hPop5, a Protein Subunit of the Human RNase MRP and RNase P Endoribonucleases*

Hans van Eenennaam, Dorien Lugtenberg, Judith H. P. Vogelzangs, Walther J. van Venrooij, and Ger J. M. Pruijn‡

From the Department of Biochemistry, University of Nijmegen, P. O. Box 9101, NL-6500 HB Nijmegen, The Netherlands

The RNase MRP and RNase P particles both function as endoribonucleases. RNase MRP has been implicated in the processing of precursor-rRNA, whereas RNase P has been shown to function in the processing of pre-tRNA. Both ribonucleoprotein particles have an RNA component that can be folded into a similar secondary structure and share several protein components. We have identified human, rat, mouse, cow, and Drosophila homologues of the Pop5p protein subunit of the yeast RNase MRP and RNase P complexes. The human Pop5p cDNA encodes a protein of 163 amino acids with a predicted molecular mass of 18.8 kDa. Polyclonal antibodies raised against recombinant hPop5 identified a 19-kDa polypeptide in HeLa cells and showed that hPop5 is associated with both RNase MRP and RNase P. Using affinity-purified anti-hPop5 antibodies, we demonstrated that the endogenous hPop5 protein is localized in the nucleus and accumulates in the nucleolus, which is consistent with its association with RNase MRP and RNase P. Catalytically active RNase P was partially purified from HeLa cells, and hPop5 was shown to be associated with it. Finally, the evolutionarily conserved acidic C-terminal tail of hPop5 appeared to be required neither for complex formation nor for RNase P activity.

The RNase MRP/RNase P ribonucleoprotein particles form one of the three families of small nucleolar ribonucleoprotein complexes that are involved in the processing of precursor rRNA to mature 5.8, 18, and 25/28 S rRNA (reviewed in Ref. 1). The RNase MRP ribonucleoprotein particle has originally been identified by virtue of its capacity to cleave a mitochondrial RNA in vitro to generate RNA primers for mitochondrial DNA replication (2). However, most of the RNase MRP complex has been shown to reside in the nucleolus (3, 4). There, it functions in the formation of the short form of the 5.8 S RNA (5.8 S(S)) by cleaving at site A3 in the internal transcribed spacer 1 (ITS1) of precursor rRNA (5–8). In many different aspects, the RNase MRP complex is related to the RNase P complex, a ribonucleoprotein complex required for the removal of the 5′ end of the precursor tRNAs. Both function as site-specific endoribonucleases, contain an RNA component that has been proposed to adopt a similar cage-shaped structure, share several protein subunits, and are predominantly localized in the nucleolus (reviewed in Ref. 9).

The biological importance of the RNase MRP function is substantiated by the recent observations that mutations in the gene encoding the RNA component of the human RNase MRP complex cause an autosomal recessive disease called cartilage-hair hypoplasia (10). In addition, RNase MRP and RNase P play a role in certain autoimmune diseases. Protein components of the RNase MRP and RNase P complexes are targeted by autoantibodies in systemic lupus erythematosus and scleroderma (11–15).

The first identified protein subunit of the human RNase MRP and RNase P complexes is the hPop1 protein, which is a homologue of the yeast Pop1p protein (16). Most of the currently known human protein subunits of the RNase MRP and RNase P complexes have been identified via purification of the RNase P complex from HeLa cells: Rpp14, Rpp20, Rpp29/hPop4, Rpp30, Rpp38, and Rpp40 (17–20). In yeast 10 proteins have been found to be associated with RNase MRP or RNase P or with both complexes (reviewed in Ref. 9). Snm1p has been reported to be associated specifically with the RNase MRP complex, whereas the Rpr2p protein is only bound to the RNase P complex (21, 22). Other subunits shared by RNase MRP and RNase P in yeast are: Pop1p, Pop7p/Rpp2p, Pop4p, Rpp1p, Pop3p, Pop5p, Pop6p, and Pop8p (7, 22–26). Although homologues for several of the yeast proteins are found in human cells (Pop1p, Pop4p, Pop7p/Rpp2p, Rpp1p), until now no homologues have been identified for Snm1p, Pop3p, Pop5p, Pop6p, Pop8p, and Rpr2p (reviewed in Ref. 9). In this report we describe the identification, cDNA cloning, and characterization of the human Pop5 protein, which, like all human protein subunits identified so far, is associated with both RNase MRP and RNase P RNAs.

