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The Cone-specific visual cycle

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ABSTRACT

Cone photoreceptors mediate our daytime vision and function under bright and rapidly-changing light conditions. As their visual pigment is destroyed in the process of photoactivation, the continuous function of cones imposes the need for rapid recycling of their chromophore and regeneration of their pigment. The canonical retinoid visual cycle through the retinal pigment epithelium cells recycles chromophore and supplies it to both rods and cones. However, shortcomings of this pathway, including its slow rate and competition with rods for chromophore, have led to the suggestion that cones might use a separate mechanism for recycling of chromophore. In the past four decades biochemical studies have identified enzymatic activities consistent with recycling chromophore in the retinas of cone-dominant animals, such as chicken and ground squirrel. These studies have led to the hypothesis of a cone-specific retina visual cycle. The physiological relevance of these studies was controversial for a long time and evidence for the function of this visual cycle emerged only in very recent studies and will be the focus of this review. The retina visual cycle supplies chromophore and promotes pigment regeneration only in cones but not in rods. This pathway is independent of the pigment epithelium and instead involves the Müller cells in the retina, where chromophore is recycled and supplied selectively to cones. The rapid supply of chromophore through the retina visual cycle is critical for extending the dynamic range of cones to bright light and for their rapid dark adaptation following exposure to light. The importance of the retina visual cycle is emphasized also by its preservation through evolution as its function has now been demonstrated in species ranging from salamander to zebrafish, mouse, primate, and human.

Contents

Abbreviations: ARAT, acyl-CoA:retinol acyltransferase; CNG, cyclic nucleotide-gated; COS, cone outer segment(s); CRALBP, cellular retinaldehyde binding protein; CRBP, cellular retinol binding protein; ERG, electroretinography; GC, guanylyl cyclase; IMH, isomerohydrolase; IPM, interphotoreceptor matrix; IRBP, interphotoreceptor retinoid binding protein; IS, inner segment(s); L-α-AAA, L-alpha-aminoadipic acid; LRAT, lecithin:retinol acyltransferase; MSP, microspectrophotometry; OS, outer segment(s); PDE, phosphodiesterase; R*, activated receptor molecules (visual pigments); RAL, retinal; RDH, retinol dehydrogenase; RE, retinyl ester; RES, retinyl ester synthase; ROL, retinol; RPE, retinal pigment epithelium; RPE65, RPE specific protein 65 kDa.

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1. Introduction

The description and discussion of the retina visual cycle requires a brief introduction of the morphology, pigment, and phototransduction in both rods and cones. The differences in these features between rods and cones either impose the requirements for a second retinoid cycle in cones, or underlie the specificity of the retina visual cycle for cones.

1.1. Photoreceptors in the retina

Photoreceptors in the retina are the neurons responsible for light detection and the initiation of our visual perception. Most vertebrates have two types of image-forming photoreceptors — rods and cones, which were initially classified based on their morphological appearance. In addition to their structural differences, rods and cones also have distinct functional properties, including light sensitivity, response kinetics, and adaptation range, that make them suitable for dim- and bright-light function, respectively (Fu and Yau, 2007; Kefalov, 2010).

Rods are so sensitive to light stimuli that they are able to detect even a single photon. This makes rods perfect for scotopic (dim light/night time) conditions where they mediate our vision. While rods function well under dim light conditions, they are saturated easily and lose their light-sensing abilities even under moderately bright conditions. Rod responses to dim light are slow and, in addition, they have a long refractory period following exposure to bright light.

In contrast to rods, cones are less sensitive and function under bright light. Together with their wide dynamic range, this makes cones perfect for photopic (bright light/day time) conditions where cones mediate vision. Cone photoresponses to dim light are fast and, in addition, they recover rapidly following exposure to bright light.

Each photoreceptor usually contains one type of opsin, the protein moiety of visual pigment, determining the absorption spectrum of its related pigment and the spectral sensitivity of the photoreceptor. Mouse cones are unusual, as S-opsin and M-opsin are co-expressed in most cones (Applebury et al., 2000).

The functional differences between rods and cones outlined above can, in part, be attributed to the structural differences in their outer segments. Photoreceptors have outer segments (OS), inner segments (IS), nuclear regions, and synaptic terminals. In the rod outer segments (ROS), disc membranes are separated from and surrounded by the plasma membrane. In contrast, cone outer segments (COS) are formed from stacked invaginations of the plasma membrane (Mustafi et al., 2009). As a result, whereas rod discs are isolated from the extracellular space by the plasma membrane, cone discs are open to the extracellular matrix. This configuration of cone outer segments greatly increases the surface of their disc membrane and the cone surface-to-volume ratio. It is believed that the continuous and open structure of cone outer segments facilitates the fast reactions of phototransduction and metabolism in cones and assures the rapid material transport between cones and interphotoreceptor matrix (IPM) (Yau, 1994).

Most vertebrates have retinas populated predominantly by rods (rod-dominant) with relatively few cones. For example, the ratio of rods to cones is 97:3 in mouse (Carter-Dawson and LaVail, 1979), 92:8 in bovine (Krebs and Krebs, 1982), and 65:35 in salamander (Mariani, 1986) retinas. Human and primate retinas are also rod-dominant with rods outnumbering cones by a ratio of 95:5. However, the central area of the human retina, the fovea, is populated exclusively by cones. Several cone-dominant animals, such as chicken with 60% cones (Meyer and May, 1973), and ground squirrel with 96% cones (West and Dowling, 1975), have been very useful for cone-specific biochemical studies. In addition, the larval zebrafish at 15 days post fertilization (dpf) or younger is also considered cone-dominant as its rods do not contribute to vision before this age (Bilotta et al., 2001; Branchek, 1984). For physiological studies of vertebrate cones, the preferred animal has long been the larval tiger salamander. More recently, methods have been developed for biochemical and physiological studies from carp cone photoreceptors that have yielded some very interesting insights into the function of cones (Miyazono et al., 2008; Takemoto et al., 2009).

1.2. Visual pigment

The photon-capturing molecule in photoreceptors is the visual pigment (rhodopsin in rods, and cone pigment in cones), which consists of light-sensing chromophore, covalently attached to a protein, opsin. Opsin, a member of the G-protein-coupled receptors family (Palczewski, 2006), has seven transmembrane helices and represents the most abundant protein on the disc membranes of both rods and cones. The pigment density is quite uniform, ~25,000 molecules μm^{-2} (corresponding to concentration of ~3.5 mM), in either type of photoreceptor across different species (Harosi, 1975). The most common chromophore in vertebrate photoreceptors is 11-*cis* retinal (11-*cis* RAL, also referred to as A1). Some aquatic animals also use 11-*cis*-3,4-dehydroretinal (also referred to as A2) (Dartnall and Lythgoe, 1965).

The apo-opsin (free opsin without chromophore) has weak constitutive activity and can trigger the transduction cascade (Cornwall and Fain, 1994; Cornwall et al., 1995). In darkness, 11-*cis* RAL, serving as an antagonist, binds to opsin via a Schiff-base linkage at a conservative lysine in opsin (K296 in mammalian rhodopsin) to form a holo-pigment, which is the inactive ground state of the visual pigment (Dartnall and Lythgoe, 1965). Photon

absorption by 11-*cis* RAL converts it to the all-*trans* form, which is a strong agonist for opsin. The photoisomerization of the retinoid induces a series of rapid conformational changes of the pigment molecule that convert it to the physiologically active state (Meta II) within ~1 ms (Lamb and Pugh, 2004; Okada et al., 2001). Meta II is the form of rhodopsin that activates the visual G-protein, transducin (Gt), and thus Meta II is also called R* (activated receptor). Eventually, Meta II decays to an inactive form, Meta III, and following the hydrolysis of the Schiff-base bond dissociates into free opsin and all-*trans* RAL. This decay takes minutes in rods but only seconds in cones (Shichida et al., 1994). The inactivation of R* in photoreceptors will be discussed below (see Section 1.4).

1.3. Phototransduction

Phototransduction, the process of converting light into electrical neural signals, takes place in the outer segments of photoreceptors. The mechanisms of phototransduction and the proteins involved are highly conserved in rods and cones across different species (Arshavsky et al., 2002; Lamb and Pugh, 1992; Pugh and Lamb, 1993). The second messenger conveying photo-signal to neural signal is cGMP, which opens nonselective cyclic nucleotide-gated (CNG) cation channels located on the outer segment plasma membrane (Yau, 1994). In darkness, when bound to cGMP, a fraction of CNG channels are open, allowing the steady influx of Na^+ and Ca^{2+} driven by the electrochemical gradient across the plasma membrane of the outer segment. This inward current (denoted as "dark current") depolarizes the photoreceptors and maintains the steady release of neurotransmitter (glutamate) from their synaptic terminals in darkness.

