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Development of the Vertebrate Eye and Retina

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Abstract

The mature, functional, and healthy eye is generated by the coordinated regulatory interaction of numerous and diverse developing tissues. The neural retina of the eye must undergo the neurogenesis of multiple retinal cell types in the correct ratios and spatial patterns. This chapter provides an overview of retinal development, and includes a summary of the process of eye organogenesis, a discussion of major principles of retinal neurogenesis, and describes some of the key molecular factors critical for retinal development. Defects in many of these factors underlie diseases of the eye, and an understanding of the process of retinal development will be critical for successful future applications of regenerative therapies for eye disease.

1. Introduction

The vertebrate retina contains seven major cell types organized into three layers of cell bodies termed nuclear layers, separated by two synaptic, or plexiform layers (Fig. 1A,B). The outermost layer (outer nuclear layer; ONL) contains the cell bodies of photoreceptors (rods and cones), which project their inner and outer segments toward the adjacent retinal pigmented epithelium (RPE). Rods and cones detect photons and convey this information by chemical signaling. The innermost layer of the retina (retinal ganglion cell layer; GCL) contains cell bodies of retinal ganglion cells (RGCs), which are the output neurons of the retina, projecting axons via the optic nerve and tract to specific targets in the brain. Bipolar cells, horizontal cells, and amacrine cells, which have cell bodies located in the inner nuclear layer (INL) between the photoreceptors and ganglion cells, process and transmit information from photoreceptors to the ganglion cells, allowing for the extraction of features from the visual field including luminance contrast, color contrast, and movement. The final major cell type derived from retinal progenitors is the Müller glial cell (MG). MGs have cell bodies which reside in the INL, and processes that span the retina, providing structure to the retina as well as supporting homeostasis. MGs are known to respond to retinal injury or disease by re-entering the cell cycle, and in some nonmammalian vertebrates can generate new neurons that functionally integrate into the retina (Bernardos, Barthel, Meyers, & Raymond, 2007; Fausett & Goldman, 2006; Sherpa et al., 2008; Sherpa et al., 2014). In addition to cells generated from retinal progenitor cells, the retina also contains astrocytes, microglia, and cells of the retinal vasculature. Each of the six major retinal neuronal categories (rod, cone, horizontal cell, bipolar cell, amacrine cell, RGC) includes numerous specific morphological and functional subtypes, and many of these subtypes are distributed across the retinal sheet in regularly spaced mosaics. In teleost fish the cone photoreceptor mosaics may be geometrically precise; Fig. 2C) (Stenkamp, 2007). This regularity of two-dimensional

neuronal pattern is likely functionally important for representative sampling of the visual field.

The mature vertebrate retina, with its stereotypical laminar and mosaic organizations, is derived from an apparently uniform, single-layered retinal neuroepithelium during development. Our understanding of the process and molecular mechanisms that result in the generation, differentiation, patterning, and subsequent wiring of numerous neuronal types continues to grow, yet remains incomplete. Great insights into these mechanisms have been revealed recently through the use of model organisms, particularly mouse, chick, and zebrafish. Mouse is a favored model because of the ability of transgenic resources. Chick offers an easy to manipulate system that is well developed upon hatching. The zebrafish model offers advantages in genetic manipulation, direct visualization, and rapid experimentation. Zebrafish generate large clutches of embryos that develop externally and remain transparent through the period of retinal neurogenesis, and a functional retina develops by 72 hours post-fertilization (hpf) (Easter & Malicki, 2002; Stenkamp, 2007). The rest of this chapter will emphasize the advancements in our understanding of retinal neurogenesis that have benefited from the use of the zebrafish and other model organisms.

