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## Retinal degeneration in animal models with a defective visual cycle

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### Abstract

Continuous generation of visual chromophore through the visual (retinoid) cycle is essential to maintain eyesight and retinal health. Impairments in this cycle and related pathways adversely affect vision. In this review, we summarize the chemical reactions of vitamin A metabolites involved in the retinoid cycle and describe animal models of associated human diseases. Development of potential therapies for retinal disorders in these animal models is also introduced.

### INTRODUCTION

Activation of rhodopsin in the retina is the initial step for light perception in vertebrates. Continuous vision depends on recycling of all-*trans*-retinal, the product of light dependent photoisomerization of the visual chromophore, back to 11-*cis*-retinal [1]. This process is enabled by the visual (retinoid) cycle, a series of biochemical reactions in photoreceptor and adjacent RPE cells [1]. Impairments in the retinoid cycle can cause retinal degeneration, and animal models exhibiting disruption of the retinoid cycle have been instrumental for testing of gene transfer therapies and pharmacological interventions aimed at restoring the function of this cyclic pathway [2]. Notably, promising gene transfer therapies for human patients with RPE65 mutations were initially developed in knockout mice [3, 4]. An in-depth mechanistic understanding of the retinoid cycle in combination with relevant animal models promises to result in the establishment of successful interventions for human retinal disorders [1, 5–8].

#### 1. Visual chromophore is regenerated by the retinoid cycle

Regeneration of the visual chromophore, 11-*cis*-retinal, through the retinoid cycle consists of several biochemical reactions taking place in photoreceptors and adjacent RPE cells (Fig.

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1). When photons are absorbed by visual pigments, the protein bound chromophore is photoisomerized from an 11-*cis* to an all-*trans* conformation, inducing a conformational change in the complex that initiates phototransduction. This process mediates the conversion of light into electrical signals that are eventually conducted to the visual and frontal cortex of the brain. All-*trans*-retinal is hydrolyzed from bleached rhodopsin generating apo-opsin. Most of all-*trans*-retinal is released from opsin into the cytoplasm of rod outer segments (ROS) of the photoreceptor cell, but a fraction is released to the intra-discal lumen. This fraction is transported to the cytosolic space of photoreceptors by the ATP-binding cassette transporter (ABCA4), to join the rest of released all-*trans*-retinal that is then reduced to all-*trans*-retinol by all-*trans*-retinol dehydrogenases (RDHs, including RDH8 and RDH12). All-*trans*-retinol is then transported to RPE cells where lecithin:retinol acyltransferase (LRAT) esterifies it to generate all-*trans*-retinyl esters which can be stored in lipid droplets called 'retinosomes'. All-*trans*-retinol is also supplied to the retina from the blood circulation through the activities of several protein factors, including serum retinol-binding protein (RBP), a cellular RBP receptor known as Stimulated by Retinoic Acid 6 (STRA6), cellular retinol-binding protein type 1 (CRBP1) and LRAT. All-*trans*-retinyl esters can be isomerized and hydrolyzed into 11-*cis*-retinal by the retinoid isomerase RPE65. 11-*cis*-retinal is subsequently oxidized into 11-*cis*-retinol by 11-*cis*-RDHs such as RDH5 and RDH11 in RPE cells. The regenerated 11-*cis*-retinal is transported back to photoreceptors by carriers including the cellular retinaldehyde-binding protein (CRALBP) and interphotoreceptor retinoid-binding protein (IRBP). After replenishing photoreceptors, 11-*cis*-retinal covalently binds to the opsin moiety of visual pigments *via* a Schiff-base linkage to regenerate ground-state rhodopsin, which completes the retinoid cycle (recently reviewed [1]). Impairment of any reaction in this cycle can lead to human visual disorders.

## 2. Animal models of human retinal diseases caused by retinoid cycle impairments

The successful application of modern scientific technology has led to breakthrough discoveries of key components for vitamin A production, transport and visual chromophore generation and recycling. Various animal models with genetic defects in these genes have been generated (Table 1). Many of these animal models essentially recapitulate blinding human retinal diseases.

**2.1. Impaired transport of retinoids from the systemic circulation to the eyes is associated with retinal diseases**—In humans,  $\beta$ -carotene is a precursor of vitamin A (all-*trans*-retinol). All-*trans*-retinal is produced after cleavage of  $\beta$ -carotene supplied from the diet by the enzymes  $\beta$ -carotene-15,15'-monooxygenase (BCMO1). A second related enzyme  $\beta$ -carotene-9',10'-dioxygenase (BCDO2) helps maintain carotenoid homeostasis more generally. Once generated and reduced in enterocytes of the intestine, all-*trans*-retinol is esterified to form retinyl esters (REs) and packaged into chylomicrons. Some RE from chylomicrons is taken up into peripheral cells in process that involves hydrolysis by lipoprotein lipase. The vast majority is delivered to the liver for storage. The liver secretes all-*trans*-retinol bound to RBP that travels through the blood circulation. From there it is transported into the eye through a process that is facilitated by STRA6, CRBP1 and LRAT in the RPE (Fig. 1).

