Endogenous regeneration of the mammalian retina: Identification of novel factors for the reprogramming of retinal glia to neurons

By Camille Boudreau-Pinsonneault

Integrated Program in Neuroscience, Faculty of Medicine
McGill University, Montreal

July 2021

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Doctor of Philosophy

Table of contents

ABSTRACT **4**

RÉSUMÉ **5**

ACKNOWLEDGEMENTS **7**

THESIS PREFACE **9**

LIST OF TABLES AND FIGURES **11**

LIST OF ABBREVIATIONS **13**

CHAPTER 1: LITERATURE REVIEW **17**

1. 1. INTRODUCTION: THE CENTRAL NERVOUS SYSTEM 18
1. 1. 1. Investigating therapies for neurodegenerative diseases *20*
1. 2. THE RETINA 21
1. 2. 1. Retinogenesis and temporal patterning *21*
1. 2. 1. 1. Temporal identity factors in Drosophila 23
1. 2. 1. 2. Temporal identity factors in mice 26
1. 2. 2. Retinal architecture *31*
1. 2. 2. 1. Photoreceptors 33
1. 2. 2. 2. Interneurons 36
1. 2. 2. 3. Retinal ganglion cells 37
1. 2. 2. 4. Müller glia 38
1. 2. 3. Retinal degenerative diseases *41*
1. 2. 3. 1. Retinal ganglion cell degeneration 41
1. 2. 3. 2. Photoreceptor degeneration 43
1. 2. 3. 2. 1. Cone photoreceptor degeneration 43
1. 2. 3. 2. 2. Rod photoreceptor degeneration 47
1. 3. THERAPIES FOR CENTRAL NERVOUS SYSTEM DEGENERATION 49
1. 3. 1. Preventive therapies *49*
1. 3. 1. 1. Neuroprotective factors 50
1. 3. 1. 2. Gene therapy 52
1. 3. 1. 3. Cell conversion: Reprogramming 54
1. 3. 2. Restorative therapies *58*
1. 3. 2. 1. Neural implants 58
1. 3. 2. 2. Optogenetics 61
1. 3. 2. 3. Cell transplantation 65
1. 3. 2. 4. Endogenous regeneration 69
1. 4. ENDOGENOUS RETINAL REGENERATION IN LOWER VERTEBRATES 71
1. 4. 1. Ciliary margin zone *71*
1. 4. 2. Retinal pigment epithelium *72*
1. 4. 3. Müller glia *73*
1. 5. MOUSE RETINAL REGENERATION FROM MÜLLER GLIA 76
1. 6. AIMS AND RATIONALES 83

CHAPTER 2: MATERIALS AND METHODS **86**

2. 1. ANIMALS 87
2. 2. TAMOXIFEN ADMINISTRATION 87
2. 3. INTRAVITREAL INJECTIONS 87
2. 4. DNA CONSTRUCTS 88
2. 5. *EX VIVO* MÜLLER GLIA REPROGRAMMING 89
2. 6. *IN VIVO* MÜLLER GLIA REPROGRAMMING 90
2. 7. PRIMARY MOUSE EMBRYONIC FIBROBLAST CULTURE 90
2. 8. LENTIVIRAL VECTORS PRODUCTION 91

2. 9. Lentiviral infection of mouse embryonic fibroblast culture ... 91
2. 10. Mouse embryonic fibroblast reprogramming assay ... 92
2. 11. Eye fixation and cryosectionning ... 93
2. 12. Immunostaining ... 93
2. 13. Microscopy and cell counts ... 94
2. 14. Fluorescent-activated cell sorting ... 95
2. 15. scRNA-sequencing ... 96
2. 16. scRNA-sequencing analysis ... 96
2. 17. Mouse embryonic fibroblast RNA extraction and sequencing ... 97
2. 18. Mouse embryonic fibroblast RNA-sequencing analysis ... 97
2. 19. Mouse embryonic fibroblast ATAC-sequencing ... 98
2. 20. ATAC-sequencing analysis ... 98
2. 21. Statistics ... 98

CHAPTER 3: GENETIC LINEAGE TRACING OF MÜLLER GLIA AFTER INJURY AND GROWTH FACTOR TREATMENT ... 99

Chapter 3 preface ... 100
3. 1. Glast-CreER;R26R-EYFP specifically labels Müller glia ... 101
3. 2. Few Müller glia incorporate EdU after NMDA and EGF treatments ... 103
3. 3. NMDA and EGF do not induce robust Müller glia neurogenesis ... 106

CHAPTER 4: COMBINED IKZF1 AND IKZF4 EXPRESSION REPROGRAMS MAMMALIAN MÜLLER GLIA AND EMBRYONIC FIBROBLASTS TO NEURONS ... 109

Chapter 4 preface ... 110
4. 1. Combined Ikzf1 and Ikzf4 expression reprograms Müller glia to cone-like cells *ex vivo* ... 112
4. 2. Ikzf1/4 do not promote Müller glia cell cycle re-entry ... 125
4. 3. Ikzf1/4 reprogram Müller glia to cone-like cells *in vivo* ... 126
4. 4. Degenerated retinas are responsive to reprogramming by Ikzf1/4 ... 129
4. 5. Detection of reprogrammed cells following Ikzf1/4 expression in Müller glia is not the result of material exchange ... 130
4. 6. Reprogrammed cells share transcriptional profiles with both cones and bipolar cells ... 133
4. 7. Ikzf1/4 convert mouse embryonic fibroblasts into neurons ... 138
4. 8. Ikzf1/4 increase chromatin accessibility and activate a neuronal program in mouse embryonic fibroblasts ... 139

CHAPTER 5: DISCUSSION ... 145

5. 1. Summary ... 146
5. 1. 1. Contributions to knowledge ... 146
5. 2. Discrepancies in Müller glia cell cycle re-entry and neurogenesis ... 147
5. 3. Reprogramming glia to neuron-like cells with Ikzf1/4 ... 149
5. 4. Eliciting the reprogramming potential of temporal identity factors ... 152
5. 5. Ikzf1/4 have classic reprogramming properties ... 154
5. 6. Implications for temporal identity factor-mediated reprogramming in the CNS ... 156
5. 7. Future perspectives ... 157
5. 7. 1. Identifying mechanisms underlying Ikzf1/4-mediated Müller glia reprogramming ... 157
5. 7. 2. Inducing cone-like cell maturation ... 158
5. 7. 3. Generating other cell types from Müller glia with temporal identity factors ... 159
5. 7. 4. Investigating the scope of temporal identity factor-mediated reprogramming ... 160
5. 7. 5. Exploring temporal identity factor reprogramming in other CNS areas ... 161
5. 8. Conclusion ... 162

REFERENCES ... 163

Abstract

The mammalian central nervous system (CNS) is mostly devoid of regenerative abilities. Injuries and degenerative disorders hence lead to irreversible loss of neurons and can result in devastating impairments. Stimulating endogenous regeneration, as occurs in lower vertebrates, would allow mammals to restore tissue integrity and function. Glia have been identified as a potential source of regeneration in the CNS since they are present in large numbers, are resistant to trauma, and some have the capacity to proliferate. Here, we take advantage of the retina as a model system to investigate glia-mediated CNS endogenous regeneration. We initially examine the regenerative potential of the main retinal glia, Müller glia, after injury and growth factor treatments. We find that these manipulations are not sufficient to induce robust regenerative capacities in Müller glia, sparking the need for novel methods to achieve this. We follow up by investigating whether temporal identity factors, which instruct, and can reprogram, the temporal competence of neural progenitors during development, could similarly reprogram the identity of differentiated cells. We find that co-expression of the temporal identity factor Ikzf1, with its family member Ikzf4, is sufficient to convert adult mouse retinal glia into neuron-like cells with mixed cone and bipolar identities. We also report that co-expression of Ikzf1 and Ikzf4, along with Brn2 and Myt1l, are sufficient to reprogram mouse embryonic fibroblasts into induced neurons by quickly increasing chromatin accessibility of neuronal-specific genes and inducing their expression. Work presented in this thesis identifies novel neuronal reprogramming factors, and uncovers new therapeutic opportunities for neurodegeneration.

Résumé

Le système nerveux central (SNC) des mammifères est principalement dépourvu de capacités régénératrices. Les blessures et maladies dégénératives génèrent donc une perte irréversible de neurones et peuvent mener à des déficiences dévastatrices. Stimuler la régénérescence endogène, comme cela se produit chez les vertébrés inférieurs, permettrait aux mammifères de rétablir l'intégrité et la fonction de leurs tissus nerveux. Les cellules gliales ont été identifiées comme source potentielle de régénérescence dans le SNC puisqu'elles sont présentes en grand nombre, sont résistantes aux traumas et certaines ont la capacité de proliférer. Ici, nous prenons avantage de la rétine comme modèle pour investiguer la régénérescence endogène du SNC par cellules gliales. Nous débutons en examinant le potentiel régénérateur des principales cellules gliales de la rétine, les cellules de Müller, après une blessure et un traitement avec un facteur de croissance. Nous trouvons que ces manipulations ne sont pas suffisantes pour induire une capacité régénératrice robuste des cellules de Müller, soulignant le besoin d'identifier de nouvelles méthodes pour y parvenir. Nous poursuivons en investiguant si les facteurs d'identité temporelle, qui instruisent et peuvent reprogrammer la compétence temporelle des progéniteurs neuraux lors du développement, pourraient similairement reprogrammer l'identité de cellules différenciées. Nous trouvons que la co-expression du facteur d'identité temporelle Ikzf1 avec un membre de sa famille, Ikzf4, est suffisante pour convertir les cellules de Müller, chez les souris adultes, en neurones avec une identité mixte entre cônes et bipolaires. Nous démontrons aussi que la co-expression d'Ikzf1 et Ikzf4 avec Brn2 et Myt1l est suffisante pour reprogrammer des fibroblastes embryonnaires de souris en neurones. La co-expression d'Ikzf1 et Ikzf4 dans les

fibroblastes augmente rapidement l'accessibilité de la chromatine aux gènes neuraux et induit leur expression. Le travail présenté dans cette thèse identifie de nouveaux facteurs de reprogrammation neuronale qui pourront mener à de nouvelles opportunités thérapeutiques dans le traitement de maladies neurodégénératives.

Acknowledgements

Work presented in this thesis has been accomplished with the support and guidance of numerous people. I am enormously grateful to have undertaken my doctoral studies in such a supportive environment.

I would like to thank all members, past and present, of the Cayouette lab for their important scientific guidance, encouragements, and friendships: Michel, Christine, Marine, Awais, Michel F., Mike, Thomas, Maéva, Sepideh, Benoit, Sarah, Fatima, Megan, Benjamin, Pierre, Basile, Carine, Adele, Yulia, Maude, Ko, Shaz, Pedro, Caroline, Therence, Marie-Claude, Milanka, and Katarina. I feel tremendously lucky to have spent the last several years with such a group of kind and passionate scientists. You have all greatly contributed to making my graduate experience a rewarding and enjoyable one. A special thank you to Marine for mentoring me and teaching me the ropes of the lab as a rotation student, and to Christine, Awais, and Michel F., with whom I have worked closely, for your expertise and dedication which have rendered this project possible. Of course, I would also like to acknowledge the remarkable supervision I have received from Michel: Thank you for trusting me with this complex project, and for guiding and cheering me along the way, as well as for the support you have provided me over the course of my Ph.D. Your unwavering optimism, scientific rigor, and compassion are true driving forces of the lab, rendering it an incredible environment in which to learn and grow. I am extremely thankful to have been trained as a scientist under your mentorship.

I am grateful to the IRCM neuro laboratories, Charron, Kania, and Takahashi labs, for their scientific input and camaraderie. A special thank you to Shannon for sharing the ups and downs of our Ph.D. experiences. It has been much more enjoyable with you! I would additionally like to thank the IRCM core facilities, including the Animal, Molecular Biology, Cytometry, Bioinformatics, and Microscopy facilities, with particular acknowledgements to Julie Lord, Dominic Filion, Odile Neyret, Jessica Barthe, Manon Laprise, Marie-Claude Lavallée, Caroline Dubé, and Julie Mercier, for their crucial expertise and help.

I would like to acknowledge and thank members of my committee, Drs. Cloutier and van Meyel, for their invaluable guidance on my degree and research, as well as our sources of funding, CIHR, FRQS, Brain Canada, Fighting Blindness Canada, and the Krembil Foundation, that have given us the means to tackle challenging questions.

My Ph.D. would not have been the same without the significant support I have also received from friends and family. *Merci à Léa et Gabrielle pour votre amitié irremplaçable et votre appui. Merci à ma famille, France, Alain, Loïc, Alexe et Liam, ainsi qu'à ma belle-famille, Sylvie, Michel et Charles, pour votre grande générosité et votre soutien constant. Maman et Papa : vous m'avez donné la capacité et les opportunités de relever les plus grands défis. Merci.*

Et finalement, merci à toi, Alexandre, pour ton écoute, tes conseils, ton optimisme inépuisable, ta joie de vivre ainsi que ton précieux et généreux soutien à travers tous les hauts et les bas des dernières années. Ce doctorat n'aurait pas été possible sans toi.

Thesis preface

This thesis is written in a traditional monograph style in accordance to McGill University guidelines and the Library and Archive Canada requirements. The candidate was the lead investigator of the work presented in this thesis and wrote the entirety of the thesis with revisions from **Dr. Cayouette**. All experiments were performed by the candidate with exceptions specified in the preface of Chapter 4.

The candidate contributed to 4 articles during their Ph.D.:

- M. Lacomme, B. Tarchini, **C. Boudreau-Pinsonneault**, C. Monat, M. Cayouette. (2016) The LGN protein promotes planar proliferative divisions in the neocortex but apicobasal asymmetric terminal divisions in the retina. Development *143*, 575-581.
- **C. Boudreau-Pinsonneault**, and M. Cayouette. (2018). Cell lineage tracing in the retina: Could material transfer distort conclusions? Developmental Dynamics *247*, 10-17.
- M. Fries, T. Brown, C. Jolicoeur, **C. Boudreau-Pinsonneault**, A. Javed, P. Abram, M. Cayouette (in preparation) Transcriptional regulation establishes the contra- versus ipsilateral fates of retinal ganglion cells during development.
- **C. Boudreau-Pinsonneault**, A. Javed, M. Fries, P. Mattar, M. Cayouette. (in preparation) Direct neuronal reprogramming by temporal identity factors.

Discoveries, described in Chapter 4, are part of a submitted patent application.

The candidate received scholarships from: the Canadian Institutes of Health Research (CIHR) and the Fonds de recherche du Québec - Santé (FRQS). Work presented in this thesis was funded by grants from Brain Canada, CIHR, Fighting Blindness Canada, and the Krembil Foundation.

List of tables and figures

Chapter 1

Figure 1: Retinal cell production during development in mice. (p. 22)

Figure 2: Temporal identity factors in *Drosophila* ventral nerve cord neuroblasts. (p. 24)

Figure 3: Temporal identity factors in mouse retinal progenitor cells. (p. 29)

Figure 4: Eye and retinal architecture. (p. 32)

Figure 5: Macular degeneration. (p.46)

Figure 6: Cell differentiation and reprogramming. (p. 56)

Figure 7: Photoreceptor conversion in rod dystrophies. (p. 57)

Figure 8: Material transfer between graft and host tissue. (p. 68)

Figure 9: Müller glia-dependent retinal regeneration in zebrafish after injury. (p. 74)

Figure 10: *In vivo* reprogramming of mouse Müller glia to neurons. (p. 81)

Figure 11: Schematic representation of aim 2 hypothesis. (p. 85)

Chapter 2

Table 1: List of primary antibodies. (p. 94)

Chapter 3

Figure 12: Glast-CreER;R26R-EYFP mouse line specifically labels Müller glia. (p. 102)

Figure 13: Few Müller glia incorporate EdU after NMDA and EGF treatments. (p. 105)

Figure 14: NMDA and EGF do not induce robust Müller glia neurogenesis. (p. 107)

Chapter 4

Figure 15: Detailed representation of experimental procedure. (p.114)

Figure 16: *Ex vivo* screen for Müller glia reprogramming to neurons. (p.116)

Figure 17: Ikzf1/4 and mCherry are expressed in YFP+ cells. (p. 118)

Figure 18: Ikzf1/4 expression alters Müller glia morphology and localization *ex vivo.* (p. 120)

Figure 19: Ikzf1/4-reprogrammed cells turn off Müller glia markers and express cone markers *ex vivo.* (p. 122)

Figure 20: Markers screened in Ikzf1/4 *ex vivo* reprogramming assay. (p. 124)
Figure 21: Ikzf1/4 do not promote Müller glia cell cycle re-entry. (p. 125)
Figure 22: Ikzf1/4 reprograms Müller glia to cone-like cells *in vivo.* (p. 128)
Figure 23: Degenerated retinas are responsive to reprogramming by Ikzf1/4. (p. 130)
Figure 24: Detection of reprogrammed cells following Ikzf1/4 expression in Müller glia is not the result of material exchange. (p. 132)
Figure 25: scRNA-seq identifies reprogrammed cells as bipolar-like. (p. 135)
Figure 26: Cone and bipolar regulons are active in Ikzf1/4 reprogrammed population. (p. 137)
Figure 27: BM/Ikzf1/4 convert MEFs into neurons. (p. 138)
Figure 28: Ikzf1/4 increase chromatin accessibility and activate a neuronal gene expression program in MEFs. (p. 141)
Figure 29: Correlation between BM/Ikzf1/4 ATAC and RNA-seq. (p. 144)

Chapter 5

Figure 30: Schematic summary of thesis results. (p. 147)

List of abbreviations

AAV: Adeno-associated virus

ADP: Adenosine diphosphate

ALS: Amyotrophic lateral sclerosis

AMD: Age-related macular degeneration

ATAC-seq: Assay for transposase-accessible chromatin sequencing

ATP: Adenosine triphosphate

BAM: Brn2 + Ascl1 + Myt1l

BDNF: Brain-derived neurotrophic factor

Bip/co: Cells with mixed bipolar and cone identities

BM: Brn2 + Myt1l

BM/Ikzf1/4: Brn2 + Myt1l + Ikzf1 + Ikzf4

BrdU: Bromodeoxyuridine

BSA: Bovine serum albumin

cas: *castor*

CCP3: cleaved caspase-3

cDNA: complementary deoxyribonucleic acid

cGMP: Guanosine 3',5'-cyclic monophosphate

ChR: Channelrhodopsin

CMZ: Ciliary margin zone

CNS: Central nervous system

CNTF: Ciliary neurotrophic factor

CRISPR: Clustered regularly interspaced short palindromic repeats

D: day

DIV: Day(s) in vitro

DMEM: Dulbecco's modified eagle medium

DNA: Deoxyribonucleic acid

DOA: Dominant optic atrophy

DPBS: Dulbecco's Buffered saline

E: Embryonic day

EdU: 2'-Deoxy-5-ethynyluridine

EGF: Epidermal growth factor

FACS: Fluorescent-activated cell sorting

FBS: Fetal bovine serum

FDA: Food and drug administration

Fig.: Figure

GABA: Gamma Aminobutyric Acid

GCL: ganglion cell layer

GDNF: Glial cell line-derived neurotrophic factor

GO: Gene ontology

grh: *grainyhead*

GWAS: Genome-wide association study

hb: *hunchback*

HB-EGF: Heparin-binding EGF-like growth factor

HT: Hydroxy-tamoxifen

IGF1: Insulin growth factor 1

Ikzf1/4: Ikzf1 + Ikzf4

ILM: Inner limiting membrane

INL: Inner nuclear layer

IP: Intraperitoneal

IPL: Inner plexiform layer

ipRGC: intrinsically photosensitive retinal ganglion cell

iPSC: induced pluripotent stem cell

kr: *kruppel*

LCA: Leber congenital amaurosis

LHON: Leber hereditary optic neuropathy

LoxP: Locus of X-over P1

MCS: Multiple cloning site

MEF: Mouse embryonic fibroblast

NGF: Nerve growth factor

NMDA: N-methyl-D-aspartate

NpHR: Halorhodopsin

n.s.: not significant

mRNA: messenger ribonucleic acid

NuRD: Nucleosome remodeling and deacetylase

ONL: Outer nuclear layer

OPL: Outer plexiform layer

P: Post-natal day

PBS: Phosphate-buffered saline

Pen/Strep: Penicillin and Streptomycin

PFA: Paraformaldehyde

QVD-OPH: Quinoline-Val-Asp-Difluorophenoxymethylketone

RdCVF: Rod-derived cone viability factor

RGC: Retinal ganglion cell

RPE: Retinal pigment epithelium

SCENIC: single-cell regulatory network inference and clustering

scRNA-seq: single cell ribonucleic acid sequencing

Seq: Sequencing

TNFα: Tumor necrosis factor alpha

TSA: Trichostatin A

TSS: Transcription start site

UMAP: Uniform manifold approximation and projection

VEGF: Vascular endothelial growth factor

YFP: Yellow fluorescent protein

Chapter 1: Literature review

1. 1. Introduction: The central nervous system

Our central nervous system (CNS) is responsible for how we perceive, reason, and behave in our environment. It is astounding that a collection of cells can mediate such intricate processes underlying cognition and be the basis of what we define as our identity. How is this possible? How can cells create such complexity? Although there is no clear answer, and much still remains to be discovered, we know that it is the assortment of a diversity of cells types, each with specific and specialised functions, underlying morphologies, and molecular identities, that is capable of accomplishing this feat.

The famous drawings of Ramon y Cajal effectively depict this morphological diversity of CNS neurons, from the elaborate branches of Purkinje cells of the cerebellum to the dense rod-shaped photoreceptors of the retina. Neurons are the computational units of the CNS and their distinct characteristics allow them to receive electrical or, in some cases, sensory information, process, and transmit this to other specialised neurons. In this way, they create a neural network capable of processing convoluted data.

Although the computational heavy lifting is performed by neurons, the CNS would not be able to function without its supportive glial cells. Glia were initially thought to be passive cells of the nervous system in place to maintain the structure of the more important neurons. Rudolf Virchow coined the term ‘neuroglia’ in the 1850s to signify ‘nerve-cement’ or ‘glue’ illustrating this inert role of glia (Allen and Lyons, 2018; Virchow, 1856). Incredible work has rebutted this passive role of glial cells, particularly in the last 30 years or so (Ndubaku and de Bellard, 2008). We now know that glia play active roles in neuronal

development, from cell differentiation to migration and formation of synaptic connections (Allen and Lyons, 2018), as well as in adult tissues in the maintenance of synapses, of the functional integrity of neurons, and of tissue homeostasis (Barres, 2008; Jakel and Dimou, 2017). Glia, like neurons, are heterogeneous and specialised cells, exquisitely tuned to the requirements of their environments and surrounding neurons. Together, neurons and glia form an elaborate neural tissue finely balanced for information processing.

The adult mammalian CNS is predominantly devoid of neurogenesis (with exceptions of small neurogenic niches in the subventricular and subgranular zones (Obernier and Alvarez-Buylla, 2019)). Loss of the specialised CNS cells due to injury or diseases is thus irreversible and can lead to, sometimes major, lifelong impediments. For instance, the loss of dopaminergic neurons of the substantia nigra leads to motor dysfunction in Parkinson's disease (Radhakrishnan and Goyal, 2018), loss of motor neurons in the cortex, brainstem, and spinal cord leads to paralysis in amyotrophic lateral sclerosis (ALS) (Hardiman et al., 2017), loss of neurons in the hippocampus leads to memory deficits in Alzheimer's disease (Masters et al., 2015), and loss of cone photoreceptors of the retina leads to blindness in age-related macular degeneration (Rattner and Nathans, 2006).

Although highly varied, neurodegenerative diseases share some common mechanisms including mitochondrial dysfunction, detrimental immune responses, and defects in protein function and processing leading to toxic aggregates (Gan et al., 2018). These defects accumulate over time and eventually lead to unhealthy neurons incapable of

performing their basic functions, and to their death. Some genetic alterations underlying these changes are well established in the case of Huntington's disease, for instance, with excessive CAG repeats in the *HD* gene (Walker, 2007). However, a majority of neurodegenerative disease cases have intricate associations between genes and phenotype which are difficult to pin point (Gan et al., 2018). Additional research is required to fully understand neurodegenerative mechanisms. Even so, therapeutic approaches to prevent some neurodegenerative hallmarks, and restore nervous system function are being investigated.

1. 1. 1. Investigating therapies for neurodegenerative diseases

It is no surprise, with such severe consequences, that finding solutions to maintain or restore nervous system function in neurodegenerative diseases is an active area of research. Age is a risk factor for these diseases and, with an ageing population, we can only expect the number of people affected and the associated financial burden to increase in the coming years (Hou et al., 2019). Many different therapeutic avenues are currently being developed (section 1.3), and some are showing great promise. Still, considerable research is required for most potential therapies to prove efficient, and, eventually, reach the clinic. Research is rendered difficult by the complexity of many CNS areas in terms of the broad diversity of cells composing these tissues and their extensive connections. Indeed, regenerative approaches must recreate normal CNS functions and, to do so, must be grounded on a deep understanding of how the targeted tissue is built under healthy conditions, which is still somewhat lacking in many CNS regions. Also, several parts of the CNS, such as the brain, cerebellum, and spinal cord, are difficult to access

since they are protected by bones of the skull or vertebral column, and by meninges. Reaching them requires invasive and complex surgeries.

Although much still remains to be discovered, retinal biology has been extensively studied and we have a decent grasp of how retinal cells are generated, organized, and how the retina functions. Its ease of accessibility, not requiring invasive procedures, and relative simple organization (described in section 1.2.2) compared to other parts of the CNS, render the retina especially well-suited as a model system to study CNS therapeutic approaches.

1. 2. The retina

1. 2. 1. Retinogenesis and temporal patterning

The vertebrate retina is a structured neural tissue located at the back of the eye responsible for transforming light from the environment in electrical signal, initial processing of this information, and its transmission to the brain. It is made of two photoreceptor types, rods and cones, three classes of interneurons, bipolar, amacrine, and horizontal cells, projections neurons, retinal ganglion cells (RGCs), and the main retinal glia, Müller glia (described in detail in section 1.2.2). This neural tissue is generated from a pool of multipotent progenitors, which give rise to all main retinal cell types (Turner et al., 1990) in a sequential and overlapping manner during development from about embryonic day 10 (E10) to post-natal day 10 (P10) in the mouse (Carter-Dawson and LaVail, 1979) (Fig. 1). Early-born cells, mostly pre-nataly in the mouse, consist of RCGs, horizontal cells, cone photoreceptors, and amacrine cells, whereas late-born cells,

predominantly generated post-nataly in the mouse, are rod photoreceptors, bipolar cells, and Müller glia (Carter-Dawson and LaVail, 1979; Turner et al., 1990; Young, 1985) (Fig. 1). In this way, retinal progenitor cells change competence over time to generate differentiated post-mitotic cell types that do not have progenitor capacities.

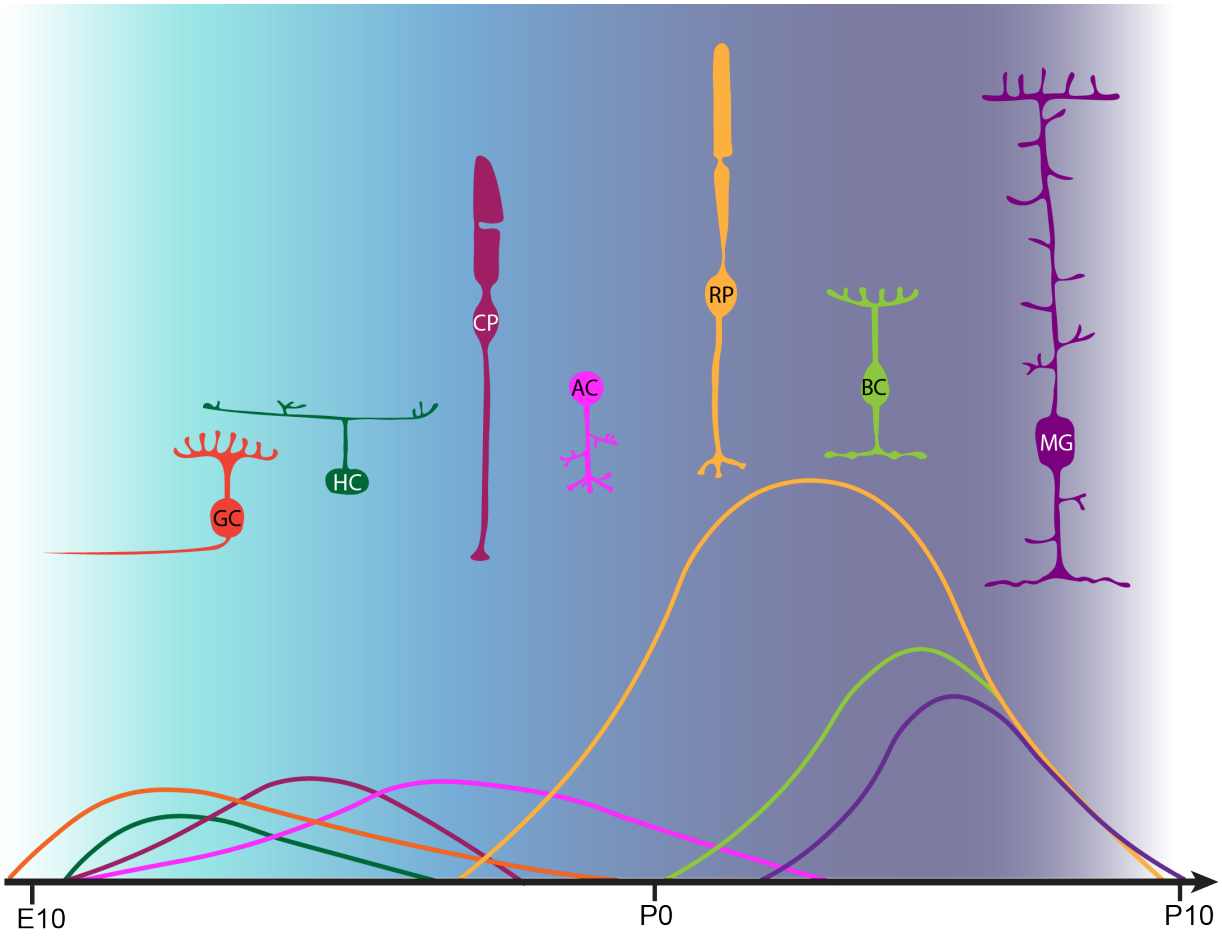


Figure 1: Retinal cell production during development in mice.

Representation of retinal cell type birth order (based on (Mattar et al., 2015)). X-axis represents time and Y-axis represents the number of cells generated. Blue to purple background gradient indicates temporal progression of progenitor cell competence to generate differentiated cells (top). Cell and genesis curve are associated by color. GC: Ganglion cell; HC: Horizontal cell; CP: Cone photoreceptor; AC: Amacrine cell; RP: Rod photoreceptor; BC: Bipolar cell; MG: Müller glia. E: Embryonic day; P: Post-natal day.

1. 2. 1. 1. Temporal identity factors in Drosophila

Such temporal patterning, changing competence over time to generate different cell types at specific stages, as seen in the retina, occurs in most neural progenitors, including those in the neocortex, hindbrain, and spinal cord (Holguera and Desplan, 2018; Oberst et al., 2019; Rossi et al., 2017). Temporal patterning is an evolutionary conserved strategy to regulate CNS progenitor output and has been extensively studied in *Drosophila melanogaster*. A well described temporal patterning occurs in ventral nerve cord neuroblasts, which generate stereotypic progenies of motor neurons, glia, and interneurons that evolve with time. Ventral nerve cord neuroblasts divide asymmetrically to give rise to one neuroblast and one ganglion mother cell (Fig. 2A). The latter subsequently divides to generate two neurons or glia. Temporal progression of embryonic ventral nerve cord neuroblasts is regulated by the sequential expression of temporal identity factors consisting of *hunchback (hb)*, *kruppel (kr)*, *pdm*, *castor (cas)*, and *grainyhead (grh)* (Brody and Odenwald, 2000; Grosskortenhaus et al., 2005; Grosskortenhaus et al., 2006; Isshiki et al., 2001; Kambadur et al., 1998; Pearson and Doe, 2003) (Fig. 2B). Progression between these factors generally occurs at each neuroblast division. However, it is not cell cycle itself that instructs cascade progression, as cytokinesis is only required for the *hb* to *kr* transition (Grosskortenhaus et al., 2005). Instead, cross-regulatory mechanisms instruct cascade progression: Each temporal identity factor activates the expression of the following factor while repressing the expression of further downstream and upstream factors (Doe, 2017) (Fig. 2B).

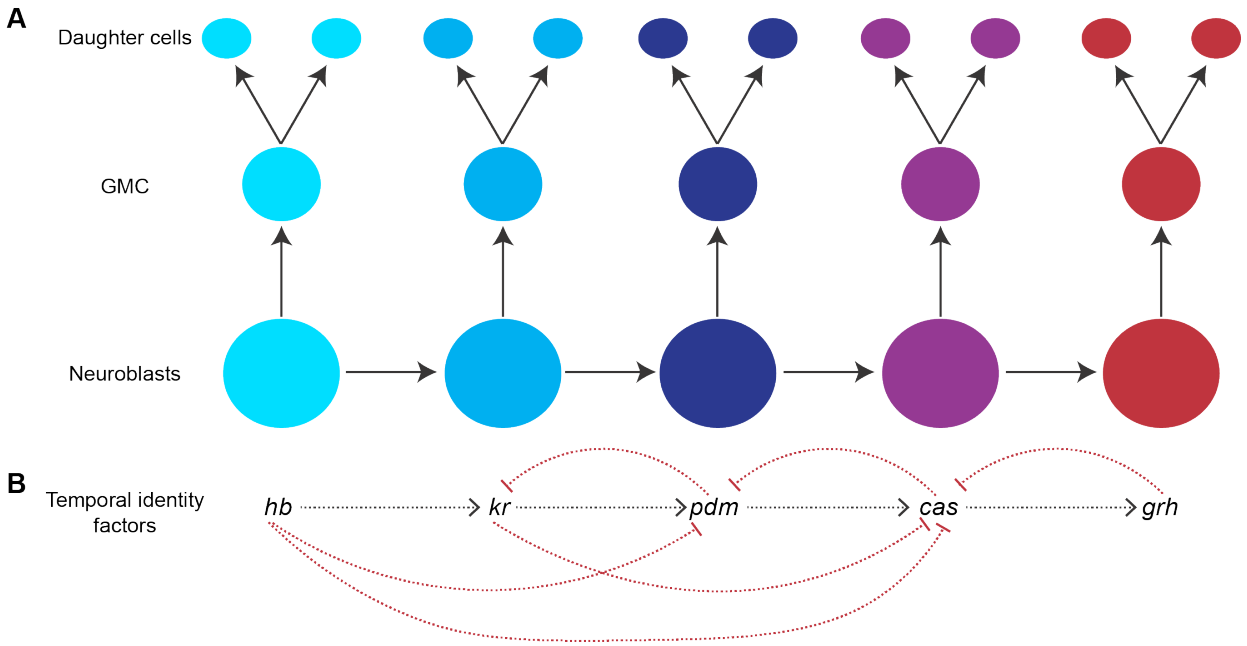


Figure 2: Temporal identity factors in *Drosophila* ventral nerve cord neuroblasts. **A.** Representation of neuroblast lineages. Each neuroblast gives rise (shown with arrows) to another neuroblast and one ganglion mother cell (GMC), which produces a pair of neurons or glia. Changing colors represent changes in neuroblast competence and their associated progeny. **B.** Temporal identity factor cascade regulating neuroblast competence shown in (A). Black dotted lines and arrows show induction of factors while red dotted lines show repression. (Information adapted from Grosskortenhaus et al. (2006))

These temporal identity factors are necessary and sufficient to confer neuroblasts the competence to generate their associated cell types within each lineage (Cleary and Doe, 2006; Novotny et al., 2002; Pearson and Doe, 2003). For instance, loss of the early factor *hb* resulted in the loss of early-born neurons, while later-born neurons were produced normally (Novotny et al., 2002). In contrast, sustained expression of *hb* indefinitely kept neuroblasts in an early competence window and maintained production of early-born neurons (Grosskortenhaus et al., 2005). Subsequent release of *hb* expression led to normal cascade progression (Grosskortenhaus et al., 2005). Similarly, ectopic expression

of *hb* in older neuroblasts, where it is not normally expressed, conferred them the capacity to generate early fates. However, neuroblast plasticity to these temporal identity factors is lost after 9-10 divisions, with ectopic expression of these factors not leading to changes in competence after this stage (Cleary and Doe, 2006; Grosskortenhaus et al., 2006). Closure of the competence window correlates with genome reorganisation, silencing temporal identity target genes and thus preventing their activity (Kohwi et al., 2013). Similarly, post-mitotic neurons were not reprogrammed by expression of *hb* or *kr* (Cleary and Doe, 2006; Pearson and Doe, 2003), suggesting that differentiated cells also lose the competence to re-specify their temporal fate with these factors.

Importantly, this same cascade of temporal identity factors regulates temporal progression of multiple neuroblast lineages. For instance in neuroblast 7-1, *hb* induces the generation of early-born U1 and U2 neurons, while in neuroblast 3-1 it promotes early-born RP1 and RP4 neurons (Doe, 2017). A recent study has identified that it is the presence of spatial factors, leading to neuroblast-specific chromatin organisation, that allows for this diversity of output from the same temporal identity factors (Sen et al., 2019). In this way, different chromatin organisation allows the same temporal identity factors to access distinctive targets in each neuroblasts in order to generate appropriate cell types. This capacity of temporal identity factors to induce different fates in different neuroblasts highlights the central notion that they are not fate, but rather general competence regulators.

1. 2. 1. 2. Temporal identity factors in mice

In vertebrates, temporal patterning is more intricate, with complex intrinsic and extrinsic cues contributing to temporal progression of neural progenitors (Oberst et al., 2019). Still, vertebrate homologs of *Drosophila* temporal identity factors have been reported to participate in temporal patterning of retinal and cortical progenitors (Alsio et al., 2013; Elliott et al., 2008; Javed et al., 2020; Mattar et al., 2015). In the mouse retina, Ikzf1, the homolog of *hb*, confers early progenitor identity (Elliott et al., 2008), Pou2f1, the homolog of *pdm*, confers mid-temporal identity (Javed et al., 2020), and Casz1, the homolog of *cas*, confers late temporal identity (Mattar et al., 2015) (Fig. 3). Foxn4, although not a homologue of a *Drosophila* temporal identity factor, has also recently been found to regulate early/mid-temporal progenitor identity (Liu et al., 2020). Similarly as in *Drosophila*, mis-expression of these temporal identity factors in mouse retinal progenitor cells alters their competence and neuronal output. This is described in more details below. Please note that, for brevity, articles identifying and describing temporal identity factors are only referred to once at the beginning of each section. Information obtained from other articles is specified throughout.

Ikzf1 (Elliott et al., 2008), a zinc finger transcription factor, is expressed in embryonic retinal progenitor cells and its expression is lost by P4. Abolishing Ikzf1 resulted in a reduction of early-born cell fates, RGCs, amacrine and horizontal cells, while late-born fates were not affected. *Ikzf1* knock-out also reduced progenitor proliferation transiently around E13, when early-born cells are normally being generated. Inversely, ectopic expression of Ikzf1 in late retinal progenitors, where it is not endogenously expressed,

provided these cells the competence to generate the early-born RGCs, amacrine and horizontal cells, at the expense of the late-born Müller glia. In this case, mis-expression of Ikzf1 did not alter progenitor clone size. Ikzf1 participates to temporal progression by repressing the expression of the late factor Casz1 (Mattar et al., 2015) and inducing the expression of the early/mid temporal factors FoxN4 (Liu et al., 2020) and Pou2f1 (Javed et al., 2020) (Fig. 3B). Ikzf1 was additionally shown to act as a temporal factor in the neocortex where it is expressed at high levels in early progenitors (Alsio et al., 2013). Sustained Ikzf1 expression in cortical progenitors prolonged the period of early-born cell fate genesis and caused a delay in late-born fate genesis (Alsio et al., 2013). Unlike in the retina, ectopic expression of Ikzf1 in late cortical progenitors did not alter their competence (Alsio et al., 2013), suggesting that there exists a window of competence for Ikzf1 activity in cortical progenitors, similarly as in *Drosophila*. Furthermore, knock-out of *Ikzf1* did not impact cortical progenitor output (Alsio et al., 2013), possibly due to redundancy with family members compensating for Ikzf1 loss of function.

Pou2f1 and Pou2f2 (Javed et al., 2020), POU-homeodomain factors, are expressed in embryonic retinal progenitor cells from E11.5 and are absent in progenitors by P0. Their downregulation in embryonic progenitors led to decreased production of the early-born cone photoreceptors and horizontal cells, and increased production of the late-born rod photoreceptors. Inversely, ectopic expression of Pou2f1 and Pou2f2 in late progenitors, where they are not endogenously expressed, led to increased production of the early-born cones and horizontal cells at the expense of late-born rods, bipolar cells, and Müller glia. Neither of these manipulations altered clone size or proliferation. Pou2f1 is integrated

in the temporal cascade by being upregulated by Ikzf1 and inhibiting Casz1 expression (Fig. 3B). As such, Pou2f1 is considered a temporal identity factor, while Pou2f2 acts as a fate determinant downstream of Pou2f1. Foxn4 (Liu et al., 2020), also expressed in embryonic stages from E11.5 and mostly absent in post-natal stages (Li et al., 2004), was found to regulate early-mid progenitor competence, somewhat redundantly with Pou2f1. Foxn4 confers progenitors the competence to generate amacrine cells, cones, horizontal cells, and rods, while inhibiting the competence to generate RGCs. Foxn4 participates to the temporal cascade by repressing Ikzf1 expression while inducing Casz1 expression (Fig. 3B). The interaction and regulation between Foxn4 and Pou2f1 remains to be investigated.

The temporal identity factor cascade identified to date, in the retina, ends with the late temporal factor Casz1 (Mattar et al., 2015) (Fig. 3B). This zinc finger transcription factor is expressed in progenitor cells in mid to late stages of retinal development, mostly from E14.5 to P4. Knock-out of *Casz1* resulted in increased production of the early-born horizontal cells, amacrine cells, and cones as well as the latest-born Müller glia, at the expense of late-born rods. Inversely, ectopic expression of Casz1 in early progenitors, where it is not normally expressed, led to increased production of late-born bipolar cells and rods at the expense of the early-born horizontal cells, amacrine cells, and cones, as well as the latest-born Müller glia. Similarly as previously, these manipulations did not alter progenitor proliferation. Casz1 hence provides progenitors the competence to generate the late-born rod photoreceptors and bipolar cells. The temporal identity factor

responsible for conferring gliogenic competence in the retina remains to be identified (Fig. 3B).

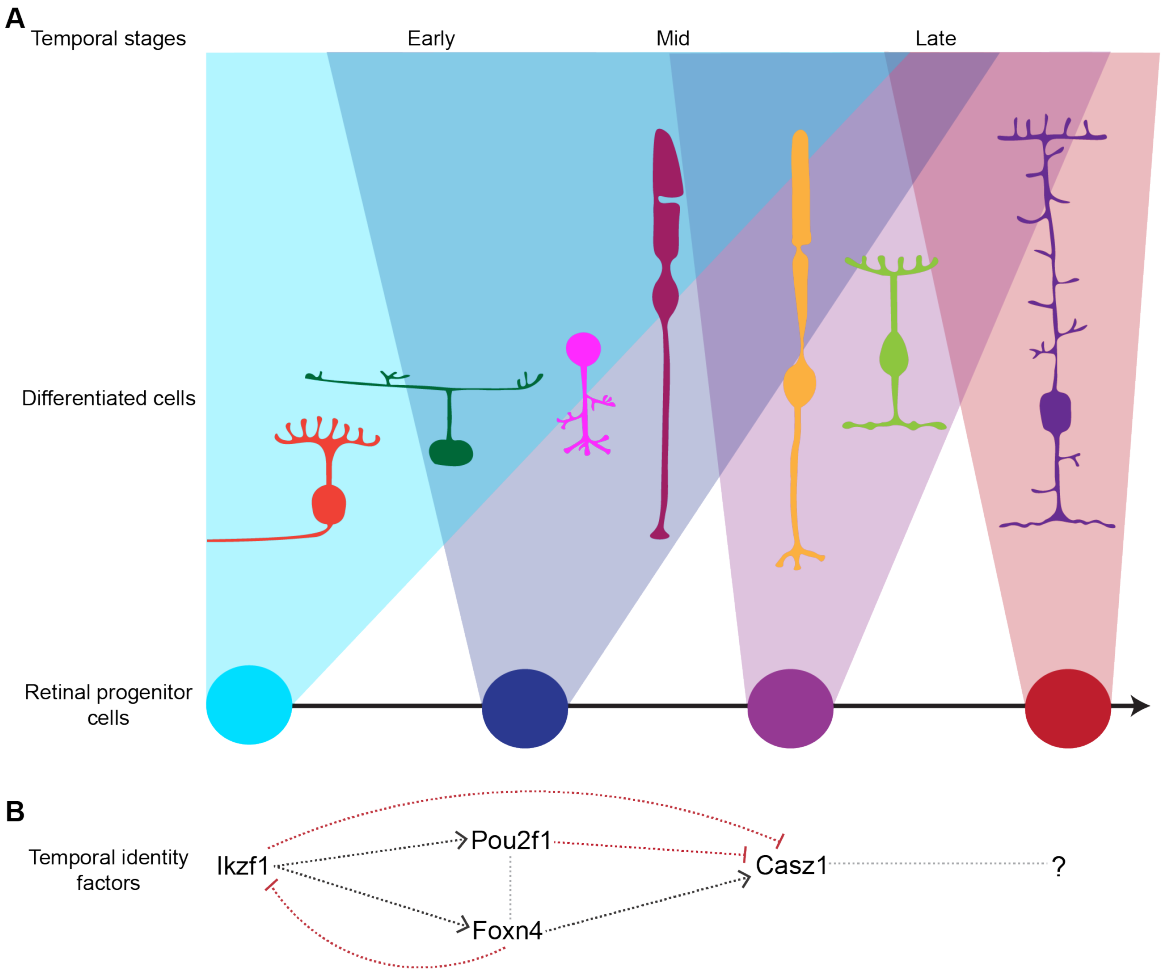


Figure 3: Temporal identity factors in mouse retinal progenitor cells.

A. Representation of retinal progenitor temporal competence. Black arrow shows time. Progenitor colors and projections represent progenitor competence and associated progeny for each stage. **B.** Temporal identity factor cascade. Factors are placed under the associated progenitor competence (A). Black dotted lines and arrows show induction, while red dotted lines show repression. Gray dotted lines show interactions remaining to be investigated.

A recent study (Mattar et al., 2021) has demonstrated that Casz1 interacts with the nucleosome remodeling and deacetylase (NuRD) complex to recruit polycomb repressive complexes to the genome. Repression of either NuRD or polycomb repressive complexes abolished Casz1-dependent induction of rod production and inhibition of gliogenesis (Mattar et al., 2021), indicating that these complexes are necessary for Casz1 function. Although mechanisms used by other temporal identity factors remain to be investigated, Ikzf1 was shown to interact with chromatin remodeling complexes such as Mi-2/NuRD and SWI/SNF (Kim et al., 1999; O'Neill et al., 2000), suggesting it could also modify chromatin landscape to alter retinal progenitor competence. This contrasts with the mode of action of temporal identity factors in *Drosophila* neuroblasts (section 1.2.1.1), where spatial factors alter neuroblast epigenome to provide access to temporal factor targets. It seems that mammalian temporal identity factors would instead modify the epigenome themselves to alter progenitor competence.

Altogether, these transcription factors generate a temporally regulated cascade to control retinal progenitor competence (Fig. 3). Gain of function experiments alter the balance of early, mid, and late cell fates without obliterating endogenous progenitor competence (still generating some of their appropriate progeny) and does not alter lineage size. Loss of function experiments decrease the production of the associated cell fates, but does not lead to complete loss of these cells. In this way, each temporal identity factor biases progenitors to generate their associated cell types in a permissive manner. Interestingly, most of these temporal identity factors are also expressed in neurons (Elliott et al., 2008; Javed et al., 2020; Mattar et al., 2015). It has been shown that Casz1 regulates rod

photoreceptor chromatin architecture (Mattar et al., 2018), demonstrating that temporal identity factors play diverse roles in different cellular contexts.

1. 2. 2. Retinal architecture

The 7 specialised retinal cell types mentioned above are organized in three distinct nuclear layers (Fig. 4B). Photoreceptors, rods and cones, located in the outer nuclear layer (ONL), capture light photons and transduce them in electrical signals. This remarkable feat is described in more details below (section 1.2.2.1). They transmit this signal to bipolar cell interneurons located in the inner nuclear layer (INL), which, in turn, transmit the information to RGCs (section 1.2.2.3), the only projection neurons of the retina, located in the ganglion cell layer (GCL). Two other types of interneurons in the INL, horizontal and amacrine cells, modulate the electrical signal at the photoreceptor-bipolar and bipolar-ganglion cell interface, respectively. The main retinal glia, Müller glia, also have their cell bodies within the INL. Although three types of glial cells are present within the rodent retina, Müller glia, astrocytes, and microglia, only Müller glia are derived from retinal progenitors (Turner and Cepko, 1987).

