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Keeping an eye on SOXC proteins

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Abstract

The formation of a mature, functional eye requires a complex series of cell proliferation, migration, induction among different germinal layers, and cell differentiation. These processes are regulated by extracellular cues such as the Wnt/BMP/Hh/Fgf signaling pathways, as well as cell intrinsic transcription factors that specify cell fate. In this review article we provide an overview of stages of embryonic eye morphogenesis, extrinsic and intrinsic factors that are required for each stage, and pediatric ocular diseases that are associated with defective eye development. In addition, we focus on recent findings about the roles of the SOXC proteins in regulating vertebrate ocular development and implicating SOXC mutations in human ocular malformations.

Keywords

Eye development; Ocular morphogenesis; SOXC

I. Introduction

Development of the vertebrate eye is a complex process consisting of a series of highly coordinated events that involve interactions between the neural ectoderm, surface ectoderm, and extraocular mesenchymal cells. Each phase of eye development depends both on inductive signaling pathways and the precise temporal expression of cell-intrinsic factors. Disruptions in these cellular signals or mutations in eye development genes result in a variety of sight-threatening pediatric ocular malformations. Therefore, to better understand anomalies in the structure and the function of the eye it is important to identify the key molecular players in each step of eye formation.

This review will provide an overview of the major stages of vertebrate eye development, highlighting some of the important molecular cues associated with each stage. In addition, we will focus on the emerging roles of SoxC proteins during vertebrate ocular morphogenesis and in human ocular malformations. Throughout the review, we will follow established nomenclature, designating human genes in all capital letters, rodent genes with the first letter capitalized, and zebrafish and Xenopus genes with all lower case letters; italic letters indicate genes, whereas non italicized names refer to proteins.

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I.1 Development of the vertebrate eye field

During gastrulation the anterior neural plate is specified into domains that eventually form the eye field, telencephalon, diencephalon, and the hypothalamus. Critical inductive events prepare the presumptive eye field present within the anterior neural plate, which eventually migrates and integrates with nearby tissues. The eye field cells are surrounded rostrally and laterally by telencephalic precursors and medially by cells that will form the hypothalamus. Therefore, one of the first critical steps that eye precursor cells must take is to define their lineage and separate themselves from the surrounding brain tissues.

Formation and specification of naive uncommitted cells into an organized eye field domain requires the contribution of many signaling pathways and transcription factors (TFs). The fibroblast growth factor (FGF), bone morphogenetic protein (BMP), and Wingless (Wnt) pathways guide movement of cells into the eye field and also help maintain the eye field territory (Esteve and Bovolenta, 2006). In addition, both canonical and non-canonical Wnt signaling pathways interact with each other to separate the eye field territory from the diencephalon and the telencephalon (Wilson and Houart, 2004; Cavodeassi et al., 2005; Esteve and Bovolenta, 2006). Balance between BMP and non-canonical Wnt (*Wnt5, Wnt11*) signaling patterns the anteroposterior axis of the neural plate, and initiates morphogenetic movements of cells in the anterior neural plate, contributing to the formation of the eye field (Wilson and Houart, 2004; Cavodeassi et al., 2005). Simultaneous inhibition of canonical Wnt signaling is also required for suppression of diencephalon fate markers, as misexpression of *Wnt8b* results in poor delineation of the border between the diencephalon and the eye field, and mutation in the Gsk3 binding domain of Axin1 results in conversion of telencephalon and eye field precursors into diencephalon due to constitutive activation of the Wnt/β-catenin pathway (Heisenberg et al., 2001; Kim et al., 2002; Wilson and Houart, 2004; Cavodeassi et al., 2005; Esteve and Bovolenta, 2006; Adler and Canto-Soler, 2007). FGF modulation of ephrinB1 phosphorylation also plays a role in inducing the prospective progenitors to migrate, coalesce, and assemble themselves as an eye field (Chong et al., 2000; Moore et al., 2004).

In addition to receiving critical signals from the surrounding forebrain tissue, eye field progenitors themselves express the eye field transcription factors (EFTFs) *Rx1/Rax, Pax6, Lhx2, Six3, Otx2, ET, tll* and *Hes1* {Figure 1; (Chow and Lang, 2001)}. Six3, Pax6, Otx2 and Rx1 specify progenitor cells to the retinal lineage, and also regulate morphogenetic cell movements that guide presumptive eye field cells the correct geographic location (Kenyon et al., 2001; Moore et al., 2004). Lhx2 is required to maintain optic identity and suppress alternative fates (Roy et al., 2013). Loss-of-function mutations in EFTFs result not only in the absence of an cup but also cause severe neuro-developmental anomalies in a variety of different animals such as mice, chicken, zebrafish, and humans (Porter et al., 1997; Winkler et al., 2000; Chow and Lang, 2001; Tucker et al., 2001; Stigloher et al., 2006; Lequeux et al., 2008). Notably, there are also TF's (e.g. *Hes1* and *Otx2)* that are expressed outside the eye field domain which influence its formation. *Otx2* is not expressed within the *Rx* positive eye field region, however *Otx2* is needed to maintain the expression of *Six3* and *Hes1* in the anterior neural plate (Simeone et al., 1993; Rhinn et al., 1998; Andreazzoli et al., 1999). *Hes1* influences eye development by controlling the formation of the forebrain. *HESX1*