*Bcmo1*<sup>-/-</sup> and *Bcdo2*<sup>-/-</sup> mice have been generated and previously characterized [9, 10]. Neither of these mouse models display a pathological eye phenotype when maintained on diets that contain preformed vitamin A. Moreover, no human retinal diseases attributed to these gene deficiencies have been reported. *Bcmo1*<sup>-/-</sup> mice dietary supplemented with carotene accumulate large quantities of this pro-vitamin in adipose tissues. Besides defects in carotene metabolism, *Bcmo1*<sup>-/-</sup> mice display general abnormalities in lipid homeostasis. Even when provided with vitamin A-sufficient chow, *Bcmo1*<sup>-/-</sup> mice develop fatty livers and display altered serum lipid levels with elevated unesterified fatty acids [9, 10]. This gene knockout mouse is also more susceptible to high fat diet-induced disruptions in fatty acid metabolism. In *Bcdo2*-deficient mice, carotenoid homeostasis is disrupted and carotenoids accumulate in several tissues, especially in hepatic tissue. These accumulated carotenoids are associated with mitochondrial dysfunction that can cause oxidative stress and disease [11].

Characterization of *Rbp4*<sup>-/-</sup> mice, reported in 1999 [12], revealed undetectable levels of serum all-*trans*-retinol. Amounts of all-*trans*-retinol in the eye are reduced to 25% at 3 weeks of age, but retinoid levels surprisingly increase with age. However, compared with WT eyes, retinoid levels are still lower in aged *Rbp4*<sup>-/-</sup> mice. Retinal function is impaired due to insufficient levels of retinoids, and ERG recordings from 4-week-old *Rbp4*<sup>-/-</sup> mice reveal a 100-fold lowered sensitivity to light. As levels of retinoids in the eye increase with age, 24 weeks-old *Rbp4*<sup>-/-</sup> mice manifest apparently normal ERGs with deep a-waves, high b-waves and a rapid falling phase. *RBP4* mutations have been found in patients with RPE and retinal atrophy [13, 14]. Additionally, elevation of serum RBP4 in patients has also been linked to type 2 diabetes, cardiovascular disease and diabetic retinopathy [15–17].

STRA6 is essential for facilitating vitamin A uptake into to the eye [18] and *Strat6*<sup>-/-</sup> mice have drastically diminished levels of retinoids in their eyes [18, 19]. Due to the lack of 11-*cis*-retinal, their retinas are not functional. The consequences of STRA6 mutations are manifested in a variety of human disorders [20, 21], which include pulmonary dysgenesis, cardiac malformations, mental retardation, and microphthalmia/anophthalmia, collectively referred to as Matthew-Wood syndrome. In contrast, *Strat6*<sup>-/-</sup> mice only display an abnormal eye phenotype.

*Crbp1*<sup>-/-</sup> mice display reduced all-*trans*-retinyl esters to 33% of WT levels, however the amount of 11-*cis*-retinal is similar to WT mice [22]. This phenotype indicates that CRBP1 plays a major role in diffusing all-*trans*-retinol into the RPE cells. Mutations in CRBP1 have not yet been reported in human retinal disorders.

**2.2. Mutations in LRAT cause LCA and RP**—LRAT esterifies all-*trans*-retinol to all-*trans*-retinyl esters which are stored in retinosomes [23] of RPE cells. Mutations in LRAT cause Leber congenital amaurosis (LCA) and retinitis pigmentosa (RP) [24] (Fig. 1). Retinoid analyses of *Lrat*<sup>-/-</sup> mouse eyes reveal only all-*trans*-retinol [25]. Due to the absence of 11-*cis*-retinal, *Lrat*<sup>-/-</sup> retinas lack visual function and exhibit nearly non-detectable ERG responses and no pupillary response to light illumination. In 2005, Lin *et al.* described *Lrat*<sup>-/-</sup> mice that do not carry a neo-cassette in the knockout target construct [26]. Similar to the previously reported *Lrat*<sup>-/-</sup> mice [25], nearly no retinoids are detected in

the eyes, demonstrating the neo-cassette insertion did not affect the phenotype in the original report. Notably, visual function loss in *Lrat*<sup>-/-</sup> mice can be reversed by either supplementation with artificial visual chromophores or *Lrat* gene transfer [27].

