Müller Glial Cell-Dependent Regeneration of the Neural Retina: An Overview Across Vertebrate Model Systems

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Retinal dystrophies are a major cause of blindness for which there are currently no curative treatments. Transplantation of stem cell-derived neuronal progenitors to replace lost cells has been widely investigated as a therapeutic option. Another promising strategy would be to trigger self-repair mechanisms in patients, through the recruitment of endogenous cells with stemness properties. Accumulating evidence in the past 15 year0s has revealed that several retinal cell types possess neurogenic potential, thus opening new avenues for regenerative medicine. Among them, Müller glial cells have been shown to be able to undergo a reprogramming process to re-acquire a stem/progenitor state, allowing them to proliferate and generate new neurons for repair following retinal damages. Although Müller cell–dependent spontaneous regeneration is remarkable in some species such as the fish, it is extremely limited and ineffective in mammals. Understanding the cellular events and molecular mechanisms underlying Müller cell activities in species endowed with regenerative capacities could provide knowledge to unlock the restricted potential of their mammalian counterparts. In this context, the present review provides an overview of Müller cell responses to injury across vertebrate model systems and summarizes recent advances in this rapidly evolving field. *Developmental Dynamics 245:727–738, 2016.* © 2015 The Authors. Developmental Dynamics published by Wiley Periodicals, Inc.

Key words: retina; stem cells; Müller cells; regeneration

Introduction

Vision loss is a major social, medical, and economical issue, affecting millions of people around the world. Neurodegenerative diseases of the retina involve the progressive and irreversible loss of retinal neurons, leading to impaired visual function. These retinal dystrophies include retinitis pigmentosa, age-related macular degeneration, glaucoma, and diabetic retinopathy. Despite being a major cause of disability, there are only limited therapeutic options that can slow down the progression of some of these diseases, but no viable treatments so far. The recent progress in stem cell research has opened new avenues for cell-based therapies. For instance, major advances regarding cell transplantation strategies have been made using exogenous photoreceptors derived from embryonic or induced pluripotent stem cells (West et al., 2009; Perron et al., 2012; Jayakody et al., 2015). However, tremendous challenges exist to achieve integration and reconstitution of the retinal network from exogenous cell sources. An

alternative and attractive therapeutic strategy is to stimulate the patient's endogenous retinal stem-(like) cells to replace lost neuronal cells. This self-repair process commonly used by several vertebrate species is the focus of the present review.

The anatomical structure of the retina, its cellular composition, as well as the order in which the various retinal cell types are produced during development, are highly conserved across all vertebrates (Livesey and Cepko, 2001). However, the regenerative modalities and capacities are tremendously different among vertebrate classes and even between species in a given class. In contrast to mammals, which have none or very limited regenerative capacities, efficient endogenous repair occurs following retinal damage in a variety of nonmammalian vertebrates (Moshiri et al., 2004; Stenkamp, 2007; Lamba et al., 2008, 2009; Karl and Reh, 2010; Brockerhoff and Fadool, 2011; Gemberling et al., 2013; Hidalgo et al., 2014). In addition to the contrasting regenerative efficiencies reported in different animal models, distinct modalities of regeneration have been identified based on the cellular sources involved. These sources include the retinal pigment epithelium (RPE, the cell layer overlying the neural retina), the ciliary margin (the peripheral region that contains retinal stem cells)
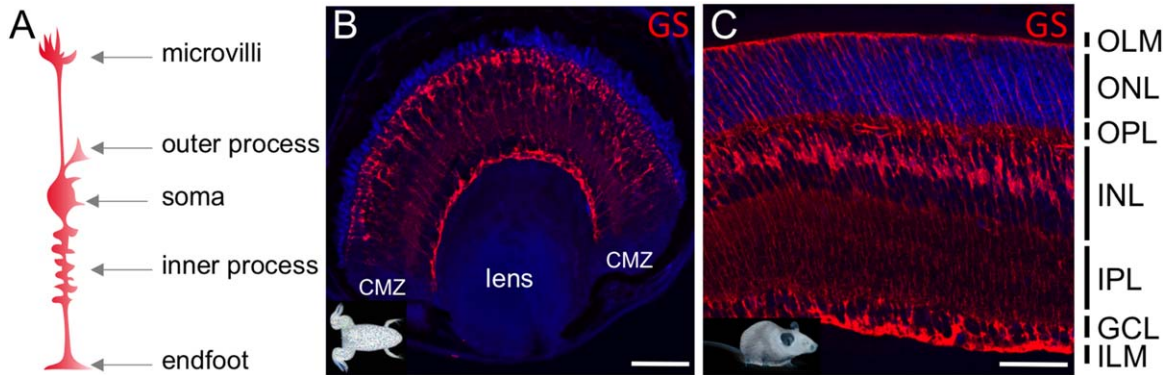
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Fig. 1. Müller glial cells. **A**: Schema of Müller glial cell morphology. **B,C**: Immunofluorescence analysis of glutamine synthetase (GS, red), a Müller glial cell marker, on transversal sections of stage 45 *Xenopus laevis* tadpole (B) and postnatal day 10 mouse (C) retina. Cell nuclei are counterstained with DAPI (blue). Although Müller cells from different species may vary in shape, these images illustrate some common features. It shows how the radially oriented processes of Müller cells span the entire thickness of the retina. Their soma resides in the inner nuclear layer (INL), while their apical (microvilli) and basal processes (endfeet) project to the outer or the inner limiting membranes (OLM and INL), respectively. CMZ, ciliary marginal zone; ONL, outer nuclear layer; OPL, outer plexiform layer; IPL, inner plexiform layer; GCL, ganglion cell layer. Scale bar: 50 μM.

and Müller glial cells. Here, we focus exclusively on the latter given the spectacular upsurge of interest in the regenerative potential of these cells across species in recent years, with the aim to provide an overview of this rapidly evolving field (Ahmad et al., 2011; Belecky-Adams et al., 2013; Karl, 2013; Gallina et al., 2014a; Goldman, 2014; Gorsuch and Hyde, 2014; Lenkowski and Raymond, 2014; Ng et al., 2014; Jayakody et al., 2015).

The Müller Cell: More than Just a Glial Cell

Müller glia, named after their discoverer, the German anatomist Heinrich Müller, were first described in 1851 as radial fibers in the fish, frog, and pigeon retina (Müller, 1851; Reichenbach and Bringmann, 2010) (Fig. 1). Müller glia represents approximately 4–5% of retinal cells (Strettoi and Masland, 1995; Jeon et al., 1998). They span the entire thickness of the retina, allowing for a symbiotic relationship with adjacent neurons. Indeed, Müller cells play a wide variety of roles that contribute to the maintenance of retinal homeostasis and visual function, including a trophic support for retinal neurons (Bringmann et al., 2006; Reichenbach and Bringmann, 2010) and as a second source of visual pigment regeneration especially for cone photoreceptors (Kaylor et al., 2013, 2014).

