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Keeping an eye on retinoic acid signaling during eye development

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Abstract

Retinoic acid is a metabolic derivative of vitamin A that plays an essential function in cell-cell signaling by serving as a ligand for nuclear receptors that directly regulate gene expression. The final step in the conversion of retinol to retinoic acid is carried out by three retinaldehyde dehydrogenases encoded by *Raldh1* (*Aldh1a1*), *Raldh2* (*Aldh1a2*), and *Raldh3* (*Aldh1a3*). Mouse *Raldh* gene knockout studies have been instrumental in understanding the mechanism of retinoic acid action during eye development. Retinoic acid signaling in the developing eye is particularly complex as all three *Raldh* genes contribute to retinoic acid synthesis in non-overlapping locations. During optic cup formation *Raldh2* is first expressed transiently in perioptic mesenchyme, then later *Raldh1* and *Raldh3* expression begins in the dorsal and ventral retina, respectively, and these sources of retinoic acid are maintained in the fetus. Retinoic acid is not required for dorsoventral patterning of the retina as originally thought, but it is required for morphogenetic movements that form the optic cup, ventral retina, cornea, and eyelids. These findings will help guide future studies designed to identify retinoic acid target genes during eye organogenesis.

Keywords

Eye; Organogenesis; Aldehyde Dehydrogenase; RALDH; Retinoic Acid

1. Vitamin A and Eye Development

Eye development involves interactions among cells derived from forebrain neuroectoderm (forming the optic cup, retina, and optic nerve), surface ectoderm (forming the lens and corneal epithelium), and neural crest-derived perioptic mesenchyme (forming the corneal stroma, eyelid folds, anterior chamber, sclera, and vitreous body). Incomplete closure of the choroid fissure (located in the ventral eye) during the final stage of optic cup formation results in ocular coloboma, a congenital eye defect that represents an important cause of childhood blindness or vision impairment [1,2]. Many cases of ocular coloboma are of unknown etiology and some may be caused by environmental influences such as vitamin A deficiency in humans [3] or animals [4]. During gestational vitamin A deficiency the eye is the most sensitive organ to malformations, thus demonstrating a major role for vitamin A in eye development [4]. Vitamin A (retinol) metabolism by alcohol- and aldehyde-metabolizing enzymes results in the production of retinoic acid (RA) which functions as a ligand for nuclear receptors that directly regulate gene expression [5]. A potential relationship may exist between impaired retinol metabolism and coloboma. For example, humans with missense mutations in the gene encoding serum retinol binding protein have been shown to exhibit ocular coloboma and retinal dystrophy [6]. These findings suggest that the availability of vitamin A in the diet and the ability to metabolize it to RA has an influence on human eye development, but the mechanism of vitamin A action is just beginning to be understood.

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Two protein families are involved in RA signal transduction, i.e. the RA receptors (RARα, β, γ) and the retinoid X receptors (RXRα, β, γ) which form RAR/RXR heterodimers when bound to a retinoic acid response element of a target gene [7,8]. RA is required as a ligand for only the RAR portion of RAR/RXR heterodimers, suggesting that RXR functions to facilitate proper DNA-binding of RAR [9]. RARα, β, γ are expressed in overlapping patterns in the eye during development resulting in significant functional redundancy [10]. Mice carrying single null mutations of each RAR exhibit very minor defects during embryogenesis and survive postnatally [11,12]. RARβ mutant mice exhibit a minor eye defect, i.e. persistence of the primary vitreous body as a retrolenticular membrane [13]. When two RARs are knocked out together many embryonic defects are observed in the eye and other organs and postnatal lethality is observed soon after birth [14,15]. Consistent defects of the eye in double RAR mutants include microphthalmia, coloboma of the retina and optic nerve, and abnormalities of the cornea, eyelids, and conjunctiva [14]. Thus, RARs mediate the functions of vitamin A as the defects observed are essentially the same as those seen during gestational vitamin A deficiency.