Above the retina lays the retinal pigment epithelium (RPE), and the choroid vasculature, which are separated by Bruch's membrane, a space filled with extracellular matrix (Fig. 4A-B). The RPE plays essential roles in retinal function by phagocytosing photoreceptor outer segments, participating to the visual cycle, and regulating exchanges between the choroid vasculature and underlying photoreceptors (Strauss, 2005). Another vasculature system, regulated by Müller glia, is present within the inner retina (from the GCL to outer

plexiform layer (OPL); not shown) to supply oxygen and nutrients to interneurons and RGCs.

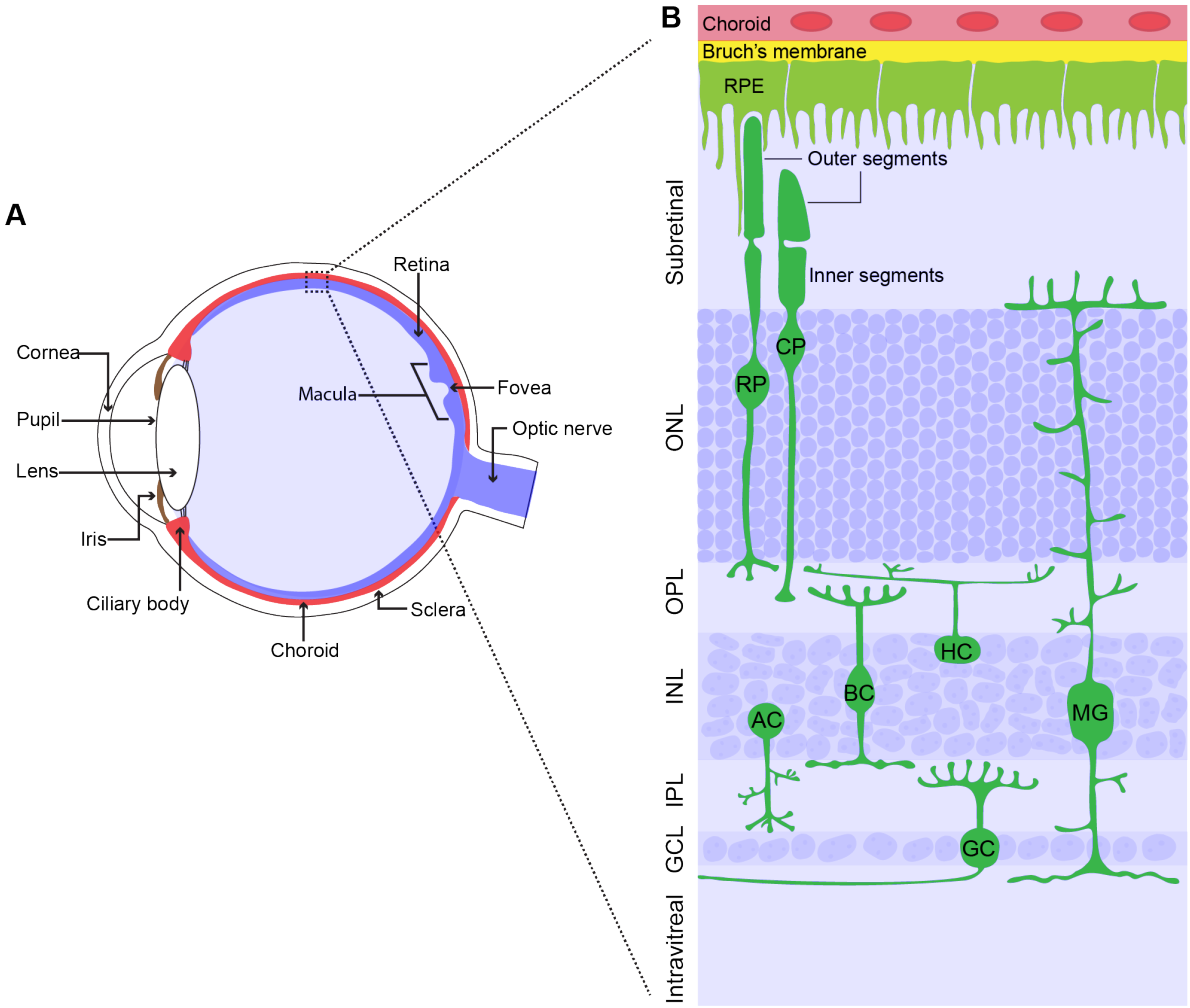


Figure 4: Eye and retinal architecture.

A. Human eye structures. **B**. Representation of retinal organisation. Layers - ONL: Outer nuclear layer, OPL: Outer plexiform layer, INL: Inner nuclear layer, IPL: Inner plexiform layer, GCL: Ganglion cell layer. Cells – RP: Rod photoreceptor, CP: Cone photoreceptor, AC: Amacrine cell, BC: Bipolar cell, HC: Horizontal cell, MG: Müller glia, GC: Retinal ganglion cell, RPE: Retinal pigment epithelium.

In humans, the central visual field is focused by the cornea and lens on a specialised high acuity region of the retina: the macula (Fig. 4A). This oval region of about 5.5mm of diameter contains a high density of retinal neurons (Bringmann et al., 2018), which results in an increased spatial resolution of the encoded light signal. The central part of the macula, the fovea, is further specialised for high acuity vision with a lack of intra-retinal vasculature and displacement of inner retinal neurons, thus reducing light scattering. Importantly, contrary to other parts of the retina where rods greatly outnumber cones, the fovea is rod-less, and solely contains cone photoreceptors and specialised Müller glia (Bringmann et al., 2018). Whereas multiple photoreceptors converge on one RGC elsewhere in the retina, in this region, one cone transfers information to one RGC (Masland, 2001). These properties permit foveal cells to encode and transmit to the brain a highly detailed representation of light. It is thanks to this tiny retinal structure that humans are able to perform many day-to-day activities as recognising faces, watching movies, and reading.

Retinal cells and their functions are described in more details below.

1. 2. 2. 1. Photoreceptors

Photoreceptors are the highly specialised light-sensing cells of the retina. Rods compose approximately 97% of mouse photoreceptors (Carter-Dawson and LaVail, 1979) and are responsible for low light vision, whereas cones compose the other 3% and mediate daylight, high acuity, and color vision. In human retinas, it is the high concentration of cones in the fovea that allow for high acuity vision (section 1.2.2). Cones can be further

subdivided based on their target wavelength (Imamoto and Shichida, 2014): humans have long, medium, and short wavelength cones responsive to red, green, and blue wavelengths respectively, and mice have medium and short-wavelength cones with some responsive to both (Applebury et al., 2000).

Photoreceptors are complex cells specialised for light detection, which can be appreciated by their particular morphologies (Fig. 4B) (Molday and Moritz, 2015; Mustafi et al., 2009). Their outer segments are made of stacks of folded double membranes embedded with visual pigments. These dense stacks allow photoreceptors to congregate high levels of pigments and capture a maximum amount of light photons. New membrane disks are continuously generated and added to outer segments, while older ones are phagocytosed by RPE cells (Young, 1967) or Müller glia (Long et al., 1986). As their names suggest, rod outer segments are thin and long rod-shaped structures, whereas cone outer segments are somewhat larger, shorter, and conical. This outer segment is attached by a connective cilium to the inner segment, which contains mitochondria, the Golgi, and endoplasmic reticulum. Their cell bodies and nuclei are located below this inner segment in the ONL. Cone nuclei locate at the apical surface of the ONL and rod nuclei fill the rest of this layer. Photoreceptor axons terminate with a synaptic pedicle for the cones or spherule for the rods on bipolar cell dendrites within the inner plexiform layer (IPL).

Phototransduction occurs within the outer segments of photoreceptors. The cascade (Kolb, 2012; Molday and Moritz, 2015) starts once a photon comes in contact with a visual

pigment, consisting of an opsin protein bound to a retinal chromophore, which dictates photoreceptor wavelength sensitivity (Imamoto and Shichida, 2014). This photon isomerizes the chromophore, activating the bound opsin, which, in turn, binds and activates the G protein transducin. Transducin initiates phosphodiesterase-mediated hydrolysis of guanosine 3',5'-cyclic monophosphate (cGMP), thus leading to the closure of cGMP-gated ion channels. As a result photoreceptors hyperpolarize and decrease the release of glutamate to their post-synaptic partners. In this way, photoreceptors hyperpolarize in light and depolarize in dark conditions. In order to maintain light sensitivity, each component of this pathway is quickly inactivated and brought back to baseline. Chromophores are re-isomerized to their photo-sensitive conformation by the RPE for rods and by both RPE and Müller glia for cones (Palczewski and Kiser, 2020). Although rod and cone photoreceptors have similar photoactivation pathways, their distinct morphologies, opsins, and second messengers confer them their divergent light sensitivities and properties.

The photoactivation pathway has low intrinsic noise, a pronounced signal amplification at each step starting from G protein activation, and is efficient even in low light conditions. Indeed, it is thought that as low as 5-14 rods detecting 1 photon each can produce a visual effect (Hecht et al., 1942). Furthermore, both cones and rod photoreceptors can adapt to light conditions through intracellular calcium levels and downstream effectors, as well as by translocation of photoactivation proteins out of the outer segment (Arshavsky and Burns, 2012). This is how we are capable of seeing both in a dark building, and when stepping outside on a sunny summer afternoon.

Unfortunately, photoreceptors are particularly susceptible to injury and genetic mutations (Stone et al., 1999b; Wright et al., 2010). The instability of their membrane-filled outer segments, their need for colossal amounts of energy to sustain outer segment function, their consequent dependence on large supplies of glucose and oxygen, and their reliance on other cells for their function and maintenance (chromophore recycling and outer segment disk phagocytosis by Müller glia and RPE) all contribute to their vulnerability. It is hence not surprising that multiple degenerative diseases (reviewed in section 1.2.3) lead to photoreceptor death and result in blindness. With their specialised properties described here, one can imagine that replacing them or their activity, for potential treatments to these disorders (section 1.3.2), is not an easy task.

1. 2. 2. 2. Interneurons

Three types of interneurons are present in the retina: Bipolar, horizontal, and amacrine cells (Fig. 4). Bipolar cells transmit photoreceptor signals to RGCs and are broadly classified as ON or OFF depending on whether they depolarize to light or dark signals. They are also categorized as cone or rod bipolar cells depending on their pre-synaptic partners, although some receive input from both photoreceptor types. In reality, over 15 bipolar cell subtypes have been identified based on single cell ribonucleic acid-sequencing (scRNA-seq) (Shekhar et al., 2016). These subtypes not only differ in their molecular identity, but also in the stratification and complexity of their axonal and dendritic arborizations and, accordingly, in the specificity and number of their pre- and post-synaptic partners. While bipolar cells, with photoreceptors and RGCs, participate to the main radial pathway of information transmission within the retina, horizontal and amacrine

cells generate lateral networks of information processing. Horizontal cells receive inputs from photoreceptors through wide lateral processes in the OPL, and are connected to each other by gap junctions, allowing them to share and regulate information from broad visual fields (Thoreson and Mangel, 2012). By regulating both photoreceptor and bipolar cell activity, horizontal cells participate to retinal light adaptation and the downstream establishment of RGC receptive fields (Chaya et al., 2017). Amacrine cells are the most diverse cell type of the retina with over 60 subtypes identified (Yan et al., 2020). They have broad and complex dendritic processes within the IPL that differ in stratification and field size between subtypes. Through their regulation of bipolar and RGC activity, amacrine cells shape spatial and temporal resolution, and are key players in motion detection (Diamond, 2017). Together, these three classes of interneurons encode diverse visual features from photoreceptor input, and transmit this information to downstream RGCs.

1. 2. 2. 3. Retinal ganglion cells

RGCs extend intricate dendrites in the IPL to gather visual features from bipolar cells and send axons along the inner limiting membrane (ILM) of the retina to the optic nerve head where they bundle to form the optic nerve (Fig. 4). RGC axons of both eyes meet at the optic chiasm: Nasal (contralateral) RGC axons cross to the other side of the brain, whereas temporal (ipsilateral) RGC axons remain on their initial side. This allows downstream brain areas to process information of the same visual field originating from both eyes. After the optic chiasm, RGC axons form the optic tracts, which project to many brain regions, including the lateral geniculate nucleus. Neurons of this structure relay

visual information to the primary visual cortex of the occipital lobe. This constitutes the vision forming pathway (Purves et al., 2001). Other optic tract projections include the Pretectum, mediating pupillary light reflex, the Suprachiasmatic nucleus, controlling circadian rhythm, and the Superior colliculus for novel stimuli detection (Dhande and Huberman, 2014). These regions receive input from a special subtype of RGCs that express melanopsin and are photosensitive, the intrinsically photosensitive retinal ganglion cells (ipRGCs) (Hattar et al., 2006; Hattar et al., 2002; Pickard and Sollars, 2010). Overall, more than 30 subtypes of RGCs haven been identified (Baden et al., 2016). These subpopulations differ in their molecular identity, the connections and stratification of their processes in the IPL, which confers them the capacity to assemble and encode distinctive visual features, and in their targets within the brain.

As the sole projection neurons of the retina, RGCs are critical for vision. Unfortunately, RGCs are particularly vulnerable to degeneration from stress to their very thin and long axons (Munemasa and Kitaoka, 2012), and from disturbances to their metabolic demands, which vastly differ between their different subcellular compartments (Yu et al., 2013) (section 1.2.3.1).

1. 2. 2. 4. Müller glia

Müller glia span the entire width of the retina with large and complex branching processes both towards the GCL, where they form the inner limiting membrane between the retina and intravitreal space, and, in the opposite direction, to the apical side of the ONL, where they form the outer limiting membrane. They also extend microvilli in the subretinal space,

next to photoreceptor segments (Fig. 4B). Müller glia, by being in contact with most retinal cells, have an ideal morphology to sense retinal environment (Wang et al., 2017). They use this information to regulate retinal homeostasis by controlling extracellular space ion concentration, water, and pH levels (Reichenbach and Bringmann, 2013). Müller glia also create the blood-retina barrier in the inner retina (Tout et al., 1993), and regulate blood vessel growth (Byrne et al., 2013; Shen et al., 2012). They play essential neuroprotective roles by producing and secreting anti-oxidants, as glutathione and pyruvate, and neuroprotective factors including brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), nerve growth factor (NGF), and ciliary neurotrophic factors (CNTF) (Bringmann et al., 2009).

Although these homeostasis regulations indirectly impact retinal function, Müller glia also participate more directly to synaptic function with the uptake and recycling of glutamate and gamma aminobutyric acid (GABA) (Bringmann et al., 2013), and with the release of adenosine triphosphate (ATP) (Newman, 2004). They additionally recycle cone chromophores (Wang and Kefalov, 2009), phagocytose cone outer segments (Long et al., 1986), and participate to outer segment formation (Jablonski and Iannaccone, 2000; Wang et al., 2005). Interestingly, Müller glia were suggested to act as optic fibers, guiding light from the intravitreal surface to photoreceptors at the back of the retina (Franze et al., 2007), possibly decreasing light scattering from the many nuclei, organelles, and processes located in the light path.

In contrast to the ‘cement’ view of Virchow (section 1.1), Müller glia were found to be elastic and soft under normal conditions, and were suggested to behave as retinal ‘shock absorbers’ (Lu et al., 2006). Under pathological conditions, however, they become reactive and stiffen by the upregulation of intermediate filaments (Lu et al., 2011). Their reactive process varies depending on the type of degeneration (Hippert et al., 2015), is heterogeneous within Müller glia population, and can be both beneficial and detrimental to the retina (Bringmann et al., 2009). By increasing the release of the neuroprotective factors mentioned above, removing waste and debris (Sakami et al., 2019), and buffering the extracellular space (Bringmann et al., 2009), Müller glia limit the extend of cell death and damage. However, over long periods of time, their reactive program can overrun and deregulate their homeostasis roles and their normal interactions with neurons, impeding their ability to support general retinal function. Müller glia can also release toxic nitric oxide (Goureau et al., 1999), and secrete proinflammatory factors, leading to the infiltration of immune cells and aggravating degeneration (Nakazawa et al., 2007). Furthermore, Müller glia can proliferate in response to injury (Dyer and Cepko, 2000; Sardar Pasha et al., 2017) and form glial scars at late stages of severe degeneration (Jones et al., 2003), hindering potential regeneration of axons or synaptic connections.

Overall, Müller glia are essential to maintain retinal integrity and function, and play important roles in both healthy and diseased states. They are remarkably resistant and resilient to perturbations, surviving many injuries and diseases (Bringmann et al., 2009), and, for this reason, can be key therapeutic targets. Remarkably, they were recently

shown to possess some neurogenic potential in mammals (section 1.5). Their use as a source of retinal regeneration is investigated in this thesis.

1. 2. 3. Retinal degenerative diseases

Degenerative diseases of the retina, as within other parts of the CNS, lead to irreversible neuronal death. Different diseases affect different subtypes of retinal cells, but all result in permanent vision impairments. Although some disorders target interneurons, for instance CLN3 Batten disease (Kleine Holthaus et al., 2020), or eventually lead to their secondary loss, the most common retinal degenerative diseases affect RGCs and photoreceptors. These latter are described below.

1. 2. 3. 1. Retinal ganglion cell degeneration

Glaucoma is a leading cause of blindness worldwide (Tham et al., 2014). It represents a set of diverse neuropathies causing optic nerve damage and RGC death (Casson et al., 2012). Generally, high intraocular pressure, due to overproduction or poor drainage of eye fluids, causes stress on the fragile RGC axons, leading to subsequent apoptosis of these cells (Krizaj, 2019). In reality, the disease is much more complex: Although it is true that high intraocular pressure is a major risk factor for glaucoma (Chauchan et al., 2008), glaucoma can be present in the absence of high intraocular pressure (Trivli et al., 2019), high intraocular pressure can be present without glaucoma (Kass et al., 2002), and relieving high intraocular pressure in glaucoma patients does not always prevent RGC death (Malihi et al., 2014). Identifying the cause of RGC degeneration in these distinct pathologies is difficult, still, glaucoma is associated with genetic (Choquet et al., 2020)

and environmental factors, including aging (Klein et al., 1992), obesity (Mori et al., 2000), and diabetes (Zhou et al., 2014). Interestingly, subtypes of glaucoma show amyloid-β and tau accumulations, typical of Alzheimer's disease, and, conversely, RGC degeneration is present in Alzheimer's disease, indicating that common neurodegenerative mechanisms are taking place in these two CNS disorders (Sen et al., 2019). It was additionally found that glaucoma not only affects RGCs, but that downstream visual brain areas also degenerate (Nucci et al., 2013), adding to the complexity of the disorder.

Hereditary optic neuropathies are a group of inherited diseases of optic nerve damage and RGC death. Although exact mechanisms of degeneration in some disease subsets are less well defined, mitochondrial dysfunction is central to most of these degenerations (Carelli et al., 2004). Hereditary optic neuropathies can occur on their own or along other disorders as Friedreich ataxia (Fortuna et al., 2009), Charcot-Marie-Tooth disease (Botsford et al., 2017), and Familial dysautonomia (Mendoza-Santiesteban et al., 2017). Two most common forms of non-syndromic hereditary optic neuropathies are Dominant optic atrophy (DOA) and Leber hereditary optic neuropathy (LHON) (Newman, 2012). Whereas DOA shows an earlier onset of vision loss, LHON vision impairments are more severe. This latter is due to mutations of mitochondrial genes, most frequently *ND1* (Howell et al., 1991; Huoponen et al., 1991), *ND4* (Wallace et al., 1988), and *ND6* (Johns et al., 1992; Mackey and Howell, 1992), implicated in mitochondria respiration. DOA is largely due to mutations in the nuclear gene *OPA1* (Alexander et al., 2000; Delettre et al., 2000), encoding a dynamin-related GTPase locating to mitochondrial inner membrane. RGC axons are particularly vulnerable to mitochondrial dysfunctions by their high energy

demands. This is especially true for axonal stretches in the retina, from their cell body to the optic nerve head, where they are not myelinated. Maintaining action potential conduction in these axonal sections requires considerable energy, which is sustained by the concentration of a large number of mitochondria in these segments (Bristow et al., 2002), rendering them especially susceptible to mitochondrial defects.

1. 2. 3. 2. Photoreceptor degeneration

1. 2. 3. 2. 1. Cone photoreceptor degeneration

A small group of inherited macular degenerative diseases cause the degeneration of the photoreceptor cells, mostly cones, in the macular region responsible for central high acuity vision (Fig. 5A). Three of these take root in the RPE and Bruch's membrane. Vitelliform macular dystrophy is due to mutations in the *VMD2* gene (Marquardt et al., 1998; Petrukhin et al., 1998), encoding the RPE chloride channel protein bestrophin (Sun et al., 2002). The exact role of this protein is still unknown. Nonetheless, altered bestrophin function creates disruptive yellow deposits in and next to the RPE. Sorsby's dystrophy and Malattia leventinese impair Bruch's matrix turnover by altering metalloproteinase activity with mutations in *TIMP3* (Weber et al., 1994) and *EFEMP* (Stone et al., 1999a) respectively. Although these three diseases have different genetic origins, they all impede diffusion of molecules from the vasculature to the RPE. This in turn leads to RPE dysfunction, and photoreceptor degeneration due to lack of RPE support. Another set of monogenic diseases, Stargardt diseases, also causes macular degeneration, but by directly impacting photoreceptors. The recessive form of this disease is caused by mutations in *ABCA4* (Allikmets et al., 1997). This gene encodes a

photoreceptor-specific ATP-binding transporter, located in photoreceptor outer segments, important for chromophore recycling (Molday et al., 2000; Sun et al., 1999; Weng et al., 1999). The dominant form of Stargardt disease is due to mutations in *ELOVL4* (Edwards et al., 2001; Zhang et al., 2001), encoding a fatty acid elongating enzyme, the role of which is still unclear in photoreceptors (Hopiavuori et al., 2019). Both of these Stargardt disease forms cause the death of macular photoreceptors and result in loss of central vision (Fig. 5A) in children and young adults.

Unlike inherited disorders described above, the most common macular degenerative disease, age-related macular degeneration (AMD), is a multifactorial disorder with a mix of both genetic susceptibility and environmental factors accounting for the emergence and progression of the disease (Wright et al., 2010). The strongest risk factors for AMD include family history (Seddon et al., 1997), and genome-wide association studies (GWAS) have identified numerous AMD-associated loci, including variants in genes implicated in the complement system, as *CHF*, *C3*, and *CFI*, lipid metabolism, as *ABCA1*, and *APOE*, and collagen pathways, as *MMP9* (Fritsche et al., 2016). Most genomic sites identified by GWAS require further investigation to confer them causal relationships to disease development (Strunz et al., 2020). Other risk factors include age, cigarette smoking, body composition, and diet (Heesterbeek et al., 2020). Identifying how these genetic and environmental factors interact to cause macular degeneration in AMD remains a major challenge.

In early stages of AMD, deposits of lipid and proteins, named drusen, accumulate between the RPE and choroid plexus without causing adverse effects on vision (de Jong et al., 2019). The origin of these drusen is still unclear. Eventually, as the number of drusen increases, the disease evolves to late AMD which can take two forms: atrophic (dry) or neovascular (wet) AMD (Mitchell et al., 2018). In atrophic AMD, the large accumulation of drusen causes the death of RPE and photoreceptor cells (Fig. 5B), similarly as described above for some monogenic diseases. Neovascular AMD consists of dysregulated blood vessel growth from the choroid plexus into the underlying RPE and retina, which leads to blood leaking in the retina and the death of fragile photoreceptor cells (Fig. 5B). A similar vascular process also leads to macular degeneration in diabetic retinopathy, a common complication of diabetes (Wong et al., 2016). Vascular Endothelial Growth Factor (VEGF) plays a central role in these vascular aberrations. Its production by retinal cells and the RPE is increased under hypoxic conditions, hence promoting excessive endothelial cell proliferation and vascular permeability (Adamis and Shima, 2005). VEGF is the main therapeutic target for these vascular disorders (section 1.3.1.1), while, unfortunately, no treatment is yet available for atrophic AMD.

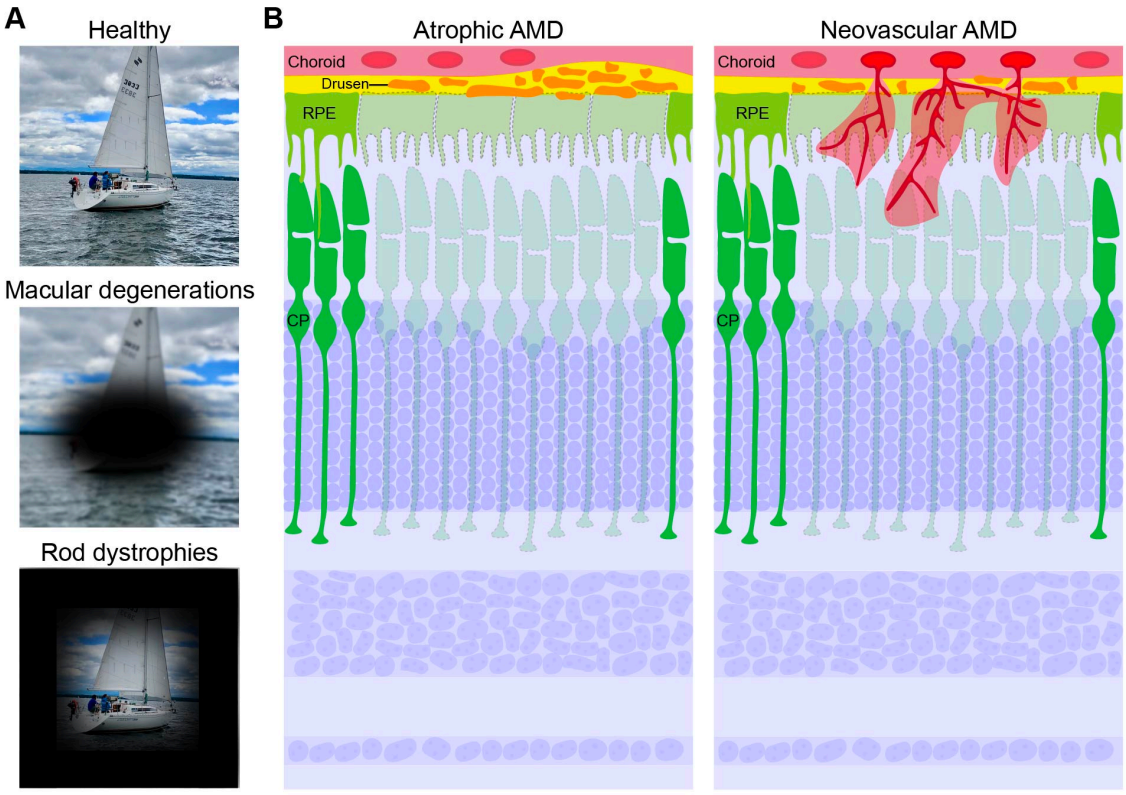


Figure 5: Macular degeneration.

A. Representations of visual fields in healthy (top), macular degenerative diseases (middle), and rod dystrophies (bottom), showing loss of central or peripheral vision. **B.** Representation of AMD. Left: In atrophic AMD, drusen accumulation between RPE and choroid leads to degeneration of RPE and underlying cone photoreceptors (CP) in the macula. Right: In neovascular AMD, blood vessels from the choroid invade underlying tissues leading to RPE and cone photoreceptor degeneration in the macular region. (Based on information from de Jong et al. (2019).)

The diseases described above cause degeneration of the macula, even in cases where proteins altered by a genetic mutation are present in cells throughout the retina (not restricted to the macula). Although degeneration eventually spreads to the rest of the retina in late stages of some diseases, it is still unclear why the macula degenerates first.

It has been suggested that the high density of photoreceptors within this region would render it more vulnerable to degeneration.

Other degenerative diseases cause cone photoreceptor death throughout the retina. This is generally followed, or sometimes concomitant, with rod degeneration leading to complete blindness in late stages of the disease. Over 30 genes have been implicated in cone dystrophies (Gill et al., 2019). These include mutations in genes important for cone function as the phototransduction genes encoding the cone phosphodiesterase *PDE6C* (Thiadens et al., 2009), or cone opsins *OPN1LW* and *OPN1MW* (Gardner et al., 2010), and neurotransmitter release *RIM1* (Michaelides et al., 2005) and *HRG4* (Kobayashi et al., 2000). It also involves genes regulating outer segment morphogenesis as *CDHR1* (Stingl et al., 2017), *PROM1* (Pras et al., 2009), and *PRPH2* (Nakazawa et al., 1996a; Nakazawa et al., 1996b), and intracellular transport as *RAB28* (Roosing et al., 2013) and *RPGR* (Yang et al., 2002). Whether present throughout the retina or limited to the macula, cone photoreceptor degeneration impairs color, daylight, and high acuity vision. Such considerable vision loss is devastating for patients.

1. 2. 3. 2. 2. Rod photoreceptor degeneration

Retinitis pigmentosa is the most common cause of inherited photoreceptor degeneration (Wright et al., 2010). It consists a broad spectrum of mutations causing rod photoreceptor death followed with secondary loss of cone photoreceptors in late stages of the disease. Generally, night blindness occurs during adolescence, followed with vision loss in the periphery in young adulthood (Fig. 5A) and complete blindness by 60 years of age

(Hartong et al., 2006). Over 50 different genes have been identified to cause retinitis pigmentosa (Daiger et al., 2013). These include genes implicated in rod phototransduction, such as *PDE6B* (McLaughlin et al., 1993) and *RHO* (Dryja et al., 1990), involved in rod structure, including *PRPH2* (Dryja et al., 1997), *RPGR* (Vervoort et al., 2000), and *RP1* (Bowne et al., 1999), and splicing, as *PRPF31* (Vithana et al., 2001). Retinitis pigmentosa can also be part of broader syndromes. For instance, Usher (Mathur and Yang, 2015) and Bardet-Biedl (Suspitsin and Imyanitov, 2016) syndromes disrupting cilia genesis and function.

Leber congenital amaurosis (LCA) is similar to retinitis pigmentosa in terms of phenotype, but has an early onset of rod degeneration occurring within the first 6 months of life. Mutations in 20 genes have been identified (Coussa et al., 2017). These include the centrosome gene *CEP290* (den Hollander et al., 2006), photoreceptor adherent junction gene *CRB1* (Lotery et al., 2001), photoreceptor differentiation gene *CRX* (Swaroop et al., 1999), and the outer segment morphogenesis gene *PRPH2* (Khan et al., 2016). LCA can also be caused by RPE dysfunction with mutations in *RPE65* (Marlhens et al., 1997), encoding a retinoid cycle enzyme (Moiseyev et al., 2005).

As exemplified here, mutations implicated in rod and cone degeneration are numerous and varied. Interestingly, there can be genetic overlap between many types of photoreceptor degeneration. For instance *PRPH2* mutations can cause LCA, retinitis pigmentosa, and cone dystrophy depending on the type and severity of the mutations. To elucidate this complexity of phenotypes, underlying degenerative mechanisms, and

potential treatments, one can take advantage of animal models. Mouse retinas are similar to human retinas in terms of cellular composition and overall structure. However, a major disadvantage of this common animal model for retinal research is the lack of a cone-rich fovea and surrounding macular region. It is therefore difficult to model macular degeneration and generally easier to model non-macular diseases, in particular, non-macular monogenic disorders since the causal mutations are known and can be inserted in the mouse genome. Also, because rods vastly outnumber cones throughout the mouse retina, mice are mostly used to study retinal degeneration through rod dystrophies. One of the first (Keeler, 1924) and most widely used mouse model of retinal degeneration is the Pde6b^{RD1} line, which contains a naturally occurring nonsense mutation in the rod phosphodiesterase gene *Pde6b* (Bowes et al., 1990; Pittler and Baehr, 1991). Rods, loosing this essential component of their phototransduction cascade, degenerate first and are completely lost by 4 weeks of age, rendering the mice blind at this early stage and effectively reproducing retinitis pigmentosa phenotypes. A slower secondary loss of cone cells follows (Carter-Dawson et al., 1978). Not surprisingly, Müller glia are strongly and permanently activated in these degenerated retinas (Ekström et al., 1988). This well-defined photoreceptor degenerative disease model can be employed to study vision restorative approaches.

1. 3. Therapies for central nervous system degeneration

1. 3. 1. Preventive therapies

Preventive therapies aim to slow or stop neurodegeneration. The main approaches to achieve this in the CNS are described below.

1. 3. 1. 1. Neuroprotective factors

A potential preventive therapeutic avenue is the use of neuroprotective factors to slow neuronal death. Although such factors would not cure disease, they may help neurons survive longer by creating a survival-prone environment. Also, since underlying causes of retinal degenerative diseases are numerous, treatments that could slow cell death in a wide-ranging set of degenerations hold great promise. Broad neuroprotective therapies are especially attractive for diseases in which the cause is unknown and to prevent secondary loss of neurons. Neuroprotective factors include anti-oxidants, anti-inflammation drugs, calcium-stabilizing compounds, and trophic factors, such as BDNF, GDNF, and NGF (Nieoullon, 2011). These neuroprotective agents can be provided to the CNS through alimentation, transplantation of cells modified to secrete these factors, viral delivery of the gene encoding them, or direct injections of these factors in the affected regions.

In the retina, many trophic factors, including GDNF, BDNF, and CNTF, have been shown to slow cell death in rodent models of degeneration (Kolomeyer and Zarbin, 2014). Recently, a clinical trial demonstrated that the release of this latter, CNTF, by a small retinal implant slowed degeneration of photoreceptors in a macular degenerative disease (Chew et al., 2019). The same approach, however, increased visual loss in retinitis pigmentosa patients (Birch et al., 2016), indicating that CNTF effects may be disease-specific and further validations are required. Indeed, while numerous trophic factors slow cell death in animal models, conclusive human data remain scarce.

Neuroprotection can also be attained by regulating other pathways implicated in cell death including, metabolism, oxidative stress, and vascular growth. Nutritional imbalance participates to secondary cone photoreceptor deaths in retinitis pigmentosa (Punzo et al., 2009). Rod-derived cone viability factor (RdCVF) is secreted by rod photoreceptors (Leveillard et al., 2004) under normal conditions to promote glucose uptake by cones (Ait-Ali et al., 2015). The loss of RdCVF in rod dystrophies causes, in part, the loss of cone photoreceptors (Narayan et al., 2016). Both subretinal injections of this protein (Yang et al., 2009) and its adeno-associated virus (AAV)-mediated expression in photoreceptors (Byrne et al., 2015) promoted cone survival in rodent models of retinitis pigmentosa. Another way to counteract energy starvation is by increasing insulin levels. Providing the retina with insulin slowed secondary loss of cone photoreceptors in a mouse model of retinitis pigmentosa (Punzo et al., 2009) and protected RGCs after optic nerve injury (Agostinone et al., 2018). As for regulating oxidative stress, Xiong et al. (2015) found that AAV-mediated NRF2 expression, a transcription factor controlling expression of antioxidant proteins, in cones or RGCs, increased their survival in different models of retinal degeneration. Similarly, the coenzyme Q10, by inhibiting production of reactive oxygen species, protected RGCs after injuries (Nakajima et al., 2008). A clinical study has also shown that increased intake of anti-oxidants, such as zinc, vitamin C and E, and Beta Carotene, decreased the risk of developing advanced degeneration in AMD patients (Kassoff et al., 2001).

Finally, limiting excessive vascular growth and stopping blood vessels from invading the RPE and retina could protect retinal cells in some neurodegenerative diseases. Anti-

VEGF molecules, including aptamers and antibodies, bind VEGF proteins and block their downstream targets, resulting in a reduction of blood vessel growth in neovascular AMD (section 1.2.3.2.1) and, importantly, improved visual acuity (Avery et al., 2006; Fish et al., 2003; Rosenfeld et al., 2006). This treatment is currently available to treat neovascular AMD and diabetic retinopathy.

1. 3. 1. 2. Gene therapy

As a general cause of degenerative diseases is altered or missing protein functions, restoring normal protein activity would prevent neurodegeneration in several disorders. This could be achieved by providing affected cells a corrected version of the mutated gene, or directly correcting endogenous mutations. Such a procedure is called gene therapy and, for now, is mostly used to express a rectified gene delivered to cells by AAV or lentiviral vectors (Piguet et al., 2017).

All genes implicated in monogenic disorders described above (section 1.2.3) could theoretically be subject to gene therapy in order to either replace the defective gene or, possibly, correct the mutations. In reality, although gene therapy has had some success in the retina, its applications remain somewhat limited. Indeed, while AAVs are generally safe and efficiently transfect retinal cells, a major limitation of their use is their small cargo capacity of about 4.7 kilobases (Carvalho and Vandenberghe, 2015). For instance, the LCA gene *CEP290* has a coding sequence of 8 kilobases and is thus too large to be inserted in AAV vectors (Carvalho and Vandenberghe, 2015). Alternatives, including splitting these genes in dual AAVs which can merge when present in the same cells

(Lopes et al., 2013), or lentivirus which allow for greater packaging capacities, are being investigated. Other issues stem from targeting the appropriate cells for infection. Virus serotypes and promoters of the encapsulated constructs can be used as tools to promote infection and expression, respectively, in specific cell types of the retina. However, although RGCs and Müller glia are infected by relatively safe intravitreal injections, more invasive subretinal injections are required to infect photoreceptors and RPE cells. Subretinal injections create transient retinal detachments from the RPE and whether this can cause additional degeneration of the diseased retinas needs to be further investigated (Trapani and Auricchio, 2018).

Still, albeit these limitations, gene therapy in the retina has led to some success stories indicating that these approaches are feasible. The first effective gene therapy was for *RPE65* in LCA. In 2008, three clinical studies (Bainbridge et al., 2008; Hauswirth et al., 2008; Maguire et al., 2008) artificially expressed *RPE65* in the RPE of LCA patients with subretinal injections of AAVs. Although these groups used varied AAV doses and different viral vectors, almost all patients self-reported vision improvements in dim light conditions. One group also reported increased visual acuity of the treated eyes (Maguire et al., 2008). These studies have been followed up by others since 2008 (Wang et al., 2020b), and *RPE65* gene therapy has been accepted by the Food and Drug Administration (FDA; USA) in 2017, becoming the first targeted gene therapy approved in North America. Other retinal gene therapy trials are underway for retinitis pigmentosa, Usher syndrome, Stargardt disease, and LHON (Trapani and Auricchio, 2018).

Direct genetic alterations of endogenous genes are not yet performed with gene therapy, but, with development of clustered regularly interspaced short palindromic repeats (CRISPR) technologies, genetic corrections should become possible (Wright et al., 2016). This will be especially important for diseases with dominant negative mutations, which would not be rescued by providing an exogenous copy of the corrected gene. Other tools have been developed to block faulty protein production in such diseases by targeting the messenger ribonucleic acid (mRNA), or the protein itself. For instance, RNA interference by delivery of short hairpin RNA or anti-sense oligonucleotides (Martinez et al., 2013) cause the degradation of the targeted mRNA and, thus, lead to decreased expression of the problematic protein. A few anti-sense oligonucleotide clinical trials for LCA, retinitis pigmentosa, and Usher syndrome (section 1.2.3.2.2) are currently underway and are showing promise (Xue and MacLaren, 2020). Another opportunity lies in targeting the protein itself. For instance, taking advantage of immune cells to eliminate the disease-causing protein by artificially generating antibodies against the toxic proteins and immunizing patients against it (Wisniewski and Goni, 2015). These potential treatments have vast implications for CNS degenerative disorders, in which toxic protein aggregates constitute a major cause of cell death, including in Alzheimer's disease and ALS (Gan et al., 2018).

1. 3. 1. 3. Cell conversion: Reprogramming

As retinal degenerative diseases are caused by numerous genetic mutations, some of which remain to be identified, the application of gene therapy to treat these varied conditions is time consuming, tedious, and costly. A broader therapy, which could

permanently stop or prevent retinal degeneration in several subclasses of diseases, may thus be optimal. One such therapy is the conversion of the diseased cell type to a closely-related cell type not affected by the disorder.

This potential therapy takes advantage of cell reprogramming. In the famous Waddington landscape model, cell fate acquisition is represented as a marble rolling down a hill, its path getting more restrictive along branching points until it reaches a final location at the bottom of the hill (Fig. 6) (Amamoto and Arlotta, 2014; Srivastava and DeWitt, 2016; Waddington, 1957). This embodies cells moving from pluripotency (mountain top), through epigenetic modifications, to a final, originally thought irreversible, identity (valley). Based on decades of work initiated by (Gurdon, 1962) and followed by numerous others (Gurdon and Melton, 2008), we now know that it is possible to alter cell identity. For instance, one can bring a differentiated cell 'back up the slope' to pluripotency (Fig. 6), as with fibroblast conversion to induced pluripotent stem cells (iPSCs) with the expression of transcription factors (Oct4, Sox2, Klf4, and c-myc) mediating pluripotency during development (Takahashi and Yamanaka, 2006). It is also possible to directly reprogram cells from one identity to another, 'across the valley' (Fig. 6), with potent lineage-specifying transcription factors. For instance fibroblasts can be converted to neurons with the pro-neural transcription factor Ascl1 (Chanda et al., 2014; Vierbuchen et al., 2010), and more efficiently along with Brn2, and Myt1l (Vierbuchen et al., 2010). For therapeutic purposes, one could reprogram tissue resident cells *in situ* to pluripotent states or directly to required neuron subtypes, thereby preventing degeneration of the reprogrammed neurons, or even restoring a lost population of cells (section 1.3.2.4).

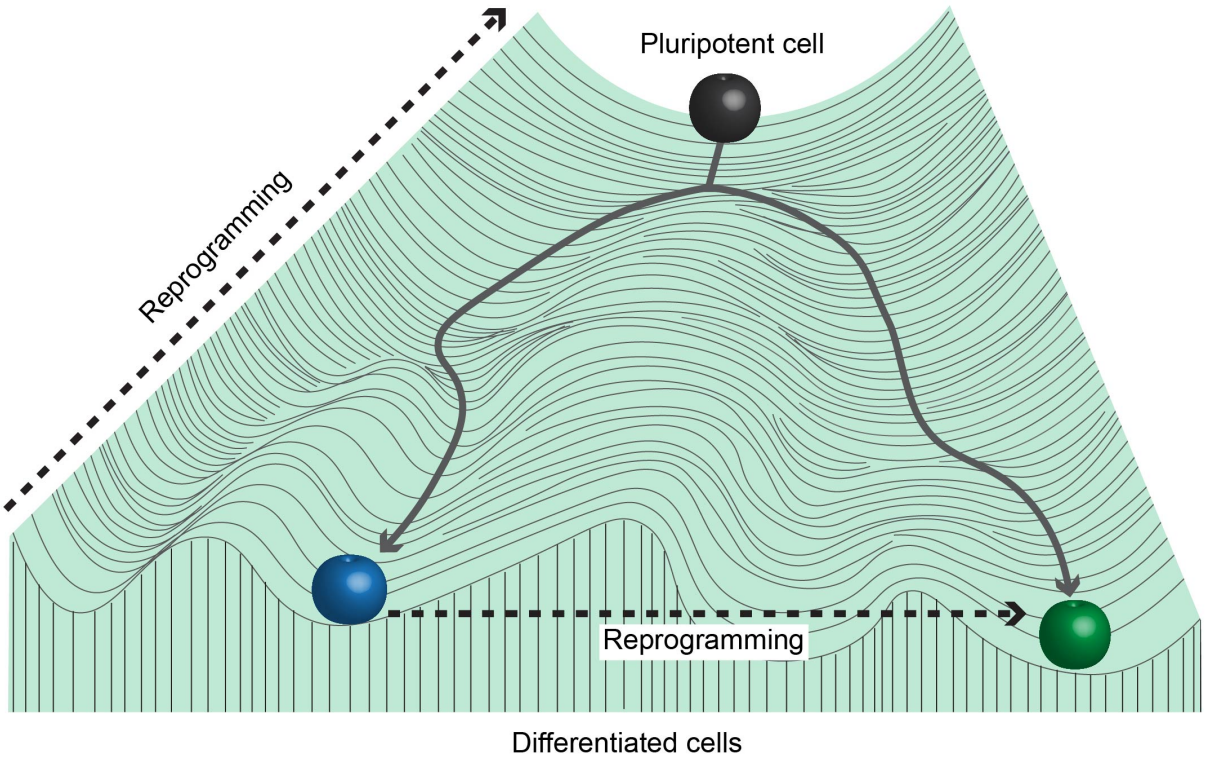


Figure 6: Cell differentiation and reprogramming.
Waddington landscape model (Waddington, 1957) of cell differentiation represented as a marble rolling down a hill, shown with full lines. Cell identity reprogramming is shown with dotted lines: Direct reprogramming (across the valley), and indirect reprogramming through a pluripotent state (up the slope) are represented.

Cell conversion therapy in the retina could use such reprogramming process to convert rods to cones in rod degenerative diseases (Fig. 7), or, inversely, cones to rods, in cone degenerative diseases. For instance, in rod dystrophies, mutations disrupt rod, but not cone function, their conversion to cone-like cells would hence render them immune to disease-causing mutations and theoretically prevent their death. Rod to cone conversions have been shown to occur when *Nrl*, the main regulator of rod genesis during development, is abolished, resulting in the generation of cone-like cells in mice (Mears et al., 2001). Remarkably, conversion of adult rods to cone-like cells by induced ablation of

Nrl was found to prevent retinal degeneration and maintain vision in mouse models of retinitis pigmentosa (Montana et al., 2013; Zhu et al., 2017) (Fig. 7).

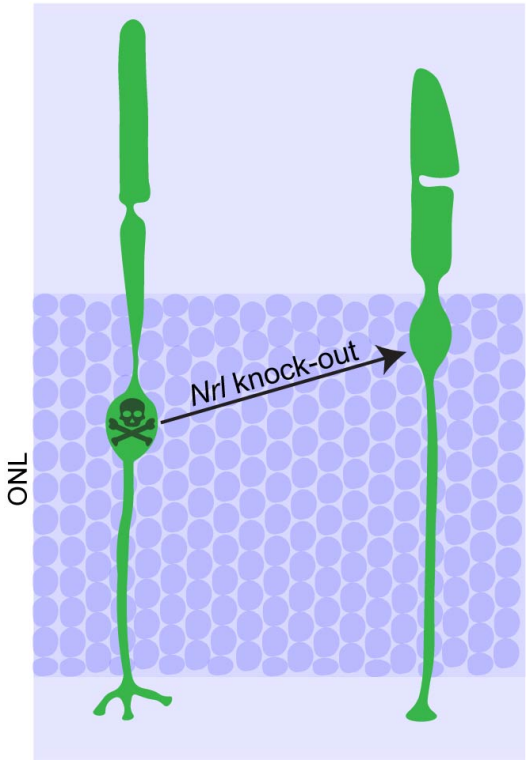


Figure 7: Photoreceptor conversion in rod dystrophies.
Rods can be converted (arrow) to cone-like cells, by abolishing *Nrl*, to save them from degeneration (skull).

Although promising, key points remain to be addressed regarding this approach. How would the rest of the retina and downstream visual cortical areas react to such a massive change of photoreceptor identity and response to light? One can image that low light vision would be lost in these patients, which may not represent a dramatic trade-off compared to the eventual complete vision loss that these patients would suffer from with normal disease progression. Still, how these newly generated cone-like cells would participate to vision and how such drastic change in overall photoreceptor composition, effectively inducing a macular-like phenotype in the entire retina, would affect vision in humans remains to be investigated. For instance, cone visual pathways rely on Müller

glia (section 1.2.2.1), and cones and Müller glia are tightly associated, being present at a ratio of about 1:1 in the retina (Reichenbach and Bringmann, 2013). How a severe change in this cone to Müller glia proportion would alter retinal function over time is unknown. Also, conversion (and thus loss) of rods may result in the loss of their cone-survival effects (Leveillard et al., 2004). Whether this would eventually cause cone photoreceptor stress and death remains to be examined.

Some of these preventive treatments show great promise to stop or slow disease progression. However, for these therapies to be effective they have to be initiated in the early stages of disease when significant cellular structures remain and damages are reversible. These treatments cannot restore nervous system function once neurons are lost. Other therapies are thus required to achieve this.

1. 3. 2. Restorative therapies

Restorative therapies try to bridge cellular and functional gaps caused by neurodegeneration in order to re-establish CNS integrity. This can be done by replacing lost neurons or providing the remaining cells the ability to perform the function of the lost ones. Both cellular and technology-based approaches are explored to achieve this.

1. 3. 2. 1. Neural implants

By receiving and/or transmitting electrical signals, electrodes can mimic some neuronal properties and can hence replace lost neurons, at least to some extent. Applications are varied depending on the type of neurodegeneration and the function emulated. For

instance, deep brain stimulation replaces lost neuronal inputs and increases dopamine release in Parkinson's disease patients, relieving some of their motor tremors (Herrington et al., 2016). Another example are cortical prostheses (Tsu et al., 2015) that can read brain signals, and could eventually relay them to an exoskeleton in order to bypass the diseased spinal cord and create movement in ALS patients otherwise paralysed.