EXPERIMENTAL PROCEDURES

cDNA Cloning and Sequence Analysis—Data base searches were performed using the BLASTN 2.0.10 program (27). The accession numbers of the human expressed sequence tags (ESTs)* are AF117232, XM-012124, NM-015918, and AF070660. The accession numbers for the overlapping mouse ESTs are BF121181 and BF583365, for rat ESTs BF567090 and AW191827, for cow BF076568, and for Drosophila mela-nogaster AAF49372.

The following oligonucleotides (based on the human EST sequence) were used to isolate the open reading frame of hPop5 by a PCR-based approach: pop51 (5′-GGG-GATT-CCC-TGG-AGA-TGG-TGC-GGT-TCA-AGC-ACA-GG-3′) and pop52 (5′-GGG-GATT-CCC-CGG-GAT-CTA-GAC-TCC-ATT-GCT-TCT-GCA-GCC-TCC-TCT-3′). The polymerase chain reaction was performed with 200 ng of denatured DNA from Agt11 human

* This work was supported in part by the Netherlands Foundation for Chemical Research with financial aid from the Netherlands Organization for Scientific Research. 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.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AJ306296.

‡ To whom correspondence should be addressed: Dept. of Biochemistry 161, University of Nijmegen, P. O. Box 9101, NL-6500 HB Nijmegen, The Netherlands. Tel.: 31-24-3616847; Fax: 31-24-3540525; E-mail: g.pruijn@bioch.kun.nl.
placenta (CLONTECH) and teratocarcinoma cDNA libraries as starting material (28). The amplified fragments were ligated in the PCR-II-TOPO vector (Invitrogen) and sequenced using the deoxynucleotide chain termination method.

Affinity Purification of hPop5 Antibodies—To obtain anti-hPop5 and anti-GST antibodies, two affinity columns were prepared by immobilizing either 1.25 mg of GST-hPop5 or 4.8 mg of GST protein to 1.5 g of CNBr-activated Sepharose 4B (Amersham Pharmacia Biotech) according to the manufacturer’s instructions. Rabbit serum raised against GST activity could be measured in the flow-through using enzyme-linked immunosorbent assay methods. Subsequently, the flow-through was incubated with affinity-purified rabbit anti-hPop5 antibodies and affinity-purified rabbit anti-GST antibodies were performed on HEp-2 cells. Fixed cells were incubated for 1 h at room temperature, washed with PBS, and subsequently incubated with Alexa Fluor® 488 goat anti-rabbit IgG conjugate (Molecular Probes, diluted 1:75 in PBS) for 1 h at room temperature. Bound antibodies were visualized by epifluorescence microscopy.

RNase P Purification from HeLa Cells—Purification of the human RNase P enzyme was performed as described previously (17, 33). HeLa S3 cells (National Cell Culture Center, Minneapolis, MN) were pelleted, disrupted, and the cell lysate was centrifuged at 10,000 × g, followed by another centrifugation at 100,000 g. The extract was loaded on a DEAE-Sepharose column, and bound material was eluted with a linear 100–500 mM KCl gradient. Fractions containing RNase P activity were pooled, concentrated, and further fractionated by centrifugation in a 15–30% glycerol gradient.

