

ROLE OF VITAMIN A IN RETINAL DISEASES

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Vitamin A has an essential role in vision in that it forms the photosensitive pigments that absorb light and initiate the visual process. However, vitamin A and its analogues also have critical roles in maintaining the structural integrity of the retina. Disruption of the metabolism of vitamin A results in several blinding diseases. This review focuses on our recent studies on the role of a protein critical to the processing of vitamin A, RPE65. The absence or dysfunction of this protein causes the childhood blinding disease Leber congenital amaurosis.

Introduction

Vitamin A is a fat soluble alcohol essential for normal growth, development and reproduction. The structure for vitamin A (all-*trans* retinol 1), with other retinoids discussed here, are shown in Figure 1. This vitamin is also essential for vision, as it is the precursor to the molecule (11-*cis* retinal 3), that forms the pigments in the eye that absorb light [1]. Indeed, the first sign of vitamin A deficiency is night blindness, that is, a failure to see under dim light conditions. Vitamin A deficiency is rarely seen in developed countries as the vitamin is readily available from the carotenoids, such as β -carotene 2, found in a healthy diet. However, it is a problem in underdeveloped countries. There are innovative programs genetically introducing carotenoids into rice, which is consumed in great quantities in many of these countries [2].

The eye

The eye contains the photosensitive cells that convert light into neuronal signals, and these cells are found in the retina, which is at the back of the eye (Fig. 2). There are two families of these photosensitive cells: rods and cones. Rods mediate dim-light vision, and cones mediate color and bright-light vision. In the human retina, the distribution patterns of rods and cones differ: rods are found throughout the retina except for a tiny central area (0.22 mm in diameter) named the fovea, but cones are localized mainly within the fovea to mediate high-acuity central vision. Although both classes of photoreceptors contribute to sight, humans exhibit a far greater dependence on the cones for daily activity. Eye diseases that result in cone death within the fovea lead to the loss of central vision and a severe detriment to the quality of life of the individual. Although the relationship of night blindness to vitamin-A deficiency was recognized many years ago [1], the understanding that many eye disorders, particularly those involving retinal degeneration, are caused by some malfunction of the retinoid cycle is relatively recent. For a recent review see Kiser et al. [3]. The purpose of our research has been to understand this cycle and to study specific areas where the process fails. Our recent studies on one of these key components, RPE65, are here discussed.

The retinoid cycle

The role of the retinoid cycle is to supply the photoreceptors with the critical retinoid, 11-*cis* retinal 3 (Fig. 1), which is

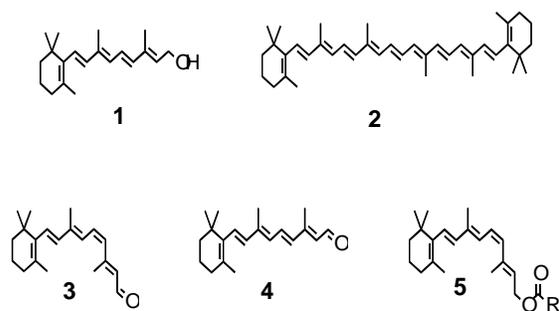


Figure 1. Retinoids important in the visual process: **1**, all-*trans* retinol (vitamin A); **2**, β -carotene; **3**, 11-*cis* retinal; **4**, all-*trans* retinal; **5**, retinyl-ester.

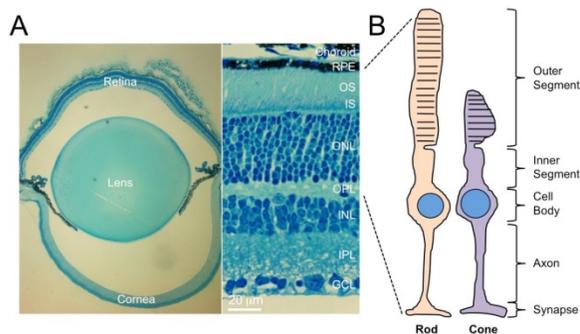


Figure 2. The vertebrate retina and rod and cone photoreceptors. (A) The left panel is a cross section of a mouse eye illustrating the retina located in the posterior portion eye (left); increasing the magnification shows that the retina is organized into multiple distinct layers (right). (B) Schematic of a vertebrate rod and cone photoreceptor cell. RPE = retina pigment epithelium; OS = outer segment; IS = inner segment; ONL = outer nuclear layer; OPL = outer plexiform layer; INL = inner nuclear layer; IPL = inner plexiform layer; GCL = ganglion cell layer. (Reprinted with permission from [5]; *Progress in Retinal Eye Research* is the copyright holder.)

