Dedication

To Hayden, Heidi, Clara and Clytie

Acknowledgements

I would like to thank all the members of the Goldman lab for the many suggestions and insights provided during frequent discussions. This work would not have been possible without the cadre of support I enjoyed while working in the lab. Matt Veldman helped me get my feet wet during my rotation and showed me how to do many of the experiments performed here. Steve Suhr taught me a great deal about cloning. Marie-Claude Senut showed me how to prepare and cut cryosections as well as immunostaining techniques. Jessica Gumerson performed the morpholino experiments in chapter III. Pete Macpherson and Huibin Tang provided helpful criticism to enhance the project. Having a good mentor in Dan greatly enhanced my learning experience. He taught me to think critically about the results and convince myself that my interpretation was sound before trying to convince others.

I would also like to thank my parents who always encouraged my scientific curiosity. This also would not have been possible without support from my wife Heidi whom I love dearly.

Table of Contents

Dedication.		ii
Acknowled	gements	iii
List of Figu	res	v
Chapter		
I.	Introduction	1
II.	A role for alpha1 tubulin-expressing Muller glia in regeneration	n of the
	injured zebrafish retina	24
	Summary	24
	Introduction	25
	Results	27
	Discussion.	36
III.	The proneural bHLH gene ascl1a is required for	retina
	regeneration	66
	Summary	66
	Introduction	67
	Results	69
	Discussion	77
IV.	Conclusion	111

List of Figures

Figure

1.1	Proneural gene expression and lateral inhibition	17
1.2	Schematic diagram of the retina	18
2.1	-1016α1T:GFP transgenic fish induce GFP expression in Müller glia follow	ing
	retinal injury	.48
2.2	Retinal ganglion cells from -1016α1T:GFP transgenic fish do not express GFP at	ter
	optic nerve crush	49
2.3	-1016α1T:GFP transgene expression in the circumferential germinal zone	50
2.4	Injury-induced cell proliferation and GFP expression	.51
2.5	-1016α1T:GFP expression is specific to Müller glia	.52
2.6	Neurogenic clusters are derived from Müller glia	53
2.7	Proliferating cells migrate to other nuclear layers	54
2.8	Neurogenic clusters are tightly apposed cells with Müller glial characteristics	56
2.9	Electron micrograph of a migrating cell	57
2.10	Cells derived from GFP ⁺ Müller glia express markers of differentiating amacr	ine
	and retinal ganglion cells	59
2.11	Cells labeled with BrdU at 4dpi become new neurons and glia	60
2.12	Cells labeled with BrdU at 4dpi exhibit little proliferation at later times	61
3.1	A 109bp region of the $\alpha 1T$ promoter is required for transgene expression	in
	dedifferentiating Müller glia	92
3.2	An E-box within the 109bp region that is required for transgene expression	in
	Müller glia binds nuclear extracts from zebrafish brain and retina	93
3.3	The E-box is required for transgene expression in vivo	94
3 4	Transgene expression in developing zehrafish	95

3.5	TG-954CA transgenic fish express GFP in axotomized retinal ganglion cells	96
3.6	Ascl1a is induced in proliferating Müller glia following retinal injury	97
3.7	Timeline of gene expression following retinal injury	98
3.8	Ascl1a regulates the α1T promoter through the E-box <i>in vitro</i>	99
3.9	Ascl1a is required for transgene expression in vivo	101
3.10	Ascl1a morpholinos do not affect GFP expression in axotomized ganglion cells.	102
3.11	Ascl1a knockdown prevents induction of α1T and pax6	104
3.12	Ascl1a is required for proliferation of Müller glia	106