

Organization of the 5' region of the rat ATP citrate lyase gene

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A genomic clone, encompassing the 5' flanking region and the first seven exons of rat ATP citrate lyase gene, was isolated from a rat genomic library and sequenced. Primer-extension analysis showed that mRNA is transcribed at 4407 nucleotides upstream from the translation start site. Primer-extension analysis and sequencing of ATP citrate lyase cDNA amplified by PCR showed that the promoter used for transcription is identical in mammary gland, lung, liver, brain and kidney. Southern-blot analysis showed that the ATP citrate lyase gene exists as a single copy.

The 5' flanking region contains several consensus sequences defined as promoter elements. These include a CAAT box and Sp1-binding sites. However, a TATA box lacks this promoter. The expression of the chloramphenicol acetyltransferase gene was induced by the 5' flanking region (–2370 to –1) in the CHO cell line. The 5' flanking region also contains several sequence elements that may be involved in the transcriptional regulation of the gene.

INTRODUCTION

Citrate transported from mitochondria is cleaved into acetyl-CoA and oxaloacetate by ATP citrate lyase for several important biosynthetic pathways, including lipogenesis and cholesterol synthesis [1]. In mammals, the activity of ATP citrate lyase is regulated by diet and hormones in a manner similar to that of other lipogenic enzymes. Generally, this regulation of enzyme activity according to the state of *de novo* lipogenesis is considered to be due to alteration of the rate of enzyme biosynthesis [2]. We reported previously that changes in ATP citrate lyase concentration correlate with changes in its mRNA concentration and the transcription rate [3]. These findings strongly suggest that ATP citrate lyase is regulated at the level of transcription.

We have now determined the structure of the ATP citrate lyase genomic promoter, as an initial step toward understanding how the regulation of this enzyme occurs at the level of transcription.

MATERIALS AND METHODS

Molecular technique

Standard procedures were used to screen phage libraries, DNA labelling, isolation of genomic DNA and total RNA and to subclone genomic DNA fragments [4]. Double-stranded genomic DNAs subcloned in the pGEM4Z vector were sequenced using [α -³⁵S]thio[dATP] and the T7 Sequencing kit (Pharmacia) according to the manufacturer's instructions. Genomic DNA concentration was measured to determine the copy number of the ATP citrate lyase gene, using Hoechst 33258 as a dye [5]. The three primers were synthesized by an Applied Biosystems 381 A DNA synthesizer and purified by denaturing PAGE [4]. The sequences of the oligonucleotides are as follows:

rACL1: GGCTGCAGAGGGACCTGGTT (reverse strand) at position +4407 to +4388;

rACL2: AAAGCCCGAGTGTGGCTCTCTGTCGTAAGC (reverse strand) at positions +67 to +37;

rACL3: GCTGCAGGCGGCTGGAGCGATCCGGGGA at position –8 to +20.

Primer-extension analysis

A 31-nucleotide oligomer, rACL2, was end-labelled with [γ -³²P]-ATP by T4 polynucleotide kinase. Total RNA was isolated from three sources: livers from young rats fed on a high-carbohydrate diet for 2 days after weaning; livers from adult rats fed on a high-carbohydrate diet for 12 h after food deprivation for 2 days; mammary glands from lactating rats. Total RNA (50 μ g) was dissolved in 100 μ l of buffer composed of 80% formamide, 40 mM Pipes, pH 6.4, and 1 mM EDTA at 90 °C and then cooled on ice. An end-labelled primer was added and hybridized at 30 °C for 15 h. Annealed primer/RNA was precipitated after the addition of 200 μ l of distilled water and 600 μ l of ethanol and resuspended in 40 μ l of 50 mM Tris/HCl, pH 8.2, containing 100 mM NaCl, 10 mM dithiothreitol (DTT), 0.5 mM each of dATP, dCTP, dGTP, dTTP and 35 units of AMV reverse transcriptase. After incubation at 40 °C for 60 min, the reaction was stopped by adding 60 μ l of 0.3 M NaOH, and the mixture incubated for an additional 60 min at 40 °C. The reaction mixture was neutralized with 10 μ l of 2 M sodium acetate, pH 4.0, followed by extraction with phenol/chloroform. The extension product was precipitated by ethanol. The final products were resuspended in 10 μ l of gel-loading solution (90% formamide, 30 mM NaOH, 1 mM EDTA, 0.1% xylene cyanol and 0.1% Bromophenol Blue), denatured by heating at 95 °C for 5 min and subjected to 8% polyacrylamide/8 M urea gel electrophoresis. Extension products were detected by autoradiography at –70 °C for 2–4 days. A sequencing ladder, obtained with the same primer on the genomic subclone template containing the exon 1 sequence, was run in an adjacent lane to determine the size of the extended product and to predict its sequence.

