

Themed Section: Midkine

REVIEW

The expression and function of midkine in the vertebrate retina

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The functional role of midkine during development, following injury and in disease has been studied in a variety of tissues. In this review, we summarize what is known about midkine in the vertebrate retina, focusing largely on recent studies utilizing the zebrafish (*Danio rerio*) as an animal model. Zebrafish are a valuable animal model for studying the retina, due to its very rapid development and amazing ability for functional neuronal regeneration following neuronal cell death. The zebrafish genome harbours two *midkine* paralogues, *midkine-a* (*mdka*) and *midkine-b* (*mdkb*), which, during development, are expressed in nested patterns among different cell types. *mdka* is expressed in the retinal progenitors and *mdkb* is expressed in newly post-mitotic cells. Interestingly, studies of loss- and gain-of-function in zebrafish larvae indicate that midkine-a regulates cell cycle kinetics. Moreover, both *mdka* and *mdkb* are expressed in different cell types in the normal adult zebrafish retina, but after light-induced death of photoreceptors, both are up-regulated and expressed in proliferating Müller glia and photoreceptor progenitors, suggesting an important and (perhaps) coincident role for these cytokines during stem cell-based neuronal regeneration. Based on its known role in other tissues and the expression and function of the *midkine* paralogues in the zebrafish retina, we propose that midkine has an important functional role both during development and regeneration in the retina. Further studies are needed to understand this role and the mechanisms that underlie it.

LINKED ARTICLES

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Abbreviations

ALK, anaplastic lymphoma kinase; Ascl1a, ascl1a achaete-scute complex-like 1a; BrdU, bromodeoxyuridine; CMZ, ciliary marginal zone; GCL, ganglion cell layer; HC, horizontal cell; hpf, hours post-fertilization; hpl, hours post-lesion; Id2a, inhibitor of DNA binding 2a; INL, inner nuclear layer; Insm1a, insulinoma-associated 1a; LRP1, low density lipoprotein receptor-related protein 1; mdka, midkine-a; mdkb, midkine-b; ONL, outer nuclear layer; Ptn, pleiotrophin; Ptp99A, protein tyrosine phosphatase 99A; RPTPβ/ζ, receptor protein tyrosine phosphatase β/ζ

Introduction

The retina is a complex neural circuit that converts photons of light into electrical impulses, which encode and process the images cast on the retina (Dowling, 2012). The retina has a long history of serving as a model tissue for discovering the mechanisms that govern brain development (Agathocleous and Harris, 2009), for investigating synaptic function (Wei and Feller, 2011) and for developing therapeutic approaches

to treating brain injury and disease (Seiler and Aramant, 2012). Among vertebrates, retinal structure and function are evolutionarily very highly conserved. The retina is a laminated tissue, formed by the orderly arrangement of six classes of neuronal cell bodies, their processes and synaptic interconnections, and one specialized type of glial cell, the Müller glia, a radial glial cell that is unique to the retina and functions, in part, similarly to astrocytes in other CNS regions (Figure 1). Neuronal cell types consist of two classes of