Müller cells are the only type of retinal glia that share a common embryonic origin with retinal neurons (Turner and Cepko, 1987; Holt et al., 1988; Wetts and Fraser, 1988). Of note, a recent lineage study in the mouse suggests that a subset of Müller cells may be derived from the neural crest (Liu et al., 2014). This is quite unexpected and thus clearly deserves further investigation and comparative studies in different vertebrate species.

Müller cells are among the latest cells to be born during development in all vertebrate retinas. Transcriptomic analyses revealed great similarities between the molecular repertoire of Müller glia and multipotent late retinal progenitors (Blackshaw et al., 2004; Livesey et al., 2004; Roesch et al., 2008; Jadhav et al., 2009). Müller glia thus acquire some specialized glial functions but maintain a molecular signature of late stage progenitor cells (Jadhav et al., 2009). Such similarity could explain the remarkable properties of these cells to acquire a stem-like state and serve as a source of retinal neurons in case of injury in certain species.

Below, we review recent advances in this area, highlighting similarities and differences in Müller cell response to retinal damage in various vertebrate classes.

Müller Cell Response to Injury in Fish

Müller Cells Are Involved in Adult Neurogenesis

As fish grow continuously throughout their lives, their retinas not only stretch but also constantly generate new neurons to keep pace with the enlarging body. It has been long understood that this adult neurogenesis occurs in a germinal zone at the margin of the retina (Johns, 1977). The presence of genuine retinal stem cells in this peripheral region, so-called ciliary marginal zone (CMZ), has recently been demonstrated (Centanin et al., 2011). The CMZ, however, is not the only site of adult neurogenesis in the fish retina. New rod photoreceptors are generated from resident proliferative cells in the inner nuclear layer of the central retina (Johns and Fernald, 1981; Johns, 1982; Julian et al., 1998; Otteson et al., 2001; Otteson and Hitchcock, 2003). The identity of these cells remained a mystery for many years until lineage tracing studies in 2006 formally revealed their Müller glial cell of origin (reviewed in Lenkowski and Raymond, 2014). In the postembryonic fish, Müller cells divide slowly and sporadically to generate fate-restricted rod progenitors that supply the growing retina with new rod photoreceptors.

Müller Cells Are Involved in Retinal Regeneration

The initial evidence of effective retinal regeneration in teleosts was provided in adult goldfish following surgical removal of one quadrant of the neural retina (Lombardo, 1968). Additional studies of this phenomenon in goldfish and zebrafish clearly demonstrated the replacement of all missing neurons after different methods of injury such as cytotoxic lesion (Maier and Wolburg, 1979; Raymond et al., 1988; Negishi et al., 1991), surgical approach (Hitchcock et al., 1992), laser or light damage (Braisted et al., 1994; Vihtelic and Hyde, 2000). As expected from the known sites of normal adult neurogenesis, two cellular sources of regeneration were identified, the CMZ and the resident proliferative cells of the inner nuclear layer that were at the time not yet

identified as Müller cells (Maier and Wolburg, 1979; Raymond et al., 1988). In the early 2000s, many studies showed that Müller glia respond to injuries, in particular through their increased proliferation (Vihtelic and Hyde, 2000; Wu et al., 2001; Yurco and Cameron, 2005; Raymond et al., 2006; Vihtelic et al., 2006). Later, using cell lineage-tracing studies in transgenic fish, Müller glia were formally recognized as the major source of endogenous stem cells for retinal regeneration (Fausett and Goldman, 2006; Bernardos et al., 2007; Fimbel et al., 2007). Although proliferating Müller cells only give rise to rod photoreceptors under physiological conditions (as mentioned above), they regenerate all types of retinal neurons following injury (Stenkamp, 2011).

Müller Cell Behavior in a Regenerating Retina

Müller glia cells respond locally and rapidly to injury. They first exhibit reactive gliosis, before initiate the regenerative program (Thomas et al., 2015). Because Müller glia in the adult zebrafish retina can be considered to be multipotent stem cells, it has been argued that they do not require a "reprogramming" event in the classic sense (Lenkowski and Raymond, 2014). Nevertheless, they undergo changes in gene expression which enable the re-acquisition of a progenitor-like state (reviewed in Goldman, 2014). However, it should be noted that Müller glia in the damaged fish retina do not dedifferentiate completely (see below). Among up-regulated genes in injury-responsive Müller cells, some well-known reprogramming factors have been identified, such as *Ascl1, Lin-28,* and *Stat3* (Kassen, 2007; Fausett et al., 2008; Ramachandran et al., 2010). They form a regulatory cascade: *Ascl1* and *Stat3* positively regulate each other while *Ascl1* acts upstream of *Lin-28,* which represses the micro-RNA *let-7,* a repressor of regeneration-associated genes (Ramachandran et al., 2010; Nelson et al., 2012). Further work is needed to shed more light on the intricate genetic networks involved in Müller cell activation since transcriptional gene profiling in isolated Müller glia revealed close to a thousand transcripts differentially regulated after retinal injury (Qin et al., 2009; Qin and Raymond, 2012).

The dedifferentiation process of Müller cells likely involves epigenetic modifications, as expected from our knowledge of somatic cell reprogramming during induced pluripotent stem (iPS) cell generation (Krishnakumar and Blelloch, 2013). Dynamic changes in DNA methylation were indeed shown to underlie Müller cell response to injury (Powell et al., 2013). However, the promoters of pluripotency factors were surprisingly found to be already hypomethylated in quiescent Müller cells (Powell et al., 2013). This appears consistent with the aforementioned idea that Müller cells retain certain progenitor-like features and are therefore poised to re-enter the cell cycle in case of injury. This hypomethylation likely facilitates the dedifferentiation process and therefore enables a quick response to retinal injury (Powell et al., 2013).