mutations in humans result in variety of defects including optic nerve hypoplasia, and in mice *Hes1* null mutants display anophthalmia and microphthalmia (Dattani et al., 1998; Chow and Lang, 2001).

I.2 From one eye field to two optic vesicles

The eye field cells undergo cellular proliferation during gastrulation and eventually split into two bilateral domains in response to secreted factors originating from the ventral midline. High-resolution dynamic fate map studies have revealed the substantial structural changes that occur to move the ventral diencephalon anlagen from a posterior to an anterior ventral position resulting in bisection of the eye field {for more in-depth discussion, see (Varga et al., 1999; England et al., 2006)}. The process of eye field segregation requires axial Nodal/TGF-β and Hedgehog (Hh) signaling, which in turn establishes optic vesicle boundaries and patterns the proximodistal and ventronasal axes of the optic vesicles by modulating expression of TFs *Pax2, Pax6, Vax1,* and Vax2 (Nornes et al., 1990; Barth and Wilson, 1995; Ekker et al., 1995; Hyatt et al., 1996; Barbieri et al., 1999; Dressler and Woolf, 1999; Muller et al., 2000; Schulte and Cepko, 2000). Loss of Nodal-related proteins such as Squint, Cyclops, or One-eyed pinhead results in cyclopia and holoprosencephaly, underscoring the importance of TGFβ/Nodal signaling for eye field segregation (Zhang et al., 1998; Pei and Feldman, 2009). Likewise, mutations in the Hh signaling ligand *SHH* result in holoprosencephaly and cyclopia in humans and mice (Belloni et al., 1996; Chiang et al., 1996; Roessler et al., 1996).

I.3 From a flat optic vesicle to a spherical optic cup

In the next phase of eye development, the symmetrical paired optic vesicles (OVs) evaginate from the ventral diencephalon and expand through the extraocular mesenchyme towards the surface ectoderm (Kessler and Melton, 1994; Li et al., 1997; Vogel-Höpker et al., 2000; Fuhrmann, 2010). This evagination step depends critically on paracrine retinoic acid (RA) signaling originating from the temporal mesenchyme (Adler and Canto-Soler, 2007; Cvekl and Wang, 2009). Upon physical contact with the overlying head surface ectoderm, a series of spatially and temporally complex structural changes ensues. The surface ectoderm at the point of contact thickens and forms a lens placode, which continues to invaginate, eventually forming the lens vesicle and detaching from the surface ectoderm. Concomitantly, the distal portion of the OV elongates laterally and undergoes invagination to form a bilayered optic cup (OC), which remains connected to the diencephalon via the optic stalk (for a detailed description see (Fuhrmann, 2010)). Dynamic migratory behavior of cells present within the optic vesicle is crucial for eye morphogenesis (Kwan et al., 2012; Ivanovitch et al., 2013). Formation of the optic cup is an important foundation on which depends the development of the iris, the ciliary body, the retina, and retina pigmented epithelium (RPE). The neural retina originates from the inner layer of the optic cup, whereas the outer layer of the optic cup differentiates into the RPE. A transient channel called the choroid fissure is present along the ventral side of the optic cup and optic stalk. This opening permits the entry of periocular mesenchymal cells (neural crest derived) that later form the blood vessels which provide nourishment to the growing eye; it also allows the axons of the ganglion cells to exit the eye and connect with the brain.

As optic cup morphogenesis and lens induction progresses, overlapping sets of TFs mark the lineage specification of distinct ocular tissues. The presumptive lens expresses *Pax6, IGF-2, Prox-1, Six3, Sox1, and Sox2*; the nascent neural retina expresses *Pax6, Pax2, Rx, Lhx2, Chx10, and Optx2*; the developing RPE expresses *Mitf and Otx2*; and the optic stalk expresses *Pax2, Vax1, Vax2, and Six3* (Jean et al., 1999; Chow and Lang, 2001). In addition to establishing the competence of progenitor cells for specific lineages, TFs interact with each other to establish boundaries within and between individual ocular tissues. Within the developing optic vesicle, *Pax6* and *Tbx5* pattern the dorso-ventral axis, and *BF-1/Foxg1* and *BF-2/Foxd2* pattern the anterior-posterior axis. Along the medial-lateral axis, *Pax2, Pax6, sox4, sox11,* and *Vax* regulate the proximo-distal patterning of optic vesicle versus optic stalk territories (Figure 1) (Hatini et al., 1994; Schwarz et al., 2000; Horsford et al., 2005; Adler and Canto-Soler, 2007).

