Molecular cloning and functional expression of human cytosolic acetyl-CoA hydrolase

Naoya Suematsu and Fumihide Isohashi

Department of Biochemistry, St. Marianna University School of Medicine, Kanagawa, Japan; e-mail: n2sue@marianna-u.ac.jp

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A cDNA encoding human cytosolic acetyl-CoA hydrolase (CACH) was isolated from a human liver cDNA library, sequenced and functionally expressed in insect cells. The human CACH cDNA encodes a 555-amino-acid sequence that is 81.4%/78.7% identical to those of the mouse/rat homologue, suggesting a conserved role for this enzyme in the human and rodent livers. Bioinformatical study further reveals a high degree of similarity among the human and rodent CACHs as follows: First, the gene is composed of 15 exons ranging in size from 56 to 157 bp. Second, the protein consists of two thioesterase regions and a C-terminal steroidogenic acute regulatory protein-related lipid transfer (START) domain. Third, the promoter region is GC-rich and contains GC boxes, but lacks both TATA and CCAAT boxes, the typical criteria of housekeeping genes. A consensus peroxisome proliferator responsive element (PPRE) present in the rodent CACH promoter regions supports marked CACH induction in rat liver by peroxisome proliferator (PP).

Keywords: acetyl-CoA hydrolase, PCR, cDNA sequence, Spodoptera frugiperda, functional expression, housekeeping-type promoter

INTRODUCTION

The cytosolic or extramitochondrial acetyl-CoA hydrolase (CACH) hydrolyzes acetyl-CoA to acetate and CoA. It seems superficially wasteful to hydrolyze the most common energy-rich metabolite acetyl-CoA without recovering any energy. So it is exciting to understand the physiological role of the enzyme comprehensibly.

The enzyme has been detected in rat liver (Prass et al., 1980) and kidney (its cytosolic CACH specific activity was 5% of that of the liver enzyme) (Matsunaga et al., 1985). Its activity in the liver increases notably in the opposite metabolic states: during enhanced fatty acid oxidation and in increased fatty acid synthesis (Matsunaga et al., 1985). Further, a marked induction in the liver was observed by thyroid hormones (Matsunaga et al., 1985) and also by 2-(p-chlorophenoxy) isobutyric acid (Ebisuno et al., 1988), a hypolipidemic drug or peroxisome proliferator (PP), which enhances β-oxidation in rat liver mitochondria and peroxisomes (Mannaerts et al., 1979) and increases cytosolic CoA level (Berge et al., 1983; Horie et al., 1986). These findings reveal that the enzyme plays a vital role specifically in fat metabolism by supplying cytosolic free CoA necessary for both fatty acid synthesis and oxidation (Matsunaga et al., 1985).

The enzyme had rejected earlier an enough of purification due to its extreme cold lability (Isohashi et al., 1983a; Suematsu et al., 1996) and little activity in the absence of ATP (Söling & Rescher, 1986).
MATERIALS AND METHODS

Chemicals. Acetyl-CoA was synthesized as described previously (Simon & Shemin, 1953). All other chemicals and reagents used were of analytical grade or better.

Enzyme assay. Enzyme activity was routinely assayed at 25°C as previously described (Prass et al., 1980). One unit of activity is that required to hydrolyze 1 µmol of acetyl-CoA × min⁻¹ under the conditions of the assay. Acetyl-CoA hydrolase activity was estimated by subtracting the nonenzymatic rate measured in the presence of 2 mM ADP, which inhibits the enzymatic activity, from that observed in the 2 mM ATP. All determinations were carried out in triplicate.

