RNA (1999), *5*:153–157. Cambridge University Press. Printed in the USA.
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REPORT

Rpp14 and Rpp29, two protein subunits of human ribonuclease P

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ABSTRACT

In HeLa cells, the tRNA processing enzyme ribonuclease P (RNase P) consists of an RNA molecule associated with at least eight protein subunits, hPop1, Rpp14, Rpp20, Rpp25, Rpp29, Rpp30, Rpp38, and Rpp40. Five of these proteins (hPop1p, Rpp20, Rpp30, Rpp38, and Rpp40) have been partially characterized. Here we report on the cDNA cloning and immunobiochemical analysis of Rpp14 and Rpp29. Polyclonal rabbit antibodies raised against recombinant Rpp14 and Rpp29 recognize their corresponding antigens in HeLa cells and precipitate catalytically active RNase P. Rpp29 shows 23% identity with Pop4p, a subunit of yeast nuclear RNase P and the ribosomal RNA processing enzyme RNase MRP. Rpp14, by contrast, exhibits no significant homology to any known yeast gene. Thus, human RNase P differs in the details of its protein composition, and perhaps in the functions of some of these proteins, from the yeast enzyme.

Keywords: human RNase P; nuclear localization sequences; RNase MRP; rRNA; tRNA

INTRODUCTION

Eukaryotic nuclear ribonuclease P (RNase P), an enzyme required for processing of the 5′ termini of precursor tRNAs, consists of several distinct proteins associated with an essential RNA subunit. In yeast, there are nine essential genes that code for protein subunits of nuclear RNase P. The requirement for these proteins in RNase P processing of tRNA precursors, as well as for the function of RNase MRP in rRNA processing (Lygerou et al., 1996a; Lee & Clayton, 1997), has been demonstrated (Lygerou et al., 1994; Chu et al., 1997; Dichtl & Tollervey, 1997; Stolc & Altman, 1997; Chamberlain et al., 1998; Stolc et al., 1998). The physical association of five proteins, hPop1 (Lygerou et al., 1996b), Rpp20, Rpp30, Rpp38, and Rpp40 (Eder et al., 1997; Jarrous et al., 1998), with human RNase P activity has previously been demonstrated by biochemical purification and by immunoprecipitation experiments using antibodies raised against their corresponding recombinant proteins. hPop1, Rpp20, and Rpp30 show 14–23% identity with their yeast homologs (Lygerou et al., 1994; Stolc & Altman, 1997; Stolc et al., 1998), yet there seem to be no homologs to Rpp38 and Rpp40 in the genome of *Saccharomyces cerevisiae*.

Here we report on the molecular cloning and immunobiochemical characterization of two additional protein subunits of HeLa RNase P, Rpp14 and Rpp29. Polyclonal rabbit antibodies raised against recombinant Rpp14 and Rpp29 recognize their corresponding endogenous proteins in HeLa cells and precipitate active RNase P complexes. Rpp29 shows 23% identity with Pop4p, a known protein subunit of yeast nuclear RNase P. In contrast, Rpp14 exhibits no significant homology to any yeast gene, as in the case of two previously characterized proteins, Rpp40 and Rpp38. Another protein that copurifies with HeLa RNase P activity, Rpp25, has not yet been shown to be physically associated with catalytic complexes.

RESULTS

Molecular cloning of Rpp14 and Rpp29 cDNAs

Human RNase P was highly purified from HeLa cells as previously described (Eder et al., 1997). Proteins of apparent molecular weights of 14 and 30 kDa, which

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copurified with RNase P activity (Eder et al., 1997), were extracted from 12% polyacrylamide-0.1% SDS preparative gel for peptide microsequencing analysis. Two peptides, PAPAATYERVVYK and LFDIKPEQQR, obtained from the 14-kDa and the 30-kDa protein bands, respectively, were used to search the GenBank Expressed Sequence Tag database (dbEST). From the several EST clones obtained, two EST clones that contained the relevant tryptic peptide sequence with large cDNA inserts were fully sequenced and designated Rpp14 and Rpp29 cDNAs (Figs. 1A and 1B, respectively). Rpp14 cDNA has one single open reading frame (ORF) that codes for a theoretical polypeptide of 124 amino acids. The predicted molecular weight of this polypeptide is 13,821 Da. Rpp29 cDNA harbors an ORF encoding a protein of 220 amino acids with a predicted molecular weight of 25,472 Da. These proteins are both basic, with a pI of 7.62 and 10.1, respectively.