2. Role of Retinaldehyde Dehydrogenase in Retinoic Acid Synthesis

The first step of RA synthesis, oxidation of retinol to retinaldehyde, is catalyzed by either alcohol dehydrogenase (ADH) or short-chain dehydrogenase/reductase (SDR), the latter often referred to as retinol dehydrogenase (RDH) [5]. Genetic studies have identified several enzymes able to oxidize retinol in vivo including *Adh1*, *Adh3*, and *Adh4* [16,17] as well as *Rdh1* [18] and *Rdh10* [19]. Although oxidation of retinol to retinaldehyde may occur at higher levels in some tissues due to tissue-specific expression of *Adh1*, *Adh4* and *Rdh10*, this reaction is not tissue-restricted as it is also stimulated by *Adh3* and *Rdh1* which are widely expressed during embryogenesis [16]. Also, it should be stressed that retinol oxidation is reversible, and that multiple enzymes (RDHs and aldoketo reductases) have been reported to participate in the reduction of retinaldehyde to retinol [20].

The second step of RA synthesis, oxidation of retinaldehyde to RA, is catalyzed by three members of the aldehyde dehydrogenase (ALDH) family also referred to as retinaldehyde dehydrogenase (RALDH) [5]. *Raldh1* (*Aldh1a1*), *Raldh2* (*Aldh1a2*), and *Raldh3* (*Aldh1a3*) have unique non-overlapping expression patterns during development [21,22]. As *Raldh* genes are expressed in unique dynamic spatiotemporal patterns, this step of RA synthesis is tissue-restricted and time-restricted.

Our understanding of what regulates synthesis of the ligand RA during embryogenesis is just beginning to emerge. The precursor retinol is made available to all cells via serum retinol-binding protein (RBP4) which can interact with a membrane receptor (STRA6) to stimulate retinol uptake [23,24]. Cellular retinol-binding protein is an intracellular protein which facilitates uptake of retinol into cells and stimulates its reversible conversion to retinyl esters for storage [25]. Metabolism of retinol to RA takes place in specific tissues as observed in embryos using a sensitive RA-reporter assay in which a retinoic acid response element (RARE) is linked to a *lacZ* reporter gene. A transgenic *RARE-lacZ* mouse strain demonstrates that RA transcriptional activity can first be detected in embryos at 7.5 days of embryonic development (E7.5); at E7.5 RA activity is detected only posteriorly, but at later stages RA is also detected anteriorly in the head [26]. At E8.25, RA is detected only in the embryonic trunk [27], but at E8.5 RA is now also detected in the head including the optic vesicles which have just formed [26]. This suggests that endogenous RA synthesis initiates in the eye field near E8.5 (10-somite stage). *Raldh2* is first expressed at E7.5 in the trunk mesoderm and by E8.5-E9.5 displays expression in the eye that appears similar to the pattern of RA localization using the *RARE-lacZ* RA-reporter mouse [21]. Studies on *Raldh2*$^{-/-}$ embryos carrying *RARE-lacZ* have shown that RALDH2 is responsible for all RA activity seen from E7.5-E8.5 [27], and for some but not all RA activity observed in the head at E9.5 [21]. Further genetic studies have demonstrated that *Raldh1* and *Raldh3* generate RA in the eye field at E9.5-E10.5 [28,29]. RA generated posteriorly in the trunk by *Raldh2* is necessary for development of the posterior portions of the central nervous system including the hindbrain [30,31] and spinal cord [32,33]. RA generated in the head from E8.5-E10.5 by all three *Raldh* genes is unnecessary for early forebrain development [29], but all three participate in development of the eye whose neural components are derived from an out-pocketing of the forebrain neuroectoderm that forms the optic vesicles. These findings suggest that RA synthesis and signaling during eye development is a complex process.

