Cloning and characterization of the 5′ end and promoter region of the chicken acetyl-CoA carboxylase gene

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Acetyl-CoA carboxylase is a rate-limiting enzyme in the biogenesis of long-chain fatty acids. In the present study, the 5′ end and flanking region of the acetyl-CoA carboxylase (ACC) gene was analysed in the chicken. A genomic clone was isolated containing the first three exons, the third one containing the ATG codon. Using nuclease-mapping experiments and primer-extension analyses, the transcription-initiation site was located 153 nucleotides upstream of the ATG codon. In contrast with rat ACC gene expression, reverse transcriptase PCR analysis performed on chicken liver mRNA did not reveal alternative splicing in the 5′-untranslated region of these messengers. The promoter region is very G+C rich, and contains no TATA or CAAT boxes. Analysis by transient transfection in a human hepatoma cell line (HepG2) demonstrates that the promoter activity requires the presence of symmetrical sequences located upstream of the GC boxes. Transcription of this gene is found to be controlled by tri-iodothyronine in HepG2 cells, but the sequence responsible for the tri-iodothyronine response is not the consensus tri-iodothyronine-responsive element localized in the promoter. These results bring new insights to the regulation of the chicken ACC gene, which differs from that of the rat.

INTRODUCTION

Acetyl-CoA carboxylase [acetyl-CoA–carbon dioxide ligase (ADP-forming) EC 6.4.1.2] (ACC) plays a central role in the *de novo* synthesis of long-chain fatty acids in mammals and birds, catalysing the conversion of acetyl-CoA into malonyl-CoA. Malonyl-CoA molecules are then condensed with one molecule of acetyl-CoA by fatty acid synthase to produce saturated fatty acids. The enzyme has been isolated from avian liver [1], rat liver [2], mammalian adipose tissues and mammary gland [3], which are all lipogenic tissues enriched in ACC. The hepatic enzyme is a polymer (molecular mass $\geqslant$ 10 MDa) composed of 40 or more identical subunits [4] of molecular mass 265 kDa [5,6], each subunit containing one biotin prosthetic group, biotin carboxylase, transcarboxylase catalytic activities and a citrate allosteric site.

ACC is subject to short- and long-term regulation. Short-term regulation can be achieved by allosteric modifications mediated by different substances. Citrate activation of crude or purified carboxylase is accompanied by polymerization of the enzyme [7,8]. Conversely, long-chain acyl-CoA esters and malonyl-CoA are very potent inhibitors of ACC and appear to oppose the activation by citrate [9,10]. This inhibition results in depolymerization of the filament. However, the main process of acute regulation *in vivo* depends on protein phosphorylation. These covalent modifications are very complex, being under the control of various metabolites. In cultured hepatoma cells, insulin rapidly leads to the activation of ACC. This activation is accompanied by inhibition of 5′-AMP-activated protein kinase [11], which is described as the major regulator of ACC [12–14]. In contrast, glucagon treatment of the cells produces further inactivation of the enzyme as the result of phosphorylation of ACC by the 5′-AMP-activated protein kinase [15]. The purified enzyme can be phosphorylated *in vitro* by at least seven distinct protein kinases [16–20] at up to seven serine residues which are now being defined from amino acid and cDNA sequencing.

Long-term regulation involves changes in the protein concentration. This concentration is mainly dependent on the synthesis rate which is itself highly correlated with mRNA concentration [21]. Several studies conducted on rat ACC gene have revealed that the transcriptional regulation of this gene is very complex. In liver and mammary gland, two spatially distinct promoters (PI and PII) generate two primary transcripts, which are differently spliced to produce five mRNAs differing in the composition of their 5′-untranslated regions [22–24]. In humans, two mRNAs differing in their 5′-untranslated region are also presumably generated by two promoters [25]. In this species a recent study suggests the presence of two genes, giving rise to two mRNAs differing in their 3′ end [26]. These two mRNAs exhibit tissue-specific distribution and are proposed to generate two enzyme isoforms [26].

In rat, the transcriptional activity of these promoters *in vivo* has been widely investigated. It is under the control of different physiological conditions. In rats fed *ad libitum*, both promoters are expressed at the basal level, i.e. PI in white adipose tissue and PII in the liver. Starvation leads to the virtual disappearance of transcriptional products from these promoters. When fatty acid synthesis is stimulated by refeeding a fat-free diet to starved animals, both PI and PII are activated in the liver. The activation of both of these promoters in the liver occurs in the absence of insulin. However, administration of insulin to streptozotocin-diabetic rats further activates both promoters, regardless of the type of diet given to the starved animals [27]. In contrast, only PI – and not PII – responds to nutritional induction in white adipose tissue [28]. It has been shown recently that the CCAAT/enhancer-binding protein (C/EBP) may be one of the factors

ACC, acetyl-CoA carboxylase; CAT, chloramphenicol acetyltransferase; RT-PCR, reverse transcriptase PCR; SV40, simian virus 40; T_3, tri-iodothyronine; TRE, tri-iodothyronine-responsive element; C/EBP, CCAAT/enhancer-binding protein.

