Retinal regeneration in birds and mice

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ABSTRACT

Retinal regeneration from resident Müller glia cells is robust in teleost fish, but is severely limited in birds and mammals. After neurotoxic injury, chick Müller glia can proliferate, and activate neurogenic genes, but they display limited capacity to differentiate into neurons. Developmental signaling molecules enhance this process. Regeneration of retinal neurons in rodents is even more limited. However, studies show evidence of proliferation and neurogenic gene expression after injury, with stronger effects in rats than mice, and differences between mouse strains. Mitogenic and Wnt signaling potentiate the proliferative response, while misexpression of the proneural transcription factor, *Ascl1*, reprograms to generate neurons from Müller glial *in vitro*, and stimulates neuronal regeneration in young mice, *in vivo*.

The regeneration of ocular tissues, including the sensory neural retina, has been known to occur in urodeles for over 200 years [1], but only recently have we begun to elucidate components of the molecular pathways that underlie the phenomenon. There are several known sources of new neurons and glia to repair damage in the retina in various species, including contributions from stem cells at the retinal margin and transdifferentiation of pigmented epithelial cells into retinal progenitors (Figure 1A) [2]. Although both of these phenomenon occur robustly in urodeles and larval anurans, neither of these two processes occurs in post-embryonic birds or mammals. Instead, in fish [3], birds [4] and to a very limited extent, mice, there is an intrinsic glial cell in the retina, the Müller glial cell, which provides a source of new neurons after retinal injury (Figure 1B). In this review, we focus on these cells.

Retinal response to injury in birds

The first report that demonstrated Müller glia could serve as a source of regenerated neurons was from work done in posthatch chicks [4]. After neurotoxic injury to the posthatch chick retina, the majority of Müller glia re-enter the mitotic cell cycle, undergo one cell division, express genes associated with neurogenic progenitors (eg. *Ascl1*, *Notch*, *FoxN4*), and produce retinal neurons [4, 5]: primarily HuCD+ amacrine cells. While most of the Müller glia adopt a progenitor-like pattern of gene expression, only a few differentiate into neurons, and of these nearly all are inner retinal neurons, not photoreceptors. The regeneration from Müller glia in birds is much more limited than that observed in zebrafish, where retinal injury stimulates the production of all types of new retinal neurons from the Müller glia [6].

The initial report of damage induced regeneration, was followed on by studies demonstrating that this process could be stimulated by injections of mitogens, like EGF, FGF, and IGF or insulin [7, 8]. More recently this has also been shown to be the case for the fish retina. Other factors that are involved in retinal regeneration in fish, have also been shown to play a role in birds, like *Shh* and *hbEGF* [9, 10]. There are other factors, like the glucocorticoid receptor[11], which

have thus far only been shown to be active in retinal regeneration in birds. Notch signaling has also been shown to be critical for both the initiation of regeneration and the percentage of Müller glial-derived progenitors that differentiate into neurons [5]. Together, these studies indicate that many of the initial steps in the regenerative response to injury in birds and fish are similar; however, there are clear limits on the numbers and types of neurons that are produced after injury in bird retina and future studies are needed to address this difference.

The retinal response to injury in mammals.

The mammalian retina reacts to injury in much the same way as other regions of the central nervous system; instead of repair and regeneration, mammalian Müller glia undergo a process called reactive gliosis [12]. Reactive gliosis has been primarily characterized in astrocytes in the brain, but Müller glia react to retinal injury in a similar manner. Reactive Müller glia increase in size and up-regulate intermediate filament genes, including Vimentin, Nestin, and Glial Fibrillary Acidic Protein (GFAP).

In the short term, reactive gliosis has a neuro-protective effect, through the release of antioxidants, neurotrophic factors, and the re-uptake of excess glutamate, which would otherwise exacerbate excitotoxic effects from the injury [12, 13]. Reactive gliosis in astrocytes is also sometimes accompanied by re-entry into the cell cycle and proliferation, resulting in the formation of a glial scar, which can negatively impact neuronal functions. This process of proliferative gliosis also results in the release of pro-inflammatory cytokines, such as $\text{TNF}\alpha$, which can have a cytotoxic effect on neurons [14]. The extensive remodeling that occurs after the loss of rods and cones in Retinitis Pigmentosa may be an extreme example of the negative consequences of reactive gliosis of Müller glia [15, 16].

