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Anterior segment development relevant to glaucoma

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ABSTRACT Development of the ocular anterior segment involves a series of inductive interactions between neural ectoderm, surface ectoderm and periocular mesenchyme. The timing of these events is well established but less is known about the molecular mechanisms involved. Various genes that participate in these processes have been identified. As the roles of more genes are determined, developmental pathways and networks will emerge. Here, we focus on recent advances made using mouse models. We summarize key morphological events in formation of anterior chamber structures, including the aqueous humor drainage structures that are involved in intraocular pressure (IOP) regulation and glaucoma. We discuss the developmental roles of genes that associate with abnormal anterior segment development and elevated IOP or glaucoma (including *Bmp4, Cyp1b1, Foxc1, Foxc2, Pitx2, Lmx1b* and *Tyr*) and how some of these genes may fit into developmental networks.

KEY WORDS: *ocular development, glaucoma, trabecular meshwork, dysgenesis*

Anterior segment dysgenesis and glaucoma

Ocular anterior segment dysgenesis (ASD) is a genetically heterogeneous group of developmental disorders (Gould and John 2002). Studying ocular development provides insight about how mis-regulated developmental processes lead to ASD. Various terms exist for human ASD including Axenfeld's anomaly, Rieger's anomaly, Peters' anomaly, aniridia, iris hypoplasia and iridogoniodysgenesis. However, clinical findings overlap within families and mutations in the same gene can cause a range of phenotypes. Considering human and mouse data, it is clear that ASD is a complex, continuous spectrum of disorders (Waring *et al.,* 1975, Shields *et al.,* 1985, Alward 2000, Gould and John 2002).

Glaucoma is a leading cause of blindness estimated to affect 70 million people (Thylefors and Negrel 1994, Quigley 1996). A major risk factor for glaucoma is elevated IOP. Patients with ASD often have malformations of the tissues responsible for IOP regulation and aqueous humor drainage. They frequently develop elevated IOP, putting them at risk for developing glaucoma. Developmental glaucomas, secondary to morphological malformations of the anterior segment are relatively rare forms of glaucoma. Importantly however, developmental abnormalities of the ocular drainage structures are not always clinically detectable and abnormal development may affect the metabolism and function of the drainage structures without disturbing morphology. Therefore, mutations in developmental genes may contribute to glaucoma more frequently than accepted, possibly contributing to common forms such as primary open angle glaucoma, which do not present with obvious developmental abnormalities.

Morphogenesis

Tissue derivations

The anterior segment consists of the cornea, iris, lens, ciliary body and ocular drainage structures (trabecular meshwork and Schlemm's canal). The drainage structures are located at the iridocorneal angle where the iris and cornea meet. This region is also known as the corneoscleral transition zone as the ocular wall transitions from cornea to sclera at this location. The anterior segment also includes the anterior chamber (space between the iris and cornea) and posterior chamber (space between iris and lens). These structures are formed by coordination of events involving induction and differentiation of three main tissue types: surface ectoderm, neural ectoderm and periocular mesenchyme (Fig. 1). Much of what we know about these events comes from

Abbreviations used in this paper: ASD, anterior segment dysgenesis; BMP, bone morphogenetic protein; ECM, extracellular matrix; FOX, forkhead box; GDF, growth and diferentiation factor; IOP, intraocular pressure; TH, tyrosine hydroxylase; Tyr, tyrosinase gene.

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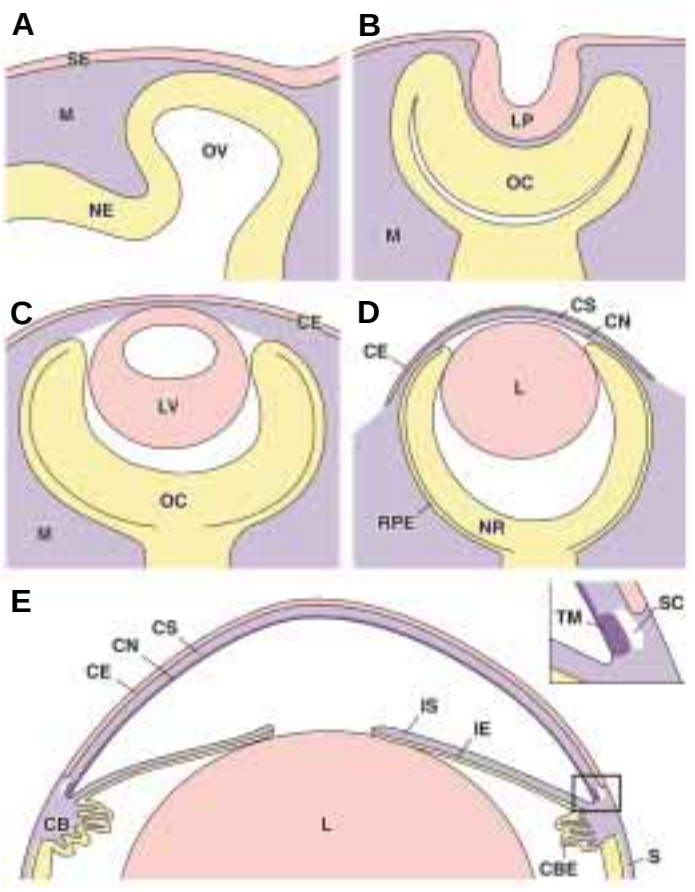

Fig. 1. Tissue derivations of developing ocular structures. *A color scheme is used to represent tissue derivations. For clarity, periocular mesenchyme derived tissues are represented by two shades of purple.* **(A)** *Neural ectoderm (NE, yellow) of the emerging optic vesicle (OV) moves through periocular mesenchyme (M, purple) until it reaches the surface ectoderm (SE, pink).* **(B)** *The surface ectoderm thickens and invaginates to form the lens pit (LP) as the optic vesicle forms the optic cup (OC).* **(C)** *The lens vesicle (LV) detaches from the surface ectoderm. The surface ectoderm becomes the epithelial layer of the future cornea (CE). Periocular mesenchyme migrates between the surface ectoderm of the corneal epithelium and the lens vesicle.* **(D)** *Lens fibers fill the lens vesicle. Mesenchyme that has migrated between the corneal epithelium and the lens forms corneal stroma (CS) and corneal endothelium (CN). Neural ectoderm in the inner layer of the optic cup will form neural retina (NR) and the outer layer will form retinal pigmented epithelium (RPE).* **(E)** *In the mature eye, the anterior rim of the optic cup has moved centrally and forms the epithelia of the iris (IE) and ciliary body (CBE). Iris stroma (IS), stroma and muscle of the ciliary body (CB), trabecular meshwork (TM) and Schlemm's canal (SC) are formed from periocular mesenchyme. Condensed periocular mesenchyme forms the sclera (S) and surrounds the posterior of the eye.*

experiments with model organisms including frog, chick, rat and mouse (Jacobson and Sater 1988, Furuta and Hogan 1998, Beebe and Coats 2000, Fuhrmann *et al.,* 2000). In this review, we concentrate on recent advances using the mouse.

Through the course of ocular development, the surface ectoderm will form the corneal epithelium and lens while the neural ectoderm derivatives will form the retina and epithelia of both the iris and ciliary body (Kaufman 1995). The corneal stroma, corneal endothelium, sclera, iris stroma, ciliary muscle, ciliary stroma and trabecular meshwork are all derived from periocular mesenchyme. Schlemm's canal forms by remodeling of vasculature in the corneoscleral transition zone (Hamanaka *et al.,* 1992, Smith *et al.,* 2001) and is likely derived from periocular mesenchyme. Thus the periocular mesenchyme, which consists of neural crest and cranial paraxial mesoderm derived cells, contributes extensively to the ocular anterior segment (Noden 1975, Johnston *et al.,* 1979, Trainor and Tam 1995). Defects of periocular mesenchyme including patterning, migration or differentiation may contribute to ASD and glaucoma (Kupfer and Kaiser-Kupfer 1978, Kupfer and Kaiser-Kupfer 1979, Kaiser-Kupfer 1989, Tripathi and Tripathi 1989).

Prenatal development

Before focusing on anterior segment development, we will briefly discuss early events of specification and morphogenesis of the optic vesicle and optic cup (for more detailed review see Freund *et al.,* 1996; Chow and Lang 2001). Specification of the eye field in neural ectoderm and lens induction in surface ectoderm are primary events in ocular development (Zuber *et al.,* 2003). Mutations in genes involved in these early events often result in anophthalmia (for examples, see Hogan *et al.,* 1986; Hill *et al.,* 1991; Mathers *et al.,* 1997; Dattani *et al.,* 1998). In the mouse, the eye field is initially specified as a dorsal strip on the anterior part of the forebrain around embryonic day (E) 8.5 (for review see Chow and Lang 2001). Soon after, the field is restricted to a lateral position where the optic pits form on the inner surface of the cephalic neural folds (Pei and Rhodin 1970, Kaufman 1995). By E9.0, the neural folds oppose each other and the optic pits deepen becoming the optic vesicles. Each optic vesicle moves through a layer of mesenchyme until it reaches the surface ectoderm at about E9.5 (Pei and Rhodin 1970). The interaction of the optic vesicle with surface ectoderm induces formation of the lens placode in the surface ectoderm and the retinal placode in the neural ectoderm. By E10.5, cellular proliferation in the lens placode contributes to formation of the lens pit and then the lens vesicle. Invagination of the lens pit coincides with folding of the neural ectoderm, which forms the optic cup (Fig. 1). The lens vesicle remains connected to surface ectoderm by the lens stalk until E11.0 when it detaches (Pei and Rhodin 1970, Kaufman 1995, Grimm *et al.,* 1998). The optic cup gives rise to the future neural retina and retinal pigment epithelium. The optic cup also contributes to the anterior segment, as the anterior rim of the optic cup will become the epithelium of the iris and ciliary body.