2. Overview of Eye Morphogenesis

The retinal neuroepithelium is itself derived from the embryonic neural tube, from a region that expresses several eye field transcription factors (EFTFs); these homeodomain-containing factors include *pax6*, one or more of the *rx/rax* genes, *six6/optx2*, *six3*, *lhx2*, and *otx2* [reviewed by (Graw, 2010)]. Mutations or knockdown of expression of these genes results in severe developmental defects of the eye; for example null mutations of the *rx3* gene in zebrafish results in the absence of eyes (Loosli et al., 2003). Similarly, mutations in *rx* in humans are associated with anophthalmia (Voronina et al., 2004). The EFTF-expressing region of the neural tube expands bilaterally to form a pair of optic vesicles. Mutations or knockdown of expression of the sonic hedgehog (Shh) gene, which is expressed at the ventral midline of the neural tube, results in cyclopia in zebrafish (Macdonald et al., 1995; Masai, Stemple, Okamoto, & Wilson, 2000; Stenkamp & Frey, 2003) and in humans (Maity, Fuse, & Beachy, 2005). Shh is a secreted signaling molecule that acts to downregulate *pax6* expression, thereby restricting *pax6* expression laterally and thus the embryonic region capable of forming an eye, to the more distal regions of the optic vesicles, and this activity results in the formation of bilateral eyes. Midline Shh signaling continues to regulate the timing of retinal neurogenesis (Kay, Link, & Baier, 2005). The most distal portion of the optic vesicle interacts with overlying surface ectoderm and surrounding periocular mesenchyme to result in the induction of the non-neural tissues of the eye: the lens and cornea, derived from surface ectoderm, and iris and ciliary body, derived from the margins of the optic cup and periocular mesenchyme [reviewed by (Graw, 2010; Harada, Harada, & Parada, 2007)] (Fig. 2).

3. Principles of Retinal Neurogenesis

At 20 hours post-fertilization (hpf) in zebrafish, the optic vesicle is collapsed into two layers, with the distal portion fated to become the neural retina, and the more proximal portion fated

to become the retinal pigmented epithelium (Schmitt & Dowling, 1994). The future neural retina is at this point a proliferative neuroepithelium, still expressing the EFTFs, which act as positive regulators of proliferation (Hsieh & Yang, 2009). This proliferative neuroepithelium then generates the neurons and glia of the retina in a process that illustrates several principles of retinal neurogenesis observed in all vertebrates. The first principle is that specific types of neurons are generated in sequential but overlapping waves. In the zebrafish this sequence is RGCs, followed by cells of the INL, followed by photoreceptor cells (Hu & Easter, 1999) (Fig. 3A). Variations on this sequence are seen in other vertebrates, but the “RGCs first, rod photoreceptors and Müller glia last” order of neuron generation is well-conserved [reviewed by (Bassett & Wallace, 2012; Centanin & Wittbrodt, 2014)].

The second principle is that neurons of each cell population are generated in a stereotyped spatiotemporal pattern. In the zebrafish, neurogenesis is initiated in a “ventral patch” immediately adjacent to the optic stalk and future position of the optic nerve head, and progresses nasally, and then dorsally and finally temporally, a pattern that has been likened to the opening of a fan (Hu & Easter, 1999; Raymond, Barthel, & Curran, 1995) (Fig. 3B). In other vertebrates the spatiotemporal pattern is manifested as a less complex central-to-peripheral gradient, with the first cells of each cell type to be generated located near the future site of the optic nerve head (McCabe, Gunther, & Reh, 1999).

A third principle of retinal neurogenesis is one that has recently been visualized directly in the zebrafish embryo: interkinetic nuclear migration. Nuclei of proliferating progenitors can be found at various apico-basal locations during most phases of the cell cycle. However, immediately prior to M-phase, nuclei migrate rapidly to the apical side of the neuroepithelium and undergo mitosis at that location (Baye & Link, 2007). The orientation of the mitotic plane of cleavage may be either circumferential or radial with respect to the retinal hemisphere. As RGCs are generated, radial divisions are more likely to generate a pair of RGCs that migrate to the appropriate layer and produce axons. Circumferential divisions are more likely to generate asymmetric fates (e.g. RGC plus a progenitor, or RGC plus some other cell type; Fig. 3C). This process has not been directly visualized in any other vertebrate, so it is not known if this is a conserved mechanism. However, factors known to establish and maintain apicobasal polarity of retinal progenitors in zebrafish – such as *Crumbs*, *nok*, and *par* (Krock & Perkins, 2014; Yamaguchi, Imai, Tonou-Fujimori, & Masai, 2010; Zou, Wen, Yang, & Wei, 2013) – are required for normal retinal neurogenesis and cell differentiation in the fish, and mutations in apicobasal polarity genes are also associated with human retinopathies (Richard et al., 2006).