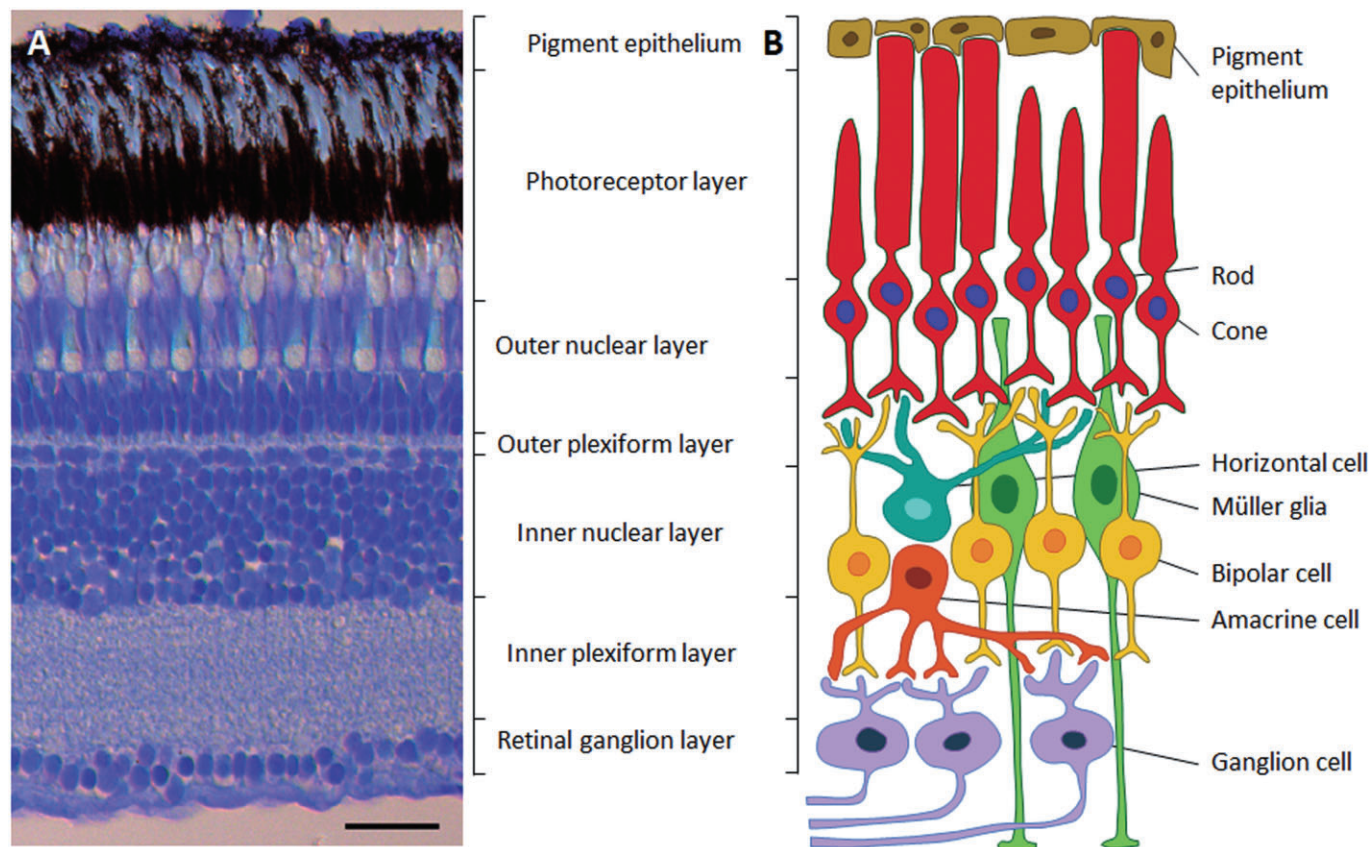


Figure 1

Structure of the retina. A: Microphotograph of a cross-section through the retina of an adult zebrafish, showing the different cellular and synaptic retinal layers. B: Diagram of the neural circuit of the retina, showing the six neuronal cell types and the two supporting cell types (Müller glia and retinal pigmented epithelium). In A, the scale bar = 25µm.

photoreceptors (rods that detect low light intensities and cones that encode colour and function during daytime vision), three classes of interneurons [horizontal cells (HCs), bipolar cells and amacrine cells], and ganglion cells, which send axons out of the eye to visual centres in the brain.

Midkine and pleiotrophin (Ptn) are the only two members of a family of small heparin-binding neurotrophic factors. Both cytokines perform multiple functions in developing and adult tissues, including the brain, and also in a variety of tissues following injury. Midkine was first discovered as a retinoic acid-inducible gene in embryonal carcinoma cells (Kadomatsu *et al.*, 1988). In the mouse, midkine is highly expressed during mid-gestation and is associated with epithelial-mesenchymal interactions (Mitsiadis *et al.*, 1995). In the developing brain, both midkine and Ptn are localized to the processes of radial glia, upon which neural progenitors both migrate and differentiate (Kadomatsu and Muramatsu, 2004; Muramatsu, 2010), supporting an important role of these cytokines in mouse neural development. Also within the nervous system, protective and reparative roles have been attributed to midkine (Muramatsu, 2011). For example, midkine knockout mice show a delay in axonal degeneration and regeneration in injured peripheral nerves (Sakakima *et al.*, 2009) and midkine expression is up-regulated following

ischaemia in the rat retina (Miyashiro *et al.*, 1998) and brain (Ishikawa *et al.*, 2009), perhaps protecting against the effects of ischaemia within these tissues (Ooboshi, 2011). Similarly, Ptn plays a neuroprotective role in the nigro-striatal pathway, in Parkinson's disease (Marchionini *et al.*, 2007) and also following neurotoxicity induced by drugs of abuse (Gramage *et al.*, 2010). Specifically, Ptn knockout mice are more vulnerable to the amphetamine-induced damage in the dopaminergic neurons of the substantia nigra and their axons in the striatum (Gramage *et al.*, 2010; see Herradón and Pérez-García, 2013). Midkine and Ptn are up-regulated in spinal motor neurons after injury (Sakakima *et al.*, 2004), in response to exposure to drugs of abuse, in both animal models and humans (Ezquerro *et al.*, 2007; Flatscher-Bader and Wilce, 2008) and in the degenerating substantia nigra of Parkinson's disease patients (Marchionini *et al.*, 2007). These and other studies provide evidence for a role for both midkine and Ptn in ameliorating the effects or effecting repair after injury to nervous tissue.

In contrast to mammals, which have a single unique *midkine* gene, the zebrafish genome encodes two *midkine* paralogues, *midkine-a* (*mdka*) and *midkine-b* (*mdkb*). These paralogues share 68% amino acid identity and are likely to result from a genome duplication event during the evolution-

ary history of teleosts (Winkler *et al.*, 2003). In the zebrafish brain, the expression of *mdka* and *mdkb* is differentially regulated during development (see below) and in adulthood. Interestingly, both paralogues are up-regulated during regeneration of multiple tissues and organs (Lien *et al.*, 2006; Schebesta *et al.*, 2006; Calinescu *et al.*, 2009a; Fujisawa *et al.*, 2011; Grotek *et al.*, 2013; Parente *et al.*, 2013), including the retina, suggesting that in fish, as in mammals, these cytokines also function to modify or repair tissue-specific injuries.