The cGMP concentration within the outer segment is equilibrated by the balance between its synthesis by guanylyl cyclase (GC) and its hydrolysis by phosphodiesterase (PDE). Upon light absorption, R* activates the G-protein transducin (Gt), which in turn activates PDE. PDE* hydrolyzes cGMP into GMP, lowering cGMP concentration. The resulting closure of the CNG channels blocks the dark current and hyperpolarizes the photoreceptor membrane. As a result, the rate of glutamate release from the synapses is reduced, thus converting and relaying the light signal to the postsynaptic neurons as electrical signal (Lamb and Pugh, 2006; Yau and Hardie, 2009).

1.4. R* inactivation

To maintain the continuous responsiveness of rods and cones, phototransduction in the outer segment has to be terminated by inactivating all the transduction components, including R*, G*, and PDE*, and finally recovering the level of cGMP. Refer to (Fu and Yau, 2007; Lamb and Pugh, 2004) for detailed review of the termination of phototransduction. Here we will focus on R* termination.

R* inactivation occurs in two steps: phosphorylation and capping with arrestin. First, the activity of R* is partially blocked when it is phosphorylated by rhodopsin kinase (GRK) (Kuhn and Wilden, 1987; Whitlock and Lamb, 1999; Wilden et al., 1986). The capping protein – arrestin then binds to the phosphorylated R* on its cytoplasmic face. The formation of this complex inactivates R* completely by blocking its access to transducin (Schleicher et al., 1989; Wilden et al., 1986; Xu et al., 1997).

Due to the conformational changes in R*, the retinyl-lysine Schiff-base bond becomes more exposed and accessible to water, and thus susceptible to hydrolysis. R* can either decay into free opsin and all-*trans* RAL or convert to the inactive Meta III form, the latter eventually also dissociating into apo-opsin and retinoid (Kolesnikov et al., 2003). Notably, this spontaneous decay is substantially slower than the inactivation of R* by GRK and arrestin and, as a result, it does not affect the flash response (Imai et al., 2007; Kefalov et al., 2003).

Exposure to bright light results in the activation and subsequent decay (bleaching) of substantial fraction of the visual pigment. This reduces the sensitivity of photoreceptors by two mechanisms: first, it reduces the amount of pigment available for subsequent light activation, and second, it produces constitutive activity due to the buildup of apo-opsin as the bleached pigment decays. Thus, complete restoration of dark-adapted sensitivity of photoreceptors requires regeneration of the bleached visual pigment with 11-*cis* RAL (Cornwall and Fain, 1994; Cornwall et al., 1995; Pepperberg et al., 1978; Surya et al., 1995). The continuous function of photoreceptors in steady background light also requires constant renewal of their visual pigment with 11-*cis* RAL.

2. The canonical RPE visual cycle

The process of recycling all-*trans* RAL, released from the bleached pigment, to 11-*cis* RAL, required for pigment regeneration, is known as the visual cycle. The canonical visual cycle involves the retinal pigment epithelium (RPE), a monolayer of epithelial cells adjacent to the outer segments of photoreceptors. In 1878, Willy Kühne found that the bleached frog retina recovers only partially its level of visual purple (Rhodopsin) if it is detached from the RPE, and photoreceptors have to contact the intact pigment epithelium to fully regenerate their pigment (Kühne, 1878). Weinstein et al. reported that no rhodopsin regeneration is observed in the isolated rat retina even if the retina is perfused with an enriched medium that maintains neuronal activity (Weinstein et al., 1967). These studies demonstrated the crucial role of RPE in pigment regeneration. In the past few decades, a lot has been learned about the RPE chromophore recycling pathway that provides 11-*cis* RAL for pigment regeneration in both rods and cones (Fig. 1).

2.1. The importance of RPE in pigment regeneration

The source of chromophore in the visual system is all-*trans* retinol (all-*trans* ROL/vitamin A), derived from the blood circulation (Dowling and Wald, 1960). The all-*trans* ROL from the choroid blood vessels is taken up by the RPE cells (Chen and Heller, 1977; Timmers et al., 1991), converted to 11-*cis* RAL and introduced into photoreceptors, where it combines with opsin to form visual pigment (Fig. 1). Following photoactivation of the pigment, the chromophore is released as all-*trans* RAL, which is reduced to all-*trans* ROL and then removed from the bleached photoreceptors and transported to the RPE. There, all-*trans* ROL is enzymatically converted into 11-*cis* RAL and transported back to photoreceptors to regenerate the visual pigment. In the dark-adapted retina, little free ROL/RAL is present in either the retina or the RPE (Dowling, 1960). As the chromophore supply from blood vessels is rather slow, pigment regeneration after a bleach is driven primarily by the recycled photolytic all-*trans* ROL released from the bleached photoreceptors (Wald, 1935).

2.2. Overview of the RPE visual cycle

The reactions of the RPE visual cycle are well characterized and outlined in Fig. 1 (reviewed in Saari (2000)). Photon absorption by the visual chromophore triggers its isomerization from 11-*cis* to all-*trans* RAL, activating the pigment. After a series of configuration transformations, the activated pigment eventually decays to free opsin and all-*trans* RAL. In the outer segments, the released all-*trans* RAL is reduced to all-*trans* ROL by NADPH-dependent retinol dehydrogenase (RDH) (Jang et al., 2000; Kolesnikov et al., 2007; Rattner et al., 2000). All-*trans* ROL is then transferred from

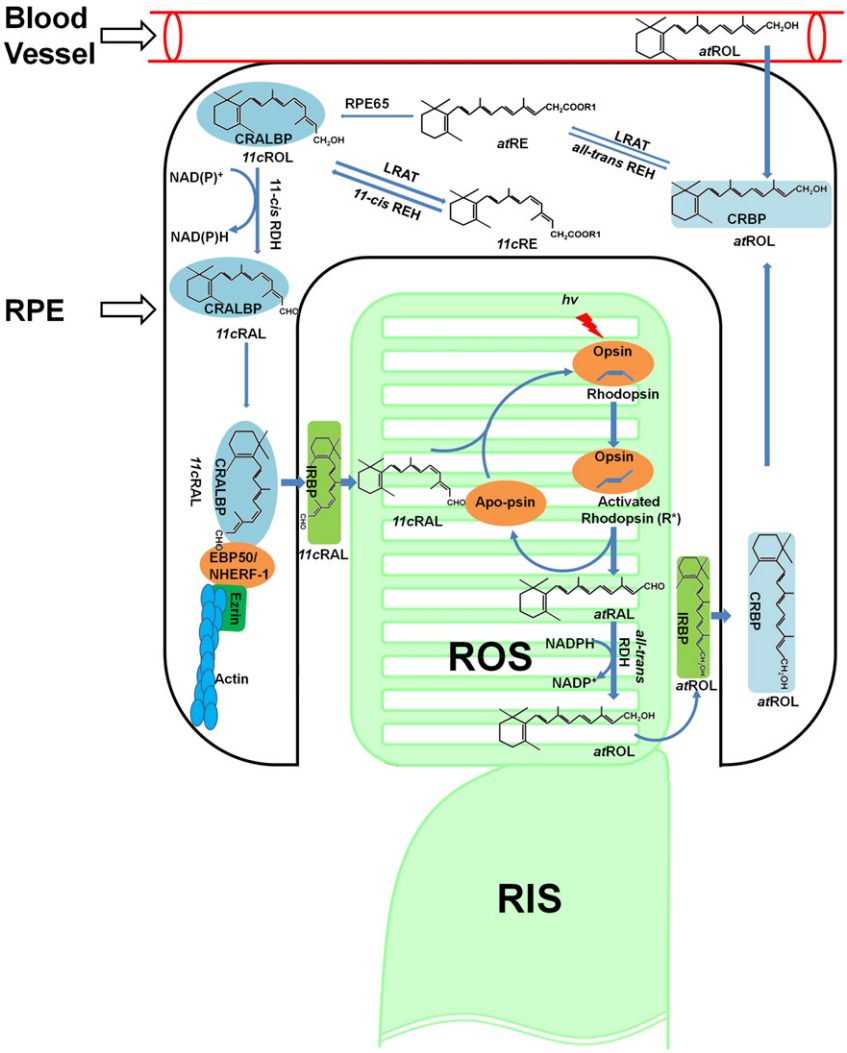

Fig. 1. Schematic representation of the pigment epithelium visual cycle. There are two sources of chromophore for pigment regeneration. All-*trans* ROL from choroid blood circulation is the original source of chromophore; all-*trans* ROL released from outer segments following light exposure is the second and the major source of chromophore for pigment regeneration. Upon photon absorption, rhodopsin is activated and then decays into free opsin and all-*trans* RAL. The latter is reduced to all-*trans* ROL by retinol dehydrogenase (RDH) in the outer segments, and is released to the interphotoreceptor matrix (IPM). A chromophore-binding protein, IRBP, in IPM facilitates transporting all-*trans* ROL to RPE apical processes, where another chromophore-binding protein, CRBP, takes over the retinoid. Bound to CRBP, all-*trans* ROL is transferred to the cell body of RPE cells. There, LRAT esterifies all-*trans* ROL to all-*trans* RE, which is then hydrolyzed and isomerized to 11-*cis* ROL by the isomerohydrolase RPE65. CRALBP removes the resultant 11-*cis* ROL from the reaction site to speed the isomerization. Bound to CRALBP, 11-*cis* ROL is oxidized to 11-*cis* RAL by RDH. It has been suggested that CRALBP, as a carrier, transports 11-*cis* RAL to the apical processes of the RPE, where the retinoid exits the RPE assisted by the scaffold proteins EBP50/NHERF-1 and Ezrin. Binding with IRBP, 11-*cis* RAL is then transferred back to photoreceptor outer segment, where it recombines with the apo-opsin to regenerate the visual pigment.

