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Stem cells in the teleost retina: persistent neurogenesis and injury-induced regeneration

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Abstract

The retina of the adult teleost fish is an important model for studying persistent and injury-induced neurogenesis in the vertebrate central nervous system. All neurons, with the exception of rod photoreceptors, are continually appended to the extant retina from an annulus of progenitors at the margin. Rod photoreceptors, in contrast, are added to differentiated retina only from a lineage of progenitors dedicated to making rods. Further, when the retina is lesioned, the lineage that produces only rods ceases this activity and regenerates retinal neurons of all types. The progenitors that supply neurons at the retinal margin and rod photoreceptors and regenerated neurons in the mature tissue originate from multipotent stem cells. Recent data suggest that the growth-associated neurogenic activity in the retina is regulated as part of the growth hormone/insulin-like growth factor-I axis. This paper reviews recent evidence for the presence of stem cells in the teleost retina and the molecular regulation of neurogenesis and presents a consensus cellular model that describes persistent and injury-induced neurogenesis in the retinas of teleost fish.

1. Introduction

In teleost fish, retinal neurogenesis continues beyond embryonic development, well into adult life. In addition, following the destruction of retinal neurons, the retina can regenerate and restore visual function. Work in multiple labs has demonstrated that all of the cellular and synaptic elements present in a normal retina can be recreated during regeneration, that the regenerated retina produces electroretinograms with normal wave forms and can mediate simple behaviors. In addition, although the number examined thus far is small, regulatory genes expressed during retinal development are re-expressed during regeneration (reviewed by Easter & Hitchcock, 2000; Raymond & Hitchcock, 1997). This striking capacity for neuronal regeneration is related to the continual growth-associated neurogenesis in the central nervous system (CNS) of fish and the presence of stem cells and their progeny within the mature retina.

In contrast to the persistent neurogenesis in the retina of teleosts, neurogenesis in the retina of mammals is completed during pre- and peri-natal development (Carter-Dawson & LaVail, 1979), and there is as yet no evidence for continual neurogenesis or regeneration in the adult retina. Recent reports of putative retinal stem cells within the ciliary epithelium of rodents (Ahmad, Tang, & Pham, 2000; Tropepe et al., 2000) have provoked interest in the possibility of developing stem cell transplantation therapies for the treatment of blinding retinopathies. Despite the excitement, the true potential for such strategies remains uncertain. Although ciliary epithelium-derived cells from mammals can proliferate in vitro, there is currently no evidence that they normally function as retinal stem cells within the ciliary epithelium in vivo. Further, although brain-derived stem cells can migrate into an injured or degenerating retina following transplantation (Kurimoto et al., 2001; Pressmar, Ader, Richard, Schachner, & Bartsch, 2001; Warfvinge, Kamme, Englund, & Wictorin, 2001; Young, Ray, Whiteley, Klassen, & Gage, 2000), their capacity to restore visual function remains unproven.

An understanding of the cellular and molecular mechanisms that regulate growth-associated and

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injury-induced neurogenesis in the retinas of teleost fish may lead to new approaches for enhancing or controlling the regenerative capacity of stem cells for future therapeutic uses. This review will examine current knowledge of retinal neurogenesis and regeneration in teleosts, including the known patterns of retinal growth, identification of the cellular sources of new retinal neurons and recent progress in understanding the mechanisms regulating retinal neurogenesis in vivo.

2. Growth-associated, persistent neurogenesis in the retina of teleost fish

As a fish grows over its lifetime, there can be substantial increase in the size of its eye and of the retina within. For example, in goldfish a 4-fold increase in body length (from 5 to 20 cm) is associated with a 6-fold increase in retinal area (from 20 to 120 mm^2) (Johns & Easter, 1977). The growth of the retina results from both hypertrophy, a balloon-like expansion of the retina and physical enlargement of the differentiated cells (Ali, 1964; Hitchcock & Easter, 1986; Johns, 1977; Johns & Easter, 1977; Lyall, 1957b) and hyperplasia, the generation of new neurons (e.g., Johns, 1977; Müller, 1952). In the retina new neurons are added in two regions. First, all neuronal types, with the exception of rod photoreceptors, are added at the retinal margin in a process that recapitulates embryonic development. Second, rod photoreceptors are added to the differentiated central retina.

