BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **243,** 101–108 (1998)
ARTICLE NO. RC978038

Cloning and Characterization of Two Human cDNAs Encoding the mRNA Capping Enzyme

Toshihiko Tsukamoto, Yoshio Shibagaki, Teruko Murakoshi, Masako Suzuki, Akiko Nakamura, Hideo Gotoh, and Kiyohisa Mizumoto[1]

Department of Biochemistry, School of Pharmaceutical Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108, Japan

Received December 26, 1997

Previous studies demonstrated that the mammalian mRNA capping enzyme is a bifunctional enzyme containing RNA 5′-triphosphatase and mRNA guanylyltransferase activities in a single polypeptide. In yeast, both the above activities are separated into two different subunits, α and β, the genes for which we have cloned recently. It is thus interesting to compare the structural and functional relationships between the mammalian and yeast capping enzymes. Here we isolated two human cDNAs encoding mRNA capping enzymes termed *hCAP1a* and *hCAP1b* which encode 597 and 541 amino acids, respectively. They are different only at the region coding for the C-terminal portion of the enzyme. Comparison of the deduced amino acid sequences with other cellular and viral capping enzymes showed that all the regions conserved among mRNA guanylyltransferases are observed in our clones except one conserved C-terminal region which was absent in the hCAP1b protein. The purified recombinant *hCAP1a* gene product, hCAP1a, exhibited both RNA 5′-triphosphatase and mRNA guanylyltransferase activities. Deletion mutant analysis of hCAP1a showed that the N-terminal 213 amino acid fragment containing a tyrosine specific protein phosphatase motif catalyzed the RNA 5′-triphosphatase activity and the C-terminal 369 amino acid fragment exhibited the mRNA guanylyltransferase activity. On the other hand, hCAP1b showed RNA 5′-triphosphatase activity, but neither enzyme-GMP covalent complex formation nor cap structure formation was detected.

Eukaryotic mRNAs contain a 5′-terminal cap structure. The cap structure plays crucial roles in the various steps of gene expression including initiation of translation (1), RNA splicing (2, 3), transport of mRNA from the nucleus to the cytoplasm (4), and mRNA turnover (5). The cap structure is formed as an early cotranscriptional modification of nascent RNA chains transcribed by RNA polymerase II in a series of enzymatic activities (for reviews, see Refs. 6, 7, 8). The first step of cap formation is the removal of the γ-phosphate from the 5′-triphosphate end of newly synthesized RNA by RNA 5′-triphosphatase to generate a diphosphate end. The GMP moiety of GTP is then transferred to the 5′-diphosphate terminus of RNA by mRNA guanylyltransferase. After these two consecutive reactions, methylation at the guanine N7 position catalyzed by mRNA (guanine-7-)methyltransferase takes place.

The capping enzyme from animal sources is a bifunctional enzyme with mRNA guanylyltransferase and RNA 5′-triphosphatase activities (6). Capping enzymes from HeLa cells, rat liver, and *Artemia salina* consist of a single polypeptide chain of 69 kDa, 69 kDa, and 73 kDa, respectively, which contain catalytic domains for both mRNA guanylyltransferase and RNA 5′-triphosphatase (6, 9, 10). In contrast, the yeast capping enzyme is composed of two subunits, α (52 kDa) and β (80 kDa), which catalyze mRNA guanylyltransferase and RNA 5′-triphosphatase, respectively (11).

Using antibodies raised against the purified yeast capping enzyme we cloned the gene (*CEG1*) coding for mRNA guanylyltransferase (α subunit) and demonstrated that it is essential for the growth of yeast cells (12). Mutational studies on *CEG1* indicated that the importance of a lysine residue (Lys70) which resides in a motif conserved among capping enzymes and polynucleotide ligases (13, 14, 15). mRNA guanylyltransferase genes from *Schiz. pombe* (*PCE1*) (16) and *C. albicans* (*CGT1*) (17) have also been cloned by complementation of mutant *CEG1* alleles and it has been shown that their products are highly homologous to Ceg1. Recently, using primers designed according to the partial amino acid sequences of the purified β subunit from

[1] To whom correspondence should be addressed. Fax: 03-3444-6198. E-mail: mizumotok@pharm.kitasato-u.ac.jp.

The abbreviations used are: *CET1,* capping enzyme triphosphatase subunit gene; *CEG1,* capping enzyme guanylyltransferase subunit gene; *hCAP1,* human capping enzyme gene; PVDF, polyvinylidene difluoride.

0006-291X/98 $25.00

the *S. cerevisiae* capping enzyme, we isolated the gene for RNA 5′-triphosphatase (*CET1*) (18). It is of interest to compare the structure-function relationships of capping enzymes from yeast and higher eukaryotes. However, so far the structure of the capping enzyme from higher organisms has not been elucidated. In this report, we isolated two cDNAs encoding human mRNA capping enzyme. The isolated cDNAs were expressed in *E. coli* and the enzymatic activities of the recombinant proteins were characterized. We also constructed deletion mutants and showed that the regions responsible for the RNA 5′-triphosphatase and mRNA guanylyltransferase activities reside in the N-terminal and the C-terminal portions, respectively.

