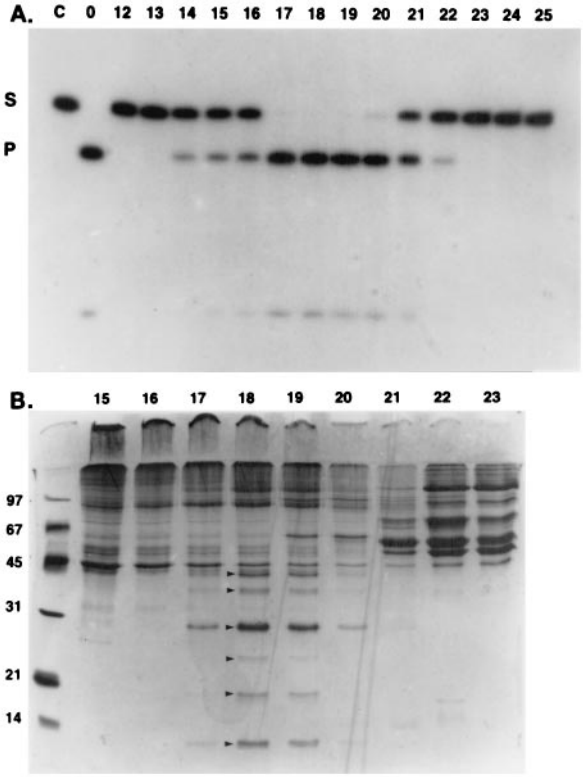


FIG. 1. Separation of polypeptides copurifying with human RNase P activity eluted from the S-12 column (*A*) in a 12% polyacrylamide gel that contains 0.1% SDS (*B*). The lane numbers indicate the fractions (0.5 ml each) from the column. (*A*) Assay for RNase P activity across the column. Lane C, no added column fraction. Lane O, load sample added. Other lanes as indicated above. S, substrate; P, tRNA-containing product. The 5′ leader portion of the product runs near the bottom of the gel. (*B*) The arrowheads indicate the positions of proteins discussed in the text. The positions of molecular weight markers are shown to the left.

Human RNase P becomes extremely unstable when the concentration of protein in preparations of the partially purified enzyme is very low. Table 1 shows the purification profile of the enzymatic activity. With the removal of the majority of the contaminating proteins in the final two column steps the enzyme became unstable, with significant losses of activity unless it was kept concentrated in 10% glycerol and stored either on ice or at −20°C. This instability could be partially alleviated on incubation of the enzyme in the presence of a stabilizing protein such as catalase or bovine serum albumin (data not shown). The purification factor cited in the table must be regarded as a minimum number, since, for example,

Table 1. Purification profile of RNase P from HeLa whole cell extracts

Step	Volume, ml	Total protein, mg	Total activity, units × 10^5	Specific activity, units/mg × 10^5	Purification, -fold
S100	450	6300	9,000	1.4	—
DEAE	180	740	32,000	43	31
Glycerol	81	140	24,000	170	121
Mono Q	7.2	2.9	2,100	720	510
S-12	2.0	0.2	640	3200	2300

One unit is defined as the amount of enzyme required to convert 1 μmol of substrate to product in 1 min at 37°C

90% of the activity is lost during the Mono Q step and a further 30% during the S-12 step. Loss of activity of the pooled holoenzyme after the DEAE or glycerol gradient velocity sedimentation steps was nearly undetectable after greater than 1 year of storage at −20°C. The half-life of the S-12 fraction was less than 6 months at −20°C. Nonetheless, the FPLC steps removed the overwhelming majority of contaminating proteins, allowing the identification of several polypeptides (p14, p20, p25, p30, p38, p40: see arrowheads in Fig. 1*B*) that copurify with the holoenzyme activity (Fig. 1*A*). In our purification scheme, high molecular weight material is not well resolved. Purification steps in addition to those shown in Table 1, including two-dimensional gel electrophoresis, did not enable us to resolve any proteins of molecular mass > 100 kDa (such as the human orthologue of Pop1) that definitively copurified with active RNase P (data not shown).

The optimal KCl concentration for RNase P activity was previously identified as 70 mM (10). However, we found that optimal activity of our present preparations of RNase P was obtained in KCl concentrations of ≈250 mM (data not shown). Further increasing the KCl concentration inhibited the enzyme significantly, with complete inhibition at 400 mM KCl. It is noteworthy that the enzyme was eluted in an active form from the FPLC columns in KCl concentrations that are higher than 400 mM, but the KCl was diluted under the assay conditions used.