2.1. Neurogenesis at the circumferential germinal zone

In the retinas of fish, the primary site of post-embryonic neurogenesis is located at the ciliary margin, the junction between the retina and the iris epithelium. Here, a remnant of the embryonic retina persists, forming a circumferential germinal zone (CGZ) that continually adds new neurons to the margin of the extant retina. The CGZ was identified 50 years ago based on cell morphology and the location of mitotic figures (Lyall, 1957b; Müller, 1952), and the role of the CGZ in retinal neurogenesis has been confirmed by multiple methods including labeling dividing cells with thymidine analogues such as H^3-thymidine (Johns, 1977; Meyer, 1978; Scholes, 1976; Sharma & Ungar, 1980) and bromodeoxyuridine (BrdU) (Johns, 1977; Meyer, 1978; Stenkamp, Barthel, & Raymond, 1997). As cells within the CGZ proliferate, they give rise to daughter cells that either renew the progenitor population within the CGZ or differentiate. Newly differentiated retinal neurons are integrated seamlessly into all layers of the existing retina in concentric annuli, much like the growth rings of a tree (Hitchcock, Macdonald, Vanderyt, & Wilson, 1996; Johns, 1977; Meyer, 1978; Stenkamp et al., 1997; Vecino, 1998; see also reviews by Easter, 1983; Fernald, 1991). This annular pattern of growth creates an spatial ordering of retinal development: the youngest and least determined cells reside within the CGZ, newly differentiated retinal neurons lie immediately adjacent to the CGZ and progressively older and more mature retinal cells are present in the fundus.

The CGZ is not unique to fish. Other cold-blooded vertebrates (notably frogs and salamanders) also have a circumferential growth zone, called the ciliary marginal zone (see reviews Harris & Perron, 1998; Reh & Levine, 1998). However, it has long been believed that warm-blooded vertebrates (such as birds and mammals) do not. This idea has been re-examined in light of the recent re-discovery of proliferating cells within a CGZ at the periphery of the retina of chicks (Fischer & Reh, 2000). These cells can be detected for at least four months post-hatching, but their capacity for neurogenesis appears more limited than in fish or frog. Cells within the chick CGZ increase proliferation when retinal growth is stimulated by form deprivation (covering of the eye with a light diffusing lens), but fail to proliferate in response to acute retinal damage, indicating that these cells are unlikely to contribute to retinal regeneration. In mammals, there is no evidence of a CGZ, although putative retinal stem cells have been cultured from the ciliary epithelium adjacent to the retinal margin (Ahmad et al., 2000; Tropepe et al., 2000). This has prompted comparisons of these cells to the CGZ in fish and frog, although clear embryological and evolutionary relationships have yet to be demonstrated. The profound capacity of the CGZ to generate retinal neurons in fish and frog has stimulated research to determine the potential of the cells in the ciliary epithelium of mammals to generate retinal neurons.

2.2. Genesis of rod photoreceptors

As the teleost retina grows by expansion rod photoreceptors are continually added to the differentiated retina. This serves to maintain a relatively constant density of rod photoreceptors, while the density of all other retinal cell types is reduced (Johns, 1982; Johns & Easter, 1977). The quest to identify the cellular origin of the new rods has provoked numerous theories, including migration or displacement of post-mitotic neurons from the CGZ (Johns, 1977; Müller, 1952) and the transdifferentiation of cone photoreceptors (Lyall, 1957a). The use of H^3-thymidine to label proliferating cells yielded no evidence for lateral migration of cells from the CGZ to mature central retina, however, these same studies did provide unambiguous evidence that proliferating cells within the outer nuclear layer (ONL), known as rod precursors, are the immediate antecedents of new rod photoreceptors (Johns, 1982; Johns & Fernald, 1981; Sandy & Blaxter, 1980).

Identifying rod precursors seemed to provide a denouement in the search for the cellular source of the new rod photoreceptors, however this prompted an important and obvious question. What was the origin of the rod precursors? The answer arose from light and electron microscopic analyses of retinal development in a variety of teleost species [goldfish (Johns, 1982); zebrafish (Branchek & Bremiller, 1984); flounder (Evans & Fernald, 1993); an African cichlid (Hagedorn, Mack, Evans, & Fernald, 1998); anchovy (Haacke, Hess, Melzar, Gebhart, & Smola, 2001)]. As larval development commences, the ONL contains cone photoreceptors only, neither rods nor rod precursors are present (see also Blaxter, 1975; Blaxter & Staines, 1970; Lyall, 1957a; Wagner, 1974). Rod photoreceptors arise from a secondary wave of neurogenesis that originates in a population of mitotically active cells located in the inner nuclear layer (INL) (Hoke & Fernald, 1997; Raymond, 1985). These cells form small radially elongated columns (called neurogenic clusters) and migrate from the INL to the ONL, seeding the ONL with the first generation of rod precursors. The migration of cells from the INL to ONL was inferred from their morphology and changes in the number of labeled nuclei in the INL vs. ONL following pulse injection of H^3-thymidine (Johns, 1982; Raymond & Rivlin, 1987). The logical conclusion was that the proliferative cells in the larval INL were remnants of the embryonic neuroepithelium that then served as the origin of rod precursors and new rod photoreceptors. In adult and juvenile fish a similar process is recapitulated adjacent to the CGZ (Fig. 1). In a narrow annulus of newly differentiated retina, which we call the circumferential larval zone (CLZ), the ONL contains cone photoreceptors only. Rod photoreceptors first appear central to this annulus in what is slightly more mature retina (Johns, 1982; Stenkamp et al., 1997) and, equivalent to rod genesis in larval fish, rod precursors originate from progenitors within the INL (Johns, 1982; Otteson et al., 2001).

