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Retinoic acid signaling in mammalian eye development

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Abstract

Retinoic acid (RA) is a biologically active metabolite of vitamin A (retinol) that serves as a signaling molecule during a number of developmental and physiological processes. RA signaling plays multiple roles during embryonic eye development. RA signaling is initially required for reciprocal interactions between the optic vesicle and invaginating lens placode. RA signaling promotes normal development of the ventral retina and optic nerve through its activities in the neural crest cell-derived periocular mesenchyme. RA coordinates these processes by regulating biological activities of a family of non-steroid hormone receptors, RAR $\alpha/\beta/\gamma$, and RXR $\alpha/\beta/\gamma$. These DNA-binding transcription factors recognize DNA as RAR/RXR heterodimers and recruit multiprotein transcriptional co-repressor complexes. RA-binding to RAR receptors induces a conformational change in the receptor, followed by the replacement of co-repressor with co-activator complexes. Inactivation of RAR $\alpha/\beta/\gamma$ receptors in the periocular mesenchyme abrogates anterior eye segment formation. This review summarizes recent genetic studies of RA signaling and progress in understanding the molecular mechanism of transcriptional co-activators that function with RAR/RXR.

Keywords

anterior segment; lens; nuclear receptors; periocular mesenchyme; optic cup; retina; retinoic acid; vitamin A

1. Introduction

Following the formation of individual retinal and lens lineage-specific progenitor cells in late gastrulation and neurulation, the subsequent stages of eye development depend on a precise coordination of cell-to-cell interactions governing the formation of the individual tissue primordia, their morphogenetic movements, and terminal differentiation. The size of the eye depends on the precise control of cell number. These processes are needed to ensure that the eye can function as a neurosensory organ with its anterior section serving as an optical instrument, and its posterior section functioning in light detection, transformation and transmission of visual signals to the brain. Embryonic development is regulated through seven major signaling pathways - receptor tyrosine kinase (e.g. FGF-MAPK and Ras), TGF- β /BMP,

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hedgehog, Notch, Wnt, nuclear receptors, and Jak/STAT (Barolo and Posakony, 2002). Mutual cell-to-cell interactions in the developing embryo establish expression patterns of lineage-specific DNA-binding transcription factors in individual embryonic germ layers, and, as development proceeds towards the organogenesis, in the individual cell type progenitors and committed precursors. These factors, in concert with chromatin remodeling complexes, control expression of genetic information stored as a DNA-nucleosome polymer, the chromatin, in the nucleus (Allis et al., 2007). Through the process of chromatin remodeling, individual epigenomes, representing the organized genomic DNA in nuclei of different cell types, originate from a single fertilized egg. This enables individual cell types to generate their unique molecular “signatures” of expressed mRNAs and ncRNAs that determine their distinct phenotypes.

The major signaling pathways are repeatedly used at multiple stages of vertebrate eye formation as well as in the formation of other organs. Controlling a variety of processes, these signals operate in the embryonic and extra embryonic space, activating different components of these pathways to perform individual regulatory steps. This review describes the RA signaling pathway and its critical molecular components at specific stages of eye development. In addition, this review provides background information on the biochemistry of RA activated nuclear receptors, the potential use of RA signaling in eye regeneration, and the recruitment of RA pathway proteins as structural components of the cornea and lens.

2. The retinoic acid signaling pathway

Vitamin A (Fig.1) is an essential dietary nutrient and precursor of retinoic acid. Carotenoids (from plants) and retinyl esters (from animal oils) are stored in the liver and other tissues as esters (retinoids, e.g. retinyl palmitate and retinyl stearate) produced by lecithin-retinol acyltransferase (LRAT) and acyl-CoA:retinol acyl-transferase (ARAT). Hydrolysis of these esters by microsomal carboxylesterases and other lipases produces retinol, which is released into the bloodstream and forms a complex with retinol-binding protein 4 (RBP4). Retinol/RBP4 complex targets cells through its interaction with nine-transmembrane receptor, STRA6 (stimulated by retinoic acid gene 6) (Kawaguchi et al., 2007), see Fig. 2. In the cytoplasm, retinol associates with retinol-binding protein 1 (CRBP1). Retinol is oxidized to retinaldehyde via the action of a family of retinol dehydrogenases such as RDH10 (Sandell et al., 2007), Adh1/3/4 and Rdh1 (Molotkov et al., 2002a; Molotkov et al., 2002b) followed by its conversion to all-trans RA catalyzed by tissue-restricted and temporally regulated retinaldehyde dehydrogenases (in human, RALDH1/2/3/4; and in mouse Aldh1a1/2/3 and Aldh8a1). Recently, RALDH-independent generation of RA by CYP1B1, a member of the cytochrome p450 family of mono-oxygenases, has been shown in chicken (Chambers et al., 2007), but its role in RA synthesis in mammals requires further experimentation. The newly synthesized RA binds to the cellular RA-binding proteins, CRABP1/2. Upon ligand binding, a conformational change exposes a nuclear localization motif in CRABP2 and the apo-protein is transported into the nucleus. When the RA/CRABP2 complex enters the nucleus, the free RA ligand interacts with the receptors (see below), followed by a transcriptional activation of the target genes. Alternatively, the cytoplasm can release RA, which other cells can acquire through unknown mechanisms. Thus, RA can signal in an autocrine or a paracrine fashion (see Fig. 2). As RA enters the nucleus, it can bind to the nuclear RA receptors (RAR $\alpha/\beta/\gamma$) and retinoid X receptors (RXR $\alpha/\beta/\gamma$) (see Table 1), assembled as specific heterodimers (RAR α /RXR α , RAR β /RXR α and RAR γ /RXR α) at the retinoic acid-response elements (RAREs) in regulatory regions of RA-direct target genes. Genetic evidence suggests that RA is bound by the RAR and not by the RXR receptors *in vivo* (Kastner et al., 1997).

Cellular concentration of RA is also regulated by RA-degrading enzymes from the CYP26 class of p450 enzymes, CYP26A1 and CYP26C1, localized in the cytoplasm (see Fig. 2).

Temporally and spatially regulated expression of individual components of the RA signaling pathway and their antagonists generate concentration gradients of RA including zones free of RA along the anterior-posterior, dorso-ventral, and medio-lateral axes of the developing embryo. The localization of cells with active RA signaling and the identification of genes important for this pathway in individual ocular tissues are presented in sections 3.2. and 3.3., respectively.

