

Like Night and Day: Rods and Cones Have Different Pigment Regeneration Pathways

Sustained vision requires continuous regeneration of visual pigments in rod and cone photoreceptors by the 11-*cis*-retinal chromophore. In this issue of *Neuron*, Mata et al. report a novel enzymatic pathway uniquely designed to keep up with the high demand for cone pigment regeneration in bright light and to preclude rods from utilizing chromophore produced in daylight, when rods are not very useful for vision.

The first events in vision take place within rod and cone cells of the retina. As a photon of light hits a molecule of visual pigment, the 11-*cis*-retinal chromophore undergoes isomerization to all-*trans*-retinal. This isomerization leads to a conformational transition within the rod or cone opsin protein, followed by the activation of the visual transduction cascade (see Pugh and Lamb, 2000, Burns and Baylor, 2001, and Arshavsky et al., 2002, for recent reviews on phototransduction).

Each opsin molecule can signal multiple light events throughout its lifetime. However, before a bleached opsin can absorb another photon, its all-*trans*-retinal must be removed and replaced by a molecule of 11-*cis*-retinal. Interestingly, neither rods nor cones can convert all-*trans*-retinal into 11-*cis*-retinal without the involvement of other ocular cells. Until now, it has been accepted that this conversion, called the retinoid (or visual) cycle, requires participation of the retinal pigment epithelium, a layer of cells located at the back of the eye, immediately behind the photoreceptors (see Figure, panels A and B; see Saari, 2000, and McBee et al., 2001, for recent reviews). In this cycle, all-*trans*-retinal dissociating from bleached opsin is reduced to all-*trans*-retinol (or vitamin A) within photoreceptors. All-*trans*-retinol is then released into the extracellular space, where it is absorbed by cells of the pigment epithelium. There, all-*trans*-retinol is esterified to form all-*trans*-retinyl ester by the transfer of a fatty acid from phosphatidylcholine. The next reaction, perhaps the most important for the whole cycle, couples hydrolysis of the retinyl ester with all-*trans* to 11-*cis* isomerization of retinol. The importance of this coupling is that the energy released by ester hydrolysis is used to power the isomerization. Finally, 11-*cis*-retinol is oxidized to 11-*cis*-retinal, which is released from the pigment epithelium and taken up by photoreceptors to regenerate bleached opsin.

In this issue of *Neuron*, the research team led by G.H. Travis (Mata et al., 2002) reports a discovery of three new enzymatic activities which are likely to represent a novel retinoid cycle responsible for regenerating visual pigments in cones. This discovery was prompted by the puzzle that the rate of visual pigment bleaching in sunlight greatly exceeds the maximal reported rate at