2.3. The lineage of rod photoreceptors

It has been inferred that in adult and juvenile fish, the progenitors in the INL divide relatively slowly, because

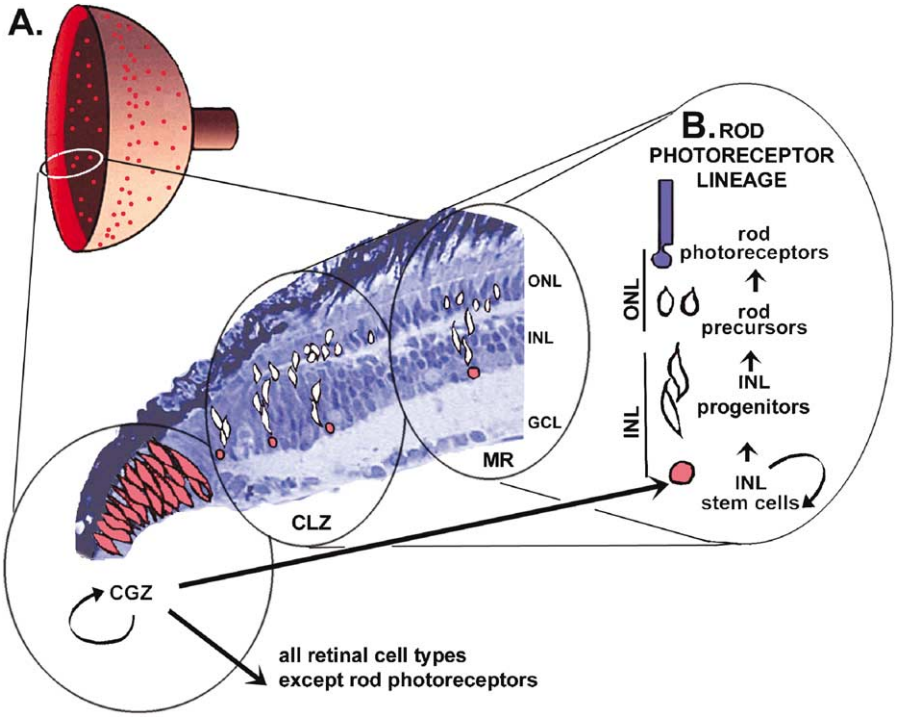

Fig. 1. Model of retinal neurogenesis and the generation of rod photoreceptors in the retinas of teleosts. (A) Cartoon of the retina depicted as a hemisphere with the sites of neurogenesis in red. Below is a photomicrograph of a radial section through circled region of retina showing growth zones and their characteristic patterns of cellular proliferation. Mitotically active, Pax6 expressing retinal progenitors (shown in red) form the CGZ and give rise to all retinal neurons, except rod photoreceptors. Stem cells are also "seeded" into the INL from the CGZ. The CLZ contains the newly differentiated retina that contains cone photoreceptors only. Within the CLZ, mitotically active cells, daughters of resident Pax6 expressing stem cells (shown in red), migrate from the INL to the ONL to produce the first generation of rod photoreceptors within the CLZ. This process continues, but at a slower pace, in the mature retina. (B) This panel illustrates a lineage model for rod genesis (adapted from Otteson, D'Costa, & Hitchcock, 2001). Within the differentiated retina, stem cells in the INL proliferate, renewing the stem cell population and giving rise to Pax6-negative INL progenitors (shown in white). Progenitors proliferate as they migrate to the ONL, where they become rod precursors and ultimately differentiate into new rod photoreceptors. INL, inner nuclear layer; ONL, outer nuclear layer; GCL, ganglion cell layer; MR, mature retina; CGZ, circumferential germinal zone; CLZ, circumferential larval zone.

