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**Isolation and Characterisation of the 5' Region Sequence for
the Bovine ATP-Citrate Lyase Gene**

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Abstract

ATP-citrate lyase (ACL) is one of the major lipogenic enzymes. It catalyzes the synthesis of acetyl-CoA from citrate in the cytosol. This is the first committed step towards the conversion of carbohydrate precursors into fatty acids. Acetyl-CoA serves as the major precursor for lipogenesis and cholestogenesis. Examination of this pathway shows that the rate of fatty acid synthesis from glucose is dependent on the activity of ACL. In rats the activity of this enzyme can be increased by feeding high carbohydrate diet and reduced to low levels by fasting. These changes are regulated at the transcriptional level.

The ruminant provides a good model to study the regulation of expression of ACL. The levels of this enzyme are high in young ruminants, but fall to very low levels once a functional rumen is developed. In adult ruminants, acetyl-CoA for fatty acid synthesis is produced directly from acetate formed by microbial fermentation in the rumen and carried to the peripheral tissues. The down-regulation of this enzyme can be reversed by the administration of glucogenic precursors by a route that bypasses their fermentation to volatile fatty acids in the rumen. An understanding of the regulation of expression of ACL in the adult ruminant and a comparison with monogastric animals will provide significant new information about the regulation of the conversion of carbohydrate into fat.

A probe containing exon 2 to exon 3 of the rat ACL gene was prepared. Its specificity to bovine genomic DNA was verified and the probe was then used to screen a bovine λ genomic library. A 17 kb clone was isolated. The restriction map of this clone was determined with several enzymes. A part of this clone (9490 base pairs) was sequenced and shown to consist of a 3 kb promoter region and downstream sequence as far as intron 3 of bovine ACL. The transcription start sites were determined by 5'RACE. Several important features of this gene were discovered by computer analysis of the sequence. Two key transcription factor binding sites were found in the promoter region. This work provided a solid basis for further investigation towards elucidating the mechanism of the transcriptional regulation of bovine ACL and the process of lipogenesis.

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Abbreviations

ACC	acetyl CoA carboxylase
ACP	acyl carrier protein
ACS	acetyl CoA synthetase
ACL	ATP citrate lyase
ADD-1	adipocyte determination and differentiation factor-1
ATP	adenosine triphosphate
b/HLH/LZ	basic/helix-loop-helix/leucine zipper
ChoRE	carbohydrate response element
cDNA	complementary DNA
CoA	coenzyme A
cpm	counts per minute
ddNTP	dideoxynucleotide triphosphate
DEPC	diethylpyrocarbonate
dH ₂ O	deionised water
Dnase	deoxyribonuclease
dNTP	deoxynucleotide triphosphate
DTT	dithiothreitol
EDTA	ethylenediamine tetraacetic acid
EEO	electroendosmosis
FAS	fatty acid synthase
GIRE	glucose response element
GLUT	glucose transporter
GSP	gene-specific oligonucleotide
HEPES	N-2-hydroxyethyl piperazine-N'-2-ethane sulfonic acid
HMC-CoA	3-hydroxy-3-methylglutaryl-CoA
IPTG	isopropyl β -D-thiogalactoside
LDL	low density lipoprotein
L-PK	L-type pyruvate kinase
λ	bacteriophage lambda

mRNA	messenger RNA
NADPH	nicotinamide adenine dinucleotide phosphaste, reduced form
NLS	n-lauryl sarcosine
nt	nucleotide
PCR	polymerase chain reaction
pfu	plaque forming units
Pol II	RNA polymerase II
PUFA	polyunsaturated fatty acids
RACE	Rapid Amplification of cDNA Ends
RNase	ribonuclease
rpm	revolutions per minute
RT	reverse transcriptase
SCD	stearoyl-CoA desaturase
SDS	sodium dodecyl sulphate
SRE	sterol regulatory element
SREBP	sterol regulatory element binding protein
SSC	sodium chloride and sodium citrate solution
TAE	tris-acetate buffer containing EDTA
<i>Taq</i>	<i>Thermus aquaticus</i>
TBP	TATA box binding protein
TdT	terminal deoxynucleotidyl transferase
TE	tris-HCl buffer containing EDTA
TSS	transcription start site
USF	upstream stimulating factor
UTR	untranslated region
UV	ultraviolet light
VFA	volatile fatty acids