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Cloning and Characterization of Three Human cDNAs Encoding mRNA (Guanine-7-)-methyltransferase, an mRNA Cap Methylase

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The mRNA cap structure is synthesized by a series of reactions catalyzed by capping enzyme and mRNA (guanine-7-)-methyltransferase. mRNA (guanine-7-)-methyltransferase catalyzes the methylation of GpppN- at the guanine N7 position, which is an essential step for gene expression in eukaryotic cells. Here we isolated three human cDNAs encoding mRNA (guanine-7-)-methyltransferase termed *hCMT1a*, *hCMT1b* and *hCMT1c*. *hCMT1a* and *hCMT1b* encode 476 and 504 amino acids, respectively, and differ only at the region coding for the C-terminal portion of the enzyme after amino acid residue 465. The third cDNA *hCMT1c* seems to encode the same polypeptide as hCMT1a, however, the 3′-noncoding region of *hCMT1c* contains sequences corresponding to part of the C-terminal coding and non-coding regions of *hCMT1b* thus consisting of a mosaic of *hCMT1a* and *hCMT1b*. RT-PCR showed that all 3 types of mRNAs were expressed in every tissue examined. Comparison of the deduced amino acid sequences with those of other viral and cellular enzymes showed the regions which are highly conserved among mRNA (guanine-7-)-methyltransferases. The recombinant hCMT1a expressed in *E. coli* exhibited mRNA (guanine-7-)-methyltransferase activity. On the other hand, neither mRNA (guanine-7-)-methyltransferase nor mRNA (nucleoside-2′-*O*-)-methyltransferase activity was detected with the recombinant hCMT1b protein. Although the biological significance of the expression of these three mRNA (guanine-7-)-methyltransferase mRNA species remains unknown at present, the nucleotide sequences suggest that they are produced by alternative RNA splicing.

Most of the cellular as well as viral mRNAs in eukaryotes contain a 5′-terminal cap structure, $m^7G(5')pppN$, in which the terminal 7-methylguanosine residue is linked to the 5′ position of the penultimate nucleotide through a 5′-5′ triphosphate bridge (1, 2). It has been shown that the cap structure plays crucial roles in the various steps of gene expression including initiation of translation (3), RNA splicing (4, 5), transport of mRNA from the nucleus to the cytoplasm (6), and. mRNA turnover (7). The cap structure is formed as an early co-transcriptional modification of nascent RNA chains transcribed by RNA polymerase II by a series of enzymatic activities (for reviews, see Refs. 2, 8, 9). The first step of cap formation is the removal of the γ-phosphate from the 5′-triphosphate end of newly-synthesized RNA to generate a diphosphate end by RNA 5′-triphosphatase. Then the GMP moiety of GTP is transferred to the 5′-diphosphate terminus of RNA by mRNA guanylyltransferase. After these two consecutive reactions, the methyl group is transferred to the the guanine N7 position from S-adenosylmethionine (AdoMet) by mRNA (guanine-7-)-methyltransferase and, in some cases, also to the ribose-2′-OH by mRNA (nucleotide-2′-*O*-)-methyltransferase.

The activity of mRNA (guanine-7-)-methyltransferase has been isolated from various eukaryotic cells as well as virus particles (for review, see Ref. 8). The cellular mRNA (guanine-7-)-methyltransferase is easily separated from mRNA capping enzyme at an early stage of purification. In contrast, a capping enzyme complex purified from vaccinia virus contains mRNA (guanine-7-)-methyltransferase activity. Vaccinia virus mRNA capping enzyme consisting of a heterodimer of D1 (95 kDa) and D12 (31 kDa) polypeptides possesses RNA 5′-triphosphatase, RNA guanylyltransferase, and RNA (guanine-7-)-methyltransferase activities, and the RNA (guanine-7-)-methyltransferase domain is localized to a 305-amino acid carboxyl-terminal segment of the D1 polypeptide (residues 540-844) which is obligatory associated with the D12 protein for its activity (9, 10). In *Saccharomyces cerevisiae*, mRNA (guanine-7-)-methyltransferase has been found to be encoded by *ABD1* gene, and its amino acid sequence displays regional similarity to the catalytic domain (the carboxyl-terminal segment of D1) of the vac-

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cinia virus mRNA (guanine-7-)-methyltransferase (11, 12, 13).

Recently, the enzymes responsible for cap synthesis, capping enzyme and mRNA (guanine-7-)-methyltransferase, were demonstrated to bind directly to the phosphorylated carboxyl-terminal domain (CTD) of the RNA polymerase II largest subunit *in vitro* (14). Therefore, it is of much interest to elucidate the structure-function relationships of mRNA (guanine 7-)-methyltransferase from yeast and higher eukaryotes. However, so far the structure of the mRNA (guanine-7-)-methyltransferase of higher organisms has not been elucidated.

In this report we isolated three cDNAs encoding human mRNA (guanine-7-)-methyltransferase and designated them *hCMT1a*, *hCMT1b*, and *hCMT1c*, respectively. The recombinant hCMT1a expressed in *E. coli* exhibited the mRNA (guanine-7-)-methyltransferase activity. However, we failed to detect any cap methyltransferase activity with the recombinant hCMT1b which differs only at the short C-terminal region. Some of the structural characteristics of these three clones as well as the motifs commonly present in many cap methyltransferases are presented.