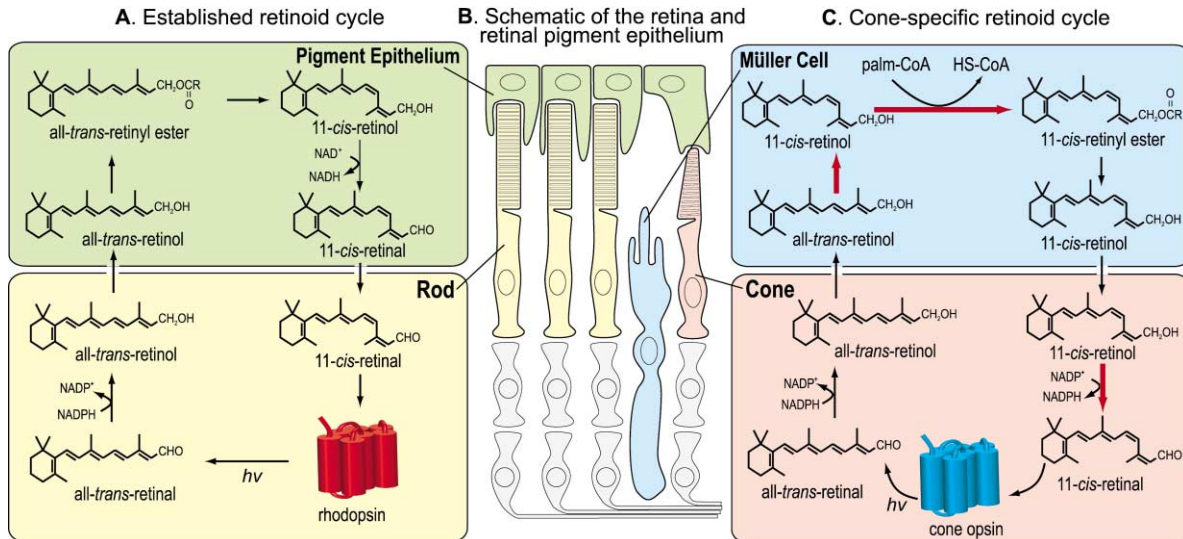
which all-*trans*-retinal is converted to 11-*cis*-retinal by the known retinoid cycle. Yet, cones remain responsive to light under these conditions. Moreover, other evidence suggested that regeneration of cone-specific visual pigments might occur via a mechanism that does not fit the pathway described above. For example, cone but not rod opsin can regenerate in isolated frog retinas separated from the pigment epithelium (Goldstein and Wolf, 1973). In addition, cultured Müller cells, the major glial cells of the retina located within the photoreceptor layer, can isomerize all-*trans*-retinol to 11-*cis*-retinol (Das et al., 1992). These observations led Mata et al. to hypothesize that cones regenerate their opsins through an alternative retinoid cycle based on the interaction between Müller cells and cones.

In experiments conducted with the retinas of two cone-dominant animals, chicken and ground squirrel, Mata et al. describe three novel catalytic activities present in cones and Müller cells, which together comprise a novel pathway responsible for regenerating cone visual pigments (see Figure, panel C). They postulate that all-*trans*-retinol leaving photoreceptors is absorbed by the Müller cells, where it is directly converted to 11-*cis*-retinol by a novel all-*trans*-retinol isomerase. 11-*cis*-retinol is then released from the Müller cells and taken up by cones where it is oxidized to 11-*cis*-retinal by another novel enzyme, a cone-specific dehydrogenase.

Although this pathway appears strikingly simple, the source of the energy required to drive the isomerization reaction in Müller cells remained to be explained. With the “traditional” retinoid cycle, this energy is drawn from hydrolysis of the ester bond in an all-*trans*-retinyl ester (see Figure, panel A). However, this step is omitted in the novel cycle, since the substrate for isomerization here is all-*trans*-retinol (see Figure, panel C). Instead, the authors found that 11-*cis*-retinol in cone-dominant retinas is esterified after isomerization by one more novel enzyme, 11-*cis*-retinyl ester synthase, which uses palmitoyl-CoA as an acyl donor. Based on these observations, they offer an elegant hypothesis regarding the energy source of this cycle. They note that the ratio between all-*trans*-retinol and 11-*cis*-retinol at the equilibrium is ~1000:1. Although this 0.1% reaction yield appears very low, the reaction can still be efficiently driven toward the 11-*cis*-retinol formation by mass action when 11-*cis*-retinol is removed from the reaction space by esterification. Therefore, it is ultimately the energy of palmitoyl-CoA hydrolysis that drives the isomerization of retinol from the all-*trans* to 11-*cis* conformation.

The formed 11-*cis*-retinyl ester is eventually hydrolyzed by retinyl ester hydrolase to yield 11-*cis*-retinol, which is taken up by the cones. This reaction must occur in a different cellular compartment from that of isomerization and esterification. Otherwise, the entire purpose of the esterification step as the mechanism of 11-*cis*-retinol removal would be defeated. Understanding how this compartmentalization is achieved on the cellular level remains an exciting area of future research.