Neural implants have also been developed for applications in the retina. Different electrical devices have been implanted in the retinas of blind patients to stimulate spared retinal neurons in the hopes of restoring vision. Argus II (Second sight medical products) (da Cruz et al., 2016; Humayun et al., 2012), an epiretinal (intravitreal) device, and Alpha AMS (Retina Implant AG) (Edwards et al., 2018), a subretinal device, receive electrical inputs from a camera located next to the eyes by a transscleral cable. Whereas epiretinal devices directly stimulate RGCs and lack interneuron signal processing, subretinal devices stimulate interneurons, thus maintaining some retinal circuitry and computation. Another subretinal device, PRIMA (Pixium vision) (Palanker et al., 2020), consists of a wireless prosthetic that can directly transduce light in electrical signal. Special glasses transform environmental light in high intensity near-infrared light and projects it on the retinal photovoltaic implant, which stimulates downstream retinal interneurons.

The retinal implants above are useful in patients with photoreceptor degeneration, but ineffective in RGC diseases, since retinal output to the brain is lost (section 1.2.3.1). A cortical implant in visual areas would circumvent this missing connection. The Orion visual cortical prosthesis system (Second sight medical products) is one such device and is

currently undergoing a small clinical trial (ClinicalTrials.gov Identifier: NCT03344848) estimated to end in 2023. Although advantageous in some cases, cortical implants are far more invasive than retinal implants, requiring the removal of the skull, and may create complications. The current clinical trial is aimed at investigating the feasibility of these procedures.

Though vision restoration with cortical implants remains to be investigated, it was shown that retinal prostheses, Argus II, Alpha AMS, and PRIMA, allow patients to see bright contrasts (Edwards et al., 2018; Humayun et al., 2012; Palanker et al., 2020). Unfortunately, vision resolution is poor and remains well beneath the legal blindness threshold. This is, in part, why both Argus II and Alpha AMS have recently been discontinued. The poor acuity generated by these devices is due to the low resolution of the microelectrode arrays. Electrodes are larger than synaptic connections, and broadly alter electrical potential of donwstream neurons. For instance, electrodes can stimulate bypassing axons of distal RGCs, impairing spatial resolution. Also, these prostheses have been implanted in the eyes of blind patients at late stages of diseases. It is possible that retinal remodelling, glia reactivity, and chronic immune activation impede retinal function in these late degenerative stages. Still, it is quite remarkable that patients who had been blind for decades were able to regain, albeit limited, visual responses, indicating that some retinal signaling is feasible even in highly degenerated retinas.

Although such applications of implants throughout the CNS are quite exciting for their regenerative potential, a general limitation of their use are the discrepancies between the

device and the tissue where they are located (Patel and Lieber, 2019). Neuronal tissues are soft, flexible, and allow for diffusion of a multitude of extracellular factors. These properties are not present in most implants, which impede normal tissue movements and homeostasis (Patel and Lieber, 2019; Prodanov and Delbeke, 2016). Also, inserting foreign materials in the CNS can lead to immune and glial responses which can aggravate neurodegeneration (Prodanov and Delbeke, 2016). Further development of these devices to confer them tissue-like properties are thus required.

1. 3. 2. 2. Optogenetics

Optogenetics refers to the expression of microbial light-sensing ion channel proteins in cells in order to manipulate their activity with light (Duebel et al., 2015). Both hyperpolarizing and depolarising channels exist. In neurodegenerative diseases, these channels could be expressed in surviving neurons to regulate their activity and re-create some parts of the lost neuronal circuitry similarly to electrodes (Ordaz et al., 2017). Importantly, whereas electrodes broadly stimulate neurons, optogenetics, by targeting specific cells to express the optogenetics channels, would create a more refined circuitry. Still, optogenetics requires the invasive implantation of a light source in the brain in order to activate or inhibit neuronal activity, at least until a light capable of transmitting through the skull and the brain without adverse effects is identified. As one can imagine, this approach would be more appropriate for a system that already uses light as source of activation i.e. the retina.

In diseased retinas, microbial or vertebrate opsins can be expressed in spared retinal cells to confer them the capacity to respond to light and replace lost photoreceptor inputs. Microbial opsins are light-sensing channels that can either hyperpolarize or depolarize cells depending on their properties. The most commonly used microbial opsins include channelrhodopsin (ChR) (Nagel et al., 2002), a sodium and calcium channel responding to blue light, and halorhodopsin (NpHR) (Sugiyama and Mukohata, 1984), a chloride pump responding to yellow light. These light-sensing channels show a fast response to light exposure and are capable of re-isomerizing after light activation. However, their light sensitivity is low and signal amplification is required for them to respond to our natural light range. Such amplification can be detrimental to the retina as bright light, especially short wavelengths, is phototoxic to retinal tissues (Glickman, 2002). Some ChR have been engineered to address this issue. For instance, CatCh increases light sensitivity approximately 70 fold (Kleinlogel et al., 2011), and ReaChR responds to red light (Lin et al., 2013), which is less toxic to cells (longer wavelength). A subtype of ChR, Chrimson (Klapoetke et al., 2014), also responds to red light and is currently in clinical trial (see below).

An alternative to microbial opsins are human opsins, which, when expressed in non-photoreceptor cells, seem to overtake resident G protein cascades to confer light-driven responses (Simunovic et al., 2019). These have better light sensitivities than microbial opsins. However, human opsins bleach, and their recovery cycle requires the RPE and Müller glia for recycling the chromophore after light-mediated isomerization. An exception to this is melanopsin, which re-isomerizes similarly to microbial opsins (Pickard and

Sollars, 2010). Still, melanopsin has a slow light-sensing kinetic and is not optimal for dynamic vision (De Silva et al., 2017).

Photoswitches are synthetic small molecules that bind intrinsic ion channels and regulate their activity with light (Tochitsky et al., 2018). Although not optogenetics per say, since they act as small drugs and cannot be genetically delivered, they have shown promise for vision restoration in mouse models of retinal degeneration (Polosukhina et al., 2012). Their use requires constant replacement and cell-specific targeting is difficult. Because of their differing properties, photoswitches are not included as part of the opsins discussed below.

To restore vision, opsins can be targeted to any spared retinal cells with AAVs. In many rod dystrophies, some cones are still present even at late stages of the disease, but are in a dormant-state, lacking outer segments. It would thus be possible to restore their light-sensitivity with optogenetics (Busskamp et al., 2010), though they can eventually degenerate and may not be an optimal target. Cells downstream of photoreceptors, bipolar cells or RGCs, can also be targeted (Kleinlogel et al., 2020). In cases of RGC degeneration, it may be possible to directly activate the visual cortex with optogenetic tools (Chernov et al., 2018), similarly to cortical prostheses described in the section above. It is important to note that visual feature computation is performed at each step of the visual pathway with increasing complexity. Consequently, reproducing the intrinsic subtype-specific patterns of cellular activation with optogenetic tools gets more challenging as cellular targets are further along the visual pathway. The brain may still be

able to interpret some artificial signals lacking normal features (for instance, vision with retinal implants stimulating RGCs), but vision, in these cases, will likely remain rudimentary.

Numerous optogenetic studies in mouse, canine, and simian models of retinal degeneration have been performed and shown promise to restore some visual responses (Simunovic et al., 2019). This vast literature has led to two clinical trials currently underway. The first, RST-001 Phase I/II trial for advanced Retinitis Pigmentosa (ClinicalTrials.gov Identifier: NCT02556736), sponsored by Allergan, was initiated in 2015. This study consists of AAV-mediated expression of ChR2 in RGCs. The second trial, PIONEER trial (ClinicalTrials.gov Identifier: NCT03326336) by GenSight Biologics, begun in 2017 and entails AAV-mediated expression of Chrimson in RGCs of late stage retinitis pigmentosa patients. This treatment is paired with goggles that transform environmental light in bright red light which can be detected by Chrimson. It will be interesting to compare vision restoration of these optogenetic approaches with implant-generated vision.

Optogenetics are promising to restore light sensitivity to the retina, especially with engineered opsins modified to better imitate retinal cell activation to light stimuli. For a perfect treatment, each subtype of targeted retinal cells would express opsins that create a response to light most closely related their endogenous response. However, subtype-specific promoters for many retinal cells are missing and further engineering of opsins would be required. A considerable limitation of this approach is its application to the fovea.

Since inner retinal neurons and RGCs are laterally displaced from the fovea, targeting these cells for foveal vision restoration in macular diseases would greatly distort visual scenes and is far from optimal.

As of now, implants and optogenetic tools are unable to recreate normal vision. Indeed, reproducing the intricate function and circuitry of highly specialised retinal cells, for instance photoreceptors (described in section 1.2.2.1), lost in degenerative diseases, is extremely challenging.

1. 3. 2. 3. Cell transplantation

Instead of imitating neurons and their circuitry with the approaches described above, why not replace the lost neurons themselves? This would circumvent many issues arising from discrepancies between tissue and devices (section 1.3.2.1). Replacing lost neurons can be performed with transplantation or endogenous regeneration (section 1.3.2.4.).

In transplantation, neurons isolated from embryos, produced either from embryonic stem cells or iPSCs, or generated directly from fibroblasts, are injected in the desired CNS region (Grade and Gotz, 2017) (see section 1.3.1.3 for cell reprogramming). Once in site, if they survive the procedure and do not induce immune rejection, they need to terminate cellular maturation, and integrate the remaining circuitry by connecting to the right synaptic partners. In this way, dopaminergic neurons could be transplanted in the brains of Parkinson's disease patients to replace lost neurons and restore normal motor functions (Parmar et al., 2020).

Although this procedure seems relatively straight forward, many challenges exist. One of them being the generation of the specific specialised neurons required to transplant. If they are collected from embryos, the targeted neurons will be closely related to normal endogenous neurons, but methods to specifically isolate only the required cells have to be developed. Furthermore, embryos provide a limited pool of donor neurons and one can imagine that large numbers of embryos would be required for this approach to be broadly applicable. Deriving neurons from embryonic stem cells or fibroblasts in culture would circumvent this limitation. However, neurons have to be specified to the right subtype and be as closely related as possible to endogenous neurons in order to achieve comparable functions, which is difficult to achieve. Additionally, transplanted neurons must integrate a circuitry already present and, although plasticity exists in adult CNS, how permissive the environment is to new neurons remains elusive, especially in degenerated regions (Grade and Gotz, 2017). These integration issues have been extensively investigated in the retina.

Modern retinal transplantation studies started in the 1980s and provided proof of concept for retinal grafting (del Cerro et al., 1997). However, although many research efforts followed, integration of transplanted cells remained inefficient and limited benefits were observed, until a major breakthrough was made for the field in the early 2000s (Santos-Ferreira et al., 2017). MacLaren et al. (2006) followed with Bartsch et al. (2008) demonstrated that the integration efficiency of photoreceptors was optimal when transplanting photoreceptor precursor cells (post-mitotic cells undergoing photoreceptor differentiation) compared to cells isolated before or after this time window. These

integrated photoreceptor cells showed normal morphologies with appropriate inner and outer segments and synaptic terminals. Remarkably, photoreceptor precursor transplantation in mouse models of retinal degeneration, or mice with genetically silenced photoreceptors, improved visual responses (Barber et al., 2013; Lamba, 2009; MacLaren et al., 2006; Pearson et al., 2012). Similarly to photoreceptors, it was shown that young RGCs, isolated from E18-P9, integrated the mouse retina more efficiently than adult RGCs (Hertz et al., 2014). Some transplanted RGCs extended axons towards the optic nerve head, even sometimes reaching brain targets, and responded to light (Venugopalan et al., 2016).

In the above studies, transplanted cells were labelled with fluorescent tags. Presence of tagged cells in the host tissue was interpreted as integration of transplanted cells. A critical re-interpretation of these results was reached by four independent groups in 2016 (Ortin-Martinez et al., 2016; Pearson et al., 2016; Santos-Ferreira et al., 2016; Singh et al., 2016), creating a major set-back for the field. Through multiple robust assays, these groups demonstrated that the great majority of labelled cells in the host retina do not represent integrated cells, but rather are host photoreceptors that have taken up the label from the graft (Fig. 8). This material exchange is not limited to fluorescent labels and includes other proteins, and possibly mRNA. Improved vision observed in the transplantation studies mentioned above was most likely due to transfer of the wild-type protein from the graft to the mutated host cells (Pearson et al., 2016), effectively rescuing the degenerative phenotype.

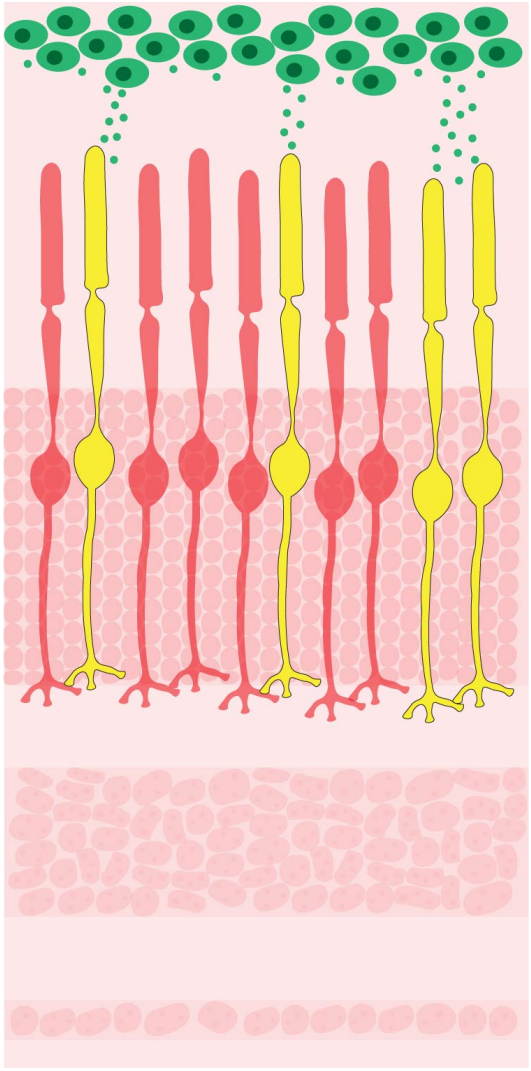


Figure 8: Material transfer between graft and host tissue.

Transplanted GFP+ photoreceptor precursor cells (top green cells) exchange material (small green circles) including fluorescent reporter with endogenous photoreceptors (red cells). Yellow photoreceptors represent endogenous photoreceptors having received material, including GFP, from graft.

Although these studies have focused on photoreceptor transplantation, whether material transfer could also impact interpretation of RGC transplantation remains a possibility. These findings also raise concerns for use of fluorescent labelling in genetic lineage tracing experiments in the retina, whether for developmental or reprogramming studies (Boudreau-Pinsonneault and Cayouette, 2018). Exchange of fluorescent label between tagged and surrounding cells in these assays could confound results, similarly as for transplantation. Furthermore, although material transfer has only been shown in the mouse retina, it is unknown whether it can also occur in other animal models or tissues.

It is important to note that a small number of transplanted cells do integrate within the host retina (Pearson et al., 2016; Santos-Ferreira et al., 2016), maintaining some promise for the potential of this therapeutic approach. Well controlled studies and the use of mouse models of complete photoreceptor loss, where label transfer to endogenous photoreceptors is thus abolished, have shown that some photoreceptor integration is feasible in degenerated retinas (Mandai et al., 2017; Ribeiro et al., 2021; Singh et al., 2013), but varies depending on the host environment (Waldron et al., 2018). Integration efficiency of transplanted cells remains a major hurdle to its application.

1. 3. 2. 4. Endogenous regeneration

Another possibility to replace lost neurons would be to elicit endogenous neurogenesis, circumventing the need for difficult transplantation procedures and the low integration rate of grafted cells in host tissues (Barker et al., 2018; Li and Chen, 2016). It has been found that some limited neurogenesis takes place in adult human hippocampus and subventricular zone (Kempermann et al., 2018; Obernier and Alvarez-Buylla, 2019). One could imagine recruiting these precursor cells or newborn neurons to degenerated regions and differentiating them to appropriate fates to restore tissue integrity. However, it seems that this adult neurogenesis is restricted, and efforts to obtain other neuronal subtypes and promote their survival have been unsuccessful (Barker et al., 2018). Additionally, adult neurogenesis is disturbed in neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases (Grade and Gotz, 2017), rendering its use for regeneration problematic.

An alternative would be to use sources of neurogenesis directly in the tissue to regenerate. These sources are not endogenously present in adult mammalian CNS, but could be generated with cell type identity reprogramming of resident cells. Glial cells would be particularly attractive as this source of neurogenesis since some can proliferate to maintain their numbers, they do not generally degenerate when neurons are lost, and they are ubiquitous in the CNS. Some groups have successfully reprogrammed mouse astrocytes, Müller glia, oligodendrocyte precursor cells, and microglia in neurons *in situ* by expressing different transcription factors (Li and Chen, 2016). These include the classic reprogramming factor Ascl1 (Liu et al., 2015; Pollak et al., 2013), and others implicated in establishing neuronal identity during development, such as Sox2 (Su et al., 2014), NeuroD1 (Guo et al., 2014; Matsuda et al., 2019), and Neurogenin2 (Grande et al., 2013). Another possibility would be to reprogram neighbouring neurons to the subtype required (Rouaux et al., 2012), but this would mean losing the initial pool of neurons and is hence a generally less attractive option than using glial cells. Limitations pertaining to the specification of appropriate neuronal subtypes and synaptic integration of novel neurons described above for transplantation (section 1.3.2.3) also apply here.

Remarkably, lower vertebrates have the endogenous capacity the regenerate their CNS, including the retina, after an injury. Endogenous retinal regeneration in lower vertebrates, and mammals is described in the following sections (1.4 and 1.5, respectively).

1. 4. Endogenous retinal regeneration in lower vertebrates

Lower vertebrates have three sources of retinal neurogenesis that can actively participate to regeneration after injury: ciliary margin zone (CMZ) cells, RPE cells, and Müller glia. The regenerative potential of this latter will be described in more details, as it is has shown most promise for mammalian regeneration.

1. 4. 1. Ciliary margin zone

The lower vertebrate CMZ is a small region containing stem cells, located between the peripheral end of the retina and the ciliary body. CMZ stem cells participate to retinogenesis during development (Fischer et al., 2013). They also generate peripheral retinal cells to support the continuous growth of the retina throughout the life of many amphibians and fish, including frogs (Straznicky and Gaze, 1971; Wetts et al., 1989), newts (Grigoryan, 2019; Mitashov et al., 2004), goldfish (Johns, 1977), and zebrafish (Wan et al., 2016). The CMZ additionally participates to retinal regeneration after injury to varying extent, depending on the animal and the type of damage. For instance, *X. tropicalis* CMZ can regenerate the entire retina after retinectomy (Miyake and Araki, 2014). In newts, while the CMZ is not implicated in retinal regeneration after retinectomy, it regenerates retinal cells after optic nerve injury (Grigoryan, 2019). CMZ stem cells, whether in development or for regeneration, divide asymmetrically to give rise to one stem cell, maintaining the regenerative cell pool, and one retinal progenitor, which migrates to the retina where it generates neurons and glia (Centanin et al., 2014; Wan et al., 2016).

Recently, in mammals, it has been demonstrated that a pool of distinct progenitors at the retinal periphery participates to retinogenesis during mouse development, reminiscent of lower vertebrate CMZ (Belanger et al., 2017; Marcucci et al., 2016). These progenitors produce both retinal neurons and glia, and ciliary epithelium cells, which populate the ciliary body (Belanger et al., 2017). However, this CMZ region is absent in mice past developmental stages and, although ciliary body cells have been reported to show some neurogenic potential in culture (Ahmad et al., 2000; Tropepe et al., 2000), whether they could mediate regeneration in vivo remains to be investigated.

1. 4. 2. Retinal pigment epithelium

Whereas fish CMZ and Müller glia regenerate their retina after injury, amphibians mainly rely on their RPE. In *X. laevis*, after retinal injury, a subset of RPE cells move to the retina where they de-differentiate, proliferate, and produce retinal cells (Yoshii et al., 2007). The RPE cells left in their original layer divide, and re-establish tissue integrity. A similar RPE regenerative process also occurs in newts. In these animals, after retinectomy, all RPE cells transdifferentiate to a stem cell state and proliferate to generate two progenitor layers, one will produce the retina and the other the RPE (Chiba, 2014). Although neurogenic potential for mammalian RPE cells was observed in culture under certain conditions (Salero et al., 2012), whether this potential could be elicited in mammals *in vivo* remains to be addressed.

1. 4. 3. Müller glia

Zebrafish Müller glia generate rod photoreceptors throughout life (Bernardos et al., 2007), and are the primary source of retinal regeneration after injury in these animals (Ail and Perron, 2017). In many other lower vertebrates, Müller glia are quiescent under normal conditions, but can show regenerative capacities, for instance in medaka fish (Lust and Wittbrodt, 2018) and Xenopus (Langhe et al., 2017), after injuries. Because of the robustness of Müller glia regenerative responses in zebrafish, it is the main animal used to study this process, and is the focus of this section.

Generally, after an injury, zebrafish Müller glia dedifferentiate to a progenitor state, divide asymmetrically to give rise to one Müller glia and a progenitor, which proliferates and eventually differentiates into retinal cells, integrating retinal circuitry and restoring vision (Goldman, 2014) (Fig. 9). This regenerative response is initiated when Müller glia sense neuronal death by their direct phagocytosis of dead cells (Bailey et al., 2010), the disruption of normal glia-neuron signaling, including decreased neurotransmitter levels (Rao et al., 2017), and the release of factors from dying neurons as well as activated immune cells (Conedera et al., 2019; White et al., 2017). Released factors include tumor necrosis factor alpha (TNFα) (Nelson et al., 2013), adenosine diphosphate (ADP) (Battista et al., 2009), FGF2, insulin growth factor 1 (IGF1), and heparin-binding EGF-like growth factor (HB-EGF) (Wan et al., 2012; Wan et al., 2014). HB-EGF is secreted by Müller glia themselves, suggesting that they are capable of regulating their own regenerative response in an autocrine and/or paracrine fashion (Wan et al., 2012).

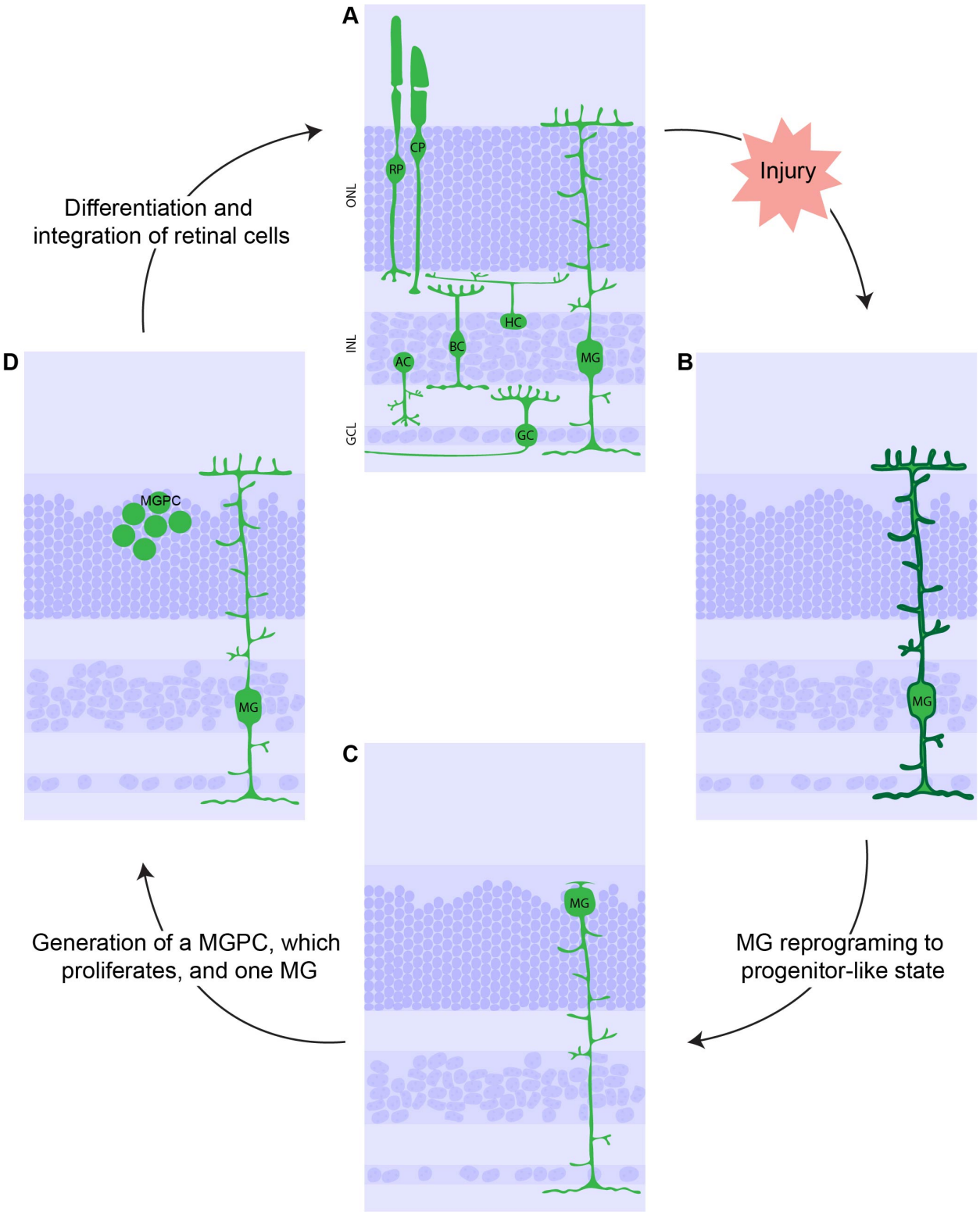


Figure 9: Müller glia-dependent retinal regeneration in zebrafish after injury.

A. Representation of a healthy retina. **B**. An injury causes neuronal death, represented

by missing nuclei, and Müller glia reactivity, represented by increased thickness. **C.** Müller glia reprogram to a progenitor-like state and migrate to the apical side of the retina. **D.** Progenitor-like Müller glia divide to give rise to one Müller glia derived progenitor cell (MGPC) and one Müller glia. The MGPC proliferates to increase the neurogenic pool, and differentiates in retinal neurons to restore tissue integrity (A). ONL: Outer nuclear layer, INL: Inner nuclear layer, GCL: Ganglion cell layer. Cells – RP: Rod photoreceptor, CP: Cone photoreceptor, AC: Amacrine cell, BC: Bipolar cell, HC: Horizontal cell, MG: Müller glia, GC: Retinal ganglion cell.

These environmental cues trigger complex Müller glia intrinsic cascades to first induce a reactive state and then quickly promote reprogramming to a progenitor identity (Hoang et al., 2020) (Fig. 9B-C). Interestingly, this reprogramming is suggested to be a reversal of the normal progenitor to Müller glia differentiation process occurring during development (Hoang et al., 2020). Many intracellular pathways that participate to this response have been identified (Lahne et al., 2020b), including the MAPK-ERK (Wan et al., 2014), PI3K/AKT (Wan et al., 2014), JAK-STAT (Zhao et al., 2014), Wnt (Kara et al., 2019; Ramachandran et al., 2011), Notch (Conner et al., 2014; Elsaeidi et al., 2018), and Shh pathways (Kaur et al., 2018; Sun et al., 2014; Thomas et al., 2018). These converge on (and often receive feedback regulations from) the activation of Ascl1 expression (Goldman, 2014), a crucial transcription factor mediating the regenerative process. Indeed, Ascl1, in turn, regulates the expression of a broad spectrum of genes implicates in progenitor identity, proliferation, and neuronal differentiation. A major component of its downstream effectors is the RNA-binding protein Lin28a, a pluripotency factor which reduces expression of the differentiation microRNA let7 (Ramachandran et al., 2010). The final step of the regenerative response, the production of retinal cells from Müller-

derived progenitors, has been shown to follow developmental transcriptional programs (Lahne et al., 2020a). The Müller glia transcriptomic modifications post-injury are concordant with epigenetic changes: an initial demethylation of deoxyribonucleic acid (DNA) when transitioning to a progenitor state, followed with re-methylation during differentiation of retinal cells (Powell et al., 2013).

Unfortunately, rather than moving from gliosis towards a progenitor state after injury, as in the zebrafish, mammalian Müller glia quickly come back to a resting state (Hoang et al., 2020). Still, as of now, Müller glia are the most promising source of endogenous regeneration for the mammalian retina. Their regenerative potential in mammals is detailed in the following section.

1. 5. Mouse retinal regeneration from Müller glia

Mammalian Müller glia were found to have a similar gene expression profile to retinal progenitors (Blackshaw et al., 2004; Jadhav et al., 2009; Roesch et al., 2008), suggesting they may have some neurogenic capacities. Indeed, under certain culture conditions *in vitro*, rodent Müller glia show stem cell properties as self-renewal, formation of neurospheres, and are capable of generating retinal neurons (Das et al., 2006). This neurogenic potential was also found in human Müller glia *in vitro* (Giannelli et al., 2011; Jayaram et al., 2014; Lawrence et al., 2007; Singhal et al., 2012).

Multiple groups have tried activating this neurogenic potential *ex vivo* or *in vivo*, and have reached varying levels of success. Surprisingly, retinal injuries alone were reported to

induce some neuron production from rodent Müller glia (Ooto et al., 2004; Wan et al., 2008). However, two other groups were unable to identify regeneration after injury (Joly et al., 2011; Kugler et al., 2015). Other studies have altered pathways implicated in zebrafish Müller glia regenerative response, including EGFR (Karl et al., 2008; Ueki and Reh, 2013), Wnt (Del Debbio et al., 2010; Osakada et al., 2007; Yao et al., 2016; Yao et al., 2018), Notch (Del Debbio et al., 2010), Shh (Wan et al., 2007), STAT (Jorstad et al., 2020), and Ascl1 (Jorstad et al., 2017; Jorstad et al., 2020; Pollak et al., 2013; Ueki et al., 2015). Key and recent studies are described in more details below to illustrate the current state of the field (Fig. 10).

The study by Karl et al. (2008) is often considered to be the first formal demonstration of mammalian Müller glia regenerative potential *in vivo*. They performed a retinal injury with an N-methyl-D-aspartate (NMDA) intravitreal injection, killing RGCs and amacrine cells by excitotoxicity, followed two days later with an intravitreal injection of epidermal growth factor (EGF) along with bromodeoxyuridine (BrdU), to label cells re-entering the cell cycle. Two days post-EGF injection, numerous cells had incorporated BrdU, many of which co-labelled with Müller glia markers. Four days later, some amacrine cells were now BrdU-positive. It was concluded that Müller glia, which initially incorporated BrdU, gave rise to these BrdU-positive amacrine cells (Fig. 10). A small proportion of Müller glia would hence give rise to these new neurons after NMDA and EGF injections.

This study, and the majority of the studies listed above, have followed Müller glia reprogramming with BrdU lineage tracing. As genetic lineage tracing was not performed,

it was not formally demonstrated that Müller glia gave rise to these neurons. Indeed, Müller glia are not the only cells incorporating BrdU at the early time point after injury, indicating that there are other potential sources of regeneration. Additionally, cells can incorporate BrdU when repairing their DNA after damage (Kuan et al., 2004). This repair-mediated incorporation of BrdU could explain the presence of the few BrdU-positive amacrine cells. Genetic lineage tracing, by permanently labelling Müller glia before manipulations, would provide a definitive answer as to whether these cells are derived from Müller glia.

Nonetheless, some subsequent studies have been able to elicit relatively strong regenerative responses in mammals. The most potent Müller glia-dependent regeneration was performed by Reh and colleagues with Ascl1 overexpression (Jorstad et al., 2017; Jorstad et al., 2020; Pollak et al., 2013; Ueki et al., 2015). Ascl1 is a strong reprogramming factor, capable of reprogramming mouse embryonic fibroblasts (MEFs) to neurons *in vitro* (section 1.3.1.3) and brain astrocytes to neurons *in vivo* (Liu et al., 2015). As mentioned previously, this factor is central to zebrafish retinal regeneration (section 1.4.3). Karl et al. (2008) had previously found that, after injury, mammalian Müller glia failed to upregulate Ascl1, which may explain their limited endogenous neurogenic properties. Accordingly, a first study demonstrated that Ascl1 overexpression promoted the production of neurons in dissociated Müller glia cultures, and of bipolar-like cells in retinal explant cultures (Pollak et al., 2013).

A follow-up study showed that Ascl1 overexpression in Müller glia *in vivo*, along with an NMDA injury, promoted the production of amacrine and bipolar-like cells from Müller glia (Ueki et al., 2015) (Fig. 10). Importantly, this regenerative process was limited to young Müller glia, with an injury at P16 or earlier. Ueki et al. (2015) suggested the lack of regenerative potential with these manipulations in adult Müller glia were due to changes in chromatin accessibility occurring around P16, restricting Ascl1 targets. This was indeed recently shown to be the case by VandenBosch et al. (2020). Accordingly, the addition of the histone deacetylase inhibitor, Trichostatin A (TSA), to Ascl1 expression and NMDA injury was shown to promote the production of bipolar-like cells from Müller glia in adult mice *in vivo* (Jorstad et al., 2017). Jorstad and colleagues have also demonstrated that STAT signaling blocks Müller glia reprogramming by directing Ascl1 away from its developmental targets (Jorstad et al., 2020). Accordingly, inhibiting this pathway increases the number of reprogrammed Müller glia and enhances regeneration *in vivo* (Jorstad et al., 2020). More recently, Todd et al. (2020) have shown that microglia reduce Ascl1-induced Müller glia neurogenic potential, possibly by activating an inflammatory response in Müller glia. The ablation of microglia increased Müller glia regenerative response in these experimental assays (Todd et al., 2020). Altogether, these studies demonstrate that the potent reprogramming factor Ascl1, which is central to zebrafish regenerative response, is capable, along additional manipulations, of conferring mammalian Müller glia some neurogenic capacities. Importantly, these studies were performed with genetic lineage tracing of Müller glia and derived cells, providing greater conviction in their interpretation than previous BrdU lineage tracing studies.

Based on gene regulatory network differences between zebrafish and mouse Müller glia responses to injury, Hoang et al. (2020) have suggested that the expression of Nfi factors, which is increased in mouse and not zebrafish reactive Müller cells, restores rest after injury in mice. Accordingly, abolishing Nfi family members in mouse Müller glia *in vivo*, along an injury, induced Müller glia to adopt proliferative or neurogenic responses, generating bipolar and amacrine-like cells (Fig. 10). Of note, this study was also performed with genetic lineage tracing. Interestingly, Nfi factors were previously shown to regulate cell cycle exit of retinal progenitors, and late retinogenesis (Clark et al., 2019), suggesting there may be parallels between the roles of these factors in progenitors and Müller glia.

Another set of recent studies have focused on the Wnt pathway, which is implicated in zebrafish retinal regeneration (section 1.4.3). Yao et al. (2016) have identified that Wnt pathway activation, through AAV-mediated expression of β-catenin, induced Müller glia proliferation without injury. Interestingly, Wnt activation increased mammalian Müller glia levels of Lin28, another central player in zebrafish retinal regenerative response. A subsequent study demonstrated that combining Wnt activation with AAV-mediated expression of three known factors critical for rod production during development, Otx2, Crx, and Nrl, in Müller glia induced the production of rod photoreceptors *in vivo* (Yao et al., 2018) (Fig. 10).

Recently, also using an AAV approach, Zhou et al. (2020) demonstrated that CRISPR-Cas knock-out of the gene encoding the RNA-binding protein Ptbp1 generated RGCs and

some amacrine cells from mouse Müller glia *in vivo* (Fig. 10). This manipulation also produced neurons from astrocytes in the striatum (Zhou et al., 2020). Whether Ptbp1 is implicated in zebrafish retinal regeneration is unknown, but it was previously identified as a regulator of the REST complex (Xue et al., 2013), which inhibits neuronal identity. Accordingly, decreasing Ptbp1 expression reduced REST activity in MEFs, and induced their reprogramming to neurons (Xue et al., 2013).

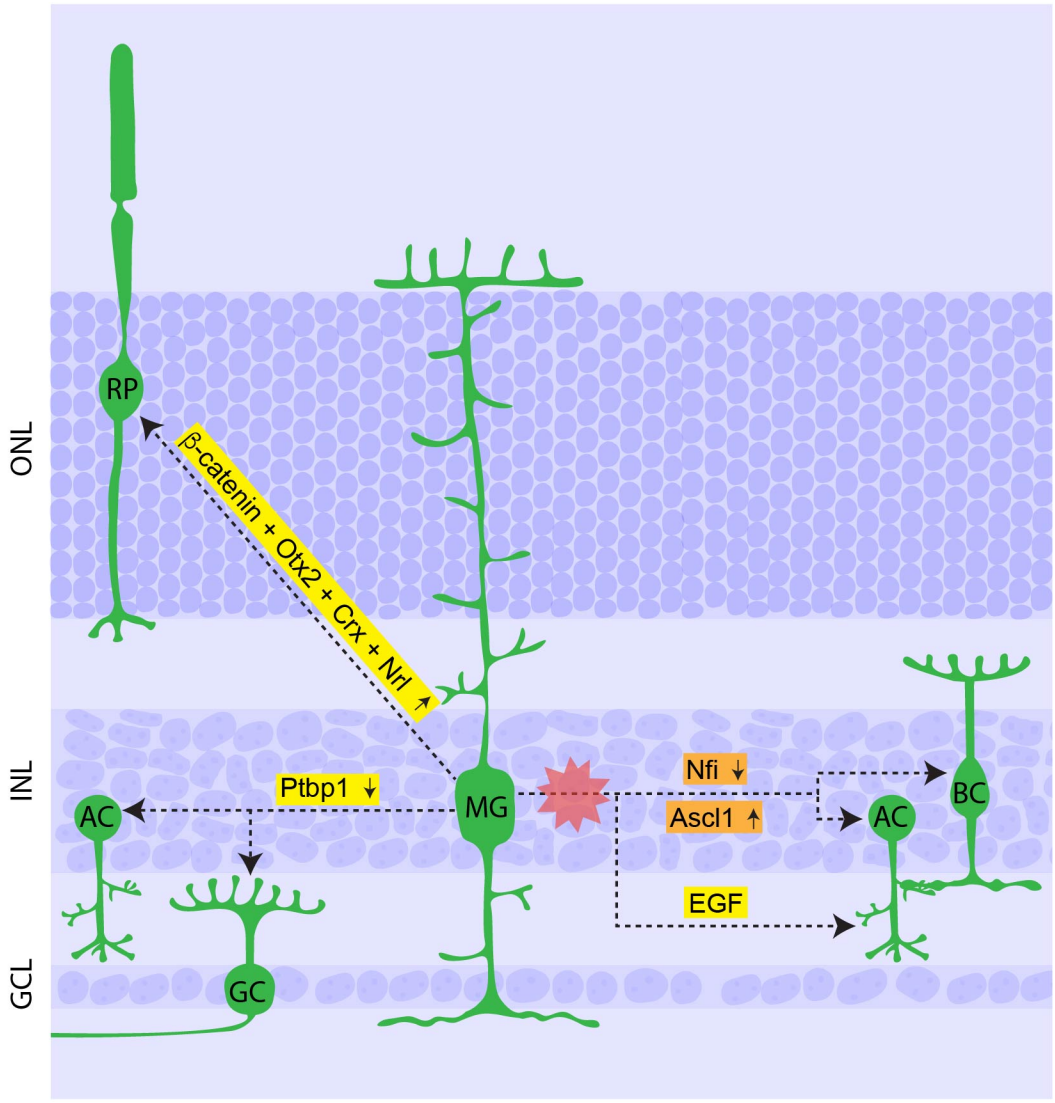


Figure 10: *In vivo* reprogramming of mouse Müller glia to neurons.
Representation of the current state of Müller glia reprograming in mice *in vivo*. Dotted lines show reprogramming to pointed cells. Conditions leading to reprogramming in yellow

and orange boxes with arrows showing upregulation (↑) or downregulation (↓) of specified factors. Red star represents a retinal injury. Yellow conditions were lineage traced with AAVs or BrdU/EdU. ONL: Outer nuclear layer, INL: Inner nuclear layer, GCL: Ganglion cell layer. RP: Rod photoreceptor, AC: Amacrine cell, BC: Bipolar cell, HC: Horizontal cell, MG: Müller glia, GC: Retinal ganglion cell.

Some reprogramming studies described above (Yao et al., 2016; Yao et al., 2018; Zhou et al., 2020) take advantage of AAV viral vectors (Fig. 10) modified to contain the glia-specific GFAP promoter. Lineage tracing of Müller glia is performed with these viral vectors containing either a fluorescent reporter or Cre, which induced the expression of a genetic reporter in the infected cells. Notably, Müller glia-targeting AAVs, even with glia-specific promoters such as the GFAP promoter, have been shown to target neurons (Lee et al., 2008; Wang et al., 2020a), and their expression pattern changes depending on the transgene they carry (Su et al., 2004). This renders their validation and the identification of appropriate controls difficult. Consequently, their use in reprogramming and, especially, for lineage tracing, must be interpreted with great caution (Martin and Poche, 2019). Concerns about these studies have been raised and findings remain to be robustly validated (Blackshaw and Sanes, 2021).

Still, these studies demonstrate that some mammalian Müller glia neurogenic potential can be elicited by taking advantage of zebrafish regenerative pathways, and known developmental and reprogramming factors. The regenerative response observed is limited in terms of cell types produced for each manipulation, and only interneurons have been robustly generated. Cone photoreceptors, lost in many degenerative diseases (section 1.2.3.2.1), have not yet been produced from Müller glia.

1. 6. Aims and rationales

Although advances have been made in recent years to stimulate mammalian Müller glia neurogenic potential, reprogramming factors identified to date and cell types generated remain limited. The main objective of this thesis consists of identifying a novel method to induce the neurogenic potential of mammalian Müller glia. This is accomplished through 2 aims.

Aim 1: Evaluate the endogenous neurogenic potential of mammalian Müller glia (Chapter 3)

Rationale: Whether mouse Müller glia show a regenerative response after injury and growth factor treatment, as suggested by Karl et al. (2008), remains unclear. This information is important to determine if work to elicit Müller glia neurogenic potential should rely on these manipulations. The first aim addresses this issue by investigating the progeny of Müller glia with genetic lineage tracing.

Objective: Establish whether mouse Müller glia generate neurons after injury and growth factor treatment.

Aim 2: Identify novel factors capable of generating neurons from mammalian Müller glia (Chapter 4)

Rationale: During retinogenesis, temporal identity factors regulate the temporal progression of progenitors and mis-expression of these factors leads to altered progenitor

competence (section 1.2.1.2). As such, temporal identity factors have competence reprogramming capacities. How conceptually similar temporal competence and cellular identity reprogramming (section 1.3.1.3) are, and, accordingly, whether temporal identity factors could reprogram the identity of fully differentiated cells remains unknown. Previous studies in *Drosophila* have shown that *hb* and *kr* are not sufficient to reprogram the identity of post-mitotic neurons (Cleary and Doe, 2006; Pearson and Doe, 2003) (section 1.2.1.1). Yet, whether vertebrate temporal identity factors have reprogramming capacities in differentiated cells remains to be addressed. Temporal identity factor-mediated reprogramming of Müller glia, conferring them the competence to generate neurons, could be central in eliciting their regenerative response in mammals.

Objective: Induce Müller glia neurogenic properties by taking advantage of the reprogramming potential of temporal identity factors.

Hypothesis: Temporal identity factors have broad reprogramming capacities and can confer Müller glia the ability to generate neurons (Fig. 11).

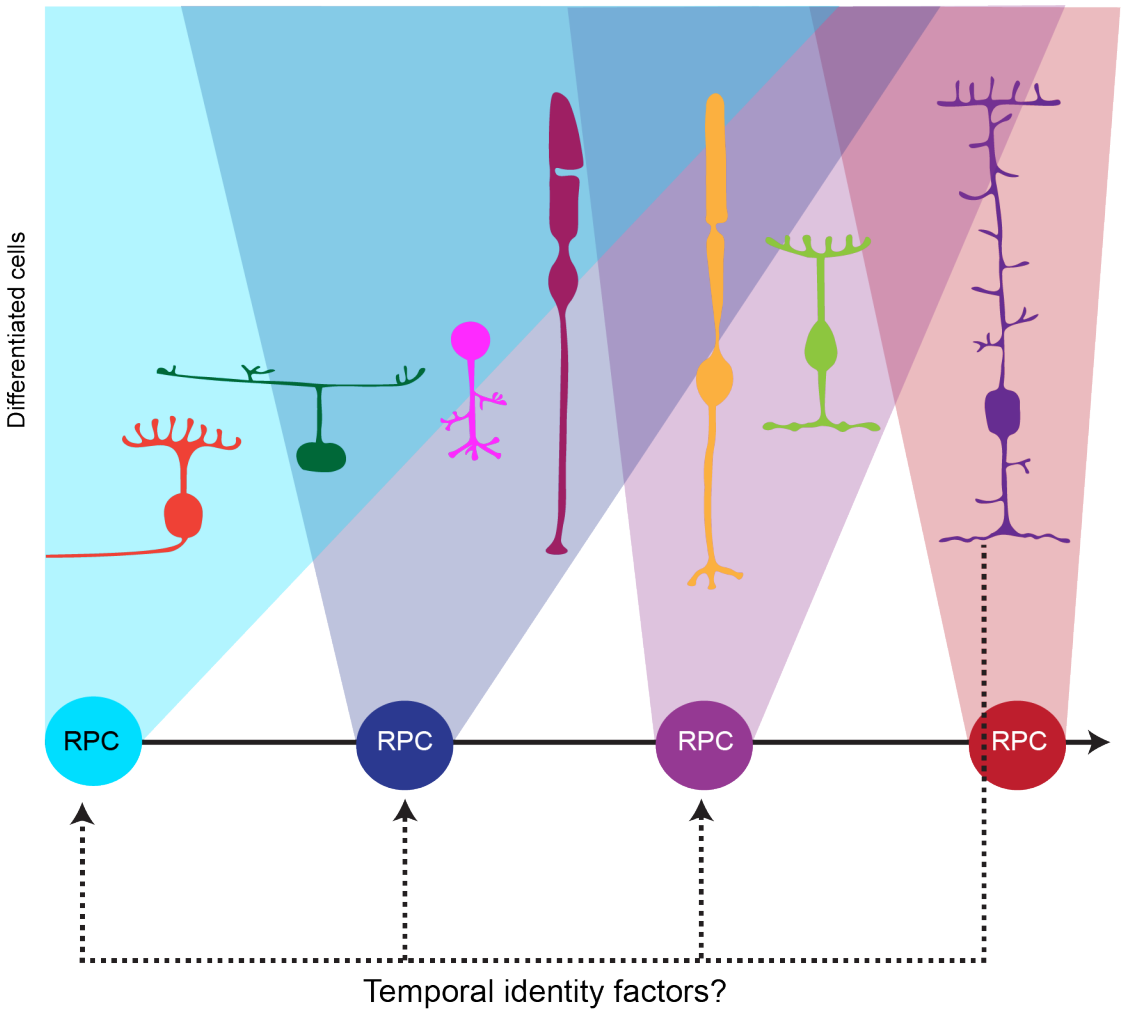


Figure 11: Schematic representation of aim 2 hypothesis.

Retinal progenitor cells (RPC) change competence over time to generate different retinal cell types and eventually give rise to Müller glia (as Fig. 3). Müller glia could be reprogrammed (shown with dotted line and arrows) with temporal identity factors to confer them neurogenic capacities.

Chapter 2: Materials and methods

2. 1. Animals

Animal work was performed in accordance with the Canadian Council on Animal Care and IRCM guidelines. Animals had water and food ad libitum and were housed with a 12 hour light/dark cycle. Tg(Slc1a3-cre/ERT)1Nat/J mice (Glast-CreER, stock 012586), B6.129X1-*Gt(ROSA)26Sor*$^{tm1(EYFP)Cos}$/J reporter mice (R26R-EYFP, stock 006148), C3H/HeJ mice (Pde6b$^{RD1/RD1}$, stock 000659), B6.Cg-*Gt(ROSA)26Sor*$^{tm1(rtTA*M2)Jae}$/J mice (R26-M2rtTA, stock 006965), C57BL/6J (stock 000664), and 129Sv/J mice (stock 000691) were all obtained from The Jackson Laboratory. Glast-CreER mice were always heterozygous, R26R-EYFP mice were fl/+ for mouse line validation, NMDA and EGF genetic lineage tracing and *ex vivo* reprogramming work, and fl/+ or fl/fl for *in vivo* reprogramming work.

2. 2. Tamoxifen administration

Tamoxifen (Toronto Research Chemicals and Cedarlane labs) was dissolved in corn oil and ethanol to a final concentration of 30mg/ml. Animals were injected intraperitoneally or gavaged for 3-4 consecutive days with 90 μg of tamoxifen per gram of body weight.