The stereotyped birth order of retinal neurons supported a model for the generation of neuronal diversity that is referred to as the “competence model” (Cepko, Austin, Yang, Alexiades, & Ezzeddine, 1996). This model states that progenitor cells pass through sequential competence states, and while in each state progenitors are capable of generating a progressively restricted set of cell types. Live imaging in the zebrafish embryo has again informed this model, revealing that, while birth order biases the fates of retinal progenitor cells, it does not restrict fates as strictly as predicted by the model (He et al., 2012; Poggi, Vitorino, Masai, & Harris, 2005).

4. Retinal Neuronal Diversity: Regulation by Intrinsic and Extrinsic Factors

Both intrinsic factors (specific transcription factors) and extrinsic factors (extracellular signals) endogenously regulate the production of specific retinal cell types in the appropriate numbers. The major intrinsic factors involved in retinal neurogenesis are transcription factors in the homeodomain-containing gene family, the basic helix-loop-helix (bHLH) family, and the nuclear hormone receptor family. Selected intrinsic factors involved in retinal neurogenesis are shown in Table 1. Some of these are required to specify lineage; for example in zebrafish the thyroid hormone receptor TR β 2 is expressed in cells that give rise to horizontal cells and long wavelength-sensitive cones (Suzuki et al., 2013). However, it is thought that intrinsic factors act in a combinatorial manner to restrict or permit progenitor cell competence to specific fates; for example the homeodomain-containing *vsx2/chx10* is required for bipolar cell development, but promotes this fate together with the bHLH genes *asc11* and *ath3* (Hatakeyama, Tomita, Inoue, & Kageyama, 2001).

Extrinsic factors known to regulate retinal neurogenesis and retinal cell differentiation include Shh, Fibroblast growth factors (Fgfs), Wnts, Bone morphogenetic proteins (Bmps/Gdfs/TGF β), Notch, and retinoic acid (RA) (Table 2). The major emphases in the field of retinal development have been centered on understanding factors regulating the neurogenesis of RGCs and of photoreceptor cells. These cell types are lost or damaged - and not replaced, resulting in permanent loss of visual function - in glaucoma (RGCs), hereditary retinal degenerations such as retinitis pigmentosa (photoreceptors), and in age-related macular degeneration (photoreceptors). These retinal disorders collectively affect millions of people in the U.S. alone. A deep understanding of the mechanisms underlying the development of these cell populations is likely to lead to treatments for these disorders that promote the survival and/or regeneration of these cells.

4.1 RGC Neurogenesis: Intrinsic and Extrinsic Factors

An important intrinsic factor for RGC neurogenesis is the bHLH transcription factor *atoh17* (*atoh7*, also referred to as *ath5*). In all vertebrates, *atoh7* is expressed immediately prior to the neurogenesis of RGCs, in a spatiotemporal gradient that anticipates the pattern of RGC genesis (Masai et al., 2000). Zebrafish with null mutations in *atoh7* fail to develop RGCs, indicating that *atoh7* is required for the development of this cell type (Kay, Finger-Baier, Roeser, Staub, & Baier, 2001). *Atoh7* has been shown to regulate a network of RGC-specific genes; for example, in mice *Atoh7* regulates the transcription factor *Bmn3b*, a factor that is required for the development of specific subpopulations of RGCs (W. Liu, Mo, & Xiang, 2001). Using an *atoh7* reporter system (*atoh7:GFP*), the fates of *atoh7*-expressing progenitor cells were tracked in living zebrafish embryos. GFP⁺ cells underwent a single cell division which generated one daughter cell that migrated to the RGC layer and differentiated as an RGC – the fate of the other daughter cell remains unknown but may include a photoreceptor fate (Poggi et al., 2005).

