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Minireview

Molecules and Cells

Compartmentalization of Vertebrate Optic Neuroephithelium: External Cues and Transcription Factors

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The vertebrate eye is a laterally extended structure of the forebrain. It develops through a series of events, including specification and regionalization of the anterior neural plate, evagination of the optic vesicle (OV), and development of three distinct optic structures: the neural retina (NR), optic stalk (OS), and retinal pigment epithelium (RPE). Various external signals that act on the optic neuroepithelium in a spatial- and temporal-specific manner control the fates of OV subdomains by inducing localized expression of key transcription factors. Investigating the mechanisms underlying compartmentalization of these distinct optic neuroepithelium-derived tissues is therefore not only important from the standpoint of accounting for vertebrate eye morphogenesis, it is also helpful for understanding the fundamental basis of fate determination of other neuroectoderm-derived tissues. This review focuses on the molecular signatures of OV subdomains and the external factors that direct the development of tissues originating from the OV.

INTRODUCTION

Axial patterning of vertebrate neuroepithelium

Vertebrate organogenesis is not simply a process of local expansion of cell number; it is also accompanied by the segregation of a certain cell population from neighboring cell populations and subsequent development into structurally and functionally different tissues. During development, local cellular characteristics therefore undergo a serial transition from an initially identical state to an intermediate state with differences across a continuous spectrum, and then finally to a structurally and functionally distinguishable state (Kiecker and Lumsden, 2005). Cells located at the border between two cell populations with different characteristics therefore must commit to one of two distinct fates to complete the segregation of the two cell populations.

The development of vertebrate central nervous system (CNS) tissues also involves multiple rounds of border formation within a continuous structure of neuroepithelium. Compartmentalization or regionalization of the neuroepithelium is controlled by secreted signaling molecules, which are produced by anterior-posterior, dorsal-ventral, and medial-lateral axial patterning centers. For instance, bone morphogenic proteins (BMPs) are mainly concentrated in the dorsal neural tube, whereas sonic hedgehog (Shh) expression is largely restricted to the ventral neural tube (Edlund and Jessell, 1999; Jessell, 2000). Fibroblast growth factor 8 (FGF8) is primarily expressed in the anterior part of the prosencephalon, where it promotes development of the forebrain (Crossley et al., 2001). Each axial patterning center produces a distinct set of signaling molecules in a combinatorial manner. Every neuroepithelial cell therefore is influenced by these axial pattern determinants at different concentrations, and consequently expresses different sets of genes that eventually define the characteristics of the cell.

Formation of the optic vesicle and its transition into the optic cup

Mouse eye development starts at E8.0 from the anterior neural plate in the prosencephalon, which gives rise to the forebrain and eye field (Chow and Lang, 2001; Fuhrmann, 2010). At E8.5, the mouse eye originates from bilaterally evaginating cells of the diencephalon that correspond to the right and left optic pits. The optic pit starts to form the optic vesicle (OV) through continued evagination (Fig. 1A). The OV is formed by a single pseudostratified neuroepithelium and is surrounded by cephalic mesenchyme. Then, the OV grows toward the overlying surface ectoderm (SE) that will ultimately give rise to the lens and cornea (Chow and Lang, 2001; Fuhrmann, 2010). The cephalic mesenchyme between the OV and the SE (apparent in mammals and chicks) is displaced later as the two tissues come into close physical contact. At E9.5, the OV and SE are tightly associated through a network of collagenous fibrils and cytoplasmic processes (de Iongh et al., 2004; Furuta and Hogan, 1998). Upon contact with the OV, the SE thickens and gives rise to the lens placode (LP; Fig. 1B). Subsequently, both the OV and the LP undergo simultaneous invagination at about E10 (Fig. 1C). The LP later becomes the lens by way of the lens vesicle (LV), whereas the OV develops into the two-layered optic cup (OC) (Figs. 1D and 1E). The outer layer of the OC becomes retinal pigment epithelium (RPE), whereas the inner layer of the OC (facing the lens) gives rise to the neural retina (NR). The NR further develops into six types of retinal neurons: photoreceptors (rods and cones), bipolar cells, horizontal cells, amacrine

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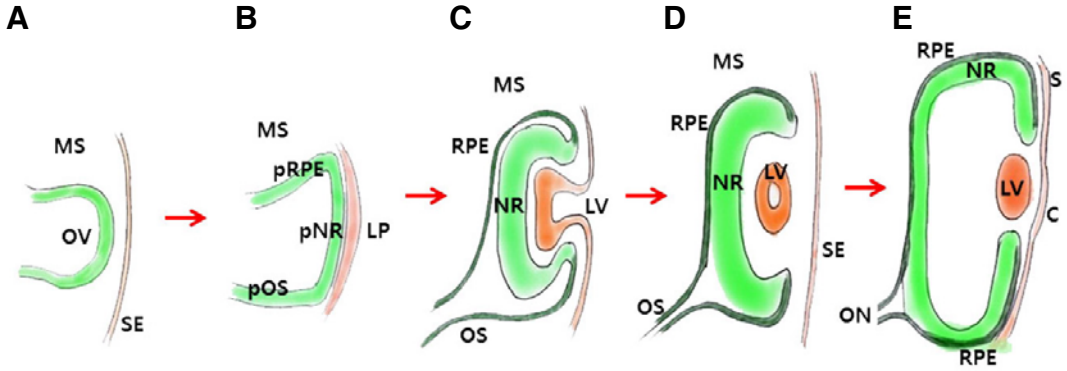

Fig. 1. Schematic diagram of mouse eye development. (A) The OV is generated as an evagination from the diencephalon at E9.5. (B) The OV start to be patterned into presumptive RPE (pRPE), presumptive NR (pNR), and OS (pOS) while the SE in turn forms the LP. (C) The OV and LP then start invaginate toward midline and form the optic cup, composed of NR and RPE, and the lens vesicle (LV), respectively at E10.5. (D-E) The NR and RPE become aligned each other and reduce ependymal cavity to a virtual space while the LV gradually also loses its cavity and becomes a solid primitive lens structure. The neuroepithelial cells at ventral OS differentiated into the astrocyte precursor cells, which further differentiate into optic nerve astrocytes. Abbreviations: C, Cornea; L, lens; LP, lens placode; LV, lens vesicle; MS, mesenchyme; NR, neural retina; ON, optic nerve; OS, optic stalk; OV, optic vesicle; RPE, retinal pigment epithelium; S, sclera; SE, surface ectoderm.

cells, ganglion cells, and a single Muller glia (Cepko et al., 1996; Harris, 1997). The axons of retinal ganglion cells (RGCs) residing in the surface layer of the NR grow proximally toward the optic disc and further extend to the brain through the OS. The ventral part of the OV undergoes even more complex structural changes, giving rise to the ventral OS (vOS), which develops into the optic fissure at the distal part and optic nerve astrocytes at the proximal part (Morcillo et al., 2006).

FATE-DETERMINING EXTERNAL CUES

The development of the three OV compartments, namely RPE, NR and OS, is regulated by a number of signaling pathways, each of which is active at different locations and time points during eye morphogenesis. The schematic diagram in Fig. 2A shows the distribution of morphogens around the mouse OV. Factors including BMP, Shh, and FGF secreted from several sources such as the head mesenchyme, surface ectoderm, ventral forebrain, and the OV itself are particularly critical for the determination of OV compartments. These inductive signals contribute to the compartmentalization of the OV by turning on or off specific transcription factors in each primitive compartment. We focus on several representative extrinsic factors of OV in more detail.