Like most regions of the CNS, the mammalian retina has long been thought to possess no ability to generate new neurons after they are lost from injury or disease; however, in light of the evidence for regeneration in fish [3] and avian retina [4], there has been a closer examination of the mouse and rat retina after injury for a

regenerative response. One of the first steps in regeneration in fish is re-entry of the Müller glia into the cell cycle [17]; the focus of many investigators has been to (1) assess Müller glial proliferation after injury, and (2) determine whether new neurons are generated from the proliferating cells.

To address these questions, a variety of different types of retinal injury have been tested. In rats, retinal injury from NMDA, MNU, NaIO₃, ouabain, or excessive light induce a small percentage of the Müller glia to re-enter the mitotic cycle, as evidenced by BrdU incorporation and mitotic markers like PCNA and Ki67 [18-20]. Interestingly, sub-toxic doses of glutamate or direct stimulation of the Müller glia by alpha-aminoadipate (AAA) also induce proliferation of the Müller glia [21]. The proliferative response of Müller glia in mouse retina is not as consistently observed as that of the rat retina. In mice, AAA, glutamate, NMDA MNU and light damage have also been shown to promote Müller glial proliferation [21-23], particularly when combined with mitogenic growth factors (see below); however, the number of Müller glia that enter the cell cycle is lower than that observed in rat retina, and in some reports few, if any, proliferating Müller glia were observed in adult mouse retina in vivo [24]. Together these studies show that mammalian Müller glia can respond to injury with a proliferative response, but the percentage of Müller glia that respond is much lower than in non-mammalian vertebrates.

The zebrafish responds to retinal injury by expression of various signaling molecules and growth factors that are necessary and/or sufficient for Müller glial regeneration [25]. Therefore, several groups have tested the hypothesis that treatment of the injured rodent retina with exogenous signaling molecules will enhance the regenerative response of Müller glia. Karl et al., (2008) [22] demonstrated that treatment of the NMDA-injured adult mouse retina with either EGF or FGF and Insulin, resulted in a significant increase in Müller glia proliferation. Transgenic lineage tracing confirmed that the Müller glia were the source of the BrdU-labeled cells, some of which later differentiated into GAD-67-GFP+ amacrine cells [22]. FGF2 and Insulin had the similar effect of enhancing proliferation in the 2-week post-natal rat retina damaged by NMDA [26], but not the adult rat [18].

Several studies have reported that the effect of mitogen stimulation is dependent on the age of the animal. EGF stimulation in rat retina induced robust Müller glia proliferation at postnatal day 10, but not at P14, due to a down-regulation of the EGF-receptor (EGFR). However, light damage caused an up-regulation of EGFR, restoring EGF stimulation of proliferation [27]. A similar age-dependent decline in Müller glia proliferation was observed in mouse retinas that were damaged by *ex vivo* culture and treated with EGF [28, 29]. The strong proliferative effect of damage and EGF treatment at P8 was markedly reduced when damage began at P12. The Müller glia may down-regulate receptors for key mitogenic factors as a way to maintain their quiescence, since there is normally little to no ongoing addition of either neurons or Müller glia in the mature mammalian retina.

In addition to EGF, several other signaling pathways have been tested for their potential to enhance the regenerative response of Müller glia. Canonical Wnt/ β -Catenin, Sonic Hedgehog, Retinoic Acid, BDNF, and VPA, have all been tested in either mouse, rat or both, for their potential to promote retinal regeneration from Müller glia (Table) [26, 30-32]. Most of these studies have been carried out in vitro and not all have ruled out the possibility that at least some of the proliferating cells were endothelial cells or retinal astrocytes. Other evidence has further complicated the interpretation of the early studies. Joly et al, 2011, found that light damage in adult mice induced cell cycle genes, including CyclinD1 and Phospho-Histone-3, in Müller glia, but the cells did not incorporate BrdU during the observation period (6-36 hrs) [24]. BrdU+ cells were present in the ONL, but these cells appeared to be undergoing DNA damage repair, not mitotic proliferation. Studies that demonstrate co-labeling between BrdU and Müller glia specific markers, or those that use lineage tracing from the Müller glia, are necessary to distinguish between these possibilities.

