Identification of a second human acetyl-CoA carboxylase gene

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Acetyl-CoA carboxylase (ACC), an important enzyme in fatty acid biosynthesis and a regulator of fatty acid oxidation, is present in at least two isoenzymic forms in rat and human tissues. Previous work has established the existence of a 265000 Da enzyme in both the rat and human (RACC265; HACC265) and a higher-molecular-mass species (275000–280000 Da) in the same species (RACC280; HACC275). An HACC265 gene has previously been localized to chromosome 17. In the present study, we report cloning of a partial-length human cDNA sequence which appears to correspond to HACC275 and its rat homologue, RACC280, as judged by mRNA tissue distribution and cell-specific regulation of mRNA/protein expression. The gene encoding this isoenzymic form of ACC has been localized to the long arm of human chromosome 12. Thus, ACC is represented in a multigene family in both rodents and humans. The newly discovered human gene and its rat homologue appear to be under different regulatory control to the HACC265 gene, as judged by tissue-specific expression *in vivo* and by independent modulation in cultured cells *in vitro*.

INTRODUCTION

Acetyl-CoA carboxylase (ACC), a rate-limiting enzyme of fatty acid biosynthesis, has been found to have a wider tissue distribution than might be predicted by rates of lipogenesis [1–4]. While the highest activity of ACC is found in the classic lipogenic tissues, such as white adipose tissue and lactating mammary gland, ACC activity is also detectable in heart and skeletal muscle, tissues which have very low lipogenic rates [1,2]. The role of ACC in the latter tissues is probably that of the provision of malonyl-CoA, the product of the ACC reaction, for the regulation of the rate of fatty acid oxidation through allosteric regulation of carnitine palmitoyltransferase I (CPT-I) [5,6].

Recent evidence has been provided to indicate that ACC is present in isoenzymic forms in different tissues. One isoenzyme, first characterized in the rat, is a 265000 Da enzyme (henceforth referred to as RACC265) that is highly expressed in rat white adipose tissue and lactating mammary gland [2,3]. A human homologue of RACC265, referred to herein as HACC265, has also been identified [7]. Based on enzyme isolation, kinetic characterization and selective immunoreactivity with a panel of anti-ACC antibodies, a second rat isoform of 280000 Da (RACC280) has also been identified and characterized [1–4,6,8,9]. RACC280 is highly expressed in rat skeletal muscle and heart, but is also expressed in rat liver, brown adipose tissue, pancreatic islets and several cell lines. A human homologue of RACC280 of 275000 Da (HACC275) has been noted and is expressed in human skeletal muscle and adipose tissue and several human breast cancer cell lines [7]. cDNAs for RACC265 and HACC265 have been cloned and sequenced and HACC265 gene(s) have been localized to chromosome 17 [10–14]. Immunological studies of RACC280 and peptide sequencing of RACC280 from liver indicate that RACC280 is probably not an alternatively spliced form of the RACC265 gene, but the product of a unique gene [2,8].

In this report, we characterize human partial-length cDNAs, isolated from a human adipose tissue library, as probably corresponding to the HACC275 gene product, the homologue of the RACC280. The gene corresponding to this cDNA has been localized to human chromosome 12. We also report the independent regulation of ACC isoform expression *in vivo* and in selected cell lines, as identified by isoform-specific immunoblotting and Northern blot analysis.

EXPERIMENTAL

cDNA library screening

A λZAP II human white adipose tissue cDNA library (gift of Dr. David Bernlohr, University of Minnesota) was screened with rat ACC probes, as previously detailed [12]. This screening yielded human ACC sequences, HACC-1 and HACC-2. HACC-2 was then used to rescreen the library to isolate overlapping sequences. Probe labelling and library screening of 2×10^6 plaques was carried out, as in [15]. Positive plaques were purified through three rounds of screening and phagemid from positive clones rescued with helper phage (Stratagene).

Plasmid preparation and DNA sequencing

Plasmid DNA was purified from bacterial culture using a standard alkaline lysis protocol [16]. DNA to be used for sequencing was further purified using Qiagen Mini or Midi-columns, according to the manufacturer's instructions. DNA was sequenced using an Applied Biosystems Prism® ready reaction Dye Deoxy Terminator Cycle Sequencing kit, and cycled in a Perkin-Elmer PCR Thermocycler, according to the manufacturers' instructions. For initial sequence analysis, T7

Abbreviations used: ACC, acetyl-CoA carboxylase; HACC, human acetyl-CoA carboxylase, as either the 275 kDa (HACC275) or 265 kDa (HACC265) isoform; RACC, rat acetyl-CoA carboxylase, as either the 280 kDa (HACC280) or 265 kDa (RACC265) isoform; DMEM, Dulbecco's modified Eagle's medium; PVDF, poly(vinylidene difluoride).

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The nucleotide sequence of HACC-2 and overlapping clones (contiguous sequence termed HACC-β) has been deposited in the GenBank under accession number U34591.