FGFs

FGFs are normally expressed in the presumptive lens ectoderm (PLE) and distal OV, and determine the NR identity of optic neuroepithelium at the distal OV (Pittack et al., 1997; Vogel-Hopker et al., 2000). FGF1 or FGF2 secreted from the PLE are sufficient to induce the NR domain by inducing the expression of Vsx2 (visual system homeobox 2; formerly Chx10) in the chick OV (Hyer et al., 1998; Miller et al., 2000; Pittack et al., 1997). The border between NR and RPE regions is disrupted in chicks in the absence of PLE, but it is rescued by treating the OV with FGF1 or FGF2 (Nguyen and Arnheiter, 2000). However, mice lacking both FGF1 and FGF2 do not show obvious ocular phenotypes, indicating that FGF1 and FGF2 are dispensable for mouse NR development (Miller et al., 2000). Instead, FGF9 is the most critical isoform for NR determination in the mouse OV. The NR transdifferentiates into RPE in FGF9-null mice (Zhao et al., 2001), whereas transgenic mice that ectopically express FGF9 in the presumptive RPE region make an additional NR in the prospective RPE region (Zhao and Overbeek, 1999). The expression of genes critical for the acquisition of RPE identity, such as orthodenticle homolog 2 (Otx2) and Mitf (microphthalmia-associated transcription factor), are down regulated upon FGF-mediated transdifferentiation of RPE into NR.

The ectopic expression of an active form of Ras or a constitutively activated allele of mitogen-activated protein kinase kinase 1 (MAPKK1)/MEK1 in the prospective RPE mimics the phenotype of FGF transgenic mice, in which RPE transdifferentiates into NR (Galy et al., 2002). This indicates that activation of the Ras-Raf-MEK-MAPK pathway plays a critical role in FGF-induced suppression of RPE development.

Wnts

The Wnt/β-catenin canonical pathway (WCP) is involved in multiple steps of eye development, including eye field establishment, maintenance of the retinal stem cell population, inhibition of neuronal specification of the ciliary marginal zone (CMZ) and RPE, and development of the cornea and lens (de Iongh et al., 2006; Lad et al., 2009). Inhibition of the WCP is required for specification of zebrafish eye field from the diencephalic region, whereas the Wnt non-canonical pathway functions to support the eye field induced from it (Cavodeassi et al., 2005; Kim et al., 2007). However, once the optic field is defined, the WCP plays a supportive role for the commitment to NR. Members of the frizzled (Fz) receptor family (Fz-4 and -5) are expressed in the distal OV and induce the development of optic neuroepithelium into NR (Van Raay and Vetter, 2004; Van Raay et al., 2005).

In the embryonic mouse retina, Wnts (Wnt-3, -5a, -5b, -7b, and -13) and Fz receptors (Fz-3 through -7) are consistently expressed in the neuroblast layer (Liu et al., 2003). However, WCP-dependent transcription remains inactive in the developing mouse retina (Liu et al., 2006). Several secreted Fz-related proteins, including Sfrp-1, -2 and -3, are also expressed in the mouse retina and possibly antagonize Wnt signaling in the retina (Esteve et al., 2011; Liu et al., 2003).

Conversely, the WCP is supportive for the development of the CMZ and RPE (Fujimura et al., 2009; Liu et al., 2007; Westenskow et al., 2009), where WCP activity is strongly enriched during development (Fujimura et al., 2009; Liu et al.,

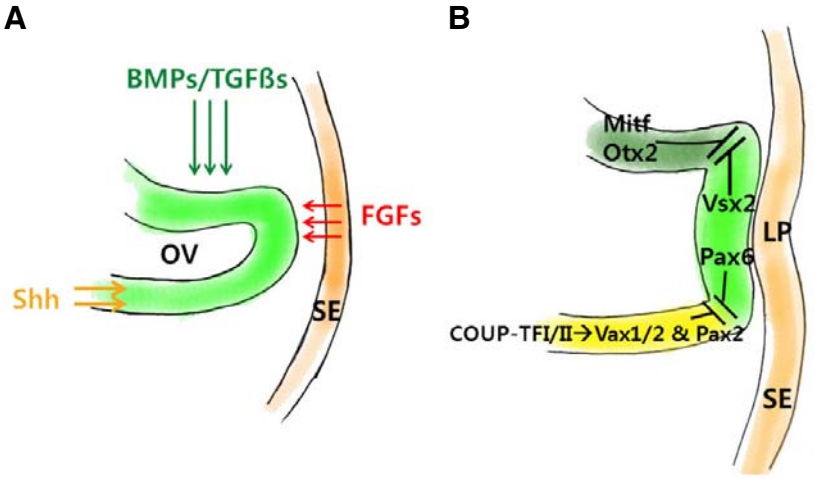

Fig. 2. Schematic representation of extrinsic factors and transcription factors involved in compartmentalization of OV. (A) Morphogens are secreted from neighboring tissues (ex. the extraocular MS, the SE, and the ventral forebrain) of the evaginating OV, and act to OV cells by forming concentration gradients. The factors determine the OV cell fates into pNR, pRPE and pOS, depending on their relative position along dorsal-ventral and proximal-distal axes. BMP/TGFβ signals from the extraocular MS favor the cells at dorsal-distal OV to become RPE, whereas FGFs from the SE activate NR identity, but repress RPE development, in the distal OV. Shh are secreted from the midline of ventral forebrain and induce OS at the ventral and proximal OV. (B) Consequence of the combinatorial distribution of the external fate determining factors listed above, distinct combination of transcriptional regulators become restricted to the pRPE, pNR and pOS. In the pRPE, Mitf and Otx2 induced by BMP/TGFβ and Wnt counteract with NR-enriched Vsx2, which is induced by FGF signaling. In the ventral-distal OV, Pax6 in the NR mutually represses Pax2 and Vax1/2, which are induced by Shh in the ventral OS. The interaction and activity of these transcriptional factors consolidate the identity and maintain the differentiated state of the RPE, NR and OS respectively.

2006). The WCP apparently activates the expression of Msx1 (msh homeobox 1) and Otx1 in the CMZ, and Otx2 and Mitf in RPE (Liu et al., 2007). Furthermore, Wnt-2b and -13 play roles in the commitment of CMZ cells to the iris and ciliary body (Cho and Cepko, 2006; Fokina and Frolova, 2006). Fz-3 and -7, and Sfrp-1 and -2 are also found in astrocyte precursor cells in the optic disc (OD) area, although the functions of Wnt components in the OD and OS are still unclear (Liu et al., 2003).

Shh

Shh, which is secreted from the ventral forebrain, controls the dorsal-ventral as well as proximal-distal polarity of the OV (Zhang and Yang, 2001). Shh is essential for the bilateral separation and proximal-distal patterning of the OV (Amato et al., 2004; Kim and Lemke, 2006). Hedgehog (Hh) signaling originating from the ventral forebrain has therefore been implicated in the specification of the ventral and proximal optic structure (i.e., vOS), but inhibits dorsal-distal fate (i.e., NR and RPE). Indeed, genetic elimination of Shh in mice and chemical inhibition of Hh signaling in Xenopus embryos impairs vOS development, resulting in proximal-distal patterning defects (Chiang et al., 1996; Perron et al., 2003).