cDNA cloning from human liver cDNA library. Oligonucleotide PCR primers were designed (Table 1) based on the two human expressed sequence tags (ESTs) AV693695 and AV685167 in the public database: sense primers S1 and S2 correspond to nucleotide sequences at 1/21 and 7/27 of the 5'-terminus region of AV693695, respectively, and antisense primers A1 and A2 correspond to those at 363/386 and 329/352 of the 3'-terminus region of AV685167, respectively. A human liver cDNA library (QUICK-Clone cDNA, CLONTECH Laboratories, Inc.), derived from whole liver mRNA from a 41-yr-old Caucasian, was used as the template for nested PCR with Ex Taq DNA Polymerase (TaKaRa), using the primer set of S1/A1 for the first step and then the nested set of S2/A2 for the second. Both strands of the amplified cDNA were directly sequenced. Then for the cDNA cloning, the nested PCR step was performed with Platinum Pfx DNA Polymerase (Life Technologies, Inc.), using a primer set of S5/A2 (Table 1). The anchor primer S5 includes a restriction site to facilitate subsequent cloning. The amplified cDNA was then inserted into the Xmal/PshAI site of the baculovirus transfer vector pTriEx-4 for expressing His-tag fusion protein, allowing a His-tag pull

Table 1. PCR primers used for cloning human acetyl-CoA hydrolase cDNA

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’-3’)</th>
<th>Position</th>
<th>Location</th>
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</thead>
<tbody>
<tr>
<td>S0</td>
<td>AAATTTGAGCCGAGACATAGGAC</td>
<td>(specific to pTriEx-4 vector)</td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td>GCCCGAGCTTACGCTCTCCG</td>
<td>-29/-9</td>
<td>5’-UTR</td>
</tr>
<tr>
<td>S2</td>
<td>GGCTTAGCGCTCTCGCCCTGG</td>
<td>-23/-3</td>
<td>5’-UTR</td>
</tr>
<tr>
<td>S3</td>
<td>TCCAGACGATGGAAGTCGGCTCC</td>
<td>548/571</td>
<td>CDS</td>
</tr>
<tr>
<td>S4</td>
<td>AAAAGGTTGGAGGAGTACCCG</td>
<td>1093/1116</td>
<td>CDS</td>
</tr>
<tr>
<td>S5</td>
<td>TCTGGCCGGGGCCATGGAGC</td>
<td>-13/7</td>
<td>anchor primer</td>
</tr>
<tr>
<td>A0</td>
<td>TCGATCTCAGTGTTTTGTTGCC</td>
<td>(specific to pTriEx-4 vector)</td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>CCAAATACGTGTTAGACAAATGC</td>
<td>1809/1832</td>
<td>3’-UTR</td>
</tr>
<tr>
<td>A2</td>
<td>CTCGGCTACTGCTTCTTTGCTAGG</td>
<td>1774/1797</td>
<td>3’-UTR</td>
</tr>
<tr>
<td>A3</td>
<td>TTTCCACAGTGGTAAACCTCC</td>
<td>1104/1127</td>
<td>CDS</td>
</tr>
<tr>
<td>A4</td>
<td>AATGTGTTTCGGTTGTGTTCG</td>
<td>580/603</td>
<td>CDS</td>
</tr>
</tbody>
</table>
Functional expression of human cytosolic acetyl-CoA hydrolase

RESULTS

Molecular cloning and sequence analysis of a human CACH cDNA covering a full-length ORF

Database searching revealed that several human expressed sequence tags (ESTs) represent high homology (around 75%) to the rodent CACH cDNAs (Suematsu et al., 2001; 2002), implicating the existence of a human homologue cDNA. One human EST clone (586 bp long, AV693695 in GenBank) shows 82% identity to the 5'-terminus of the rat cDNA in a 114 amino acid overlap, and another (394 bp long, AV685167 in GenBank) shows 70% identity to the 3'-terminus in a 73 amino acid overlap. Based on the nucleotide sequences of the two ESTs, oligonucleotide PCR primers were designed (Table 1) and used to clone a human homologue cDNA from a human liver cDNA library. As outlined in Fig. 2, the amplified human homologue

Figure 1. Schematic depiction of the strategy for recombinant expression of a full-length ORF of human cytosolic acetyl-CoA hydrolase.

A. Construction of the recombinant baculovirus transfer vector containing the complete coding region of human cytosolic acetyl-CoA hydrolase (hCACH). The full-length ORF encoding hCACH was inserted into the multiple cloning site of the baculovirus transfer vector pTriEx-4 at the XmaI/PshAI site. The resultant vector was 6939 bp long and designated as pTriEx-4/hCACH. B. A schematic representation of the recombinant CACH containing a polyhistidine (His6) tag.

