Review
The role of homeobox genes in retinal development and disease

Jamie L. Zagozewski, Qi Zhang, Vanessa I. Pinto, Jeffrey T. Wigle, David D. Eisenstat

Keywords: Retina, Vertebrate, Homeobox, Transcription factor, Coloboma, Anophthalmia

Abstract
Homeobox genes are an evolutionarily conserved class of transcription factors that are critical for development of many organ systems, including the brain and eye. During retinogenesis, homeodomain-containing transcription factors, which are encoded by homeobox genes, play essential roles in the regionalization and patterning of the optic neuroepithelium, specification of retinal progenitors and differentiation of all seven of the retinal cell classes that derive from a common progenitor. Homeodomain transcription factors control retinal cell fate by regulating the expression of target genes required for retinal progenitor cell fate decisions and for terminal differentiation of specific retinal cell types. The essential role of homeobox genes during retinal development is demonstrated by the number of human eye diseases, including colobomas and anophthalmia, which are attributed to homeobox gene mutations. In the following review, we highlight the role of homeodomain transcription factors during retinogenesis and regulation of their gene targets. Understanding the complexities of vertebrate retina development will enhance our ability to drive differentiation of specific retinal cell types towards novel cell-based replacement therapies for retinal degenerative diseases.

Introduction
The retina is a remarkably complex, yet structurally simple laminar tissue made up of seven distinct retinal cell types (6 neuronal and 1 glial), that are born sequentially in a temporally ordered and overlapping manner (Young, 1985). These cells are organized into three cellular layers, which include the ganglion cell layer (GCL), the inner nuclear layer (INL) and the outer nuclear layer (ONL) (Fig. 1). As mouse embryonic retinal development proceeds, retinal ganglion cells (RGC) are generated first, followed by cone photoreceptors, horizontal cells and amacrine cells. During the postnatal period, rod photoreceptors, bipolar cells and Müller glia are specified and complete differentiation. Retinal cells arise from a common pool of multipotent retinal progenitor cells (RPC) (Livesey and Cepko, 2001; Turner and Cepko, 1987; Wets and Fraser, 1988). RPC competence gradually becomes restricted to produce the correct retinal cell types within the appropriate temporal window. Initiation of intrinsic transcription factor expression in the RPC population plays a critical role in directing these multipotent cells to adopt specific retinal cell fates (Marquardt, 2003; Ohsawa and Kageyama, 2008). Transcription factors of the homeodomain, basic helix-loop-helix (bHLH) and forkhead box (FOX) families work in concert to direct retinal cell fate specification and differentiation. Homeobox genes encode transcription factors containing a 60 amino acid DNA-binding domain and are evolutionarily conserved from invertebrates to vertebrates. The binding of individual transcription factors is modulated by the adjacent conserved motifs, which imparts specificity of binding and function. In addition, homeobox genes can be further classified based on the presence of additional conserved domains, such as the paired and LIM domains. Homeobox genes play a number of critical roles during vertebrate forebrain (Wigle and Eisenstat, 2008) and eye development including specification of the eye field and the optic stalk, progenitor cell fate determination, and retinal cell differentiation and survival. Due to these critical roles, mutations in human homeobox genes can lead to a number of ocular abnormalities, ranging from colobomas to anophthalmia (Table 1). We will focus...
our review on members of the homeobox gene family, and the
critical role each gene plays in RPC fate specification, retinal cell
type differentiation and inherited eye diseases.

**Rax**

Retina and anterior neural fold homeobox gene (Rax), previously
referred to as the retinal homeobox gene (Rx), belongs to
the paired-like (Prd-L) homeodomain (HD) family. In addition to
the paired-like homeodomain, the RAX protein contains a con-
served OAR domain in the C-terminus named for conservation of
this region between otx, aristless and rax (Furukawa et al., 1997).
Rax genes have been identified in a number of model systems
including mice, zebrafish, chick, Xenopus, and Drosophila with the
number of Rax genes varying depending on the species. Both
the structure and the expression pattern of the Rax genes are highly
conserved across these species (Chuang and Raymond, 2001; Furukawa et al., 1997; Mathers et al., 1997; Muranishi et al., 2012).
Rax is among the earliest genes expressed in the developing
retina. During zebrafish gastrulation, r3 expression in the anterior
forebrain permits specification of the eye field over telencephalic
fate (Stigloher et al., 2006). Murine Rax is first expressed at E7.5 in
the cephalic neural fold (Furukawa et al., 1997; Mathers et al., 1997).
At E9.5, Rax expression initiates in the optic vesicle and is
subsequently localized to the neural retina. Rax is expressed
uniformly throughout the developing neuroepithelial layer (NBL),
with peak expression observed at E16.5. With the onset of retinal
cell differentiation, Rax expression progressively decreases but is
maintained in photoreceptors of the developing retina until the
second postnatal week.

Rax is essential for early specification of eye development as
demonstrated by the loss of eye structures in vertebrate models
lacking Rax expression, including mice and zebrafish (Furukawa et al., 1997; Loosli et al., 2003; Mathers et al., 1997). In addition, Rax
null mice have reduced brain structures, which can range from a
lack of ventral forebrain or the complete absence of the forebrain.
Conversely, Rax gain-of-function experiments in Xenopus and
zebrafish result in ectopic retinal tissue (Mathers et al., 1997;
Terada et al., 2006). In the absence of Rax, upregulation of Pax6
expression is not induced in the ventral neuroectoderm, suggest-
ing that Rax is upstream of Pax6 and required to specify RPCs
(Zhang et al., 2000). Similar to the loss of eye structures observed in different model organisms, human RAX mutations
have been associated with both anophthalmia and microphthal-
mia (Table 1) (Abouzeid et al., 2012; Bardakjian and Schneider,
2011; Vörösné et al., 2004). Mutations are typically located in
either the DNA binding homeodomain or the OAR domain and
result in impaired expression of RAX target genes (Lequeux et al.,
2008).

In addition to a role in early eye development, Rax regulates
expression of genes required for photoreceptor development
(Fig. 2). Knockdown of zebrafish and Xenopus Rax orthologs results in
reduction of photoreceptor-specific gene expression (Nelson et al.,
2009; Pan et al., 2010). Early in vitro studies demonstrated that
RAX can directly bind to labeled oligonucleotide probes of the
arrestin promoter (a photoreceptor specific gene) through the
photoreceptor conserved element (PCE-1) in vitro and activate
expression of arrestin in reporter gene assays (Kimura et al., 2000).
More recently, the Xenopus Rax gene was demonstrated to occupy
PCE-1 containing promoter elements of rhodopsin and red cone
opsin in vivo (Pan et al., 2010). Rax has also been shown to regulate
the expression of the murine homolog of orthodonticle (Otx2), a
homeobox gene essential for determination of photoreceptor cell
fate (Muranishi et al., 2011). A conserved enhancer upstream of the
OTX2 initiation codon was identified and termed the embryonic
enhancer locus for photoreceptor Otx2 transcription (EELPOT).
RAX was shown to bind to EELPOT in vivo by chromatin immuno-
precipitation (ChIP), and significantly activates EELPOT-luciferase
reporters in vitro. Additionally, Otx2 expression is dramatically
decreased with concomitant decrease in cone–rod homeobox (Crx)
expression (a transcriptional target of OTX2) upon conditional
knockout of Rax in RPC (Muranishi et al., 2011).

**Pax2 and Pax6**

The paired box (Pax) gene family encodes transcription factors
that are important for many developmental processes. In total,
nine mammalian Pax genes have been identified; all contain a
paired domain (Chi and Epstein, 2002; Mansouri et al., 1999; Noll,
1993). The Pax gene family is further subdivided into four groups
based on the presence or absence of additional structural domains,
which include either partial or complete homeodomains. PAX3,
PAX4, PAX6 and PAX7 are among the PX genes that contain
complete homeodomains; PAX2 is unique within this transcription
factor family since it does not contain a homeodomain.