MATERIALS AND METHODS

Isolation of cDNAs. From the nucleotide sequences of human EST (GenBank accession number C04619), the following sense and antisense primers were synthesized: hMT-F, 5′-TTT TTG GAA AGG TAC GAC AGA-3′ and hMT-R, 5′-GTC AGC CTG CTC ATA AGA CTC-3′. The total RNA was extracted from human leukocytes using a single step procedure (15). 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), 50 mM KCl, 2 mM $MgCl_2$, 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 above primers and the synthesized cDNA. The amplification was carried out for 35 cycles, each consisting of a denaturing step at 94° C for 20 s, annealing at 52°C for 20 s, and extension at 72°C for 20 s. The last cycle was followed by a 7 min-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). The amplified band obtained by PCR was used as a probe to screen a λ ZAP II cDNA libraries derived from a human colon adenocarcinoma cell line COLO 205 (Stratagene) and HeLa cells (Stratagene). Bacteriophage plaques bound to a nylon membrane were hybridized to a digoxigenin-labeled probe in a hybridization buffer containing 50% formamide at 42°C and washed in standard saline citrate (SSC)/0.1% SDS at room temperature. The cDNAs were sequenced by primer walking using a dRhodamine terminator cycle sequencing kit (Perkin-Elmer) on an ABI PRISM 310 genetic analyzer (Perkin-Elmer).

RT-PCR. mRNAs and cDNAs of various human tissues were kindly provided by Drs. Hitoshi Endoh and Fumino Konishi of Jichi Medical School. Primers used for the amplification of *hCMTa* were CMT-F10 (5′-GAA CAT GCA GCA AAG TAC ATG AAG-3′) and CMTa-R1(5′-AGA GGC AAA TGG AAT TCT TAG AAC-3′). For the amplification of *hCMT1b* and *hCMT1c*, MT-F10 and MTb-R1 (5′-ACA CGG CTC CCA CAG AAG TGG TTA-3′) were used.

Recombinant proteins. For the recombinant protein expression in *E. coli*, the coding sequence of *hCMT1a* was amplified from a cloned plasmid DNA by PCR using a sense primer, 5′-ATC ACA TAT GGC AAA TTC TGC AAA AG-3′ (*Nde*I site underlined), corresponding to the upstream of initiating AUG generating 5′ *Nde*I site, and a primer corresponding to the vector sequence. The amplified fragment was digested with *Nde*I and *Bam*HI and ligated into pT7-7(His) plasmid (16) to yield pT77CMT1a for the expression of His-tagged recombinant protein, His-hCMT1a. For the expression of recombinant hCMT1b, the fragment corresponding to the C-terminal region specific to hCMT1b was isolated from the positive clone and ligated into pT77CMT1a to yield pT77CMT1b for the expression of His-hCMT1b. The recombinant His-hCMT1a and His-hCMT1b were expressed in *E. coli* strain BL21(DE3)pLysS and purified on a Ni-nitrilo-triacetic acid (NTA) column. For the recombinant protein expression of yeast (guanine-7-)-methyltransferase (ABD1), the coding region was amplified from yeast genomic DNA using primers of reported sequence (GenBank accession number L12000) and inserted into pT7-7(His) plasmid. Recombinant *S. cerevisiae* gunanylyltransferase (Ceg1) was expressed and purified as described (17).

Assays for mRNA (guanine-7-)-methyltransferase and mRNA (nucleoside-2′-O-)-methyltransferase. For the preparation of diphosphate-ended RNA (ppG-RNA), a DNA fragment containing the sequence, 5′-GGG CGC CTT TTT TTT TCT TTC CTT TTC CTT TTT TTT CA-3′, was inserted downstream of the T7 promoter of pGEM plasmid and transcribed in a reaction mixture containing 40 mM Tris-HCl (pH 8.0), 15 mM $MgCl_2$, 5 mM dithiothreitol, 1 mM spermidine, 0.01% Triton X-100, 1 mM CTP, 1 mM GTP, 4 mM UTP, 4 mM GDP, and 3000 units of T7 RNA polymerase at 37°C for 8 h. To prepare ^{32}P-cap-labeled RNA ([^{32}P]GpppG-RNA), ppG-RNA of length 37 nucleotides was incubated in a reaction mixture containing 40 mM Tris-HCl (pH 7.9), 3 mM $MgCl_2$, 8 mM dithiothreitol, 20% glycerol, 0.1 mg/ml BSA, 50 mM GTP, 1 μCi [α-^{32}P]GTP, and 0.3 mg/ml of recombinant Ceg1 at 30°C for 60 min, and then EDTA was added to stop the reaction. Cap methyltransferases were assayed in a reaction mixture containing 50 mM Tris-HCl (pH 7.5), 5 mM dithiothreitol, 40 mM NaCl, 50 mM S-adenosylmethionine, ^{32}P-cap-labeled RNA synthesized as above and an appropriate amount of enzyme fraction. The reaction mixture was incubated at 37°C for 5 min and then heated at 95°C for 3 min to inactivate the enzyme. The synthesized cap-methylated RNA was digested in a reaction mixture containing 500 mM sodium acetate (pH 5.4), 1 mM $ZnCl_2$, and 10 mg/ml nuclease P1 at 50°C for 60 min. The digests were then spotted on a polyethyleneimine cellulose TLC plate and developed with 350 mM $(NH_4)_2SO_4$. The plate was dried and subjected to autoradiography or analyzed using a Bio-Imaging System BAS2000 (FUJIX) for [^{32}P]m^{7}GpppG, [^{32}P]GpppGm, or [^{32}P] m^{7}GpppGm.