Key extrinsic factors that regulate RGC neurogenesis include the extracellular signaling proteins FGF and Shh. FGF has been shown to be produced in the most distal region of the optic stalk, immediately adjacent to the retinal region where neurogenesis is initiated. Pharmacological inhibition of FGF signaling in zebrafish embryos prevents the expression

of *atoh7*, and hence the production of RGCs (Picker & Brand, 2005). Mutants lacking functional *Fgf8* and *Fgf3* genes (double mutants) display a similar phenotype (Martinez-Morales et al., 2005). Shh signaling has similarly been investigated in zebrafish using both gain- and loss-of-function approaches. Unlike FGF, Shh is expressed by the RGCs themselves, as well as their *atoh7*⁺ progenitors, and there is evidence for two specific roles for Shh signaling in regulating RGC production. The first role is in propagating the wave of RGC neurogenesis; the zebrafish *shh* mutant displays arrested RGC differentiation, and reduced cell proliferation (Neumann & Nuesslein-Volhard, 2000; Stenkamp & Frey, 2003). The second role is in regulating RGC number; in other animal models, defects in Shh signaling result in overproduction of RGCs, suggesting that Shh acts as a negative feedback regulator of RGC neurogenesis (Zhang & Yang, 2001). Interestingly, reduced Shh signaling during retinal regeneration in zebrafish also results in overproduction of neurons (Sherpa et al., 2014). Shh signals are likely integrated with other signals (such as FGF and Notch) via intracellular signaling cascades to influence cell cycle progression and fate (Bassett & Wallace, 2012). Retinal progenitor cells express *ptch2* (Bibliowicz & Gross, 2011; Stenkamp, Frey, Prabhudesai, & Raymond, 2000), a transmembrane component of the Shh receptor complex, and likely integrate this information.

4.2 Photoreceptor Neurogenesis: Intrinsic and Extrinsic Factors

Numerous specific transcription factors have been demonstrated as important for the neurogenesis of photoreceptors, including those that regulate rod vs. cone fate. This “photoreceptor transcription factor network” includes the homeobox genes *crx* and *rx* and the bHLH gene *NeuroD*, all of which are essential for photoreceptor development [reviewed by (Swaroop, Kim, & Forrest, 2010)]. Mutations or knockdown of these genes result in failure of rod and cone development in animal models (Hennig, Peng, & Chen, 2008; Nelson, Park, & Stenkamp, 2009; Shen & Raymond, 2004), and human mutations in *crx* are associated with hereditary retinal degenerations (Huang et al., 2012). The orphan nuclear receptors *Nr2e3* and *RORa*, and the leucine zipper-containing transcription factor *nrl* are all key regulators of rod fate and rod-specific gene expression (Swaroop et al., 2010). When these genes are mutated in mice, retinas contain no rods and instead show an abundance of photoreceptors with an unusual cone-like phenotype most similar to short wavelength-sensitive (S; blue) cones (Mears et al., 2001; Webber et al., 2008). In the zebrafish, the T-box transcription factor *tbx2b* is required for the genesis of ultraviolet-sensitive (UV) cones (the zebrafish UV opsin is orthologous to the mouse S opsin), and functional nulls instead have supernumerary rods (Alvarez-Delfin et al., 2009). We have recently observed in the zebrafish that the nuclear hormone receptor *RARα* is needed for a retinoic acid-mediated cone-to-rod fate change of progenitor cells (Stevens, Cameron, & Stenkamp, 2011). In addition, the nuclear hormone receptors *RXRγ* and *TRβ2* promote the production of cones expressing a medium or long-wavelength-sensitive opsin rather than S or UV opsin (Roberts, Hendrickson, McGuire, & Reh, 2005; Roberts, Srinivas, Forrest, Morreale de Escobar, & Reh, 2006; Suzuki et al., 2013).