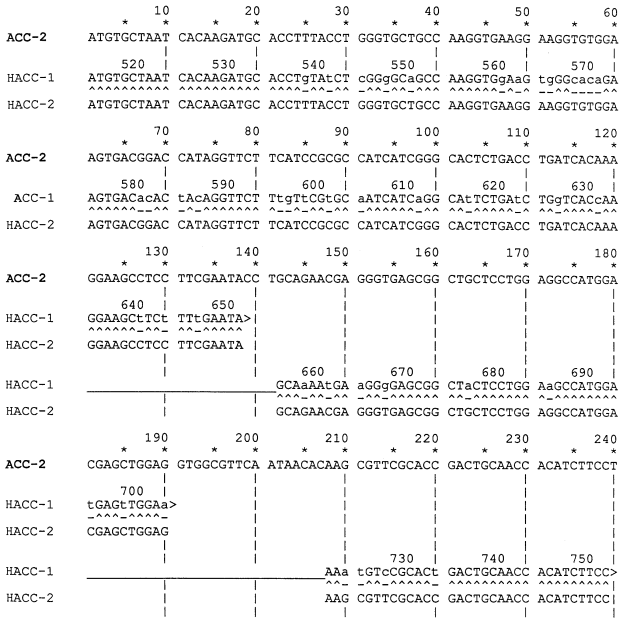

Figure 1 Nucleotide overlap of HACC-2 and HACC-1

Shown are the first 240 bp of the HACC-2 nucleotide sequence and its overlap with bp 514–752 of the HACC-1 sequence. The sequences were aligned and formatted with MacVector®. ∧ indicates nucleotide identity; non-identical nucleotides are shown in lower-case type in the HACC-1 sequence and are denoted with an underbar.

promoter and M13 reverse primers were used to prime sequence reactions. Subsequently gene-specific primers were employed to completely sequence both DNA strands of each plasmid. Extraneous dye terminators were then removed from the resulting sequence reactions using a Centri-Sep column (Princeton Separations, Inc). The purified sequencing reactions were then dried in a Speed-Vac and analysed on an automated DNA sequencer (Applied Biosystems Model 373). DNA sequences were analysed and aligned using MacVector® software and the GCG software package. Sequences were additionally formatted using an Excel® macro, as in [15].

Southern blot analysis and analysis of somatic cell hybrid DNA

Southern blotting was carried out by a standard method [16]. Genomic DNA from human, mouse and hamster (CHO cells) and from two somatic cell hybrid panels (Coriell; Bios Laboratories) was digested with various restriction enzymes prior to gel loading (see Figure legends). All membranes were washed at high stringency [2 × SSC for 30 min (2 changes) at room temperature; 0.1 × SSC/0.2 % SDS for 60 min (2 changes) at 65 °C; SSC: 0.15 M NaCl/0.015 M sodium citrate].

Analysis of ACC protein and mRNA in selected cell lines

Two principal cell lines were chosen for the current investigations, the H4IIE rat hepatoma cell and the rat H9c2 embryonic cardiomyocyte, the latter of which differentiates from myoblasts to myotubes in the presence of low serum concentrations [17].

H4IIE Reuber hepatoma cells were cultured in 10-cm-diam. Corning dishes to 80 % confluency at 37 °C in a humidified atmosphere of 5 % CO_2/95 % air with Dulbecco's α-minimal essential medium containing 2.5 % (v/v) fetal bovine serum, 2.5 % (v/v) newborn-calf serum, 5 % (v/v) donor-calf serum, penicillin and streptomycin. For the ciprofibrate experiments, cells in the presence of this serum were exposed to either control vehicle or ciprofibrate (0.5 mM; obtained from Sterling-Winthrop, Inc.) for 72 h, followed by digitonin and/or SDS harvests (see below). For the glucose depletion/repletion experiments, cells were washed in PBS and switched to a substrate-free, serum-free medium [Dulbecco's modified Eagle's medium (DMEM) without Phenol Red]. After 48 h in this medium, nutrient additions (glucose; 10 mM) were made and harvests were performed 24 h later.

H9c2 cells were plated in 10-cm-diam. Corning dishes in DMEM/Ham's medium supplemented with glutamine, streptomycin/penicillin and 10 % (v/v) fetal bovine serum. The cells were incubated in an atmosphere of 5 % CO_2/95 % air at 37 °C and grown to a confluency between 80 and 90 %. Upon reaching this degree of confluency, some plates were harvested to serve as pre-fusion samples which could be compared with the later lysates. The remaining plates were washed twice with sterile PBS, incubated for 1 h in serum-free medium and placed in DMEM/Ham's medium with 1 % (v/v) horse serum and insulin (10^{-7} M). Fresh medium was supplied every 2 to 3 days. In the standard differentiation medium, myotube formation and the appearance of creatine phosphokinase activity can both be noted by day 4 and is near maximal by day 6 (results not shown).

For both cell lines, at the time of cell harvest, cells were rinsed in ice-cold PBS and cell lysates were prepared either by digitonin lysis into a buffer (0.5 ml per 10-cm-diam. plate) containing Tris/HCl (50 mM, pH 7.5), EDTA (1 mM), NaF (0.1 M), 2-mercaptoethanol (10 mM), sucrose (0.25 M), digitonin (0.4 mg/ml), and seven protease inhibitors, or by solubilization of cells directly into the same lysis buffer (omitting digitonin), but including 1 % SDS [18]. For analysis of cellular mRNA by Northern blot analysis, total RNA was prepared from parallel plates, as detailed below.

Electroblotting of ACC

After separation of proteins on a 5 % SDS/acrylamide gel and transfer to poly(vinylidene difluoride) (PVDF) membrane, lysate proteins were probed with either a monoclonal antibody specific for RACC280 (ACC 7AD3) [2], an anti-(N-terminal peptide) antibody (N) specific for RACC265 [2], or streptavidin–peroxidase, which detects both ACC isoenzymes [2,7]. Antibody binding was detected with peroxidase-labelled second antibodies and chemiluminescence (ECL; Amersham).

Northern blot analysis and RNA preparation

Total RNA was isolated from tissues and cell lines using a guanidinium isothiocyanate–lithium chloride method, as described in [19]. Northern blot analysis was carried out, as previously detailed [15]. Filters were washed at high stringency in all experiments [2 × SSPE for 20 min (2 changes) at room temperature followed by 0.1 × SSPE/0.1 % SDS at 55 °C for 1.5 h (3 changes); SSPE: 0.15 M NaCl/10 mM sodium phosphate (pH 7.4)/1 mM EDTA].