Pre-tRNA Processing Assay—To assay for RNase P activity in the immunoprecipitates and column fractions, an internally 32P-labeled pre-tRNA substrate (Schizosaccharomyces pombe rRNA™ SupS5), was transcribed in vitro and gel-purified. This 110-nucleotide-long substrate contains a 5′ end extension of 28 nucleotides in comparison with the mature tRNA. The immunoprecipitates or fractions were incubated with equal amounts of substrate in assay buffer (20 mM Tris-HCl, pH 8.0, 10 mM MgCl2, 1 mM dithioerythritol, 10 mM KCl, 50 μg/ml bovine serum albumin, 60 units/ml RNasin) for 10 min at 37 °C. Reaction products were analyzed by denaturing polyacrylamide gel electrophoresis and autoradiography.

Transfection Constructs—VSV G epitope (34) (VSV-G)-tagged (hereafter referred to as VSV-tagged) cDNAs were constructed as follows. 55k-VSV cDNA constructs (31), containing either a XhoI or a XbaI site between the 5′-kDa open reading frame (ORF) and the VSV tag sequence (positioned at the N- and C-terminal sides, respectively) were cleaved with XhoI and XbaI releasing the 55-kDa cDNA from this plasmid. The VSV-tagged constructs of hPop5 were constructed in isolation of the hPop5 ORF from the hPop5/PCR-II-TOPO construct by PCR-based approach. Besides the deletion introduced by the
PCR strategy, an XhoI site was introduced at the 5' end of the translational start codon, whereas an XbaI site was introduced at the 3' end of the coding sequence. The transcription construct was generated by ligating the XhoI/XbaI fragment into the XhoI/XbaI-digested 5k-VSV constructs. The integrity of the resulting construct was confirmed by DNA sequencing. The pcT-neo vector (Promega), which has been used to prepare 5k-VSV previously, was used as a control in the transfection experiments.

**RESULTS**

**Identification of Homologues of Yeast Pop5p**—Purification of the yeast RNase P holoenzyme has resulted in the identification of nine protein subunits (22). Although some of these were shown to have homologues in humans, no human homologues have been reported for Snm1p, Pop3p, Pop5p, Pop6p, Pop8p, and Rpr2p. We have searched protein and nucleic acid sequence databases to identify sequences that might represent human homologues of these yeast protein subunits.

Four virtually identical nucleic acid entries were found that encode a putative human homologue of the yeast Pop5p protein (hereafter designated hPop5). The latter polypeptide consists of 173 amino acids and has a predicted molecular mass of 19.6 kDa. The corresponding human sequence (data base entry AF117232) is 785 nucleotides long and contains an ORF encoding a polypeptide of 162 amino acids with a predicted molecular mass of 18.7 kDa. The corresponding human sequence (data base entry AF117232) is 785 nucleotides long and contains an ORF encoding a polypeptide of 162 amino acids with a predicted molecular mass of 18.7 kDa. For reasons documented below, this protein will be referred to as hPop5.

Besides the human ESTs retrieved from the sequence data bases, two mouse ESTs, two rat ESTs, one cow EST, and one *D. melanogaster* entry that encode putative human Pop5p protein were identified. These entries contain ORFs of 169, 169, 170, and 145 amino acids, respectively.

**Cloning of the Human Pop5 cDNA**—To obtain a cDNA encoding the putative human Pop5 protein, two oligonucleotides were designed based on the identified human EST sequence. These oligonucleotides were used as PCR primers to isolate hPop5 cDNAs from human teratocarcinoma and placenta cDNA libraries. Sequence analysis of several clones obtained by this procedure revealed that cDNAs derived from both sources were identical, thereby ruling out the introduction of PCR artifacts. Two minor differences were found in all sequenced cDNAs in comparison with the corresponding EST described above (AF117232). Nucleotides 154–162 in the cloned cDNA sequence (numbering according to data base accession no. AJ306296) are 5'-GCA GCC GCC-3', whereas the EST contains 5'-GCA CCC-3' at this position, resulting in the replacement of a Pro codon by two Ala codons in the cloned cDNA in comparison with the EST. Furthermore, a single nucleotide substitution was detected at position 461, where a T residue was found rather than a C residue, which is observed at this position in the human EST.