Abbreviations used: 1 \times SSC, 0.15 M NaCl/0.015 M sodium citrate, pH 7.0; DTT, dithiothreitol; CAT, chloramphenicol acetyltransferase; FSE, fat-specific element.

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The nucleic acid sequence data have been deposited in the EMBL/Gen Bank/DBJ Nucleotide Sequence Databases under accession number L27075.

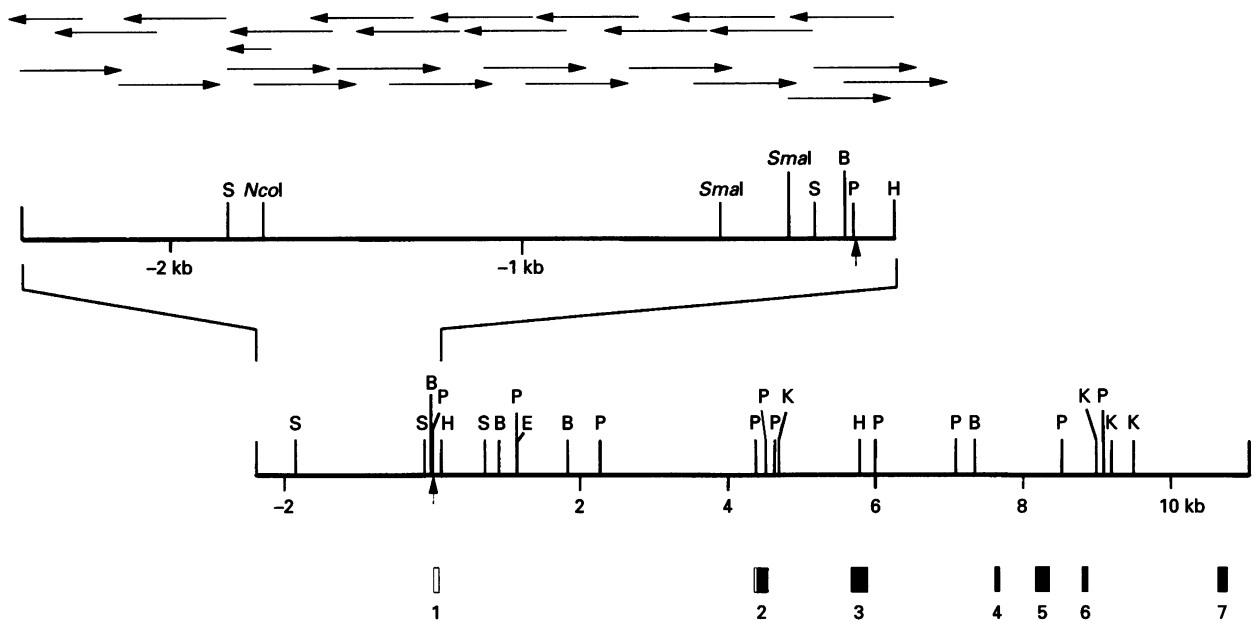


Figure 1 Structure of the 5' portion of the rat ATP citrate lyase gene

λ ACL8 (13.5 kb) was sequenced and the restriction sites are mapped as indicated. E, B, H, K, P and S represent the restriction sites of *EcoRI*, *BamHI*, *HindIII*, *KpnI*, *PstI* and *SacI* respectively. The vertical arrow indicates the transcription-initiation site. The open and closed boxes show the location of the 5' untranslated and translated exons respectively. The strategy used to sequence the 5' flanking region is indicated with horizontal arrows above the expanded map at the top.

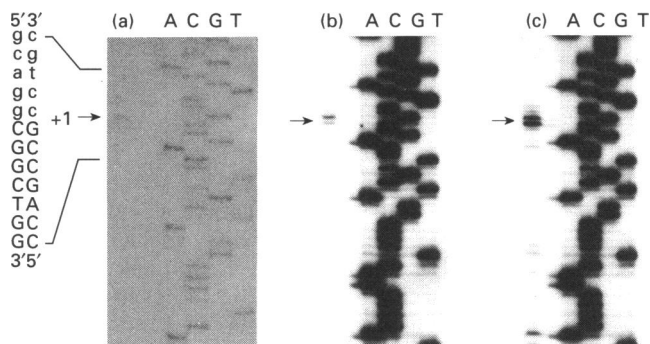


Figure 2 Determination of the transcription-initiation site of the ATP citrate lyase gene

Primer-extension analysis with rACL2 was performed on RNA from mammary glands (a), livers from weaned young rats (b) and livers from refed adult rats (c) as described in the Materials and methods section. The size of the fragment was determined by A, C, G and T (lane A, C, G and T respectively) dideoxynucleotide sequencing ladder of a genomic DNA containing the exon 1 sequence, primed with the same rACL2 oligomer. Autoradiography was performed at -70°C for 2–4 days. The +1 denotes the transcription-start site.