MATERIALS AND METHODS

RT-PCR. From the nucleotide sequences of mouse EST (GenBank accession number AA096583), the following sense and antisense primers were synthesized: CEF1, 5′-TTT TCA GCC TAT TGG AAA ATA C-3′ and CER1, 5′-GTC AGG GTC CAG GGG ATA CTT-3′. Total RNA was extracted from mouse spleen using a single step procedure (19). First strand cDNA was synthesized using an oligo(dT) primer and used as the template for amplification. PCR was performed in a 50 μl volume of reaction mixture containing 10 mM Tris-HCl (pH 8.3), 2 mM $MgCl_2$, 50 mM KCl, 0.01% (w/v) gelatin, 200 μM each of dGTP, dATP, dTTP, and dCTP, 2.5 units of *Taq* DNA polymerase, 0.2 μM of the above primers and the synthesized cDNA. The amplification was carried out for 35 cycles, each consisting of a denaturing step at 94°C for 30 seconds, annealing at 52°C for 30 seconds, and extension at 72°C for 1 minute. The last cycle was followed by a 7 minutes-extension at 72°C. The products were separated on a 2% agarose gel run in 0.5 × TBE buffer (1× TBE: 89 mM Tris base, 89 mM boric acid, 2 mM EDTA).

Library screening. The amplified band obtained by PCR was used as a probe to screen a λ ZAP II cDNA library derived from the human colon adenocarcinoma cell line COLO 205 (Stratagene). Bacteriophage plaques bound to a nylon membrane were hybridized to a digoxigenin-labeled probe in 30% formamide at 42°C and washed under low-stringency conditions (final wash, 2×SSC/0.1% SDS at room temperature). The inserts of positive clones were sequenced by cycle sequencing using AmpliTaq DNA polymerase, FS (PE Applied Biosystems) on the Perkin-Elmer GeneAmp PCR System 2400.

Recombinant proteins. For the recombinant hCAP1a protein expression in *E. coli*, the coding sequence of *hCAP1a* was amplified from a cloned plasmid DNA by PCR using a sense primer corresponding to the region upstream of the initiator AUG generating 5′ *Nde*I site, and an antisense primer located downstream of the stop codon containing *Bam*HI site. The primers used were: CF1, 5′-CAA CAT ATG GCT CAC AAC AAG ATC-3′ (*Nde*I site underlined); CBR1, 5′-TCG GAT CCA GGT CTT AGG TTA AAG-3′ (*Bam*HI site underlined). The amplified fragment was digested with *Nde*I and *Bam*HI and ligated into the pT7-7(His) plasmid (20). For the expression of hCAP1b, amplification of the coding sequence was performed using CF1 and CHR1, 5′-TCA AGC TTC TGG GCT CAA GTG ATC-3′ (*Hin*dIII site underlined). The amplified fragment was digested and ligated into the pT7-7(His) plasmid. The recombinant hCAP1a and hCAP1b were expressed in a *E. coli* strain BL21(DE3)pLysS and purified on a Ni^{2+}-nitrilotriacetic acid (Ni-NTA) column (QIAGEN). For the construction of C-terminal deletion mutant of hCAP1a, the *Sma*I-*Hin*dIII fragment was removed from the constructed plasmid and self-ligated to generate His-CAP1a (1-213). The primers used for the construction of N-terminal deletion mutant, His-CAP1a (229-597) were: DFN1, 5′-TTA CAT ATG GGC GCT ATT TTC TTG-3′ (*Nde*I site underlined) and CBR1. The amplified fragment was digested with *Nde*I and *Bam*HI and ligated into the pT7-7 (His) plasmid. Recombinant Ceg1 (15) and Cet1 (18) proteins were expressed in *E. coli* and purified as described.

Western blotting. Proteins were separated by SDS-PAGE and transferred to PVDF membrane. After blocking the blots were incubated with horseradish peroxidase-conjugated Ni-NTA (QIAGEN), and then visualized by the ECL system (Amersham).

mRNA guanylyltransferase activity. mRNA guanylyltransferase activity was assayed by the enzyme-[^{32}P]GMP complex formation as well as the cap formation as previously described (21).

RNA 5′-triphosphatase activity. RNA 5′-triphosphatase acitivity was assayed by the procedure described earlier using [γ-^{32}P]pppG-RNA (9,18).

RESULTS

In our previous work, we isolated the yeast gene designated *CEG1* encoding mRNA guanylyltransferase (mRNA capping enzyme α subunit) (12). Homology search of the GenBank using the *CEG1* sequence revealed a high predicted amino acid similarity to mouse EST (GenBank accession number AA096583). We then synthesized a pair of primers to amplify this sequence from mouse mRNA. RT-PCR using these primers and mouse spleen RNA as a template yielded a 451-bp fragment. Using this PCR fragment as a probe, we isolated 2 clones, λhC41 and λhC42, from a cDNA library of a human colon adenocarcinoma cell line. DNA sequence analysis of the isolated cDNA clones showed that λhC42 contained an open reading frame of 1,791 nucleotides coding for 597 amino acids with a calculated M_r of 68,557. Whereas λhC41 contained an open reading frame of 1,623 nucleotides encoding 541 amino acids with a calculated M_r of 61,705. We termed λhC42 and λhC41 genes *hCAP1a* and *hCAP1b*, respectively. Figure 1 shows the nucleotide and predicted amino acid sequences of *hCAP1a* and *hCAP1b*. The nucleotide sequences of *hCAP1a* and *hCAP1b* were identical from the 5′ flanking region to codon 503, but were entirely different thereafter. The N-terminal portion of the predicted amino acid sequence contained the motif of the tyrosine specific protein phosphatase active site, [LIVMF]-H-C-x(2)-G-x(3)-[STC]-[STAG]-x-[LIVMFY] (PROSITE accession number PS00383) (boxed with a solid line in Fig. 1). The C-terminal portion of the protein is highly homologous to the known mRNA guanylyltransferases and contained all the motifs, *i.e.*, motifs I, III, IIIa, IV, V, VI, and P, which are commonly observed in viral and cellular mRNA guanylyltransferases (22) except that the motif VI was missing in hCAP1b (Fig. 1). In order to examine whether these two capping enzyme mRNAs are actually expressed in human tissues we performed RT-PCR using mRNAs from various human organs and tissues including cerebrum, cerebellum, thyroid, lung, heart, liver, kidney, spleen, large intestine, testis, skin and muscle. *hCAP1a and hCAP1b* mRNAs were detected in all tis-