To determine whether the first ATG present in the human EST represents the translational start codon, we synthesized cDNA from several sources of mRNA. DNA sequencing of the resulting clones revealed that no additional sequence information was obtained (data not shown). The cloned cDNA encodes a protein of 163 amino acids, with a predicted molecular mass of 18.8 kDa and a predicted pI of 7.9.

In Fig. 1 an alignment of the amino acid sequences derived from the human, cow, rat, mouse, *Drosophila*, and yeast cDNAs/ESTs is shown. This illustrates the high level of homology between the mammalian Pop5 polypeptides (88–91% identity, 93–95% similarity), whereas the homology between the human, *Drosophila*, and yeast proteins is much lower (23–27% identity and 40–43% similarity). The conservation of the yeast and human Pop5 amino acid sequences is most extensive in the N terminus. Except for the acidic C terminus, the primary sequence of hPop5 does not reveal established sequence motifs. The acidic nature of the C terminus is conserved from human to yeast (in hPop5, 9 out of 15 residues are acidic), although it appears to be absent in the *Drosophila* sequence.

**Anti-hPop5 Antibodies Immunoprecipitate Both RNase MRP and RNase P Particles**—To determine whether the hPop5 protein is associated with the human RNase MRP and RNase P ribonucleoprotein particles, a polyclonal antibody was raised against recombinant hPop5. The hPop5 protein was expressed...
Cloning and Characterization of the hPop5 Protein

As a fusion protein with GST in E. coli, the recombinant GST-hPop5 fusion protein was purified using glutathione-Sepharose 4B beads (Fig. 2, lane 2) and used for immunization of rabbits. Western blot analysis showed that the resulting rabbit serum is reactive with the GST-hPop5 protein (lane 4), whereas the pre-immune serum is not (lane 3). Because the rabbit serum failed to recognize the hPop5 protein in HeLa extracts (data not shown), anti-hPop5 antibodies were purified by affinity selection with recombinant GST-hPop5 from the rabbit serum. Prior to the GST-hPop5 selection, the rabbit serum was depleted of anti-GST activity by performing affinity selection with recombinant GST. Western blot analysis of HeLa cell extracts with the affinity-purified antibodies against hPop5 revealed a single protein with an estimated size of 19 kDa (lane 6), whereas the affinity-purified antibodies against GST failed to detect any protein in HeLa extracts (lane 5).

To investigate whether the hPop5 protein is associated with the RNase MRP and RNase P complexes, immunoprecipitations with the anti-hPop5 serum using total HeLa cell extract were performed. The co-precipitating RNAs were isolated and analyzed by Northern blot hybridization using riboprobes specific for RNase MRP, RNase P, and U3 RNA. As depicted in Fig. 3 (lane 6), the anti-hPop5 antiserum efficiently immunoprecipitated both RNase MRP and RNase P RNAs, whereas no detectable precipitation of U3 RNA was observed. The specificity of this assay was demonstrated by the observation that pre-immune serum did not precipitate any of the RNAs analyzed (lane 5) and that anti-55K antibodies specifically precipitated U3 RNA (lane 3) in accordance with previous observations (31). In addition, the RNase MRP and RNase P RNAs were co-immunoprecipitated by a patient anti-Th/To antiserum and by anti-hPop4 antibodies (lanes 2 and 4). These results indicate that the 19-kDa hPop5 protein is associated with both RNase MRP and RNase P RNAs in HeLa cells.

hPop5 Accumulates in the Nucleoli—To investigate the subcellular localization of the hPop5 protein, the affinity-purified antibodies directed against GST-hPop5 and GST were used for immunolocalization experiments in HEP-2 cells. The affinity-purified anti-hPop5 antibodies strongly stained the nucleoli and showed, in addition, a fine-speckled cytoplasmic staining (Fig. 4, panel A). Using affinity-purified anti-GST antibodies, a similar fine-speckled cytoplasmic staining pattern, but no nucleolar staining, was observed (panel C), strongly suggesting that the cytoplasmic staining is caused by anti-GST reactivity. Although these results do not completely rule out a cytoplasmic localization of the hPop5 protein, they clearly show that the hPop5 protein accumulates in the nucleoli, consistent with its association with RNase MRP and RNase P.