2. 3. Intravitreal injections

Animals were anesthetised with isoflurane (Fresenius Kabi) and intravitreally injected with 2μl of solution with a glass micro-needle (Drummond scientific). NMDA (Tocris) was injected at a concentration of 100mM, EGF (PreproTech) at a concentration of 1μg/μl, and 2'-Deoxy-5-ethynyluridine (EdU, Abcam) at a concentration of 1mg/ml.

2. 4. DNA constructs

pCALL2 was digested with ClaI and SphI to insert mCherry (amplified from MSCV-mCherry) in the locus of X-over P1 (LoxP) cassette. IRES-EGFP was removed with SmaI and NotI digestions. A Gateway cassette was added within the multiple cloning site (MCS) to allow insertions of some coding sequences with Gateway Cloning System (Thermo Fisher), while others were inserted directly in the MCS by restriction digestions or with In-Fusion cloning (Clontech). Empty pCALL2-MCS was used as control for retinal work. Construct was tested in HEK 293T (ATCC) cells by jetPRIME (Polyplus) transfection alone or with pCIG-Cre following instructions from manufacturer.

psPAX2 (Addgene plasmid # 12260; http://n2t.net/addgene:12260; RRID:Addgene_12260) and pMD.2G (Addgene plasmid # 12259; http://n2t.net/addgene:12259; RRID:Addgene_12259) were provided by Dr. Trono. TeT-O-FUW-Ascl1 (Addgene plasmid # 27150; http://n2t.net/addgene:27150; RRID:Addgene_27150), TeT-O-FUW-Brn2 (Addgene plasmid # 27151; http://n2t.net/addgene:27151; RRID:Addgene_27151) and TeT-O-FUW-Myt1L (Addgene plasmid # 27152; http://n2t.net/addgene:27152; RRID:Addgene_27152) were provided by Dr. Wernig (Vierbuchen et al., 2010), whereas TeT-O-FUW-Ikzf1 and TeT-O-FUW-Ikzf4 were generated by cloning the Ikzf1 and Ikzf4 coding sequences into TeT-O-FUW (obtained by removing Brn2 from TeT-O-FUW-Brn2) by standard techniques. These plasmids were transformed in Stbl2 competent cells (Thermo Fisher) to prevent recombination with bacterial DNA due to the repetitive sequences of the lentiviral backbones.

2. 5. *Ex vivo* Müller glia reprogramming

Eyes from P0-1 Glast-CreER;R26R-EYFP mice were collected in Dulbecco's Buffered saline (DPBS; Gibco) under sterile conditions. Retinas were electroporated with single or combined plasmids, with pCALL2-MCS as control. Vectors (1µl at 3µg/µl) described above were injected in the sub-retinal space and a current (50millisec duration, 950 millisec interval, 40-50 volts, unipolar electrodes; BTX ECM 830) was applied over the eye with the positive electrode facing the cornea. Retinas were then dissected in DPBS and placed on a culture insert (Millicell) in a 6-well plate (Falcon) containing 1.3ml of equilibrated medium: Dulbecco's modified eagle medium (DMEM; supplemented with glutaMAX; Gibco) with 10% fetal bovine serum (FBS; Sigma) and 1x Penicillin/Streptomycin (Pen/Strep; Gibco). At day in vitro (DIV)12, hydroxy-tamoxifen (Cayman Chemical Co.) and EGF (PreproTech) were added to the culture medium at a final concentration of 5µM and 100ng/ml, respectively. Two to three days later (DIV14/15), the solution was removed and replaced with fresh culture medium (DMEM/10%FBS/Pen/Strep) after rinsing the well with DPBS. When indicated, EdU was added to the culture medium at a concentration of 10µg/ml and left for three days. At DIV26, the solution was removed and replaced with 1ml of 4% Paraformaldehyde (PFA; Electron microscopy sciences) in phosphate-buffered saline (PBS) for five minutes at room temperature. 1ml of 4% PFA was then added over the culture insert and left for another 5-minute incubation at room temperature. Explants were quickly washed with PBS and left in 20% sucrose in PBS at 4°C for two to five hours before being detached from the insert with curved forceps and frozen in a 20% sucrose:OCT (1:1, Sakura).

2. 6. *In vivo* Müller glia reprogramming

Glast-CreER;R26R-EYFP or Glast-CreER;R26R-EYFP;Pde6b$^{RD1/RD1}$ P0-2 mice were anesthetized on ice, injected sub-retinally with 1µl of DNA vectors (3µg/µl; pCALL2-MCS or pCALL2-Ikzf1 + pCALL2-Ikzf4) in one eye and subjected to an electrical current as previously described (de Melo and Blackshaw, 2011). When indicated, some animals were injected intraperitoneally with EdU at 50µg/g of body weight daily from P3-7. Animals were given tamoxifen (section 2.2) for four consecutive days between P21 and P35 for Glast-CreER;R26R-EYFP or between P87 and P140 for Glast-CreER;R26R-EYFP;Pde6b$^{RD1/RD1}$. Animals were euthanized by CO_2 three to five weeks post-tamoxifen, as specified. Eyes were collected and the retinas dissociated for scRNA-seq (section 2.14 and 2.15) or fixed as described below and processed for immunofluorescence (sections 2.11 and 2.12).

2. 7. Primary mouse embryonic fibroblast culture

Homozygous R26-M2rtTA male mice were crossed with wildtype C57BL/6J females. Plugged females were sacrificed at E13.5 and embryos extracted. Primary MEF culture was performed as previously described (Jozefczuk et al., 2012) with some modifications: 0.25% Trypsin-EDTA (Thermo Fisher) was used for dissociation, and dissociated cells were left for 15 minutes at room temperature to let cell debris and remaining tissue sink to the bottom of the tube, the supernatant was collected and centrifuged. MEFs were cultured in MEF medium: DMEM, 10% Heat-inactivated Cosmic Calf Serum (Cytiva), 1% MEM non-essential amino acids solution (100X stock; Thermo Fisher), 1nM Sodium-Pyruvate (Thermo Fisher), 100U/ml Pen/Strep (Thermo Fisher), 0.114mM 2-

Mercaptoethanol (Sigma). When cells reached 90-100% confluency, they were either passaged or frozen in liquid nitrogen. Cells were passaged at least three times before lentiviral vector infection.

2. 8. Lentiviral vectors production

293FT cells (Thermo Fisher) were plated onto 10cm dishes and transfected at 70% confluency. For transfection, Polyethylenimine (PEI 25K, Polysciences) was added to 1ml of DMEM at a final concentration of 45ng/ul with 5µg of psPAX2, 10µg of pMD.2G, and 10µg of TeT-O-FUW-Ascl1, TeT-O-FUW-Brn2, TeT-O-FUW-Myt1L, TeT-O-FUW-Ikzf1 or TeT-O-FUW-Ikzf4, incubated for 15 minutes at room temperature, and the solution was added drop-wise to the cells. Six hours later, the culture medium was replaced with fresh DMEM supplemented with 5% bovine serum albumin (BSA; sigma Aldrich). Lentiviral collection and spindown was performed at 24 hours and 48 hours after initial change of medium with Lenti-X-concentrator (Clontech) according to the manufacturer protocol. Viral pellet was resuspended in DMEM and divided into 10-20µl aliquots before storing at -80°C. Lentiviral vector aliquots were sequentially thawed rapidly at room temperature, centrifuged at 3000g for three minutes and the supernatant was used for infection.

2. 9. Lentiviral infection of mouse embryonic fibroblast culture

MEFs were seeded in a 6-well plate at 200 000 cells/well. Infection was carried out about 12 hours post-plating if the cells had reached a confluency of at least 50%. Prior to infection, cells were incubated at 37°C for 30-45 minutes in MEF medium (section 2.7) containing 8µg/ml of Polybrene (Santa Cruz Biotechnology). Brn2, Ascl1, Ikzf1 and Ikzf4

and Myt1L lentiviral vectors were added to each corresponding condition at a MOI of 2. Medium containing lentiviral vectors was discarded and replaced with fresh MEF medium 12-18 hours after infection. 24 hours later, cells for immunostaining were trypsinized and replated on acid-washed glass coverslips coated with 0.1% bovine gelatin (Sigma) in 24-well plates (each well of a 6-well plate was divided in three wells of a 24-well plate), while cells for ATAC and RNA-sequencing were maintained in 6-well plates. MEF medium containing 10μM of the caspase-inhibitor quinoline-val-asp-difluorophenoxymethylketone (QVD-OPH; MedChem Express) was added to the cells at this time.

2. 10. Mouse embryonic fibroblast reprogramming assay

MEF reprogramming assay followed a previously published protocol (Vierbuchen et al., 2010) with some modifications. 24 hours after initial addition of QVD-OPH, the culture medium was changed to MEF medium containing 10μM QVD-OPH and 2μg/ml doxycycline (Sigma), corresponding to D0 of the assay. 48 hours later, MEF medium was replaced with reprogramming medium for the rest of the assay, with changes every two days: DMEM/F12 (Gibco) with 1/50 B27-Supplement without vitamin A (50X stock; Thermo Fisher), 1/100 N2-Supplement (homemade, as previously reported by (Vierbuchen et al., 2010)), 2μg/ml doxycycline, 10μM QVD-OPH, 5ng/ml BDNF, 10ng/ml CNTF, and 10ng/ml NT-3 (PeproTech). At D14, cells were washed with PBS and incubated for 15 minutes in 4% PFA/PBS solution at room temperature.

2. 11. Eye fixation and cryosectionning

Eyes were collected and fixed five minutes at room temperature for reprogramming work or three to six hours at 4°C for mouse line validation in 4% PFA/PBS, washed with PBS, and immersed in 20% sucrose for four hours to overnight at 4°C, then frozen in 20% sucrose:OCT (1:1) for cryosectionning. Retinal explants and eyes were sectioned with a cryostat (Leica) at 16 µm for mouse line validation and genetic lineage tracing work (Chapter 3) or 25µm for other assays.

2. 12. Immunostaining

Immunostaining for retinal explant and eye sections were performed as previously described (Javed et al., 2020). Slides stained for CyclinD3 underwent a two hour antigen retrieval in sodium citrate buffer at 54°C before blocking incubation. See Table 1 for primary antibody list. When specified, slides were processed for EdU Click-It reaction following the manufacturer protocol (Abcam; modified to use half of recommended AlexaFluor-647). Chicken anti-GFP primary antibody was used whenever mCherry was present.

MEFs were incubated for 1 hour in blocking solution: 3% BSA and 0.5% Triton in PBS, and then incubated at room temperature for three hours in blocking solution with the primary antibodies against Tau (Table 1). Cells were then washed 3x in PBS and incubated in blocking solution with secondary antibodies (1/1 000) for one hour, washed 3x in PBS. Slides were washed in PBS and incubated in a Hoechst in PBS (1/10 000)

solution at room temperature for five minutes. Cells were then washed 3x in PBS and the coverslips mounted on a microscopy slide with Mowiol for analysis.

Table 1. List of primary antibodies.

Antigen	Species	Company	Cat. number	Concentration
Brn3b	Goat	Santa Cruz biotech.	SC-6026	1/200
Chx10	Sheep	Exalpha Biologicals	X1180P	1/200
Cleaved caspase 3	Rabbit	New England Biolabs	9661	1/100
Cone arrestin	Rabbit	Millipore Sigma	AB15282	1/1000
CyclinD3	Mouse	Santa Cruz biotech.	SC-6283	1/100
Gad6	Mouse	DSHB	GAD-6	1/100
GFP	Chicken	Abcam	AB13970	1/1000
GFP	Rabbit	Thermo Fisher	A11122	1/500
Glutamine synt.	Mouse	BD Bioscience	610517	1/200
Glutamine synt.	Mouse	Chemicon	MAB-302a	1/200
Ikzf1	Goat	Santa Cruz biotech.	discontinued	1/200
Ikzf4	Mouse	Sigma	SAB1407877	1/500
L/M-Opsin	Rabbit	Millipore Sigma	AB5405	1/1000
Lhx2	Rabbit	Thermo Fisher	PA5-78287	1/200
Map2	Mouse	Sigma	A0024	1/10000
Nrl	Goat	R&D Systems	AF2945-SP	1/500
Otx2	Goat	R&D Systems	AF1979	1/1000
Rxrg	Rabbit	Abcam	AB15518	1/100-1/200
S-opsin	Goat	Santa Cruz biotech.	SC-14363P	1/1000
Sox2	Rabbit	Abcam	AB97959	1/200
Tau	Rabbit	Dako	M4403	1/10000
Tuj1	Mouse	Covance	MMS-435P	1/1000

2. 13. Microscopy and cell counts

For Glast-CreER;R26R-EYFP line validation, three to four images per animal and each marker were randomly acquired using a 40x objective with a Leica DM6000 microscope and analyzed on Volocity™ software (Perkin Elmer) to quantify the number of yellow

fluorescent protein+ (YFP+) cells co-labelling with cell-type specific markers. For genetic lineage tracing experiments, all sections were analyzed and cells counted at a Leica DM6000 fluorescent microscope. For retinal reprogramming assays, images were acquired using a 20x or 63x objective on an SP8 confocal microscope (Leica), analyzed on Volocity™ software, and processed with Adobe™ Illustrator (Adobe). Electroporated (mCherry+) regions with normal retinal morphology were selected for quantification. For cell counts, YFP+ mCherry+ cells were analyzed. Images in figures are z-projections or single planes, as specified in figure legends, for optimal representation of cell morphologies.

2. 14. Fluorescent-activated cell sorting

Glast-CreER;R26R-EYFP retinas were isolated and mCherry regions were dissected out using a mini scalpel under a fluorescent microscope (Leica MZ16FA). Retina pieces were dissociated for 16 minutes in papain (82.5U; Worthington) at 37°C. The reaction was stopped by rinsing the retinas once with Lo-Ovo (DPBS, 1.5% BSA, 1.5% Trypsin inhibitor (Roche Diagnostics), pH 7.4) solution with DNase (Worthington Biochemical; 0.3U/μl), and then re-suspended in Lo-Ovo with DNase solution for gentle trituration by pipetting up and down slowly with a P1000 pipet (Gilson). The cell suspension was then passed through a 70μm filter and Hoechst 33258 (Invitrogen) was added to the solution to label dying cells. Fluorescent cells were sorted with a FACSAria III Cell Sorter (BD Biosciences) with a 100μm nozzle. Viable YFP+ cells (single cells) were collected in PBS with 0.15% BSA (Millipore). YFP+ cells from mCherry+ regions of 7 retinas (n=7) were pooled for the Ikzf1/4 condition. As control, YFP+ cells from both mCherry+ (n=4 retinas) or mCherry-

(n=5 retinas) regions were pooled. Cells were then spun at 300g for 10 minutes at 4°C and re-suspended in 0.15% BSA/PBS.

2. 15. scRNA-sequencing

Cells were loaded on a 10xGenomics Single Cell 3' chip and processed according to manufacturer V3 pipeline 3.1.0 kit. Complementary deoxyribonucleic acid (cDNA) libraries we sequenced with Illumina NovaSeq 6000 at an estimated 20 000 reads per cell. A total of 4 207 cells were sequenced for the control sample and 4 608 cells for the Ikzf1/4 sample. Data can be found on GEO #GSE169519.

2. 16. scRNA-sequencing analysis

scRNA-seq fastq files were processed with Cellranger version 4.0 to generate counts with default parameters and reference index provided by 10x Genomics (refdata-cellranger-mm10-2020-A). Loom files were generated with Velocyto with run10x function on the cellranger folder and repeat mask for mm10 genome from UCSC genome browser (La Manno et al., 2018). Scanpy was used to analyze loom files and generate UMAP clusters, and scVelo was used to analyze spliced and unspliced mRNA abundance, RNA-velocity and cell trajectories (Bergen et al., 2020; Wolf et al., 2018).

Single cell regulatory network inference and clustering (SCENIC) analysis was conducted on the same loom files generated from Velocyto. Default parameters for mm10 RcisTarget database were used according to the SCENIC vignette

(https://github.com/aertslab/SCENIC) (Van de Sande et al., 2020). Cell type clustering and regulon AUC UMAPs were generated with Scanpy scatter plot.

2. 17. Mouse embryonic fibroblast RNA extraction and sequencing

RNA extraction from MEF cultures was performed using the RNeasy Plus Micro Kit (Qiagen). At D2 of the reprogramming assay, cells were washed briefly with room temperature DPBS and cells collected by scrapping the well surface with a 1ml pipette tip containing 450µl cold RLT buffer. Cell suspension was transferred in 1.5ml DNA-LoBind Eppendorf tubes. Further extraction steps were performed according to manufacturer guidelines. mRNA was isolated by Poly(A) mRNA magnetic isolation (NEBNext; E7490S), cDNA libraries were generated with KAPA RNA HyperPrep kits (Roche; 08098197702), and sequenced on the Illumina NovaSeq 6000 sequencing system. Data can be found on GEO #GSE169519.

2. 18. Mouse embryonic fibroblast RNA-sequencing analysis

Salmon quant function was used to quantify effective length of transcripts and transcript per million value from RNA-seq raw fastq reads (Patro et al., 2017) and the Galaxy platform was used to perform downstream RNA-seq analysis (Afgan et al., 2016). Counts normalization and differential expression analysis was performed with DESeq2 (Love et al., 2014) and heatmaps were generated with Complex Heatmap (Gu et al., 2016). GOrilla was used to classify genes in GO classification terms (Eden et al., 2009).

2. 19. Mouse embryonic fibroblast ATAC-sequencing

48 hours after doxycycline, 50 000 MEF nuclei were isolated as previously reported (Buenrostro et al., 2015), with modifications described in (Mayran et al., 2018), to perform assay for transposase accessible chromatin (ATAC) sequencing. Isolated nuclei underwent transposase reaction: 2.5µl of 10x TD buffer, 10µl of water, and 12.5µl of enzyme (Illumina Nextera kit; FC-121–1031). DNA was then purified with GeneRead Purification columns (Qiagen), enriched by PCR, and purified again with GeneRead Purification columns before being sequenced with Illumina NovaSeq 6000. Data can be found on GEO #GSE169519.

2. 20. ATAC-sequencing analysis

Raw ATAC-sequencing fastq reads were aligned with bowtie2 on the Galaxy platform (Afgan et al., 2016; Langmead and Salzberg, 2012) and peak calling was performed with MACS2 with the following parameters: --nomodel --shift -37 --extsize 73 (Feng et al., 2012). Bamcoverage was used to generate bigwig files and Integrative Genomics Viewer (IGV) for visualization of ATAC peaks (Ramirez et al., 2016; Robinson et al., 2011). Deeptools was used to compute peaks in a matrix using computeMatrix and heatmaps were generated using plotHeatmap (Ramirez et al., 2016). GO term classification +/-2kb from TSS was performed with GREAT algorithm (McLean et al., 2010).

2. 21. Statistics

Statistical analyses are described in figure legends. All statistical tests were performed using Prism (GraphPad) software.

Chapter 3: Genetic lineage tracing of Müller glia after injury and growth factor treatment

Chapter 3 preface

This unpublished work was entirely performed by the candidate under the guidance of **Dr. Michel Cayouette**.

Of note, this work was completed at the beginning of the doctoral studies of the candidate, when the mammalian Müller glia reprogramming field was relatively new, and NMDA and EGF treatments were the most robust manipulations for Müller glia-derived neurogenesis *in vivo*.

Summary

To evaluate the endogenous neurogenic potential of mouse Müller glia, we performed genetic lineage tracing of these cells after injury and growth factor treatment. Using the Glast-CreER;R26R-EYFP line, we show that, although NMDA and EGF injections induce broad retinal cell cycle re-entry, few of these cells are Müller glia. In addition, we find that Müller glia-derived neurogenesis is a very rare event, which occurred only once in 17 animals. These results indicate that other manipulations are required to induce a neurogenic response from mouse Müller glia.

3. 1. Glast-CreER;R26R-EYFP specifically labels Müller glia

To perform genetic lineage tracing of Müller glia, we took advantage of Glast-CreER;R26R-EYFP mice. These mice express tamoxifen-inducible CreER under the *GLAST* promoter (Nathans, 2010), which is specific to Müller glia and astrocytes in the adult rodent retina (Lehre et al., 1997), and the Cre-dependent R26R-EYFP reporter (Fig. 12A). Upon tamoxifen administration, CreER proteins expressed in Müller glia translocate to the nuclei, where they recombine the Rosa locus loxP sites, and initiate permanent YFP expression. This allows for genetic lineage tracing of Müller glia and any derived cells with the YFP reporter.

We validated this mouse line in our hands by injecting tamoxifen in adult mice and analyzing the proportion of YFP+ cells expressing Müller glia markers 7-8 days after initial tamoxifen administration. While only ~40% of all Müller glia (CyclinD3+ cells) were YFP+ (Fig. 12B-C), indicating that not all Müller cells were targeted after tamoxifen injection, we found that over 95% of YFP+ cells expressed CyclinD3, and 100% expressed glutamine synthetase and Sox2 (Fig. 12B,D), three Müller glia markers. Importantly, YFP+ cells were not observed in Glast-CreER;R26R-EYFP animals that did not receive tamoxifen (Fig. 12E). These results confirm the high specificity of this line for labelling Müller glia, and validate its use for genetic lineage tracing of these cells.

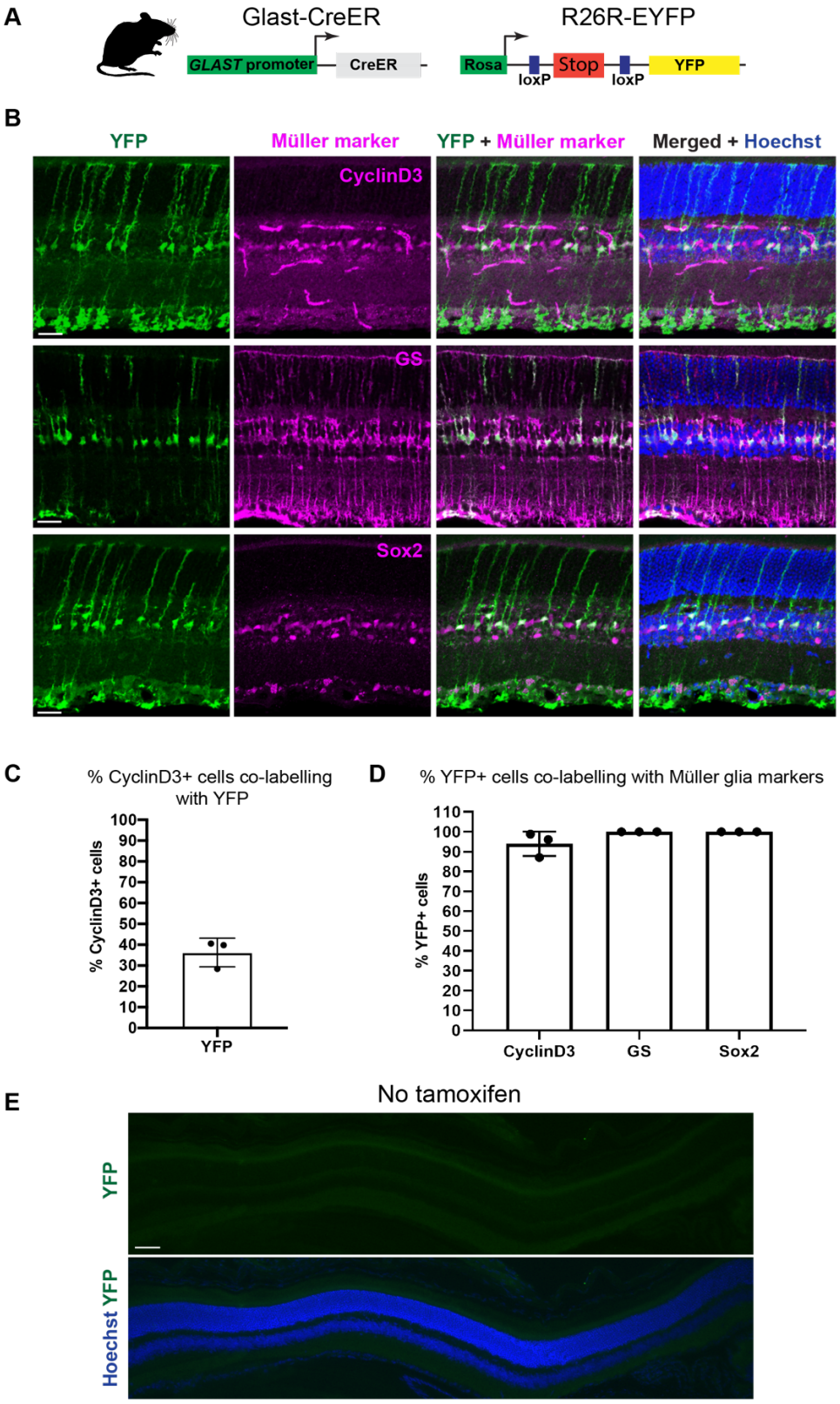


Figure 12: Glast-CreER;R26R-EYFP mouse line specifically labels Müller glia.

A. Representation of mouse line strategy. **B.** Co-immunostaining for YFP and the Müller

glia markers CyclinD3, glutamine synthetase (GS), or Sox2 on retinal sections from the Glast-CreER;R26R-EYFP mouse line 7-8 days after tamoxifen injection. Scale bars: 25µm. **C.** Graph representing the proportion of CyclinD3+ cells co-labelled with YFP (n=3). **D.** Graph representing the proportion of YFP+ cells co-labelled with Müller glia markers (n=3). Quantifications (C-D) done in the Glast-CreER;R26R-EYFP mouse line 7-8 days after tamoxifen injection. **E.** Representative images of retinal sections, immunostained for YFP, of Glast-CreER;R26R-EYFP mice that did not received tamoxifen. Scale bars: 50µm. All images are z-projections except, in B: GS images are single planes. Graphs represent mean +/- standard deviation.

3. 2. Few Müller glia incorporate EdU after NMDA and EGF treatments

One week after tamoxifen administration, we intravitreally injected P32 Glast-CreER;R26R-EYFP animals with NMDA in one eye and saline in the other as control. Two days later, we intravitreally injected the injured eye with EGF and EdU, and the control eye with EdU, as previously performed by Karl et al. (2008). We sacrificed the animals one day post-EdU to investigate Müller glia cell cycle re-entry (Fig. 13A).

Because it was shown that more Müller glia re-enter the cell cycle after injury in mice of the 129SvJ strain compared to C57BL/6J strain (Suga et al., 2014), we backcrossed both Glast-CreER and R26R-EYFP lines, which were maintained in a C57BL/6J background, separately to the 129SvJ strain for 5 generations. We then crossed them together to obtain the 129SvJ Glast-CreER;R26R-EYFP line. We performed the experimental condition above with this 129SvJ line (Fig. 13A) to compare cell cycle re-entry with C57BL/6J mice.

Injured retinas could be easily identified with reduced INL and GCL layers (Fig. 13B), concordant with the loss of amacrine cells and RGCs due to NMDA excitotoxicity, thus validating our delivery method and NMDA activity. To obtain a representation of pan-retinal as well as Müller glia cell cycle re-entry, we quantified the total number of EdU+ and YFP+/EdU+ cells per retinas for control and experimental conditions (Fig. 13C). Whereas only ~25 cells per retina on average were EdU+ in control retinas, treated retinas contained ~2 000 EdU+ cells in C57BL/6J, and ~700 EdU+ cells in 129SvJ. These manipulations hence successfully induced broad retinal cell cycle re-entry, significantly more in C57BL/6J compared to both control and 129SvJ treated retinas, contrary to what has been previously reported for 129SvJ mice (Suga et al., 2014) (see section 5.2 for discussion). While no EdU+ cells co-labeled with YFP in the control condition, we could identify a few YFP+/EdU+ cells in injured retinas, significantly more in C57BL/6J treated retinas compared to control (Fig. 13C-D). Still, YFP+/EdU+ cells consisted of less than 0.5% of all EdU+ cells in treated C57BL/6J retinas. These results demonstrate that, although NMDA and EGF induce broad retinal cell cycle re-entry, few of these cells are Müller glia. Since C57BL/6J Müller glia re-entered the cell cycle more robustly than 129SvJ mice (Fig. 13C), we performed further experiments with the C57BL/6J strain.

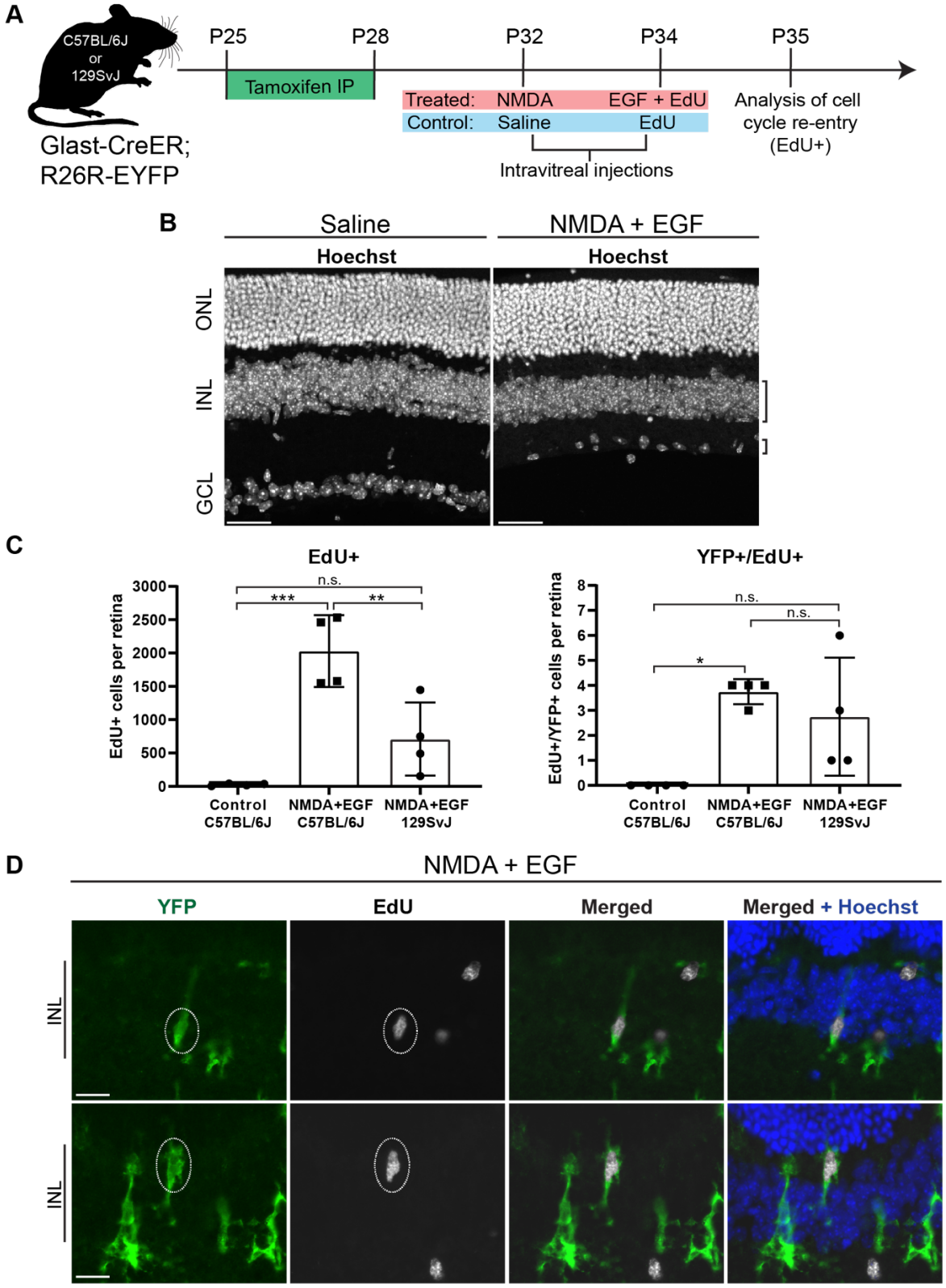


Figure 13: Few Müller glia incorporate EdU after NMDA and EGF treatments.

A. Representation of experimental protocol. **B.** Retinal sections with Hoechst staining of

treated (right) and control eyes (left). Black lines (right) show INL and GCL thickness in treated retina. Scale bar: 32 μm. **C.** Graph representing average number of EdU+ (left) and YFP+/EdU+ (right) cells per retina in control (n=4), experimental C57BL/6J (n=4), and experimental 129SvJ (n=4) retinas. Left: **p<0.001, **p<0.01, One way ANOVA and Tukey's post-hoc tests. Right: *p<0.05, Kruskal-Wallis followed with Dunn's post-hoc test. Not significant: n.s. **D.** Representative images of retinal sections stained for YFP and EdU showing YFP+/EdU+ cells (circled) in NMDA and EGF treated retinas. Scale bar: 18μm. Pictures are single planes.

3. 3. NMDA and EGF do not induce robust Müller glia-derived neurogenesis

We next investigated whether some YFP+/EdU+ cells would give rise to neurons by repeating experiments described in 3.2, and analyzing the retinas at 6, 8, 15, and 30 days post-injury (Fig. 14A). We performed immunofluorescence for the early neuronal marker Tuj1, the amacrine cell marker Gad6, and the RGC marker Brn3b. Most of our analysis was completed with Gad6, as this was the marker previously used to identify newly generated neurons (Karl et al., 2008). We searched for YFP+/EdU+/neuronal marker+ cells in retinas of 17 animals (Fig. 14B).

Most retinas did not have triple labelled cells. We did find rare EdU+/YFP+/Brn3b+ or EdU+/YFP+/Gad6+ cells (Fig. 14C), but these were not convincing neurons: consisting of dense YFP+ nuclei without any apparent cytoplasm or processes, and with abnormal localization of cytoplasmic neuronal markers to the nucleus (Fig. 14C'), likely dying cells. We only identified one EdU+/YFP+/Gad6+ cell, in the GCL, that showed normal Gad6 localization (Fig. 14C''). It is difficult to conclude with certainty whether this corresponds

to a Müller-derived amacrine cell or rather to Müller glia endfeet partially surrounding a displaced amacrine cell.

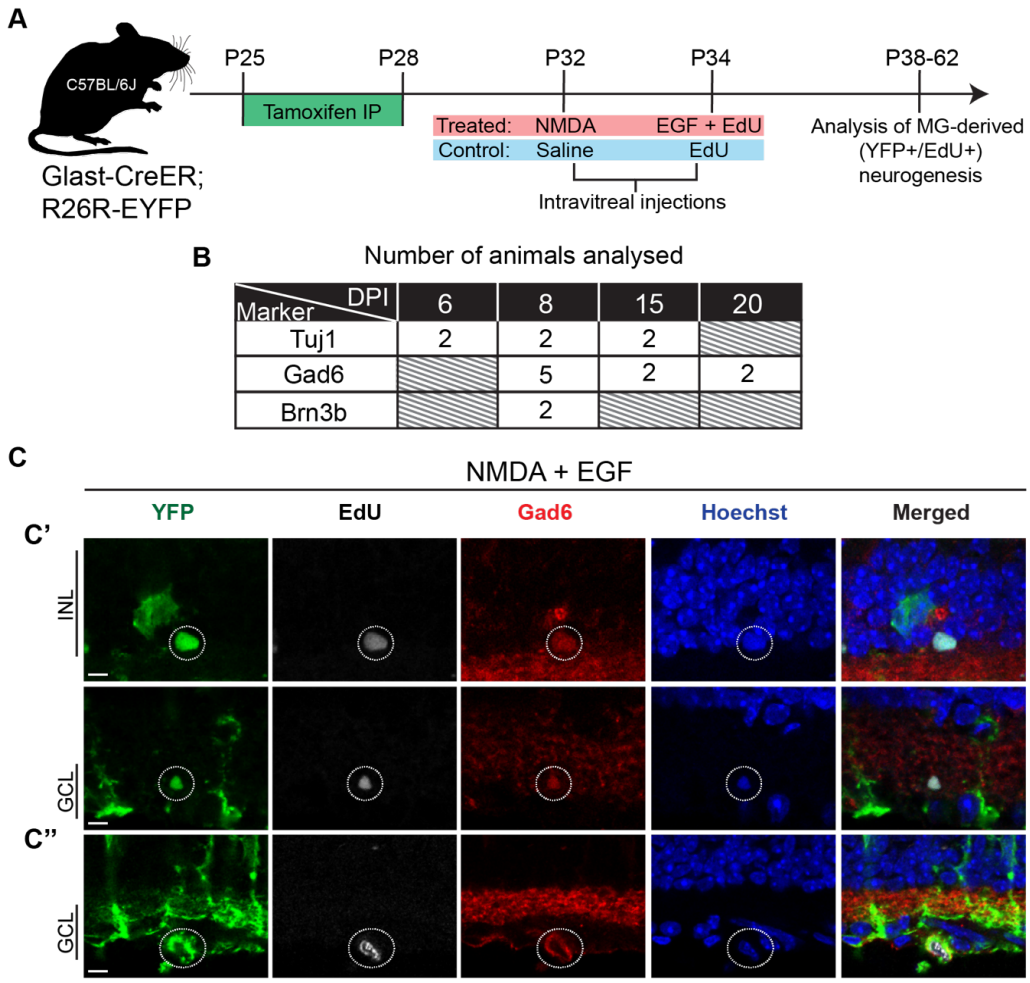


Figure 14: NMDA and EGF do not induce robust Müller glia-derived neurogenesis. **A.** Representation of experimental protocol. MG: Müller glia. **B.** Number of animals analysed by neuronal marker (row) and time point (column). DPI: Days post-injury. **C.** Retinal sections stained for YFP, EdU, and Gad6 showing YFP+/EdU+/Gad6+ cells (circled). C'. Representative images of cells with abnormal morphologies and Gad6 staining. C". Potential Müller-derived amacrine cell. Scale bars: 6μm. All pictures are single planes.

Still, in 17 retinas analyzed, only one potential neuron would have been generated from Müller glia after NMDA and EGF injections, demonstrating that these manipulations are not sufficient to induce robust Müller glia neurogenic potential. This contrasts with previous interpretations of BrdU lineage tracing (Karl et al., 2008), indicating that genetic lineage tracing likely underlies these discrepancies, and may allow for more precise analysis of Müller glia lineage. Overall, these data demonstrate that other techniques to stimulate neuron production from mammalian Müller glia are required.

Chapter 4: Combined Ikzf1 and Ikzf4 expression reprograms mammalian Müller glia and embryonic fibroblasts to neurons

Chapter 4 preface

Results presented in this chapter are part of an article posted on bioRxiv (https://doi.org/10.1101/2021.07.05.451124) and in preparation for submission to a peer-reviewed journal:

C. Boudreau-Pinsonneault, A. Javed, M. Fries, P. Mattar, M. Cayouette. (in preparation) Direct neuronal reprogramming by temporal identity factors.

All experiments were performed under the guidance of **Dr. Michel Cayouette**.

Awais Javed, along with the candidate, performed scRNA-seq (Fig. 24 and 25), RNA-seq, and ATAC-seq (Fig. 27 and 28) computational analyses.

Michel Fries extracted RNA for MEF RNA-seq, and, with the candidate and **Christine Jolicoeur**, performed MEF reprogramming assays (Fig. 26).

cDNA libraries for sequencing experiments were generated by the **Molecular Biology platform**, and fluorescent activated cell sorting (FACS) was performed with the **Cytometry platform** of the IRCM.

All other experiments were performed by the candidate.

The candidate would also like to acknowledge **Dr. Pierre Mattar** for his work initiating MEF reprogramming experiments.

Summary

NMDA and EGF injections are not sufficient to induce a robust neurogenic response from mouse Müller glia (Chapter 3), sparking the need for additional manipulations to achieve this. As neuronal reprogramming factors identified to date are generally involved in progenitor cell fate decisions, developmental regulators represent good candidates to identify factors with reprogramming abilities. Temporal identity factors are sufficient to reprogram developmental competence of neural progenitors, but whether they could also reprogram the identity of fully differentiated cells is unknown. To address this question, we designed a conditional gene expression system combined with genetic lineage tracing that allows rapid screening of potential reprogramming factors in the mouse retina. Using this assay, we report that co-expression of the early temporal identity transcription factor Ikzf1, together with Ikzf4, another Ikaros family member, is sufficient to directly convert adult Müller glial cells into neuron-like cells *in situ*, without inducing a proliferative state. scRNA-seq analysis shows that the reprogrammed cells share some transcriptional signatures with both cone photoreceptors and bipolar cells. Furthermore, we show that co-expression of Ikzf1 and Ikzf4 can reprogram mouse embryonic fibroblasts to induced neurons by remodeling chromatin and promoting a neuronal gene expression program. This work uncovers general neuronal reprogramming properties for temporal identity factors in differentiated cells, opening new opportunities for cell therapy development.

4. 1. Combined Ikzf1 and Ikzf4 expression reprograms Müller glia to cone-like cells *ex vivo*

As NMDA and EGF treatment was not found to induce Müller glia neurogenesis, we next aimed to find novel ways to achieve this (section 1.6). To identify factors that can reprogram retinal glia to neurons, we designed an assay to conditionally express any gene of interest specifically in Müller cells, while genetically tracing the lineage of the potential progeny. We relied on electroporation of conditional expression constructs (pCALM) in which a loxP-mCherry-STOP-loxP cassette is excised in a Cre-dependent manner, allowing expression of a downstream gene of interest (Fig. 15A-B). We tested the specificity of this construct by transfecting a version containing GFP after the loxP cassette (pCALM-GFP) into HEK 293T cells, alone or together with a Cre-expressing construct. We found that only mCherry is expressed when pCALM-GFP is transfected alone, whereas mCherry is turned off and GFP is expressed when it is co-transfected with a Cre construct (Fig. 15C), validating the conditional expression strategy. To achieve expression of Cre in Müller glia and permanently label them, we used Glast-CreER;R26R-EYFP mice described previously (Chapter 3).

To screen for potential neuronal reprogramming factors, we electroporated the Cre-dependent expression constructs in P0-1 Glast-CreER;R26R-EYFP retinas, which were then explanted and cultured for 12 days to allow neurogenesis to complete. When electroporating retinas at P0, the great majority of transfected cells are progenitors, and approximately 5% of these progenitors go on to generate Müller glia (Javed et al., 2020; Matsuda and Cepko, 2004), which will inherit the transfected plasmids. Addition of

hydroxytamoxifen in the culture medium at day 12 will activate Cre recombinase specifically in Müller cells, allowing expression of the transfected construct and permanent expression of the YFP reporter. Two weeks later, the retinas were fixed and analyzed for any potential Müller cell state changes by examining cell morphology, soma position within the retinal layers, and expression of cell type specific markers (Fig. 15A-B).

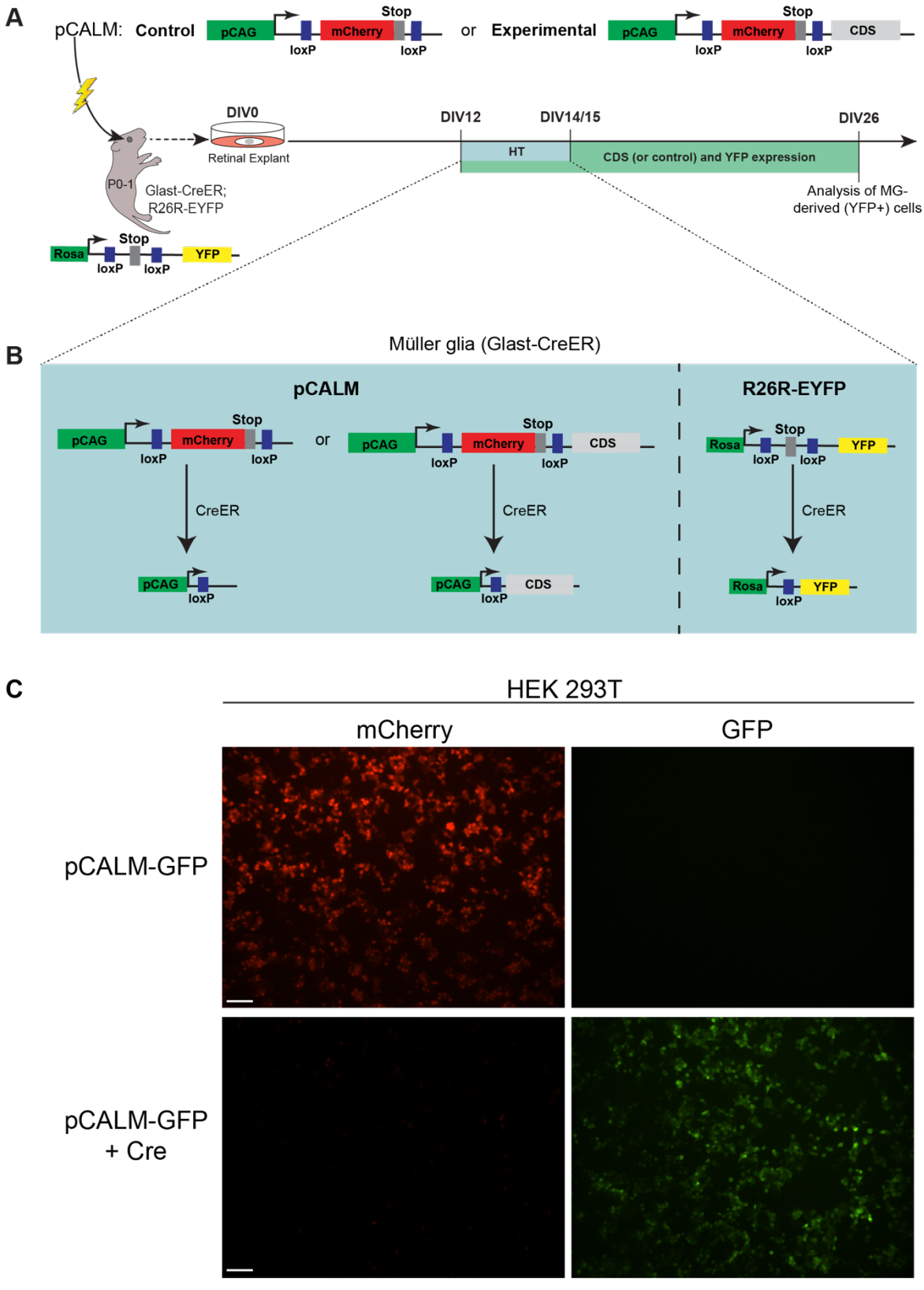


Figure 15: Detailed representation of experimental procedure.

A. pCALM plasmids, control or experimental, are electroporated (lightning bolt) in the eyes of neonate Glast-CreER;R26R-EYFP animals, which are explanted and cultured for 26 days. Hydroxytamoxifen (HT) is added to culture medium from DIV12 to 14/15. CDS: Coding sequence. **B.** Representation of CreER activity in Müller glia when HT is present. pCALM constructs (left) are recombined at the loxP cassettes to induce expression of the gene coding sequence (experimental) or nothing (control). Rosa locus (right) loxP cassette is also removed to induce permanent expression of EYFP. **(C)** HEK 293T cells transfected with pCALM-GFP (top row) or pCALM-GFP + Cre (bottom row). Scale bars: 100µm.

Using this assay, we expressed 22 different gene combinations in Müller glia (Fig. 16A), primarily focusing on various temporal identity factors previously shown to control retinal progenitor cell competence (section 1.2.1.2), such as Ikzf1, Pou2f1, Pou2f2, and Casz1 (Elliott et al., 2008; Javed et al., 2020; Mattar et al., 2015). Taking advantage of the flexibility of our assay, we also included several other factors, including another Ikaros family member (Ikzf4) expressed in the retina (Clark et al., 2019; Elliott et al., 2008), factors found to promote Müller glial reprogramming in fish (Apobec2b) (Powell et al., 2012) or mice (Ascl1) (Pollak et al., 2013; Ueki et al., 2015), or found to potentiate MEF reprogramming (Brn2 and Myt1l) (Vierbuchen et al., 2010). In 20 out of 22 combinations tested, similarly to the control condition consisting of the empty pCALM construct (Fig. 15A), YFP+ cells had typical Müller glia morphologies, with cell bodies located in the INL, complex processes extending apically and basally (Fig. 15C), and expressed glia markers (Fig. 16B-C), suggesting that Müller glia did not change identity following expression of these factors. Upon Ascl1 expression, however, we identified some YFP+ cells in the INL that also expressed the bipolar cell marker Otx2, suggesting that some Müller glia

reprogrammed into bipolar-like cells, as previously reported (Pollak et al., 2013) (Fig. 16D).

