Genomic distribution of three promoters of the bovine gene encoding acetyl-CoA carboxylase α and evidence that the nutritionally regulated promoter I contains a repressive element different from that in rat

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The enzyme acetyl-CoA carboxylase α (ACC-α) is rate-limiting for the synthesis of long-chain fatty acids *de novo*. As a first characterization of the bovine gene encoding this enzyme, we established the entire bovine ACC-α cDNA sequence (7041 bp) and used experiments with 5′ rapid amplification of cDNA ends to determine the heterogeneous composition of 5′ untranslated regions, as expressed from three different promoters (PI, PII and PIII). The individual locations of these promoters have been defined within an area comprising 35 kbp on *Bos taurus* chromosome 19 ('BTA19'), together with the segmentation of the first 14 exons. Primer extension analyses reveal that the nutritionally regulated PI initiates transcription from at least four sites. PI transcripts are much more abundant in adipose and mammary-gland tissues than in liver or lung. A 2.6 kb promoter fragment drives the expression of reporter genes only weakly in different model cells, irrespective of stimulation with insulin or dexamethasone. Thus bovine PI is basically repressed, like its analogue from rat. Finely graded deletions of PI map two separate elements, which have to be present together in *cis* to repress bovine PI. The distal component resides within a well-preserved Art2 retroposon element. Thus sequence, structure and evolutionary origin of the main repressor of PI in bovines are entirely different from its functional counterpart in rat, which had been identified as a $(CA)_{28}$ microsatellite. We show that, in different mammalian species, unrelated genome segments of different origins have been recruited to express as functionally homologous PI the ancient and otherwise highly conserved ACC-α-encoding gene.

Key words: *Bos taurus*, cDNA, fat synthesis, gene expression, promoter.

INTRODUCTION

The enzyme acetyl-CoA carboxylase α (ACC-α, EC 6.4.1.2) catalyses the formation of malonyl-CoA from acetyl-CoA in the cytoplasm. This is the rate-limiting step in the formation of long-chain fatty acids in all organisms (reviewed in [1]). The enzyme is therefore equipped with three highly conserved domains to bind the metabolites biotin, ATP and CoA. More than 40 molecules of the large enzyme (more than 2300 amino acid residues, 265 kDa) multimerize to exert their catalytic function [2]. The enzyme is active in (1) any cell, (2) adipose tissues, (3) liver cells and (4) the lactating mammary gland. This accommodates the variable demands of different cells, cell types and tissues for fatty acids, which serve as precursors for the synthesis of membranes but also for fat as a food storage component. The regulation of activity of the enzyme is accordingly complex. Regulatory levels include short-term regulation via allosteric interactions with metabolites [3] and reversible phosphorylation [4,5] triggered extracellularly by various hormones (insulin, glucagon, catecholamines, steroid hormones, thyroxine and prolactin) (reviewed in [1]), but are also influenced by variations in glucose concentration in the serum [6]. Long-term regulation throughout differentiation is exerted at the transcriptional level [7,8].

First molecular characterizations of a gene encoding ACC-α in rat [9] revealed quickly that tissue-specific gene expression is driven by two different promoters, PI and PII [10]. Exon 5 harbours the translational start codon. The 5′ untranslated region (UTR) of the mRNA might be heterogeneous, including a variable number of exons [11]. PI is very active in adipose tissue, but is repressed in this tissue during lactation [12]. This lactation-associated repression of PI could be relieved in primary explants of adipose tissue from sheep by the administration of insulin and glucocorticoid hormones (such as dexamethasone [13]). PI is also the main promoter driving ACC-α expression in liver, but under tight nutritional control, as observed in human and rat. It is virtually blocked unless a high-carbohydrate diet is available after a fasting period [14]. The repressive element in rat is bipartite in structure. It is composed of a distal $(CA)_{28}$-repeat microsatellite interacting with a CCAAT box in the proximal promoter area. Repression is relieved if the transcription factor CCAAT-enhancer-binding protein (C/EBP) binds to this CCAAT box [15]. PII is considered to be the housekeeping promoter [16] but is also lactationally stimulated in the mammary

Abbreviations used: ACC, acetyl-CoA carboxylase; BAC, bacterial artificial chromosome; C/EBP, CCAAT-enhancer-binding protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PI, PII and PIII, promoters I, II and III; PPAR, peroxisome-proliferator-activated receptor; RACE, rapid amplification of cDNA ends; RT–PCR, reverse-transcriptase-mediated PCR; STAT, signal transduction and activators of transcription; tsp, transcriptional start point(s); UTR, untranslated region.

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The nucleotide sequence data reported will appear in DDBJ, EMBL, GenBank® and GSDB Nucleotide Sequence Databases under the accession numbers AJ132890 (cDNA) and AJ276223 (PI promoter).

gland of the rat [14,17]. The human homologue of ACC-α has also been characterized by cDNA cloning [18,19] and it was shown that the large gene spans 200–480 kbp on human chromosome 17q12 [19]. The human gene is also expressed from two different promoters [18].

A third promoter driving ACC-α expression (PIII) has been identified in sheep [20]. The activity of PIII leads to the formation of an N-terminally modified isoform of the enzyme. PIII is active in the mammary gland, where its activity increases approx. 15-fold during lactation [20,21].

Nevertheless, our knowledge of the molecular controls regulating the tissue-specific expression of ACC-α is still very limited. Although the transcription factor peroxisome-proliferator-activated receptor (PPAR)γ and various members of the C/EBP family [22–24] have recently been identified as key elements of the signal transduction pathway leading to adipocyte differentiation, no studies have been reported on the impact of these factors for the tissue-specific expression of ACC-α. Moreover, considerable evidence is emerging that several members of signal transduction and activators of transcription (STAT) factors are acting downstream to mediate the C/EBP and PPARγ signals [22,25]. Severely decreased fat deposition in STAT5A and STAT5B double knock-out mice [26] supports the relevance of this signal transduction pathway for ACC-α gene expression. The lack of knowledge on the impact of these factors for the regulation of ACC-α gene expression might be due in part to the very limited data available on the sequences and functional organizations of the various promoters of ACC-α. The structure of PI has been analysed from rat only; no sequence data are publicly available, and no other comparable studies have been reported on PI from other species or on the other promoters of the gene encoding ACC-α.

In view of the significance of fat metabolism for breeding farm animals, we started to characterize the gene encoding ACC-α from cattle. Here we report the entire cDNA sequence and the genomic locations and sequences of all exons contributing to the 5′ UTR. We limit the functional characterization of PI to the delineation of key elements acting in *cis* to control the basal repression of this promoter. Unexpectedly, the first comparison of PI repressors between two mammalian species shows that both the whole promoters and the main components of the repressor elements are of entirely different origins and sequences.

EXPERIMENTAL

Establishment of cDNA and genomic sequences

All cDNA clones of the bovine ACC-α were established by reverse-transcriptase-mediated PCR (RT–PCR) amplifications (see below). Amplicons were subcloned into the vectors pGEM-Teasy (Promega) or pKS+ (Stratagene) and sequenced (310A DNA Sequencer from Applied Biosystems, or LICOR4000L from MWG-Biotech). Genomic isolations were retrieved from our bovine genomic libraries established in bacteriophage λ [27] or bovine bacterial artificial chromosome (BAC) [28]. The λ library was screened with ^{32}P-labelled cDNA subclones. PCR screening with exon-5-specific primers served to isolate the BAC clones. The GenomeWalker kit and application protocol (ClonTech) were used to establish subclones from the BAC isolates.

