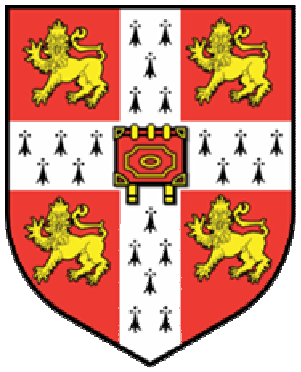
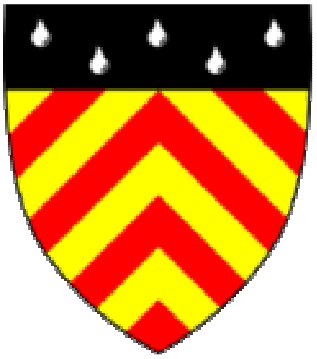
CHARACTERISATION OF BCL11 FUNCTIONS IN DEVELOPMENT USING GENETICALLY MODIFIED MICE

A Dissertation submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

By Song Choon Lee





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DECLARATION

I hereby declare that this dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration, except where specially indicated in the text. None of the material presented herein has been submitted previously for the purpose of obtaining another degree. I confirm that this thesis does not exceed 300 single sided pages of double spaced text, or 80,000 words.

Song Choon Lee

For always being there,

Dad, Mum, Angel and Shia

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ABSTRACT

Bcl11a and Bcl11b are two transcription factors that are essential for lymphocyte development and cell-fate decisions. To study the spatial expression patterns of these genes, I generated the *Bcl11-lacZ* tagged reporter mice. Using X-gal and FDG staining, the expression patterns of *Bcl11* genes in hematopoietic and mammary lineages were fully characterized. I found that *Bcl11a* and *Bcl11b* exhibited dynamic and contrasting expression patterns throughout mammary gland development. Both genes were among the earliest genes that were expressed specifically in the embryonic mammary placodes in the mouse. In the adult gland, *Bcl11a* was expressed in luminal progenitors and their differentiated derivatives while *Bcl11b* expression was predominantly restricted to basal cells and a small number of luminal progenitors.

Absence of *Bcl11a* and *Bcl11b* caused embryonic mammary placode defects. Deletion of *Bcl11a* in the virgin gland disrupted the mammary epithelial architecture and led to a decrease in Gata-3$^+$ cells and an increase in the number of ERα^+ cells. Loss of *Bcl11a* in the lactation gland led to the loss of secretory cells in the lobulo-alveoli, lactation failure, and premature onset of involution. This suggests that *Bcl11a* is essential for maintenance of terminally differentiated luminal secretory cell fate. In contrast, deletion of *Bcl11b* in the virgin gland led to precocious alveologenesis and a basal to luminal lineage switch in the basal cells. Transient over-expression of *Bcl11b* was sufficient to induce expression of basal cell specific genes. These results demonstrate that *Bcl11b* promotes and maintains basal identity, and also suppresses the luminal lineage.

At the molecular level, deletion of *Bcl11a* in the lactation glands resulted in dysregulation of JAK-Stat and Notch signalling pathways. In the *Bcl11a*-deficient lactation gland, there was an absence of phosphorylated Stat5-positive cells and a dramatic increase in phosphorylated Stat3-positive cells. Interestingly, loss of *Bcl11a* in lactation gland resulted in over-expression of *Notch1* but down-regulation of *Notch3* expression. These results demonstrate that different Notch receptors/ligands play different roles in maintaining mammary cell fate and that *Bcl11a* might potentially regulate JAK-Stat signalling via the Notch signalling pathway. This study thus identified *Bcl11a* and *Bcl11b* as critical regulators of the mammary epithelium.

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LIST OF ABBREVIATIONS

4-OHT	4-hydroxytamoxifen
AGM	Aorta-gonand-mesonephros
Amp	Ampicillin
BAC	Bacterial artificial chromosome
Bcl	B-cell lymphoma/leukaemia
BLG	β-lactoglobulin
BM	Bone marrow
Bmp	Bone morphogenic protein
BSA	Bovine serum albumin
Bsd	Blasticidin
C/EBP	CCAAT/enhancer-binding protein
cDNA	Complementary deoxyribose nucleic acid
ChIP	Chromatin immunoprecipitation
Cko	Conditional knockout
CLP	Common lymphoid progenitor
CMP	Common myeloid progenitor
CNS	Central nervous system
COUP-TF	Chicken ovalbumin upstream promoter transcription factor
Cre-ERT	Cre-estrogen receptor (Tamoxifen-inducible Cre)
CTIP	COUP-TF interacting protein
ddH_2O	Double-distilled H_2O
DMEM	Dulbecco's modified Eagle's medium
DMSO	Dimethyl sulfoxide
DN	Double negative ($CD4^-CD8^-$)
dNTP	Deoxyribonucleotide triphosphate
Dox	Doxycycline
DP	Double positive ($CD4^+CD8^+$)
Dpc	Days post-coitum
DTT	Dithiothreitol