**2.3. Defects in RPE65 are associated with LCA and RP**—RPE65 is an RPE-specific isomerase which generates 11-*cis*-retinol from an all-*trans*-retinyl ester substrate [28–30]. RPE65 mutations are associated with human retinal disorders including LCA and RP [31]. Redmond *et al.* generated an *Rpe65*<sup>-/-</sup> mouse and reported its visual phenotype in 1998 [32]. Since then, this mouse model has been widely used for understanding the pathogenesis of human RPE65-associated diseases and testing potential interventions. In 2005, a causal gene for retinal degeneration 12 (*rd12*) mice with naturally occurring retinal degeneration was identified as a RPE65 mutation [33]. Sequence analysis of *Rpe65* cDNA from *rd12* mice shows a single base substitution at position 130 (C to T) of exon 3, which leads to a stop codon at amino acid position 44 (CGA to TGA). *Rpe65*<sup>-/-</sup> and *rd12* mice show very similar phenotypes with early cone degeneration, slow progressive rod degeneration, lipid inclusions in RPE cells, retinyl ester accumulation, leading to exacerbated retinosome formation [23], and no endogenous production of 11-*cis*-retinal. Due to lack of 11-*cis*-retinal, *Rpe65*<sup>-/-</sup> mice and *rd12* mice display only barely detectable ERG responses to high intensity light impulses at various wavelengths. It was also found that an RPE65 mutation spontaneously occurs in blind dogs [34]. Samardzija *et al.* generated a mouse with the R91W mutation in RPE65 to study LCA phenotypes observed in humans with the same mutation [35]. C57BL/6 mice naturally carry the *Rpe65* mutation that results in a Leu to Met substitution at position 450 [36], resulting in reduced enzymatic activity. Decreased production of 11-*cis*-retinal results in a milder light-induced retinal degeneration phenotype in mice expressing this RPE65 variant. In 2001, the first gene transfer therapy was conducted with Swedish Briard dogs carrying an RPE65 mutation [37]. To date, many gene transfer studies involving RPE65 dogs, *Rpe65*<sup>-/-</sup> mice and *rd12* mice have been performed. In addition to gene transfer therapies, these RPE65 animals have been used for developing pharmacological treatments, including supplementation with the visual chromophores 9-*cis*-retinal and 11-*cis*-retinal [38].

**2.4. RDH5 mutations cause fundus albipunctatus and late-onset cone dystrophy**—Oxidation of 11-*cis*-retinol to 11-*cis*-retinal is catalyzed by 11-*cis*-retinol dehydrogenases in the RPE. Currently RDH5, RDH11 and RDH10 have detectable RPE expression and 11-*cis*-RDH activity (Fig. 1). RDH5 (one of the 11-*cis*-RDHs of the RPE) mutations are found in patients with fundus albipunctatus (FA) which is characterized by white dots in fundus images and night blindness [39]. Some patients with FA have been reported to develop cone dystrophy after their 4th decade of life [40]. Unexpectedly, *Rdh5*<sup>-/-</sup> mice do not recapitulate any human visual phenotype except a mild delay in dark adaptation [41]. *Rdh5*<sup>-/-</sup>*Rdh11*<sup>-/-</sup> mice show slightly slowed dark adaptation, and late-onset cone dysfunction at 12 months of age [42]. Because *Rdh5*<sup>-/-</sup>*Rdh11*<sup>-/-</sup> mice manifest only a slightly abnormal retinal phenotype, a contribution of other RDHs, most likely RDH10, to generation of 11-*cis*-retinal from 11-*cis*-retinol has been suspected. However, because knockout of *Rdh10* gene produces mice that are not viable [43], this hypothesis can only be tested by conditional knockout of *Rdh10*. Cone dysfunction of *Rdh5*<sup>-/-</sup>*Rdh11*<sup>-/-</sup>

mice can be prevented by supplementation with 9-*cis*-retinal [44], indicating that retinal degeneration associated with 11-*cis*-RDH deficiency is characterized by lower levels of visual chromophore. Defects in RDH function in mice is also associated with accumulation of 13-*cis*-retinyl ester in RPE cells [45].

**2.5. RLBP1 defects are associated with RP**—CRALBP, encoded by the retinaldehyde-binding protein 1 (*RLBP1*) gene, transports 11-*cis*-retinal to photoreceptor cells. A complex consisting of RPE65, RDH5 and CRALBP can form *in vitro* and CRALBP increases the production of 11-*cis*-retinol by RPE65 in biochemical assays [46]. Mutations in *RLBP1* are associated with RP, Bothnia dystrophy and retinitis punctata albescens (RPA) [47–49]. Although *Rlbp1*<sup>-/-</sup> mice do not show retinal degeneration under room light conditions, acute retinal degeneration can be induced in this mouse model by intense light illumination [50]. Moreover, delayed 11-*cis*-retinal regeneration also occurs after intense light exposure.

**2.6. ABCA4 mutations are associated with human macular degeneration**—ABCA4 mutations are associated with Stargardt disease, a juvenile form of macular dystrophy [51]. Higher risk of AMD has been also reported in individuals with ABCA4 mutations [52, 53]. ABCA4 translocates all-*trans*-retinal from the intra-discal lumen of photoreceptor discs to the cytosolic space of outer segments [54]. *Abca4*<sup>-/-</sup> mice show accelerated age-related di-retinoid-pyridinium-ethanolamine (A2E) accumulation in their RPE cells [55]. A2E is an important component of autofluorescent lipofuscin granules. Mild rod photoreceptor degeneration was reported in albino *Abca4*<sup>-/-</sup> mice [56]. Unexpectedly, *Abca4*<sup>-/-</sup> mice do not mimic the retinal degeneration observed in humans with ABCA4 defects until crossbred with *Rdh8*<sup>-/-</sup> mice (described in section 2.8). Pharmacological interventions by retinoid cycle modulators and gene transfer therapies have been tested in both *Abca4*<sup>-/-</sup> and the latter double knockout models [57–62].