cells within the INL are rarely labeled following brief exposures to ^{3}H-thymidine or BrdU. In an effort to label and characterize these slowly dividing INL progenitors, Julian, Ennis, and Korenbrot (1998) devised a method to achieve long-term exposure of mitotically active cells to BrdU without resorting to repeated intraocular injections, which can damage the eye and confound any observations. It was surprisingly simple. Fish were housed in dilute solutions of BrdU for a period of hours to days. BrdU is presumed to enter the vasculature through the gills and achieve systemic levels sufficient to label all cells passing through S-phase during the period of exposure. Using this technique, they could readily label mitotically active cells in the INL of juvenile and adult trout. Similarly, we used this systemic-labeling method to identify and characterize mitotically active cells within the INL of goldfish (Otteson et al., 2001). Following nine days of BrdU exposure, several populations of dividing cells were labeled within the retina. As expected, the relatively rapidly cycling cells of the CGZ and rod precursors within the ONL were labeled. In addition, two populations of slowly dividing cells within the INL were labeled. These cells were few in number, relative to labeled rod precursors, and could be subdivided by nuclear morphology: the majority had radially elongated, fusiform nuclei, whereas the remaining cells had spherical nuclei. The labeled cells in the INL were most abundant in areas of rapid addition of rod photoreceptors, notably within the all-cone CLZ, as predicted by Johns (1982) (Fig. 1). BrdU labeled cells were also present in the INL in more central retina suggesting that here too rod precursors originate from progenitors within the INL (Otteson et al., 2001; see also Julian et al., 1998).

The fusiform cells in the INL were frequently in radially elongated clusters that clearly resembled present in larval goldfish (Johns, 1982; Raymond & Rivlin, 1987) and juvenile trout (Julian et al., 1998). Julian and colleagues, unequivocally demonstrated that in trout these cells migrate from the INL to the ONL, where they become new rod photoreceptors. Our observations suggest that the fusiform cells present in the INL of goldfish behave similarly. This is based, in part, on their morphological similarity to the migrating cells present in the INL of larval goldfish and trout, and our results from fish allowed to survive long-term following BrdU labeling. In animals exposed to BrdU for 9 days and subsequently transferred to fresh water (without BrdU) for 30 days, there was a significant reduction in the proportion of BrdU-labeled fusiform cells in the INL, concomitant with a dramatic increase in the number of labeled cells in the ONL, consistent with their migration from the INL to the ONL.

In addition to the fusiform cells, a second population of cells in the INL of goldfish were labeled after long-term systemic exposure to BrdU. These cells were rare, had spherical nuclei, and a subset expressed Pax6 (Otteson et al., 2001), a marker of retinal stem cells (Hitchcock et al., 1996; Perron, Kanekar, Vetter, & Harris, 1998). In addition, these cells persisted in the INL, indicating that they did not migrate, and they did not express markers of differentiated retinal neurons, indicating they had not differentiated. Interestingly, Johns (1982) observed persistently labeled cells in the INL in larval fish. In an electron micrographic study Johns described two [^{3}H]-thymidine labeled cells in the INL that had spherical nuclei, homogeneous chromatin and pale cytoplasm that were associated with (but distinct from) the fusiform cells and Müller glia that formed the neurogenic clusters. Based on these observations, Otteson et al. (2001) proposed that the rare, Pax6-expressing cells that could be labeled with BrdU are retinal stem cells that are the origin of a lineage of cells that normally generates rod photoreceptors.

3. Retinal regeneration in teleosts

It was first shown more than a quarter century ago that the retinas of teleost fish can regenerate following injury (Lombardo, 1968, 1972). Throughout the intervening years, numerous methods have been used to destroy retinal neurons (surgical removal of a portion of the retina (Cameron, 2000; Cameron, Cornwall, & MacNichol, 1997, 1999; Cameron & Easter, 1995; Hitchcock, Myhr, Easter, Mangione-Smith, & Jones, 1992; Hitchcock et al., 1996; Hitchcock & Vanderyt, 1994; Lombardo, 1968, 1972), intraocular injection of metabolic poisons (Maier & Wolburg, 1979; Mensinger & Powers, 1999; Raymond, Reifler, & Rivlin, 1988), intraocular injection of neurotoxins (Braisted & Raymond, 1992, 1993; Negishi, Teranishi, Kato, & Nakamura, 1987; Negishi et al., 1991a,b), laser photocoagulation (Braisted, Essman, & Raymond, 1994; Wu et al., 2001) and light damage (Vihtelic & Hyde, 2000)), and in each instance the lesion initiates injury-induced neurogenesis. For example, surgical removal of a small (1–2 mm^{2}) portion of the retina stimulates mitotically active cells in the INL and ONL to form radially elongated clusters that organize into a regenerative blastema that caps the wound margin (Hitchcock et al., 1992). Morphologically and functionally, the blastema resembles the CGZ and adds new neurons to the surviving retina, filling in the gap created by the surgical lesion (Hitchcock et al., 1992).