photoreceptors to the apical processes of RPE cells in a process facilitated by a carrier protein – Interphotoreceptor Retinoid Binding Protein (IRBP) (Adler and Martin, 1982; Lai et al., 1982; Liou et al., 1982). In the RPE, all-*trans* ROL binds to Cellular Retinol Binding Protein Type I (CRBPI) (Napoli, 2000; Noy, 2000) and diffuses from the apical processes to the RPE cell body (Huang et al., 2009), where it is esterified to all-*trans* retinyl ester (all-*trans* RE) by lecithin:retinol acyltransferase (LRAT) (Mondal et al., 2000; Ruiz et al., 1999; Saari and Bredberg, 1988, 1989). The all-*trans* RE is then hydrolyzed and isomerized into 11-*cis* ROL by RPE65 (Isomerohydrolase (IMH), Isomerase I) (Gollapalli et al., 2003; Gollapalli and Rando, 2003; Jin et al., 2005; Moiseyev et al., 2006; Redmond et al., 2005). Bound to another protein called Cellular Retinaldehyde Binding Protein (CRALBP), 11-*cis* ROL is oxidized into RAL by 11-*cis* RDH. Still bound to CRALBP, 11-*cis* RAL diffuses to the apical processes of RPE cells which are close to the photoreceptors outer segments. Next, 11-*cis* RAL exits the RPE to the extracellular interphotoreceptor matrix (IPM), possibly facilitated by CRALBP, EBP50/NHERF-1, ezrin and actin complex (Nawrot et al., 2006; Saari et al., 2009). IRBP is believed to carry 11-*cis* RAL and transfer it back to the outer segments for pigment regeneration (Bok, 1985; Pepperberg and Clack, 1984; Perlman et al., 1982).

In addition to converting to 11-*cis* RAL, 11-*cis* ROL can also be esterified by LRAT to form 11-*cis* RE (Mata and Tsin, 1998; Saari et al., 1993), which is stored as a pool of 11-*cis* retinoid in the RPE and later released by 11-*cis* retinyl ester hydrolase (REH) (Blaner et al., 1987; Mata et al., 1998a,b, 1992; Tsin et al., 2000) to supply chromophore for pigment regeneration (Mata et al., 1998a,b).

3. The cone-specific retina visual cycle

Rods and cones function in different light conditions that impose different requirements for the properties of their visual pigments. Yet, light exposure bleaches rod and cone pigment at equal rates because the pigment concentration and density in both photoreceptors are similar across species (Harosi, 1975). The

quantum efficiency, defined as the ratio of the number of photo-activated pigment molecules to the number of such molecules that absorbed a photon, is also identical in rods and cones (Dartnall, 1972). Finally, the extinction coefficients for rod and cone pigments are also very similar (Shichida et al., 1994), suggesting rod and cone pigments have the same ability to capture photons. Yet, despite the comparable levels of pigment bleach following exposure to bright light, cones dark adapt within 3–4 min whereas rods take over 30 min to fully restore their sensitivity (Hecht et al., 1937). As pigment regeneration is required for the restoration of dark-adapted sensitivity following exposure to bright light, this dramatic difference in the rates of dark adaptation of rods and cones implies that visual pigment is regenerated substantially faster in cones compared to rods. This, together with the observation that pigment regeneration is rate-limited by the supply of recycled chromophore to the photoreceptors (Imai et al., 2007; Lamb and Pugh, 2004), suggests that chromophore is supplied substantially faster to cones than to rods, possibly with the help of a second, cone-specific visual cycle. Furthermore, in contrast to the rods, which saturate in light conditions close to twilight (Penn and Hagins, 1972), cones remain functional and cannot be saturated even in very bright light (Baylor and Hodgkin, 1974; Schnapf et al., 1990; Weale, 1961). As the bright light would be activating and bleaching the visual pigment at a high rate, the ability of cones, but not rods, to remain responsive under these conditions also suggests a preferential rapid supply of recycled 11-*cis* RAL to cones. Notably, recent biochemical studies show that the RPE visual cycle, which supplies chromophore to both rods and cones, is too slow to support cone function under bright conditions (Mata et al., 2002). The requirement for a rapid supply with recycled chromophore in order to sustain cone function throughout the day points to the possible existence of a second, cone-specific retinoid cycle.

Despite the requirement for rapid supply with chromophore to sustain the function of cones throughout the day, when it comes to being supplied with recycled chromophore by the RPE, cones are at a disadvantage in rod-dominant retinas, such as mouse and human. First, cones account for only 3–5% of the photoreceptors in these retinas and, as a result they have to compete with overwhelming number of rods for the 11-*cis* RAL supplied from the RPE. Although rods are saturated under bright light, their pigment still undergoes repetitive decay-regeneration cycles, and thus consumes the recycled retinoid at a high rate. Second, rods are physically closer to the apical processes of RPE (Dowling, 1967) and, as a result, the traffic of chromophore to the retina would favor rods over cones. Finally, whereas once formed rod pigment is stable, cone pigments can dissociate spontaneously into opsin and 11-*cis* RAL (Kefalov et al., 2005; Wald et al., 1955). Thus, even after cone pigment is formed, it might lose its chromophore to the adjacent rods. These pitfalls of the RPE visual cycle relating to cone function also point to the possible existence of a second cone-specific retinoid cycle.

3.1. Early evidence implying an RPE-independent visual cycle

By recording early receptor potential (ERP), which is linearly related to the amount of photopigment (Cone, 1964, 1967), Goldstein found that bleached frog cone ERP recovered to more than 80% of its dark-adapted amplitude in retina isolated from RPE with $t_{1/2} \approx 5$ min (Fig. 2), whereas bleached rod ERP did not recover under the same condition (Goldstein, 1970). These results were the first experimental demonstration that the retina itself might have the capacity to drive cone pigment regeneration, suggesting the likely existence of an RPE-independent cone visual cycle (Goldstein, 1967, 1968, 1970; Goldstein and Wolf, 1973).

However, Goldstein and Wolf also reported regeneration of green rod pigment ($\lambda_{max} = 433$ nm) in isolated frog retina

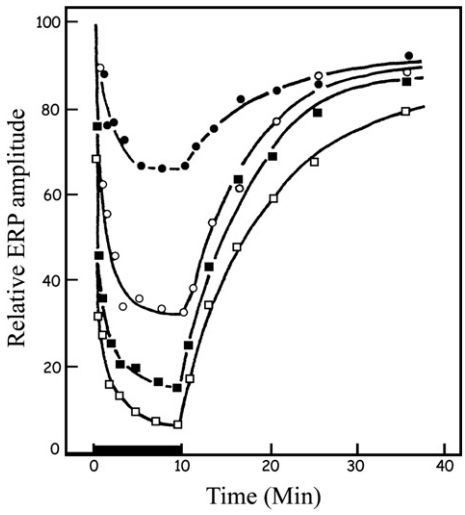

Fig. 2. Red cone ERP amplitudes recover within the isolated frog retina after bleach. Recovery of frog cone ERP elicited by 630 nm in the isolated retina after 10-min bleaches of increasing intensity. The bleaching light intensity increases in relative logarithmic units. 0.0 (filled circles), 0.54 (open circles), 0.88 (filled squares), and 1.12 (open squares), ($n = 5$). Each recovery phase is fitted with an exponential function; the values of $t_{1/2}$ for amplitude recovery are uniformly about 5–6 min. Adapted from Goldstein (1970).

(Goldstein and Wolf, 1973). In addition, Cone and Brown reported that rhodopsin was regenerated spontaneously in both rat and frog isolated retinas, when only a small area of the retina was bleached (Cone and Brown, 1969). These findings challenged the long-known and well established notion that rod pigment regeneration requires the pigment epithelium (Crescitelli and Sickel, 1968; Goldstein, 1967; Hood and Hock, 1973; Kühne, 1878; Weinstein et al., 1967). Such contradictory findings puzzled scientists and cast doubt on the possible existence of a cone-specific RPE-independent pigment regeneration pathway. As a result, Goldstein's findings about the possible retina-driven cone pigment regeneration did not draw researchers' attentions for decades following his work.