RT–PCR

For RT–PCR, 2–5 μg of total RNA [29] was primed with 10 pmol of either a gene-specific reverse primer or an oligo(dT) oligonucleotide and transcribed in reverse with SuperScript RNaseH$^-$ Reverse Transcriptase (Life Technologies), as prescribed by the manufacturer. Usually, 10% of the yield from first-strand cDNA synthesis was used as template for subsequent PCR amplifications (AmpliTaq polymerase and buffers from Appligene). 'Touch-down' amplification protocols were used throughout [30]. An initial denaturation (4 min at 94 °C) preceded 10 cycles in which the annealing temperature was lowered from 70 to 60 °C in 1 °C decrements. These were followed by 40 standard cycles (1 min at 94 °C, 1 min at 60 °C and 3 min at 70 °C). The following primers were used for the RT–PCR analysis of the 5′ UTR of the bovine ACC-α mRNA: Ac_ex1f, 5′-GTCTGTCCATCTGTGAAGTATC-3′; Ac_ex6rn, 5′-CAAATTCTGCTGGAGAGGCTACA-3′; Ac_ex3f, 5′-TCCTCGGAGATGCTTAGTGAC-3′.

Quantification of transcripts with Real Time PCR

Real Time RT–PCR with a Light-Cycler (Roche) was used to quantify and compare in different tissues the abundance of transcripts either derived from PI or comprising all ACC-α mRNA molecules. Within tissues, transcript levels in different animals were normalized to the simultaneously recorded abundance of transcripts from glyceraldehyde-3-phosphate dehydrogenase (GAPDH). All amplification primers were placed on different exons. For any assay, 1 μg of total RNA was doubly primed with 10 pmol of oligo(dT) and 10 pmol of the ACC-α-specific reverse primer Ac_990r (5′-TTTCAAGAGAAGTTCT-GGGAGCT-3′). After first-strand synthesis (Superscript-H$-$) products were purified (High Pure PCR Product Purification Kit; Roche) and aliquots were distributed into different vials representing an input of 12.5 ng of total RNA. Transcripts from PI were amplified with primers ex1f (5′-GTCTGTCCA-TCTGTGAAGTATC-3′) and ex5r (5′-GAAGAAGGTTCAT-CCATTGCTTC-3′). Total ACC-α molecules were amplified with ex7f (5′-GTTCTCATTGCCAACAATGGCA-3′) and ex8_9r (5′-CAGCCAGCCCAAACTGCTTGCAC-3′) bridging the splice boundary between exons 8 and 9. The primers for the amplification of GAPDH cDNA molecules were 5′-CATTGACCTTCACTACATGGT-3′ (forward) and 5′-ACCCTTCAAGTGAGCCCCAG-3′ (reverse). Products were amplified with the LightCycler-FastStart DNA Master Syber GreenI kit (Roche). An initial denaturation at 95 °C (10 min) was followed by 40 cycles consisting of annealing for 5 s at 60 °C, elongation at 72 °C (20 s) and denaturation at 95 °C (15 s). Syber Green fluorescence was assayed in each cycle at 79 °C during ramping-up of the temperature from 70 to 96 °C. Samples were assayed in duplicate. A dilution series (10^6–10^2 copies) of appropriate cDNA subclones was included in each run and served to calculate the copy numbers of transcripts. PI and total ACC-α transcripts were calibrated against the same cDNA clone, harbouring exons 1, together with exons 5–9. Values were normalized within tissues for equal GAPDH copy numbers.

Because Real Time PCR amplifications revealed large individual differences in ACC-α mRNA concentrations within the same tissues during this study, we controlled the validity of the Real Time PCR measurements in a model experiment. Seven different samples spanning a 27-fold difference in the concentrations of total ACC-α mRNA molecules as measured by Real Time PCR quantifications were also quantified by slot-blot analyses. A ^{32}P-labelled subclone of the ACC-α cDNA was hybridized with two dilutions (15 and 7.5 μg) of total RNA from any one of these samples. Quantification of the hybridization signals with a STORM PhosphorImager (Molecular Dynamics) reflected a 32-fold difference between the extreme samples.

Rapid amplification of cDNA ends (RACE) PCR

5′-RACE amplifications were performed with a Marathon cDNA Amplification Kit and protocols (ClonTech). Total RNA (2 μg) from various tissues was primed for first-strand synthesis of the cDNA with the gene-specific primer Ac_ex6–7r (5′-TGGCAATGAGAACCTTCTCAATC-3′), spanning exons 6 and 7. Transcripts were ligated to the adaptor and amplified with the forward adaptor primer 1 of the kit in combination with Ac_ex6rn, to amplify all transcripts. Transcripts from PI were amplified from the products of the first amplification, with primer Ac_ex1r (5′-ACTCAGAGACCTCTCTGCTTC-3′), whereas those from PII were obtained with oligonucleotide Ac_ex3r (5′-GCAGCGCACCGAACAAGTCAC-3′). Each of these primers is located close to the 3′ boundary of the respective exon.

Primer extension

Primer extension was performed as described [31]. Extension products were analysed on a 6% (w/v) polyacrylamide/8 M urea sequencing gel next to the sequencing ladder of the corresponding genomic subclone. The same batch of radiolabelled primer was used for the extension and sequencing reactions (dsCycle Sequencing System; Life Technologies).

PI deletion series and analyses of reporter gene constructs

PI was retrieved as a 2.9 kbp PCR amplification product (Expand Long Template PCR System; Boehringer Mannheim) from λ-clone Ac10a, with a λ-EMBL3-specific forward primer (5′-CTCGTGAAAGGTAGGCGGATC-3′) and Ac_ex1r reverse primer. The fragment was digested with *Mst*II (within exon 1), filled in with Klenow enzyme, digested with *Sal*I (restriction site from λ-EMBL3 cloning site) and subcloned into pKS+ (*Sal*I/*Sma*I). The *Bam*HI-retrievable fragment was ligated into the *Bgl*II site of the promoterless luciferase expression vector pGL3 basic (Promega). The proper orientations and sequences of this and all other expression clones were verified by sequencing.

Deletion series were established from this longest expression clone by exploiting either suitable restriction sites or suitable primers for PCR amplifications of the desired segments. The unique *Mst*II site from exon 1 at position +82 was used throughout for the necessary subclonings.

Cell culture, transient transfection and luciferase assay

Constructs were analysed either in the murine mammary epithelial cell line HC11 or murine HepR1 hepatoma cells (a gift from Dr R. Jesenofsky, Department of Medicine, University of Rostock, Germany). HC11 cells were grown in RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated fetal calf serum, 5 μg/ml insulin, 10 ng/ml epidermal growth factor and 50 μg/ml gentamicin as described [32]; HepR1 cells were propagated in Dulbecco's modified Eagle's medium containing 10% (v/v) heat-inactivated fetal calf serum and 50 μg/ml gentamicin.

For transient transfections, cells were split into 24-well plates (5×10^4 cells per well). At 60% confluence, a mixture of 500 ng of the respective ACC-α PI luciferase reporter construct and 50 ng of the control plasmid pCMV-β-galactosidase (in which CMV stands for cytomegalovirus) (Stratagene) was co-transfected with LIPOFECTAMINE PLUS™ (Life Technologies), as prescribed; 2 days after transfection, luciferase and β-galactosidase activities were determined from cell extracts. For this, cells were washed with ice-cold PBS, scraped into 50 μl of lysis buffer [100 mM potassium phosphate (pH 7.8)/0.2% (v/v) Triton X-100/0.5 mM dithiothreitol] and centrifuged for 5 min at 11000 ***g*** (13000 rev./min); the supernatants were removed by aspiration. Luciferase and β-galactosidase activities were determined from aliquots with the Dual Light Chemiluminescent Reporter Gene Assay System (Tropix). This reagent kit permits the measurement, in one tube, of the luciferase activity with luciferin as a substrate and then, after luminescence has ceased (approx. 1 h), that of the β-galactosidase activity with Galacton-Plus as substrate. Differences in the individual transfection efficiencies were compensated for by normalizing the luciferase activities with the simultaneously recorded β-galactosidase activity. In each experiment, each construct was transfected in triplicate and the experiments were repeated at least three times. The means ± S.E.M. of their averages are given.