I.4 Optic cup morphogenesis

1. The anterior segment: lens, cornea, iris and ciliary body—All sensory placodes possess the capacity to form the lens before they acquire unique identities (reviewed extensively by (Streit, 2007)). The preplacodal region gets patterned along the anterior-posterior axis, and precursors for the lens placode reside in the anterior region of the surface ectoderm. *Pax6* expression is critical for lens formation from the early preplacodal phase, and its expression is controlled at various stages of lens development by a variety of pathways. BMP and FGF signaling regulate P*ax*6 expression during placode formation; *Six3* and *Meis* family members regulate *Pax6* activity in the presumptive lens ectoderm. Additionally, *Pax6* regulates the critical expression of *Sox2* in the presumptive lens epithelium, and as the placode is formed there is role reversal and *Pax6* controls the expression of *Six3* (Kamachi et al., 1998; Wawersik et al., 1999; Liu et al., 2006; Streit, 2007; Zhao et al., 2008). The last stages of lens formation lead to inductive events resulting in the formation of the cornea endoderm, iris, and the ciliary body stroma. Soon after the lens separates from the overlying surface ectoderm, periocular mesenchymal cells of neural crest origin invade the space between the lens and the surface ectoderm. The mesenchymal cells differentiate and contribute to the formation of the cornea, iris, and the ciliary body (Piatigorsky, 2001; Graw, 2010).

2. The RPE and retina—Concomitant with lens development, profound changes are occurring in the bi-layered optic cup. As mentioned above, the outer layer of the optic cup forms the RPE, and the inner layer forms the neural retina. The cells in the inner layer proliferate (accompanied by some pruning due to programmed cell death) to generate six classes of retinal neurons and one intrinsic glial cell type: retinal ganglion cells (RGCs), amacrine cells, horizontal cells, bipolar cells, cone photoreceptors, rod photoreceptors, and the Müller glia (for more extensive reviews on retinal development, see (Livesey and Cepko, 2001; Fadool and Dowling, 2008; Bassett and Wallace, 2012; He et al., 2012; Gregory-Evans et al., 2013)). The RGCs are present in the ganglion cell layer (GCL), the interneurons (amacrine, horizontal and bipolar cells) and the cell bodies of the Müller glia are present in the inner nuclear layer (INL), and the photoreceptors are present in the outer nuclear layer (ONL). The genesis of the different retinal cell types occurs in a conserved temporal order but species-specific spatial order. In general, RGCs are the first cells born in

the neural retina, followed by amacrine, horizontal cells, and cone photoreceptors. The last retinal cell types to differentiate are the bipolar cells, rod photoreceptors, and the Müller glia. Importantly, in mammals retinal neurogenesis occurs only during embryonic/perinatal development; however cold-blooded vertebrates such as teleost fish and some amphibians experience growth-associated retinal neurogenesis throughout their lifespan. Retinal progenitor cells (RPCs) attain competence to become RGCs by expressing a bHLH TF called *ath5*. In addition, Hh and FGF signaling are required for the proper temporal expression of *ath5* and the generation of RGCs (Martinez-Morales et al., 2005; Masai et al., 2005). Amacrine, horizontal, and bipolar cells are generated by an overlapping network of TFs such as *Prox1*, *Math5*, NeuroD, *Math3, Ptf1*, Pax6, *Six3, Mash1*, and *Foxn4* (for an extensive review see (Tomita et al., 2000; Dyer et al., 2003; Ohsawa and Kageyama, 2008; DEMB and SINGER, 2012)). Photoreceptors are generated from a pool of *Crx*- and *Otx2*-positive cells, which later acquire competence to form either cone or rod photoreceptors by expressing the TFs *TRβ2* and *RXRγ* for cones, or *Nrl*, Nr2e3, and *Ascl1* for rods {for a more detailed review see (Swaroop et al., 2010)}.

The RPE is characterized by the presence of melanosomes, which produce and store melanin pigment. The RPE lies between the neural retina and the vascular choroid, and is sensitive to signals emanating from the adjacent periocular mesenchyme. The RPE is indispensable for retinal function. It forms part of the blood-retina barrier, facilitates adhesion between the neural retina and the surrounding choroid, captures free radicals, absorbs background light, is critical for retinoid metabolism, and phagocytoses the spent outer segment tips of the photoreceptors (Boulton and Dayhaw-Barker, 2001; Martinez-Morales et al., 2005). Surprisingly, only a handful of TFs have been identified that are required for the development and differentiation of the RPE. These include *Mitf*, *Otx1*/*Otx2*, and *Pax6* (Goding, 2000; Baumer et al., 2003; Martinez-Morales et al., 2005; Bharti et al., 2012; Raviv et al., 2014). TGFβ, FGF, BMP, and Hh signals from the surrounding mesenchyme also help induce the RPE fate during development (Dohrmann et al., 1993; Feijen et al., 1994; Zhang and Yang, 2001; Müller et al., 2007; Fuhrmann et al., 2014).