3. *Raldh* Gene Expression in the Developing Eye

Raldh1, *Raldh2*, and *Raldh3* are expressed in unique non-overlapping tissues in the mouse embryonic eye field, and RA activity can be detected in those tissues plus surrounding tissues using embryos carrying the *RARE-lacZ* RA-reporter transgene (Table 1) [28]. *Raldh2* is the first source of RA synthesis for the eye field. By E9.0 it is clear that *Raldh2* is generating RA in the mesenchyme next to the temporal (lateral) side of the optic vesicle but not the nasal (medial) side prior to its invagination to form the optic cup. Also prior to invagination of the optic vesicle, *Raldh3* begins to generate RA in the surface ectoderm over the eye field at E8.75 and later in the dorsal retinal pigment epithelium (RPE) starting at E9.5. Between E9.5-E10.5, invagination of the optic vesicle occurs resulting in an optic cup with separate layers for neural retina and RPE folded around the lens vesicle that developed from invagination of the surface ectoderm which occurs at the same time. Expression of *Raldh2* and *Raldh3* changes during optic cup formation. *Raldh2* expression in the perioptic mesenchyme terminates at E10.0, and *Raldh3* expression initiates in the ventral neural retina at E10.5. In addition, *Raldh1* expression begins to generate RA in the dorsal neural retina at E10.5. From E10.5 onwards to birth (approximately E19.5), *Raldh1* and *Raldh3* continue to be expressed in the dorsal and ventral neural retina, respectively. *Raldh1* and *Raldh3* are the only sources of RA from E11.5–13.5 when the ventral folds of the optic cup fuse to form the choroid fissure at E13.5.

4. Effects of *Raldh* Gene Knockouts on Embryonic Eye Development

Genetic studies have demonstrated that *Raldh* gene knockouts are quite useful for studying the function of RA signaling during embryogenesis as they produce embryos that completely lack RA activity in certain tissues [21,27,28,34–36]. *Raldh* gene knockout mice have been used to sort out the individual contributions of each enzyme for eye development (Table 1). *Raldh1*$^{-/-}$ mice survive to adulthood and exhibit no noticeable defects in eye development [35]. *Raldh1* mutants initially do not completely lose RA activity in the dorsal retina due to compensation by *Raldh3*, but even though they completely lose dorsal RA activity from E16.5 onwards, retinal lamination is normal in adult mice and retinal ganglion axons reach the brain both dorsally and ventrally [35]. Thus, RA generated by *Raldh1* does not appear to be necessary for late stages of retina or optic nerve development. However, *Raldh1/Raldh3* double mutants exhibit mesenchymal overgrowth in the cornea and eyelids that is associated with a defective apoptosis program in perioptic mesenchyme [28,37]. Thus, *Raldh1* and *Raldh3* have completely redundant functions in generating RA that travels from the retina to the perioptic mesenchyme to control the amount of mesenchyme that reaches the anterior eye to form the cornea and eyelids.

Studies in this laboratory [28] and another [36] have demonstrated that *Raldh3*$^{-/-}$ embryos exhibit eye and nasal defects. *Raldh3*$^{-/-}$ mice die at birth due to a blockage of the nasal passages [36]. *Raldh3*$^{-/-}$ embryos begin the process of optic cup formation, but they exhibit a shortening of the ventral retina. These findings suggest that RALDH3 synthesizes RA required to complete ventral optic cup formation (including closure of the choroid fissure) and that RALDH1 cannot fulfill this function. As *Raldh1/Raldh3* double mutants still undergo most of the morphogenetic movements needed for optic cup formation, further studies have indicated that RA generated by *Raldh2* allows these movements to initiate but not proceed to completion [28].

Raldh2$^{-/-}$ embryos and *Raldh1/Raldh2* double mutants do not develop optic cups, but optic cup formation can be rescued by maternal RA administration from E6.75–E8.25 which rescues a lethal developmental defect that stalls eye development at the optic vesicle stage [38]. *Raldh3* expressed in rescued *Raldh2* mutants may be responsible for allowing optic cup formation. RA administered to *Raldh2*$^{-/-}$ embryos does not itself induce RA signaling in the eye field, but this treatment does result in relatively normal *Raldh3* expression in the eye field from E8.75–E10.5 which then results in relatively normal detection of RA signaling [28]. Examination of *Raldh2/Raldh3* double mutants and *Raldh1/Raldh2/Raldh3* triple mutants treated with RA to E8.25 (to rescue the early developmental functions of *Raldh2*) revealed that such embryos fail to produce RA in the eye field and do not form a complete optic cup; interestingly, the dorsal portion of the optic cup invaginates but the ventral portion does not invaginate resulting in an incomplete optic cup [28]. Thus, *Raldh2* and *Raldh3* both generate RA that stimulates the ventral morphogenetic movements of the optic vesicle that begin ventral optic cup formation. However, *Raldh3* must be present in order to complete the ventral morphogenetic movements that culminate in closure of the choroid fissure at E13.5, presumably due to its continued expression in the eye after *Raldh2* expression ends at E10.0.