Figure 2. Nucleotide and deduced amino-acid sequences of the cDNA encoding full-length ORF of human cytosolic acetyl-CoA hydrolase.

The nucleotide and predicted amino-acid residues are numbered on the right from the first base of the ATG start codon (shown in bold face). The underlined nucleotide sequences correspond to the gene-specific primers used for sequencing (Table 1). The asterisk denotes the TAA stop codon. Sequence data have been submitted to DDBJ under the accession number AB078619.
cDNA is 1820 bp long and comprises the entire coding region of 1668 bp. The open reading frame (ORF) for a polypeptide of 555 amino-acid residues with a calculated molecular mass of 62033 Da, starts with an ATG initiation codon (Fig. 2, shown in bold face), whose first nucleotide is numbered as +1, and terminates with a TAA stop codon (at positions 1669/1671, shown with an asterisk). The first AUG agrees pretty well with the Kozak’s consensus sequence GCCGCC(A/G)CC AUGG for initiation in higher eukaryotes (Kozak, 1987; 1991). As shown in Fig. 3, the deduced amino-acid sequence of the human homologue cDNA exhibited extensive homology throughout the entire ORF to those of the mouse and rat CACH (81.4% and 78.7% identity, respectively, see Table 2). The human cDNA sequence is available from DDBJ/EMBL/GenBank under the accession number AB078619.

Expression and purification of recombinant CACH in Sf9 insect cells

Recombinant expression of the human CACH cDNA in Sf9 insect cells resulted in overproduction of a His-tag fusion protein with the expected enzymatic activity. The activity was detected as early as two days after infection in the lysate prepared from infected cells, and was maximal at four days post-infection. The expressed human recombinant enzyme hydrolyzed acetyl-CoA in the presence of 2 mM ATP and the activity was completely inhibited by ADP. Further, it exhibited cold lability and its cold-inactivation could be partially abolished through the incubation at 37°C in the presence of 0.16 mM Triton X-100 just as the rodent enzymes (Suematsu et al., 2001; 2002). The expressed His-tag

| Table 2. Comparison of nucleotide and deduced amino-acid sequence of cytosolic acetyl-CoA hydrolase ORF among human and the rodents. |
|---|---|
| Length | Identical residues with human CACH |
| hCACH | 555 aa |
| mCACH | 556 aa |
| rCACH | 556 aa |
| 1371/1665 bp (82.3%) | 452/555 aa (81.4%) |
| 1356/1665 bp (81.4%) | 437/555 aa (78.7%) |

Figure 3. Comparison of the deduced amino-acid sequence of cytosolic acetyl-CoA hydrolase among human and the rodent species.

The amino-acid residues are numbered on the right. Residues identical with those in the first sequence were denoted by dots. A dash has been introduced to maximize alignment. Exons I–XV are presented with their boundaries indicated by broken line. Arrows represent the extent of functional domains revealed by a publicly available database at the NCBI Conserved Domain Search: Acyl-CoA hydrolase (thioesterase); 4HBT (4-hydroxybenzoyl-CoA thioesterase); START (steroidogenic acute regulatory (STAR)-related lipid-transfer) domain. The compared sequences are as follows: hCACH, human cytosolic acetyl-CoA hydrolase (GenBank accession AB078619); mCACH, mouse cytosolic acetyl-CoA hydrolase (GenBank accession AB078618); rCACH, rat cytosolic acetyl-CoA hydrolase (GenBank accession AB040609).

Figure 4. SDS/PAGE analysis of purified recombinant hCACH protein.