RNase P activity in the optimal KCl concentration (250 mM) was completely inhibited in the absence of a reducing agent. However, in the presence of 1 mM DTT, the activity was restored. This result led to the supposition that one or more protein subunits contain essential sulfhydryl groups. This hypothesis was supported by the observation that the activity is inhibited by *N*-ethylmaleimide: RNase P activity is inhibited 50% in 1.3 mM *N*-ethylmaleimide and 100% in 3.2 mM *N*-ethylmaleimide (data not shown).

Identification of Polypeptides That Cross-React with Th Antiserum. Sera from patients with scleroderma was tested for their ability to immunodeplete RNase P activity from purified fractions. Fig. 2 shows that two different sera, ThFi (*A*, lane 5) and ThLo (*B*, lane 5), could immunodeplete RNase P from glycerol gradient-purified holoenzyme, whereas serum ThMi (*C*, lane 5) was less effective. In contrast, antibodies against the *E. coli* RNase P protein, C5, were even less potent (Fig. 2*A*, lanes 3 and 4; *B*, lane 4; *C*, lane 4). The two sera that displayed efficient immunodepletion of RNase P activity from partially purified preparations were then used in immunoblot analyses to identify proteins that cross-reacted with them.

Fractions spanning the peak of highly purified RNase P activity that was eluted from the S-12 size exclusion column were tested with ThLo serum in immunoblots. A strong signal was observable (Fig. 3) only in the fractions that contained the peak of enzymatic activity. The signal corresponded to cross-reaction with Rpp30 and Rpp38. Immunoblots of fractions spanning the peak of RNase P activity in glycerol gradients also showed cross-reaction of Rpp30 and Rpp38 with ThFi and ThLo. In contrast, serum ThMi, which was relatively ineffective in immunodepleting RNase P activity from partially purified preparations of the holoenzyme, did not cross-react with either Rpp30 or Rpp38 in immunoblots (data not shown). Therefore, Rpp30 and Rpp38 were then isolated by preparative SDS/PAGE, and tryptic fragments from each were sequenced.

Characterization of Nucleotide Sequences Coding for p30 (*RPP30*) and p38 (*RPP38*). The amino acid sequences of the tryptic peptides derived from Rpp30 and Rpp38 were reverse translated and used to identify cDNA clones coding for these sequences as described in the *Materials and Methods*. cDNA clones that encompassed the entire coding sequence for each protein were constructed as described above. The nucleotide

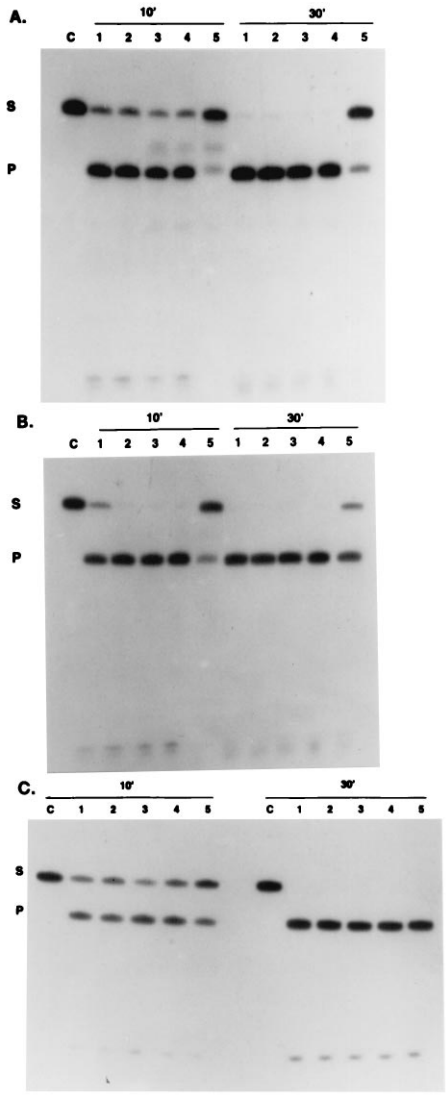


FIG. 2. Immunoprecipitation of RNase P activity in the second glycerol gradient fractions that contained the peak of activity with sera from SSc patients labeled as ThFi (*A*), ThLo (*B*), and ThMi (*C*). RNase P activity was assayed as indicated for either 10 or 30 min. (*A*) Lane C, no added antiserum or enzyme. Lanes 1, RNase P added; no antiserum. Lanes 2, protein A-Sepharose beads with no added antiserum; RNase P added. Lanes 3 and 4, beads coated with a different rabbit anti-C5 protein (*E. coli*) in each case and RNase P added. Lanes 5, beads coated with ThFi serum and RNase P added. (*B*) Same as *A*, except lanes 3 contain beads coated with rabbit preimmune serum and lanes 5 contain beads coated with ThLo serum. (*C*) Same as *B*, except lanes 5 contain beads coated with ThMi serum.

sequences of these clones and the corresponding amino acid sequences are shown in Fig. 4.

