Biochemistry of Visual Pigment Regeneration
The Friedenwald Lecture

John C. Saari

Rods bleached in vivo therefore do not regenerate their visual purple from material within themselves or in the tissues proximal to them, but from the distally placed pigment epithelium: the pigment epithelium functions in the regeneration of visual purple; it exerts a regenerative action on the rods.1

Phototransduction and the visual cycle play complementary roles in vertebrate vision. Phototransduction is initiated by the photoisomerization of 11-cis-retinal bound to opsin and ultimately results in a change in the release of neurotransmitter by photoreceptor cells. The visual cycle restores the product of photoisomerization, all-trans-retinal, to the 11-cis configuration and allows the regeneration of bleached visual pigments (Fig. 1). The biochemical mechanism of phototransduction has been extensively studied during the past 2 decades, and as a result, the process serves as the paradigm for understanding G-protein-coupled receptors in general. In contrast, molecular understanding of the visual cycle is poorly developed, and many fundamental questions regarding reactions, enzymes, and control mechanisms remain unanswered. Sequences of cDNAs encoding three visual cycle enzymes have been published2–4; however, molecular information is unavailable for the other three presumed enzymes of the cycle, including retinol isomerase (isomerohydrolase).

It has been reported that regeneration of visual pigments is a slow process and that photoisomerization of 11-cis-retinal in rhodopsin is very rapid. In fact, complete dark adaptation in humans requires approximately 40 minutes,5–7 and conversion of rhodopsin to photorhodopsin requires only 200 fsec.8 However, this is not a fair comparison, because it is clear that the photolysis and regeneration rates in the living eye must be equal and opposite in sign at ambient levels of illumination. Alpern has equal and opposite in sign at ambient levels of illumination. Although the molecular identity of the desensitizing intermediate(s) remains a matter of active investigation,9,10,12–16 it is clear that visual cycle reactions are important in determining the steady state level of bleached visual pigment and thus the sensitivity of the retina.

The critical role of the retinal pigment epithelium (RPE) in visual pigment regeneration is apparent from the studies of 19th century investigators who demonstrated that dissected frog retina could regenerate its bleached visual pigment only when in contact with the RPE.1,17–19 The reader is referred to Marmor and Martin20 for a depiction of some of their insightful experiments. It is fortunate that the early physiologists used frog eyes for their experiments because rodent eyes do not regenerate their visual pigments when removed from the animal, as will be discussed later. Fifty years later, Wald21 used extraction techniques to show that vitamin A was involved in the visual process and formulated the first modern version of the visual cycle including the participation of the RPE (Fig. 2). The role of the RPE in the visual cycle became more clear after the classic study by Dowling,22 which demonstrated movement of retinoid out of the neural retina and into RPE during extensive bleaching and return during recovery in the dark. Later studies by other investigators with techniques offering more resolution verified the fundamental observation.23–26 Bernstein et al.27 and Rando28 provided a molecular explanation for the necessity of the RPE with the demonstration that the critical enzymatic regeneration of the 11-cis configuration occurred within this tissue.25–29 The transcellular migration of the retinoids during bleaching and regeneration is all the more remarkable, considering the anatomy of the journey (Fig. 3). The relatively insoluble retinoid must leave the disc membranes, diffuse through a cytosolic compartment to reach the plasma membrane of the rod outer segment, traverse the plasma membrane, diffuse across the subretinal space to reach the plasma membrane of the RPE cell, enter into the reactions of the visual cycle in this cell, and make the return journey!

In humans the progress curves for the regain of visual threshold and for the regeneration of visual pigment coincide when displayed on a semi-log plot.6,7 The molecular explanation for this log-linear relationship is not well understood, and the relationship may be fortuitous, but most agree that a photoproduct is responsible for desensitization of the visual system.6,10,11 Although the molecular identity of the desensitizing intermediate(s) remains a matter of active investigation,10,12–16 it is clear that visual cycle reactions are important in determining the steady state level of bleached visual pigment and thus the sensitivity of the retina.

