Sox3 dosage regulation is important for roof plate specification during central nervous system development

A thesis submitted to the University of Adelaide for the degree of Doctor of Philosophy

By

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June 2011

Errata

The following corrections have been made in the final version of the thesis: --

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“5.4.1 Bmp and Wnt RP expression was diminished transgenic embryos” to “5.4.2 Bmp and Wnt RP expression was diminished in transgenic embryos”

“5.4.2” to “5.4.3”

“5.4.3” to “5.4.4”

“5.4.4” to “5.4.5”

“5.4.5” to “5.4.6”

“APPENDIX IV: AN OUTLINE OF THE MICROARRAY EXPERIEMNT” to
“APPENDIX IV: AN OUTLINE OF THE MICROARRAY EXPERIMENT”

Summary

Third paragraph, line 8: “have similar role” to “have a similar role”

Abbreviations

Title: “Abbreviations Abbreviations” to “Abbreviations

Anatomical terms: “MZ mantel zone” to “MZ mantle zone”

Chapter 1: Introduction

Page 3, line 6: “subfamily” to “subfamilies”

Page 4, line 14: “to minor groove of DNA helix” to “to the minor groove of the DNA helix”

Page 5, line 6: “activate” to “activates”

Page 5, line 11: “These indicate” to “These data indicate”

Page 5, line 29: “target genes” to “target gene”

Page 6, line 1: “target genes” to “target gene”

Page 6, line 1-2: “function specialisation” to “the functional specialisation”

Page 7, line 7: “study on the abnormal development” to “study the abnormal development”

Page 7, line 8: “the discussion” to “the following discussion”

Page 7, line 25: “the formation of DV is” to “the formation of DV axis is”

Page 8, line 16: “with respects to” to “with respect to”

Page 8, line 21: :”(blastocoels)” to “(blastocoel)”

Page 8, line 24: “process which some epithelial cells” to “process by which some epithelial cells”

Page 10, line 13: “mesenchyme” to “mesoderm”

Page 10, line 26: “a node explants from a late-streak embryo has” to “node explants from late-streak embryos have”

Page 10, line 27: “the expression of neural marker” to “the expression of the neural marker”

Page 11, line 14: “The following few section” to “The following section”

Page 13, line 10: “binds to Ephrin” to “binds to Eph’

Page 14, line 3: “NC cells migration” to “NC cell migration”

Page 15, line 21: “as well as constitutive” to “as well as constitutively active”

Page 15, line 25: “dysplasic” to “dysplastic”

Page 16, line 4: “part of” to “part of the”

Page 16, line 7: “with onset of” to “with the onset of”

Page 16, line 18: “domain of dorsal” to “domain of the dorsal”

Page 17, line 2: “may involve in” to “may be involved in”

Page 18, line 1: “CNS needs to be” to “the CNS needs to be”

Page 18, line 5: “referred as” to “referred to as”

Page 18, line 6: “controversial that whether” to “controversial whether”

Page 18, line 9: “is not incorporated in” to “is not incorporated into”

Page 18, line 23: “with diminished AP spanning as progressing dorsally” to “with diminishing AP spanning towards the dorsal domain”

Page 18, line 30: “In cooperation of” to “In cooperation with”

Page 18, line 31: “within diencephalon” to “within the diencephalon”

Page 19, line 3: “Division along AP” to “Division along the AP”

Page 19, line 4: “composing” to “comprised”

Page 20, line 20: “present at” to “present in”

Page 21, line 1” “inner cell mast” to “inner cell mass”

Page 22, line 8: “a role of” to “a role for”

Page 24, line 7: “portion” to “proportion”

Page 24, line 29: “pharyngeal arches” to “pharyngeal arch”

Page 25, line 5: “(Minoux and Rijli)” to “(Minoux and Rijli, 2010)

Page 25, line 20: “Together, these” to “Together, these studies”

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Page 26, line 2: “the discussion *Sox3*” to “the discussion of *Sox3*”

Page 26, line 21: “there lack any” to “there is lack of any”

Page 26, line 30: “transcription regulation” to “the transcriptional regulation”

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Page 27, line 4: “secrets” to “secretes”

Page 27, line 16: “throughout CNS” to “throughout the CNS”

Page 27, line 23-24: “when both ectopically expressed, *xSox3* counteracted *POU class V protein oct-91* (*xOct91*) neuralising” to “when both *xSox3* and *POU class V protein oct-91* (*xOct91*) are ectopically expressed, *xSox3* counteracted *xOct91* neuralising”

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Page 46, line 26: “characteristic to” to “characteristic of”

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Page 87, line 20: “brought by” to “brought about by”

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Page 89, line 17-18: “there cannot rule out the possibility of a positional effect whereby integration site residing transcription enhancing element might act on the transgene and upregulate its expression.” to “it is possible that local transcription enhancing element at the integration site might act on the transgene and upregulate its expression.”