**2.7. Mutations in RDH12 cause LCA and severe types of RP**—Delayed clearance of all-*trans*-retinal plays a leading role in the pathogenesis of retinal degeneration because this retinoid is a reactive aldehyde. All-*trans*-retinal can also form condensation products such as A2E and all-*trans*-retinal dimers which also are closely associated with retinal disease pathology. Conversion of all-*trans*-retinal to all-*trans*-retinol is catalyzed by all-*trans*-RDHs including RDH8 and RDH12. RDH8 is found in the outer segments of rod and cone photoreceptors [63], whereas RDH12 is located in the inner segments of rod and cone cells [64, 65]. In mice, RDH8 is responsible for ~70% of the all-*trans*-RDH activity and RDH12 carries out ~30% [66]. However, mice with a double deletion of RDH8 and RDH12 still display residual all-*trans*-RDH activity *in vivo*. Both *Rdh8*<sup>-/-</sup> and *Rdh12*<sup>-/-</sup> mice accumulate large amounts of A2E in their RPE cells but don't display obvious degenerative changes of the eyes under room lighting conditions. But these mice do exhibit light-induced retinal degeneration after exposure to intense illumination. *Rdh8*<sup>-/-</sup>*Rdh12*<sup>-/-</sup> mice display slowly progressive cone and rod photoreceptor degeneration with A2E over-accumulation in the RPE cells. Currently no causal mutations of RDH8 are known in patients with retinal disorders but mutations in RDH12 are found in patients with LCA and RP [67].

### 2.8. Double knockout of ABCA4 and RDH8 in mice recapitulates human retinal pathology

**—***Abca4*<sup>-/-</sup>*Rdh8*<sup>-/-</sup> mice, deficient in the clearance of all-*trans*-retinal from photoreceptor cells, display progressive rod-cone degeneration with A2E over-accumulation, RPE atrophy and complement deposition at Bruch's membrane [68]. Additionally, some *Abca4*<sup>-/-</sup>*Rdh8*<sup>-/-</sup> mice develop choroidal neovascularization. The phenotype of this mouse shows many similarities to human AMD and Stargardt disease. Notably, these mice develop age-related retinal degeneration as well as acutely accelerated degeneration in response to intense light illumination (Fig. 2). Most photoreceptors undergo apoptosis with TUNEL-positive staining observed 24 h after bright light exposure [69]. Importantly, retinal degeneration in this mouse can be prevented by pre-treatment with a retinoid cycle inhibitor, retinylamine (Fig. 2). Thus, this mouse has already proven valuable for testing the efficacy of drug candidates that ameliorate retinal dystrophies [61, 62].

**2.9. IRBP defects are associated with RP**—Interphotoreceptor retinoid-binding protein (IRBP), or interstitial retinol-binding protein 3 (RBP3), is proposed to be important for the intercellular exchange of retinoids, serving to prevent the potentially cytotoxic effects of these compounds. As early as postnatal day (PND) 11, histological examination of the retinas of *Irbp*<sup>-/-</sup> mice reveals a loss of photoreceptor nuclei and changes in the structural integrity of the outer segments [70]. At PND30, the photoreceptor abnormalities become more severe and electroretinographic recordings reveal a marked loss in photosensitivity. Other studies with *Irbp*<sup>-/-</sup> mice suggest a role for IRBP in the rapid regeneration of cone pigments. Under photopic ERG conditions with 10 Hz flickering light (a condition used to isolate cone responses), the *Irbp*<sup>-/-</sup> mouse does not regenerate cone pigments as quickly as C57BL/6J (WT) mice [71]. These observations indicate that IRBP plays roles in both photoreceptor development and function and knockout of IRBP can result in a slowly progressive degeneration of retinal photoreceptors.

**2.10. RGR mutations are associated with human RP**—Retinal G protein-coupled receptor (RGR) was first cloned from a retinal epithelium enriched-cDNA library as an opsin homolog bound with 11-*cis*-retinal [72]. RGR localizes specifically to intracellular membranous compartments of RPE and Müller cells. *Rgr*<sup>-/-</sup> mice were characterized in 2001, as displaying less efficient light-dependent 11-*cis*-retinal production from all-*trans*-retinal. From this finding it was concluded that RGR is involved in an alternative pathway of 11-*cis*-retinal regeneration by harnessing light energy in an RPE65-independent manner [73]. However, other groups failed to confirm the contribution of RGR to rhodopsin regeneration, suggesting that RGR contributes only slightly to the retinoid cycle, potentially as an enhancer of isomerization or a retinal flow mediator [74, 75]. RGR mutations have been detected in a sub-population of RP patients [76].