Southern-blot hybridization

Rat liver DNA (10 μg) was digested with restriction enzymes, electrophoresed on 0.8% agarose gels, transferred to a nylon membrane and immobilized to the support by cross-linking by u.v. light. The blots were hybridized to a 739 bp *SmaI*–*KpnI* restriction fragment (+8230 to +9022) labelled with [α - ^{32}P]dCTP (3000 Ci/mmol) by random priming in hybridization solution [$6\times\text{SSC}$ (where $1\times\text{SSC}$ is 0.15 M NaCl/0.015 M sodium citrate), 1.5% SDS, $5\times\text{Denhardt's}$ 100 $\mu\text{g}/\text{ml}$ herring sperm DNA] at 68°C for 18 h. Blots were washed in $2\times\text{SSC}/0.01\%$

SDS at room temperature, and then in $0.5\times\text{SSC}/0.1\%$ SDS at 65°C for 1 h, followed by exposure at -70°C for 4 days.

Direct sequencing of amplified cDNA

Total RNA (1 μg) isolated from various tissues was dissolved in 20 μl of RT buffer (50 mM Tris/HCl, pH 8.4, 10 mM MgCl_2 , 10 mM DTT, 0.5 mM spermidine and 1 mM dNTP) containing 40 pmol of downstream primer, rACL1, and 35 units of avian-myeloblastosis-virus reverse transcriptase. The reaction mixture was incubated at 42°C for 1 h and then at 52°C for 30 min, followed by heat inactivation at 90°C for 5 min. PCR mixture was made with 80 μl of PCR buffer (50 mM KCl, 10 mM Tris/HCl, pH 9.0) containing 40 pmol of upstream primer, rACL3, and 2 units of *Taq* polymerase. PCR was performed in a Gene ATAQ Controller (Pharmacia) with the following temperature profile: denaturation at 94°C for 30 s, primer annealing at 63°C for 30 s, and primer extension at 72°C for 30 s. The cycles were repeated 30 times followed by a final extension step for 10 min at 72°C . Amplified cDNA was isolated, using a GeneClean kit II (Bio101) according to the manufacturer's instructions. Purified PCR products were sequenced by the method of Bachmann et al. [6] using internal primer, rACL2. Autoradiography was performed at room temperature for 15 h.

Expression plasmid construction and transfection

A 2370 bp fragment (-2370 to -1) flanking the 5' side of exon 1 was inserted in front of the CAT gene of the pCAT-Basic plasmid (Promega, WI, U.S.A.). CHO cells were cultured in 60 mm Petri dishes to about 80% confluence in Ham's F12 medium supplemented with 10% fetal bovine serum. The cells were transfected with 7 μg of test plasmid and 3 μg of pCMV- β -gal, using the Lipofectin reagent (Gibco-BRL) according to the manufacturer's instructions. After 15 h, the medium was replaced