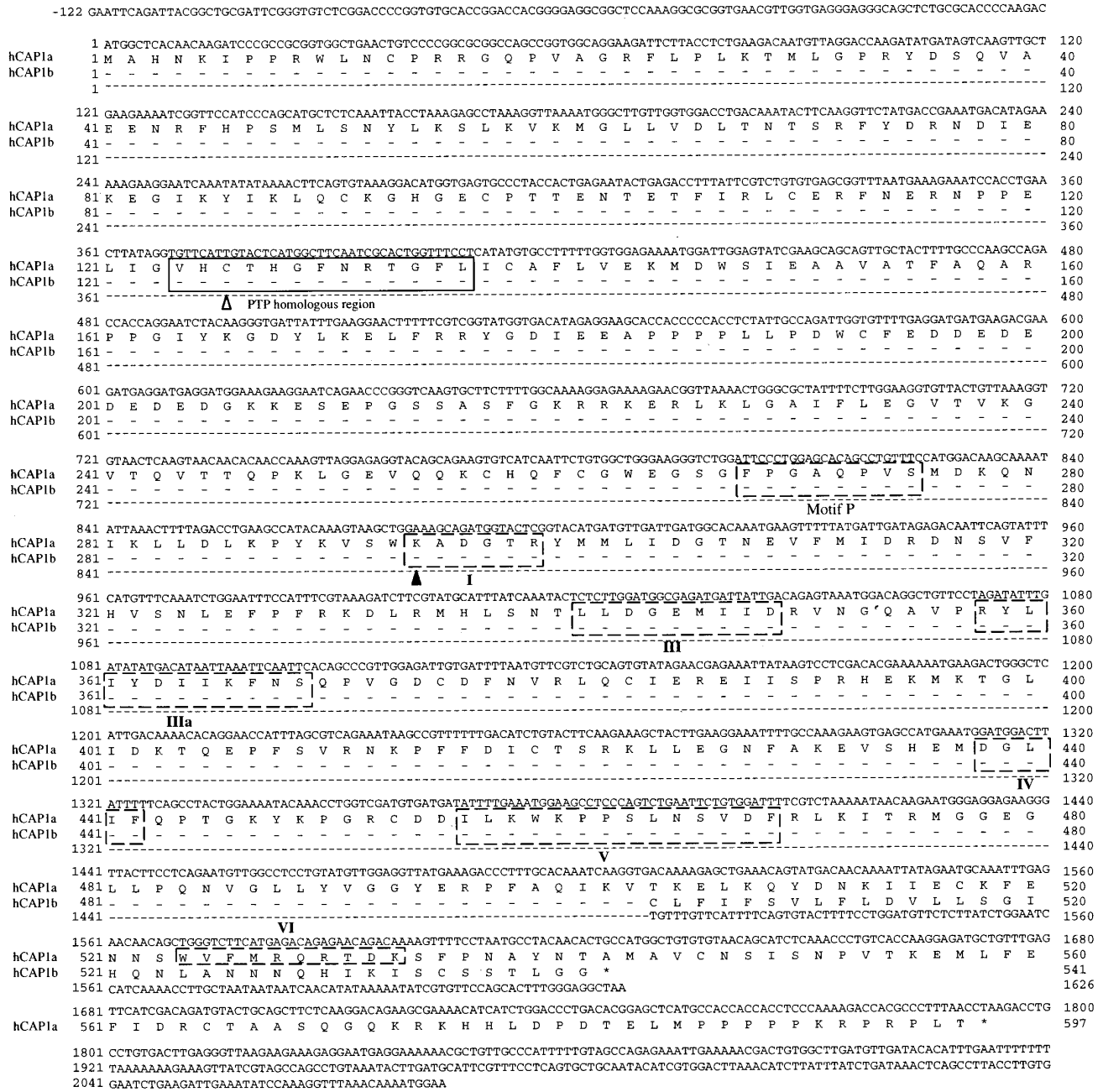

FIG. 1. Nucleotide and deduced amino acid sequences of human mRNA capping enzyme cDNAs (*hCAP1a* and *hCAP1b*). Hyphens represent the nucleotide and amino acid residues in hCAP1b that are identical to those in hCAP1a. Amino acid sequences compatible with the tyrosine specific protein phosphatase (PTP) active site, [LIVMF]-H-C-x(2)-G-x(3)-[STC]-[STAG]-x-[LIVMFY] (PROSITE accession number PS00383), are boxed with a solid line. Open arrowhead indicates the putative cysteine residue to form the phosphocysteine intermediate in tyrosine specific protein phosphatases. Closed arrowhead indicates the lysine residue of the putative GMP binding site (15,16). Amino acid sequences conserved among viral and cellular mRNA guanylyltransferases are boxed with broken lines. Roman numerals and P indicate the number of motifs as defined by Wang *et al.* (22).

sues tested, although *hCAP1b* was expressed to a lesser extent (data not shown).