RESULTS

cDNA isolation and sequence

Initial cDNA isolations were obtained by the cloning of RT–PCR products of bovine liver RNA. Products were established with a pair of consensus primers derived from the sequences of the biotin-binding domain from rat and chicken. However, the main part of the bovine ACC-α cDNA was amplified and subcloned on the basis of primers derived from the homologous sheep sequence [33]. The final sequence was established from 11 overlapping clones based exclusively on authentic bovine primers (Figure 1). The reading frame of the bovine cDNA sequence comprises 7041 bp (Figure 1, Table 1). The translated part of the nucleotide sequence is highly similar (Table 1) not only to the closely related sheep sequence (97.6%) but also to those from human [18,19], rat [9] and chicken [34]. The degree of similarity of the derived amino acid sequence (2346 residues; molecular mass 265 kDa) is even higher (Table 1). The entire protein sequence of the bovine enzyme is 97.9% similar to its analogue from rat enzyme and 92.2% similar to the sequence of the evolutionarily very distinct chicken.

Genomic isolations

A subcloned cDNA segment was used to isolate two overlapping λ clones (Ac2, Ac3; results not shown), spanning from intron 8 over 35 kbp into intron 14 of the bovine gene encoding ACC-α, as became apparent throughout the study. Exon 1 was amplified from bovine genomic DNA with primers derived from the sheep cDNA sequence, and used to isolate a bovine genomic λ-clone harbouring exon 1 (Ac10a; Figure 1). Two overlapping BAC clones (BAC91, BAC478) were isolated, based on bovine primers amplifying exon 5 (Figure 1), which encodes the translational start for the most widely distributed isoform of ACC-α. These BAC clones served as templates to characterize the entire 5′ region of the bovine gene encoding ACC-α. Direct sequencing of BAC91 was used to determine the sequences of the 5′-terminal first 14 exons of the gene (Figure 1, Table 1).

Determination of the heterogeneous 5′-termini of the mRNA

5′-RACE techniques were used to analyse the composition of the 5′-terminal mRNA molecules. Knowledge of the exon segmentation allowed us to place a highly specific reverse primer for the first strand of cDNA synthesis on the splice boundary of exons 6 and 7, and a reverse 'nested' primer on exon 6. Preparations of total RNA from a variety of tissues (liver, adipose tissue, kidney, brain, mammary gland, muscle and lung) were used to amplify a heterogeneous collection of 5′-RACE products. Subcloning and sequencing revealed cDNA clones of six types of transcripts differing in the composition of the 5′ UTR regions of the ACC-α mRNA. They are designated as class 1 and class 2 transcripts, in keeping with the literature [11].

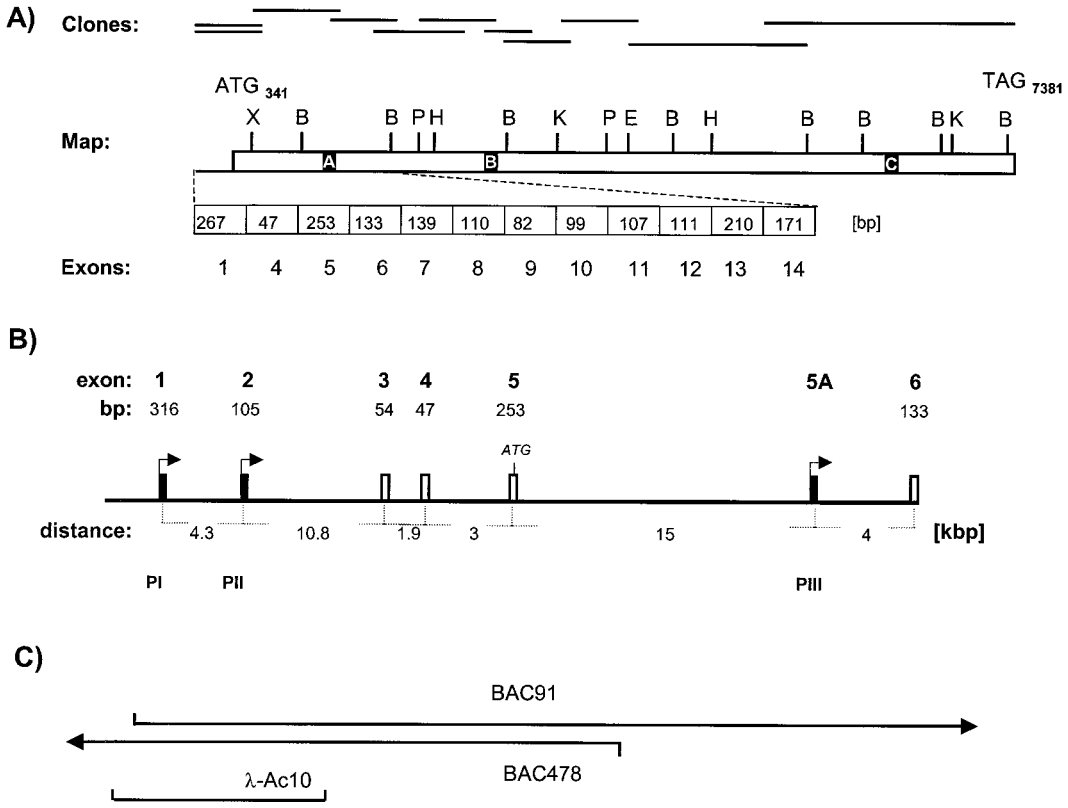

Figure 1 Isolation and arrangement of cDNA and genomic sequences of bovine ACC-α

(**A**) cDNA isolation and partial exon segmentation. Position of cDNA clones projected on the restriction map of the bovine cDNA sequence (EMBL file AJ132890). Nucleotide file positions of translational start and stop codons (ATG and TAG respectively) are indicated. A, B, C in the open bar represent the positions of the coding regions for the domains binding ATP (A), biotin (B) and CoA (C). Exon numbers and the respective sizes of the 5′-terminal 14 exons are indicated. Abbreviations: B, *Bam*HI; E, *Eco*RI; K, *Kpn*I; P, *Pst*1; X, *Xho*I. (**B**) Genomic organization of promoters. The relative positions of exons 1–6 were deduced from long-span PCR amplifications with exon-specific primers and BAC91 DNA as template. The exon sizes are indicated. The size of exon 1 (316 bp) is calculated on the basis of the 3′-most tsp (Figure 3). (**C**) Position of genomic isolates used to characterize the promoter area. The flanking regions of both BAC clones have not been determined precisely. Amplifications based on BAC91 were consistent with all other sources for areas comprising exon 1 and locations nearer the 3′ end. However, PI sequence areas 5′ of position −1241 could not be amplified from this BAC clone, whereas those based on λ-Ac10 and BAC478 as templates were identical. No exon 3′ of exon 5 could be amplified from BAC478.

Class 1 transcripts harbour exons (1:4:5) and [1:5]; all class 2 transcripts are 5′-preceded by six nucleotides (5′-CTTGAG-3′) followed by exons (3:4:5), or (3:5), or (4:5), or (5) only. The various transcripts were found in all tissues analysed with RT–PCR amplifications. Possible tissue-specific differences in the abundance of class II transcripts have not been examined. We detected two splice variants of exon 3, comprising 54 or 47 nt (see Figure 4C). Whereas the longer version is prominent in almost all tissues, the short version has only been found in mammary gland.

An additional type of transcript was detected in the mammary gland of cattle, class 3 messages. They consist of exon-5A-encoded RNA spliced to exon 6, just as observed in sheep [20].