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The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide sequence Databases under the accession number X77240.

that is responsible for the activation of promoter PI under lipogenic conditions [29]. At present, the mechanisms involved in the regulation of chicken ACC gene expression are only partly elucidated. Tri-iodothyronine (T_3) enhances the level of chicken ACC mRNA to about the same extent as the increase in ACC activity brought about by this hormone [30]. It has been suggested that protein phosphorylation is required for the stimulation of ACC gene transcription by T_3 [31].

In order to improve our understanding of the regulation of ACC gene expression, we isolated and characterized a genomic fragment corresponding to the 5′ region of this gene. Its sequence was then compared with the cDNA previously described by Takai et al. [5]. The transcription-start site was localized from primer-extension analysis and nuclease-mapping experiments. A promoter activity within the 2 kb upstream region was identified by transient transfection experiments in the human HepG2 hepatoma cell line [32,33]. Further investigations allowed the localization of sequences important for promoting transcription. In addition, T_3 was shown to modulate the promoter activity in the HepG2 cells.

EXPERIMENTAL

Screening of genomic library and clone amplification

A ^{32}P-labelled oligonucleotide (PE1; Figure 1B) encompassing the ATG codon of the chicken ACC cDNA sequence [5] was used as a probe for screening a chicken genomic library kindly provided by Dr. Chambon. One positive clone was selected. Large-scale preparation of the genomic clone was carried out using a modification of the method of Sambrook et al. [34]. The phage DNA was not precipitated but pelleted by centrifugation at 25000 ***g*** for 3 h.

Plasmid preparation and analysis

The genomic DNA clone was digested by *Eco*RI (Boehringer-Mannheim), and fragments corresponding to the inserts were subcloned into the pBluescript KS$^+$ plasmid (Stratagene). These subclones were then amplified and purified by ultracentrifugation in a CsCl gradient [34]. Insert sequences were determined on both strands by the dideoxynucleotide-chain-termination procedure [35] using sequenase (USB) and T3 and T7 universal primers. In addition, specific oligonucleotide primers were used for internal sequences of the *Xho*I–*Bam*HI fragment. Sequence analyses were performed using the Bisance package [36]. The subclones were also analysed by Southern blot [37] with ^{32}P-labelled oligonucleotide, localized in the 5′-non-coding region of the published cDNA.

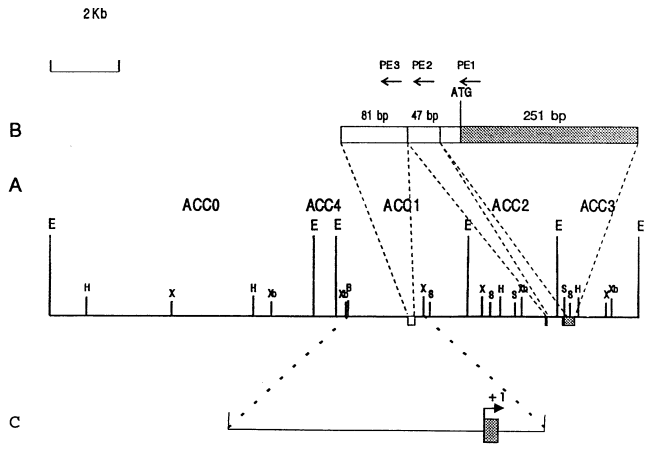

Figure 1 Structural organization of genomic DNA and cDNA around the 5′ end of the chicken ACC gene

(**A**) Restriction map of the 18 kb λACC genomic clone, E, *Eco*RI; B, *Bam*HI; X, *Xho*I; Xb, *Xba*I; H, *Hin*dIII; S, *Sac*I. ACC0 to ACC4 are the five subclones obtained after subcloning of *Eco*RI fragments in pBluescript KS$^+$ plasmid. (**B**) cDNA map showing region surrounding the transcription-start site including the untranslated (open boxes) and translated (shaded box) regions of the first three exons. The arrows indicate the oligonucleotide (PE1) used for screening the chicken genomic library, and PE2 and PE3 used in genomic clone analyses. (**C**) The *Bam*HI–*Xho*I fragment used in the promoter studies, including the first exon (shaded box).

RNA extraction

Total RNA was purified from the liver of 15-day-old chickens from a line selected for high abdominal fat pad [38]. Animals were deprived of food for 48 h and then re-fed a high-carbohydrate diet for 36 h in order to stimulate lipogenesis [5,39,40]. Total RNA was purified by the method of Auffray and Rougeon [41].