3. Retinoic acid signaling in mouse eye development

3.1. Vertebrate eye development

The vertebrate eye is formed from cells of neural ectoderm origin, surface ectoderm, and neural crest derived periocular mesenchyme (POM). The earliest stages of eye development can be traced to the neural plate stage, where a single bilateral region, the *eye field*, is established as a result of subdivisions of the anterior neural plate directed by specific patterns of expression of BMP antagonists in concert with FGF and Wnt signaling pathways that pattern the neural plate (Fuhrmann, 2008; Yamamoto et al., 2005; Zuber et al., 2003; Zuber and Harris, 2006). The sonic hedgehog (Shh) signaling divides the eye field into two bilateral *eye primordia* (Chiang et al., 1996; Li et al., 1997). Elevation of the neural plate accompanies the formation of optic grooves. Following the process of neural tube formation, optic grooves balloon outwards to form optic vesicles from the walls of the developing forebrain. A combination of Shh, TGF- β and FGF signaling pathways subdivide the optic vesicle, causing the neuroepithelium of the optic vesicle to become the prospective retinal pigment epithelium (RPE), neuroretinal and optic stalk (Mui et al., 2002; Mui et al., 2005). Lens progenitor cells are formed from a transient population of cells, the *pre-placodal region* (PPR) (Streit, 2002, 2004). The surface ectoderm-derived PPR encircles the anterior neural plate, and, in addition to the lens lineage, gives rise to other cell types such as the anterior pituitary, the olfactory epithelium and the inner ear. The PPR is established as a result of a unique combination of signals (e.g. FGFs, antagonists of BMP and Wnt signaling pathways) from the underlying dorsal midline mesoderm, lateral mesoderm, and ectoderm (Bailey and Streit, 2006; Streit, 2007). The emergence of converged lens progenitors from the pre-placodal region is believed to result from a broad specification of various cell types originating from the PPR, followed by cell-sorting events combined with directed cellular migration. Morphologically, lens progenitor cells are visible as thickened surface ectoderm known as the lens placode which spatially corresponds to the area where the optic vesicle is contact with the surface ectoderm. A reciprocal interaction between the lens placode and the optic vesicle, gives rise to the lens vesicle and the optic cup. This process enables the prospective neuroretina to form the inner part of the optic cup. The prospective RPE is placed to the outer region of the optic cup (Mui et al., 2005). The partial optic cup grows circumferentially, eventually sealing over the choroidal fissure. The prospective neuroretina gives rise to retinal ganglions, cone photoreceptors, amacrine, horizontal, bipolar, Muller and rod photoreceptor cells. The most distal region of the optic cup assists in the formation of the iris. A regional distribution of components of the Wnt, sonic hedgehog, BMP and Notch signaling is thought to participate in the proximal-distal patterning of the optic cup (Baumer et al., 2002). From the marginal zone between the neuroretina and the RPE, the future ciliary body and a part of the iris are formed (Bharti et al., 2006).

The anterior segment is constructed from the surface ectoderm and the periocular mesenchyme (Chauhan et al., 2004). As the lens placode grows and sinks below the level of the surrounding ectoderm, the lens pit is formed. Following lens pit formation, proliferation of ectodermal cells restores its external surface. This layer of cells gives rise to the corneal epithelium. Finally, the lens vesicle detaches from the surface ectoderm and becomes fully invaginated into the partial optic cup (see above). The mesenchymal cells initially accumulate in the space between the neuroepithelium of the optic cup and the surface ectoderm. During lens placode formation, any

presence of mesenchymal cells inhibits morphogenesis of the lens placode. However, shortly after the lens vesicle is formed, mesenchymal cells start to migrate into the narrow space between the anterior epithelium of the lens vesicle and the surface ectoderm. The lens vesicle is polarized with its anterior cells retaining their epithelial morphology and proliferative capacity. In contrast, the posterior lens precursor cells exit the cell cycle and initiate terminal differentiation, there upon forming the primary lens fibers. Interestingly, the primary fiber cell differentiation proceeds asymmetrically, likely as a result of regionalized lens fiber cell differentiation signals, FGFs (Robinson, 2006), and BMPs (Faber et al., 2002). The earliest differentiating primary fiber cells are localized in the temporal side of the ventral lens while late differentiating primary lens fiber cells are localized on the nasal side. It is hypothesized that asymmetry in BMP signaling from the optic cup is responsible for this aspect of lens primary fiber cell differentiation (Faber et al., 2002).

The developing lens serves as a local organizer of the subsequent anterior segment development through the production of TGF- β and other signaling molecules (Ittner et al., 2005). As the number of cells between the lens and ectoderm continuously increases, the cells condense to form several layers of flat mesenchymal cells. Following the completion of lens primary fiber cell differentiation, the posterior mesenchyme cells closest to the lens flatten, connect with apicolateral contacts, and form a prospective corneal endothelium. Mesenchyme between the corneal endothelium and epithelium differentiate into keratocytes, the specific cell type of corneal stroma. These cells are responsible for the synthesis of the highly specialized and optically transparent extracellular matrix of the corneal stroma. During corneal endothelium terminal differentiation, the lens detaches from the corneal endothelium and a fluid-filled cavity, the anterior chamber, is generated.

Two additional morphogenetic events that employ the migrating mesenchymal cells are needed to complete the formation of the anterior segment. First, the anterior edge of the optic cup enlarges to form the epithelium of the iris and ciliary body. As the second wave of migrating cells reaches the angle between the developing cornea and anterior edge of the optic cup, these neural crest-derived cells differentiate into the stroma of the iris and ciliary body. The remaining mesenchymal cells reach the chamber angle and undergo a series of changes including additional migration, cell elongation, extracellular fiber and matrix production to form the trabecular meshwork (Chauhan et al., 2004).

3.2. Expression patterns of RA signaling components in the eye

Retinoic acid signaling is important for the embryonic development of a number of organs such as the central nervous system, heart, pancreas and pituitary, and sensory organs such as the eye, the ear, and the olfactory. Here, we summarize recent data on RA signaling in mouse eye development; general reviews on other systems can be found elsewhere (Hebert and Fishell, 2008; Maden et al., 2007; Mark et al., 2006; Niederreither and Dolle, 2008; Pan and Baker, 2007; Rawson and LaMantia, 2006; Romand et al., 2006).

The individual components of RA signaling show temporally and spatially restricted patterns of their expression. In many instances, there are at least two proteins/enzymes encoded by separate genes that perform similar functions in specific tissues. A possibility of paracrine RA signaling implies that the production of RA takes place elsewhere in the embryo. Taken together, it is not possible to predict how RA signaling actually operates without having a direct *in vivo* assay that can identify those specific cells/tissues with active signaling and synthesis of RA.

3.2.1. RA signaling activity in the developing mouse eye—The *in vivo* reporter systems employed to visualize RA signaling activity are based on the ability of multiple copies of RAREs to direct expression of the linked *lacZ* reporter gene in transgenic mice (Balkan et

al., 1992; Enwright and Grainger, 2000; McCaffery and Drager, 1993; Rossant et al., 1991), and green fluorescent protein (GFP) in transgenic zebrafish (Perz-Edwards et al., 2001). These studies show active RA signaling around neural plate formation and define the posterior section of the mouse embryo. Subsequently, the second expression domain is established in the developing eye and its surrounding cells, followed by new expression patterns during organogenesis of other systems (e.g. inner ear, gut, pharyngeal arches/heart, spinal cord, and telencephalon).