3.2. Biochemical studies supporting an alternative cone visual cycle in the retina

About two decades later, the likely existence of an alternate visual cycle interested scientists again after the discovery of retinoid derivatives in cone-dominant retinas. Using HPLC assays, the concentration and localization of retinoids in different species were measured. In rod-dominant frog and cow retinas, retinyl ester is localized predominantly in the RPE with a prevalence of all-*trans* isomer. In cone-dominant chicken and ground squirrel, in contrast, retinyl esters are concentrated in the retina, instead of RPE, with a prevalence of the 11-*cis* RE (Berman et al., 1980; Bridges, 1976; Bridges et al., 1987; Rodriguez and Tsin, 1989). The ratio of 11-*cis* to all-*trans* retinoids (RE, ROL) was found to increase with the cone proportion in the neural retina (from rat to chicken) (Das et al., 1992). More recent studies from Andrew Tsin's laboratory found the rates of accumulation and depletion of 11-*cis* RE in chicken retina to be much higher than these of all-*trans* RE in RPE and to be dependent on the light intensity and duration of light exposure (Trevino et al., 2005; Villazana-Espinoza et al., 2006). Based on these studies, Tsin and colleagues suggested that the 11-*cis* RE pool in the retina may be a source for cone pigment regeneration (Rodriguez and Tsin, 1989).

One of the most significant steps in establishing the retina cone visual cycle was identifying retinoid enzymatic activities in the cone-dominant chicken and ground squirrel retina membrane

fractions. When incubated with all-*trans* ROL, the chicken retina membrane fraction could produce 11-*cis* RE, 11-*cis* ROL and all-*trans* RE (Das et al., 1992), indicating the existence of at least three enzymatic activities in the retina: 11-*cis* retinyl ester synthase (RES), isomerase, and all-*trans* RES. Retinyl ester hydrolase (REH) activity was also reported in chicken retina to hydrolyze RE (11-*cis* and all-*trans* forms) into free ROL (Bustamante et al., 1995; Rodriguez and Tsin, 1989; Tsin and Lam, 1986). Subsequently, an elegant study done by Mata et al. identified clearly these three visual cycle enzymatic activities in the chicken and ground squirrel retina membrane fractions and proposed a model for the cone-specific visual cycle in cone-dominant retina (Mata et al., 2002).

The first enzymatic activity found in cone-dominant retinas is that of Isomerase II, catalyzing all-*trans* to 11-*cis* retinol isomerization. Isomerase II catalyzes the isomerization of retinol (Mata et al., 2002, 2005). In contrast, RPE65, the isomerase in the canonical RPE pathway, has coupled activities of isomerase and hydrolase, catalyzing the simultaneous isomerization and hydrolysis of retinoid esters (Jin et al., 2005; Moiseyev et al., 2005; Redmond et al., 2005). Notably, similar to their role in the isomerase reaction in RPE, CRALBP and CRBPI might be also required for the reaction catalyzed by Isomerase II in the retina (Mata et al., 2002, 2005).

The second enzymatic activity found in the retina is that of retinyl ester synthase (RES), catalyzing the production of RE from ROL. The retina RES activity is not affected by LRAT inhibitors and can be increased by palmitoyl CoA, suggesting that the retina RES is an acyl-CoA:retinol acyltransferase (ARAT), which uses acyl coenzyme A as donor (Kaschula et al., 2006; Mata et al., 2002). The rate of 11-*cis* RE synthesis is dependent on the concentration of all-*trans* ROL (Mata et al., 2005), suggesting that the two reactions might be correlated.

The third enzymatic activity found in the retina is that of retinol dehydrogenase (RDH) that oxidizes 11-*cis* ROL to RAL (Mata et al., 2002). This reaction requires $NADP^+$ as a cofactor rather than NAD^+, which is the preferable cofactor for 11-*cis* RDH in the RPE (Jang et al., 2000). Notably, when bleached salamander cones are exposed to 11-*cis* ROL, they regenerate their visual pigment, while rods do not (Ala-Laurila et al., 2009; Jones et al., 1989). This result indicates that the retina 11-*cis* RDH activity is localized in cones. Whether the oxidation of 11-*cis* ROL takes place in the inner segment or in the outer segment has not been established yet. An interesting recent study in carp cone showed that the oxidation of 11-*cis* ROL to 11-*cis* RAL is coupled with the reduction of *all-trans* RAL to *all-trans* ROL (Miyazono et al., 2008), suggesting that the oxidation of 11-*cis* ROL might take place in the cone outer segments.

Taken together, these biochemical studies provided clear evidence for the existence of an alternate chromophore recycling pathway in the cone-dominant neural retinas of chicken and ground squirrel. The chromophore supplied by the retina was likely to be 11-cis ROL, which only cones would be able to utilize, restricting the retina visual cycle to cones and excluding rods. However, the lack of functional evidence for the presence of such a visual cycle and negative results from rod-dominant species cast doubt on the physiological relevance of these studies.

4. Potential involvement of Müller cells in the retinoid recycling pathway

The expression of the retinoid binding proteins CRBP and CRALBP in Müller (radial glial) cells suggested that Müller cells might be involved in the chromophore processing pathway (Bok et al., 1984; Bunt-Milam and Saari, 1983; Eisenfeld et al., 1985) as both retinoid binding proteins are critical in the RPE visual cycle. The soma of Müller cells is located in the inner nuclear layer (INL) and gives rise to two opposite trunk processes spanning the retina from ganglion cell layer (GCL) to the outer limiting membrane (OLM) where they form elaborate sheaths around the neuronal somata (Dreher et al., 1992; Lessell and Kuwabara, 1963; Reichenbach et al., 1989; Robinson and Dreher, 1990).

The first evidence supporting the involvement of Müller cells in retinoid recycling came from primary culture studies of chicken Müller cells (Das et al., 1992). Cultured Müller cells were able to synthesize 11-*cis* ROL and 11-*cis* retinyl palmitate from radiolabeled all-*trans* ROL added into the culture medium, indicating that the chicken Müller cells contain isomerase and RES activities. In 2006, Muniz et al. further confirmed that the RES activity in chicken Müller cells is ARAT (Muniz et al., 2006). Its activity is expected to be enhanced by CRALBP and, indeed, addition of apo-CRALBP strongly stimulates the synthesis of 11-*cis* ROL from all-*trans* ROL in the retina fraction (Mata et al., 2002). Based on these results, Mata et al. proposed that Müller cells in the retina is where the recycling of chromophore takes place, and Müller cells provide cones with 11-*cis* ROL (Mata et al., 2002). Interestingly, the ratio of cones to Müller cells in the fovea of primates is 1:1, implying the close relationship between Müller cells and cone function (Ahmad et al., 2003; Burris et al., 2002).

5. The traffic of chromophore in the retina

Both the canonical RPE visual cycle and the putative retina visual cycle involve two cellular compartments, one is the photoreceptors and the other is RPE or Müller cells. Thus, transport of the highly hydrophobic retinoid to and from photoreceptors is critical for the function of both visual cycles and for the regeneration of visual pigment in rods and cones. The mechanism for traffic of retinoid between Müller cells and cones is unknown. Notably, the processes of Müller cells end at the outer limiting membrane (OLM) and only reach the inner segment of photoreceptors. Therefore, the chromophore exiting from Müller cells would most likely be transported to the inner segments of cones, from where it will have to move to their outer segments. This scheme is consistent with the observation that, unlike in rods, in cones the chromophore can traffic from the inner to the outer segment to drive pigment regeneration (Jin et al., 1994). The inability of rods to transport chromophore from the inner to the outer segment might be related to the separation of plasma and disc membranes in their outer segments. In contrast, the single membrane in the invaginated disks of the cone outer segment is likely to promote the diffusion of chromophore and its movement along the outer segment. The uptake of chromophore by cone inner segment and the utilization of 11-*cis* ROL by cone outer segment would make 11-*cis* ROL recycled from Müller cells accessible for pigment regeneration selectively in cones.

The importance of IRBP in trafficking retinoid in the RPE visual cycle has been established (Jin et al., 2009; Parker et al., 2009). $Irbp^{-/-}$ mice have reduced cone ERG responses and their cone photoreceptors undergo degeneration (Jin et al., 2009; Parker et al., 2009). This result suggests that IRBP plays an important role in delivering chromophore to cones. It is not clear at the moment whether IRBP facilitates the traffic of chromophore only in the RPE visual cycle or it also plays a role in the retina visual cycle. Further studies will be needed to determine the function of IRBP in the traffic of retinoid specifically between Müller cells and cones.