Please cite this article as: Zagozewski, J.L., et al., The role of homeobox genes in retinal development and disease. Dev. Biol. (2014), http://dx.doi.org/10.1016/j.ydbio.2014.07.004
Table 1
Retinal expression and mutant phenotypes of selected homeobox genes.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Retinal expression</th>
<th>Retinal phenotype of knockout mice</th>
<th>Transcriptional targets</th>
<th>Human disease</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rax</strong></td>
<td>RPC</td>
<td>Eyeless</td>
<td>Pax6(+) Otx2(+) (\beta)-arrestin(+) rhodopsin(+)</td>
<td>Anophthalmia</td>
</tr>
<tr>
<td><strong>Pax2</strong></td>
<td>Ventral optic vesicle, optic fissure, optic stalk</td>
<td>Failure to close optic fissure, guidance defects of optic nerve</td>
<td>Pax2(−) Atoh7(+) Ngn2(+) Crx(−)</td>
<td>Coloboma syndrome and renal hypoplasia</td>
</tr>
<tr>
<td><strong>Pax6</strong></td>
<td>RPC, amacrine cells</td>
<td>Small Eye (heterozygotes); Anophthalmia in homozygotes</td>
<td>Pax2(−) Atoh7(+) Ngn2(+) Crx(−)</td>
<td>Aniridia, cataracts, Peter’s anomaly, coloboma, optic nerve hypoplasia, foveal hypoplasia</td>
</tr>
<tr>
<td><strong>Lhx1</strong></td>
<td>Post-mitotic horizontal cells</td>
<td>Anophthalmia, reactive gliosis in MG CKO</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Lhx2</strong></td>
<td>Optic cup, optic stalk, MG</td>
<td>Failure of proper horizontal cell lamination</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Vsx2</strong></td>
<td>RPC in early retinal development, Bipolar and MG in late development</td>
<td>Reduced RPC proliferation and failure of bipolar cell differentiation</td>
<td>Pax6(−)</td>
<td>Microphthalmia</td>
</tr>
<tr>
<td><strong>Meis1/Meis2</strong></td>
<td>RPC</td>
<td>Microphthalmia due to impaired RPC proliferation, partial retina ventralization</td>
<td>smad7(+) thbs1(+) vux2(−)</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Vsx1</strong></td>
<td>Cone bipolar cells</td>
<td>Differentiation defects in type 7 bipolar cells</td>
<td>Recoverin(+) NefI(+) NK3R(+) CaB5(−) Vux2(−) Cdbp5(−) In(−)</td>
<td>Coloboma</td>
</tr>
<tr>
<td><strong>Vsx2</strong></td>
<td>Ventral retina</td>
<td>Ventral retina dorsalization, ventral RGC axon pathfinding defects; retina replaces optic nerve in the Vox1/Vox2 double knockout</td>
<td>gdf6(+) aldha12a2(+) thbs1(+)</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Pbx</strong></td>
<td>RGC</td>
<td>No mouse phenotype is described. Zebrash</td>
<td>hmx(+) atoh7(+)</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Dlx1/Dlx2</strong></td>
<td>RGC, horizontal, amacrine</td>
<td>~30% loss of late-born RGC</td>
<td>Bm32(−) Cdx(−) TrkB(−) Dlx5(−) Nrp-2(−)</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Dlx5/Dlx6</strong></td>
<td>RGCs, horizontal, amacrine</td>
<td>ND</td>
<td>Bm32(+) Bm31(+) Dlx1/Dlx2(−) Otx2(−) Crx(−)</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Bm32/Bm3c</strong></td>
<td>RGCs</td>
<td>~60–80% loss of RGC</td>
<td>Bm32(+)</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Bm3a/Bm3c</strong></td>
<td>RGC</td>
<td>Altered RGC dendritic stratification in Bm3a CKO; No retinal phenotypes in Bm3c knockouts</td>
<td>Bm3a(+) Bm3b(+)</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Isl1</strong></td>
<td>RGC</td>
<td>Apoptosis of ~67% of RGCs</td>
<td>Bm3a(+)</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Isl2</strong></td>
<td>RGC</td>
<td>Aberrant ipsilateral projection of RGC axons</td>
<td>Zic2(−) EphB1(−)</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Oc1</strong></td>
<td>RGC (E12.5 – P16), horizontal cells</td>
<td>75% reduction in horizontal cells</td>
<td>Ush1(+) Pax6(+)</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Oc2</strong></td>
<td>RGC (E12.5 – P16) horizontal cells</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Prox1</strong></td>
<td>Horizontal, bipolar, amacrine</td>
<td>Embryonic lethal, absence of horizontal cells, increase in rods and Muller glial cells</td>
<td>p27KIP1(−) p57KIP2(−)</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Irx5</strong></td>
<td>Type 2 and Type 3a/b bipolar cells, MG</td>
<td>Defects in Type 2 and Type 3a/b bipolar cell development</td>
<td>CaRBP5(+) PMCA4(+) Recoverin(+)</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Irx6</strong></td>
<td>Type 2 and Type 3a bipolar cells, MG</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Otx2</strong></td>
<td>RPE, cones, rods, bipolar cells</td>
<td>Microphthalmia, increase of amacrine cells, decrease in photoreceptors (CKO); lack of head structures in conventional KO</td>
<td>Crx(+) Otx2(+) Bhlh1(+)</td>
<td>Microphthalmia</td>
</tr>
<tr>
<td><strong>Crx</strong></td>
<td>Cones, rods, bipolar</td>
<td>No gross abnormalities; photoreceptor degeneration postnatally</td>
<td>Crx(+) Cone Opsin(+) rhodopsin(+) Nrl3e(+) Otx2(−)</td>
<td>Leber’s Congenital Amaurosis, cone–rod dystrophy, retinitis pigmentosa</td>
</tr>
</tbody>
</table>

Both Pax2 and Pax6 are expressed in the developing eye with Pax6 expression found in the neural retina, the retinal pigmented epithelium (RPE) and the lens surface ectoderm. In contrast, Pax2 expression is restricted to the optic stalk and optic cup. Pax2 expression is observed in the optic stalk structures of both mice and zebrafish. During early embryonic development (E9.0–E11.0), Pax2 expression is localized to ventral optic cup and optic stalk structures (Macdonald et al., 1997; Nornes et al., 1990; Torres et al., 1996). Sonic Hedgehog (Shh) signaling from the midline demarcates the division between optic stalk from neural retina structures by regulating expression of Pax2 and Pax6, which are expressed in these domains (Cai et al., 2013; Macdonald et al., 1995). During axonal outgrowth from the neural retina, Pax2 expression remains elevated in the optic disc and optic nerve, but is excluded from the neural retina. Loss of Pax2 function in mice and the zebrafish results in the failure to close the choroid fissure, significant axonal pathfinding defects and coloboma (Macdonald et al., 1997; Torres et al., 1996). Similarly, mutations in human PACX2 result in papillorenal syndrome where patients have both optic nerve colobomas and renal hypoplasia (Table 1) (Sanyanusin et al., 1995; Schimenti et al., 1997). Recently, fibroblast growth factor (FGF) signaling has been observed to regulate Pax2 expression in the optic disc and optic fissure (Cai et al., 2013). Mutation of FGF receptors, Fgfr1 and Fgfr2, results in failure to close the optic fissure with significant down-regulation of Pax2 expression. Expression of the RPE marker microphthalmia-associated transcription factor (Mitf) was upregulated in the open fissure, suggesting a cell fate switch from optic disc to RPE.

