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Review

Iris development in vertebrates; genetic and molecular considerations

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ABSTRACT

The iris plays a key role in visual function. It regulates the amount of light entering the eye and falling on the retina and also operates in focal adjustment of closer objects. The iris is involved in circulation of the aqueous humor and hence functions in regulation of intraocular pressure. Intriguingly, iris pigmented cells possess the ability to transdifferentiate into different ocular cell types of retinal pigmented epithelium, photoreceptors and lens cells. Thus, the iris is considered a potential source for cell-replacement therapies. During embryogenesis, the iris arises from both the optic cup and the periocular mesenchyme. Its interesting mode of development includes specification of the peripheral optic cup to a non-neuronal fate, migration of cells from the surrounding periocular mesenchyme and an atypical formation of smooth muscles from the neuroectoderm. This manner of development raises some interesting general topics concerning the early patterning of the neuroectoderm, the specification and differentiation of diverse cell types and the interactions between intrinsic and extrinsic factors in the process of organogenesis. In this review, we discuss iris anatomy and development, describe major pathologies of the iris and their molecular etiology and finally summarize the recent findings on genes and signaling pathways that are involved in iris development.

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1. Introduction

The iris, one of the most visually impressive and colorful organs of the human body, is aptly named after Iris, the goddess of the rainbow and the messenger for the Olympian gods. It is a thin, contractile disk that is located between the lens and the cornea and regulates the amount of light that passes through them and falls on the retina.

In the last century, iris development has been studied as a model for understanding the complex interactions between the neuroectoderm and the periocular mesenchyme, both are involved in iris morphogenesis. In the last 10 years, iris research was based on advanced technologies of molecular biology, transgenic animal studies and cell-fate tracing methods, which have unraveled some of the molecular players regulating these interactions.

2. The anatomy of the iris

The iris is made up of several different cell types: the most posterior layer, closest to the lens, is the iris pigmented

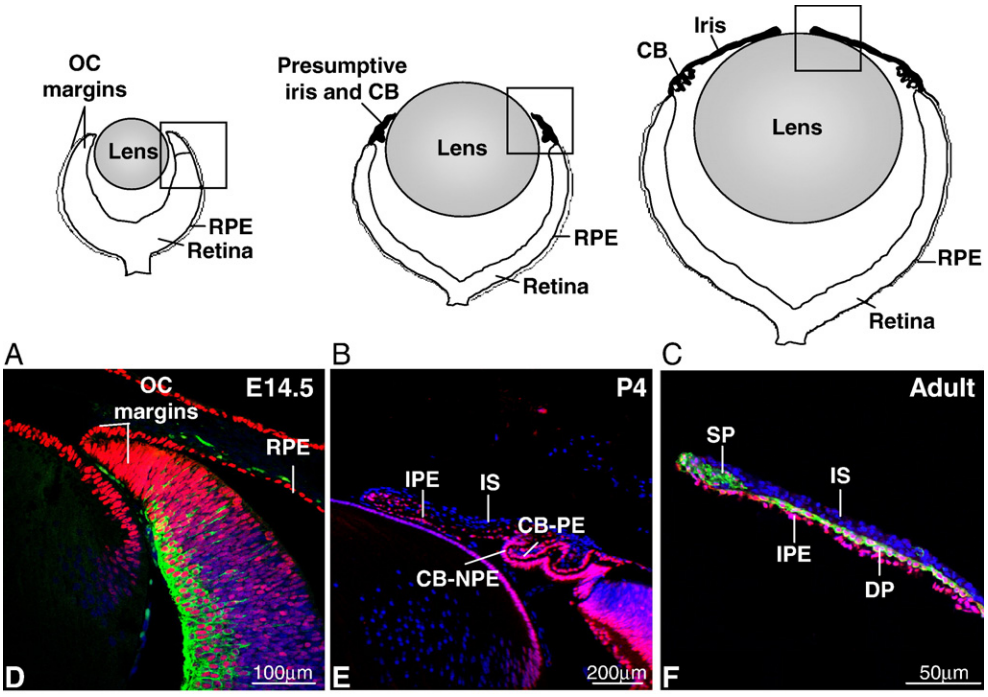

Fig. 1 – Iris morphogenesis. (A–C) are diagrams of the murine iris throughout its development. (A) shows the iris and CB progenitor pool located at the OC margins at around embryonic day (E) 14.5, (B) shows the developing structures of the iris and CB at postnatal (P) day 4 and (C) shows the adult forms. (D–F) Immunofluorescent images of the boxed regions in (A–C). (D) *Pax6* (red) is differentially upregulated in the OC periphery, which is distinct from the rest of the neuroretina by the absence of the neuronal marker *βIII-tubulin* (green). (E) *Pax6* (red) is strongly expressed in the epithelial layers of the developing iris and CB. (F) In the adult iris, *Pax6* (red) is expressed in the pigmented epithelium but not in the stroma. In addition, it is intensively expressed in the sphincter and dilator muscles that are labeled for smooth muscle actin (αSma, green). Abbreviations: CB-NPE, ciliary body non-pigmented epithelium; CB-PE, ciliary body pigmented epithelium; DP, dilator pupillae, IPE, iris pigmented epithelium; IS, iris stroma; OC, optic cup; RPE, retinal pigmented epithelium; SP, sphincter pupillae.

epithelium. Above these pigmented cells are the iridial muscles, and anteriorly lies the iris stroma. The iris root is attached to the ciliary body (CB) and to the corneal–sclera junction. This region is known as the iridocorneal angle. The CB shares a common embryonic origin with the iris but develops into a functionally different structure; here we discuss the CB in the context of iris development. Other aspects of CB development and physiology have been reviewed elsewhere (Beebe, 1986; Tamm et al., 1996).

