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Rod Progenitor Cells in the Mature Zebrafish Retina

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1 Introduction

In mammals, neuronal cell death during retinal degenerative diseases or following acute retinal injury often leads to irreversible visual impairment because, as is true for most regions of the adult central nervous system, the mammalian retina harbors little capacity for regeneration. In contrast, the neural retinas of urodele amphibians and teleost fishes show remarkable regenerative ability in response to many types of experimental damage. In teleost fishes, retinal regeneration is mediated in part by the proliferation of a specialized population of cells called the rod progenitor cells. These cells possess some of the properties characteristic of transit amplifying cells, such as steady-state proliferation and a commitment to the rod photoreceptor cell fate. However, these cells are also able to respond to rod photoreceptor damage by increasing their rate of proliferation. Therefore, an understanding of the signals that induce rod progenitor cells to proliferate and differentiate following retinal damage may help us to understand the barriers to photoreceptor regeneration in higher vertebrate organisms.

Although it has been many years since the discovery that rod progenitor cells are a source for new neurons in the regenerating fish retina, the *in vivo* factors that regulate rod progenitor proliferation and differentiation, as well as the source of the proliferative cues, remain poorly defined. This chapter will review the phenomena of persistent and injury-induced neurogenesis in the zebrafish retina, with particular emphasis on the role of rod progenitor cells, and will describe a new genetic model that should further our understanding of the regulation of rod progenitor proliferation.

2 Barriers to Retinal Regeneration in Mammals

Neuronal degeneration associated with ocular diseases such as retinitis pigmentosa, macular degeneration, and retinal detachment is a significant cause of visual impairment and blindness. In recent years, there has been tremendous interest in the potential of neural stem/progenitor cells to repopulate the damaged retina and/or to rescue degenerating retinal neurons. This interest has been sparked both by the discovery of putative neural stem/progenitor cells in the adult mammalian eye (Ahmad et al., 2000; Haruta et al., 2001; Tropepe et al., 2000), and by the demonstration that neural stem/progenitor cells isolated from adult ocular tissues could be incorporated into the retina upon transplantation and could express proteins consistent with their laminar position (Ahmad et al., 2000; Tropepe et al., 2000; Chacko et al., 2003). Recent work indicates that newly post-mitotic rod progenitor cells isolated from the post-natal mouse retina are able to integrate more effectively than stem cells upon transplantation, suggesting that the ontogenetic state of transplanted cells is critical for their successful incorporation into the degenerating retina (MacLaren et al., 2006). While these results are very exciting, several basic developmental questions remain: What are the intrinsic and extrinsic properties of neural progenitor cells in the eye? How do environmental signals direct migration and differentiation of these cells? Can the intrinsic and extrinsic properties be manipulated to direct neural

replacement? The retinas of teleost fish have long been known to possess the capacity to regenerate following injury, owing to the presence of two distinct populations of stem/progenitor cells in the adult visual system. Therefore, the teleost retina represents a valuable model system in which to study the mechanisms of neural stem/progenitor proliferation, differentiation, and neuronal regeneration.

3 The Anatomy of the Zebrafish Retina

Like most classes of extant vertebrates, the zebrafish retina is composed of seven major cell types (six classes of neurons and one glial cell type) organized into three nuclear layers (the ganglion cell, inner, and outer nuclear layers) and two synaptic layers (the inner and outer plexiform layers). However, this simplified view grossly understates that true diversity of neuronal types that contribute to the complex circuitry of the vertebrate retina. Unlike rodents, the zebrafish is diurnal and its retina contains a large number of diverse cone subtypes in addition to rod photoreceptors [reviewed in Morris and Fadool (2005)]. The cones are subdivided into four classes based upon spectral sensitivity and morphology (Raymond et al., 1993; Robinson et al., 1993.) The four cone subtypes are stereotypically tiered within the outer nuclear layer. Just below the level of the cone nuclei, the rod cell bodies reside closest to the outer plexiform layer. In the tangential plane, the cone and rod photoreceptors form a highly ordered mosaic that serves to ensure an even sampling of the visual field (Robinson et al., 1993; Fadool, 2003.) One of our goals is to make use of this highly ordered spatial arrangement as a model to explore the importance of cell-cell interactions during normal retinal development and in retinal dystrophies.

4 Persistent Neurogenesis in the Teleost Retina

Zebrafish, similar to many teleost fish and amphibians, continue to grow throughout their life, and the increase in body size is matched by an increase in the size of the eye and the area of the retina [reviewed in Hitchcock et al. (2004), Fadool (2003)]. As the animal grows, enlargement of retinal area is accomplished by two mechanisms. First, new retinal neurons are added to the existing retina from a population of mitotic progenitor cells that reside in a specialized region termed the circumferential germinal zone (CGZ), at the junction between the neural retina and the iris epithelium (Fig. 1). The newly differentiated retinal neurons at the retinal periphery are integrated into the existing retina in an annular fashion (Fadool, 2003; Otteson and Hitchcock, 2003).