Materials

H9c2 cells were obtained from the American Type Culture Collection. H4IIE cells were a kind gift from Dr. Daryl Granner (Vanderbilt). Cell media and sera were obtained from Gibco/BRL. PVDF membranes were purchased from Millipore. Secondary antibodies and most chemical reagents were purchased from Sigma. α-[^{32}P]CTP for cDNA probe labelling was purchased from Dupont/New England Nuclear. Nitrocellulose for North-

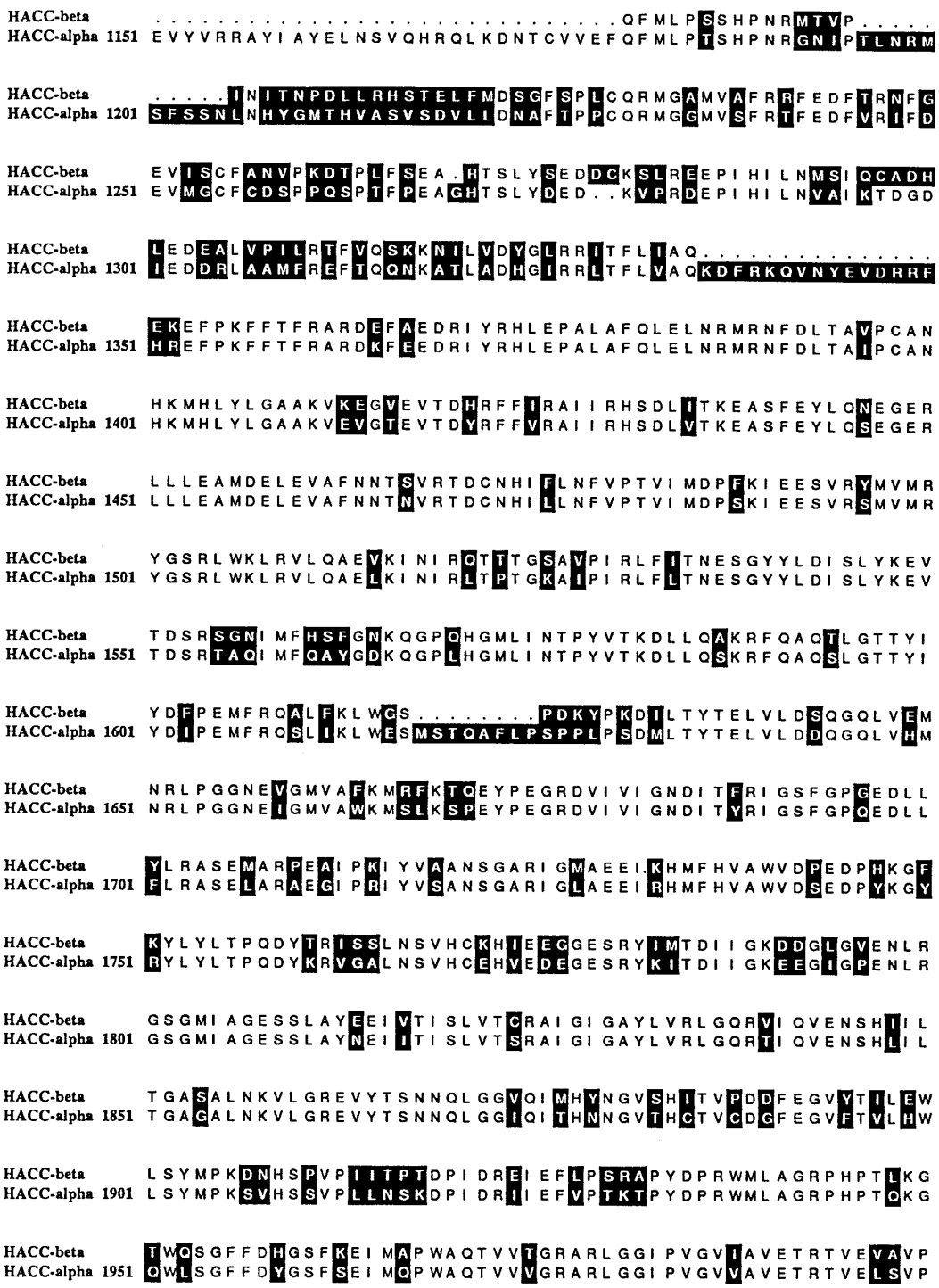

Figure 2 Comparison of HACC-α and HACC-β deduced amino acid sequences

Shown is the alignment of the deduced HACC-β amino acid sequence with residues 1181–2000 of the HACC-α (HACC265) [14]. Alignments with gap assignments were performed using the Pileup program of the GCG software package. Non-identical amino acids are indicated by shading.

ern blots and library screening was purchased from MSI (Westboro, MA, U.S.A.) and from Schleicher and Schull (Keene, NH, U.S.A.). Zetabind membranes for Southern blots was purchased from AMF Cuno (Meriden, CT, U.S.A.). Gene-specific primers for DNA sequence analysis were synthesized by Midland Certified Reagent Co. (Midland, TX, U.S.A.).