Anti-hPop5 Antibodies Immunoprecipitate Catalytically Active RNase P—To obtain further evidence that the hPop5 protein is part of the complete RNase P complex, we tested whether hPop5 is associated with catalytically active RNase P. First, we partially purified the active RNase P complex from a HeLa cell extract by DEAE-Sepharose chromatography and glycerol gradient centrifugation. The fractions of the glycerol gradient were tested for RNase P activity by monitoring its ability to specifically cleave an internally labeled precursor tRNA substrate to tRNA and its 5’-leader. Products of this reaction were separated on a denaturing polyacrylamide gel and visualized by autoradiography. In agreement with previous observations (17,33), the RNase P activity was only detected in relatively fast sedimenting fractions (Fig. 5A). To study the immunoprecipitates of the hPop5 protein in the active RNase P fractions of the glycerol gradient, these fractions were analyzed by immunoblotting. As shown in the lower part of Fig. 5A, hPop5 (bottom panel), hPop4 (middle panel), and Rpp38 (top panel) co-fractionated with the RNase P activity, suggesting that hPop5 indeed is associated with catalytically active RNase P. To confirm this, immunoprecipitations were performed on RNase P containing glycerol gradient fractions using affinity-purified anti-hPop5 and anti-GST antibodies and the immunoprecipitates were assayed for RNase P activity. As shown in Fig. 5B (lanes 2 and 3), the immunoprecipitate obtained with the affinity-purified anti-hPop5 antibodies was able to cleave pre-tRNA into mature tRNA and its 5’-leader, whereas the immunoprecipitate of the affinity-purified anti-GST antibodies was not. The RNase P activity in the anti-hPop5 immunoprecipitate was indistinguishable from that obtained with anti-hPop4 antibodies, which functioned as a positive control (lane 1). These results indicate that the hPop5 protein is part of the catalytically active RNase P complex. Although these data do not show that the hPop5 protein is associated with catalytically active RNase MRP as well, the strong evolutionary relationship between the RNase P and RNase MRP complexes suggests that hPop5 is also associated with RNase MRP.

The Acidic C Terminus of hPop5 Is Not Essential for Complex Association—As described above, the only amino acid sequence element that could be detected in the primary structure of hPop5 is its acidic C terminus. Therefore, we investigated...
whether this sequence element is required for complex association. A VSV tag sequence (34) was fused to the 3' end of the hPop5 ORF and to the 3' end of a truncated cDNA lacking the sequence corresponding to the acidic C terminus (designated hPop5-VSV and hPop5-H9004 (145–163), respectively). HEp-2 cells were transfected with these constructs or with the corresponding “empty” vector as a negative control. After culturing the cells for 16-h extracts were prepared and used for immunoprecipitation with anti-VSV tag antibodies. Co-precipitating RNAs were isolated and analyzed via Northern blot hybridization using riboprobes for RNase MRP and RNase P RNA. As is shown in Fig. 6A (lanes 1 and 2), RNase MRP and RNase P RNA were both immunoprecipitated from a cell extract containing the hPop5-VSV protein, but not from an extract from cells transfected with the empty vector. This result confirms that hPop5 associates with the RNase MRP and RNase P RNAs in human cells and demonstrates that this approach is suitable to analyze hPop5 mutants. As depicted in lane 3, the hPop5 Δ(145–163) mutant was able to associate with both RNase MRP and RNase P RNA, indicating that the evolutionarily conserved acidic C terminus is not required for association with these ribonucleoproteins.

To investigate whether this element plays a role in the endoribonuclease activity of RNase P, HEp-2 cells were trans-

FIG. 4. Subcellular localization of the hPop5 protein. HEp-2 cells were fixed and stained with affinity-purified anti-hPop5 (panel A) or affinity-purified anti-GST (panel C) antibodies. The corresponding phase-contrast images are shown in panels B and D, respectively.