The Rpp30 polypeptide is 268 amino acids in length and has a theoretical pI of 9.4. The predicted molecular weight is 29,447. Sixteen of 18 cDNA clones examined contained an uninterrupted coding sequence for Rpp30 as shown in Fig. 4 *Left*. One clone contained two introns separated by a small exon and one other clone contained only the first intron (data not shown).

The Rpp38 polypeptide is 283 amino acids long and has a theoretical pI of 9.9. The predicted molecular weight is 31,845, which is different from the molecular mass (38 kDa) as judged by its electrophoretic mobility in 12% acrylamide/0.1% SDS gels. This discrepancy can be explained in a number of ways, of which three are (*i*) anomalous migration of a very basic protein in the gels; (*ii*) posttranslational modifications of

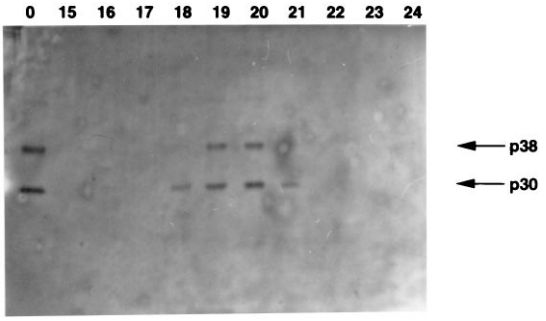


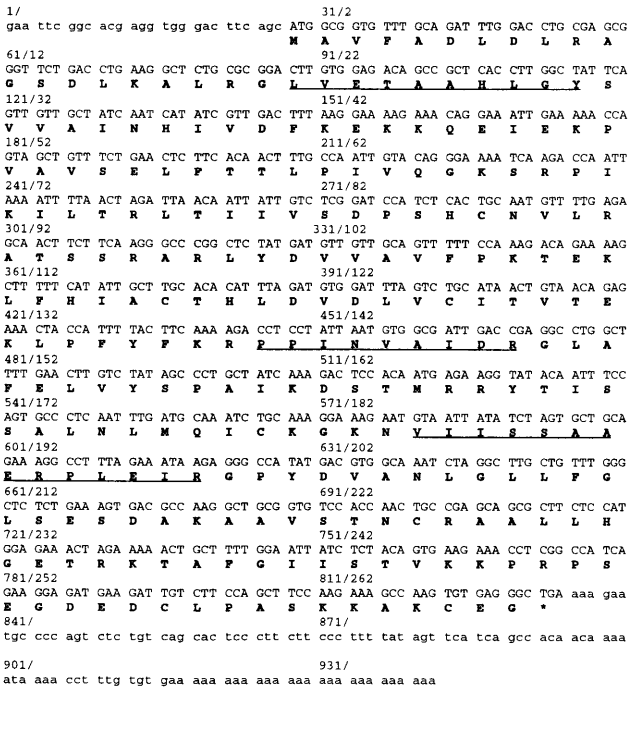
FIG. 3. Immunoblot using ThLo serum of S-12 fractions that spanned the peak of RNase P activity eluted from the column. Molecular weight markers were run in the gel but are not shown in the figure. The markers were used to determine the approximate molecular weights of the species indicated in the figure. Lane 0 refers to the sample (from the Mono Q column) loaded on the S-12 column and the other lanes refer to fractions eluted from the column: the peak of RNase P activity was in fractions 19 and 20.

Rpp38 [extensive carbohydrate modification would be compatible with the anomalous, low buoyant density of the holoenzyme complex (10)]; and (*iii*) an undetected error in the cloning and sequence determination of the cDNA clones that led to the absence of additional coding sequence upstream from the first methionine residue shown in Fig. 4 *Right*. The tryptic peptides are in phase with the first methionine as shown in the figure. None of these possible explanations affect the main conclusions of this report (see below).

DISCUSSION

Human RNase P from HeLa cells is a ribonucleoprotein complex that behaves on a size exclusion column (S-12) as a large complex and has a sedimentation coefficient of 15 S (10). Several proteins have been isolated from highly purified RNase P and we have identified cDNA clones coding for some of them using reverse translation of tryptic peptides derived from these proteins. Both basic and acidic proteins (data not shown) are found in the complex. Two of the basic proteins (Rpp38 and Rpp30), which copurify with RNase P activity, cross-react with Th antiserum from some patients with the autoimmune disease, SSc. These antisera also immunodeplete the enzymatic activity. Additionally, Th antiserum cross-reacts in immunoblots with recombinant Rpp30 (encoded by the sequence shown in Fig. 4 *Left*) overexpressed in *E. coli* (P.S.E., N. Jarrous, and S.A., unpublished work) and rabbit antisera to recombinant Rpp30 or recombinant Rpp38 immunoprecipitate partially purified human RNase P (N. Jarrous and S.A., unpublished work). Other Th antisera, described elsewhere (31, 35, 43, 44), also precipitate an antigen with a molecular mass of ≈38 kDa, and have been used to show that such an antigen is localized in the nucleolus (31, 45, 46) and binds to human RNase P RNA *in vitro* in the domain between nucleotides 20–70 and to a region of similar secondary structure in RNase MRP RNA (35, 47).