To characterize the enzymatic activities of the proteins by hCAP1a and hCAP1b, the coding regions were amplified from the cloned plasmid DNAs by PCR using the primers synthesized according to the respective sequences. They were then ligated to the expression vector to produce His-tagged recombinant proteins and expressed in *E. coli* (Fig. 2). When the purified recombinant His-tagged hCAP1a (His-hCAP1a) protein was subjected to western blotting (Fig. 2A), it migrated at the position of about 70 kDa (Fig. 2A, lane 1) which

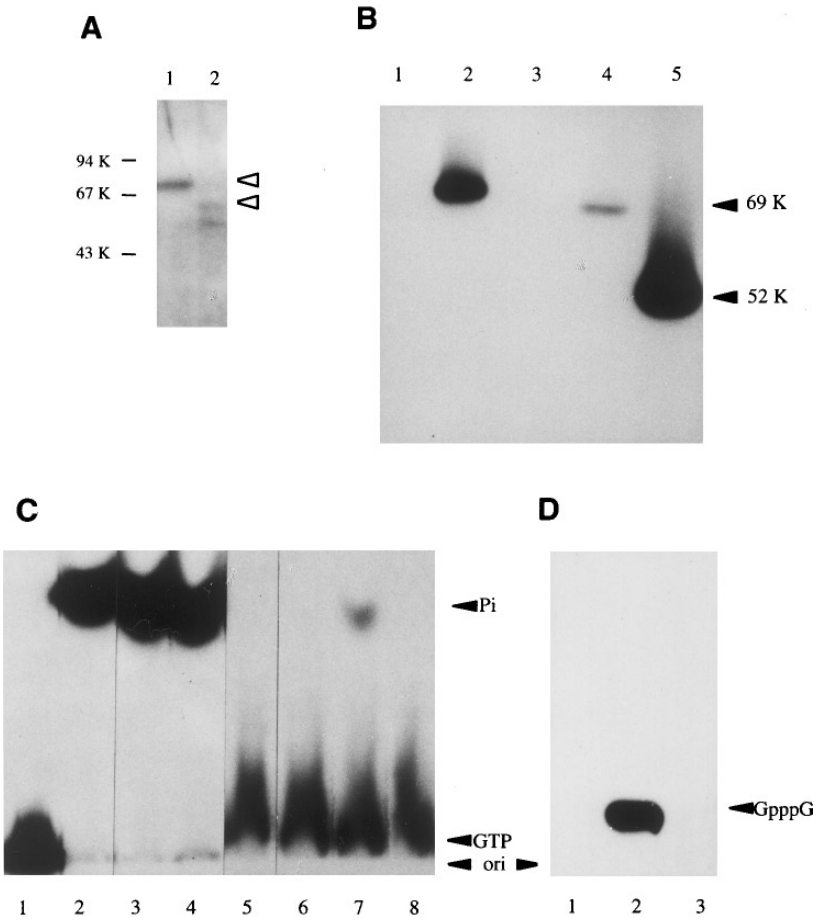

FIG. 2. Expression and enzymatic activities of the recombinant human capping enzyme proteins. (A) Western blot analysis. Recombinant proteins were prepared as described in Materials and Methods. Bacterially expressed His-tagged hCAP proteins (His-hCAP1a and His-hCAP1b) were subjected to SDS-PAGE and transferred to PVDF membrane, before being probed with horseradish peroxidase-conjugated Ni-NTA (QIAGEN). Lane 1, 0.5 μg of His-hCAP1a. Lane 2, 2 μg of His-hCAP1b. Open triangles indicate the expected sizes of recombinant proteins. (B) Enzyme-[^{32}P]pG complex formation assay. Recombinant His-tagged hCAP1 proteins, recombinant yeast capping enzyme α subunit (Ceg1) (15) and HeLa whole cell extract were assayed for the enzyme-[^{32}P]pG formation as described in Materials and Methods. Lane 1, no protein added. Lane 2, 0.05 μg of His-hCAP1a. Lane 3, 0.5 μg of His-hCAP1b. Lane 4, 30 μg of HeLa whole cell extract. Lane 5, 0.1 μg of Ceg1. (C) RNA 5′-triphosphatase activity. Recombinant yeast capping enzyme β subunit (Cet1) and hCAP1 proteins were subjected to phosphohydrolase assay using [γ-^{32}P]triphosphate-terminated RNA (lanes 1-4) or [γ-^{32}P]GTP (lanes 5-8). Lanes 1 and 5, no protein added. Lanes 2 and 6, 0.05 μg of His-hCAP1a. Lanes 3 and 7, 0.1 μg of His-hCAP1b. Lanes 4 and 8, 0.05 μg of His-tagged Cet1. (D) Cap (GpppG) formation assay. Recombinant hCAP1 proteins were subjected to GpppG formation assay using ppG-terminated RNA. Lane 1, no protein added. Lane 2, 0.1 μg of His-hCAP1a. Lane 3, 0.5 μg of His-hCAP1b.