3. Iris pigmented epithelium (IPE)

The IPE is composed of two cellular layers that are continuous with the pigmented and non-pigmented epithelial layers of the CB (Figs. 1E, F). Interestingly, the IPE cells are known for their outstanding plasticity: in the newt, for instance, a structurally and functionally normal lens can regenerate from the dorsal margin of the IPE (Wolff, 1895). This regeneration includes reorganization of the extracellular matrix, re-entry into the cell cycle and dedifferentiation of the dorsal IPE cells (reviewed in Tsonis et al., 2004). This fact should be viewed in light of the capability of Urodela to self-regenerate; however, recent studies have demonstrated that IPE cells of other vertebrates also posses the ability to transdifferentiate into completely different cell types. Dissociated chick IPE cells, for example, have been shown to dedifferentiate and form lentoids in culture (Kosaka et al., 1998) and in appropriate culture conditions these cells form neurospheres that may differentiate to retinal cell types. The latter finding suggests that chick IPE cells maintain progenitor/stem-cell properties and neurogenic potential, similar to the mammalian retinal stem cells of the pigmented CB (Ahmad et al., 2000; Sun et al., 2006; Tropepe et al., 2000). In mammals, iris-derived cells from rodents or primates were shown to transdifferentiate into cells expressing photoreceptor-specific markers only upon *Crx*/*Otx2* or *Crx*/*NeuroD* viral transduction (Akagi et al., 2004, 2005; Haruta et al., 2001). Importantly, these photoreceptor-like cells showed a rod-specific electrophysiological response to light and managed to integrate into embryonic retinal explants (Akagi et al., 2005). This remarkable plasticity is already being implemented in cell-replacement therapy (reviewed in Thumann, 2001). It has been shown that subretinal transplantation of IPE cells inhibits pathologic choroidal neovascularization in rat models of retinopathy and increases the survival of photoreceptor cells (Semkova et al., 2002). Other studies have shown that IPE cells can replace the retinal pigmented epithelium (RPE) in patients with age-related macular degeneration (Thumann et al., 2000).

4. The muscles of the iris

Two different sets of involuntary muscles, the sphincter pupillae and the dilator pupillae (Fig. 1F), act in opposition to cause miosis (constriction) or mydriasis (dilation) of the pupil in response to different levels of light or during focal adjustment. The sphincter muscle is under the control of the parasympathetic nervous system and is innervated by fibers from the oculomotor nerve, which originate in the Edinger–Westphal nucleus of the midbrain. The dilator muscle is controlled by the sympathetic system and is innervated by post-ganglionic neurons from the superior cervical ganglion (Smith, 2002).

5. Iris stroma

The stroma of the iris (Figs. 1E, F) consists of cells and connective fibers that create delicate meshes, in which the blood vessels and nerves are integrated (Smith, 2002).

Individual variations in iris color are typically attributed to the melanin content within the iris stroma and IPE. In albino eyes, pigment is entirely absent, in various shades of blue eyes the pigment cells are confined to the IPE, whereas in gray, brown and black eyes, pigment is also found in the cells of the stroma.

6. The iridocorneal angle

Within the angle between the cornea and the iris lies the region through which the aqueous humor constantly drains out of the eye. Aqueous humor is a transparent liquid that has a chemical composition which is slightly hypertonic to blood plasma with a much lower protein content (Kinsey, 1951). It provides nutrition for the lens and cornea and removes waste products of metabolism as both of these tissues are devoid of blood vessels. The aqueous humor is secreted continuously by the CB into the posterior chamber, flows through the pupil into the anterior chamber and drains out through the trabecular meshwork and Schlemm's canal, located in the iridocorneal angle. Some iris pathologies involve elevation of the intraocular pressure, the major risk factor for glaucoma (Gould et al., 2004). For instance, in the pigment-dispersion syndrome, pigment granules from the IPE are deposited onto various ocular structures, including the trabecular meshwork. As a result of these obstructions, approximately 50% of patients develop increased intraocular pressure and degeneration of the optic nerve, causing permanent loss of sight (Richter et al., 1986).

It should be noted that most congenital glaucomas develop as a consequence of maldevelopment of the drainage structures themselves (Barishak et al., 1978; Fine, 1964). Indeed, in many iridial pathologies, iris malformations are also accompanied by dysgenesis of the anterior segment, including the trabecular meshwork. Good examples of this are the nail-patella or the iridogoniodysgenesis syndromes, glaucomatous conditions that include a combined dysgenesis of the iris and iridocorneal angle (Berg, 1932; Weatherill and Hart, 1969). Thus, it is reasonable to speculate that the elevation of intraocular pressure in these patients is a result of malfunction of the angle structures rather than of the iris itself. However, it is possible that in some cases the iris is directly involved in pressure elevation, due to structural changes that lead to narrowing and closure of the iridocorneal angle. This can potentially affect the drainage ability of the trabecula, as happens in acute angle-closure glaucoma, in which the angle closes and covers the trabecular meshwork and Schlemm's canal.