Protein samples from purification steps for the recombinant hCACH protein were subjected to SDS/PAGE on a 10–20% polyacrylamide gradient gel followed by silver staining. The samples, prepared as described in Materials and Methods, were the clarified cell lysate from the infected insect cells [1], the flow through from the Ni²⁺-charged resin [2], the eluted His-tag fusion protein [3] and the hCACH protein free of the fused peptide [4]. Numbers to the left and right denote the molecular mass (in kDa) of the markers (lane M) and the purified proteins (lanes [3] and [4]). The molecular mass markers are phosphorylase b (97400), bovine serum albumin (66300), aldolase (42400), carbonic anhydrase (30300), soybean trypsin inhibitor (20100) and lysozyme (14400).
Table 3. Affinity purification and characterization of a recombinant form of human cytosolic acetyl-CoA hydrolase.

(A) Recombinant human cytosolic acetyl-CoA hydrolase protein expressed in infected Sf9 insect cells (4 µl cell pellet) was purified as described in the text. (B) Constants representing catalytic activity of purified human recombinant enzyme (hCACH) and purified rat counterpart (rCACH, Suematsu et al., 2001). Proteins were determined according to the method of Bradford.

<table>
<thead>
<tr>
<th>Step</th>
<th>Total activity (U)</th>
<th>Total protein content (μg)</th>
<th>Specific activity (U mg⁻¹)</th>
<th>Purification (fold)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysate</td>
<td>5.2</td>
<td>655</td>
<td>7.9</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>Eluate</td>
<td>5.0</td>
<td>1.51</td>
<td>3300</td>
<td>419</td>
<td>97</td>
</tr>
</tbody>
</table>

(B)

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Specific activity (U mg⁻¹)</th>
<th>Michaelis constant (Kₐ (M))</th>
<th>Catalytic constant (kₜ (s⁻¹))</th>
<th>Catalytic efficiency (kₜkat/Kₐ (M⁻¹ s⁻¹))</th>
</tr>
</thead>
<tbody>
<tr>
<td>hCACH</td>
<td>3300</td>
<td>1.5 × 10⁻⁴</td>
<td>7.0 × 10⁷</td>
<td>4.6 × 10⁷</td>
</tr>
<tr>
<td>rCACH</td>
<td>3100</td>
<td>1.5 × 10⁻⁴</td>
<td>6.6 × 10⁷</td>
<td>4.3 × 10⁷</td>
</tr>
</tbody>
</table>

fusion protein was purified to homogeneity with 97% yield by affinity purification using Ni²⁺-charged resin (Table 3A) and then relieved of the fused peptide by thrombin cleavage (Fig. 4). The purified human CACH represented comparable specific activity (3300 U/mg), Michaelis constant for acetyl-CoA (Kₐ = 1.5 × 10⁻⁴ M), catalytic constant (kₜ = 7.0 × 10⁷/s) and catalytic efficiency (kₜkat/Kₐ = 4.6 × 10⁷ /M·s) to those of the rat enzyme (Table 3B).

Highly conserved genomic organization of mammalian CACHs

We here describe a comparative analysis of the genomic organization of human, mouse and rat CACHs. It was verified by mRNA-genomic alignments and published in established databases that the CACH gene is mapped at human chromosome 5q14.1, mouse chromosome 13C3 and rat chromosome 2q12 and covers about 64-kb, 45-kb and 50-kb genomic regions, respectively (Fig. 5). The mammalian CACH genes are similarly composed of 15 exons, the size of each of which is identical among the three mammalian species with an exception that exon I codes for 43 amino-acid residues in human and 44 residues in the rodents (Fig. 3). Introns are generally longer in human than in the rodents with the exceptions of introns I, IX, and X (Fig. 5).

Putative domain structure of mammalian CACH proteins

Bioinformatical work using the NCBI Conserved Domain Search website revealed that human, mouse and rat CACH proteins commonly consist of uniquely aligned functional domains as follows: two acyl-CoA hydrolase (thioesterase) regions (accession: COG1607 (Dillon & Bateman, 2004)) encoded by exons I–V and VI–IX, respectively, and a C-terminal Steroidogenic acute regulatory (STAR) related lipid transfer (START) domain (accession: pfam01852; (Tsujishita & Hurley, 2000)) encoded by exons X–XV (Fig. 3). Because the rest of the domains might be too small, ranging in length from only 10 to 23 amino-acid residues, to contribute to the enzymatic function, all or some of the three functional domains presumably contribute to the CACH activity. As shown in Fig. 3, 4-hydroxybenzoyl-CoA thioesterase (4HBT) domains (accession: pfam03061) I, II, encoded by exons I–IV and VI–VIII, respectively, were also demarcated in the midst of the thioesterase regions I and II, respectively. The 4HBT domains I and II show especially high amino-acid sequence homology between human and mouse/rat (I: 87.5%/86.1% identity; II: 93.2%/93.2% identity). On the other hand, the C-terminal START domain shows a relatively low amino-acid sequence homology between human and mouse/rat (77.1%/74.6% identity).