These reporter mice were crossed with a number of mice deficient in various components of RA signaling such as *Raldh1*^{-/-} (Fan et al., 2003), *Raldh2* (Mic et al., 2002; Molotkov et al., 2006; Niederreither et al., 2002), *Raldh1*^{-/-} and *Raldh1*^{-/-};*Raldh2*^{-/-} (Mic et al., 2004), *Aldh1a1*^{-/-}, *Aldh1a3*^{-/-}, and *Aldh1a1*^{-/-};*Aldh1a3*^{-/-} (Matt et al., 2005a), *CRBP1*^{-/-} (Matt et al., 2005b), *RAR* (Matt et al., 2008)), *Rdh10*^{-/-} (Sandell et al., 2007) or key genes that control eye development such as *Pax6* (Enwright and Grainger, 2000), to localize precisely cells with impaired RA signaling. Collectively, these studies show active RA signaling in the optic vesicle but not in the surface ectoderm of a seven-somite stage embryo (E8.5). The surface ectoderm exhibits active RA signaling from E8.75 (8-12 somite stage), through the lens placode, lens pit and lens vesicle stages. RA signaling is attenuated during primary lens fiber cell differentiation (E13.5). In the developing retina, RA signaling is active in the optic vesicle (E8.5-E8.75), in the prospective neuroretina and the RPE of the optic cup (E10.5), and in the developing retina (E11.5 and E13.5). From E10.25, RA signaling is active in the periocular mesenchyme (POM) prior to its migration to the anterior segment (see 3.1.) as well as in the surface ectoderm, the prospective corneal epithelium. These studies show where RA signaling is active in the embryonic eye; however, they do not show where the RA is produced, and whether it signals via autocrine or paracrine mechanisms. These issues have recently been resolved using conditional gene targeting of *RARα/β/γ* and gene ablation studies of *Raldh1/2/3* enzymes as described in section 3.3.

3.2.2. Expression pattern of RA nuclear receptors in mouse eye—Systematic immunolocalization studies of all six receptors, *RARα/β/γ* and *RXRα/β/γ* (see Table 1) in the embryonic (E10.5, 12.5, 14.5 and 17.5), neonatal (P1, 4, 7, 10 and 14) and adult mouse eyes were completed (Mori et al., 2001) after earlier genetic studies demonstrated that these receptors play major roles in mouse eye development (Kastner et al., 1994; Lohnes et al., 1994). *In situ* hybridization data (from E15.5 embryos to 2 month old adults) are also available (Grondona et al., 1996). Expression of *RXRβ* was ubiquitous at all examined stages (Mori et al., 2001). The main features of expression of the remaining five genes are subsequently described for the lens, the retina, the cornea and the POM and its derivative tissues (Table 1).

In lens, *RARα* is also expressed in all embryonic and postnatal stages described above. In contrast, *RARβ*, *RXRα* and *RXRγ*, are only expressed till E12.5 stage, i.e. in early differentiating primary lens fibers. No expression of *RARγ* was found in the mouse lens (Mori et al., 2001). In neuroretina, *RARα* and *RXRγ* are expressed at much higher levels compared to *RXRα*. Both these proteins show dynamic expression patterns during retinal embryogenesis and are eventually localized to specific retinal types, e.g. *RXRγ* in cone photoreceptors, and *RARα* in a subset of retinal ganglion cells (Mori et al., 2001). In the RPE, *RXRα* and *RXRγ* are expressed during the initial formation of this tissue (E10.5-E12.5). In contrast, *RARα* is expressed at all examined stages of RPE development (Mori et al., 2001).

RARα, *RARβ*, *RARγ* and *RXRα* nuclear receptors are abundant in the periocular mesenchyme (E10.5-E14.5). Subsequently, *RARα*, *RARγ* and *RXRα*, but not *RARβ*, are expressed both in the corneal epithelium and corneal stroma. *RARα*, *RARβ* and *RXRα* are expressed in the iris and ciliary body. *RARγ* and *RXRα* are both expressed in the conjunctival epithelium. *RARγ* is the prevailing receptor expressed in mouse sclera (Mori et al., 2001). Taken together, each

embryonic, postnatal and adult mouse ocular tissue expresses RXR β , and various combinations of individual RAR $\alpha/\beta/\gamma$ and RXR α/γ genes. Their functional significance for mouse eye development is evident from gene targeting studies of their combinations as described below (see section 3.3.).

3.2.3. Expression patterns of enzymes involved in the production and degradation of RA in mouse eye—Two sequential reactions convert retinol into retinaldehyde and RA (Figs. 1 and 2). Genetic evidence demonstrates that retinol dehydrogenase RDH10 is the key enzyme to catalyze the formation of retinaldehyde (Sandell et al., 2007). Rdh10 is expressed in specific tissues, i.e. in the neural tube and head mesenchyme of the E8.5 mouse embryo, and in the optic vesicle of the E10.5 embryo (Sandell et al., 2007). The complete expression pattern of Rdh10 in the whole eye remains to be determined; however, it is expressed in the RPE and in retinal Muller (Wu et al., 2002; Wu et al., 2004).

The oxidation of retinaldehyde to all-trans RA is catalyzed by retinaldehyde dehydrogenases (in human, RALDH1/2/3/4; and in mouse Aldh1a1/2/3 and Aldh8a1). RALDHs show dynamic expression patterns in mouse embryo that can be correlated with the expression of the *RARE-lacZ* reporter in transgenic mice as described above (see 3.2.1.). RALD2/Aldh1a2 is the first enzyme from this family of dehydrogenases, and is expressed in the primitive streak and mesodermal cells, and is restricted to the posterior part of the embryo. In the developing eye, Aldh1a2 is transiently expressed in the optic vesicle neuroepithelium of the E8.5 (10-somite stage) mouse embryo until E9.0, but not in the optic cup of the E9.5 embryo (Mic et al., 2004; Molotkov et al., 2006). Although Aldh1a2 is expressed in the developing muscles around the eye (at E11.5 and E13.5), it is not expressed in the adjacent POM (Matt et al., 2005b; Molotkov et al., 2006).

RALDH1/Aldh1a1 is expressed from E9.5 mainly in the dorsal part of the retina through E13.5, in the lens pit, lens vesicle and both in the lens epithelium and the primary lens fibers, and in the prospective corneal epithelium (Matt et al., 2005b). In contrast, RALDH3/Aldh1a3 is mainly expressed in the ventral retina, in the peripheral portion of the dorsal retina, in the RPE, in the future optic nerve, prospective lens ectoderm, lens placode/pit (Molotkov et al., 2006), and corneal epithelium (E10.5 to E13.5) (Matt et al., 2005a) but not in the optic vesicle neuroepithelium (Molotkov et al., 2006).