6. Physiological evidence for the retina visual cycle in rod-dominant species

A recent review described in detail the parallel visual cycles in zebrafish (Fleisch and Neuhauss, 2010), thus we will focus on the

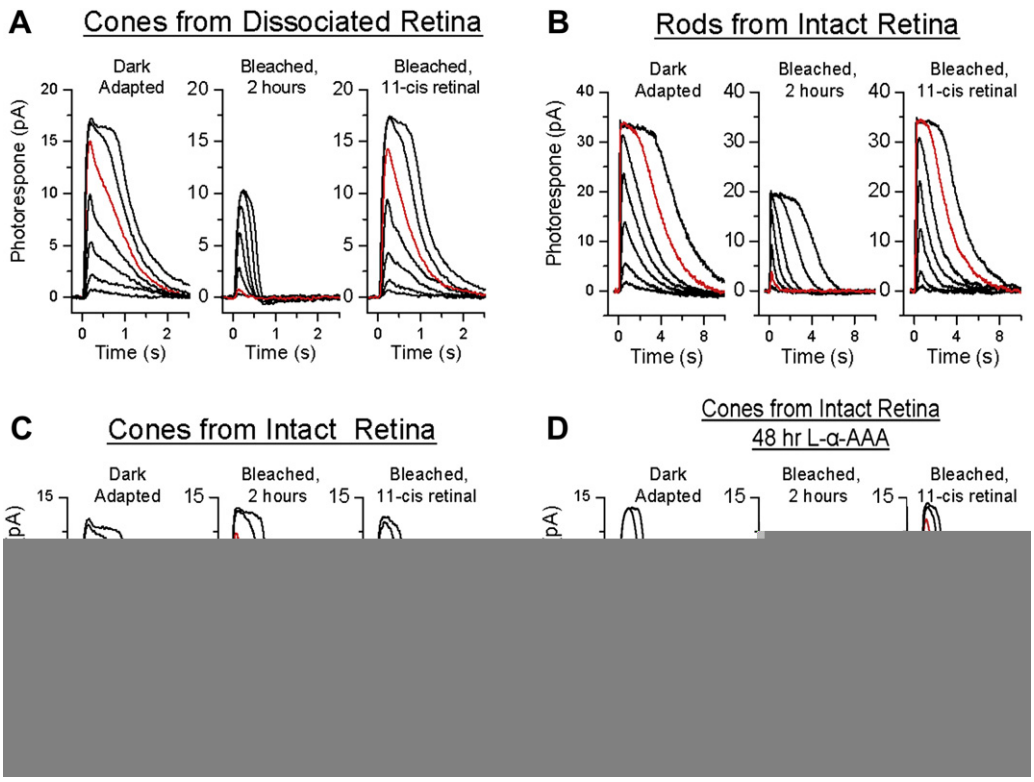

Fig. 3. Salamander retina promotes cone, not rod, dark adaptation, with the help of Müller cells. Suction recordings of flash response families from single dissociated cones (A), rods from intact retina (B), cones from intact retina (C), and cones from intact retina treated with the Müller cell inhibitor L-α-AAA (D). Each panel shows test flash responses from cells in dark-adapted state (left), bleached with a 40 s white light followed by 2 h dark incubation (middle), and after treatment with exogenous 11-*cis* retinal (right). For red cones (A, C, and D), red traces represent photoresponses to 6550 photons μm^{-2}, 620 nm. For rods (B), red traces represent photoresponses to 119 photons μm^{-2}, 520 nm. Note the recovery of sensitivity of bleached cones from intact retina (C) but not of cones from dissociated retina (A) or rods from intact retina (B). When the function of Müller cells is inhibited by 48 h L-α-AAA incubation prior to the bleach, the cone sensitivity recovery driven by the intact isolated retina is eliminated (D). Adapted from Wang et al. (2009).

cone-specific visual cycle in other vertebrates and in mammals. Because of the difficulties of investigating biochemically or physiologically the function of mammalian cones in rod-dominant species, all the evidence discussed above in support of a retina visual cycle came from *in vitro* biochemical studies and only from cone-dominant species. The small fraction of cones and the homology between rod and cone transduction proteins makes unfeasible the execution of similar biochemical studies with rod-dominant species, such as mouse and human. The lack of evidence supporting the validity of these results for rod-dominant species created controversy regarding the existence of a visual cycle in their retinas. In addition, two recent studies failed to find evidence for the function of a retina visual cycle in mouse retina (Feathers et al., 2008; Wenzel et al., 2007) further bringing into question its existence. Finally, previous biochemical studies from mouse retina also failed to detect enzymatic activities consistent with a retina visual cycle (Mata et al., 2002). This issue was settled by our recent physiological studies from rod-dominant animals, including salamander, mouse, primate, and human (Wang et al., 2009; Wang and Kefalov, 2009).

6.1. A functional visual cycle in the salamander retina

We first used salamander as a rod-dominant model, with cones in its retina accounting for 35% of all photoreceptors (Mariani, 1986). Salamander retina has large and robust photoreceptors, making it suitable for long-lasting experiments and for physiological and microspectrophotometric (MSP) studies from individual cones.

By combining MSP and single-cell suction recordings (Wang et al., 2009), we demonstrated that following a bleach salamander red cones in intact isolated retina were able to regenerate over 90% of their pigment and dark adapt, while rods from the same retina could not (Fig. 3). In contrast, isolated cones, removed from the retina, were not able to regenerate their pigment and dark adapt following a bleach. These results indicate that the isolated salamander retina can promote cone, but not rod, pigment regeneration independently of the RPE. The less than complete cone pigment restoration might be due to dissection damage of the retina or to loss of chromophore from the retina to the perfusion solution. We also found that preserving the contact between cones and the retina is critical for proper function of the retina visual cycle as even gently drawing the outer segment of a cone, still sitting in the retina, into the suction pipette did block the retina-driven cone pigment regeneration and dark adaptation (Fig. 4A). This manipulation did not affect otherwise the function of the cones and if they were pre-incubated with 11-*cis* RAL prior to bleach, their sensitivity recovered readily and so rapidly that the desensitization induced by the bleach was not observable at all (Fig. 4B).

6.2. Cone-specific visual cycle in the mouse retina

Having demonstrated that the retina visual cycle exists in salamander retina, we investigated its possible presence in mouse. The small number of cones in the mouse retina (3%) makes identifying them and performing single-cell recording from them extremely challenging. To circumvent this technical issue, we used rod transducin alpha subunit deficient ($T\alpha^{-/-}$) mice. While the retina of these animals appears normal, its rods do not generate light

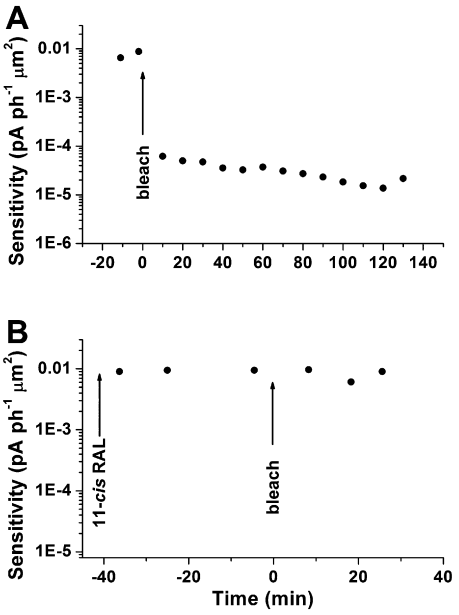

Fig. 4. Importance of the intact cone–retina interaction in the retina-driven cone dark adaptation. The cone sensitivity recovery following a bleach is blocked by drawing the outer segment gently into a suction electrode; no recovery is observed up to 130 min post-bleach, limited by the stability of the recordings (A). In contrast, if the retina is pre-incubated with exogenous 11-*cis* RAL prior to the bleach, cone recovery is complete and too rapid to be easily observed with suction recording (B). Adapted from Wang et al. (2009).

responses (Calvert et al., 2000). This makes it possible to identify cones as the only photoreceptors responding to light (Nikonov et al., 2006; Shi et al., 2007; Wang and Kefalov, 2010). By using this approach, we were able to obtain single-cell recordings from mouse cones and to investigate whether in the intact isolated retina cone photosensitivity recovers after bleach (Wang and Kefalov, 2009). We illuminated briefly the isolated mouse retina to bleach over 90% of the pigment in both rods and cones and then allowed the retina to recover in darkness for 2 h. We found that following such a treatment, the sensitivity of cones would recover substantially indicating regeneration of cone pigment in the absence of RPE (Fig. 5B). In contrast, rods remained permanently desensitized unless supplied with exogenous chromophore (Fig. 5A). These results clearly demonstrated that the mouse neural retina promotes cone, but not rod, pigment regeneration and established the function of a visual cycle in the mouse retina. The lack of pigment regeneration in rods ruled out the possibility that the regeneration we observed in cones was due to residual RPE in the isolated retina. Similar to what we found in the salamander, mouse cone recovery was blocked by drawing the inner segment into electrode (Fig. 5B), confirming that the function of the retina visual cycle requires efficient interaction of cones with the retina.