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The enzymes are depicted as part of one continuous internal membrane compartment for simplicity. Absorption of light by rhodopsin in the disc membrane converts 11-cis-retinal to all-trans-retinal and generates the active photoprotein, metarhodopsin II (Rho*). The G-protein–stimulating activity of Rho* is quenched by phosphorylation and by the binding of arrestin (not shown in the figure). The Schiff base linking all-trans-retinal and opsin is hydrolyzed to release free all-trans-retinal. Recent reports suggest that an adenosine triphosphate (ATP)–binding cassette transporter (ABCR) is involved in moving all-trans-retinal from the intradiscal to the cytosolic aspect of the disc membrane, perhaps as an adduct with phosphatidyl ethanolamine. all-trans-Retinol dehydrogenase (RDH) catalyzes the reduction of all-trans-retinal to all-trans-retinol by reduced nicotinamide adenine dinucleotide phosphate (NADPH). all-trans-Retinol leaves the photoreceptor cell, traverses the interphotoreceptor matrix space (the subretinal space) where it encounters interphotoreceptor retinoid-binding protein (IRBP), and enters the RPE where it is esterified by lecithin-retinol acyltransferase (LRAT). all-trans-Retinyl ester is converted to 11-cis-retinol and free fatty acid by an isomerase reaction (11-RDH). 11-cis-Retinol can be taken up from the blood and esterified by LRAT in RPE cells (not shown). 11-cis-Retinyl esters can be hydrolyzed and used for visual pigment regeneration by 11-cis-retinyl ester hydrolase (11-REH). Cellular retinaldehyde-binding protein (CRALBP), a water soluble retinoid-binding protein, is shown (Fig. 4) with its high-affinity ligands, 11-cis-retinal or 11-cis-retinol. Cellular retinol-binding protein (CRBP) is shown associated with all-trans-retinol, although the protein also binds 11-cis-retinol in vitro. IRBP is present in the photoreceptor matrix. Its role in retinoid transport is uncertain (discussion to follow). Opsin is shown on the lower side of the disc only for reasons of symmetry.

Evidence leading to this working hypothesis of the rod visual cycle has been presented in several recent reviews. Each of the enzymatic reactions shown in the RPE or rod outer segments (ROSs) has been demonstrated in RPE microsome or ROS preparations, respectively. The water-soluble retinoid-binding proteins (CRALBP, CRBP, and IRBP) have been localized to the compartments in which they are shown by immunocytochemistry. CRBP and CRALBP are also found in Müller cells. Mammalian rods cannot use exogenous 11-cis-retinol for regeneration of visual pigments.
Thus, 11-cis-retinal is the presumed product of RPE retinoid metabolism for the mammalian rod visual cycle.

**ANALYSIS OF THE FLOW OF RETINOIDS IN THE MOUSE VISUAL CYCLE**

Dowling determined the flow of retinoids in and out of the RPE by analysis of their temporal appearance during prolonged, total bleaching and recovery in the dark. Several fundamental questions remained regarding the flux of the cycle. What step of the cycle determines the rate of visual pigment regeneration? Is there biochemical evidence to suggest that the cycle is regulated? Do the several retinoid-binding proteins that have been characterized in RPE and Müller cells play essential roles in the visual cycle? We addressed these questions by analyzing the composition of visual cycle retinoids during recovery from a flash or from steady illumination. We chose mice as the experimental animals because of the increasing availability of animals with targeted disruption of genes encoding putative visual cycle components.