The second mechanism of retinal growth in teleost fish involves the gradual stretching of the existing retina within the expanding optic cup in a balloon-like manner (Fadool, 2003; Otteson and Hitchcock, 2003). This results in a corresponding reduction in the packing density of the retinal neurons. While visual acuity is preserved through an increase in the size of the retinal image (proportional to the increase in size of the eye), visual sensitivity must be maintained by the addition of new rod photoreceptors in the central retina. These rod photoreceptors are generated from another population of mitotically active neural progenitors, the rod progenitor cells.

Rod progenitor cells were first described over 20 years ago in the goldfish and cichlid retina (Johns and Fernald, 1981; Johns, 1982; Sandy and Blaxter, 1980), and have since been observed in numerous species of teleost fish (Otteson and Hitchcock, 2003). These germinal cells are interspersed throughout the outer nuclear layer, positioned at the level of the rod photoreceptor nuclei. Labeling experiments with various markers of mitotic activity have demonstrated that rod progenitors are actively dividing cells which also produce daughter cells that go on to differentiate into retinal neurons (Johns and Fernald, 1981; Johns, 1982). As their name implies, the differentiated progeny of the rod progenitors are normally only rod photoreceptors. It was formerly thought that rod progenitors were also capable of producing multiple retinal cell types

in response to retinal damage (see below). This view has been revised in light of more recent work suggesting that stem cells of the inner nuclear layer (INL) are the source for new neurons in the regenerating retina (Wu et al., 2001; Yurco and Cameron, 2005; Fausett and Goldman, 2006). The rod progenitors themselves appear to arise from a population of more slowly dividing cells in the INL (Julian et al., 1998; Otteson et al., 2001). These rounded, stationary cells give rise to radially arranged, fusiform-shaped cells that appear to migrate from the INL to the outer nuclear layer (ONL) where they develop into the rod progenitors (Fig. 1). Furthermore, the proliferative cells of the INL express Pax6, a marker of retinal progenitor cells, whereas the rod progenitor cells are Pax6-negative (Otteson et al., 2001; Hitchcock et al., 1996). Therefore, the slowly-dividing INL cells have been suggested to be the true neuronal stem cells of the retina that in the intact retina give rise to the cells of the rod lineage, whereas the rod progenitor may be thought of as a transit amplifying cell. However, as with the rod progenitor cells, the putative INL stem cells have not been purified or propagated in vitro, preventing a thorough examination of their developmental potential.

As mentioned above, the mechanisms that regulate the proliferative capacity of rod progenitor cells are poorly understood. Because the generation of new rods is associated with the continued growth of the animal, it is not surprising that stimulation of the growth hormone/IGF-I signaling pathway induces an increase in rod progenitor proliferation (Mack et al., 1995; Otteson et al., 2002; Zygar et al., 2005). However, IGF-I regulation of rod progenitor cells has not yet been demonstrated in vivo. Recently, expression of the bHLH transcription factor NeuroD was demonstrated in the rod progenitors and putative INL stem cells of the adult goldfish and larval zebrafish retina (Hitchcock et al., 2004; Hitchcock and Kakuk-Atkins, 2004; Ochocinska and Hitchcock, 2007). However, not all of the rod progenitor cells were positive for NeuroD expression, so it may not be a ubiquitous marker of rod progenitors, nor is it known whether NeuroD plays a role in regulating rod progenitor proliferation or differentiation.

5 Retinal Regeneration in Fish

The ability of the retina of teleost fish to regenerate following injury has been known for over 30 years [reviewed in Fadool (2003), Otteson and Hitchcock (2003)]. Numerous methods have been used to damage retinal neurons in fish, including surgical excision of a portion of the retina (Yurco and Cameron, 2005; Cameron, 2000; Cameron et al., 2005), cytotoxic destruction with chemicals such as ouabain, tunicamycin, or 6-hydroxydopamine (Raymond et al., 1988; Negishi et al., 1991; Braisted and Raymond, 1993; Braisted and Raymond, 1992), laser ablation (Wu et al., 2001; Braisted et al., 1994), phototoxicity (Vihtelic and Hyde, 2000; Vihtelic et al., 2005) and heat lesioning (Raymond et al., 2006). In each experimental paradigm, the damaged retina regenerates within several days to weeks. While these studies were able to demonstrate regeneration of retinal neurons, it should be recognized that the reintegration of these neurons into the existing retinal circuitry, as well as the functionality of the newly generated neurons, has not been fully examined. In fact, in many cases, regenerated photoreceptors fail to reform a correctly patterned mosaic (Braisted et al., 1994; Vihtelic and Hyde, 2000; Raymond et al., 2006).