RESULTS

Homology search of the GenBank using yeast mRNA (guanine-7-)-methyltransferase (*ABD1*) sequence revealed a high predicted amino acid similarity to a human EST (GenBank accession number C04619). Then, we synthesized a pair of primers to amplify this sequence from human mRNA. RT-PCR using these primers and human leukocyte mRNA as a template yielded a 335-bp fragment. Using this PCR fragment as a probe, we isolated 2 clones, λMT44 and λMT101 from cDNA libraries derived from a human colon adenocarcinoma cell line and HeLa cells, respectively. DNA sequence analyses of the isolated cDNA clones showed that λMT101 contained an open reading frame of 1,128 nucleotides coding for 476 amino acids. Whereas, λMT44 encoded a truncated protein starting from amino acid 69 of λMT101, however, containing a dif-

ferent C-terminal portion. RT-PCR using human colon mucosa mRNA as a template and a pair of primers corresponding to the 5′-flanking region of λMT101 and to the 3′-noncoding region of λMT44 demonstrated the expression of an mRNA encoding 504 amino acids. The nucleotide sequence matched with λMT101 from the 5′-flanking region to codon 464 but completely differed thereafter. We designated the genes encoding 476 amino acids and 504 amino acids as *hCMT1a* and *hCMT1b*, respectively. Figure 1 shows the nucleotide and predicted amino acid sequences of *hCMT1a* and *hCMT1b*. The calculated *M*rs of hCMT1a and hCMT1b were 54,844 and 57,725, respectively. In the 3′-noncoding sequence of *hCMT1a*, no consensus polyadenylatin signal was observed, but a closely related sequence, ATAAAA, was found 15 nucleotides upstream of the poly(A) site (Fig. 1). In contrast to *hCMT1a*, *hCMT1b* possessed an extremely long 3′-noncoding region and a putative polyadenylation signal located 2,372 nucleotides downstream of the stop codon (Fig. 1). RT-PCR using primers corresponding to the coding and 3′-noncoding regions of *hCMT1a* showed an additional larger band which we termed cDNA *hCMT1c*. Figure 2A shows the partial nucleotide and deduced amino acid sequences of *hCMT1c*. As underlined in Figure 2A, *hCMT1c* possessed a coding sequence encoding the same C-terminal amino acids as *hCMT1b* and a part of the 3′-noncoding sequence of *hCMT1b*. However, as indicated by shading in Figure 2A and schematically represented in Figure 2B, 230 nucleotides downstream of the stop codon of hCMT1c, a sequence from *hCMT1b* corresponding to its downstream region from codon 466 including the 3′-noncoding sequence was joined.

To examine whether the three mRNA (guanine-7-)-methyltransferase mRNAs are actually expressed in human tissues, we designed primers to amplify the 3 types of *hCMTs* separately and performed RT-PCR using mRNAs from various human organs and tissues including cerebrum, cerebellum, thyroid, heart, liver, pancreas, large intestine, kidney, testis, muscle, and skin. As shown in Figure 3, *hCMT1a* (Fig. 3A), *hCMT1b* (Fig. 3B, lower bands) and *hCMT1c* (Fig. 3B, upper bands) mRNAs were detected in all tissues examined.

Predicted amino acid sequences of *hCMT1a* and *hCMT1b* were aligned with the yeast enzyme Abd1, vaccinia virus D1 subunit (residues 540-843), and *C. elegans* putative gene product C25A1.f (GenBank accession number Z81038), a hypothetical protein showing high similarity with known mRNA (guanine-7-)-methyltransferases (Figure 4). Two motifs common to DNA, RNA, and small molecule S-adenosylmethionine-dependent methyltransferases (18) were observed in every protein including hCMT1a (shaded in Fig. 1 and underlined in Fig. 4). The similarities of hCMT1a to C25A1.f and Abd1 were 48.7% and 34.4%, respectively. In Abd1, it was reported that the residues 130 to 426 are enough for full enzymatic activity (13). Sequence alignment in Figure 4 showed that this region is well conserved among mRNA (guanine-7-)-methyltransferases, in which six essential amino acids were identified in Abd1 by mutational analysis (12, 13). Whereas, lengths and sequences of N-terminal regions are highly variable. In human mRNA (guanine-7-)-methyltransferase, The N-terminal region is rich in hydrophilic amino acids. At amino acid residues 57 to 60, 80 to 83, 103 to 107, and 194 to 197, four or five consecutive arginines or lysines, putative nuclear localization signal (NLS) (19, 20), were observed (indicated in Figs. 1 and 4).