6.3. Müller cells are part of the retina visual cycle

It has been suggested by biochemical studies that Müller cells might be involved in the retina visual cycle. However, transformed rat Müller cells failed to isomerize all-*trans* to 11-*cis* retinoid (Kanan et al., 2008). Although this might be due to the possible loss of some critical enzymes in the transformed cell line, these results also led to doubt of the role of Müller cells in the visual cycle.

To study the role of Müller cells in chromophore recycling, we used a Müller cell-specific inhibitor – L-alpha-aminoadipic acid (L-α-AAA), to selectively and acutely destroy Müller cells (Jablonski

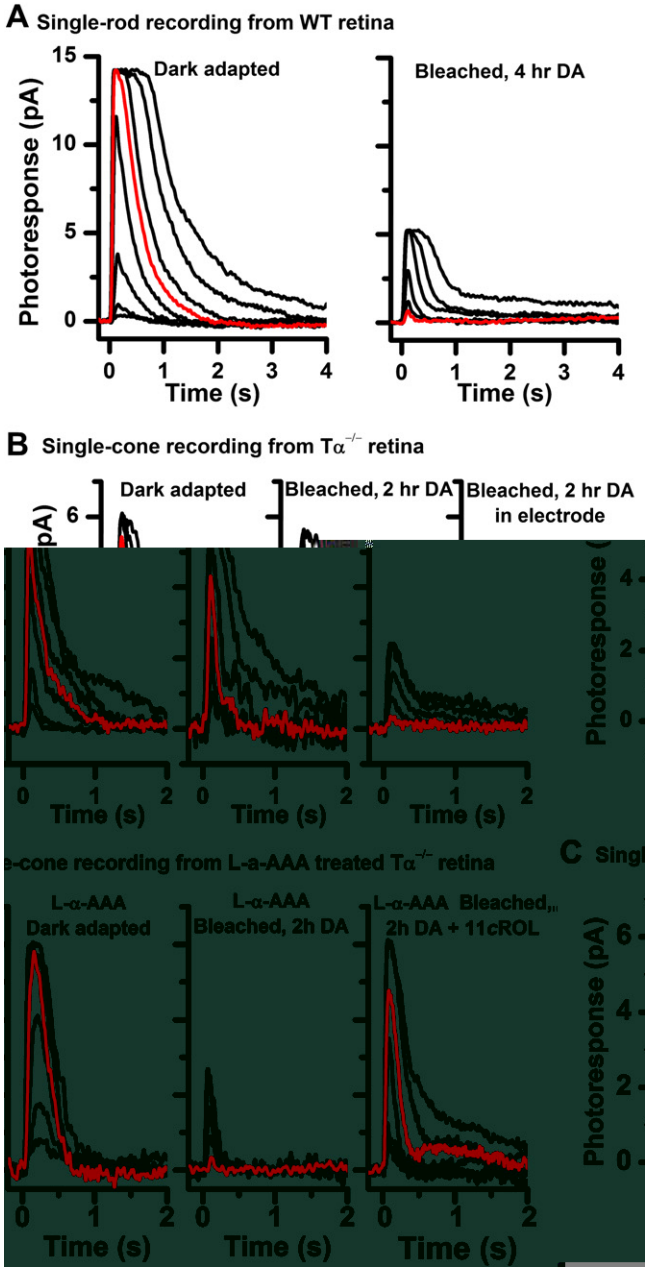

Fig. 5. The mouse retina promotes cone pigment regeneration. Single-cell suction recordings from rods of wild type mice (A), cones of Trα$^{-/-}$ mice (B), and cones of Trα$^{-/-}$ retina pre-incubated with L-α-AAA (C). Test flash responses of individual cells are from dark-adapted state (left panels), or bleached followed by 4-h (rod, right panel in A) or 2-h (cone, middle panels in B and C) dark recovery period. Disturbing the interaction of cone with retina by drawing the inner segment into electrode blocks the retina visual cycle (right panel in B). The right panel in (C) shows that mouse cones from L-α-AAA-treated retina are able to utilize 11-*cis* ROL to restore their sensitivity. Red traces represent photoresponses to 291 photons μm^{-2}, 500 nm for rods (A) and to 75,814 photons μm^{-2}, 500 nm for M cones (B and C). The desensitization produced by the bleach is persistent in rods but largely reversed in cones from isolated retina in the absence of pigment epithelium, and cone sensitivity recovery within the retina is inhibited by blocking Müller cells function with L-α-AAA. Adapted from Wang and Kefalov (2009).

and Iannaccone, 2000). L-α-AAA functions by inhibiting cysteine uptake through the cysteine/glutamate antiporter, which is primarily localized in glial cells. This, in turn, blocks the synthesis of the antioxidant glutathione, leading to glial cell damage (Kato et al., 1993).

The retina-driven cone pigment regeneration, in both salamander and mouse, was inhibited by incubating the retina with L-α-AAA prior to the bleach, clearly demonstrating the involvement of the Müller cells in the retina visual cycle (Figs. 3D and 4C). Notably, the drug incubation did not affect the morphology of the retina or the function of cones. In addition, the gliotoxin did not affect the RPE visual cycle, as salamander rod pigment regeneration within the eyecups, where the retina is still attached to the RPE, was not affected by L-α-AAA treatment (Wang et al., 2009).

The block of the retina visual cycle by gliotoxin allowed us to then test the hypothesis that 11-*cis* ROL is the form of chromophore produced by the Müller cells and utilized by cones for pigment regeneration. We incubated salamander and mouse retinas with gliotoxin, then bleached the pigment in both rods and cones with bright light, and applied exogenous 11-*cis* ROL. As previously reported (Jones et al., 1989), in the case of salamander photoreceptors, rods were not affected by the 11-*cis* ROL and remained desensitized following the bleach, whereas cones recovered their sensitivity and dark-adapted (Fig. 3D). A similar result was observed in the case of mouse and human retinas (Figs. 5C and 7D; see also Wang et al. (2009); Wang and Kefalov (2009)). This ability of cones, but not rods, to regenerate their pigment with 11-*cis* ROL is consistent with the cone specificity of the retina visual cycle. It also indicates that cones, but not rods, must have the ability to oxidize 11-*cis* ROL to the 11-*cis* RAL needed for pigment regeneration. The nature of this cone-specific enzyme is currently unknown.

6.4. Role of the retina visual cycle in cone function

Having established the function of the cone-specific visual cycle in the rod-dominant neural retina, we next investigated its possible physiological role. To address this question, we adopted the *in vitro* trans-retinal ERG recording technique to record cone ERG a-wave, which represents the photoreceptors' light response (Green et al., 2004; Nymark et al., 2005). To obtain the complete ERG a-wave response, we used a cocktail of synaptic transmission inhibitors to block the propagation of signals beyond the photoreceptors (Wang et al., 2009). Using this method, we could observe in real time the recovery of cone sensitivity following exposure to bright bleaching light and investigate how this cone dark adaptation is affected by the retina visual cycle. We found that both salamander and mouse isolated retinas were able to promote a rapid cone dark adaptation. Notably, the rate of cone dark adaptation in the isolated retina, driven only by the retina visual cycle, was comparable to the rate of cone dark adaptation in vivo (Wang et al., 2009; Wang and Kefalov, 2009). Thus, the retina visual cycle could explain the substantially faster dark adaptation of cones compared to that of rods, which rely only on the RPE visual cycle for chromophore.

Using recordings from eyecup, with the retina still attached to the RPE, we could also compare the rates of cone pigment regeneration driven by the RPE and the retina visual cycles. Cone pigment regeneration in the intact eyecup should result from the combined function of the two visual cycles. Incubating the eyecup with the gliotoxin L-α-AAA would block the retina visual cycle while leaving the RPE visual cycle intact. Using a previously derived relation between the level of pigment bleach and the decrease in sensitivity in salamander cones (Kefalov et al., 2005), we could then convert the measured sensitivity at different time points following the bleach into level of regenerated pigment in each condition. By subtracting the pigment regenerated via the RPE visual cycle alone (eyecup in the presence of L-α-AAA) from the total pigment regenerated by the RPE and retina visual cycles combined (eyecup in control solution), we derived the pigment regenerated via the retina visual cycle alone (Wang et al., 2009). Surprisingly, contrary to expectations from previous biochemical work in the chicken suggesting that the retina visual cycle might be as much as 20-fold faster than the RPE pathway (Mata et al., 2002), we found that, in salamander, the initial cone pigment regeneration rates from the two visual cycles are virtually identical (Fig. 6A). However, due to the nonlinear relation between pigment content and sensitivity (Jones et al., 1989; Kefalov et al., 2005), this effective doubling of the amount of chromophore with both cycles working simultaneously results in 4-fold increase in cone sensitivity compared to the case of RPE cycle alone and substantially speeds up cone dark adaptation. Unfortunately, the rapid detachment of the retina in mouse eyecup has prevented us so far from performing similar experiments and comparing the rates of the RPE and retina visual cycles in mouse.