corresponded to the calculated M_r of the tagged protein. Similarly, His-hCAP1b gave a 64 kDa band corresponding to the tagged full length protein. A faster migrating band was also observed with His-hCAP1b, indicating some degradation during expression (Fig. 2A, lane 2). To demonstrate the bifunctional enzymatic activities of the capping enzyme (6), mRNA guanylytransferase and RNA 5′-triphosphatase activities were examined using these recombinant proteins. The mRNA guanylyltransferase activity was assayed by enzyme-[^{32}P]-GMP covalent complex formation. After incubation of the recombinant proteins with [α-^{32}P]GTP, the reaction mixtures were subjected to SDS-PAGE along with the enzyme-GMP complex formed with the yeast capping enzyme α subunit (52 kDa) (Fig. 2B). The reaction with the recombinant His-hCAP1a gave a single ^{32}P-labeled protein band with a M_r of 70 kDa (Fig. 2B, lane 2). However, no ^{32}P-labeled band was observed with His-hCAP1b even with an increased amount (Fig. 2B, lane 3). It may be possible that hCAP1b which lacks the domain VI catalyzes cap formation without forming a stable covalent intermediate. Therefore, we tested capping activity using ppG-terminated RNA as the substrate. As shown in Fig. 2D, hCAP1a catalyzed the synthesis of G(5′)pppG (lane 2), however hCAP1b exhibited no cap formation (lane 3). Then, to demonstrate the RNA 5′-triphosphatase activity of hCAP1 proteins, [γ-^{32}P]triphosphate-terminated RNA was prepared

and tested for the release of [^{32}P]Pi (Fig. 2C). Both of the recombinant proteins, hCAP1a and hCAP1b, released [^{32}P]Pi from [γ-^{32}P]triphosphate-terminated RNA (Fig. 2C, lanes 2 and 3), but they were less active with [γ-^{32}P]GTP (Fig. 2C, lanes 6 and 7), indicating that they recognize the RNA chain.

To assign the functional domains for mRNA guanylyltransferase and RNA 5′-triphosphatase activities in the hCAP1a protein, His-tagged C- and N-terminal deletion mutants, His-hCAP1a(1-213) and His-hCAP1a(229-597), respectively, were prepared and assayed for these two activities (Fig. 3). mRNA guanylyltransferase activity was examined by the enzyme-[^{32}P]GMP complex formation (Fig. 3C) as well as the cap formation (Fig. 3E). When mutant proteins were incubated with [α-^{32}P]GTP, His-hCAP1a(229-597) gave a single ^{32}P-labeled protein band (Fig. 3C, lane 4), whereas His-hCAP1a(1-213) did not (Fig. 3C, lane 3). Incubation with ppG-terminated RNA showed that His-hCAP1a(229-597) catalyzed G(5′)pppG formation (Fig. 3E, lane 4) to a level comparative to the intact His-hCAP1a (Fig. 3E, lane 2), however, His-hCAP1a(1-213) was again inert (Fig. 3E, lane 3). On the other hand, when RNA 5′-triphosphatase activity was assayed using [γ-^{32} P]pppG-RNA, His-hCAP1a(1-213) released [^{32}P]Pi (Fig. 3D, lane 7), while no [^{32}P]Pi was released by hCAP1a(229-597) (Fig. 3D, lane 8). Furthermore, the N-terminal half molecule, hCAP1a(1-213) exhibited no NTPase activity (Fig. 3, D, lane 3), indicating that this half molecule still retains the specificity for RNA. These results indicate that the active site for RNA 5′-triphosphatase resides in the N-terminal 213 amino acids fragment, whereas mRNA guanylyltransferase activity resides in the C-terminal 289 amino acid fragment.

DISCUSSION

Our previous studies demonstrated that the purified yeast capping enzyme is composed of two subunits of α (52 kDa) and β (80 kDa), catalyzing mRNA guanylyltransferase and RNA 5′-triphosphatase activities, respectively (11). In contrast, capping enzymes from higher eukaryotes consist of a single polypeptide chain of about 70 kDa, which contains catalytic domains for both mRNA guanylyltransferase and RNA 5′-triphosphatase (9, 10). We further demonstrated that the domains for mRNA guanylyltransferase (44 kDa) and RNA 5′-triphosphatase (20 kDa) were separated from a single polypeptide chain of 73 kDa by limited tryptic digestion of the capping enzyme from *Artemia salina* (10). In the present study, we have isolated two human capping enzyme cDNA clones which code for 597 and 541 amino acids, and we designated these genes as hCAP1a and hCAP1b, respectively. They are different from each other only at the region encoding the C-terminal portion of the enzyme. The predicted amino acid sequences of hCAP1a and hCAP1b contain regions which are highly homologous to known viral and cellular mRNA guanylyltransferases in their C-terminal two thirds (amino acids 211 to 597 in hCAP1a, and 211 to 541 in hCAP1b) except that one motif is missing in hCAP1b (Figs. 1 and 4). We expressed two deletion mutants of hCAP1a protein, hCAP1a (1-213) and hCAP1a (229-597), and demonstrated that they exhibited RNA 5′-triphosphatase and mRNA guanylyltransferase activities, respectively. Interestingly, the region responsible for RNA 5′-triphosphatase activity contains the tyrosine specific protein phosphatase (PTP) active site motif which is composed of 13 amino acids, [LIVMF]-H-C-x(2)-G-x(3)-[STC]-[STAG]-x-[LIVMFY], and is found in the capping enzyme of *C. elegans* (CEL-1) (23). A sequence comparison of the region with RNA 5′-triphosphatase showed high similarity to the N-terminal portion of CEL-1 (Fig. 4, TPase domain). Recently, we have cloned the gene encoding the *S. cerevisiae* capping enzyme β subunit which catalyzes RNA 5′-triphosphatase (*CET1*) and shown that the active site of the triphosphatase resides in the C-terminal 60% of the Cet1 molecule (18). Surprisingly, comparison of the C-terminal region of Cet1 with hCAP1 showed no similarity, and Cet1 has no PTP active site motif, although they catalyze almost the same reaction. Further studies are needed to identify the active site of Cet1, but mRNA 5′-phosphohydrolysis may be catalyzed by different mechanisms in mammalian and yeast capping enzymes. In this regard, it is worth testing whether hCAP1 or its TPase domain can complement the yeast *CET1*Δ.