Pax6 plays numerous critical roles in development of the eye, including the retina and the lens. Pax6 structure and function is

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highly conserved among vertebrates and Drosophila (Quiring et al., 1994). The eyeless (ey) mutant phenotype was first described in Drosophila, which as the name suggests, results in complete lack of eyes due to mutations in the ey gene, subsequently identified as the Drosophila homolog of the vertebrate gene Pax6. Similar mutations in the vertebrate Pax6 homologs are observed in both humans and mice, leading to congenital aniridia and the semidominant “Small Eye” (Sey) mutation phenotype, respectively (Table 1) (Brown et al., 1998; Hill et al., 1991; Tzoulaki et al., 2005). Homozygous null mutations of Pax6 are lethal and result in complete absence of eye structures in mice. Over-expression of Pax6 also leads to abnormalities of the eye, including microphthalmia and retinal dysplasia (Manuel et al., 2008; Schedl et al., 1996). Pax6 expression initiates at E8.0 in both the optic pits and a broad region of surface ectoderm (Grindley et al., 1995; Walther and Gruss, 1991). In the neural ectoderm, expression becomes progressively localized to the distal optic vesicles. From E9.5, Pax6 is highly expressed in the optic cup and the developing lens placodes, followed by continued expression in the prospective neural retina and lens. Pax6 is key to establishing the optic cup/optic stalk boundaries during ocular development through reciprocal control of Pax2 expression (Schwarz et al., 2000). Pax6 expression is expanded into the optic stalk in the absence of Pax2, where this tissue acquires neural retina fate. Conversely, in the absence of Pax6, ectopic Pax6 expression is found in the optic cup margin prior to degeneration of the optic cup, demonstrating mutual repressive interactions between Pax6 and Pax2 during optic development. In addition, molecular interactions were observed between Pax2 and Pax6 and Pax6 and Pax2 enhancers, respectively in vitro. In the developing neural retina, Pax6 is expressed in early RPC and required for maintenance of RPC multipotency. In the absence of Pax6, RPC competence is dramatically restricted, producing exclusively amacrine cells at the expense of all remaining retinal cell types (Marquardt et al., 2001). Loss of RPC competency in the absence of Pax6 can be partially explained by failure to activate expression of proneural bHLH genes, such as Atoh7 (formerly Math5) and neurogenin-2 (Ngn2), which are both direct targets of PAX6 regulation, and required for RGC development and retina neurogenesis, respectively (Marquardt et al., 2001; Riesenberg et al., 2009; Wang et al., 2001). Pax6 plays dual roles in RPC fate specification, depending on the spatial location of the RPC. Cells located in the peripheral retina require Pax6 for completion of neurogenesis and restriction of Crx expression (Fig. 2) while centrally localized RPC require Pax6 to retain multipotency (Oron-Karni et al., 2008). Recently, Pax6 was identified as being downstream of suppressor-of-fused (Sufu), a negative regulator of the Hedgehog signaling pathway (Cwinn et al., 2011; Svard et al., 2006; Varjosalo et al., 2006). Conditional loss of Sufu in the proximal retina results in Pax6 down-regulation and phenocopies the Pax6 conditional knockout described above.
44. Chx10 (Cwinn et al., 2011). In addition to its role in the neural retina, Pax6 is required for lens development. Conditional knockout of Pax6 in the surface ectoderm at the lens induction stage (~E9.5) results in failed lens placode formation, with a lack of surface ectoderm thickening and invagination, and the absence of lens placode-specific gene expression (Ashery-Padan et al., 2000). In addition to aniridia, mutations in human Pax6 contribute to several retina disorders including coloboma, optic nerve hypoplasia, foveal hypoplasia, and Peter’s anomaly (Azuma et al., 1996, 2003; Hanson et al., 1994).

Lhx1 and Lhx2

Lhx1 (also referred to as Lim1) and Lhx2 belong to the LIM-homeodomain (LIM-HD) subfamily of homeobox genes. In addition to a DNA-binding homeodomain, LIM-HD proteins contain two zinc finger LIM domains, which participate in protein–protein interactions (Hobert and Westphal, 2000; Porter et al., 1997; Sanchez-Garcia and Rabbitts, 1994). In the developing retina, Lhx1 is expressed exclusively in postmitotic horizontal cells with expression initiating at E14.5 (Edqvist and Hallbook, 2004; Liu et al., 2000b). Thinning of the outer plexiform layer is observed in retinas with conditional mutations for Lhx1 (Poche et al., 2007). Horizontal cells of the Lhx1 null retina are properly specified, but fail to localize to the correct laminar position in the outer INL. In retinas lacking the One-cut transcription factor, Otf1, 80% of horizontal cells are lost with down-regulation of Lhx1 expression, demonstrating upstream regulation of Lhx1 by Otf1 (Wu et al., 2013). An early role for Lhx1 in optic vesicle development has also been demonstrated in chick (Kawaue et al., 2012). Lhx1 expression is observed in the proximal optic vesicles. Lhx1 expression in chick optic vesicle induces formation of ectopic neural retina tissue in the outer optic cup, demonstrated by increased expression of neural retina specific genes.

Lhx2 can function either as a transcriptional activator or repressor by interacting through its LIM domain with co-activators and co-repressors (Agulnick et al., 1996; Tetreault et al., 2009). Lhx2 plays a key role in a number of developmental processes including erythropoiesis, forebrain and eye development (Porter et al., 1997). Lhx2 belongs to a group of transcription factors collectively referred to as the eye field transcription factors (EFTFs), which are critical for early specification of the eye field (Zuber et al., 2003). Lhx2 expression is first observed in the prospective eye field of the neural anterior plate (Porter et al., 1997; Tetreault et al., 2009; Yun et al., 2009) and by E10, Lhx2 expression is observed throughout the optic neuroectoderm where it is localized to the optic stalk and most, if not all RPC (Gordon et al., 2013). In the mature neural retina, Lhx2 is expressed in a small number of amacrine cells as well the Müller glia localized to the medial INL (Fig. 1) (de Melo et al., 2012; Gordon et al., 2013). Eye development is halted at the optic vesicle stage in Lhx2 mutant embryos prior to formation of the optic cup, resulting in anophthalmia (Porter et al., 1997; Tetreault et al., 2009; Yun et al., 2009). However, mutations in the zebrafish Lhx2 ortholog Lhx2b do not result in anophthalmia, likely due to functional redundancies with Lhx2a (Seth et al., 2006). Despite the anophthalmic phenotype observed in Lhx2 knockout mouse models, Lhx2 mutations have not yet been identified in human cases of anophthalmia (Desmaison et al., 2010).

Lhx2 has been shown to play an important role in early optic development by initiating the expression of a number of important EFTFs, including Rax, Pax6 and Six3 (Tetreault et al., 2009). As retinal development progresses from the optic vesicle to optic cup stages, Lhx2 is required for regionalization and patterning of the optic neuroepithelium (Yun et al., 2009). In the optic vesicle, Vsx2 expression demarcates the presumptive neural retina while Mitf and Pax6 become localized to the RPE, and the optic stalk and neural retina, respectively. In Lhx2−/− optic vesicles, initiation of Vsx2 and Mitf expression fails, while expression of Pax6 does not persist past E9.5. These results demonstrate that in the absence of Lhx2, there is a failed regionalization of the optic vesicle and eye development is arrested prior to regional specification (Yun et al., 2009). Dorsoventral patterning in Lhx2 mutant optic vesicles is also disrupted. Vsx2 is a target of Lhx2 and its expression is required to maintain ventral identity and restrict Pax6 expression to the ventral optic vesicle (Horsford et al., 2005; Rowan et al., 2004). Lhx2−/− optic vesicles are dorsalized since they lack Vsx2 expression and consequently, Pax6 expression expands into the ventral optic vesicle (Yun et al., 2009). Lhx2 was recently demonstrated to be required continuously for optic identity from vesicle stages to neural retinal development, by shutting down alternative thalamic fates (Roy et al., 2013).