CYP26A1 and CYP26C1 are RA-degrading enzymes expressed in a narrow region between expression territories of RALDH1 and RALDH3 in the optic cup/retina (Sakai et al., 2004). This spatial distribution of enzymes producing and degrading RA creates three regions along the dorsoventral axis of the developing retina: a ventral region with high RA levels, a dorsal region with lower amounts of RA, and a transitional zone free of RA (Matt et al., 2005a). It is hypothesized that this mechanism controls local concentration of morphogen, as both insufficient concentrations of RA or its excess can cause specific developmental abnormalities.

3.2.4. Expression patterns of retinol-, retinaldehyde- and RA-binding proteins in mouse eye—The retinol-binding proteins 1 to 3 (CRBP1/2/3, official symbols RBP1, RBP2 and RBP3) and cellular RA-binding proteins, CRABP1/2 are members of a superfamily of fatty acid binding proteins with molecular weights of ~16 kDa. These are ubiquitously expressed proteins in the mouse embryo localized primarily in the cytoplasm. However, CRABP2 can transport its ligands into the nucleus (see Fig. 2). Cellular retinaldehyde-binding protein (RLBP1) is a 36.5 kDa cytoplasmic protein highly expressed in the RPE, retinal Muller cells (Vogel et al., 2007), and the pineal gland. RLBP1 belongs to the lipid-binding SEC14-like protein family. Structurally similar proteins include the retinaldehyde-binding protein 1-like proteins 1 and 2 (RLBP1L1 and RLBP1L2) with ~45% amino acid identity to RLBP1. RLBP1L1 is mostly expressed in brain (Kong et al., 2006).

3.3. Mechanism of RA signaling in the eye: Lessons from mouse embryonic development

Gene targeting studies in mouse embryonic stem cells combined with the recent development of conditional knock out models of nearly all known genes involved in RA signaling have amassed a wealth of data for early mouse development, organogenesis, and tissue homeostasis (Mark et al., 2006; Niederreither and Dolle, 2008). The depths of these studies reached a critical level which led to the resolution of earlier ambiguities and controversies. Multiple independent studies uncovered that the most prevailing mode of RA signaling is paracrine but not autocrine (Fig. 2) function in mouse eye development (Matt et al., 2005a; Matt et al., 2008; Molotkov et al., 2006). Recent genetic studies additionally revealed two distinct phases of RA signaling required for eye development: optic cup formation and anterior segment formation (Duester, 2008).

During the period of growth of the optic vesicle neuroepithelium, formation of the lens placode, and the optic cup, *Raldh2* appears to be responsible for much of the RA synthesis in the optic vesicle (E8.5 to E9.0) until expression of *Raldh1* and *Raldh3* begins at E9.5 developing eye. RA signaling monitored in *RARE-lacZ* transgenic mice is active in both the optic vesicle and the surface ectoderm as well as in the periocular mesenchyme (Enwright and Grainger, 2000; Mic et al., 2004). In *RALDH2/Aldh1a2*^{-/-} mouse embryos, eye development is arrested when the reciprocal invagination between the lens placode and optic vesicle is not initiated (Mic et al., 2004). The primary defect of this process could be potentially tracked to a lack of RA signaling in the optic vesicle. However, studies of RA signaling in compound *Raldh1*^{-/-};*Raldh3*^{-/-} mouse embryos combined with the analysis of expression domains of *Raldh2* during the optic cup formation show that RA, produced by *Raldh2* in the mesenchyme adjacent to the temporal portion of the optic vesicle, acts in a paracrine fashion on the optic vesicle (Molotkov et al., 2006). As comprehensive markers of both lens lineage and retinal progenitors cells were not examined, we do not have additional information about the causal relationship concerning this abrogated developmental process. Nevertheless, from analysis of the *RARE-lacZ* reporter in *RARα*^{-/-} embryos, we do know that *RARα* nuclear receptor is a critical mediator of RA signaling in the optic cup (both in the prospective neuroretina and RPE) and in the lens pit, but not in the surface ectoderm and periocular mesenchyme (POM) of E10.5 mouse embryo (Matt et al., 2008). However, since adult *RARα*^{-/-} mutants have normal eyes (Li et al., 1993; Lohnes et al., 1993), this feature puts at issue whether the RA signaling observed at E10.5 embryonic eye has any significant functional relevance to eye development.

The early role of RA signaling in optic cup/lens placode invagination was corroborated by inhibiting retinol binding protein 1 (CRBP1) by yolk-sac injections of antisense oligonucleotides into 3- to 12-somite pair mouse embryos (Bavik et al., 1996). The phenotype of these embryos at E9.5 is similar to the *Pax6*^{Sey/Sey} mouse model which does not form the lens placode, although the optic vesicle reaches the surface ectoderm (Enwright and Grainger, 2000). In addition, RA signaling is reduced in *Pax6*^{Sey/Sey} embryos analyzed at E8.75, E9.5 and E10.5 (Enwright and Grainger, 2000). Expression of *Raldh3/Aldh1a3* is absent from the surface ectoderm, retina and POM in rat *Pax6*^{rSey/rSey} embryos (Suzuki et al., 2000). No data is available on *Raldh1* and *Raldh2* expression domains in *Pax6*^{-/-} embryos. In compound *Raldh1*^{-/-};*Raldh2*^{-/-} mouse embryos, RA produced by *Raldh3* in RPE, but not by the prospective lens ectoderm/lens placode, is the source of RA that acts on the neural retina for its invagination (Molotkov et al., 2006). Taken together, paracrine RA signaling generated by *Raldh2* from the temporal mesenchyme reaches the optic vesicle, and is required for lens pit/optic cup reciprocal invagination.

Given the dynamic expression patterns of individual *Raldhs* in the optic vesicle and optic cup, it has been proposed that RA signaling is involved in the dorso-ventral patterning of the optic cup (McCaffery et al., 1999; Wagner et al., 2000). However, E10.5 *Raldh1*^{-/-};*Raldh3*^{-/-} embryos maintain normal dorsal and ventral expression of *Tbx5*, and *Pax2* and *Vax2* (Matt et al.,

2005a; Molotkov et al., 2006). In addition, expression domains of Pax2 (Torres et al., 1996), Tbx5 (Chapman et al., 1996) and Vax2 (Mui et al., 2002) in the retina are established prior to the onset of RA function at E9.5 (Mic et al., 2004). Studies on *Raldh1*^{-/-}; *Raldh2*^{-/-}; *Raldh3*^{-/-} triple mutants that lack RA activity reveal that RA is required for ventral invagination of the optic vesicle that defines the junction between the ventral retina and optic stalk, but not for the dorsal invagination that defines the junction between the dorsal retina and RPE (Molotkov et al., 2006). The ultimate proof of paracrine RA signaling from the POM to the optic cup is based on the genetic ablation of all three *RARα/β/γ* genes by the neural crest cell-specific Wnt1-cre deleter (Matt et al., 2008). Complete removal of *RARα/β/γ* genes only in the neural crest derived POM (Matt et al., 2008) recapitulates all eye malformations observed in earlier gene targeting studies in ES cells of various combinations of two inactivated RA nuclear receptors (Grondona et al., 1996; Kastner et al., 1994; Lohnes et al., 1994).