RNA analysis

Nuclease mapping

Double-stranded genomic probes were 5′-^{32}P-labelled and hybridized with 100 μg of chicken liver total RNA or yeast tRNA as described by Berk [42]. After hybridization, digestion was performed with 100 units of S1 nuclease or 100 units of mung bean nuclease at 37 °C for 30 min. Digestion was stopped by adding 0.4 M NaOH and the reaction mixture was incubated at 45 °C for 1 h. After precipitation and denaturation, the nucleic acids were analysed on a denaturing polyacrylamide/urea sequencing gel and then autoradiographed.

Primer-extension analysis

Primer-extension analysis was performed as described by Boorstein and Craig [43]. Liver total RNA (30 μg) was hybridized to a 21-mer 5′-^{32}P-labelled oligonucleotide in 80% formamide solution overnight at the oligonucleotide melting temperature. Primer extension was performed using avian myeloblastoma virus reverse transcriptase (Stratagene). The extension products were analysed by denaturing sequence gel electrophoresis and autoradiography.

Reverse transcriptase PCR (RT-PCR)

RT-PCR was performed using the RNA PCR kit (Perkin–Elmer–Cetus Company) using rTth reverse transcriptase. Total RNA (250 ng) was submitted, after a 'hot start' (95 °C for 1 min), to 35 cycles of PCR (95 °C for 1 min; 60 °C for 1 min). The amplified fragments were analysed by electrophoresis and ethidium bromide staining.

Analysis of promoter activity

Bacterial chloramphenicol acetyltransferase (CAT) vector constructs

The pJFCAT1 vector containing the bacterial CAT gene was kindly provided by J. L. Fridovich-Keil [44]. This promoterless/enhancerless basic vector contains a polylinker upstream from the coding regin of the CAT gene, which is preceded by a trimer cassette of the simian virus 40 (SV40) major late polyadenylation site in order to block any plasmid-initiated read-through expression of CAT. Different parts of the ACC gene sequences upstream of the ATG codon were cloned into the pJFCAT1

polylinker. The resulting constructs were amplified and purified by two successive ultracentrifugations with CsCl gradients [34].

Cell culture and transfection

Samples of the confluent human HepG2 hepatoblastoma cell line [32,33] were cultured in a 75 cm² flask in William's E medium (Gibco/BRL) supplemented with 10% foetal calf serum and then passaged 1:4 into 25 cm² flasks. The next day each flask was transfected for 8 h with 2 μg of CAT plasmid, 2 μg of pSV40LacZ (Pharmacia) and 2.25 μg of carrier DNA (salmon sperm DNA; Sigma) using the calcium phosphate coprecipitation method [45]. After 48 h of culture, the cells were harvested and either stored at -20 °C or analysed directly after lysis by three freeze–thaw cycles.

Assays of CAT and β-galactosidase

β-Galactosidase activity was assessed by the modified method of Sanes et al. [46]. CAT activity was analysed broadly as described by Gorman et al. [47]. In brief, cell lysates corresponding to 1 m-unit of β-galactosidase activity were incubated in the presence of 0.04 μCi of [^{14}C]chloramphenicol (Amersham) and 4 mM acetyl-CoA (Boehringer-Mannheim). Reaction products were then analysed on silica-gel TLC plates. The radioactive spots were scraped off and counted by liquid scintillation. The β-galactosidase-normalized CAT activities are expressed as percentages of the highest value obtained with pXBCAT (the longest construct) taken as 1. Statistical tests were performed using the SAS statistical package [48].

RESULTS

Isolation and restriction map of the 5′ end of the chicken ACC gene

An oligonucleotide (PE1; Figure 1B), encompassing the ATG codon of the chicken ACC cDNA sequence [5], was used as a probe to screen the chicken genomic library. One 18 kb genomic clone, λACC, was isolated and characterized. The results are summarized in Figure 1. Southern-blot and partial-sequencing analyses enabled us to establish a partial restriction map (Figure 1A). The five *Eco*RI fragments, designated ACC0 to ACC4 with respective sizes 8, 4.1, 2.9, 2.3 and 0.7 kb (Figure 1A) were subcloned in pBluescript KS$^+$ plasmid.

The oligonucleotide PE1 hybridized to the ACC3 subclone. Sequence analysis led to the localization of a 251 bp exon which contains the first 226 bp of the coding region as well as 25 bp of the upstream non-coding region. An oligonucleotide named PE2 (Figure 1B), which is located upstream of this exon in the cDNA sequence, was used as a probe for Southern-blot analysis (results not shown). PE2 was hybridized to ACC2 (Figure 1B). A 47 bp exon sequence homologous to the published cDNA was characterized within this subclone. Finally, the first 81 bases of the published cDNA were localized in ACC1 with the PE3 oligonucleotide (Figure 1B) selected upstream of the 47 bp exon.