Rpp29 has a homolog in yeast, Pop4p. *POP4* is an essential gene that codes for a protein shared by the yeast nuclear RNase P and RNase MRP (Chu et al., 1997). Rpp29 and Pop4 showed 23% identity at the level of protein sequence (Fig. 2). Similar identities were exhibited by hPop1 and Rpp30 to their yeast counterparts (Lygerou et al., 1996b; Stolc & Altman, 1997). By contrast, Rpp14 seems to have no gene ortholog in the *Saccharomyces cerevisiae* genome, as was the case with Rpp38 and Rpp 40 (Jarrous et al., 1998).

Figure 3 shows silver-stained gels that contain purified, histidine-tagged Rpp14 and Rpp29, obtained from the expression of Rpp14 and Rpp29 ORFs in *Escherichia coli* (see Materials and Methods). These tagged proteins were purified on nickel-charged Hi-Trap FPLC columns. Because of the 2–3-kDa histidine tag fused to the amino terminal of the translated products, their molecular sizes are larger by 2–3 kDa than Rpp14 and Rpp29 that copurify with HeLa RNase P (Eder et al., 1997). Rpp29, but not Rpp14, showed an anomalous migration, a property that is shared with several other Rpp subunits, including Rpp20, Rpp38, and Rpp40 (Jarrous et al., 1998). Thus, Rpp14 and Rpp29 ORFs code for proteins with molecular weights similar to the endogenous HeLa Rpp14 and Rpp29 proteins. These highly purified, recombinant Rpp14 and Rpp29 proteins (Fig. 3) were then used to raise polyclonal antibodies in rabbits (see Materials and Methods).

A

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1                                       31
AAT TCG GCA CGA GGG AGA AGC CAA ACG TAA AGA CAC CAG GAG TTT CTC GGG CCC AGC TGT
61                                      91
GGC TGC TGC CGG GGA GCC CCA AGC CTT GGC GGT CCT TGC TGC GAA TAG GAG TCT GGT CAG
121                                     151
GCG TCA GGC TAG TCC GAC GAA GAG TGG GTG TGA TCA GCA CTG GAA AAG ATG CCT GCC CCT
                                                                M   P   A   P

181/5                                  211
GCT GCC ACA TAT GAA AGA GTA GTT TAC AAA AAC CCT TCC GAG TAC CAC TAC ATG AAA GTC
A   A   T   Y   E   R   V   V   Y   K   N   P   S   E   Y   H   Y   M   K   V