Role of midkine during retinal development

The retina develops from a neuroepithelial sheet comprised of undifferentiated progenitor cells undergoing active proliferation. As for all complex organs, cellular differentiation in the retina is governed by complex signalling events (see Agathocleous and Harris, 2009). In zebrafish, neuronal differentiation in the retina is amazingly rapid (Stenkamp, 2007). Retinal progenitors first begin to withdraw from the cell cycle and initiate programmes of differentiation around 28–32 h post-fertilization (hpf; Schmitt and Dowling, 1996; Hu and Easter, 1999). The initial differentiation begins within a nas-ventral patch. This is then followed by waves of differentiation that, analogous to the hands on a clock face, sweep circumferentially from ventronasal to ventrotemporal domains (Easter and Malicki, 2002). During retinal morphogenesis in zebrafish, the expression of *mdka* and *mdkb* are differentially regulated (Calinescu *et al.*, 2009a; Figure 2). Starting at about 30 hpf (Calinescu *et al.*, 2009a), *mdka* is expressed in the mitotic retinal progenitors, though the level of expression appears greatest at the ciliary marginal zone (CMZ; Figure 2), which contains the retinal stem cell niche

(Raymond *et al.*, 2006). As retinal morphogenesis proceeds, *mdka* expression is down-regulated in cells that exit the cell cycle. Starting at about 72 hpf and persisting through about 120 hpf, *mdka* is transiently expressed in Müller glia. The final adult pattern emerges between 72 and 120 hpf and consists of the exclusive expression of *mdka* in HCs (cf. Figures 2 and 3A). In contrast, *mdkb* expression temporally lags behind that of *mdka*, and *mdkb* is expressed in newly post-mitotic cells within the inner nuclear and ganglion cell layers (GCLs). This expression pattern persists into and throughout adulthood (Calinescu *et al.*, 2009a; Figure 3B).

The cellular expression of *mdka* in the embryonic retina suggests that during retinal development, midkine-a (Mdka) may govern aspects of retinal neurogenesis. With this as an underlying hypothesis, Luo *et al.* (2012) used reverse genetics approaches to investigate the function of Mdka during early retinal development in the zebrafish. This study found that Mdka regulates proliferation among retinal progenitors by governing cell cycle kinetics (Luo *et al.*, 2012). When Mdka synthesis is blocked with *mdka*-targeted morpholino-oligonucleotides, retinal progenitors fail to exit the cell cycle and neuronal differentiation is delayed, although the underlying neurogenic programme within the retina is initiated at the correct developmental stage. This delay in neuronal differentiation following Mdka loss-of-function results from the increased length of the cell cycle, largely a consequence of an increase in the duration of the S-phase. The delay of neuronal differentiation results in a mild microphthalmia. Complementing the results from Mdka loss-of-function, over-expression of Mdka in a line of transgenic fish carrying an inducible *mdka* allele results in an acceleration of the cell cycle, an excess number of retinal progenitors and a mild macrophthalmia. This acceleration of the cell cycle does not lead to premature cell cycle exit, however. Expression levels of several core cell cycle regulators remain unchanged after