FIG. 5. hPop5 associates with catalytically active RNase P. The RNase P holoenzyme was purified from a total HeLa extract by DEAE-Sepharose column chromatography followed by glycerol gradient centrifugation. A, top panel, fractions of the glycerol gradient (fractions 1–29) were assayed for RNase P activity by incubation with a 32P-labeled pre-tRNA substrate (input). Subsequently, the reaction products were analyzed by denaturing polyacrylamide gel electrophoresis and autoradiography. On the right, the positions of the pre-tRNA, the mature tRNA, and the 5' leader are indicated. Bottom panel, detection of RNase MRP/RNase P proteins in glycerol gradient fractions. Reactivity with affinity-purified anti-hPop5 (bottom), anti-hPop4 (middle), and anti-Rpp38 (top) antisera with proteins from glycerol gradient fractions was analyzed by Western blotting. On the left the positions of molecular weight markers are indicated, and on the right the antisera that were used. B, anti-hPop4 (lanes 1 and 5), affinity-purified anti-GST (lanes 2 and 6), and affinity-purified anti-hPop5 (lanes 3 and 7) antibodies were used for immunoprecipitations using fraction 5 of the glycerol gradient. Immunoprecipitates (IPP) and supernatants were assayed for RNase P activity (lanes 1–3 and 5–7, respectively). On the right, the positions of the pre-tRNA, the mature tRNA, and the 5'-leader are indicated.
Cloning and Characterization of the hPop5 Protein

Fig. 6. The C terminus of hPop5 is neither required for complex association nor for RNase P activity. HEp-2 cells were transfected with constructs encoding hPop5, hPop5 Δ(145–163), and the corresponding “empty” vector as a negative control, and extracts of these cells were subjected to immunoprecipitation with anti-VSV antibodies. A, association of VSV-tagged hPop5 and the C-terminal deletion mutant of hPop5 with RNase MRP and RNase P complexes. Lanes 1 and 4, empty vector; lanes 2 and 5, hPop5; lanes 3 and 6, hPop5 Δ(145–163). RNA isolated from extract (input; lanes 4–6) and immunoprecipitates (IPP; lanes 1–3) was analyzed by Northern blotting using riboprobes for RNase MRP and RNase P RNA, as indicated on the right. B, RNase P activity was assayed as described in the legend of Fig. 5. Lane 1, material from cells expressing hPop5-VSV; lane 2, hPop5 Δ(145–163); lane 3, empty vector; lane 4, substrate pre-trNA; lane 5, glycerol gradient fraction 5. On the right, the positions of the pre-trNA, the mature RNA, and the 5′-leader are indicated.

hPop5 Is Evolutionarily Conserved—Besides the human homologue of Pop5p, our data base searches identified two ESTs (accession nos. AI751395 and AA834933) encoding a putative homologue of the RNase P-specific protein subunit: Rpr2p (9). No homologues were identified for the Snm1p, Pop3p, Pop6p, and Pop8p protein, suggesting that only 6 of the 10 yeast RNase MRP/RNase P protein subunits have human counterparts. A high degree of sequence conservation was observed among the mammalian Pop5 proteins, whereas only a moderate level of conservation exists between human, yeast, and Drosophila Pop5 (23–27% identity). A similar level of sequence conservation has been reported for other RNase MRP and RNase P protein subunits: hPop1/Pop1p (22% identity), Rpp30/Rpp1p (23% identity), Rpp20/Rpp2p (14% identity), and hPop4/Pop4p (29% identity) (16–20). Recently, the analysis of the genomes of archaea bacteria allowed the detection of hPop5 and Rpp30 counterparts also in these organisms (35) and in addition showed that the secondary structures of Pop5 and Rpp14 are related. A similar conservation from humans to archaea has been suggested for the Rpp29/hPop4 and Rpp21 subunits (36). A comparison of the secondary structures of RNase MRP RNA and RNase P RNA from various organisms has led to the hypothesis that RNase MRP evolved from RNase P in an early eukaryote (37). The association of the four highly conserved proteins with MRP RNA would be explained by the hypothesis that proteins associated with RNase P RNA have the capacity to bind MRP RNA as well.