RESULTS

Isolation of unique HACC cDNAs

During screening of a human adipose tissue cDNA library with a partial-length RACC265 cDNA, two partially overlapping but non-identical cDNAs, termed HACC-2 (785 bp) and HACC-1

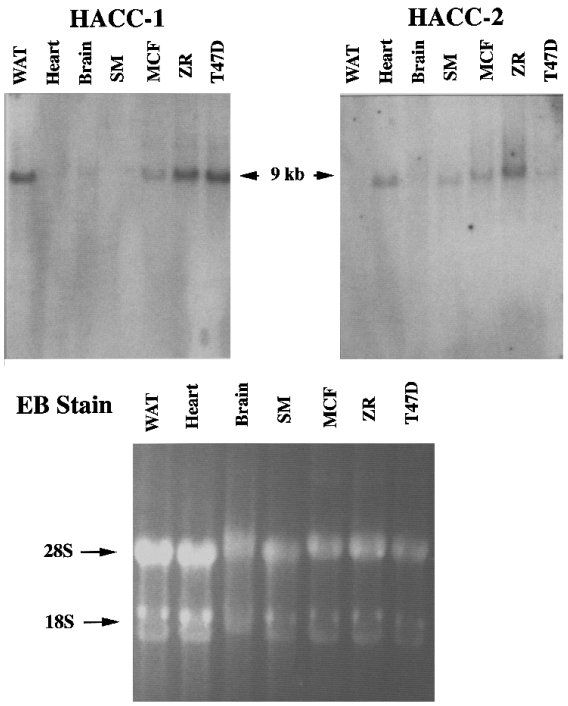

Figure 3 Northern blot analysis of rat tissue and human breast cancer cell mRNA

Shown is a representative Northern blot analysis, performed as in the Experimental section, of total RNA prepared from rat white adipose tissue (WAT), rat heart, rat brain, rat skeletal muscle (SM) and from three human breast cancer cell lines (MCF7, ZR-75 and T47D). A 20 μg aliquot of total RNA is loaded in each lane. The two upper panels are radioautographs performed after probing with either ^{32}P-HACC-1 (upper left) or ^{32}P-HACC-2 (upper right). The bottom panel shows the ethidium bromide stain of these RNA preparations to indicate the equivalency of loading as identified by the 28 S and 18 S ribosomal RNA bands.

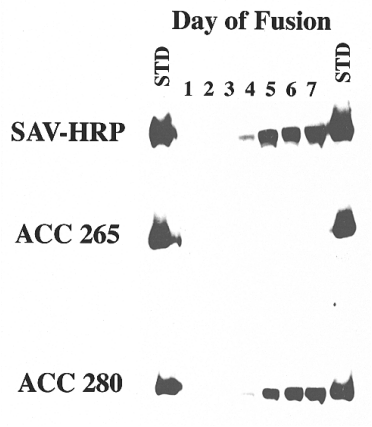

Figure 4 RACC280 induction with myogenic differentiation

Shown is a composite series of blots from a single representative experiment of lysates (20 μg of lysate protein per lane) prepared from H9c2 myocytes harvested on successive days after switching to the fusion medium on day 1. The upper panel is a streptavidin–horseradish peroxidase (SAV-HRP) blot, the middle panel an immunoblot with RACC265-specific antibody [7] and the bottom panel an immunoblot with RACC280-specific antibody [7]. STD refers to purified ACC standards (0.5 μg) from heart (upper and lower panels) and white adipose tissue (middle panel).

(752 bp) were isolated (Figure 1). The DNA sequence of HACC-1 corresponds to nt 3672 to 4423 of the reported HACC265 cDNA [14]. The HACC-2, as a putative new ACC sequence, was used to rescreen the library to isolate overlapping clones; two such clones were identified that extended this sequence in both the 5′ and 3′ directions, yielding a contiguous cDNA sequence of about 2.5 kb. An open reading frame was identified within this contiguous sequence, which encodes for a protein sequence highly homologous to, yet significantly different from, the reported HACC265 deduced amino acid sequence (Figure 2) [14]. In this alignment, we have assigned the name HACC-alpha (α) to the HACC265 sequence and HACC-beta (β) to the new sequence; the reasons for this designation are detailed in the Discussion. We were unable with further screening to extend the HACC-β sequence in either the 3′ or 5′ direction. While thus lacking a full-length coding sequence, sufficient sequence has been obtained to indicate that this unique sequence encodes an ACC. Comparison with the database of the HACC-β contiguous sequence yields alignment with ACC species from several organisms (results not shown).

Identification of unique mRNAs recognized by HACC-2

In order to characterize further this new ACC sequence, Northern blot analysis was performed. The hybridization profile of HACC-2 was compared with that of HACC-1 against RNA isolated from several human cell lines, from representative rat tissues and from rat cell lines after manipulation of ACC polypeptide content.

We have reported heterogeneity of HACC isoenzyme expression in several human breast cancer cell lines [7]. As previously determined by immunoblotting and streptavidin blotting, the MCF7 and ZR-75 lines express both HACC265 and HACC275 polypeptides, while the T47D line expresses only HACC265 [7]. In parallel to these observations of expressed protein, HACC-1, corresponding to HACC265 sequence, recognizes a 9 kb mRNA in all three of these cell lines with the highest apparent expression in the T47D line (Figure 3). However, HACC-2 hybridizes with a similar sized mRNA predominantly in the MCF7 and ZR-75 cells, corresponding to the distribution of HACC275.

Since HACC275 appears to be the human homologue of RACC280 and HACC265 the human homologue of RACC265 [7], the hybridization patterns of HACC-2 and HACC-1 to rat tissue RNAs were compared in an effort to further distinguish these cDNAs. As shown in Figure 3, HACC-1 hybridizes to a 9 kb mRNA in rat white adipose, but no hybridization to any mRNA in either rat skeletal muscle or heart is seen. This parallels the expected distribution of RACC265 polypeptide [2,3]. In contrast, HACC-2 hybridizes to a 9 kb mRNA in both heart and skeletal muscle, but not in white adipose tissue, corresponding to the polypeptide distribution of RACC280 [2,3]. Weak hybridization was seen in this experiment to either probe with total brain RNA; in other experiments (results not shown) the HACC-1 reactive species appeared to predominate. A pattern of hybridization identical to that of HACC-2 was seen with both the 3′- and 5′-overlapping clones in the HACC-β contiguous sequence that were obtained by further library screening (results not shown). Taken together, these data suggest that HACC-1 is highly homologous to the RACC265 sequence, while HACC-2 is highly homologous to the RACC280 sequence.