Roles for Lhx2 outside of the optic vesicle to optic cup transition have recently been identified. Conditional knockout of Lhx2 function demonstrates that Lhx2 is required for RPC maintenance (Gordon et al., 2013). Conditional Lhx2 mutant retinas have significantly decreased RPC populations with a consequent increase in neurogenesis. Lhx2 loss in early RPC populations results in excess RGC production while late removal of Lhx2 in RPC results in supernumerary rod photoreceptor formation. Production of these cells occurs at the expense of all other retinal cell types.

Recently, Lhx2 was found to be critical in maintaining Müller glia in a non-reactive state (de Melo et al., 2012). Conditional loss of Lhx2 in mature Müller glial cells induces reactive gliosis as demonstrated by increased expression of the intermediate filament glial fibrillary acidic protein (GFAP) and glial hypertrophy. Lhx2 null Müller glia are compromised in their ability to up-regulate neuroprotective factors in response to light damage.

Vsx2 and Vsx1

Similar to Pax6 and Rax, Vsx2 (formerly Chx10), is a member of Prd-L HD transcription factor family, and plays major roles in eye development. In addition to the paired homeodomain, VSX2 contains an additional conserved region known as the CVC domain, located immediately adjacent to the C-term of the HD, and named after the identification of Chx10 and three other genes: Vsx1/Vsx2 and Celt-10, which are the Chx10 homologs in goldfish and Caenorhabditis elegans, respectively (Levine et al., 1994; Liu et al., 1994). The CVC domain was reported to be required for the homeodomain of VSX2 to bind to DNA with high affinity and specificity (Zou and Levine, 2012). The HD and CVC domains are both required for VSX2 mediated repression (Dorval et al., 2005). The Prd-L HD:CVC proteins can be further classified into two groups, depending on the presence of a RV region, which is exclusive to Vsx1 group, or the OAR region specific for Vsx2 group (Chow et al., 2001). Vsx2 and Celt-10 belong to the Vsx2 group, which is highly conserved. However, the Vsx1 group is considered to be a rapidly evolving gene and shares only 71% identity with murine Vsx1 and human VSX1 orthologs. Vsx2 is expressed in the interneurons of the retina, hindbrain and spinal cord and expression is initiated in RPCs by E9.5 (Liu et al., 1994). During optic cup formation, contact between the optic vesicle and presumptive lens ectoderm is hypothesized to induce Vsx2 expression (Nguyen and Armitrlet, 2000). Vsx2 is down-regulated when the RPCs exit the cell cycle and differentiate (Green et al., 2003). During late retinal development and in mature retinas, Vsx2 expression is restricted to the INL, where it is predominantly expressed in bipolar interneurons and a subset of Müller glia (Fig. 1) (Liu et al., 1994; Rowan and Cepko, 2004).

Consistent with its expression pattern, two major phenotypes are observed in Vsx2 mutant mouse models: defects in RPC proliferation...
and bipolar cell differentiation (Burmeister et al., 1996). In the mature mouse retina there are 12 bipolar cell sub-types: 11 cone bipolar and one rod bipolar (Wassle et al., 2009). The ocular retardation (or) mouse model carries a spontaneous mutation with a premature stop codon at the midpoint of Vsx2, and therefore no Vsx2 is detected in the Chx10\textsuperscript{fl/fl} homozygote (Chx10\textsuperscript{fl/fl}) retina (Burmeister et al., 1996). Chx10\textsuperscript{fl/fl} retinas demonstrate significant reduction of RPC proliferation (up to 83% loss of bromodeoxyuridine (BrDU) incorporating RPCs in the peripheral retina). Studies on Vsx2 bacterial artificial chromosome (BAC) reporter mice have linked the reduction of RPC proliferation to RPE trans-differentiation, with increased expression of Mitf, a gene associated with onset and maintenance of pigmentation (Rowan et al., 2004). The severe RPC loss observed in Chx10\textsuperscript{fl/fl} can also be attributed to de-repression of the cyclin-dependent kinase inhibitor 1b (Cdkn1b), also known as p27(KIP1), which subsequently silences cyclin D1 (Green et al., 2003). Genetic deletion of Cdkn1b on a Vsx2 null background is able to partially rescue RPC proliferation. Complete loss of bipolar cells is observed in the postnatal Chx10\textsuperscript{fl/fl} retina (Burmeister et al., 1996). In contrast to the role of Vsx2 in early retinal development, Vsx2 is dispensable for RPC proliferation in later development and instead, plays a critical role in bipolar cell fate determination (Livne-Bar et al., 2006). This role is supported by the observation that no bipolar cells are detected in the Chx10\textsuperscript{fl/fl}; Cdkn1b\textsuperscript{−/−} double mutant retina, where the progenitor cell numbers are partially increased (Green et al., 2003). In addition, shRNA knockdown of Vsx2 in postnatal retinas dramatically reduces bipolar cell production, without influencing RPC proliferation (Livne-Bar et al., 2006). Bipolar cells and rod photoreceptors are born in a temporally overlapping window during postnatal murine retinal development. Vsx2 promotes a bipolar fate over a rod fate by repressing photoreceptor specific genes, while conversely, transcription factors critical to photoreceptor development repress Vsx2 to promote rod development (Fig. 2) (Brzezinski et al., 2010; Dorval et al., 2005; Katoh et al., 2010). For example, conditional loss of Blimp1, a zinc-finger transcription factor, results in increased expression of Vsx2 and increased production of bipolar cells with a concomitant reduction in photoreceptor numbers (Brzezinski et al., 2010; Katoh et al., 2010). Other phenotypes observed with Vsx2 mutation include microphthalmia in both human and mice, and lack of optic nerves (Table 1) (Burmeister et al., 1996; Ferda Percin et al., 2000).

In contrast to the early embryonic expression of Vsx2 in RPCs, Vsx1 is expressed predominantly in the postnatal retina (Chow et al., 2001, 2004). In situ hybridization and reporter gene studies revealed that Vsx1 expression is initiated within the INL no earlier than P5. By P12, Vsx1 expression is restricted to the outer INL, where it is expressed in a subset of cone bipolar cells, but not in Müller glia or rod bipolar, amacrine, and horizontal cells (Fig. 1) (Chow et al., 2001). Vsx1 is also a transcriptional target of Vsx2 (Fig. 2) (Clark et al., 2008). In Vsx2 deficient RPCs in both mice and zebrafish, Vsx1 mRNA is up-regulated. In addition, Vsx2 represses luciferase expression driven by the Vsx1 promoter (Clark et al., 2008). Interestingly, the transcriptional role of Vsx1 varies depending on the subtype of bipolar cell in which Vsx1 is expressed (Chow et al., 2004; Shi et al., 2011). Vsx1 is required to activate expression of genes required for differentiation and function of OFF bipolar cells, including recoverin, Neto1, NK3R and CaB5 (Chow et al., 2004). Conversely, Vsx1 negatively regulates expression of Vsx2 and CaB5 in type 7 ON bipolar cells (Shi et al., 2011). Unlike Vsx2, Vsx1 is not altered in human microphthalmic phenotypes.

**Meis Genes**

The Meis family proteins are the vertebrate homologs of Homothorax (Hth) in *Drosophila*. In the invertebrate retina, Hth promotes proliferation of RPC along with the Pax6 homolog (Ey) and an additional transcription factor, Tsh (Bessa et al., 2002). Resembling its role in invertebrates, the vertebrate Meis family is involved in RPC proliferation. The Meis family consists of three members, which includes Meis1, Meis2 and Meis3. Of these three factors, Meis1 and Meis2 expression is observed in the vertebrate retina. In mouse, chick and zebrafish, Meis1/2 expression is observed in proliferating RPC and is downregulated upon the onset of neurogenesis (Bessa et al., 2008; Heine et al., 2008). Microphthalmic eyes are observed with Meis1/2 knockdown (Bessa et al., 2008; Heine et al., 2008). This phenotype is the result of reduced cell numbers due to the downregulation of cell cycle regulatory proteins, including cyclin D1 and c-myc.