The defective vOS development of Shh-deficient mice can be partially rescued by concomitant loss of the *Gli3* (GLI family zinc finger 3) allele, suggesting that the transcriptional regulation of Hh target genes is critical for vOS development (Furimsky and Wallace, 2006; Kim and Lemke, 2006). Supporting this hypothesis, the expression of genes important for vOS development, such as *Pax2* (paired box gene 2) and ventral anterior homeobox 1 (*Vax1*), is induced by Shh, whereas retinal determining genes, such as *Pax6* and *Rx/Rax* (retina and anterior neural fold homeobox), are repressed by ectopically expressed Shh (Perron et al., 2003). Shh not only ventralizes the OV through transcriptional activation of OS determinants, it also regulates them at the post-translational level. Shh facilitates nuclear accumulation of Vax2 in the mouse vOS (Kim and Lemke, 2006). As a consequence, it increases Vax protein content in the ventral distal OV where the border between vOS and NR develops.

BMP/TGFβ family

Several BMPs, members of the tumor growth factor (TGF)-β superfamily, and their receptors are found in developing vertebrate eyes and adjacent tissues (Chow and Lang, 2001). In mice, BMP4 expression is detected in the distal OV, whereas BMP7 is enriched in SE and the dorsal OV (Dudley and Robertson, 1997; Furuta and Hogan, 1998; Muller et al., 2007). Distal OV-enriched BMP4 is especially crucial for lens induction, and BMP7 is essential for eye morphogenesis by facilitating OV-PLE interaction.

In the optic cup stage, BMPs are expressed in the dorsal NR and support the expression of Tbx5 (T-box 5), which in turn represses EphBs in the dorsal retina, as opposed to Vax2, which represses ephrinBs expression in the ventral retina (Behesti et al., 2006; Koshiba-Takeuchi et al., 2000). In contrast, the BMP antagonist ventrophin is enriched in the ventral NR, where it restricts the influence of BMP only at the dorsal NR (Sakuta et al., 2001). These reports suggest that the function of BMPs in eye morphogenesis follows a general feature of BMP signaling as a critical dorsal axial determinant, as shown in other CNS tissues such as forebrain and spinal cord.

Gain-of-function experiments have shown that BMPs are also sufficient to elicit RPE development. Ectopic BMPs induce transdifferentiation of the presumptive OS and NR into RPE (Muller et al., 2007). By contrast, interfering with BMP signaling at OV stages inhibits RPE formation and induces NR-specific gene expression in the outer OC layer. The TGFβ family member activin, which is expressed in the extraocular MS, also plays critical roles in eye morphogenesis in the chick by supporting RPE development through the induction of Mitf in the OV (Fuhrmann et al., 2000). These observations provide physiological evidence to support the idea that BMP signaling participates in establishing RPE identity and is necessary and sufficient for RPE development.

Cross-talk between external signals for OV patterning

The external factors listed above impose their actions not only by activating their specific intracellular signal cascades, but also by reciprocally regulating the activity of other signaling path-

Table 1. Transcription factors involve in eye development

Transcription factors	Expression in the embryonic eye	Organism	References
Rx	OV, NR	Mouse, Xenopus, zebrafish	Andreazzoli et al. (1999); Furukawa et al. (1997); Kennedy et al. (2004); Mathers et al. (1997)
Pax6	NR, RPE	Mouse, chick, Xenopus, zebrafish	Hirsch and Harris (1997); Liu et al. (1994); Macdonald et al. (1995); Stoykova and Gruss (1994)
Pax2	OS, RPE	Mouse, chick, Xenopus, zebrafish	Heller and Brändli (1997); Herbrand et al. (1998); Macdonald et al. (1995); Nornes et al. (1990)
Otx1	OV, RPE	Mouse, chick, zebrafish	Bellipanni et al. (2000); Martinez-Morales et al. (2001); Simeone et al. (1993)
Otx2	OV, RPE, NR	Mouse, chick, zebrafish	Bellipanni et al. (2000); Bovolenta et al. (1997); Martinez-Morales et al. (2001); Simeone et al. (1993)
Six3	OV	Mouse, chick, zebrafish	Bovolenta et al. (1998); Kondoh et al. (2000); Oliver et al. (1995); Zhou et al. (2000)
Lhx2	OV	Mouse, Xenopus, zebrafish	Ando et al. (2005); Porter et al. (1997); Zuber et al. (2003)
Six6	OV, OS	Mouse, Xenopus	Toy et al. (1998); Zuber et al. (1999)
Vsx2	NR	Mouse, chick, zebrafish	Barabino et al. (1997); Belecky-Adams et al. (1997); Liu et al. (1994)
Mitf	OV, RPE	Mouse, chick, Xenopus, zebrafish	Dorsky et al. (2000); Hodgkinson et al. (1993); Kumasaka et al. (2004); Mochii et al. (1998)
Vax1	OS > NR	Mouse, Xenopus, zebrafish	Hallonet et al. (1998); Take-uchi et al. (2003)
Vax2	NR > OS	Mouse	Barbieri et al. (1999); Take-uchi et al. (2003)
COUP-TFI/II	OS	Mouse	Tang et al. (2010)

ways directly or indirectly. For instance, Shh expressed in the ventral forebrain supports the expression of FGFs in the anterior neural plate of the telencephalon, where BMPs inhibits FGF expression (Ohkubo et al., 2002). FGF8 from the anterior neural plate then cooperates with Shh in the development of the vOS in zebrafish by inducing key determinants, such as Pax2 and Vax (Take-uchi et al., 2003). In contrast, Hh-activated Gli1 targets secreted Fz-related protein 1 (sFrp1), which interferes with the binding of Wnt to Fz receptors and consequently inactivates Wnt signaling around Hh-activated cells (He et al., 2006). Therefore, the level of Wnt signaling in the Hh-enriched OV region is gradually diminished, consequently resulting in segregation of Hh-restricted ventral-proximal OV versus Wnt-restricted dorsal-distal OV.

In addition to the direct cross-talk between signaling pathways, these external signals also converge on common intracellular components in either a positive or negative manner. For instance, in *Drosophila* retinal neurogenesis, Hh signaling inactivates adenylate cyclase (AC) via inhibitory G-protein (Gi)-coupled Smoothened (Smo), whereas Wnt signaling activates AC *via* stimulatory G-protein (Gq) coupled Fz (Katanaev et al., 2005; Ogden et al., 2008; Pan and Rubin, 1995). Consequently, vertebrate Shh inactivates the cAMP-dependent protein kinase A (PKA) signaling pathway, whereas Wnt activates it (Chen et al., 2005; Hammerschmidt et al., 1996; Suzuki et al., 2008; Waschek et al., 2006). In developing vertebrate eyes, PKA activity in the mouse OV is inversely correlated with Hh concentration through formation of a dorsal-distal high and ventral-proximal low gradient (Kim and Lemke, 2006). Furthermore, activation of PKA dorsalizes zebrafish OV, whereas inhibition of PKA ventralizes it (Masai et al., 2005). However, whether the cAMP-PKA signaling pathway is an indispensable intracellular convergence point of Hh and Wnt in vertebrates remains to be determined.