To characterize the enzymatic activities of the proteins encoded by the *hCMTs*, the coding regions of *hCMT1a* and *hCMT1b* were ligated into the expression vector to produce His-tagged recombinant proteins. When purified recombinant His-hCMT1a and His-hCMT1b were subjected to SDS-PAGE, they migrated at the positions of about 56 kDa and 59 kDa (Fig. 5A), respectively, which corresponded well to the calculated *M*rs of the His-tagged proteins. mRNA (guanine-7-)-methyltransferase activity was assayed using the conversion of ^{32}P-cap-labeled RNA ([^{32}P]GpppG-RNA) to methylated capped RNA by the transfer of methyl group from S-adenosylmethione to the N7 position of 5′ terminal guanine residue. Figure 5B shows the enzymatic activities of the recombinant proteins, His-hCMT1a, His-hCMT1b, and His-Abd1. When the recombinant His-hCMT1a was incubated with [^{32}P]GpppG-RNA and digested with nuclease P1, radio-labeled spot of m^{7}G(5′)pppG was observed (Fig. 5, lanes 2, 3, 4), as it was using His-Abd1 (Fig. 5, lane 5). In this experiment, the activity obtained using 0.5 pmol of His-hCMT1a was roughly comparable to that with 1.5 pmol of His-Abd1. The fact that the methylation reaction with hCMT1a gave a single product of m^{7}GpppG without yielding any labeled spot of GpppGm or m^{7}GpppGm indicates that the reaction is specific to the N7 position. It is also noted that hCMT1b which differs from hCMT1a only in the C-terminal portion of the molecule could not give any methylated spot (Fig. 5, lane 6) suggesting the importance of the C-terminal region of hCMT1a in the enzymatic activity.

DISCUSSION

In the present study, we have isolated 3 types of human mRNA (guanine-7-)-methyltransferase cDNAs, *hCMT1a*, *hCMT1b*, and *hCMT1c*. *hCMT1a* and *hCMT1b* encode 476 and 504 amino acids, respectively, and differ only in the region encoding C-terminal peptide downstream from codon 465 (Fig. 1). Also shown in Figure 1, the lengths of the 3′-noncoding region of *hCMT1a* and *hCMT1b* differ