RACC280 isoenzyme content can be selectively altered in both H4IIE hepatoma cells and in the differentiating H9c2 cell, providing other sources of mRNA for cDNA characterization. As shown in Figure 4, during differentiation of the H9c2 cell

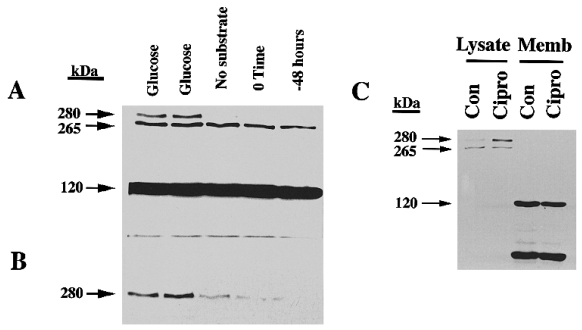

Figure 5 Induction of RACC280 in response to glucose and ciprofibrate in hepatoma cells

Shown (**A** and **B**) are blots of SDS lysates (20 μg of protein per lane) prepared from H4IIE hepatoma cells during the glucose starvation/repletion protocol, as described in the Experimental section. —48 hours time represents cells on the day of switch to the glucose-free medium and 0 time represents cells harvested at the beginning of glucose repletion. The left-hand three lanes represent cells then incubated for an additional 24 h in the presence of glucose (10 mM; replicate incubations shown) or no substrate addition. (**A**) is a blot with streptavidin–peroxidase and (**B**) a blot of the same samples with RACC280-specific antibody [2]. In (**C**) is shown a representative experiment of ciprofibrate induction. H4IIE cells, in the presence of serum, were exposed to either control vehicle (Con) or ciprofibrate (Cipro; 0.5 mM) for 72 h. Cells were then lysed with a digitonin-containing buffer to release soluble contents (lysate; left-hand two lanes); the residual cells were then solubilized in the same volume of the SDS-containing buffer to examine non-soluble proteins (memb; right-hand two lanes). Gels were loaded at equal volumes of each type of extract per lane, so that the relative distribution of reactive species can be easily envisaged. In this representative experiment, cell proteins were blotted with streptavidin–peroxidase, as described in the Experimental section.

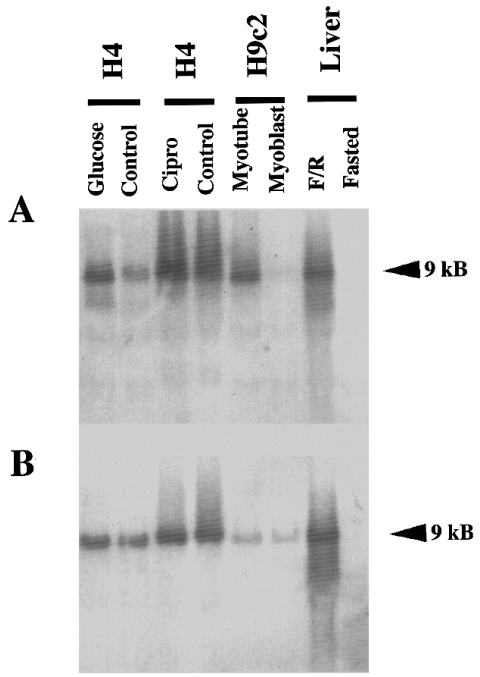

Figure 6 Northern blot analysis of rat cell line mRNA

Shown is a representative Northern blot analysis, performed as described in the Experimental section, of total RNA prepared from rat cell lines and from rat liver after experimental manipulation of ACC polypeptide content (see legends to Figures 4 and 5). Shown are representative samples from experiments performed in H4IIE heptoma cells (H4) in control (lane 2) and glucose stimulation (24 h induction, lane 1), in H4IIE cells during control (lane 3) or ciprofibrate stimulation (0.5 mM for 24 h; lane 4), and in H9c2 embryonic cardiomyocytes before (myoblast; lane 6) and after differentiation (myotube; lane 5) in the presence of low serum and insulin. Lanes 7 and 8 show liver RNA prepared from either 48-h-fasted rats (lane 8) or similarly fasted rats after an additional 24 h of feeding with a high carbohydrate diet (lane 7). Twenty μg of total RNA is loaded in each lane. (**A**) was probed with ^{32}P-HACC-2 and (**B**) with ^{32}P-HACC-1. All lanes had equivalent amounts of total RNA loaded as judged by ethidium bromide staining (results not shown).

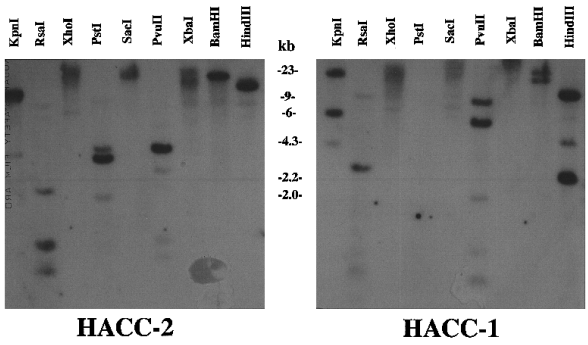

Figure 7 Southern blot analysis of human genomic DNA

Shown are two Southern blots of human genomic DNA (5 μg per lane) after digestion with nine different restriction enzymes. Blots were probed either with ^{32}P-HACC-2 (left) or ^{32}P-HACC-1 (right). The DNA fragment sizes indicated refer to the ethidium bromide-stained DNA ladder used.

from myoblast to myotube, RACC280 is selectively induced. As determined by streptavidin blotting of cell lysates, ACC induction is first detectable on day 4 of differentiation and plateaus between days 6 and 7 (Figure 4, upper panel). With the use of ACC isoenzyme-specific antibodies, this ACC species is identified as the RACC280 isoenzyme (Figure 4, middle panel); no reactivity with an RACC265 species can be observed (Figure 4, bottom panel).