TRANSCRIPTION FACTOR CODE

The initial phase of OV patterning by external factors described above is next converted into the combinatorial code of transcription factors, which are differentially expressed in each OV compartment and establish the cellular identity of the compartment. The key transcription factors involved in vertebrate eye morphogenesis are list in Table 1; several representative examples are reviewed in more detail below.

Otx2

Otx2 is not only essential for the development of fore/midbrain structures, it also plays key roles in vertebrate eye development (Martinez-Morales et al., 2001; 2003). Otx2 is initially expressed in the entire OV, but its expression soon becomes restricted to the presumptive RPE, where it is maintained throughout adulthood (Bovolenta et al., 1997; Bumsted and Barnstable, 2000). Otx2 is also detectable in bipolar cells and photoreceptors of the retina, and supports the survival of these cell populations (Bumsted and Barnstable, 2000; Decembrini et al., 2006; Koike et al., 2007). $Otx2^{+/-}$ mice carrying additional loss of Otx1 alleles show clear defects in the patterning of the RPE, which is replaced by a NR-like structure (Martinez-Morales et al., 2001). In contrast, Otx2 overexpression induces a pigmented phenotype in cultured NR cells (Martinez-Morales et al., 2004). Otx2 binds and activates the promoter region of the melanogenic genes, QNR-71, tyrosinase (*Tyr*), tyrosinase related protein 1 (*Tyrp1*) and *Tyrp2*, which contain the specific Otx-binding site TAATCC/T in their promoters (Martinez-Morales et al., 2003; Takeda et al., 2003). Furthermore, Otx2 together with Wnt/β-catenin signaling turns on the expression of Mitf, which in cooperation with Otx2

is sufficient to drive differentiation of the RPE.

Mitf

Mitf is a basic helix-loop-helix (bHLH) leucine zipper transcription factor that is crucial for the acquisition and maintenance of RPE cell identity (Martinez-Morales et al., 2004). Ectopic expression of Mitf in cultured avian NR cells results in the induction of pigmentation by initiating the expression of two markers of differentiated pigment cells: melanosomal matrix protein 115 kDa (MMP115) and tyrosinase (Mochii et al., 1998; Planque et al., 1999). By contrast, inhibition of Mitf by small interfering RNAs (siRNAs) decreases MMP115 expression and promotes dedifferentiation of the RPE (Iwakiri et al., 2005). In Mitf mutants, the RPE remains unpigmented and displays areas that develop into a second NR (Bumsted and Barnstable, 2000; Nguyen and Arnheiter, 2000).

Mitf expression in RPE can be regulated by redundant functions of Pax2 and Pax6 (Baumer et al., 2003). Mitf can be also induced through cooperative transcriptional regulation by the β-catenin/Tcf complex and Otx2, both of which are able to bind the Mitf promoter (Westenskow et al., 2009; 2010). Mitf expression is therefore impaired in β-catenin-deficient RPE, which consequently transforms into NR (Fujimura et al., 2009; Westenskow et al., 2009). However, it is still unclear whether Otx2 is also indispensable for Mitf maintenance in RPE.

Vsx2

The paired-like homeobox gene Vsx2 is a specific marker of retinal progenitor cells and functions to repress Mitf expression in the distal OV (Horsford et al., 2005; Rowan et al., 2004). Moreover, overexpression of Vsx2 in the chick RPE causes down regulation of Mitf expression and other pigment markers, leading to a non-pigmented RPE (Rowan et al., 2004). Initially, dorsal and distal OV cells express Mitf, but cells in close proximity to the lens placode, receiving FGF signals, start to express Vsx2, which subsequently leads to the repression of Mitf, and possibly also of Otx2, in the presumptive NR (Horsford et al., 2005). Vsx2 negatively regulates *Mitf* expression by binding to its promoter, thereby ensuring NR development in the distal portion of the OV (Horsford et al., 2005; Rowan et al., 2004). However, OV cells located farther away from the LP maintain Mitf expression and specify the RPE fate.

Pax6

Pax6 is an evolutionary conserved transcription factor that is crucial for eye development (Chow and Lang, 2001; Graw, 2010). Mutations in Pax6 result in a small-eye phenotype in mice and rats (Hill et al., 1991; Matsuo et al., 1993). Pax6 contributes to eye development by mediating the induction of lens and cornea from SE as well as by supporting the development of the NR and RPE from OV. Pax6 is first expressed in the optic primordium, whereas later it is found in all cells of the prospective NR, RPE, and lens epithelium (Ashery-Padan and Gruss, 2001). Upon formation of the border between NR and vOS, Pax6 is repressed by Shh and becomes restricted to the dorsal and distal domain of OC, where it supports the development of RPE and NR, whereas Shh targets, such as Pax2 and Vax1, induce vOS development at the ventral-proximal OV. Therefore, in the absence of Shh in $Shh^{-/-}$;$Gli3^{+/-}$ mice, Pax6 is elevated in the vOS, which is transformed into NR (Mui et al., 2005). Ectopically expressed Pax6 was also shown to inhibit the development of the vOS in chick OV, consequently posi-tioning the eyes in a more ventral direction (Leconte et al., 2004). These results therefore suggest that Shh-dependent suppression of Pax6 expression in the vOS is critical for the development of the vOS and proper eye positioning.

Pax2

Pax2 is primarily expressed in the ventral half of the OV, and becomes restricted to astrocyte precursor cells and astrocytes of the OS soon after invagination of the OC (Baumer et al., 2003; Sehgal et al., 2008). Pax2 is induced by Shh and is consistently expressed in ventral and proximal OV cells, including the ventral NR cells surrounding the optic fissure and cells of the OS.

In Pax2-deficient mice, no glial cells develop in the optic nerve (ON) and the optic chiasma fails to form (Torres et al., 1996). The NR/vOS and RPE/dorsal OS (dOS) borders are also positioned more proximally in Pax2-deficient mice, resulting in coloboma at the ventral OC and expansion of the RPE into the dOS territory. Apparently, the Pax6 expression domain expands proximally and invades over the NR/OS border in Pax2-deficient mice. Importantly, Pax2 and Pax6 are suggested to act in a mutually exclusive manner to regulate the regionalization of different presumptive eye tissues. Accordingly, Pax2 restricts Pax6 expression to the more distal portion of the OV by directly binding to a NR-specific intronic enhancer element, whereas Pax6 represses Pax2 expression in the NR by binding to a *Pax2* promoter sequence (Schwarz et al., 2000).

Vax1 and Vax2

Vax1 and Vax2 are two-related homeodomain transcription factors that are expressed in the ventral OV. Mouse Vax1 is broadly expressed in the ventral domain of the forebrain, whereas Vax2 is specifically restricted to the ventral distal part of the OV (Barbieri et al., 1999; Hallonet et al., 1998). Between E11.5 and E14.5, Vax1 becomes restricted to the OS, whereas Vax2 is expressed in the ventral half of the NR. Ectopic expression of Vax1 or Vax2 results in expansion of the OS into NR territory, suggesting that Vax gene functions overcome the default neural fate of the vOS (Barbieri et al., 1999; Kim and Lemke, 2006). Conversely, the RPE and NR expand proximally into the OS territory of Vax1-deficient mice, which also exhibit broad-spectrum ventral forebrain developmental defects (Bertuzzi et al., 1999; Hallonet et al., 1999). In contrast, Vax2 is dispensable for OS development, but it is critical for dorsal-ventral polarization of the NR (Mui et al., 2002).