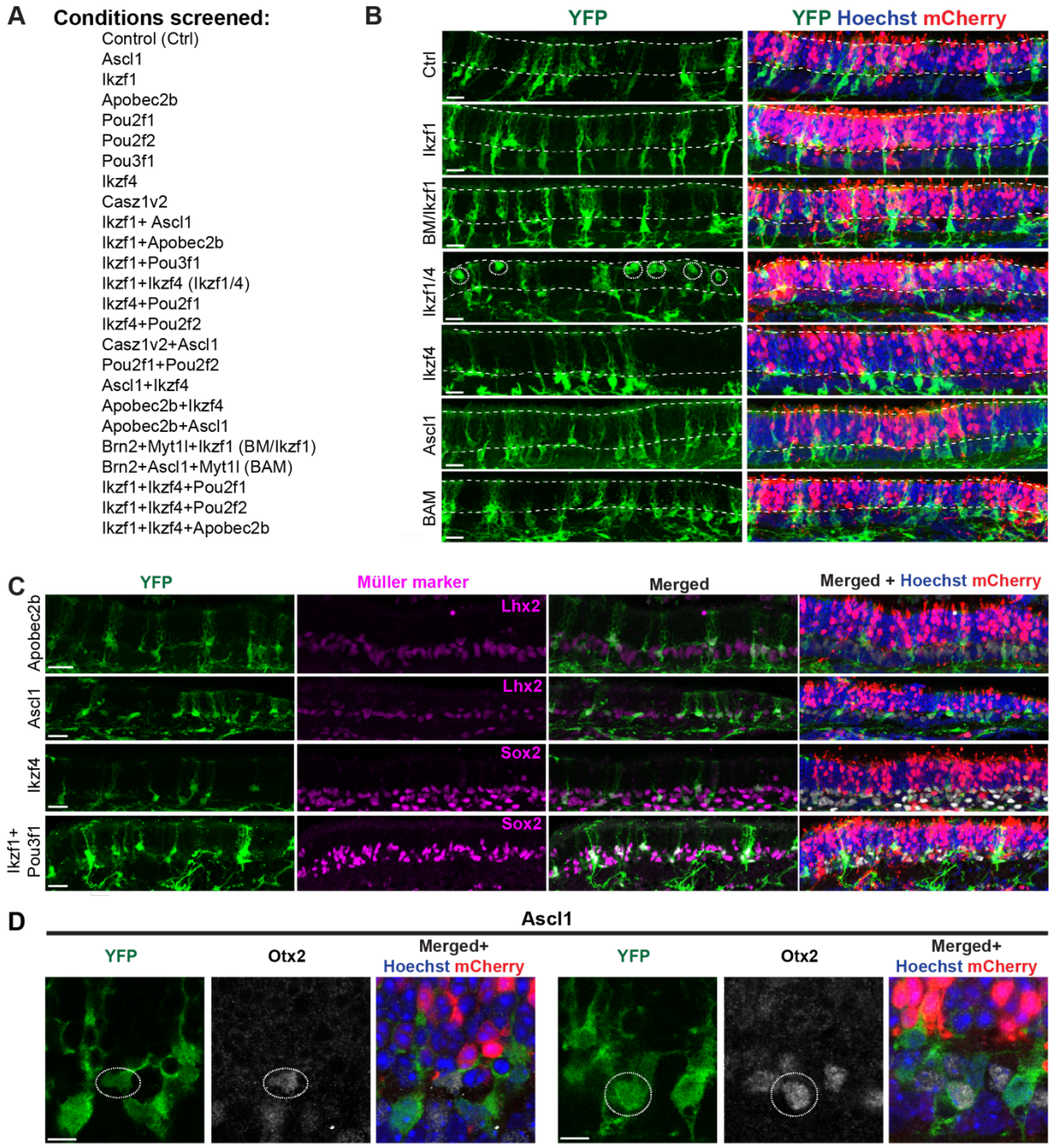


Figure 16: *Ex vivo* screen for Müller glia reprogramming to neurons.

A. List of conditions screened for Müller glia reprogramming. **B.** Representative images of retinal sections electroporated with various conditions included in the screen immunostained for YFP. Dotted lines show ONL, where photoreceptors reside. Circles in

the Ikzf1/4 condition point to cells with altered morphology located in the ONL. Scale bars: 17µm. **C.** Representative images of co-immunostaining for YFP and the Müller glia markers Lhx2 (top) and Sox2 (bottom) on retinal sections from additional conditions tested in the screen. Scale bars: 24µm. **D.** Co-immunostaining for YFP and Otx2 after electroporation and expression of Ascl1. Circles show co-labelled cells. Scale bars: 7µm. All images are z-projections except for E.

However, the only condition that elicited widespread changes in this assay, was the combined expression of Ikzf1 and Ikzf4 (Ikzf1/4), which gave rise to many YFP+ cells with morphologies distinct from Müller glia that relocated to the ONL, where only photoreceptor cells reside (Fig. 16B, 18). Of note, morphologically-reprogrammed YFP+ cells were still mCherry+, most likely due to Cre-mediated recombination of a fraction of all plasmid constructs transfected in each cell. We validated that mCherry+ Müller glia expressed Ikzf1/4 after tamoxifen, by co-immunostaining YFP, Ikzf1, and Ikzf4 on retinal sections of animals electroporated with Ikzf1/4 5 weeks post-tamoxifen (Fig. 17A). We hence took advantage of mCherry to focus our analysis on transfected cells for both Ikzf1/4 and control conditions (YFP+: Müller glia and their progeny; mCherry+: transfected cells).

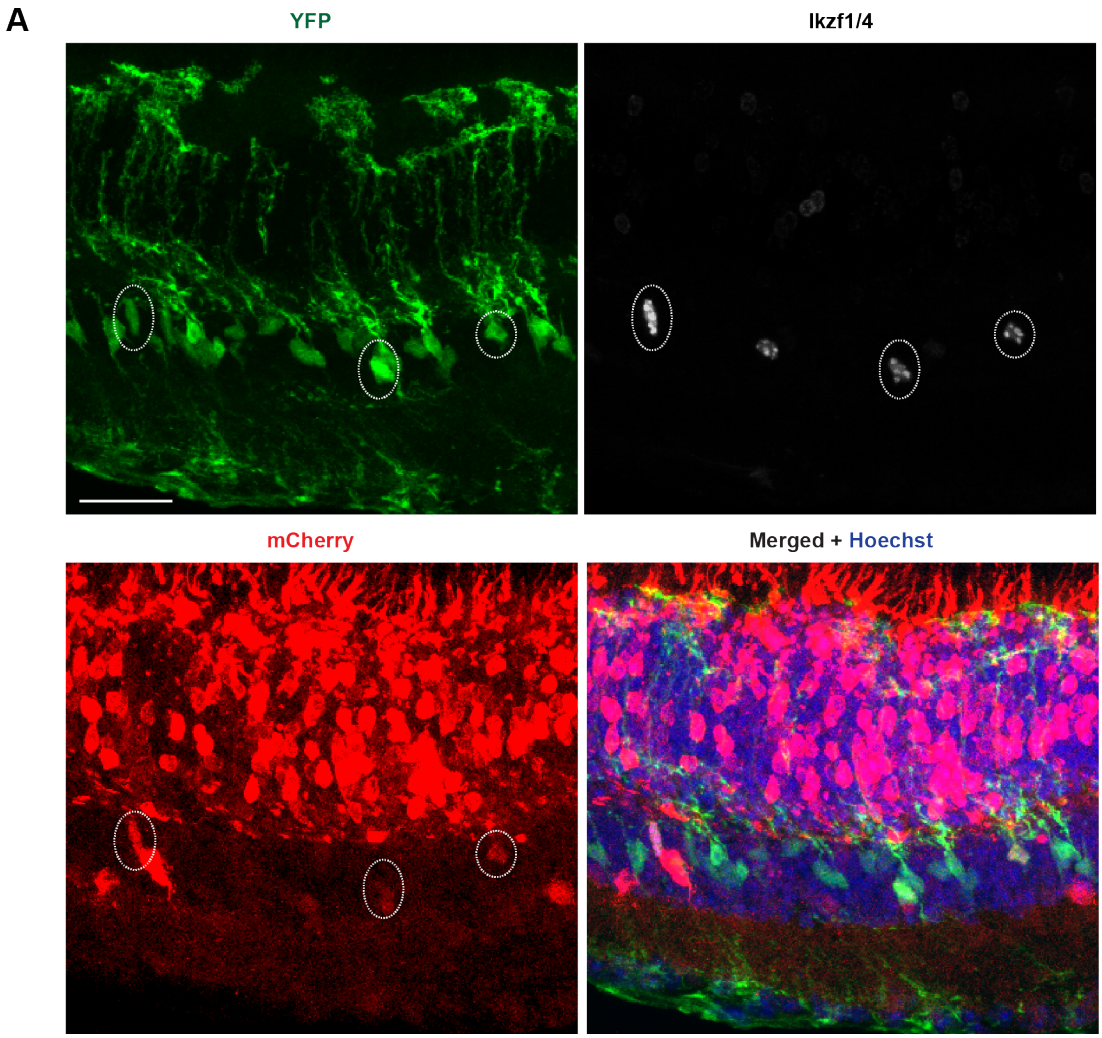


Figure 17: Ikzf1/4 and mCherry are expressed in YFP+ cells.

A. Retinal section of *in vivo* Ikzf1/4 electroporated retina 5 weeks post-tamoxifen immunostained for YFP and Ikzf1/4. Circles show YFP+/mCherry+/kzf1/4+ cells. Scale bar: 25 µm.

The majority of Ikzf1/4 cells in the ONL were located at the apical side of this layer, where cone photoreceptors usually reside (Fig. 18A-B). Most relocated cells had a round morphology without apparent cell processes, but some resembled immature photoreceptors with an oval cell body, a simple process extending towards the plexiform layer, and a small protrusion towards the apical surface (Fig. 18C-D). Interestingly, Ikzf1

or Ikzf4 expression alone did not alter Müller glia morphology or cell soma position (Fig. 16B), indicating that combined expression of Ikzf1 and Ikzf4 is necessary to induce morphological reprogramming. To possibly enhance Ikzf1/4 reprogramming, we also tested the co-expression of Ikzf1/4 together with other factors (Pou2f1, Pou2f2, or Apobec2b), by transfecting three constructs (Fig. 16A). We found, however, that triple transfections reduced the number of reprogrammed cells, potentially due to the dilution of each transfected construct and a reduction of the number of cells inheriting both Ikzf1 and Ikzf4.

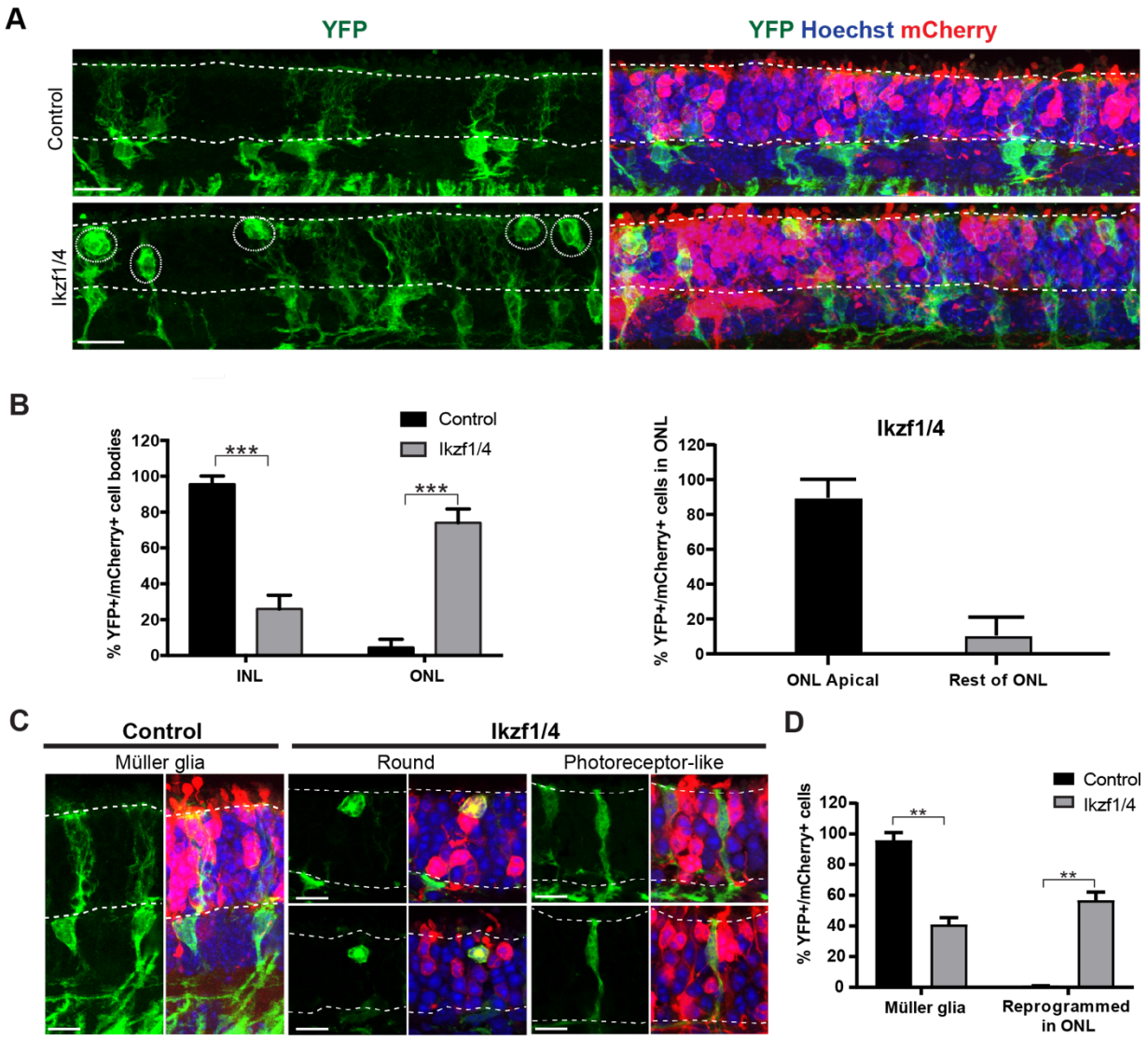


Figure 18: Ikzf1/4 expression alters Müller glia morphology and localization *ex vivo.* **A.** Representative images of retinal sections from electroporated regions with control (top) and Ikzf1/4 (bottom) constructs immunostained for YFP. Circles point to Müller glia-derived cells in the ONL. Dotted lines show the ONL. Scale bars: 38μm. **B.** Left: Localization of cell bodies of YFP+/mCherry+ cells for control (n=6) and Ikzf1/4 (n=6) conditions. ***p<0.001, unpaired t-test. Right: Graph representing location of Ikzf1/4 cell bodies within ONL. **C.** Representative images, immunostained for YFP, of YFP+ cells in control electroporation showing Müller glia morphologies, and in Ikzf1/4 electroporation with rounded and photoreceptor-like morphologies. Dotted lines show the ONL. Scale bars: 10μm. **D.** Graph representing proportion of YFP+/mCherry+ cells with Müller or

reprogrammed morphologies in control and Ikzf1/4 conditions. ** p=0.0022, Mann-Whitney test, control (n=6) and Ikzf1/4 (n=6). All images are z projections, except for (F) Ikzf1/4 images are single planes. Graphs represented as means + standard deviation.

To determine whether the apparently reprogrammed cells actually changed molecular identity, we performed immunofluorescence for the Müller glia markers Sox2 and Lhx2. Whereas virtually all Müller cells in the control condition expressed these markers, most Ikzf1/4 morphologically-reprogrammed cells in the ONL did not (Fig. 19A-B), suggesting that they lost their glial identity. A few cells that had repositioned their soma to the photoreceptor layer still expressed Müller glia markers, however, likely representing cells at intermediate stages of reprogramming (Fig. 19A arrow).

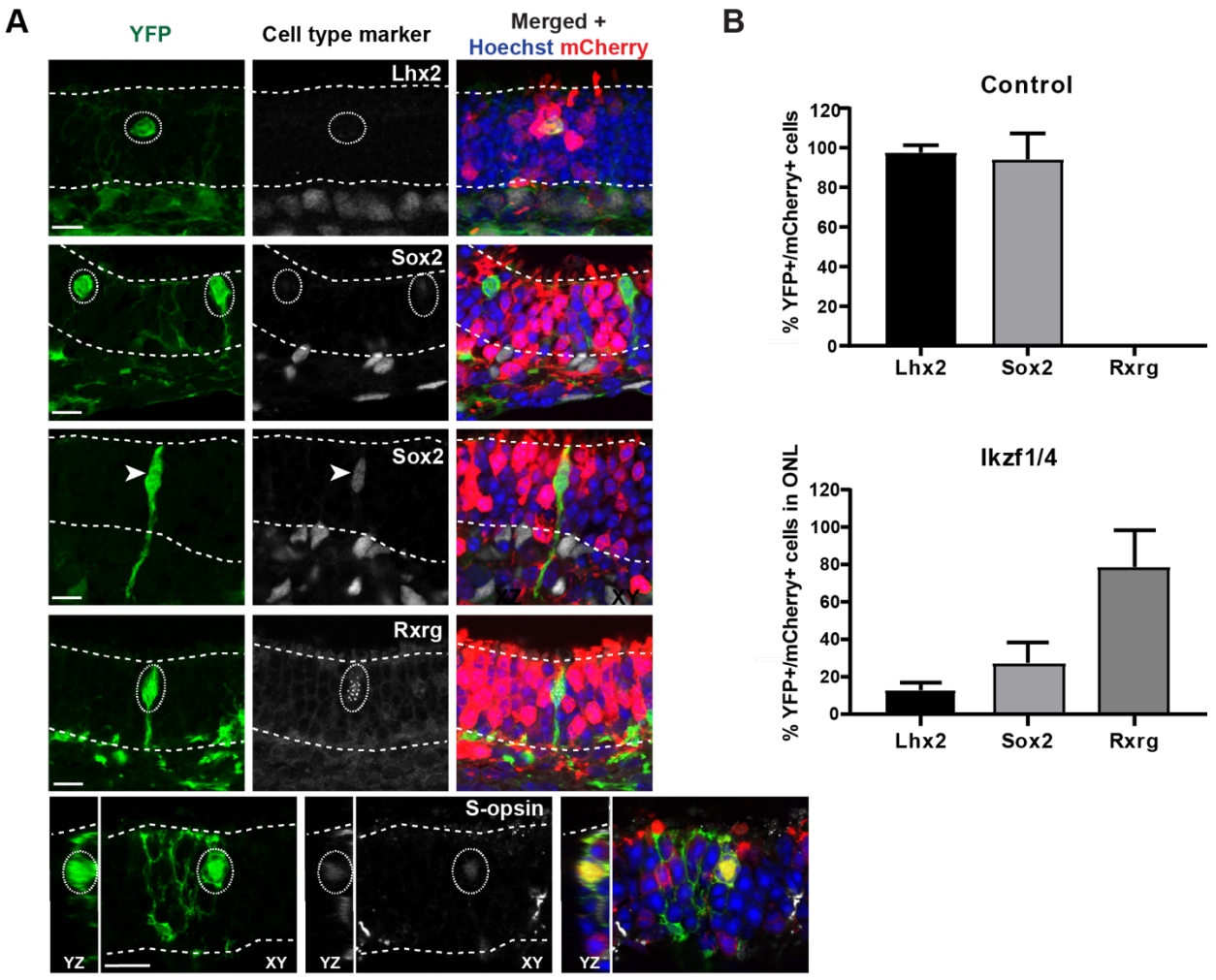


Figure 19: Ikzf1/4-reprogrammed cells turn off Müller glia markers and express cone markers *ex vivo*.

A. Immunostaining on Ikzf1/4-electroporated retinas *ex vivo* with YFP and various cell type specific markers, as indicated. Most reprogrammed cells in the ONL (circled) are negative for the Müller glia markers Lhx2 or Sox2, but are immunostained for the cone marker Rxrg. Arrow points to Sox2-positive cell in the ONL. Bottom pictures shows YFP+/S-opsin+ cell (circled). Left panels are YZ view. Dotted line define the ONL thickness. Scale bars: 10µm. **B.** Quantification of marker expression for all YFP+/mCherry+ cells in control- (top) and YFP+/mCherry+ cells located in ONL in Ikzf1/4-electroporated retinas (bottom). Images show z projections in (A) for Lhx2 and Rxrg images, all others are single planes. Graphs show means + standard deviation.

.

To establish whether the cells that changed morphology, moved to the ONL, and down regulated glia markers might have reprogrammed into neurons, we stained Ikzf1/4-transfected retinas for various cell-type specific markers (Fig. 20). YFP+ reprogrammed cells did not stain for markers of rod photoreceptors and various retinal interneurons (Fig. 20A-B) and were also negative for cleaved caspase 3, a marker of apoptosis (Fig. 20B). Remarkably, most YFP+ reprogrammed cells turned on the cone photoreceptor marker Rxrg (Fig. 19A-B), which was never observed in controls (Fig. 19B). Rare Ikzf1/4-reprogrammed cells also expressed low levels of S-opsin, albeit in an unusual cytoplasmic pattern (Fig. 19A), suggesting incomplete differentiation. As Rxrg is also expressed in RGCs, we asked whether the reprogrammed cells could be RGCs by staining for Brn3b. However, did not find any Brn3b+/YFP+ cells (Fig. 20B), and since the reprogrammed cells moved to the photoreceptor layer, we conclude that these cells are not RGCs. Together, these results indicate that expression of Ikzf1/4 in Müller glia promotes reprogramming into cone-like photoreceptor cells.

A

Markers screened	Cell types labelled	Present in reprogrammed cells
Lhx2	Müller glia	Few cells
Sox2	Müller glia	Few cells
CyclinD3	Müller glia	Few cells
Rxrg	Cone photoreceptors and RGCs	Yes
S-opsin	Cone photoreceptors	Rare cells
L/M-opsin	Cone photoreceptors	No
Cone arrestin	Cone photoreceptors	No
Nrl	Rod photoreceptors	No
Otx2	Photoreceptors & Bipolar cells	No
Chx10	Bipolar cells	No
Brn3b	RGCs	No

B

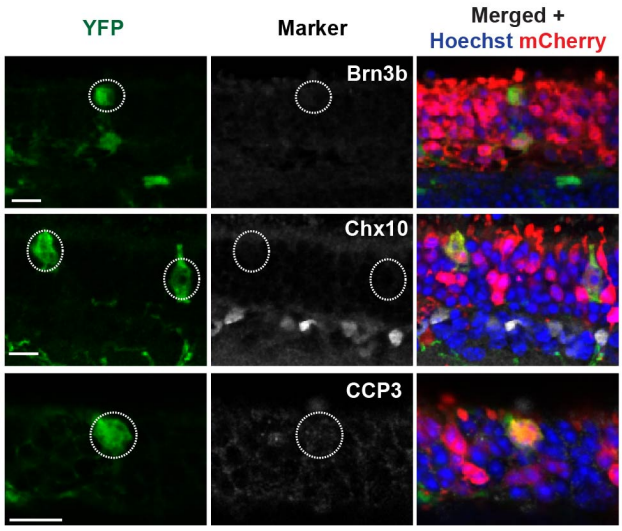


Figure 20: Markers screened in Ikzf1/4 *ex vivo* reprogramming assay.

A. Table listing cell type markers screened in Ikzf1/4 *ex vivo* electroporations and the presence or absence of co-labelling with YFP+ reprogrammed cells. **B.** Co-immunostaining for YFP and Brn3b, Chx10, or cleaved caspase-3 (CCP3) in Ikzf1/4-electroporated retinas. Reprogrammed cells (circled) are negative for all three markers. Scale bars: 12μm. Images are single planes.

4. 2. Ikzf1/4 do not promote Müller glia cell cycle re-entry

As *in situ* reprogramming was previously reported to sometimes trigger Müller glia proliferation (Jorstad et al., 2020), we wondered whether expression of Ikzf1/4 might stimulate cell division. To test this, we repeated the experiments as described above, but added EdU to the culture medium either from DIV12 to 15 and 18 to 21 or DIV15 to 18 and 21 to 24, spanning the culture time between initiation of Ikzf1/4 expression and fixation (Fig. 21A). We found no difference between the number of YFP+/mCherry+/EdU+ cells in Ikzf1/4 compared to controls in any condition (Fig. 21B), indicating that Ikzf1/4 do not promote cell cycle re-entry.

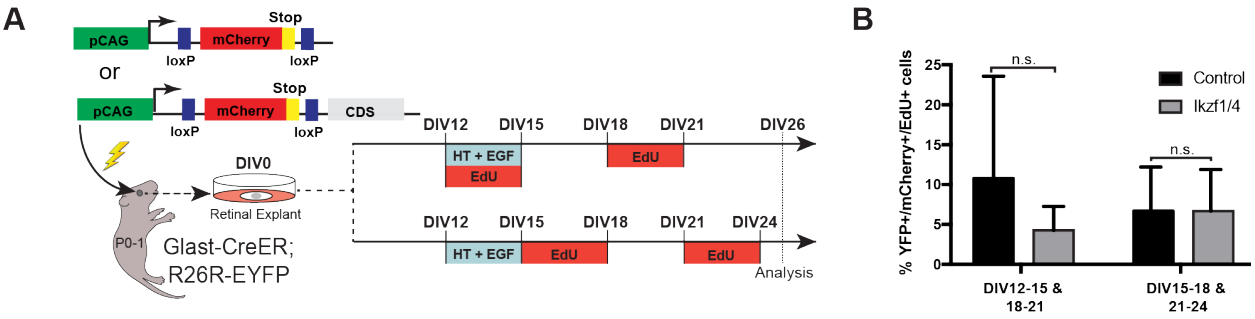


Figure 21: Ikzf1/4 do not promote Müller glia cell cycle re-entry.

A. Schematic representation of the cell cycle re-entry analysis protocol. DIV: days in vitro. HT: hydroxy-tamoxifen. CDS: coding sequence. Lightning bolt represents electroporation.

B. Quantifications of the number of YFP+/mCherry+ cells that incorporated EdU in control and Ikzf1/4-electroporated retinas in both conditions. Graph shows mean + standard deviation. Unpaired t-tests, n=4 in control DIV12-15 18-21, n=5 in control DIV15-18 21-24, n=5 in both Ikzf1/4 conditions; n.s.: not significant.

4. 3. Ikzf1/4 reprogram Müller glia to cone-like cells *in vivo*

We next investigated whether co-expression of Ikzf1/4 was sufficient to convert adult Müller glia to cone-like photoreceptor cells *in vivo*. Using a similar approach as described above for retinal explants, we electroporated empty pCALM or pCALM- Ikzf1/4 constructs in the retinas of neonate (P0-P2) Glast-CreER;R26R-EYFP mice. When the mice reached adult ages (≥P21), we injected tamoxifen to induce YFP and Ikzf1/4 expression in adult Müller glia. Three or five weeks later, the retinas were collected for analysis (Fig. 22A).

In control electroporations, YFP+/mCherry+ cells had normal Müller glia morphology with cell soma in the INL and expression of glia markers, as expected (Fig. 22B). Three weeks post-tamoxifen, in Ikzf1/4 electroporations, about one fifth of all YFP+/mCherry+ cells had changed morphology, relocated to the ONL, and expressed the cone marker Rxrg (Fig. 22C). Unlike *ex vivo*, their cell bodies did not reach the apical-most part of the ONL, but rather spanned the entire ONL thickness. These cells did not express the Müller glia markers Lhx2 and Sox2 (Fig. 22D), and were still present 5 weeks post-tamoxifen, albeit in lower numbers (Fig. 22C). Remarkably, and in contrast to *ex vivo* experiments, Ikzf1/4-reprogrammed cells very rarely showed rounded morphologies and mostly had complex photoreceptor-like morphologies with some pedicle-like structures in the outer plexiform layer (Fig. 22F), suggesting that the *in vivo* environment allows a more complete reprogramming of Müller glia into cone-like cells, at least in terms of morphology. We also identified YFP+/mCherry+ cells in the INL that did not stain for, or showed varying levels of, the glia marker Sox2 (Fig. 22E). Additionally, some YFP+/mCherry+ INL cells stained for Rxrg (Fig. 22C arrow), while others did not, suggesting that there might be cells in

intermediate stages of reprogramming or, alternatively, reprogrammed cells of different subtypes that did not stain with the antibodies used. Altogether, these results show that Ikzf1/4 are sufficient to induce the conversion of adult Müller glia into cone-like cells *in vivo*.

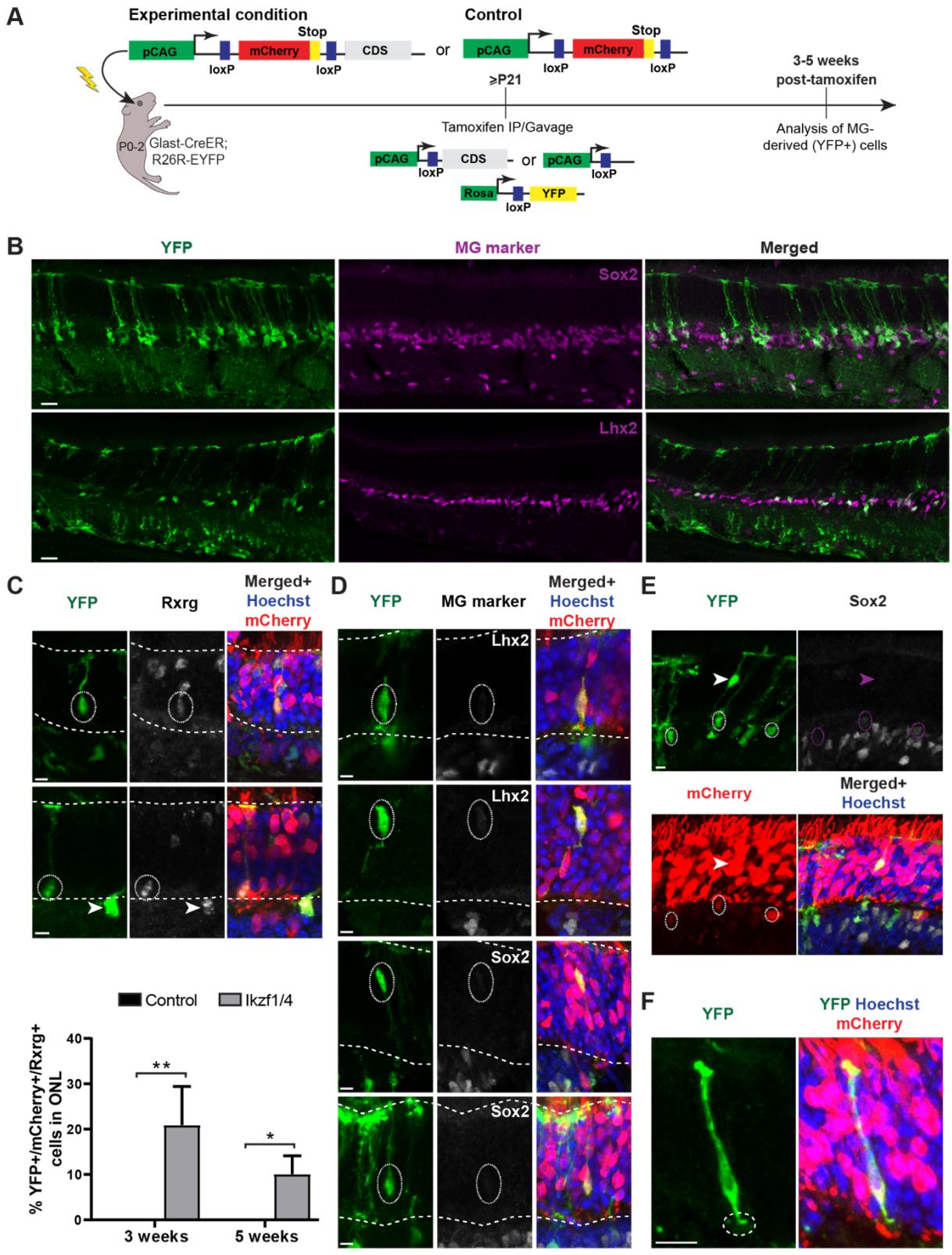


Figure 22: Ikzf1/4 reprograms Müller glia to cone-like cells *in vivo.*

A. Summary diagram of the experimental protocol. IP: intraperitoneal injection. CDS:

coding sequence. Lightning bolt represents electroporation. **B.** Retinal sections from control electroporations stained for YFP and the Müller glia markers Sox2 and Lhx2 (B). YFP+ cells display typical Müller glia morphology and express Müller glia markers. Scale bars: 38µm. **C.** Representative images of retinal sections immunostained for YFP and the cone marker Rxrg 3 weeks after Ikzf1/4-expression *in vivo*. Reprogrammed cells in the ONL (circled) stain positive for Rxrg. Arrow shows intermediate cells in INL. Dotted lines show ONL thickness. Scale bars: 5µm. Bottom: Proportion of reprogrammed cells in control- and Ikzf1/4-electroporated retinas at 3 and 5 weeks after tamoxifen injection. Graph represents mean + standard deviation. * $p< 0.05$; ** $p<0.01$; Mann-Whitney test. Control: 3 weeks n=5, 5 weeks n=4; Ikzf1/4: 3 weeks n=5, 5 weeks n=5. **D.** Several representative images of retinal sections immunostained for YFP and the Müller glia markers Lhx2 and Sox2 3 weeks after Ikzf1/4 expression *in vivo*. YFP+ Ikzf1/4 reprogrammed cells in the ONL (circled) do not express Müller glia markers. Scale bars: 5µm. **E.** Immunostaining for YFP and Sox2 on retinal sections 3 weeks after tamoxifen. Some YFP+/mCherry+ cells (circled) in the INL show varying levels of Sox2, whereas reprogrammed cell in the ONL (arrowhead) is negative for Sox2. Scale bar: 5µm. **F.** High magnification image of an Ikzf1/4-reprogrammed cell, stained for YFP, showing pedicle-like structure in the outer plexiform layer (circled). Scale bars: 10µm. All images shown are z projections except for (D; top row), which is a single plane.

4. 4. Degenerated retinas are responsive to reprogramming by Ikzf1/4

A key question for future use of cell reprogramming as therapy for retinal degeneration is whether Müller glia of diseased retinas are responsive to reprogramming factors. To address this question, we crossed Glast-CreER and R26R-EYFP mice to Pde6b$^{RD1/RD1}$ mice (section 1.2.3.2.2), a mouse model of retinitis pigmentosa in which glial cells are strongly and permanently activated (Ekström et al., 1988), to generate a Glast-CreER;R26R-EYFP;Pde6b$^{RD1/RD1}$ mouse line. We electroporated Ikzf1/4 in these mice at P0-1 and waited until they reached at least 12 weeks of age, when all rods have died and

only some cones remain (Carter-Dawson et al., 1978), to inject tamoxifen (Fig. 23A). Even in these highly degenerated retinas, Ikzf1/4 expression in Müller glia induced a number of YFP+/mCherry+ cells to migrate to the apical side of the retina, change morphology, and start expressing Rxrg (Fig. 23B). This demonstrates that Müller glia can be reprogrammed with Ikzf1/4 expression in diseased environments.

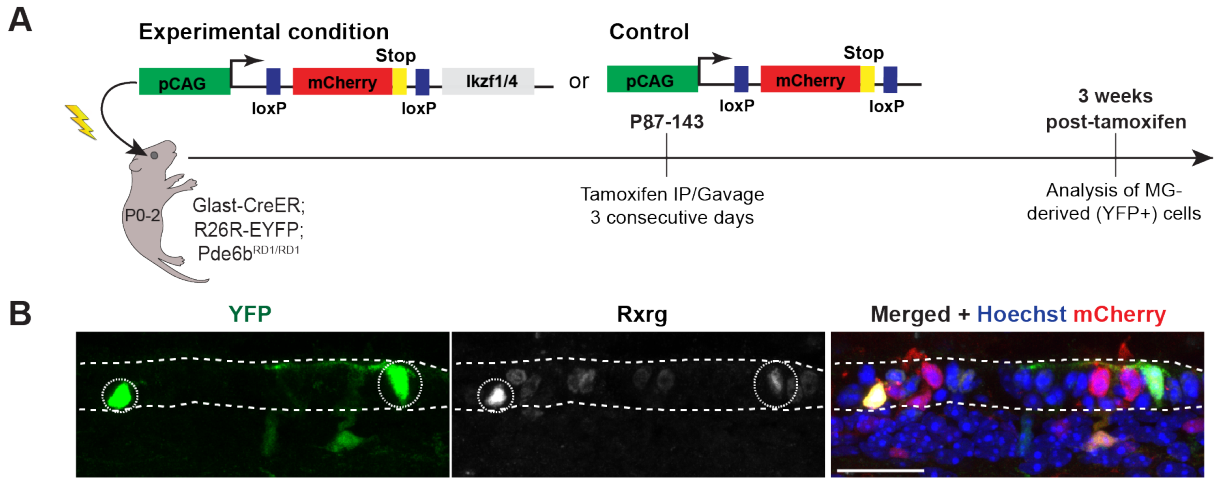


Figure 23: Degenerated retinas are responsive to reprogramming by Ikzf1/4.
A. Representation of experimental protocol performed in Glast-CreER;R26R-EYFP;Pde6b$^{RD1/RD1}$ animals. IP: intraperitoneal injection. CDS: coding sequence. Lightning bolt represents electroporation. **B.** Image of retinal section co-immunostained for YFP and Rxrg. YFP+/Rxrg+ cells are circled. Dotted lines show ONL thickness. Images are z-projections. Scale bar: 20µm.

4. 5. Detection of reprogrammed cells following Ikzf1/4 expression in Müller glia is not the result of material exchange

Previous studies showed that transplanted photoreceptor precursor cells readily exchange cytoplasmic material, including fluorescent reporters, with host photoreceptors (Ortin-Martinez et al., 2016; Pearson et al., 2016; Santos-Ferreira et al., 2016; Singh et

al., 2016) (section 1.3.2.3) sparking the need for additional controls in lineage tracing studies with fluorescent reporters in the retina (Boudreau-Pinsonneault and Cayouette, 2018). As Müller glia are tightly associated with cones (Reichenbach and Bringmann, 2013), we wondered whether the YFP+ cone-like cells observed after Ikzf1/4 expression might be explained by transfer of YFP from Müller glia to endogenous cones. Our observation that reprogrammed cells did not express all cone markers argued against this interpretation, but we could not exclude the possibility that transfer of Ikzf1/4 might occur and lead to downregulation of these markers. To directly address this question, we repeated the experiments *in vivo* as described in Figure 22, but additionally gave a daily intraperitoneal injection of EdU to the pups from P3 to P7 (Fig. 24A). This corresponds to the peak production period of Müller glia from retinal progenitors, and is well past when the last cone photoreceptors are born. Accordingly, in control electroporations, many Müller glia (YFP+) incorporated EdU, but cone photoreceptors did not (Fig. 24B) Following expression of Ikzf1/4, however, we found several reprogrammed YFP+ cone-like cells in the ONL that were also EdU+ (Fig. 24C), indicating that these are not endogenous cones and providing additional support to our conclusion that they are derived from Müller glia that expressed Ikzf1/4.

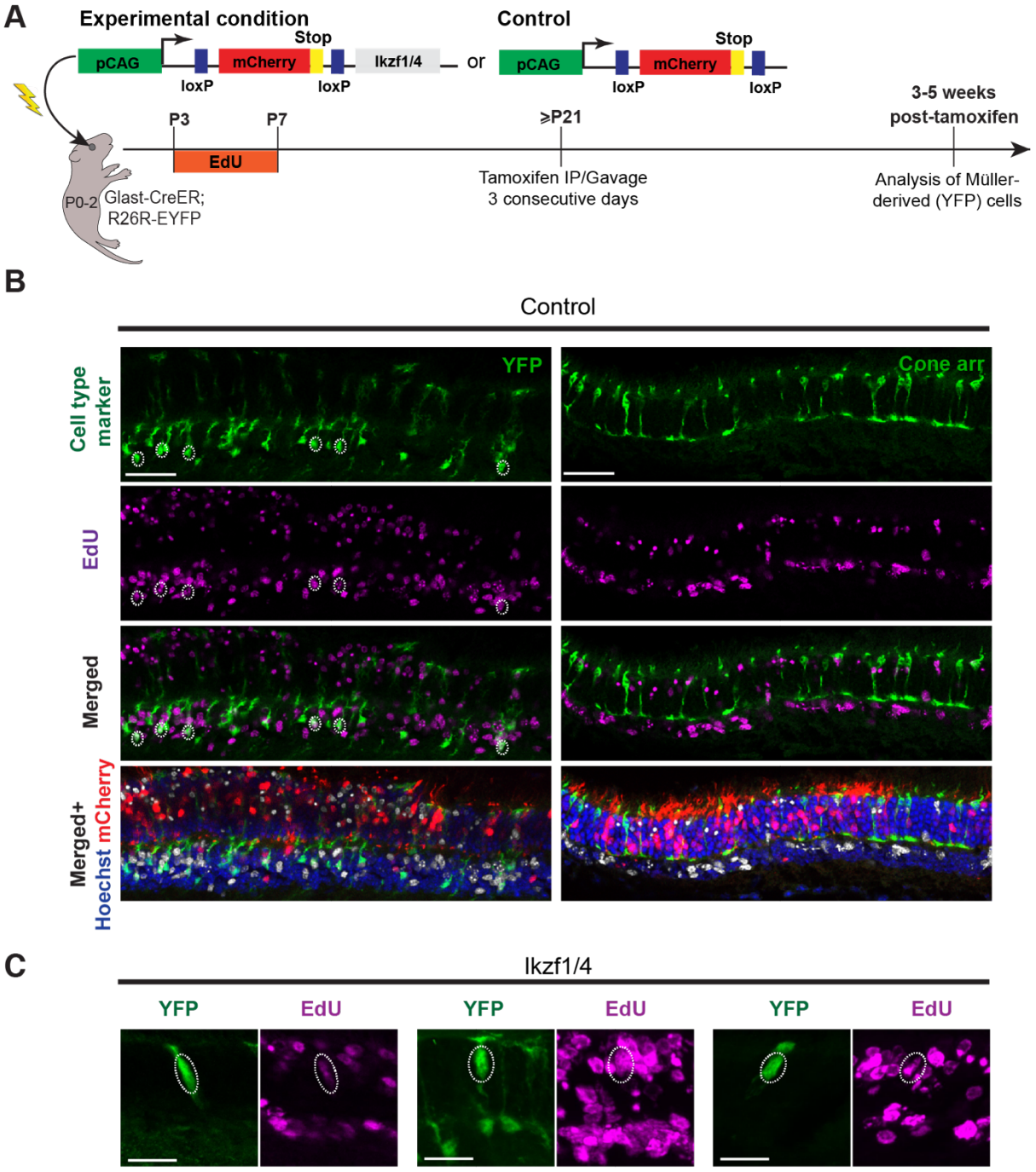


Figure 24: Detection of reprogrammed cells following Ikzf1/4 expression in Müller glia is not the result of material exchange.

A. Schematic representation of the experimental protocol. IP: intraperitoneal injection. CDS: coding sequence. Lightning bolt represents electroporation. **B.** Co-immunostaining for EdU and YFP (left) or cone arrestin (cone arr.; right) on retinal sections from control-electroporated retinas. YFP+ Müller glia (circled) stained for EdU, whereas cone arrestin+

cone photoreceptors did not (n=3). Scale bars: 33µm. **C.** Co-immunostaining for EdU and YFP on retinal sections after Ikzf1/4 electroporation. Reprogrammed cells (circled) in the ONL co-label with EdU. Scale bars: 15µm. All images are single planes except (C; middle and right), which are z projections.

4. 6. Reprogrammed cells share transcriptional profiles with both cones and bipolar cells

The low throughput of immunofluorescence analyses in the above experiments rendered the precise characterization of reprogrammed cell types difficult. To circumvent this issue and obtain a more complete molecular profiling of the reprogrammed cells, we performed single cell RNA-sequencing (scRNA-seq) on sorted YFP+ cells from control- and Ikzf1/4-electroporated retinas three weeks after tamoxifen injection in adult Glast-CreER; R26R-EYFP mice (≥P21). The specificity of cell collection was tested by sorting YFP+ cells and analyzing the sorted population by flow cytometry. We found that, on average, 90% +/- 3% (n=3) of collected cells are YFP+, indicating that, although some non-fluorescent contaminating cells are present in the sorted population, we strongly enrich YFP+ cells with this method.

We sequenced 4207 cells from the control condition and 4608 cells from the Ikzf1/4 condition. As expected, the main Uniform Manifold Approximation and Projections (UMAPs) cell cluster observed in both conditions was composed of MG. Interestingly, we found a clear increase in the number of cell clusters in the Ikzf1/4 condition compared to control, and the number of cells in the MG clusters was proportionally reduced (Fig. 25A). Surprisingly, the additional clusters in the Ikzf1/4 condition were identified as bipolar cells

based on top gene expression (Fig. 25B). Bipolar clusters 1, 2, 4, and 5 express markers of various bipolar cell subtypes and correspond to cone bipolar cells, whereas bipolar cluster 3 expresses rod bipolar markers (Shekhar et al., 2016) (Fig. 25C). Interestingly, we noticed that the bipolar clusters 2 and 4 additionally express *Thrb*, a cone photoreceptor gene (Fig. 25C), suggesting that these cells share transcriptional profiles with both cones and bipolars. In contrast to our immunostaining results, we did not detect Rxrg expression in these bipolar clusters, suggesting that Rxrg transcript levels may be below the detection threshold. Based on these data, we estimate that the MG to bipolar-like cell conversion rate is around 40%, which is about twice as much as the reprogramming efficiency observed by immunostaining *in vivo*, suggesting that a greater percentage of cells adopt a bipolar/cone transcriptomic identity than what we observed by immunostaining. Of note, we did not find progenitor-like clusters in the Ikzf1/4 condition, suggesting direct transdifferentiation of MG.

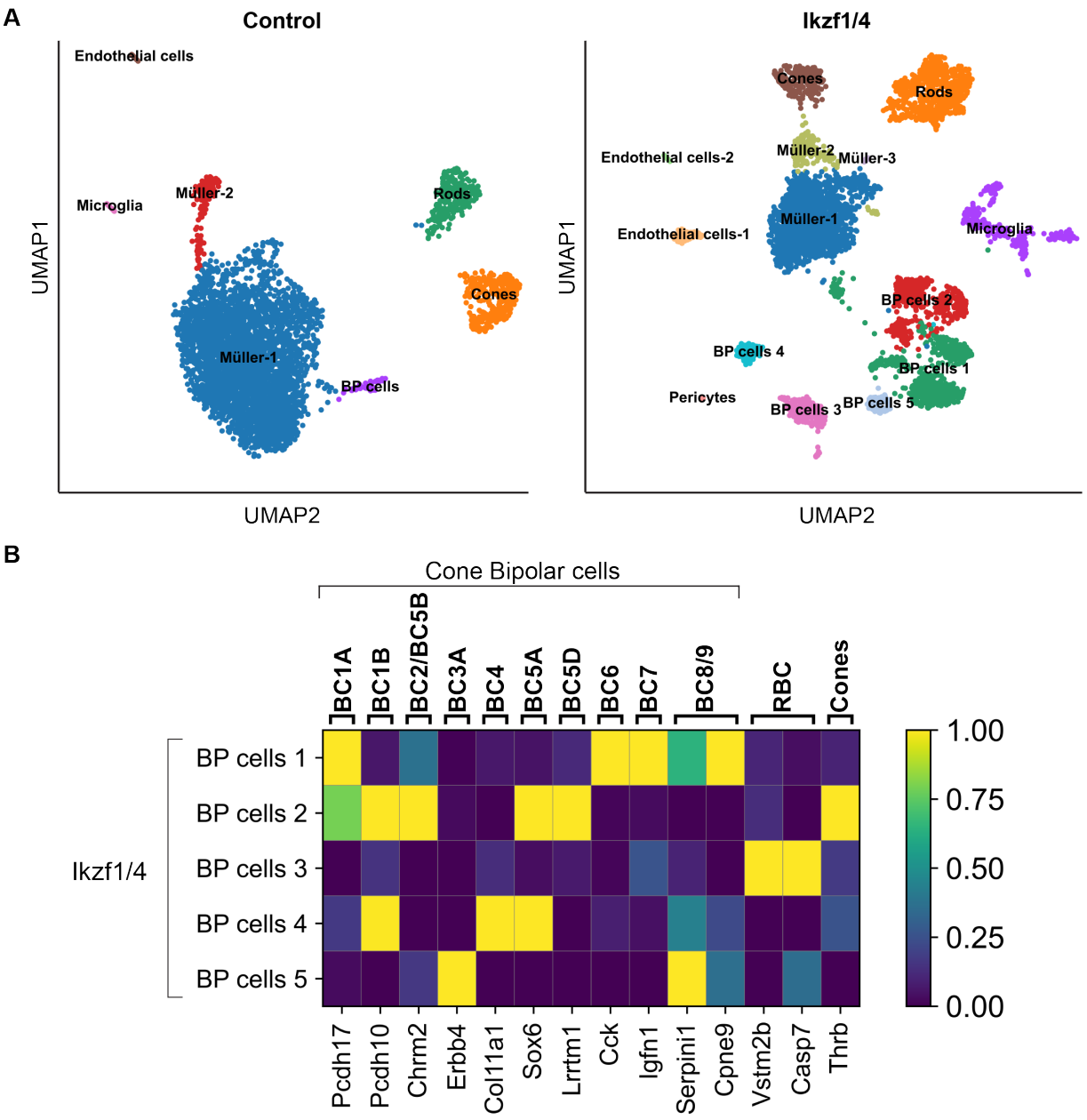


Figure 25: scRNA-seq identifies reprogrammed cells as bipolar-like cells

A. Control UMAP (left) and Ikzf1/4 UMAP (right). Cell types were identified based on top marker expression (Clark et al., 2019). BP: Bipolar. **B.** Expression matrix of bipolar subtypes (top label) markers (bottom label) and the cone marker Thrb for Ikzf1/4 bipolar (BP) clusters based on Shekhar et al. (Shekhar et al., 2016). Yellow denotes high expression and blue no expression. RBC: Rod bipolar cell.

