# ANALYSIS OF PATHWAYS AND PROTEINS THAT PATTERN *OLIG2*<sup>+</sup> CELLS WITHIN THE ZEBRAFISH CENTRAL NERVOUS SYSTEM

By

Karen A. McFarland

Dissertation

Submitted to the Faculty of the

Graduate School of Vanderbilt University

in partial fulfillment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

in

**Biological Sciences** 

December 2007

Approved:

Bruce Appel

Lilianna Solnica-Krezel

Michael Cooper

Douglas McMahon

#### **ACKNOWLEDGEMENTS**

I would like to thank my mentor Bruce for being such a positive influence in my scientific career and my life, without his guidance I would have been flopping around like a fish out of water. I also want to thank my committee members whose advice and support lifted my spirits every time we had a meeting and were continually there for me outside of our meetings. I would especially like to thank Lila whose words of kindness and wisdom have had more of an impact on me than she will ever know. I would also like to thank my lab mates, my colleagues, who not only provided scientific advice but were also a constant source of encouragement and laughs. To my friends outside of the lab, thank you for helping me feel like I had a life outside of science (although I know this is a farce). Most importantly I would like to thank my mother father and sisters, Genevieve, Taylor and my fiancé, Ben whose constant love and support never let me give up and helped me accomplish what they apparently knew I was capable of all along. Thank you and I love you.

## TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	ii
LIST OF FIGURES	vi
LIST OF ABBREVIATIONS	xii
Chapter	
I. INTRODUCTION	1
Description of cerebellum Signaling Pathways that Pattern Hedgehog Signaling Bone Morphogenetic Proteins The Wnt Pathway A Model for Dorsoventral Patterning Antagonistic Gradients Zebrafish as a Model System Neuronal Circuitry of the Zebrafish Cerebellum versus Mammalian Function of the Cerebellum Disease Implications	5 6 10 21 21
PURKINJE NEURONS IN THE ZEBRAFISH CEREBELLUM	29 32 32 33 34 35 35 35

	signaling	.39
	Expression patterns of Hh pathway genes do not coincide w	
	olig2 <sup>+</sup> PNs	
	Discussion	
	olig2 expression marks a subset of PNs and reveals	
	cerebellar DV pattern	.52
	Hh signaling and cerebellar patterning	
	Wnt signaling is necessary for olig2 <sup>+</sup> PN development	
	Acknowledgements	
II.	A GENETIC SCREEN IDENTIFIES A MUTANT DEFECTIVE IN	
	PURKINJE NEURON DEVELOPMENT	.58
	Introduction	
	Materials and Methods	
	Zebrafish staging and strains	
	ENU mutagenesis and screening	
	Immunohistochemistry	
	Whole mount imaging	
	Results	
	Genetic screen for mutations that disrupt formation of olig2 <sup>+</sup>	
	PNs	
	Phenotypic characterization of vu225	
	Analysis of EGFP expression in the vu225 cerebellum	
	Specification of cerebellar cell types in vu225 mutant	.69
	Discussion	
	Cerebellar specific mutation	
	Behavioral defects in vu225	
	Future Directions	.78
	Acknowledgements	.79
V.	TREATMENT WITH A BMP ANTAGONIST PRODUCES EXCESS	
	DORSALLY LOCATED OLIGODENDROCYTES IN THE ZEBRAFISH	
	SPINAL CORD	.80
	Introduction	
	Materials and Methods	
	Zebrafish strains and staging	
	Immunohistochemistry	
	Results	.85
	Treatment with HY compound at 24 hpf results in excess	
	dorsal OPCs	
	Sox 10 labeling indicates HY causes early specification	.87
	Blocking BMPs results in improperly positioned	

	oligodendrocytes	88
	Discussion	92
	Could BMPs inhibit a different origin of oligodendrocytes? . BMPs may be necessary for proper positioning of	92
	oligodendrocytes	93
	Acknowledgements	93
V.	CONCLUDING REMARKS	94
REFE	RENCES	97