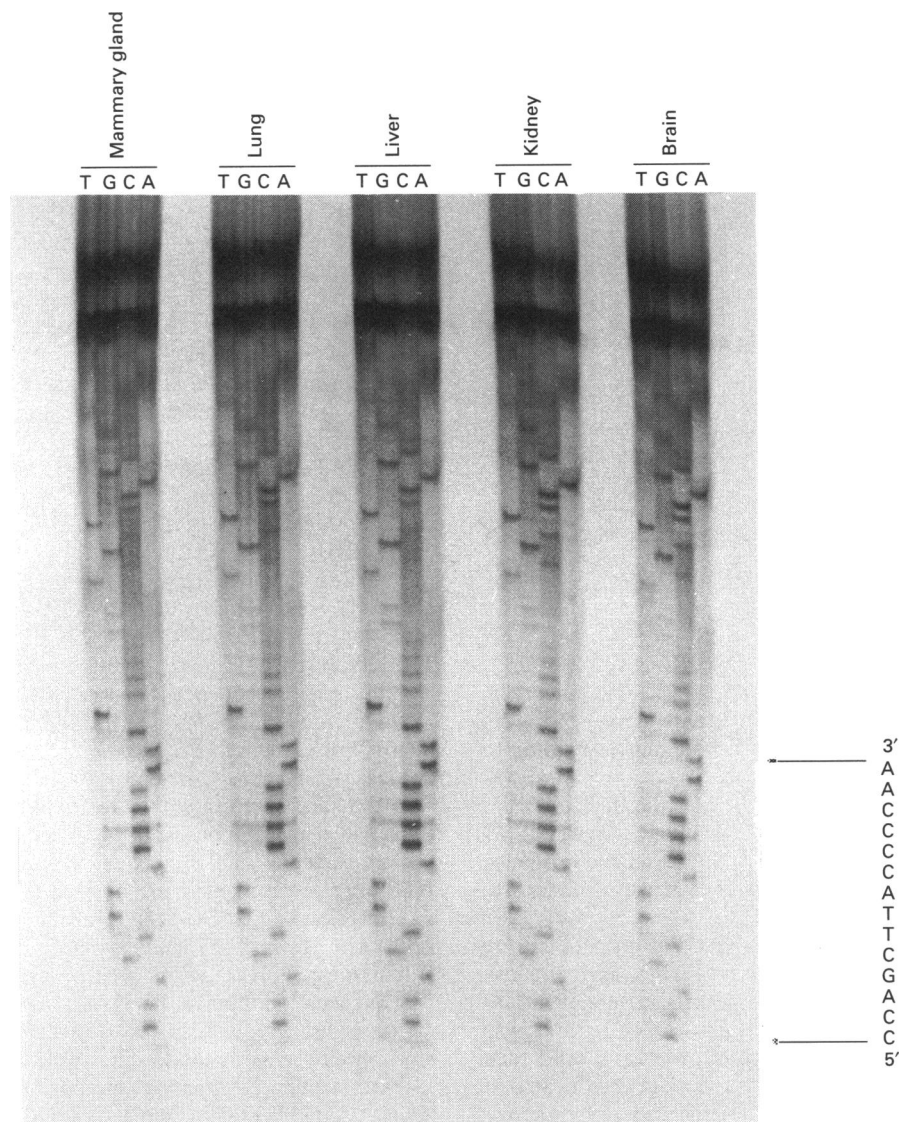


Figure 3 Direct sequencing of amplified ATP citrate lyase cDNA

ATP citrate lyase cDNA was amplified by reverse-transcriptase PCR from total RNA isolated from mammary gland, lung, liver, kidney and brain. The sense primer (rACL3) and the antisense primer (rACL1), which were complementary to the sequences in exon 1 and exon 2 respectively, were used. Amplified cDNA was sequenced using the internal antisense primer (rACL2) by the dideoxynucleotide-chain-termination method. The reverse sequence from +35 to +22 is indicated.

with Ham's F12 medium supplemented with 10% fetal bovine serum. The activity of chloramphenicol acetyltransferase (CAT) [7] was measured 48 h after the medium change, and normalized against β -galactosidase activity [8] for transfection efficiency.

RESULTS AND DISCUSSION

ATP citrate lyase is the main enzyme that supplies acetyl-CoA for the biosynthesis of fatty acid and cholesterol [1,9]. We have previously reported that the biosynthesis of rat liver ATP citrate lyase was dramatically increased by refeeding a high-carbohydrate diet, and this increase was mainly the result of transcriptional regulation [3]. In the present study, we isolated the promoter region of the ATP citrate lyase gene which might be involved in the control of expression by several hormones. By screening a rat genomic library, six clones containing the ATP

citrate lyase gene were isolated. However, only the λ ACL8 clone hybridized to the 100 bp *EcoRI*-*Bgl*II restriction fragment which is the 5' end of cDNA from the pGACL1 clone [3], whereas the other clones hybridized to the 3' remainder sequence of pGACL1 (results not shown). Therefore we sequenced the 13.6 kb genomic DNA of λ ACL8. The sequence has been deposited in the GenBank database under accession number L27075. Figure 1 shows the restriction map, the location of the exons on λ ACL8 and the sequencing scheme of the 5' flanking region of the ATP citrate lyase gene.

The transcription-initiation site was determined by primer-extension analysis with RNA from tissues expressing a high level of ATP citrate lyase mRNA (mammary glands of lactating rats, livers from young rats fed on a high-carbohydrate diet for 2 days after weaning, and livers from adult rats fed on a high-carbohydrate diet after food deprivation for 2 days). Auto-

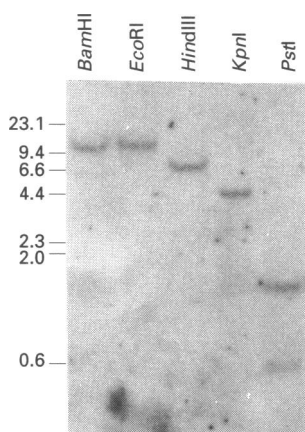


Figure 4 Southern-blot hybridization of rat genomic DNA

High-molecular-mass genomic DNA from rat livers was digested with *Bam*HI, *Eco*RI, *Hind*III, *Kpn*I and *Pst*I. DNA fractionated by 0.8% agarose gel electrophoresis was transferred to a nylon membrane and hybridized to a 793 bp *Sma*I–*Kpn*I fragment. Size markers are indicated by kb on the left.

radiograms obtained from this experiment are shown in Figure 2. The intensity of a 67-nucleotide-long band, corresponding to a cytosine, was strong in all extended products. This base was designated as position +1 for numbering of the nucleotides in the ATP citrate lyase gene. However, minor bands 65–69 nucleotides long were also observed in primer-extended products, suggesting that transcription starts over a range of these five bases.

