ISOLATION OF 5' REGULATORY SEQUENCES FOR RUMINANT ATP CITRATE LYASE

A thesis presented in partial fulfilment of the requirements for the degree of
Master of Science in Biochemistry
at Massey University

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1998

ABSTRACT

ATP citrate lyase is an essential enzyme in the pathway for conversion of glucose to fatty acids in mammalian tissues. The enzyme catalyses the cleavage of cytosolic citrate to acetyl CoA and oxaloacetate in an ATP-dependent reaction. The sequence of the cDNAs for both rat and human ATP citrate lyase have been published and have 96.3% identity at the amino acid level. This high level of identity may also extend to other mammals, including ruminants. The ruminant presents a unique system in which to study the regulation of ATP citrate lyase as levels of expression of the enzyme change during the development of a functional rumen. An analysis of the 5'-regulatory region of ruminant ATP citrate lyase will be important in determining factors that contribute to the developmental regulation of this enzyme in ruminants.

In order to analyse the 5'-regulatory region of ruminant ATP citrate lyase, a probe was constructed with which to screen an amplified bovine genomic library. The probe was produced by cloning a 282 bp PCR product containing rat ATP citrate lyase exon II sequence, amplified from rat genomic DNA. This clone was sequenced to verify that it contained rat ATP citrate lyase exon II sequence.

This probe was then used for northern and Southern blotting, and for screening an amplified bovine genomic library. Northern blotting of rat and lamb total RNA showed that the probe hybridised with rat RNA, but not with lamb RNA. Conditions for hybridisation were not optimised, as hybridisation between the probe and rat RNA was not as specific as expected. The quality of RNA used for preparing the northern blots could have also affected the specificity of hybridisation.

Southern blotting experiments were also inconclusive, as the hybridisation signals seen were not specific. However the probe was shown to hybridise to rat and human genomic DNA.

Screening of the bovine genomic library was unsuccessful, but once conditions for hybridisation are optimised, then the probe could be used to rescreen the amplified bovine genomic library, and isolate a clone containing the 5'-regulatory sequences for bovine ATP citrate lyase.

ACKNOWLEDGEMENTS

I would like to thank Kathryn Stowell and John Tweedie for their supervision, guidance and encouragement during my thesis. I would also like to thank the past and present members of the Twilight Zone for all their help and friendship provided during my time in the lab - it has been a truly memorable experience. Finally, I would like to thank my family and friends for their love and support.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

A	adenine
Amp	ampicillin
bp	base pair
BRL	Bethseda Research Laboratories
cDNA	complementary deoxyribonucleic acid
C	cytosine
cpm	counts per minute
Da	dalton
DNA	deoxyribonucleic acid
DNAse	deoxyribonuclease
dCTP	deoxycytidine triphosphate
E. coli	*Escherichia coli*
EDTA	ethylene diamine tetra-acetate
EEO	electroendosmosis
G	guanine
IPTG	isopropyl β-D-thiogalactopyranoside
kb	kilobase
kDa	kilodalton
λ	Bacteriophage lambda
LMP	low melting point
mRNA	messenger ribonucleic acid
µg	microgram
µl	microlitre
mM	millimole
ng	nanogram
nm	nanometres
nt	nucleotide
OD	optical density
PCR	polymerase chain reaction
pfu	plaque forming units
pmol	picomole
RNA	ribonucleic acid

RNAase	ribonuclease
SDS	sodium dodecylsulphate
SSC	standard saline citrate
T	thymine
TAE	Tris acetate EDTA
Taq	*Thermus aquaticus*
TE	Tris (10 mM) EDTA (1 mM) pH 8.0
TEMED	N, N, N', N'-Tetramethylethlyenediamine
U	unit
UV	ultraviolet
X-Gal	5-bromo-4-chloro-3-indolyl β-D-galactopyranoside