We also investigated the role of the retina visual cycle in background adaptation (Wang et al., 2009). By again comparing the function of cones in control solution, with functional retina visual cycle, and in L-α-AAA, with the retina visual cycle blocked, we found that the retina visual cycle expands the functional dynamic range of cones nearly 10-fold (Fig. 6B). This result implies that by recycling chromophore at a high rate and supplying it to cones, the retina visual cycle enables the rapid regeneration of cone pigment in bright light, thus extending their dynamic range.

In summary, our results demonstrate that the retina visual cycle is required for the rapid and complete dark adaptation of cones and it extends the dynamic functional range of cones under bright light.

6.5. The retina visual cycle in primate and human

The ultimate goal of our studies is to determine the mechanisms of human vision and eye diseases and to provide insights for their possible clinical treatment. Thus, a key point was to determine whether a cone-specific visual cycle is functional in the primate and human retina (Wang and Kefalov, 2009). We addressed this question first in primate retina using experiments similar to the ones described above for salamander and mouse. As the primate eyeballs were enucleated under bright surgical light from euthanized animals, their photoreceptors were initially bleach-adapted. When such primate retina was dark-adapted for 2 h while still attached to the RPE, both rod and cone responses could readily be observed using whole retina ERG recordings (Fig. 7A). In contrast, when the primate retina was removed from the RPE immediately after dissection and put in the dark for 2 h, only cone responses were observed (Fig. 7B). In both cases cone sensitivity recovered following a bleach, while the rod sensitivity did not. Similarly, recordings from the foveal region of freshly removed human retina demonstrated that after dark adaptation, cones recover their sensitivity (Fig. 7C). Rod responses could not be observed, indicating that the rods were greatly desensitized following the bleach and did not recover in the isolated retina. Notably, the recovery of cone sensitivity following a bleach could be blocked by the Müller cell inhibitor L-α-AAA and was reinstated by exogenous 11-*cis* ROL (Fig. 7D). Taken together, all these results demonstrate that the retina visual cycle is evolutionarily preserved from amphibians to humans. The characterization of a retina visual cycle in the human retina calls attention to its possible clinical implication for visual disorders. Its abnormal function could potentially affect selectively the cones and could lead to cone visual disorders. On the other hand, the retina visual cycle could play an important role in preserving cone vision in the case of disorders affecting selectively the canonical RPE visual cycle.

7. Overview of the cone-specific retina visual cycle

Based on the current knowledge from biochemical and physiological studies, the cone-specific visual cycle could be depicted briefly as follows (Fig. 8). After photolysis, all-*trans* RAL is reduced to all-*trans* ROL, which is then released and transported across the interphotoreceptor matrix to Müller cells, possibly with the help of

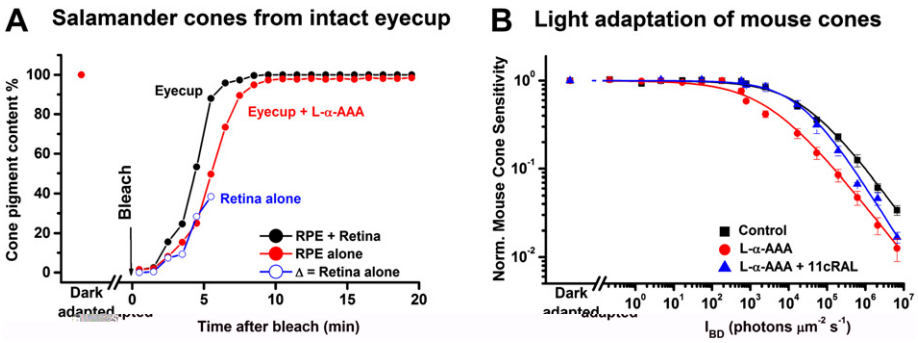

Fig. 6. The role of the retina visual cycle in cone function. (A) Recovery of salamander cone pigment content in eyecup driven by both retina and RPE visual cycles (black), or in L-α-AAA-pre-incubated eyecup driven by the pigment epithelium alone (red). Pigment content following a bleach is estimated from sensitivity recovery, using the relation between cone pigment loss and desensitization (Kefalov et al., 2005). The initial rate of pigment regeneration by the retina visual cycle (open circles) is calculated by subtracting the pigment regenerated by the pigment epithelium (red) from the total regenerated pigment (black). Note the comparable rates of pigment regeneration by the two cycles (red circles vs. open circles). The regeneration of cone pigment was significantly accelerated and driven to completion by the addition of the retina visual cycle. (B) The role of the retina visual cycle in mouse cone light adaptation. The decrease of cone sensitivity in background light in each case is fitted with the Weber–Fechner equation $S/S_{DA} = (1 + I_B/I_O)^{-1}$. Blocking the retina visual cycle with L-α-AAA results in a substantial shift to the left in the cone background adaptation curve (red), with I_O reduced from 25,165 photons μm^{-2} s^{-1} in control retinas to 2747 photons μm^{-2} s^{-1} in L-α-AAA treated retinas. Exogenous 11-*cis* retinal reverses the effect of gliotoxin, with I_O = 20,749 photons μm^{-2} s^{-1}, except for the brightest backgrounds. Adapted from Wang et al. (2009) and Wang and Kefalov (2009).

IRBP (Crouch et al., 1992; Jin et al., 2009; Okajima et al., 1989; Parker et al., 2009; Pepperberg et al., 1991). In Müller cells, binding with CRBP, all-*trans* ROL is isomerized directly to 11-*cis* ROL by Isomerase II (Bunt-Milam and Saari, 1983; Eisenfeld et al., 1985; Mata et al., 2002, 2005). 11-*cis* ROL is released from Müller cells to the IPM. Perhaps bound to IRBP, 11-*cis* ROL in the IPM is transported to cone inner segment (Jin et al., 2009; Parker et al., 2009). 11-*cis* ROL moves freely from cone inner segment to outer segment, where it is oxidized to 11-*cis* RAL by an unidentified 11-*cis* RDH and/or by coupling to all-*trans* RAL reduction, for pigment regeneration (Jones et al., 1989; Mata et al., 2002; Miyazono et al., 2008).

8. Future directions

The mechanisms underlying the retina visual cycle are still not well understood and its molecular components have not been identified. Specifically, the role of chromophore-binding proteins

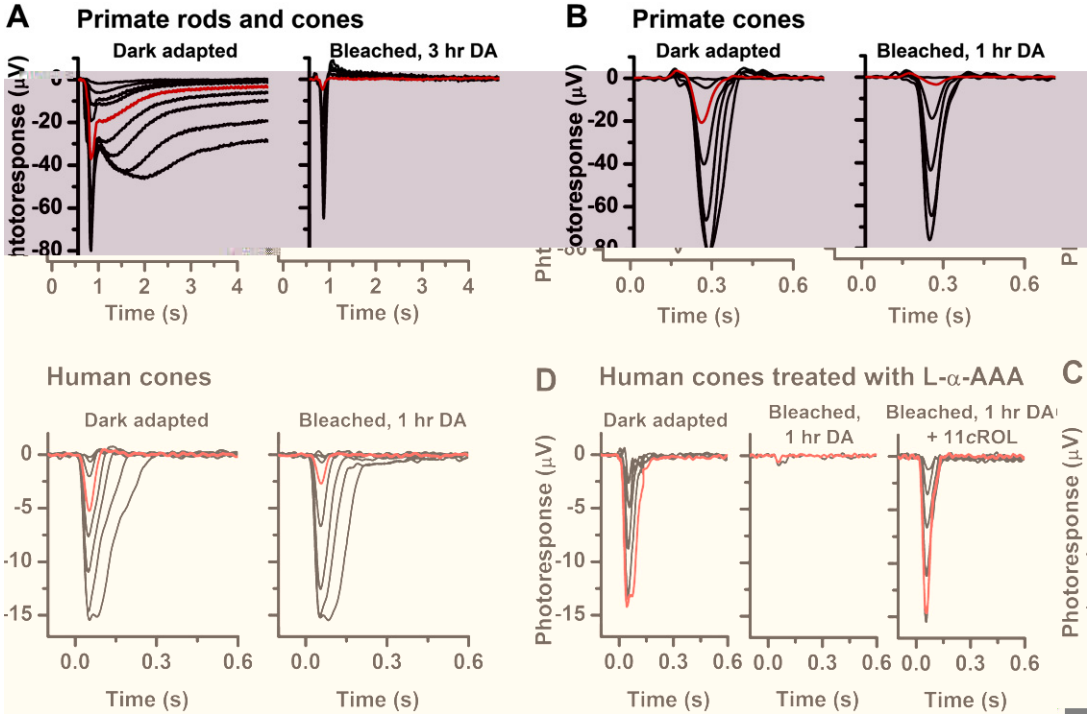

Fig. 7. Primate and human retinas promote cone pigment regeneration. (A) Primate rod and cone ERG responses from retina dark-adapted in eyecup after enucleation. Exposure to bright light abolishes the rod component (slow, high sensitivity), whereas the cone component (fast, low sensitivity) recovers substantially. (B) Primate cone ERG responses from retina, isolated immediately after enucleation from the RPE. No rod response is observed if the retina is dark-adapted in the absence of RPE (left). Following subsequent bleach and 1-h dark incubation (right), cone response amplitude and sensitivity recover substantially. (C) Only cone responses are observed in human retina, isolated under surgical light from the RPE prior to dark adaptation (left). Significant cone recovery is observed following subsequent bleach and 1-h dark incubation (right). (D) Human cone sensitivity and amplitude recovery following a bleach was blocked by preincubating the retina in Müller cell inhibitor (middle). The effect of L-α-AAA was reversed by exogenous 11-*cis* ROL (right). Red traces represent photoresponses to 18,560 photons μm^{-2} 560 nm (A–C) and to 663,500 photons μm^{-2}, 560 nm (D). Adapted from Wang and Kefalov (2009).