While some experimental methods for injuring retinal neurons cause more damage than others – for example, ouabain destroys all retinal neurons whereas photodamage kills only the rod and cone photoreceptors – generation of new retinal neurons appears in many cases to involve an increase in proliferation of the rod progenitor cells (Raymond et al., 1988; Negishi et al., 1991; Braisted and Raymond, 1993; Braisted and Raymond, 1992; Vihtelic and Hyde, 2000; Hitchcock et al., 1992). This originally led to the hypothesis that the rod progenitors are the primary source of regenerating retinal neurons. However, this view has recently been challenged by work indicating that Muller cells respond to retinal damage by proliferating and producing the INL stem cells (Wu et al., 2001; Yurco and Cameron, 2005; Fausett and

Goldman, 2006). It is possible that both the INL stem cells and the rod progenitor cells can serve as sources of regenerating retinal neurons and the choice of cell population depends on the extent of damage (see below).

Just as we know little about the regulation of rod progenitor proliferation in the intact fish retina, our knowledge of the factors that regulate the increase in rod progenitor proliferation in the regenerating retina is correspondingly thin. It has been shown that some of the genes, such as the *Notch* and *Delta* genes, *N-cadherin*, *rx1*, and *vsx2*, that are expressed during embryonic development of the retina, are also expressed during regeneration of photoreceptors in the goldfish and zebrafish (Wu et al., 2001; Raymond et al., 2006), supporting the idea that retinal regeneration in the adult fish recapitulates the developmental program of the embryonic retina. However, the factors involved in inducing the regenerative program remain to be discovered. A comparison of the gene expression profiles of intact and regenerating zebrafish retinas was recently reported (Cameron et al., 2005). This study identified several genes whose expression is increased in the regenerating zebrafish retina. However, because the retina was damaged by surgical excision, many of the identified factors were involved in processes that are most likely unrelated to proliferation of rod progenitor cells, such as activation of the immune system, clearance of cellular debris, and wound healing.

6 XOPS-mCFP Transgenic Zebrafish

As mentioned above, studies of rod progenitor cell proliferation in response to retinal injury are complicated by the extent of damage caused by the acute injury methods typically employed. Furthermore, the behavior of the rod progenitors must be studied during a discrete window of time following injury. Clearly, these studies would benefit from the development of genetic models of photoreceptor degeneration in zebrafish. Our laboratory has characterized a transgenic line of zebrafish, the XOPS-mCFP line, which experiences selective degeneration of the rod photoreceptor cells (Morris et al., 2005) due to the toxic effect of a rod-targeted fluorescent reporter gene. This rod degeneration is accompanied by a significant increase in rod progenitor activity, leading to an ongoing cycle of rod photoreceptor birth and death, mediated by the continued proliferation of the rod progenitor cells. Therefore, this line offers a unique opportunity to study the rod progenitors in more detail.

Because the XOPS-mCFP line is a genetic model, we are able to examine rod progenitor responses to photoreceptor degeneration continually throughout the life of the animal as opposed to during a discrete window of time following acute injury. For example, we have found that rod progenitor activity is detectable in XOPS-mCFP animals as early as 7 days post fertilization, long before rod progenitor proliferation is observable in wild type animals (in preparation).

The XOPS-mCFP line is also currently the only transgenic zebrafish line that exhibits selective degeneration of the rod photoreceptors, without any other secondary cell loss. Therefore, we can compare the response to rod degeneration alone with that observed by others after loss of rods and cones and/or multiple retinal neurons. Interestingly, we have found that selective rod degeneration in XOPS-mCFP animals does not lead to an increase in proliferation of either Muller cells or the putative stem cells in the INL (in preparation). This suggests that the rod progenitors possess an intrinsic ability to respond to cues in the ONL, and to upregulate their own proliferation when needed. Further studies of the XOPS-mCFP line should yield important insights into the intrinsic and extrinsic factors that regulate proliferation of the rod progenitors in response to rod degeneration.

7 Summary

The zebrafish is an excellent model organism in which to study the retina's response to photoreceptor degeneration and/or acute injury. While much has been learned about the retinal stem and progenitor cells that mediate the damage response, several questions remain that cannot be addressed by acute models of injury. The development of genetic models, such as the XOPS-mCFP transgenic line, should further efforts to understand the nature of the signals that promote rod progenitor proliferation and differentiation following photoreceptor loss. This in turn may help to refine future approaches in higher vertebrates aimed at enhancing retinal progenitor cell activity for therapeutic purposes.

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Fig. 1.
Persistent neurogenesis in the tectal retina and injury-induced regeneration