In H4IIE hepatoma cells, glucose and a fibric acid derivative, ciprofibrate, both lead to the selective induction of RACC280 with no change in RACC265 content (Figure 5). As judged by streptavidin–horseradish peroxidase blotting, RACC265 is the predominant form of ACC present in these cells in basal medium (Figure 5A). No change in ACC content is observed over the 48 h of substrate deprivation. However, after 24 h of glucose repletion, there is a marked induction of RACC280 with no change in RACC265. The identification of this band as RACC280 is indicated by its reactivity with the RACC280-specific antibody (Figure 5B). It should be noted that the biotin-containing protein at 120 kDa is unchanged over this time period (Figure 5A); this protein is mitochondrial pyruvate carboxylase, which is solubilized in the presence of SDS [4]. In the H4IIE hepatoma cells, ciprofibrate also leads to a selective induction of RACC280 with no change in RACC265 after 72 h of drug stimulation (Figure 5C). This increase is observed in the digitonin lysate, which contains all of the ACC. No ACC is observed in the membrane fractions remaining after digitonin lysis, although other biotin-containing proteins of mitochondrial origin (e.g. pyruvate carboxylase) are seen in this fraction.

To reinforce the conclusion that HACC-1 is highly homologous to RACC265 sequence, while HACC-2 is highly homologous to RACC280 sequence, we examined the hybridization patterns of HACC-2 and HACC-1 cDNAs with the rat cell RNAs after manipulation of ACC polypeptide expression. As shown in Figure 6, a 9 kb mRNA, recognized by HACC-2, is increased in all of these cell lines after RACC280 induction, but

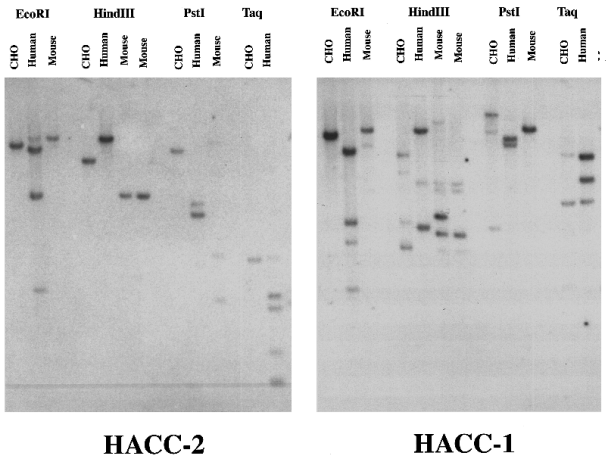

Figure 8 Southern analysis of mouse, hamster and human genomic DNA

Genomic DNA from hamster (Chinese hamster ovary cell line; CHO), mouse and human were digested with *Eco*RI, *Hin*dIII, *Pst*I and *Taq* and then analysed by Southern blotting. The blot on the left, after hybridization with ^{32}P-HACC-2, was stripped and then reprobed with ^{32}P-HACC-1 to allow overlay alignment.

the 9 kb mRNA recognized by HACC-1 is unchanged under all three conditions of induction. Both mRNAs are, however, induced in liver by fasting/refeeding of the intact rat *in vivo*, a nutritional manipulation known to induce both ACC species [2,9].

Given the unique hybridization patterns of these cDNAs in the human cell lines, rat tissues and in rat cell lines, we conclude that the HACC-2 sequence (and overlapping extension sequences in the HACC-β contiguous sequence) represents the homologue of RACC280, namely HACC275, while HACC-1 sequence, corresponding to partial HACC265 sequence [14], represents the homologue of RACC265. These conclusions, based on available sequence information and mRNA distribution, must be regarded as tentative until such time as the full-length cDNA sequence, *in vitro* expression of both isoforms and human enzyme isolation are completed. However, irrespective of the completion of this work in progress, as discussed below, a second and novel human ACC gene has clearly been identified in the present study.

Identification of a second human ACC gene

Inspection of the sequences of HACC265 and the partial sequence of HACC275 suggests strongly that they are derived from different genes and are not related by alternative splicing of a single gene (Figure 2). Southern blot analysis of human genomic DNA digested with nine different restriction enzymes and probed with either HACC-2 or HACC-1 reveals non-overlapping patterns of hybridization, also strongly suggesting a multigene family (Figure 7). Similar analysis of rat genomic DNA by this same technique was also consistent with more than one rat ACC gene (results not shown).

The gene encoding HACC275 was localized by hybridization of DNA fragments derived from two separate somatic cell hybrid panels (Bios and Coriell). Before these studies, Southern blot hybridization of human, mouse and hamster genomic DNA was carried out in order to assure that human DNA sequence could be recognized against a background of either mouse or hamster DNA. As shown in Figure 8, both the HACC265 and HACC275 gene sequences can be recognized against this rodent background