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Page 120, line 4: “statistically” to “statistical”

Page 120, line 16: “(assumed most gene expressions did not change between samples)” to “(assuming most gene expressions did not change between samples)”

Page 120, line 22: “but the other” to “but absent from the other”

Page 121, line 15: “analysed” to “analysis”

Page 121, line 22: “the direction for future investigation” to “the direction of future investigation”

Page 125, line 12: “might due” to “might be due”

Page 126, line 20-21: “In contrast, *Sr/+; Nr/+* SCO *Dab2* expression had a 1.25-fold decrease from the two microarray samples. This did not correlate with the microarray, which indicated a 3-fold decrease in expression.“ to “On the other hand, the microarray experiment detected a 3-fold decrease of *Dab2* expression in *Sr/+; Nr/+* SCO . However, qRT-PCR analysis using identical SCO samples as the microarray indicated that *Dab2* expression has only downregulated for 1.25-fold.”

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Page 129, line 27: “SMAD complex” to “the SMAD complex”

Page 131, line 10: “*in vitro* study” to “an *in vitro* study”

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Page 142, line 1: “within the dorsal neural developing RP” to “within the developing dorsal neural RP”

Page 142, line 5: “unlikely due to” to “unlikely to be due to”

Page 142, line 12: “These suggest” to “These observations suggest”

Pate 142, line 13: “functional redundant with *Sox2* such that the function of *Sox3* is substituted by *Sox2* in *Sox3* null embryos” to “functionally redundant with *Sox2*”

Page 143, line 20: “these indicate” to “these data indicate”

Page 143, line 23: “SOX3 expression difference were found” to “SOX3 expression were found”

Page 143, line 25: “intensity that those” to “intensity than those”

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Page 146, line 4: “Since the RP is an organising centre” to “The RP is an organising centre”

Page 146, line 16: “recently study” to “a recent study”

Page 146, line 18: “the transcription activity of *Hes1,* which *Hes1* then acts” to “the transcription activity of *Hes1*. In return, *Hes1* then acts”

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Page 148, line 25: “leaving the dorsal NT with cells making up the definitive RP” to “and only cells that give rise to the definitive RP are left at the dorsal NT”

Page 149, line 15: “understanding this defect” to “understanding of this defect”

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Page 149, line 17: “begin migrate” to “begin to migrate”

Pag e149, line 23: “were observed” to “was observed”

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Page 152, line 10: “lethally” to “lethality”

Page 152, line 24: “immuneprecipitation” to “immunoprecipitation”

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Figure 1.18, legend, line 3: “trasngenic” to “transgenic”

Figure 1.6, legend, line 1: “within an developing” to “within a developing”

Figure 3.5 A, the note at the bottom of the figure, line 1: “frequecny” to “frequency”

Figure 3.14, the figure at the top right corner is labelled as (K)

Figure 4.1, legend, line 7: “ependymals” to “ependymal”

Figure 4.15, legend, line 1: “at a varing low level” to “at varying low levels”

Figure 4.16, bottom of the figure: “12.5 dpc” to “15.5 dpc”

Summary

The central nervous system (CNS) is one of the most complicated organs in the body. Its development requires spatially and temporally dynamic but yet coordinated proliferation and differentiation of neural progenitors in order to allow the formation of the neural tube (NT) from a sheet-like neural plate, as well as the specification of different CNS domains from a homogeneous group of neural precursors. A number of genes have been identified to be important for the robust genetic and molecular regulation of the CNS development. One of these genes is *SOX3*, which encodes a *SOX* family transcription factor within a single exon. In humans, *SOX3* duplications and mutations have been implicated in X-linked hypopituitarism (XH) with variable mental retardation, suggesting that CNS development is sensitive to *SOX3* dosage. To recapitulate the condition in XH patients with *SOX3* duplications, a transgenic *Sox3* overexpression mouse model was generated in the Thomas laboratory. Two *Sox3* transgenic lines were established. Intriguingly, they presented not with XH associated phenotypes, but with hallmarks of hydrocephalus.