Meis1 also plays a key role in retinal patterning. *meis1* knockdown in zebrafish embryos lead to decreased dorsal *tbx5* expression in the developing retina, with a concomitant increase in the ventral *vasx* expression domain (Erickson et al., 2010). This patterning defect in the *meis1* mutant is due to the downregulation of *smad*1, a component of BMP signaling which is required for dorsal retinal patterning.

**Vax genes**

Vax genes are a family of homeodomain subfamily closely related to the *Enx* and *Not* genes, sharing sequence homology, similar chromosomal location and expression patterns (Hallonet et al., 1998). Vax genes are named for their highly specialized expression pattern during early neuroventrulation (Ventral anterior homeobox-containing gene). In the mouse, Vax1 mRNA is first detected at E8.0, in the anterior neural ridge and adjacent ectoderm. During embryogenesis, Vax1 expression is restricted ventrally to the derivatives of these regions, including the basal forebrain, ventral optic vesicle, optic stalk and optic chiasm (Hallonet et al., 1998, 1999). Mice targeted for deletion of Vax1 show defects in RGC axonogenesis and axonal-glial associations, without influencing expression of *Pax2* or *Bf1*. In addition, *Vax1*−/−axons fail to fasciculate and extend toward the midline of the hypothalamus, leading to an absence of the optic chiasm. These RGC axon pathfinding defects are partially due to the loss of important axon guidance cues, including *Netrin-1* and *EphB3*, but not *Slit1* (Bertuzzi et al., 1999). *Vax1* mutant mice also have coloboma due to failed choroid fissure closure. *Pax6* and *Rax* are ectopically expressed in the *Vax1* mutant optic nerve, whereas *Pax2* expression is unchanged.

VAX2 shares an identical homeodomain with VAX1 (Hanson et al., 1994). By E9.0, Vax2 transcripts are detected in the ventral optic vesicle, with lower expression in the optic nerve and stalk (Barbieri et al., 1999). Within a short time, *Vax1* and *Vax2* expression overlaps in the ventral retina and optic stalk (Hanson et al., 1994). By E12.0, *Vax2* expression is restricted to the ventral neural retina in all retinal neuroblasts while during late embryonic retinal development, Vax2 is only detected in the ventral RGCs (Barbieri et al., 2002; Mui et al., 2002). Vax2 is not expressed in the adult retina.

Consistent with its expression pattern, Vax2 plays a major role in ventralizing the embryonic retina. Mis-expression of Vax2 in the dorsal retina alters the expression of putative dorsal–ventral (D/V) markers, including up-regulation of *EphB2/EphB3*, *Pax2* and *Vax2* itself, and down-regulation of the dorsally restricted transcription factor *Tbx5* (Barbieri et al., 1999; Schulte et al., 1999). In addition, ectopic Vax2 expression in dorsal retina induces profound axonal pathfinding defects in dorsal RGCs (Schulte et al., 1999). In agreement with Vax2 gain-of-function studies, ventral RGCs from Vax2 null mice show complete dorsalization (Barbieri et al., 2002; Mui et al., 2002). The RGC axons from Vax2−/−ventral retina aberrantly project into the lateral rostral edge of the superior colliculus (SC) together with all the dorsal RGC axons, instead of into the medial rostral SC which are innervated by ventral RGCs.
axons. The ventral expression domain of EphB2/EphB3 is absent in Vax2−/− ventral retina. However, in contrast to Vax2 gain-of-function studies, the loss of Vax2 function failed to alter the expression of early regulators of D/V polarization, such as Pax2 and Tlx5 (Barbieri et al., 2002; Mui et al., 2002). Interestingly, while ipsilateral projecting axons originate primarily from the ventral temporal retina, complete loss of ipsilateral projecting axons was observed in only one of two studies to examine Vax2 mutant RGC axons (Barbieri et al., 2002). The coloboma phenotype is mild in Vax2 mutants (Barbieri et al., 2002; Mui et al., 2005).

Despite their complementary expression pattern, Vax1 and Vax2 interact and function in concert in retina and optic nerve formation (Mui et al., 2005). Vax1/Vax2 double knockout mice have more severe retinal colobomas when compared to the single mutants, and the optic nerve is transformed into a differentiated and laminated retina. By directly acting on the α-enhancer of Pax6, VAX1 and VAX2 negatively regulate Pax6 expression in the optic neuroepithelium (Fig. 2). Of significance, the repression of Pax6 transcripts in retinal development is closely related to the subcellular location of VAX2. Phosphorylation of Serine-170, which is C-terminal to the VAX2 homeodomain, sequesters VAX2 into the cytoplasm after E12.5, and in turn disables VAX2 repression of Pax6 expression (Kim and Lemke, 2006). By antagonizing Serine-170 phosphorylation, Shh promotes VAX2 nuclear localization.

Pbx genes

With the Meis homeobox genes, the Pre-B cell leukemia homeobox (Pbx), homeobox transcription factors belong to the TALE class of homeobox genes. Pbx genes are critical in specifying and patterning the midbrain and hindbrain by cooperating with Engrailed, and in complex with Hox and Meinox, respectively (Erickson et al., 2007; Waskiewicz et al., 2001, 2002). The role of Pbx genes in vertebrate retinal development has largely been studied in zebrafish. Pbx gene expression is observed throughout retinal development with pbx2 and pbx4 expression initiated during early zebrafish optic cup development, while pbx1 and pbx3b expression is observed shortly thereafter (French et al., 2007). In the developing retina, pbx2 and pbx4 play a role in RGC axon outgrowth. RGC axons from embryos lacking pbx2 and pbx4 project toward but fail to enter the optic tectum. This phenotype is thought to result from mis-regulation of pbx target genes required for dorsal retina (aldh1a2, tbx5 and hm4x) and tectal patterning (efna2, fabp7a and nat10) (Fréchéc et al., 2007). In addition, atoh7 expression is downregulated in the dorsal retina of pbx2/pbx4 mutants, which may also contribute to the observed pathfinding defects due to altered RGC differentiation. Pbx also plays a role in dorsal/temporal retina patterning, gdf6a, a BMP family growth factor, regulates dorsal/temporal expression of aldha1a2 and tbx5 in the zebrafish retina. In pbx2/pbx4 mutants, gdf6a and its targets are strongly downregulated (French et al., 2007).

Dlx genes

Distal-less (Dll) is required for Drosophila limb development. Dlx genes are the vertebrate orthologs of Dll. There are six murine Dlx genes, which are arranged into three bicanic clusters (Dlx1/Dlx2, Dlx5/Dlx6, and Dlx3/Dlx7), and are localized to mouse chromosomes 2, 6 and 11, respectively (Ghanem et al., 2003). Within the intergenic regions of Dlx1/Dlx2 and Dlx5/Dlx6, several cis-acting regulators have been characterized, including 112a and 112b within Dlx1 and Dlx2 genes, and 156i and 156ii separating Dlx5 and Dlx6 genes (Poitras et al., 2007). Two conserved enhancer elements, URE1 and URE2, have also been found in the 5′ flanking region of Dlx1 (Hamilton et al., 2005). These cis-acting elements are important for cross-regulatory interactions between the Dlx gene family members. For instance, DLX1 and DLX2 activate Dlx5/Dlx6 expression by directly binding to 156i (Zhou et al., 2004). Of the six Dlx genes, four (Dlx1, Dlx2, Dlx5 and Dlx6) are expressed in the developing forebrain, in differing, but temporally and spatially overlapping patterns.

