

New developments in the visual cycle: functional role of 11-*cis* retinyl esters in the retinal pigment epithelium

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Abstract

Although both 11-*cis* and all-*trans* retinyl esters exist in the retinal pigment epithelium, the relative importance of each in the visual cycle has been unclear. Recent data indicate that there are 2 biochemical pathways leading to the formation of 11-*cis* retinoids from the retinal pigment epithelium pool of retinyl esters. One well-established pathway is located in the endoplasmic reticulum where all-*trans* retinyl esters are hydrolyzed, isomerized, and then oxidized to form 11-*cis* retinal (endoplasmic reticulum pathway). A more recently identified pathway resides within the plasma membrane where 11-*cis* retinyl esters are hydrolyzed directly to 11-*cis* retinol (plasma membrane pathway). Either or both pathways may provide 11-*cis* retinoids for regeneration of rod and cone visual pigments. Recent reports have suggested that the regeneration of rod and cone pigments are carried out by different mechanisms, and that 11-*cis* retinyl esters (plasma membrane pathway) in the retinal pigment epithelium may be specific to cone pigment regeneration. In this paper we review both visual pathways and consider data in support of the hypothesis that 1 of these 2 pathways' (the plasma membrane pathway) functions to provide visual pigment chromophores selectively for cone photoreceptors.

Background

The visual cycle delineates a cascade of reactions which regulate the supply of 11-*cis* retinal, the chromophore to form visual pigments.¹ The precursor of 11-*cis* retinal is all-*trans* retinol, which is available either from blood circulation (via the choroidal vessels at the basal side of the retinal pigment epithelium [RPE]), or from bleached photoreceptors (via the interphotoreceptor matrix at the apical side of the RPE). All-*trans* retinol is first esterified with fatty acids in the RPE to become all-*trans* retinyl ester and, following ester hydrolysis, is isomerized to 11-*cis* retinol.^{2,3} Both 11-*cis* retinal and 11-*cis* retinyl esters are derived from this 11-*cis* retinol and their rates of formation are dependent on the availability of a retinol binding protein and the activity of the esterifying enzyme, lecithin retinol acyl transferase (LRAT).^{4,5} The functional significance of 11-*cis* retinyl esters in the RPE is not clear and has been a major focus of investigation in our laboratory.

Retinyl esters in the retinal pigment epithelium

Although many different retinoids (or retinol derivatives), are found in the RPE of various vertebrate species, retinyl esters constitute the major molar form of all retinoids in this tissue (87% in bovine RPE^{6,7} and 99% in humans⁸). Two major isomers, all-*trans* and 11-*cis* retinol, are esterified to palmitate, stearate, and/or oleate, forming different retinyl esters (the type and ratios of fatty acids being species-dependent).⁹ The proportion of the 2

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ester isomers varies with the degree of light/dark adaptation¹⁰ and have been found in the RPE of many species from tadpoles,^{11,12} frogs,^{6,11} goldfish,^{12,13} chickens,^{10,14} rabbits,⁹ and cows,^{10,15} to baboons¹⁶ and humans.^{17,18} However, there are 2 notable exceptions: dark-adapted albino rats¹⁹ and lake char fish¹¹ store almost no vitamin A esters in their ocular tissues.

Functional role of retinyl esters in the retinal pigment epithelium

The size of the retinyl ester pools in the RPE is significantly affected by the state of light or dark adaptation. Using albino rats, Dowling was the first to show that the decrease of retinal in the retina followed the same time course as the rise of retinol/retinyl esters in the RPE during light adaptation, and likewise, the decrease of retinol/retinyl esters in the RPE mirrored the increase of retinal in the retina.¹⁹ This suggested that retinoids in the RPE served as precursors for chromophore of visual pigments. More compelling evidence for the role of retinyl esters in the RPE to supply visual chromophore came later when tritiated retinyl acetate was injected into rats with radio-labelled retinyl esters in the RPE.^{20,21} During dark adaptation, the level of tritiated retinyl esters in the RPE decreased as the level of tritiated 11-*cis* retinal in the retina increased. These studies provided strong evidence to show that retinyl esters supply retinal chromophore for visual pigment formation. Bridges, using high performance liquid chromatography (HPLC), further demonstrated that regeneration of rhodopsin in the frog eye derived its retinal chromophore from retinyl esters in the RPE.²²