Strain differences also complicate the interpretation of retinal regeneration experiments in rodents. Suga et al. [31] observed differences in the proliferative capacity of Müller glia between mouse strains: C57BL/6 (BL6), 129x1/SvJ (129),

and BDNF. After injury, BrdU+ Müller glia were rare in BL6 retinas, but 30-fold higher in retinas from 129 mice. A Wnt signaling activator induced a further increase in Müller glia proliferation, but had no effect in retinas from BL6 mice. Thus, different mouse strains can differ greatly in their inherent capacity to respond to signaling molecules and re-enter the cell cycle.

Taken together, these studies show that some amount of Müller glial proliferation occurs after retinal injury, in rats and some strains of mice, and that this can be stimulated by many of the same growth factors that act in the regeneration pathway in fish. However, the evidence that new neurons are generated from glia is much less robust. Although some neural markers were co-localized with BrdU in some studies, most of these early studies did not use confocal imaging and/or nuclear markers of neurons to definitively demonstrate co-labeling. In addition, these studies did not use lineage tracing to demonstrate that the origin of BrdU+ neuronal cells was actually the Müller glia. Going forward, these caveats will be critical for the evaluation of retinal regeneration in mammals.

Stimulating retinal regeneration with proneural transcription factors

Another potentially powerful strategy for stimulating regeneration is reprogramming Müller glia to a neurogenic state by experimentally expressing proneural transcription factors [18]. A variety of protocols have now been developed to directly reprogram fibroblasts or astrocytes to neurons using proneural transcription factors [33, 34]. One of the crucial factors in retinal regeneration in zebrafish is activation of the proneural transcription factor, *ascl1a* [35]. The mammalian homolog, *Ascl1*, is expressed in neural progenitors during development and important for progenitor proliferation as well as proper neuronal differentiation [36]. It has recently been shown that *Ascl1* is capable of reprogramming mammalian fibroblasts [37] and astrocytes [38] directly into neurons *in vitro*, presumably through its ability to 'pioneer' closed chromatin [39]. In the adult mouse, *Ascl1* is not expressed following damage or growth factor treatment [22]. To test whether the lack of expression of this critical factor was

sufficient to convert mouse Müller glia to a neurogenic state, Pollak et al (2013) used a lentivirus to drive expression of *Ascl1* in dissociated cultures of Müller glia. They found that the cells could be reprogrammed to a neural progenitor state with high efficiency [40]. *Ascl1* transduction caused the Müller glia to express neural progenitor genes, including Notch pathway components and other proneural transcription factors, while glial genes were down-regulated. The reprogrammed Müller glia generated cells that expressed neuronal morphology, neuronal markers (Figure 1), and showed a response to NMDA and kainate similar to inner retinal neurons. These results suggest that proneural transcription factors might be sufficient to stimulate neural regeneration in the mammalian retina from Müller glia.

To test the hypothesis that proneural transcription factors could induce regeneration in mammalian retina, Ueki et al. [41] used a transgenic approach. By directing *Ascl1* specifically to Müller glia with a *Glast-CreER/tetO-Ascl1* transgene *in vivo* they found that *Ascl1* was sufficient to promote regeneration of new retinal neurons after NMDA induced damage in mice up to two weeks old (Figure 2). Markers of mature neuronal cell types were induced, including those of amacrine cells (*HuC/D*, *Pax6*), bipolar cells (*Otx2*, *Cabp5*), and photoreceptors (*Otx2*, *Rcvn*), which had migrated to the ONL. In addition, reprogrammed cells often exhibited cell type specific morphology, lamination, and even showed evidence for synaptic connections. These studies indicate that expression of the proneural transcription factor *Ascl1* is sufficient to convey a neurogenic progenitor state to mammalian Müller glia *in vivo* and the cells respond to injury much like they do in birds and fish, regenerating new neurons.