By E12.5, the developing corneal epithelium consists of one to two cell layers of surface ectoderm that rest on a basal lamina (Fig. 2A). Over the next two days, migration of periocular mesenchyme between the corneal epithelium and anterior lens epithelium establishes the presumptive corneal stroma and presumptive corneal endothelium (Fig. 2 A,B) (Pei and Rhodin 1970, Haustein 1983). After migration, the mesenchyme of the posterior cornea begins differentiating to form corneal endothelial cells and keratocytes (Pei and Rhodin 1970, Haustein 1983).

By E14.5-15.5, the corneal endothelium is present and the anterior chamber first appears as a small space (Fig. 2B). Development of a functional corneal endothelium is proposed to be a prerequisite for formation of the anterior chamber (Kidson *et al.,* 1999, Reneker *et al.,* 2000). The anterior rim of the optic cup, which will form the iris and ciliary epithelia, is pigmented and

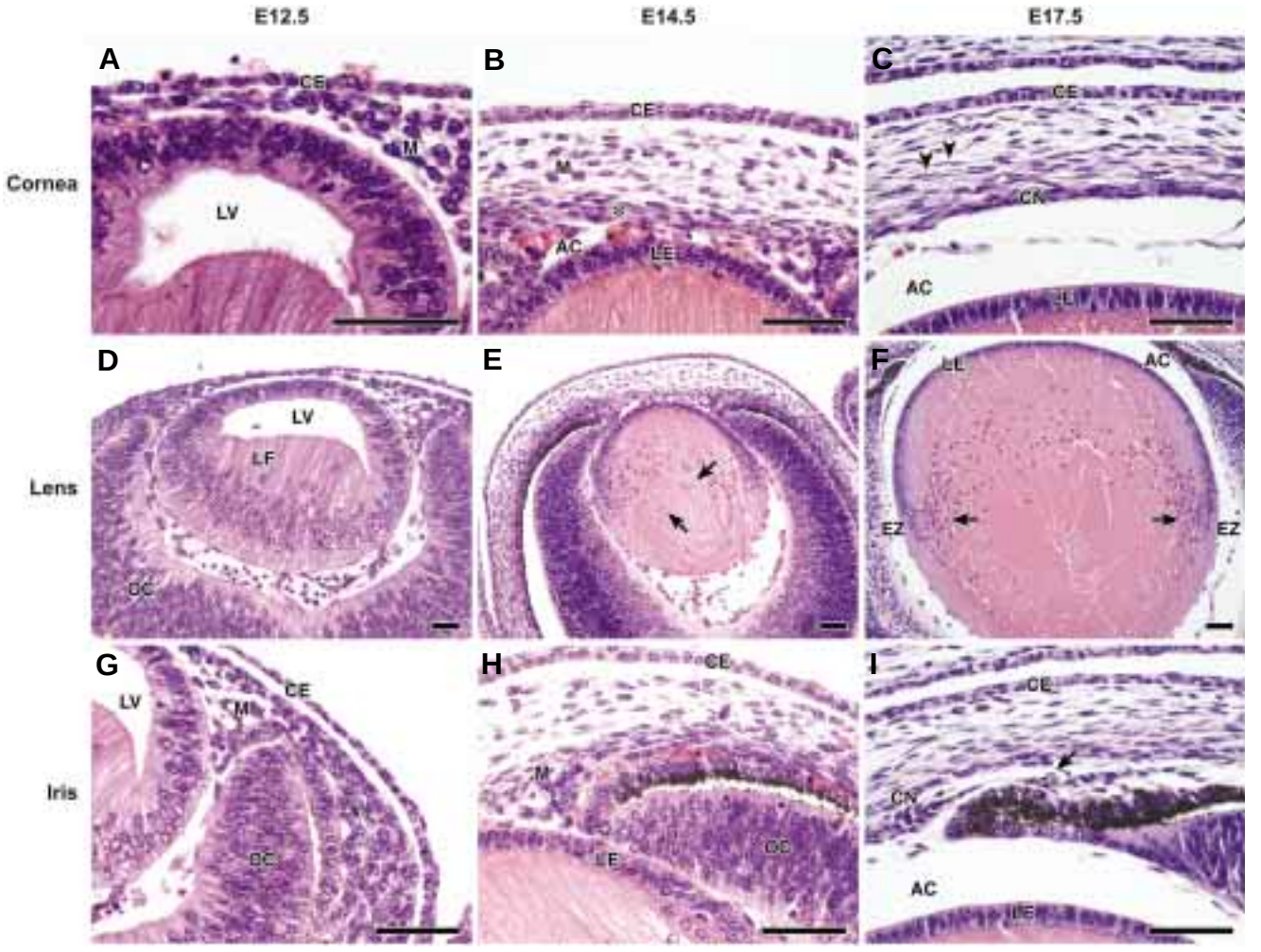

Fig. 2. Prenatal development of cornea, lens and iris. *All analyses were performed using C57BL/6J mice. Hemotoxylin and eosin stained sections of eyes at indicated ages. (A-C) Cornea.* **(A)** *At E12.5, the corneal epithelium (CE) is 1-2 cells thick. Mesenchyme (M) has started to migrate between the corneal epithelium and the lens vesicle (LV).* **(B)** *At E14.5, the corneal epithelium is two cell layers resting on a basal lamina. A thick presumptive corneal stroma has formed from migrating mesenchyme. The posterior mesenchyme has started to condense (*) and form corneal endothelium. The anterior chamber (AC) first appears as a small space above the lens epithelium (LE).* **(C)** *At E17.5, differentiating keratocytes become flattened (arrowheads), especially near the endothelium (CN). Extracellular matrix and keratocytes give the stroma a lamellar appearance. The anterior chamber is well established. (D-F) Lens.* **(D)** *At E12.5, the cavity of the lens vesicle (LV) is partially filled with elongating primary lens fibers (LF).* **(E)** *By E14.5, the primary lens fibers have filled the lens vesicle. Nuclei of the lens fibers are near the center of the lens and become translucent (arrows).* **(F)** *At E17.5, the emergence of nuclei of secondary lens fibers (arrows) can be seen near the equatorial zone (EZ). (G-I) Iris.* **(G)** *At E12.5, the periocular mesenchyme is migrating into the eye at the rim of the optic cup (OC). It is not possible to distinguish the mesenchyme of the presumptive cornea from that of the iris.* **(H)** *By E14.5, the anterior rim of the optic cup is extending anteriorly and the mesenchyme (M) adjacent to the optic cup is condensed.* **(I)** *By E17.5, the presumptive iris stroma can be identified and its mesenchyme synthesizes pigment (arrow). The developing iris stroma is bordered anteriorly by the corneal endothelium (CN) and posteriorly by the anterior optic cup that will become the iris pigment epithelium. Panel D is reproduced with permission from Smith* et al., *(Smith* et al., *2001). All other panels are previously unpublished data. Scale bars represent 50 microns.*

begins to extend anteriorly and centrally (compare Fig. 2 G,H). This provides a base for the periocular mesenchyme that will form the iris stroma and ciliary body stroma. Primary lens fibers fill the lens vesicle and their nuclei arrange near the center of the lens (Fig. 2E). By E16.5, the anterior chamber is formed and the presumptive iris stroma is no longer apposed to the cornea (Fig. 2I). The number of keratocytes in the corneal stroma steadily increases in number until it plateaus at E16.5. By E17.5, the corneal stroma takes on a lamellar arrangement (Fig. 2C). Secondary lens fibers are produced at the equatorial zone of the lens (Fig. 2F).

Postnatal development

Although many studies have detailed prenatal ocular development, few reports document postnatal development. Significant development of the anterior chamber structures occurs in the postnatal period (Smith *et al.,* 2001, Baulmann *et al.,* 2002). This is especially true for the drainage structures that affect IOP and glaucoma.