**The Rate-Limiting Step in the Mouse Visual Cycle**

Lightly pigmented mice were dark adapted and subjected to either a flash or to steady illumination that bleached approximately 40% of their visual pigment. Retinoids were extracted and analyzed before bleaching (dark adapted) and during the recovery period in the dark. The high-performance liquid chromatography traces from an experiment using flash illumination are shown in Figure 5, and the results are summarized in Figure 6. Surprisingly, the only retinoid that accumulated in substantial amounts during the recovery period was all-trans-retinal. In other words, all processes after reduction of all-trans-retinal by NADPH determines the rate of entry of retinoid into the visual cycle and emphasizes the importance of this reaction. These results refer to the experimental situation in which approximately 40% of the mouse visual pigment was bleached. It is possible that intermediates other than all-trans-retinal would appear if the visual system were challenged with larger

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**Figure 4.** A schematic illustrating a working hypothesis for the mammalian rod visual cycle. The reactions and compartments are discussed in more detail in the text. The abbreviations used are: ACR, ATP-binding cassette, retina; CRBP, cellular retinol-binding protein; CRALBP, cellular retinaldehyde-binding protein; IMH, isomerohydrolase; IRBP, interphotoreceptor retinoid-binding protein; LRAT, lecithin:retinol acyltransferase; RDH, all-trans-retinol dehydrogenase; 11-RDH, 11-cis-retinol dehydrogenase; Rho, rhodopsin; Rho*, activated rhodopsin; 11-Ral, 11-cis-retinal; 11 Rol, 11-cis-retinol; at-Ral, all-trans-retinal; at-Rol, all-trans-retinol; at-RE, all-trans-retinyl ester.
fractional bleaches. At the limit of 100% bleach, Dowling\textsuperscript{22} and Zimmerman\textsuperscript{24} observed in rats the progressive appearance and disappearance of all the visual cycle intermediates that could be resolved. In addition, Perlman et al.\textsuperscript{41} observed that the time constant for the regeneration of visual pigment in rats is inversely proportional to the amount of visual pigment bleached. Because of the complex nature of the chemical reactions and transport processes involved, it is possible that another step could become rate limiting as flux through the pathway is increased.

Flash illumination of animals and humans has been used with great success in a number of experimental situations. However, it could be argued that physiological conditions are more closely approximated with steady illumination. Thus, we thought it important to verify that our observation of the accumulation of all-trans-retinal during recovery from a flash was not an artifact of the illumination conditions. Dark-adapted mice were subjected to illumination from two 60-W fluorescent bulbs (50 foot-candles). Retinoids were extracted and analyzed at various times after onset of the lights and during the recovery period in the dark. Again, all-trans-retinal was the only retinoid that accumulated in substantial amounts during bleaching and recovery. Figure 7 depicts the amount of all-trans-retinal accumulated during steady state bleaching and during recovery in the dark. The constant light resulted in a steady state with approximately 35% of the visual pigment bleached. When the light was turned off, the all-trans-retinal rapidly decayed to the original dark-adapted value. We compared the rate of decay of all-trans-retinal produced by steady illumination with that produced by a flash. Approximately the same amount of visual pigment was bleached in each case. The

![Figure 5](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933586/)

**Figure 5.** HPLC [high-performance liquid chromatography] separation of visual cycle retinoids. (A) dark adapted mice; (B) mice immediately following a flash; (C) mice 60 minutes in the dark after a flash. The numbers indicate the elution positions of 1, retinyl palmitate; 2, all-trans-retinyl acetate (internal standard); 3, syn 11-cis-retinal oxime; 4, syn all-trans-retinal oxime; 5, 11-cis-retinol; 6, anti 11-cis-retinal oxime; 7, all-trans-retinol; 8, anti all-trans-retinal oxime. Reprinted, with permission, from Van Hooser JP, Garwin GG, Saari JC. Analysis of the visual cycle in transgenic mice. *Methods Enzymol.* 2000, Academic Press. In press.

![Figure 6](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933586/)

**Figure 6.** Composition of polar retinoids in dark-adapted mice recovering from a flash. (○) 11-cis-Retinal; (●) all-trans-retinal; (■) all-trans-retinol; (□) 11-cis-retinol. Arrow: a flash. Retinyl esters did not change significantly with this amount of bleaching (not shown). Modified, with permission, from Palczewski K, Van Hooser JP, Garwin GG, Saari JC. Kinetics of visual pigment regeneration in excised mouse eyes and in mice with a targeted disruption of the gene encoding interphotoreceptor retinoid-binding protein or arrestin. *Biochemistry.* 1999;39:12012-12019. American Chemical Society.