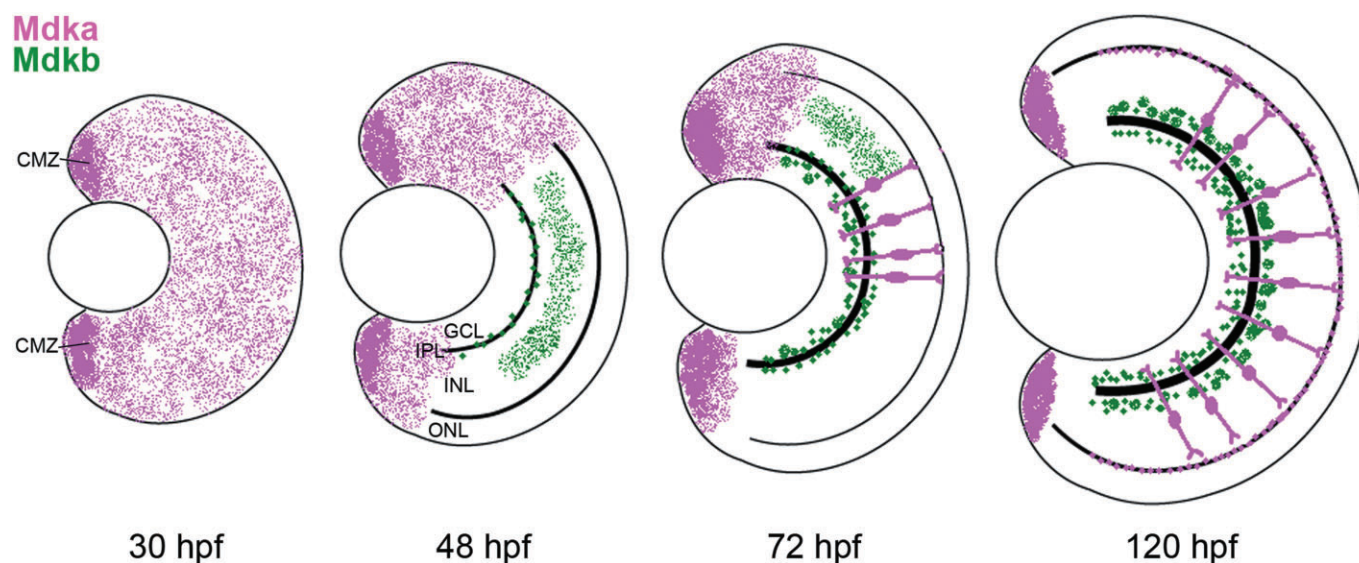


Figure 2

Expression of *mdka* and *mdkb* during retinal development. *mdka* (pink) is expressed in proliferating retinal progenitors, whereas *mdkb* (green) is expressed in newly post-mitotic cells. Between 72 and 120 hpf, *mdka* is transiently expressed in Müller glia.

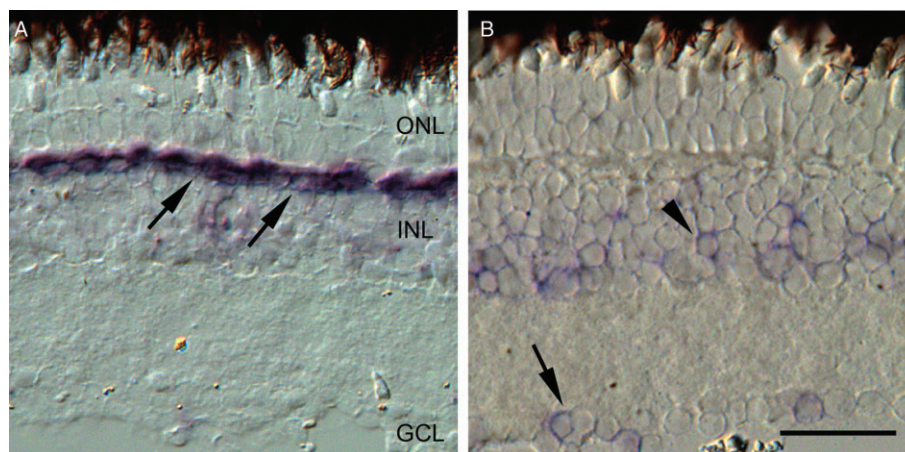


Figure 3

Expression of *mdka* and *mdkb* in the adult zebrafish retina. A: *In situ* hybridization showing the expression of *mdka* in horizontal cells (arrows). B: *In situ* hybridization showing the expression of *mdkb* in amacrine cells (arrowhead) in the inner tier of the inner nuclear layer and ganglion cells (arrow). GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer. Scale bar = 25 μ m.

Mdka loss- or gain-of-function, though the possibility exists that Mdka could regulate the translation, duration or phosphorylation state of these proteins.

The retinal phenotypes observed following Mdka loss- and gain-of-function were reminiscent of those observed following loss- and gain-of-function of the putative transcription factor-binding protein, Id2a (inhibitor of DNA binding 2a) (Uribe and Gross, 2010). Id2a loss-of-function lengthens the cell cycle, delays neuronal differentiation and leads to microphthalmia (Uribe and Gross, 2010), whereas Id2a gain-of-function shortens the cell cycle, accelerates the S- to M-phase transition and leads to a mild macrophthalmia. Testing for potential epistasis between Mdka and Id2a found that Id2a functions downstream of Mdka in a shared signalling pathway. Injecting *id2a* mRNA into embryos is sufficient to rescue the Mdka loss-of-function and restores the normal timing of cellular differentiation, and systematically altering Mdka levels induces corresponding changes in the expression of *id2a* (Luo *et al.*, 2012). Based on these data, it was concluded that Mdka and Id2a reside in a shared signalling pathway that functions to govern cell cycle kinetics and neuronal differentiation in the vertebrate retina. There were slight differences in the retinal phenotypes in embryos following alterations in the expression of Mdka and Id2a, respectively; however, the basis for these phenotypic differences awaits further clarification.

Unlike *mdka*, the function of *mdkb* during retinal development is completely unexplored. Ubiquitous Mdkb gain-of-function results in the absence of eyes or severely reduced eye size (Winkler and Moon, 2001; Lim *et al.*, 2013) and is likely to be due to secondary effects resulting from the development defects in the forebrain. In future studies, it will be important to develop tissue-specific approaches to knockdown or over-express Mdkb in the retina in order to examine its functional role in the developing retina.

The function of midkine in the developing mammalian retina has yet to be investigated. The evidence that midkine may be functionally important, however, is based on a screen

for transcripts expressed in CNS progenitors in the mouse (Livesey *et al.*, 2004). In this study, *midkine* was identified as a member of the core set of transcripts enriched in retinal progenitors. Given the functional role of Mdka in the zebrafish retina, one can hypothesize that in the developing retina (and brain) of mammals, midkine may also play an important functional role governing neurogenesis.