Regeneration from Human Müller Glia

What are the prospects that Müller glia can serve as a source for new neurons? Müller cells can be grown from adult human retinas in dissociated culture and a subpopulation of these cells undergoes spontaneous immortalization [42]. Several studies have shown some ability of these cells to be reprogrammed to express neural genes *in vitro*, and when transplanted to animals with retinal

degenerations, can provide some benefit [43-46]. However, it is not clear from these reports whether the cells were actually differentiating into specific types of retinal neurons in vivo after transplantation, or provided trophic support to the host neurons. Future studies demonstrating neuronal morphology and connectivity of lineage traced cells will be critical to determine the potential of human MG for reprogramming and regeneration.

Conclusions/Perspective

The field of retinal regeneration has evolved over the past ten years from demonstrations that small numbers of new neurons could be made after injury to a systematic study of the restriction points to glial reprogramming and the molecular mechanisms underlying these constraints. It is becoming apparent that many of the initial stages in the response to injury are conserved among animals like fish that regenerate well, and those like mice that have little capacity to regenerate. For example, some of the changes described as reactive gliosis in mammals (eg. GFAP expression and hypertrophy) [47] also occur in fish after injury and are associated with regeneration; growth factors, like CNTF and HB-EGF are increased spontaneously in mammalian retina after injury, as they are in fish, and in mice have been implicated as survival factors, rather than stimulants of regeneration. However, these molecular responses ultimately diverge. In adult mice, these growth factors do not induce robust Müller glial proliferation, though they do in birds and fish (and possibly some other mammals or mouse strains). Future studies of mitotic inhibitors might provide new targets for stimulating regeneration.

Another key difference between birds and fish on the one hand and mammals on the other is in the activation of the proneural program. *Ascl1* is expressed spontaneously in fish and birds after injury, but not at appreciable levels in mice and rats. Very little is known about the factors that regulate *Ascl1* expression. A recent report suggests that the REST complex is active in Müller glia and may repress *Ascl1* expression in these cells [48]. Further analysis of miRNAs in Müller glia will likely identify additional candidates in mice, since work in zebrafish has shown *Lin28* and *let-7* play an important role in regulating *Ascl1* expression in

that species [49]. BMP signaling also seems to be important in mammalian Müller glial differentiation [50], and increases after injury, along with BMP targets Id1/3 (potential inhibitors of Ascl1). Id1/3 are rapidly downregulated in fish regeneration [51, 52], suggesting that BMP signaling is not active in the injured fish retina. The regulation of Ascl1 both transcriptionally and post-transcriptionally is not well understood in any species.

Transcription factors, like Ascl1, are potent activators of downstream targets of neural progenitors, and studies with mouse Müller glia have now shown that Ascl1 can drive proneural reprogramming sufficient to generate neurons in vitro and in vivo. However, like the bird retina, mouse Müller glia forced to express Ascl1 generate primarily inner retinal neurons, eg. bipolar cells and amacrine cells. While many of the transcriptional regulators that control cell fate in the developing retina have been identified, we are far from a complete understanding of the developmental network that controls the production of the various cell types in appropriate ratios. It is likely that a better understanding of the normal developmental processes of cell fate specification will lead to more effective strategies for stimulating regeneration in this system. It is also important to understand the role that epigenetic factors, like chromatin modifications, play in restricting the potential of mammalian Müller glia to generate multipotent retinal progenitors, as they do in fish. The chromatin in Müller glia is much less accessible at key neuronal differentiation genes than in retinal progenitors [41]. Targeted epigenetic engineering strategies could be critical in effectively regulating reprogramming for regeneration in the retina. Most importantly, no study has yet demonstrated functional integration of Müller glial-derived neurons in mouse retina; it remains an open question as to how well newly-generated neurons will integrate into a mature retinal circuitry in adult mammals. A better understanding of the factors that regulate circuit formation in the retina will also be important in translating the findings from recent reprogramming studies to ultimately restore function to the damaged human retina.