Promoters are distributed over approx. 35 kbp

We determined the relative positions of the 5′-terminal exons from exon 1 to exon 6 in long-span PCR amplifications, with BAC91 as template. The primers were derived from the sequences of the 5′-RACE clones. Exon 6 is located approx. 40 kbp downstream of exon 1 (Figure 1B). Eventually, all the exons contributing to the 5′ UTR region were isolated from the GenomeWalker libraries and sequenced. Exon 2 was detected in the sequence of a subclone from the genomic λ isolate Ac10a, owing to its sequence similarity to the human and rat analogue (Table 1). The bovine sequence is very rich in GC nucleotides (77% in GC content; see Figure 3B), explaining why we had obtained the only 3′-most six nucleotides of this exon in 5′-RACE experiments. Expression and sequence of exon 2 (105 bp) was confirmed from RT–PCR amplicons obtained with an exon 2 forward primer Ac_ex2f (5′-TGTCAGCCATCGCCGGAGC-3′) and reverse primer Ac_ex6rn. The exact size of exon 2 of bovine ACC-α has yet to be determined by primer extension analyses.

Transcripts expressed from PI comprise a similar fraction of total ACC-α messages in different tissues

We surveyed differences in the tissue-specific abundance of transcripts as derived from PI in five different tissues (mammary gland, liver, lung, subcutaneous adipose tissue and muscle) and compared it with the concentration of transcripts generated by the combined activity of all three different promoters (total ACC-α). Tissues had been collected from five healthy lactating cows (3–5 years old) and of five virgin pubertal (14–16 months old) female animals. The concentration of transcripts derived

Table 1 Interspecies comparison of ACC-α cDNA sequences and 5′-UTR-encoding exons

The bovine cDNA sequence (AJ132890) as derived from liver is compared with the respective sequences from sheep [33], rat [9,11], human [18,19] and chicken [34]. Bovine sequences of exons 2 and 3 are represented in Figure 4. Abbreviation: n.a., not reasonably analysable (see the text).

		Similarity (%)			
Sequence or exon	Length (nt)	Sheep	Human	Rat	Chicken
Total cDNA coding sequence					
Nucleotide	7041	97.6	90.3	88.8	81.3
Amino acid	2346	99.4	96.8	97.9	92.2
5′ UTR					
Exon 1	267	92.3	48.3	64.6	–
Exon 2	105	–	77.7	73.9	61.4
Exon 3	47/54	–	–	n.a.	–
Exon 4	47	100	95.7	93.6	83.0
Exon 5	253	95.7	85.4	84.6	73.3

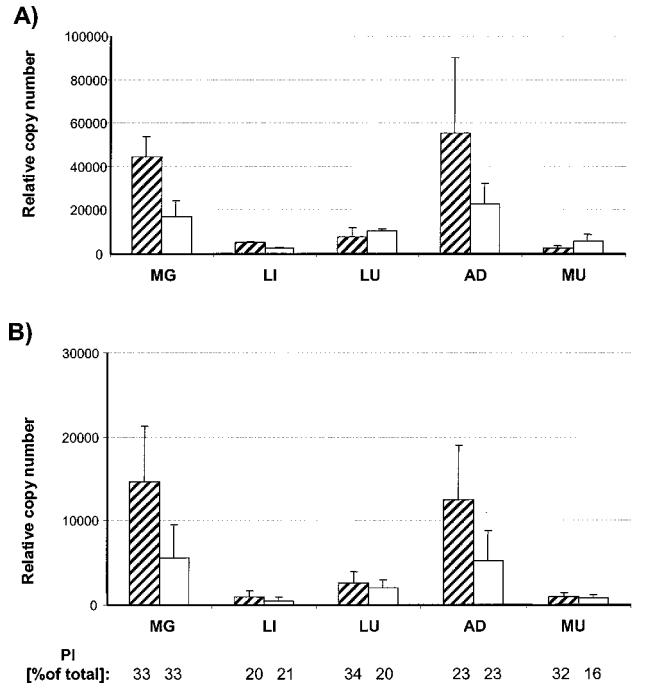

Figure 2 Relative abundances of total ACC-α mRNA and transcripts derived from PI in different tissues

The concentrations of mRNA molecules from either total ACC-α messages (**A**) or those derived from PI activity (**B**) were measured in different tissues with Real Time PCR amplifications. Values are means $\pm$ S.E.M. from lactating cows and pubertal virgins (five individuals each, hatched and open columns respectively). Individual copy numbers of ACC-α transcripts were normalized within tissues on the basis of the simultanously recorded GAPDH values. The percentages of PI-derived transcripts from the total amount of ACC-α messages are indicated at the bottom. Abbreviations: MG, mammary gland; LI, liver; Lu, lung; AD, subcutaneous adipose tissue; MU, muscle.

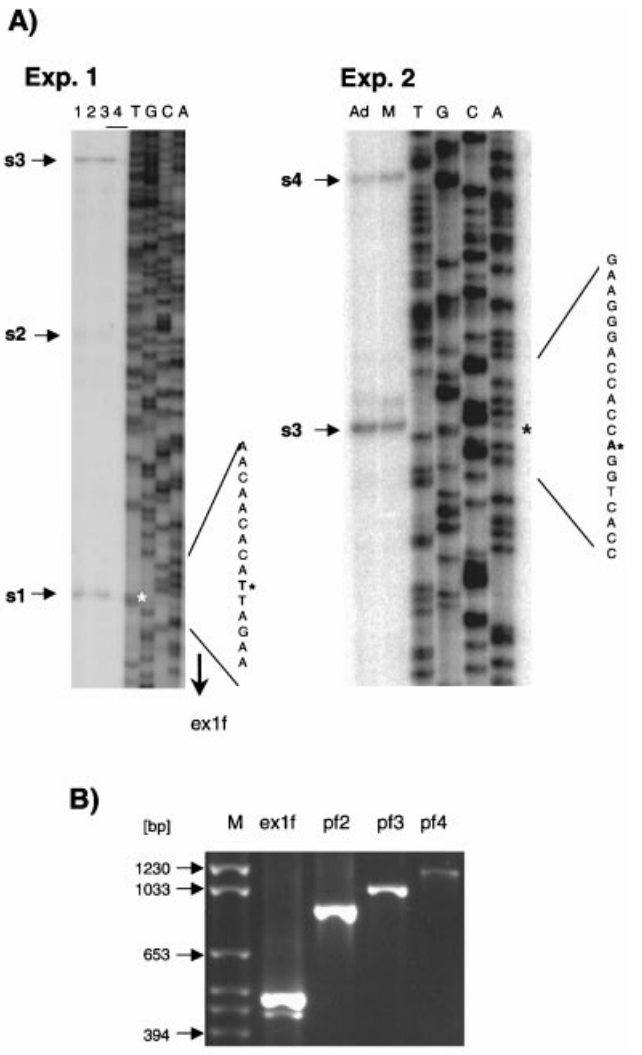

Figure 3 Primer extension analyses of PI

(**A**) Primer extension products obtained in two independent experiments were resolved on sequencing gels, alongside sequencing products (lanes T, G, C and A) of the respective genomic subclone of PI. Four tsps are resolved (s1–s4). The nucleotides complementary to tsp1 and tsp3 are indicated (asterisks) together with a short string of the respective sequence. Products resolved in lanes 1–4 in experiment 1 (Exp. 1) were obtained with total RNA from adipose tissue from an embryo (input 3 μg, lane 1), lactating mammary gland (2 μg, lane 2), white adipose tissue from an adult cow (3 μg, lane 3) and for lane 4 same as in lane 3, but 0.4 μg input only. In experiment 2 (Exp. 2) products obtained with 2 μg of adipose tissue (Ad) and mammary gland (M) from adult cows were resolved. (**B**) Resolution of RT–PCR products, as obtained from adipose tissue RNA with the respective forward primer and an exon 6 reverse primer. The positions of the forward primers are described in the text.

from PI, like those comprising all ACC-α messages, was highest in adipose tissues and mammary gland (Figure 2), approx. 10–20-fold higher than in liver or muscle. The concentrations differ between individuals to a large extent, notably in adipose and mammary-gland tissues. However, PI-derived transcripts comprise quite consistently 20–30 % of the total messages of the ACC-α mRNA molecules. Regarding the different lactational statuses, we note that (1) ACC-α expression in pubertal animals is significantly lower in mammary gland and liver ($P < 0.05$; t test) and (2) lactation does not increase the concentration of PI-derived transcripts in the mammary gland.