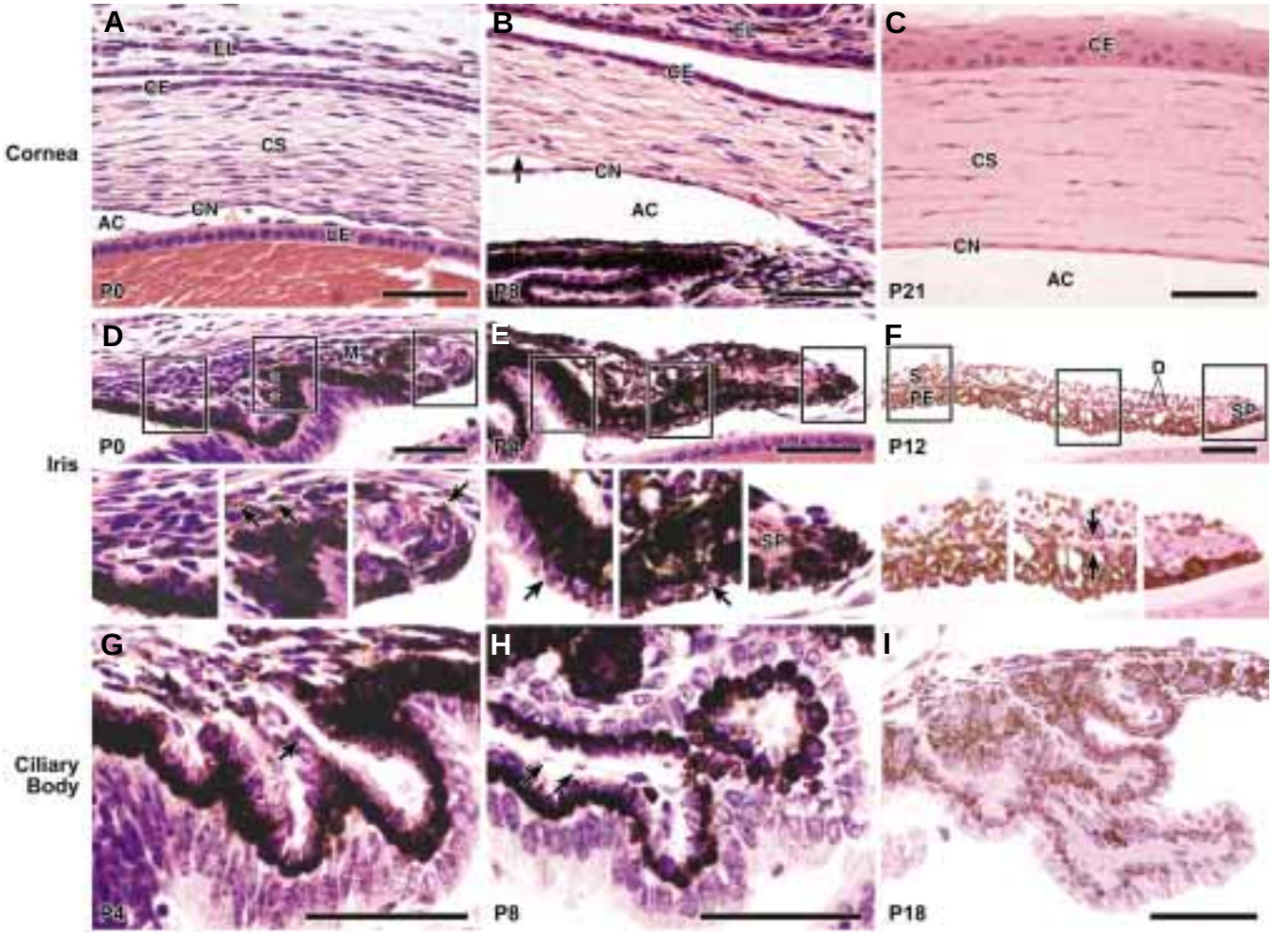

Fig. 3. Postnatal development of cornea, iris and ciliary body. *All analyses were performed on C57BL/6J mice. (A-C) Cornea.* **(A)** *At P0, the corneal epithelium (CE) is two cell layers thick and covered by eyelid (EL). The corneal stroma (CS) has a lamellar arrangement of extracellular matrix and flattened keratocytes and the corneal endothelium (CN) is present. The anterior chamber (AC) is the space defined by the corneal endothelium and lens epithelium (LE).* **(B)** *By P8, the corneal stroma is less cellular. Descemet's membrane (arrow) is clearly visible in an area of artifactual separation from the corneal endothelium (CN).* **(C)** *By P21, the cornea is mature. The eyelids are open and the corneal epithelium is five cell layers thick. (D-F) Iris.* **(D)** *Dense mesenchyme (M) is present on the anterior of the developing iris and will give rise to the iris stroma. Small folds in the pigment epithelium (asterisks and middle inset) indicate early morphogenesis of ciliary processes. The cells of the presumptive iris stroma begin to synthesize pigment (arrows in insets) and this distinguishes them from the cells that give rise to the trabecular meshwork (left inset).* **(E)** *By P4, the iris has extended centrally. The posterior layer of iris pigment epithelium is only slightly pigmented peripherally (arrow in left inset) but more so centrally (arrow in middle inset). The entire anterior layer of pigmented epithelium is already pigmented. The stroma is also pigmented by this time (middle inset) and the iris sphincter muscle (SP) is present (right inset).* **(F)** *At P12.5 the iris is mature with a robust stroma (S) that is separated from the bi-layered pigment epithelium (PE) by a thin dilator muscle (D) (between arrows in inset). The sphincter muscle is located at the pupilary margin. (G-I) Ciliary Body.* **(G)** *Compared to the P0 ciliary body shown in (D), the ciliary body at P4 has well formed ciliary processes. Endothelial cells are present indicating that the vascular core is being established (arrow).* **(H)** *By P8, the ciliary body is completely developed with a vascular core (arrows).* **(I)** *Lower magnification image of a mature ciliary body at P18. All panels are of previously unpublished data. Scale bars represent 50 microns.*

At birth, the corneal epithelium is one to two cells thick. The corneal stroma is filled with keratocytes surrounded by extracellular matrix (ECM) and the corneal endothelium is clearly defined (Fig. 3A). Descemet's membrane (a specialized basal lamina of the corneal endothelium) is still not evident by light microscopy but can be detected by electron microscopy (Kidson *et al.,* 1999, Smith *et al.,* 2001). The mesenchyme of the presumptive iris stroma begins to synthesize pigment distinguishing it from the trabecular meshwork mesenchyme (Fig. 3D middle inset compared to left inset). Future ciliary processes are visible as slight folds in the ciliary pigment epithelium (Fig. 3D). By postnatal day (P) 2 to P4, there is a decrease in cellularity of the corneal stroma. The iris and ciliary body are now clearly distinguishable from each other. There are prominent ciliary folds (Fig. 3G), the iris stroma is darkly pigmented and there is clear evidence of iris sphincter muscle development (Fig. 3E). The mesenchyme of the presumptive trabecular meshwork is densely packed (Fig. 4A) (Smith *et al.,* 2001). By P6-P8, Descemet's membrane is distinct from the corneal stroma (Fig. 3B). The iris stroma, pigment epithelium, dilator muscle and sphincter muscle are mature by P8-P10 (Fig.

3F). The processes of the ciliary body are completely developed and have a vascular core, but ciliary muscle fibers are not yet present (Fig. 3H).

At this stage, the presumptive trabecular meshwork is a mass of mesenchymal cells in the iridocorneal angle. To allow aqueous humor drainage, this mesenchymal mass remodels (see below) to form a functional trabecular meshwork (Smith *et al.,* 2001). A major component of the functional trabecular meshwork is ECM organized into a network of beams that are covered by trabecular cells. The ECM includes collagen, laminin, elastin, fibronectin and vitronectin (Yue 1996). Intertrabecular spaces, between the beams of the functioning meshwork, allow aqueous humor to flow to Schlemm's canal. The aqueous humor passes through the endothelial wall of Schlemm's canal in drainage structures known as giant vacuoles. The lumen of Schlemm's canal connects to the venous system through collector channels. Abnormal development of these iridocorneal angle structures can lead to elevated IOP and glaucoma.

In the mouse, the process of forming the functional trabecular meshwork from the mesenchymal mass involves morphogenesis of closely packed beams followed by tissue remodeling to open up the intertrabecular spaces, without cell death or atrophy (Smith *et al.,* 2001). At P10, the mesenchymal cells align parallel to the cornea. Between P10 and P14 trabecular beams appear but are still closely apposed (Fig. 4 B,C) (Smith *et al.,* 2001, Baulmann *et al.,* 2002). Ciliary muscle fibers are observed and Schlemm's canal appears as a small lumen lined with endothelial cells (Fig. 4C). Intertrabecular spaces first form between the trabecular beams nearest the anterior chamber. As the channels between the trabecular beams form, aqueous humor can enter the drainage structures and flow towards Schlemm's canal. Abundant giant vacuoles are present at P18. By P21, the anterior segment is fully developed except for minor remodeling that increases the extent of the intertrabecular spaces (Fig. 4D) (Smith *et al.,* 2001).

Molecular development

Although the developmental networks are largely undefined, some of the molecules and pathways contributing to anterior segment development are known. We now discuss developmentally important genes and pathways that mediate anterior segment development and are associated with IOP and glaucoma. We include the recently identified role of tyrosinase/L-dopa, since it modifies phenotypes in mice with mutations in the orthologues of human glaucoma genes. We do not include all genes known to cause ASD. We discuss recurring themes with multiple genes and suggest potential interactions between the different genes and pathways.