Interestingly, Ptn, the other member of this family of heparin-binding growth factors, is expressed in the post-natal rat retina and functions to determine the fates of late-born neurons (Roger *et al.*, 2006). Ptn is expressed in retinal progenitors within the outer neuroblastic layer and post-mitotic cells within the inner nuclear layer (INL). The over-expression of Ptn diminishes the genesis of rod photoreceptors and promotes the genesis of bipolar cells.

Finally, *miple*, the *Drosophila* orthologue of vertebrate *midkine/pleiotrophin*, plays a role in the development of the invertebrate eye (Muñoz-Soriano *et al.*, 2013). The compound eye of *Drosophila* is a highly organized structure composed of about 750 ommatidia, each containing a cluster of eight photoreceptor cells (Hsiao *et al.*, 2012). Each cluster of photoreceptors develops from a precluster of five cells that, as the photoreceptors differentiate, undergo a precise 90° rotation, creating a mirror image between the dorsal and ventral ommatidia along the dorsal-ventral midline (Wolff and Ready, 1993; Mlodzik, 1999). Miple regulates this ommatidial rotation by binding to the midkine receptor Ptp99A (protein tyrosine phosphatase 99A) (Muñoz-Soriano *et al.*, 2013), the *Drosophila* orthologue of PTP ζ (see below).

The expression of midkine in adult zebrafish retina

In the retina of the adult zebrafish, as in the developing retina, the expression of *mdka* and *mdkb* is differentially regulated. *mdka* is expressed exclusively in HCs, whereas

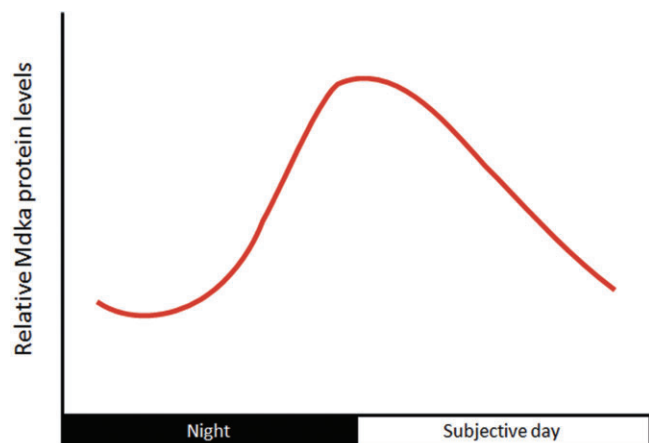


Figure 4

Circadian regulation of Mdk levels in the adult zebrafish retina. The expression of Mdk increases in anticipation of light onset and decreases throughout the daylight hours, reaching a minimum during the night.

mdkb is more broadly expressed in cells within the inner nuclear and GCLs (Calinescu *et al.*, 2009a; Figure 3). In the midst of studies determining the adult expression of these midkine paralogues, it was discovered that the expression of *mdka* (and possibly of *mdkb*) is regulated by circadian rhythms (Calinescu *et al.*, 2009b). Circadian rhythms are biological oscillations entrained by the environmental light/dark cycle, and the regulatory and metabolic networks that direct the body's adjustments to variations of external and internal environment (Anderson *et al.*, 2013). Using both *in situ* hybridization and Western blot analysis, it was discovered that both Mdk mRNA and protein reach peak levels shortly after light onset, and the levels of both decrease as the day progresses, reaching a minimum several hours after the onset of darkness (Figure 4). It was confirmed that this pattern of expression is governed by circadian rhythms, because the cyclical expression of both mRNA and protein persists for at least 48 h in the complete absence of light. The expression of *mdkb* might also be governed by circadian rhythms, but the data supporting this conclusion were less consistent than for Mdk (Calinescu *et al.*, 2009b). It is unknown if the expression of midkines in other brain regions of zebrafish is similarly governed by circadian rhythms, but this possibility must be considered when interpreting the significance of experimentally induced changes in levels of expression.

The function of midkine in neuroprotection and retinal regeneration

It is well established that midkine is involved in the protection and repair in a variety of neural tissues (Muramatsu, 2010). The protective role of midkine in the CNS was first demonstrated in the retina, where midkine promotes photoreceptor survival following constant light exposure (Unoki

et al., 1994). In rats, an intraocular injection of midkine is sufficient to rescue photoreceptors that would otherwise be killed by exposure to constant light. This rescue of the photoreceptors is both anatomical and functional (Masuda *et al.*, 1995). Interestingly, the normal levels of endogenous midkine in the rat retina are up-regulated after pressure-induced retinal ischaemia (Miyashiro *et al.*, 1998). The fact that midkine is up-regulated after retinal damage and prevents light-induced death of photoreceptors leads to the hypothesis that in the injured nervous system, midkine might play an important role in neuroprotection.