The isolation of two human capping enzyme cDNAs, *hCAP1a* and *hCAP1b* is particularly interesting and unexpected. RT-PCR showed that these two species are expressed in every tissue examined, although hCAP1b to a lesser extent (data not shown). The nucleotide sequences of hCAP1a and hCAP1b are identical from the initiation codon up to codon 503 , where they suddenly diverge, resulting in the loss of motif VI in hCAP1b. This might indicate, although the genome structure has not yet been examined, that the two mRNAs are produced by alternative splicing. The biological significance of hCAP1b, however, remain unknown at present. Despite of our efforts, we failed to detect mRNA guanylyltransferase activity in the hCAP1b protein expressed in *E. coli*. It has been reported that single amino acid changes in the motif VI of yeast Ceg1 abolish its enzymatic activity (22). We also expressed a recombinant yeast Ceg1 deletion mutant lacking motif VI and found no mRNA guanylyltransferase activity (data not shown). It is tempting to speculate that, in the cell, the hCAP1b may somehow work in mRNA metabolism in a different way from capping reaction *per se*, but with its RNA 5′-triphosphatase activity. It should be noted that, in yeast cells, the β subunit (RNA 5′-triphosphatase) is expressed to at least a several times higher degree than the α subunit (mRNA guanylyltransferase) when analyzed by western blotting