Transforming Growth Factor Beta Super-Family

The TGFβ super-family of secreted signaling molecules influences a spectrum of biological processes including pattern determination, cell proliferation, cell differentiation, cell death, bone morphogenesis and wound repair (Graham *et al.,* 1994, Hogan 1996, Hogan 1999, Mabie *et al.,* 1999). Abnormal TGFβ signaling contributes to a wide variety of disease processes

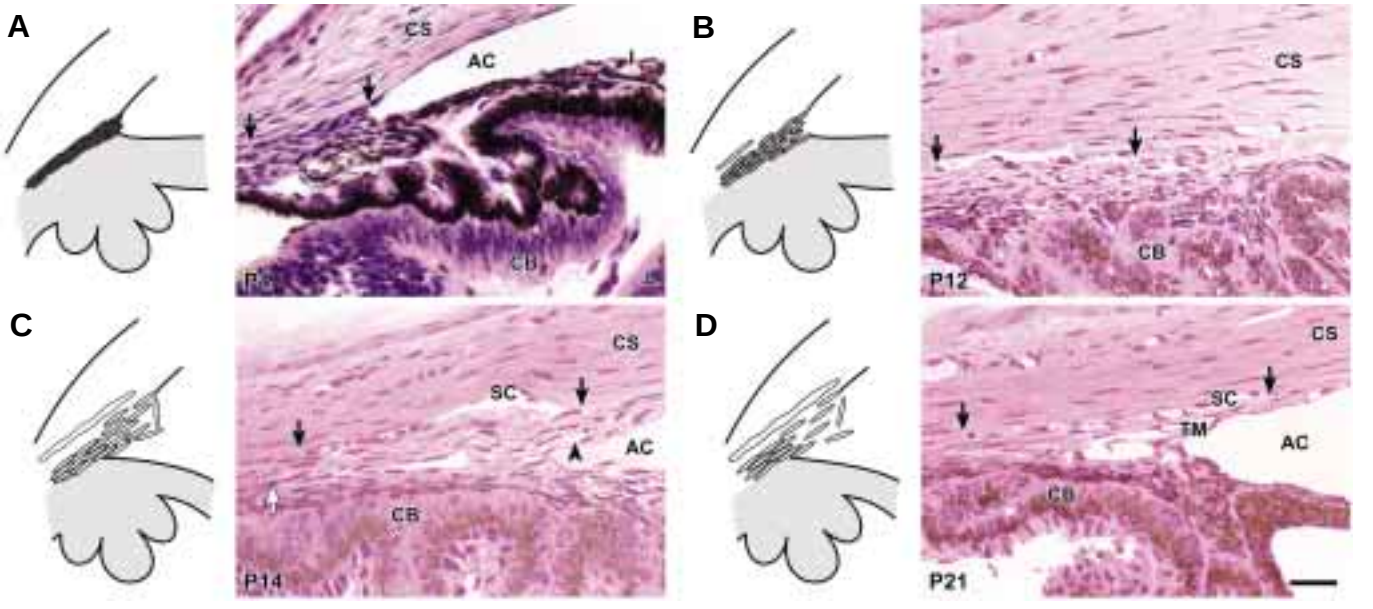

Fig. 4. Postnatal development of the iridocorneal angle. *The diagrams represent the relative developmental states of trabecular meshwork and Schlemm's canal in C57BL/6J mice at each stage. They are based on analysis of multiple mice and are not direct representations of the adjacent histologic images. The diagrams indicate the enlargement of Schlemm's canal and opening of aqueous humor drainage channels (intratrabecular spaces) in the trabecular meshwork.* **(A)** *At P4, the anlage of the trabecular meshwork is recognizable as condensed mesenchyme (arrows) between the corneal stroma (CS) and ciliary body (CB). AC, anterior chamber and I, iris.* **(B)** *By P12, trabecular meshwork cells have differentiated and trabecular beams are present but are still packed close together. Schlemm's canal is present by this age (between arrows).* **(C)** *By P14, trabecular beams nearest the anterior chamber begin to separate as intertrabecular spaces appear (arrowhead) but aqueous humor access to Schlemm's canal (SC, between arrows) is still restricted. Ciliary muscle fibers are present (open arrow).* **(D)** *The angle continues to remodel with more intratrabecular spaces forming between the trabecular beams. At P21, the major morphogenesis is complete with only minor remodeling occurring afterwards (up to approximately P35). Adapted with permission from (Smith* et al., *2001). Scale bars represent 50 microns.*

(Miyazono *et al.,* 2001). The TGFβ super-family is divided into two similar but separate branches: the BMP/GDF (bone morphogenetic protein/growth and differentiation factor) branch and the TGFβ/activin/nodal branch. Each branch works through extracellular ligand binding to a membrane bound receptor complex consisting of Type I and Type II serine/threonine kinase receptors. Ligand binding initiates a cytoplasmic signal cascade that activates SMAD (also called MADH) proteins. Activated SMAD proteins enter the nucleus where they associate with transcription factors and participate in transcriptional regulation of target genes (Cho and Blitz 1998, Chang *et al.,* 2001b, Miyazono *et al.,* 2001, Balemans and Van Hul 2002).

Multiple TGFβ super-family pathway members are important in ocular development. *Bmp4* is expressed in the distal portion of the emerging optic vesicle and in the overlying surface ectoderm. Expression becomes restricted to the dorsal distal tip of the optic vesicle at the site of contact with the surface ectoderm and later to the dorsal rim of the optic cup (Furuta and Hogan 1998). Postnatally, *Bmp4* is expressed in the pigmented epithelia of the iris, ciliary body and the retina that all derive from the optic cup (Chang *et al.,* 2001a). Two BMP4 receptors, BMPR1A and BMPR1B are present in the developing eye. *Bmpr1a* is expressed throughout most ocular tissues up to at least E11.5. *Bmpr1b* is expressed in head mesenchyme as early as E9.5 (Furuta and Hogan 1998).

Completely *Bmp4* deficient mice (homozygous mutant, *Bmp4*$^{-/-}$) are not viable and do not develop eyes due to the absence of lens induction (Furuta and Hogan 1998). Partially *Bmp4* deficient mice (heterozygous mutant, *Bmp4*$^{+/-}$) develop eyes but often have severe ASD (Chang *et al.,* 2001a). Although the phenotype is variable and dependent on genetic background, all *Bmp4*$^{+/-}$ mice have some degree of ASD. All anterior segment tissues are affected including lens, iris, cornea, trabecular meshwork and Schlemm's canal. The trabecular meshwork is hypoplastic, compressed, has a reduction in number of trabecular beams and has less ECM (Chang *et al.,* 2001a). ASD in *Bmp4*$^{+/-}$ mice is similar to ASD in human patients.

Molecular details of how BMP4 influences ocular development are starting to be revealed. *Bmp4* is expressed in the distal optic vesicle and has a receptor ubiquitously expressed in ocular tissues. Complete BMP4 deficiency results in a loss of *Sox2* expression in the surface ectoderm and loss of *Msx2* expression in the distal optic vesicle (Furuta and Hogan 1998). SOX2 is required for lens induction and mutations in *SOX2* have recently been reported in human patients with anophthalmia (Fantes *et al.,* 2003). It is possible that BMP4 from the optic vesicle binds BMPR1A in surface ectoderm and induces *Sox2* expression via SMAD transcriptional complexes. Alternatively or additionally, *Msx2* in the optic vesicle may be the target of BMP4 signaling which then plays a role in supplying a second signal for *Sox2* expression. Either of these paradigms would explain the loss of both *Sox2* expression and lens induction in the absence of BMP4 (Furuta and Hogan 1998).

Even though there are no known targets for BMP4 in the periocular mesenchyme, mesenchyme-derived tissues are malformed in *Bmp4*$^{+/-}$ mice and BMP4 receptors are present in mesenchyme. Therefore, it is possible that BMP4 has a direct effect on the mesenchyme. In *Bmp4*$^{+/-}$ mice, the mesenchyme-derived trabecular meshwork has profound ECM deficiencies. ECM is known to be developmentally important for many tissues and provides cues for cellular metabolism and various developmental processes such as migration and differentiation (Adams and Watt 1993, Lin and Bissell 1993, Perris 1997). Thus, BMP4 may influence trabecular meshwork morphogenesis by regulating ECM features (including composition and signaling) that are important for mesenchymal migration, differentiation and/or remodeling.

TGFβ2 is another signaling ligand involved in anterior segment development. The corneas of *TGFβ2*$^{-/-}$ mice have reduced accumulation of ECM and are thin with densely packed keratocytes. The corneal endothelium fails to differentiate and an anterior chamber never forms (Saika *et al.,* 2001). The trabecular meshwork in these mice has not been closely studied. Like BMP4, the relevant downstream targets of TGFβ2 signaling are not known. However, the BMP4 and TGFβ2 pathways do converge and overlap (Miyazono *et al.,* 2001). Given the convergence of the pathways and the paucity of ECM in the eyes of *Bmp4*$^{+/-}$ and *Tgfb2*$^{-/-}$ mice, it is possible that ECM components are important downstream targets of both of these signaling molecules.