Characterization of the putative promoter region of mammalian CACH genes

The putative promoter regions of human, mouse and rodent CACH genes are unexpectedly found to be of the housekeeping-type, namely GC-rich with GC box(es), but lacking both TATA and CCAAT boxes, as described below. The 5′-flanking nucleotide sequences 100 bp long show high GC contents (human, 83%; mouse, 74%; rat, 70%). A GC box (GGCGG) is commonly found in the upstream regions of the three mammalian species, especially in human, where six tandemly arranged GC boxes are found in the region −107/−54 (Fig. 6). On the other hand, although we searched the 5′-

![Figure 5. Comparison of exon-intron structure of cytosolic acetyl-CoA hydrolase genes among human and the rodents.](image)
flanking region of the CACH genes up to the position –1200, neither a canonical core promoter motif TATA box (TATAAA) nor a CCAAT box was found in mouse and rat. These cis-elements were also absent in the proximal promoter region of the human gene, whereas in a farther upstream region, a sequence TTTATA at –824/–819, corresponding to the inverted sequence of TATAAA, a typical CCAAT box at –394/–390, and its inverted sequence ATTGG at –767/–763, were found.

A peroxisome proliferator responsive element (PPRE) motif (TGACCTcTGACCT) (Kliewer et al., 1992) was, as expected, found in the rodent CACH promoter regions (Fig. 6), consistent with the marked CACH induction by peroxisome proliferator (PP) observed in rat liver (Prass et al., 1980). In human the PPRE was found not in the putative promoter region but in introns II and III and so forth of the CACH gene (Table 4). Further, a computer analysis using TESS revealed that possible well-characterized cis-elements are commonly found in the upstream regions of the mammalian CACH genes (Fig. 6). These features observed are discussed later in relation to the CACH expression.

**DISCUSSION**

A human cytosolic acetyl-CoA hydrolase (CACH) cDNA was cloned from a liver cDNA library and characterized for the first time. Cytoplasmic localization of the human CACH was predicted by PSORT WWW Server from the cDNA nucleotide sequence not containing any targeting signals for organella including peroxisome, mitochondria or nucleus, as well as the rodent enzymes (Suematsu et al., 2001; 2002). The extensive homology of its deduced amino-acid sequence with the rodent enzymes (Suematsu et al., 2001; 2002) implies that the obtained human homologue cDNA encodes no other acyl-CoA thioesterase (Broustas et al., 1996; Hunt et al., 1999) but CACH. The authenticity of the human cDNA was further confirmed by its functional expression. The expressed recombinant form of human CACH was found to be a cold labile allosteric enzyme activated by ATP and completely inhibited by ADP, and shared comparable enzymatic characteristics with the rat enzyme (Table 3B). Thus, our previous and current studies clearly demonstrate that the mammalian CACH proteins have been highly conserved.

Here we proposed putative functional domains of CACH (Fig. 3). Among them, the highly conserved 4HBT thioesterase domains (Benning et al., 1998) presumably contribute greatly to the enzymatic activity of active CACH homooligomers. Although the actual domain functions remain to be established, the highly conserved unique alignment of the putative functional domains (Fig. 3) together with the overall high amino-acid sequence similarities of the cis-elements are commonly found in the upstream regions of the mammalian CACH genes (Fig. 6). These features observed are discussed later in relation to the CACH expression.