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Figure Legends

Figure 1. The neural retina (NR) is a thin sheet of neural tissue derived from the optic cup, an evagination of the neural tube. In fish, amphibians, and young birds, new retinal neurons are produced by a zone of stem cells in the ciliary marginal zone (blue, CMZ, also called circumferential germinal zone). After retinal injury, amphibians have the capacity to regenerate new retinal neurons from the CMZ, or alternatively from the pigmented epithelial cells (brown, RPE), a layer of cells adjacent to the neural retina and also derived from the optic cup. Fish and birds do not regenerate neurons from the RPE (except in early development), but instead a glial cell within the retina, called the Müller glia (B, red), has the potential in fish to regenerate all types of neurons, and in birds to regenerate bipolar cells and amacrine cells (B, red).

Figure 2. Müller glia can be maintained in dissociated culture (A) and grow much like astrocytes. When infected with a lentivirus to induce *Ascl1* expression, the cells are reprogrammed to produce cells with a neuronal morphology (B) and express neural markers, like *Map2* (C). The Müller glial-specific *Rbp1-CreER* allows lineage tracing of the cells to demonstrate directly they can differentiate into neurons after viral-expression of *Ascl1* (modified from Pollak et al).

Figure 3. Transgenic expression of *Ascl1* in Müller glia provides them with competence to regenerate retinal neurons after injury in young mice in vivo. (A, A') Undamaged retina; normal Müller glial morphology (*Glast-CreER/Ascl1-IRES-GFP*, green) and expression of *Sox9* (red). (B, B') Light damage to the retina causes some of the *Ascl1*-expressing Müller glia to express cell cycle markers (PH3, white). (C, D, E) NMDA damage to the retina causes some *Ascl1*-expressing Müller glia to differentiate into cells with distinctive neuronal morphologies of amacrine cells (C, arrow), bipolar cells (D) and rod photoreceptors (E, E').

References

1. Bermingham-McDonogh, O., and Reh, T.A. (2011). Regulated reprogramming in the regeneration of sensory receptor cells. *Neuron* 71, 389-405.
2. Moshiri, A., Close, J., and Reh, T.A. (2004). Retinal stem cells and regeneration. *Int J Dev Biol* 48, 1003-1014.
3. Goldman, D. (2014). Müller glial cell reprogramming and retina regeneration. *Nat Rev Neurosci* 15, 431-442.
4. Fischer, A.J., and Reh, T.A. (2001). Müller glia are a potential source of neural regeneration in the postnatal chicken retina. *Nat Neurosci* 4, 247-252.
5. Hayes, S., Nelson, B.R., Buckingham, B., and Reh, T.A. (2007). Notch signaling regulates regeneration in the avian retina. *Dev Biol* 312, 300-311.
6. Raymond, P.A., and Hitchcock, P.F. (1997). Retinal regeneration: common principles but a diversity of mechanisms. *Adv Neurol* 72, 171-184.
7. Fischer, A.J., and Reh, T.A. (2002). Exogenous growth factors stimulate the regeneration of ganglion cells in the chicken retina. *Dev Biol* 251, 367-379.
8. Fischer, A.J., McGuire, C.R., Dierks, B.D., and Reh, T.A. (2002). Insulin and fibroblast growth factor 2 activate a neurogenic program in Müller glia of the chicken retina. *J Neurosci* 22, 9387-9398.
9. Todd, L., Volkov, L.I., Zelinka, C., Squires, N., and Fischer, A.J. (2015). Heparin-binding EGF-like growth factor (HB-EGF) stimulates the proliferation of Müller glia-derived progenitor cells in avian and murine retinas. *Mol Cell Neurosci* 69, 54-64.
10. Todd, L., and Fischer, A.J. (2015). Hedgehog signaling stimulates the formation of proliferating Müller glia-derived progenitor cells in the chick retina. *Development* 142, 2610-2622.
11. Gallina, D., Zelinka, C., and Fischer, A.J. (2014). Glucocorticoid receptors in the retina, Müller glia and the formation of Müller glia-derived progenitors. *Development* 141, 3340-3351.
12. 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 Müller cell gliosis: neuroprotective and detrimental effects. *Prog Retin Eye Res* 28, 423-451.
13. Bringmann, A., Pannicke, T., Biedermann, B., Francke, M., Iandiev, I., Grosche, J., Wiedemann, P., Albrecht, J., and Reichenbach, A. (2009). Role of retinal glial cells in neurotransmitter uptake and metabolism. *Neurochem Int* 54, 143-160.
14. Bringmann, A., and Wiedemann, P. (2009). Involvement of Müller glial cells in epiretinal membrane formation. *Graefes Arch Clin Exp Ophthalmol* 247, 865-883.
15. Jones, B.W., Watt, C.B., and Marc, R.E. (2005). Retinal remodelling. *Clinical & experimental optometry* 88, 282-291.