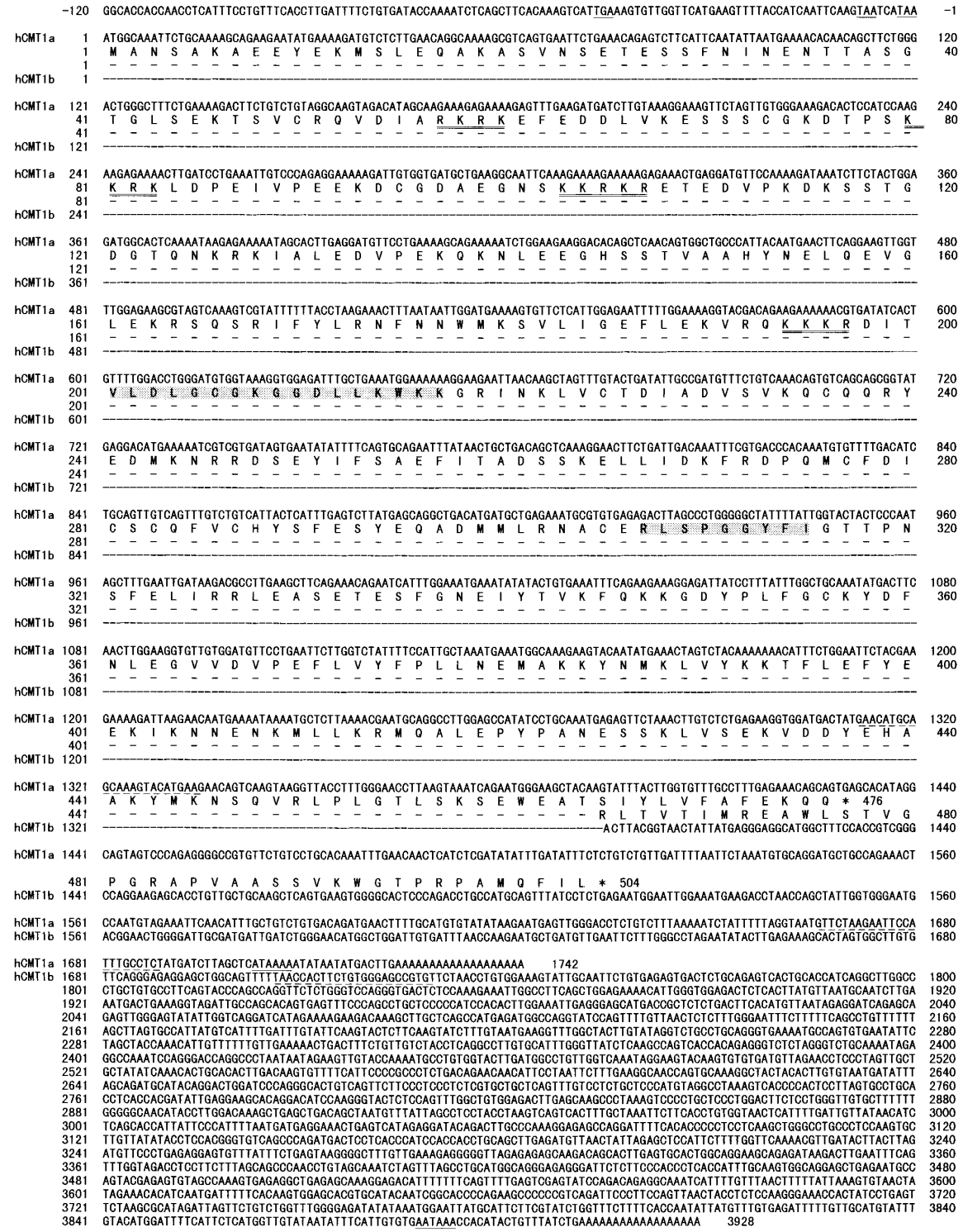

FIG. 1. Nucleotide and deduced amino acid sequences of two human mRNA (guanine-7-)-methyltransferase cDNAs (*hCMT1a* and *hCMT1a*). Hyphens represent the nucleotide and deduced amino acid residues of *hCMT1b* that are identical to those of *hCMTa.* In-frame termination codons in the 5′-noncoding region and putative polyadenylation signals are underlined. Conserved motifs common in protein, DNA, RNA, and small molecule S-adenosylmethionine-dependent methyltransferases (20) are shaded. Double underlines indicate the locations of putative nuclear localizing signals. Broken underlines show the regions corresponding to primers used for RT-PCR in Figure 3.

