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Review

Key enzymes of the retinoid (visual) cycle in vertebrate retina ☆

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ABSTRACT

A major goal in vision research over the past few decades has been to understand the molecular details of retinoid processing within the retinoid (visual) cycle. This includes the consequences of side reactions that result from delayed all-*trans*-retinal clearance and condensation with phospholipids that characterize a variety of serious retinal diseases. Knowledge of the basic retinoid biochemistry involved in these diseases is essential for development of effective therapeutics. Photoisomerization of the 11-*cis*-retinal chromophore of rhodopsin triggers a complex set of metabolic transformations collectively termed phototransduction that ultimately lead to light perception. Continuity of vision depends on continuous conversion of all-*trans*-retinal back to the 11-*cis*-retinal isomer. This process takes place in a series of reactions known as the retinoid cycle, which occur in photoreceptor and RPE cells. All-*trans*-retinal, the initial substrate of this cycle, is a chemically reactive aldehyde that can form toxic conjugates with proteins and lipids. Therefore, much experimental effort has been devoted to elucidate molecular mechanisms of the retinoid cycle and all-*trans*-retinal-mediated retinal degeneration, resulting in delineation of many key steps involved in regenerating 11-*cis*-retinal. Three particularly important reactions are catalyzed by enzymes broadly classified as acyltransferases, short-chain dehydrogenases/reductases and carotenoid/retinoid isomerases/oxygenases. This article is part of a Special Issue entitled: Retinoid and Lipid Metabolism.

1. Introduction: regeneration of the chromophore: retinoid cycle

The pioneering studies of Boll and Kühne ca. 1877 demonstrated that exposure of frog retinas to light resulted in a series of color changes from purplish-red to yellow and then from yellow to white [1]. This process is known as photochemical bleaching and results from the sequential photoisomerization and hydrolysis of the rhodopsin chromophore [2]. A critical discovery made by Kühne was that the bleached retina could regain its purplish-red hue when repositioned in the back of the eye on top of the monolayer of cells known as the RPE and incubated in the dark [1]. This finding provided the first evidence that the RPE contained enzymatic activities necessary for regeneration of visual chromophore. The principle reactions comprising the retinoid (visual) cycle were delineated by George Wald in the 1940's [3] but several more decades of research were required before the actual enzymes that catalyze these reactions began to be identified.

Today we know that to sustain vision, all-*trans*-retinal released from light-activated rhodopsin must be enzymatically isomerized back to the 11-*cis* isomer. This process occurs by a sequence of reactions catalyzed by membrane-bound enzymes of the retinoid cycle located in rod and cone photoreceptor cell outer segments (OS) and the retinal pigment epithelium (RPE) (Fig. 1) [4–7]. The first step in the retinoid cycle involves RDH-catalyzed reduction of all-*trans*-retinal released from light-activated visual pigments to all-*trans*-retinol (see also [8]). A portion of the freed all-*trans*-retinal is released into the disk lumen and must be transferred to the cytosol by the ATP-binding cassette transporter 4 (ABCA4) in order to be reduced (reviewed in [9]).

Several critically important reactions take place during this series of metabolic transformations. The first is the lecithin:retinol acyltransferase (LRAT) reaction that catalyzes esterification of retinols by fatty acid moieties in the RPE [10]. This reaction is important both for retaining ocular retinol generated from photoactivated visual pigments in photoreceptors and capturing retinol present in the circulation [11]. This process is facilitated by two retinoid-binding proteins: interphotoreceptor retinoid-binding protein (IRBP), which binds retinoids in the extracellular space, and cellular retinol-binding protein-1 (CRBP1) located within RPE cells [12,13]. Resulting retinyl esters are substrate for isomerization [14]. Because of the propensity of fatty acid retinyl esters to cluster, these compounds are retained in lipid droplets of the RPE called retinosomes [15–20].

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Two important redox reactions take place in photoreceptors and the RPE. Retinal released from visual pigments must be reduced to retinol in the photoreceptor OS, which then diffuses into RPE where it is esterified by LRAT. This reduction is extremely important for the health of the retina, because retinal, which can reach millimolar concentrations following a strong photobleach, is extremely toxic to photoreceptor cells and the RPE [21–27]. A high flux of retinoids through the retinoid cycle, as occurs during intense light exposure, can lead to both elevated levels of toxic retinoid intermediates, especially all-*trans*-retinal, and photoreceptor degeneration. However, the mechanisms by which all-*trans*-retinal and/or its condensation products lead to photoreceptor cell death have not been completely elucidated. The second redox reaction, in this case an oxidation, generates 11-*cis*-retinal from the 11-*cis*-retinol isomerization product. This reaction occurs in the RPE.

RPE65 catalyzes the formation of 11-*cis*-retinol from all-*trans*-retinyl esters [28–30]. This unique biochemical reaction consists of an atypical ester cleavage coupled to a *trans*-to-*cis* double bond isomerization. The reaction occurs in the RPE and is thought to be the rate-limiting step of the retinoid cycle. Although, regeneration of rhodopsin requires 11-*cis*-retinal supplied from the RPE, but cones are not exclusively dependent on RPE65-mediated isomerization [7,31–36]. Biochemical studies in cone-dominant ground-squirrels and chickens [33], as well as genetic studies in zebrafish (*Danio rerio*), support the existence of a separate "cone visual cycle" [34,35]. Confirmation of this alternative visual cycle awaits identification of genes that encode proteins responsible for its key enzymatic steps.

Here we focus on the three key retinoid cycle reactions catalyzed by LRAT, RDHs and RPE65 with an updated analysis of published results.