16. Marc, R.E., Jones, B.W., Watt, C.B., Vazquez-Chona, F., Vaughan, D.K., and Organisciak, D.T. (2008). Extreme retinal remodeling triggered by light damage: implications for age related macular degeneration. *Mol Vis* *14*, 782-806.
17. Lenkowski, J.R., and Raymond, P.A. (2014). Müller glia: Stem cells for generation and regeneration of retinal neurons in teleost fish. *Prog Retin Eye Res* *40*, 94-123.
18. 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. *Proc Natl Acad Sci U S A* *101*, 13654-13659.
19. Wan, J., Zheng, H., Chen, Z.L., Xiao, H.L., Shen, Z.J., and Zhou, G.M. (2008). Preferential regeneration of photoreceptor from Müller glia after retinal degeneration in adult rat. *Vision Res* *48*, 223-234.
20. Wan, J., Zheng, H., Xiao, H.L., She, Z.J., and Zhou, G.M. (2007). Sonic hedgehog promotes stem-cell potential of Müller glia in the mammalian retina. *Biochem Biophys Res Commun* *363*, 347-354.
21. Takeda, M., Takamiya, A., Jiao, J.W., Cho, K.S., Trevino, S.G., Matsuda, T., and Chen, D.F. (2008). alpha-Aminoadipate induces progenitor cell properties of Müller glia in adult mice. *Invest Ophthalmol Vis Sci* *49*, 1142-1150.
22. 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. *Proc Natl Acad Sci U S A* *105*, 19508-19513.
23. Harada, C., Guo, X., Namekata, K., Kimura, A., Nakamura, K., Tanaka, K., Parada, L.F., and Harada, T. (2011). Glia- and neuron-specific functions of TrkB signalling during retinal degeneration and regeneration. *Nat Commun* *2*, 189.
24. Joly, S., Pernet, V., Samardzija, M., and Grimm, C. (2011). Pax6-positive Müller glia cells express cell cycle markers but do not proliferate after photoreceptor injury in the mouse retina. *Glia* *59*, 1033-1046.
25. Hamon, A., Roger, J.E., Yang, X.J., and Perron, M. (2015). Müller glial cell-dependent regeneration of the neural retina: An overview across vertebrate model systems. *Dev Dyn*.
26. 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 Müller glia in the mammalian retina: regulation by Notch and Wnt signaling. *Dev Biol* *299*, 283-302.
27. Close, J.L., Liu, J., Gumuscu, B., and Reh, T.A. (2006). Epidermal growth factor receptor expression regulates proliferation in the postnatal rat retina. *Glia* *54*, 94-104.
28. Löffler, K., Schafer, P., Volkner, M., Holdt, T., and Karl, M.O. (2015). Age-dependent Müller glia neurogenic competence in the mouse retina. *Glia* *63*, 1809-1824.
29. Ueki, Y., Karl, M.O., Sudar, S., Pollak, J., Taylor, R.J., Loeffler, K., Wilken, M.S., Reardon, S., and Reh, T.A. (2012). P53 is required for the developmental restriction in Müller glial proliferation in mouse retina. *Glia* *60*, 1579-1589.