7. Embryonic development of the murine iris

Formation of the complex structure of the iris is morphologically evident at mid-gestation and is completed in postnatal stages (reviewed in Cvekl and Tamm, 2004). This process is dependent on proper development of the embryonic structures from which the iris originates, the neuroectoderm and the periocular mesenchyme. Furthermore, signals from the adjacent lens are essential triggers for iris development.

The first morphological evidence of eye development in vertebrates is bilateral evaginations of the forebrain that lead to the formation of the optic vesicles (Chow and Lang, 2001). These vesicles move through a layer of mesenchyme until they reach the surface ectoderm; the interaction with the surface ectoderm leads to an invagination of the vesicles into cup-like structures termed the optic cups (OCs). The peripheral margins of the OC are the embryonic source of the iris and CB (Figs. 1B, C, E), while the rest of the cup forms the neuroretina and the overlaying RPE (Figs. 1A, D).

The murine iris and CB start to develop around mid-gestation. At this stage, the adjacent retinal progenitor cells divide extensively and differentiate into the six neuronal cell types and glia composing the mature neuroretina (reviewed in Marquardt and Gruss, 2002). This neuronal differentiation progresses from the central retina toward the periphery, until it reaches the margins of the OC, which contain the non-neuronal progenitors of the iris and CB. At around embryonic day (E) 15.5, these progenitors are well distinguished from the rest of the neuroretina as they do not express typical neuronal markers (e.g. *βIII-tubulin*, Fig. 1D) and most of them are mitotically inactive. At around E17, the margins of the OC extend, and small subsets of cells located near the presumptive pupil start to express smooth muscle-specific markers (Davis-Silberman et al., 2005; Link and Nishi, 1998). Iris muscle development is worthy of note as it is a very rare example of ectodermally, rather than mesodermally, derived muscles (Cvekl and Tamm, 2004; Imaizumi and Kuwabara, 1971; Szili, 1901). Interestingly, in the chick, iris muscles originate from neural crest cells (Creuzet et al., 2005; Johnston et al., 1979; Nakano and Nakamura, 1985). In view of this, it would be important to re-assess the origin of mammalian iris muscles using modern techniques that can trace the fate of genetically labeled cells (Lobe et al., 1999; Zinyk et al., 1998).

At around the time of birth, the epithelia of the iris and the CB differentiate: the outer margins of the OC, which are continuous with the RPE, give rise to the anterior pigmented layers, while the inner margins, which are continuous with the neuroretina, form the posterior pigmented layer of the iris and the non-pigmented layer of the CB (Figs. 1E, F) (Beebe, 1986; Thumann, 2001).

The stroma of the iris is composed of migratory cells that move into the eye from the periocular mesenchyme (reviewed in Cvekl and Tamm, 2004). These cells are first apparent at the angle between the future cornea and the extended margins of the OC. When the margins extend to form the iris, the mesenchymal cells proliferate and migrate along the iris epithelial layers and differentiate into the stroma. The iridocorneal structures are similarly produced from mesenchymal cells and develop mostly during postnatal life (reviewed in Cvekl and Tamm, 2004).

Much effort has been invested in tracing the embryonic origin of the periocular mesenchyme. Historically, this mesenchyme was thought to arise from the head mesoderm (Mann, 1928). However, fate-mapping experiments performed in birds, such as chick–quail chimeras, vital dye-labeling and neural crest-specific antibodies have all demonstrated the contribution of neural crest cells to the periocular mesenchyme (Johnston et al., 1979; Le Douarin, 1999; Noden, 1982). In mammals, this issue is still under debate. Gage and colleagues traced the movement of mesenchymal cells into the eye using a *floxed* reporter gene with either the *Wnt1-Cre*, which is specific to neural crest cells, or the *αGSU-Cre*, which is mesoderm-specific. Their analysis supported the hypothesis of mesodermal derivation of the iris stroma in mice (Gage et al., 2005). In contrast, Kanakubo and associates employed a different neural crest-specific *Cre* line, the *P0-Cre*, and reported that the iris stroma is composed of neural crest migratory cells (Kanakubo et al., 2006). The discrepancies between the above studies may reflect transgenic aberrations or different genetic backgrounds and need to be reconciled. Nevertheless, considering the two reports, it is most likely that the iris stroma in mice originates from both neural crest and mesoderm.

There is a long line of evidence emphasizing the role of the lens in the development of the anterior segment structures, including the iris (reviewed in Beebe, 1986). For example, an ablation of the lens, either mechanical or by lens-specific expression of the cytotoxic diphtheria *toxin A*, disrupted the development of the iris, CB and cornea (Beebe and Coats, 2000; Harrington et al., 1991). In addition, lens phenotypes that are caused by mutations in lens-specific genes (e.g. *FoxE3* Semina et al., 2001) or by lens-specific changes in the expression of ubiquitous genes (e.g. *Tgfβ* Flugel-Koch et al., 2002) are sometimes accompanied by anterior segment dysgenesis. Finally, it has been shown that chick lens could induce the expression of iris and CB specific markers in cultured embryonic neuroretina of mouse (Thut et al., 2001).

Another interesting evidence for this essential role of the lens in iris development came from the *Astyanax mexicanus*, a teleost with surface and cave forms. The cave-fish are blind: the lens vesicle is initially formed but later degenerates, and the cornea, iris and other optic tissues are absent or rudimentary. Intriguingly, transplantation of a surface-fish lens into the blind cave-fish eye stimulates the growth of the iris and cornea, suggesting that the lens secretes factors that are important for the development of the anterior segment (Yamamoto and Jeffery, 2000). However, the identity of these signaling molecules is currently unknown.