Therefore, transforming the OS entirely into NR requires disruption of both Vax1 and Vax2 in mice and zebrafish (Mui et al., 2005; Take-uchi et al., 2003). Vax1 and Vax2 repress the expression of Pax6 by directly binding to a NR-specific intronic enhancer of *Pax6* (Mui et al., 2005). However, the Vax binding site is distinct from the *Pax6* auto-regulatory element, which is also a target of Pax2 (Schwarz et al., 2000). Although Pax6 is abnormally elevated in ectopic NR in the OS territory of *Vax1/2*-double-knockout (dko) mice, Pax2 expression is still detectable in the OV and ectopic NR of *Vax1/2*-dko embryos (Mui et al., 2005), suggesting that Vax and Pax2 might function independently in the optic neuroepithelium in the acquisition of OS fate.

Similar to *Vax1/2*-dko mice, COUP-TFI/II-dko mouse OS also fails to develop and transform into NR (Tang et al., 2010). The expression of Vax1/Vax2 and Pax2 is impaired in the COUP-TFI/II-dko mouse OS, whereas Shh expression in the ventral forebrain is intact. These results therefore indicate that COUP-TFI/II is located downstream of Shh and upstream of Vax1/2 and Pax2. COUP-TFs can also regulate RPE development by directly regulating the expression of Otx2.

CONCLUSIONS

A current model that takes into account the findings described above is schematically illustrated in Fig. 2. In this simplified scheme, exogenous signals (e.g., BMP, Shh, Wnt and FGF) from adjacent tissues are instrumental for the initial regionalization of the OV into RPE, NR, and OS. Subsequently, specific transcription factors, including Mitf, Otx2, Vsx2, Pax6, Pax2 and Vax1, turn on in each domain. Mutual regulation among these domain-specific transcription factors then permanently segregates eye territories into the OS, NR, and RPE.

The molecular mechanisms underlying dOS and RPE are not yet as clearly defined as those involved in border formation between RPE and NR or NR and vOS. However, reciprocally regulating external signals and key transcription factors also appear to be involved. Further study of intracellular signaling components that enable exclusive dominance of fate-determining transcription factors as well as signaling pathways that function during the binary fate segregation of cells at the optic borders is warranted.

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REFERENCES

Ando, H., Kobayashi, M., Tsubokawa, T., Uyemura, K., Furuta, T., and Okamoto, H. (2005). Lhx2 mediates the activity of Six3 in zebrafish forebrain growth. Dev. Biol. *287*, 456-468.

Andreazzoli, M., Gestri, G., Angeloni, D., Menna, E., and Barsacchi, G. (1999). Role of Xrx1 in Xenopus eye and anterior brain development. Development *126*, 2451-2460.

Amato, M.A., Boy, S., and Perron, M. (2004). Hedgehog signaling in vertebrate eye development: a growing puzzle. Cell Mol. Life Sci. *61*, 899-910.

Ashery-Padan, R., and Gruss, P. (2001). Pax6 lights-up the way for eye development. Curr. Opin. Cell Biol. *13*, 706-714.

Barabino, S.M., Spada, F., Cotelli, F., and Boncinelli, E. (1997). Inactivation of the zebrafish homologue of Chx10 by antisense oligonucleotides causes eye malformations similar to the ocular retardation phenotype. Mech. Dev. *63*, 133-143.

Barbieri, A.M., Lupo, G., Bulfone, A., Andreazzoli, M., Mariani, M., Fougerousse, F., Consalez, G.G., Borsani, G., Beckmann, J.S., Barsacchi, G., et al. (1999). A homeobox gene, vax2, controls the patterning of the eye dorsoventral axis. Proc. Natl. Acad. Sci. USA *96*, 10729-10734.

Baumer, N., Marquardt, T., Stoykova, A., Spieler, D., Treichel, D., Ashery-Padan, R., and Gruss, P. (2003). Retinal pigmented epithelium determination requires the redundant activities of Pax2 and Pax6. Development *130*, 2903-2915.

Behesti, H., Holt, J.K., and Sowden, J.C. (2006). The level of BMP4 signaling is critical for the regulation of distinct T-box gene expression domains and growth along the dorso-ventral axis of the optic cup. BMC Dev. Biol. *6*, 62.

Belecky-Adams, T., Tomarev, S., Li, H.S., Ploder, L., McInnes, R.R., Sundin, O., and Adler, R. (1997). Pax-6, Prox 1, and Chx10 homeobox gene expression correlates with phenotypic fate of retinal precursor cells. Invest Ophthalmol. Vis. Sci. *38*, 1293-1303

Bellipanni, G., Murakami, T., Doerre, O.G., Andermann, P., and Weinberg, E.S. (2000). Expression of Otx homeodomain proteins induces cell aggregation in developing zebrafish embryos. Dev. Biol. *223*, 339-353.

Bertuzzi, S., Hindges, R., Mui, S.H., O'Leary, D.D., and Lemke, G. (1999). The homeodomain protein vax1 is required for axon guidance and major tract formation in the developing forebrain. Genes Dev. *13*, 3092-3105.

Bovolenta, P., Mallamaci, A., Briata, P., Corte, G., and Boncinelli, E. (1997). Implication of OTX2 in pigment epithelium determination and neural retina differentiation. J. Neurosci. *17*, 4243-4252.

Bovolenta, P., Mallamaci, A., Briata, P., Corte, G., and Boncinelli, E. (1997). Implication of OTX2 in pigment epithelium determination and neural retina differentiation. J. Neurosci. *17*, 4243-4252.

Bumsted, K.M., and Barnstable, C.J. (2000). Dorsal retinal pigment epithelium differentiates as neural retina in the microphthalmia (mi/mi) mouse. Invest. Ophthalmol. Vis. Sci. *41*, 903-908.

Cavodeassi, F., Carreira-Barbosa, F., Young, R.M., Concha, M.L., Allende, M.L., Houart, C., Tada, M., and Wilson, S.W. (2005). Early stages of zebrafish eye formation require the coordinated activity of Wnt11, Fz5, and the Wnt/beta-catenin pathway. Neuron *47*, 43-56.

Cepko, C.L., Austin, C.P., Yang, X., Alexiades, M., and Ezzeddine, D. (1996). Cell fate determination in the vertebrate retina. Proc. Natl. Acad. Sci. USA *93*, 589-595.

Chen, A.E., Ginty, D.D., and Fan, C.M. (2005). Protein kinase A signalling via CREB controls myogenesis induced by Wnt proteins. Nature *433*, 317-322.

Chiang, C., Litingtung, Y., Lee, E., Young, K.E., Corden, J.L., Westphal, H., and Beachy, P.A. (1996). Cyclopia and defective axial patterning in mice lacking Sonic hedgehog gene function. Nature *383*, 407-413.

Cho, S.H., and Cepko, C.L. (2006). Wnt2b/beta-catenin-mediated canonical Wnt signaling determines the peripheral fates of the chick eye. Development *133*, 3167-3177.

Chow, R.L., and Lang, R.A. (2001). Early eye development in vertebrates. Annu. Rev. Cell Dev. Biol. *17*, 255-296.