The second phase of the anterior segment formation dependent on RA signaling involves completion of corneal, lens and vitreous body development. For corneal development, the sources of RA are *Raldh1* (*Raldh3*) expressed in the dorsal (ventral) neural retina (Molotkov et al., 2006). In addition, *Raldh1* is expressed in the lens, albeit at lower levels (Matt et al., 2005a; Molotkov et al., 2006). The RA target tissue through the paracrine mechanism is the POM that migrates into the space between the lens epithelium and prospective corneal epithelium (Matt et al., 2005a; Matt et al., 2008; Molotkov et al., 2006). RA produced by *Raldh1* and *Raldh3* controls apoptosis in the POM (Matt et al., 2005a; Molotkov et al., 2006) by regulating expression of *Eya2* and *Pitx2* (Matt et al., 2005a). This process, in turn, limits cell number and/or morphogenetic movements of the POM prior to the formation of corneal endothelium and stroma (Matt et al., 2005a; Molotkov et al., 2006). In addition, loss of *RARβ* and *RARγ* in the periocular mesenchyme via Wnt1-cre generates similar ocular defects in the anterior segment such as a ventral rotation of lens, a thick layer of mesenchyme between the lens and surface ectoderm, and an abnormal corneal stroma, similar to those found in *Raldh1*^{-/-}; *Raldh3*^{-/-} embryos (Matt et al., 2005a). In summary, three types of paracrine RA signaling processes function in the mouse eye: First, *Raldh2* produced in the mesenchyme signals to the neuroepithelium for optic cup formation; second, *Raldh3* from the RPE produces RA for the prospective neuroretina; and, third, *Raldh1* and *Raldh3* both produce RA in epithelial tissues (neuroretina, lens and prospective corneal epithelium) that reaches the mesenchymal cells required for anterior segment formation (Molotkov et al., 2006).

The potential role of *Raldh3* expression in the lens placode for the reciprocal invagination that forms the optic cup/lens pit requires additional experimentation. The invaginating lens pit is an earlier and a stronger expressor of *Raldh3* compared to the RPE (Molotkov et al., 2006). Exclusion of mesenchymal cells from the space between the lens placode and optic vesicle is critical for the initiation and completion of this invagination (Furuta and Hogan, 1998). Additional roles for *Raldh3* in eye development are supported by its genetic control via Pax6 (Suzuki et al., 2000).

Less attention was given to the potential role of RA signaling in later stages of lens development. The most severe lens defects, i.e. abnormal lens fibers, corneal-lenticular stalk and agenesis of lens, were observed in *RARα/RARγ* double null embryos (Lohnes et al., 1994). Ventral rotation of lens was a hallmark of *RXRα*^{-/-}; *RARα*^{+/-} and *RXRα*^{+/-}; *RARγ*^{-/-} E14.5 double mouse embryos (Kastner et al., 1994). Lens cataracts associated with posterior lenticonus were observed in *RARβ2*^{-/-}; *RARγ2*^{-/-} eyes (Grondona et al., 1996). Using a *αA*-crystallin promoter fragment, lens-specific expression of *RARα* from E12.5 generated microphthalmia, i.e. reduced lens/eye size, which caused abnormal lens fiber cell differentiation and cataract formation (Balkan et al., 1992). Ectopic expression of cellular retinol binding protein I (CRABP1) in differentiating lens fibers resulted in abnormal

secondary lens fiber cell differentiation (Perez-Castro et al., 1993) likely through the increase of cellular capacity to sequester retinol.

4. Structural and functional properties of retinoic acid receptors and their co-activators and co-repressors

4.1. RA nuclear receptors

RARs and RXRs have conserved modular structures similar to other nuclear receptors as shown in Fig. 3 (Alvarez et al., 2007; Chambon, 1996; Mangelsdorf et al., 1995). Their variants differ from one another in the variable region A/B. These variants arise from the differential use of two promoters and the presence of alternative splicing (Chambon, 1996). The core DNA binding sequence of 66 amino acid residues contains two zinc finger-like motifs (Lee et al., 1993). Most of the RAREs are composed of two hexameric motifs, 5'-(A/G)G(G/T)TCA-3', arranged as palindromes, direct repeats (DR), or inverted repeats (IR) as shown in Fig. 3B. The most frequent are direct repeats with 1, 2 or 5 nucleotide spacing (see Fig. 3C), termed DR1, DR2 and DR5 elements (Bastien and Rochette-Egly, 2004; Glass, 1996). DR1 exhibit different polarity of binding of the liganded RAR subunit compared to the DR2 and DR5 elements (see Fig. 3D). In the DR1 RARE type, an upstream half site is recognized by a RAR and this type of RARE-bound complex acts as a transcriptional repressor (Bastien and Rochette-Egly, 2004). In contrast, in DR2/DR5 sites, a RAR molecule occupies the downstream halves of these RAREs and the nuclear receptor complex functions as transcriptional activator (Glass, 1996). Additional arrangements of two or three hexameric motifs with variable spacing have also been identified (Chambon, 1996).

RA nuclear receptors can also be recruited to DNA via transcription factors Sp1 and Sp3 that employ GC-rich promoter binding sites (Chen et al., 2007; Lehmann et al., 1992). Studies of transcriptional regulation of the BLR1 gene suggest that ligand-activated RAR/RXR can also bind two adjacent GT-rich sequences (boxes) approximately 1 kb upstream from its promoter and that a DNA loop is formed between these RA receptors and Oct1, NFATc3 and CREB2 factors bound between the promoter and the more distal GT-boxes (Wang and Yen, 2004). Thus, RA signaling is mediated both by a variety of RAREs (Fig. 3) and through other alternate mechanisms that include transcription factors Sp1 and Sp3, or GT-rich sites.

The ligand-binding domain is organized as a canonical “antiparallel α -helical sandwich” fold comprised of 12 α -helices and one antiparallel β -sheet arranged in three layers (Alvarez et al., 2007). In the absence of RA, the RAR/RXR heterodimer generates a surface recognized by co-repressor proteins NCoR1 or NCoR2 (SMRT), and their associated chromatin remodeling enzymes, such as histone deacetylases (HDACs) or protein methyl transferases (PMTs). NCoRs interact with RARs via two nuclear-receptor-interacting domains that share a core motif LXX(I/H)IXXX(I/L) (Perissi and Rosenfeld, 2005). Binding of RA ligand induces a series of conformational changes with the most prominent role played by helix 12. The “conformational switch” enables the receptor to recruit histone acetyltransferases (HATs) and other complexes (see 4.2) that activate gene expression.