To identify the presence of exon 1 sequences on ATP citrate lyase mRNA expressed in various tissues, such as mammary gland, lung, liver, kidney and brain, the first exon region was directly sequenced using PCR. The first cDNA strand was synthesized with antisense primer located in exon 2, and then PCR was performed by adding upstream sense primer located in exon 1. Amplified DNA products were sequenced with dideoxynucleotide-chain-termination methods, using internal primer. ATP citrate lyase cDNA (107 bp) was amplified in all RNAs isolated from mammary gland, lung, liver, kidney and brain (results not shown), and these cDNAs contain the first exon sequence (Figure 3). Acetyl-CoA carboxylase, one of the representative lipogenic enzymes, is known to have two different promoters which are used in a tissue-specific manner [10,11]. However, in the case of ATP citrate lyase, primer-extension analysis and direct sequencing of its cDNA showed that the 5' untranslated region of its mRNA is always the same and that the identical promoter is used in various organs.

Southern-blot hybridization of rat genomic DNA was performed to determine whether there were multiple ATP citrate lyase genes or other closely related genes. A *Sma*I–*Kpn*I restriction fragment (793 nucleotides) of the ATP citrate lyase genomic subclone, containing exon 5 and 6, was used to probe *Eco*RI, *Bam*HI, *Hind*III, *Kpn*I and *Pst*I restriction digests of rat genomic DNA. As shown in Figure 4, all restriction digests, except that of *Pst*I, showed a single DNA band. The 4.3 kb band shown in the *Kpn*I digest corresponded to the expected size from the restriction map of the genomic clone. In the *Pst*I digest, two bands (1.44 kb and 553 bp) were seen, which were expected because of a single *Pst*I restriction site on the 793 bp *Sma*I–*Kpn*I fragment. These results, along with the quantitative analysis of the ATP citrate lyase gene in genomic DNA (results not shown)

suggest that this gene is present in the rat genome as a single copy.

The sequences of the 5' flanking region and the first seven exons with their boundaries are shown in Figure 5. GT and AG dinucleotides are present at the beginning and end of all introns. The first exon including the 5' untranslated region has an additional 27-nucleotide sequence not present in the ATP citrate lyase cDNA sequence reported by Elshourbagy et al. [13]. The capping site is located 4407 bp upstream from the ATG codon on genomic DNA and 99 bp upstream on cDNA. The sequences of genomic DNA differed from that of the published cDNA sequence [13] at codon 116. The sequence reported previously for this codon (Ala) was GCG, but our results revealed it to be GTG (Val). It was verified that the sequence at codon 116 on both genomic and complementary DNA amplified by PCR is GTG (Val) (Figure 6).

The TATA box is considered to be an important element for the precise positioning of transcription initiation [14]. However, the promoter region of the ATP citrate lyase gene lacks the TATA box. The absence of a TATA box could explain the observation that transcription of the ATP citrate lyase gene is initiated over several bases. A CAAT sequence (GCCCAATCG) is located at positions –95 to –87 in the ATP citrate lyase gene. The region from –310 to –30 is very rich in GC content and contains several sequence elements of the consensus binding site [(G/T)GGGCGG(G/A)(G/A)(C/T)] of the Sp1 transcription factor [15]. Sequence analysis revealed two sites that matched the consensus binding site in nine of ten bases, and the other two sites that are complementary to the consensus binding site in eight and ten of ten bases. In order to prove the activity of the promoter, the 5' flanking sequence from –2370 to –1 of the ATP citrate lyase gene was fused to the CAT gene, such that transcription of the CAT gene was directed by the promoter of ATP citrate lyase. CAT activity was dramatically induced by the 5' flanking region of the ATP citrate lyase gene (Figure 7).

Sequences common to the 5' flanking regions of several genes whose transcription increased during adipocyte differentiation have been termed fat-specific elements (FSEs). The two 13-base sequences starting at positions –370 and –175 (CGCACTGGTTTGG, GCCCCGGGTCAAG) match the FSE-1 consensus [GGC(T/A)CTGGTCA(G/T)G] in ten positions (underlined) [16]. This FSE-1-like element may play a role in mediating induction of the ATP citrate lyase gene in adipocytes. Hepatocyte nuclear factor I-binding site (ATTAAC) [17] involved in tissue-specific expression is located at position –1654 with two repeats.