A key step in regulation of TGFβ super-family signaling is inhibitory binding of the ligand by extracellular antagonists such as noggin (NOG) (Cho and Blitz 1998, Balemans and Van Hul 2002). Transgenic over-expression of *Nog* demonstrates the importance of BMP signaling in the development of the ciliary body. Ectopic, lens-specific expression of *Nog*, presumed to antagonize BMP4 and BMP7, decreases levels of activated SMAD1 in lens epithelium, ciliary epithelium, corneal epithelium and mesenchymal cells (Zhao *et al.,* 2002). These data show that SMAD1 is an important mediator of BMP signaling in ciliary body development. Furthermore, inhibiting BMP signaling with NOG significantly down regulates expression of the downstream targets *Msx1* and *Otx1* in the ciliary epithelium. *Otx1* is necessary for development of the ciliary body (Acampora *et al.,* 1996). By re-introducing transgenic *Bmp7* (presumably re-establishing a ratio of ligand/antagonist) normal ciliary body development is restored (Zhao *et al.,* 2002).

It is clear that members of the TGFβ signaling super-family pathway are important for normal ocular development. Therefore, any genes that influence the balance of TGFβ/BMP signaling are candidates to affect anterior segment development and glaucoma phenotypes.

PAX6

PAX6 is a transcription factor with paired-class and homeobox DNA binding domains. In neural ectoderm derivatives, *Pax6* is expressed in the distal optic vesicle, rim of the optic cup and then in the epithelia of iris and ciliary body, which arise from the rim of the optic cup. *Pax6* expression in surface ectoderm is broad at E8.5 but becomes restricted to the lens placode by E9.5. It is retained throughout development in ocular tissues derived from surface ectoderm (Walther and Gruss 1991, Grindley *et al.,* 1995, Koroma *et al.,* 1997, Baulmann *et al.,* 2002). *Pax6* is also expressed in the developing iris stroma, ciliary stroma and trabecular meshwork, consistent with a regulatory function during the morphogenesis of these mesenchymal derived tissues (Baulmann *et al.,* 2002).

The small eye mutant mouse has a null allele of *Pax6* (*Pax6*^{Sey}) (Hill *et al.,* 1991). Homozygous *Pax6*$^{Sey/Sey}$ mice, have morphological abnormalities of the optic vesicle and lens induction fails (Hogan *et al.,* 1986). Mice heterozygous for the *Pax6*^{tm1Pgr} targeted null allele have ocular defects including small eyes, small

anterior chambers, corneal haze, iris hypoplasia, iridocorneal adhesions, dysgenesis of the trabecular meshwork and absence of Schlemm's canal (Baulmann *et al.,* 2002). The trabecular meshwork is hypoplastic and undifferentiated. Null mutations or duplications of *PAX6* can cause anterior segment dysgenesis indicating the importance of PAX6 dosage for normal development (Ton *et al.,* 1991, Jordan *et al.,* 1992, Hanson *et al.,* 1993, Glaser *et al.,* 1994, Hanson *et al.,* 1994).

There is conservation of PAX6 regulated gene function between flies and vertebrates (Halder *et al.,* 1995). Many of the same genes and interactions are conserved between flies and vertebrates (Oliver *et al.,* 1995, Pignoni *et al.,* 1997, Xu *et al.,* 1997, Heanue *et al.,* 1999, Kozmik *et al.,* 1999, Xu *et al.,* 1999a, Xu *et al.,* 1999b, Heanue *et al.,* 2002, Hsieh *et al.,* 2002). Therefore, mammalian orthologues of the fly genes are good candidates to participate in anterior segment development and may contribute to ASD and glaucoma. The *Drosophila Pax6* paralogues *eyeless* (*ey*) and *twin of eyeless* (*toy*) participate in a complex regulatory hierarchy of gene expression. During ocular development in *Drosophila*, they regulate the genes *eyes absent* (*eya*), *sine oculus* (*so*) and *dachshund* (*dac*). Mutations in a human *eyes absent* orthologue *EYA1* associate with anterior segment malformations (Azuma *et al.,* 2000). The genetic interactions within this pathway have been the subject of considerable study in flies and vertebrates (for review see Treisman 1999; Wawersik and Maas 2000; Pichaud and Desplan 2002; van Heyningen and Williamson 2002).

FOXC1 and FOXC2

FOXC1 (forkhead box C1) is a transcription factor with a forkhead/winged-helix DNA binding domain. In the developing eye, *Foxc1* is primarily expressed in the periocular mesenchyme and mesenchymal cells that have migrated into the eye (Kume *et al.,* 1998, Kidson *et al.,* 1999). At E12.5, the presumptive cornea between the surface ectoderm and the lens no longer expresses *Foxc1*. By E16.5, expression of *Foxc1* is further restricted to the region of the future trabecular meshwork (Kidson *et al.,* 1999). *Foxc1*$^{-/-}$ mice die at birth with multiple congenital abnormalities including skeletal defects and hydrocephalus (Kume *et al.,* 1998, Hong *et al.,* 1999). They also have severe anterior segment developmental defects. In corneas of *Foxc1*$^{-/-}$ mice the epithelium is thickened, the stroma is disorganized and there is no differentiation of the endothelium. The lens fails to separate from the cornea and as a result there is a complete absence of the ocular anterior chamber (Kidson *et al.,* 1999).

Foxc1 $^{+/-}$ mice are viable and have milder anterior segment defects than *Foxc1*$^{-/-}$ mice (Hong *et al.,* 1999, Smith *et al.,* 2000). Development of periocular mesenchyme derived tissues is abnormal in *Foxc1* $^{+/-}$ mice. Abnormalities include iris malformations, iridocorneal adhesions and corneal opacity. Angle malformations include small or absent Schlemm's canal and hypoplastic, compressed trabecular meshwork. Abnormal iridocorneal angle structures have a paucity of ECM including collagen and elastic tissue and cells that have the appearance of undifferentiated precursor cells. Even though clinically obvious iris and corneal abnormalities do not occur in *Foxc1* $^{+/-}$ mice on some genetic backgrounds, all mice have histologically detectable malformations of the trabecular meshwork and Schlemm's canal. This indicates that genetic modifiers have a differential influence on iris and corneal development compared to iridocorneal angle development and it suggests that iridocorneal angle development may be more sensitive to levels of FOXC1.

FOXC2 is a forkhead/winged-helix transcription factor with strong homology to FOXC1. *Foxc1* and *Foxc2* have largely overlapping expression patterns including similar expression in the periocular mesenchyme and in tissues derived from periocular mesenchyme (Winnier *et al.,* 1997, Hiemisch *et al.,* 1998). These genes have overlapping function, at least in the developing heart (Winnier *et al.,* 1999). FOXC2 affects ocular development in a very similar fashion to FOXC1 suggesting that they also have overlapping function in the developing eye (Smith *et al.,* 2000).

All double heterozygous (*Foxc1* $^{+/-}$ *Foxc2* $^{+/-}$) mice have malformations of the ciliary body not usually seen in mice heterozygous for either mutant gene alone (Smith *et al.,* 2000). The fact that a phenotype that is not present in either single heterozygote is revealed in double heterozygotes strongly supports overlapping functions of FOXC1 and FOXC2 in the developing eye. It also suggests that the ciliary body requires a certain threshold level of FOXC transcription factors for normal development (total FOXC1 and FOXC2) that often is not maintained in double heterozygotes.

Patients with mutations in *FOXC1* have a spectrum of ASD and glaucoma phenotypes (Mears *et al.,* 1998, Nishimura *et al.,* 1998, Mirzayans *et al.,* 2000, Nishimura *et al.,* 2001, Honkanen *et al.,* 2003). Similar to PAX6, normal development is very sensitive to FOXC1 dosage and both duplications or deletions can cause ASD (Kume *et al.,* 1998, Lehmann *et al.,* 2000, Nishimura *et al.,* 2001, Saleem *et al.,* 2001, Saleem *et al.,* 2003a, Saleem *et al.,* 2003b).

No upstream regulators or downstream targets of FOXC1 or FOXC2 are known. *Foxc1* and *Foxc2* have very similar expression patterns and their DNA binding domains are nearly identical. Thus, it is likely that FOXC1 and FOXC2 share at least some downstream target genes. Given the phenotypic similarity of *Bmp4, Tgfb2*, *Foxc1* and *Foxc2* mutant mice, it is possible that TGFβ family members and FOXC transcription factors act in the same pathway. Other members of the winged-helix family of transcription factors are known to facilitate TGFβ super-family signaling in Xenopus and mouse. FOXH1 (formerly FAST for forkhead activin signal transducer) is a forkhead/winged-helix transcription factor that associates with SMAD proteins activated by the TGFβ signaling ligand activin (Chen *et al.,* 1996, Chen *et al.,* 1997, Weisberg *et al.,* 1998, Liu *et al.,* 1999, Germain *et al.,* 2000). It is possible that FOXC1 and FOXC2 interact with SMAD proteins to mediate BMP4 (or other TGFβ super-family ligand) signaling.