A large number of extrinsic factors have been shown regulate photoreceptor neurogenesis and differentiation, including Fgfs, Wnts, Shh, retinoic acid (RA) and thyroid hormone (T3). The latter two are particularly interesting because they act directly upon the nuclear

hormone receptors (RARs, RXRs, and TRs) mentioned above. Gradients in the concentration of these lipophilic signaling molecules are associated with gradients in photoreceptor-specific opsin expression (Mey, McCaffery, & Klemmeit, 2001; Roberts et al., 2006; Stevens et al., 2011), and while the patterns of rod and cone photoreceptors are distinct in specific animal models, the use of these gradients appears to be a conserved mechanism for regulating photoreceptor fate decisions and therefore photoreceptor diversity and ratios.

5. Persistent Retinal Neurogenesis and Regeneration

The field of retinal development aspires not only to understand the developmental processes and mechanisms underlying the generation of cell diversity in the retina, but also to translate this knowledge into treatments for retinal disorders. Potential treatments include gene therapy, cell transplantation, and regenerative therapies that utilize endogenous sources of new neurons. Regenerative therapies have become of high interest as the National Eye Institute has named as a priority area (the “Audacious Goal”) the regeneration of retinal neurons and their connections to restore vision in retinal disorders. To accomplish this goal, several laboratories are taking advantage of the zebrafish animal model using approaches other than the direct study of embryonic retinal development. This is possible because zebrafish, like other teleost fishes, display persistent retinal neurogenesis as well as the capacity for retinal regeneration (Stenkamp, 2007). Persistent neurogenesis in zebrafish includes the postembryonic addition of new retina at the periphery, generated by a population of stem cells known as the ciliary marginal zone (CMZ) or circumferential germinal zone (CGZ; Fig. 4). This persistent neurogenesis allows the fish retina to grow in concert with body size. CGZ-like characteristics have been described to exist at the retinal margin in mammals (Kubota, Hokoc, Moshiri, McGuire, & Reh, 2002), although their location in the periphery makes them inconvenient as potential sources of new neurons in treatment of any retinal disease that affects central vision. A second aspect of persistent neurogenesis in zebrafish is the continuous generation of new rod photoreceptors by a dedicated lineage of cells known as the rod lineage (Stenkamp, 2011). At the apex of this lineage – the stem cell – is the Müller glial cell (MG), which generates progenitors that proliferate within the INL (a transit-amplifying population), migrate to the ONL, then undergo terminal mitosis and differentiate as rods (Bernardos et al., 2007) (Fig. 4). MG in mammals have very limited potential for cell cycle re-entry, and do not contribute to a rod lineage. However, an understanding of this lineage and its regulation in zebrafish may lead to rod replacement treatments in humans. Finally, in cases of retinal damage, the MG of zebrafish not only contribute to the rod lineage, but also contribute to the replacement of all retinal cell types (Bernardos et al., 2007; Qin, Barthel, & Raymond, 2009; Ramachandran, Reifler, Parent, & Goldman, 2010; Yurco & Cameron, 2005) (Fig. 4), resulting in the restoration of vision (Sherpa et al., 2008; Sherpa et al., 2014). Normally, MG proliferation in mammals gives rise to gliosis; however, it has been observed that in special circumstances and with specific treatments the MG of mammals can re-enter the cell cycle and generate at least some new neurons (Karl et al., 2008; Pollak et al., 2013). These are exciting findings that the vision research community plans to build upon to develop regenerative therapies for retinal disorders (Fischer & Bongini, 2011).

6. Summary

This chapter has provided an overview of retinal development, summarizing the process of organogenesis, principles of retinal neurogenesis, and describing some of the major molecular factors critical for retinal development. The field is actively engaged in deciphering the mechanisms through which intrinsic and extrinsic factors are integrated to regulate progenitor cell competence, identifying additional important players such as chromatin remodeling factors and micro RNAs, and understanding the mechanisms underlying retinal regeneration. This information can be applied for treatment of developmental disorders of the eye, and the development of stem cell and regenerative therapies for retinal degenerative disease and damage.

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Figure 1.