![Figure 7](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933586/)

**Figure 7.** Recovery from steady state bleaching. The bar at the top of the figure illustrates the lighting regimen used (filled bar, dark; open bar, light). In the dark (0 on the abscissa) ~5% of the retinals are in the all-trans configuration. The onset of light increased the amount of all-trans-retinal to ~40%. When the light was turned off (90 minutes) all-trans-retinal rapidly decreased to the dark-adapted value.
A Block of the Visual Cycle at the RDH Reaction

Based on the results obtained with constant illumination of excised mouse eyes, it could be predicted that flash illumination would produce a similar metabolic pattern—namely, accumulation of all-trans-retinyl ester. However, the actual experimental result we obtained was completely unanticipated. All-trans-Retinol generated by the flash was not further metabolized (Fig. 9B). No reduction was evident, nor did any other metabolites appear. This striking result indicates that the reaction catalyzed by RDH is more complicated than had been anticipated and that simply supplying one of the substrates (all-trans-retinol) is not sufficient to activate the reaction. Why does reduction of all-trans-retinol not occur, and is this related to the differences we have observed in the rates of regeneration after flash or constant illumination? Several possibilities will be discussed in the subsequent sections.

Reduction of all-trans-retinol requires a source of reducing power, and numerous studies have demonstrated that in the visual cycle this source must be NADPH. The pentose phosphate pathway (also known as the hexose monophosphate shunt) supplies most of the NADPH in most tissues. This pathway is considered to be constitutive in most quiescent cells except in neutrophils where NADPH is required for superoxide production, in adipose tissue where NADPH is used for fatty acid biosynthesis, and in dividing cells that require ribose-P for DNA synthesis. Activation of glucose 6-phosphate dehydrogenase (G6PD), the first enzyme of the pentose phosphate pathway, by epidermal growth factor has been studied in detail in growing cells where it appears to involve release of the enzyme from structural elements within the cell. Studies of glucose metabolism emphasized that ROSs were capable of producing NADPH through the pentose phosphate pathway in amounts sufficient to account for reduction of all-trans-retinol. However, in dark-adapted rabbit and monkey retina, the ratio of NADPH to nicotinamide adenine dinucleotide phosphate (NADP) was reported to be 0.3, indicating that the pathway would have to be activated for

The Block in the Visual Cycle in Excised Mouse Eyes

Dissected frog eyes, which contain preformed 11-cis-retinyl ester in their RPE, regenerate their visual pigment after bleaching. However, mouse eyes, with little if any preformed 11-cis-retinyl ester, do not regenerate their visual pigment once removed from the animal. What step in the visual cycle is blocked in excised mouse eyes? The visual cycle, as currently postulated, does not include any obvious step that requires metabolic energy except for the ABCR transporter reaction (see Fig. 4). Formation of the 11-cis configuration is an endergonic process, but the energy required for formation of the hindered 11-cis configuration has been postulated to come from the hydrolysis of the ester bond of all-trans-retinyl ester. Thus, it would not be anticipated that depriving an eye of its source of blood would prevent the formation of 11-cis-retinoids. We addressed this question by analyzing the composition of visual cycle retinoids in bleached, excised mouse eyes. Eyes were removed from dark-adapted mice and subjected to constant illumination (Fig. 9A). 11-cis-Retinal steadily disappeared during the illumination period. All-trans-Retinal transiently appeared, and ultimately all-trans-retinol and all-trans-retinyl ester accumulated. No 11-cis-retinoids were formed. Based on current ideas about the visual cycle (Fig. 4), this suggests that the isomerization reaction is not functional in excised mouse eyes. This block in the cycle could result from several causes. Perhaps the isomerization reaction requires metabolic energy (e.g., ATP), contrary to what has been suggested, and the ATP stores in these excised eyes are rapidly depleted. In support of this are reports that the electrical responses of rabbit eyes rapidly decay in the absence of oxygen and glucose and that excised mouse eyes do not regenerate their visual pigment unless they are perfused with oxygen and glucose. However, it is also possible that changes in cellular pH, ionic concentrations, or oxidative stress results in inhibition of the isomerase.