Various hormones are known to be responsible for the transcriptional regulation of ATP citrate lyase [18–21]. The mechanism of regulation of these hormones at the transcriptional level has been explained as interaction with hormone-responsive elements of many other genes [22]. A search of the ATP citrate lyase gene has revealed a number of sequences similar to known elements. The two sequences starting at positions –1288 and –288 match the thyroid hormone-responsive element consensus sequence GATCANNNNNN-TGACC in eight out of ten positions [23]. These elements may be involved in the amplified effect of thyroid hormone on the induction of ATP citrate lyase during differentiation to adipocytes [20]. The sequences starting at –1602 and –842 match the glucocorticoid-responsive element consensus sequence (AGAACANNNTGTTCT) [23] in ten and nine of twelve positions respectively. Glucocorticoid is known to induce lipogenesis in lung and hepatocyte [18,24]. Thus expression of the ATP citrate lyase gene seems to be regulated by glucocorticoid through these elements. Several studies have suggested that cyclic cAMP

-2370 ATCCTTCCTGAGGCTCCTGAATGCTGAATA
-2340 CCTGAATCCCTTCCCACAGCTGTTCTCCATCCGTGAGTGGGGACCCTTTAGGGGGCTTTGAATGACCCTTTCATAGGAGTTGCATGTAC
-2250 AGATATTCGTGCATATAAGATATTACATTATATTTATAACAGTATAACAGTAGCAAATATAGTTATAAAGTAGCAACAAAATAATTTT
-2160 ATGGTTGAAGGTTGCCACAACCTTGATTATTAAGGGTACAGTATTAGGTAGGTTGAGAACACTGCTTTACTACATTCCTGCTGAAGGTTCT
-2070 TTTTCTTTCTATTGTTTTTGTGTTGTTGTTGTTGTTTCTGTTGTTAGCCCTGGTGGTCTGGAACTTGGTTGTAGACCAGGCTG
-1980 GCCTTAAACTCACAGAGATCCACCTCGCTCTGGCTCGCAAGTGTGGAACTAAAGATATGTGCCACCATGCTGGACTAAAGGTTATTCTT
-1890 TTCTAACCAAAGGGATAACCAAATTCATAGAGCTTACTGCTCTATAAAAAGTTATTAGTGTGACATGTAGGGGGCACAGTACTACA
-1800 GTGTATGTGTGGAGGCTGAGGACAATTTGTGGAGCCGTTTCTTAAATTGCCTATATGTGGGTTAGCCATGGAACCTCATCTT

