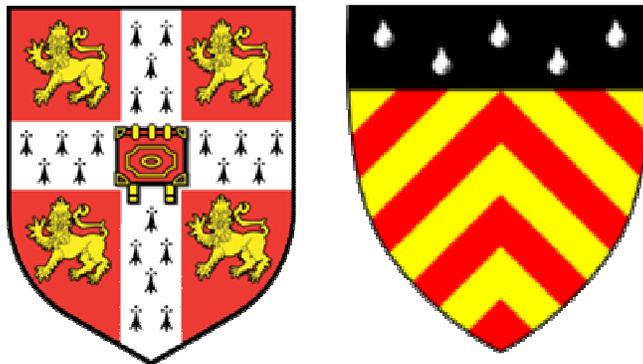


# **CHARACTERISATION OF BCL11 FUNCTIONS IN DEVELOPMENT USING GENETICALLY MODIFIED MICE**

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

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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<sup>+</sup> cells and an increase in the number of ERα<sup>+</sup> 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 alveologensis 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-gonad-mesonephros                                  |
| Amp                | Ampicillin   |
| BAC                | Bacterial artificial chromosome                          |
| Bcl                | B-cell lymphoma/leukaemia                                |
| BLG                | $\beta$ -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 <sub>2</sub> O | Double-distilled H <sub>2</sub> O                        |
| DMEM               | Dulbecco's modified Eagle's medium                       |
| DMSO               | Dimethyl sulfoxide                                       |
| DN                 | Double negative (CD4 <sup>-</sup> CD8 <sup>-</sup> )     |
| dNTP               | Deoxyribonucleotide triphosphate                         |
| Dox                | Doxycycline  |
| DP                 | Double positive (CD4 <sup>+</sup> CD8 <sup>+</sup> )     |
| 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- $\beta$ -D-galactopyranoside                  |
| Fgf    | Fibroblast growth factor                                      |
| FIAU   | 1-2-deoxy-2-fluoro- $\beta$ -D-arabinofuranosyl)-5-iodouracil |
| GAS    | $\gamma$ -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 <sup>+</sup> Sca1 <sup>+</sup> 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 <sub>3</sub> | Phosphatidylinositol-3,4,5-triphosphate  |
| PtdIns-4,5-P <sub>2</sub>   | 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-J $\kappa$ | Recombination-binding protein-J $\kappa$                     |
| 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- $\beta$ -D-galactopyranoside     |

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