Although basic retinal structure and function are evolutionarily conserved among all vertebrates, there exist striking differences between vertebrate species in their ability to regenerate retinal neurons following injury (Karl and Reh, 2010). The mammalian retina has a negligible ability for neuronal regeneration, but retinas of non-mammalian vertebrates, amphibians and fish (and birds to a lesser extent), can fully regenerate the retina and restore function (Hitchcock and Raymond, 2004). For zebrafish, any lesion that kills retinal neurons is sufficient to induce complete neuronal regeneration (see Hitchcock *et al.*, 1992; Fausett *et al.*, 2008; Sherpa *et al.*, 2008; Montgomery *et al.*, 2010). This regenerative neurogenesis relies upon Müller glia, which also serves as the intrinsic retinal stem cell. Regardless of the nature of the injury, the response of Müller glia and their progeny is relatively stereotyped (Raymond *et al.*, 2006). At about 24 h post-lesion (hpl), Müller glia re-enter the cell cycle. Progeny from individual Müller glia then divide rapidly to form neurogenic clusters that envelope the parent Müller glia. This proliferative phase peaks at about 72–80 hpl (Figure 5). Retinal progenitors within neurogenic clusters then migrate to the sites of cell death, exit the cell cycle and differentiate to replace the missing neurons (Hitchcock *et al.*, 1992; Fausett and Goldman, 2006; Bernardos *et al.*, 2007; Thummel *et al.*, 2008; Nelson and Hyde, 2010). Each type of regenerated neuron then re-establishes its characteristic synaptic connections (Hitchcock and Cirenza, 1994; Hitchcock, 1997).

One of the most utilized injury paradigms is a photolytic lesion that selectively kills photoreceptors (Vihtelic *et al.*, 2006; Taylor *et al.*, 2012; Thomas *et al.*, 2012; Figure 5). The advantage of this approach is that light-induced damage selectively kills only photoreceptors and leaves other neuronal classes undamaged. The selective death of photoreceptors serves as a model for aspects of human photoreceptor dystrophies. As for all retinal injuries in zebrafish, the death of photoreceptors (Figure 5A–D) stimulates Müller glia to de-differentiate, enter the cell cycle and give rise to photoreceptor progenitors that migrate to the depleted outer nuclear layer (ONL) (Figure 5E). These progenitor cells then exit the cell cycle and differentiate into rod and cone photoreceptors. Interestingly, the order of photoreceptor regeneration (cone regeneration precedes rod regeneration) recreates the temporal pattern of photoreceptor genesis during retinal development (Raymond *et al.*, 2006).

Relatively little is known about the molecular mechanisms that govern the ability of Müller glia to de-differentiate and re-enter the cell cycle, though this is presently being intensely investigated (Qin *et al.*, 2009; Calinescu *et al.*, 2009a; Craig *et al.*, 2010; Meyers *et al.*, 2012; Nelson *et al.*,