## LIST OF FIGURES

Figure	Pa	ge
1.1	Schematic diagram depicting the neuronal circuitry in a mammalian cerebellum	3
1.2	All cerebellar neurons arise from either the rhombic lip or the ventricular zone	
1.3	Schematic diagram of the Hh pathway7	',8
1.4	Schematic diagram of the BMP pathway11,	12
1.5	Schematic diagram of the Wnt pathway14,	15
1.6	A schematic representing progenitor domains and corresponding transcription factors within each domain along the entire DV axis of the spinal cord	17
1.7	Schematic for ventral specification along the DV axis of spinal cord	18
1.8	Schematic for dorsal specification along the DV axis of spinal cord	20
1.9	Images of live development modified from ZFIN	23
1.10	Schematic diagram representing the zebrafish cerebellum neuronal circuitry	25
2.1	Transgenic reporter gene expression recapitulates endogenous <i>olig2</i> RN expression in zebrafish cerebellum	
2.2	olig2 expression identifies a subset of Purkinje neurons	38
2.3	Hh signaling restricts cerebellar <i>olig2</i> expression	41
2.4	Temporal regulation of <i>olig2</i> <sup>+</sup> PN number by Hh signaling	42
2.5	Zebrafish cerebellum expresses wnt1 but not Hh pathway genes	44
2.6	Wnt signaling is necessary for cerebellar olig2 expression	47
2.7	Embryos lacking Hh signaling result in a ventral expansion of Wnt1 expression	49

2.8	atoh1a expression reveals a normally formed cerebellum	.50
2.9	Expansion of <i>ptc1</i> expression in <i>Df(LG01:lef1)</i> <sup>x8</sup> mutants but not in embryos with activated Dkk1 indicate two potential roles for Wnt signaling in regulating cerebellar formation	
3.1	Schematic of ENU mutagenesis screen	.62
3.2	DIC images of WT compared to <i>vu225</i> mutant embryos show morphological presence of cerebellum, flattened head and missing swin bladder	
3.3	A developmental time course comparison of EGFP expression in <i>Tg(olig2:egfp)</i> WT embryos and <i>vu225</i> mutants reveal the complete absence or severe decrease in EGFP <sup>+</sup> cells within the cerebellum	.68
3.4	Transverse sections of $Tg(olig2:egfp)$ embryos through the cerebellum reveal improperly positioned and a general decrease in EGFP <sup>+</sup> and Calretinin <sup>+</sup> cells in $vu225$ when compared to WT	.71
3.5	Transverse sections of <i>Tg(olig2:egfp)</i> embryos through the cerebellum reveal a decrease in EGFP <sup>+</sup> and Zebrin II <sup>+</sup> PNs	.72
3.6	Transverse sections of <i>Tg(olig2:egfp)</i> embryos through the cerebellum reveal a decrease in EGFP <sup>+</sup> and PV <sup>+</sup> PNs	.74
3.7	Model of a transverse section through the cerebellum depicting WT cerebllar cell type expression versus <i>vu225</i>	.75
4.1	EGFP expression in the spinal cord reveals a dose dependant increase dorsally located $olig2^+$ OPCs when $Tg(olig2:egfp)$ embryos are treated with HY compound at 24 hpf	
4.2	sox10 antibody labeling sox10 <sup>+</sup> ;EGFP <sup>-</sup> dorsally located OPCs at 48 hpf	89
4.3	Graph representing the increase in sox10 <sup>+</sup> and EGFP <sup>+</sup> dorsally located OPCs within the spinal cord	.90
4.4	sox10 antibody labeling reveals an increase of OPCs in the grey matter embryos treated with HY	

#### LIST OF ABBREVIATIONS

AP Anterior- Posterior

BMP Bone Morphogenetic Protein

CA Cyclopamine

CNS Central Nervous System

dpf days post fertilization

DV Dorsal-Ventral

HB Hindbrain

Hh Hedgehog

hpf hours post fertilization

Ihh Indian hedgehog

MB Midbrain

MBHB Midbrain-hindbrain

MO Medulla Oblongata

OPC Oligodendrocyte Progenitor Cell

PN Purkinje Neuron

Ptc Patched

PV Parvalbumin

Shh Sonic hedgehog

Smo Smoothened

TGFß Transforming Growth Factor ß

Twhh Tiggy winkle hedgehog