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**Regulation of tight junction proteins during  
engorgement of the mammary gland**

**Claire Vanessa Cooper Phyn**

2006

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# **Regulation of tight junction proteins during engorgement of the mammary gland**

A thesis presented in partial fulfilment of the requirements for  
the degree of

**Doctor of Philosophy  
in Animal Science**

at

**Massey University**  
Palmerston North, New Zealand.

**Claire Vanessa Cooper Phyn**

2006



## Abstract

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Extended periods of milk accumulation result in loss of secretory activity, increased apoptosis and eventually, involution of mammary glands. This process is associated with increased permeability of the tight junction (TJ) complexes between adjacent mammary epithelial cells (MECs). The change in cell shape during mammary engorgement from a cuboidal to a flattened morphology may initiate changes in protein and gene expression (mechanotransduction) that trigger these processes. Therefore, this study examined the regulation of the major TJ protein components during mammary engorgement, and in particular the role of physical distension of the mammary epithelium in the regulatory process. Expression of the integral transmembrane TJ proteins, occludin and claudin-1, and the cytoplasmic TJ protein, ZO-1, were down-regulated in both bovine and rat mammary glands during the early stages of mammary apoptosis and involution following the abrupt cessation of milk removal. In the rat, these responses were locally regulated as they occurred only in teat-sealed glands in a hemi-suckled model. Furthermore, the down-regulation of TJ proteins is consistent with a loss of TJ integrity during mammary engorgement. Induced physical distension of rat mammary glands *in vivo* transiently up-regulated the expression levels of occludin protein and mRNA, and ZO-1 mRNA, followed by an accelerated decrease in expression compared with the effects of milk accumulation alone. This was associated with the initiation of apoptosis, the up-regulation of the pro-apoptotic factor pSTAT3, and the down-regulation of the cell-ECM survival factor  $\beta$ 1-integrin. An *in vitro* model was also developed to stretch MECs, mimicking the flattening in cell shape during mammary engorgement *in vivo*. While stretching MECs *in vitro* did not conclusively alter TJ protein expression, the overall results of this project support further investigation into the role of the TJ complex in mechanotransduction pathways. In addition, the results point to crosstalk between cell-ECM survival signalling and STAT3 death signalling as a candidate for regulation by physical distension of the mammary epithelium. In conclusion, this study supports the hypothesis that physical distension during engorgement of the mammary glands with milk is a primary trigger initiating apoptosis of MECs through changes in the regulation of gene pathways controlling cell survival and death, and the disruption of TJ function.

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To my husband Derek,

even though he may not understand a word of it...

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## List of Abbreviations

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S.I. (Système International d'Unités) abbreviations for units, and standard notations for chemical elements, formulae and chemical abbreviations are used in the text. Other abbreviations are listed below.

$A_{260}$	absorbance at 260 nm
$A_{280}$	absorbance at 280 nm
$A_{260}/A_{280}$	ratio of absorbance at 260 nm to absorbance at 280 nm
ABHA	p-aminobenzoyl-gly-pro-D-leu-D-ala hydroxamic acid
ANOVA	analysis of variance
APS	ammonium persulphate
bp	base pairs
BCA	bicinchoninic acid
BCIP	5-Bromo-4-chloro-3-indolyl-phosphate, 4-toluidine salt
BME	$\beta$ -mercaptoethanol
BSA	bovine serum albumin
CAPS	3-[cyclohexylamino]-l-propanesulfonic acid
cDNA	complementary DNA
contig	contiguous sequence
$C_T$	threshold cycle
DAB	3, 3'-diaminobenzidine tetrahydrochloride
dATP	deoxyadenosine triphosphate
dCTP	deoxycytidine triphosphate
$\Delta$ MSA	change in membrane surface area
DEPC	diethylpyrocarbonate
dGTP	deoxyguanosine triphosphate
DIG-11-dUTP	digoxigenin-11-2'-deoxy-uridine-5'-triphosphate
DMEM	Dulbecco's modified Eagle's medium
DMEM:F12	Dulbecco's modified Eagle's medium F12 nutrient mix
DMSO	dimethyl sulphoxide

DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
dNTP	deoxynucleotide triphosphate
DPX	DePeX mounting agent
dsDNA	double-stranded DNA
DTT	1, 4-dithiothreitol
dTTP	deoxythymidine triphosphate
ECL	enhanced chemiluminescence
ECM	extracellular matrix
EDTA	ethylenediaminetetraacetic acid
EGF	epidermal growth factor
EST	expressed sequence tag
FAK	focal adhesion kinase
FCS	foetal calf serum
FIL	feedback inhibitor of lactation
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GAR	goat anti-rabbit
H&E	haematoxylin and eosin
HEPES	4-[2-hydroxyethyl]-1-piperazineethanesulphonic acid
IAA	isoamyl alcohol
i.d.	internal diameter
IgA	immunoglobulin A
IGF-1	insulin-like growth factor 1
IgG	immunoglobulin G
ISEL	<i>in situ</i> end labelling
i.v.	intravenous
Kb	kilobase
kDa	kiloDaltons
kHz	kilohertz
LB	Luria broth
mA	milliamperes
MDCK	Madin-Darby canine kidney cells
MEC	mammary epithelial cell
MOPS	3-[N-morpholino]propane-sulphonic acid

MQ	Milli-Q filter-purified water
mRNA	messenger RNA
MSA	membrane surface area
MSAA	mammary serum amyloid A3
MW	molecular weight
NBT	4-Nitro-Blue tetrazolium chloride
NFR	nuclear fast red
NP-40	Nonidet P-40
ODM	once-daily milking
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PMSF	phenylmethanesulfonyl fluoride
polyA	polyadenylated
PRL	prolactin
PVP	polyvinylpyrrolidone
rep.	replicates
RmT	room temperature
RNA	ribonucleic acid
RNase	ribonuclease
rpm	revolutions per minute
RT	reverse transcriptase
RT-PCR	reverse transcription-polymerase chain reaction
rRNA	ribosomal RNA
SCC	somatic cell count
SED	standard error of the difference
SEM	standard error of the mean
SDS	sodium dodecyl sulphate
SSC	salt/sodium citrate
ssDNA	single-stranded DNA
TAE	Tris-acetate EDTA
TB	Terrific broth
TBS	Tris buffered saline
TBST	Tris buffered saline containing Tween-20

TDM	twice-daily milking
TE	Tris/EDTA
TEMED	N, N, N', N'-tetra-methylenediamine
TER	transepithelial resistance
TGF	transforming growth factor
TJ	tight junction
T <sub>m</sub>	melting temperature
TNF	tumor necrosis factor
Tris	Trizma Base or Tris[hydroxymethyl]aminomethane
Tris HCl	2-amino-2-(hydroxymethyl)-1,3-propanediol
U	Units
UV	ultraviolet
V	volts
VEA	vesicle-engorged alveoli
v/v	volume per volume
w/v	weight per volume
ZO	zonula occludens