Mapping the transcription-initiation site

The transcription-initiation site of the ACC gene was determined by nuclease mapping and primer-extension analysis, using chicken liver total RNA as described above.

Nuclease mapping experiments were carried out with *Hinc*II–*Sau*I and *Eco*RI–*Nde*I 5′-^{32}P-end-labelled double-stranded DNA probes (Figure 2A). These two probes were annealed to chicken liver RNA (lanes 1, 2, 5 and 6) and then

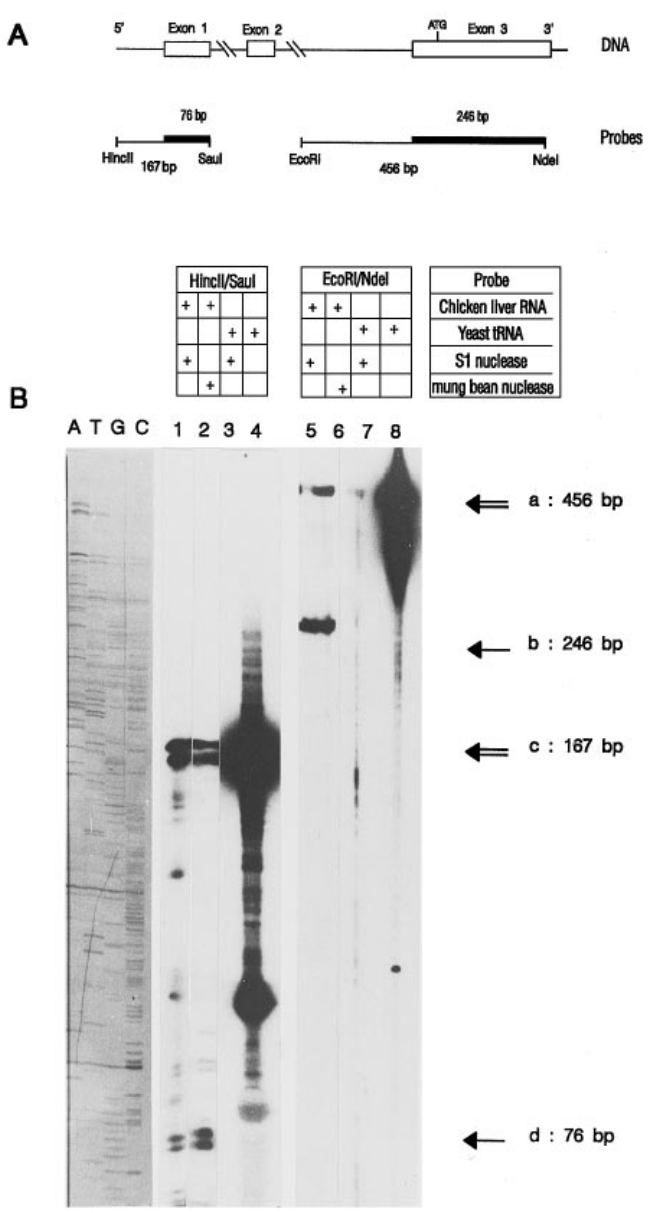

Figure 2 Nuclease assay analyses of chicken liver ACC mRNA

(**A**) *Hinc*II–*Sau*I and *Eco*RI–*Nde*I fragments used as probes. Portions corresponding to exons are indicated as black bars. (**B**) *Hinc*II–*Sau*I (lanes 1–4) and *Eco*RI–*Nde*I (lanes 5–8) probes were annealed with 100 μg of chicken liver total RNA extracted from re-fed animals (lanes 1, 2, 5 and 6) or with 100 μg of yeast tRNA (lanes 3, 4, 7 and 8), then digested with 300 units of S1 nuclease (lanes 1, 3, 5 and 7), or 300 units of mung bean nuclease (lanes 2 and 6), or left undigested (lanes 4 and 8). b and d, protected fragments; a and c, undigested probes; ATGC, size markers corresponding to a DNA sequence.

treated with S1 nuclease (lanes 1, 3, 5 and 7) or mung bean nuclease (lanes 2 and 6). The results are shown in Figure 2(B). A 246 bp nuclease-protected fragment (band b) was observed (lane 6), as expected from sequence analysis. The *Hinc*II–*Sau*I probe, which contains the 76 bp upstream of the 81 bp previously assigned to the first exon, was used to map this exon (lanes 1 to 4). In fact, chicken liver RNA gives rise to a 76 bp protected fragment (band d). The result was even more pronounced when mung bean nuclease was used (lane 2), suggesting that this enzyme has a more specific action than S1 nuclease; this is