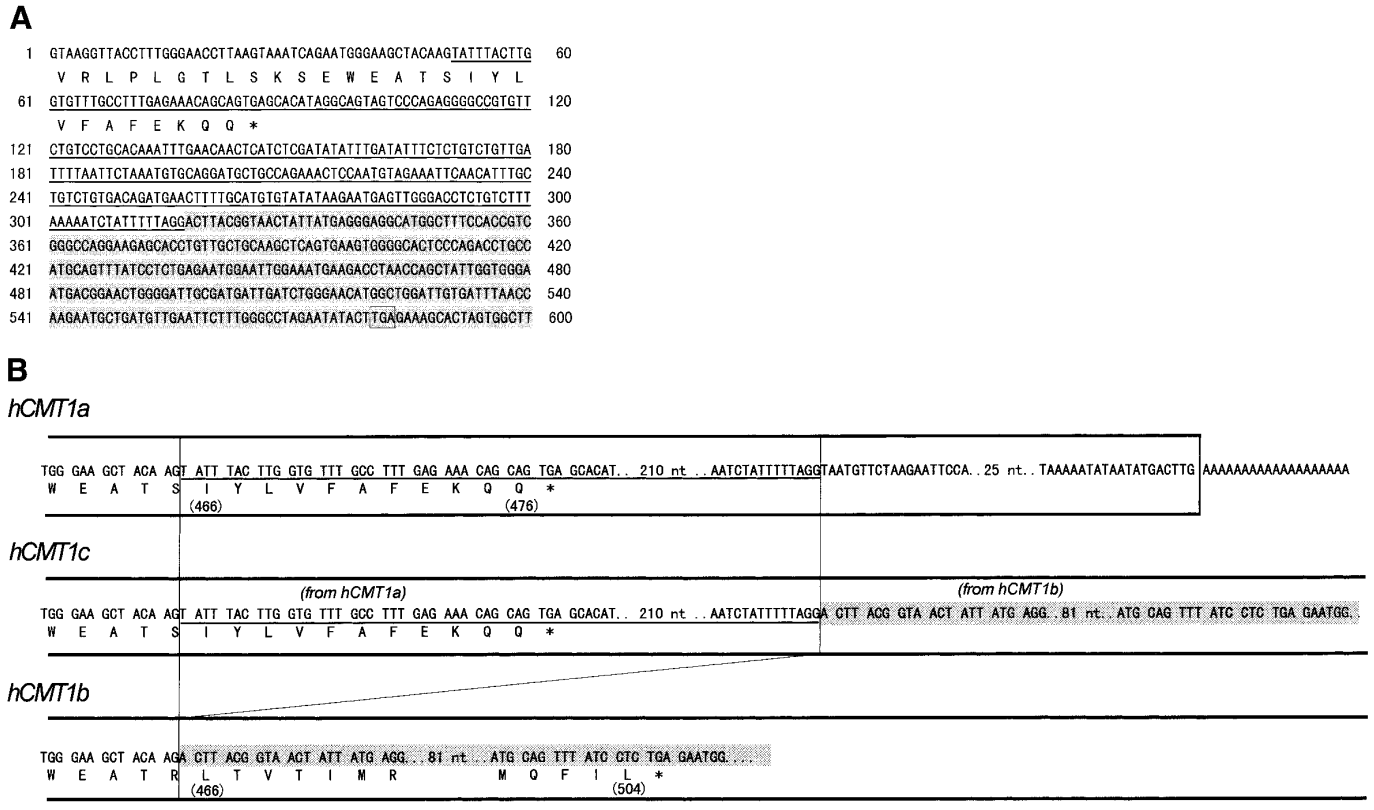

FIG. 2. Three human mRNA (guanine-7-)-methyltransferase cDNAs (*hCMT1a*, *hCMT1b*, and *hCMT1c*). (A) The mosaic structure of the C-terminal region (600 nucleotides) of h*CMT1c*. Nucleotide sequence identical to the coding sequence for amino acid residues 465 to 476 and the 3′-noncoding sequence of *hCMT1a* is underlined. Shading indicate the region identical to the C-terminal coding (amino acid residues 465 to 504) and the 3′-noncoding sequences of *hCMT1b*. The stop codon used in *hCMT1b* is boxed. Numbers (1-600) on both sides are arbitrary. (B) Schematic representatin of the C-terminal coding and 3′-noncoding regions of *hCMT1a*, *hCMT1b*, and *hCMT1c*.

markedly. Another cDNA candidate, *hCMT1c*, encodes the same C-terminal polypeptide as *hCMT1a*. However, in the 3′-noncoding region of *hCMT1c*, the nucleotide sequence corresponding to the region downstream from codon 465 of *hCMT1b* including its 3′-noncoding sequence is joined in tandem (Fig. 2). The comparison of the sequences downstream from codon 465 of three classes of *hCMT1* clones may indicate, although the genomic DNA has not been analyzed, that the three *hCMT1* mRNAs are produced by alternative splicing. In summary, the hGM1a-type C-terminal peptide is encoded by two mRNAs, *hCMT1a* and *hCMT1c*, which contain different 3′-noncoding sequences. Although the biological significance of this difference in the 3′-noncoding region of mRNAs to express the hCMT1a-type protein is unclear, it might be related to the differences in the stability or these mRNAs. Similarly, clarification of an important issue still remains with respect to the presence of *hCAP1b* which encodes, so far as in *in vitro* assay, an inactive protein. It should be noted that the three types of mRNAs are ex-

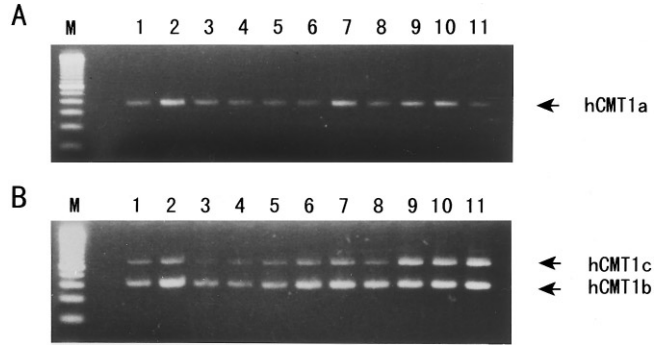

FIG. 3. Expression of three human mRNA (guanine-7-)-methyltransferases (*hCMT1a, hCMT1b, and hCMT1c*). (A) RT-PCR of *hCMT1a*. (B) RT-PCR of *hCMT1b* and *hCMT1c*. Arrows indicate the positions of *hCMT1a*, *hCMT1b*, and *hCMT1c*. Lane 1, cerebrum. Lane 2, cerebellum. Lane 3, thyroid. Lane 4, heart. Lane 5, liver. Lane 6, pancreas. Lane 7, large intestine, Lane 8, kidney. Lane 9, testis. Lane 10, skeletal muscle. Lane 11, skin. M, molecular mass markers. The primers used are as indicated in Fig. 1 (broken underlines). The antisense primer for *hCMT1c* is identical to that for *hCMT1b*. hCMT1c mRNA is expected to give a longer product than *hCMT1b*.