Possible role of 11-*cis* retinyl esters in the eye

The ratio of 11-*cis* to all-*trans* retinyl esters is also species-dependent. Bridges et al were the first to show that unlike most other animals studied, where all-*trans* retinyl esters predominate, the chicken eye (with a cone-dominated retina) was unusual in that it stores a preponderance (>70%) of 11-*cis* retinyl esters.¹⁴ Rodriguez and Tsin explored this further when they compared the amounts of 11-*cis* and all-*trans* retinyl esters in both light- and dark-adapted chicken eyes.¹⁰ This study showed that cone-rich chicken eyes always contain a significantly higher amount of 11-*cis* than all-*trans* retinyl esters, in both the RPE and the retina, while the reverse was observed in frog and bovine eyes, whose retinas are comprised mostly of rods (cone/rod ratio = 0.1 in the bovine eye). Das et al measured retinyl esters in the RPE of chickens (cone-dominant retina, with a cone/rod ratio of 3.6), rats (rod-dominant retina, with a cone/rod ratio of 0.009), cats, and humans (mixed rods and cones, with a cone/rod ratio of 0.5).²³ These researchers found that the ratio of 11-*cis* to all-*trans* isomers of retinyl palmitate in the RPE increases progressively with the increase in 'diurnality' (cone/rod ratio) of the species, from rats to chickens. Recently, we have also observed that RPE had a high proportion of 11-*cis* retinyl esters under both light and dark adaptation in

a cone-dominated mammalian species (the 13-lined ground squirrel, *Citellus sp.*, with a cone/rod ratio of 5.9). (N Mata, Unpublished data.) This association between 11-*cis* retinyl esters and cone-dominated retinas strongly suggests a functional role of 11-*cis* retinyl esters in the formation of cone pigments.

Visual pathways in the retinal pigment epithelium and pigment regeneration in the retina

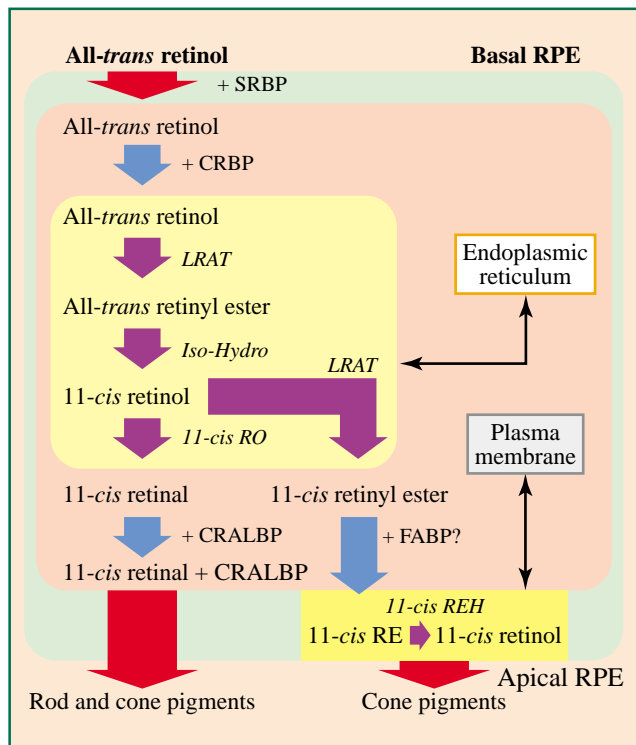
Visual pathways in the retinal pigment epithelium

Based on reports in the literature and recent research results from our laboratory, there are 2 spatially-separated visual pathways in the bovine RPE that supply 11-*cis* retinoids for visual pigment synthesis.^{3,24-26} In our studies, we observed the co-localization of 11-*cis* retinyl esters and 11-*cis*-specific retinyl ester hydrolase in the plasma membrane of the RPE, while all-*trans* retinyl esters and their corresponding hydrolase enzymes were found mainly in the endoplasmic reticulum.²⁴ Therefore, all-*trans* retinyl esters must be hydrolyzed (and then isomerized) to 11-*cis* retinol in one location in the RPE (in the endoplasmic reticulum [ER]),³ while 11-*cis* retinyl esters are hydrolyzed to release 11-*cis* retinol at another site (in the plasma membrane [PM]).²⁴ It is now apparent that 11-*cis* retinol dehydrogenase is located in the ER, thereby leading to the formation of 11-*cis* retinal from the ER pathway.^{25,27} In contrast, the end product of the PM pathway is mainly 11-*cis* retinol, as a much lower level of the 11-*cis* retinol dehydrogenase activity is recovered in the PM.²⁵ Based on in vitro enzyme kinetic data, we further showed that 11-*cis* retinyl esters in the RPE PM constitute a more readily available source of 11-*cis* retinoids for visual pigment biosynthesis.²⁴ However, the functional role of these 2 visual pathways in the RPE remains unknown.