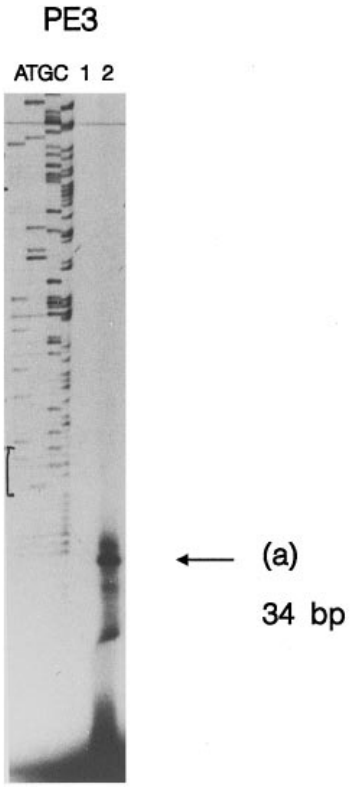

Figure 3 Primer-extension analysis of chicken liver ACC mRNA

Primer-extension experiment was performed with oligonucleotide PE3. Primer was applied to 30 μg of liver total RNA extracted from re-fed chickens (lane 2) or yeast tRNA (lane 1). The expected primer-extension product is indicated as a. ATGC, size markers corresponding to a DNA sequence.

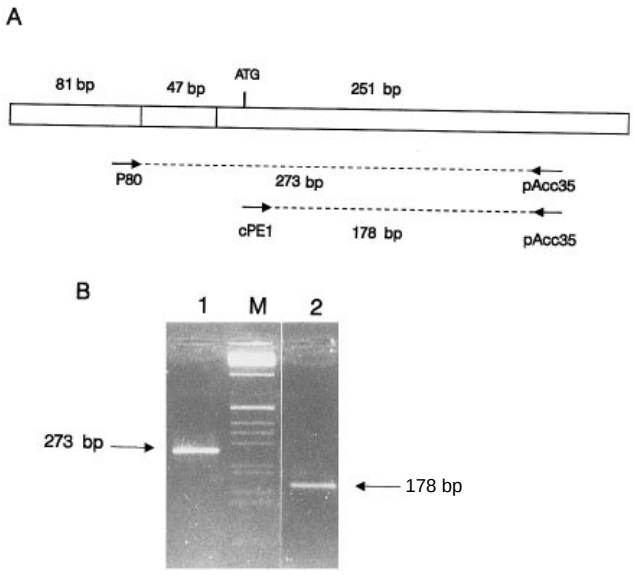

Figure 4 RT-PCR analysis of chicken liver ACC mRNA

(**A**) Primer positions on the ACC mRNA, with distances indicated. (**B**) Amplifications were performed with primers p80 and pACC35 (lane 1) or cPE1 and pACC35 (lane 2). PCR products are indicated by arrows and their size given in bp. Lane M is a size marker (DNA 1 kb; Gibco/BRL).

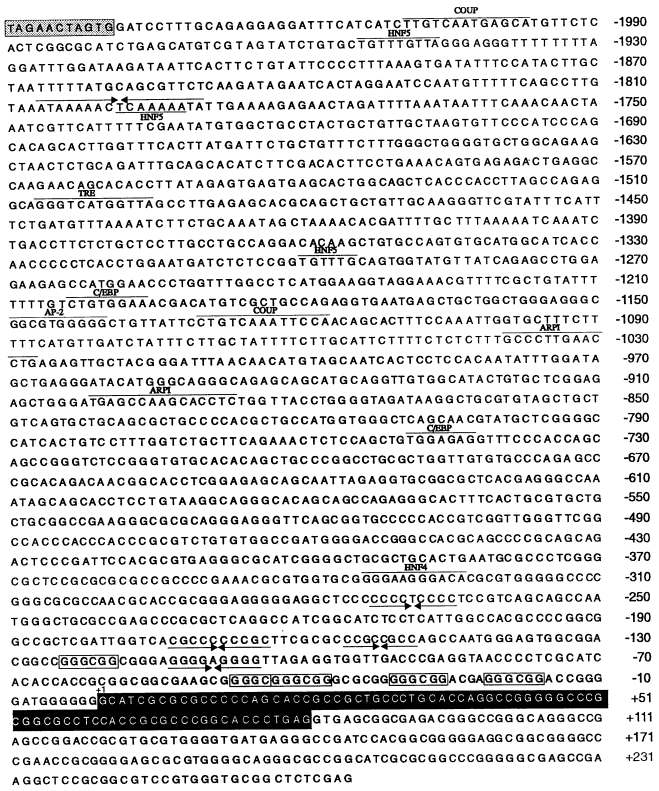

Figure 5 Nucleotide sequence of the 5′-flanking region of the chicken ACC gene

The transcription-initiation site is at +1. The exon 1 sequence is in the black box. The open boxes show the putative Sp1-binding sites. Arrows indicate symmetrical motifs. Potential *cis*-regulating elements on the coding strand are overlined, and *cis*-regulating elements on the non-coding strand are underlined. The sequence in the shaded box is from the cloning vector (pBluescript). The sequence has been deposited with the EMBL, GenBank and DDBJ Nucleotide sequence Databases under accession number X77240.

consistent with an assumed size of 81 bp for this exon. In order to confirm this result and to localize precisely the cap site, we performed a primer-extension experiment using PE3 oligonucleotide, located in the more upstream exon (Figure 1B).