We focused next on a functional characterization of PI. A segment of PI comprising 2.6 kbp was retrieved together with exon 1, then sequenced; the promoter was then defined in primer extension analyses. The promoter fragment was also used to drive reporter gene expression in model cells.

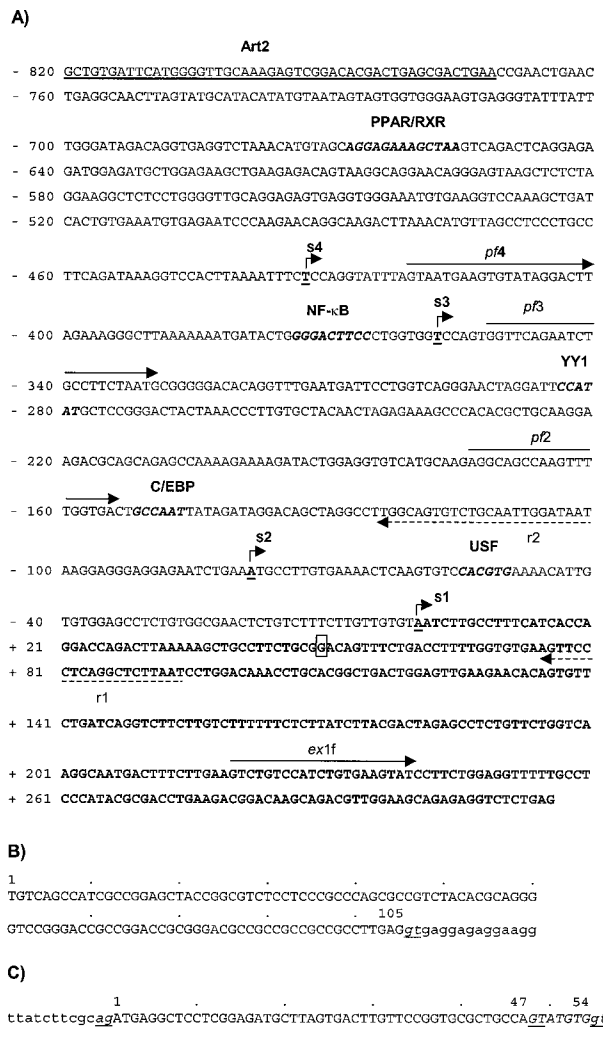

Figure 4 Sequence of PI and untranslated exons

(**A**) Sequence of PI and section of exon 1 (bold). The 'A' nucleotide identified as tsp1 (s1) has been defined as the 5′ start of exon 1. The 5′-starting nucleotide of the longest 5′-RACE clone is boxed. The other tsps (s2–s4) are indicated, together with DNA-binding boxes of transcription factors (USF, upstream stimulating factor; YY1, Yin and Yang 1; NF-κB, nuclear factor κB; PPAR/RXR, PPAR/retinoic X receptor heterodimer-binding site). Arrows labelled ex1f, pf2–4, r1 and r2 denote the positions of oligonucleotides used for RT–PCR amplifications to verify the tsp sites (see Figure 3). Note that the sequence begins at the 5′ end with a well-preserved (92.6% similarity) Art2 element (underlined), extending 5′- of position −1297. (**B**) Sequence of exon 2. Although the 5′ end has not been determined experimentally, the 3′ splice boundary has been defined by RT–PCR clones (GT splice donor underlined). (**C**) Sequence of exon 3. Splice acceptor and donor dinucleotides are underlined. The splice variant is indicated in italics.

PI initiates transcription from multiple starts

Transcriptional start points (tsps) of PI were identified in primer extension experiments on the basis of RNA from adipose and mammary-gland tissue. PI initiates transcription from various tsps (Figures 3 and 4). We first placed a reverse primer located in exon 1 (primer r1; Figure 4) to initiate cDNA synthesis. Resolution of the products revealed at least three tsps (s1, s2 and s3; Figure 3). The comparison of their positions with the sequencing ladder of the appropriate genomic subclone allowed us to identify precisely the nucleotide serving as tsp1 (s1, Figure 3A, Figure 4A), and s2 was also identified in the original autoradiogram. Our longest 5′-RACE clone (from five examined) ended 49 bp in the 3′ direction from this tsp (Figure 4A). More 5′-located tsps were examined in a second primer extension experiment. cDNA synthesis was therefore initiated from a location closer to the 5′ end (primer r2; Figure 4A). This experiment allowed us to identify tsp3 and tsp4 (Figures 3 and 4). The use of these tsps was verified in RT–PCR experiments. Different forward primers (ex1f and pf2–pf4; Figures 3B and 4A) were combined with a reverse primer placed on exon 6 and products amplified from total RNA preparations of adipose tissue. All these amplifications yielded amplicons of the expected sizes (Figure 3B). We subcloned the longest product derived from pf4 and used sequencing to verify its authenticity. The other amplification products were verified in diagnostic restriction digestions exploiting unique cutting sites for *Eco*RV and *Xho*I localized in exons 4 and 5 respectively.

Art2 retroposon element resides within the proximal promoter area of PI

The entire 2.6 kbp promoter segment of PI was sequenced. The promoter does not harbour a TATA-box (Figure 4A). However, the DNA sequence in the vicinity of tsp1 conforms to the consensus for a transcriptional initiator element for transcriptional initiation [35]. We therefore define the respective 'A' nucleotide as position +1 of exon 1. Computer analyses [36] of the proximal promoter sequence feature several attachment sites for transcription factors (Figure 4A) known to be relevant for PI activation in other species. These include binding sites for PPAR/retinoic X receptor heterodimers (at −664) [37], nuclear factor κB (at −374) [38], C/EBPα (at −152), Yin and Yang 1 ('YY1') (at −284) [39] and upstream stimulating factor [40] (at −55) and several binding sites for the hepatic nuclear factors 1 and 4 (at −429, −536, −1126, −1298, −1458 and −1562) (see [41]). Upstream stimulating factor is known to interact with transcriptional-initiator-binding factors [35], but also to mediate the signalling of insulin to promoters of genes involved in fatty acid synthesis [42].

An Art2 retroposon element reaches from position −1297 into the proximal area of the promoter to position −762. The DNA sequence of this retroposon element is 92.6% similar to the reported Art2 element prototype sequence [43]. Another short retroposon element resides further upstream, between positions −2138 and −2022.

Deletion of the retroposon elements relieves the repression of PI

We fused the 2.6 kbp promoter segment of PI to the reporter gene luciferase to permit the characterization of the regulatory properties of PI. Transient transfection of this construct into either murine hepatoma cells (HepRI) or murine mammary epithelia cells (HC11) revealed a low activity of this promoter segment (Figure 5), barely higher than the expression level of the promoterless vector (1.2-fold above pGL3 basic; mean value from many experiments). The main repressive elements were delineated on the basis of three sets of deletion constructs established from PI. The first, coarse mapping, series showed in both cell lines (Figure 5A) that (1) truncation of the long promoter segment to only approx. 1.7 kb results in a doubling of the promoter activity in both cell lines, (2) further shortening (to 1.3 kbp) decreases expression to the basal level, but (3) the very short promoter fragment (127 bp only) is a strong driver of gene expression, 4–9-fold higher than the long segment.