To follow up on the idea that some Ikzf1/4 bipolar clusters might contain cells with both cone and bipolar transcriptional identity, we performed SCENIC analysis (Aibar et al., 2017; Van de Sande et al., 2020), which identifies broad gene regulatory networks, or 'regulons', active within different cell populations. We found that, in addition to bipolar regulons (Fig. 26B''), cone regulons are active in Ikzf1/4 reprogrammed cell clusters 1-7 (Fig. 26B'). Importantly, we found that the Rxrg regulon is active in Ikzf1/4 bipolar clusters (Fig. 26B'), consistent with our immunostaining data. Since we could not compare this population to endogenous bipolar cells, as they were not enriched in our control condition (Fig. 26A-A''), we took advantage of a P14 retina scRNA-seq dataset previously published (Clark et al., 2019). As expected, bipolar cells from this dataset did not show activity of Thrb or Rxrg regulons, and showed low activity of other cone regulons (Fig. 26C'), supporting the interpretation that the Ikzf1/4 reprogrammed population represents a distinct cell type. We hence labelled these clusters as Bip/Co. Interestingly, we could not detect all MG regulons in our Ikzf1/4 dataset (Fig. 26B''), suggesting that MG transcriptional programs are altered in this condition. Of note, the Rxrg regulon was not detected in both control datasets (Fig. 26A', C') potentially due to the low number of cones present and/or the low expression of Rxrg targets within this small population. Altogether, these data demonstrate that Ikzf1/4 expression in MG induces reprogramming to bipolar/cone cells.

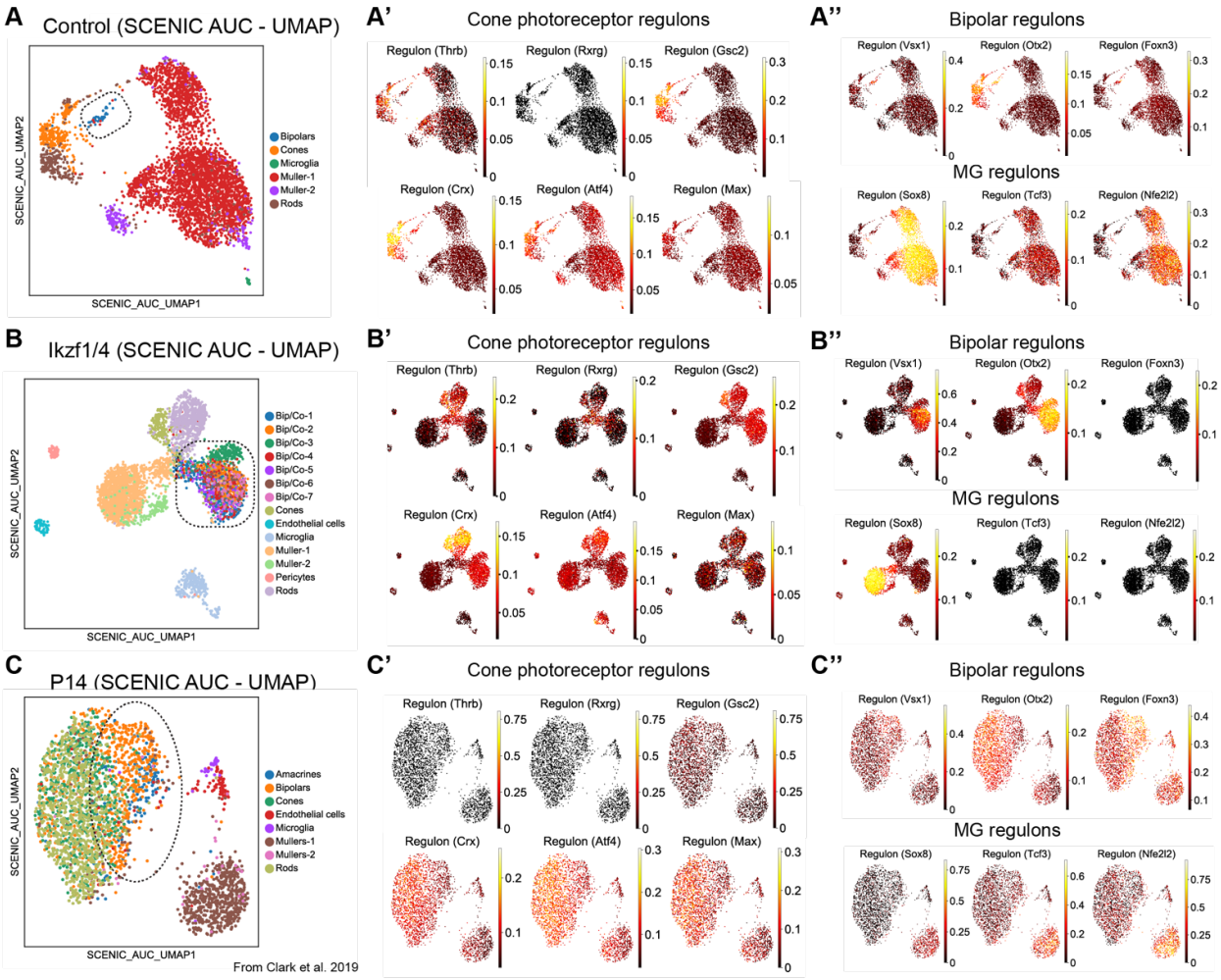


Figure 26: Cone and bipolar regulons are active in Ikzf1/4 reprogrammed population

A-C''. SCENIC analysis of control (A-A''), Ikzf1/4 (B-B''), and (Clark et al., 2019) data of P14 retinas (C-C'') for cone photoreceptor, bipolar and MG regulons. Left: UMAP for each dataset with bipolar (or Bip/co) clusters circled. Top regulons per cluster were found by calculating the regulon specificity scores for each cluster. Shown are the top 5 enriched regulons per cluster for the P14 dataset, and Rxrg and Thrb regulon to highlight cone regulons active in the Bip/Co population. Ikzf1/4 Bip/Co have active cone (B') and bipolar (B'') regulons, which are not detected or present in low levels in control bipolar datasets (A'', C''). Scales represent AUC values (black indicates no regulon activity; Yellow represents high regulon activity).

4. 7. Ikzf1/4 convert mouse embryonic fibroblasts into neurons

To investigate whether Ikzf1 and Ikzf4 have general neuronal reprogramming capacities in non-neural cells, we asked if they were sufficient to convert MEFs to neurons, as previously reported for Ascl1 (Vierbuchen et al., 2010) (section 1.3.1.3). Using a doxycycline-inducible lentiviral expression system (Fig. 27A), we expressed Ikzf1/4, together with Brn2 and Myt1l (BM/Ikzf1/4), which are known to support neuronal differentiation (Mall et al., 2017; Vierbuchen et al., 2010; Wapinski et al., 2013), and used Brn2/Ascl1/Myt1l (BAM) as positive control, and Brn2/Myt1l (BM) as negative control. As expected, neurons were present in the BAM condition, but not in the BM control condition (Fig. 27B). Remarkably, we also found neurons when BM/Ikzf1/4 was expressed in MEFs. These neurons expressed Tau, and had clear neuronal morphologies (Fig. 27B). These data demonstrate that Ikzf1/4 can generate neurons from MEFs, and thus have broad neuronal reprogramming capacities.

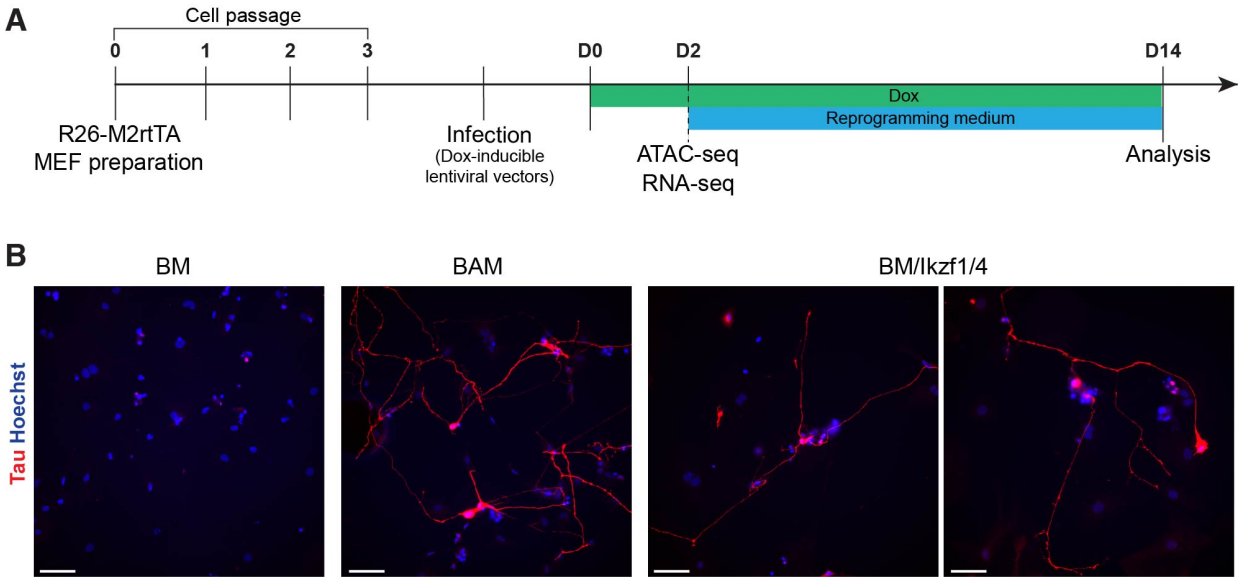


Figure 27: BM/Ikzf1/4 convert MEFs into neurons.

A. Schematic representation of the MEF reprogramming protocol described in sections 2.9 and 2.10. Day 0 (D0) of reprogramming assay corresponds to the first day of doxycycline-induced (dox) expression of lentiviral vectors. **B.** Representative images of MEF cultures expressing BM, BAM, or BM/Ikzf1/4 at D14 of reprogramming assay immunostained for the neuronal marker Tau. Scale bars: 50µm.

4. 8. Ikzf1/4 increase chromatin accessibility and activate a neuronal program in mouse embryonic fibroblasts

To gain insights on the molecular mechanisms underlying Ikzf1/4 reprogramming function, we next carried out epigenetic and transcriptomic analyses on MEFs 48 hours after doxycycline-induced expression of Brn2 and Myt1l only (BM), as control, or together with Ikzf1/4 (BM/Ikzf1/4) (Fig. 27A). As classical reprogramming factors generally modify the chromatin landscape, we first investigated chromatin accessibility by ATAC-seq. We found that BM/Ikzf1/4 expression leads to a general increase in chromatin accessibility compared to the BM control condition, with open chromatin in clusters 2 and 4 forming about two thirds of all significantly altered peaks (Fig. 28A). Peak clusters 1 and 3 represent closed chromatin in BM/Ikzf1/4 compared to BM, with cluster 1 peaks showing a partial reduction in signal, and cluster 3 peaks showing almost complete loss of signal (Fig. 28A). To identify genes associated with closed and opened chromatin regions, we focused our analysis +/- 2kb from transcription start sites (TSS). Interestingly, we found that many chromatin regions that become closed after expression of Ikzf1/4 are in the cis-regulatory region of fibroblast genes, such as *Tead2*, *Ednra*, *Ogn*, *Fibin* and *Matn4*, which correspond to *transmembrane transport* gene ontology (GO) terms (Fig. 28B). In contrast, many chromatin regions that become opened after expression of Ikzf1/4 are associated

with neuronal genes, such as *Gabra4*, *Gabrg1*, *Sox2*, *Neurod2*, *Pou4f1* and *Lhx5*, and are classified as general *nervous system* GO terms (Fig. 28C). Of note, numerous olfactory receptor genes were also associated with opened peaks. These data demonstrate that expression of Ikzf1/4 in MEFs promotes chromatin accessibility of neuronal genes while reducing accessibility of fibroblast genes.

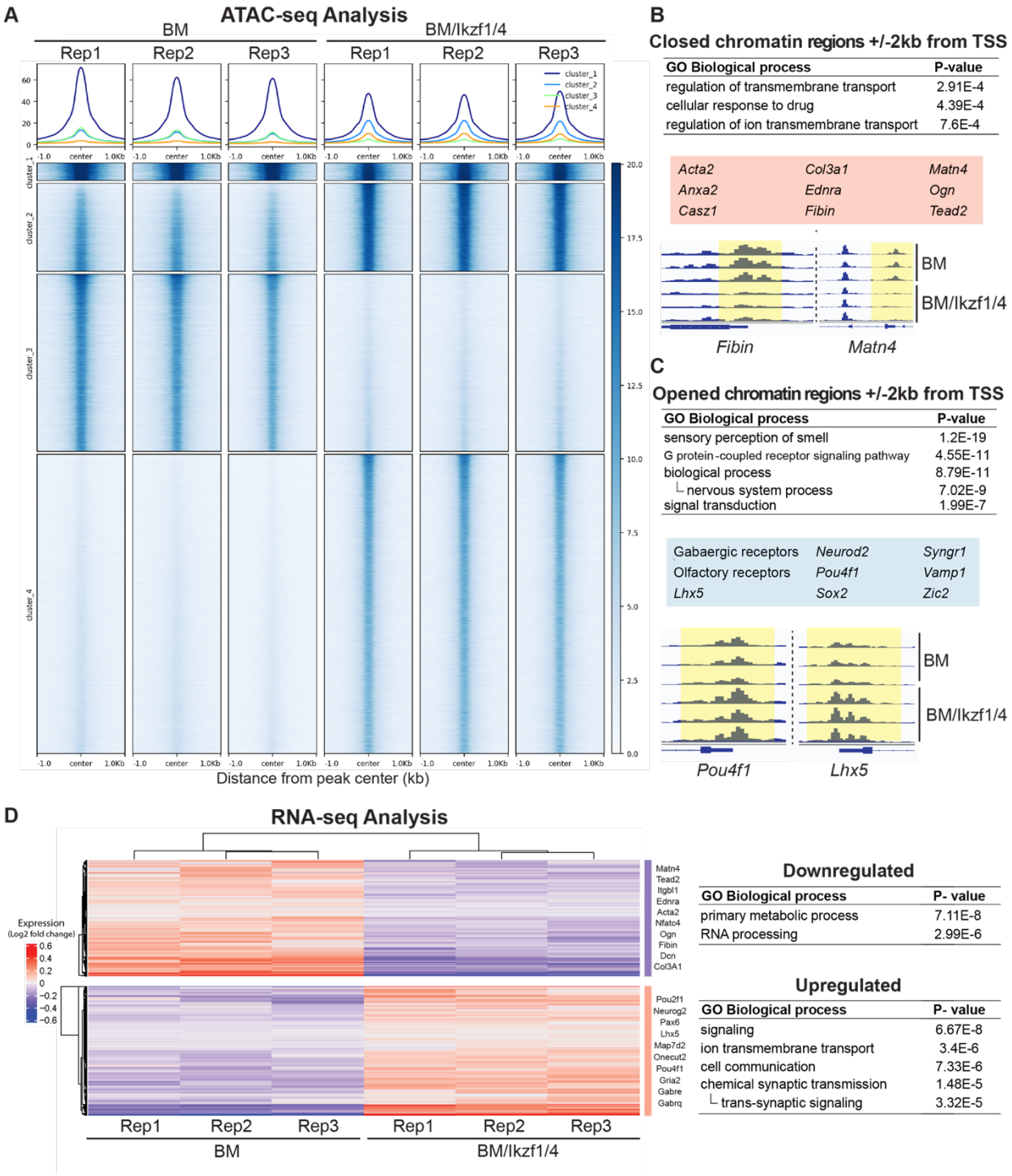


Figure 28: Ikzf1/4 increase chromatin accessibility and activate a neuronal gene expression program in MEFs

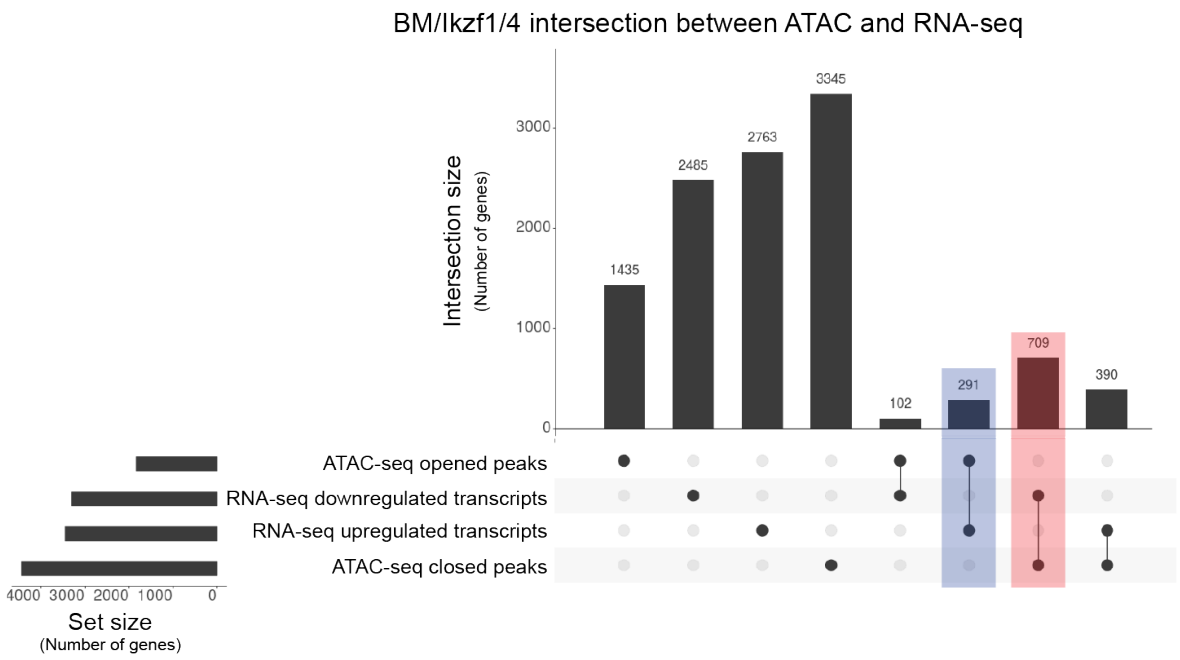
A. ATAC signal aligned at peak center of all significantly ($p<0.05$) enriched or depleted ATAC peaks in BM/Ikzf1/4 (right) compared to BM (left). Clusters 1 and 3 show peaks

with decreased signal in BM/Ikzf1/4 (closing of chromatin), and clusters 2 and 4 show peaks with increased signal in BM/Ikzf1/4 (opening of chromatin). Top graphs show mean signal for each cluster. Scale represents peak coverage with no coverage in white and maximum coverage in dark blue. Columns represent replicates (Rep) per infection condition. **B, C.** Closed (B) or opened (C) chromatin regions located +/- 2kb from the transcription start site (TSS) represented as a table of GO term classification. Genes were classified using GREAT algorithm. Examples of genes associated with closed or opened chromatin regions are shown in red (B) and blue (C) boxed areas, respectively. TSS genomic tracks for *Fibin* and *Matn4* (B) or *Pou4f1* and *Lhx5 (C)* are shown in the bottom panels. **D.** Heatmap of log2 expression fold change and GO term classification of the most significantly upregulated and downregulated genes in MEFs 48 hours after BM (left) or BM/Ikzf1/4 (right) induced expression. Parameters used for scoring significant genes were Log2FC>0.25 and p-value<0.05. High expression is denoted by red whereas low expression is denoted by blue. Each replicate is represented as a column (n=3). Examples of downregulated fibroblast genes and upregulated neuronal genes in BM/Ikzf1/4 compared to BM are listed on the right side of the heatmap. GOrilla classification of GO terms are represented as a table on the right.

We next assessed whether this change in chromatin architecture resulted in altered gene expression by RNA-seq. We found that expression of BM/Ikzf1/4, compared to BM, leads to significant upregulation of several neuronal genes like *Neurog2*, *Pax6*, *Lhx5*, *Pou4f1*, and some GABA and glutamate receptors (Fig. 28D), suggesting that both gabaergic and glutamatergic neurons are generated. Notably, Ikzf1/4 also upregulates expression of *Sall3*, *Pou2f1* and *Onecut2*, three genes that were previously linked to cone photoreceptor development (de Melo et al., 2011; Javed et al., 2020; Sapkota et al., 2014), as well as *Lhx4* and *Isl1*, which are involved in bipolar cell specification (Dong et al., 2020; Elshatory et al., 2007). *Ascl1* expression was not upregulated in the BM/Ikzf1/4 condition, suggesting that Ikzf1/4 does not require Ascl1 expression to induce neuronal

reprogramming. Upregulated genes were associated with GO terms like *cell-cell signaling, ion transmembrane transport,* and *chemical synaptic transmission* (Fig. 28D). Conversely, we observed downregulation of several fibroblast genes like *Matn4*, *Tead2*, *Nfatc4*, and *Fibin* (Fig. 28D). These genes belong to GO terms associated with various *metabolic processes* (Fig. 28D). Of note, olfactory receptors that were found to have opened chromatin after Ikzf1/4 expression did not generally show an increase in transcript levels, suggesting they are not involved in reprogramming. Overall, approximately 17% of genes associated with BM/Ikzf1/4-enriched ATAC peaks show increased transcript levels, including the neuronal genes *Sall3*, *Gabra4*, *Lhx5*, *Pou4f1*, and *Zic2* (Fig. 29). A similar percentage of genes associated with reduced ATAC peaks show decreased transcript levels, including MEF genes *Ednra*, *Fibin, Matn4*, and *Tead2* (Fig. 29). Together, these results demonstrate that Ikzf1/4 quickly inhibit fibroblast and activate neuronal transcriptional programs in MEFs.

A



B

Camk2b	*Acta2*
Gabra4	*Collagen genes*
Lhx5	*Ednra*
Lhx4	*Fibin*
Pou4f1	*Matn4*
Sall3	*Mmp23*
Sncg	*Ogn*
Zic2/5	*Tead2*

Figure 29: Correlation between BM/Ikzf1/4 ATAC and RNA-seq.

A. Upset plot showing correlation between ATAC and RNA-seq hits for BM/Ikzf1/4 in MEFs. Y-axis represents intersection size as number of genes, and x-axis represents conditions (black dots indicating condition, with connected black dots indicating intersection). Set size represents the total number of genes associated with each condition. ATAC-seq opened and closed genes correspond to genes associated with peaks +/- 2kb from TSS. Blue highlights intersection of genes associated with opened BM/Ikzf1/4 peaks and BM/Ikzf1/4 upregulated transcripts. Red highlights intersection of genes associated with closed BM/Ikzf1/4 peaks and BM/Ikzf1/4 downregulated transcripts. **B.** Example of genes included in both intersections. Colors correspond to (A).

Chapter 5: Discussion

5. 1. Summary

Work presented in this thesis has shown that simple manipulations of injury and growth factor treatment are not sufficient to induce robust neurogenic potential of mammalian Müller glia (Fig. 30A), highlighting the need for novel methods to achieve this. Taking inspiration from developmental processes, we postulated that temporal identity factors could mediate Müller glia reprogramming to neurons. We show that the early temporal identity factor Ikzf1, when co-expressed with its family member Ikzf4, is a potent reprogramming factor capable of converting retinal glia and MEFs, with Brn2 and Myt1l co-expression, to neuron-like cells (Fig. 30B).

5. 1. 1. Contributions to knowledge

Chapter 3 (Fig. 30A) makes a small, but important contribution to the field of retinal regeneration by demonstrating the low endogenous regenerative capacity of mouse Müller glia, effectively completing **aim 1**, and showcasing the importance of genetic lineage tracing. Work presented in Chapter 4 (Fig. 30B) contributes to the field of cellular reprogramming by identifying novel neuronal reprogramming factors, capable of generating neurons from Müller glia, thus fulfilling **aim 2**, and establishing a previously unappreciated relationship between temporal and cell identity reprogramming. This work also represents a first step to generate cone-like cells from Müller glia, which have never been produced from mouse Müller glia and are important targets for regenerative therapies in numerous retinal degenerative diseases. Altogether, Chapter 3 and 4, by identifying a temporal identity factor-mediated method to induce the generation of neurons from mammalian Müller glia, reach the main objective of this thesis.

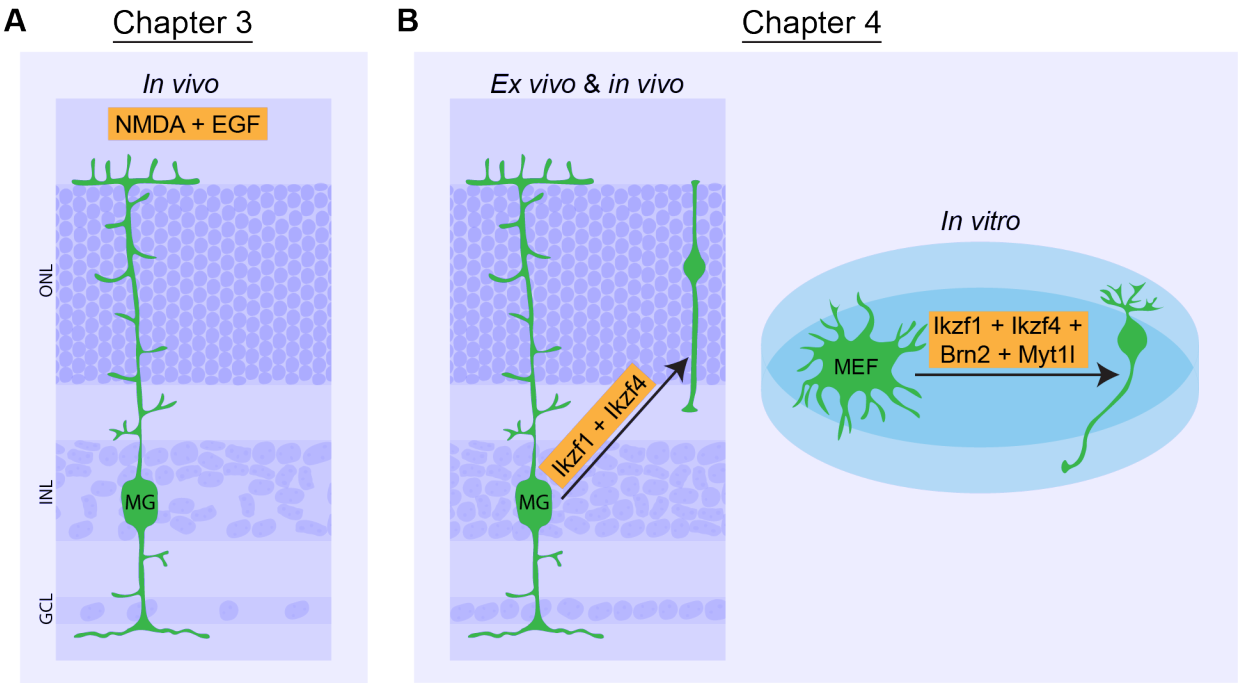


Figure 30: Schematic summary of thesis results.

A. Chapter 3. NMDA injury with EGF treatment (orange box) did not induce robust Müller glia neurogenic potential *in vivo*. Representation of a retina after treatment with fewer INL and GCL cells (nuclei) due to NMDA injury. Müller glia keep their identity. **B.** Chapter 4. Left: Ikzf1 and Ikzf4 co-expression (orange box) reprograms (arrow) Müller glia to neuron-like cells *ex vivo* and *in vivo*. Right: Ikzf1, Ikzf4, Brn2 and Myt1l co-expression (orange box) reprograms MEFs to neurons in culture. ONL: Outer nuclear layer, INL: Inner nuclear layer, GCL: Ganglion cell layer, MG: Müller glia, MEF: Mouse embryonic fibroblast.

5. 2. Discrepancies in Müller glia cell cycle re-entry and neurogenesis

In contrast to what was previously reported (Suga et al., 2014), we did not find increased cell cycle re-entry of Müller glia in 129SvJ compared to C57BL/6J mice. In this prior study, increased proliferation in 129SvJ animals was observed after explanting the retina, leading mostly to photoreceptor death, whereas the NMDA injury performed in this thesis targeted RGCs and interneurons. This implies that differences exist in Müller glia cell cycle re-entry between mouse strains depending on injury types.

Our genetic lineage tracing of Müller glia after NMDA and EGF injections indicate that these manipulations are not sufficient to efficiently induce neurogenesis from Müller glia in the mammalian retina. This contrasts with the interpretation of previously published results (Karl et al., 2008). BrdU+ neurons observed in this latter study may have been dying cells or cells repairing their DNA, two processes which have been shown to induce BrdU incorporation (Kuan et al., 2004), and may not correspond to Müller-derived cells.

Because our genetic lineage tracing method labels approximately 35% of Müller glia, cell cycle re-entry events may be underrepresented in our data. If we extrapolate our findings to all Müller glia, we conclude that a total of about 11-12 Müller glia per retina would re-enter the cell cycle after injury and growth factor injection, still representing a minute population. This labelling limitation may also impact our ability to identify Müller glia-derived neurons. Still, in 17 replicates, we have only observed evidence of one potential Müller glia-derived neuron. It seems unlikely that neurogenesis would have been entirely outside of the labelled cell population in 16 out of 17 replicates.

Of note, differences between our manipulations and the ones previously published may account for some disparities in the results obtained. Karl et al. (2008) provided BrdU to animals both by intravitreal injections and IP injections, whereas we injected EdU intravitreally only. Although, in our hands, these methods seemingly gave comparable numbers of labelled cells, we cannot exclude the possibility that there were BrdU/EdU incorporation differences between administration techniques. Additionally, Karl et al. (2008) gave multiple injections of growth factors and BrdU to some animals after NMDA

injury, while we focused our analysis on single injections (also suggested to induce neurogenesis).

That only a small subset of Müller glia re-enter the cell cycle after these manipulations suggests that these cells have varying susceptibility to proliferate. Although it is known that Müller glia show heterogeneous responses to injury within a single retina (Bringmann et al., 2009), subtypes of Müller glia were not found by scRNA-sequencing analyses (Clark et al., 2019), suggesting that, under normal conditions, these cells form a homogeneous population. What mediates divergent responses of Müller glia to injury and could account for variability in cell cycle re-entry remains to be determined.

Overall, our results highlight the importance of performing lineage tracing with multiple techniques to confirm the source and presence of regeneration, and suggests that previous studies using BrdU/EdU to lineage trace Müller glia may have overestimated the number of neurons derived from these cells. Although genetic lineage tracing is generally more convincing than BrdU/EdU lineage tracing, even this technique has limitations and potential pitfalls as found with label transfer in retinal transplantation (section 1.3.2.3) (Boudreau-Pinsonneault and Cayouette, 2018). A combination of lineage tracing methods is hence optimal for regenerative studies whether in the CNS or other systems.

5. 3. Reprogramming glia to neuron-like cells with Ikzf1/4

During development Ikzf1 regulates the early temporal identity of retinal progenitors, providing them the competence to generate early-born RGCs, horizontal and amacrine

cells (section 1.2.1.2). Although cone photoreceptors are also early-born, they are not considered part of Ikzf1 competence. Knock-out of *Ikzf1* during retinal development did not alter the number of cone photoreceptors, suggesting that it is not required for cone genesis (Elliott et al., 2008). Yet, whether Ikzf1 could induce cone photoreceptors remains somewhat unclear, since its overexpression was found to cause photoreceptor apoptosis (Elliott et al., 2008). This renders the detection of potential roles for Ikzf1 in photoreceptor genesis difficult. Still, this raises the possibility that Ikzf4, and not Ikzf1, is responsible for cone genesis in our reprogramming context. Of note, Ikzf1 toxicity may also explain why the number of Ikzf1/4 reprogrammed cone-like cells *in vivo* was found to decrease with time (section 4.3). Generating transient Ikzf1 expression, with a tetracycline inducible expression system for instance, could address this point.

We were surprised to find that Ikzf1/4-reprogrammed cells were of a mixed cone and bipolar identity as revealed by scRNA-seq analyses. During retinal development, certain precursor cells have the potential of generating both bipolar cells and photoreceptors (Brzezinski and Reh, 2015). It is therefore possible that Ikzf1/4 reprogram Müller glia to this precursor state, but that factors that allow fate selection between photoreceptor or bipolar cells are missing, leading to the production of a mixed cell type. We failed to find reprogrammed cells positive for bipolar markers by immunofluorescence. This may stem from the low throughput of these analyses or from low translation levels of mRNA into detectable proteins.

Whether reprogrammed cells integrate retinal circuitry, are functional, and could improve vision in models of retinal degeneration remains to be addressed. As reprogrammed cells were not found to express mature cone markers by immunofluorescence analyses, it seems unlikely that they would be responsive to light. We have tried to induce the maturation of reprogrammed cells *ex vivo* and *in vivo* with various manipulations that were shown to promote photoreceptor maturation. These include treatments with taurine (Altshuler et al., 1993), retinoic acid (Kelley et al., 1994), Fgf8 (da Silva and Cepko, 2017), and/or Notch inhibition with DAPT (Kaufman et al., 2019). We also tested factors known to increase epigenetic plasticity and reprogramming efficiency, including NMDA injury (Ueki et al., 2015), and treatment with the histone deacetylase inhibitor TSA (Jorstad et al., 2017). Unfortunately, none of these manipulations were successful. Whether this was due to the factors tested being inefficient at inducing maturation, a suboptimal timing of administration, the continuous expression of Ikzf1/4, potentially maintaining reprogrammed cells in an immature state, or the limited number of cells available to analyze, which renders the detection of small, but potentially important effects difficult, is hard to conclude.

The novel technique developed here, electroporation of conditional overexpression constructs (section 4.1), is optimal for screening different conditions of gene expression. However, it is limited in terms of the number of cells targeted: a small region of the retina is electroporated with the construct, of which a subset of transfected progenitors will give rise to Müller glia, which will express the transfected gene(s) of interest if CreER is efficiently activated, leading to about one fifth of transfected glia undergoing

reprogramming *in vivo*. This results in a small population of reprogrammed cells. As mentioned above, identifying factors which could enhance reprogramming efficiency or induce full cone photoreceptor differentiation of the reprogrammed cells is difficult because of this limited cell population available for analysis. Investigating the function of reprogrammed neuron-like cells with pan-retinal recordings or behavioral tests is also problematic, since broad visual changes are not expected from a small pool of *de novo* cells. Inducing expression of Ikzf1/4 in a larger population of Müller glia, resulting in a bigger population of reprogrammed cells, by generating a mouse line to conditionally express Ikzf1/4 or with AAV vectors, would circumvent this limitation and be optimal to address these points. Such wider targeting of Müller glia would additionally provide a larger population of cells to investigate mechanisms underlying Ikzf1/4-mediated reprogramming with transcriptomic and epigenetic approaches.

5. 4. Eliciting the reprogramming potential of temporal identity factors

It was previously shown that, in *Drosophila*, *hb* and *kr* cannot reprogram late-born neurons, or progenitors past the tenth division, to earlier fates (Cleary and Doe, 2006; Pearson and Doe, 2003), probably due to chromatin remodeling that renders older cells unresponsive to temporal identity factor expression (Kohwi et al., 2013). Similarly, we show that Ikzf1 cannot reprogram mammalian glia to neurons when expressed on its own. However, we find that co-expression with Ikzf4 elicits a reprogramming response in the retina, converting Müller glia to Bip/Co cells. Ikzf1 and Ikzf4 are known to physically interact (Honma et al., 1999), and this interaction may potentiate Ikzf1 reprogramming ability in Müller cells. As Ikzf4 is also expressed in the developing retina, these results

raise the possibility that it might cooperate with Ikzf1 to control early temporal identity of retinal progenitor cells, potentially for cone photoreceptor genesis.

We also screened several other factors for glia to neuron conversion, including other temporal identity factors like Casz1v2 and Pou2f1, but did not observe reprogramming. It is possible that appropriate potentiating factors were missing, limiting our ability to detect the reprogramming potential of these factors. It will be interesting to extend the screen to identify co-factors, as this might help induce Müller glia reprogramming into several other cell types. Identifying such potentiating factors could provide a robust method for temporal identity factor-mediated regeneration of diverse cell types.

Interestingly, Fezf2, a transcription factor regulating cortical progenitor output, was shown to reprogram late-born neurons to an early-born neuronal cell type (De la Rossa et al., 2013; Rouaux and Arlotta, 2013). Although Fezf2 reprogramming was limited to young neurons and consisted of changing neuronal subtype rather than cell identity, the ability of this transcription factor to alter the identity of differentiated cells parallels our work. Still, it remains to be determined whether cell identity reprogramming can be achieved by other temporal factors.

Another type of temporal reprogramming has recently been identified for regenerative purposes: Expressing Oct4, Sox2, and Klf4 in old or injured RGCs restores their youthful epigenetic landscape conferring them the capacity to regenerate their axons and leading to visual improvements (Lu et al., 2020). These three factors can hence act both as cell

type reprogramming, when expressed in somatic cells in culture (Takahashi and Yamanaka, 2006) (section 1.3.1.3), and competence reprogramming factors (Lu et al., 2020), similarly as Ikzf1. This supports the notion that cell identity and temporal competence are closely related. It will be interesting to investigate how the environment in which these reprogramming factors are expressed modulates their activity to induce cell identity or competence reprogramming effects.

5. 5. Ikzf1/4 have classic reprogramming properties

Ikzf1/4 expression in MEFs leads to widespread chromatin reorganization, as observed with most classical reprogramming factors studied to date (Dall'Agnese et al., 2019; Koche et al., 2011; Wapinski et al., 2017; Wapinski et al., 2013). Reprogramming factors usually have pioneer activity, binding and opening chromatin regions that are normally not accessible to transcription factors (Iwafuchi-Doi and Zaret, 2016). While it remains unclear whether Ikzf1/4 function as pioneer factors, the widespread changes in chromatin accessibility observed 48 hours after their expression in MEFs is consistent with this possibility. In such context, Ikzf1 might play a critical role, as it is known to interact with the chromatin remodeling complexes Mi-2/NuRD and SWI/SNF (Kim et al., 1999; O'Neill et al., 2000). Mattar et al. (2021) recently reported that the late temporal identity factor Casz1 also interacts with the NuRD complex and requires polycomb activity to control the neurogenesis to gliogenesis transition in retinal progenitors, suggesting that altering chromatin state is a common theme for vertebrate temporal factors. Conversely, in *Drosophila*, spatial patterning factors, rather than temporal factors, alter chromatin accessibility, suggesting that temporal factors use different mechanisms to alter

progenitor competence in vertebrates and invertebrates (section 1.2.1.2). The chromatin-modifying ability of vertebrate temporal identity factors might explain why Ikzf1/4 can elicit neuronal reprogramming, whereas *hb* and *kr* cannot.

Importantly, our data indicate that Ikzf1/4 expression not only alters chromatin accessibility, but that this quickly translates into activation of a neuronal transcriptional program and repression of fibroblast gene expression. Whether Ikzf1 and Ikzf4 co-expression is required for MEF to neuron conversion, as observed for Müller glia to neuron conversion, remains to be addressed. Single expression of these factors, with or without Brn2 and Myt1l, will provide a clearer picture of their individual roles in reprogramming and allow for deeper investigation of underlying mechanisms.

Ikaros family members, including Ikzf1 and Ikzf4, are well known for their implication in the hematopoiesis system (Heizmann et al., 2017; John and Ward, 2011). Accordingly, we found that some upregulated genes in MEFs are associated with two neutrophil differentiation GO terms. Still, most upregulated genes were found to be associated with neuronal and not hematopoiesis processes. It is possible that Brn2 and Myt1l direct Ikzf1/4 towards neuronal rather than hematopoiesis targets. Indeed, Myt1l has been shown to suppress non-neuronal gene expression (Mall et al., 2017). Interestingly, we also find that Ikzf1/4 control chromatin conformation at olfactory receptor loci. Although this did not result in altered expression of olfactory receptor genes in MEFs, it suggests that Ikzf1/4 could be involved in olfactory neuron specification by regulating chromatin accessibility of olfactory receptor promoters. Ikzf1 binding sites were previously identified

at promoter regions of olfactory receptors (Lane et al., 2001; Plessy et al., 2012), but its role in olfactory neurons is unknown.

Similar reprogramming properties to Ikzf1/4 were identified for Onecut factors in MEFs (van der Raadt et al., 2019). We observed rapid upregulation of Onecut2 in MEFs 48 hours after Ikzf1/4 expression, suggesting that these factors might act through a common program. In contrast, we find that Ikzf1/4 expression does not upregulate *Ascl1* in MEFs, supporting an Ascl1-independent reprogramming mechanism.

5. 6. Implications for temporal identity factor-mediated reprogramming in the CNS

Temporal identity factor-mediated neuronal reprogramming is a potentially broadly applicable technique. As Ikzf1 also regulates early temporal identity in cortical progenitors (Alsio et al., 2013), it is tempting to speculate that it might also act as a reprogramming factor in the cortex. Our findings indicate that Ikzf1 can reprogram both MEFs and glia to neurons, when co-expressed with appropriate potentiating factors. This argues against a cell-specific predisposition to reprogramming by Ikzf1. Still, it should be noted that Müller glia have a similar transcriptome to late retinal progenitors (Blackshaw et al., 2004; Jadhav et al., 2009; Roesch et al., 2008), which may render them more prone to temporal factor-mediated reprogramming than other cell types. Reactive astrocytes also show some progenitor-like properties and gene expression profile (Gotz et al., 2015), suggesting they could similarly be susceptible to reprogramming with temporal identity factors.

Regenerative responses elicited in the mammalian CNS to date are still far from reaching the regenerative levels of lower vertebrates. Inducing glia to adopt progenitor-like states, thus conferring them the capacity to generate all types of neurons present in a tissue, remains a major challenge. We had proposed that temporal identity factors would confer glia broad neurogenic potential. Although Ikzf1/4 reprogram glia to an early-born neuronal-like cell type, which aligns with our initial hypothesis (section 1.6), whether this is due to a temporal reprogramming of glia, through a progenitor-like state, or to a direct cell type conversion is unknown at this time. Still, the identification of novel neuronal reprogramming factors consists of an important breakthrough. Additionally, that these factors are capable of generating cells with cone properties for the first time from retinal glia, does suggest that they confer glia some neurogenic potential that had not been achieved previously. We hope that work presented in this thesis will serve as a building block for investigations of the reprogramming potential of temporal identity factors and contribute to the advancement of CNS regenerative approaches.

5. 7. Future perspectives

Numerous avenues of research could be undertaken in order to enhance Ikzf1/4 reprogramming, and further investigate the reprogramming potential of temporal factors.

5. 7. 1. Identifying mechanisms underlying Ikzf1/4-mediated Müller glia reprogramming

As mentioned previously (section 5.6), whether Ikzf1/4-mediated glia reprogramming induces an intermediate progenitor-like state remains to be addressed. Generating a method to express Ikzf1/4 in a larger pool of Müller glia (discussed in section 5.3) would

render the investigation of the underlying mechanism of these transcription factors feasible. To achieve this, we could perform a time course analysis of transcriptomic and epigenetic changes occurring during glia reprogramming, for instance at 48 hours, one week, and two weeks post-expression of Ikzf1/4. Investigating intermediate stages of glia reprogramming would allow us to generate a comprehensive trajectory of their reprogramming, and to identify key players in this reprogramming process. These data could also be compared to transcriptomic changes induced by Ikzf1 expression in late retinal progenitors (unpublished data of the laboratory), which results in temporal reprogramming (section 1.2.1.2), as well as to MEF transcriptomic and epigenetic data described in this thesis (section 4.8). Such analysis, by identifying shared effectors of competence and identity reprogramming in progenitors and differentiated cells, should help uncover wide-ranging regulators of cell state and neurogenic competence.

5. 7. 2. Inducing cone-like cell maturation

A major challenge to obtain functional regeneration from Müller-derived neuron-like cells remains their full differentiation and maturation in cone photoreceptors. Although we have tested several factors to achieve this (section 5.3), none were successful. Other manipulations are thus required to induce cone-like cell maturation. Since these Ikzf1/4 reprogrammed cells express high levels of Rxrg, one possibility would be to increase or transiently block Rxrg activity with agonists or antagonists, respectively. During retinal development, Rxrg is implicated in the induction of cone opsin expression, with maintained expression promoting m-opsin cones and transient reduction in expression

generating s-opsin cones (Roberts et al., 2005). Increasing or blocking Rxrg activity with drugs may hence induce opsin expression in Müller-derived cone-like cells.

The addition of transcription factors, along Ikzf1/4, that are important in normal cone photoreceptor specification and maturation could also induce reprogrammed hybrid cells to adopt a cone identity. For instance, expression of the key photoreceptor differentiation factors Crx and Otx2 (Chen et al., 1997; Furukawa et al., 1997; Nishida et al., 2003) and cone differentiation factor Sall3 (de Melo et al., 2011) may allow for a more complete cone-like cell specification from Ikzf1/4 expression. Knock-down of bipolar differentiation genes, as *Chx10* (Livne-Bar et al., 2006), could also direct Müller-derived Bip/Co cells towards a cone identity. More in depth analysis of the Müller glia Ikzf1/4 reprogramming trajectory (section 5.7.1) may also provide other targets to improve this regenerative process.

5. 7. 3. Generating other cell types from Müller glia with temporal identity factors

Different co-factors may be required to broadly elicit the regenerative potential of temporal identity factors. Retinal cells affected by most degenerative diseases, including rod photoreceptors and RGCs (section 1.2.3), would be important to generate for eventual regenerative therapies. The late temporal identity factor Casz1 strongly promotes rod photoreceptor fate during development (Mattar et al., 2015), raising the possibility that co-expression of rod differentiation factors may be necessary to unravel Casz1 reprogramming ability. In this way, co-expression of Casz1 with the photoreceptor differentiation factors Otx2, Crx, and/or Nrl (Chen et al., 1997; Furukawa et al., 1997;

Mears et al., 2001; Nishida et al., 2003) in Müller glia may elicit a regenerative response to produce rod photoreceptors.

Other co-factors may also allow Ikzf1 reprogramming towards different early-born cell types, such as RGCs, horizontal cells, or amacrine cells, which are ectopically produced by late retinal progenitors upon expression of Ikzf1 (Elliott et al., 2008). In particular, co-expression of Ikzf1 with factors implicated in RGC differentiation during retinal development, as Brn3b, Atoh7, and Isl1 (Brown et al., 2001; Gan et al., 1996; Pan et al., 2008), may reprogram Müller glia to RGC-like cells. These different conditions for rod and RGC production from Müller glia could be easily tested with the assay developed in chapter 4.

5. 7. 4. Investigating the scope of temporal identity factor-mediated reprogramming

A major question remaining is whether broad reprogramming capacities are inherent to all vertebrate temporal identity factors. It will be important to test this by expressing temporal identity factors in MEFs, along Brn2 and Myt1l, as done in section 4.7 for Ikzf1/4, and examining the generation of neurons. This could be performed for Pou2f1, Foxn4, and Casz1. These experiments, along with the one described in the previous section for Casz1, would establish whether broad neuronal reprogramming properties are common for vertebrate temporal identity factors or limited to Ikzf1.

To further investigate the reprogramming potential of Ikzf1/4, we could test whether these factors can reprogram retinal cells, other than Müller glia, to bipolar/cone-like cells. For

instance, expressing Ikzf1/4 in mature rod photoreceptors to convert them in cone-like cells, similarly as described for potential therapies in section 1.3.1.3. An approach to target rods with electroporations, as described for Müller glia in chapter 4, could be developed by replacing the Müller specific Glast-CreER line with another line expressing CreER in rods instead. Alternatively, adeno-associated viral vectors with a rod-specific promoter could be used to transfect and express Ikzf1/4 in rods, while still genetically lineage tracing these cells. This experiment would provide another therapeutic avenue for retinal degenerative diseases. It would additionally address whether Ikzf1/4 reprogramming of Müller glia is made possible due to their transcriptomic resemblances with progenitors cells (sections 1.5 and 5.6), or whether Ikzf1/4 are capable of reprogramming varied mature retinal cells independent of their similarity to a progenitor identity.

5. 7. 5. Exploring temporal identity factor reprogramming in other CNS areas

Finally, it will be important to investigate whether Ikzf1 could reprogram other CNS glia to neurons. As described in section 5.6, astrocytes would be appealing targets for reprogramming. Cortex and spinal cord astrocytes could be transfected with Ikzf1 and Ikzf1/4 with adeno-associated viral or lentiviral vectors, as done previously (Liu et al., 2015; Niu et al., 2013; Su et al., 2014), while lineage tracing of these cells could be achieved with the same Glast-CreER;R26R-EYFP line as used in this thesis. If successful, this reprogramming method could be performed in cortical and spinal cord injury models to investigate astrocyte reprogramming in degenerated environments, and potential rescue of tissue integrity and function by the astrocyte-derived neurons.