Similar to retinal progenitor cells during development, responsive Müller cells exhibit interkinetic nuclear movements, with their nuclei migrating apically to undergo mitosis (Lahne et al., 2013; Nagashima et al., 2013). Activated Müller cells undergo a single asymmetric division to self-renew and produce a proliferating retinal progenitor (Nagashima et al., 2013; Lenkowski and Raymond, 2014) (Fig. 2). The newly formed daughter cell divides rapidly and repeatedly to generate a cluster of progenitor cells. These migrate, basally or apically according to the cell type that need to be replaced, along the radial processes that the dedifferentiated Müller cell has preserved (Fausett and Goldman, 2006; Bernardos et al., 2007; Stenkamp, 2011; Nagashima et al., 2013). The fact that these activated Müller cells retain a radial shape throughout the regenerative response likely participates in preserving the cytoarchitecture of the retina (Lenkowski and Raymond, 2014). Cells in the active Müller cell-derived clusters then differentiate and generate new neurons for retinal repair, retinal ganglion cells in case of ouabain-induced cytotoxicity or photoreceptors in case of light damages (Nagashima et al., 2013).

Influence of the Injury Paradigm

The location and extent of Müller cell activation has been compared in multiple injury models affecting different classes of retinal neurons (reviewed in Ng et al., 2014). Müller cells re-enter the cell cycle at the site of the injury but proliferation differentially spreads out depending on the lesional paradigm (Yurco and Cameron, 2005). Furthermore, there is a correlation between the amount of cell proliferation and the extent of photoreceptor cell death (Vihtelic et al., 2006; Thomas et al., 2012). In the case of severe and extensive damages, new neurons are overproduced by Müller cells, leading to histological defects (Sherpa et al., 2014). In addition, Müller glia response is slower after the loss of inner neurons than after photoreceptor cell death (Nagashima et al., 2013). Taken together, these observations strongly suggest that the type and extent of retinal insults likely lead to the release of different types/amounts of signals at the injury site and that Müller cell are differentially sensitive and responsive to this changing microenvironment.

Extrinsic Signals Triggering Müller Cell Activation

Although the molecular mechanisms driving Müller glia response to injury in fish are still poorly understood, advances these last few years allowed the identification of a variety of extrinsic cues and signaling pathways involved in this process (Fig. 3). Apoptotic cells constitute a potential source of diffusible molecules in the damaged retina. Following zebrafish retina injury, the secreted pro-inflammatory cytokine tumor necrosis factor alpha (TNFα) is produced by dying retinal neurons and triggers Müller cell dedifferentiation and proliferation (Nelson et al., 2013). Moreover, it has been proposed that the subsequent increase of TNFα expression in Müller cells themselves drives the generation of a secondary population of proliferating Müller glia. Regarding the downstream pathway, TNFα was shown to impinge on *Stat3* and *Ascl1* genes in Müller cells (Nelson et al., 2013).

The phagocytic capacity of fish Müller glial cells was first demonstrated in vitro but such activity had not been observed in vivo (Wagner and Raymond, 1991). It has since been shown that Müller cells respond to photoreceptor cell death by engulfing remnant debris of apoptotic rods in vivo (Morris et al., 2005; Thomas et al., 2015), as previously reported in mammals (Egensperger et al., 1996). Such phagocytic activity was shown to be essential to trigger Müller glia proliferation but independently of *Ascl1* and *Stat3* expression regulation (Bailey et al., 2010). An alternate underlying molecular mechanism (or interpretation) thus remains to be discovered.

Recent studies demonstrated that Müller glia cells are a source of growth factors and cytokines that regulate their own regenerative response in an autocrine/paracrine manner (Wan et al., 2014; Zhao et al., 2014a). These include interleukin-6 family
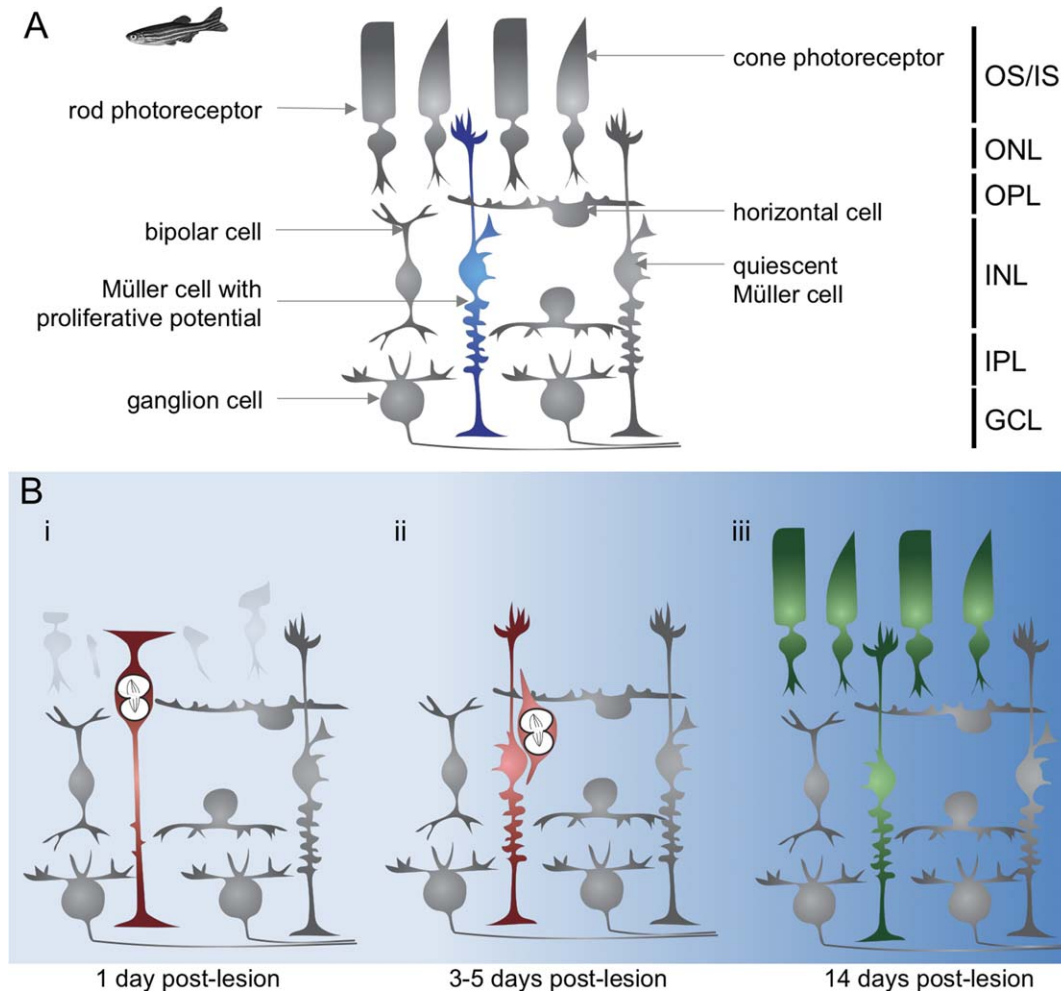
Fig. 2. Müller cell response to light-induced retinal damage in zebrafish. **A**: Schematic of the different cell type organization in the zebrafish retina. OS/IS, outer and inner segments of rods and cones; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer; NFL, optic nerve fiber layer. **B: (i)** Intense light exposure causes selective loss of photoreceptors (light grey) in the larval or adult zebrafish retina. Such injury triggers the dedifferentiation of a subset of Müller cells (in blue in panel A), which re-enter the cell cycle (red). The mitosis occurs apically as the injury-induced Müller nuclei undergo interkinetic nuclear migration. **(ii)** The injury-induced Müller glia divides only once, asymmetrically, in a self-renewing mode that preserves the differentiated Müller glia cell and produces a multipotent retinal progenitor (both in red). This progenitor divides rapidly and repeatedly to generate a neurogenic cluster of cells tightly associated with the radial processes of the dedifferentiated Müller cell. **(iii)** The daughter cells in the cluster migrate and differentiate to replace lost photoreceptor cells (in green). Given that injury-induced Müller cells self-renew (in green), the total number of Müller glia returns to normal in the regenerated retina.

