The Bovine Lactoferrin Gene;

Defining the Minimal Promoter Region

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1997

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

Dedication

This thesis is dedicated to the memory of my grandfather,

Ted Bonney.

Acknowledgements

I would like to thank John Tweedie and Kathryn Stowell for the opportunity to work on this project and for all the help and guidance they have provided in the course of this study.

I thank my parents Philip and Tina for supporting my decision to attend University and helping me get there, and also my sisters Celeste and Aletta, my brother Matthew and my extended family for their interest in what I was doing.

Thanks to all the members of the Twilight Zone and my classmates for their help and making the lab a fun place to work.

I would also like to thank the NZSBMB for providing funding to attend the 1996 NZIC/NZSBMB conference in Dunedin to present this research in a student paper competition.

A special thanks to Rachel Williamson for her patience and help in getting this finished.

Abstract

Lactoferrin is an 80 kDa glycoprotein with two lobes, each of which bind a single iron atom. Originally isolated from milk, lactoferrin has since been identified in a variety of exocrine secretions and in the secretory granules of neutrophils. A number of functions have been proposed for lactoferrin, some of which are related to the capacity of the protein to bind iron tightly but reversibly. The proposed functions include iron transport in the gut, antimicrobial activity and modulation of the activity of the immune system.

The synthesis of lactoferrin in the mammary gland is developmentally regulated with changes in protein concentration in milk being correlated with changes in lactoferrin mRNA in mammary tissue. Most species studies have identified high levels of lactoferrin during involution and late pregnancy into early lactation. The amounts of lactoferrin during the lactational phase are much lower, especially in bovine milk. The regulation of bovine lactoferrin expression was studied in the belief that knowledge of the factors influencing expression will provide insight into the function of lactoferrin in the mammary gland.

A 2.5 kb fragment of bovine genomic DNA, including the region immediately upstream of the transcription start point, has been subcloned into luciferase reporter gene vectors. The 2.5 kb fragment has been sequenced and a number of putative response elements identified. Promoter activity was tested by transient expression in the human endometrial carcinoma cell line RL 95-2. 5′- and 3′- deletion analysis of the promoter was used to establish regions which confer transcriptional regulation and the minimal promoter region.

A recent report on the sequence of the cDNA for caprine (goat) lactoferrin suggests that the transcription start point for the mRNA for this protein may be further upstream than that reported for the mRNA of bovine lactoferrin. In view of the high level of sequence identity between the two cDNA's in this region an attempt was made to reinvestigate the transcriptional start point for bovine lactoferrin using DNA footprinting.

Abbreviations

AP-1	activator protein 1
bp	base pair
β-Gal	beta galactosidase
BRL	Bethesda research laboratories
BSA	bovine serum albumin
CAP	calf alkaline phosphatase
CAT	chloramphenicol acetyl transferase
COUP-TF	chicken ovalbumin upstream promoter-transcription factor
Da	Dalton
DES	diethlstilbestrol
DMEM	Dulbecco's modification of Eagle's medium
DMSO	dimethyl sulphoxide
DNA	deoxyribose nucleic acid
DNAse	deoxyribonuclease
dNTP	deoxyribonucleotide triphosphate
DTT	dithiothreitol
EDTA	ethylene diamine tetra-acetate
EGF	epidermal Growth Factor
ERE	estrogen response element
ERM	estrogen response module
FCS	foetal Calf Serum
IL-6	interleukin 6
IL-8	interleukin 8
kb	kilobase
kDa	kilo Dalton
MGF	mammary gland factor
mRNA	messenger RNA
NF-IL 6	nuclear factor interleukin 6
NF-IL 8	nuclear factor interleukin 8

NF-κB	nuclear factor kappa B
nt	nucleotide
Oct-1	octamer transcription factor-1
OD	optical density
ONPG	*o*-nitrophenyl-β-galactoside
PBS	phosphate buffered saline
PCR	polymerase Chain Reaction
RNA	ribose nucleic acid
RNase	ribonuclease
SDS	sodium dodecylsulphate
STAT	signal transducer and activator of transcription
TAE	Tris acetate EDTA
TBE	Tris borate EDTA
TBP	TATA-box binding protein
TE	Tris EDTA
tRNA	transfer ribose nucleic acid
UTR	untranslated region
UV	ultraviolet

List of Tables

List of Figures

Page

Table of Contents

Page