241/25                                  271/91
TGC CTA GAA TTT CAA GAT TGT GGA GTT GGA CTG AAT GCT GCA CAG TTC AAA CAG CTG CTT
C   L   E   F   Q   D   C   G   V   G   L   N   A   A   Q   F   K   Q   L   L
301/45                                  331/111
ATT TCG GCT GTG AAG GAC CTG TTT GGG GAG GTT GAT GCC GCC TTA CCT TTG GAC ATC CTA
I   S   A   V   K   D   L   F   G   E   V   D   A   A   L   P   L   D   I   L
361/65                                  391/131
ACC TAT GAA GAG AAG ACC TTG TCA GCC ATC TTG AGA ATA TGT AGC AGT GGT CTT GTC AAA
T   Y   E   E   K   T   L   S   A   I   L   R   I   C   S   S   G   L   V   K
421/85                                  451/151
TTG TGG AGC TCT TTG ACC CTG TTA GGA TCC TAT AAA GGC AAA AAA TGT GCT TTC CGG GTG
L   W   S   S   L   T   L   L   G   S   Y   K   G   K   K   C   A   F   R   V
481/105                                 511/171
ATT CAG GTT TCT CCA TTT CTT CTT GCA TTA TCT GGT AAT AGT AGG GAA CTA GTA TTG GAT
I   Q   V   S   P   F   L   L   A   L   S   G   N   S   R   E   L   V   L   D
541                                     571
TGA ATG AAT AGT CTT CCA TTT TGG AAA CGT TCA TCC ACT CTC ATA TTT ATT TTT TCG GTG
601                                     631
CCT GCA TGT TTG AAG ACT GAA GCA GGC TAA AAG CTC TTG ATG AAA TTT GAG GGT GCT GAA
661                                     691
GAT GTT CCC ACT AAT TTC CAG CCA TCA CCT TTG GTG GGG TGG GCT TCG GAG GAC AGT CTG
721                                     751
TCT GAA CCT GCC AGT GCT GAC CCT GCA GCA CTT TCA GCA TAT GCA CAT CAA AGT TGG AGA
781                                     811
CCG CGC TGA ACT TAG GAG GGC CTT CAC ACA GAC TGA TGT GGC TAC CTT CTC AGA ATT AAC
841                                     871
AGG GGA TGT TAA TCC TTT GCA TTT GAA TGA AGA CTT TGC AAA ACA CAC CAA GTT TGG AAA
901                                     931
TAC AAT TGT ACA TGG AGT TTT GAT CAA CGG ACT TAT CTC AGC TCT CCT AGG AAC TAA AAT
961                                     991
GCC AGG GCC AGG CTG TGT ATT TCT TTC CCA GGA AAT TAG CTT TCC AGC CCC TTT ATA TAT
1021                                    1051
TGG AGA AGT TGT TTT AGC TTC TGC AGA AGT GAA AAA GCT GAA GCG GTT CAT TGC TAT TAT
1081                                    1111
TGC AGT GTC ATG TTC TGT AAT AGA AAG TAA AAA GAC TGT TAT GGA AGG CTG GGT TAA AGT
1141                                    1171
TAT GGT TCC AGA AGC TTC CAA ATC CTG AAA TAG ATG TTT TAA AGA TGC AAC CTC AAA CAC
1201                                    1231
CAA TGC TGT TGT TAA AGA GCC TAT GGG GAA TTG CTG CTC TTT ACC AAA GAA TGG TTG ATA
1261                                    1291
GGC CCA GAA GCC CAT GTT AGT TAG GGG AAG GGA GCA GGA AGA GGG TTG TTC AAA TGC CCA
1321                                    1351
CTT TCC AGT TTG GCC TTA TGC TTT ATG CAG ACT TGA GTG TAT GCA GGA TTT CAT TAT CTG
1381                                    1411
CCT GGG TTT TTT TGT TTG TTT TTT GTT TTT TAA TTC AAG AAG TAG GCT GGG CCC GGT GGC
1441                                    1471
TCA TGC CTG TAA TCC TGG CAC TTT GGG AGG CTG CGG CAG GCG GAT CAC TTG AGG TCA GGA
1501                                    1531
GTC CAA GAC CAG CCT GGC CAA CAT GGT GAA ACC CCA TCT CTA CCA AAA AAA AAA AAA AAA
1561
AAA AAA
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B

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1                                       31/2
cta gag agc gcc gga agc ggt ccg aga ATG AAG AGT GTG ATC TAC CAT GCA TTG TCT CAG
                                    M   K   S   V   I   Y   H   A   L   S   Q
61/12                                   91/22
AAA GAG GCG AAT GAC TCC GAT GTC CAG CCT TCA GGA GCA CAG CGG GCC GAG GCC TTC GTG
K   E   A   N   D   S   D   V   Q   P   S   G   A   Q   R   A   E   A   F   V
121/32                                  151/42
AGG GCC TTC CTG AAG CGC ACG ACG CCC CGC ATG AGC CCG CAG GCC CGC GAG GAC CAG CTG
R   A   F   L   K   R   T   T   P   R   M   S   P   Q   A   R   E   D   Q   L
181/52                                  211/62
CAG CGC AAG GCG GTG GTC CTG GAG TAC TTC ACC CGC CAC AAG CGC AAG GAG AAG AAG AAG
Q   R   K   A   V   V   L   E   Y   F   T   R   H   K   R   K   E   K   K   K
241/72                                  271/82
AAA GCC AAA GGC TTC TCT GCC AGG CAA AGG AGG GAG CTG CGG CTC TTT GAC ATT AAA CCA
K   A   K   G   F   S   A   R   Q   R   R   E   L   R   L   F   D   I   K   P