Dlx1 and Dlx2 were initially detected in the retinal neuroepithelium at E12.5 (Eisenstat et al., 1999). Our group has detected DLX2 expression by immunostaining in E11.5 retina, where DLX2 is expressed in a clear high-dorsal to low-ventral gradient (de Melo et al., 2008). At E13.5, both DLX1 and DLX2 are expressed throughout the retina. Almost 25% of all retinal cells express DLX2 at E13.5. The proportion of DLX2 expressing cells in the total retinal population declines during development. By E18.5, DLX1 and DLX2 expression is restricted to the GCL and the inner NBL, where they are co-expressed with markers for RGC, amacrine and horizontal cells (Fig. 1). DLX1 expression resembles that of DLX2 in embryonic retinas, but decreases dramatically after birth (de Melo et al., 2003). DLX1 is not detected in the adult retina. However, DLX2 expression is maintained in the GCL and the INL throughout adulthood.

Homozygous deletion of Dlx1 and Dlx2 is perinatally lethal, and leads to a 33% reduction of RGC numbers, in part due to enhanced apoptosis of late-born RGCs (de Melo et al., 2005). TrkB, a neurotrophin receptor family member, is a downstream target of DLX2 during murine retinal development and may contribute to Dlx1/Dlx2 dependent survival of RGCs (de Melo et al., 2008). Similar to the Dlx1/Dkx2 double mutant, mice lacking Dlx1 or Dlx2 die at birth, but no retinal phenotype has been reported in the single gene knockouts (Panganiban and Rubenstein, 2002). Although DLX2 is co-expressed with GAD65, GAD67 and GABA in the developing retina, we have not discovered any defects in GABAergic interneuron differentiation in the Dlx1/Dlx2 null retinas (Zhang, Q., de Melo, J., and Eisenstat, D.D., unpublished observations).

The role of Dlx5 and Dlx6 in retinogenesis remains undetermined. In situ hybridization revealed Dlx5 mRNA expression in retina by E16.5 (Zhou et al., 2004). In the P0 and adult retinas, Dlx5 mRNA is co-expressed with DLX2 in the GCL and INL. The Dlx5/Dlx6 intergenic enhancer (MI56) co-expresses with DLX1, DLX2 and DLX5 in the RGCs, amacrine and horizontal cells.

The regulatory network of homeobox genes during retinogenesis is complex. Dlx1/Dlx2 and Brn3b function in parallel but cross-regulatory pathways to determine RGC cell fate. While the Atoh7−/−Brn3b RGC pathway is critical for RGC specification and differentiation, another pathway exists whereby Dlx1/Dlx2 cross-regulates expression of Brn3b for differentiation and survival of late-born retinal ganglion cells (Zhang et al., submitted for publication, Fig. 2). DLX2 may function as a transcriptional repressor of Crx during photoreceptor differentiation, since in the Dlx1/Dlx2 double mutant retinas there is increased Crx expression in the outer NBL and ectopic Crx expression in the GCL (Fig. 2) (de Melo et al., 2005). To date, no human eye diseases have been linked to mutations in the Dlx gene family.

Brn-3 genes

The POU-domain transcription factors were named for the identification of three mammalian homeodomain coding genes, Pit1, Oct1/Oct2, and one similar C. elegans gene Unc-86. The POU-domain is a bipartite DNA-binding protein domain, which contains a POU-specific region and a POU-homeodomain region. The class IV POU-domain proteins, BRN3a, BRN3b and BRN3c (POU4f1, POU4f2 and POU4f3, respectively) are the homologs of Unc-86 in C. elegans. Brn-3 genes are expressed in the embryonic and adult central nervous system, and are required for the sensorineural development and survival.
Each of the Brn-3 POU-homeodomain genes is expressed specifically in postmitotic RGCs (Fig. 1) (Xiang et al., 1995). In the mouse, Brn3b expression is first detected in the RGCs at E11.5 in the central inner retina, followed by Brn3a and Brn3c expression two days later (Pan et al., 2005; Xiang et al., 1995). Through E15.5 to adulthood, Brn3a and Brn3b expression partially overlaps in 80% of RGCs. Although Brn3a and Brn3b share a similar global expression pattern, their expression is distinct, particularly in the postnatal retina. In P5 RGCs, Brn3a is predominately expressed with few RGCs expressing Brn3b (Quina et al., 2005). Only ~15% of RGCs express Brn3c (Xiang et al., 1995).

The overlapping expression pattern and a similar specific DNA-binding site ([(A/G)CTATATA(T/C)] of the Brn-3 proteins suggest a potential for functional redundancy in retinogenesis. However, targeted mutations of Brn-3 genes show distinct developmental defects. Only the Brn3b<sup>−/−</sup> mouse shows an obvious retinal phenotype. Targeted deletion of Brn3b results in a 60–80% loss of RGCs in adult retinas, depending on the genetic mouse strain (Erkman et al., 1996; Gan et al., 1996). RGC loss in this model is due to enhanced apoptosis after E15.5. Initial cell fate specification and migration of RGCs in Brn3b mutant retinas is not affected. Brn3a is also required for RGC axon pathfinding and fasciculation (Erkman et al., 2000). Brn3a-null mutants die at birth, with loss of dorsal root and trigeminal neurons (Erkman et al., 1996; Xiang et al., 1996). Brn3c<sup>−/−</sup> mice display balance deficits and complete deafness, due to loss of vestibular and auditory hair cells (Erkman et al., 1996; Xiang et al., 1997). Neither Brn3a nor the Brn3c mutant mice display obvious retinal defects.

Brn3b is downstream of Atoh7, a bHLH transcription factor that is required for RGC development (Brown et al., 2001; Wang et al., 2001). The Atoh7–Brn3b regulation pathway promotes RGC differentiation by repressing a group of retinal development genes, including Dlx1/Dlx2, Otx2 and Crx (Qi et al., 2008). Recent work has shown that under the regulation of ATOH7, ISEL1, a LIM-HD TF, defines a distinct but overlapping sub-population of RGCs expressing Brn3b (Mu et al., 2008; Pan et al., 2008). Brn3a and Brn3c are downstream of Brn3b as Brn3a expression is reduced in Brn3b mutant retinas (Fig. 2) (Erkman et al., 1996). Despite the essential requirement for Brn3b in RGC development, several independent research groups have reported that all Brn-3 genes are functionally equivalent during retinogenesis. Over-expression of Brn3a, Brn3b or Brn3c in chick RGC has similar effects in promoting RGCs differentiation (Liu et al., 2000a). Knocking-in the Brn3a coding sequence into a Brn3b null background rescues RGCs from apoptosis and restores the proper RGC axon pathfinding process (Pan et al., 2005). Conditional deletion of Brn3a alters RGC dendritic stratification without influencing RGC axon central projections, while Brn3b conditional knockouts have reduced RGC number, loss of axon projections to medial terminal nuclei (MTN), lateral terminal nuclei (LTN) and corresponding visual sensory defects (Badea et al., 2009).

Islet1 and Isl1

Named for their role in binding to an enhancer of the insulin gene (Tanizawa et al., 1994), the Islet transcription factors belong to the LIM homeodomain family, and include Islet1 (ISL1) and ISL2. ISL2 is required for the correct lateral projection of RGC axons (Pak et al., 2004). ISL2 expression in the retina is first observed at E13.5 and becomes localized to contralaterally projecting RGCs. ISL2 down regulates Zic2 and EphB1 expression, which are factors required for ipsilateral projection. Consistent with this observation, ISL2 loss increases ipsilateral projections and upregulates Zic2 and EphB1 expression in ISL2 null RGCs.

ISL1 plays a critical role in vertebrate neurogenesis. ISL1 is required for the generation of motoneurons and its loss results in early embryonic lethality (Paff et al., 1996). ISL1 expression is localized to post-mitotic RGC during early retinogenesis and maintained into adulthood (Mu et al., 2008; Pan et al., 2008). In addition, cholinergic amacrine cell and bipolar cells express ISL1 postnatally (Elshatory et al., 2007). Similar to Brn3b, ISL1 is key for differentiation and survival of RGCs, but dispensable for initial RGC generation (Mu et al., 2008; Pan et al., 2008). Prior to RGC axoposis, axon pathfinding is disrupted in ISL1 null retinas as RGC axons fail to reach the midline (Pan et al., 2008). ISL1 and BRN3B expression co-localizes in postmitotic RGCs and are regulated in parallel by Atoh7 (Pan et al., 2008). As described for Brn3b genes above, ISL1 regulates distinct downstream RGC targets and shares targets with Brn3b in vivo (Mu et al., 2008; Pan et al., 2008).