3. Ocular vasculature and optic nerve—A mature eye has the highest oxygen demand per unit weight of any human tissue (Saint-Geniez and D'Amore, 2004). Early on, the developing eye is nourished by intraocular vasculature generated by the hyaloid system and the choroid vessels. The hyaloid artery enters the optic cup through the choroid fissure and traverses the primitive vitreous to land on the posterior portion of the developing lens. The hyaloid arteries rapidly spread out and form a dense capillary network and connect with a venous system at the anterior portion of the optic cup. The venous system is provided by the choroidal vasculature derived from the periocular mesenchyme. As soon as the hyaloid system and choroid vasculature merge, the hyaloid vasculature is formed and it provides all the nutrients and metabolites to the growing eye. Around the same time the more permanent retinal vasculature formation is initiated and in mammals hyaloid vasculature starts to regress (for excellent description see (Saint-Geniez and D'Amore, 2004)).

The proper connection between the arterial-venous network is crucial for functional circulation of nutrients and oxygen within the ocular tissue (Haigh et al., 2003; Bussmann et al., 2011). Development and maturation of choroidal blood vessels depends on expression of

the angiogenic factors VEGF, bFGF, PDGF and PEDF from the RPE, and neutralization of bFGF and VEGF within the RPE results in incomplete formation of choroid vessels (Rousseau et al., 2003; Le et al., 2010). Misexpression of FGFR1 results in immature choroidal vessels and mutation in VEGF causes severe vascular defects (Carmeliet et al., 1996; Ferrara et al., 1996). Notch signaling is important for capillary bed formation in mice, humans, and zebrafish. VEGF and Delta-like 4 (DII4) interact dynamically to regulate vascular patterning (Shawber and Kitajewski, 2004; Hellstrom et al., 2007). Finally, genetic studies in zebrafish suggest that Hh signaling specifies endothelial cell fate to directly regulate arterial differentiation (Williams et al., 2010).

In summary, organogenesis of the eye is a conserved process that is driven by the complex interaction between the surface ectoderm, neuroepithelia and the extraocular mesenchyme (Chow and Lang, 2001; Fuhrmann, 2010). While the events of ocular morphogenesis are broadly conserved across vertebrates, various stages exhibit species specific differences. For example, in zebrafish, lens development does not progress through a hollow lens vesicle stage as it does in mammals (Greiling et al., 2010). Additionally, unlike mammals, zebrafish retinal vasculature development does not involve regression of the hyaloid vessels (Alvarez et al., 2007). Despite these small differences, the information gathered from different vertebrate animal models has enriched our understanding of the key molecular players and signaling pathways in embryonic eye development.

4. Congenital ocular malformations—Estimates of the prevalence of congenital ocular defects vary by region and method of ascertainment, but in general range from 1-10 cases per 10,000 births (Royal National Institute of Blind People, RNIB; (Gregory-Evans et al., 2004)). Improper execution of any stage of early eye development can result in sight-threatening ocular malformations. For example, abnormal signaling from the midline (caused by defects in Shh, Nodal, and RA signaling or mutations in *Six3*) can cause failure of the eye field to properly segregate into two bilateral domains, resulting in ocular hypotelorism or cyclopia in extreme cases, as well as holoprosencephaly (Belloni et al., 1996; Roessler et al., 1996). Another group of ocular defects associated with abnormal morphogenesis includes microphthalmia (small eye), anophthalmia (absence of an eye), and coloboma (failure of choroid fissure closure), collectively called MAC. Mutations or altered gene dosage of several of the TFs and signaling molecules active during early eye development, such as *VAX*, *SOX10*, *OTX2*, *RAX*, *SOX2*, *PAX2*, *PAX6*, *SIX3*, and *SHH* can result in MAC phenotypes in humans (Bondurand et al., 1999; Dressler and Woolf, 1999; Lequeux et al., 2008). Although genetic factors contribute significantly to the aetiology of MAC, the causative mutations identified to date account for less than 20% of all cases (Gregory-Evans et al., 2004). Further investigations in animal models should help to identify novel MAC-causing genes.