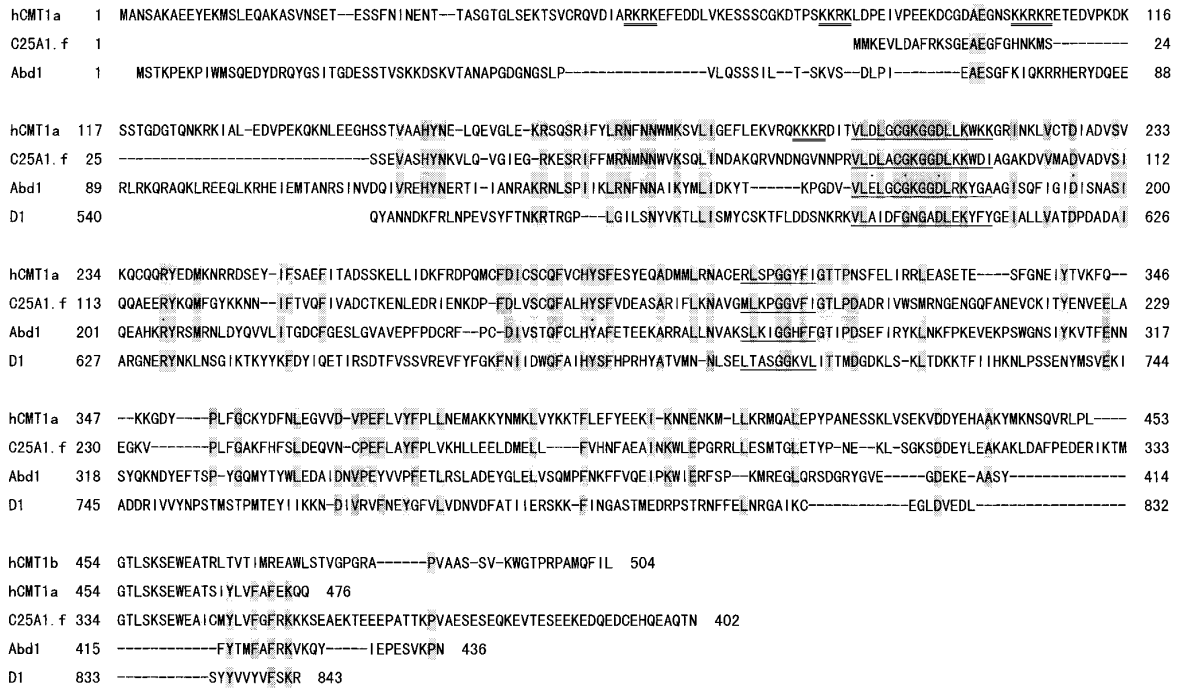

FIG. 4. Comparison of the amino acid sequences of human mRNA (guanine-7-)-methyltransferase with those of other cellular and viral capping enzymes. hCMT1a and the C-terminal portion of hCMT1b are aligned with mRNA (guanine-7-)-methyltransferases of *S. cerevisiae* (Abd1) (GenBank accession number L12000), *C. elegans* (C25A1.f) (Z81038), and vaccinia (D1) (M15058). Hyphens specify gaps in the sequences. Conserved motifs common in protein, DNA, RNA, and small molecule S-adenosylmethionine-dependent methyltransferases (20) are indicated by underlines. Identical amino acid residues observed in three out of three or four different species are shaded. Double underlines indicate the positions of putative nuclear localization signals. The residues with dots of Abd1 indicate the amino acids essential for the activity (14, 15).

pressed in various human tissues and there were no marked differences in the expression patterns of these mRNAs in any tissue when examined by RT-PCR (Fig. 3).

The mRNA (guanine-7-)-methyltransferase activity was demonstrated with His-tagged hCMT1a. The fact that the recombinant protein alone exhibited the enzymatic activity which is comparable to or greater than that of yeast counterpart Abd1 indicates that, in contrast to vaccinia enzyme (21), there may be no obligatory requirement for interaction with other protein for enzymatic activity. On the other hand, we did not detect any methyltransfrase activity in hCMT1b. The C-terminal region consisting of 40 amino acids of hCMT1b is completely different from that of hCMT1a in which a conserved YXXFXFXK motif among cellular cap methyltransferase is present, thus indicating the importance of the C-terminal short stretch (12 amino acid residues) of hCMT1a for the enzymatic activity.

The alignment of the predicted amino acid sequences of hCMT1, other known cap methyltransfereses, and *C. elegans* putative gene product C25A1.f whose enzymatic activity has not yet been examined revealed high similarity in the putative region essential for mRNA (guanine-7-)-methyltransferase activity deduced from the results obtained by mutational studies of the yeast counterpart Abd1 (13). In contrast, the lengths and sequences of the N-terminal region of mRNA (guanine-7-)-methyltransferases vary markedly (Fig. 4). In hCMT1, the N-terminal region is rich in hydrophilic amino acids and there are four putative nuclear localization signals (NLSs) which are rich in basic residues in amino acid residues 57 through 197. In particular, it is interesting to note as shown in Figure 6, that there are four redundant sequences in the region spanning residues 43 to 133, three of which includes putative NLSs. These redundant sequences are unique to human enzyme. The yeast *ABD1* gene was demonstrated to be essential for cell growth (11). The N-terminal residues 1 to 129 of Abd1 were reported to be dispensable for the enzymatic activity (13). However it was also reported that the amino acid residues 110-129 are needed for the complementation (13). Although no mutational study was performed with hCMT1, from the sequence analysis, it appears the N-terminal region of hCMT1 is likewise dispensable for the enzymatic activity. We speculate, however, that the unique region of residues 43 to 133 of hCMT1, with a predicted structure of helix-loop-