The pattern of repression and derepression was similar in both cell lines, although the HC11 cells had been propagated in the

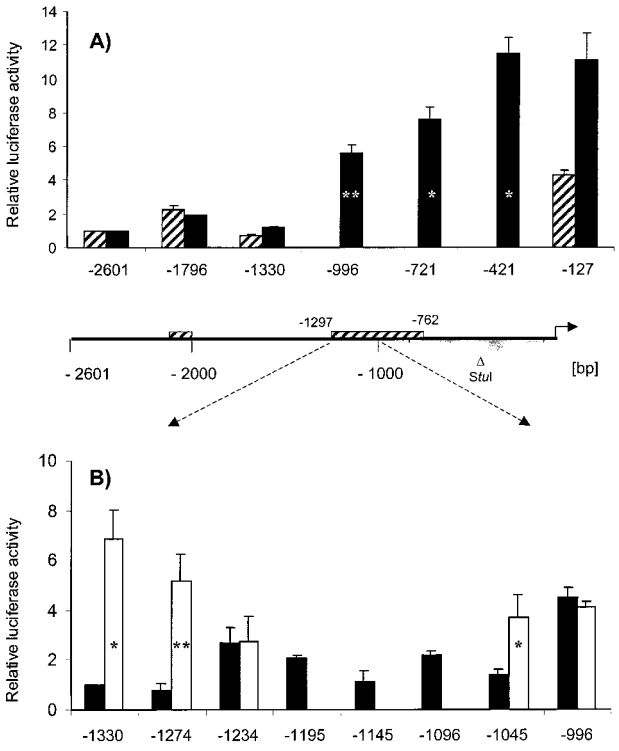

Figure 5 Delineation of repressive elements in PI

(**A**) Coarse delineation of repressor element. Upper panel: the longest segment of PI (−2601) or deletions of the promoter (5′-terminal positions indicated) were used to drive luciferase expression in transient transfections of HC11 or HepRI cells (black and hatched columns respectively). Results are means ± S.E.M. for three independent experiments for each construct, represented as fold differences relative to the longest promoter segment. Transfection efficiencies were normalized on the basis of the co-transfected β-galactosidase expression construct. Significant increases in activity were observed on truncating further the −1330 or −721 bp constructs (*t* test; $^{*}P < 0.05$, $^{**}P < 0.01$). Lower panel: the locations of both retroposon elements within the promoter sequence are indicated by hatched boxes. Note that the deletion of the 5′ half of the Art2 element results in a relief of repression. (**B**) Fine mapping of the distal PI repressive element and evidence for two separate repressive elements. The crucial portion of the Art2 element was finely mapped in HC11 cells by placing six more deletion constructs into this area (−1274 to −1045) and examining their expression potential relative to construct −1330 (just 5′ of the Art2 element) taken as 1 (filled columns). The strongest increase is seen between positions −1045 and −996, which is highly significant (*t* test, $P < 0.001$). Note the good quantitative agreement of values from (**A**) and (**B**) for constructs −1330 and −996 respectively. The expression levels of five of these constructs (−1330, −1274, −1234, −1045 and −996) were compared with those obtained with constructs that were similar (open columns) but from which 801 bp had been deleted between the *Stu*I sites (**A**, lower panel). These clones are derepressed. Statistically significant differences between the basic and the respective *Stu*I-deleted construct are indicated as for (**A**).

presence of insulin and dexamethasone, whereas HepRI cells were grown without these additives. Hence neither of these components known to induce PI in adipocytes *in vivo* [13] could relieve repression in these transient transfections. These constructs have also been stably transfected into HC11 cells to examine whether, in the presence of insulin, their expression might be induced by the lactogenic hormones dexamethasone and prolactin. Neither of these components resulted in relief of repression or induction (results not shown).

The next set of deletions (examined in HC11 cells only) mapped the main repressive element between positions −1330 and −996 (Figure 5A). Deletion of this 334 bp of the promoter segment increased expression more than 5-fold. This elevated promoter activity increased by a further approx. 60% on deletion of the segment between positions −721 and −421.

The third set of promoter deletions was designed to map more precisely the crucial area for repression, eventually between positions −1330 and −996. This set of six finely graded deletions reproducibly revealed a modulated pattern of considerable shifts in promoter activity (Figure 5B, filled bars). However, a crucial element resided between positions −1045 and −996. Deleting these nucleotides caused a steep increase in reporter gene activity. A main repressive element therefore resides within the central part of the Art2 retroposon element, which is located between positions −1297 and −772.

However, this sequence element alone was not sufficient to block transcription: it required the presence of another more proximal part of the promoter. This feature became evident in the analysis of five of these reporter gene constructs, from which we had deleted 801 bp from the proximal promoter region, between the *Stu*I sites at positions −828 and −127. These constructs (Figure 5B, open bars) revealed approximately the same activity as that measured in the construct from which that main distal repressor had been deleted (clone −996; Figure 5B). Repression of the basal activity of the bovine PI therefore requires the presence of two separated sequence elements, neither of which functions alone.

DISCUSSION

We present the first DNA sequence data of the gene from cattle encoding ACC-α. The reported DNA sequences comprise the entire cDNA sequence, the promoter sequences of PI and those of all exons contributing to the 5′ UTR region of the mRNA. These DNA sequence data are relevant for research into farm animal breeding because they provide an inevitable and necessary basis for a systematic investigation into the molecular controls regulating the expression of a candidate gene for two commercially interesting traits, the production of body fat and milk fat, in that commercially significant farm animal species. Our fine mapping data of *cis* elements regulating the basal activity of PI are of general biochemical interest because a comparable analysis has until now been conducted only in the rat [15,44]. Our data therefore now permit, for the first time, a comparative evaluation of sequence elements and promoter structures necessary to control the activity of PI.

cDNA sequence and 5′ UTR structure are highly conserved, whereas exons 1 and 3 have divergent evolutionary histories

The cDNA sequence of bovine ACC-α is highly similar to the sheep sequence [33], as expected. Remarkable, though, is the exceptionally high degree of evolutionary preservation of the protein sequence of the large enzyme ACC-α. The 97.9% sequence similarity of the ACC-α protein observed between the bovine and rat enzyme exceeds, for instance, the 95.6% of similarity between the STAT5A transcription factors of the same species [45]. This highlights the fact that the ancient enzyme common to all eukaryotes has been functionally optimized and was structurally fixed a long time ago.

The observed heterogeneity of the 5′ UTR regions of the bovine ACC-α mRNA conforms well to that what is known from rat and human [17,18]. However, neither has a splice variant of exon 3 been reported previously, nor has a class 2 message been found of the composition (2:3:5). We detected this type of a class 2 message in all tissues analysed, but not, however, in adipose tissue and brain.

The high degree of evolutionary conservation of the ACC-α protein sequence is in stark contrast with the divergent origins of exons 1 and 3 in different species. Whereas the sequences of exons 4 and 5 are very well preserved among mammals, exons 1 and 3 are different in this regard (Table 1). The sequence of the bovine exon 1 is homologous with the closely related sheep species (92.3 %) but its degree of relatedness is only 64.6 % and 48.3 % to exon 1 of rat and human respectively. These are also not related to each other (49.5 % similarity). This indicates an absence of DNA sequence similarity of exon 1 (Table 1). The only other exon 3 sequence known has been reported from rat [10,11]; it comprises 61 bp and is completely unrelated to the bovine analogue. The best fit of the alignments is obtained in reverse orientation, resulting in 57.8 % sequence 'similarity' in a 45 nt overlap. The interspecies comparison of the exon sequences therefore suggests that in different species previously unrelated genomic segments have been recruited to serve as functional homologues for exons 1 and 3 respectively. However, exon 1 is conserved between cattle and sheep, indicating that it shares a common evolutionary history in the Bovidae. In contrast, exons 2, 4 and 5 stem from conserved segments of a primordial gene.