Defects in ocular morphogenesis may also cause anterior segment dysgenesis, resulting in malformations of the lens, iris, cornea, or ciliary body. Mutations in *PAX2* and *PAX6* can cause aniridia (absence of the iris), isolated cataracts, and Peters anomaly (persistent adhesion between the cornea and lens) (Glaser et al., 1992; Hill et al., 1992; Otteson et al., 1998; Abouzeid et al., 2009; Bower et al., 2012). Mutations in *PITX*, *FOX* and *MAF* family members cause cataracts, anterior segment mesenchymal dysgenesis, and severe iris

anomaly (Blixt et al., 2000; Semina et al., 2001; Komatireddy et al., 2003; Cheong et al., 2007; Shaham et al., 2009). Congenital cataracts are also highly prevalent in patients carrying mutations in lens intrinsic membrane proteins, gap-junction membrane channel proteins, and crystallins (Shiels and Bassnett, 1996; Berry et al., 2000; Graw, 2003).

Finally, ocular malformations are often associated with more extensive syndromes that involve defects in the development of the heart, nervous system, skeleton, or other tissues. Some examples are CHARGE syndrome (coloboma, heart anomaly, choanal atresia, retardation, genital and ear anomalies), Anophthalmia-Esohageal-Genital (AEG) syndrome, and Coffin-Siris syndrome (systemic abnormalities like skeletal defects, neurologic defects, cardiac malformations, renal and genital defects) (Onwochei et al., 2000; Hornby et al., 2003; Gregory-Evans et al., 2004; Kelberman et al., 2006).

II. SOXC proteins

II.1 SOX subfamilies in vertebrates

The SOX proteins are transcription factors named after a shared motif called the SRY box, a high-mobility-group (HMG) DNA binding domain homologous to the DNA-binding domain of the mammalian sex determining region on the Y chromosome (*SRY)*. SOX proteins are found extensively throughout the animal kingdom, including nematodes, arthropods, birds, fish, reptiles, amphibians, and mammals (Bowles et al., 2000). In vertebrates about 30 *SOX* genes have been identified that are placed into 8 subgroups (A-H) on the basis of sequence similarity and genomic arrangements (Bowles et al., 2000; Schepers et al., 2002). Two new subgroups I and J have been introduced to include *Xenopus sox31* and a new invertebrate *C. elegans sox* gene. The HMG box allows SOX proteins to recognize a specific linear DNA sequence 5′-(A/T)(A/T)CAA(A/T)G-3′in the minor grove of the DNA helix, and it also helps SOX proteins to bind four-way DNA junctions without any sequence specificity (Ferrari et al., 1992). The DNA bending causes the width of the minor groove to widen and also results in possible unstacking of base pairs.

By regulating target gene transcription or posttranslational modification, SOX proteins are involved in multiple developmental processes (Wegner, 2010), including embryonic eye development. Mutations in SOX2, which belongs to the SOXB subfamily, cause anophthalmia or microphthalmia (Faivre et al., 2006). Sox2 is also required for the induction of the first lens specific gene *δ-crystallin* (Kamachi et al., 2001). Sox9, a SOXE family member, is required for expression of several retinal genes (such as c*alb2a, calb2b, crx, neurod, rx1, sox4a* and *vsx1)* and for the differentiation of Müller glia and photoreceptors (Yokoi et al., 2009). In addition, Sox9 can also regulate visual cycle gene expression in the RPE (Masuda et al., 2014), and a recent study in *Sox9*$^{-/-}$ mice demonstrates that Sox9 and Sox10 are both required for the formation of lacrimal gland (Chen et al., 2014).

II.2 SOXC proteins and their known target genes

Recently, a role for the SOXC proteins in regulating eye development has emerged from studies in animal models and in humans. The SOXC family in invertebrate animal species consists of a single member (Cremazy et al., 2001). In most vertebrates, the SOXC family includes three intronless genes: *SOX4* [MIM: 184430], *SOX11* [MIM: 600898], and *SOX12*

[MIM: 601947] (Penzo-Mendez, 2010). SOXC proteins are known as transcriptional activators. Apart from the HMG domain in the N-terminus, a transactivation domain (TAD) in the C-terminus is present among the SOXC members. Like other *SOX* members, the HMG domain of *SOX4*, *SOX11*, and *SOX12* is greater than 50% identical to the corresponding domain of SRY, both at nucleotide and amino acid level. Within the subgroup, SOXC proteins share a high degree of similarity within the HMG box domain as well as flanking sequences and the TAD domain (Koopman et al., 1991; Bhattaram et al., 2010), and are strongly conserved across vertebrate species. Among SOXC proteins, SOX4 is the most efficient in DNA binding due to the lack of the acidic domain and SOX11 has the highest transactivation activity because of its continuous α-helical structure (van de Wetering et al., 1993; Wiebe et al., 2003; Dy et al., 2008; Hoser et al., 2008; Penzo-Mendez, 2010).