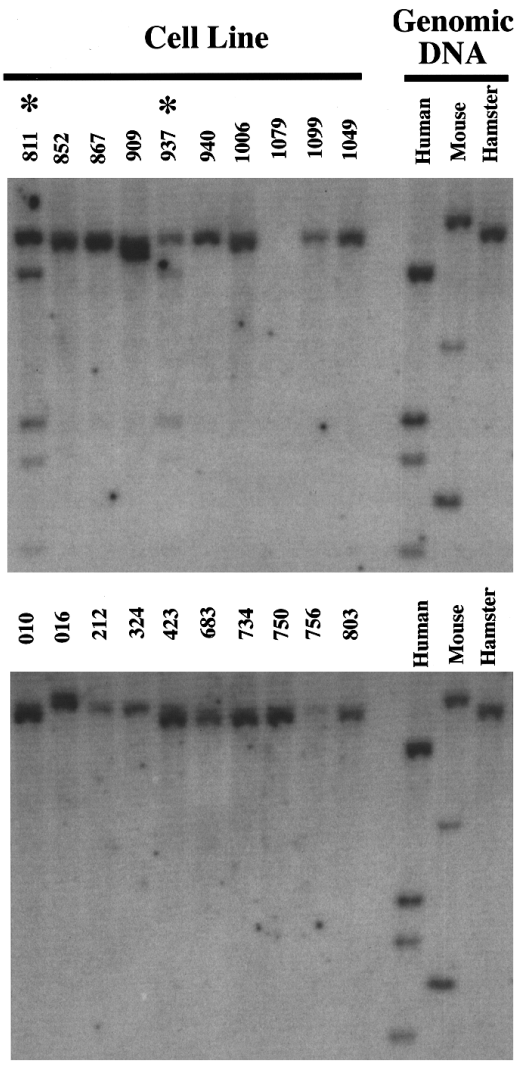

Figure 9 Localization of HACC-1 to chromosome 17

Shown is a Southern blot of somatic cell hybrid genomic DNA (5 μg per lane) from 20 cell lines (Bios) after digestion with *Eco*RI. These membranes were probed with ^{32}P-HACC-1. The hybridization pattern of these DNAs is compared with total genomic DNA from human, mouse and hamster, shown in the right-hand three lanes of each panel. The asterisk indicates that only cell lines 811 and 937 contain hybridizing human genomic sequence.

after digestion with four different restriction enzymes. These results further indicate the unique hybridization patterns of these two cDNAS, not only in the human, but in the mouse and hamster, consistent with a multigene family in these species as well. We previously documented the existence of more than one ACC isoenzyme in cell lines derived from both mouse and hamster [3,4].

Hybridization of the *Eco*RI-digested Bios cell panel DNA with HACC-1 showed recognizable human sequence only in cell lines 811 and 937 (Figure 9). Concordance analysis showed that this corresponds to hybridization with human chromosome 17 sequences, confirming the previous localization of HACC265 to chromosome 17 by *in situ* hybridization [13,14]. An identical chromosomal localization was assigned after analysis of the DNA from the Coriell cell panel (results not shown).

Hybridization of the *Eco*RI-digested Coriell cell panel DNA

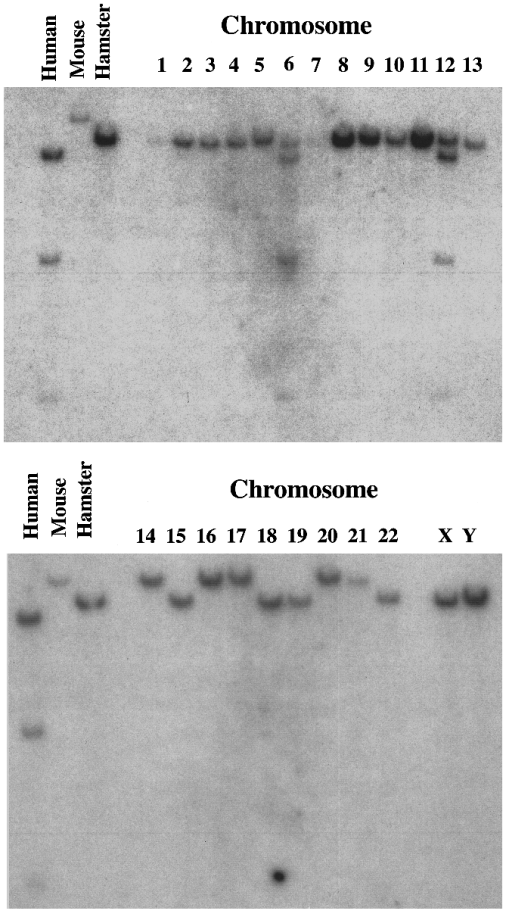

Figure 10 Localization of HACC-2 to chromosome 12

Shown is a Southern blot of somatic cell hybrid genomic DNA (5 μg per lane) from 24 cell lines (Coriell) after digestion with *Eco*RI. These membranes were probed with ^{32}P-HACC-2. The hybridization pattern of these DNAs is compared with total genomic DNA from human, mouse and hamster, shown in the left-hand three lanes of each panel. The lane designations correspond to the predominant or exclusive chromosome represented in each of the cell lines. Hybridizing human genomic DNA is recognized only in the cell line chromosome 12 and chromosome 6; the latter is known to contain a fragment of chromosome 12 (see text).

with HACC-2 showed recognizable human sequence only in cell lines corresponding to chromosomes 12 and 6 (Figure 10). The latter cell line is known to contain a piece of chromosome 12, represented by the mitochondrial aldehyde dehydrogenase gene at the 12q24.2 locus [20,21]. In contrast, the chromosome 12 cell line is not known to contain any chromosome 6 fragments. Analysis of the Bios cell panel DNA with this same probe also assigned this gene to chromosome 12 (results not shown). Thus, the HACC-2 hybridizing gene [termed HACC-β] can be assigned to chromosome 12. In addition, both the 3′- and 5′-extension cDNAs of HACC-2 gave the identical localization pattern on Southern blot analysis of Coriell cell line DNA (results not shown); thus, all the cDNAs in the 2.5 kb HACC-β contiguous sequence appear to be derived from this chromosome 12 gene.

DISCUSSION

These studies indicate that ACC is represented in the human genome by a multigene family of related sequences. In addition, we have presented evidence that a multigene family is also present in the rat, mouse and hamster.