2. Lecithin:retinol acyltransferase (LRAT) — structure, catalysis, and physiological significance

Retinyl esters are bioactive storage metabolites of vitamin A. Because of their chemical stability and hydrophobicity, these compounds serve as a transport and storage form of vitamin A in vertebrates and therefore play an essential role in maintenance of retinoid homeostasis. Vitamin A esters can be formed *in vivo* by enzymatic transfer of activated fatty acyl moieties from acyl-CoAs or directly from a phospholipid donor. However, phospholipid-dependent synthesis is quantitatively the most dominant pathway of retinyl ester production [11,37,38]. An acyl-CoA-independent retinol esterification enzymatic activity was described for the first time in a microsomal fraction isolated from rat small intestine [39]. Shortly thereafter, LRAT activity was reported to be the main contributor to retinyl ester formation in liver and the RPE, and the fatty acid moiety in the sn-1 position of phosphatidylcholine (PC) was identified as an endogenous acyl source for retinyl ester formation [10,40]. These initial studies opened the avenue for further detailed biochemical characterization of lipid-dependent acyltransferase enzymatic activity. Particularly the discovery of suicidal inhibitors such as N-Boc-l-biocytinyl-11-aminoundecane chloromethyl ketone permitted specific labeling of the protein and consequently molecular identification of this enzyme and its corresponding gene in 1999 [41].

2.1. Molecular characterization of LRAT

The *LRAT* gene is located in human chromosome 4 at locus 4q32.1 and encodes a membrane bound protein with a molecular weight of

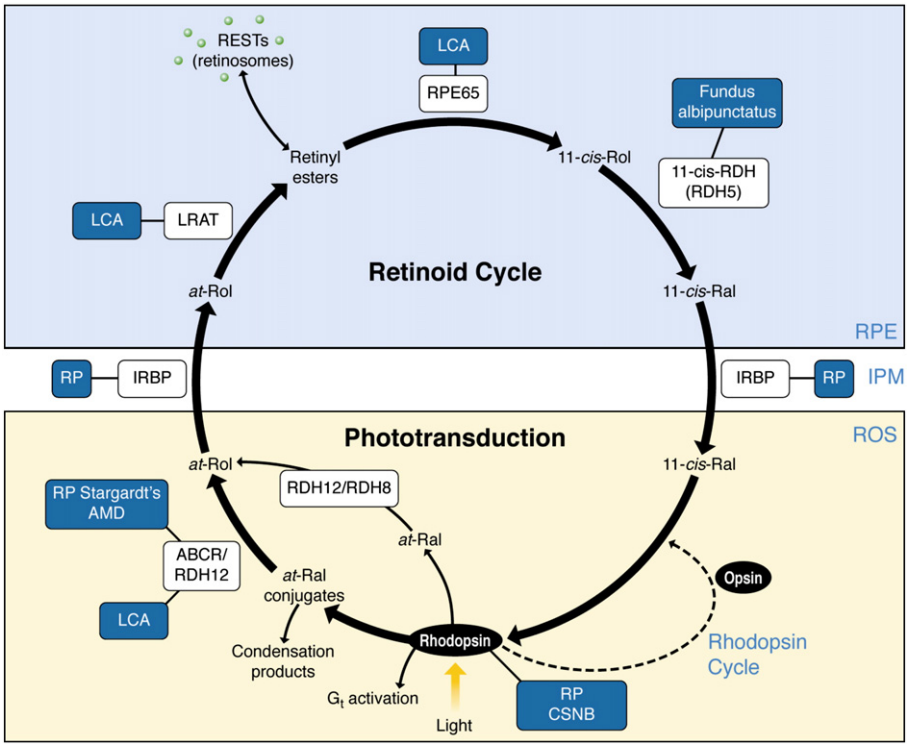

Fig. 1. The retinoid cycle regenerates 11-*cis*-retinal. In rod outer segments (ROS), 11-*cis*-retinal (11-*cis*-Ral) couples to a protein opsin, forming rhodopsin [2]. Absorption of a photon of light by rhodopsin causes photoisomerization of 11-*cis*-Ral to all-*trans*-retinal (at-Ral) leading to its release from the chromophore-binding pocket of opsin. The movement of at-Ral and certain at-Ral conjugates from the intradiscal face to the cytosolic face of disc membranes is accomplished by the ABC transporter ABCR (also known as ABCA4). At-Ral then is reduced to all-trans-retinol (at-Rol) in a reversible reaction catalyzed by an NADPH-dependent all-*trans*-retinol dehydrogenase (RDH). At-Rol diffuses across the interphotoreceptor matrix (IPM) facilitated by the interphotoreceptor retinoid-binding protein (IRBP) into the retinal pigment epithelium (RPE) where it is esterified in a reaction catalyzed by lecithin: retinol acyltransferase (LRAT). There, all-*trans*-retinyl esters may be stored in retinyl ester storage particles (RESTs), also known as retinosomes, or may serve as the substrate for RPE65 that converts them to 11-*cis*-retinol (11-*cis*-Rol), which is further oxidized back to 11-*cis*-Ral by RDH5, RDH11 and other RDHs. 11-*cis*-Ral formed in the RPE diffuses back into the rod and cone outer segments, where it completes the cycle by recombining with opsins to form rhodopsin and cone pigments. Diseases that result from mutations in proteins involved in the retinoid cycle are indicated in blue boxes. AMD — age-related macular degeneration, CSNB — congenital stationary night blindness, LCA — Leber congenital amaurosis and RP — retinitis pigmentosa. Reproduced with permission from Trends in Biochemical Sciences from reference [4]. See reviews [4–7] for more details.