Although they are essential and play multiple roles in embryonic development and tissue differentiation, very few SOXC target genes have been identified. One known target gene is classIII β-tubulin (known as *Tubb3* or *Tuj1*). It is a pan-neuronal gene expressed specifically in the nervous system and is critical for neurogenesis (Memberg and Hall, 1995). Sox4 and Sox11 can transactivate the lacZ reporter driven by the *Tubb3* promoter and directly interact with the sequence upstream of *Tubb3* in DNA-binding gel shift assays (Bergsland et al., 2006). Sox12 can also bind to *Tubb3* but with a relatively lower transactivation activity (Hoser et al., 2008). Another SoxC target gene is *Tead2*, a downstream mediator of the Hippo signaling pathway. Several SoxC binding motifs are present in the promoter region and first exon of *Tead2*, and direct binding has been detected both in vitro and in vivo by EMSA and chromatin immunoprecipitation assay (ChIP) (Bhattaram et al., 2010). In addition, Sox4 can bind to the T-cell specific enhancer at the 3′ end of the *CD2* gene and transactivate *CD2* (Wotton et al., 1995). Moreover, SoxC proteins are also involved in target gene stability. For example, in response to DNA damage, Sox4 can directly bind to p53 and enhance its acetylation, thus inhibiting Mdm2-mediated p53 ubiquitination (Pan et al., 2009).

SOXC family members are expressed extensively in the developing vertebrate nervous system and are known to regulate neuronal and mesenchymal progenitor survival, neuronal cell fate determination and differentiation in a highly redundant manner (Dy et al., 2008; Bhattaram et al., 2010; Bergsland et al., 2011). Expression of *SoxC* is strong in the ventricular region of the brain, particularly in cells that have exited the cell cycle but have not yet differentiated into mature neurons (Bhattaram et al., 2010). As development progresses, *SoxC* expression is limited to the forebrain and caudal part of the spinal cord suggesting that *SoxC* is downregulated as the nervous system matures (Jankowski et al., 2006).

III. SOXC proteins and their functions during vertebrate eye development

III.1 SOXC expression in the developing vertebrate eye

In the embryonic vertebrate eye, *SoxC* genes are expressed in a partially overlapping pattern in both time and space (Maschhoff et al., 2003; Dy et al., 2008; Cizelsky et al., 2013; Pillai-Kastoori et al., 2014; Uy et al., 2014) (W.Wen, unpubl.data, 2014). They are expressed in

the neuroepithelium of the optic cup, in the cells of the surface ectoderm and lens placode, and also in the surrounding neural crest derived mesenchymal cells (Table 1). Their expression is initiated in the retina in the first group of cells that exit cell cycle to differentiate into RGCs. Their expression subsequently spreads throughout the ganglion cell layer and into the inner nuclear layer, along with the progression of retinal cell differentiation (Cizelsky et al., 2013; Jiang et al., 2013; Usui et al., 2013; Pillai-Kastoori et al., 2014) Expression of *Sox4* and *Sox11* coincides with the onset of expression of the ganglion cell marker *Brn3b* and amacrine cell marker *Islet1* (Jiang et al., 2013; Usui et al., 2013) (W.Wen, unpubl.data, 2014). In addition, *sox11* is also expressed in the developing lens (Pillai-Kastoori et al., 2014). As retinal neurogenesis progresses, *SoxC* expression is downregulated in mature retinal neurons. Very little expression is detected in the mammalian adult eye. In vertebrates that display persistent retinal neurogenesis, such as *Xenopus* and zebrafish, *sox4* and *sox11* continue to be expressed in the retinal progenitor cell niche called the ciliary marginal zone (CMZ) throughout adulthood (Cizelsky et al., 2013; Pillai-Kastoori et al., 2014) (W.Wen, unpubl.data, 2014).

III.2 Functional studies of SOXC proteins in animal models

In mice, global knockout of *Sox11* and/or *Sox4* is lethal due to common trunk defect in the ventricular chamber of the heart. However, *Sox12* knockouts are viable and fertile (Schilham et al., 1996; Dy et al., 2008; Hoser et al., 2008). Compound $Sox11^{+/-}; Sox4^{+/-}$ heterozygotes also die at birth, suggesting that optimal gene dosage of both *Sox4* and *Sox11* is critical for cardiac development.

$Sox11^{-/-}$ homozygous mutant mice embryos exhibit a range of ocular abnormalities, including Peter's anomaly, open eyelids, microphthalmia, and coloboma (Sock et al., 2004; Wurm et al., 2008). No eye defects were reported in $Sox4^{-/-}$ mutant mice, however this may be due to the early embryonic lethality of this model. Morpholino-mediated knockdown of *sox4* and/or *sox11* in zebrafish also causes ocular coloboma, indicating a conserved role for soxC proteins in regulating early ocular morphogenesis. Experiments in zebrafish revealed that the ocular phenotypes in Sox4-and Sox11-deficient embryos are caused by elevated Hedgehog signaling, and further demonstrated that Sox4 and Sox11 are required to negatively regulate expression of the Hh pathway ligands *shha* and *ihhb* (Pillai-Kastoori et al., 2014) (W.Wen, unpubl.data, 2014).