Many nuclear receptor binding proteins such as NCoA1, NCoA2, NCoA6 and CBP use their LXXLL helical motifs to recognize a structural pocket in the C-terminal region of the nuclear receptor (Heery et al., 1997; Perissi et al., 1999). Similarly, three subunits of the SWI/SNF complex, BAF250/OSA1, BAF170 and BAF60c, also contain LXXLL nuclear receptor interaction motifs (Flajollet et al., 2007). Two 13 amino acid extended LXXLL motifs are also found in the TRAP220 (PPAR-binding protein, PBP), a mediator complex subunit 1 (Coulthard et al., 2003). The mediator complex is required for induction of RNA polymerase II transcription by DNA-binding transcription factors (Sato et al., 2004).

4.2. Co-activators and co-repressors and chromatin remodeling

RA nuclear receptors regulate transcription via at least two distinct mechanisms, through chromatin remodeling (Niederreither and Dolle, 2008; Perissi and Rosenfeld, 2005; Rosenfeld et al., 2006) and via recruitment of RNA polymerase II, mediator complex, and TBP-associated factors to the promoter (Bastien and Rochette-Egly, 2004; Ito and Roeder, 2001). These are not mutually exclusive as they collaborate with each other. Typically, the *unliganded* RAR/RXR heterodimer interacts with a variety transcriptional co-repressors assembled as multiprotein complexes with molecular weights significantly larger than the core histone octamer (~ 108 kDa). The major direct partners of the RAR/RXR/DNA ternary complex are two large proteins, NCOR1 (270 kDa) and NCOR2 (SMRT, 274 kD) that recruit histone deacetylases HDAC1 and HDAC2 via the SIN3A/B proteins. Evidence exists that histone methyltransferases that methylate H3 K9, K20 and K27 residues are also recruited to unliganded RARs. In addition, protein demethylases such as JMJD3 co-localize with NCOR2 (Jepsen et al., 2007). In contrast, NRIP1 (RIP140), a 127 kDa protein with nine LXXLL motifs, functions as a ligand-dependent co-repressor. NCoRs are ubiquitously expressed proteins as consistent with their roles to repress RA target genes in the absence of the RA ligands.

Ligand-bound RARs exert their chromatin remodeling function via recruitment of histone acetyltransferases (HATs), histone methyltransferases (HMTs), and ATP-dependent chromatin remodellers (Niederreither and Dolle, 2008). Use of an *in vitro* reconstituted system with a chromatin associated DR5-regulated promoter suggested that chromatin remodeling is initiated by acetylation of histones followed by ATP-driven chromatin remodeling (Dilworth et al., 2000). Mapping of physical interactions suggest that BAF60c proteins, subunits of the SWI/SNF complex, associate with RAR/RXR heterodimers via the LXXLL motif, and this interaction is ligand-dependent (Flajollet et al., 2007). RAR/RXR heterodimers also bind p300, CBP, and PCAF HATs. Expression of p300 and CBP has been shown in the mouse embryonic eye (Yang et al., 2007). Lens-specific expression of fragments of viral oncogenes that inhibit the function of p300 and CBP interferes with normal lens development and crystallin gene expression (Chen et al., 2002a; Chen et al., 2002b; Chen et al., 2004).

NCoA6 (other synonyms AIB3, ASC-2 and NRC) is a multifunctional 250 kDa coactivator that interacts with nuclear receptors via its two LXXLL motifs, and with other proteins such as AP-1, CREB, E2F-1, NF- κ B and p53 (Mahajan and Samuels, 2008). Expression of the peptide encompassing the N-terminal LXXLL fragment in the transgenic mouse interfered with normal eye development resulting in microphthalmia, abnormal lens fiber cell differentiation, and posterior lenticonus (Kim et al., 2002). NCoA6 is a subunit of at least three multiprotein complexes, ASCOM, ALR and PTIP (Mahajan and Samuels, 2008). Although the number of individual proteins forming these complexes varies from 8 to 12, they always contain histone-lysine N-methyltransferases, MLL1/2/3/4. MLLs are large enzymes with molecular weights > 500 kDa with a 122 amino acid catalytical SET-domain localized near their C-termini. They specifically methylate H3 K4 residues. MLL3 is expressed in the developing eye, particularly in the optic vesicle (E9.0), in the lens vesicle, and in primary lens fibers (Brun et al., 2006).

TRAP220 (PPAR-binding protein, PBP), a subunit of the mediator complex, associates with not only nuclear receptors (Yuan et al., 1998) but also with the GATA family of transcription factors (Crawford et al., 2002). Mouse embryos engineered with PBP null mutation develop until stage E11.5 when they die due to cardiac failure. Their eyes show abnormal production of pigment by the RPE, a defective lens, and eye vasculature development (Crawford et al., 2002).

In conclusion, regulation of gene expression via RA signaling requires molecular mechanisms that can establish sharp differences in expression levels of important genes in adjacent but

differently specified-cell types during embryonic development. It is reasonable to postulate that RAREs are surrounded by additional *cis*-regulatory sites that interact with local, i.e. tissue-specific and/or tissue-restricted, transcription factors (Barolo and Posakony, 2002). The unliganded RAR/RXR/NCoR complex, assembled at the RARE, represents a robust block to gene activation, placing the transcriptional unit to the default state of repression (see Fig. 4). Binding of RA elicits conformational changes that shed off the NcoR from the RAR/RXR following recruitment of chromatin remodeling activatory complexes. This process may also allow another regulatory factor to gain access to DNA. Synergistic interactions between multiple DNA-bound transcription factors allow recruitment of different types of chromatin remodelers to proximal sites in the same or adjacent nucleosomes. These three principles, activator insufficiency, cooperative interactions between signal regulated transcription factors and local activators, and default repression together ensure gene activation at the right place and time (Barolo and Posakony, 2002). The precise order of the recruitment of chromatin remodeling complexes to the gene regulatory regions may vary depending on the position of *cis*-elements and the selectivity of DNA-bound transcription factors towards various complexes. In any case, current studies show cyclic recruitment and release of these complexes as well as proteasome-dependent degradation of the transcriptional activators (Bastien and Rochette-Egly, 2004; Perissi and Rosenfeld, 2005; Rosenfeld et al., 2006).