5. 8. Conclusion

Stimulation of endogenous regeneration in the CNS is a promising therapeutic approach to restore tissue integrity after neurodegeneration. The identification of factors which could reprogram resident cells to a desired identity is required to achieve this. Work presented in this thesis has demonstrated that the early temporal identity factor Ikzf1, when co-expressed with its family member Ikzf4, can reprogram retinal glia to neuron-like cells. This work identifies a novel method for neuronal reprogramming with potential implications for nervous system regeneration.

References

Adamis, A.P., and Shima, D.T. (2005). The role of vascular enfothelial growth factor in health and disease. Retina *25*, 111-118.

Afgan, E., Baker, D., van den Beek, M., Blankenberg, D., Bouvier, D., Cech, M., Chilton, J., Clements, D., Coraor, N., Eberhard, C.*, et al.* (2016). The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2016 update. Nucleic acids research *44*, W3-W10.

Agostinone, J., Alarcon-Martinez, L., Gamlin, C., Yu, W.Q., Wong, R.O.L., and Di Polo, A. (2018). Insulin signalling promotes dendrite and synapse regeneration and restores circuit function after axonal injury. Brain *141*, 1963-1980.

Ahmad, I., Tang, L., and Pham, H. (2000). Identification of neural progenitors in the adult mammalian eye. Biochemical and biophysical research communications *270*, 517-521.

Aibar, S., Gonzalez-Blas, C.B., Moerman, T., Huynh-Thu, V.A., Imrichova, H., Hulselmans, G., Rambow, F., Marine, J.C., Geurts, P., Aerts, J.*, et al.* (2017). SCENIC: single-cell regulatory network inference and clustering. Nature methods *14*, 1083-1086.

Ail, D., and Perron, M. (2017). Retinal Degeneration and Regeneration-Lessons From Fishes and Amphibians. Curr Pathobiol Rep *5*, 67-78.

Ait-Ali, N., Fridlich, R., Millet-Puel, G., Clerin, E., Delalande, F., Jaillard, C., Blond, F., Perrocheau, L., Reichman, S., Byrne, L.C.*, et al.* (2015). Rod-derived cone viability factor promotes cone survival by stimulating aerobic glycolysis. Cell *161*, 817-832.

Alexander, C., Votruba, M., Pesch, U.E.A., Thiselton, D.L., Mayer, S., Moore, A., Rodriguez, M., Kellner, U., Leo-Kottler, B., Auburger, G.*, et al.* (2000). OPA1, encoding a dynamin-related GTPase, is mutated in autosomal dominant optic atrophy linked to chromosome 3q28. Nature Genetics *26*, 211-215.

Allen, N.J., and Lyons, D.A. (2018). Glia as architects of central nervous system formation and function. Science, 181-185.

Allikmets, R., Singh, N., Sun, H., Shroyer, N.F., Hutchison, A., Chidambaram, A., Gerrard, B., Baird, L., Stauffer, D., Peiffer, A.*, et al.* (1997). A photoreceptor cell-specific AlP-binding transporter gene (ABCR) is mutated in recessive Stargardt macular dystrophy. Nature Genetics *15*, 236-246.

Alsio, J.M., Tarchini, B., Cayouette, M., and Livesey, F.J. (2013). Ikaros promotes early-born neuronal fates in the cerebral cortex. Proceedings of the National Academy of Sciences of the United States of America *110*, E716-725.

Altshuler, D., Lo Turco, J.J., Rush, J., and Cepko, C. (1993). Taurine promotes the differentiation of a vertebrate retinal cell type in vitro. Development, 1317-1328.

Amamoto, R., and Arlotta, P. (2014). Development-inspired reprogramming of the mammalian central nervous system. Science *343*, 1239882.

Applebury, M.L., Antoch, M.P., Baxter, L.C., Chun, L.L.Y., Falk, J.D., Farhangfar, F., Kage, K., Krzystolik, M.G., Lyass, L.A., and Robbins, J.T. (2000). The murine cone photoreceptor: a single cone type expresses both S and M opsins with retinal spatial patterning. Neuron *27*, 513-523.

Arshavsky, V.Y., and Burns, M.E. (2012). Photoreceptor signaling: supporting vision across a wide range of light intensities. The Journal of biological chemistry *287*, 1620-1626.

Avery, R.L., Pieramici, D.J., Rabena, M.D., Castellarin, A.A., Nasir, M.A., and Giust, M.J. (2006). Intravitreal bevacizumab (Avastin) for neovascular age-related macular degeneration. Ophthalmology *113*, 363-372 e365.

Baden, T., Berens, P., Franke, K., Roman Roson, M., Bethge, M., and Euler, T. (2016). The functional diversity of retinal ganglion cells in the mouse. Nature *529*, 345-350.

Bailey, T.J., Fossum, S.L., Fimbel, S.M., Montgomery, J.E., and Hyde, D.R. (2010). The inhibitor of phagocytosis, O-phospho-L-serine, suppresses Muller glia proliferation and cone cell regeneration in the light-damaged zebrafish retina. Experimental eye research *91*, 601-612.

Bainbridge, J.W., Smith, A.J., Barker, S.S., Robbie, S., Henderson, R., Balaggan, K., Viswanathan, A., Holder, G.E., Stockman, A., Tyler, N.*, et al.* (2008). Effect of Gene Therapy on Visual Function in Leber's Congenital Amaurosis. The New England Journal of Medicine *358*, 2231-2239.

Barber, A.C., Hippert, C., Duran, Y., West, E.L., Bainbridge, J.W., Warre-Cornish, K., Luhmann, U.F., Lakowski, J., Sowden, J.C., Ali, R.R.*, et al.* (2013). Repair of the degenerate retina by photoreceptor transplantation. Proceedings of the National Academy of Sciences of the United States of America *110*, 354-359.

Barker, R.A., Gotz, M., and Parmar, M. (2018). New approaches for brain repair-from rescue to reprogramming. Nature *557*, 329-334.

Barres, B.A. (2008). The mystery and magic of glia: a perspective on their roles in health and disease. Neuron *60*, 430-440.

Bartsch, U., Oriyakhel, W., Kenna, P.F., Linke, S., Richard, G., Petrowitz, B., Humphries, P., Farrar, G.J., and Ader, M. (2008). Retinal cells integrate into the outer nuclear layer and differentiate into mature photoreceptors after subretinal transplantation into adult mice. Experimental eye research *86*, 691-700.

Battista, A.G., Ricatti, M.J., Pafundo, D.E., Gautier, M.A., and Faillace, M.P. (2009). Extracellular ADP regulates lesion-induced in vivo cell proliferation and death in the zebrafish retina. Journal of neurochemistry *111*, 600-613.

Belanger, M.C., Robert, B., and Cayouette, M. (2017). Msx1-Positive Progenitors in the Retinal Ciliary Margin Give Rise to Both Neural and Non-neural Progenies in Mammals. Developmental cell *40*, 137-150.

Bergen, V., Lange, M., Peidli, S., Wolf, F.A., and Theis, F.J. (2020). Generalizing RNA velocity to transient cell states through dynamical modeling. Nat Biotechnol *38*, 1408-1414.

Bernardos, R.L., Barthel, L.K., Meyers, J.R., and Raymond, P.A. (2007). Late-stage neuronal progenitors in the retina are radial Muller glia that function as retinal stem cells. The Journal of neuroscience : the official journal of the Society for Neuroscience *27*, 7028-7040.

Birch, D.G., Bennett, L.D., Duncan, J.L., Weleber, R.G., and Pennesi, M.E. (2016). Long-term Follow-up of Patients With Retinitis Pigmentosa Receiving Intraocular Ciliary Neurotrophic Factor Implants. Am J Ophthalmol *170*, 10-14.

Blackshaw, S., Harpavat, S., Trimarchi, J., Cai, L., Huang, H., Kuo, W.P., Weber, G., Lee, K., Fraioli, R.E., Cho, S.H.*, et al.* (2004). Genomic analysis of mouse retinal development. PLoS biology *2*, E247.

Blackshaw, S., and Sanes, J.R. (2021). Turning lead into gold: reprogramming retinal cells to cure blindness. Journal of Clinical Investigation *131*, e146134.

Botsford, B., Vuong, L.N., Hedges, T.R., III, and Mendoza-Santiesteban, C.E. (2017). Characterization of Charcot-Marie-Tooth optic neuropathy. J Neurol *264*, 2431-2435.

Boudreau-Pinsonneault, C., and Cayouette, M. (2018). Cell lineage tracing in the retina: Could material transfer distort conclusions? Dev Dyn *247*, 10-17.

Bowes, C., Li, T., Danciger, M., Baxter, L.C., Applebury, M.L., and Farber, D.B. (1990). Retinal degeneration in the rd mouse is caused by a defect in the β subunit of rod cGMP-phosphodiesterase. Nature *347*, 677-680.

Bowne, S.J., Daiger, S.P., Hims, M.M., Sohocki, M.M., Malone, K.A., McKie, A.B., Heckenlively, J.R., Birch, D.G., Inglehearn, C.F., Bhattacharya, S.S.*, et al.* (1999). Mutations in the RP1 gene causing autosomal dominant retinitis pigmentosa. Human molecular genetics *8*, 2121-2128.

Bringmann, A., Grosche, A., Pannicke, T., and Reichenbach, A. (2013). GABA and Glutamate Uptake and Metabolism in Retinal Glial (Muller) Cells. Front Endocrinol (Lausanne) *4*, 48.

Bringmann, A., Iandiev, I., Pannicke, T., Wurm, A., Hollborn, M., Wiedemann, P., Osborne, N.N., and Reichenbach, A. (2009). Cellular signaling and factors involved in Muller cell gliosis: neuroprotective and detrimental effects. Progress in retinal and eye research *28*, 423-451.

Bringmann, A., Syrbe, S., Gorner, K., Kacza, J., Francke, M., Wiedemann, P., and Reichenbach, A. (2018). The primate fovea: Structure, function and development. Progress in retinal and eye research.

Bristow, E.A., Griffiths, P.G., Andrews, R.M., Johnson, M.A., and Turnbull, D.M. (2002). The Distribution of Mitochondrial Activity in Relation to Optic Nerve Structure. ARCH OPHTHALMOL *120*, 791-796.

Brody, T., and Odenwald, W.F. (2000). Programmed transformations in neuroblast gene expression during Drosophila CNS lineage development. Dev Biol *226*, 34-44.

Brown, N.L., Patel, S., Brzezinski, J.A., and Glaser, T. (2001). Math5 is required for retinal ganglion cell and optic nerve formation. Development *128*, 2497-2508.

Brzezinski, J.A., and Reh, T.A. (2015). Photoreceptor cell fate specification in vertebrates. Development *142*, 3263-3273.

Buenrostro, J.D., Wu, B., Chang, H.Y., and Greenleaf, W.J. (2015). ATAC-seq: A Method for Assaying Chromatin Accessibility Genome-Wide. Curr Protoc Mol Biol *109*, 21 29 21-29.

Busskamp, V., Duebel, J., Balya, D., Fradot, M., Viney, T.J., Siegert, S., Groner, A.C., Cabuy, E., Forster, V., Seeliger, M.*, et al.* (2010). Genetic Reactivation of Cone Photoreceptors Restores Visual Responses in Retinitis Pigmentosa. Science *329*, 413-417.

Byrne, L.C., Dalkara, D., Luna, G., Fisher, S.K., Clerin, E., Sahel, J.A., Leveillard, T., and Flannery, J.G. (2015). Viral-mediated RdCVF and RdCVFL expression protects cone and rod photoreceptors in retinal degeneration. The Journal of clinical investigation *125*, 105-116.

Byrne, L.C., Khalid, F., Lee, T., Zin, E.A., Greenberg, K.P., Visel, M., Schaffer, D.V., and Flannery, J.G. (2013). AAV-mediated, optogenetic ablation of Muller Glia leads to structural and functional changes in the mouse retina. PloS one *8*, e76075.

Carelli, V., Ross-Cisneros, F.N., and Sadun, A.A. (2004). Mitochondrial dysfunction as a cause of optic neuropathies. Progress in retinal and eye research *23*, 53-89.

Carter-Dawson, L.D., and LaVail, M.M. (1979). Rods and cones in the mouse retina: Structural analysis using light and electron microscopy. The Journal of comparative neurology *188*, 245-262.

Carter-Dawson, L.D., LaVail, M.M., and Sidman, R.L. (1978). Differential effect of the rd mutation on rods and cones in the mouse retina. Investigative ophthalmology & visual science *17*, 489-498.

Carvalho, L.S., and Vandenberghe, L.H. (2015). Promising and delivering gene therapies for vision loss. Vision research *111*, 124-133.

Casson, R.J., Chidlow, G., Wood, J.P., Crowston, J.G., and Goldberg, I. (2012). Definition of glaucoma: clinical and experimental concepts. Clin Exp Ophthalmol *40*, 341-349.

Centanin, L., Ander, J.J., Hoeckendorf, B., Lust, K., Kellner, T., Kraemer, I., Urbany, C., Hasel, E., Harris, W.A., Simons, B.D.*, et al.* (2014). Exclusive multipotency and preferential asymmetric divisions in post-embryonic neural stem cells of the fish retina. Development *141*, 3472-3482.

Chanda, S., Ang, C.E., Davila, J., Pak, C., Mall, M., Lee, Q.Y., Ahlenius, H., Jung, S.W., Sudhof, T.C., and Wernig, M. (2014). Generation of induced neuronal cells by the single reprogramming factor ASCL1. Stem cell reports *3*, 282-296.

Chauchan, B.C., Mikelberg, F.S., Balaszi, A.G., LeBlanc, R.P., Lesk, M., and Trope, G.E. (2008). Canadian Glaucoma Study 2. Risk Factors for the Progression of Open-angle Glaucoma. ARCH OPHTHALMOL *126*, 1030-1036.

Chaya, T., Matsumoto, A., Sugita, Y., Watanabe, S., Kuwahara, R., Tachibana, M., and Furukawa, T. (2017). Versatile functional roles of horizontal cells in the retinal circuit. Scientific reports *7*, 5540.

Chen, S., Wang, Q.-L., Nie, Z., Sun, H., Lennon, G., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., and Zack, D.J. (1997). Crx, a Novel Otx-like Paired-Homeodomain Protein, Binds to and Transactivates Photoreceptor Cell-Specific Genes. Neuron *19*, 1017-1030.

Chernov, M.M., Friedman, R.M., Chen, G., Stoner, G.R., and Roe, A.W. (2018). Functionally specific optogenetic modulation in primate visual cortex. Proceedings of the National Academy of Sciences of the United States of America *115*, 10505-10510.

Chew, E.Y., Clemons, T.E., Jaffe, G.J., Johnson, C.A., Farsiu, S., Lad, E.M., Guymer, R., Rosenfeld, P., Hubschman, J.P., Constable, I.*, et al.* (2019). Effect of Ciliary Neurotrophic Factor on Retinal Neurodegeneration in Patients with Macular Telangiectasia Type 2: A Randomized Clinical Trial. Ophthalmology *126*, 540-549.

Chiba, C. (2014). The retinal pigment epithelium: an important player of retinal disorders and regeneration. Experimental eye research *123*, 107-114.

Choquet, H., Wiggs, J.L., and Khawaja, A.P. (2020). Clinical implications of recent advances in primary open-angle glaucoma genetics. Eye *34*, 29-39.

Clark, B.S., Stein-O'Brien, G.L., Shiau, F., Cannon, G.H., Davis-Marcisak, E., Sherman, T., Santiago, C.P., Hoang, T.V., Rajaii, F., James-Esposito, R.E.*, et al.* (2019). Single-Cell RNA-Seq Analysis of Retinal Development Identifies NFI Factors as Regulating Mitotic Exit and Late-Born Cell Specification. Neuron *102*, 1-16.

Cleary, M.D., and Doe, C.Q. (2006). Regulation of neuroblast competence: multiple temporal identity factors specify distinct neuronal fates within a single early competence window. Genes & development *20*, 429-434.

Conedera, F.M., Pousa, A.M.Q., Mercader, N., Tschopp, M., and Enzmann, V. (2019). Retinal microglia signaling affects Muller cell behavior in the zebrafish following laser injury induction. Glia *67*, 1150-1166.

Conner, C., Ackerman, K.M., Lahne, M., Hobgood, J.S., and Hyde, D.R. (2014). Repressing Notch Signaling and Expressing TNFalpha Are Sufficient to Mimic Retinal Regeneration by Inducing Muller Glial Proliferation to Generate Committed Progenitor Cells. The Journal of neuroscience : the official journal of the Society for Neuroscience *34*, 14403-14419.

Coussa, R.G., Lopez Solache, I., and Koenekoop, R.K. (2017). Leber congenital amaurosis, from darkness to light: An ode to Irene Maumenee. Ophthalmic Genet *38*, 7-15.

da Cruz, L., Dorn, J.D., Humayun, M.S., Dagnelie, G., Handa, J., Barale, P.O., Sahel, J.A., Stanga, P.E., Hafezi, F., Safran, A.B.*, et al.* (2016). Five-Year Safety and Performance Results from the Argus II Retinal Prosthesis System Clinical Trial. Ophthalmology *123*, 2248-2254.

da Silva, S., and Cepko, C.L. (2017). Fgf8 Expression and Degradation of Retinoic Acid Are Required for Patterning a High-Acuity Area in the Retina. Developmental cell.

Daiger, S.P., Sullivan, L.S., and Bowne, S.J. (2013). Genes and mutations causing retinitis pigmentosa. Clin Genet *84*, 132-141.

Dall'Agnese, A., Caputo, L., Nicoletti, C., di Iulio, J., Schmitt, A., Gatto, S., Diao, Y., Ye, Z., Forcato, M., Perera, R.*, et al.* (2019). Transcription Factor-Directed Re-wiring of Chromatin Architecture for Somatic Cell Nuclear Reprogramming toward trans-Differentiation. Mol Cell *76*, 453-472 e458.

Das, A.V., Mallya, K.B., Zhao, X., Ahmad, F., Bhattacharya, S., Thoreson, W.B., Hegde, G.V., and Ahmad, I. (2006). Neural stem cell properties of Muller glia in the mammalian retina: regulation by Notch and Wnt signaling. Dev Biol *299*, 283-302.

de Jong, E.K., Geerlings, M.J., and den Hollander, A.I. (2019). Age-related macular degeneration. Genetics and genomics of eye disease, 155-180.

De la Rossa, A., Bellone, C., Golding, B., Vitali, I., Moss, J., Toni, N., Luscher, C., and Jabaudon, D. (2013). In vivo reprogramming of circuit connectivity in postmitotic neocortical neurons. Nat Neurosci *16*, 193-200.

de Melo, J., and Blackshaw, S. (2011). In vivo electroporation of developing mouse retina. J Vis Exp, 2847.

de Melo, J., Peng, G.H., Chen, S., and Blackshaw, S. (2011). The Spalt family transcription factor Sall3 regulates the development of cone photoreceptors and retinal horizontal interneurons. Development *138*, 2325-2336.

De Silva, S.R., Barnard, A.R., Hughes, S., Tam, S.K.E., Martin, C., Singh, M.S., Barnea-Cramer, A.O., McClements, M.E., During, M.J., Peirson, S.N.*, et al.* (2017). Long-term restoration of visual function in end-stage retinal degeneration using subretinal human melanopsin gene therapy. Proceedings of the National Academy of Sciences of the United States of America *114*, 11211-11216.

del Cerro, M., Lazar, E.S., and Diloreto, D. (1997). The First Decade of Continuous Progress in Retinal Transplantation. Microscopy research and technique *36*, 130-141.

Del Debbio, C.B., Balasubramanian, S., Parameswaran, S., Chaudhuri, A., Qiu, F., and Ahmad, I. (2010). Notch and Wnt signaling mediated rod photoreceptor regeneration by Muller cells in adult mammalian retina. PloS one *5*, e12425.

Delettre, C., Lenaers, G., Griffoin, J.-M., Gigarel, N., Lorenzo, C., Belenguer, P., Pelloquin, L., Grosgeorge, J., Turc-Carel, C., Perret, E.*, et al.* (2000). Nuclear gene OPA1, encoding a mitochondrial dynamin-related protein, is mutated in dominant optic atrophy. Nature Genetics *26*, 207-210.

den Hollander, A.I., Koenekoop, R.K., Yzer, S., Lopez, I., Arends, M.L., Voesenek, K.E., Zonneveld, M.N., Strom, T.M., Meitinger, T., Brunner, H.G.*, et al.* (2006). Mutations in the CEP290 (NPHP6) gene are a frequent cause of Leber congenital amaurosis. Am J Hum Genet *79*, 556-561.

Dhande, O.S., and Huberman, A.D. (2014). Retinal ganglion cell maps in the brain: implications for visual processing. Curr Opin Neurobiol *24*, 133-142.

Diamond, J.S. (2017). Inhibitory Interneurons in the Retina: Types, Circuitry, and Function. Annu Rev Vis Sci, 1-24.

Doe, C.Q. (2017). Temporal Patterning in the Drosophila CNS. Annu Rev Cell Dev Biol *33*, 219-240.

Dong, X., Yang, H., Zhou, X., Xie, X., Yu, D., Guo, L., Xu, M., Zhang, W., Liang, G., and Gan, L. (2020). LIM-Homeodomain Transcription Factor LHX4 Is Required for the Differentiation of Retinal Rod Bipolar Cells and OFF-Cone Bipolar Subtypes. Cell reports *32*, 108144.

Dryja, T.P., Hahn, K.M., Kajiware, K., and Berson, E.L. (1997). Dominant and digenic mutations in the peripherin/RDS and ROM1 genes in Retinitis Pigmentosa. Investigative ophthalmology & visual science *38*, 1972-1982.

Dryja, T.P., McGee, T.L., Reichel, E., Han, L.B., Cowley, G.S., Yandell, D.W., Sandberg, M.A., and Berson, E.L. (1990). A point mutation of the rhodopsin gene in one form of retinitis pigmentosa. Nature *343*, 364-366.

Duebel, J., Marazova, K., and Sahel, J.A. (2015). Optogenetics. Curr Opin Ophthalmol *26*, 226-232.

Dyer, A.M., and Cepko, C.L. (2000). Control of Müller glial cell proliferation and activation following retinal injury. Nature Neuroscience *3*.

Eden, E., Navon, R., Steinfeld, I., Lipson, D., and Yakhini, Z. (2009). GOrilla: a tool for discovery and visualization of enriched GO terms in ranked gene lists. BMC Bioinformatics *10*, 48.

Edwards, A.O., Donoso, L.A., and Ritter, R. (2001). A Novel Gene for Autosomal Dominant Stargardt-like Macular Dystrophy with Homology to the SUR4 Protein Family. Investigative ophthalmology & visual science *42*, 2652-2663.

Edwards, T.L., Cottriall, C.L., Xue, K., Simunovic, M.P., Ramsden, J.D., Zrenner, E., and MacLaren, R.E. (2018). Assessment of the Electronic Retinal Implant Alpha AMS in Restoring Vision to Blind Patients with End-Stage Retinitis Pigmentosa. Ophthalmology *125*, 432-443.

Ekström, P., Sanyal, S., Narfström, K., Chader, G.J., and van Veen, T. (1988). Accumulation of Gliol Fibrillary Acidic Protein in Muller Radial Glia During Retinal Degeneration. Investigative ophthalmology & visual science *29*, 1363-1371.

Elliott, J., Jolicoeur, C., Ramamurthy, V., and Cayouette, M. (2008). Ikaros confers early temporal competence to mouse retinal progenitor cells. Neuron *60*, 26-39.

Elsaeidi, F., Macpherson, P., Mills, E.A., Jui, J., Flannery, J.G., and Goldman, D. (2018). Notch Suppression Collaborates with Ascl1 and Lin28 to Unleash a Regenerative Response in Fish Retina, But Not in Mice. The Journal of neuroscience : the official journal of the Society for Neuroscience *38*, 2246-2261.

Elshatory, Y., Everhart, D., Deng, M., Xie, X., Barlow, R.B., and Gan, L. (2007). Islet-1 controls the differentiation of retinal bipolar and cholinergic amacrine cells. The Journal of neuroscience : the official journal of the Society for Neuroscience *27*, 12707-12720.

Feng, J., Liu, T., Qin, B., Zhang, Y., and Liu, X.S. (2012). Identifying ChIP-seq enrichment using MACS. Nat Protoc *7*, 1728-1740.

Fischer, A.J., Bosse, J.L., and El-Hodiri, H.M. (2013). The ciliary marginal zone (CMZ) in development and regeneration of the vertebrate eye. Experimental eye research *116*, 199-204.

Fish, G., Haller, J.A., Ho, A.C., Klein, P.A., Loewenstein, J., Martin, D., Orth, D., Rosen, R.B., Sanisho, S., Schwartz, S.D.*, et al.* (2003). Anti-vascular endothelial growth factor therapy for subfoveal choroidal neovascularization secondary to age-related macular degeneration. Ophthalmology *110*, 979-986.

Fortuna, F., Barboni, P., Liguori, R., Valentino, M.L., Savini, G., Gellera, C., Mariotti, C., Rizzo, G., Tonon, C., Manners, D.*, et al.* (2009). Visual system involvement in patients with Friedreich's ataxia. Brain *132*, 116-123.

Franze, K., Grosche, J., Skatchkov, S.N., Schinkinger, S., Foja, C., Schild, D., Uckermann, O., Travis, K., Reichenbach, A., and Guck, J. (2007). Muller cells are living optical fibers in the vertebrate retina. Proceedings of the National Academy of Sciences of the United States of America *104*, 8287-8292.

Fritsche, L.G., Igl, W., Bailey, J.N., Grassmann, F., Sengupta, S., Bragg-Gresham, J.L., Burdon, K.P., Hebbring, S.J., Wen, C., Gorski, M.*, et al.* (2016). A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants. Nat Genet *48*, 134-143.

Furukawa, T., Morrow, E.M., and Cepko, C. (1997). Crx, a Novel otx-like Homeobox Gene, Shows Photoreceptor-Specific Expression and Regulates Photoreceptor Differentiation. Cell *91*, 531-541.

Gan, L., Cookson, M.R., Petrucelli, L., and La Spada, A.R. (2018). Converging pathways in neurodegeneration, from genetics to mechanisms. Nat Neurosci *21*, 1300-1309.

Gan, L., Xiang, M., Zhou, L., Wagner, D.S., Klein, W.H., and Nathans, J. (1996). POU domain factor Brn-3b is required for the development of a large set of retinal ganglion cells. Proceedings of the National Academy of Sciences of the United States of America *93*, 3920-3925.

Gardner, J.C., Webb, T.R., Kanuga, N., Robson, A.G., Holder, G.E., Stockman, A., Ripamonti, C., Ebenezer, N.D., Ogun, O., Devery, S.*, et al.* (2010). X-linked cone dystrophy caused by mutation of the red and green cone opsins. Am J Hum Genet *87*, 26-39.

Giannelli, S.G., Demontis, G.C., Pertile, G., Rama, P., and Broccoli, V. (2011). Adult human Muller glia cells are a highly efficient source of rod photoreceptors. Stem cells *29*, 344-356.

Gill, J.S., Georgiou, M., Kalitzeos, A., Moore, A.T., and Michaelides, M. (2019). Progressive cone and cone-rod dystrophies: clinical features, molecular genetics and prospects for therapy. Br J Ophthalmol.

Glickman, R.D. (2002). Phototoxicity to the Retina: Mechanisms of Damage. International Journal of Toxicology *21*, 473-490.

Goldman, D. (2014). Muller glial cell reprogramming and retina regeneration. Nature reviews Neuroscience *15*, 431-442.

Gotz, M., Sirko, S., Beckers, J., and Irmler, M. (2015). Reactive astrocytes as neural stem or progenitor cells: In vivo lineage, In vitro potential, and Genome-wide expression analysis. Glia *63*, 1452-1468.

Goureau, O., Régnier-Ricard, F., and Courtois, Y. (1999). Requirement for Nitric Oxide in Retinal Neuronal Cell Death Induced by Activated Müller Glial Cells. Journal of neurochemistry *72*, 2506-2515.

Grade, S., and Gotz, M. (2017). Neuronal replacement therapy: previous achievements and challenges ahead. NPJ Regen Med *2*, 29.

Grande, A., Sumiyoshi, K., Lopez-Juarez, A., Howard, J., Sakthivel, B., Aronow, B., Campbell, K., and Nakafuku, M. (2013). Environmental impact on direct neuronal reprogramming in vivo in the adult brain. Nature communications *4*, 2373.

Grigoryan, E.N. (2019). Endogenous Cell Sources for Eye Retina Regeneration in Vertebrate Animals and Humans. Russian Journal of Developmental Biology *49*, 314-326.

Grosskortenhaus, R., Pearson, B.J., Marusich, A., and Doe, C.Q. (2005). Regulation of temporal identity transitions in Drosophila neuroblasts. Developmental cell *8*, 193-202.

Grosskortenhaus, R., Robinson, K.J., and Doe, C.Q. (2006). Pdm and Castor specify late-born motor neuron identity in the NB7-1 lineage. Genes & development *20*, 2618-2627.

Gu, Z., Eils, R., and Schlesner, M. (2016). Complex heatmaps reveal patterns and correlations in multidimensional genomic data. Bioinformatics *32*, 2847-2849.

Guo, Z., Zhang, L., Wu, Z., Chen, Y., Wang, F., and Chen, G. (2014). In vivo direct reprogramming of reactive glial cells into functional neurons after brain injury and in an Alzheimer's disease model. Cell Stem Cell *14*, 188-202.

Gurdon, J.B. (1962). The Developmental Capacity of Nuclei taken from Intestinal Epithelium Cells of Feeding Tadpoles. J Embryol exp Morph *10*, 622-640.

Gurdon, J.B., and Melton, D.A. (2008). Nuclear Reprogramming in Cells. Science *322*, 1811-1815.

Hardiman, O., Al-Chalabi, A., Chio, A., Corr, E.M., Logroscino, G., Robberecht, W., Shaw, P.J., Simmons, Z., and van den Berg, L.H. (2017). Amyotrophic lateral sclerosis. Nat Rev Dis Primers *3*, 17071.

Hartong, D.T., Berson, E.L., and Dryja, T.P. (2006). Retinitis pigmentosa. The Lancet *368*, 1795-1809.

Hattar, S., Kumar, M., Park, A., Tong, P., Tung, J., Yau, K.W., and Berson, D.M. (2006). Central projections of melanopsin-expressing retinal ganglion cells in the mouse. The Journal of comparative neurology *497*, 326-349.

Hattar, S., Liao, H.-W., Takao, M., Berson, D.M., and Yau, K.W. (2002). Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. Science *295*, 1065-1070.

Hauswirth, W.W., Aleman, T.S., Kaushal, S., Cideciyan, A.V., Schwartz, S.B., Wang, L., Conlon, T.J., Boye, S.L., Flotte, T.R., Byrne, B.J.*, et al.* (2008). Treatment of leber congenital amaurosis due to RPE65 mutations by ocular subretinal injection of adeno-associated virus gene vector: short-term results of a phase I trial. Hum Gene Ther *19*, 979-990.

Hecht, S., Shlaer, S., and Pirenne, M.H. (1942). Energy, Quanta, and Vision. The Journal of General Physiology, 819-840.

Heesterbeek, T.J., Lores-Motta, L., Hoyng, C.B., Lechanteur, Y.T.E., and den Hollander, A.I. (2020). Risk factors for progression of age-related macular degeneration. Ophthalmic Physiol Opt *40*, 140-170.

Heizmann, B., Kastner, P., and Chan, S. (2017). The Ikaros family in lymphocyte development. Curr Opin Immunol *51*, 14-23.

Herrington, T.M., Cheng, J.J., and Eskandar, E.N. (2016). Mechanisms of deep brain stimulation. J Neurophysiol *115*, 19-38.

Hertz, J., Qu, B., Hu, Y., Patel, R.D., Valenzuela, D.A., and Goldberg, J.L. (2014). Survival and integration of developing and progenitor-derived retinal ganglion cells following transplantation. Cell Transplant *23*, 855-872.

Hippert, C., Graca, A.B., Barber, A.C., West, E.L., Smith, A.J., Ali, R.R., and Pearson, R.A. (2015). Muller glia activation in response to inherited retinal degeneration is highly varied and disease-specific. PloS one *10*, e0120415.

Hoang, T., Wang, J., Boyd, P., Wang, F., Santiago, C., Jiang, L., Yoo, S., Lahne, M., Todd, L.J., Jia, M.*, et al.* (2020). Gene regulatory networks controlling vertebrate retinal regeneration. Science *370*, eabb8598.

Holguera, I., and Desplan, C. (2018). Neuronal specification in space and time. Science *363*, 176-180.

Honma, Y., Kiosawa, H., Mori, T., Oguri, A., Nikaido, T., Kanazawa, K., Tojo, M., Takeda, J., Tanno, Y., Yokoba, S.*, et al.* (1999). Eos: a novel member of the Ikaros

gene family expressed predominantly in the developing nervous system. FEBS letters *447*, 76-80.

Hopiavuori, B.R., Anderson, R.E., and Agbaga, M.P. (2019). ELOVL4: Very long-chain fatty acids serve an eclectic role in mammalian health and function. Progress in retinal and eye research *69*, 137-158.

Hou, Y., Dan, X., Babbar, M., Wei, Y., Hasselbalch, S.G., Croteau, D.L., and Bohr, V.A. (2019). Ageing as a risk factor for neurodegenerative disease. Nat Rev Neurol *15*, 565-581.

Howell, N., Bindoff, L.A., McCullough, D.A., Kubacha, I., Poulton, J., Mackey, D., Taylor, L., and Turnbull, D.M. (1991). Leber Hereditary Optic Neuropathy: Identification of the Same Mitochondrial NDI Mutation in Six Pedigrees. Am J Hum Genet *49*, 939-950.

Humayun, M.S., Dorn, J.D., da Cruz, L., Dagnelie, G., Sahel, J.A., Stanga, P.E., Cideciyan, A.V., Duncan, J.L., Eliott, D., Filley, E.*, et al.* (2012). Interim results from the international trial of Second Sight's visual prosthesis. Ophthalmology *119*, 779-788.

Huoponen, K., Vilkki, J., Aula, P., Nikoskelainen, E.K., and Savontaus, M.-L. (1991). A New mtDNA Mutation Associated with Leber Hereditary Optic Neuroretinopathy. Am J Hum Genet *48*.

Imamoto, Y., and Shichida, Y. (2014). Cone visual pigments. Biochim Biophys Acta *1837*, 664-673.

Isshiki, T., Pearson, B., Holbrook, S., and Doe, C.Q. (2001). Drosophila Neuroblasts Sequentially Express Transcription Factors which Specify the Temporal Identity of Their Neuronal Progeny. Cell *106*, 511-521.

Iwafuchi-Doi, M., and Zaret, K.S. (2016). Cell fate control by pioneer transcription factors. Development *143*, 1833-1837.

Jablonski, M.M., and Iannaccone, A. (2000). Targeted Disruption of Müller Cell Metabolism Induces Photoreceptor Dysmorphogenesis. Glia *32*, 192-204.

Jadhav, A.P., Roesch, K., and Cepko, C.L. (2009). Development and neurogenic potential of Muller glial cells in the vertebrate retina. Progress in retinal and eye research *28*, 249-262.

Jakel, S., and Dimou, L. (2017). Glial Cells and Their Function in the Adult Brain: A Journey through the History of Their Ablation. Frontiers in cellular neuroscience *11*, 24.

Javed, A., Mattar, P., Lu, S., Kruczek, K., Kloc, M., Gonzalez-Cordero, A., Bremner, R., Ali, R.R., and Cayouette, M. (2020). Pou2f1 and Pou2f2 cooperate to control the timing of cone photoreceptor production in the developing mouse retina. Development *147*, dev188730.

Jayaram, H., Jones, M.F., Eastlake, K., Cottrill, P.B., Becker, S., Wiseman, J., Khaw, P.T., and Limb, G.A. (2014). Transplantation of photoreceptors derived from human Muller glia restore rod function in the P23H rat. Stem Cells Transl Med *3*, 323-333.

John, L.B., and Ward, A.C. (2011). The Ikaros gene family: transcriptional regulators of hematopoiesis and immunity. Mol Immunol *48*, 1272-1278.

Johns, D.R., Neufeld, M.J., and Park, R.D. (1992). An ND-6 mitochondrial DNA mutation associated with leber hereditary optic neuropathy. Biochemical and biophysical research communications *187*, 1551-1557.

Johns, P.R. (1977). Growth of the adult goldfish eye. III. Source of the new retinal cells. The Journal of comparative neurology *176*, 343-358.

Joly, S., Pernet, V., Samardzija, M., and Grimm, C. (2011). Pax6-positive Muller glia cells express cell cycle markers but do not proliferate after photoreceptor injury in the mouse retina. Glia *59*, 1033-1046.

Jones, B.W., Watt, C.B., Frederick, J.M., Baehr, W., Chen, C.K., Levine, E.M., Milam, A.H., Lavail, M.M., and Marc, R.E. (2003). Retinal remodeling triggered by photoreceptor degenerations. The Journal of comparative neurology *464*, 1-16.

Jorstad, N.L., Wilken, M.S., Grimes, W.N., Wohl, S.G., VandenBosch, L.S., Yoshimatsu, T., Wong, R.O., Rieke, F., and Reh, T.A. (2017). Stimulation of functional neuronal regeneration from Muller glia in adult mice. Nature *548*, 103-107.

Jorstad, N.L., Wilken, M.S., Todd, L., Finkbeiner, C., Nakamura, P., Radulovich, N., Hooper, M.J., Chitsazan, A., Wilkerson, B.A., Rieke, F.*, et al.* (2020). STAT Signaling Modifies Ascl1 Chromatin Binding and Limits Neural Regeneration from Muller Glia in Adult Mouse Retina. Cell reports *30*, 2195-2208.e2195.

Jozefczuk, J., Drews, K., and Adjaye, J. (2012). Preparation of mouse embryonic fibroblast cells suitable for culturing human embryonic and induced pluripotent stem cells. J Vis Exp, e3854.

Kambadur, R., Koizumi, K., Stivers, C., Nagle, J., Poole, S.J., and Odenwald, W.F. (1998). Regulation of POU genes by castor and hunchback establishes layered compartments in the Drosophila CNS. Genes & development *12*, 246-260.

Kara, N., Kent, M.R., Didiano, D., Rajaram, K., Zhao, A., Summerbell, E.R., and Patton, J.G. (2019). The miR-216a-Dot1l Regulatory Axis Is Necessary and Sufficient for Müller Glia Reprogramming during Retina Regeneration. Cell reports *28*, 2037-2047.e2034.

Karl, M.O., Hayes, S., Nelson, B.R., Tan, K., Buckingham, B., and Reh, T.A. (2008). Stimulation of neural regeneration in the mouse retina. Proceedings of the National Academy of Sciences of the United States of America *105*, 19508-19513.

Kass, M.A., Heuer, D.K., Higginbotham, E.J., Johnson, C.A., Keltner, J.L., Miller, P.J., Parrish, R.K., Wilson, M.R., and Gordon, M.O. (2002). The Ocular Hypertension Treatment Study
A Randomized Trial Determines That Topical Ocular Hypotensive Medication Delays or Prevents the Onset of Primary Open-Angle Glaucoma. ARCH OPHTHALMOL *120*, 701-713.

Kassoff, A., Kassoff, J., Buehler, J., Eglow, M., …, Ferris III, F.L., Sperduto, R.D., Kurinji, N., and Chew, E.Y. (2001). A Randomized, Placebo-Controlled, Clinical Trial of High-Dose Supplementation With Vitamins C and E, Beta Carotene, and Zinc for Age-Related Macular Degeneration and Vision Loss. ARCH OPHTHALMOL *119*, 1417-1436.

Kaufman, M.L., Park, K.U., Goodson, N.B., Chew, S., Bersie, S., Jones, K.L., Lamba, D.A., and Brzezinski, J.A.t. (2019). Transcriptional profiling of murine retinas undergoing semi-synchronous cone photoreceptor differentiation. Dev Biol *453*, 155-167.

Kaur, S., Gupta, S., Chaudhary, M., Khursheed, M.A., Mitra, S., Kurup, A.J., and Ramachandran, R. (2018). let-7 MicroRNA-Mediated Regulation of Shh Signaling and the Gene Regulatory Network Is Essential for Retina Regeneration. Cell reports *23*, 1409-1423.

Keeler, C.E. (1924). The inheritance of a retinal abnormality in white mice. PNAS *10*, 329-333.

Kelley, M.W., Turner, J.K., and Reh, T.A. (1994). Retinoic acid promotes differentiation of photoreceptors in vitro. Development, 2091-2102.

Kempermann, G., Gage, F.H., Aigner, L., Song, H., Curtis, M.A., Thuret, S., Kuhn, H.G., Jessberger, S., Frankland, P.W., Cameron, H.A*., et al.* (2018). Human Adult Neurogenesis: Evidence and Remaining Questions. Cell Stem Cell.

Khan, A.O., Al Rashaed, S., Neuhaus, C., Bergmann, C., and Bolz, H.J. (2016). Peripherin mutations cause a distinct form of recessive Leber congenital amaurosis and dominant phenotypes in asymptomatic parents heterozygous for the mutation. Br J Ophthalmol *100*, 209-215.

Kim, J.S., Sif, S., Jones, B., Jackson, A., Koipally, J., Heller, E., Winandy, S., Viel, A., Sawyer, A., Ikeda, T*., et al.* (1999). Ikaros DNA-Binding Proteins Direct Formation of Chromatin Remodeling Complexes in Lymphocytes. Immunity *10*, 345-355.

Klapoetke, N.C., Murata, Y., Kim, S.S., Pulver, S.R., Birdsey-Benson, A., Cho, Y.K., Morimoto, T.K., Chuong, A.S., Carpenter, E.J., Tian, Z*., et al.* (2014). Independent optical excitation of distinct neural populations. Nature methods *11*, 338-346.

Klein, B.E.K., Klein, R., William, E.S., Franke, T.F., Cantor, L.B., Martone, J., and Menage, M.J. (1992). Prevalence of Glaucoma: The Beaver Dam Eye Study. Ophthalmology *99*, 1499-1504.

Kleine Holthaus, S.M., Aristorena, M., Maswood, R., Semenyuk, O., Hoke, J., Hare, A., Smith, A.J., Mole, S.E., and Ali, R.R. (2020). Gene Therapy Targeting the Inner Retina Rescues the Retinal Phenotype in a Mouse Model of CLN3 Batten Disease. Hum Gene Ther *31*, 709-718.

Kleinlogel, S., Feldbauer, K., Dempski, R.E., Fotis, H., Wood, P.G., Bamann, C., and Bamberg, E. (2011). Ultra light-sensitive and fast neuronal activation with the Ca(2)+-permeable channelrhodopsin CatCh. Nat Neurosci *14*, 513-518.

Kleinlogel, S., Vogl, C., Jeschke, M., Neef, J., and Moser, T. (2020). Emerging approaches for restoration of hearing and vision. Physiol Rev.

Kobayashi, A., Higashide, T., Hamasaki, D., Kutoba, S., Sakuma, H., An, W., Fujimaki, T., McLaren, M.J., Weleber, R.G., and Inana, G. (2000). HRG4 (UNC119) Mutation Found in Cone–Rod Dystrophy Causes Retinal Degeneration in a Transgenic Model. Investigative ophthalmology & visual science *41*, 3268-3277.

Koche, R.P., Smith, Z.D., Adli, M., Gu, H., Ku, M., Gnirke, A., Bernstein, B.E., and Meissner, A. (2011). Reprogramming factor expression initiates widespread targeted chromatin remodeling. Cell Stem Cell *8*, 96-105.

Kohwi, M., Lupton, J.R., Lai, S.L., Miller, M.R., and Doe, C.Q. (2013). Developmentally regulated subnuclear genome reorganization restricts neural progenitor competence in Drosophila. Cell *152*, 97-108.

Kolb, H. (2012). Photoreceptors. Webvision: The Organization of the Retina and Visual System [Internet].

Kolomeyer, A.M., and Zarbin, M.A. (2014). Trophic factors in the pathogenesis and therapy for retinal degenerative diseases. Surv Ophthalmol *59*, 134-165.

Krizaj, D. (2019). What is glaucoma? Webvision: The Organization of the Retina and Visual System [Internet].

Kuan, C.Y., Schloemer, A.J., Lu, A., Burns, K.A., Weng, W.L., Williams, M.T., Strauss, K.I., Vorhees, C.V., Flavell, R.A., Davis, R.J*., et al.* (2004). Hypoxia-ischemia induces DNA synthesis without cell proliferation in dying neurons in adult rodent brain. The Journal of neuroscience : the official journal of the Society for Neuroscience *24*, 10763-10772.

Kugler, M., Schlecht, A., Fuchshofer, R., Kleiter, I., Aigner, L., Tamm, E.R., and Braunger, B.M. (2015). Heterozygous modulation of TGF-beta signaling does not influence Muller glia cell reactivity or proliferation following NMDA-induced damage. Histochemistry and cell biology *144*, 443-455.

La Manno, G., Soldatov, R., Zeisel, A., Braun, E., Hochgerner, H., Petukhov, V., Lidschreiber, K., Kastriti, M.E., Lonnerberg, P., Furlan, A*., et al.* (2018). RNA velocity of single cells. Nature *560*, 494-498.

Lahne, M., Brecker, M., Jones, S.E., and Hyde, D.R. (2020a). The Regenerating Adult Zebrafish Retina Recapitulates Developmental Fate Specification Programs. Front Cell Dev Biol *8*, 617923.

Lahne, M., Nagashima, M., Hyde, D.R., and Hitchcock, P.F. (2020b). Reprogramming Muller Glia to Regenerate Retinal Neurons. Annu Rev Vis Sci *6*, 171-193.

Lamba, D.A.K., M. O.; Reh, T. A. (2009). Strategies for retinal repair: cell replacement and regeneration. Progress in Brain Research *175*, 23-31.

Lane, R.P., Cutforth, T., Young, J., Athanasiou, M., Friedman, C., Rowen, L., Evans, G., Axel, R., Hood, L., and Trask, B.J. (2001). Genomic analysis of orthologous mouse and human olfactory receptor loci. Proceedings of the National Academy of Sciences of the United States of America *98*, 7390-7395.

Langhe, R., Chesneau, A., Colozza, G., Hidalgo, M., Ail, D., Locker, M., and Perron, M. (2017). Muller glial cell reactivation in Xenopus models of retinal degeneration. Glia *65*, 1333-1349.

Langmead, B., and Salzberg, S.L. (2012). Fast gapped-read alignment with Bowtie 2. Nature methods *9*, 357-359.

Lawrence, J.M., Singhal, S., Bhatia, B., Keegan, D.J., Reh, T.A., Luthert, P.J., Khaw, P.T., and Limb, G.A. (2007). MIO-M1 cells and similar muller glial cell lines derived from adult human retina exhibit neural stem cell characteristics. Stem cells *25*, 2033-2043.

Lee, Y., Messing, A., Su, M., and Brenner, M. (2008). GFAP promoter elements required for region-specific and astrocyte-specific expression. Glia *56*, 481-493.

Lehre, K.P., Davanger, S., and Danbolt, N.C. (1997). Localization of the glutamate transporter protein GLAST in rat retina. Brain research *744*, 129-137.

Leveillard, T., Mohand-Said, S., Lorentz, O., Hicks, D., Fintz, A.C., Clerin, E., Simonutti, M., Forster, V., Cavusoglu, N., Chalmel, F.*, et al.* (2004). Identification and characterization of rod-derived cone viability factor. Nat Genet *36*, 755-759.

Li, H., and Chen, G. (2016). In Vivo Reprogramming for CNS Repair: Regenerating Neurons from Endogenous Glial Cells. Neuron *91*, 728-738.

Li, S., Mo, Z., Yang, X., Price, S.M., Shen, M.M., and Xiang, M. (2004). Foxn4 controls the genesis of amacrine and horizontal cells by retinal progenitors. Neuron *43*, 795-807.

Lin, J.Y., Knutsen, P.M., Muller, A., Kleinfeld, D., and Tsien, R.Y. (2013). ReaChR: a red-shifted variant of channelrhodopsin enables deep transcranial optogenetic excitation. Nat Neurosci *16*, 1499-1508.

Liu, S., Liu, X., Li, S., Huang, X., Qian, H., Jin, K., and Xiang, M. (2020). Foxn4 is a temporal identity factor conferring mid/late-early retinal competence and involved in

retinal synaptogenesis. Proceedings of the National Academy of Sciences of the United States of America *117*, 5016-5027.

Liu, Y., Miao, Q., Yuan, J., Han, S., Zhang, P., Li, S., Rao, Z., Zhao, W., Ye, Q., Geng, J.*, et al.* (2015). Ascl1 Converts Dorsal Midbrain Astrocytes into Functional Neurons In Vivo. The Journal of neuroscience : the official journal of the Society for Neuroscience *35*, 9336-9355.