**2.11. DES1 is associated with cone pigment regeneration**—Based on biochemical results, cone retinoid cycle was proposed to supplement chromophore for cone pigment regeneration in addition to the canonical retinoid cycle [77, 78]. Dihydroceramide desaturase-1 (DES1) is a potential isomerase that supports the cone retinoid cycle [79]. *Des1*<sup>-/-</sup> mice reveal an incompletely penetrant lethality and surviving animals are small in size with a complex phenotype [80]. This dysfunction includes decreased body weight, scaly



skin, hematological abnormalities, and abnormal liver function. The *Des1*<sup>-/-</sup> animals die 8 to 10 weeks after birth. The eye phenotype has not been examined. No DES1 mutations are known to be causal for retinal disorders in patients.

### 3. Therapies for animal models of retinal degeneration pertaining to defective retinoid cycle

Two major pharmacological concepts underlie the treatment of retinal disorders that are associated with a defective retinoid cycle, namely the supplementation of visual chromophore or drug analogs thereof and the prevention of the formation of toxic of retinoid cycle biproducts including all-*trans*-retinal and A2E. Gene replacement therapy has been also tested in animal models as a method to restore the healthy state and thus vision in degenerative diseases of the retina.

**3.1. Pharmacological therapies**—Supplementation of visual chromophore has been performed with both 11-*cis*-retinal and 9-*cis*-retinoids [38, 81]. Impaired production of the 11-*cis*-retinal chromophore is found in *Rpe65*<sup>-/-</sup>, *Lrat*<sup>-/-</sup>, *Rlbp1*<sup>-/-</sup> and *Rdh5*<sup>-/-</sup>*Rdh11*<sup>-/-</sup> mice [27, 38, 44, 71]. Supplementation with the visual chromophore successfully reverses retinal dysfunction and degeneration in these mice [82].

Preventive approaches against toxic side effects of components of the retinoid cycle, all-*trans*-retinal and its condensation product A2E, have been implemented in animals which show delayed clearance of all-*trans*-retinal after light exposure, including *Abca4*<sup>-/-</sup> and *Abca4*<sup>-/-</sup>*Rdh8*<sup>-/-</sup> mice. The first approach to reduce toxicity of retinoid cycle byproducts relied on slowing down the retinoid cycle. Such an approach could theoretically reduce production of all-*trans*-retinal and subsequently A2E. Isotretinoin (Accutane) (which can decrease RDH5 activity [57]), N-(4-hydroxyphenyl) retinamide (HPR) and A1120 (which reversibly reduce serum retinol [58, 59]) and retinylamine which inhibits RPE65 activity [83] have all been tested. All of these compounds can mitigate retinal pathology; however, they all also cause delayed dark adaptation due to 11-*cis*-retinal deficiency. In 2011, all-*trans*-retinal-associated retinal degeneration was prevented using FDA-approved drugs containing primary amino groups. It was demonstrated that these drugs form a transient Schiff-base intermediate with all-*trans*-retinal, thereby reducing the toxicity by this reactive aldehyde [61]. In addition to drugs with primary amino groups, inhibition of downstream pathways involved in all-*trans*-retinal-induced toxicity, including phospholipase C and NADPH oxidase, also successfully prevents retinal degeneration [62]. Unlike inhibition of the retinoid cycle, such indirect approaches do not grossly affect the retinoid cycle and can avoid drug-induced delayed dark adaptation.

**3.2. Gene transfer therapies**—Gene transfer therapies for RPE65-deficient dogs [37], as well as *Rpe65*<sup>-/-</sup> [84], *rd12* [85] and RPE65-R91W/R91W [86] mice have been intensely pursued based on adeno-associated and Lenti viruses. Over the years more than two dozen studies have been reported [37, 84, 87]. These efforts have contributed to successful treatment of human patients with RPE65 mutations [3, 4]. Gene transfer therapies with Lenti virus in *Lrat*<sup>-/-</sup> mice also rescued visual functions [27]. In *Abca4*<sup>-/-</sup> mice, the use of non-

viral nanoparticles to deliver a 6.8 kb ABCA4 cDNA ameliorates the visual phenotype of this mouse mutant [60].

**3.3 Other approaches**—Retinal cell death is the final consequence of retinal degeneration associated with a defective retinoid cycle. Pharmacological interventions have been employed to target the mechanisms of cellular death, as has been done for other types of neural degeneration. These interventions include inhibition of apoptosis pathways and suppression of oxidative stress [88, 89]. Thus, several parallel approaches are currently being employed to develop therapies.

## CONCLUSIONS

Continuous regeneration of the visual chromophore through the retinoid cycle is essential to vision. Impairment of this pathway can lead to human retinal disorders. The reactions of these pathways have been well-documented, especially in rod photoreceptor cells. Transgenic and knockout mice lacking retinoid metabolic enzymes, such as *Rpe65*<sup>-/-</sup> and *Lrat*<sup>-/-</sup> animals, recapitulate many features of the human diseases. These animal models allow testing of novel therapeutics for retinal diseases. Effective gene transfer and pharmacologic therapies developed in these animal models have successfully been applied to humans. Progress from the test tube to the bed-side has become a reality for retinal diseases involving the retinoid cycle.