EDTA	Ethylene-diamine-tetra-acetic acid
ER	Estrogen receptor
ERK	Extracellular signal-regulated kinase
Evi	Ecotopic virus integration site
FACS	Fluorescent-activated cell sorting
FAM	6-carboxyfluorescein
FCS	Fetal calf serum
FDG	Fluorescein di-β-D-galactopyranoside
Fgf	Fibroblast growth factor
FIAU	1-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouracil
GAS	γ-interferon activation sites
GH	Growth hormone
Hes	Hairy enhancer of split
Hh	Hedgehog
HSC	Hematopoietic stem cell
HSVtk	Herpes Simplex Virus thymidine kinase
Hygro	Hygromycin
ICN	Notch intracellular domain
IGF	Insulin-like growth factor
IGFBP	Insulin-like growth factor binding protein
IL	Interleukin
IRES	Internal ribosome entry site
JAK	Janus kinase
Kan	Kanamycin
KLS	Lineage-negative c-kit$^+$ Sca1$^+$ cells
Lef	Lymphoid enhancing factor
LIF	Leukemia inhibitory factor
LMPP	Lymphoid-primed multi-potent progenitor
Ma-CFC	Mammary colony-forming-cell
MAML	Mastermind-like protein
MAP	Mitogen-activated protein

MaSC	Mammary stem cell
MEF	Mammary epithelial cell
MEP	Megakaryocyte/erythroid progenitor
MMTV	Mouse mammary tumour virus
MPP	Multi-potent progenitor
MSCV	Murine stem cell virus
MTA	Metastasis-associated protein
NBT/BCIP	Nitro blue tetrazolium chloride/5-Bromo-4-chloro-3-indolyl phosphate, toluidine salt
NCoR	Nuclear receptor co-repressor
Neo	Neomycin
NP-40	Nonidet P-40
Nrg	Neuregulin
NuRD	Nucleosome remodelling and deacetylase
PAC	P1 artificial chromosome
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PBST	Phosphate buffered saline with 0.1% Tween-20
PCR	Polymerase chain reaction
PDK1	Phosphoinositide-dependent kinase 1
PFA	Paraformaldehyde
PH	Pleckstrin homology
PI3K	Phosphatidylinositol-3-OH-kinase
PIAS	Protein inhibitor of activated Stat
PKB	Protein kinase B
PR	Progesterone receptor
Prl	Prolactin
PtdIns-3,4,5-P_3	Phosphatidylinositol-3,4,5-triphosphate
PtdIns-4,5-P_2	Phosphatidylinositol-4,5-biphosphate
PTHrP	Parathyroid hormone related peptide
Puro	Puromycin

QPCR	Quantitative real-time PCR
qRT-PCR	Quantitative real-time Reverse Transcription PCR
QTL	Quantitative trait locus
RBP-Jκ	Recombination-binding protein-Jκ
Rit	Radiation induced tumour suppressor
RT-PCR	Semi-quantitative Reverse Transcription PCR
rtTA	Reverse tetracycline-controlled transactivator
SA	Splice acceptor
Sca1	Stem cell antigen 1
SDS	Sodium dodecyl sulphate
SH2	Src-homology 2
SHP	SH2 containing phosphatase
Ska	Scaramanga
SMA	Smooth muscle actin
SMRT	Silencing mediator for retinoid and thyroid hormone receptor
SOCS	Suppressor of cytokine signalling
Stat	Signal transducer and activator of transcription
TAM	Tamoxifen
TAMRA	Tetramethyl-6-carboxyrhodamine
Tbx	T-box
TE	Tris-EDTA
TEB	Terminal end bud
Tet	Tetracycline
TetR	Tetracycline repressor protein
TGF	Transforming growth factor
TNF	Tumour necrosis factor
TRE	Tetracycline response element
tTA	Tetracycline-controlled transactivator
WAP	Whey acidic protein
Wnt	Wingless and Int
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside

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