The discovery of a novel cone-specific retinoid cycle



Two Retinoid Cycles in Vertebrate Retina

See text for details. The established retinoid cycle, illustrated in panel (A), serves as the only source of rod opsin regeneration; however, it provides for the cone opsin regeneration as well. The novel pathway from panel (C) serves to regenerate exclusively cone opsin. The three novel enzymatic reactions described by Mata et al. are highlighted by red arrows. Modified from Figures 1 and 7 of Mata et al. (2002).

brings us to question the biological significance of having two separate cycles in the same retina. Rods and cones work at different conditions of ambient light intensity. While rods are mostly used for sensing single-photon events in conditions of limited illumination, cones are used for daytime vision. Cones remain responsive at practically any level of illumination encountered in nature, because at least some of their visual pigment is always regenerated. Two features of the novel retinoid cycle make it very useful in supporting an adequate degree of cone pigment regeneration at high levels of light. First, it works about 20-fold faster than the pigment epithelium-mediated pathway. Second, the novel pathway can only be used by cones, because rods cannot oxidize 11-*cis*-retinol to 11-*cis*-retinal. This feature prevents rods from “stealing” the isomerized retinoids from cones under daylight conditions when rods are not very useful for vision.

The latter feature of the cone retinoid cycle seems to be even more important for rod-dominant species, including humans (assuming that we also have this pathway), than for the cone-dominant animals. Consider the following: only ~5% of all photoreceptors in our retinas are cones (Osterberg, 1935). Provided that photon capture efficiencies of rods and cones are similar, light of any given intensity bleaches approximately the same fractions of visual pigments in either cell type. As a result, only ~5% of the total 11-*cis*-retinol produced by the pigment epithelium would appear to be available to cones at any illumination. In contrast, every molecule of 11-*cis*-retinol produced by the Müller cells would end up regenerating cone pigments after being oxidized to 11-*cis*-retinal by the novel 11-*cis*-retinol dehydrogenase in cones. With this new cycle in place, rods actually assist rather than impede the regeneration of cone pigments by providing all-*trans*-retinol substrate for the Müller cell isomerase.

Is the capacity of the novel retinoid cycle sufficient

to support cone vision at any lighting condition that an animal can encounter? Mata et al. calculate that it is sufficient for rapid regeneration of the entire pool of the bleached cone pigment in all but the brightest light encountered in nature. Interestingly, even this apparent limitation could be advantageous for vision. It has been shown that cones are able to remain responsive to light with the vast majority of their pigment bleached (Burkhardt, 1994). Not only do they produce normal photoreponses under these conditions, but a reduction in the amount of regenerated pigment allows them to absorb fewer photons, thus rescuing cones from saturation caused by the bright light (see Pugh and Lamb, 2000, Burns and Baylor, 2001, and Fain et al., 2001, for recent reviews on photoreceptor light adaptation).

In summary, the data by Mata et al. indicate that cone vision is supported by a novel pathway for regenerating all-*trans*-retinol into 11-*cis*-retinol. This pathway, involving enzymes located in cones and Müller cells, is uniquely designed to keep up with the high demand for cone opsin regeneration in bright light. Furthermore, it precludes rods from using 11-*cis*-retinol produced in this pathway.

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Open the Loop: Dissecting Feedback Regulation of a Second Messenger Transduction Cascade

Single photon responses were compared in wild-type and transgenic retinal rods with and without guanylate cyclase activating protein (GCAP) to disrupt Ca²⁺-dependent feedback regulation of guanylate cyclase (see Burns et al. in this issue of *Neuron*). The results provided new insights into the molecular mechanisms underlying phototransduction.