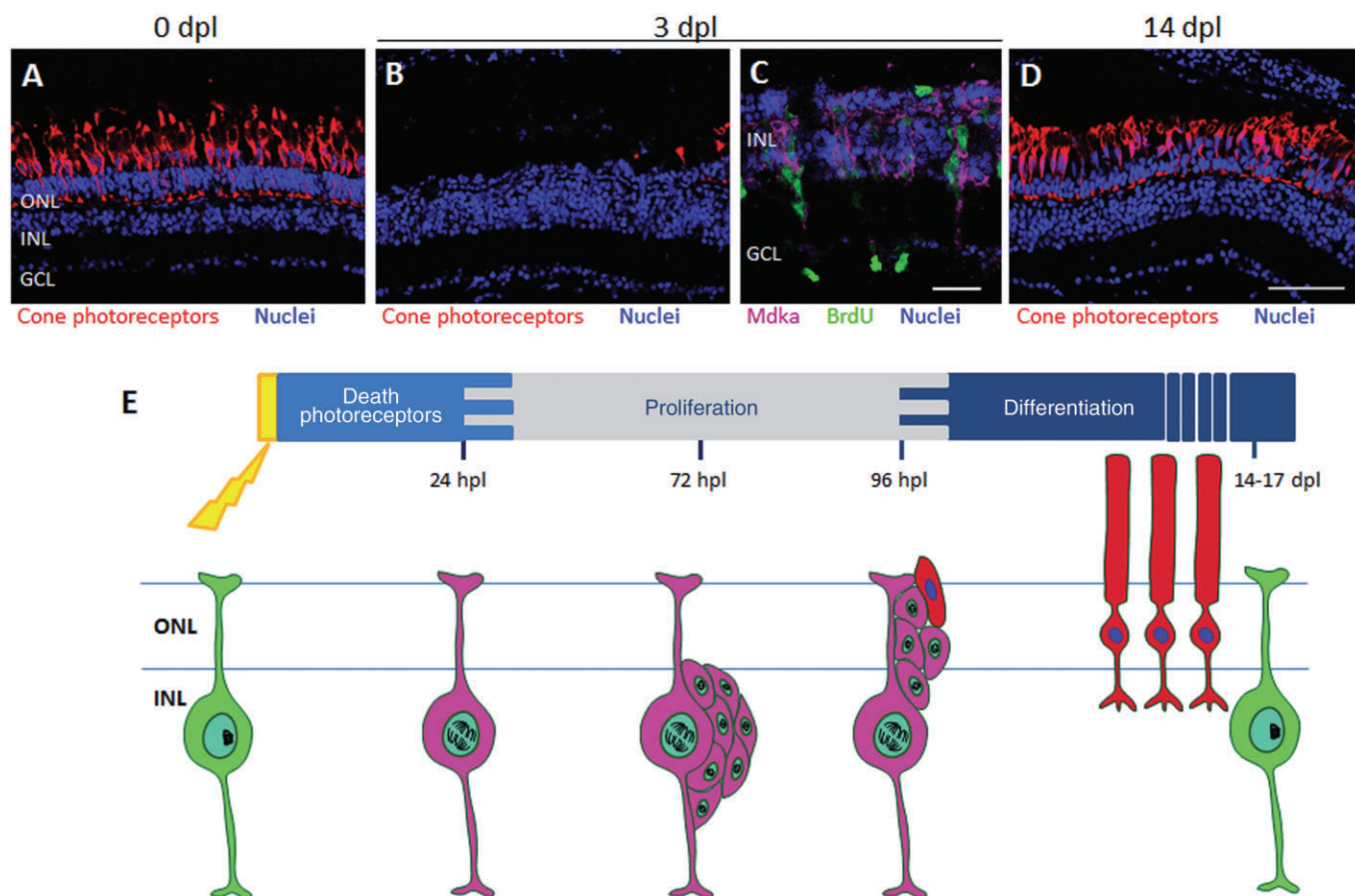


Figure 5

Midkine expression in zebrafish retinal regeneration. A: Cone photoreceptors are immunolabelled (red signal) in an unlesioned retina. B: Photoreceptors are nearly completely killed following exposure to a photolytic lesion. C: *In situ* hybridization showing that *mdka* is expressed in proliferating photoreceptor progenitors (pink label) following photoreceptor death. The *mdka* message co-localizes with antibody staining for bromodeoxyuridine (BrdU, green signal). D: Fourteen days following the onset of a photolytic lesion, rod (not shown) and cone photoreceptors (red signal) are regenerated. E: Timeline of the different processes that occur in the adult zebrafish retina after light-induced damage and the proliferative response of Müller glia. The pink colour represents the onset and duration of *mdka* expression in Müller glia and photoreceptor progenitors. Scale bar = 25 μ m (C) and 50 μ m (A, B and D). GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer; dpl: days post-lesion.

2012; 2013; Ramachandran *et al.*, 2012; Wan *et al.*, 2012). Studies to date indicate that dying neurons are likely to signal to Müller glia their imminent demise, and key transcriptional regulators and signalling pathways within Müller glia govern their ability to re-enter the cell cycle. For example, it was recently discovered that dying photoreceptors synthesize TNF- α (Nelson *et al.*, 2013), which serves as a potential paracrine signal to Müller glia (see also Rattner and Nathans, 2005; Zhou *et al.*, 2012). Similarly, recent studies have shown that induction of the transcription factors ascl1a achaete-scute complex-like 1a (Ascl1a) (Ramachandran *et al.*, 2010), Stat3 (Nelson *et al.*, 2012) and Insm1a (insulinoma-associated 1a) (Ramachandran *et al.*, 2012) are required for Müller glia to re-enter the cell cycle.

Though they have yet to be studied experimentally, midkines are hypothesized to also be important in neuronal regeneration in the zebrafish retina. The presence of *midkines* in the zebrafish retina was first discovered in a microarray screen for genes whose expression is induced by photorecep-

tor death and during photoreceptor regeneration (Calinescu *et al.*, 2009a). The microarray data were validated both by qRT-PCR and *in situ* hybridizations. Following photoreceptor death, *mdka* expression expands from its restricted expression in HCs (Figure 3A) to cells throughout the INL and ONL (Figure 5C). Similarly, *mdkb* expression expands from the inner tier of the INL to cells throughout this cellular layer, including HCs (not shown). As shown by *in situ* hybridization, this broadening of the expression within the INL appears to be at a relatively low level. Markedly, however, the expression of both midkine paralogues is induced at high levels in Müller glia that have re-entered the cell cycle and in dividing photoreceptor progenitors (Figure 5C,E). This *de novo* induction of *mdka* and *mdkb* in Müller glia suggests an important role for these cytokines in photoreceptor regeneration. As discussed above, separate functions have been identified for *Mdka* and *Mdkb* in the developing brain of zebrafish (Liedtke and Winkler, 2008; Luo *et al.*, 2012). The fact that both paralogues of *midkine* have coincident expression in the

proliferating Müller glial suggests that both cytokines may govern common cellular functions during neuronal regeneration. Further studies are needed to understand the function of these midkines during retinal regeneration.

Midkine receptors in the retina

As a growth factor, midkine exerts its function through binding with a variety of receptors (see Sakamoto and Kadomatsu, 2012), including anaplastic lymphoma kinase (ALK) (Stoica *et al.*, 2002), low density lipoprotein receptor related protein 1 (LRP1) (Muramatsu *et al.*, 2000), integrins (Muramatsu *et al.*, 2004), Notch2 (Huang *et al.*, 2008), and cell surface proteoglycans such as syndecan, glypican (Kurosawa *et al.*, 2001), neuroglycan-C (Ichihara-Tanaka *et al.*, 2006) and the receptor protein tyrosine phosphatase β/ζ (RPTP β/ζ) (Maeda *et al.*, 1999).