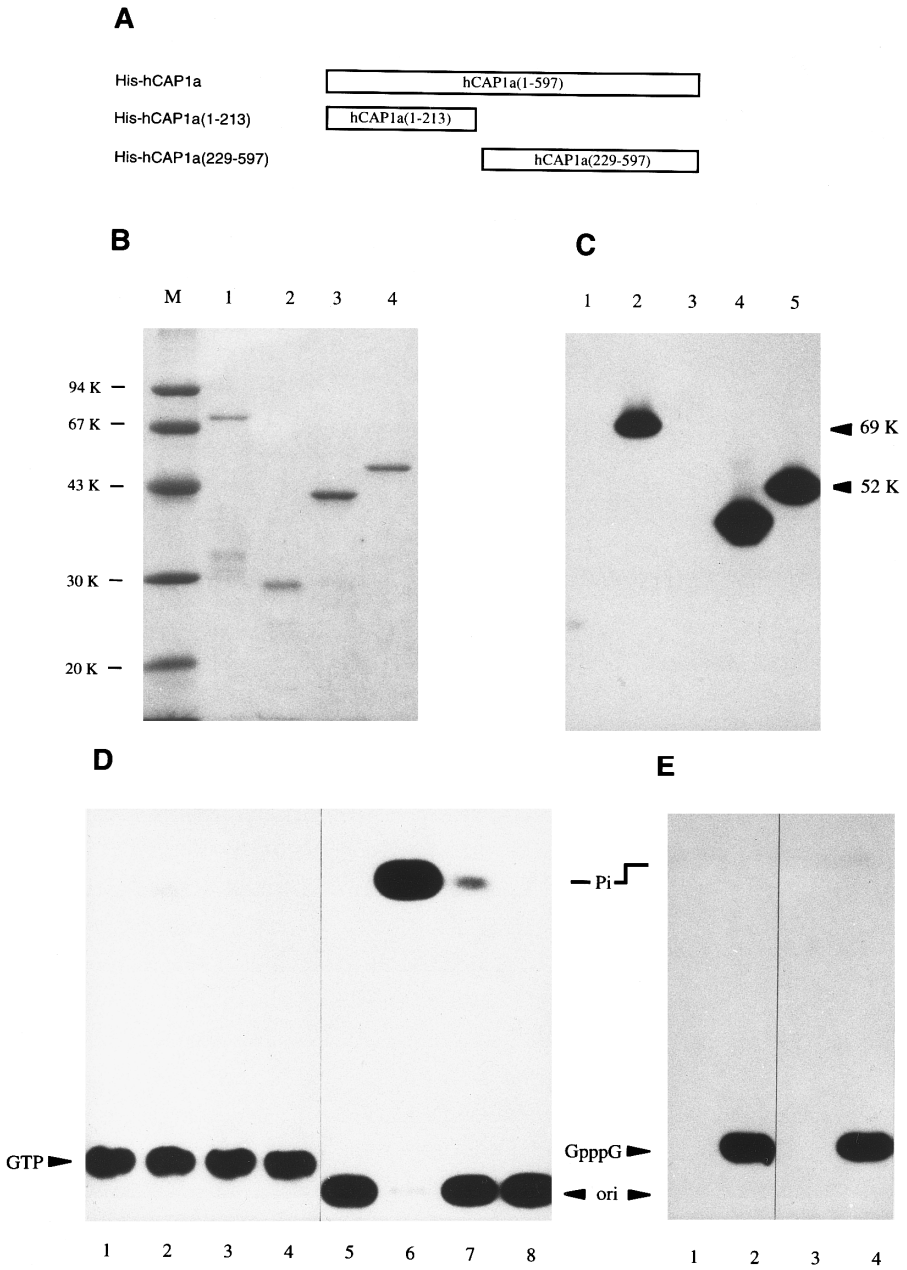

FIG. 3. Expression of C- and N-terminal deletion mutant proteins of hCAP1a. (A) Schematic representation of wild-type and C- and N-terminal deletion mutants of hCAP1a. (B) His-hCAP1a, His-hCAP1a (1-213) and His-hCAP1a (229-597) were expressed in *E. coli* as described in Materials and Methods, and following SDS-PAGE were stained with Coomassie Brilliant Blue. Lane 1, 0.3 μg of His-hCAP1a. Lane 2, 0.3 μg of His-hCAP1a (1-213). Lane 3, 0.6 μg of His-hCAP1a (229-597). Lane 4, 0.5 μg of recombinant yeast capping enzyme α subunit (Ceg1). M, molecular mass markers. (C) Enzyme-[^{32}P]pG complex formation. Lane 1, no protein added. Lane 2, 0.1 μg of His-hCAP1a. Lane 3, 0.3 μg of His-hCAP1a (1-213). Lane 4, 0.2 μg of His-hCAP1a (229-597). Lane 5, 0.15 μg of Ceg1. (D) Substrate specificity of RNA 5′-triphosphatase. Recombinant proteins were assayed for phosphohydrolase activity using [γ-^{32}P]GTP (lanes 1-4) or [γ-^{32}P]triphosphate-terminated RNA (lanes 5-8) as the substrate. Lanes 1 and 5, no protein added. Lanes 2 and 6, 0.1 μg of His-hCAP1a. Lanes 3 and 7, 0.1 μg of His-hCAP1a (1-213). Lanes 4 and 8, 0.2 μg of His-hCAP1a (229-597). (E) Cap formation assay using ppG-terminated RNA as the substrate. Lane 1, no protein added. Lane 2, 0.1 μg of His-hCAP1a. Lane 3, 0.1 μg of His-hCAP1a (1-213). Lane 4, 0.1 μg of His-hCAP1a (229-597).

(our unpublished observation), and that there is actually a free form of the β chain present not associated with the α subunit (11). Experiments are in progress to address the significance of the hCAP1b protein in mRNA metabolism, focusing especially on the regulatory functions in capping as well as in transcription processes.