A number of possible sources for the regenerated neurons have been considered. In early studies, the CGZ was proffered as the likely contributor because it was the only site where mitotic figures were observed prior to the formation of the blastema (Lombardo, 1968, 1972). More recent studies have found no evidence that cells migrate from the CGZ to the central retina as a part of

either normal or regenerative neurogenesis (Hitchcock et al., 1992, 1996; Johns, 1982; Raymond et al., 1988). Although pigmented epithelial cells are the source for retinal regeneration in amphibians (Mitashov, 1996, 1997; Reh, Nagy, & Gretton, 1987) and in embryonic chick (Coulombre & Coulombre, 1965), there is no evidence that pigmented epithelial cells contribute to retinal regeneration in fish (Knight & Raymond, 1994; see reviews by Hitchcock & Raymond, 1992; Raymond & Hitchcock, 1997). Müller glia in the retina of goldfish respond to focal lesions by upregulating glial fibrillary acidic protein expression, translocating their nuclei to the ONL and incorporating BrdU (Braisted et al., 1994; Wu et al., 2001), however there is no evidence supporting these cells as the source of regenerated neurons. In the retina of the post-hatch chick, the Müller glia demonstrate the ability to de-differentiate into retinal progenitors (Fischer & Reh, 2001), but significant regeneration of retinal neurons does not occur. Since Müller glia derive from the same lineage as the retinal neurons (Cepko, 1993) they remain a possible source for retinal regeneration in teleosts.

Following any form of retinal lesion, the bulk of mitotic activity is in the ONL; therefore rod precursors were considered a likely cellular source for regenerated neurons (Braisted & Raymond, 1992, 1993; Hitchcock et al., 1992, 1996; Negishi et al., 1991a,b; Raymond et al., 1988; Sullivan, Barthel, Largent, & Raymond, 1997). The most compelling evidence favoring a role for rod precursors is the observation that for regeneration to occur, the ONL must sustain cell loss regardless of the extent of cell death in other retinal layers (Braisted & Raymond, 1992; Negishi et al., 1987; Raymond et al., 1988). Injury-induced proliferation is not limited to the ONL, however. Radially elongated clusters of proliferating cells are also observed in INL following a retinal lesion (Braisted et al., 1994; Cameron, 2000; Hitchcock et al., 1996; Negishi et al., 1991a,b; Raymond et al., 1988; Sullivan et al., 1997; Vihtelic & Hyde, 2000; Wu et al., 2001). Morphologically, these clusters resemble the neurogenic clusters that contribute to rod photoreceptor neurogenesis during larval development and the fusiform INL progenitors present in the mature retina (Julian et al., 1998; Otteson et al., 2001; Raymond et al., 1988).

4. Stem cells in the teleost retina

So far, we have used the term ''stem cells'' sparingly. This is for two reasons. First, by the most rigorous criteria, only those cells capable of producing all cell types in the body (e.g. embryonic stem cells) might be considered true stem cells. However, this distinction is rarely used. Clearly, many self-renewing tissues have intrinsic stem-like populations that are capable of producing all cell types within a more limited lineage and thus can be called stem cells (reviewed by Doe, Fuerstenberg, & Peng, 1998; Weiss et al., 1996). Second, the proposal that stem cells are present within the CNS has provoked some criticism that these studies lack the rigor that has been applied to stem cell biology in other systems (Weiss & Van der Kooy, 1998).

The very characteristics that define stem cells often make them difficult to identify (Doe et al., 1998; Reh & Fischer, 2001; Temple & Alvarez-Buylla, 1999). By classical definitions, stem cells constitute a very small population of undifferentiated cells that persists within a differentiated tissue. They are self-renewing, multi-potent cells that are mitotically quiescent in mature tissues, dividing only frequently enough to supply the demand for new cells associated with growth. They can increase proliferation in response to injury and cell death, ultimately repopulating their target tissue. When stimulated to divide, stem cells undergo either symmetric divisions that amplify the population of stem cells or, more typically, asymmetric cell divisions that give rise to two intrinsically different daughter cells. In the latter case, one daughter cell replaces the original stem cell and the other begins a progression down a path toward differentiation. This second cell, often referred to as a transit amplifying cell or progenitor cell, is capable of limited, but typically more frequent cell divisions and ultimately all of its daughter cells differentiate.

There is ample empirical evidence indicating the presence of stem cells in the retinas of teleost fish. First, there is ongoing generation of all retinal cell types. Second, the loss of retinal neurons can stimulate the regeneration of all cell types from progenitors within the retina (Braisted et al., 1994; Hitchcock et al., 1992; Lombardo, 1968, 1972; Raymond et al., 1988). Third, these retinal progenitors must be self-renewing as growth-related and regenerative neurogenesis occur even in adults. The original source of all retinal cells is the embryonic retina, and, as fish mature, retinal stem cells persisting within the CGZ are responsible for the bulk of growth-associated neurogenesis (Johns, 1977; Meyer, 1978). These are relatively easy to identify by their position, by their morphological similarity to undifferentiated cells of the embryonic retinal neuroepithelium (Johns, 1982) and by their patterns of gene expression (see Perron et al., 1998).