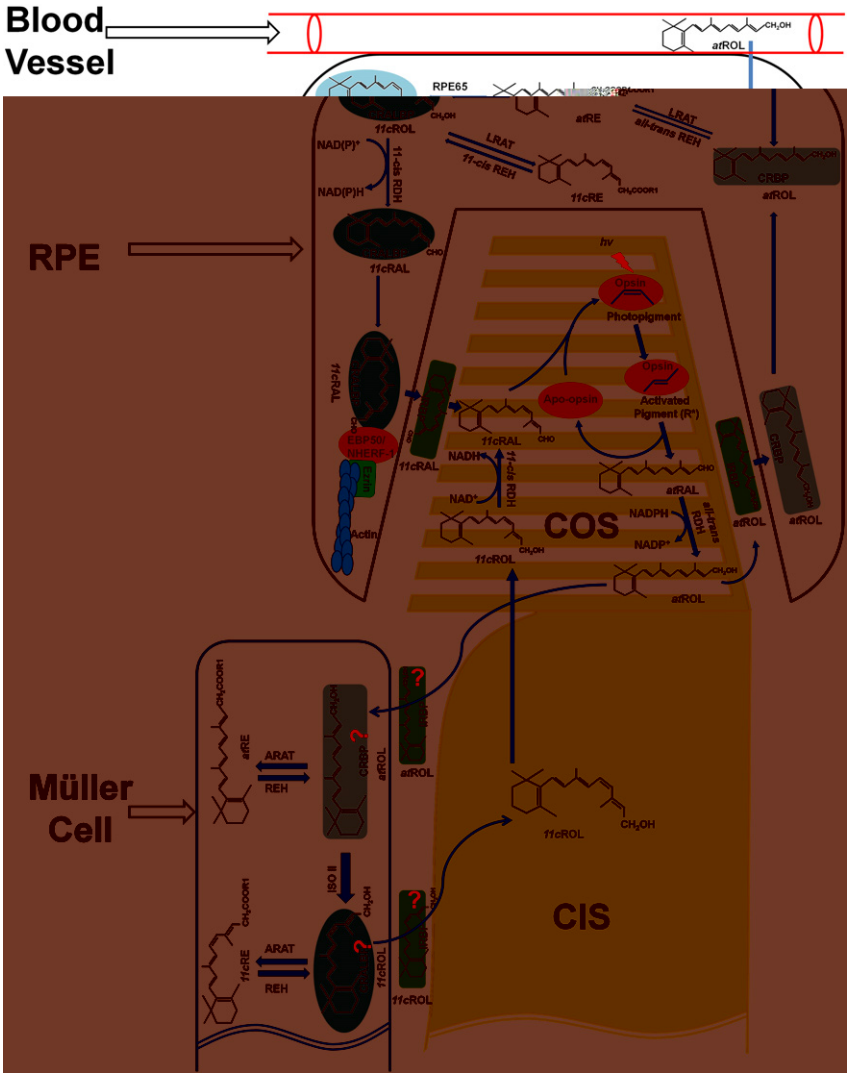

Fig. 8. Retina and RPE visual cycles for cones. After photolysis, all-*trans* RAL is reduced to all-*trans* ROL by RDH in cone outer segment. All-*trans* ROL can be either transported to the RPE cells and recycled to 11-*cis* RAL via the canonical visual cycle (refer to text and Fig. 1), or transported to Müller cells where it is enzymatically converted to 11-*cis* ROL. 11-*cis* ROL is transferred back to cone inner segment, and then moves along the inner segment to the outer segment, where it is oxidized by an unidentified RDH to 11-*cis* RAL for pigment regeneration. The key enzymes, such as RDH and Isomerase II, in this pathway have not been identified. The role of the retinoid binding proteins CRALBP, CRBP, and IRBP in the retina visual cycle still needs to be determined.

expressed in the retina is not known and the enzymes involved in the recycling of retinoid in the retina have not been identified.

8.1. 11-*cis* RDH

A key enzyme in the retina visual cycle is 11-*cis* retinol dehydrogenase (RDH), oxidizing the 11-*cis* ROL produced by Müller cells to the 11-*cis* RAL form, used for pigment regeneration. Although biochemical and physiological studies have demonstrated the RDH activity in cones, the nature of this enzyme remains unknown. In the RPE and retina, a number of RDHs are expressed (Chrispell et al., 2009). It is not clear whether one of the known RDH enzymes is responsible for the oxidation of 11-*cis* ROL in cones or the reaction is driven by a novel, yet unidentified enzyme.

8.2. CRALBP

CRALBP is expressed in both RPE and Müller cells. Biochemical studies have shown that CRALBP is required for the activities of Isomerase II and ARAT in the retina, implying a possible important role for CRALBP in the retinal visual cycle (Mata et al., 2002; Muniz et al., 2006). Recent studies in zebrafish lavae have demonstrated that there are two orthologues of CRALBP, expressed in the RPE and Müller cells, respectively (Collery et al., 2008; Fleisch et al., 2008). CRALBP *a* is expressed exclusively in the RPE, whereas CRALBP *b* is localized in Müller cells. ERG recordings from antisense morpholino-injected zebrafish demonstrated that CRALBP *b* in Müller cells is essential for cone vision, and suggested that a parallel visual cycle functions in zebrafish retina (Collery et al., 2008; Fleisch et al., 2008).

In contrast to zebrafish, in the mouse the involvement of CRALBP in its cone-specific retinoid cycle has not been investigated with loss of function studies, although CRALBP-deficient mice were generated several years ago (Saari et al., 2001). Thus, the role of this retinoid binding protein in the mammalian retina visual cycle remains unknown. It has been suggested that CRALBP in the RPE might be involved in releasing 11-*cis* RAL to IPM by the aid of EBP50/NHERF-l and Ezrin (Saari et al., 2009). Whether CRALBP is also responsible for the release of 11-*cis* ROL from Müller cells to

IPM is still unknown. Loss of function studies currently under way in our lab will be helpful in elucidating the role of CRALBP in the retina visual cycle.

8.3. RPE65

Although RPE65 is a key enzyme of the canonical RPE visual cycle, its possible involvement in the retina visual cycle is controversial. On one hand, in addition to its expression in the pigment epithelium cells, RPE65 is also expressed in amphibian and mammalian cones (Ma et al., 1998; Znoiko et al., 2002). On the other hand, the targeted knockdown of RPE65 in cone-dominant zebrafish larvae (5-dpf) does not appear to affect the retina visual cycle as their cone vision remains functional (Schonthaler et al., 2007). One possible explanation for these results is that RPE65 in cones is not required for the function of the retina visual cycle but plays a modulatory role, perhaps accelerating the turnover of chromophore by functioning not as an isomerohydrolase but simply as a chromophore-binding protein. This hypothesis is currently being tested as the function of RPE65 in the retina visual cycle needs to be elucidated.

8.4. CRBP

CRBP is another retinoid binding protein expressed both in the RPE and Müller cells. In the RPE cells, CRBP serves as a carrier of all-*trans* ROL and transfers it from RPE apical processes to their cell bodies, where CRBP further facilitates the esterification of the retinoid (Herr and Ong, 1992; Huang et al., 2009; Napoli, 2000; Ong et al., 1988; Yost et al., 1988). Consistent with this idea, CRBP mutant mice have reduced amounts of hepatic and RPE retinyl esters (Ghyselinck et al., 1999; Saari et al., 2002). The possible function of CRBP in Müller cells is still unknown. One possibility is that there the protein also serves as the carrier for all-*trans* ROL and facilitates the chromophore isomerization.

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