Crossley, P.H., Martinez, S., Ohkubo, Y., and Rubenstein, J.L. (2001). Coordinate expression of Fgf8, Otx2, Bmp4, and Shh in the rostral prosencephalon during development of the telencephalic and optic vesicles. Neuroscience *108*, 183-206.

de Iongh, R.U., Chen, Y., Kokkinos, M.I., and McAvoy, J.W. (2004). BMP and activin receptor expression in lens development. Mol. Vis. *10*, 566-576.

de Iongh, R.U., Abud, H.E., and Hime, G.R. (2006). WNT/Frizzled signaling in eye development and disease. Front Biosci. *11*, 2442-2464.

Decembrini, S., Andreazzoli, M., Vignali, R., Barsacchi, G., and Cremisi, F. (2006). Timing the generation of distinct retinal cells by homeobox proteins. PLoS Biol. *4*, e272.

Dorsky, R.I., Raible, D.W., and Moon, R.T. (2000). Direct regulation of nacre, a zebrafish MITF homolog required for pigment cell formation, by the Wnt pathway. Genes Dev. *14*, 158-162

Dressler, G.R., Deutsch, U., Chowdhury, K., Nornes, H.O., and Gruss, P. (1990). Pax2, a new murine paired-box-containing gene and its expression in the developing excretory system. Development *109*, 787-795.

Dudley, A.T., and Robertson, E.J. (1997). Overlapping expression domains of bone morphogenetic protein family members potentially account for limited tissue defects in BMP7 deficient embryos. Dev. Dyn. *208*, 349-362.

Edlund, T., and Jessell, T.M. (1999). Progression from extrinsic to intrinsic signaling in cell fate specification: a view from the nervous system. Cell *96*, 211-224.

Esteve, P., Sandonis, A., Ibanez, C., Shimono, A., Guerrero, I., and Bovolenta, P. (2011). Secreted frizzled-related proteins are required for Wnt/beta-catenin signalling activation in the vertebrate optic cup. Development *138*, 4179-4184.

Fokina, V.M., and Frolova, E.I. (2006). Expression patterns of Wnt genes during development of an anterior part of the chicken eye. Dev. Dyn. *235*, 496-505.

Fuhrmann, S. (2010). Eye morphogenesis and patterning of the optic vesicle. Curr. Top Dev. Biol. *93*, 61-84

Fuhrmann, S., Chow, L., and Reh, T.A. (2000). Molecular control of cell diversification in the vertebrate retina. Results Probl. Cell Differ. *31*, 69-91.

Fujimura, N., Taketo, M.M., Mori, M., Korinek, V., and Kozmik, Z. (2009). Spatial and temporal regulation of Wnt/beta-catenin signaling is essential for development of the retinal pigment epithelium. Dev. Biol. *334*, 31-45.

Furukawa, T., Kozak, C.A., and Cepko, C.L. (1997). rax, a novel paired-type homeobox gene, shows expression in the anterior neural fold and developing retina. Proc. Natl. Acad. Sci. USA *94*, 3088-3093.

Furuta, Y., and Hogan, B.L. (1998). BMP4 is essential for lens induction in the mouse embryo. Genes Dev. *12*, 3764-3775.

Furimsky, M., and Wallace, V.A. (2006). Complementary Gli activity mediates early patterning of the mouse visual system. Dev. Dyn. *235*, 594-606.

Galy, A., Neron, B., Planque, N., Saule, S., and Eychene, A. (2002). Activated MAPK/ERK kinase (MEK-1). induces transdifferentiation of pigmented epithelium into neural retina. Dev. Biol. *248*, 251-264.

Graw, J. (2010). Eye development. Curr. Top Dev. Biol. *90*, 343-386.

Hallonet, M., Hollemann, T., Wehr, R., Jenkins, N.A., Copeland, N.G., Pieler, T., and Gruss, P. (1998). Vax1 is a novel homeobox-containing gene expressed in the developing anterior ventral forebrain. Development *125*, 2599-2610.

Hallonet, M., Hollemann, T., Pieler, T., and Gruss, P. (1999). Vax1, a novel homeobox-containing gene, directs development of the basal forebrain and visual system. Genes Dev. *13*, 3106-3114.

Hammerschmidt, M., Bitgood, M.J., and McMahon, A.P. (1996). Protein kinase A is a common negative regulator of Hedgehog signaling in the vertebrate embryo. Genes Dev. *10*, 647-658.

Harris, W.A. (1997). Cellular diversification in the vertebrate retina. Curr. Opin. Genet. Dev. *7*, 651-658.

He, J., Sheng, T., Stelter, A.A., Li, C., Zhang, X., Sinha, M., Luxon, B.A., and Xie, J. (2006). Suppressing Wnt signaling by the hedgehog pathway through sFRP-1. J. Biol. Chem. *281*, 35598-35602.

Heller, N., and Brandli, A.W. (1997). Xenopus Pax-2 displays multiple splice forms during embryogenesis and pronephric kidney development. Mech. Dev. *69*, 83-104.

Herbrand, H., Guthrie, S., Hadrys, T., Hoffmann, S., Arnold, H.H., Rinkwitz-Brandt, S., and Bober, E. (1998). Two regulatory genes, cNkx5-1 and cPax2, show different responses to local signals during otic placode and vesicle formation in the chick embryo. Development *125*, 645-654.

Hill, R.E., Favor, J., Hogan, B.L., Ton, C.C., Saunders, G.F., Hanson, I.M., Prosser, J., Jordan, T., Hastie, N.D., and van Heyningen, V. (1991). Mouse small eye results from mutations in a paired-like homeobox-containing gene. Nature *354*, 522-525.

Hirsch, N., and Harris, W.A. (1997). Xenopus Pax-6 and retinal development. J. Neurobiol. *32*, 45-61.

Hodgkinson, C.A., Moore, K.J., Nakayama, A., Steingrimsson, E., Copeland, N.G., Jenkins, N.A., and Arnheiter, H. (1993). Mutations at the mouse microphthalmia locus are associated with defects in a gene encoding a novel basic-helix-loop-helix-zipper protein. Cell *74*, 395-404.

Horsford, D.J., Nguyen, M.T., Sellar, G.C., Kothary, R., Arnheiter, H., and McInnes, R.R. (2005). Chx10 repression of Mitf is required for the maintenance of mammalian neuroretinal identity. Development *132*, 177-187.

Hyer, J., Mima, T., and Mikawa, T. (1998). FGF1 patterns the optic vesicle by directing the placement of the neural retina domain. Development *125*, 869-877.

Iwakiri, R., Kobayashi, K., Okinami, S., and Kobayashi, H. (2005). Suppression of Mitf by small interfering RNA induces dedifferentiation of chick embryonic retinal pigment epithelium. Exp. Eye Res. *81*, 15-21.

Jessell, T.M. (2000). Neuronal specification in the spinal cord: inductive signals and transcriptional codes. Nat. Rev. Genet. *1*, 20-29.

Katanaev, V.L., Ponzielli, R., Semeriva, M., and Tomlinson, A. (2005). Trimeric G protein-dependent frizzled signaling in Drosophila. Cell *120*, 111-122.

Kennedy, B.N., Stearns, G.W., Smyth, V.A., Ramamurthy, V., van Eeden, F., Ankoudinova, I., Raible, D., Hurley, J.B., and Brockerhoff, S.E. (2004). Zebrafish rx3 and mab21l2 are required during eye morphogenesis. Dev. Biol. *270*, 336-349.

Kiecker, C., and Lumsden, A. (2005). Compartments and their boundaries in vertebrate brain development. Nat. Rev. Neurosci. *6*, 553-564.