Table 4. Putative peroxisome proliferator responsive elements (PPRE) in human, mouse and rat cytosolic acetyl-CoA hydrolase genes.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence (5'-3')</th>
<th>Position in gene</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>hCACH</td>
<td>ggtAGGTCAAGATCA</td>
<td>–39,165/–39,149</td>
<td>5'-upstream region</td>
</tr>
<tr>
<td></td>
<td>aggTGTCAAAGGTCA</td>
<td>15,930/15,946</td>
<td>intron II</td>
</tr>
<tr>
<td></td>
<td>agtACGGTCACAGGTA</td>
<td>22,527/22,543</td>
<td>intron III</td>
</tr>
<tr>
<td>mCACH</td>
<td>TGACCTCTGACCTGAT</td>
<td>–266/–249</td>
<td>5'-flanking region</td>
</tr>
<tr>
<td></td>
<td>TGACCTCTGACCTccta</td>
<td>–101/–85</td>
<td>5'-flanking region</td>
</tr>
<tr>
<td>rCACH</td>
<td>TGACCTCTGACCTccta</td>
<td>–105/–89</td>
<td>5'-flanking region</td>
</tr>
<tr>
<td></td>
<td>attcAGGTCAAGGTCA</td>
<td>33,614/33,630</td>
<td>intron V</td>
</tr>
</tbody>
</table>

The nucleotide sequences are described in the legend to Fig. 5. PPRE was searched using TESS. Nucleotide positions are numbered as in Fig. 2. Boldface letters represent highly conserved sequences in agreement with the consensus sequences for PPRE (Kliewer et al., 1992).
ity among the three mammalian species (Table 2) implies a crucial role of CACH in mammalian metabolism.

In the mammalian CACH genes, all the introns are found to be bordered by consensus GT-AG splice sites, which are usually excised by the major spliceosome utilizing U2 snRNA (Sharp & Burge, 1997), and further their splicing donor and acceptor sites share common pyrimidine stretches (Breathnach et al., 1978; Breathnach & Chambon, 1981). In the human CACH gene, as shown in Table 5, exons range in length from 56 bp (exon IX) to 157 bp (exon VI), while the introns from 270 bp (intron XIV) to 13946 kb (intron II). As commonly found in the mammalian CACH genes, introns I–V are generally larger, whereas introns VI–X are distinctly compact (Fig. 5), implying separate origins of the two thioesterase domains, composed of exons I–V and VI–IX, respectively, and their conjugation by domain shuffling during evolution of the genomes. This interesting inference is further supported by the finding that intron V located at the domain border is of phase I, which is classified according to the number of bases of the final codon generated in the previous exon (Cawley et al., 2001). It has been proposed that domain shuffling within phase I introns played an important role in the evolution of the human genome (Kaessmann et al., 2002).

The three major core promoter motifs containing binding sites for RNA polymerase II have been reported to be the TATA box, the initiator and the downstream promoter element (Burke & Kadonaga, 1997). In the present paper, we analyzed mammalian CACH putative promoter regions for the first time. To estimate the transcription start sites of CACH, each 5′-end of mammalian CACH transcripts and EST entries extending into the 5′-direction available from the GenBank was marked on the 5′-upstream region of the mammalian CACH genes (Fig. 6). Unexpectedly, we found that none of them matched the consensus sequence of mammalian initiator Py-Py-Λ1-N-T/A-Py-Py, whose Λ1 is the transcription start site (Burke & Kadonaga, 1997). We here report that the CACH putative promoters do not have any of the three known core promoter motifs, suggesting the presence of other core promoter motifs responsible for accurate positioning of RNA polymerase relative to the initiation site.