Similar to *Bmp4* $^{+/-}$ and *Tgfb2* $^{+/-}$ mutant mice, *Foxc1* $^{+/-}$ and *Foxc2*$^{+/-}$ mutants have major deficiencies of ECM in the iridocorneal angle structures. Additionally, *Foxc1*$^{-/-}$ mice have ECM abnormalities in other tissues including the arachnoid layer of the meninges and the prechondrogenic mesenchyme (Kume *et al.,* 1998, Hong *et al.,* 1999). Thus, FOXC1 and FOXC2 may control development by directly or indirectly affecting synthesis and/or metabolism of ECM (Fig. 5).

FOXE3

A third winged-helix/fork head gene, *Foxe3* is also involved in ocular development. In contrast to mesenchymal expression of *Foxc1* and *Foxc2*, *Foxe3* is expressed in the lens placode, lens pit and anterior lens vesicle before becoming restricted to the anterior lens epithelium (Blixt *et al.,* 2000, Brownell *et al.,* 2000). Mice homozygous for a *Foxe3* mutation (*dy*/ for dysgenetic lens) have

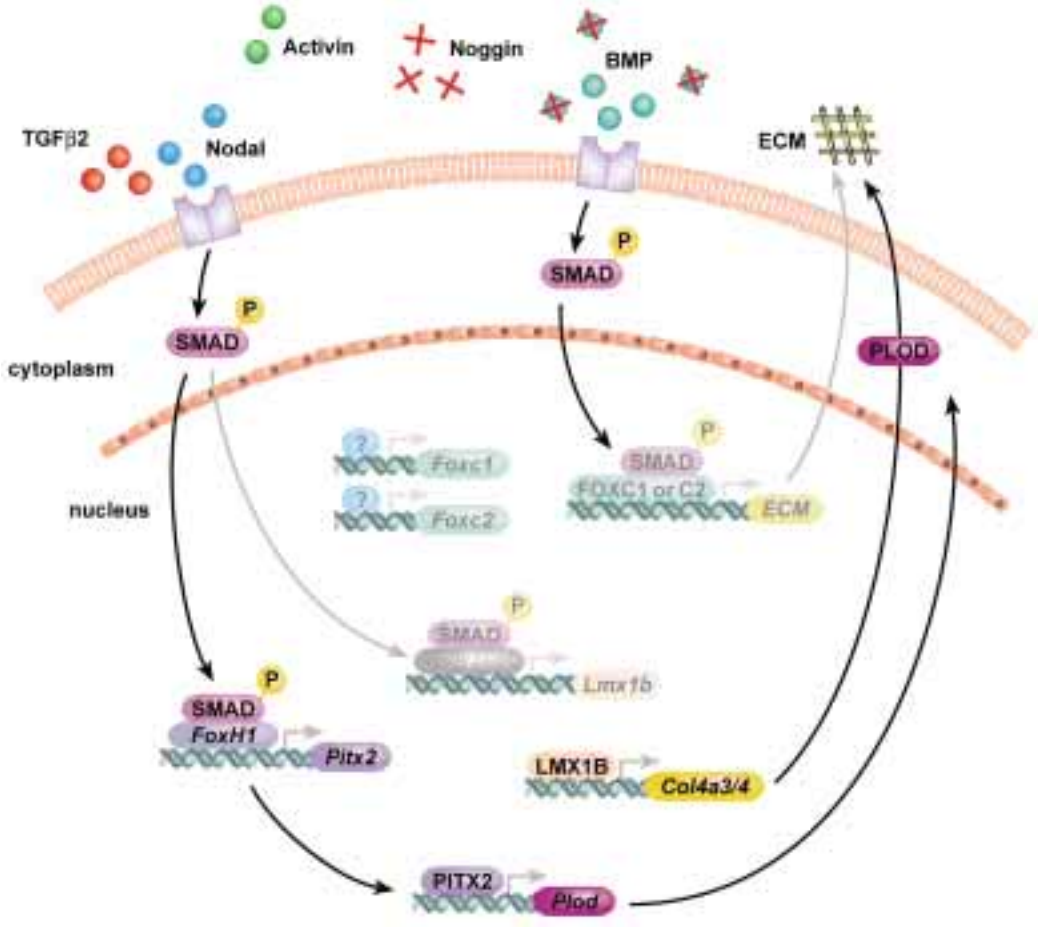

Fig. 5. TGFβ-superfamily/BMP signaling and anterior segment developmental genes. *The ways in which known anterior segment developmental genes interact is largely unknown. In an attempt to link the functions of known genes, this figure suggest that TGFβ-superfamily signaling may regulate developmentally important roles of ECM by modulating the expression of genes that are known to affect both anterior segment development and ECM composition/abundance in the eye. Dark arrows indicate steps supported by experiments in various developmental systems. Proposed interactions are depicted as fainter arrow and symbols. TGFβ signaling ligands (Activin, Nodal, BMPs, TGFβ2) bind transmembrane receptors and activate SMAD proteins by phosphorylation, while Noggin is an antagonist. Phosphorylated SMAD proteins translocate to the nucleus where they associate with DNA binding transcription factors (e.g. FOXH1) to regulate the transcription of target genes. Some of these target genes are known to affect ECM synthesis or modification as described in the text. Others, such as* Foxc1*, are known to affect ECM abundance and structure but it is not known if this is a direct or indirect effect. Although drawn in a sequential linear sequence, it is possible that these interactions do not occur in the same cell or developing tissue. For example, PITX2 induction by nodal may not occur at the same time or place as PITX2 activation of* Plod *genes. Similarly the depicted events may not be sequentially dependent. For example, Nodal activation of* Pitx2 *may not be necessary for* Plod *activation since* Pitx2 *may also be regulated by other factors. PLODs hydroxylate lysine residues in collagen that are important for intermolecular crosslinks.*

small eyes and persistent adhesion of the anterior lens epithelium to the cornea (Sanyal and Hawkins 1979, Blixt *et al.,* 2000, Brownell *et al.,* 2000). Atrophy of the lens epithelium and vacuolization of the lens body occurs.

Foxe3 acts downstream of *Pax6*. Homozygous mutant *Pax6*$^{Sey/Sey}$ mice develop an optic vesicle but fail to induce a lens placode. There is no induction of *Foxe3* in the surface ectoderm of the *Pax6*$^{Sey/Sey}$ presumptive lens placode (Brownell *et al.,* 2000). Furthermore, a surface ectoderm specific decrease in *Pax6* is sufficient to ablate *Foxe3* expression (Dimanlig *et al.,* 2001). This suggests that *Foxe3* is directly or indirectly induced by PAX6 within the surface ectoderm rather than *Foxe3* responding to PAX6 induced signals from the optic vesicle.

Dosage levels of both PAX6 and FOXE3 are important. Patients heterozygous for mutations in *PAX6* or *FOXE3* have ASD phenotypes including failure of lens and cornea to separate (Hanson *et al.,* 1994, Semina *et al.,* 2001, Ormestad *et al.,* 2002). Similarly, *Foxe3*$^{+/dyl}$ mice have lenticorneal adhesions and swollen, misshaped corneas (Ormestad *et al.,* 2002). Although no downstream targets of FOXE3 are known, it appears to be involved in maintenance of proliferation and inhibition of differentiation. In *Foxe3*$^{dyl/dyl}$ mice, lens epithelial cells exit from a proliferative state and enter a differentiated state followed by apoptosis (Blixt *et al.,* 2000, Brownell *et al.,* 2000).

PITX2

PITX2 is a paired-like homeodomain transcription factor. During normal ocular development, *Pitx2* is expressed in the periocular mesenchyme by E9.5 (Gage *et al.,* 1999). Expression persists in the mesenchyme and in the presumptive corneal stroma at E13.5 (Semina *et al.,* 1996, Lu *et al.,* 1999, Hjalt *et al.,* 2000). By E18.5, *Pitx2* expression is restricted to the presumptive iris and iridocorneal angle (Hjalt *et al.,* 2000). This expression pattern is very similar to the expression patterns of *Foxc1* and *Foxc2*. *Pitx2*$^{-/-}$ mice die by E15.5 (Lin *et al.,* 1999, Lu *et al.,* 1999). At this stage, *Pitx2*$^{-/-}$ mice have thickened, undifferentiated corneas that do not develop corneal endothelium or anterior chambers (Gage *et al.,* 1999, Lu *et al.,* 1999) similar to the eyes of *Tgfb*$^{-/-}$ or *Foxc1*$^{-/-}$ mice. Similar to humans with *PITX2* mutations, *Pitx2* $^{+/-}$ mice have variable degrees of ASD (Gage *et al.,* 1999).

Indicating the importance of PITX2 activity levels, both hypomorphic and over-activating alleles of *PITX2* cause human

ASD (Semina *et al.,* 1996, Alward *et al.,* 1998, Kulak *et al.,* 1998, Doward *et al.,* 1999, Kozlowski and Walter 2000, Priston *et al.,* 2001, Phillips 2002). Together with the dosage sensitivity for other transcription factors (discussed above), this demonstrates the importance of a narrow range of activity of various transcription factor for normal anterior segment development and reflects a delicate balance of signaling by interacting pathways.