Bovine and rat PI conceivably have different origins

Our primer extension analyses reveal considerable heterogeneity in the length of transcripts initiated from PI, seen reproducibly in three experiments with RNA preparations from two different tissues. The RT–PCR control amplifications verify the natural occurrence of all of these transcripts. They also suggest that there is no other promoter possibly located further upstream. Hence PI, as defined by this study, is the farthest 5′-located ACC-α promoter in cattle. The promoter does not contain a TATA-box DNA sequence motif, either in the vicinity of the various tsps or in the whole proximal promoter area reaching 5′ upstream to the Art2 retroposon element (Figure 4). TATA-box-less promoters are known to initiate transcription from multiple tsps. The functionally analogous PI of the rat does contain a TATA-box [15]. Hence PI in rat relies on a TATA-box, and thus might exploit the TATA-box-binding protein (TBP) [46] to activate transcription, whereas bovine PI is conceivably activated by basal transcription factors other than TBP. This major difference in the key feature of a promoter indicates that not only are exons 1 evolutionarily unrelated between rat and cattle (Table 1), but also the respective gene segments serving as promoters 1 are of different evolutionary origins. A direct sequence comparison of the bovine PI sequence with those of other species is impossible because, to the best of our knowledge, no other PI sequence is publicly available, from rat or any other species.

Tissue-specific activity of bovine PI conforms to other species

The abundance of transcripts derived from bovine PI is highest in adipose tissue and mammary gland. We based our survey of the abundance of transcripts derived from PI exclusively on RT–PCR amplifications as measured with Real Time PCR, because this method permits the analysis of the authentic transcript from this promoter, unbiased by any other transcript possibly derived from the activity of the other two promoters. Although our data represent only a first evaluation of gross differences in the relative abundance of these transcripts in various, they reveal two aspects very clearly. First, PI transcripts are very abundant in adipose tissue, as in homologous transcripts from other species [14,17,19]. Taking the abundance of transcripts as a parameter for promoter activity thus indicates that the bovine PI is very active in adipose tissue, like PI from other species. Secondly, transcripts from PI are also very prominent in mammary gland, but their concentration does not increase during lactation. Mammary-gland tissue contains many adipocytes. Hence it is not surprising that the bovine mammary gland is a rich source for transcripts from PI. Our amplifications show that their concentration does not increase during lactation, although the total concentration of the ACC-α mRNA increases during lactation in this tissue (Figure 2) [13,14,21]. PI might not be the promoter driving the lactationally stimulated expression of the ACC-α encoding gene in mammary epithelial cells, either in cattle (this study) or in rat [17]. Rather, PIII is the lactationally stimulated bovine ACC-α promoter, as has previously been reported from sheep [20].

Functional structure of the bovine PI repressive element is similar to that from rat, but is mediated by entirely different DNA sequences

The PI variants from both cattle and rat are basically repressed by distal sequence elements. They reveal very high activity in reporter gene assays if only short proximal segments are used {bovine, 421 or 127 bp (this study); rat, 204 or 68 bp [15]}. The reported ACC-α promoter from chicken [47] is indeed a PII homologue and it loses its activity if truncated to less than 194 bp. Hence the active repression of an otherwise strongly driving element of transcription seems to be a common property of ACC-α PI.

The reporter gene assays of our PI promoter deletion series show that two different sequence elements together need to be present for the repression of PI. Although the proximal element has not been finely delineated, we narrowed down the location of the crucial segment of the distal element to between positions -1045 and -996. Thus it resides in the centre of a well-preserved Art2 retroposon element, just as it is found in at least 100000 copies in the bovine genome [37]. Thus the bipartite structure of the repressive element of the bovine PI resembles that of the functional counterpart in rat. However, in rat a $(CA)_{28}$-type microsatellite serves as main structural component of this repressor, the effect of which is relieved by the binding of C/EBP protein to a GCAAT sequence motif in the proximal promoter [15,44]. The evolutionary origin, primary sequence and structure of Art2 retroposon elements are entirely different from a microsatellite repeat sequence. The only feature shared by both elements is their widespread occurrence in the genome. Hence unrelated genome segments have been recruited in different mammalian species into the promoter region of this ancient gene, belonging to the core genome of all live organisms.

It is an unprecedented situation that both PI promoters include and apparently make functional use of unrelated but highly abundant sequence elements. These elements of divergent evolutionary origin display physiologically quite similar regulatory properties. However, the unrelated nature of the regulatory elements of PI demands that great care must be exercised if conclusions on molecular controls of this promoter are to be inferred from one species to another, despite the physiological similarities of regulatory properties of PI in different species. Our promoter deletion series permits the dissection of components of the signal transduction pathways regulating bovine PI expression in adipocytes in further experiments.