301/92                                 331/102
GAG CAG CAG AGA TAC AGC CTT TTC CTC CCT CTC CAT GAA CTC TGG AAA CAG TAC ATC AGG
E   Q   Q   R   Y   S   L   F   L   P   L   H   E   L   W   K   Q   Y   I   R

361/112                                 391/122
GAC CTG TGC AGT GGG CTC AAG CCA GAC ACG CAG CCA CAG ATG ATT CAG GCC AAG CTC TTA
D   L   C   S   G   L   K   P   D   T   Q   P   Q   M   I   Q   A   K   L   L
421/132                                 451/142
AAG GCA GAT CTT CAC GGG GCT ATT ATT TCA GTG ACA AAA TCC AAA TGC CCC TCT TAT GTG
K   A   D   L   H   G   A   I   I   S   V   T   K   S   K   C   P   S   Y   V
481/152                                 511/162
GGT ATT ACA GGA ATC CTT CTA CAG GAA ACA AAG CAC ATT TTC AAA ATT ATC ACC AAA GAA
G   I   T   G   I   L   L   Q   E   T   K   H   I   F   K   I   I   T   K   E
541/172                                 571/182
GAC CGC CTG AAA GTT ATC CCC AAG CTA AAC TGC GTG TTC ACT GTG GAA ACC GAT GGC TTT
D   R   L   K   V   I   P   K   L   N   C   V   F   T   V   E   T   D   G   F
601/192                                 631/202
ATT TCC TAC ATT TAC GGG AGC AAA TTC CAG CTT CGG TCA AGT GAA CGG TCT GCG AAG AAG
I   S   Y   I   Y   G   S   K   F   Q   L   R   S   S   E   R   S   A   K   K
661/212                                 691
TTC AAA GCG AAG GGA ACG ATT GAC CTG TGA ATT CTT TGC CGT CTA AGG CAG TTG TTT ATG
F   K   A   K   G   T   I   D   L   *
721                                     751
ACA GCT GAA AAC TGG ACA CTC CCT AAA TGT CCA CCT TTC AGT GAA GAG ATA GTT AAG CCA
781                                     811
ATT CCA TTT ATA GAC CAC CTC CAG CCA GTG ACG CTC CGA GTT GAG GAT GTT GAA CAA CAT
841                                     871
GGG AAG GTC GCA GCG TAC TAA GTG AAG AAG TCA GAG GAC AGA GGA ATT TCT CTT TCT AGG
901                                     931
AGA TTT TCA TTT TGT GTG ACT CCC ATG GGG AGG AAC AGA CTG GCA GGA AGC ACA CCG GGG
961                                     991
TTA ACA CTG GTT GAC TTG AAT AGG ATT ATT CGA TTT TTA AAA ATA CTT TTC CAT GTT TTC
1021                                    1051
TGA GTG CTC TAT GAT AAA TCA GTT GCA TCT GTG ATA ATA CAG TAC ATA TGT GGA CAT AAA
1081                                    1111
CAG GGA TCA AAT AAA GGA GGT ATT GCT GCA AAA AAA AAA AAA AAA AAA AA
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FIGURE 1. cDNA sequence and deduced amino acid sequence for Rpp14 (**A**) and Rpp29 (**B**). Nucleotide sequences are numbered from the start of each cDNA. Amino acids are numbered from the first methionine residue and are shown in bold. Portions of the peptide sequences that correspond to tryptic peptide fragments derived from HeLa Rpp14 and Rpp29 are underlined. GenBank accession number for Rpp14 and Rpp29 are AF001175 and AF001176, respectively.