Recently, ISL1 and BRN3B were shown to form complexes with each other that can bind to and regulate RGC specific gene expression (Li et al., 2014).

**Barhl2**

The BarH-like homeobox genes, Barhl1 and Barhl2, are homologs of the Drosophila BarH genes, which play a role in the development of the compound eye (Higashijima et al., 1992). Vertebrate BarH transcription factors contain a homeodomain and one or two FII domains, named for the presence of phenylalanine, isoleucine and leucine, and are required for in vivo transcription repression (Reig et al., 2007; Smith and Jaynes, 1996). Barhl1 and Barhl2 each contain two FII domains.

**Barhl2** expression is first observed in the vertebrate retina in the inner NBL at E14.5 and is maintained in adulthood with expression localized to both the GCL and the INL, where it is expressed in a subpopulation of postmitotic RGCs, amacrine cells and horizontal cells (Mo et al., 2004) (Fig. 1). Barhl1 is not expressed in the developing or mature retina. In Barhl2 null mice, a decrease in RGCs is observed while initial RGC specification is unaffected (Ding et al., 2009). Decreases in Barhl2 expression in Atoh7 and Brn3b null retinas demonstrate Barhl2 functions downstream of the Atoh7–Brn3b RGC developmental pathway. Barhl2 also plays a key role in amacrine cell subtype specification. Loss of Barhl2 results in significant reduction of both glycinergic and GABAergic amacrine cells and concomitant increase in cholinergic amacrine cells. Using morpholino knockdown, ptf1a was shown to be functionally upstream of barhl2 in specification of zebrafish amacrine cells (Jusuf et al., 2012).

**Oncet1 and Oncet2**

The Oncut (Oc) class of transcription factors have recently been demonstrated to play a role in vertebrate retinal development. Of the three Oc family members (Oc1, Oc2 and Oc3), Oc1 and Oc2 are highly expressed in the developing retina (Wu et al., 2012). In addition to an atypical homeodomain, unique in amino acid composition compared to traditional homeodomains, Oc transcription factors contain an additional DNA binding domain known as the CUT domain (Jacquemin et al., 1999; Lemaigre et al., 1996; Vanhorenbeeck et al., 2002). From E12.5 to P0, Oc1 and Oc2 are highly expressed in RGC (Wu et al., 2012). Oc1 and Oc2 expression is also observed in RPCs during prenatal stages of retina development. Postnatally (P5–P16), Oc1 and Oc2 expression is observed in horizontal cells (Fig. 1). Despite the high level of expression of Oc1 in RGC, RGC development in the Oc1<sup>−/−</sup> retina is unaffected (Wu et al., 2013). However, Oc2 expression levels increase in the GCL of Oc1<sup>−/−</sup> mutants, suggesting that Oc2 and Oc1 may be functionally redundant for RGC development. Loss of Oc1 significantly affects horizontal cell genesis with an 80% reduction in horizontal cells in the mature retina. Prox1 and Lhx1 expression is also reduced, demonstrating a genetic pathway whereby Oc1 acts upstream of
Prox1 and Uhx1 during horizontal cell development (Fig. 2). OC1 has also been shown to cooperate with OTX2 in RPC to specify cone and horizontal cell fates through co-regulation of thyroid receptor hormone beta (Thrβ) (Emerson et al., 2013). Cone precursors subsequently reduce Otx1 while maintaining Otx2 expression, whereas horizontal cells decrease Otx2 and maintain Otx1 expression.

Prox1

Prox1 is the vertebrate ortholog of the Drosophila gene prospero. PROX1 contains a sequence-divergent homeodomain adjacent to a carboxyl terminal Prospero domain, which facilitates proper alignment of PROX1 binding to DNA. Though PROX1 is a homeodomain-containing transcription factor, the homeodomain and the prospero domain of PROX1 combine to form a unique single-structural unit referred to as the Homeo-Prosporo domain through which it binds to DNA (Elslir et al., 2012; Ryter et al., 2002; Yousef and Matthews, 2005). Prox1 expression is first observed in the developing eye at E9.5 in the lens placode, followed by expression in the lens vesicle and the lens fibers at E10.0 and E12.5 (Wigle et al., 1999). Prox1 expression is observed in the neural retina by E14.5 where it is expressed in RPC and newly postmitotic horizontal cells (Dyer et al., 2003). Prox1 knockout mice die at mid-gestation and have a number of developmental ocular defects. In the lens of the Prox1−/− mice, abnormal proliferation and down-regulation of cell cycle inhibitors, p27kip1 and p57kip2 results in failed elongation and polarization of the lens (Wigle et al., 1999). In the neural retina, Prox1 loss results in significant horizontal cell loss and failure of early RPC to exit the cell cycle (Dyer et al., 2003). Failed cell cycle exit in early RPC produces a lower proportion of early-born cell types and an increased proportion of late-born cell types. Conversely, ectopic Prox1 induces horizontal cell production and premature cell cycle exit, producing smaller clones. Two transcription factors have been identified upstream of Prox1 in horizontal cell production: Foxn4 and Onecut1 (Oc1) (Li et al., 2004; Wu et al., 2013). Knockouts of Foxn4 and Oc1 both demonstrate significant reduction in Prox1 expression and horizontal cell production. Direct regulation of Prox1 expression by these transcription factors has yet to be established.

Irx genes

The Iroquois (Irx) genes were first discovered in Drosophila where they control bristle patterning on the dorsal mesothorax by regulating proneuralachaete-scutecan genes (Leys et al., 1996). Irx genes belong to the TALE superclass of homeodomain transcription factors and also contain an Iro box specific to the Irx family. Irx genes in Drosophila, mice and humans are organized into tri-gene clusters. Drosophila has one Irx cluster, which includes the genes araucan, caupolican and mirror. Mice and humans possess two clusters of Irx genes: Cluster A (IrxA) containing Irx1, Irx2 and Irx4 and Cluster B (IrxB) containing Irx3, Irx5 and Irx6 (Cavodeassi et al., 2001; Gomez-Skarmeta and Modoell, 2002; Peters et al., 2000). Zebrafish contain 11 Irx orthologous that are grouped according to their similarity to mammalian vertebrate Irx genes (Dildrop and Ruthner, 2004; Feijoo et al., 2004).

Expression of all vertebrate Irx genes is first observed in the GCL during early retinal development (Cohen et al., 2000). Postnatally, subsets of Irx transcription factors are required for development of specific bipolar cell subtypes. In addition to GCL expression, Irx5 expression is observed in the INL at P14 (Cheng et al., 2005) (Fig. 1). In mature retinas, Irx5 expression is found in Müller glia and type 2 OFF and type 3 OFF cone bipolar cells. Irx5 null retinas lack expression of a number of markers expressed in type 2 OFF (recoverin) and type 3 OFF (CaBP5 and PMCA1) cone bipolar cells, respectively, indicating that Irx5 plays a role in development of these bipolar cell subtypes.

Irx6 also plays a role in the development of bipolar cell subtypes (Star et al., 2012). Co-localization of Irx6 expressing cells with bipolar subtype specific markers demonstrates Irx6 is localized to both type 2 and type 3a bipolar cells (Fig. 1). Loss of Irx6 in the murine retina results in loss of type 3a bipolar cells and axon stratification defects in remaining 3a bipolar cells in the IPL. Resembling Irx5, Irx6 expression is also observed in the GCL, but development of this cell type is unaffected in mutants. Mis-regulation of Vsx1 and Bhlhb5 is also observed in the absence of Irx6 (Fig. 2). Vsx1 is ectopically expressed in the type 3a bipolar cells while Bhlhb5 expression was reduced in both type 2 and 3a bipolar cells, demonstrating a requirement for Irx6 mediated repression of Vsx1 in type 3a cells and activation of Bhlhb5 in type 2 and 3a bipolar cells.