There is evidence that *Pitx2* is at least partially controlled by TGFβ super-family signaling. Multiple studies show that TGFβ super-family member, NODAL, can induce *Pitx2* expression (Logan *et al.,* 1998, Piedra *et al.,* 1998, Ryan *et al.,* 1998, Yoshioka *et al.,* 1998). *Pitx2* has a NODAL response element in its promoter that contains binding sites for SMAD-associating transcription factor FOXH1 (Shiratori *et al.,* 2001). Furthermore, mice deficient for NODAL receptor *Acvr2b* (Oh and Li 1997) have phenotypes very similar to mice deficient for *Pitx2* (Lin *et al.,* 1999, Lu *et al.,* 1999). PITX2 can also determine expression boundaries of BMP4 and therefore, may interact in a regulatory feedback mechanism (Lu *et al.,* 1999).

Procollagen lysyl hydroxylase (*Plod*) genes are potential downstream targets for PITX2. The promoter regions of *PLOD1* and *Plod2* have multiple PITX2 binding sites. PITX2 can bind the promoters *in vivo* and induce expression of a reporter gene driven by this promoter *in vitro* (Hjalt *et al.,* 2001). PLOD1 and PLOD2 belong to a family of enzymes responsible for hydroxylating lysine residues in collagens. Hydroxylysine residues provide stability to intermolecular collagen cross-links (Kivirikko and Myllyla 1985). Therefore, disturbances of ECM stability and function may underlie PITX2 phenotypes.

PITX3

A second paired-like homeodomain transcription factor, PITX3, can also cause ASD. Expression of *Pitx3* is first seen at the late lens placode stage around E9.5. Expression continues in the lens pit and primary lens fibers until E16.5 when it is most strongly expressed in the equatorial zone of the lens (Semina *et al.,* 1998, Semina *et al.,* 2000). A recessive mutation in mice, aphakia (*ak*), causes a massive reduction of *Pitx3* expression. *Pitx3*$^{ak/ak}$ mice have small eyes without lenses. Lens morphogenesis is disrupted at approximately E10.5 (Grimm *et al.,* 1998, Semina *et al.,* 2000). The lens vesicle is filled with abnormal cells and primary lens fibers never develop. The lens never detaches from surface ectoderm and eventually dissolves. Anterior segment development is arrested at this point (Grimm *et al.,* 1998, Semina *et al.,* 2000). Dominant mutations in human *PITX3* can cause ASD including cataracts, corneal opacities and iridocorneal adhesions (Semina *et al.,* 1998).

The mouse aphakia mutation provides insight into the regulation of *Pitx3* expression. *Pitx3*^{ak} is caused by a deletion in the *Pitx3* regulatory region. Within the deletion are the binding sites for transcription factor AP2, alpha (TCFAP2A) and avian musculoaponeurotic fibrosarcoma AS42 oncogene homolog (MAF). TCFAP2A and MAF are transcription factors present in the developing lens at times coincident with *Pitx3* expression (Yoshida *et al.,* 1997, Ogino and Yasuda 1998, Kawauchi *et al.,* 1999, Kim *et al.,* 1999, West-Mays *et al.,* 1999). Mutations of *Tcfap2a* or *Maf* can result in abnormal lens development and ASD consistent with their role in induction of *Pitx3* (Kim *et al.,* 1999, West-Mays *et al.,* 1999, Jamieson *et al.,* 2002, Lyon *et al.,* 2003).

LMX1B

LMX1B is a LIM homeodomain class transcription factor expressed throughout the periocular mesenchyme by E10.5 and in the presumptive cornea until E14.5 (Pressman *et al.,* 2000). At birth, corneal expression is still observed in keratocytes, corneal endothelium and mesenchyme of the presumptive iris, ciliary body and trabecular meshwork. Expression is later maintained in the iris and trabecular meshwork but not in the ciliary body (Pressman *et al.*, 2000).

Lmx1b$^{-/-}$ mice have multiple developmental defects including ocular malformations (Chen *et al.,* 1998, Pressman *et al.,* 2000). The first morphological abnormality is observed at E15.5. Corneal keratocytes are less densely packed and there is a reduction in the depth of the anterior chamber. After birth, eyes are small and have significant iris and ciliary body hypoplasia including a lack of ciliary folds in the ciliary epithelium. Lens development appears normal except that cells in the lens epithelium have altered morphology. Homozygous null mice die as neonates precluding analysis of any effects of absence of LMX1B on postnatal anterior segment development including morphogenesis of the trabecular meshwork and Schlemm's canal. Detailed analysis of *Lmx1b*$^{+/-}$ mice has not been reported. Mutations in *LMX1B* have dominant, pleiotrophic effects and cause nail-patella syndrome (NPS) (Dreyer *et al.,* 1998, McIntosh *et al.,* 1998, Vollrath *et al.,* 1998). In addition to dysplastic nails and hypoplastic patellae, NPS is also associated with nephropathy and glaucoma (Lichter *et al.,* 1997).

Compared to wild type mice, *Lmx1b*$^{-/-}$ mice have molecular differences by E13.5, before there are morphological differences. *Foxc1* expression is altered by *Lmx1b* deficiency. Normally, when periocular mesenchyme cells migrate into the presumptive cornea, *Foxc1* expression is turned off and the presumptive corneal cells will start to express keratocan (an ECM molecule) as the keratocytes differentiate. In *Lmx1b*$^{-/-}$ mice, there is a persistence of *Foxc1* expression in the presumptive cornea and these cells do not express keratocan by E15.5 (Pressman *et al.,* 2000). Thus, LMX1B is not required for the migration of mesenchyme into the presumptive cornea but is necessary for the normal differentiation of these cells.

In addition to keratocan, LMX1B also affects expression of other ECM molecules. *Lmx1b*$^{-/-}$ mice and some NPS patients have renal defects. These defects involve the loss of two subtypes of type IV collagen, COL4A3 and COL4A4, in the glomerular basement membrane. *Col4a3* and *Col4a4* are direct transcriptional targets of LMX1B (Morello *et al.,* 2001). Thus, regulation of ECM may be a major role of LMX1B in ocular development.

CYP1B1

The *Cyp1b1* gene (Cytochrome P450, family 1, subfamily b, polypeptide 1) encodes an enzyme that participates in iridocorneal angle development. In the developing mouse eye, *Cyp1b1* is reported to be most highly expressed in the ciliary body after birth (Bejjani *et al.,* 2002). *Cyp1b1*$^{-/-}$ mice are grossly normal, but have focal malformations of the iridocorneal angle (Libby *et al.,* 2003). In affected regions, malformations may include hypoplastic trabecular meshwork, abnormally located basal lamina in the trabecular meshwork and iridocorneal adhesions. Recessive mutations in *CYP1B1* associate with human congenital glaucoma (Stoilov *et al.,* 1997, Bejjani *et al.,* 1998, Plasilova *et al.,* 1999, Bejjani *et al.,* 2000, Belmouden *et al.,* 2002). Developmental malformations in some children with congenital glaucoma resemble those observed in