30. Osakada, F., Ooto, S., Akagi, T., Mandai, M., Akaike, A., and Takahashi, M. (2007). Wnt signaling promotes regeneration in the retina of adult mammals. *J Neurosci* 27, 4210-4219.
31. Suga, A., Sadamoto, K., Fujii, M., Mandai, M., and Takahashi, M. (2014). Proliferation potential of Müller glia after retinal damage varies between mouse strains. *PLoS One* 9, e94556.
32. 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 Müller cells in adult mammalian retina. *PLoS One* 5, e12425.
33. Ang, C.E., and Wernig, M. (2014). Induced neuronal reprogramming. *J Comp Neurol* 522, 2877-2886.
34. Heinrich, C., Blum, R., Gascon, S., Masserdotti, G., Tripathi, P., Sanchez, R., Tiedt, S., Schroeder, T., Gotz, M., and Berninger, B. (2010). Directing astroglia from the cerebral cortex into subtype specific functional neurons. *PLoS Biol* 8, e1000373.
35. Fausett, B.V., Gumerson, J.D., and Goldman, D. (2008). The proneural basic helix-loop-helix gene *ascl1a* is required for retina regeneration. *J Neurosci* 28, 1109-1117.
36. Castro, D.S., Martynoga, B., Parras, C., Ramesh, V., Pacary, E., Johnston, C., Drechsel, D., Lebel-Potter, M., Garcia, L.G., Hunt, C., et al. (2011). A novel function of the proneural factor *Ascl1* in progenitor proliferation identified by genome-wide characterization of its targets. *Genes Dev* 25, 930-945.
37. 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.
38. Berninger, B., Costa, M.R., Koch, U., Schroeder, T., Sutor, B., Grothe, B., and Gotz, M. (2007). Functional properties of neurons derived from in vitro reprogrammed postnatal astroglia. *J Neurosci* 27, 8654-8664.
39. 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.
40. 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 Müller glia into neurogenic retinal progenitors. *Development* 140, 2619-2631.
41. 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 Müller glia stimulates retinal regeneration in young mice. *Proc Natl Acad Sci U S A* 112, 13717-13722.
42. 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 Müller glial cell lines derived from adult human retina exhibit neural stem cell characteristics. *Stem Cells* 25, 2033-2043.
43. 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 Müller 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.
44. Bhatia, B., Jayaram, H., Singhal, S., Jones, M.F., and Limb, G.A. (2011). Differences between the neurogenic and proliferative abilities of Müller glia with stem cell characteristics and the ciliary epithelium from the adult human eye. *Exp Eye Res* 93, 852-861.
 45. 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 Müller glia restore rod function in the P23H rat. *Stem Cells Transl Med* 3, 323-333.
 46. Giannelli, S.G., Demontis, G.C., Pertile, G., Rama, P., and Broccoli, V. (2011). Adult human Müller glia cells are a highly efficient source of rod photoreceptors. *Stem Cells* 29, 344-356.
 47. Thomas, J.L., Ranski, A.H., Morgan, G.W., and Thummel, R. (2016). Reactive gliosis in the adult zebrafish retina. *Exp Eye Res* 143, 98-109.
 48. Wohl, S.G., and Reh, T.A. (2016). miR-124-9-9* potentiates Ascl1-induced reprogramming of cultured Müller glia. *Glia*.
 49. Powell, C., Elsaiedi, F., and Goldman, D. (2012). Injury-dependent Müller glia and ganglion cell reprogramming during tissue regeneration requires Apobec2a and Apobec2b. *J Neurosci* 32, 1096-1109.
 50. Ueki, Y., Wilken, M.S., Cox, K.E., Chipman, L.B., Bermingham-McDonogh, O., and Reh, T.A. (2015). A transient wave of BMP signaling in the retina is necessary for Müller glial differentiation. *Development* 142, 533-543.
 51. Qin, Z., and Raymond, P.A. (2012). Microarray-based gene profiling analysis of Müller glia-derived retinal stem cells in light-damaged retinas from adult zebrafish. *Methods Mol Biol* 884, 255-261.
 52. Qin, Z., Barthel, L.K., and Raymond, P.A. (2009). Genetic evidence for shared mechanisms of epimorphic regeneration in zebrafish. *Proc Natl Acad Sci U S A* 106, 9310-9315.