Livne-Bar, I., Pacal, M., Cheung, M.C., Hankin, M., Trogadis, J., Chen, D., Dorval, K.M., and Bremner, R. (2006). Chx10 is required to block photoreceptor differentiation but is dispensable for progenitor proliferation in the postnatal retina. Proceedings of the National Academy of Sciences of the United States of America *103*, 4988-4993.

Long, O.K., Fisher, S.K., Fariss, R.N., and Anderson, D.H. (1986). Disc Shedding and Autophagy in the Cone-dominant Ground Squirrel Retina. Experimental eye research *43*, 193-205.

Lopes, V.S., Boye, S.E., Louie, C.M., Boye, S., Dyka, F., Chiodo, V., Fofo, H., Hauswirth, W.W., and Williams, D.S. (2013). Retinal gene therapy with a large MYO7A cDNA using adeno-associated virus. Gene therapy *20*, 824-833.

Lotery, A.J., Jacobson, S.G., Fishman, G.A., Weleber, R.G., Fulton, A.B., Namperumalsamy, P., Héon, E., Levin, A.V., Grover, S., Rosenow, J.R.*, et al.* (2001). Mutations in the CRB1 Gene Cause Leber Congenital Amaurosis. ARCH OPHTHALMOL *119*, 415-420.

Love, M.I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol *15*, 550.

Lu, Y., Brommer, B., Tian, X., Krishnan, A., Meer, M., Wang, C., Vera, D.L., Zeng, Q., Yu, D., Bonkowski, M.S.*, et al.* (2020). Reprogramming to recover youthful epigenetic information and restore vision. Nature *588*, 124-129.

Lu, Y.B., Franze, K., Seifert, G., Steinhauser, C., Kirchhoff, F., Wolburg, H., Guck, J., Janmey, P., Wei, E.Q., Kas, J.*, et al.* (2006). Viscoelastic properties of individual glial cells and neurons in the CNS. Proceedings of the National Academy of Sciences of the United States of America *103*, 17759-17764.

Lu, Y.B., Iandiev, I., Hollborn, M., Korber, N., Ulbricht, E., Hirrlinger, P.G., Pannicke, T., Wei, E.Q., Bringmann, A., Wolburg, H.*, et al.* (2011). Reactive glial cells: increased stiffness correlates with increased intermediate filament expression. FASEB journal : official publication of the Federation of American Societies for Experimental Biology *25*, 624-631.

Lust, K., and Wittbrodt, J. (2018). Activating the regenerative potential of Muller glia cells in a regeneration-deficient retina. eLife *7*.

Mackey, D., and Howell, N. (1992). A Variant of Leber Hereditary Optic Neuropathy Characterized by Recovery of Vision and by an Unusual Mitochondrial Genetic Etiology. Am J Hum Genet *51*, 1218-1228.

MacLaren, R.E., Pearson, R.A., MacNeil, A., Douglas, R.H., Salt, T.E., Akimoto, M., Swaroop, A., Sowden, J.C., and Ali, R.R. (2006). Retinal repair by transplantation of photoreceptor precursors. Nature *444*, 203-207.

Maguire, A.M., Simonelli, F., Pierce, E.A., Pugh, E.N., Mingozzi, F., Bennicelli, J.L., Banfin, S., Marshall, K.A., Testa, F., Surace, E.M.*, et al.* (2008). Safety and Efficacy of Gene Transfer for Leber's Congenital Amaurosis. The New England Journal of Medicine *358*, 2240-2248.

Malihi, M., Moura Filho, E.R., Hodge, D.O., and Sit, A.J. (2014). Long-term trends in glaucoma-related blindness in Olmsted County, Minnesota. Ophthalmology *121*, 134-141.

Mall, M., Kareta, M.S., Chanda, S., Ahlenius, H., Perotti, N., Zhou, B., Grieder, S.D., Ge, X., Drake, S., Euong Ang, C.*, et al.* (2017). Myt1l safeguards neuronal identity by actively repressing many non-neuronal fates. Nature *544*, 245-249.

Mandai, M., Fujii, M., Hashiguchi, T., Sunagawa, G.A., Ito, S., Sun, J., Kaneko, J., Sho, J., Yamada, C., and Takahashi, M. (2017). iPSC-Derived Retina Transplants Improve Vision in rd1 End-Stage Retinal-Degeneration Mice. Stem cell reports *8*, 69-83.

Marcucci, F., Murcia-Belmonte, V., Wang, Q., Coca, Y., Ferreiro-Galve, S., Kuwajima, T., Khalid, S., Ross, M.E., Mason, C., and Herrera, E. (2016). The Ciliary Margin Zone of the Mammalian Retina Generates Retinal Ganglion Cells. Cell reports *17*, 3153-3164.

Marlhens, F., Bareil, C., Griffoin, J.-M., Zrenner, E., Amalric, P., Eliaou, C., Liu, S.-Y., Harris, E., Redmond, T.M., Arnaud, B.*, et al.* (1997). Mutations in RPE65 cause Leber's congenital amaurosis. Nature Genetics *17*, 139-141.

Marquardt, A., Stohr, H., Passmore, L.A., Kramer, F., Rivera, A., and Weber, B.H.F. (1998). Mutations in a novel gene, VMD2, encoding a protein of unknown properties cause juvenile-onset vitelliform macular dystrophy (Best's disease). Human molecular genetics *7*, 1517-1525.

Martin, J.F., and Poche, R.A. (2019). Awakening the regenerative potential of the mammalian retina. Development *146*.

Martinez, T., Wright, N., Lopez-Fraga, M., Jimenez, A.I., and Paneda, C. (2013). Silencing human genetic diseases with oligonucleotide-based therapies. Hum Genet *132*, 481-493.

Masland, R.H. (2001). The fundamental plan of the retina. Nature Neuroscience *4*, 877-886.

Masters, C.L., Bateman, R., Blennow, K., Rowe, C.C., Sperling, R.A., and Cummings, J.L. (2015). Alzheimer's disease. Nat Rev Dis Primers *1*, 15056.

Mathur, P., and Yang, J. (2015). Usher syndrome: Hearing loss, retinal degeneration and associated abnormalities. Biochim Biophys Acta *1852*, 406-420.

Matsuda, T., and Cepko, C.L. (2004). Electroporation and RNA interference in the rodent retina in vivo and in vitro. Proceedings of the National Academy of Sciences of the United States of America *101*, 16-22.

Matsuda, T., Irie, T., Katsurabayashi, S., Hayashi, Y., Nagai, T., Hamazaki, N., Adefuin, A.M.D., Miura, F., Ito, T., Kimura, H.*, et al.* (2019). Pioneer Factor NeuroD1 Rearranges Transcriptional and Epigenetic Profiles to Execute Microglia-Neuron Conversion. Neuron *101*, 472-485 e477.

Mattar, P., Ericson, J., Blackshaw, S., and Cayouette, M. (2015). A conserved regulatory logic controls temporal identity in mouse neural progenitors. Neuron *85*, 497-504.

Mattar, P., Jolicoeur, C., Dang, T., Shah, S., Clark, B.S., and Cayouette, M. (2021). A Casz1-NuRD complex regulates temporal identity transitions in neural progenitors. Scientific reports *11*, 3858.

Mattar, P., Stevanovic, M., Nad, I., and Cayouette, M. (2018). Casz1 controls higher-order nuclear organization in rod photoreceptors. Proceedings of the National Academy of Sciences of the United States of America *115*, E7987-E7996.

Mayran, A., Khetchoumian, K., Hariri, F., Pastinen, T., Gauthier, Y., Balsalobre, A., and Drouin, J. (2018). Pioneer factor Pax7 deploys a stable enhancer repertoire for specification of cell fate. Nat Genet *50*, 259-269.

McLaughlin, M.E., Sandberg, M.A., Berson, E.L., and Dryja, T.P. (1993). Recessive mutations in the gene encoding the B-subunit of rod phosphodiesterase in patients with retinitis pigmentosa. Nature Genetics *4*, 130-134.

McLean, C.Y., Bristor, D., Hiller, M., Clarke, S.L., Schaar, B.T., Lowe, C.B., Wenger, A.M., and Bejerano, G. (2010). GREAT improves functional interpretation of cis-regulatory regions. Nat Biotechnol *28*, 495-501.

Mears, A.J., Kondo, M., Swain, P.K., Takada, Y., Bush, R.A., Saunders, T.L., Sieving, P.A., and Swaroop, A. (2001). Nrl is required for rod photoreceptor development. Nature Genetics *29*, 447-452.

Mendoza-Santiesteban, C.E., Palma, J.A., Hedges, T.R., 3rd, Laver, N.V., Farhat, N., Norcliffe-Kaufmann, L., and Kaufmann, H. (2017). Pathological Confirmation of Optic Neuropathy in Familial Dysautonomia. J Neuropathol Exp Neurol *76*, 238-244.

Michaelides, M., Holder, G.E., Hunt, D.M., Fitzke, F.W., Bird, A.C., and Moore, A.T. (2005). A detailed study of the phenotype of an autosomal dominant cone-rod dystrophy (CORD7) associated with mutation in the gene for RIM1. Br J Ophthalmol *89*, 198-206.

Mitashov, V.I., Panova, I.G., and Koussoulakos, S. (2004). Transdifferentiation Potential of Ciliary and Pigment Epithelial Cells in Lower Vertebrates and Mammals. Biology Bulletin *31*, 395-403.

Mitchell, P., Liew, G., Gopinath, B., and Wong, T.Y. (2018). Age-related macular degeneration. The Lancet *392*, 1147-1159.

Miyake, A., and Araki, M. (2014). Retinal stem/progenitor cells in the ciliary marginal zone complete retinal regeneration: a study of retinal regeneration in a novel animal model. Developmental neurobiology *74*, 739-756.

Moiseyev, G., Chen, Y., Takahashi, Y., Wu, B.X., and Ma, J.X. (2005). RPE65 is the isomerohydrolase in the retinoid visual cycle. Proceedings of the National Academy of Sciences of the United States of America *102*, 12413-12418.

Molday, L.L., Rabin, A.R., and Molday, R.S. (2000). ABCR expression in foveal cone photoreceptors and its role in Stargardt macular dystrophy. Nature Genetics *25*, 257-258.

Molday, R.S., and Moritz, O.L. (2015). Photoreceptors at a glance. J Cell Sci *128*, 4039-4045.

Montana, C.L., Kolesnikov, A.V., Shen, S.Q., Myers, C.A., Kefalov, V.J., and Corbo, J.C. (2013). Reprogramming of adult rod photoreceptors prevents retinal degeneration. Proceedings of the National Academy of Sciences of the United States of America *110*, 1732-1737.

Mori, K., Ando, F., Nomura, H., Sato, Y., and Shimokata, H. (2000). Relationship between intraocular pressure and obesity in Japan. International Journal of Epidemiology *29*, 661-666.

Munemasa, Y., and Kitaoka, Y. (2012). Molecular mechanisms of retinal ganglion cell degeneration in glaucoma and future prospects for cell body and axonal protection. Frontiers in cellular neuroscience *6*, 60.

Mustafi, D., Engel, A.H., and Palczewski, K. (2009). Structure of cone photoreceptors. Progress in retinal and eye research *28*, 289-302.

Nagel, G., Ollig, D., Fuhrmann, M., Kateriya, S., Musti, A.M., Bamberg, E., and Hegemann, P. (2002). Channelrhodopsin-1: a light-gated proton channel in green algae. Science *296*, 2395-2398.

Nakajima, Y., Inokuchi, Y., Nishi, M., Shimazawa, M., Otsubo, K., and Hara, H. (2008). Coenzyme Q10 protects retinal cells against oxidative stress in vitro and in vivo. Brain research *1226*, 226-233.

Nakazawa, M., Kikawa, M., Chida, Y., Wada, Y., Shiono, T., and Tamai, M. (1996a). Autosomal dominant cone-rod dystrophy associated with mutations in codon 244 (Asn244His) and codon 184 (Tyr184Ser) of the peripherin:RDS gene. ARCH OPHTHALMOL *114*, 72-78.

Nakazawa, M., Naoi, N., Wada, Y., Nakazaki, S., Maruiwa, F., Sawada, A., and Tamai, M. (1996b). Autosomal dominant cone-rod dystrophy associated with a Val200Glu mutation of the peripherin/RDS gene. Retina *16*, 405-410.

Nakazawa, T., Hisatomi, T., Nakazawa, C., Noda, K., Maruyama, K., She, H., Matsubara, A., Miyahara, S., Nakao, S., Yin, Y*., et al.* (2007). Monocyte chemoattractant protein 1 mediates retinal detachment-induced photoreceptor apoptosis. Proceedings of the National Academy of Sciences of the United States of America *104*, 2425-2430.

Narayan, D.S., Wood, J.P., Chidlow, G., and Casson, R.J. (2016). A review of the mechanisms of cone degeneration in retinitis pigmentosa. Acta Ophthalmol *94*, 748-754.

Nathans, J. (2010). Generation of an inducible Slc1a3-cre/ERT transgenic allele. MGI Direct Data Submission *MGI: J:157151*.

Ndubaku, U., and de Bellard, M.E. (2008). Glial cells: old cells with new twists. Acta Histochem *110*, 182-195.

Nelson, C.M., Ackerman, K.M., O'Hayer, P., Bailey, T.J., Gorsuch, R.A., and Hyde, D.R. (2013). Tumor necrosis factor-alpha is produced by dying retinal neurons and is required for Muller glia proliferation during zebrafish retinal regeneration. The Journal of neuroscience : the official journal of the Society for Neuroscience *33*, 6524-6539.

Newman, E.A. (2004). Glial modulation of synaptic transmission in the retina. Glia *47*, 268-274.

Newman, N.J. (2012). Treatment of hereditary optic neuropathies. Nat Rev Neurol *8*, 545-556.

Nieoullon, A. (2011). Neurodegenerative diseases and neuroprotection: current views and prospects. Journal of Applied Biomedicine *9*, 173-183.

Nishida, A., Furukawa, A., Koike, C., Tano, Y., Aizawa, S., Matsuo, I., and Furukawa, T. (2003). Otx2 homeobox gene controls retinal photoreceptor cell fate and pineal gland development. Nat Neurosci *6*, 1255-1263.

Niu, W., Zang, T., Zou, Y., Fang, S., Smith, D.K., Bachoo, R., and Zhang, C.L. (2013). In vivo reprogramming of astrocytes to neuroblasts in the adult brain. Nature cell biology *15*, 1164-1175.

Novotny, T., Eiselt, R., and Urban, J. (2002). Hunchback is required for the specification of the early sublineage of neuroblast 7-3 in the *Drosophila* central nervous system. Development *129*, 1027-1036.

Nucci, C., Martucci, A., Cesareo, M., Mancino, R., Russo, R., Bagetta, G., Cerulli, L., and Garaci, F.G. (2013). Brain involvement in glaucoma: advanced neuroimaging for understanding and monitoring a new target for therapy. Curr Opin Pharmacol *13*, 128-133.

O'Neill, D.W., Schoetz, S.S., Lopez, R.A., Castle, M., Rabinowitz, L., Shor, E., Krawchik, D., Goll, M.G., Renz, M., Seelig, H.-P*., et al.* (2000). An Ikaros-Containing Chromatin-Remodeling Complex in Adult-Type Erythroid Cells. Molecular and cellular biology *20*, 7572-7582.

Obernier, K., and Alvarez-Buylla, A. (2019). Neural stem cells: origin, heterogeneity and regulation in the adult mammalian brain. Development *146*.

Oberst, P., Agirman, G., and Jabaudon, D. (2019). Principles of progenitor temporal patterning in the developing invertebrate and vertebrate nervous system. Curr Opin Neurobiol *56*, 185-193.

Ooto, S., Akagi, T., Kageyama, R., Akita, J., Mandai, M., Honda, Y., and Takahashi, M. (2004). Potential for neural regeneration after neurotoxic injury in the adult mammalian retina. Proceedings of the National Academy of Sciences of the United States of America *101*, 13654-13659.

Ordaz, J.D., Wu, W., and Xu, X.M. (2017). Optogenetics and its application in neural degeneration and regeneration. Neural Regen Res *12*, 1197-1209.

Ortin-Martinez, A., Tsai, E.L., Nickerson, P.E., Bergeret, M., Lu, Y., Smiley, S., Comanita, L., and Wallace, V.A. (2016). A Reinterpretation of Cell Transplantation: GFP Transfer from Donor to Host Photoreceptors. Stem cells *35*, 932-939.

Osakada, F., Ooto, S., Akagi, T., Mandai, M., Akaike, A., and Takahashi, M. (2007). Wnt signaling promotes regeneration in the retina of adult mammals. The Journal of neuroscience : the official journal of the Society for Neuroscience *27*, 4210-4219.

Palanker, D., Le Mer, Y., Mohand-Said, S., Muqit, M., and Sahel, J.A. (2020). Photovoltaic Restoration of Central Vision in Atrophic Age-Related Macular Degeneration. Ophthalmology.

Palczewski, K., and Kiser, P.D. (2020). Shedding new light on the generation of the visual chromophore. Proceedings of the National Academy of Sciences of the United States of America *117*, 19629-19638.

Pan, L., Deng, M., Xie, X., and Gan, L. (2008). ISL1 and BRN3B co-regulate the differentiation of murine retinal ganglion cells. Development *135*, 1981-1990.

Parmar, M., Grealish, S., and Henchcliffe, C. (2020). The future of stem cell therapies for Parkinson disease. Nature reviews Neuroscience *21*, 103-115.

Patel, S.R., and Lieber, C.M. (2019). Precision electronic medicine in the brain. Nat Biotechnol *37*, 1007-1012.

Patro, R., Duggal, G., Love, M.I., Irizarry, R.A., and Kingsford, C. (2017). Salmon provides fast and bias-aware quantification of transcript expression. Nature methods *14*, 417-419.

Pearson, B.J., and Doe, C.Q. (2003). Regulation of neuroblast competence in Drosophila. Nature *425*, 624-628.

Pearson, R.A., Barber, A.C., Rizzi, M., Hippert, C., Xue, T., West, E.L., Duran, Y., Smith, A.J., Chuang, J.Z., Azam, S.A.*, et al.* (2012). Restoration of vision after transplantation of photoreceptors. Nature *485*, 99-103.

Pearson, R.A., Gonzalez-Cordero, A., West, E.L., Ribeiro, J.R., Aghaizu, N., Goh, D., Sampson, R.D., Georgiadis, A., Waldron, P.V., Duran, Y.*, et al.* (2016). Donor and host photoreceptors engage in material transfer following transplantation of post-mitotic photoreceptor precursors. Nature communications *7*, 13029.

Petrukhin, K., Koisti, M.J., Bakall, B., Li, W., Xie, G., Marknell, T., Sandgren, O., Forsman, K., Holmgren, G., Andreasson, S.*, et al.* (1998). Identification of the gene responsible for Best macular dystrophy. Nature Genetics *19*, 241-247.

Pickard, G.E., and Sollars, P.J. (2010). Intrinsically photosensitive retinal ganglion cells. Sci China Life Sci *53*, 58-67.

Piguet, F., Alves, S., and Cartier, N. (2017). Clinical Gene Therapy for Neurodegenerative Diseases: Past, Present, and Future. Hum Gene Ther *28*, 988-1003.

Pittler, S.J., and Baehr, W. (1991). Identification of a nonsense mutation in the rod photoreceptor cGMP phosphodiesterase beta-subunit gene of the rd mouse. Proceedings of the National Academy of Sciences of the United States of America *88*, 8322-8326.

Plessy, C., Pascarella, G., Bertin, N., Akalin, A., Carrieri, C., Vassalli, A., Lazarevic, D., Severin, J., Vlachouli, C., Simone, R.*, et al.* (2012). Promoter architecture of mouse olfactory receptor genes. Genome Res *22*, 486-497.

Pollak, J., Wilken, M.S., Ueki, Y., Cox, K.E., Sullivan, J.M., Taylor, R.J., Levine, E.M., and Reh, T.A. (2013). ASCL1 reprograms mouse Muller glia into neurogenic retinal progenitors. Development *140*, 2619-2631.

Polosukhina, A., Litt, J., Tochitsky, I., Nemargut, J., Sychev, Y., De Kouchkovsky, I., Huang, T., Borges, K., Trauner, D., Van Gelder, R.N.*, et al.* (2012). Photochemical restoration of visual responses in blind mice. Neuron *75*, 271-282.

Powell, C., Elsaeidi, F., and Goldman, D. (2012). Injury-dependent Muller glia and ganglion cell reprogramming during tissue regeneration requires Apobec2a and Apobec2b. The Journal of neuroscience : the official journal of the Society for Neuroscience *32*, 1096-1109.

Powell, C., R., G.A., Cornblath, E., and Goldman, D. (2013). Analysis of DNA methylation reveals a partial reprogramming of the Muller glia genome during retina regeneration. Proceedings of the National Academy of Sciences *110*, 19814-19819.

Pras, E., Abu, A., Rotenstreich, Y., Avni, I., Reish, O., Morad, Y., Reznik-Wolf, H., and Pras, E. (2009). Cone-rod dystrophy and a frameshift mutation in the PROM1 gene. Molecular vision *15*, 1709-1716.

Prodanov, D., and Delbeke, J. (2016). Mechanical and Biological Interactions of Implants with the Brain and Their Impact on Implant Design. Frontiers in neuroscience *10*, 11.

Punzo, C., Kornacker, K., and Cepko, C.L. (2009). Stimulation of the insulin/mTOR pathway delays cone death in a mouse model of retinitis pigmentosa. Nat Neurosci *12*, 44-52.

Purves, D., Augustine, G.J., Fitzpatrick, D., al., e., and editors (2001). Neuroscience, 2nd edition edn (Sunderland (MA): Sinauer Associates).

Radhakrishnan, D.M., and Goyal, V. (2018). Parkinson's disease: A review. Neurol India *66*, S26-S35.

Ramachandran, R., Fausett, B.V., and Goldman, D. (2010). Ascl1a regulates Muller glia dedifferentiation and retinal regeneration through a Lin-28-dependent, let-7 microRNA signalling pathway. Nature cell biology *12*, 1101-1107.

Ramachandran, R., Zhao, X.F., and Goldman, D. (2011). Ascl1a/Dkk/β-catenin signaling pathway is necessary and glycogen synthase kinase-3β inhibition is sufficient for zebrafish retina regeneration. PNAS *108*, 15858-15863.

Ramirez, F., Ryan, D.P., Gruning, B., Bhardwaj, V., Kilpert, F., Richter, A.S., Heyne, S., Dundar, F., and Manke, T. (2016). deepTools2: a next generation web server for deep-sequencing data analysis. Nucleic acids research *44*, W160-165.

Rao, M.B., Didiano, D., and Patton, J.G. (2017). Neurotransmitter-Regulated Regeneration in the Zebrafish Retina. Stem cell reports.

Rattner, A., and Nathans, J. (2006). Macular degeneration: recent advances and therapeutic opportunities. Nature reviews Neuroscience *7*, 860-872.

Reichenbach, A., and Bringmann, A. (2013). New functions of Muller cells. Glia *61*, 651-678.

Ribeiro, J., Procyk, C.A., West, E.L., O'Hara-Wright, M., Martins, M.F., Khorasani, M.M., Hare, A., Basche, M., Fernando, M., Goh, D.*, et al.* (2021). Restoration of visual function in advanced disease after transplantation of purified human pluripotent stem cell-derived cone photoreceptors. Cell reports *35*, 109022.

Roberts, M.R., Hendrickson, A., McGuire, C.R., and Reh, T.A. (2005). Retinoid X receptor (gamma) is necessary to establish the S-opsin gradient in cone photoreceptors of the developing mouse retina. Investigative ophthalmology & visual science *46*, 2897-2904.

Robinson, J.T., Thorvaldsdóttir, H., Winckler, W., Guttman, M., Lander, E.S., Getz, G., and Mesirov, J.P. (2011). Integrative genomics viewer. Nat Biotechnol *29*, 24-26.

Roesch, K., Jadhav, A.P., Trimarchi, J.M., Stadler, M.B., Roska, B., Sun, B.B., and Cepko, C.L. (2008). The transcriptome of retinal Muller glial cells. The Journal of comparative neurology *509*, 225-238.

Roosing, S., Rohrschneider, K., Beryozkin, A., Sharon, D., Weisschuh, N., Staller, J., Kohl, S., Zelinger, L., Peters, T.A., Neveling, K.*, et al.* (2013). Mutations in RAB28, encoding a farnesylated small GTPase, are associated with autosomal-recessive cone-rod dystrophy. Am J Hum Genet *93*, 110-117.

Rosenfeld, P.J., Heier, J.S., Hantsbarger, G., and Shams, N. (2006). Tolerability and efficacy of multiple escalating doses of ranibizumab (Lucentis) for neovascular age-related macular degeneration. Ophthalmology *113*, 623 e621.

Rossi, A.M., Fernandes, V.M., and Desplan, C. (2017). Timing temporal transitions during brain development. Curr Opin Neurobiol *42*, 84-92.

Rouaux, C., and Arlotta, P. (2013). Direct lineage reprogramming of post-mitotic callosal neurons into corticofugal neurons in vivo. Nature cell biology *15*, 214-221.

Rouaux, C., Bhai, S., and Arlotta, P. (2012). Programming and reprogramming neuronal subtypes in the central nervous system. Developmental neurobiology *72*, 1085-1098.

Sakami, S., Imanishi, Y., and Palczewski, K. (2019). Muller glia phagocytose dead photoreceptor cells in a mouse model of retinal degenerative disease. FASEB journal : official publication of the Federation of American Societies for Experimental Biology *33*, 3680-3692.

Salero, E., Blenkinsop, T.A., Corneo, B., Harris, A., Rabin, D., Stern, J.H., and Temple, S. (2012). Adult human RPE can be activated into a multipotent stem cell that produces mesenchymal derivatives. Cell Stem Cell *10*, 88-95.

Santos-Ferreira, T., Llonch, S., Borsch, O., Postel, K., Haas, J., and Ader, M. (2016). Retinal transplantation of photoreceptors results in donor–host cytoplasmic exchange. Nature communications *7*, 13028.

Santos-Ferreira, T.F., Borsch, O., and Ader, M. (2017). Rebuilding the Missing Part-A Review on Photoreceptor Transplantation. Front Syst Neurosci *10*, 1-14.

Sapkota, D., Chintala, H., Wu, F., Fliesler, S.J., Hu, Z., and Mu, X. (2014). Onecut1 and Onecut2 redundantly regulate early retinal cell fates during development. Proceedings of the National Academy of Sciences of the United States of America *111*, E4086-4095.

Sardar Pasha, S.P.B., Munch, R., Schafer, P., Oertel, P., Sykes, A.M., Zhu, Y., and Karl, M.O. (2017). Retinal cell death dependent reactive proliferative gliosis in the mouse retina. Scientific reports *7*, 9517.

Seddon, J.M., Ajani, U.A., and Mitchell, B.D. (1997). Familial Aggregation of Age-related Maculopathy. American Journal of Ophthalmology *123*, 199-206.

Sen, S.Q., Chanchani, S., Southall, T.D., and Doe, C.Q. (2019). Neuroblast-specific open chromatin allows the temporal transcription factor, Hunchback, to bind neuroblast-specific loci. eLife *8*, e44036.

Shekhar, K., Lapan, S.W., Whitney, I.E., Tran, N.M., Macosko, E.Z., Kowalczyk, M., Adiconis, X., Levin, J.Z., Nemesh, J., Goldman, M.*, et al.* (2016). Comprehensive Classification of Retinal Bipolar Neurons by Single-Cell Transcriptomics. Cell *166*, 1308-1323 e1330.

Shen, W., Fruttiger, M., Zhu, L., Chung, S.H., Barnett, N.L., Kirk, J.K., Lee, S., Coorey, N.J., Killingsworth, M., Sherman, L.S.*, et al.* (2012). Conditional Mullercell ablation causes independent neuronal and vascular pathologies in a novel transgenic model. The Journal of neuroscience : the official journal of the Society for Neuroscience *32*, 15715-15727.

Simunovic, M.P., Shen, W., Lin, J.Y., Protti, D.A., Lisowski, L., and Gillies, M.C. (2019). Optogenetic approaches to vision restoration. Experimental eye research *178*, 15-26.

Singh, M.S., Balmer, J., Barnard, A.R., Aslam, S.A., Moralli, D., Green, C.M., Barnea-Cramer, A., Duncan, I., and MacLaren, R.E. (2016). Transplanted photoreceptor precursors transfer proteins to host photoreceptors by a mechanism of cytoplasmic fusion. Nature communications *7*, 13537.

Singh, M.S., Issa, P.C., Butler, R., Martin, C., Lipinski, D.M., Sekaran, S., Barnard, A.R., and MacLaren, R.E. (2013). Reversal of end-stage retinal degeneration and restoration of visual function by photoreceptor transplantation. Proceedings of the National Academy of Sciences of the United States of America *110*, 1101-1106.

Singhal, S., Bhatia, B., Jayaram, H., Becker, S., Jones, M.F., Cottrill, P.B., Khaw, P.T., Salt, T.E., and Limb, G.A. (2012). Human Muller glia with stem cell characteristics

differentiate into retinal ganglion cell (RGC) precursors in vitro and partially restore RGC function in vivo following transplantation. Stem Cells Transl Med *1*, 188-199.

Srivastava, D., and DeWitt, N. (2016). In Vivo Cellular Reprogramming: The Next Generation. Cell *166*, 1386-1396.

Stingl, K., Mayer, A.K., Llavona, P., Mulahasanovic, L., Rudolph, G., Jacobson, S.G., Zrenner, E., Kohl, S., Wissinger, B., and Weisschuh, N. (2017). CDHR1 mutations in retinal dystrophies. Scientific reports *7*, 6992.

Stone, E.M., Lotery, A.J., Munier, F.L., Héon, E., Piguet, B., Guymer, R.H., Vandenburgh, K., Cousin, P., Nishimura, D., Swiderski, R.E*., et al.* (1999a). A single EFEMP1 mutation associated with both Malattia Leventinese and Doyne honeycomb retinal dystrophy. Nature Genetics *22*, 199-202.

Stone, J., Maslim, J., Valter-Kocsi, K., Mervin, K., Bowers, F., Chu, Y., Barnett, N., Provis, J., Lewis, G., Fischer, S.K*., et al.* (1999b). Mechanisms of Photoreceptor Death and Survival in Mammalian Retina. Progress in retinal and eye research *18*, 689-735.

Strauss, O. (2005). The retinal pigment epithelium in visual function. Physiol Rev *85*, 845-881.

Straznicky, K., and Gaze, R.M. (1971). The growth of the retina in Xenopus laevis: an autoradiographic study. J Embryol exp Morph *26*, 67-79.

Strunz, T., Kiel, C., Sauerbeck, B.L., and Weber, B.H.F. (2020). Learning from Fifteen Years of Genome-Wide Association Studies in Age-Related Macular Degeneration. Cells *9*.

Su, M., Hu, H., Lee, Y., d'Azzo, A., Messing, A., and Brenner, M. (2004). Expression Specificity of GFAP Transgenes. Neurochemical Research *29*, 2075-2093.

Su, Z., Niu, W., Liu, M.L., Zou, Y., and Zhang, C.L. (2014). In vivo conversion of astrocytes to neurons in the injured adult spinal cord. Nature communications *5*, 3338.

Suga, A., Sadamoto, K., Fujii, M., Mandai, M., and Takahashi, M. (2014). Proliferation potential of Muller glia after retinal damage varies between mouse strains. PloS one *9*, e94556.

Sugiyama, Y., and Mukohata, Y. (1984). Isolation and characterization of halorhodopsin from *Halobacterium halobium*. J Biochem *96*, 413-420.

Sun, H., Molday, R.S., and Nathans, J. (1999). Retinal Stimulates ATP Hydrolysis by Purified and Reconstituted ABCR, the Photoreceptor-specific ATP-binding Cassette Transporter Responsible for Stargardt Disease. Journal of biological chemistry *274*, 8269-8281.

Sun, H., Tsunenari, T., Yau, K.W., and Nathans, J. (2002). The vitelliform macular dystrophy protein defines a new family of chloride channels. Proceedings of the National Academy of Sciences of the United States of America *99*, 4008-4013.

Sun, L., Li, P., Carr, A.L., Gorsuch, R., Yarka, C., Li, J., Bartlett, M., Pfister, D., Hyde, D.R., and Li, L. (2014). Transcription of the SCL/TAL1 interrupting Locus (Stil) is required for cell proliferation in adult Zebrafish Retinas. The Journal of biological chemistry *289*, 6934-6940.

Suspitsin, E.N., and Imyanitov, E.N. (2016). Bardet-Biedl Syndrome. Mol Syndromol *7*, 62-71.

Swaroop, A., Wang, Q.-L., Wu, W., Cook, J., Coats, C., Xu, S., Chen, S., Zack, D.J., and Sieving, P.A. (1999). Leber congenital amaurosis caused by a homozygous mutation (R90W) in the homeodomain of the retinal transcription factor CRX: direct evidence for the involvement of CRX in the development of photoreceptor function. Human molecular genetics *8*, 299-305.

Takahashi, K., and Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell *126*, 663-676.

Tham, Y.C., Li, X., Wong, T.Y., Quigley, H.A., Aung, T., and Cheng, C.Y. (2014). Global prevalence of glaucoma and projections of glaucoma burden through 2040: a systematic review and meta-analysis. Ophthalmology *121*, 2081-2090.

Thiadens, A.A., den Hollander, A.I., Roosing, S., Nabuurs, S.B., Zekveld-Vroon, R.C., Collin, R.W., De Baere, E., Koenekoop, R.K., van Schooneveld, M.J., Strom, T.M.*, et al.* (2009). Homozygosity mapping reveals PDE6C mutations in patients with early-onset cone photoreceptor disorders. Am J Hum Genet *85*, 240-247.

Thomas, J.L., Morgan, G.W., Dolinski, K.M., and Thummel, R. (2018). Characterization of the pleiotropic roles of Sonic Hedgehog during retinal regeneration in adult zebrafish. Experimental eye research *166*, 106-115.

Thoreson, W.B., and Mangel, S.C. (2012). Lateral interactions in the outer retina. Progress in retinal and eye research *31*, 407-441.

Tochitsky, I., Kienzler, M.A., Isacoff, E., and Kramer, R.H. (2018). Restoring Vision to the Blind with Chemical Photoswitches. Chem Rev *118*, 10748-10773.

Todd, L., Finkbeiner, C., Wong, C.K., Hooper, M.J., and Reh, T.A. (2020). Microglia Suppress Ascl1-Induced Retinal Regeneration in Mice. Cell reports *33*, 108507.

Tout, S., Chan-Ling, T., Holländer, H., and Stone, J. (1993). The role of müller cells in the formation of the blood-retinal barrier. Neuroscience *55*, 291-301.

Trapani, I., and Auricchio, A. (2018). Seeing the Light after 25 Years of Retinal Gene Therapy. Trends in molecular medicine *24*, 669-681.

Trivli, A., Koliarakis, I., Terzidou, C., Goulielmos, G.N., Siganos, C.S., Spandidos, D.A., Dalianis, G., and Detorakis, E.T. (2019). Normal-tension glaucoma: Pathogenesis and genetics. Exp Ther Med *17*, 563-574.

Tropepe, V., Coles, B.L.K., Chiasson, B.J., Horsford, J.D., Elia, A.J., McInnes, R.R., and van der Kooy, D. (2000). Retinal Stem Cells in the Adult Mammalian Eye. Science *287*, 2032-2036.

Tsu, A.P., Burish, M.J., GodLove, J., and Ganguly, K. (2015). Cortical neuroprosthetics from a clinical perspective. Neurobiol Dis *83*, 154-160.

Turner, D., and Cepko, C. (1987). A common progenitor for neurons and glia persists in rat retina late in development. Nature *328*, 131-136.

Turner, D., Snyder, E.Y., and Cepko, C. (1990). Lineage-independent determination of cell type in the embryonic mouse retina. Neuron *4*, 833-845.

Ueki, Y., and Reh, T.A. (2013). EGF stimulates Muller glial proliferation via a BMP-dependent mechanism. Glia *61*, 778-789.

Ueki, Y., Wilken, M.S., Cox, K.E., Chipman, L., Jorstad, N., Sternhagen, K., Simic, M., Ullom, K., Nakafuku, M., and Reh, T.A. (2015). Transgenic expression of the proneural transcription factor Ascl1 in Muller glia stimulates retinal regeneration in young mice. Proceedings of the National Academy of Sciences of the United States of America *112*, 13717-13722.

Van de Sande, B., Flerin, C., Davie, K., De Waegeneer, M., Hulselmans, G., Aibar, S., Seurinck, R., Saelens, W., Cannoodt, R., Rouchon, Q.*, et al.* (2020). A scalable SCENIC workflow for single-cell gene regulatory network analysis. Nat Protoc *15*, 2247-2276.

van der Raadt, J., van Gestel, S.H.C., Nadif Kasri, N., and Albers, C.A. (2019). ONECUT transcription factors induce neuronal characteristics and remodel chromatin accessibility. Nucleic acids research *47*, 5587-5602.

VandenBosch, L.S., Wohl, S.G., Wilken, M.S., Hooper, M., Finkbeiner, C., Cox, K., Chipman, L., and Reh, T.A. (2020). Developmental changes in the accessible chromatin, transcriptome and Ascl1-binding correlate with the loss in Muller Glial regenerative potential. Scientific reports *10*, 13615.

Venugopalan, P., Wang, Y., Nguyen, T., Huang, A., Muller, K.J., and Goldberg, J.L. (2016). Transplanted neurons integrate into adult retinas and respond to light. Nature communications *7*, 10472.

Vervoort, R., Lennon, A., Bird, A.C., Tulloch, B., Axton, R., Miano, M.G., Meindl, A., Meitinger, T., Ciccodicola, A., and Wright, A.F. (2000). Mutational hot spot within a new RPGR exon in X-linked retinitis pigmentosa. Nature Genetics *25*, 462-466.

Vierbuchen, T., Ostermeier, A., Pang, Z.P., Kokubu, Y., Sudhof, T.C., and Wernig, M. (2010). Direct conversion of fibroblasts to functional neurons by defined factors. Nature *463*, 1035-1041.

Virchow, R. (1856). Gesammelte Abbildung zur wissenschaftlichen Medizin. Verlag von Meidinger Sohn & Comp.

Vithana, E.N., Abu-Safieh, L., Allen, M.J., Carey, A., Papaioannou, M., Chakarova, C., Al-Maghtheh, M., Ebenezer, N.D., Willis, C., Moore, A.T.*, et al.* (2001). A Human Homolog of Yeast Pre-mRNA Splicing Gene, PRP31, Underlies Autosomal Dominant Retinitis Pigmentosa on Chromosome 19q13.4 (RP11). Mol Cell *8*, 375-381.

Waddington, C. (1957). The Strategy of the Genes; a Discussion of Some Aspects of Theoretical Biology (London: Allen & Unwin).

Waldron, P.V., Di Marco, F., Kruczek, K., Ribeiro, J., Graca, A.B., Hippert, C., Aghaizu, N.D., Kalargyrou, A.A., Barber, A.C., Grimaldi, G.*, et al.* (2018). Transplanted Donor- or Stem Cell-Derived Cone Photoreceptors Can Both Integrate and Undergo Material Transfer in an Environment-Dependent Manner. Stem cell reports.

Walker, F.O. (2007). Huntington's disease. The Lancet *369*, 218-228.

Wallace, D.C., Singh, G., Lott, M.T., Hodge, J.A., Schurr, T.G., Lezza, A.M.S., Elsas II, L.J., and Nikoskelainen, E.K. (1988). Mitochondrial DNA Mutation Associated with Leber's Hereditary Optic Neuropathy. Science *242*, 1427-1430.

Wan, J., Ramachandran, R., and Goldman, D. (2012). HB-EGF is necessary and sufficient for Muller glia dedifferentiation and retina regeneration. Developmental cell *22*, 334-347.

Wan, J., Zhao, X.F., Vojtek, A., and Goldman, D. (2014). Retinal Injury, Growth Factors, and Cytokines Converge on beta-Catenin and pStat3 Signaling to Stimulate Retina Regeneration. Cell reports.

Wan, J., Zheng, H., Chen, Z.L., Xiao, H.L., Shen, Z.J., and Zhou, G.M. (2008). Preferential regeneration of photoreceptor from Muller glia after retinal degeneration in adult rat. Vision research *48*, 223-234.

Wan, J., Zheng, H., Xiao, H.L., She, Z.J., and Zhou, G.M. (2007). Sonic hedgehog promotes stem-cell potential of Muller glia in the mammalian retina. Biochemical and biophysical research communications *363*, 347-354.

Wan, Y., Almeida, A.D., Rulands, S., Chalour, N., Muresan, L., Wu, Y., Simons, B.D., He, J., and Harris, W.A. (2016). The ciliary marginal zone of the zebrafish retina: clonal and time-lapse analysis of a continuously growing tissue. Development *143*, 1099-1107.

Wang, J., O'Sullivan, M.L., Mukherjee, D., Punal, V.M., Farsiu, S., and Kay, J.N. (2017). Anatomy and spatial organization of Muller glia in mouse retina. The Journal of comparative neurology *525*, 1759-1777.

Wang, J.S., and Kefalov, V.J. (2009). An alternative pathway mediates the mouse and human cone visual cycle. Current biology : CB *19*, 1665-1669.

Wang, L.-L., Garcia, C.S., Zhong, X., Ma, S., and Zhang, C.-L. (2020a). Rapid and efficient in vivo astrocyte-to-neuron conversion with regional identity and connectivity? bioRxiv.

Wang, X., Iannaccone, A., and Jablonski, M.M. (2005). Contribution of Müller cells toward the regulation of photoreceptor outer segment assembly. Neuron Glia Biology *1*, 1-6.

Wang, X., Yu, C., Tzekov, R.T., Zhu, Y., and Li, W. (2020b). The effect of human gene therapy for RPE65-associated Leber's congenital amaurosis on visual function: a systematic review and meta-analysis. Orphanet J Rare Dis *15*, 49.

Wapinski, O.L., Lee, Q.Y., Chen, A.C., Li, R., Corces, M.R., Ang, C.E., Treutlein, B., Xiang, C., Baubet, V., Suchy, F.P.*, et al.* (2017). Rapid Chromatin Switch in the Direct Reprogramming of Fibroblasts to Neurons. Cell reports *20*, 3236-3247.

Wapinski, O.L., Vierbuchen, T., Qu, K., Lee, Q.Y., Chanda, S., Fuentes, D.R., Giresi, P.G., Ng, Y.H., Marro, S., Neff, N.F.*, et al.* (2013). Hierarchical mechanisms for direct reprogramming of fibroblasts to neurons. Cell *155*, 621-635.

Weber, B.H.F., Vogt, G., Pruett, R.C., Stohr, H., and Felbor, U. (1994). Mutations in the tissue inhibitor of metalloproteinases-3 (TIMP3) in patients with Sorsby's fundus dystrophy. Nature Genetics *8*, 352-356.

Weng, J., Mata, N.L., Azarian, S.M., Tzekov, R.T., Birch, D.G., and Travis, G.H. (1999). Insights into the Function of Rim Protein in Photoreceptors and Etiology of Stargardt's Disease from the Phenotype in abcr Knockout Mice. Cell *98*, 13-23.

Wetts, R., Serbedzija, G.N., and Fraser, S.E. (1989). Cell lineage analysis reveals multipotent precursors in the ciliary margin of the frog retina. Dev Biol *136*, 254-263.

White, D.T., Senhupta, S., Saxena, M.T., Xu, Q., Hanes, J., Ding, D., Ji, H., and Mumm, J.S. (2017). Immunomodulation-accelerated neuronal regeneration following selective rod photoreceptor cell ablation in the zebrafish retina. PNAS, E3719-E3728.

Wisniewski, T., and Goni, F. (2015). Immunotherapeutic approaches for Alzheimer's disease. Neuron *85*, 1162-1176.

Wolf, F.A., Angerer, P., and Theis, F.J. (2018). SCANPY: large-scale single-cell gene expression data analysis. Genome Biol *19*, 15.

Wong, T.Y., Cheung, C.M., Larsen, M., Sharma, S., and Simo, R. (2016). Diabetic retinopathy. Nat Rev Dis Primers *2*, 16012.

Wright, A.F., Chakarova, C.F., Abd El-Aziz, M.M., and Bhattacharya, S.S. (2010). Photoreceptor degeneration: genetic and mechanistic dissection of a complex trait. Nature reviews Genetics *11*, 273-284.

Wright, A.V., Nunez, J.K., and Doudna, J.A. (2016). Biology and Applications of CRISPR Systems: Harnessing Nature's Toolbox for Genome Engineering. Cell *164*, 29-44.

Xiong, W., MacColl Garfinkel, A.E., Li, Y., Benowitz, L.I., and Cepko, C.L. (2015). NRF2 promotes neuronal survival in neurodegeneration and acute nerve damage. The Journal of clinical investigation *125*, 1433-1445.

Xue, K., and MacLaren, R.E. (2020). Antisense oligonucleotide therapeutics in clinical trials for the treatment of inherited retinal diseases. Expert Opin Investig Drugs *29*, 1163-1170.

Xue, Y., Ouyang, K., Huang, J., Zhou, Y., Ouyang, H., Li, H., Wang, G., Wu, Q., Wei, C., Bi, Y.*, et al.* (2013). Direct conversion of fibroblasts to neurons by reprogramming PTB-regulated microRNA circuits. Cell *152*, 82-96.

Yan, W., Laboulaye, M.A., Tran, N.M., Whitney, I.E., Benhar, I., and Sanes, J.R. (2020). Mouse Retinal Cell Atlas: Molecular Identification of over Sixty Amacrine Cell Types. The Journal of neuroscience : the official journal of the Society for Neuroscience *40*, 5177-5195.

Yang, Y., Mohand-Said, S., Danan, A., Simonutti, M., Fontaine, V., Clerin, E., Picaud, S., Leveillard, T., and Sahel, J.A. (2009). Functional cone rescue by RdCVF protein in a dominant model of retinitis pigmentosa. Mol Ther *17*, 787-795.

Yang, Z., Peachey, N., Moshfeghi, D.M., Thirumalaichary, S., Chorich, L., Shugart, Y.Y., Fan, K., and Zhang, K. (2002). Mutations in the RPGR gene cause X-linked cone dystrophy. Human molecular genetics *11*, 605-611.

Yao, K., Qiu, S., Tian, L., Snider, W.D., Flannery, J.G., Schaffer, D.V., and Chen, B. (2016). Wnt Regulates Proliferation and Neurogenic Potential of Muller Glial Cells via a Lin28/let-7 miRNA-Dependent Pathway in Adult Mammalian Retinas. Cell reports *17*, 165-178.

Yao, K., Qiu, S., Wang, Y.V., Park, S.J.H., Mohns, E.J., Mehta, B., Liu, X., Chang, B., Zenisek, D., Crair, M.C.*, et al.* (2018). Restoration of vision after de novo genesis of rod photoreceptors in mammalian retinas. Nature *560*, 484-488.

Yoshii, C., Ueda, Y., Okamoto, M., and Araki, M. (2007). Neural retinal regeneration in the anuran amphibian Xenopus laevis post-metamorphosis: transdifferentiation of retinal pigmented epithelium regenerates the neural retina. Dev Biol *303*, 45-56.

Young, R.W. (1967). The renewal of photoreceptor cell outer segments. J Cell Biol *33*, 61-72.

Young, R.W. (1985). Cell differentiation in the retina of the mouse. Anat Rec *212*, 199-205.

Yu, D.Y., Cringle, S.J., Balaratnasingam, C., Morgan, W.H., Yu, P.K., and Su, E.N. (2013). Retinal ganglion cells: Energetics, compartmentation, axonal transport, cytoskeletons and vulnerability. Progress in retinal and eye research *36*, 217-246.

Zhang, K., Kniazeva, M., Han, M., Li, W., Yu, Z., Yang, Z., Li, Y., Metzger, M.L., Allikmets, R., Zack, D.J.*, et al.* (2001). A 5-bp deletion in ELOVL4 is associated with two related forms of autosomal dominant macular dystrophy. Nature Genetics *27*, 89-93.

Zhao, X.F., Wan, J., Powell, C., Ramachandran, R., Myers, M.G., Jr., and Goldman, D. (2014). Leptin and IL-6 Family Cytokines Synergize to Stimulate Muller Glia Reprogramming and Retina Regeneration. Cell reports.

Zhou, H., Su, J., Hu, X., Zhou, C., Li, H., Chen, Z., Xiao, Q., Wang, B., Wu, W., Sun, Y.*, et al.* (2020). Glia-to-Neuron Conversion by CRISPR-CasRx Alleviates Symptoms of Neurological Disease in Mice. Cell.

Zhou, M., Wang, W., Huang, W., and Zhang, X. (2014). Diabetes mellitus as a risk factor for open-angle glaucoma: a systematic review and meta-analysis. PloS one *9*, e102972.

Zhu, J., Ming, C., Fu, X., Duan, Y., Hoang, D.A., Rutgard, J., Zhang, R., Wang, W., Hou, R., Zhang, D.*, et al.* (2017). Gene and mutation independent therapy via CRISPR-Cas9 mediated cellular reprogramming in rod photoreceptors. Cell Res *27*, 830-833.