This experiment gave rise to a major 34 bp extension product (Figure 3, band a). Hence, the primer-extension experiment as well as the nuclease-protection assays provide strong evidence that the cap site of chicken ACC mRNA should be located 153 nt upstream of the first translated codon.

Chicken ACC mRNA splicing analysis by RT-PCR

A strong similarity (about 80 %) is observed betewen the ACC cDNA sequence in the rat [6] and chicken [5]. Moreover, the 5′ portions of the gene greatly resemble each other in these two species. The sizes of rat exons 2, 4 and 5 [24] are very similar to those of chicken exons 1, 2 and 3 respectively.

Five mRNAs are observed in the rat, expressed from two promoters by alternative splicing [22–23,49]. To check whether different types of ACC mRNA are also present in chicken liver, we performed RT-PCR experiments on total RNA extracted from the liver of 15-day-old chickens in which lipogenesis was stimulated by diet. First, RNA was reverse-transcribed from pACC35 oligonucleotide (Figure 4A) and then PCR-amplified with the same primer and either cPE1 (complementary to PE1 as

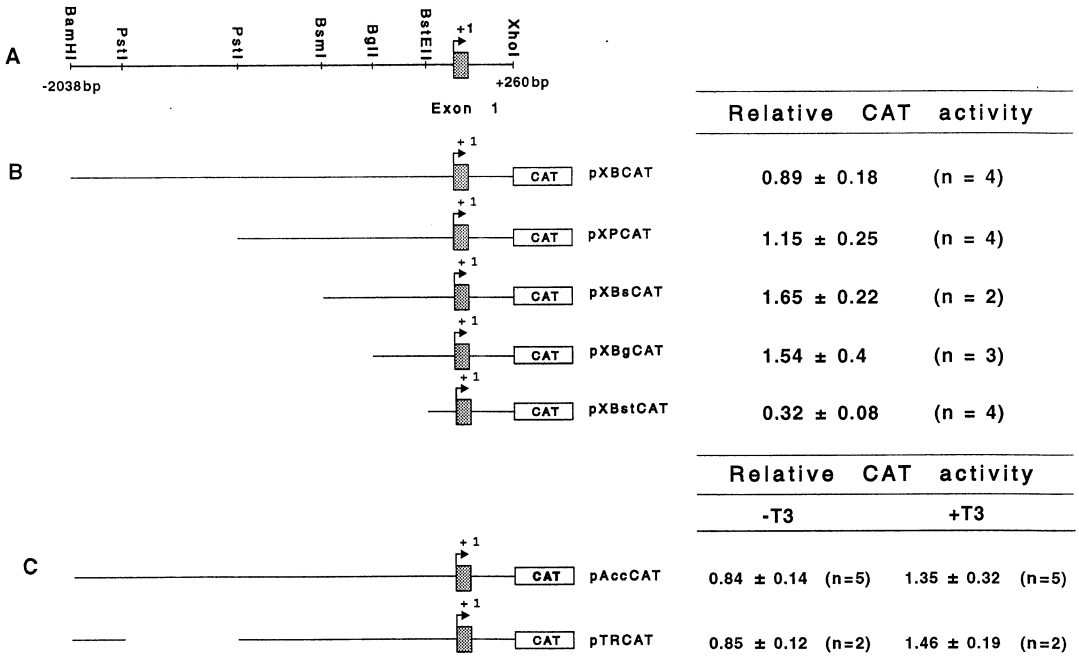

Figure 6 CAT analysis of promoter activity

(**A**) DNA fragment studied. (**B**) On the left side of the diagram, variously sized gene fragments generated from 2.3 kb ACC gene ligated to the promoterless/enhancerless CAT basic vector (pJFCAT1) are shown. The open box represents the CAT gene and the shaded box the first exon. On the right hand side is shown CAT activities observed in HepG2 cells transfected with constructs. CAT activity was normalized to β-galactosidase activity. Results are expressed relative to CAT activity. The higher value obtained with the pXBCAT consctuct was used as standard (value fixed arbitrarily at 1) and results are means $\pm$ S.D., with *n* representing the number of independent transfections carried out in each case. In each experiment, duplicate plates were transfected. (**C**) Relative promoter activity of the ACCCAT chimaeric plasmids in the presence or absence of T_3. For all the experiments, the relative CAT activities are expressed relative to the higher value obtained with plasmid pXBCAT in the absence of T_3 (as described above).

positive control) or p80 oligonucleotide localized in the first 81 bp exon (Figure 4A). As expected, a band at 178 bp was detected (Figure 4B, lane 2) in the positive control. The RT-PCR performed with primers p80 and pAC35 enabled the amplification of a unique 273 bp band (Figure 4B, lane 1), suggesting that, in chicken liver, all the ACC mRNA contains the first three exons. No alternative splicing was detected in adult chicken muscle, uropygial gland, heart, spleen, adipose tissues or liver (results not shown).