cytokines and growth factors, such as insulin, insulin-like growth factor-1, fibroblast growth factor (FGF), and HB-EGF. All these factors are present only in reactivated, not quiescent, zebrafish Müller cells (Wan et al., 2014; Zhao et al., 2014a). These factors exhibit extensive crosstalk and converge through the MAPK–Erk, PI3K/Akt, and Jak–Stat signaling cascades on *Ascl1* and *Stat3* gene regulation to promote Müller glia dedifferentiation and proliferation (Kassen et al., 2009; Wan et al., 2012, 2014; Zhao et al., 2014a).

Wnt signaling appears to be another major pathway mediating growth factors and cytokines effect on Müller cell response to injury (Wan et al., 2014). Canonical Wnt/ß-catenin signaling pathway is activated in Müller glial cells following retinal damage and is necessary for their dedifferentiation and proliferation (Ramachandran et al., 2011; Meyers et al., 2012). A positive feedback loop occurs between Wnt signaling and *Ascl1*: ASCL1

inhibits the expression of the Wnt signaling inhibitor Dkk and stimulates the expression of Wnt4a ligand, while Wnt signaling pathway promotes *Ascl1* expression in activated Müller cells (Ramachandran et al., 2011). The transcriptional repressor Insm1 is likely the molecular effector of the *Ascl1*-dependent repression of Dkk (Ramachandran et al., 2012; Gorsuch and Hyde, 2014). Yet, Insm1 seems to play multiple roles during regeneration as it has also been shown to restrict the response of Müller cells by negatively regulating *hb-egf* expression (Ramachandran et al., 2012). Notch signaling was also reported to be part of a negative feedback loop in injury-responsive Müller cells (Wan et al., 2012). Notch is known to be important for the stabilization of the glial identity of Müller cells in the intact zebrafish retina (Nelson et al., 2011). In the injured adult retina, it is activated by the HB-EGF/MAPK/ASCL1-signalling cascade while inhibiting *Stat3*, *Ascl1*, and *hb-egf* expression (Wan et al., 2012). By repressing
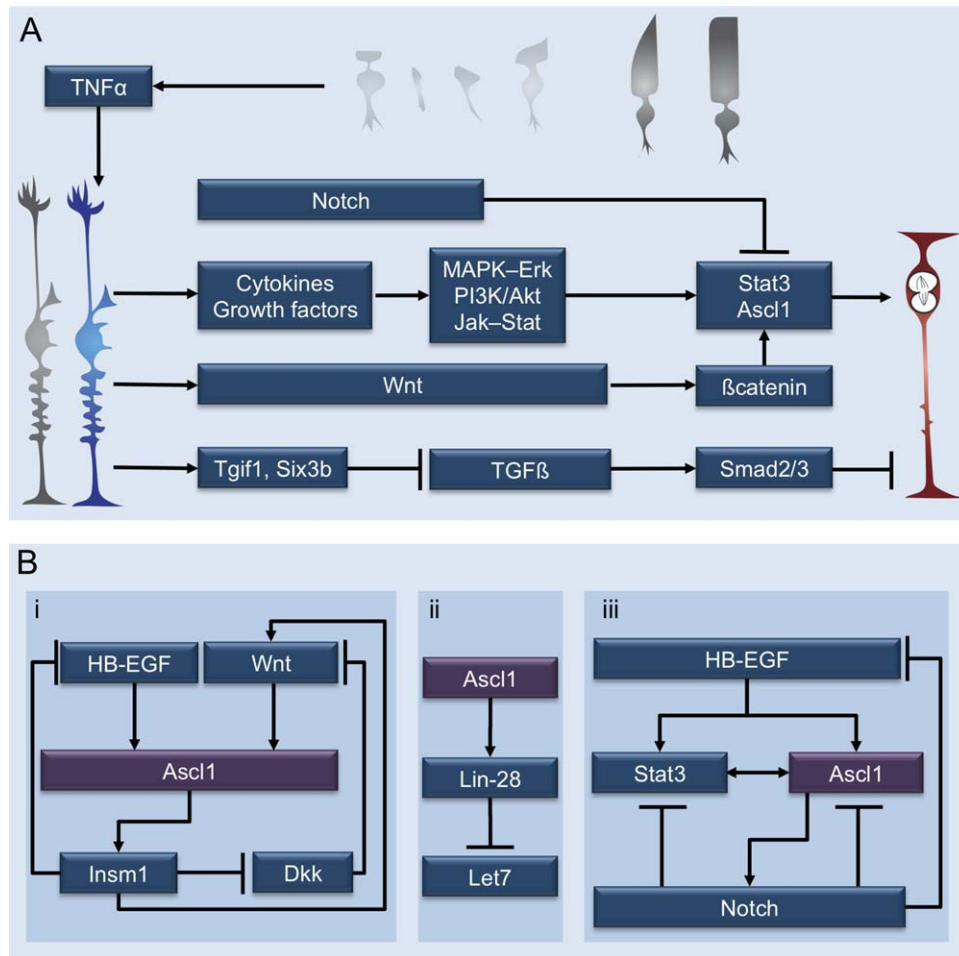
Fig. 3. Signaling pathways regulating Müller glia cell dedifferentiation and proliferation following retinal injury in zebrafish. **A**: Dying photoreceptors (light grey) secrete TNFα, which triggers Müller cell response to injury. Activated Müller glia cells (in blue) are a source of growth factors and cytokines, which impinge on *Stat3* and *Ascl1* genes through the MAPK–Erk, PI3K/Akt, and Jak–Stat signaling cascades. *Ascl1* and *Stat3* promote Müller glia dedifferentiation and proliferation (in red). Wnt/ß-catenin signaling pathway is also activated in Müller glia cells following retinal damage and promotes *Ascl1* expression. On the other hand, Notch inhibits *Ascl1/Stat3* expression and thereby preserves a pool of quiescent Müller glia in the injured retina. Smad2/3-mediated TGFβ signaling also inhibits the proliferative response of Müller glia following photoreceptor destruction. Although an initial increase in TGFβ signaling occurs in Müller cells following injury, its subsequent inhibition by the corepressors Tgif1 and Six3b promotes Müller glia proliferation. **B**: Illustration of regulatory cascades involving *Ascl1* gene in zebrafish Müller cells following injury. **i**: Wnt signaling and ASCL1 are part of a feedback loop: Wnt signaling promotes *Ascl1* expression in activated Müller cells. ASCL1 is upstream the transcriptional repressor Insm1, which inhibits the expression of the Wnt signaling inhibitor Dkk and stimulates the expression of Wnt4a ligand. On the other hand, Insm1 also restricts the response of Müller cells by negatively regulating *hb-egf* expression. **ii**: ASCL1 acts upstream of *Lin-28*, which represses the micro-RNA *let-7*, a repressor of regeneration-associated genes. **iii**: Notch signaling and ASCL1 are part of a negative feedback loop. Notch signaling is activated by the HB-EGF/MAPK/ASCL1-signalling cascade but inhibits *Stat3, Ascl1* and *hb-egf* expression.