This thesis focuses on the characterisation of the hydrocephalus phenotype and the elucidation of the molecular pathogenesis that underlines the defect. Comprehensive morphological and expression analyses of single hemizygous and double hemizygous (mice with two transgene alleles, one from each *Sox3* transgenic line) *Sox3* transgenic mice demonstrated that the hydrocephalus has a congenital origin (congenital hydrocephalus, CH) and is associated with defective development of the subcommissural organ (SCO), a diencephalic roof plate (RP) derivative that has previously been implicated in CH. Moreover, both the CH and the SCO dysmorphology of *Sox3* transgenic mice are dependent on *Sox3* dosage. In addition to the SCO, defective development was also identified in other diencephalic RP derivatives in *Sox3* double hemizygous embryos. In fact, SCO maldevelopment appears to be the consequence of a general RP defect, in which the identity of the RP cells (or SCO precursors) is lost and is coupled with an elevation of cellular proliferation. Further molecular analysis of the SCO development supported that *Sox3* overexpression leads to the loss of RP cellular identity as the expression of genes implicated in both Bmp and Wnt signalling pathways, which are important for normal RP development, were significantly downregulated in *Sox3* transgenic embryos. In addition to its role as the precursor of RP derivatives, the RP is also essential to maintain dorsal identity for proper DV

patterning within the NT. Moreover, dorsal NT patterning was disrupted within the developing *Sox3* transgenic embryos. Together these suggest that the *Sox3* overdosage leads to the loss of RP identity and the failure of RP dependent processes.

In summary, this thesis indicates that, at least in the mouse, *Sox3* dosage regulation is important for proper RP development. In fact, a low level of *Sox3* is permissive for RP specification. It is not known what cellular identity is taken up by the RP cells in the *Sox3* double hemizygous embryos. However, given endogenous *Sox3* is highly expressed in the lateral tissue that flanks the RP, it is logical to propose that the RP cells of *Sox3* double hemizygous embryos may take on a cellular identity similar to their laterally neighbouring tissue. Interestingly, a CNS defect that may be due to RP dysfunction has been identified in some XH patients with *SOX3* duplications, suggesting that *SOX3* may have similar role in humans. CH is a heterogeneous disorder in which identification of its genetic basis in humans is difficult. This thesis proposes *SOX3* as a candidate gene for CH and other RP associated defects in humans, which may assist the development of relevant prognostic techniques for clinical application.

Statement

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Signed: ……..……(Kristie Pan Yu Lee)

Date: ……..…

Acknowledgments

My PhD project would not be possible without my supervisor, Paul Thomas, for offering me a position in his laboratory, as well as his support for the last four years. Here, I would like to thank him for the numerous hours that he spent in reading my manuscript and thesis, in our marathon meeting where he gave me valuable advice, and in undertaking double-blinded tests of my many *in situ* hybridisation experiments. Also, I would like to thank him for his moral support and faith during times in my PhD when experiments did not work as well as we hoped for. In addition, I would like to thank my co-supervisor Kirk Jensen, whose scientific advice has been greatly beneficial to my PhD project.

I am deeply grateful to those who provided professional help and opinion that made me to become a better scientist. To James Hughes, who had frequent lengthy discussion with me about the SCO development; and to Mike Morris, who gave me great assistance and valuable opinion in those last experiments of my PhD. To Sandie, who performed the BrdU injection and made my mouse work so much easier for looking after the colonies; to Dale, who provided his unlimited pool of knowledge in my microarray statistical analysis, to Pike-See, Rob Moyer from Obstetrics and Gynaecology and Gail Hermanis from Anatomical Sciences who offered great help in the my histology work; and to Rosalie Kenyon from the Adelaide Microarray Centre for performing my Microarray experiment. Moreover, I would like to express my appreciation to those whose data I presented in this thesis, Chloe Shard and again Mike Morris and James Hughes.

Doing a PhD means that you probably spend most of your conscious time in the lab, and you probably see your colleagues at work more often than your rabbit at home. Thus, I would like to thank everyone who shared their time (and laughter and tears if there were any) with me in the past four years, especially James, Sandie, Bryan, Dale, Nick, Anne, Sophie, Pete and Adrienne (and Shwetha and Edwina who are greatly missed!), all current and past member of the Thomas lab, as well as my fellow PhD candidates. I would like to extend my appreciation to everyone on the third floor whose help I received throughout the years.

Last but not least, I would like to thank my friends and family. To Angela, Rosalie, Wee-ching, Tracy and Zoe, thank you for all the support that you have given me, prior and during my PhD (and hopefully years from now on).Your friendship is and will be mostly

appreciated. To my mum and dad, thank you very much for your unconditional support, both emotionally and financially, since I was born and of course during my PhD. I would never make to where I am without you. Thanks for being there whenever I needed and for making sure that I was properly fed all the time. To my brother Eric, thanks for your patience to listen to my work and to put up with an occasional grumpy sister. Finally, to Nick, thank you very much for your patience, encouragements and understanding in the past years. Thanks for being there and giving me a reason to smile for even at the end of a day when none of my experiment worked.