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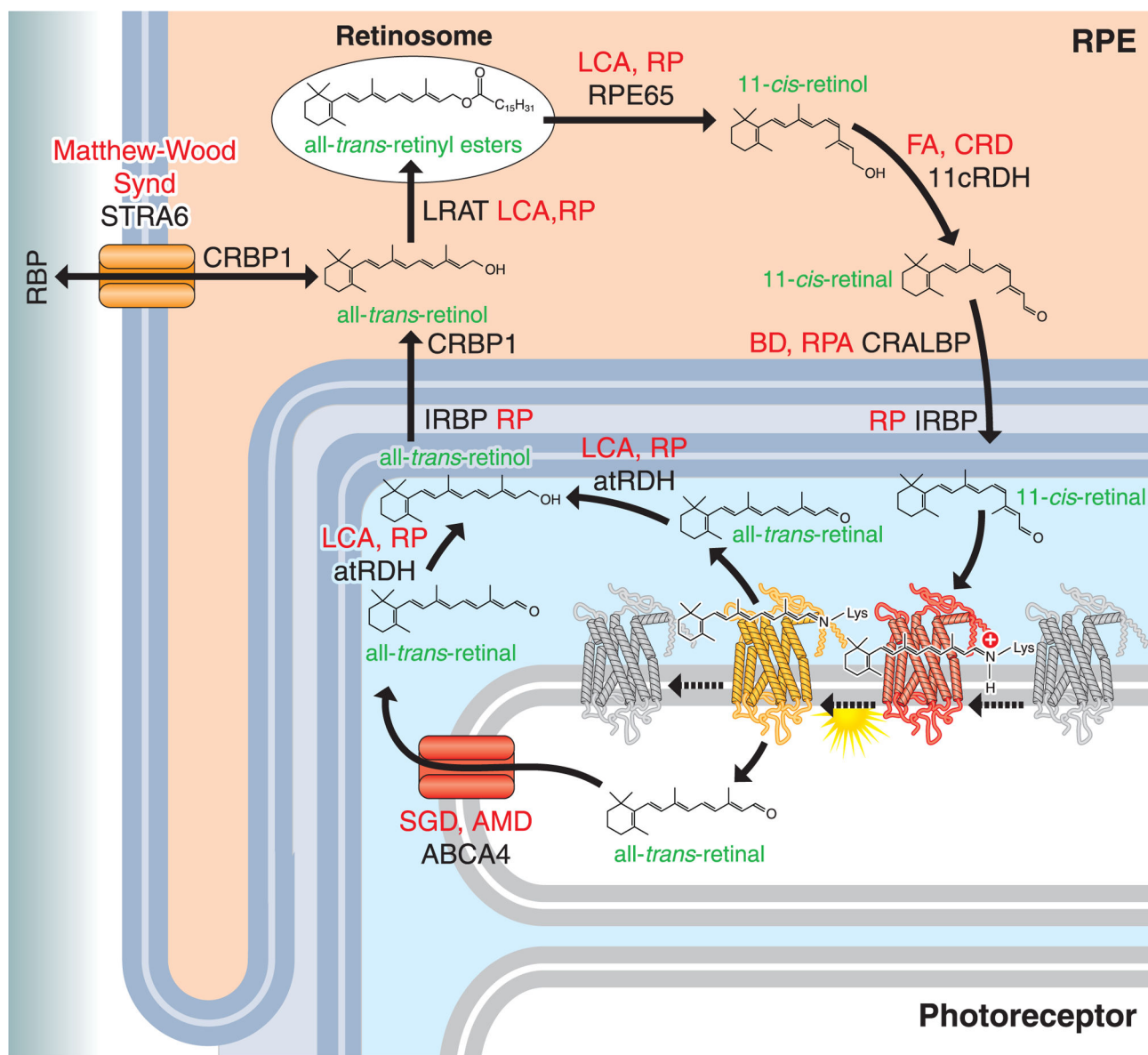
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### Highlights

- The visual (retinoid) cycle is a critical enzymatic pathway that sustains vision
- Mutations in genes encoding visual cycle proteins cause retinal degeneration
- Animal models were generated with faulty visual cycles that mimic human pathologies
- These animals are instrumental for testing new therapies to prevent blindness



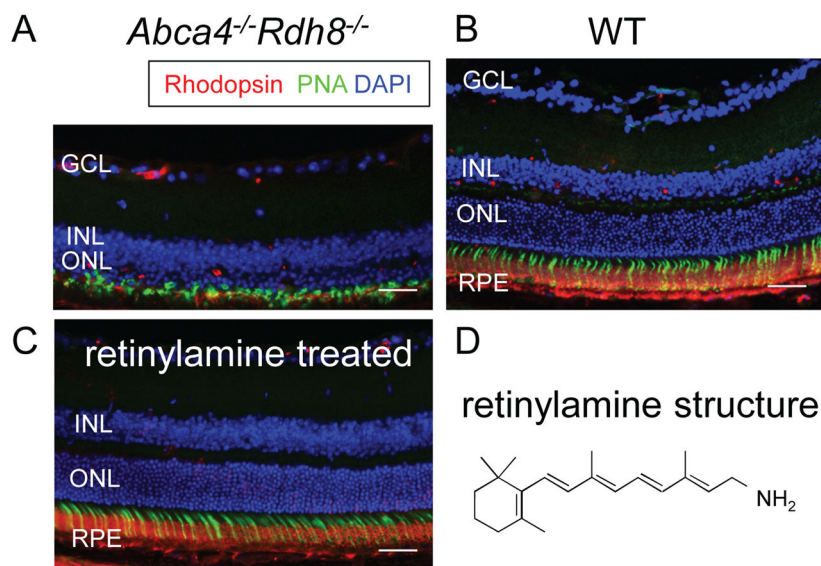
**Figure 1. Vitamin A metabolism in the eye and retinal disorders**