Analysis of the 5′-flanking region of the ACC gene

The nucleotide sequence of the 5′-flanking region of the ACC gene up to nucleotide −2038 is presented in Figure 5. No consensus TATA or CAAT boxes were detected upstream of the transcription-start site. The region between positions −310 and +1 is 80% G+C rich and contains five GC boxes known as potential binding sites for the nuclear transcription factor Sp1 [50] at positions −16, −26, −38, −42 and −119. Two of them are embedded at positions −38 and −42. This region displays the typical features of a housekeeping promoter [51–53]. The whole region contains several potential binding sites for transcription factors such as HNF4, HNF5, ARP1 and C/EBP. A potential T_3-responsive element (TRE) is located at −1495.

Analysis of chicken ACC promoter activity

Identification of sequences required for promoter activity

Promoter activity of the 5′-flanking region of the ACC gene was investigated by transient transfection assays in the human hepatoma cell line HepG2 using the CAT gene as reporter. Different fragments corresponding to series of deletions in this 5′ region (Figure 6B) were subcloned in the pJFCAT1 vector. Noticeable CAT activity was expressed in cells transfected by the largest fragment (pXBCAT; Figure 6B), proving its status of promoter. Deletion of the region −837 to −377 (pXBsCAT; Figure 6B) increases CAT activity by about 40% (Figure 6B). CAT activities remain relatively constant until deletion of bases up to −191 (pXBstCAT). Further deletion of the 107 bp between −191 and −84 leads to a dramatic decrease (about 80%) in CAT activity (Figure 6B). Examination of the primary structure in this region (Figure 5) reveals the existence of three symmetrical sequences: CGCCCCCCGC (−173 to −164), CCGCCGCC (−156 to −149) and GGGGAGGGG (−113 to −105).

Effects of T_3 on ACC promoter activity

The sequence analyses enable us to locate a putative TRE-binding site in chicken ACC promoter (−1506, −1495; Figure 5). In order to test the functionality of this sequence, HepG2 cells were transfected with either pXBCAT (−2038/+260) or pTRCAT, in which the −1618/−837 segment was deleted so as to omit the TRE-like sequence (Figure 6C). Transfected cells were cultured with or without T_3 (1 μg/ml), and the CAT activity was assayed 2 days after transfection. The presence of T_3 caused a 60% increase in CAT activity in the cells transfected with the largest construct (pXBCAT). Contrary to expectations, the deleted construct (pTRCAT) keeps the ability to be stimulated by T_3 (about 70%). The same results were observed in the LMH cell line [54] (results not shown). These results strongly suggest that the removed region does not correspond to an actual TRE-

binding site. The region responsible for T_3 stimulation is localized at a site other than this putative TRE site.

DISCUSSION

To study the molecular basis of the transcriptional control of chicken ACC gene, we isolated a chicken genomic ACC fragment (18 kb) which encodes the first three exons (1, 2 and 3), introns 1 and 2 and part of intron 3, and 11 kb upstream from the first exon.

The size of the 5′-untranslated region was determined to be 153 bp upstream of the ATG codon by primer-extension experiments and nuclease digestion. When primer-extension experiments were performed with primer PE1 (localized in exon 3) and primer PE2 (localized in exon 2), in addition to the expected band, some other longer bands were observed (results not shown). These upper bands could reflect non-specific elongation or the presence of multiple species of ACC mRNA in chicken liver, transcribed upstream and coding for an additional exon. The structure of rat [22–23] and human [25] liver mRNA supports the latter hypothesis, since in these species two classes of primary transcripts are generated by two promoters PI and PII [25,49].

Furthermore, a comparison between the 5′ region of rat and chicken ACC genes reveals structural similarity in the region downstream of the PII rat promoter [49]. If we assume that this similarity exists upstream as well, the upper extended bands become compatible with the presence of an additional exon about 240 bp in length, similar to exon 1 described in the rat and human ACC genes [25,49].

Primer PE1 (Figure 1B), which is located in the chicken exon containing the ATG codon (exon 3), was able to reveal all the ACC mRNA speces present in chicken liver. Therefore the bands in the higher-molecular-mass range were able to match the rat class-1 ACC mRNA. PE2, which is localized in exon 2 (Figure 1B), was also able to reveal the different species of ACC mRNA, and a situation similar to the case of PE1 may be proposed. However, primer PE3 can only reveal mRNAs such as the rat class-2 mRNA species. Indeed, primer PE3 gave rise to a single major band in our experiments.