Identifying the stem cells in the central retina that are responsible for regenerative neurogenesis has proven more challenging. Rod precursors have received the most attention, in part because they have been the only population of mitotically active cells that could be consistently labeled within the central retina. However, rod precursors do not fit some of the classical definitions of a stem cell. First, they are labeled by short-term exposure to H^3-thymidine (Johns & Fernald, 1981; Raymond et al., 1988) or BrdU (Braisted et al., 1994;

Hitchcock et al., 1996; Mack & Fernald, 1995, 1997; Stenkamp et al., 1997), indicating that they divide relatively frequently. Second, they have a restricted lineage in the normal retina, giving rise only to rod photoreceptors (Johns & Fernald, 1981; Mack & Fernald, 1995, 1997; Stenkamp et al., 1997). Finally, although stem cells at the CGZ and in the injury-induced blastema express Pax6, rod precursors do not (Hitchcock et al., 1996). This suggests that rod precursors may function more as transit amplifying cells than as stem cells.

Following the initial identification of the fusiform INL progenitors, it was proposed that they could be retinal stem cells (Julian et al., 1998). Their relatively quiescent nature fits well with our concept of stem cells, and the increased proliferation in the INL following retinal damage demonstrates an injury response. However, we have found that like the rod precursors, the migrating, fusiform INL progenitors do not express Pax6 and thus may be transit amplifying cells that have already entered the rod photoreceptor lineage (Otteson et al., 2001). We have shown that a subset of the spherical INL cells labeled only by long-term systemic BrdU do, in fact, express Pax6 and persist in the INL without differentiating. These observations are consistent with the hypothesis that these rare, slowly dividing cells may be intrinsic retinal stem cells. This remains an area of active research and we anticipate that further characterization will provide a more definitive identification.

5. Regulation of persistent neurogenesis in the teleost retina

Many teleost species demonstrate indeterminate growth; if they can obtain a nutritious diet, good environmental conditions and avoid predation, they will continue to grow throughout life (Fig. 2). Therefore, the regulation of mitotic activity of retinal progenitors in the teleost retina can be viewed as a growth-associated phenomenon. Proliferation of retinal progenitors is more robust in animals that are well nourished and growing rapidly than in animals that are in poor condition and growing slowly (Johns, 1982; Otteson et al., 2001). Recent experimental evidence suggests that growth hormone (GH) and insulin-like growth factor-I (IGF-I) regulate retinal neurogenesis and coordinate retinal and somatic growth in the fish (Boucher & Hitchcock, 1998; Otteson et al., 2002; summarized in Fig. 2).

In fish, as in other vertebrates, somatic growth is controlled by GH, and manipulating GH levels has profound effects on body size (reviewed by Duan, 1998; Moriyama, Ayson, & Kawauchi, 2000). GH functions primarily by increasing synthesis of IGF-I in target tissues, both hepatic and non-hepatic. IGF-I, in turn, then

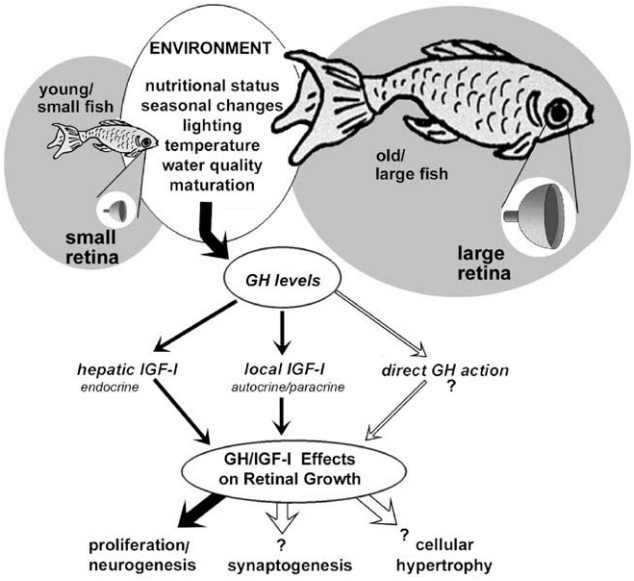

Fig. 2. Model of coordinated retinal and somatic growth regulated by the GH/IGF-I axis. Environmental factors that effect growth impact GH release from the pituitary and somatic growth of the fish. Growth-associated neurogenesis within the retina is yoked to the somatic growth and regulated by the same molecular mechanisms. Black arrows indicate demonstrated actions of the GH/IGF-I axis. White arrows indicate proposed actions of GH and IGF-I (Otteson, Cirenza, & Hitchcock, 2002), including the potential direct effect of GH on retinal progenitors.