Vitamin A (all-*trans*-retinol) is supplied from blood circulation by the concerted action of serum retinol-binding protein (RBP), stimulated by retinoic acid 6 (STRA6), cellular retinol-binding protein type 1 (CRBP1) and lecithin:retinol acyl transferase (LRAT). Vitamin A is stored as all-*trans*-retinyl ester in the retinosomes of RPE cells. With all-*trans*-retinyl ester as substrate, RPE65 isomerizes the ester to 11-*cis*-retinol and then 11-*cis*-retinol dehydrogenases (RDHs) such as RDH5 and RDH11 oxidize 11-*cis*-retinol to 11-*cis*-retinal. 11-*cis*-Retinal binds to retinoid binding proteins including cellular retinaldehyde-binding protein (CRALBP) and inter-photoreceptor retinoid-binding protein (IRBP), and is then transported to photoreceptors to regenerate light-sensitive rhodopsin. When light hits the light-sensitive form of rhodopsin with 11-*cis*-retinal, 11-*cis*-retinal is photo-isomerized to all-*trans*-retinal. This conformational change initiates rhodopsin activation. All-*trans*-retinal



is largely released from rhodopsin into the cytosolic lumen of rod photoreceptor cell outer segments, but a fraction remains in the intra-discal lumen. This portion of the aldehyde is flipped into the cytosolic lumen of photoreceptors by the ATP-binding cassette transporter (ABCA4), to join the rest of released all-*trans*-retinal that then becomes reduced by all-*trans*-retinol dehydrogenases (RDHs, including RDH8 and RDH12) to all-*trans*-retinol. All-*trans*-retinol is transported back to RPE cells by binding with IRBP and CRBP1. Impairment of any reaction in this cycle is closely associated with human retinal disorders (red). LCA, Leber congenital amaurosis; RP, retinitis pigmentosa; CRD, cone-rod dystrophy; FA, fundus albipunctatus; BD, Bothnia dystrophy; RPA, retinitis punctata albescens; SGD, Stargardt disease; AMD, age-related macular degeneration; Matthew-Wood Synd, Matthew-Wood Syndrome. This current work focuses mainly on models in mouse, which is rod dominant species, but identification of supplementary retinoid transformations in Müller cells could open the possibility to study the cone retinoid cycle that, in addition to the canonical retinoid cycle pathway, supports cone pigment regeneration [1, 5–8].

Note that Stecher *et al.* [91] showed two pools of retinyl esters in the RPE, only one of which were substrates for retinoid isomerase. This discovery was followed by findings made by 1) Imanishi *et al.* who showed that a large amount of the esters are stored in retinosomes [23, 92]; and 2) Orban *et al.* and others showed that RPE65 does not localize retinosomes [93]. Thus, the fraction of retinyl esters embedded in the smooth ER are like the source of substrate for RPE65 (not distinct from retinosomes on the figures).



**Figure 2. Acute bright light-induced retinal degeneration could be prevented by treatment of retinylamine in *Abca4*<sup>-/-</sup> *Rdh8*<sup>-/-</sup> mice**

Light exposure at 10,000 lux for 30 min can cause light-induced retinal degeneration in *Abca4*<sup>-/-</sup> *Rdh8*<sup>-/-</sup> mice (A), whereas the stimulus has virtually no impact on wild-type (WT) mice (B). Light-induced retinal degeneration in *Abca4*<sup>-/-</sup> *Rdh8*<sup>-/-</sup> mice can be prevented by pre-treatment with retinylamine at 1 mg/ml given by oral gavage (C). The chemical structure of retinylamine is presented (D). Retinal sections were prepared 7 days after light exposure. Rod photoreceptors, cone photoreceptors and nuclei were stained by anti-rhodopsin antibody (red), peanut agglutinin (PNA, green) and DAPI (blue), respectively. GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer; RPE, retinal pigmented epithelium. Scale bars indicate 30  $\mu$ m.

Table 1

Animal models with impaired retinoid cycle.