8. Molecular mechanisms regulating iris development

A review of iris development exposes two major events in its formation. The first is the central to peripheral patterning of the OC, leading to the production of an iris and CB progenitors in the OC margins. The second is the cellular migration that builds the iris stroma. Recent studies characterizing gene-expression patterns in the OC, as well as analyses of anterior

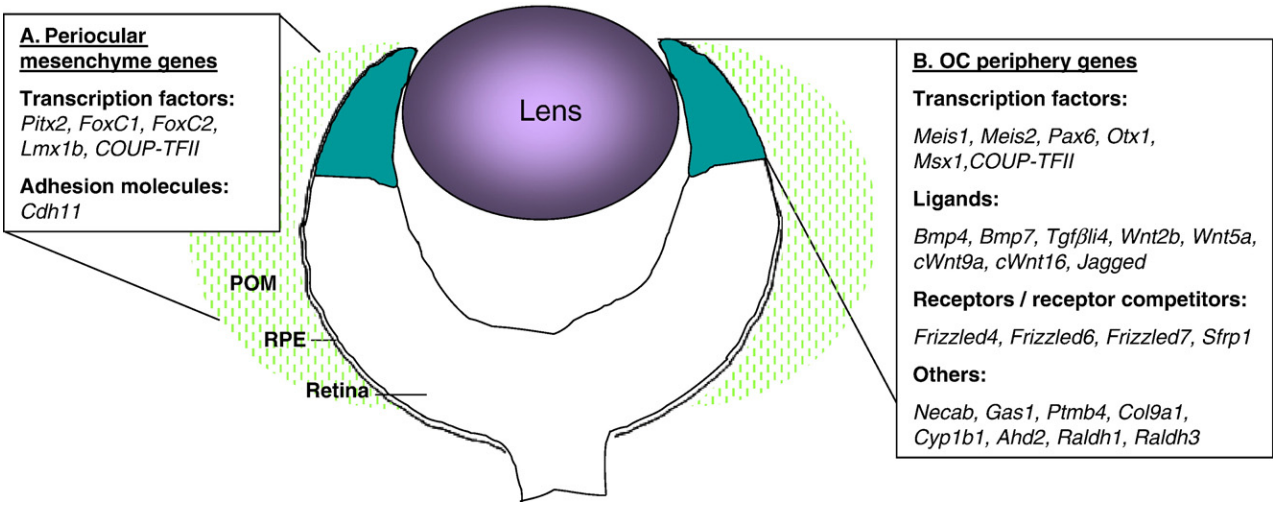

Fig. 2 – Genes involved in iris development. A summary of the iris-related gene expression at around mid-gestation. (A) includes genes that are expressed in the periocular mesenchyme and were suggested to be involved in the development of the iris. (B) includes genes that are either upregulated in the OC periphery or expressed exclusively in this region. The prefix "c" indicates that the expressions of these genes were examined only in chick. References for genes that do not appear in the text: Cyp1b1 (Stoilov et al., 2004), COUP-TFII and Ahd2 (McCaffery et al., 1999), Wnt5a, Frizzled4, Frizzled6, Frizzled7 and Sfrp1 (Liu et al., 2003a), Necab (Bernier et al., 2001), Ptmb4, Col9a1, Cdh11 (Thut et al., 2001), Lmx1b (Pressman et al., 2000). Abbreviations: OC, optic cup; POM, periocular mesenchyme; RPE, retinal pigmented epithelium.

segment abnormalities, have revealed new insights into the molecular mechanisms regulating these major events in relation to morphogenesis of the iris.

The compartmentalization of the OC into neuronal/central versus non-neuronal/peripheral progenitors occurs days before the genesis of the iris and CB. This patterning is first evident by the enhanced peripheral expression of transcription factors such as the homeobox genes *Meis1*, *Meis2*, *Pax6* and *Otx1* (Baumer et al., 2002; Martinez-Morales et al., 2001; Zhang et al., 2002) and the growth-arrest-specific protein *Gas1* (Lee et al., 2001). Soon after, additional factors are upregulated in the OC periphery. Among them are members of signaling pathways such as the Wnt ligand *Wnt2b* (Liu et al., 2003a), the Notch ligand *Jagged* (Bao and Cepko, 1997) and the TGFβ family members *Tgfβ1i4* and *Bmp4* (Thut et al., 2001; Zhao et al., 2002). This unique molecular composition of key developmental regulators distinguishes the peripheral OC from the adjacent neuroretina and RPE. These and additional genes that are upregulated in the OC periphery are listed in Fig. 2. It could be that the expression of some of these factors implies an undifferentiated state of the OC-margin cells rather than differentiation into non-neuronal progenitors. In addition, it should be considered that this progenitor pool is common to both iris and CB and that the exact point of the commitment to either one of the two fates has not yet been determined. Taking together, it is not clear which of these factors is truly iridogenic. Nonetheless, it is most probable that some of these genes, such as *Pax6*, *Bmp4* and *Wnt2b* are essential for the specification and morphogenesis of the iris, as suggested by human syndromes or functional studies.

Iris pathologies usually result from mutations in genes that are expressed either in the OC margins or in the periocular mesenchyme. In the following sections we discuss two such iris pathologies, whose molecular basis has been extensively studied.