Separate rod and cone pigment regeneration in the retina

It is now fully established that rods and cones are different photoreceptors with significantly distinct anatomical and functional features.^{28,29} Specifically, these photoreceptors make different structural contacts with RPE cells at the apical membrane of RPE³⁰ and their light intensity thresholds for visual excitation differ by several log units.³¹ Furthermore, there are distinctly different mechanisms of regeneration for rod and cone pigments^{23,32,33} and the rate of cone pigment regeneration far exceeds that of rod pigment.³⁴⁻³⁶

Recent studies on key proteins of the visual cycle have also supported the idea that rods and cones utilize separate mechanisms for visual pigment regeneration. In the first study, targeted disruption of the RPE65 gene, which was believed to encode the retinol isomerase enzyme, was used to study the function of this protein in the RPE.³⁷ It was found that RPE65 was necessary for the production of 11-*cis* vitamin A in the visual cycle. Deletion of this protein resulted in a selective loss of rod outer segments,



Abbreviations: SRBP = serum retinol binding protein; CRBP = cellular retinol binding protein; LRAT = lecithin:retinol acyl transferase; Iso-Hydro = isomerohydrolase; 11-cis RO = 11-cis retinol oxidase; CRALBP = cellular retinal binding protein; FAPB = fatty acid binding protein; RPE = retinal pigment epithelium; 11-cis REH = 11-cis retinyl ester hydrolase; 11-cis RE = 11-cis retinyl esters.

Figure 1. Hypothetical pathways for the biosynthesis of rod and cone visual pigments using vitamin A from the retinal pigment epithelium. In the retinal pigment epithelium, 11-cis retinoids are derived from 2 sub-cellular compartments — the endoplasmic reticulum and the plasma membrane. It is possible that retinoid from the plasma membrane pathway is specific for the cone pigment synthesis.

together with a loss of rod function (as measured by electroretinography), as well as increasing levels of all-trans retinyl esters. Meanwhile, cone cells and cone function remained intact. These authors concluded that "... cone pigment regeneration may be dependent on a separate pathway."³⁷

In another study, patients with a hereditary defect in serum retinol binding protein (SRBP) were found to have an elevated dark-adaptation threshold with reduced mixed rod electroretinogram (ERG) responses.³⁸ Meanwhile, the cone ERG was normal in 1 of the 2 patients examined. Similarly, selective degeneration of rod function occurred in RBP knockout mice given a vitamin A-deficient diet. After 3 to 4 weeks of the vitamin A-deficient diet, all rod components in the ERG were lost in the knockout strain. In contrast, only a reduced cone ERG was detectable after 3 months of the diet. These data clearly show that "... cones are more resistant than rods to vitamin A deficiency",³⁹ suggesting that cones may be privileged to use an additional (PM) pathway which is not utilized by rods.

Hypothesis and possible mechanisms

Development of hypothesis

Based on the information already presented, it is clear that there are 2 pathways in the RPE deriving 11-cis retinoids from all-trans retinyl esters (the ER pathway) and from 11-cis retinyl esters (the PM pathway) to form visual pigments (**Figure 1**). It is also evident that there are 2 separate mechanisms of visual pigment regeneration for rods and cones, with the rate of cone regeneration being much faster. Therefore an appropriate question is: Is there a selective utilization of 11-cis retinoids (from the 2 visual pathways in the RPE) for the 2 (rod and cone) visual pigment regenerations?