Abbreviations Abbreviations

Standard terms

%	percentage
ºC	degree Celsius
cDNA	complementary deoxyribonucleic acid
DNA	deoxyribonucleic acid
g	gravitational force
kb	kilobase
kDa	kilodalton
kg	kilogram
RNA	ribonucleic acid
M	molar
mg	milligram
ml	millilitre
µl	microlitre
mm	millimetre
µm	micrometer (micron)
mM	millimolar
µM	micromolar
ms	millisecond

ng	nanogram
N	normality
w/v	weight per volume
v/v	volume per volume

Non-standard terms

a.a.	amino acid
AH	acquired hydrocephalus
BAC	bacterial artificial chromosome
bp	base pair
CH	congenital hydrocephalus
dpc	days post coitum
ES	embryonic stem
HCNE(s)	highly conserved non-coding element(s)
IRES	internal ribosome entry site
NC	neural crest
ORF	open reading frame
P	postnatal
RF	Reissner's fibre
RIN	RNA integrity number
SCBE	SOX core binding sequence
UTR	untranslated region

WT	wildtype
XH	X-linked hypopituitarism

Mouse lines

Nr	*Sox3 non-sex reversal line*
Sr	*Sox3 sex reversal line*

Materials and methods

BrdU	bromodeoxyuridine
DIG	dioxygenin
EtOH	ethanol
gDNA	genomic DNA
H & E	haematoxylin and eosin staining
IgG	immunoglobulin G
MeOH	methanol
MQ	milliQ
NaAc	sodium acetate
OCT	optimal cutting temperature
O/N	overnight
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PFA	paraformaldehyde

qPCR	quantitative polymerase chain reaction
qRT-PCR	quantitative real-time polymerase chain reaction
RT	room temperature
TUNEL	terminal deoxynucleotidyl transferase dUTP nick end labelling
U	unit

Statistical and bioinformatics terms

ANOVA	analysis of variance
BLAST	Basic Local Alignment Search Tool
FDR	false discovery rate
Limma	linear models for microarray analysis
MGI	Mouse Genome Database
NCBIm37	*mus musculus* genome assembly NCBIm37

Anatomical terms

3V	third ventricle
4V	fourth ventricle
AP	anterior-posterior
Aq	Sylvian aqueduct
AVE	anterior visceral endoderm
BBB	blood-brain barrier
BCSFB	blood-CSF barrier

CCx	cerebral cortex
Cer	cerebellum
ChP	choroid plexus
CNS	central nervous system
CSF	cerebrospinal fluid
CSFBB	CSF-brain barrier
CVO	circumventricular organs
DLHP	dorsolateral hinge point
DTh	dorsal thalamus
DV	dorsoventral
EpTh	epithalamus
FP	floor plate
GO	gastrula organiser
HbC	habenular commissure
Hpc	hippocampus
LGE	lateral ganglionic eminence
LV	lateral ventricles
MGE	medial ganglionic eminence
MHP	medial hinge point
MR	mesocoelic recess
MZ	mantel zone

NT	neural tube
PC	posterior commissure
PG	pineal gland
PR	pineal recess
PS	primitive streak
Pt	pretectum
RP	roof plate
SCO	subcommissural organ
TrG	trigeminal ganglia
VTh	ventral thalamus
VZ	ventricular zone
ZLI	zona limitans intrathalamica

Cell lines

293T	human embryonic kidney 293 cells
HeLa	human cervical cancer cells
NTERA2	human embryo carcinoma cells

Protein families and protein domains

APC	*adenomatous polyposis coli*
Bmp	bone morphogenic protein
Fgf	fibroblast growth factor

Gli	GLI-kruppel family member
GSK3	glycogen synthase kinase
HMG	high motility group
JAK	Janus family of tyrosine kinases
MAP	mitogen associated protein
mTOR	mammalian target of rapamycin
NICD	notch-intracellular domain
Shh	sonic hedgehog
SNARE	N-ethylmaleimide sensitive fusion attachment protein receptors
SOX	sry-related HMG box
STAT	signal transducers and activators of transcription
TA	transactivation
Tgfβ	transforming growth factor beta
Wnt	wingless-related MMTV integration site

Gene Nomenclature

Human

Gene: italic, all capital, e.g. *SOX3*

Protein: non-italic, all capital, prefixed with ‘h’ indicative of human origin, e.g. hSOX3

Mouse

Gene: italic, first letter capital, e.g. *Sox3*

Protein: non-italic, all capital, e.g. SOX3

Other model organisms

Gene: italic, first letter capital, prefixed with a relevant letter indicative of animal origin, i.e. ‘c’ for chicken; ‘z’ for zebrafish; ‘x’ for *Xenopus*, e.g. *cSox3, zSox3, xSox3*

Protein: non-italic, first letter capital, prefixed with a relevant letter indicative of animal origin, i.e. ‘c’ for chicken; ‘z’ for zebrafish; ‘x’ for *Xenopus*, e.g. cSox3, zSox3, xSox3

Note: The nomenclature of the highest organism is used if describing a gene or protein from more than one model organisms.