37

(** of outstanding interest)

Ueki, Y., Wilken, M.S., Cox, K.E., Chipman, L.B., Bermingham-McDonogh, O., and Reh, T.A. (2015). A transient wave of BMP signaling in the retina is necessary for Müller glial differentiation. *Development* 142, 533-543.

Demonstrated generation of bipolar and photoreceptor-like cells by transgenic overexpression of Ascl1 in lineage-traced Müller glia in vivo, requiring neurotoxic injury and young age.

36

(** of outstanding interest)

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 Müller glia into neurogenic retinal progenitors. *Development* 140, 2619-2631.

Viral misexpression of Ascl1 in lineage-traced Müller glia in vitro caused reprogramming to a neurogenic state and genome-wide characterization of gene expression changes.

35

(** of outstanding interest)

Fausett, B.V., Gumerson, J.D., and Goldman, D. (2008). The proneural basic helix-loop-helix gene ascl1a is required for retina regeneration. *J Neurosci* 28, 1109-1117.

Demonstrated the crucial importance of the proneural gene Ascl1a in regeneration of neurons in zebrafish.

31

(** of outstanding interest)

Suga, A., Sadamoto, K., Fujii, M., Mandai, M., and Takahashi, M. (2014). Proliferation potential of Müller glia after retinal damage varies between mouse strains. PLoS One 9, e94556.

Illuminating study indicating that different mouse strains have different intrinsic regenerative capacities, with implications for how regeneration studies should be reported and standardized.

22

(* of special interest)

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. Proc Natl Acad Sci U S A 105, 19508-19513.

Demonstrated that NMDA neurotoxic injury in the adult mouse retina, combined with mitogenic growth factors, caused Müller glia re-entry into the cell cycle and generation of a small number of amacrine cells.

18

(* of special interest)

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. Proc Natl Acad Sci U S A 101, 13654-13659.

This study showed that NMDA neurotoxic injury in the adult rat retina caused a subset of Müller glia to re-enter the cell cycle and possibly generate bipolar cells.

4

(* of special interest)

Fischer, A.J., and Reh, T.A. (2001). Müller glia are a potential source of neural regeneration in the postnatal chicken retina. Nat Neurosci 4, 247-252.

First demonstration of Müller glial proliferation, neurogenic gene re-activation, and generation of some amacrine cells in the post-hatch chick retina after neurotoxic injury.

figure 01

figure 02

P12 MG GFP

P12 MG Ascl1

Rlbp1-cre^{ERT2} ; tdTomato P12 ASCL1

figure 3

table 1

	Age	Damage > Proliferation	GFs > Proliferation	Strain Difference	TFs > Neurons
Rat	Adult	Very Low	Shh, Wnt	No Data	NeuroD, Math3
	Post-natal	Yes	EGF, FGF Shh, Wnt	No Data	No Data
Mouse	Adult	Extremely low	EGF, FGF, Insulin, Wnt	Yes	No Data
	Post-natal	Yes	EGF, Wnt	No Data	Ascl1
Chick	Post-hatch	Yes	EGF, FGF, Insulin, IGF, Notch	No Data	No Data
<p>Species and age specific differences in regenerative response of Muller glia to retinal injury. The columns indicate: whether Muller glia proliferation is observed after damage; which growth factors or signaling molecules have been observed to potentiate the proliferative response; if there are observed strain differences within the indicated species; and which, if any, transcription factors (TFs) lead to production of neuronal-like cells from Muller glia. See text for details.</p>					