4.3. Orphan receptors and thyroid receptors in eye development

Several other nuclear receptors including orphan (Jetten et al., 2001) and thyroid (Harpavat and Cepko, 2003) receptors play major roles during eye development. The retinoid-related orphan receptors ROR α / β / γ comprise one orphan receptor subfamily (Jetten et al., 2001). RORs bind as monomers to the consensus binding motif 5'-TAA(A/T)NTAAGGTCA-3'. Ligand (to be discovered) binding to, or phosphorylation of ROR induces conformation changes like those described earlier for RAR/RXR complexes. Alternatively, RORs forms heterodimeric complexes with RXRs and associate with direct, inverted, or palindromic repeats of "core" motifs, 5'-AGGTCA-3' spanned by 0-7 nucleotides (Jetten et al., 2001). There is an established role for ROR β in the adult retina. ROR $\beta^{-/-}$ retina exhibit defects in cellular differentiation and exhibit massive cell death (Andre et al., 1998). Recently, ROR α has been shown to synergize with cone-rod homeobox transcription factor (Crx), to activate the short wave opsin (Opn1sw) promoter and associate *in vivo* with the locus control region (LCR) of medium wave opsin, Opn1mw (Fujieda et al., 2009).

Thyroid hormone receptor β 2 (TR β 2 encoded by *Thrb* gene), a member of the thyroid subfamily of nuclear receptors, is a ligand-activated transcription factor that is expressed in the outer nuclear layer of the embryonic retina. *Thrb* gene is required for the development of green (M-) cone photoreceptors (Ng et al., 2001) and for suppression of S-opsin expression (Roberts et al., 2006). Thyroid receptors (TRs) can bind to DNA as homodimers or heterodimers with RXRs, preferentially recognizing DR4 binding sites with core sequences AGGTCA (Ribeiro et al., 2001). Expression of TR β 2 is regulated by proneural basic helix-loop-helix transcription factor Neurod1 (Liu et al., 2008) and indirectly via Pax6 (Marquardt et al., 2001; Oron-Karni et al., 2008).

5. Genes regulated by RA signaling in the eye

Given the significance of RA signaling in eye development, the important question is to identify those genes that are directly regulated by RAR/RXR complexes. In 2002, a compilation study identified 532 genes regulated by RA signaling (Balmer and Blomhoff, 2002). However, only 27 of them were considered proven direct targets with functional RAREs (Balmer and Blomhoff, 2002). Expression of several RA receptors, RAR α 2, RAR β 2, RAR β 4 and RAR γ 2, is controlled via one or more RAREs in their promoters. In addition, cellular retinoic acid binding protein Crabp2 (Durand et al., 1992) and cellular retinol-binding protein Rbp1

(Husmann et al., 1992; Smith et al., 1991) are regulated via evolutionary conserved RAREs. Pertinent to the eye, RA direct target genes are also α B-crystallin (Gopal-Srivastava et al., 1998) expressed mostly in the lens, Ets1 transcription factor (Raouf et al., 2000), phosphoenolpyruvate carboxykinase (Scott et al., 1996), and FGF8, a growth factor expressed in the early telencephalon and regulator of the prospective neuroretina (Brondani et al., 2002).

Experimental data also support lens-specific γ F-crystallin (Chauhan et al., 2004; Kralova et al., 2002; Tini et al., 1993; Yang et al., 2004) as another RA-regulated gene. In the retina, complement factor H (Munoz-Canoves et al., 1990), ATP-binding cassette transporter 1 (Abca1) (Costet et al., 2003), and methionine sulfoxide reductase A (MSRA) (Pascual et al., 2009) are also considered genes directly regulated by RA signaling. CFH, ABCA1 and MSRA are thought to play major roles in degenerative retinal diseases. The link between RA signaling and the expression of these genes raise the possibility of therapies of retinal diseases via retinoids, their agonists, and antagonists.

6. RA signaling in eye evolution and during lens regeneration

During the eye evolution, development of the lens and the cornea is viewed as a major upgrade of the simple eye comprised of photoreceptors and pigment cells (Jonasova and Kozmik, 2008). Lens structural proteins, the crystallins, comprise a diverse family of multifunctional proteins. It has been proposed that elevated expression of these proteins in lens via lens-preferred DNA-binding transcription factors was a key step necessary to promote preexisting proteins into their “crystallin status” (Cvekl and Piatigorsky, 1996; Piatigorsky, 1992, 2003; Piatigorsky and Wistow, 1991). More recent studies of proteins highly expressed in cornea also showed their structural diversity and linked their expression with transcription factors preferentially expressed in the cornea (Swamynathan and Piatigorsky, 2008). Interestingly, expression of several aldehyde dehydrogenases including Raldh1 (Aldh1a1/Aldh1), Aldh3a1 (Aldh3) and Aldh1a9, and a I-crystallin, a closely related protein to the cellular retinol binding protein 1 (CRBP1), reached high levels in lenses and corneas of various species. These proteins functioned both in the RA signaling pathway and as UV light filters because of their NADP cofactors (E.C. 1.2.1.5), and likely as detoxifying enzymes.

Analysis of Aldh1a1^{-/-}; Aldh3a1^{-/-} mouse eyes revealed anterior and posterior subcapsular cataracts as well as cortical opacities (Lassen et al., 2007). Aldh3a1 represents 5-50% of water-soluble proteins in corneas of various species. This enzyme protects the eye from UV-B induced oxidative stress (Estey et al., 2007). Crystallin-level expression of Aldh1a1/Raldh1 was found in elephant shrew lenses, and this enzyme was called η -crystallin (Graham et al., 1996). Similarly, Ω -crystallin/Aldh1a9, abundant in both scallop lenses and corneas, is 64% identical to human Raldh1 and 61% identical to elephant shrew η -crystallin (Piatigorsky et al., 2000). Lenses of various diurnal geckos contain up to 12% of I-crystallin (Roll et al., 1996). I-crystallin binds 3,4-didehydroretinol, rather than retinol, as a ligand (Werten et al., 2000). The 3,4-didehydroretinol gives the lens a protective yellow color by absorbing short-wave radiation. These studies show that retinaldehyde dehydrogenases and CRBP1/2 genes represent a pool of genes from which natural selection can choose to elevate their expression for specific protective functions in both cornea and lens (Piatigorsky, 1998, 2003).

Lens regeneration is a transdifferentiation process that can take place in the eye of selected species. The lens is either formed from the dorsal iris via dedifferentiation of the pigment epithelium (“Wolffian regeneration” in a few species of salamanders) (Tsonis et al., 2004) or from the corneal epithelium (in *Xenopus laevis* and *tropicalis*) (Henry, 2003). Inhibition of retinal dehydrogenases by disulfiram inhibits lens regeneration in over 50% of cases (Tsonis et al., 2000). Similar results are found with the RAR antagonist AGN192890 which inhibits

all three RARs. Furthermore, weak expression of newt RAR δ , a homologue of mammalian RAR γ 1, is limited to the retinal ganglion cells of the adult eye. However, its expression is significantly induced in the regenerating lens (Tsonis et al., 2000)). Interestingly, both these treatments induce lens regeneration from ectopic places such as the cornea and the ventral iris. The follow-up use of a more specific RAR α antagonist, AGI-X2, also inhibits lens regeneration (Tsonis et al., 2002)). In addition, RAR α transcripts were found in dedifferentiated cells and in transdifferentiated lens epithelial cells. Taken together, RA signaling plays an important role in Wolffian lens regeneration.