these key reprogramming factors, Notch signaling preserves a pool of quiescent Müller glia in the injured retina. This may ensure a fine-tuning of the number of activated Müller cells able to re-enter the cell cycle, according to the extent of the injury (Wan et al., 2012; Conner et al., 2014). Finally, it was shown that Smad2/3-mediated TGFß signaling inhibits the proliferative response of Müller glia following photoreceptor loss (Lenkowski et al., 2013). The authors proposed that although an initial increase in TGFβ signaling is important for the damage response of Müller cells, its subsequent inhibition by the corepressors Tgif1 and Six3b is necessary to promote their proliferation and therefore support the regeneration process.

Triggering cell proliferation is a key event in regeneration but the cessation of cell division is just as important to prevent uncontrolled progenitor cell expansion. In addition to its roles

mentioned above, Insm1 also promotes progenitor cell-cycle exit in the regenerative retina by stimulating expression of the cyclin–cdk inhibitor, p57kip2 (Ramachandran et al., 2012).

Müller Cell Response to Injury in Amphibians

Urodele amphibians, such as the newt or the salamander, have the fascinating capacity to regenerate organs, including the retina, following ablation (Mitashov, 1996). Anuran amphibians are less renowned in the field as their regenerative ability was thought to be restricted to the larval stages and disappear after metamorphosis (Hitchcock et al., 2004). However, it was demonstrated in 2007 that the frog *Xenopus* actually retains the
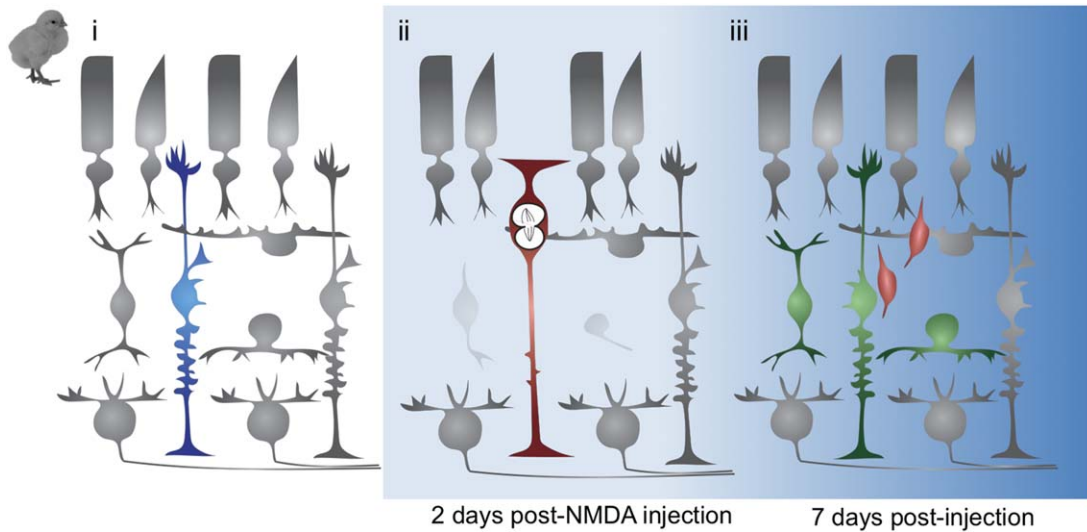
Fig. 4. Müller cell response to NMDA-induced retinal damage in the chick. **i**: In the chick, as in zebrafish (see Fig. 2), a subset of Müller cells (in blue) have the potential to re-enter the cell cycle upon retinal injury. **ii**: Acute retinal damage induced by NMDA treatment leads to amacrine and bipolar cell apoptosis (light grey) and subsequent dedifferentiation and proliferation of some Müller cells (in red). Such injury-induced Müller cells undergo interkinetic nuclear migration with their nuclei moving toward the outer nuclear layer when entering M phase. **iii**: Proliferative Müller cells self-renew and generate amacrine and bipolar cells (in green). However, most of the newly formed cells remain as undifferentiated progenitor-like cells (in red).

capacity to regenerate its retina at postmetamorphic stages following retinectomy (Yoshii et al., 2007). Although contrasting modes of regeneration in different amphibian species have been documented, in all cases, RPE cells and/or retinal stem cells from the ciliary margin have been reported as the cellular sources of newly generated neurons (Vergara and Del Rio-Tsonis, 2009; Hidalgo et al., 2014; Miyake and Araki, 2014).