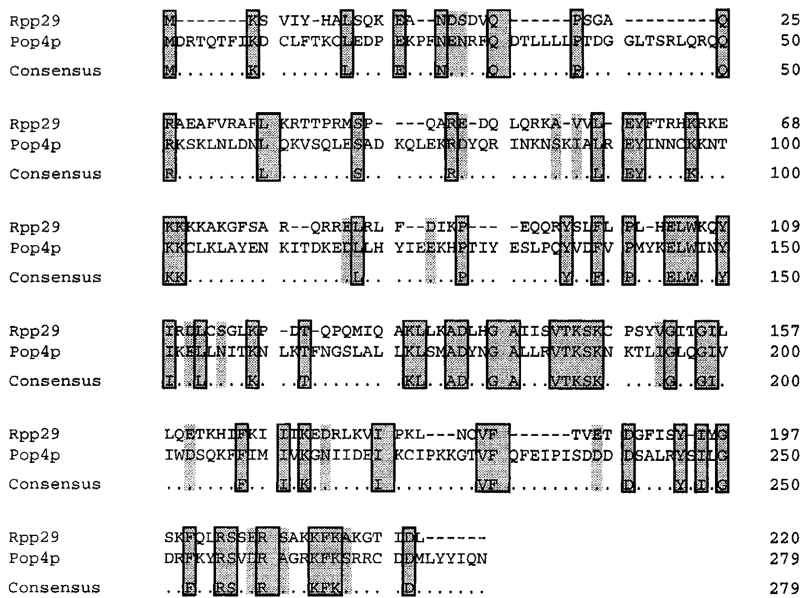

FIGURE 2. Alignment of amino acid sequence of Rpp29 with the yeast Pop4p. Identical amino acids in both proteins are shown in shaded boxes. 23% of residues are identical in both polypeptides. Amino acids with conserved properties are shaded.

Immunobiochemical analysis of Rpp14 and Rpp29

Human RNase P was purified through the MonoQ FPLC step (Eder et al., 1997) and fractions across the peak of enzyme activity for tRNA precursor processing (Fig. 4A) were subjected to Western blot analysis (see Materials and Methods) using the anti-Rpp14 and anti-Rpp29 antibodies described above. Each antibody recognized its corresponding HeLa Rpp14 (Fig. 4B) or Rpp29 (Fig. 4C) protein that copurified with the enzymatic activity (Fig. 4A). Some nonspecific cross-reaction was also observed in lanes 6 and 7 of Figure 4C. We detected human RNase P RNA, H1 RNA, in the same fractions that contained Rpp14 and Rpp29, using Northern blot hybridization analysis (data not shown). These results show that Rpp14 and Rpp29 coexist in fractions containing RNase P.

The ability of the anti-Rpp14 and anti-Rpp29 antibodies described above to precipitate MonoQ-purified RNase P was tested. Both antibodies efficiently brought down active RNase P (Fig. 5, lanes 7 and 9), as did the anti-Rpp20 and anti-Rpp38 antibodies (Fig. 5, lanes 8 and 9), described previously (Jarrous et al., 1998). Anti-Rpp20 and anti-Rpp38 specifically recognized their corresponding proteins in this fraction as well (data not shown). Together, both biochemical purification and immunoprecipitations indicate that Rpp14 and Rpp29 are associated with highly purified, catalytically active human RNase P.