9. Aniridia and PAX6/Pax6

Aniridia, which literally means "without iris", was first described by Barrata in 1818. It is characterized by complete or partial iris hypoplasia that is apparent at birth (Elsas et al., 1977). Frequently associated ocular abnormalities include optic nerve and macular hypoplasia, cataract, glaucoma, nystagmus, strabismus and corneal defects (Hittner et al., 1980; Shaw and Neel, 1960). Most cases of the aniridia are the result of heterozygous mutations in the transcription factor PAX6, a member of the PAX (Paired Box) gene family (Glaser et al., 1992; Ton et al., 1991; Walther and Gruss, 1991). To date, 309 *PAX6* mutations have been described, most of them resulting in aniridia (http://Pax6.hgu.mrc.ac.uk/).

Like other members of the PAX gene family, *PAX6/Pax6* is highly conserved among the Metazoan (reviewed in Gehring, 2001; Kozmik, 2005). In vertebrates, *Pax6* is essential for the development of the eyes, the olfactory system, the pancreas and the central nervous system (reviewed in Simpson and Price, 2002). Most notably, *Pax6* is considered a key regulator of eye development. It is invariably essential for eye formation in different species and has the intriguing capacity to induce ectopic eyes upon misexpression in flies and amphibian embryos (Chow et al., 1999; Grindley et al., 1995; Halder et al., 1995; Quiring et al., 1994). Interestingly, *Pax6* in the newt is required for the proper regeneration of the lens from the IPE (Madhavan et al., 2006).

Human aniridia, or the parallel phenotype in mice, designated "small eye" (*Sey*) (Baulmann et al., 2002; Hill et al., 1992;

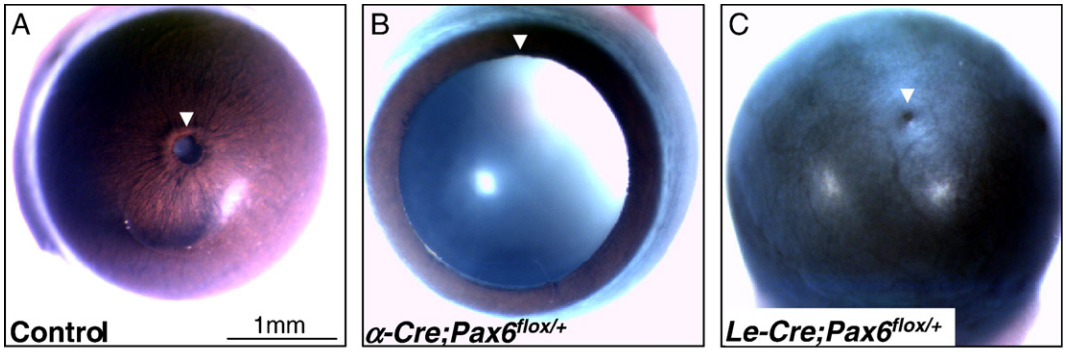

Fig. 3 – Iris phenotypes of Pax6 somatic mutants. (A–C) are images of enucleated eyes treated with the parasympathetic agonist pilocarpine that acts to constrict the pupil (marked in arrowhead). In comparison to the control eye (A), *Pax6$^{flox/+}$;α-Cre* eye (B) exhibits severe iris hypoplasia. In contrast, *Pax6$^{flox/+}$;Le-Cre* eye shows over-constricted pupil without gross iris malformations.

Hogan et al., 1986), are the manifestations of the intolerance of ocular tissues to a reduction in *Pax6* dosage. However, until recently it was not obvious that the iris itself displays this kind of sensitivity. In fact, the analysis of *Pax6$^{+/+}$↔Pax6$^{+/-}$* chimeric eyes demonstrated that the iris was asymptomatic even when 80% of its cells were *Pax6$^{+/-}$*. Therefore, it was suggested that iris hypoplasia in aniridia patients or in *Sey* mice may be secondary consequences of primary defects in the lens (Collinson et al., 2001). Nevertheless, there are some limitations to the use of chimera analysis in studying this complex tissue of the iris. First, the proportion of *Pax6$^{+/-}$* cells was evaluated only in the whole iris, with no reference to particular layers; thus, it is possible that the *Pax6$^{+/-}$* cells were concentrated in the iris stroma, which does not express *Pax6* (Figs. 1E, F). Secondly, a direct assessment of Pax6 dosage required by the lens for proper development of the iris was not feasible in this system due to the existence of negative selection against *Pax6$^{+/-}$* cells.

Direct examination of *Pax6* dosage requirements in the lens for iris morphogenesis was accomplished by using a conditional mutagenesis approach for somatic inactivation of single allele of *Pax6* in the lens (*Le-Cre;Pax6$^{flox/+}$*) or in the OC periphery (*α-Cre;Pax6$^{flox/+}$*) (Davis-Silberman et al., 2005). The lens phenotype of the *Le-Cre;Pax6$^{flox/+}$* mice mimicked the *Sey* mice lens phenotype but, surprisingly, iris hypoplasia was not observed (Fig. 3C). This demonstrated that the lens phenotype arising from *Pax6* dosage reduction is not sufficient to induce iris maldevelopment. Moreover, it suggests that the iris itself is sensitive to *Pax6* dosage. Indeed, the *α-Cre;Pax6$^{flox/+}$* mice display an eye morphology that is grossly normal, aside from an obvious iris hypoplasia (Fig. 3B). Together, the above observations led to the conclusion that *Pax6* dosage plays an important and cell-autonomous role in the developing iris.