What about Müller cells? Under physiological conditions, they are quiescent in amphibians, unlike the adult fish situation where a subset of Müller glia is slowly cycling and constantly generating rod precursors. Although amphibians are famous for regeneration, it is still unknown whether their Müller cells participate in retinal repair. Because retinal regeneration was mostly studied following large ablation or even retinectomy, resident Müller cells response could not be investigated. However, the presence of some proliferating cells in the newt retina after retinal detachment could be detected not only in the RPE and ciliary margin but also in Müller cells (Grigorian and Poplinskaia, 1999; Novikova et al., 2008). Such observation strongly suggests that Müller cells are able to re-enter the cell cycle upon damage. In accordance with this, retinal precursor cells were found at the wound site after partial retinal resection in premetamorphic *Xenopus laevis* (Martinez-De Luna et al., 2011). In contrast, conditional nitroreductase-mediated rod cell ablation was reported to trigger Müller cell hypertrophy but cell cycle re-entry was not observed (Choi et al., 2011). As in fish, such differences strongly suggest that Müller cell response in the amphibian retina may also differ according to the lesional paradigm, the extent of damage and/or the developmental stage of the animal (Vihtelic et al., 2005; Ng et al., 2014). We have recently established two injury models in *Xenopus* premetamorphic tadpoles: mechanical retinal injury and nitroreductase-mediated photoreceptor cell ablation. We found in both models that a subset of Müller cells do indeed re-enter the cell cycle (Perron lab, unpublished data). Therefore, it should henceforth be possible to elucidate whether these activated *Xenopus* Müller glia divide asymmetrically and self-renew, whether

their progeny differentiate into new neurons, whether such reactivation also holds true in the adult, and whether this might be generalized to other amphibian species such as urodeles. By consequence, such novel amphibian models with targeted and limited retinal injury, instead of large ablations, are likely to provide an opportunity to explore the underlying molecular mechanisms of Müller cell–dependent retinal regeneration in vertebrates.

Müller Cell Response to Injury in Birds

Müller Cells Are Involved in Retinal Regeneration

The chick retina was thought to be incapable of regeneration after embryonic stages of development (Reh and Pittack, 1995). This was re-evaluated when a neurogenic region similar to the fish and amphibian ciliary margin was discovered in 2000 in the posthatched chick retina (Fischer and Reh, 2000). However, unlike the fish or amphibian CMZ, this area does not contribute to regeneration after retinal injury even when stimulated by exogenous growth factors (reviewed in Fischer, 2005; Fischer et al., 2013). Nevertheless, the regeneration of some types of retinal neurons has been reported in the posthatched chicken following acute neurotoxic damage, with a proliferative response observed in the central retina. These proliferative cells were identified as Müller cells (Fischer and Reh, 2002). For the first time, posthatched chicken Müller glial cells were thus shown to be capable of re-entering the cell cycle, dedifferentiating into retinal progenitors and generating new retinal neurons and Müller cells (Fig. 4). These injury-induced Müller cells generate different classes of neurons according to the types of cells that have been destroyed. For instance, amacrine and bipolar neurons are produced following their destruction by the neurotoxin N-methyl-D-aspartic acid (NMDA) while ganglion cells can be generated when these cells are ablated by injections of colchicine or kainic acid (Fischer and Reh, 2002). So far, given the limited number of rod or cone degenerative models, it has been difficult to assess

whether photoreceptors are able to regenerate from Müller cells in the chicken retina. In a model of retinal detachment, Müller cells re-enter the cell cycle following photoreceptor loss but their ability to regenerate photoreceptors still remains an open question (Cebulla et al., 2012).

Extrinsic Signals Triggering Müller Cell Activation

In the injured chicken retina, some signals triggering Müller cell response and their cellular sources have been recently identified. Dying neurons themselves may produce factors that elicit Müller cell regenerative response. Other potential sources of diffusible molecules, such as microglia or infiltrating macrophages, have also raised interest. Unlike in fish, the phagocytic ability of chicken Müller glia has not been demonstrated yet. On the other hand, microglia and macrophages become activated and function as phagocytes for the clearance of dying retinal cells following injury (Fischer et al., 2014). A recent study revealed that their activation also stimulates Müller cell dedifferentiation and cell cycle re-entry (Fischer et al., 2014). Although signals mediating these effects remain to be identified, they may include components of the complement system and pro-inflammatory cytokines (Fischer et al., 2014).

Additional extrinsic signals triggering Müller cell activation may also originate from an atypical glial cell type identified in the avian retina and named the nonastrocytic inner retinal glial cells (NIRG) (Fischer et al., 2010). Indeed, acute retinal damage causes their accumulation, migration and reactivity. NIRG cell behavior is actually linked to that of microglia (Zelinka et al., 2012). Both cell types could thus act in concert to communicate with Müller cells to initiate their cellular response following injury.

The inflammatory response of Müller cells may also influence their own reprogramming efficiency (Gallina et al., 2014b). Glucocorticoid receptors, usually associated with anti-inflammatory effects, were shown to have an inhibitory influence on Müller glia reactivation. These receptors are expressed by Müller cells and are dynamically regulated upon retinal damage, showing an initial decrease followed by a subsequent increase. The transient drop of glucocorticoid receptor signaling just after the injury may permit the formation of proliferating Müller glia (Gallina et al., 2014b).

The response of Müller glia to retinal damage is also most likely dependent on changes in the levels of secreted growth factors. This is supported by the fact that some combinations of exogenous growth factors, such as insulin and FGF2, synergistically stimulate Müller cell dedifferentiation and proliferation (Fischer and Reh, 2002; Fischer et al., 2002). It has been proposed that FGF2, through MAPK-signaling, exerts its effect directly on Müller glia to promote their dedifferentiation while Insulin would act in an indirect manner, affecting microglia and NIRG cell reactivity, which in turn would stimulate Müller cell proliferation (Fischer et al., 2009a,b; Fischer et al., 2010; Gallina et al., 2014a).

Although the Notch pathway is essential for maintaining Müller cell quiescence in zebrafish (see above), different conclusions were drawn from studies in the avian retina. As in the fish, Notch pathway components are up-regulated in chicken Müller glia after retinal damage. However, such Notch signaling activation appears necessary for the dedifferentiation and proliferation of Müller glia (Hayes et al., 2007; Ghai et al., 2010). This apparent disparity observed between the two species may contribute to their different regenerative potentials or may reflect the complex and dynamic role of Notch signaling during regeneration in different cell types, i.e. quiescent Müller cells, dedifferentiated Müller cells or progenitor cells. Indeed, a biphasic role of Notch was uncovered during chicken retinal regeneration: although Notch activation is necessary for the initial steps of the regeneration, it is detrimental to successful completion of the process (Hayes et al., 2007).

Finally, recent work also demonstrated the involvement of Hedgehog signaling in Müller glia cell cycle re-entry (Todd and Fischer, 2015). The proposed model is that retinal injury triggers the release of Hedgehog from retinal ganglion cells rendering Müller cells receptive to the ligand. Subsequent activation of Hedgehog signaling then stimulates the dedifferentiation and proliferation of Müller cells.