It should be noted that a number of promoters lack classical TATA or CCAAT boxes but have an increased GC content with specificity protein 1 (Sp 1) binding site(s), features typical of ‘housekeeping’ genes (La Thangue & Rigby, 1988; Rundlöf et al., 2001). The ubiquitously expressed TATA-less genes have been reported to be controlled by Sp proteins (Dyanan et al., 1986; Suske, 1999; Müller et al., 1999), which have been shown to bind the GC box, the hexanucleotide sequence GGGCGG (Lania et al., 1997). Interestingly, the mammalian CACHs have a typical housekeeping-type promoter (Fig. 6), which presumably stimulates constitutive transcription of CACH, implying that the enzyme might be generally essential for mammalian cells. The ubiquitously expressed Sp1 was reported as a member of a transcriptionally active multiprotein–DNA complex (Foti et al., 2003), and also as a repressor by recruiting histone deacetylase 1 (HDAC1) to the growth-regulated murine thymidine kinase gene (TK) promoter (Doetzlhofer et al., 1999). Thus, it is likely that Sp1 acts in a combinatorial manner with other transcription factors, which may have more pronounced temporally or spatially restricted expression patterns. It has been also shown that the Sp1-related BTEB (basic transcription element binding protein) protein is a repressor of a promoter containing a single GC box, however, when the GC box was repeated five times, BTEB turned out to be an activator (Lania et al., 1997). Thus, Sp1-related proteins like BTEB could possibly influence the CACH transcription differently between the rodents and human, since the former contains a single GC box and the latter contains six tandemly arranged GC boxes in their putative promoter regions of CACH. How the Sp proteins actually control the CACH gene expression remains to be established.

Although the mammalian CACHs have a typical housekeeping-type promoter, the enzyme activity is detectable only in liver and kidney of the rat tissues tested (Matsunaga et al., 1985). The tissue-specific expression must arise from cis-acting elements other than a GC box. Web-based search using TESS revealed several other possible cis-elements commonly found in the mammalian CACH putative promoter regions (Fig. 6) as described below. First, consensus (C/T)GGGGT sequence, known as a binding site of yeast Adr1p, locates at –42/–37 (human), –22/–17 (mouse) and –145/–140 (rat). Adr1p has been reported to govern fatty acid degradation and peroxisome proliferation (Gurvitz et al., 2001). Second, consensus CCTGC sequence, known as a binding site of LVC (leukemia virus factor c), lies at –26/–22 (human), –37/–33 (mouse) and –43/–39, –92/–88 (rat). LVC interacts with leukemia virus enhancer (Speck & Baltimore, 1987). Third, an E box motif (consensus CANNTG) exists at –134/–129 (human), –101/–96 (mouse) and –106/–101 (rat). It is usually found in promoter or enhancer regions and is known as a binding site of ubiquitous basic helix-loop-helix (bHLH) transcription factors, which induce the expression of a number of genes in the appropriate cell type, resulting in a tissue-specific phenotype (Murre et al., 1994). The bHLH proteins can be categorized into three classes: Class A and B function as transcriptional activators, while Class C as transcrip-
tional repressors (Azmi et al., 2003). Since E box sites appear in a wide variety of promoters and enhancer regions (Murre et al., 1994), other flanking factors would specify their functions. In the rodent CACH promoter region, an E box is located close to a PPRE element, and in rat it is additionally found directly adjacent to a GC box (Fig. 6), suggesting possible synergistic transactivation of the CACH gene by their ligands. A PPRE element found in the rodent CACHs putative promoter region (Fig. 6) must be responsible for the marked enzyme induction by a peroxisome proliferator (PP) (Prass et al., 1980). On the other hand, the human PPRE elements found outside of the putative promoter region (Table 4) remain to be determined whether active or not. A significant number of studies have suggested that human hepatocytes are non-responsive to PPs — this includes epidemiological studies using hypolipidemic drugs, and in vitro experiments with human hepatocytes (Tugwood et al., 1996). To address the question whether hCACH is induced by PPs, further in vitro experiments with human hepatocytes should be carried out. Precise demarcation of the promoter and identification of all the elements involved in the gene transcription awaits further experiments including in vivo transfection.

In conclusion, this paper for the first time describes the entire cDNA sequence of human cytosolic acetyl-CoA hydrolase and its overproduction, allowing future studies on its physiological functions and physicochemical characteristics. Although a detailed characterization of its promoter remains to be established, the bioinformatical promoter analysis presented here has provided insights into the regulation of the gene expression. Transgenic approach is currently underway to explore physiological roles of the enzyme. The in vivo study would provide direct evidence on the enzyme functions and facilitate conclusive establishment of its significance.

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