Following the analysis of the *α-Cre;Pax6$^{flox/+}$* phenotype, several steps in iris development are now recognized as sensitive to reductions in *Pax6* dosage. This sensitivity is first apparent at the very early stages of iris development. High levels of Pax6 protein in the OC periphery are required for specification of the iris progenitor cells as in *α-Cre;Pax6$^{flox/+}$* embryos there are less non-neuronal progenitors (Davis-Silberman et al., 2005). Furthermore, the correct dosage of *Pax6* is essential for onset of differentiation of the iris muscles, and later on for proper development of the sphincter. Finally, a non-cell-autonomous effect of *Pax6* dosage reduction in the OC is evidenced by the maldevelopment of iris stroma in the *α-Cre;Pax6$^{flox/+}$* iris. The latter finding is in agreement with the observation that the correct dosage of *Pax6* is essential for the distribution and integration of neural crest cells into various ocular tissues (Kanakubo et al., 2006).

Pax6 involvement in cell migration to the iris seems to be non-cell-autonomous as its expression is mostly restricted to the iris epithelia and musculature (Figs. 1E, F). *Pax6* may regulate the expression of molecules required for the guidance or adhesion of the migratory cells to the developing iris. *Pax6* has indeed been implicated in the regulation of cell-adhesion molecules, including *N-cadherin* in the lens (van Raamsdonk and Tilghman, 2000) and *R-cadherin* in the brain (Stoykova et al., 1997). However, the expression of other cell-adhesion molecules, including *NCAM*, *L1*, *β-integrin* and *HNK1*, was recently reported to be unchanged in *Sey* mice (Kanakubo et al., 2006).

Interestingly, the expression of the retinoic acid-synthesizing enzyme *Raldh3* was found to be reduced in *Sey* rats (Suzuki et al., 2000). Retinoic acid is secreted from the retina, RPE and cornea and appears to function in a paracrine manner on the morphogenesis of the periocular mesenchyme-derived structures (Matt et al., 2005). *Raldh1/Raldh3* are upregulated in the OC periphery (Fig. 2 and Matt et al., 2005) and the double-mutant mice (*Raldh1$^{-/-}$;Raldh3$^{-/-}$*) display several malformations of the anterior segment structures, including the iris stroma. The association between *Pax6* and *Raldh3* suggests that abnormal retinoic acid signaling might explain at least part of the iris defect observed in *α-Cre;Pax6$^{flox/+}$* mice.

10. Axenfeld–Rieger syndrome, PITX2/Pitx2 and FOXC1/FoxC1

As the iris stroma consists of migratory cells, hypoplasia of the iris is a rather frequent feature of genetic disorders that involve cell-migration failure, such as in Axenfeld–Rieger syndrome (ARS).

ARS is a genetically heterogeneous, autosomal dominant disorder that is characterized by anterior segment defects, glaucoma and other extraocular anomalies (Rieger, 1935; Shields, 1983). Iris malformation includes stromal hypoplasia, distorted or displaced pupils and extra holes in the iris, symptoms that are referred to as Rieger anomaly. When iris strands bridge the iridocorneal angle to the trabecular meshwork, patients are considered to have Axenfeld anomaly (Axenfeld, 1920). Shields proposed that an arrest in the development of neural crest derived-tissues in the anterior segment causes the ocular features of ARS. This leads to retention of primordial endothelial tissue on the iris and across the anterior chamber angle, which produces the iridic changes (Shields, 1983).

Mutations in two main genes have been described in ARS: in the bicoid-like homeobox gene *PITX2* (Semina et al., 1996) and in the forkhead/winged helix transcription factor *FOXC1* (Mears et al., 1998). In addition, there has been one report of a complete form of ARS resulting from a small deletion on 11p13, where the *PAX6* gene resides (Riise et al., 2001). However, as the expression pattern of *PAX6* cannot explain the extraocular symptoms of ARS, it is possible that other putative genes, linked to the same region, were also involved. Furthermore, as 40% of ARS patients were not diagnosed with mutations in *PITX2*, *FOXC1* or *PAX6*, the potential for discovering new ARS genes has not yet been fulfilled (reviewed in Hjalt and Semina, 2005).

PITX2/Pitx2 is a member of the *PITX* homeobox gene family that is involved in the determination of left–right asymmetry. It plays a pivotal role in the normal development of the heart, lung, brain, eyes and craniofacial structures in vertebrates (Gage and Camper, 1997; Gage et al., 1999; Kitamura et al., 1999; Lin et al., 1999; Logan et al., 1998; Lu et al., 1999; Yoshioka et al., 1998). *PITX2/Pitx2* encodes three isoforms in mice and four in humans (*PITX2a-d* (Cox et al., 2002)). These isoforms partially overlap in their expression patterns, and at least few of *Pitx2* targets are regulated by distinct isoforms (Cox et al., 2002; Kitamura et al., 1999; Yu et al., 2001).

Heterozygous mutations in human *PITX2* result in ARS (Semina et al., 1996). The ocular phenotype of these patients correlates with the normal expression of *Pitx2* in the murine developing eye: it is first expressed in the periocular mesenchyme (Fig. 2) and then in the mesenchymal cells that migrate to the corneal and iris stroma and to the iridocorneal angle (Hjalt et al., 2000; Lu et al., 1999; Semina et al., 1996). This expression pattern implies that *Pitx2* is involved in the migration of periocular mesenchyme cells into the eye. Indeed, both cell-culture and *in vivo* studies have provided evidence for this view. For example, expression of *Pitx2a* in HeLa cells induces actin-myosin reorganization, which leads to increased cell spreading, suppression of cell migration and strengthening of cell–cell adhesion (Wei and Adelstein, 2002). Fate-mapping studies with a *Pitx2-Cre* recombinase knock-in allele revealed that *Pitx2* functions to regulate local cell movement during development of the heart and craniofacial structures (Liu et al., 2002, 2003b).