The aforementioned pathways, FGF2/MAPK, Notch, and Hedgehog, likely function in a signaling network with multiple crosstalks to modulate the formation of reactive Müller glia upon injury. For instance, FGF2 was shown to activate components of the Notch-pathway (Ghai et al., 2010) and to render Müller glia responsive to Hedgehog-signaling (Todd and Fischer, 2015), while the Notch pathway was reported to function downstream of Hedgehog signaling (Todd and Fischer, 2015).

Müller Cell Response to Injury in Mammals

Müller Cells Have a Neurogenic Potential

In contrast to the above species, the mammalian retina is not able to self-repair. Thus, human retinal diseases triggering neuronal cell death lead to permanent visual disorders. Nonetheless, several cell types in different regions of the adult mammalian retina can exhibit some degree of neurogenic potential under pathological circumstances. These include cells of the ciliary margin, RPE and Müller glial cells (Kiyama et al., 2012; Salero et al., 2012; Wang and Yan, 2014; Jayakody et al., 2015). Müller cells are quiescent in the adult healthy mammalian retina but have long been known to be reactive in disease or following injury. In such pathological situations, they undergo reactive gliosis, which includes changes in morphology, up-regulation of various markers, dedifferentiation, nuclear migration to the apical surface, and in some cases, proliferation (Dyer and Cepko, 2000; Bringmann et al., 2009). This process thus exhibits striking similarities with the early stages of the regenerative process observed in the fish or chick retina. However, given the lack of spontaneous neuron replacement in diseased eyes, it was believed that mammalian Müller cells could only undergo reactive gliosis and not neurogenesis. This view was challenged in 2004, when some Müller glia cells were shown to be able to dedifferentiate, re-enter the cell cycle, and produce new bipolar cells and rod photoreceptors, in response to NMDA-induced excitotoxic retinal damage (Ooto et al., 2004). Regeneration of photoreceptors from rodent Müller glia has subsequently been reported in models of photoreceptor degeneration: following N-methyl-N-nitrosourea (MNU) administration (Wan et al., 2008) or in retinal explants (Osakada et al., 2007). However, this Müller cell potential remains controversial as other teams found no dedifferentiation nor proliferation following NMDA-induced damage (Kugler et al., 2015). Similarly, photoreceptor damage due to intense light exposure does not seem to promote Müller cell proliferation (Joly et al., 2011). The authors suggest that the few cells that incorporate

bromodeoxyuridine are actually cells with on-going DNA repair rather than proliferative cells. Nevertheless, it was demonstrated that NMDA-induced damage coupled with growth factor treatment (see below) provides rodent Müller cells with the ability to proliferate and regenerate amacrine cells (Karl et al., 2008). Although the number of newly produced neurons remains quite small in rodent models, these studies unveiled the regenerative potential of the adult mammalian retina, which could be stimulated for therapeutic retinal repair purposes.

In vitro studies revealed that rodent and human Müller cells in culture can generate both glial cells and neurons (Das et al., 2006; Lawrence et al., 2007; Zhao et al., 2014b). Of interest, human Müller glial cells in culture differentiate toward rods four to six times faster than conventional differentiating pluripotent stem cells (Giannelli et al., 2011). When transplanted into a photoreceptor or ganglion cell depleted retina, these Müller cell-derived neurons migrate, integrate into the appropriate retinal layer and lead to improvement in rod or ganglion cell function, respectively (Singhal et al., 2012; Jayaram et al., 2014).

As mentioned above, a recent study suggested that a subset of Müller cells in the mouse retina may derive from the neural crest (Liu et al., 2014). This is quite surprising and contrasts with results of most neural crest lineage tracing studies and with previous evidence that Müller glia is exclusively derived from the retinal neuroepithelium. Nevertheless, the authors raised the intriguing possibility that the regenerative capacity of only a subpopulation of Müller glia may be associated with their neural crest origin. If this is indeed the case, there are many issues that could be addressed in the future such as whether or not the proportion of neural crest-derived Müller cells differs in the retina of different species, and whether this could underlie their contrasting regenerative abilities.

Müller Cell Reprogramming

The in vivo study of mammalian Müller cell regenerative response to injury has been challenging given their very limited and inefficient capacity for retinal repair. A recent detailed analysis of Müller cell behavior was reported ex vivo in mouse retinal explants, a system where neuronal loss spontaneously occurs (Löffler et al., 2015). In these explants, Müller glia present different states that recapitulate the major phases described in regenerating fish and chick Müller cells: from a quiescent state, they dedifferentiate/reprogram, proliferate and then generate neuronal progenies, in particular amacrine neurons. Under appropriate growth factors stimulation (see the paragraph below on extrinsic signals triggering Müller cell activation), more than half of the Müller cells can re-enter the cell cycle (Löffler et al., 2015).

Regarding the molecular mechanisms underlying mammalian Müller cell reprogramming, ASCL1 was a potential candidate, given its key role in the conversion of fish Müller cells into retinal progenitor cells. Of interest, *Ascl1* is not up-regulated in the mouse retina after NMDA-induced damage (Karl et al., 2008), which could account for the limited regenerative capacity of mammalian Müller cells. On the other hand, using the mouse retina organ culture approach, it was shown that *Ascl1* is up-regulated following retinal damage in a subset of Müller glia in young mice (Löffler et al., 2015). Of interest, this differential expression according to the age of the animal correlates with the higher potential for Müller cells to reprogram in young mice than in old mice (Ueki et al., 2012; Löffler et al., 2015). Moreover, it

was shown that ASCL1 overexpression is sufficient to reprogram mouse Müller cells in vitro and activate a neurogenic program (Pollak et al., 2013). More recently, this issue was addressed in vivo in adult Müller glia (Ueki et al., 2015). Although *Ascl1* overexpression in undamaged retina does not affect Müller cell behavior, it promotes their cell cycle re-entry and neurogenic potential when the retina is injured. Again, this Müller cell response is even more pronounced in young mice. Together, this supports the hypothesis that a deficit in *Ascl1* up-regulation in adult Müller cells might restrict their ability to reprogram. Thus, a potential difference between mammalian and fish Müller glia underlying their difference in regenerative potential is their unique levels of the transcription factor ASCL1 (Ueki et al., 2015). *Ascl1* overexpression-dependent reprogramming involves chromatin remodelling of ASCL1 targets from a repressive to an active configuration (Pollak et al., 2013). Of interest, some chromatin remodelling factors were shown to be differentially expressed in two mouse strains exhibiting different degrees of Müller cell proliferation upon damage (Suga et al., 2014). Further experiments are needed to provide a deeper understanding of epigenetic regulation in Müller cell reprogramming.