The dorsal versus ventral expression patterns of *Raldh1* and *Raldh3* led to the hypothesis that RA may be involved in dorsoventral patterning of the retina and its axonal projections into the brain [39]. RA signaling in chick embryos has been reported to be necessary for expression of the retinal topographic guidance molecules *ephrinB2* (dorsally) and *EphB2* (ventrally) that function downstream of *Tbx5* (dorsally) and *Vax2* (ventrally) to provide dorsoventral patterning of the retina [40]. However, we found that *ephrinB2* and *EphB2* were still expressed in their correct locations in *Raldh1*$^{-/-}$;*Raldh3*$^{-/-}$ mouse embryos that lack retinal RA activity [28]. Thus, the previous studies which relied upon addition of a dominant-negative RA receptor may have affected *ephrinB2* and *EphB2* expression by disturbing an RA-independent mechanism that controls their expression [40]. In addition, *Raldh* compound mutants retain dorsal expression of *Tbx5* and ventral expression of *Vax2* in the optic cup while losing all RA activity, indicating that RA signaling is not required for establishing dorsoventral polarity in the retina [28,37].

Studies on gestational vitamin A deficiency have demonstrated that embryonic eye defects can be prevented or reduced by addition of RA to the maternal diet [41]. Similarly, eye defects in mouse *Raldh* mutants can be rescued by maternal dietary RA supplementation [28]. Thus, tools are now becoming available to manipulate both the enzymes controlling endogenous RA synthesis and the availability of dietary RA, making it possible to examine in more detail the mechanism of RA action in the eye.

4. Summary

The investigations reported here illustrate the usefulness of *Raldh* gene knockouts in revealing the mechanism of RA signaling in the developing eye. These findings indicate that RA signaling is initially required for ventral morphogenetic movements that allow a transition from optic vesicle to optic cup and closure of the choroid fissure. Immediately after optic cup formation RA is required to stimulate apoptosis in the perioptic mesenchyme needed to correctly generate the cornea and eyelids. In both of these processes *Raldh* genes generate RA that functions in a paracrine but not autocrine fashion (Table 1). For optic cup formation the bending of the ventral neural retina is controlled by RA generated outside the neural retina in the perioptic mesenchyme, dorsal retinal pigment epithelium, and surface ectoderm over the eye. Later, the perioptic mesenchyme becomes the RA target tissue but as *Raldh2* is no longer expressed there, the source of RA is *Raldh1* and *Raldh3* expressed in the neural retina. Although the three *Raldh* genes do show distinct expression patterns, one must keep in mind that RA synthesized in one region by one RALDH may diffuse to another region, and the extent to which this occurs has been extensively documented [28]. Thus, *Raldh2* and *Raldh3* are redundant for generation of RA needed to initiate optic cup formation, and *Raldh1* and *Raldh3* are redundant for generation of RA needed to control perioptic mesenchyme movements during anterior eye formation. Further studies on compound *Raldh* mutants should provide insight into the target genes regulated by RA that are needed to control eye morphogenetic movements. This information will be useful in evaluating potential roles for RA signaling in eye development and congenital eye disease.

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Table 1

Roles for retinaldehyde dehydrogenase genes in eye development determined by defects observed in compound *Raldh* gene knockouts. These results support a paracrine function for RA signaling in eye development as the target of RA action is adjacent to the site of RA synthesis.

Stage	Gene	Tissue Expression	Function for Eye Development
E8.5–E9.0	*Raldh2*	Optic vesicle	None detected
E8.75–E10.5	*Raldh3*	Surface ectoderm over eye field	Ventral invagination of optic cup
E9.0–E10.0	*Raldh2*	Perioptic mesenchyme (temporal side of optic vesicle)	Ventral invagination of optic cup
E9.5–E10.5	*Raldh3*	Dorsal retinal pigment epithelium	Ventral invagination of optic cup
E10.5-birth	*Raldh1*	Dorsal neural retina	Cornea and eyelid morphogenesis (apoptosis in perioptic mesenchyme)
E10.5-birth	*Raldh3*	Ventral neural retina	Cornea and eyelid morphogenesis (apoptosis in perioptic mesenchyme)