Interestingly, *Pitx2* is expressed in both the neural crest- and the mesoderm-derived precursors of the periocular mesenchyme. To gain an insight into *Pitx2*'s role in the neural crest-derived mesenchyme, Evans and Gage generated neural crest-specific *Pitx2* knockout mice (*Pitx2-ncko* Evans and Gage, 2005). The ocular phenotype of the *Pitx2-ncko* mice showed a variety of symptoms in structures derived from the neural crest, including complete absence of the sclera and the corneal endothelium and stroma. Interestingly, optic stalk development was abrogated suggesting that *Pitx2* regulates the expression of factors within the periocular mesenchyme that are required for the development of the adjacent optic nerve (Evans and Gage, 2005). The severe developmental ocular abnormality of the *Pitx2-ncko* mutants has thus precluded the analysis of the iris phenotype resulting from neural crest inactivation of *Pitx2*. The use of temporally controlled somatic mutagenesis (Hayashi and McMahon, 2002) for the functional study of *Pitx2* is expected to reveal its roles in iris development.

FOXC1/FoxC1 is a forkhead/winged helix type of transcription factor. It is expressed in many embryonic tissues, including prechondrogenic mesenchyme, periocular mesenchyme, meninges, endothelial cells and kidney. Homozygous null mice die at birth exhibiting phenotype of hydrocephalus, multiple skeletal abnormalities and eye defects (Kume et al., 1998). Heterozygous mutations in *FOXC1* result in ARS; mutations in the murine homologous gene lead to similar ocular abnormalities, including iris hypoplasia with severely eccentric pupils, corneal opacities, small or absent Schlemm's canal and aberrantly developed trabecular meshwork (Kidson et al., 1999; Smith et al., 2000). Interestingly, normal development of the murine anterior segment requires an additional member of the forkhead/winged helix family together with *FoxC1* as the ocular phenotype of $FoxC1^{+/-}$;$FoxC2^{+/-}$ mice is broader than that of either single heterozygote. The role of *FOXC2* in human eye development is currently unknown (Smith et al., 2000).

Common to all of the ocular structures affected in *FoxC1* and *FoxC2* heterozygous mice is their derivation from the periocular mesenchyme, where these two genes are mainly expressed (Fig. 2). Thus, it is reasonable to speculate that this phenotype results from a primary defect in the migration or differentiation of the mesenchymal cells. In particular, it may be the outcome of aberrant extracellular matrix synthesis or organization as mutant eyes show abnormally thin collagen bundles (Smith et al., 2000).

Co-expression of *FoxC1* and *Pitx2* in the periocular mesenchyme, together with the similar phenotypes of their mutants, implies a functional interaction between the two transcription factors. Indeed, *Pitx2a* and *FoxC1* were recently found to interact in common protein complex on the chromatin (Berry et al., 2006). This type of protein interaction may explain the strict dosage sensitivity for both transcription factors as cell-culture studies demonstrate that changes in the stoichiometry of these two factors lead to an alteration in their transcriptional activity. The next challenge will be to identify the direct targets of *Pitx2*, *FoxC1* and *FoxC2*, which mediates their roles in the formation of the anterior segment of the eye. Interestingly, the expressions of *FoxC1* and *Pitx2* appeared to be reduced in *Raldh1/Raldh3* null mice. Possibly, this finding could potentially correlate *Pitx2*, *FoxC1* and *Pax6* since *Raldh3* expression was found to be reduced in *Sey rats*, as noted above (Suzuki et al., 2000).

11. Signal transduction pathways involved in iris development

In general, organogenesis is regulated by a relatively small number of evolutionarily conserved signaling pathways. Here we describe components of two signaling pathways, BMP and Wnt, which have been recognized as participants in the development of the iris and CB.

12. The bone morphogenic proteins (BMPs)

The BMPs are growth factors that belong to the TGFβ superfamily. These secreted factors have been shown to be essential for cell differentiation and morphogenesis of numerous tissues during embryonic development (Zhao, 2003).

In the eye, *Bmp4* and *Bmp7* are first expressed in the lens and optic vesicle and then in the dorsal OC (Zhao et al., 2002). At around mid-gestation, the expression of *Bmp4* and *Bmp7* and the putative downstream target, the transcription factor *Msx1*, is restricted to the OC margins, and later to the developing CB and the adult iris (Jensen, 2005; Monaghan et al., 1991; Zhao et al., 2002). *Bmp* receptors, including all six type-I receptors, *ActR-II* and the alternatively spliced long version of *BmpR-II* are expressed throughout the eye (Obata et al., 1999). Knockout studies have shown that *Bmp4* and *Bmp7* are essential for early morphogenesis of the eye, but due to the lethality of these mice, information regarding the later roles of *Bmp4* could not be provided (Furuta and Hogan, 1998; Wawersik et al., 2005). To circumvent this, Zhao and colleagues generated mice expressing the BMP inhibitor *Noggin* in an ectopic manner, suppressing both *Bmp4* and *Bmp7* expression in the developing CB and iris. This inhibition of BMP signaling resulted in a complete loss of the CB. The iris of these transgene mice was also malformed, with thinning of the epithelial layers and reduction in size of the smooth muscles (Zhao et al., 2002).