During the preparation of this manuscript, we

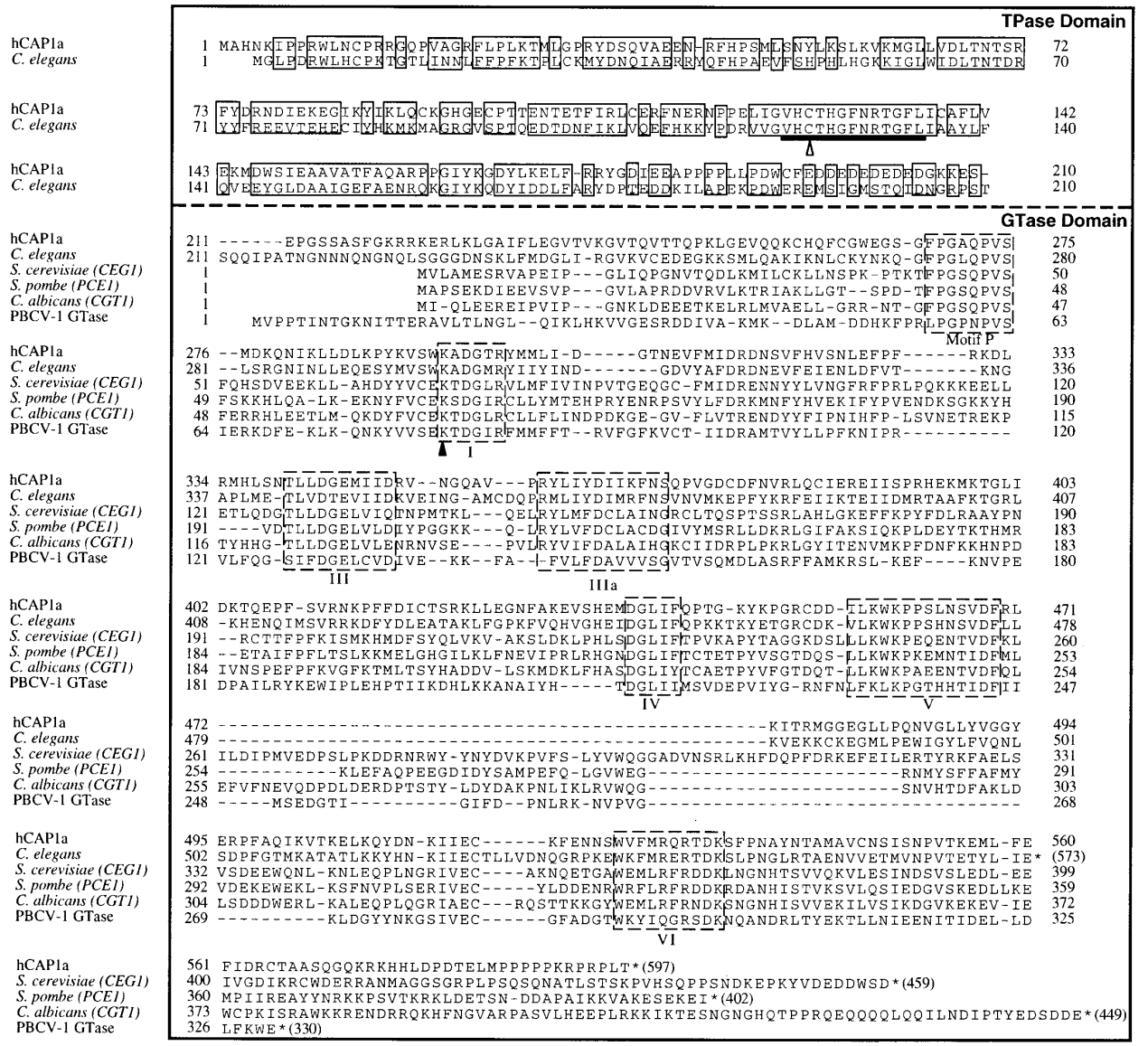

FIG. 4. Comparison of the amino acid sequence of hCAP1a with those of other cellular and viral capping enzymes. The predicted amino acid sequence of *hCAP1a* was divided into the N-terminal RNA 5′-triphosphatase (TPase) domain and the C-terminal mRNA guanylyltransferase (GTase) domain according to the results obtained using deletion mutants. Hyphens specify gaps in the sequences. In the TPase domain, the amino acid sequence of hCAP1a is aligned with the sequence of the *C. elegans* capping enzyme (CEL-1) (23). Identical and similar amino acids are boxed. The PTP motif is underlined and the putative active site cysteine residue is indicated by an open arrowhead. In the C-terminal GTase domain, hCAP1a is aligned with CEL-1 (23) and mRNA guanylyltransferases from *S. cerevisiae* (Ceg1) (12), *Schiz. pombe* (PCE1) (16), *C. albicans* (CGT1) (17), and PBCV-1 (24). Amino acid sequences conserved in viral and cellular capping enzymes are boxed and numbered according to Wang *et al.* (22). Closed arrowhead indicates the lysine residues of the putative nucleotide binding site.

learned that Yue *et al.* (25) also isolated a human capping enzyme cDNA clone, whose amino acid sequence was identical with our hCAP1a.

REFERENCES

1. Filipowicz, W. (1978) *FEBS Lett.* **96,** 1–11.
2. Konarska, M. M., Padgett, R. A., and Sharp, P. A. (1984) *Cell* **38,** 731–736.
3. Izaurralde, E., Lewis, J., McGuigan, C., Jankowska, M., Darzynkiewicz, E., and Mattaj, I. W. (1994) *Cell* **78,** 657–668.
4. Izaurralde, E., Lewis, J., Gamberi, C., Jarmolowski, A., MacGuigan, C., and Mattaj, I. W. (1994) *Nature* **376,** 709–712.
5. Caponigro, G., and Parker, R. (1996) *Microbiological Reviews* **60,** 233–249.
6. Mizumoto, K., and Kaziro, Y. (1987) *Progress in Nucleic Acid Research and Molecular Biology* **34,** 1–28.
7. Banerjee, A. K. (1980) *Microbiological Reviews* **44,** 175–205.

8. Shuman, S. (1995) *Progress in Nucleic Acid Research and Molecular Biology* **50,** 101–129.
9. Yagi, Y., Mizumoto, K., and Kaziro, Y. (1983) *EMBO J.* **2,** 611–615.
10. Yagi, Y., Mizumoto, K., and Kaziro, Y. (1984) *J. Biol. Chem.* **259,** 4695–4698.
11. Itoh, N., Yamada, H., Kaziro, Y., and Mizumoto, K. (1987) *J. Biol. Chem.* **262,** 1989–1995.
12. Shibagaki, Y., Itoh, N., Yamada, H., Nagata, S., and Mizumoto, K. (1992) *J. Biol. Chem.* **267,** 9521–9528.
13. Schwer and Shuman (1994) *Proc. Natl. Acad. Sci. USA* **91,** 4328–4332.
14. Fresco and Buratowski (1994) *Proc. Natl. Acad. Sci. USA* **91,** 6624–6628.
15. Shibagaki, Y., Gotoh, H., Kato, M., and Mizumoto, K. (1995) *J. Biochem.* **118,** 1303–1309.
16. Shuman, S., Liu, Y., and Schwer, B. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 12046–12050.
17. Yamada-Okabe, T., Shimmi, O., Doi, R., Mizumoto, K., Arisawa, M., and Yamada-Okabe, H. (1996) *Microbiology* **142,** 2515–2523.
18. Tsukamoto, T., Shibagaki, Y., Imajoh-Ohmi, S., Murakoshi, T., Suzuki, M., Nakamura, A., Gotoh, H., and Mizumoto, K. (1997) *Biochem. Biophys. Res. Commun.* **239,** 116–122.
19. Chomczynski, P., and Sacchi, N. (1987) *Anal. Biochem.* **162,** 156–159.
20. Goulaouic, H., and Chow, S. A. (1996) *J. Viol.* **70,** 37–46.
21. Itoh, N., Mizumoto, K., and Kaziro, Y. (1984) *J. Biol. Chem.* **259,** 13923–13929.
22. Wang, S. P., Deng, L., Ho, C. K., and Shuman, S. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 9573–9578.
23. Takagi, T., Moore, C. R., Diehn, F., and Buratowski, S. (1997) *Cell* **89,** 867–873.
24. Ho, C. K., Van Etten, J. L., and Shuman, S. (1996) *J. Virology* **70,** 6658–6664.
25. Yue, Z., Maldonado, E., Pillutla, R., Cho, H., Reinberg, D., and Shatkin, A. J. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 12898–12903.