Kim, J.W., and Lemke, G. (2006). Hedgehog-regulated localization of Vax2 controls eye development. Genes Dev. *20*, 2833-2847.

Kim, H.S., Shin, J., Kim, S.H., Chun, H.S., Kim, J.D., Kim, Y.S., Kim, M.J., Rhee, M., Yeo, S.Y., and Huh, T.L. (2007). Eye field requires the function of Sfrp1 as a Wnt antagonist. Neurosci. Lett. *414*, 26-29.

Koike, C., Nishida, A., Ueno, S., Saito, H., Sanuki, R., Sato, S., Furukawa, A., Aizawa, S., Matsuo, I., Suzuki, N., et al. (2007). Functional roles of Otx2 transcription factor in postnatal mouse retinal development. Mol. Cell. Biol. *27*, 8318-8329.

Kondoh, H., Uchikawa, M., Yoda, H., Takeda, H., Furutani-Seiki, M., and Karlstrom, R.O. (2000). Zebrafish mutations in Gli-mediated hedgehog signaling lead to lens transdifferentiation from the adenohypophysis anlage. Mech. Dev. *96*, 165-174.

Koshiba-Takeuchi, K., Takeuchi, J.K., Matsumoto, K., Momose, T., Uno, K., Hoepker, V., Ogura, K., Takahashi, N., Nakamura, H., Yasuda, K., et al. (2000). Tbx5 and the retinotectum projection. Science *287*, 134-137.

Kumasaka, M., Sato, H., Sato, S., Yajima, I., and Yamamoto, H. (2004). Isolation and developmental expression of Mitf in Xenopus laevis. Dev. Dyn. *230*, 107-113.

Lad, E.M., Cheshier, S.H., and Kalani, M.Y. (2009). Wnt-signaling in retinal development and disease. Stem Cells Dev. *18*, 7-16.

Leconte, L., Lecoin, L., Martin, P., and Saule, S. (2004). Pax6 interacts with cVax and Tbx5 to establish the dorsoventral boundary of the developing eye. J. Biol. Chem. *279*, 47272-47277.

Liu, I.S., Chen, J.D., Ploder, L., Vidgen, D., van der Kooy, D., Kalnins, V.I., and McInnes, R.R. (1994). Developmental expression of a novel murine homeobox gene (Chx10): evidence for roles in determination of the neuroretina and inner nuclear layer. Neuron *13*, 377-393.

Liu, H., Mohamed, O., Dufort, D., and Wallace, V.A. (2003). Characterization of Wnt signaling components and activation of the Wnt canonical pathway in the murine retina. Dev. Dyn. *227*, 323-334.

Liu, H., Thurig, S., Mohamed, O., Dufort, D., and Wallace, V.A. (2006). Mapping canonical Wnt signaling in the developing and adult retina. Invest. Ophthalmol. Vis. Sci. *47*, 5088-5097.

Liu, H., Xu, S., Wang, Y., Mazerolle, C., Thurig, S., Coles, B.L., Ren, J.C., Taketo, M.M., van der Kooy, D., and Wallace, V.A. (2007). Ciliary margin transdifferentiation from neural retina is controlled by canonical Wnt signaling. Dev. Biol. *308*, 54-67.

Macdonald, R., Barth, K.A., Xu, Q., Holder, N., Mikkola, I., and Wilson, S.W. (1995). Midline signalling is required for Pax gene regulation and patterning of the eyes. Development *121*, 3267-3278.

Martinez-Morales, J.R., Signore, M., Acampora, D., Simeone, A., and Bovolenta, P. (2001). Otx genes are required for tissue specification in the developing eye. Development *128*, 2019-2030.

Martinez-Morales, J.R., Dolez, V., Rodrigo, I., Zaccarini, R., Leconte, L., Bovolenta, P., and Saule, S. (2003). OTX2 activates the molecular network underlying retina pigment epithelium differentiation. J. Biol. Chem. *278*, 21721-21731.

Martinez-Morales, J.R., Rodrigo, I., and Bovolenta, P. (2004). Eye development: a view from the retina pigmented epithelium. Bioessays *26*, 766-777.

Masai, I., Yamaguchi, M., Tonou-Fujimori, N., Komori, A., and Okamoto, H. (2005). The hedgehog-PKA pathway regulates two distinct steps of the differentiation of retinal ganglion cells: the cell-cycle exit of retinoblasts and their neuronal maturation. Development *132*, 1539-1553.

Mathers, P.H., Grinberg, A., Mahon, K.A., and Jamrich, M. (1997). The Rx homeobox gene is essential for vertebrate eye development. Nature *387*, 603-607.

Matsuo, T., Osumi-Yamashita, N., Noji, S., Ohuchi, H., Koyama, E., Myokai, F., Matsuo, N., Taniguchi, S., Doi, H., Iseki, S., et al. (1993). A mutation in the Pax-6 gene in rat small eye is associated with impaired migration of midbrain crest cells. Nat. Genet. *3*, 299-304.

Miller, D.T., Read, R., Rusconi, J., and Cagan, R.L. (2000). The Drosophila primo locus encodes two low-molecular-weight tyrosine phosphatases. Gene *243*, 1-9.

Mochii, M., Mazaki, Y., Mizuno, N., Hayashi, H., and Eguchi, G. (1998). Role of Mitf in differentiation and transdifferentiation of chicken pigmented epithelial cell. Dev. Biol. *193*, 47-62.

Morcillo, J., Martinez-Morales, J.R., Trousse, F., Fermin, Y., Sowden, J.C., and Bovolenta, P. (2006). Proper patterning of the optic fissure requires the sequential activity of BMP7 and SHH. Development *133*, 3179-3190.

Mui, S.H., Hindges, R., O'Leary, D.D., Lemke, G., and Bertuzzi, S. (2002). The homeodomain protein Vax2 patterns the dorsoventral and nasotemporal axes of the eye. Development *129*, 797-804.

Mui, S.H., Kim, J.W., Lemke, G., and Bertuzzi, S. (2005). Vax genes ventralize the embryonic eye. Genes Dev. *19*, 1249-1259.

Muller, F., Rohrer, H., and Vogel-Hopker, A. (2007). Bone morphogenetic proteins specify the retinal pigment epithelium in the chick embryo. Development *134*, 3483-3493.

Nguyen, M., and Arnheiter, H. (2000). Signaling and transcriptional regulation in early mammalian eye development: a link between FGF and MITF. Development *127*, 3581-3591.

Nornes, H.O., Dressler, G.R., Knapik, E.W., Deutsch, U., and Gruss, P. (1990). Spatially and temporally restricted expression of Pax2 during murine neurogenesis. Development *109*, 797-809.

Ogden, S.K., Fei, D.L., Schilling, N.S., Ahmed, Y.F., Hwa, J., and Robbins, D.J. (2008). G protein Galphai functions immediately downstream of Smoothened in Hedgehog signalling. Nature *456*, 967-970.

Ohkubo, Y., Chiang, C., and Rubenstein, J.L. (2002). Coordinate regulation and synergistic actions of BMP4, SHH and FGF8 in the rostral prosencephalon regulate morphogenesis of the telencephalic and optic vesicles. Neuroscience *111*, 1-17.

Oliver, G., Mailhos, A., Wehr, R., Copeland, N.G., Jenkins, N.A., and Gruss, P. (1995). Six3, a murine homologue of the sine oculis gene, demarcates the most anterior border of the developing neural plate and is expressed during eye development. Development *121*, 4045-4055.