essential for the formation of the photosensitive pigments that detect light. This isomeric form is extremely reactive and hard to synthesize in a test tube. There are many steps in the process and the mechanism is still not fully understood. The 11-*cis* retinal forms a covalent bond with the pigment apoprotein (opsin) specific to that photoreceptor and the resulting pigment is then light sensitive. The light sensitivity of the photoreceptor depends on the particular opsin. In

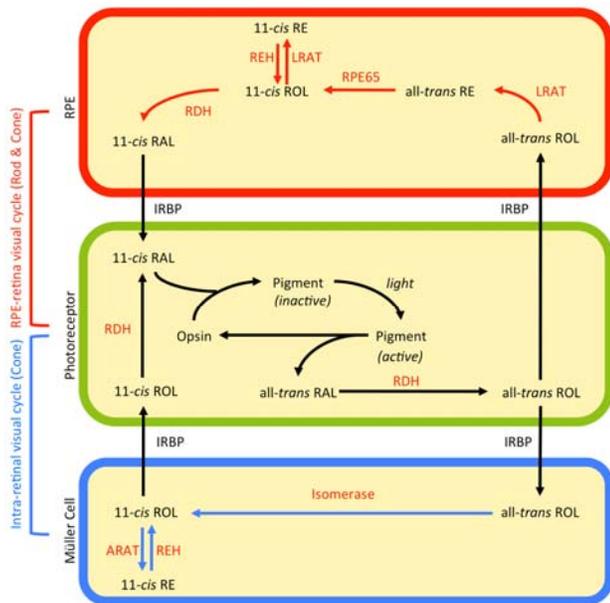


Figure 3. Retinoid cycles for rods and cones. The rods depend solely on the retina pigment epithelium (RPE) cycle for 11-*cis* retinal (11-*cis* RAL). The cones depend on both the RPE and the intraretinal cycles. RE = retinyl-ester; REH = retinyl ester hydrolase; RDH = retinol dehydrogenase; RAL = retinal; ROL = retinol; LRAT = lecithin-retinol acyltransferase; IRBP = interphotoreceptor retinoid binding protein; ARAT = acyl CoA-retinol acyltransferase. (Reprinted with permission from [8]; *Journal of Neuroscience* is the copyright holder.)

humans, there are three cone opsins and one rod opsin. The absorption of a photon causes an isomerization of the 11-*cis* bond to the *trans* form, all-*trans* retinal 4 (Fig. 1), activating the opsin protein, which is a G- protein coupled receptor, and initiating the visual process. The retinoid cycles for both rods and cones are outlined in Figure 3. For many years it was believed that a single cycle operated for both. However, the need for 11-*cis* retinal by the two classes of photoreceptors is different. The cones function under bright light conditions and need a constant, immediately available supply of the critical 11-*cis* retinal. The rods have a much slower process. Studies by many groups have resulted in the understanding that the classical retinoid process proceeding through the retinal pigment epithelium is not adequate to supply both the cone and the rod processes. The cones depend on both cycles, whereas the rods use only the rod cycle (for recent reviews, see Kiser et al. [3], Sari [4] Tang et al. [5]).

RPE65

The critical step in the visual process is the formation of the photosensitive pigments and this requires 11-*cis* retinal. RPE65 is an abundant protein in the retinal pigment epithelium. Its function was unknown, but when an animal model which was missing the protein was studied, it was recognized that no 11-*cis* retinal was being generated [6]. Thus, this protein is an essential component of the complex that isomerizes the all-*trans* form of retinal to the 11-*cis* form. The exact mechanism is still not fully elucidated, but RPE65 is key to the process of generating 11-*cis* retinal by the retinal pigment epithelium. It was initially proposed that RPE65 is a protein specific to the retinal pigment epithelium. However,

our group has identified the protein in the cones from several species (Fig. 4) [7] and recently in the human cones (Fig. 5) [8]. The protein is in the outer segment of the cones and unlikely to have a direct role in the isomerization process. Our studies support a role as a retinoid binding protein, possibly promoting hydrolysis of the 11-*cis* ester.

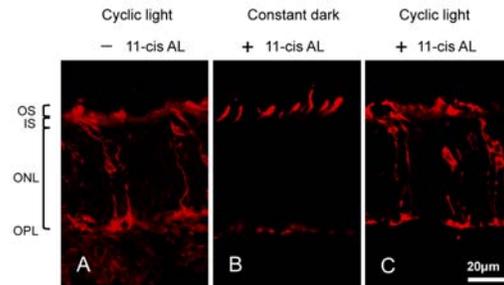


Figure 4. Lack of RPE65 results in visual pigment mislocalization in cones. Co-localization in cone photoreceptors of cross-sectioned, post-natal day 21, Rpe65^{-/-}Rho^{-/-} retinas. (A) Untreated mice; (B) 11-*cis* retinal-treated mice maintained in the dark; (C) 11-*cis* retinal-treated mice maintained in 12 hours light/12 hours dark conditions. The sections were stained with antibody for the mouse S cone opsin (red). The locations of the photoreceptor outer segment (OS), photoreceptor inner segment (IS), outer nuclear layer (ONL) and outer plexiform layer (OPL). The scale bar represents 20 µm. (Reprinted with permission from [7]; *Investigative Ophthalmology and Visual Science* is the copyright holder.)