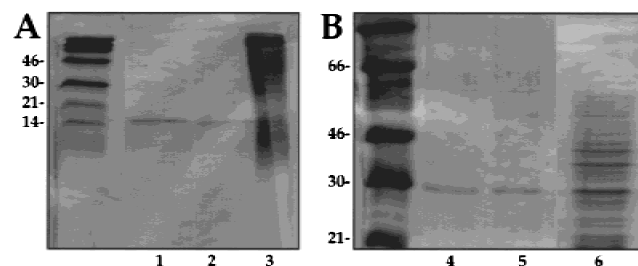

FIGURE 3. Molecular weights and affinity purification of histidine-tagged Rpp14 and Rpp29 proteins. Histidine-tagged Rpp14 and Rpp29 proteins were overexpressed in *E. coli* (lane 3 in **A** and lane 6 in **B**, respectively). These were then affinity purified on a nickel-charged Hi-Trap FPLC column and two fractions eluted with the peak of protein content are presented (lanes 1 and 2 in **A** and lanes 4 and 5 in **B**, respectively). Proteins were separated in 18% (**A**) or 12% (**B**) SDS/PAGE and silver stained. Protein size marker in kDa is indicated.

DISCUSSION

We report here on the cDNA cloning and immunobiochemical characterization of Rpp14 and Rpp29. As with several other previously identified proteins (hPop1, Rpp20, Rpp30, Rpp38, and Rpp40), Rpp14 and Rpp29 are physically associated with RNase P complexes that are catalytically active in the processing of 5′ termini of tRNA precursors in vitro.

Our results suggest that the identity between the human and yeast RNase P protein subunits is relatively limited. Rpp20, Rpp30, Rpp29, and hPop1p have 16–23% identity to their yeast protein homologs and an equal high conservation of biochemical properties of amino acids at given positions. Rpp14, Rpp38, and Rpp40 have no such conservation of identity with yeast genes, including the four RNase P subunits, Pop8p, Pop6p, Pop5p, and Pop3p.

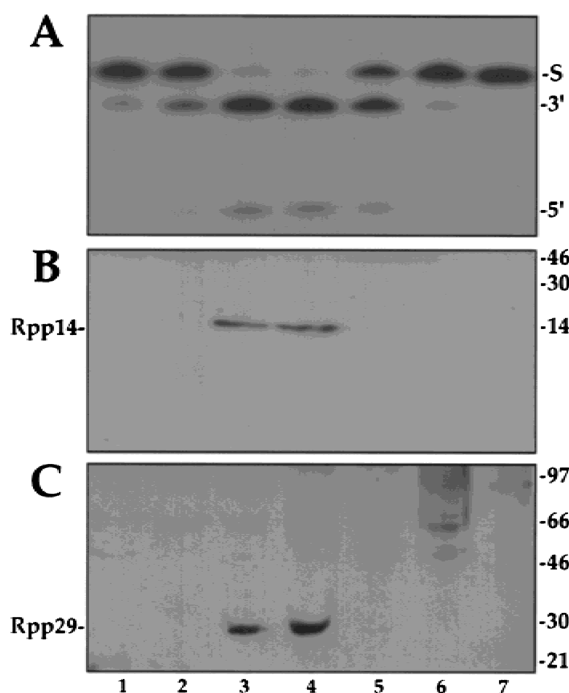

FIGURE 4. Rpp14 and Rpp29 coexist in highly purified HeLa RNase P. Fractions (0.5 mL) eluted from a MonoQ FPLC column were tested for RNase P activity on yeast precursor $tRNA^{Ser}$ (S) (**A**) and subjected to Western blot analyses using anti-Rpp14 (**B**) and anti-Rpp29 (**C**) polyclonal rabbit antibodies. For Western blots, the proteins were separated by 12% SDS/PAGE. The size markers in kDa are shown. RNase P reactions were carried out in 1×PA (see Materials and Methods) for 10 min at 37 °C and the cleavage products, mature tRNA (3′) and 5′ leader sequence (5′), were separated on an 8% polyacrylamide/7 M urea gel. Fractions across the peak of RNase P activity are presented.