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results, shown in Figure 8, illustrate that the recovery from steady illumination is approximately 3.5 times more rapid (half-life \( t_{1/2} \), 5 minutes with constant illumination; \( t_{1/2} \) = 17 minutes with flash illumination). Similar results were reported in a study of phosphorylation of rhodopsin.

What can account for the difference in the rates of decay of all-trans-retinal (and rate of 11-cis-retinal formation) generated by the two different bleaching regimens? Because all-trans-retinal was the only visual cycle intermediate that accumulated in both cases, the difference in rates must result from a difference in rate of reduction of all-trans-retinal. It is possible that the reaction is subject to control at the level of the enzyme, all-trans-retinol dehydrogenase. Alternatively, it is possible that flash illumination fails to fully activate the pentose phosphate pathway, which is largely responsible for generation of NADPH for retinal reduction. Modulation of the rate of all-trans-retinal reduction will be discussed further in subsequent sections.

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reduction to occur. In vivo $[^{13}\text{C}]$ nuclear magnetic resonance studies of rabbit retina have demonstrated an activation of the pentose phosphate pathway in constant light.\textsuperscript{56} Perhaps flash illumination does not fully activate the pentose phosphate pathway in normal mouse eyes and not at all in excised mouse eyes.

The Schiff base linking all-trans-retinal and opsin must be hydrolyzed before the retinoid can be reduced to all-trans-retinol. Differences in the rates of reduction in vivo or the absence of reduction in excised eyes could be due to differences in the rates of hydrolysis of the Schiff bases formed with intermediates generated by a flash or constant illumination. Flash illumination is known to result in phosphorylation primarily of ser334 of opsin, whereas constant illumination results in phosphorylation primarily of ser338.\textsuperscript{42} Complex formation of these differently phosphorylated opsins with arrestin could further affect the rates of hydrolysis of the Schiff base, although our studies of arrestin knockout mice indicate otherwise\textsuperscript{44} (see following discussion).

It is also possible that NADPH and all-trans-retinal are generated in separate compartments, and an active process must occur to unite them. The rim protein of ROSs\textsuperscript{57} has recently been identified as a member of the ABC-transporter family (ABCR).\textsuperscript{58,59} The rate of hydrolysis of ATP by this protein is stimulated by addition of 11-cis- or all-trans-retinal,\textsuperscript{28} suggesting that one or both of these retinoids are substrates for the transporter. The investigators propose that the function of the protein is to pump all-trans-retinal from inside the disc, where it is generated, to the cytosolic side, where it can be reduced by NADPH. Recent reports of the accumulation of condensation products of phosphatidylethanolamine and retinal in the ABCR knockout mouse suggests that these may be the actual substrates for the transporter.\textsuperscript{29} Thus, depletion of ATP in excised mouse eyes could prevent the two substrates of the reaction from uniting. However, this explanation seems unlikely to account for the absence of reduction of all-trans-retinal that we have observed in flashed, excised mouse eyes because reduction occurs in the same experimental system with constant illumination (Fig. 9A). In addition, a normal rate of visual pigment regeneration was observed in ABCR−/− animals, indicating that the visual cycle is not dependent on the transporter.

Finally, it is possible that the enzyme is directly regulated by unknown mechanisms. The lack of structural information about rod RDH considerably hinders further progress in this area.