Direct evidence of *Bmp4* role in iris development came from the phenotype of the $Bmp4^{+/-}$ mice (Chang et al., 2001). As in the cases of *Pax6* and *FoxC1/2* heterozygous mice, the reduction in *Bmp4* levels resulted in hypoplasia of the iris. The iris phenotype of the $Bmp4^{+/-}$ mice consisted of large, irregularly shaped and often eccentric pupils. Abnormalities were also observed in the iridocorneal angle, including a small or absent Schlemm's canal and a hypoplastic trabecular meshwork. Subsequently, these defects lead to elevated intraocular pressure, which implicate *BMP4* as a candidate contributor to congenital ocular syndromes in humans such as ARS and other glaucomas.

The absence of detectable *Bmp4* expression in the drainage structures suggests that *Bmp4* produced by the CB or iris acts as a growth and differentiation factor during angle development. It should be noted that, in contrast to mice, RT-PCR experiments clearly showed that, in humans, *BMP4* is expressed in the trabecular meshwork (Wordinger et al., 2002). This implies that *BMP4*'s mode of action is slightly different in human and might involve some aspects of autocrine activity, at least in the trabecular meshwork.

13. Wnt pathway

The Wnt signaling pathway regulates a wide range of developmental processes such as proliferation, cell migration, axon guidance and cell-fate determination (Clevers, 2006). Activity of the canonical Wnt signaling results in the stabilization of the cytoplasmic β-catenin which is then associated with the Lef/TCF-type of transcription factors to regulate the expression of downstream targets. The Wnt pathway has been implicated in retinal development with multiple roles (Kubo et al., 2003, 2005; reviewed in Van Raay and Vetter, 2004).

Recent studies have demonstrated a novel role for the canonical Wnt pathway in the specification of retinal progenitors to the non-neuronal fates of the anterior structures, the iris and CB. This proposed function was based on several observations: firstly, some of the Wnt pathway components are highly expressed in the developing iris and CB (Fig. 2) and in the adult structures (Jasoni et al., 1999; Kubo et al., 2003; Liu et al., 2003a). Second, transgenic reporters designed for the detection of canonical Wnt signaling demonstrate activity of this pathway in the iris and CB progenitors in both chick and mouse embryos (Cho and Cepko, 2006; Liu et al., 2003a). Finally, direct evidence for the role of Wnt signaling in the formation of the anterior structures was obtained by recent loss- and gain-of-function studies in avian embryos (Cho and Cepko, 2006; Kubo et al., 2003). Forced expression of *Wnt2b* or constitutively active *β-catenin* in the retina caused a dramatic change in the specification of proximal retinal progenitor cells that adopt non-neuronal fate. Coinciding with these findings, blockage of Wnt signaling by expressing dominant-negative *Lef1* resulted in an inhibition of the expression of peripheral markers such as *Collagen-9* and *Bmp7* and consequently led to an iris hypoplasia with a severe reduction in muscle size (Cho and Cepko, 2006). Together, these findings suggest an autonomous function for the Wnt pathway in the specification of the iris and CB progenitors. It should be noted that, although Wnt signaling is fundamental for the development of the iris and CB, it is probably not sufficient to execute their full developmental program as the expression of *Pax6* was downregulated following forced expression of *β-catenin* (Cho and Cepko, 2006).

Co-expression of BMP and Wnt family members in the OC periphery suggests a possible interaction of these two pathways in the development of the iris and CB. The convergence of the two pathways could come about via the regulation of common target genes such as the *Msx1* and *Msx2*. These transcription factors that have been shown to be downstream of both pathways (Foerst-Potts et al., 1997; Furuta and Hogan, 1998; Hussein et al., 2003; Willert et al., 2002; Zhao et al., 2002) are expressed in the OC margins and may participate in the initial acquisition of the non-neuronal fate of the progenitor cells.

Furthermore, it should be considered that Wnts and BMPs may have paracrine effect on the periocular mesenchyme and the morphogenesis of the derivative structures. Supporting this notion are the findings that *Pitx2* transcription is modulated by the two pathways in other developmental systems (Kioussi et al., 2002; St Amand et al., 2000).

14. Future prospects

Work done in the last few years has revealed genes that are involved in the specification of non-neuronal structures originating from the OC periphery. One of the emerging questions is what the convergence points of the different signaling pathways are and how these pathways interact with the tissue-specific genes. Another intriguing question concerns the cross-talk between the OC periphery and the adjacent periocular mesenchyme and how each influences the other's morphogenesis. The third question concerns the partitioning of the non-neuronal progenitors of the OC periphery to CB and iris fates. The latter issue is also interesting in terms of the regeneration properties of the adult CB and iris epithelia. As already mentioned, the mammalian CB pigmented cells are considered retinal stem cells as they form neurospheres and can give rise to multiple retinal cell types (Ahmad et al., 2000; Tropepe et al., 2000). The adjacent IPE is recognized to possess the capacity to transdifferentiate to lens and RPE cell types (Haruta et al., 2001; Tropepe et al., 2000) but its neurogenic potential has not yet been demonstrated in mammals. Learning the possible differences between these two adjacent epithelia may shed light on the mechanisms that maintain regeneration and stem-cell properties in the adult.

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