Despite the copurification and coimmunoprecipitation of Rpp38 with RNase P under most conditions, this protein depleted in the active complex in some fractions purified using a different regimen from that reported here (data not shown). Accordingly, Rpp38 may be associated transiently with RNase P RNA as a factor involved in the transport of H1 RNA to the putative site of its assembly in the cell, the nucleolus (48). This possibility is supported by the recent observations that only a small subpopulation of RNase P is coimmunoprecipitated and associated with RNase MRP as determined by cytological methods (31). Furthermore, the carboxyl-terminal end of the polypeptide sequence (Fig. 4 *Right*) is especially rich in basic



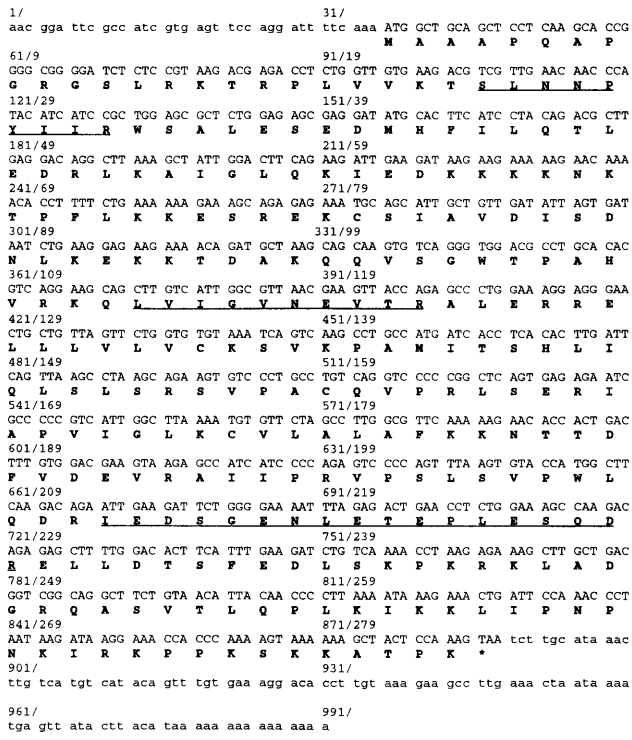


FIG. 4. cDNA sequence and translated polypeptide sequence for *RPP30* (*Left*) and *RPP38* (*Right*). The nucleotide sequence is numbered from the start of the total sequence determined. The amino acid sequence is numbered from the first methionine residue and is shown in boldface letters. The portions of the peptide sequences that correspond to tryptic peptide fragments derived from authentic Rpp30 and Rpp38 are underlined. Note that for *RPP30*, the cDNA sequence has one base substitution difference from the predicted sequence and the cDNA sequence for *RPP38* has two base substitution differences from the predicted sequence. These differences, which might well be expected from cDNA clones derived from human tissue different from HeLa cells, give rise to an I → S change in the peptide sequence at position 189 in Rpp30 and to an A → S change in the peptide sequence at position 24 and an A → V change at position 114 in Rpp38.

amino acids and may be part of a nuclear localization signal (49).

Rpp30 has not been separated from the holoenzyme under any conditions we tested. The size of Rpp30 immediately suggests that it may be related to proteins of ≈30 kDa found in complexes immunoprecipitated from HeLa extracts with some other Th antisera (43, 44). In one of those studies there was a strong correlation between the abundance of a 30-kDa protein immunoprecipitated by the Th antiserum and the efficiency with which that particular serum immunodepleted RNase P activity (44). Genetic and biochemical studies of an orthologue of Rpp30 in *S. cerevisiae* indicate that this protein is an essential subunit of the mature RNase P complex (V.S. and S.A., unpublished work). The newly detailed characterization of Rpp30 and Rpp38 presented here is a useful step both in the definition of subcategories of autoimmune sera and of the protein subunits of human RNase P.

Note Added in Proof We have now shown that Rpp38 has an anomalous, slow migration in our SDS/PAGE system and that the amino acid sequence of this protein has a region similar to a leucine zipper RNA binding motif (N. Jarrous and S.A., unpublished work).

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