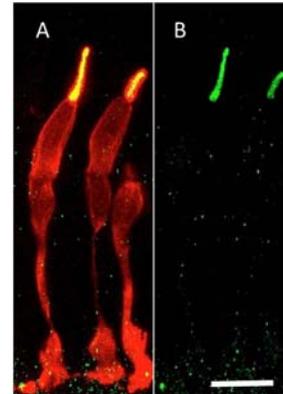


Figure 5. Localization of RPE65 in human cones. Cones are visualized by immunostaining for cone arrestin (CAR) in red. RPE65 is immunostained in green and found to be co-localized within the outer segment (yellow). (A) RPE65 staining alone in the same cone appears green. Images are acquired from sections prepared from a young donor eye (age 11 years). Scale bar = 10 µm. (Reprinted with permission from [8]; *Journal of Neuroscience* is the copyright holder.)

The role of RPE65 in humans

Mutations of the RPE65 protein are known to result in a childhood blinding disease, Leber congenital amaurosis Type 2 (LCA2) [9]. These mutations impair the ability of the RPE65 protein to generate 11-*cis* retinal and degeneration of the retina occurs. Animal studies gave us the unexpected finding that the cones actually degenerate more rapidly than

the rods in the absence of RPE65. We found this to be due to the mistrafficking of the cone visual pigment proteins (Fig. 5) [10]. This can be corrected by the administration of 11-*cis* retinal, but only if the animals are kept in the dark [11]. Interestingly, the cone opsins apparently require 11-*cis* retinal to move the opsin into the outer segment, while the rods do not. The purpose of our recent studies has been to understand the protein RPE65 and its involvement in promoting cone visual function in humans. The results of these studies have clinical implications for the treatment of Leber congenital amaurosis Type 2. This disease has been the focus of much attention as successful gene therapy to replace the defective *Rpe65* gene has been accomplished [12, 13]. However, for patients to have useful vision, early degeneration of cones must be prevented so that when the protein is introduced, the patients can function under normal daylight. Challenges certainly remain for the future.

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References

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1. G. Wald, *Science* 113, 287 (1951).
2. G. Tang, Y. Hu, S. A. Yin, Y. Wang, G.E. Dallal, *et al.*, *Am. J. Clin. Nutr.* **96**, 658 (2012).
3. P. D. Kiser, M. Golczak, K. Palczewski, *Chem. Rev.* (Epub ahead of print) (2013).
4. J. C. Saari, *Annu. Rev. Nutr.* **32**, 125 (2012).
5. P. H. Tang, M. Kono, Y. Koutalos, Z. Ablonczy, R. K. Crouch, *Prog. Retin. Eye Res.* **32**, 48 (2013).
6. T. M. Redmond, S. Yu, E. Lee, D. Bok, D. Hamasaki, *et al.*, *Nat. Genet.* **20**, 344 (1998).
7. S. L. Znoiko, R. K. Crouch, G. Moiseyev, J. X. Ma, *Invest. Ophthalmol. Vis. Sci.* **43**, 1604 (2002).
8. P. H. Tang, M. C. Buhusi, J. X. Ma, R. K. Crouch, *J. Neurosci.* **31**, 18618 (2011).
9. F. Marlhens, J. M. Griffoin, C. Bareil, B. Arnaud, M. Claustres, C. P. Hamel, *Eur. J. Hum. Genet.* **6**, 527 (1998).
10. B. Rohrer, H. R. Lohr, P. Humphries, T. M. Redmond, M. W. Seeliger, *et al.*, *Invest. Ophthalmol. Vis. Sci.* **46**, 3876 (2005).
11. J. Fan, R. K. Crouch, M. Kono, *Invest. Ophthalmol. Vis. Sci.* **52**, 2412, (2011).
12. R. B. Hufnagel, Z. M. Ahmed, Z. M. Correa, R. A. Sisk, *Graefes Arch. Clin. Exp. Ophthalmol.* **250**, 1117 (2012).
13. A. V. Cideciyan, S. G. Jacobson, W. A. Beltran, A. Sumaroka, M. Swider, *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **110**, E517 (2013).