7. Conclusions and perspectives

In this review, we summarized the most recent advances in our understanding of RA signaling during mouse eye development. The biology of retinaldehyde serving as a chromophore in photoreceptors, its recycling in the RPE, and its function in eye diseases are described elsewhere (Bok, 1999; Strauss, 2005; Travis et al., 2007). Three examples of human diseases associated with mutations in genes in RA and retinaldehyde metabolism include Matthew-Wood syndrome, fundus albipunctatus and Leber congenital amaurosis with mutations in STRA6 (15q24.1), RDH5 (12q13-14), and RDH12 (14q23.3), respectively (Golzio et al., 2007; Kawaguchi et al., 2007; Yamamoto et al., 1999). Thus, studies of vitamin A and its derivatives as signaling molecules and chromophores are important both for the understanding of eye embryonic development, ocular cell physiology, and homeostasis.

Despite the major progress in the understanding of eye development at the molecular level during the last 25 years, a number of interesting questions remain to be addressed. Efforts should be focused on the role of RA signaling in early eye development. RA signaling has been shown to control reciprocal formation of the optic cup and lens pit. For optic cup formation, the RA signaling acts non-cell autonomously as the RA is produced by the adjacent POM. It is not clear if RA signaling has any cell autonomous function in the invaginating lens placode. There is also another potential role of cell autonomous RA signaling in the POM as these cells inhibit lens placode formation. Conditional gene targeting of various combinations of participating genes should provide more definitive answers to these questions.

Although we do know a relatively small number of genes directly regulated by RA signaling in eye tissues, a comprehensive catalogue of these genes in individual ocular tissues can be generated using ChIP-on-chip and ChIP-seq studies as excellent antibodies recognizing individual RAR α / β / γ , RXR α / β / γ , ROR α / β and TR β 2 are already available. These methods are currently being explored to map interactions of Pax6 (Wolf et al., 2009) and other DNA-binding factors with chromatin in various ocular cell types. Studies of core histone modifications of eye-specific genes in individual cell types will clarify the relationship between specific DNA-binding factors such as RAR/RXR and surrounding nucleosomes as these factors serve as anchoring points for the recruitment of chromatin remodellers.

Better understanding of RA signaling will also require extensive research into two areas related to other signaling pathways. First, nuclear receptors not only respond to the RA ligand, but they also can integrate information derived from a plethora of external stimuli such as the phosphorylation, acetylation, sumoylation, ubiquitination and methylation of RA-activated nuclear receptors (Perissi and Rosenfeld, 2005). Postranslational modifications of RA-activated nuclear receptors enable a cross-talk of multiple signaling pathways, e.g. a cross-talk with receptor tyrosine kinase signaling (FGF/MAPK pathway) implicated in phosphorylation of nuclear receptors (Perissi and Rosenfeld, 2005; Rosenfeld et al., 2006). Second, some RA target genes are important signaling molecules such as FGF8 in the retina (Brondani et al., 2002) and in the developing inner ear (Hans and Westerfield, 2007). TGF- β receptor II and Smad2 are also regulated by RA signaling in this tissue (Butts et al., 2005). Thus, the cross-

talks between nuclear hormone signaling and the FGF-MAPK, TGF- β /BMP, hedgehog, Notch, Wnt, and Jak/STAT pathways as found in other developmental systems (e.g. central nervous system, ear, heart, olfactory, and pituitary) have to be examined in the eye.

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Abbreviations used in this paper

CBP, CREB-binding protein; HAT, histone acetyltransferase; POM, periocular mesenchyme; PPR, pre-placodal region; RA, retinoic acid; RPE, retinal pigmented epithelium; RARE, retinoic acid responsive element.

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All-trans-retinol (vitamin A)

All-trans-retinoic acid (tRA)

9-cis retinoic acid (9cRA)

Figure 1.
Chemical structures of retinol, all-trans RA, and 9-cis RA.

Figure 2. Schematic diagram of pathways involved in the retention, metabolism and function of retinoic acid (RA)

RA signaling can function as an autocrine and paracrine process.

Figure 3. Molecular structure of RA nuclear receptors and their DNA-binding mechanisms
 (A) RA nuclear receptors, like other members of the nuclear receptor superfamily, contain a variable N-terminal region (A/B), a conserved DNA binding domain (C, 66 amino acid residues), a hinge domain (D), a ligand binding domain (E, 220 amino acid residues), and a variable C-terminal region (F). Their overall length varies from 454 to 533 amino acid residues (Germain et al., 2006a,b). Forty-eight members of the nuclear receptor family were found in the human genome (Perissi and Rosenfeld, 2005).

Figure 4.

A schematic diagram illustrating the switch between repressed to activated gene expression. A hypothetical gene is regulated via three distinct *cis*-elements, one of them is RARE. A1 and A2 are target sequences for two distinct tissue-restricted local activators A1 and A2, respectively. The occupancy of site A1 depends on the type of complex assembled at the RARE. In contrast, site A2 associates with a weak activator, insufficient to activate the linked gene expression on its own. (A) Default repression. The RARE occupied by unliganded RAR/RXR complex and NCoRs form a strong repressive complex as different types of repressive chromatin remodelling activities (HDACs and enzymes that methylate H3 K9 and H3 K27 residues) are recruited to the local chromatin. (B) A conformation change in the RAR/RXR

complex induced by RA binding allows recruitment of chromatin remodeling complex(es) with specific chromatin remodeling enzymes (ChRE), and this process allows the local activator A1 to recognize its DNA-binding site. (C) Additional chromatin remodeling complexes (enzymes, ChRE1 and ChRE2) may be recruited to this regulatory region to generate a strong and cell-specific response to the RA signal.

Table 1

Retinoic acid (RAR) and retinoid X (RXR) receptors.

Gene	Major variants	Chromosomal Localization (human)	Prevailing expression domains in mouse eye (Mori et al. 2001)
RAR α	$\alpha 1, \alpha 2$	17q21.1	Neuroretina, RPE, periocular mesenchyme, lens, cornea, iris/ciliary body, choroid, sclera, conjunctiva
RAR β	$\beta 1, \beta 2, \beta 3, \beta 4$	3p24	Periocular mesenchyme, lens, iris/ciliary body, sclera
RAR γ	$\gamma 1, \gamma 2$	12q13	Periocular mesenchyme, choroids, sclera, cornea, conjunctiva
RXR α	$\alpha 1, \alpha 2$	9q34.3	Neuroretina, RPE, iris/ciliary body, cornea, conjunctiva
RXR β	$\beta 1, \beta 2$	6p21.3	Ubiquitous
RXR γ	$\gamma 1, \gamma 2$	1q22-q23	Neuroretina