HNF-1

-1710 TTTGCATAGTAAGTCATTACCCACTGAGCCATTTGTCTGGGACACTCCACTGGTGATTCACATTAACATAGTTTCTAACTAGCCTGTGA
GRE (10/12)
-1620 AGACCAGCTAGTCCCAAGGTTTCATGTTGTTATCCAGCATTGGGAAGCTGAGGCAGGAGAATAGACATTTGAAGTTGTTCTCAATTTA
-1530 TACAACGAGTTTACAAACAACCTGGGCTACATATTACCCTGTCTCAAACAGAAAAATAAATGACCGTAAAAGCTCTGATTTCTGCTCTCC
-1440 AGGGCCTGAATTTGAGCAACTTCATAGAAGACATTTACTTAGGGAGAAGTGATCCCTCACAGAGATTTGGGTAGGTTCTAGGCCTTTCTC
TRE (8/10) CRE (7/8)
-1350 TCAACTTCAGGAGCAAGGGGCACTATAAACTGTTATTCTAAAGAATAAGTATGAGGGAAGGGACCAAAAAATGACATCAAGGAGC
-1260 ACACGCTGGTCAAGCATGGGTGTAACAAGTTCAGCCAGCCTTTGGAGGAGGGCTTGGAGTGGGGAGGGGGCTTGGGAAAAGTGTGTTG
-1170 CCCAGGAGAGTGTGCAACATTGTTGCAGGGAGCTTTTGACCAGGGTTTTTGGCCAGGGCATAGCCATGCCACGCTGATTCTCTGCACTC
-1080 TGGGCTGATGCATCACCTGAGCCGATTAGAGGATGATTCTGGCCTCCTCTAGAGCTGGAATAATTGTTGTTTCTGGGACCGTCTGGGA
-990 AGACATACTGGGACTAAGGTACAAGCAAAAGAGGCTTTCACTCCAGGAAGCAGTAAATCGGAGTCTGATTTCCAGGACGTTCTGATTT
GRE (9/12)
-900 TATATATCTGCTCCACAAGTCCGGCACCCTGTAGACTGGGTGCTCCACTTTTGGATTGGAAAATATGTTCTACCTCTCATATGTAACA
-810 GAAAAGAAGAGGAAGCTGTTCAATGCTTTCACTGTCTTAGTTGAGTGTGGAGGGAGCAAGAGGTTGGACAGGGATGGCCCCAGTGTCT
-720 AGGTTTTAATCTCAGATAAATGATCAGCTCCGCCAGATCCGCGCTCAGGTGAGCCCGTAAGAGGACTCGCGTTGGAACCTGGCGTCTCTC
-630 TGGCCTCACTAGTTTACCTCTTCTCCTATCTATGTTCTACGTCGGGAGCTGATTGTGTAGCGTGGTCTAGTCTGATTTCTAGTAA
FSE (10/13)
-540 ATTCTATGGGTGGGTTTCAGGAGCGGAGAGGCGCCAGTGTGAAAGGAAAGCAGACGTAGCTTTCCAAAGCAGGTCTACCGCACTGGTT
-450 TGGTGTGGCGACTTACAAACGCCAGAGATAAGAACGTCAGTTGCTCCAGGGGAGGGAGGGAGGGGACGCCAGGTCCCGGGAGAAGTGCT
TRE (8/10)
-360 ACTGCCTTTTGGGCCAACAAACCCCTCCCACAGCTACCAATCCAGCAGCTGGCCGGCCAGCCCCGTAAGCGATCAGGCCACAGCCCC
Sp1 (10/10) Sp1 (8/10)
-270 AGCCTCGTAGGTTCTCCTCCTAGAGCTGCCCGCCCTCTCCCCATCTAGGCCGCCACGGCCCCCTCCACTTCCAAGACTACAAGTCC
FSE (10/13) Sp1 (9/10) CAAT
-180 CGTCAGCCCCGGGTGAGCATGAGGGCGGGCCACCCGAGCAGCGAATGGGGAGGAGCCTAGAGCTCAGGCTAGGGAACGCGTGTGCCA
Sp1 (9/10)
-90 ATCGCCAGGCTGCATGGCTGTGAGCTGATGGGGCGGGAGGAGCCCGCTTGGGCCGGGACAAAAGCCGGATCCCGGCAGCTGCAGG

Exon 1

+1 CGGCTGGAGCGATCCGGGGAGTTGGGGTAAGCTGGTGCTTACGGACAGAGGCCACACTCGGGCTTTCTCGAAGAG GTGAGT... 4.3 kb..

*** Exon 2

CTTGCAG GTAAACCAGGTCCTCTGCAGCAATGTCAGCAAGGCAATTTACAGCAGACCCGGCAAAGAACTCCTTTACAAGTACATCTG
TACCACCTCAGCATCCAGAACCGGTTCAAGTATGCCCGGGTACTCCCGACAGACTGGGCCATCTCCTGCAGGACCACCCCTGGCT
GCTTAGCCAG GTAAGC 1.2 kb

Exon 3

TTCCAG AGCTTGGTAGTCAAGCCGACCAGCTGATCAAACGTCGAGGAAAGCTTGGTCTAGTCGGGGTCAACCTCTCTCTGGATGGAGTC
AAATCCTGGCTGAAACCTCGACTGGGACATGAGGCCACC GTGAGT 1.8 kb

Exon 4

CCTTAG GTCGGCAAGGCCAAAGGCTTCTCAAGAATTTCTGATTGAGCCCTTCGTCACCCACAGTCAG GTATGT 0.5 kb ...

*** Exon 5

TGAAAG GTGGAGGAGTTCTACGTTGTCATCTATGCTACCCGGGAAGGAGACTACGTCCTGTTCCACCATGAAGGGGGTGTGGATGTGGG
GATGTGGACACCAAAGCCAGAAGCTGCTGTGGGTGTGGACGAGAACTGAACGCTGAAGACATTAAGAGACACCTGTTGGTCCACGCC
CCGAAGACAAGAAAGA GTTGAG 0.5 kb

Exon 6

TTCTAG AATCCTGGCCAGCTTCATCTCCGGCCTATTCAATTTCTACGAAGATCTTTACTTCACCTACCTTGAGATCAACCCCTTG

Exon 7

GTAACA 1.7 kb TCCTAG TGGTGACCAAAGATGGTGTCTACATCCTTGACCTGGCGGCAAGGTGGACGCCACTGC
TGACTACATCTCAAAGTCAAGTGGGGTATATAGAGTTCCTCCCCCTTTGGGCGTGAGGCATACCAGAG GTAAGG

Figure 5 Sequence of promoter region, the first seven exons and their boundaries for the ATP citrate lyase gene