A persistent lens stalk and delayed lens maturation are also observed in SoxC knockout mice as well as in zebrafish and *Xenopus soxC* morphants (Wurm et al., 2008; Cizelsky et al., 2013; Pillai-Kastoori et al., 2014) (W.Wen, unpubl.data, 2014). Immunohistochemical analysis of $Sox11^{-/-}$ embryos revealed reduced mitotic profiles in the lens placode during lens invagination, suggesting that Sox11 is required for the separation of the lens vesicle from the surface ectoderm (Wurm et al., 2008). A recent study using lens-specific *Pax6* conditional knockout mice suggests that as lens development progresses, *Pax6* is required to suppress the expression of *Sox11* in the lens via *miR-204* (Shaham et al., 2013).

In addition to ocular morphogenesis and lens defects, retinal neurogenesis is also disrupted in SoxC-deficient animals. Jiang and others created *Sox4*, *Sox11*, and *Sox4/Sox11* conditional knockout mice using a *Six3*-Cre line, thereby removing *Sox4* and/or *Sox11* in the

eye field and ventral forebrain from E9 onwards. They observed a modest reduction in RGCs in the single knockouts, and a complete loss of RGCs as well as significant reductions in other retinal neurons in the *Sox4*/*Sox11*-null retina (Jiang et al., 2013). Loss of Sox4 and Sox11 function in mice also resulted in reduction in the expression of histone H3 acetylation at proneural genes such as *NeuroD*, suggesting that Sox4 and Sox11 may influence retinal progenitor cell competence and differentiation by creating a specific epigenetic state. The expression of the RGC marker *pouf4f1* was absent in the retinas of both Sox4- and Sox11-deficient *Xenopus* embryos, indicating a defect in RGC differentiation (Cizelsky et al., 2013). Apoptosis was also significantly increased in Sox4- and Sox11- deficient *Xenopus* retinas, indicating a potential cause for the smaller eye phenotype and the retinal disorganization in these animals (Cizelsky et al., 2013). Surprisingly, Sox4- and Sox11-deficient zebrafish embryos did not exhibit significant changes in the neurogenesis of RGCs in zebrafish, although ectopic retinal progenitor proliferation in the GCL was observed (Pillai-Kastoori et al., 2014) (W.Wen, unpubl.data, 2014).

Sox4 and *sox11* are both upregulated in a zebrafish model of chronic rod photoreceptor degeneration and regeneration (Morris et al., 2011), suggestive of their potential involvement in rod photoreceptor differentiation. Sox4- and Sox11-deficient zebrafish also display a reduction mature rod photoreceptors in the developing retina, supporting a role for SoxC factors in photoreceptor differentiation (Pillai-Kastoori et al., 2014). In contrast, gain-of-function studies using mouse retinal explants demonstrated that overexpression of SoxC factors interferes with the maturation and terminal differentiation of rods and cones (as well as Müller glia), suggesting that SoxC factors inhibit photoreceptor differentiation (Usui et al., 2013). It may be that SoxC factors have species-specific functions in photoreceptor development, or that photoreceptor progenitors are exquisitely sensitive to *SoxC* expression levels, such that too much or too little significantly compromises terminal differentiation.

IV. SOXC and human ocular defects

In conjunction with the data from animal models, evidence implicating SOXC factors in human diseases that affect eye development is beginning to emerge. In a sequencing screen of 79 patients with MAC phenotypes, we identified two coloboma patients with novel heterozygous mutations within the *SOX11* coding region (a G145C missense mutation and an in-frame 4 amino acid duplication at S351-354). We also defined a small segmental deletion, within which *SOX11* is the only protein-coding locus, in a patient with microphthalmia and agenesis of the optic nerve (Lo-Castro et al., 2009; Pillai-Kastoori et al., 2014). Tsurusaki et al. performed whole-exome sequencing on DNA samples from 92 patients with Coffin-Siris syndrome, a disorder characterized by developmental delay abnormalities of the fingers and/or toes, and abnormal facial features). Two patients were identified with two novel *de novo* heterozygous mutations in the HMG domain of *SOX11*. One of the patients exhibited vision defects, along with several other clinical features of Coffin-Siris syndrome (Tsurusaki et al., 2014).