Nucleolar Accumulation of hPop5—We showed that the hPop5 protein is localized in the nucleus and accumulates in the nucleolus (Fig. 4). Previously, it has been proposed that nucleolar entry is a two-step mechanism (38–40). First, a nucleolar protein is transported to the nucleus and subsequently it accumulates in the nucleolus. Recently, we demonstrated that clusters of basic amino acids are important for the localization of protein subunits of RNase MRP and RNase P in the nucleolus.2 The absence of a nuclear localization signal as well as clusters of basic residues in the hPop5 amino acid sequence suggests that hPop5 is targeted to the nucleus/nucleolus in a different manner. The hPop5 protein might be transported to the nucleus and nucleolus by a piggyback mechanism. In that case hPop5 binds in the cytoplasm to another (RNase MRP/RNase P) protein, which carries the hPop5 protein to the nucleus and subsequently to the nucleolus. Transport of the hPop5 protein from the nucleoplasm to the nucleolus might also be dependent on its association with the (partially assembled) RNase MRP/RNase P ribonucleoprotein complexes. A similar situation has been proposed for the Rpp14 protein (41).

hPop5 Is the Sixth Protein Subunit Shared by the Human

2 H. van Eenennaam, A. van der Heijden, R. J. R. J. Janssen, W. J. van Venrooij, and G. J. M. Pruijn, submitted for publication.
RNase MRP and RNase P Complexes—The results in Figs. 3 and 6 demonstrate that the hPop5 protein subunit, like its yeast counterpart, is associated with both RNase MRP and RNase P. The association of protein subunits with both RNase MRP and RNase P has been previously reported for hPop1, Rpp30, Rpp38, and hPop4/Rpp29 (reviewed in Ref. 9) and has been observed for the Rpp20 protein subunit as well.3 The sharing of protein subunits by these endoribonuclease complexes might be explained at least in part by the conservation of the secondary structure of their RNA components and the suggestion that the RNase MRP RNA has evolved from the RNase P RNA in early eukaryotes (see above). In yeast two protein subunits have been described to be specifically associated with either the RNase MRP complex or the RNase P complex (21, 22). Such complex-specific protein subunits have been observed for the Rpp20 protein subunit as well.3 The Rpp30, Rpp38, and hPop4/Rpp29 (reviewed in Ref. 9) and has been previously reported for hPop1, and G. J. M. Pruijn, unpublished observations.

An alternative explanation for extensive subunit sharing is the possibility that RNase MRP and RNase P assemble into a single complex in human cells. This possibility is supported by the finding that a subset of the cellular RNase MRP and RNase P complexes can be physically separated and they function separately from each other (6, 17, 20, 22). The exact function and architecture of this human macromolecular complex and its relation to the individual RNase MRP and RNase P complexes remain to be elucidated and provide interesting topics for further research.

Acknowledgments—We thank Drs. Cecilia Guerrier-Takada and Sidney Altman for sharing their knowledge on the purification of the RNase P holoenzyme and for their kind gift of anti-Rpp38 antibodies and Dona Wesolowski for technical assistance (Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT). The patient serum was kindly provided by Dr. Frank van den Hoogen (De-
hPop5, a Protein Subunit of the Human RNase MRP and RNase P Endoribonucleases
Hans van Eenennaam, Dorien Lugtenberg, Judith H. P. Vogelzangs, Walther J. van Venrooij and Ger J. M. Pruijn

J. Biol. Chem. 2001, 276:31635-31641.
doi: 10.1074/jbc.M103399200 originally published online June 18, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M103399200

Alerts:
  • When this article is cited
  • When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 41 references, 25 of which can be accessed free at http://www.jbc.org/content/276/34/31635.full.html#ref-list-1