**Blocked Transport of Retinoids in the Visual Cycle**

Constant illumination of mouse eyecups immersed in buffer revealed a third pattern of metabolism. 11-cis-Retinal steadily disappeared concomitant with a transient increase in the amount of all-trans-retinal and an eventual accumulation of all-trans-retinol (Fig. 9C). No retinyl esters were produced. This result suggests that the transport of all-trans-retinol to the RPE, where LRAT is localized\textsuperscript{60,61} did not occur. Examination of the eyecups at the end of the experiment provides the explanation for this result. The retinas became detached during the incubation. This somewhat trivial explanation nonetheless provides information relative to retinoid transport in detached retinas and illustrates the power of retinoid analysis in detecting abnormal visual cycle function.
In summary, the metabolic inertness of all-trans-retinal in excised mouse eyes generated by a flash and the differences in the rates of regeneration after flash or steady bleaching point out how poorly we understand the processes by which the visual cycle is controlled. Continued study will lead to a solution to this problem and very likely to enhanced understanding of the control of the visual cycle.

**ANALYSIS OF THE FLOW OF RETINOIDS IN KNOCKOUT MICE**

**The Visual Cycle in Arrestin−/− Mice**

Photoactivated Rho is quenched in a two-step process involving opsin phosphorylation by rhodopsin kinase and binding of arrestin to phosphorylated opsin. Two studies have suggested that arrestin affects the activity of RDH in ROS preparations. Direct addition of arrestin to washed, flashed ROS membranes results in a 40% inhibition of the rate of reduction of all-trans-retinal, whereas we observed inhibition of RDH in whole ROS preparations (containing rhodopsin kinase and arrestin) when ATP and guanosine triphosphate were added. We attribute the ATP effect to phosphorylation of Rho* by rhodopsin kinase, formation of a complex with arrestin, and a reduction of the accessibility of all-trans-retinal to RDH. These results led to the prediction that reduction of all-trans-retinal and regeneration of visual pigments would be more rapid in a mouse without functional arrestin than in normal subjects.

To address these possibilities, we examined the composition of retinoids during bleaching and regeneration in arrestin−/− mice. These animals were born and raised in the dark and subjected to flash bleaching followed by a recovery period in the dark. The distribution of visual cycle retinoids observed before and after a flash is very similar to that seen with normal mice (not shown). The recovery of 11-cis-retinal in the dark was slower in arrestin−/− mice than in normal mice (0.6 and 1.1% per minute, respectively; Fig. 10). However, the rates of rhodopsin regeneration were similar (0.8% per minute and 1% per minute, respectively).

The failure to detect major changes in the rates of rhodopsin and 11-cis-retinal regeneration in arrestin−/− mice is surprising in view of the in vitro results mentioned earlier. Perhaps the experimental situation in which we observed the ATP effect poorly approximates the conditions found within the rod outer segment. An alternative possibility is that our interpretation of the in vitro results was incorrect and that phosphorylation of opsin alone was sufficient to alter the rate of release of all-trans-retinal. Perhaps an examination of the kinetics of visual pigment regeneration in rhodopsin kinase knockout mice will resolve this issue.

**The Visual Cycle in IRBP−−/− Mice**

There is much circumstantial evidence to support a role for IRBP in the diffusion of retinoids between RPE and photoreceptor cells: 1) IRBP possesses two or more high-affinity binding sites for retinoids, whereas we observed inhibition of RDH in whole ROS preparations (containing rhodopsin kinase and arrestin) when ATP and guanosine triphosphate were added. We attribute the ATP effect to phosphorylation of Rho* by rhodopsin kinase, formation of a complex with arrestin, and a reduction of the accessibility of all-trans-retinal to RDH. These results led to the prediction that reduction of all-trans-retinal and regeneration of visual pigments would be more rapid in a mouse without functional arrestin than in normal subjects.

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search toward other potential roles for IRBP in visual physiology.

