The Regulation of Bovine ATP citrate Lyase Promoter

By

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Abstract

The synthesis of fatty acids is important for many house keeping functions such as the formation of cell membranes and as energy storage. This process occurs mostly in the adipose tissues and liver of monogastric animals.

The regulation of fatty acid biosynthesis in monogastric animals such as human and rat have been studied intensively. Several lines of experimental evidences have shown that fatty acid biosynthesis is dependent on the nutritional state of the animal and other hormonal influences, such as insulin and glucagon. However the molecular regulation of fatty acid biosynthesis is relatively unknown in ruminants.

Ruminants are large mammals that have a predominantly herbivorous diet and therefore have a very different metabolism to monogastric animals. Although a large percentage of ruminant feed is carbohydrate, very little of these dietary carbohydrates are available for *de novo* fatty acid biosynthesis and therefore many of the enzymes involved in the conversion of glucose to fat such as ATP citrate lyase may be down-regulated as a mean of physiological adaptation for glucose conservation.

ATP citrate lyase (ACLY) is a lipogenic enzyme that catalyses the cleavage of cytosolic citrate into acetyl CoA and oxaloacetate and it is unique to the fatty acid biosynthesis pathway. The molecular regulation of the bovine ACLY gene is unknown, however approximately 10 Kb of bovine ACLY gene has been sequenced and characterised. To investigate the molecular regulation of the bovine ATP citrate lyase gene, several experimental methods were used in this study such as reporter gene assays and electrophoretic mobility shift assays.

Abbreviations

A	Adenine
ACC	Acetyl CoA Carboxylase
ACLY	ATP citrate lyase
Amp	Ampicillin
ATP	Adenine triphosphate
BLAST	Basic local alignment search tool
bp	Base pair (DNA)
BSA	Bovine serum albumin
C	Cytosine
cAMP	Cylic Adenine monophosphate
ChREBP	Carbohydrate response element binding protein
CLUSTAL W	General purpose multiple sequence alignment program
Cpm	Count per minutes
CRE	cAMP response element
DMSO	Dimethyl sulfoxide
DNA	Deoxyribose nucleic acid
DTT	Dithiothrethiol
EDTA	Ethylene diamine tetra-acetic acid
EMSA	Electrophoretic mobility shift assay
FAD^-	Flavin adenine dinucleotide (oxidised)
FADH	Flavin adenine dinucleotide (reduced)
FAS	Fatty acid synthase
FCS	Foetal calf serum
G	Guanine
GRE	Glucocorticoid response element
HCL	Hydrochloric acid
HeLa	Human cervical carcinoma cells
HNF	Hepatocyte nuclear factor
IPTG	Isopropyl thiogalactosidase
Kb	Kilo base (DNA)

LB	Luria Bertani
L-PK	Liver pyruvate kinase
luc	Luciferase
MCS	Multiple cloning site
MFPK	Multifunctional protein kinase
MgCl	Magnesium chloride
mt	Mutant or mutated
MWT	Molecular Weight
NaCl	Sodium chloride
NAD^+	Nicotinamide adenine dinucleotide (oxidised)
NADH	Nicotinamide adenine dinucleotide (reduced)
$NADP^+$	Nicotinamide adenine dinucleotide phosphate (oxidised)
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced)
NFY	Nuclear factor Y
OAA	Oxaloacetate
ONPG	O-Nitrophenol β-D-galactosidase-pyranoside
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PBSE	Phosphate buffered saline plus EDTA
PCR	Polymerase chain reaction
PEG	Polyethelyne glycol
pGL3B	pGL3 basic plasmid
PKA	Protein kinase A
PKB	Protein kinase B
RNA	Ribose nucleic acid
RNase	Ribonuclease
Rpm	Revolution per minute
RT	Reverse transcriptase
RT-PCR	Reverse transcription polymerase chain reaction
SDS	Sodium dodecyl sulphate
SP1	Specificity protein 1
SP3	Specificity protein 3
SRE	Sterol regulatory element

SREBP	Sterol regulatory element binding protein
STET	Sucrose, tris base, EDTA and triton X buffer
T	Thymine
TAE	Tris base, Acetic acid and EDTA
Taq	*Thermus aquaticus* DNA polymerase enzyme
TBE	Tris borate EDTA buffer
TE	Tris-EDTA buffer
TEMED	N,N,N',N'-Tetramethylethylenediamine
TEN	Tris-EDTA buffer with sodium
Tet	Tetracycline
TRE	Thyroid hormone receptor response element
UV	Ultra violet light
wt	Wild type
X-gal	5-bromo-4-chromo-3-indolyl-β-D-galactosidase
β Gal	β galactosidase

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