Histology and cell arrangements in the mature vertebrate retina. A. Cartoon showing the major cell types of the retina. B. Nomarski (DIC) image of a cryosection of the adult zebrafish retina; rpe, retinal pigmented epithelium; os/is, outer segments and inner segments of photoreceptors; onl, outer nuclear layer; opl, outer plexiform layer; inl, inner nuclear layer; ipl, inner plexiform layer; gcl, ganglion cell layer; nfl, nerve fiber layer. C. Dual *in situ* hybridization of zebrafish retinal wholemount with cRNA probes complementary to mRNA encoding SWS2 (blue-sensitive; b) opsin and SWS1 (UV-sensitive; uv) opsin, showing geometrically precise mosaic. Figure panels reproduced from (Stenkamp, 2007), with permission.

Figure 2. Formation of the major tissues of the eye. Forebrain neuroectoderm (blue) contributes to RPE, iris, and neural retina; surface ectoderm contributes to lens and cornea (green); neural crest contributes to cornea, choroid, and sclera (pink), and mesoderm contributes to extraocular muscles and cornea (yellow). Figure from (Harada et al., 2007), with permission.

Figure 3.

Patterns and principles of retinal neurogenesis. A. Nomarski (DIC) image of a cryosection of the embryonic zebrafish retina, with patterns of neurogenesis of cells of the ganglion cell layer (gcl, in green), inner nuclear layer (inl, in blue), and outer nuclear layer (onl, in red). B. Wholemounted embryonic zebrafish eye, with patterns of neurogenesis as in A. C. Nomarski (DIC) image of a cryosection of an embryonic zebrafish retina earlier in neurogenesis, with orientation of terminal mitotic division predictive of symmetric vs. asymmetric fates; gc, ganglion cell. Figure panels reproduced from (Stenkamp, 2007), with permission.

Figure 4.

Persistent neurogenesis and retinal regeneration in adult teleost fish. Top: Stem cells of the circumferential germinal zone (CGZ) generate all retinal cells except rod photoreceptors. Bottom, left: Müller glia are stem cells of the rod lineage (Pathway A). Bottom, right: Following damage, Müller glia become multipotent, and contribute to the neurogenesis of all retinal neuronal types (Pathway B). Figure reproduced from (Stenkamp, 2011), with permission.

Table 1Selected Intrinsic Factors Regulating Retinal Neurogenesis.¹

Family	Factor	Regulatory Role		
		Eye Morphogenesis	Retinal Progenitor Cell Cycle Control	Retinal Cell Determination and Differentiation
Homeo-domain-containing	Pax6	(Macdonald et al., 1995; Nornes et al., 1998)	(Hsieh & Yang, 2009)	(P. F. Hitchcock, Macdonald, VanDeRyt, & Wilson, 1996; Otteson, D'Costa, & Hitchcock, 2001)
	Otx2	(Bernard et al., 2014; Martinez-Morales, Signore, Acampora, Simeone, & Bovolenta, 2001)		
	Six3	(Loosli, Winkler, & Wittbrodt, 1999)	(Dyer, 2003)	(Dyer, 2003)
	Six6	(Bernier et al., 2000)		
	Lhx2	(Tetreault, Champagne, & Bernier, 2009)		
	Pax2	(Macdonald et al., 1995; Macdonald et al., 1997)		
	Vax	(Take-uchi, Clarke, & Wilson, 2003)		(Vitorino et al., 2009)
	Chx20/Vsx		(Barabino, Spada, Cotelli, & Boncinelli, 1997; Dyer, 2003; Passini, Raymond, & Schechter, 1998)	(Passini et al., 1998)
	Crx		(Shen & Raymond, 2004)	(Hennig et al., 2008; Y. Liu, Shen, Rest, Raymond, & Zack, 2001; Shen & Raymond, 2004)
	Rx/Rax	(Chuang & Raymond, 2001; Loosli et al., 2003; Rojas-Munoz, Dahm, & Nusslein-Volhard, 2005)	(Nelson et al., 2009)	(Chuang, Mathers, & Raymond, 1999; Nelson et al., 2009)
bHLH	Atoh7		(Poggi et al., 2005)	(Kay et al., 2001; Kay et al., 2005; Masai et al., 2000; Poggi et al., 2005; Stenkamp & Frey, 2003)
	Ascl1			(Brzezinski, Kim, Johnson, & Reh, 2011; Hatakeyama et al., 2001; Mazurier et al., 2014)
	Math3			(Inoue et al., 2002)
	NeuroD		(Ochocinska & Hitchcock, 2009)	(P. Hitchcock & Kakuk-Atkins, 2004; Korzh, Sleptsova, Liao, He, & Gong, 1998)
Nuclear hormone receptor or orphan receptor	RAR α			(Stevens et al., 2011; Tallafuss et al., 2006)
	RXR γ			(Roberts et al., 2005)
	PNR/Nr2e3			(Chen, Rattner, & Nathans, 2005; Nelson, Frey, Wardwell, & Stenkamp, 2008)
	TR β 2			(Roberts et al., 2006; Suzuki et al., 2013)