Although all the proposed midkine receptors are expressed in the vertebrate retina (Table 1), at present it remains unclear which receptor, or combination of receptors (see below), transduce midkine function in this tissue. One possibility is that, as in other tissues, ALK is involved in the midkine-induced proliferation (Reiff *et al.*, 2011). Also, a recent study has shown that in the developing mouse retina, *notch2* is expressed in progenitors and Müller glia (Zhu *et al.*, 2013), an expression pattern that is reminiscent of that for *mdka* in the developing zebrafish. In addition, in zebrafish the cellular expression of *mdka* and *notch2* are similar during

retinal regeneration (Cameron *et al.*, 2005; Calinescu *et al.*, 2009a). Based on these data, it would be reasonable to evaluate the potential functional relationship between *Mdka* and either ALK or Notch2 both during development and regeneration in the zebrafish retina. Interestingly, in mice it has been shown that RPTP β/ζ (receptor protein tyrosine phosphatase β/ζ) inhibits the proliferation of oligodendrocyte precursor cells and promotes their development into mature oligodendrocytes (Lamprianou *et al.*, 2011). Whether or not *Mdka* regulates cell cycle kinetics in the developing retina through binding RPTP β/ζ is still unclear. A potential complexity is that midkine receptors may function as components in a molecular complex. For example, RPTP β/ζ and LRP6 can be co-immunoprecipitated (Muramatsu *et al.*, 2004). Clearly, initiatives are needed to identify and functionally characterize midkine receptors and additional downstream signalling pathways in both the developing and regenerating retina.

Conclusions

As in other regions of the mammalian brain, retinal neurons that are lost through diseases or injury are not replaced. In distinct contrast, in the zebrafish, spontaneous stem cell-based regeneration occurs to replace lost neurons and restore lost function. In zebrafish, Müller glia can re-enter the cell cycle and regenerate all neuronal types – a process in which midkine may play a central role. A better understanding of this regulated reprogramming by Müller glia could provide

Table 1

Receptors for midkine and their expression patterns in retina

Receptor	Known expression pattern in retina	References
ALK	Neural layer of E11.5 and E13.5 mouse retina	Vernersson <i>et al.</i> , 2006.
Notch2	Mouse Müller glia	Roesch <i>et al.</i> , 2008;
	Mouse E9.5 optical vesicle, E11.5 and E14.5 retinal progenitors, presumptive future Müller glial cells at P6, adult Müller glia	Zhu <i>et al.</i> , 2013;
	Weak expression in adult zebrafish retina but up-regulated after lesion	Cameron <i>et al.</i> , 2005.
LRP1	RGC in normal rat retina	Shi <i>et al.</i> , 2008.
Integrins	Developing chick retina ($\alpha 4$; $\beta 1$; $\alpha 6$ $\beta 1$ integrins)	Leu <i>et al.</i> , 2004; Cann <i>et al.</i> , 1996; de Curtis <i>et al.</i> , 1991)
	Mouse RGC and undifferentiated retinal neuroblasts during axon extension and migration ($\alpha 4$ $\beta 1$ integrin)	Hikita <i>et al.</i> , 2003.
	Tiger salamander retina ($\alpha 1$ –6 integrins)	Sherry and Proske, 2001.
Syndecan-3 (N-Syndecan)	Transiently expressed in the neural fibres at early post-natal stages and in the axons of RGCs in rat retina	Inatani <i>et al.</i> , 2002.
Glypican	Optic nerve, NFL of the optic cup and weakly in the INL at E16 rat retina	Karthikeyan <i>et al.</i> , 1994.
NGC	NFL and IPL of post-natal rat retina	Inatani <i>et al.</i> , 2000.
RPTP β/ζ	Chick Müller glia culture	Shock <i>et al.</i> , 1995;
	Mouse E13 retina	Horvat-Bröcker <i>et al.</i> , 2008;
	Mouse E13 retina (precursor cells) and mature retina (RGC, HC, NFL, IPL, OPL)	Klausmeyer <i>et al.</i> , 2007.

ALK, anaplastic lymphoma kinase; HC, horizontal cell; INL, inner nuclear layer; IPL, inner plexiform layer; LRP1, low-density lipoprotein receptor-related protein 1; NFL, nerve fibre layer; NGC, neuroglycan C; OPL, outer plexiform layer; RGC, retinal ganglion cells; RPTP β/ζ , receptor protein tyrosine phosphatase β/ζ .

insights into reprogramming potential stem cells in the mammalian retina and brain. It is not too speculative to infer that midkine, or drugs acting on midkine signalling pathways, may have therapeutic use for stimulating neuronal regeneration in the mammalian CNS. Key to this approach will be knowledge of which receptor or combination of receptors mediate midkine function in the brain. An immediate goal will be to link the intrinsic neuronal regeneration in zebrafish and the molecular biology of midkine signalling so that potential therapeutic approaches for treating neuronal degeneration in the human retina can be developed.

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Conflict of interest

We have no conflicts of interest to declare.

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