Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

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

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

**Xingzhang Tong**

**2001**

# 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 doenstream seqence 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.

# Acknowledgement

I sincerely thank my supervisor, Associate Professor John Tweedie, for his remarkable guidance and supervision. His encouragement, patience and enthusiasm throughout the course of this project were deeply appreciated. I would also like to gratefully thank Carole Flyger for her help in preparing the probe and general guidance, Carolyn Young for her help in the library screening, and all members of the Twilight Zone for their help and advice in the lab.

# TABLE OF CONTENTS

# LIST OF FIGURES

# LIST OF TABLES

# 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 |
| $dH_2O$ | deionised water |
| Dnase | deoxyribonuclease |
| dNTP | deoxynucleotide triphosphate |
| DTT | dithiothreitol |
| EDTA | ethylenediamine tetraacetic acid |
| EEO | electroendosmosis |
| FAS | fatty acid synthase |
| GlRE | 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 |
| *Taq* | *Thermus aquaticus* |
| 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 |