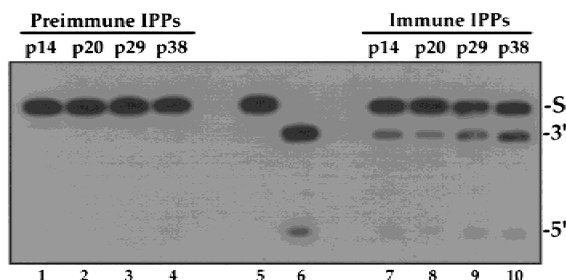

FIGURE 5. Immunoprecipitation of HeLa RNase P. Aliquots of 10 μL from the fraction with the peak of MonoQ RNase P activity shown in lane 4 of Figure 4 were subjected to immunoprecipitation analysis using Protein A beads coupled to sera obtained from rabbits immunized with recombinant Rpp14 (lane 7), Rpp20 (lane 8), Rpp29 (lane 9), or Rpp38 (lane 10). Immunoprecipitates (IPPs) were tested for RNase P activity on $ptRNA^{ser}$ (S) as described in Figure 4. RNase P activity in precipitates obtained with beads coupled to the preimmune rabbit sera (before immunization with the corresponding proteins p14, p20, p29, and p38) are shown on the left side in lanes 1–4, respectively. Control reactions of $ptRNA^{ser}$ incubated with (Lane 6) and without (Lane 5) highly purified RNase P are also shown.

Most of the proteins associated with the yeast (Chamberlain et al., 1998, and references therein) and human nuclear RNase P are basic. The acidic protein subunits of the two holoenzymes, Pop8p and Rpp40, do not share significant homology at the amino acid sequence level. It thus seems possible that shared biochemical and structural characteristics, rather than mere primary sequence, of several human and yeast proteins govern the roles they play in RNase P function in eukaryotes, as is found with the RNase P protein cofactors in eubacteria.

There is, however, extensive similarity between nuclear RNase P and RNase MRP ribonucleoproteins at the level of RNA subunit structure and protein subunit composition. The yeast protein Pop4p is associated with nuclear RNase P and RNase MRP, and is required for their function in stable RNA processing (Chu et al., 1997). Human Rpp29 is a homolog to yeast Pop4p. Although further work is required to establish if Rpp29 is a protein subunit of human RNase MRP, the evolutionary conservation of Rpp29 supports the conclusion that nuclear RNase P and RNase MRP in human cells could share several protein components, as in the case of yeast.

Rpp29 is a highly basic protein in which lysine and arginine residues constitute 20.5% of the amino acid content. Rpp29 has both a putative bipartite nuclear localization sequence, found between positions 53 and 67, and a monopartite nuclear localization sequence at positions 69–74 (Dingwall & Laskey, 1991), as does the human protein, Rpp38 (Jarrous et al., 1998). Fluorescent microscopy studies using green fluorescent protein fused to Rpp38 or Rpp29 indicate that both proteins do indeed contain functional nuclear and nucleolar localization signals (N. Jarrous, J. Wolenski, D. Wesolowski, & S. Altman, in prep.). Accordingly, Rpp38 and Rpp29 may function together to coordinate the nucleolar targeting and/or assembly of RNase P in human cells.

MATERIALS AND METHODS

Molecular cloning of Rpp14 and Rpp29

The purification of human RNase P from HeLa S3 cells through the MonoQ FPLC step has been previously described (Eder et al., 1997). Fractions enriched with RNase P activity were separated on 12% polyacrylamide-0.1% SDS preparative gels, and protein bands of 14 and 30 kDa were extracted after visualization with Coomassie brilliant blue staining. Approximately, 100 pmol of protein were analyzed by the W. M. Keck Biotechnology Resource Center at Yale University.

The tryptic peptides PAPAATYERVVYK and LFDIKPEQQR, obtained from the 14- and 30-kDa protein bands, respectively, were searched in the dbEST using the tblastn program. Several partial human EST clones were found (I.M.A.G.E. consortium cDNA clones) and were completely sequenced. Clone 112976 (accession #T83597) from a fetal liver-spleen

human cDNA library was found to have an insert of approximately 1.5 Kbp with an ORF containing the 14-kDa tryptic peptide. Clone 213567 (accession #H71709), also from a fetal liver-spleen human cDNA library, was found to have an insert of approximately 1.1 Kbp with an ORF containing the 29-kDa tryptic peptide.