Theoretically, it is possible to calculate the rate of retinal formation from each pathway in the RPE and compare them to the rate of pigment regeneration in an attempt to match an appropriate RPE pathway to a visual pigment regeneration process. However, such a comparison cannot be readily accomplished at this time because quantitative data on the RPE pathways remain to be collected. For example, the turnover numbers (Kcat) in most metabolic steps of these RPE pathways are not known since pure proteins are not yet available. Without such values, it is not possible to calculate the rate of retinal formation (moles of retinal formed per unit time). In the ER pathway, 11-cis retinal, after being derived from all-trans retinyl ester, is bound to cellular retinal binding protein (CRALBP), which carries it to the apical membrane of the RPE. In contrast, 11-cis retinyl esters in the ER pathway are hydrolyzed to form 11-cis retinol, which does not require CRALBP for intracellular transport. Therefore, it is possible that the 11-cis retinyl ester pool (the PM pathway) in the RPE is utilized for rapid formation of 11-cis retinoid for visual pigment synthesis.²⁴ The many distinctive features of these 2 retinal pathways in the RPE render the possibility that a specific RPE pathway may provide visual chromophore for a specific (rod or cone) pigment regeneration process. Different selective mechanisms in support of the suggestion that the PM pathway may provide chromophore specific for the regeneration of cone pigments are presented below.

Possible mechanisms

In order for 11-cis retinoids from the RPE to be specific for 1 of the 2 visual pigment regeneration processes, some selective mechanisms must exist for the supply, transfer and utilization of these retinoids to form visual pigments.

Retinoid supply from the retinal pigment epithelium

In the RPE, we have identified 2 visual pathways from which chromophore may be selectively derived.²⁴ It is likely that one of these pathways (the PM pathway) proceeds at a fast rate thus being capable of supporting the higher rate of chromophore demand for cone pigment regeneration.²⁴ Furthermore, 11-cis retinol, the major product of the PM pathway, can only be utilized by isolated

cones, but not rods, for visual pigment regeneration.³² Additionally, both 11-*cis* retinol and 11-*cis* retinal are found to be endogenous ligands of interphotoreceptor retinoid binding protein (IRBP),^{40,41} indicating a regular flow of 11-*cis* retinol from the PM pathway to the photoreceptors.

Transfer of retinoids between retinal pigment epithelium and retina

It is also possible that the mechanism of transfer of retinal from RPE to the retina results in a selective delivery of retinal to support rod versus cone pigment regeneration. For example, rod and cone photoreceptors make different structural contacts with the RPE apical membrane.^{28,30} These different anatomical features may contribute to differential transfer rates or transfer pathways of retinal from the RPE to the photoreceptors. Furthermore, the rates of retinoid delivery by extracellular binding protein (such as IRBP in the photoreceptor matrix) to cones versus rods have not been determined. It is possible that the binding affinity between the membrane receptor and/or the rate of internalization of the receptor-protein complex differs between rod and cone cells. Better understanding of this retinal transfer is needed to fully explore whether the retinal supply from the RPE is affected in a photoreceptor-specific manner.^{42,43}

Selective utilization of retinoids by rod and cones

It has also been reported that neural retina and Müller cells from cone-dominated chicken retina (in comparison to rod-dominated bovine retina) can form 11-*cis* retinoids from all-*trans* retinol.²³ It is therefore possible that some retinoids from the RPE may be utilized by cones

(but not rods) to form visual pigment. Furthermore, Jones et al reported that isolated cones (but not rods) are capable of utilizing 11-*cis* retinol for pigment regeneration, suggesting “separate pathways of visual pigment regeneration for rods and cones.”³² Since 11-*cis* retinol is a major product of the PM pathway in the RPE,²⁵ this retinol is transported to the retina where cones (but not rods) can use it for pigment regeneration.

Conclusions

The recovery of visual sensitivity following light exposure is dependent on the regeneration of bleached visual pigments.⁴⁴ Many common disorders such as retinitis pigmentosa, age-related maculopathy, diabetic retinopathy, glaucoma, vitamin A deficiency, hypoxia, and alcohol intoxication are all associated with some form of abnormality of visual recovery.^{45,46} Mutations in the gene encoding a visual cycle enzyme (11-*cis* retinol dehydrogenase), for example, cause delayed dark-adaptation and fundus albipunctatus, a congenital night-blindness disorder.^{47,48} Therefore, the relationship between visual pathways in the RPE and pigment regeneration in the retina provides important information for the understanding, as well as the basis of treatment, of many ocular disorders.

This review discusses 2 separate cone and rod regeneration mechanisms as well as 2 pathways of visual chromophore biosynthesis (the ER and PM pathways) in the RPE. Whereas the ER pathway provides 11-*cis* retinal for both rod and cone pigment regeneration, 11-*cis* retinol from the PM pathway may be selective for cone pigment regeneration.

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