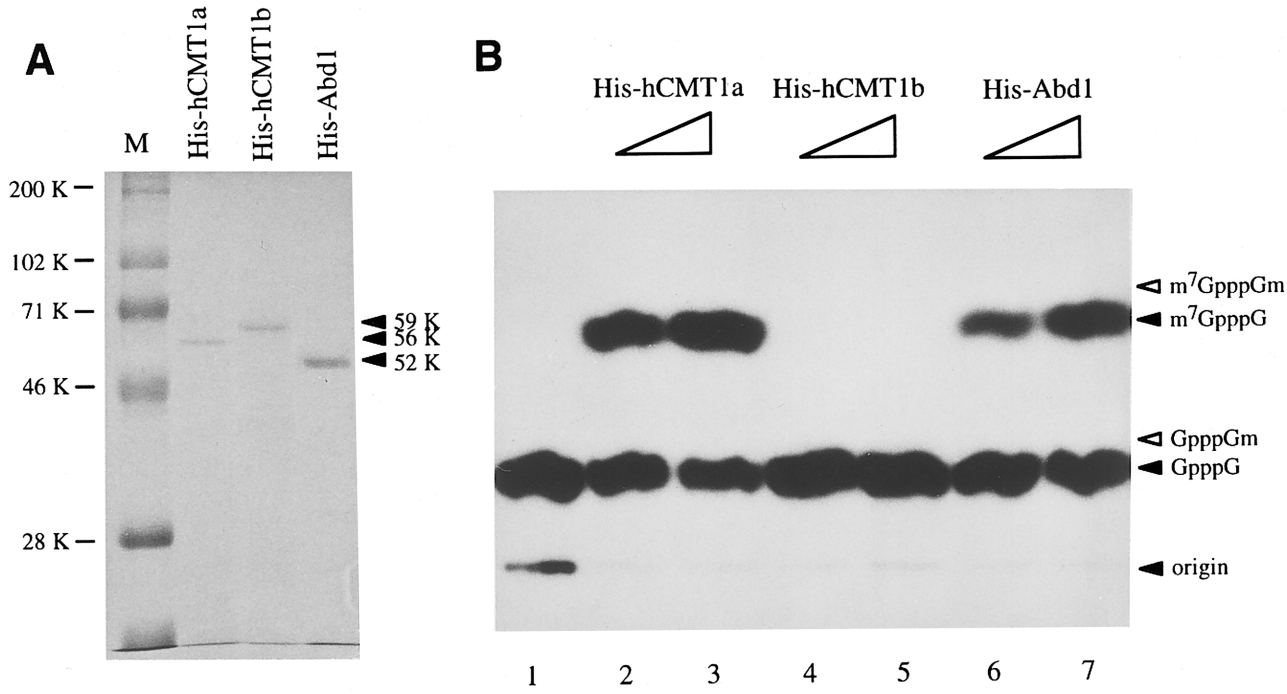

FIG. 5. Expression and enzymatic activities of recombinant human mRNA (guanine-7-)-methyltransferase. (A) His-hCMT1a and His-hCMT1b were expressed in *E. coli* as described in Materials and Methods and subjected to SDS-PAGE and then stained with Coomassie Brilliant Blue. His-hCMT1a, 300 ng of His-hCMT1a. His-hCMT1b, 300 ng of His-hCMT1b, and His-Abd1, 600 ng of His-tagged recombinant yeast mRNA (guanine-7-)-methyltransferase. M, molecular mass markers. (B) mRNA (guanine-7-)-methyltransferase activities. Enzyme activity was assayed as described in Materials and Methods using [^{32}P]GpppG-RNA as the substrate. The electrophorogram on DE81 paper is shown. Lane 1, no protein added. Lanes 2 (0.5 pmol) and 3 (1.5 pmol), His-hCMT1a. Lanes 4 (1 pmol) and 5 (3 pmol), His-hCMT1b. Lanes 6 (0.5 pmol) and 7 (1 pmol), His-Abd1.

helix, is involved in the interaction with other protein(s) in the nucleus.

Recently, the enzymes responsible for cap synthesis, capping enzyme and mRNA (guanine-7-)-methyltransferase, were demonstrated to bind directly to the phosphorylated form of the carboxy-terminal domain (CTD) of RNA polymerase II largest subunit *in vitro* (14). However, whether mRNA (guanine-7-)-methyltransferase binds to the CTD *in vivo* directly or indirectly, *i.e.*, via other protein(s), still remains to be elucidated. In this regard, identification of the protein(s) interacting with hCMT1a using a recombinant protein or antibody might be important.

In the present study, we isolated three types of mRNA (guanine-7-)-methyltransferase cDNAs. Mutational analysis for the assignment of the catalytic domain, a search for the interacting protein(s), and assignment of the substrate specificity of isoforms using recombinant proteins are under way for the elucidation of the mechanism of cap methylation as well as the significance of the presence of three different species of hCMT1 in mammalian mRNA metabolism.

43- 65 LS-E-KTSVCRQVDI---ARKRK-EFEDD
66- 87 LVKE-SSS-CGK-DTP--SKKRKLDPE
88-111 IVPEEKD--CG--DAEGNSKKRKRETED
112-133 VPKDKSST-G--DGTQN--KRKIALED

FIG. 6. Repeated sequences in the N-terminal region of hCMT1a and hCMT1b. Numbers to the left indicate the amino acid positions of hCMT1a and hCMT1b. Hyphens specify gaps in the sequences. Putative nuclear localization signals (NLSs) with consecutive lysines or arginines are underlined. Identical amino acids observed in more than three of four repeats are shaded.

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