Otx2

Otx genes, the mammalian orthologs of the Drosophila gene orthodenticle (Otd) family, belong to the paired-class of homeodomain proteins which are required for proper anterior–posterior patterning during embryogenesis. Otx2 belongs to a family of transcription factors, which includes Otx1, Otx2 and the Otx-like protein, Crx. The homeodomain of Otx2 differs from that of Otd by only two amino acids (Simeone et al., 1993). Otx2 plays a critical role in specification of the rostral central nervous system, as Otx2 deletions result in embryonic lethality due to a lack of anterior head structures, including the forebrain, midbrain and rostral hindbrain (Acampora et al., 1995; Gonzalez-Rodriguez et al., 2010; Matsuo et al., 1995). During development of the murine retina, Otx2 expression initiates at E11.5 where it is strongly expressed in the RPE and weakly expressed in the NBL of the neural retina (Martinez-Morales et al., 2003; Martinez-Morales et al., 2001; Nishida et al., 2003). At E12.5, Otx2 expression increases in the neural retina. During late embryonic retinal development, Otx2 is localized to the outer NBL, which includes the prospective photoreceptor layer. During adulthood, Otx2 expression significantly decreases in both the neural retina and RPE (Fig. 1).

Otx2 plays critical roles in both photoreceptor cell fate determination and bipolar cell development (Koike et al., 2007; Nishida et al., 2003). In the absence of Otx2 expression in the developing retina, a cell fate switch from photoreceptors to amacrine cells is observed. Microarray analysis of the Otx2 CKO retina compared to WT controls also demonstrated decreases in a number of transcription factors specific to photoreceptor development, including Crx, Nr2e3 and Nrl (Omori et al., 2011). Otx2 is reported to be genetically upstream of Crx based on reporter gene assays (Fig. 2) (Nishida et al., 2003). However, transcriptional regulation by OTX2 of the Crx promoter in vivo has yet to be identified. Conditional knockout of Otx2 in postnatal bipolar cells results in decreased expression of the mature bipolar cell marker, protein kinase C (PKC), demonstrating a requirement for Otx2 in bipolar cell development (Koike et al., 2007). Consistent with this observation, increases in bipolar specific genes, including Vsx2 and Bhlhb4, are observed in Otx2 CKO retinas (Omori et al., 2011).

In addition to its role in photoreceptor and bipolar cell development, Otx2 is also required for proper development of the RPE (Lane and Lister, 2012; Martinez-Morales et al., 2003, 2001). Interestingly, in mice both Otx2 and Mitf are required for normal development of the RPE, whereas zebrafish only require expression of otx1a and otx2 (Lane and Lister, 2012).

Mutations in human Otx2 result in a number of ocular disorders including microphthalmia, optic nerve hypoplasia, optic
nerve aplasia, colobomas and anophthalmia (Bardakjian and Schneider, 2011; Beby and Lamonerie, 2013; Ragge et al., 2005; Tajima et al., 2009; Verma and Fitzpatrick, 2007; Wyatt et al., 2008). OTX2 mutations are autosomal dominant and account from 5% of all (bilateral) microphthalmia and anophthalmia cases (Bardakjian and Schneider, 2011; Verma and Fitzpatrick, 2007). In addition to ocular diseases, OTX2 plays a role in the malignant childhood brain tumor, medulloblastoma, where its expression is amplified in both cell lines and primary medulloblastoma tumors (Boon et al., 2005; de Haas et al., 2006).

Crx

The cone–rod homeobox-containing gene (Crx) also belongs to the orthodenticle family of homeobox genes. The CRX homeodomain shares 88% and 86% homology with the homeodomains of OTX1 and OTX2, respectively, whereas the overall protein homology between CRX and OTX1 and OTX2 is 40% and 44%, respectively (Freund et al., 1997). Crx is specifically expressed in both photoreceptors and pinealocytes (Furukawa et al., 1999). Crx is first expressed at E12.5 in the outer NBL, corresponding to birth of cone photoreceptors. Crx expression peaks at postnatal day 6 (P6) of retinal development, which coincides with birth and maturation of rod photoreceptors. Crx expression is maintained in adulthood in the mature ONL (Fig. 1). Knockout mouse models of Crx fail to form photoreceptor outer segments from P14 when these structures are established and lack rod and cone electroretinogram (ERG) activity. Contrary to Otx2, Crx is not required for specification or photoreceptor cells fate, but rather for development and maintenance of photoreceptors by controlling the expression of photoreceptor specific genes, including rhodopsin, cone opsins, Nrl2e3, Nrl and Trp2 (Corbo et al., 2010; Furukawa et al., 1999; Hennig et al., 2008). In addition to binding the above gene promoters, Crx binds its own promoter in vivo and autoregulates its expression in a dose-dependent manner (Fig. 2) (Furukawa et al., 2002; Hennig et al., 2008). Crx also binds to the promoter of Otx2, which as described above, lies genetically upstream of Crx (Hennig et al., 2008; Nishida et al., 2003). This interaction results in repression of Otx2 expression, as demonstrated by a greater than two-fold increase in Otx2 expression in Crx−/− mouse models (Hennig et al., 2008). The genetic interactions of Crx suggest a regulatory mechanism for development of photoreceptors whereby Otx2 is first required for photoreceptor cell fate specification, subsequently leading to the downstream activation of Crx (Hennig et al., 2008). Expression of Crx increases in committed photoreceptor precursors and auto-regulates its expression while concomitantly repressing the expression of Otx2. Crx then activates expression of photoreceptor-specific genes required for the terminal differentiation and survival of rod and cone photoreceptors (Blackshaw et al., 2001; Hennig et al., 2008; Swaroop et al., 2010).

Consistent with Crx regulating a number of photoreceptor specific gene targets, CRX mutations have been implicated in a number of retinal diseases including retinitis pigmentosa, cone–rod dystrophy and Leber congenital amaurosis (LCA) (Freund et al., 1997; Rivolta et al., 2001; Sohocki et al., 1998).

Summary

Homeobox genes are critical for many aspects of retinal development including specification of the eye field, and retinal cell fate specification and differentiation. This review highlights a number of homeobox transcription factors and their contribution to retinal development through downstream target gene expression. Due to the critical contributions of these transcription factors to the proper development of the vertebrate retina, mutations in homeobox genes have been shown to result in a wide range of human ocular disorders. With the emergence of cell-based therapies as a potential treatment for some degenerative retinal disorders, furthering our understanding of how homeodomain-containing transcription factors direct specific retinal cell fates from stem cells or committed progenitor pools will be critical for the generation of the desired replacement retinal cells.

Acknowledgments

JLZ received graduate student funding from the Manitoba Health Research Council (MHRC), and from the Women and Children’s Health Research Institute (WCHRI) and the University of Alberta; QZ received MHRC, Manitoba Institute of Child Health (MICU) and CancerCare Manitoba Foundation (CCMF) graduate student scholarships; VIP was supported by MHRC and CCMF graduate student awards. JTW was funded by an MHRC Chair. DDE has received funding from the Canadian Institutes for Health Research (CIHR), the Foundation Fighting Blindness (Canada) and the Muriel & Ada Hole Kids with Cancer Society Chair in Pediatric Oncology (University of Alberta) in support of his retina research programs.

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Please cite this article as: Zagozewski, J.L., et al., The role of homeobox genes in retinal development and disease. Dev. Biol. (2014), http://dx.doi.org/10.1016/j.ydbio.2014.07.004


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