In conclusion, the present experiments reveal the location of the transcription-initiation site to be 153 bp upstream from the ATG codon. It is also proposed that there are several ACC mRNA species differing in their 5′-untranslated regions. Moreover, class-2 ACC mRNAs are subject to differential splicing in the rat and mouse [25]. They result from the loss of the 47 bp exon or the appearance in the rat of a new 61 bp exon [24]. Analyses of such splicings were performed by RT-PCR on chicken liver total RNA. The p80 and pACC35 primers should reveal all ACC mRNA species assumed to be similar to the class-2 rat mRNA. However, only a single 273 bp band was detected with these two primers. The same result was obtained with RT-PCR experiments performed in the presence of [α-^{32}P]dCTP, which increased the sensitivity of detection (results not shown). The absence of splicing has also been observed in other tissues, suggesting the same transcriptional regulation as that observed in liver. Thus we may conclude that the splicings observed in different class-2 rat ACC mRNAs are not present in chicken liver or other tissues. The same situation occurs in humans [25].

Sequencing and computer analysis of the region localized upstream of the transcription-initiation site revealed a very high G+C content – a potential binding site for nuclear factor Sp1 – but no apparent TATA or CAAT box sequences. This structure is frequently observed in housekeeping gene promoters [51–53]. This kind of promoter controls RNA synthesis at relatively low levels, with little tissue specificity.

The same structure has been described for the PII promoter in rat [49]. In agreement with the structure, functional analysis of chicken ACC promoter indicates that it is active in HepG2 [32,33] and LMH [54] – two cell lines isolated from liver tissues – as well as in QT6, a quail fibroblastic cell line [55] (results not shown). Besides their resemblance to the housekeeping promoter, sequences for liver-specific nuclear factors or factors involved in cell differentiation (e.g. HNF4, HNF5 and C/EBP) [56] are unexpectedly located in the 5′-flanking region of the ACC gene. Other binding sites for nuclear factors that are involved in gene response to various hormones are also located in the *Bam*HI–*Xho*I region. The presence of two potential sequences for ARP1 binding, a novel member of the steroid receptor superfamily [57] and a potential thyroid-hormone-responsive element (TRE), suggests that the expression of the chicken ACC gene could be modulated by hormones.

Indeed, a 60 % enhancement of chicken ACC promoter activity was observed in HepG2 cells cultured with T_3. This observation agrees with the increased accumulation (5–7-fold) of ACC protein and mRNA observed in chick embryo hepatocytes cultured in the presence of T_3 [31]. Our results suggest that part of the increase in the rate of synthesis of ACC mRNA induced by T_3 is due to the regulation of gene transcription. Some of the increase may also be due to mRNA stabilization by T_3 as observed in rat livers [58]. However, the present transfection experiments with constructs lacking the putative TRE sequence show that this precise region is not directly involved in T_3 stimulation of the ACC gene. This fits with the hypothesis advanced by Swierczysnki et al. [31], whereby T_3 stimulation of the ACC gene implies the phosphorylation of a protein, with T_3 behaving as a *cis*-acting factor.

Functional analysis of chicken ACC promoter points to two important sequences: a negative element located between −837 and −377, and a positive element between −191 and −84. When the negative fragment is deleted, promoter activity is increased by almost 40 %. In this fragment, only a putative C/EBP-binding site was identified (Figure 5) that could be responsible for hepatic regulation of the gene. The most important sequence involved in ACC gene transcription seems to be the positive element between −191 and −84. When this fragment is deleted (pXBstCAT; Figure 6), promoter activity decreases by about 80 %. This loss of promoter activity occurs even though the construct still contains four GC boxes, which are putative sequences for the Sp1 nuclear-factor-binding site. The 107 bp positive element is composed of three symmetrical sequences (Figure 5). Similar results have been reported for the rat ACC promoter PII, where the deletion of 115 bp in a region containing three 13-mer sequences, localized just upstream of the CG boxes, leads to a decrease of almost 94 % in promoter activity [49]. Further studies are necessary to determine the role of these symmetrical sequences in the regulation of chicken ACC gene expression.

In conclusion, a housekeeping-like promoter has been isolated in the present study which actually regulates chicken ACC gene expression, in contrast with other housekeeping promoters that are more involved in the basal expression of genes than in regulation. The regulation of chicken ACC mRNA expression appears to be different from that observed in the rat; this is because no alternative splicing was detected in chicken ACC mRNA and the three different rat ACC mRNAs under the control of the PII promoter were not observed in either chicken or human liver [25].

However, some results suggest that the presence of an additional upstream promoter is possible. Further studies are necessary to test this hypothesis. The identification and study of

the chicken ACC gene provide very useful data for the understanding of the regulation and action of ACC in lipogenesis in birds.

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