The 265000 Da form of ACC, present in both the rat and human (RACC265 and HACC265, respectively), has previously been extensively characterized [10–14]. The deduced amino acid sequence of HACC265 has been in some dispute, based on results obtained from different laboratories [12,14]. Recently, Abu-Elheiga and co-workers have reported a cDNA sequence for human ACC265 that is different at the 3′-end from that reported earlier by Ha and co-workers in one of our laboratories (K.H.K.) [12,14]. The former suggested the possibility of the earlier sequence being a hybrid sequence between the two different forms of ACC. Subsequent work to clarify this discrepancy, as reported herein by independent cloning of HACC sequences in one of our laboratories (L.A.W.), indicates that this is indeed the case. The sequence from nt 4404 to 7020, reported by Ha et al. [12], is not that of HACC-α (HACC265) as originally reported, but that of HACC-β. Therefore, in comparing the HACC-β sequence to the HACC-α sequence, we have compared our sequences in the studies reported herein to that to Abu-Elheiga, which we now believe to be the correct full-length HACC265 sequence.

Given that full-length ACC sequences, which have regions of absolute nucleotide identity, have been assembled by cDNA library walking and/or PCR extension techniques, it becomes important to ascertain that each piece of overlapping cDNA is aligned in its correct full-length clone. As we have demonstrated in this study, the profiling of select tissue and cell mRNAs may be useful in making the correct contiguous alignment of large cDNAs assembled in this manner. Southern blot hybridization of total genomic DNA and/or DNA derived from somatic cell hybrid panels can also be used to maintain the proper assignments.

The partial-length cDNA contiguous sequence that has been assembled in the present study from HACC-2 appears to correspond to the 275000 Da form of HACC (HACC275) and its rat homologue, RACC280, based on patterns of RNA hybridization. Its gene has been localized to chromosome 12, and, based on hybridization to genomic DNA in one somatic cell hybrid line (Coriell Chromosome 6; HHW484), probably to its distal long arm [20,21]. The mRNA derived from this human gene (and from its rat homologue) appears to be highly expressed both in tissues that are classically either lipogenic (liver) or non-lipogenic (heart, skeletal muscle) [1–3]. All of these tissues are mitochondria-rich and can have high rates of fatty acid oxidation. It is therefore tempting to speculate that this gene product is perhaps most important in the provision of malonyl-CoA, not for fatty acid synthesis, but for the allosteric regulation of fatty acid oxidation through modulation of carnitine palmitoyltransferase I [4,5].

The discovery of isoforms increases the complexity of the understanding of the role and regulation of ACC. One factor contributing to this complexity is the tissue-specific expression of the isoenzymes [1–4]. In rat, both isoforms (RACC265 and RACC280) are expressed in the liver, lactating mammary gland, pancreatic islets and brown adipose tissue. The heart and skeletal muscle predominantly express RACC280 and the brain and white adipose tissue solely express RACC265. In the human, two isoenzymes (HACC265 and HACC275) are expressed in adipose tissue, while only the higher-molecular-mass form is expressed in skeletal muscle. Human breast cancer cell lines have varying phenotypic expression of one or both isoenzymes [7]. The ACC isoenzymes also differ in their enzyme kinetics. RACC280 has increased citrate dependence (higher K_a) and a 2-fold higher K_m for acetyl-CoA [2,22]. In tissues where both isoenzymes are expressed, they are also associated in a complex composed of both types of polypeptide chains [2,3]. It seems possible that

numerous forms of total ACC actually exist, depending on the relative mix of isoenzyme content in each tissue.

In addition to tissue-specific expression, the two isoforms can be co-ordinately or independently regulated *in vivo* [2,23] and *in vitro* in cultured cell lines. *In vivo* in the liver, starvation (where lipogenesis is low) represses the content of both isoenzymes, and high carbohydrate feeding (in which lipogenesis is increased) induces both. However, in heart and skeletal muscle, nutritional state does not alter RACC280 content [2]. Similarly, the induction of diabetes mellitus in the rat by streptozotocin markedly diminishes expression of both isoenzymes in the liver and RACC265 in adipose tissue, but the cellular content of RACC280 in heart and skeletal muscle is unaltered (A. Bianchi and L. A. Witters, unpublished work). In cell lines cultured *in vitro*, shown in the present study, RACC280 expression can be independently regulated by myodifferentiation, glucose and ciprofibrate. These *in vitro* observations provide some model systems where the dissection of the individual roles of ACC isoforms and the mechanisms underlying their unique regulation can be addressed in future studies.

There is adequate precedent for multigene families of metabolic enzymes with selective expression in liver, heart and skeletal muscle. Several of the enzymes of glycogen metabolism are prime examples of such multigene families. Given this precedent, it is possible that the HACC275/RACC280 expressed in liver may not necessarily be the product of the same gene as the enzyme expressed in heart or skeletal muscle. In order to avoid confusion with the designation of ACC isoenzymes based solely on molecular mass, we propose a nomenclature change. We propose that the 265000 Da form, as extensively characterized in the rat and human and represented by an HACC265 sequence reported in a HepG2 clone [14], be referred to as ACC-alpha (α) and that the larger-molecular-mass form, that is the subject of the present report (275000 Da in the human; 280000 in the rat), be referred to as ACC-beta (β). Human species are thus designated as HACC-α and HACC-β. Such a designation leaves open the possibility that there are other as yet unidentified ACC genes encoding enzymes of similar/identical molecular masses. Indeed, by *in situ* chromosomal hybridization, there are conflicting reports as to the localization of the human gene encoding for the 265000 Da enzyme. One group has reported localization to 17q12, using an HACC265 PCR-derived genomic probe [14], while another has reported localization to 17q21 using an RACC265 cDNA [13]. This suggests the possibility of three HACC genes, two on chromosome 12 and one on chromosome 17.

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