The sequence of the 2370 bases of DNA that flank the 5' side of the transcription-initiation site, and the sequence of the first seven exons and their boundaries are shown. The location of the CAAT box is indicated with a double underline. The putative Sp1 sites and sequences similar to the glucocorticoid- (GRE), thyroid hormone- (TRE) and cyclic AMP- (CRE) responsive elements and to FSE-1 and hepatocyte nuclear factor 1 (HNF-1) are indicated by underlining. The sequences of the exons are enclosed in boxes. The initiating ATG codon in exon 2 and codon 116 in the exon 5 are indicated with asterisks. The sequences of the beginning and end of the intervening introns are shown with their sizes.

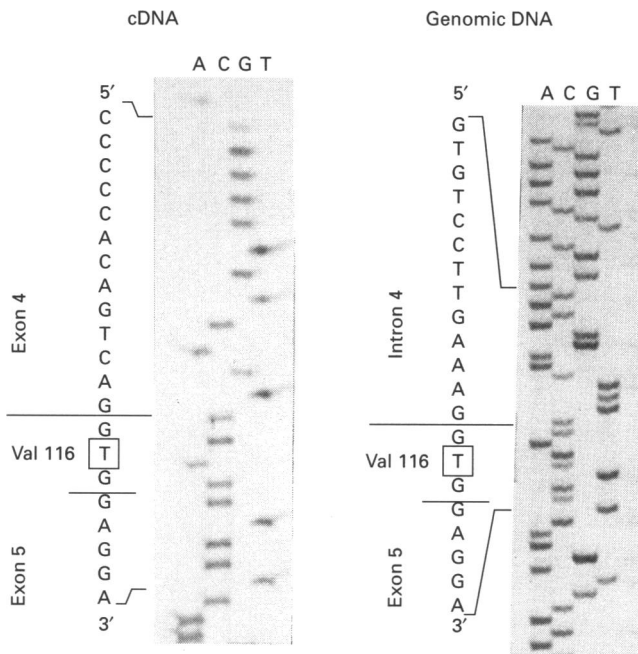


Figure 6 Sequences around codon 116 on cDNA and genomic DNA of ATP citrate lyase amplified by PCR

cDNA and genomic DNA of ATP citrate lyase containing codon 116 were amplified and subcloned into GEM4Z vector, followed by dideoxynucleotide sequencing. The sequence of the sense strand around codon 116 (Val) is denoted on the left.

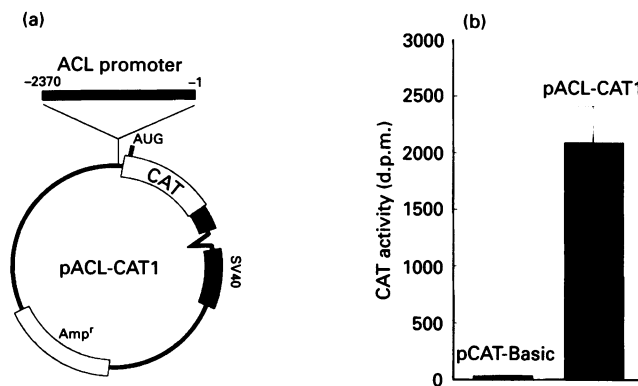


Figure 7 Expression of the CAT gene directed by the 5' flanking regions of the ATP citrate lyase gene

(a) Structure of pACL-CAT1. The 5' flanking fragment containing the sequences from -2370 to -1 of the ATP citrate lyase gene was inserted in front of the CAT gene. SV40, Simian virus 40. (b) CAT activity in the cell transfected with pACL-CAT1 and pCAT-Basic. Results are means \pm S.E.M.

decreases the expression of the lipogenic enzymes in liver [25,26]. Transcriptional regulation by cyclic AMP is known to be mediated via a cyclic AMP-responsive element (TGACGTCA) [27]. There is one of these sequences at -1283 conserved in seven of eight positions. Insulin is known to regulate the transcription of several genes, such as phosphoenolpyruvate carboxykinase [28], glucokinase [29], glyceraldehyde-3-phosphate dehydro-

genase [30], *c-fos* [31] and amylase [32]. It is thought to be the main hormone that induces the expression of lipogenic enzymes in liver after refeeding a high-carbohydrate diet [21,25,26]. However, no consensus insulin-responsive element has been identified. It would be interesting to localize this element in the 5' flanking region of the ATP citrate lyase gene, and to compare them from genes either stimulated or inhibited by insulin.

In conclusion, the transcription-initiation site and CAAT box of the rat ATP citrate lyase gene have been identified in the present study. However, direct experimental evidence for the existence of regulatory elements is required.

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