Family	Factor	Regulatory Role		
		Eye Morphogenesis	Retinal Progenitor Cell Cycle Control	Retinal Cell Determination and Differentiation
	RORa			(Forrest & Swaroop, 2012)
Other	Brn3b			(DeCarvalho, Cappendijk, & Fadool, 2004)
	Tbx2b			(Alvarez-Delfin et al., 2009)
	Nrl			(Mears et al., 2001)
Chromatin remodeling	Brg1			(Gregg, Willer, Fadool, Dowling, & Link, 2003; Link, Fadool, Malicki, & Dowling, 2000)
	Add		(Yamaguchi et al., 2005)	

¹ Selected citations emphasize literature pertaining to the zebrafish/teleost fish models.

Table 2Selected Extrinsic Factors Regulating Retinal Neurogenesis.¹

Factor	Regulatory Role		
	Eye Morpho-genesis	Retinal Progenitor Cell Cycle Control	Retinal Cell Determination and Differentiation
IGFs		(Boucher & Hitchcock, 1998; Mack & Fernald, 1993; Otteson, Cirenza, & Hitchcock, 2002)	
FGFs	(Heisenberg, Brennan, & Wilson, 1999; Picker & Brand, 2005)		(Mack & Fernald, 1993; Martinez-Morales et al., 2005) (Hochmann et al., 2012; Qin et al., 2011)
Wnts	(Veien, Rosenthal, Kruse-Bend, Chien, & Dorsky, 2008)	(Masai, Yamaguchi, Tonou-Fujimori, Komori, & Okamoto, 2005; Yamaguchi et al., 2005)	(Meyers et al., 2012)
Hh	(Macdonald et al., 1995; Stenkamp & Frey, 2003; Take-uchi et al., 2003)	(Masai et al., 2005; Shkumatava & Neumann, 2005; Stenkamp, Frey, Mallory, & Shupe, 2002)	(Masai et al., 2005; Neumann & Nusslein-Volhard, 2000; Shkumatava, Fischer, Muller, Strahle, & Neumann, 2004; Stenkamp & Frey, 2003; Stenkamp et al., 2000)
RA	(Hyatt, Schmitt, Marsh-Armstrong, et al., 1996; Hyatt, Schmitt, Marsh-Armstrong, & Dowling, 1992; Marsh-Armstrong, McCaffery, Gilbert, Dowling, & Drager, 1994)		(Biehlmaier, Lampert, von Lintig, & Kohler, 2005; Hyatt, Schmitt, Fadool, & Dowling, 1996; Meyers et al., 2012; Prabhudesai, Cameron, & Stenkamp, 2005; Stevens et al., 2011)
T3			(Pessoa et al., 2008; Suliman & Novales Flamarique, 2014)
Notch		(Yamaguchi et al., 2005)	(Bernardos, Lentz, Wolfe, & Raymond, 2005; Scheer, Groth, Hans, & Campos-Ortega, 2001)

¹ Selected citations emphasize literature pertaining to the zebrafish/teleost fish models.