Purification of recombinant proteins and preparation of polyclonal antibodies

Two primers, one encompassing the ATG translation initiation codon and upstream sequences of Rpp14 cDNA, and the other encompassing the translation stop codon and downstream sequences, were used for PCR with the Rpp14 cDNA as a template. The PCR product was digested with *Pst*I and *Bst*BI (sites located in the primers) and then subcloned downstream from the T7 promoter and in-frame with six histidine residues of pHTT7K, which was first digested with *Pst*I and *Bst*BI.

Two primers, one encompassing the ATG translation initiation codon and upstream sequences of Rpp29 cDNA, and the other covering the translation stop codon and downstream sequences, were utilized for PCR using the Rpp29 cDNA as a template. The PCR product was digested with *Nde*I and *Bam*HI and subcloned in-frame with the six histidine residues of pHTT7K, which was first digested with *Nde*I and *Bam*HI.

Overexpression of recombinant Rpp14 and Rpp29 polypeptides in *E. coli* strain BL21 (DE3) was performed essentially as described (Jarrous et al., 1998). Overexpressed Rpp14 is a soluble polypeptide in 1×PA buffer. Rpp29 required the addition of 7 M urea to the buffer for it to be soluble. The two proteins were affinity purified on a nickel-charged Hi-Trap FPLC column (Pharmacia). The purity of the proteins was confirmed by SDS/PAGE followed by silver staining.

Polyclonal rabbit antibodies raised against the purified recombinant Rpp14 and Rpp29 proteins were obtained from the Pocono Rabbit Farm (Canadensis, Pennsylvania).

Western blot analysis

Proteins in fractions eluted from the MonoQ FPLC column were separated in 12–18% polyacrylamide-0.1% SDS gels and then electrotransferred to a nitrocellulose membrane and immunoblotted with a 1:200 dilution of sera from immunized rabbits as described (Eder et al., 1997). As a secondary antibody, a 1:5,000 dilution of anti-rabbit mouse IgG antibody (Vector Laboratories) was used. Blots were washed and antibody-antigen complexes were visualized using the ECL-Plus detection system (Amersham), following manufacturer's instructions.

Immunoprecipitation and assay of RNase P

Polyclonal rabbit antibodies (40 μL of sera) were mixed with 5 mg washed protein A Sepharose CL-4B (Pharmacia) in NET-2 buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 0.05% NP-40). Coupling was made by nutating beads overnight at 4 °C, followed by four washes of beads with NET-2 buffer and two washes with 1×PA buffer (50 mM Tris-HCl, pH 7.5, 100 mM NH_4Cl, 10 mM $MgCl_2$) that contained 10 U/mL RNasin. Ten microliters from MonoQ fractions enriched with RNase P were added to the beads in a final volume of 150 μL and mutated over night at 4 °C. Beads were then collected by a short centrifugation, supernatant was removed, and the beads were washed six times with 1×PA buffer. The immunoprecipitates were assayed for the presence of RNase P activity. Cleavage of the 5′ leader of the yeast suppressor precursor $tRNA^{Ser}$ (SupS1) was performed as described (Jarrous et al., 1998), except that the 1×PA buffer contained 2 mM Tris-HCl, pH 7.5, 35 mM NaCl, 0.1 mM Na_2EDTA, 1 mM β-mercaptoethanol, 0.01% Triton X-100 and 60 U of RNasin.

ACKNOWLEDGMENTS

We thank Craig Crews (Yale University) for the use of FPLC apparatus and columns, and our colleagues, especially Cecilia Guerrier-Takada, for assistance and helpful discussions. This work was supported by USPHS grant GM 19422 and HFSP grant RG 02N1 1997M to S.A., and by postdoctoral fellowships to N.J. from Innovir Laboratories and to P.S.E. from the National Institutes of Health.

Received October 7, 1998; returned for revision October 23, 1998; revised manuscript received November 4, 1998

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