**Perspective**

**Outstanding Problems**

The visual cycle shown in Figure 4 is consistent with the available evidence regarding the regeneration of rod visual pigments. However, this model must be regarded as a working hypothesis for several reasons. First, several “orphan” retinoid-binding components have been described, and some are likely to be involved in the visual cycle. For instance, RPE65 may play a major role in retinoid metabolism, based on the inability of RPE65/−/− mice to make 11-cis-retinoids,82 but its function is not understood at this time. Other proteins such as peropsin83 and RGR opsin84 have amino acid sequences related to that of opsin. What are their functions? Peropsin has been localized to the apical plasma membrane of RPE.85 Could it be involved in the export of 11-cis-retinal from RPE or in the import of all-trans-retinol from the interphotoreceptor matrix? Eicosanoids such as prostaglandins are secreted from cells through a transmembrane protein called the prostaglandin transporter, which facilitates secretion and uptake of the hydrophobic signaling molecules.85 There is insufficient information to draw any conclusions at this time, but the phenotype associated with knockout mice may provide an answer. Second, the cone visual cycle differs from the rod visual cycle in several quantitative aspects (e.g., faster turnover) and perhaps qualitative aspects as well. Müller cells are known to contain several enzymes, and intercellular flow of retinoids. However, the questions of further experimentation. However, the answers to these and other fascinating questions await the results of future experimentation. However, the questions emphasize our rudimentary understanding of the complex process of visual pigment regeneration.

**Why Is the Visual Cycle So Complex?**

Two general systems are used for regeneration of bleached visual pigments. Invertebrates rely on the establishment of photoequilibrium, in which the first photon absorbed converts 11-cis-retinal to all-trans-retinal (Fig. 11). A second photon can then convert all-trans-retinal back to 11-cis-retinal. At constant levels of illumination a steady state level of bleached visual pigment is generated, which is a factor in determining the sensitivity of the visual system, as has been discussed. Thus, the chromophore does not dissociate from the opsin to which it is covalently bound and the meta II species absorbs in the visible range of the spectrum. In contrast, invertebrates there is a complicated system involving dissociation of the chromophore and regeneration of the 11-cis configuration in a neighboring nurse cell. What are the advantages and disadvantages of the two systems? The invertebrate system is inherently more simple and elegant in design. However, the bleaching rate and the regeneration rate are tied to the photon flux, a feature perhaps disadvantageous in rapidly changing light conditions. In contrast, the vertebrate regeneration system is more complex, because it involves the participation of two different cell types, several enzymes, and intercellular flow of retinoids. However, the regeneration rate is independent of the photon flux, allowing relatively rapid restoration of visual sensitivity even in the dark.

**Is Night Blindness Caused by Defects in the Visual Cycle?**

Night blindness is a common hallmark of many inherited retinal diseases and nutritional disorders. In general, the condition appears to result from two phenomena: decreased photon catch resulting from diminished rhodopsin content and/or the generation of a species that actively desensitizes the retina. The former mechanism results in relatively mild elevations of the scotopic threshold. For instance, a 25% reduction in rhodopsin level increases the scotopic threshold by 1.5. However, active species produced by mutation, photobleaching or vitamin A deficiency, result in very large elevations in scotopic threshold. For example, 25% bleaching of rhodopsin increases the scotopic threshold by 104. The night blindness reported in many retinitis pigmentosa cases results simply from the reduced photon catch associated with decreased amounts of rhodopsin in the affected retina.87,88 However, several mutations in the opsin gene result in constitutively active species that further desensitize the retina.89,97,90 In addition, mutations in other genes that produce constitutive activation of phototransduction91 or that affect photointermediate quenching pathways92,93 result in delayed dark adaptation.