acts locally through both endocrine and autocrine/paracrine pathways (reviewed by Butler & LeRoith, 2001). IGF-I is a small peptide growth factor that acts through its cognate receptor (IGF-IR) to mediate multiple biological activities and metabolic functions, including stimulation of synthesis of DNA, proteoglycans, glycosaminoglycans and proteins (see reviews by Benito, Valverde, & Lorenzo, 1996; Froesch, Hussain, Schmid, & Zapf, 1996). In the CNS, activation of the IGF-I signaling pathway has been implicated in a variety of functions: IGF-I stimulates proliferation of neuroepithelial cells, influences recruitment and differentiation of post-mitotic neurons, regulates process outgrowth and synaptogenesis and can be neuroprotective following CNS injury (reviewed by D'Ercole, Ye, Calikoglu, & Gutierrez-Ospina, 1996).

A role for IGF-I in regulating neurogenesis in the retina has been suggested by in vitro studies showing that IGF-I stimulates proliferation of retinal progenitors. IGF-I increases incorporation of H^3-thymidine by neuroepithelial cells isolated from retinas of embryonic chick (Calvaruso et al., 1996; de la Rosa et al., 1994; Hernandez-Sanchez, Lopez-Carranza, Alarcon, de La Rosa, & de Pablo, 1995). IGF-I also increases proliferation in primary cultures of Müller glia isolated from human retina (Ikeda, Waldbillig, & Puro, 1995). In cultured slices from the retinas of cichlids, IGF-I stimulates proliferation of rod precursors in the ONL

(Mack, Balt, & Fernald, 1995; Mack & Fernald, 1993) and in ex vivo cultured eyecups from goldfish, IGF-I stimulates proliferation of retinal progenitors within the CGZ (Boucher & Hitchcock, 1998).

Recent evidence indicates that the GH/IGF-I axis regulates the persistent, growth-associated neurogenesis in teleost retina. Components of the GH and IGF-I signaling pathways are present in the retina. IGF-I and the IGF-I receptor mRNA are present in the retina, and in situ hybridization demonstrates that differentiated neurons and retinal progenitors within the CGZ express the receptor (Otteson et al., 2002). Further, an intraperitoneal injection of GH elevates IGF-I expression in the retina (Otteson et al., 2002), indicating that the GH receptor is also expressed in the retina (this result has been confirmed by reverse transcriptase-polymerase chain reaction (Hitchcock, unpublished observation)), and that the retina is a target of GH action. Further, intraperitoneal injections of GH also stimulate mitotic activity of retinal progenitors within the CGZ, and the cells of the rod-photoreceptor lineage, including the rare, putative stem cells within the INL (Otteson et al., 2002).

6. A consensus cellular model of persistent and injury-induced neurogenesis in the teleost retina

Based on the current concepts of retinal stem cells and studies describing the persistent neurogenesis in teleosts, we propose a consensus model for the lineage of rod photoreceptors in the teleost retina and suggest that cells within this lineage are the source of regenerated neurons (Otteson et al., 2001). In this model (summarized in Fig. 1), a population of slowly dividing stem cells persists in the INL of the mature retina, and these cells are the source of the migrating INL progenitors and rod precursors (Fig. 1B). Stem cells are initially sequestered in the INL during embryonic development and lie in association with the Müller glia (Raymond & Rivlin, 1987). During larval development, stem cells self-renew and give rise to INL progenitors. The progenitors acquire a fusiform morphology as they migrate along the radial processes of the Müller glia to the ONL, and undergo additional and presumably symmetric cell divisions en route to the ONL. Upon reaching the ONL, the INL progenitors become committed (or constrained) to a rod photoreceptor fate, becoming rod precursors which undergo additional but rapid cell divisions. Ultimately, the daughter cells differentiate into new rod photoreceptors that are intercalated into the pre-existing mosaic of cones and the space-filling lawn of rods (Hagedorn & Fernald, 1992; Hagedorn et al., 1998; Johns, 1982; Johns & Fernald, 1981).

During subsequent post-larval growth, this pattern of rod photoreceptor genesis is recapitulated. At the CGZ, as new annuli of neurons are added to the retina, stem cells are also seeded into the INL. The newly formed, all-cone retina is subsequently transformed into a mature retina containing both rods and cones by the same process that occurs during larval development: the INL stem cells divide, renewing their own population and giving rise to INL progenitors that migrate to the ONL to produce rod precursors and ultimately new rod photoreceptors. This same process appears to continue within the central retina, albeit at a much slower pace that is more consistent with the lower demand for rod photoreceptors. The difference between rod photoreceptor genesis in the retinas of larval goldfish, in the larval growth zone and in the mature central retina in older animals is thus one of scale, not of substance. Differences in the apparent abundance of mitotically active cells in the retinas of larval fish vs. older fish seem to reflect differential demands for new rod photoreceptors, rather than intrinsic differences in the mechanisms for rod photoreceptor production.