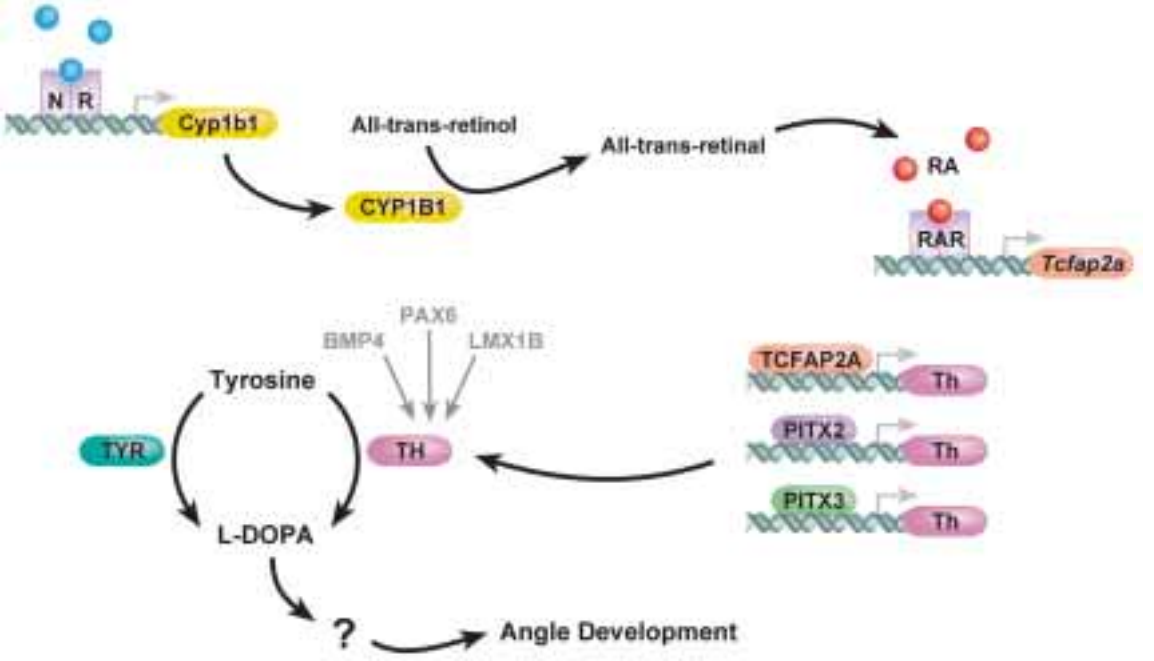

Fig. 6. Multiple genes implicated in anterior segment development and glaucoma may modulate L-dopa levels. *Many of the genes implicated in anterior segment dysgenesis, elevated IOP and glaucoma may affect L-dopa levels. Most can be linked to L-dopa through tyrosine hydroxlase (TH, as discussed in the text). The dark arrows represent known direct relationships. TCFAP2, PITX2 and PITX3 can all directly bind to the tyrosine hydroxylase promoter. The fainter arrows and text indicate that the represented genes affects on TH and L-dopa may not be direct. BMP4, PAX6 and LMX1B can promote either tyrosine hydroxylase expression or the number of TH expressing neural crest cells during the development of other tissues, but how they do so is not known. How L-dopa modulates angle development also is not known. It is possible that either L-dopa itself or a catecholamine metabolite(s) of L-Dopa mediates an important signaling event(s).*

Cyp1b1$^{-/-}$ mice (Allen *et al.,* 1955, Maumenee 1958, Libby *et al.,* 2003).

CYP1B1 belongs to a family of monomeric, mixed function mono-oxygenases (Sutter *et al.,* 1994). *Cyp1b1* expression can be induced by aromatic hydrocarbons acting as ligands for a nuclear receptor complex (Denison *et al.,* 1989, Shehin *et al.,* 2000). The receptor complex consists of two basic helix-loop-helix proteins, the aryl hydrocarbon receptor (AHR) and the aryl hydrocarbon nuclear translocator (ARNT) (Reyes *et al.,* 1992, Dolwick *et al.,* 1993). It is hypothesized that CYP1B1 is involved in metabolism of signaling molecules important in ocular development (Sarfarazi and Stoilov 2000). It is possible that in the absence of CYP1B1, a key signaling molecule is not produced/activated or alternatively not degraded/deactivated. One possibility is that CYP1B1 influences anterior segment development through a mechanism involving retinoic acid signaling. CYP1B1 oxidizes all-*trans*-retinol to all-*trans*-retinal which is a rate-limiting step in retinoic acid biosynthesis (Chen *et al.,* 2000). However, the exact role of CYP1B1in ocular development is not known. *Cyp1b1*$^{-/-}$ mice are a valuable tool to address these possibilities.

Tyrosinase modifies anterior segment dysgenesis

The tyrosinase gene (*Tyr*) was recently identified as a modifier of iridocorneal angle defects present in *Cyp1b1*$^{-/-}$ mice (Libby *et al.,* 2003). *Cyp1b1*$^{-/-}$ mice, that are also *Tyr* deficient, have more severe iridocorneal angle malformations than *Cyp1b1*$^{-/-}$ mice with functional *Tyr*. *Tyr* is necessary for melanin synthesis and so *Tyr*$^{-/-}$ mice are albino. The demonstration that *Tyr* participates in iridocorneal angle development may explain the increased incidence of ASD in people with albinism (van Dorp *et al.,* 1984). Importantly, *Tyr* also modifies angle phenotypes in another mouse model of ASD. Albino *Foxc1*$^{+/-}$ mice had more severe iridocorneal angle malformations than pigmented *Foxc1*$^{+/-}$ mice (Libby *et al.,* 2003). Thus, the effect of *Tyr* is not specific to *Cyp1b1* deficiency.

Tyrosinase, tyrosine hydroxylase, dopa and ASD

TYR converts tyrosine to dihydroxyphenylalanine (L-dopa). L-dopa affects exit from cell cycle and is a precursor of developmentally important catecholamines (Thomas *et al.,* 1995, Zhou *et al.,* 1995, Ilia and Jeffery 1999). Therefore, TYR may contribute to angle development by producing L-dopa. It is possible that *Tyr* deficiency exacerbates iridocorneal angle malformations due to an L-dopa deficiency. Supplementation of L-dopa to albino *Cyp1b1*$^{-/-}$ mice greatly alleviated the developmental defects. This proves that *Tyr* affects anterior segment development through a mechanism involving L-dopa or an L-dopa metabolite (Libby *et al.,* 2003).

Other genes influencing L-dopa signaling may also participate in anterior segment development including dopamine receptors, genes affecting catecholamine metabolism or signaling and genes affecting L-dopa levels. Tyrosine hydroxylase is another enzyme that converts tyrosine to L-dopa. Therefore, tyrosine hydroxylase and genes affecting levels of tyrosine hydroxylase are excellent candidates to affect ocular development.

Many of the anterior segment development genes discussed in this review can directly or indirectly affect levels of tyrosine hydroxylase. BMP4, PAX6 and LMX1B can promote tyrosine hydroxylase activity or proliferation of tyrosine hydroxylase expressing neural crest cells (Varley and Maxwell 1996, Dellovade *et al.,* 1998, Smidt *et al.,* 2000, Vitalis *et al.,* 2000). Mutations of these genes could affect the supply of L-dopa available to the developing ocular structures. Additionally, tyrosine hydroxylase expression can be regulated by PITX2, PITX3 and TCFAP2 (Cazorla *et al.,* 2000, Kim *et al.,* 2001, Lebel *et al.,* 2001). Finally, CYP1B1 can catalyze a rate limiting step in retinoic acid biosynthesis. Retinoic acid induces *Tcfap2a*, which can regulate tyrosine hydroxylase and also promotes proliferation of a subset of avian neural crest cells that express tyrosine hydroxylase (Rockwood and Maxwell 1996).

Together, these observations open an exciting new avenue for investigating the role of L-dopa in anterior segment development and glaucoma caused by multiple genes. Since L-dopa participates in angle development and various ocular development genes potentially influence L-dopa (via tyrosine hydroxylase), it is possible that a metabolic defect involving L-dopa is a common theme in ASD and glaucoma.

Common themes and developmental networks

Normal development requires cross-talk between networks of interacting pathways that have synergistic or opposing effects. The dosage sensitivity of anterior segment development to many of the genes/pathways discussed above implies that a delicate balance of signaling by interacting pathways is required for normal ocular development. Another effect of many of the genes discussed above is a disturbance of ECM composition and abundance. Considering the developmental importance of ECM and the substantial structural and physiological role it has in the trabecular meshwork, it is likely that ECM mis-regulation will be a common consequence of mutations in many genes that cause ASD and glaucoma. In addition to altered ECM regulation, mutations in ECM genes themselves may contribute to ASD. For example, *Col18a1*$^{-/-}$ mice have malformations of the iris and ciliary body (Ylikarppa *et al.,* 2003). Thus, ECM signaling is one important component of the developmental networks that regulate anterior segment formation.

Despite the genes and pathways discussed above, the developmental networks that regulate anterior segment formation are largely undefined. Many components remain to be identified and the ways in which the known genes interact are not well characterized. As an initial attempt to link the actions of known genes and pathways in anterior segment formation, we suggest how they may relate to TGFβ family signaling and ECM regulation (Fig. 5). Extending this, TGFβ family member signaling interacts with other signaling pathways, including fibroblast growth factor signaling, during optic vesicle morphogenesis (Ohkubo *et al.,* 2002). Additionally, Sonic hedgehog signaling can induce competence of a cell to respond to BMP signaling by regulating SMAD expression (Dick *et al.,* 1999, Murtaugh *et al.,* 1999). As an example of how various genes involved in anterior segment formation may converge to affect signaling by a common molecule/pathway, we indicate known developmental genes that can affect L-dopa or catecholamine mediated developmental events (Fig. 6).

As discussed above, many components of the complicated regulatory/signaling networks controlling anterior segment development remain undefined. Collection of extensive datasets of pertinent biological information in a stage and tissue specific fashion is needed (including mutant phenotypes, gene expression, protein abundance and modification). The production of mouse mutants by gene targeting and transgenic technologies, along with the many new mutants provided by mutagenesis efforts, will be a substantial help in defining network components. To reach an in depth understanding of these networks and how independent networks interact with each other, it will be essential to combine the tools of genomics, molecular biology, developmental biology, bioinformatics and computational biology. The availability of the genome sequence from various species will allow candidate identification of network members *in silico* and subsequent role testing *in vivo*. Combining tissue specific gene expression studies with computational methods can identify important configurations of cis-regulatory elements for coordinately regulated genes, (as recently reported for genes responding to similar thresholds of developmental gradients (Stathopoulos *et al.,* 2002). Knowledge of these regulatory elements and genome sequence analysis can then be used to predict roles of other genes for subsequent testing. Continued investment in appropriate electronic and biological resources will poise the field for an unprecedented molecular understanding of anterior segment development.

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