Complex machinery depends on feedback to keep it running smoothly and capable of doing its job without spiraling out of control or oscillating wildly. In biology, examples of this exist at scales ranging from populations to molecules. A prototypic case of feedback regulation at the cell and molecular level is the G protein-coupled enzyme cascade in retinal photoreceptors that detects light by converting photon absorption into an electrical signal that begins the process of vision. The molecular details of the phototransduction cascade are well known, probably more so than for any other cellular signaling pathway (see Burns and Baylor, 2001; Arshavsky et al., 2002 for reviews). Its operation is tightly controlled by an exquisite second messenger feedback loop that was recognized years ago (Hodgkin and Nunn, 1988) and has been studied extensively ever since. In spite of a wealth of information about feedback in vertebrate photoreceptors, it is still not known exactly how it works. The elegant experiments and clever analysis described by Burns, Mendez, Chen, and Baylor in this issue of *Neuron* supply much of the missing information. They do this by delivering straightforward and clear answers to a number of fundamental questions about feedback in retinal rods: how fast does it act? How strong is its control? What does it do for rod performance? What are its targets? These are points that are basic to understanding the operation of any feedback control system. So, in addition to providing important new insight into the molecular operation of the transduction process in vision, Burns et al. present a beautiful example of how to dissect a feedback pathway, which could be used as a template for studies of other biological systems that are under feedback control.

In rods, light triggers changes in two intracellular second messengers, cGMP and Ca²⁺, which are tightly interconnected in a feedback loop. In darkness, a current carried by Na⁺ and Ca²⁺ circulates into the photoreceptor through cyclic nucleotide-gated (CNG) channels that are opened by cGMP and then out again through transport pumps. Calcium is pumped out vigorously by Na⁺: Ca²⁺, K⁺ exchange, which is strong enough to insure that in the resting (steady) state, Ca²⁺ efflux matches its influx via CNG channels (several hundred μM Ca²⁺/s in mouse rods). Light absorption excites the cascade by photoisomerizing rhodopsin to produce an activated intermediate (Rh*). In its lifetime, Rh* serially stimulates many G proteins (hundreds), each of which subsequently bind to single phosphodiesterase (PDE) molecules and stimulate cGMP hydrolysis. The drop in cGMP closes CNG channels and the reduction in the normal inward current generates both an electrical signal and an intracellular Ca²⁺ signal. The Ca²⁺ signal, which is a decrease in Ca²⁺, is produced when CNG channel closure decreases Ca²⁺ influx without affecting its efflux via the continued operation of the exchangers. The resulting fall in Ca²⁺ is sensed by GCAP (guanylate cyclase activating protein), which increases cGMP synthesis by stimulating guanylate cyclase (GC). Thus, feedback acts to counteract the fall cGMP that would otherwise follow increases in PDE activity from either its spontaneous activation in darkness or in response to light; e.g., a decrease cGMP leads to a decrease in Ca²⁺, which leads to an increase in cGMP. Hence at all times the rate of change of cGMP is given simply by the difference in the rates of cGMP synthesis (α) and hydrolysis (β), which can be expressed as:

$$\frac{d(\text{cGMP})}{dt} = \alpha(t) - [\text{cGMP}]\beta(t), \quad (1)$$

where α(t) is the sum of a constant basal dark rate α₀ and a rate dependent on Ca²⁺ feedback, α*(t).

Using single cell electrical recording, Burns et al. studied the feedback process by comparing the properties of rods from wild-type (wt) mice with feedback intact (closed loop) and transgenic mice in which GCAP was knocked out (KO) to disable feedback control of cGMP synthesis (open loop). They found that the resting dark current and the initial rate of rise of the light response was the same in wild-type and knockout rods, making the important point that loss of GCAP had no compensatory effect on expression levels of other proteins in the cascade nor on their activation by light.

Further analysis of the results exploited two unique features of rod physiology. First, the electrically recorded current involves only CNG channels and thus provides an accurate and instantaneous measure of intracellular free [cGMP]. Second, dim light flashes generate reproducible responses to minimal activation, i.e., single photon responses.

By knowing the dose-response properties of CNG channel activation by cGMP, the light-evoked changes current recorded from wild-type and GCAP knockout rods could be expressed as changes in cGMP, i.e., d(cGMP)/d(t). Since single photon activation of PDE is reproducible and not influenced by the absence of