As discussed above, the promoters of pluripotency factors, including that of *Ascl1*, are hypomethylated in quiescent zebrafish Müller cells (Powell et al., 2013). Unexpectedly, these promoters also exhibit permissive methylation levels in mammals. This suggests that, as their zebrafish counterparts, mouse Müller cells may be poised for reprogramming in case of injury (Powell et al., 2013). Therefore, it seems that methylation status of pluripotency genes is not a limiting factor that could explain the ineffective regenerative capacity of Müller cells in mammals compared with fish.

Extrinsic Signals Triggering Müller Cell Activation

The identification of factors that could be used to recruit Müller cells and stimulate their regenerative potential is obviously subject of intense interest. For instance, the release of glutamate from dying neurons may serve as a signal to activate Müller glia. Indeed, it has been shown that subtoxic levels of glutamate are sufficient to directly stimulate Müller glia to re-enter the cell cycle and induce neurogenesis (Takeda et al., 2008).

As in nonmammalian species, the proliferation of rodent Müller cells can be greatly stimulated by exogenous growth factors, such as FGF, EGF, or insulin (Ooto et al., 2004; Close et al., 2006; Karl et al., 2008; Fischer and Bongini, 2010). However, the action of these factors and the involved downstream genetic cascades remain poorly understood. Recent evidence has revealed that activation of both MEK/ERK1/2 and PI3K/AKT pathways is required for Müller glia to proliferate in response to EGF (Ueki and Reh, 2013). Bone morphogenetic protein (BMP) signaling is also activated by the EGF pathway, downstream of PI3K/AKT activation, and is essential for Müller glia proliferation (Ueki and Reh, 2013).

Several studies demonstrated that the proliferative and neurogenic properties of mammalian Müller cells following injury can also be stimulated by key signaling pathways, such as Wnt (Das et al., 2006; Osakada et al., 2007; del Debbio et al., 2010; Liu et al., 2013), Notch (Das et al., 2006; del Debbio et al., 2010), and Hedgehog (Wan et al., 2007). How these pathways relate to one another in reactive murine Müller cells remains to be investigated.

DEVELOPMENTAL DYNAMICS

Maintenance of Müller Cell Quiescence

The limited proliferative response to injury of Müller glia in mammals compared with nonmammalian species may be due to limited pro-mitogenic factors and/or inhibitory mechanisms. Consequently, the identification of molecular cues limiting mammalian Müller cell proliferation may provide hints to unlock their latent regenerative capacity. For instance, p53 has recently been identified as a factor restricting Müller glia potential for retinal regeneration (Ueki et al., 2012; Zhao et al., 2014b). TGFβ signaling, which stimulates the expression of the cyclin-dependent kinase inhibitor p27^{kip1}, is essential for the establishment and the maintenance of the mitotic quiescence of rodent Müller cells at post-natal stages (Close et al., 2005). Whether TGFβ also has cytostatic effects in the damaged retina and could counterbalance mitogenic factor activity was a tempting hypothesis. However, a recent report showed that moderate modulation of TGFβ signaling was not sufficient to influence Müller glia cell proliferation following NMDA excitotoxic damage (Kugler et al., 2015). Yet, the consequences of the complete inhibition of TGFβ signaling in Müller cells remains to be investigated to fully exclude the involvement of this pathway in the maintenance of their quiescence. As mentioned above, the BMP pathway is activated in mammalian reactive Müller glia. Given the known gliogenic function of BMP, the question is raised as to whether it could thus interfere with the neurogenic potential of mammalian Müller cells (Ueki and Reh, 2013).

Finally, the ability to regenerate may be linked to changes in the immune response across evolution. Indeed, regenerative capacity is inversely correlated with the maturation of the immune system (Mescher and Neff, 2005; Godwin and Brockes, 2006; Godwin and Rosenthal, 2014). Of interest, it has recently been shown in the early embryonic chick, at a stage that precedes the appearance of Müller glia, that the complement fragment C3a is sufficient to induce complete retinal regeneration from the ciliary margin (Haynes et al., 2013). Whether or not this effect is mediated through an innate cellular immune response remains to be addressed. Further studies are needed to elucidate the relationship between immune mechanisms and the regenerative capacity of Müller cells in mammalian retinal regeneration.

Conclusions and Future Prospects

Much interest has been generated lately by the stemness potential of Müller glial cells. Whether they should be considered as stem cells is a matter of debate, as they do not function as such in the noninjured retina. Nonetheless, in response to neuronal loss, Müller cells can exhibit stem-like properties as they can undergo a reprogramming process to re-acquire a progenitor state, self-renew, and generate new neurons. This offers much hope for treatments aimed at invigorating Müller glia-dependent retinal regeneration in mammals. Consequently, and as summarized in this review, discovering the cellular events and molecular mechanisms underlying Müller cell behavior in species with different regenerative capacities is an active area of investigation. Although a wealth of data has been accumulated this past decade, it is a nascent field and there is still a long way to go.

To obtain further insights into the signaling network underlying the different steps of Müller cell–dependent regeneration, many key questions remain to be addressed. Why does only a subset of Müller cells respond to retinal injury? Are there distinct subpopulations endowed with different regenerative capabilities? Molecular fingerprint indeed suggested some heterogeneity within Müller glial cells (Roesch et al., 2008). Why is the Müller cell–dependent regeneration so constrained in mammals compared with other species? Are there extrinsic inhibitors, intrinsic differences, epigenetic constraints, and halting immune mechanisms? Answering those questions should contribute to the design of therapeutic strategies to enhance Müller cell potential in patients afflicted with degenerative diseases. Such regenerative therapy from endogenous Müller glia is very appealing. Indeed, although clinical trials based on transplantation of retinal cells derived from embryonic stem cells or iPS cells are underway and offer tremendous hope to patients suffering from presently untreatable retinal diseases, substantial challenges still lie ahead (Wright et al., 2014). For instance, the process to produce retinal cells in vitro is costly, time-consuming and complex, the surgical procedure is delicate, there may be residual pluripotent stem cells with a tumorigenic potential within donor cells, the integration into the existing neural network is extremely challenging, and there may be immune rejection and potential ethical objections. On the other hand, unlocking mammalian Müller glia regenerative potential would circumvent all the issues related to the in vitro cell derivation and transplantation procedures. Moreover, Müller cells seem to lack any tumorigenic potential (Giannelli et al., 2011). Therefore, the stemness potential of Müller cells warrants intensive investigations not only to increase our knowledge of the fascinating regenerative process but also for potential medical applications.

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