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REFERENCES

1 Kim, K. H. (1997) Regulation of mammalian acetyl-coenzyme A carboxylase. Annu. Rev. Nutr. **17**, 77–99
2 Thampy, K. G. and Wakil, S. J. (1988) Regulation of acetyl-coenzyme A carboxylase. II. Effect of fasting and refeeding on the activity, phosphate content, and aggregation state of the enzyme. J. Biol. Chem. **263**, 6454–6458
3 Wakil, S. J., Stoops, J. K. and Joshi, V. C. (1983) Fatty acid synthesis and its regulation. Annu. Rev. Biochem. **52**, 537–579
4 Carlson, C. A. and Kim, K. H. (1974) Regulation of hepatic acetyl coenzyme A carboxylase by phosphorylation and dephosphorylation. Arch. Biochem. Biophys. **164**, 478–489
5 Hardie, D. G. (1989) Regulation of fatty acid synthesis via phosphorylation of acetyl-CoA carboxylase. Prog. Lipid Res. **28**, 117–146
6 Brownsey, R. W., Zhande, R. and Boone, A. N. (1997) Isoforms of acetyl-CoA carboxylase: structures, regulatory properties and metabolic functions. Biochem. Soc. Trans. **25**, 1232–1238
7 Batenburg, J. J. and Whitsett, J. A. (1989) Levels of mRNAs coding for lipogenic enzymes in rat lung upon fasting and refeeding and during perinatal development. Biochim. Biophys. Acta **1006**, 329–334
8 Eritani, N., Fukuda, H. and Matsumura, Y. (1993) Lipogenic enzyme gene expression in rat liver during development after birth. J. Biochem. (Tokyo) **113**, 519–525
9 Lopez-Casillas, F., Bai, D. H., Luo, X. C., Kong, I. S., Hermodson, M. A. and Kim, K. H. (1988) Structure of the coding sequence and primary amino acid sequence of acetyl-coenzyme A carboxylase. Proc. Natl. Acad. Sci. U.S.A. **85**, 5784–5788
10 Lopez-Casillas, F., Luo, X. C., Kong, I. S. and Kim, K. H. (1989) Characterization of different forms of rat mammary gland acetyl-coenzyme A carboxylase mRNA: analysis of heterogeneity in the 5′ end. Gene **83**, 311–319
11 Luo, X. C., Park, K., Lopez-Casillas, F. and Kim, K. H. (1989) Structural features of the acetyl-CoA carboxylase gene: mechanisms for the generation of mRNAs with 5′ end heterogeneity. Proc. Natl. Acad. Sci. U.S.A. **86**, 4042–4046
12 Travers, M. T., Vernon, R. G. and Barber, M. C. (1997) Repression of the acetyl-CoA carboxylase gene in ovine adipose tissue during lactation: the role of insulin responsiveness. J. Mol. Endocrinol. **19**, 99–107
13 Travers, M. T. and Barber, M. C. (1999) Insulin-glucocorticoid interactions in the regulation of acetyl-CoA carboxylase-α transcript diversity in ovine adipose tissue. J. Mol. Endocrinol. **22**, 71–79
14 Lopez-Casillas, F., Ponce-Castaneda, M. V. and Kim, K. H. (1991) *In vivo* regulation of the activity of the two promoters of the rat acetyl coenzyme-A carboxylase gene. Endocrinology **129**, 1049–1058
15 Tae, H. J., Luo, X. and Kim, K. H. (1994) Roles of CCAAT/enhancer-binding protein and its binding site on repression and derepression of acetyl-CoA carboxylase gene. J. Biol. Chem. **269**, 10475–10484
16 Luo, X. C. and Kim, K. H. (1990) An enhancer element in the house-keeping promoter for acetyl-CoA carboxylase gene. Nucleic Acids Res. **18**, 3249–3254
17 Ponce-Castaneda, M. V., Lopez-Casillas, F. and Kim, K. H. (1991) Acetyl-coenzyme A carboxylase messenger ribonucleic acid metabolism in liver, adipose tissues, and mammary glands during pregnancy and lactation. J. Dairy Sci. **74**, 4013–4021
18 Ha, J., Daniel, S., Kong, I. S., Park, C. K., Tae, H. J. and Kim, K. H. (1994) Cloning of human acetyl-CoA carboxylase cDNA. Eur. J. Biochem. **219**, 297–306
19 Abu-Elheiga, L., Jayakumar, A., Baldini, A., Chirala, S. S. and Wakil, S. J. (1995) Human acetyl-CoA carboxylase: characterization, molecular cloning, and evidence for two isoforms. Proc. Natl. Acad. Sci. U.S.A. **92**, 4011–4015
20 Barber, M. C. and Travers, M. T. (1998) Elucidation of a promoter activity that directs the expression of acetyl-CoA carboxylase α with an alternative N-terminus in a tissue-restricted fashion. Biochem. J. **333**, 17–25
21 Travers, M. T. and Barber, M. C. (1997) Tissue-specific control of the acetyl-CoA carboxylase gene. Biochem. Soc. Trans. **25**, 1215–1219
22 Stephens, J. M., Morrison, R. F., Wu, Z. and Farmer, S. R. (1999) PPARγ ligand-dependent induction of STAT1, STAT5A, and STAT5B during adipogenesis. Biochem. Biophys. Res. Commun. **262**, 216–222
23 Oberfield, J. L., Collins, J. L., Holmes, C. P., Goreham, D. M., Cooper, J. P., Cobb, J. E., Lenhard, J. M., Hull-Ryde, E. A., Mohr, C. P., Blanchard, S. G. et al. (1999) A peroxisome proliferator-activated receptor γ ligand inhibits adipocyte differentiation. Proc. Natl. Acad. Sci. U.S.A. **96**, 6102–6106
24 Shi, X. M., Blair, H. C., Yang, X., McDonald, J. M. and Cao, X. (2000) Tandem repeat of C/EBP binding sites mediates PPARγ2 gene transcription in glucocorticoid-induced adipocyte differentiation. J. Cell Biochem. **76**, 518–527
25 Stewart, W. C., Morrison, R. F., Young, S. L. and Stephens, J. M. (1999) Regulation of signal transducers and activators of transcription (STATs) by effectors of adipogenesis: coordinate regulation of STATs 1, 5A, and 5B with peroxisome proliferator-activated receptor-γ and C/AAAT enhancer binding protein-α. Biochim. Biophys. Acta **1452**, 188–196
26 Teglund, S., McKay, C., Schuetz, E., van Deursen, J. M., Stravopodis, D., Wang, D., Brown, M., Bodner, S., Grosveld, G. and Ihle, J. N. (1998) Stat5a and Stat5b proteins have essential and nonessential, or redundant, roles in cytokine responses. Cell **93**, 841–850
27 Koczan, D., Hobom, G. and Seyfert, H. M. (1991) Genomic organization of the bovine α-S1 casein gene. Nucleic Acids Res. **19**, 5591–5596
28 Cai, L., Taylor, J. F., Wing, R. A., Gallagher, D. S., Woo, S. S. and Davis, S. K. (1995) Construction and characterization of a bovine bacterial artificial chromosome library. Genomics **29**, 413–425
29 Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. and Rutter, W. J. (1979) Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry **18**, 5294–5299
30 Don, R. H., Cox, P. T., Wainwright, B. J., Baker, K. and Mattick, J. S. (1991) 'Touchdown' PCR to circumvent spurious priming during gene amplification. Nucleic Acids. Res. **19**, 4008
31 Henke, M., Hobom, G., Senft, B. and Seyfert, H. M. (1996) Structural deviations in a bovine low expression lysozyme-encoding gene active in tissues other than stomach. Gene **178**, 131–137
32 Welte, T., Garimorth, K., Philipp, S. and Doppler, W. (1994) Prolactin-dependent activation of a tyrosine phosphorylated DNA binding factor in mouse mammary epithelial cells. Mol. Endocrinol. **8**, 1091–1102
33 Barber, M. C. and Travers, M. T. (1995) Cloning and characterisation of multiple acetyl-CoA carboxylase transcripts in ovine adipose tissue. Gene **154**, 271–275
34 Takai, T., Yokoyama, C., Wada, K. and Tanabe, T. (1988) Primary structure of chicken liver acetyl-CoA carboxylase deduced from cDNA sequence. J. Biol. Chem. **263**, 2651–2657
35 Javahery, R., Khachi, A., Lo, K., Zenzie-Gregory, B. and Smale, S. T. (1994) DNA sequence requirements for transcriptional initiator activity in mammalian cells. Mol. Cell Biol. **14**, 116–127
36 Quandt, K., Frech, K., Karas, H., Wingender, E. and Werner, T. (1995) MatInd and MatInspector: new fast and versatile tools for detection of consensus matches in nucleotide sequence data. Nucleic Acids Res. **23**, 4878–4884
37 Palmer, C. N., Hsu, M. H., Griffin, H. J. and Johnson, E. F. (1995) Novel sequence determinants in peroxisome proliferator signaling. J. Biol. Chem. **270**, 16114–16121
38 Gilmore, T. D. (1990) NF-κB, KBF1, dorsal, and related matters. Cell **62**, 841–843
39 Guo, B., Odgren, P. R., van Wijnen, A. J., Last, T. J., Nickerson, J., Penman, S., Lian, J. B., Stein, J. L. and Stein, G. S. (1995) The nuclear matrix protein NMP-1 is the transcription factor YY1. Proc. Natl. Acad. Sci. U.S.A. **92**, 10526–10530
40 Bendall, A. J. and Molloy, P. L. (1994) Base preferences for DNA binding by the bHLH-Zip protein USF: effects of MgCl2 on specificity and comparison with binding of Myc family members. Nucleic Acids Res. **22**, 2801–2810
41 McNair, A., Cereghini, S., Brand, H., Smith, T., Breillat, C. and Gannon, F. (2000) Synergistic activation of the Atlantic salmon hepatocyte nuclear factor (HNF) 1 promoter by the orphan nuclear receptors HNF4 and chicken ovalbumin upstream promoter transcription factor I (COUP-TFI). Biochem. J. **352**, 557–564
42 Wang, D. and Sul, H. S. (1997) Upstream stimulatory factor binding to the E-box at −65 is required for insulin regulation of the fatty acid synthase promoter. J. Biol. Chem. **272**, 26367–26374
43 Duncan, C. H. (1987) Novel Alu-type repeat in artiodactyls. Nucleic Acids Res. **15**, 1340–1340
44 Tae, H. J., Zhang, S. and Kim, K. H. (1995) cAMP activation of CAAT enhancer-binding protein-beta gene expression and promoter I of acetyl-CoA carboxylase. J. Biol. Chem. **270**, 21487–21494
45 Seyfert, H. M., Pitra, C., Meyer, L., Brunner, R. M., Wheeler, T. T., Molenaar, A., McCracken, J. Y., Herrmann, J., Thiesen, H. J. and Schwerin, M. (2000) Molecular characterization of STAT5A- and STAT5B-encoding genes reveals extended intragenic sequence homogeneity in cattle and mouse and different degrees of divergent evolution of various domains. J. Mol. Evol. **50**, 550–561
46 Kim, J. L., Nikolov, D. B. and Burley, S. K. (1993) Co-crystal structure of TBP recognizing the minor groove of a TATA element. Nature (London) **365**, 520–527
47 El Khadir-Mounier, C., Le Fur, N., Powell, R. S., Diot, C., Langlois, P., Mallard, J. and Douaire, M. (1996) Cloning and characterization of the 5′ end and promoter region of the chicken acetyl-CoA carboxylase gene. Biochem. J. **314**, 613–619

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