Pan, D., and Rubin, G.M. (1995). cAMP-dependent protein kinase and hedgehog act antagonistically in regulating decapentaplegic transcription in Drosophila imaginal discs. Cell *80*, 543-552.

Perron, M., Boy, S., Amato, M.A., Viczian, A., Koebernick, K., Pieler, T., and Harris, W.A. (2003). A novel function for Hedgehog signalling in retinal pigment epithelium differentiation. Development *130*, 1565-1577.

Pittack, C., Grunwald, G.B., and Reh, T.A. (1997). Fibroblast growth factors are necessary for neural retina but not pigmented epithelium differentiation in chick embryos. Development *124*, 805-816.

Planque, N., Turque, N., Opdecamp, K., Bailly, M., Martin, P., and Saule, S. (1999). Expression of the microphthalmia-associated basic helix-loop-helix leucine zipper transcription factor Mi in avian neuroretina cells induces a pigmented phenotype. Cell Growth Differ. *10*, 525-536.

Porter, F.D., Drago, J., Xu, Y., Cheema, S.S., Wassif, C., Huang, S.P., Lee, E., Grinberg, A., Massalas, J.S., Bodine, D., Alt, F., and Westphal, H. (1997). Lhx2, a LIM homeobox gene, is required for eye, forebrain, and definitive erythrocyte development. Development *124*, 2935-2944.

Rowan, S., Chen, C.M., Young, T.L., Fisher, D.E., and Cepko, C.L. (2004). Transdifferentiation of the retina into pigmented cells in ocular retardation mice defines a new function of the homeodomain gene Chx10. Development *131*, 5139-5152.

Sakuta, H., Suzuki, R., Takahashi, H., Kato, A., Shintani, T., Iemura, S., Yamamoto, T.S., Ueno, N., and Noda, M. (2001). Ventroptin: a BMP-4 antagonist expressed in a double-gradient pattern in the retina. Science *293*, 111-115.

Schwarz, M., Cecconi, F., Bernier, G., Andrejewski, N., Kammandel, B., Wagner, M., and Gruss, P. (2000).. Spatial specification of mammalian eye territories by reciprocal transcriptional repression of Pax2 and Pax6. Development *127*, 4325-4334.

Sehgal, R., Karcavich, R., Carlson, S., and Belecky-Adams, T.L. (2008). Ectopic Pax2 expression in chick ventral optic cup phenocopies loss of Pax2 expression. Dev. Biol. *319*, 23-33.

Simeone, A., Acampora, D., Mallamaci, A., Stornaiuolo, A., D'Apice, M.R., Nigro, V., and Boncinelli, E. (1993). A vertebrate gene related to orthodenticle contains a homeodomain of the bicoid class and demarcates anterior neuroectoderm in the gastrulating mouse embryo. EMBO J. *12*, 2735-2747.

Stoykova, A., and Gruss, P. (1994). Roles of Pax-genes in developing and adult brain as suggested by expression patterns. J. Neurosci. *14*, 1395-1412.

Suzuki, A., Ozono, K., Kubota, T., Kondou, H., Tachikawa, K., and Michigami, T. (2008). PTH/cAMP/PKA signaling facilitates canonical Wnt signaling via inactivation of glycogen synthase kinase-3beta in osteoblastic Saos-2 cells. J. Cell Biochem. *104*, 304-317

Take-uchi, M., Clarke, J.D., and Wilson, S.W. (2003). Hedgehog signalling maintains the optic stalk-retinal interface through the regulation of Vax gene activity. Development *130*, 955-968.

Takeda, K., Yokoyama, S., Yasumoto, K., Saito, H., Udono, T., Takahashi, K., and Shibahara, S. (2003). OTX2 regulates expression of DOPAchrome tautomerase in human retinal pigment epithelium. Biochem. Biophys. Res. Commun. *300*, 908-914.

Tang, K., Xie, X., Park, J.I., Jamrich, M., Tsai, S., and Tsai, M.J. (2010). COUP-TFs regulate eye development by controlling factors essential for optic vesicle morphogenesis. Development *137*, 725-734.

Torres, M., Gomez-Pardo, E., and Gruss, P. (1996). Pax2 contributes to inner ear patterning and optic nerve trajectory. Development *122*, 3381-3391.

Toy, J., Yang, J.M., Leppert, G.S., and Sundin, O.H. (1998). The optx2 homeobox gene is expressed in early precursors of the eye and activates retina-specific genes. Proc. Natl. Acad. Sci. USA *95*, 10643-10648.

Van Raay, T.J., and Vetter, M.L. (2004). Wnt/frizzled signaling during vertebrate retinal development. Dev. Neurosci. *26*, 352-358.

Van Raay, T.J., Moore, K.B., Iordanova, I., Steele, M., Jamrich, M., Harris, W.A., and Vetter, M.L. (2005). Frizzled 5 signaling governs the neural potential of progenitors in the developing Xenopus retina. Neuron *46*, 23-36.

Vogel-Hopker, A., Momose, T., Rohrer, H., Yasuda, K., Ishihara, L., and Rapaport, D.H. (2000). Multiple functions of fibroblast growth factor-8 (FGF-8). in chick eye development. Mech. Dev. *94*, 25-36.

Waschek, J.A., Dicicco-Bloom, E., Nicot, A., and Lelievre, V. (2006). Hedgehog signaling: new targets for GPCRs coupled to cAMP and protein kinase A. Ann. N Y Acad. Sci. *1070*, 120-128

Westenskow, P., Piccolo, S., and Fuhrmann, S. (2009). Beta-catenin controls differentiation of the retinal pigment epithelium in the mouse optic cup by regulating Mitf and Otx2 expression. Development *136*, 2505-2510.

Westenskow, P.D., McKean, J.B., Kubo, F., Nakagawa, S., and Fuhrmann, S. (2010). Ectopic Mitf in the embryonic chick retina by co-transfection of beta-catenin and Otx2. Invest. Ophthalmol. Vis. Sci. *51*, 5328-5335.

Zhang, X.M., and Yang, X.J. (2001). Temporal and spatial effects of Sonic hedgehog signaling in chick eye morphogenesis. Dev. Biol. *233*, 271-290.

Zhao, S., and Overbeek, P.A. (1999). Tyrosinase-related protein 2 promoter targets transgene expression to ocular and neural crest-derived tissues. Dev. Biol. *216*, 154-163.

Zhao, S., Hung, F.C., Colvin, J.S., White, A., Dai, W., Lovicu, F.J., Ornitz, D.M., and Overbeek, P.A. (2001). Patterning the optic neuroepithelium by FGF signaling and Ras activation. Development *128*, 5051-5060.

Zhou, X., Hollemann, T., Pieler, T., and Gruss, P. (2000). Cloning and expression of xSix3, the Xenopus homologue of murine Six3. Mech. Dev. *91*, 327-330.

Zuber, M.E., Perron, M., Philpott, A., Bang, A., and Harris, W.A. (1999). Giant eyes in Xenopus laevis by overexpression of XOptx2. Cell *98*, 341-352.

Zuber, M.E., Gestri, G., Viczian, A.S., Barsacchi, G., and Harris, W.A. (2003). Specification of the vertebrate eye by a network of eye field transcription factors. Development *130*, 5155-5167.