We believe that variations in mitotic activity of retinal progenitors are regulated at least in part by the GH/IGF-I axis (summarized in Fig. 2). Changes in IGF-I levels appear to integrate nutritional status, age, maturation and environmental conditions and serve to maintain retinal growth that is scaled to overall somatic growth. Thus, a young, well nourished animal would have higher GH and IGF-I levels, leading to rapid growth. The GH and IGF-I levels would also stimulate neurogenesis in the retina in order to maintain visual acuity. There is good evidence that changes in GH result in changes in IGF-I levels both in serum (the hepatic, endocrine IGF-I) and in non-hepatic tissues (autocrine/paracrine IGF-I). IGF-I is mitogenic for cells within the CGZ and rod photoreceptor lineage. Given its known effects on protein synthesis, cellular hypertrophy and synaptogenesis, IGF-I may also participate in regulation of retinal hypertrophy. However, we currently have no direct evidence regarding this hypothesis.

Implicit in this model is the idea that the quiescent INL stem cells are the cellular source not only for growth-associated neurogenesis, but for regenerative neurogenesis as well (Fig. 3). Various injuries to the retina stimulate proliferation of clusters of cells in the INL that morphologically resemble the INL progenitors. In the case of surgical lesion to the retina, where all cell types are regenerated, the clusters of injury-induced progenitors are Pax6+ (Hitchcock et al., 1996). We cannot yet distinguish the relative contributions of the various cell populations within the rod lineage. However, we do know that following surgical lesions, Pax6-expressing cells are found in both the outer and inner nuclear layers. The proposed Pax6-expressing retinal stem cells within the INL constitute a very small population and could be a source of the injury-induced cells. We cannot exclude a direct contribution from the other

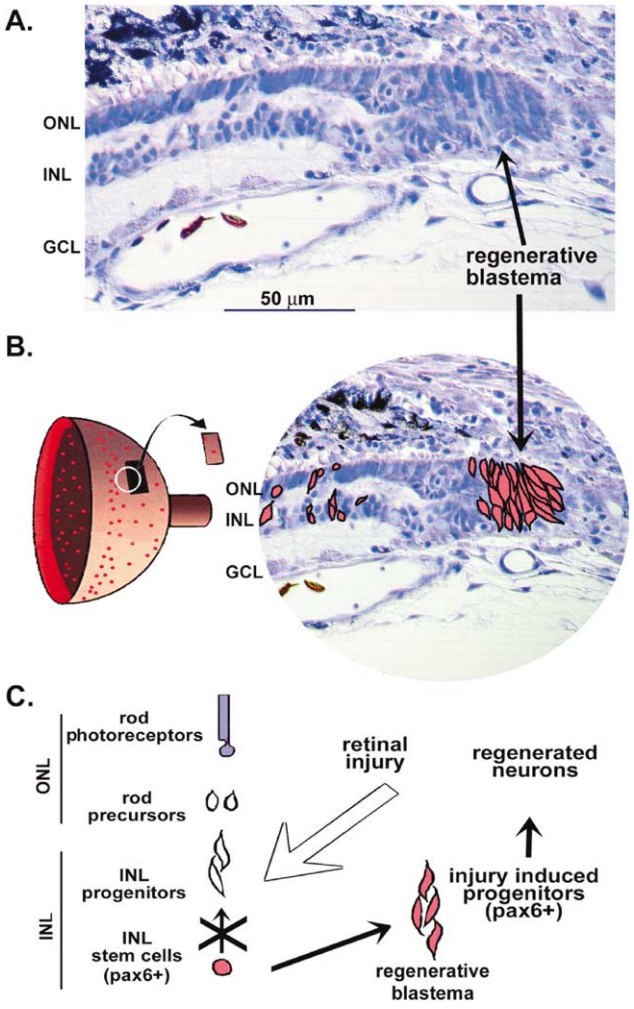

Fig. 3. (A) Cartoon illustrating the regenerative response to surgical removal of a piece of retina. Panel A illustrates a photomicrograph of the blastema two weeks post-lesion. Panel B illustrates the injury-induced proliferation within the regenerative blastema and adjacent retina. Panel C models the response of stem cells in the INL following a lesion. Pax6 expressing retinal stem cells are indicated in red, Pax6-negative progenitors within the rod photoreceptor lineage are indicated in white.

populations within the rod lineage—the fusiform INL progenitors and the rod precursors. However, before contributing to regenerative neurogenesis, INL progenitors and rod precursors would have to de-differentiate and re-initiate Pax6 expression before they regain their potential to produce all retinal cell types.

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