Animal models	Species	Localization	Photoreceptor changes	RPE changes	Retinoids	Other changes	Ref.
<i>Bemo1</i> <sup>-/-</sup>	mouse	RPE				pro-vitamin in adipose tissues, fatty liver	[9]
<i>Bcdo2</i> <sup>-/-</sup>	mouse	RPE				carotenoids acml <sup>7</sup> , mitochondrial dysfunction	[11]
<i>Rbp4</i> <sup>-/-</sup>	mouse	Plasma, RPE			low atROL <sup>2</sup> in the eye		[12]
<i>Strab6</i> <sup>-/-</sup>	mouse	RPE	rapid cone deg. <sup>1</sup> , slow progressive rod deg.	no retinosomes	no 11cRAL <sup>3</sup> , 95% loss of RE <sup>4</sup>		[18]
<i>Crbp1</i> <sup>-/-</sup>	mouse	RPE			low RE		[22]
<i>Lrat</i> <sup>-/-</sup>	mouse	RPE	rapid cone deg., slow progressive rod deg.	no retinosomes	no 11cRAL, no RE		[25, 26]
<i>Rpe65</i> <sup>-/-</sup>	mouse	RPE	rapid cone deg., slow progressive rod deg.	large retinosomes	atRE <sup>4</sup> acml, no 11cRAL		[32]
<i>Rpe65 (R91W/R91W)</i>	mouse	RPE	rapid cone deg., slow progressive rod deg.		atRE acml, no 11cRAL		[35]
<i>Rpe65-rd12</i>	mouse	RPE	rapid cone deg., slow progressive rod deg.		atRE acml, no 11cRAL		[33]
<i>RPE65</i> <sup>-/-</sup> ( <i>4bp-deletion</i> )	dog	RPE	rapid cone deg., slow progressive rod deg.		atRE acml, no 11cRAL		[34]
<i>C57BL/6 (Leu450 Met)</i>	mouse	RPE			slow 11cRAL reg. <sup>8</sup>		[36]
<i>Rdh5</i> <sup>-/-</sup>	mouse	RPE			13cRE <sup>5</sup> acml, slow 11cRAL reg.	delayed DA <sup>9</sup>	[41]
<i>Rdh11</i> <sup>-/-</sup>	mouse	RPE and IS <sup>11</sup>					[42, 90]
<i>Rdh5</i> <sup>-/-</sup> <i>Rdh11</i> <sup>-/-</sup>	mouse		slow cone deg.		13cRE acml, slow 11cRAL reg.	delayed DA	[42, 45]
<i>Rdh10</i> <sup>-/-</sup>	mouse	RPE and Müller cells				Embryonic lethality	[43]
<i>Rbp1</i> <sup>-/-</sup>	mouse	RPE and Müller cells			slow 11cRAL reg.	LD <sup>10</sup> , delayed DA	[50]
<i>Abca4</i> <sup>-/-</sup>	mouse	OS <sup>12</sup>	slow rod deg. in albino	lipofuscin	A2E <sup>6</sup> acml, delayed atRAL clearance	LD, delayed DA	[55, 56]
<i>Rdh12</i> <sup>-/-</sup>	mouse	IS		lipofuscin	A2E acml	LD, delayed DA	[64, 65]

Animal models	Species	Localization	Photoreceptor changes	RPE changes	Retinoids	Other changes	Ref.
<i>Rdh8</i> <sup>-/-</sup>	mouse	OS		lipofuscin	A2E acml, delayed atRAL clearance	LD, delayed DA	[63]
<i>Rdh8</i> <sup>-/-</sup> <i>Rdh12</i> <sup>-/-</sup>	mouse		slow cone and rod deg.	lipofuscin	A2E acml, delayed atRAL clearance	LD, delayed DA	[66]
<i>Abca4</i> <sup>-/-</sup> <i>Rdh8</i> <sup>-/-</sup>	mouse		rod and cone deg.	lipofuscin, RPE atrophy	A2E acml, delayed atRAL clearance	LD, delayed DA	[68]
<i>Irbp</i> <sup>-/-</sup>	mouse	extracellular matrix of photoreceptors	slow rod and cone deg.				[70, 71]
<i>Rgr</i> <sup>-/-</sup>	mouse	RPE and Müller cells			low 11cRAL under light adapted eyes		[73]
<i>Rgr</i> <sup>-/-</sup> <i>Rpe65</i> <sup>-/-</sup>	mouse						[74, 75]
<i>Des1</i> <sup>-/-</sup>	mouse	Müller cells and RPE (widely expressed)				Small size, scaly skin, liver dysfunctions, die at 8-10 weeks old	[80]

deg<sup>1</sup>, degeneration; atROL<sup>2</sup>, all-*trans*-retinol; 11cRAL<sup>3</sup>, 11-*cis*-retinal; RE<sup>4</sup>, retinyl esters; atRE<sup>4</sup>, all-*trans*-retinyl ester; 13cRE<sup>5</sup>, 13-*cis*-retinyl ester; A2E<sup>6</sup>, di-retinoid-pyridinium-ethanolamine; acml<sup>7</sup>, accumulation; reg<sup>8</sup>, regeneration; DA<sup>9</sup>, dark adaptation; LD<sup>10</sup>, light-induced damage; IS<sup>11</sup>, photoreceptor inner segment; OS<sup>12</sup>, photoreceptor outer segment.