One of the most common syndromes among coloboma patients is CHARGE syndrome. Mutations in Chromatin remodeler chromodomain-helicase-DNA-binding protein 7 (*CHD7)* are identified in 65% of patients with CHARGE syndrome (Vissers et al., 2004; Chang et

al., 2006). CHD7 regulates neurogenesis by epigenetically changing the chromatin structure of target genes and modifying their transcription activity (Kim and Roberts, 2013). Loss of CHD7 in mouse neural stem cells results in a significant reduction in neurogenesis as well as a loss of *SoxC* gene expression (Feng et al., 2013). Computational analysis of gene expression profiling data from the Cancer Genome Atlas Project (TCGA) revealed that *SOX4* and *SOX11* display the strongest correlation with *CHD7* expression (Feng et al., 2013). Several lines of evidence suggest that SOX4 and SOX11 are direct targets of CHD7. Given the coloboma phenotype present in Sox11 mutant mice and sox4/11-deficient zebrafish, it is tempting to speculate that altered expression of *SOX4* and *SOX11* also underlies the coloboma observed in CHARGE syndrome patients.

V. Conclusions and Perspectives

Extensive work has across several vertebrate models has begun to unravel the intricacies of ocular morphogenesis. One thing that we have learned from these studies is that a handful of signaling pathways control various aspects of oculogenesis and they are deployed re-iteratively throughout the course of embryonic eye development. These signaling pathways regulate the expression of several key TFs to pattern the developing eye into tissue-specific domains, and to control the precise and timely specification of progenitor cells for differing fates. SOX family members are critical regulators of embryonic development, and the SOXC family has been recently implicated in eye development in a variety of animal models. Although it is clear that mutation or loss of SOXC proteins results in defects in ocular morphogenesis, lens development, and retinal neurogenesis, we do not know all of the transcriptional targets of SOXC proteins in the eye. The future lies in the investigation and identification of SOXC target genes, and in understanding their mechanism of action during ocular development.

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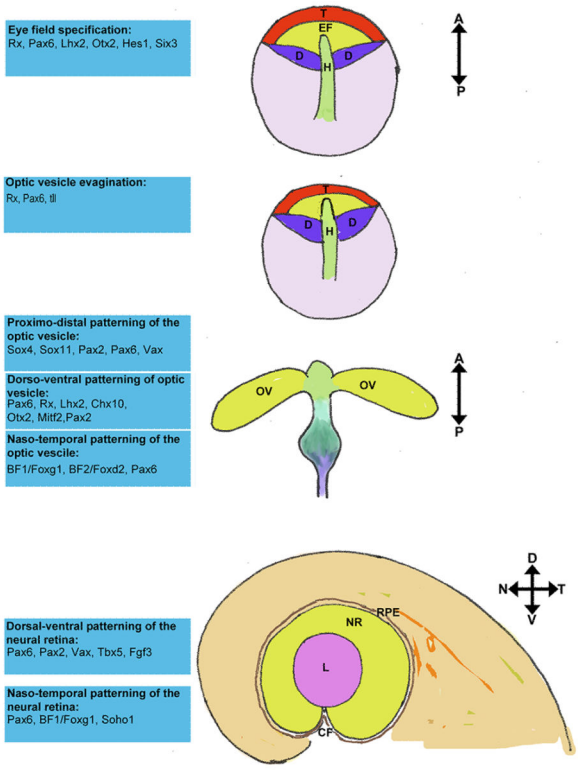

Figure 1.

Vertebrate ocular morphogenesis. A schematic representation of the major stages of eye development is shown, with the presumptive telencephalon (T, red), eye field (EF, yellow), hypothalamus (H, green), and diencephalon (D, purple) indicated within the anterior neural plate. The transcription factors (TF's) associated with each stage of eye development are indicated in blue boxes on the left of the figure. OV, optic vesicle; L, lens; NR, neural retina; RPE, retinal pigmented epithelium; CF, choroid fissure; A, anterior; P, posterior; N, nasal; T, temporal.

Table 1

SoxC expression in the developing vetebrate eye

	Eye (in general)	Lens	GCL	INL	ONL	CMZ	POM
Mouse	*Sox4* *Sox11* *Sox12*	*Sox11* *Sox12*	*Sox4* *Sox11* *Sox12*	*Sox4* *Sox11*			ND
Xenopus	*sox4* *sox11*	*sox11*	*sox4* *sox11*	*sox4*	ND	*sox4* *sox11*	ND
Zebrafish	*sox4* *sox11*	*sox11b*	*sox4b* *sox11a*	*sox4a* *sox4b* *sox11a*	*sox11a* *sox11b* *(regeneration)*	*sox4a* *sox4b* sox11a *sox11b*	*sox4a*
Chick	*Sox4*						
Lamprey	*Sox4* *Sox11* *Sox12*						

GCL: ganglion cell layer; INL: inner nuclear layer; ONL: outter nuclear layer; CMZ: ciliary marginal zone; POM: periocular mesenchyme; ND: not described.