**Visual Cycle Defects and Inherited Retinal Conditions**

Mutations in several genes encoding presumptive visual cycle components have recently been implicated in several inherited retinal diseases. The roles of ABCR in Stargardt disease84 and of RPE65 in Leber’s congenital amaurosis95,96 and in other retinal diseases have been discussed in detail elsewhere and will not be covered here. Recently, missense mutations in the gene encoding 11-cis-retinol dehydrogenase have been found in patients with fundus albipunctatus.97 This form of congenital stationary night blindness results in delayed dark adaptation and delayed regeneration of visual pigments.98–100
CRALBP has several distinguishing characteristics that strongly suggest its participation in the visual process. First, the protein has a high-affinity binding site for either 11-cis-retinal ($K_D = 10$ nM) or 11-cis-retinol ($K_D = 60$ nM). Second, CRALBP purifies from RPE saturated with 11-cis-retinal and from neural retinal saturated with 11-cis-retinal and 11-cis-retinol, retinoids of the visual cycle. Third, in retina, CRALBP is found in Müller and RPE cells. The RPE, of course, is the site of intense retinoid metabolism related to the visual cycle. Fourth, CRALBP affects the enzymatic activity of four enzymes of the visual cycle in vitro. The binding protein reduces LRAT-mediated esterification of 11-cis-retinol by 90% and modestly stimulates oxidation of 11-cis-retinol by 11-RDH. Apo-CRALBP is required for retinol isomerase (isomerohydrolase) activity and for release of 11-cis-retinol from endogenous 11-cis-retinyl esters by 11-REH. The development of CRALBP knockout mice should resolve the question of the role of CRALBP in visual physiology.

Mutations in the gene encoding CRALBP have been associated with several forms of retinal degeneration. Affected siblings in a consanguineous pedigree segregating for nonsyndromic autosomal recessive retinitis pigmentosa were homozygous for a G4763A nucleotide substitution in the CRALBP gene. Recombinant CRALBP bearing this substitution (R150Q) did not bind 11-cis-retinal in vitro, stressing the importance of the retinoid-binding site. Four mutations in the gene encoding CRALBP were found in three unrelated patients with recessively inherited retinitis punctata albescens. Twenty patients from seven families with features of retinitis punctata albescens and macular degeneration (Bothnia dystrophy) were homozygous for a missense mutation (R234W) in the CRALBP gene. These results suggest that CRALBP plays an important role in visual physiology.

Müller Cells and Cone Visual Pigment Regeneration

The literature contains many intriguing suggestions that the visual cycle in cones differs from that in rods and that Müller cells may be involved. Two retinoid-binding proteins, CRALBP and CRBP, are found in Müller and RPE cells in retinas from several species. The presence of CRALBP is particularly intriguing, because the binding protein, as mentioned above, has been demonstrated to purify from neural retina as a mixture of complexes with 11-cis-retinol or 11-cis-retinal. The all-trans- and 9-cis-retinoids modulate many biologic processes but 11-cis-retinoids are only known to be involved in the visual process or in the absorption of light (pincal). Cultured chick Müller cells take up exogenous all-trans-retinol and convert it to all-trans- and 11-cis-retinyl palmitate and 11-cis-retinol. The latter retinoid has been found in the culture medium. Although no oxidation to 11-cis-retinal has been observed, other investigators have noted that isolated amphibian cone cells resensitize with exogenous 11-cis-retinol, whereas rods require 11-cis-retinal. Goldstein noted that the amplitude of the cone but not the rod early receptor potential reaches a steady state in illuminated, isolated frog retina and recovers with a $t_{1/2}$ of 5 to 6 minutes in the dark. Thus, it appears that cones can regenerate their visual pigment in frog retina in the absence of RPE. However, the interpretation of these provocative studies hinges on the validity of the demonstration that early receptor potential amplitude is directly proportional to the amount of unbleached visual pigment. Thus, it seems clear that some interesting retinoid metabolism occurs in Müller cells, and the presence of 11-cis-retinoids strongly suggests that it is related to the visual cycle.

CONCLUSION

This is an exciting time in visual cycle research. During the past few years the number of published studies related to visual pigment regeneration has increased dramatically. Some well-established proteins have been characterized at the molecular level (for instance, cone RDH, 11-RDH, and LRAT), and several other proteins have been shown to play very important roles in the visual cycle (RPE65 and ABCR). Further indication of the sophistication of the cycle has been shown by studies that point out the complexity of a seemingly simple step such as the reduction of all-trans-retinal. We can anticipate the elaboration of further complexities and further medical relevance as investigators obtain the tools and molecular information necessary for precise dissection of individual and integrated steps of the visual cycle.

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References


