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Visual cycle proteins: Structure, function, and roles in human retinal disease

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Here, we seek to summarize the current understanding of the biochemical and molecular events mediated by visual cycle molecules in the eye. The structures and functions of selected visual cycle proteins and their roles in human retinal diseases are also highlighted. Genetic mutations and malfunctions of these proteins provide etiological evidence that many ocular diseases arise from anomalies of retinoid (vitamin A) metabolism and related visual processes. Genetic retinal disorders such as retinitis pigmentosa, Leber's congenital amaurosis, and Stargardt's disease are linked to structural changes in visual cycle proteins. Moreover, recent reports suggest that visual cycle proteins may also play a role in the development of diabetic retinopathy. Basic science has laid the groundwork for finding a cure for many of these blindness-causing afflictions, but much work remains. Some translational research projects have advanced to the clinical trial stage, while many others are still in progress, and more are at the ideas stage and remain yet to be tested. Some examples of these studies are discussed. Recent and future progress in our understanding of the visual cycle will inform intervention strategies to preserve human vision and prevent blindness.

Classic rod/cone visual cycle

The classic visual cycle is initiated by the conversion of a single photon of light energy into an electrical signal in the retina. This signal transduction occurs due to a G protein–coupled receptor (GPCR)[2] called opsin, which contains an 11-*cis*-retinal chromophore. When activated by a photon, 11-*cis*-retinal undergoes photoisomerization to all-*trans*-retinal leading to a change in the conformation of opsin GPCR and a signal transduction cascade to close cGMP-gated cation channels resulting in hyperpolarization of the photoreceptor cell. The collective change in the receptor potentials of rods and cones triggers nerve impulses that our brain interprets as vision. Following isomerization and release from opsin, all-*trans*-retinal is reduced to all-*trans*-retinol and then transferred to the adjacent retinal pigment epithelium. It is esterified by lecithin–retinol acyltransferase to retinyl ester and then converted to 11-*cis*-retinol by the isomerohydrolase RPE65 (also known as isomerase I). It is oxidized to 11-*cis*-retinal before returning to the photoreceptors to combine with opsin to form rhodopsin (Fig. 1) (1–6).

Intraretinal cone visual cycle

Cones and their photopigments are responsible for daylight vision (photopic) and the perception of colors. There are three types of cones in the retina that respond to short-, medium-, and long-wavelength light, also called S-cones, M-cones, and L-cones, respectively. The intraretinal cone visual cycle begins after photoisomerization, and all-*trans*-retinal is released from cone pigments. After reduction to all-*trans*-retinol, it is transported from the cone outer segments to Müller cells in the retina where it is isomerized to become 11-*cis*-retinol and then esterified to retinyl ester. Upon hydrolysis, 11-*cis*-retinol is returned to the cone photoreceptors where it is oxidized to 11-*cis*-retinal to conjugate with cone opsins to form cone pigments. The cone visual cycle is supported by isomerase II, dihydroceramide desaturase 1 (DES1), and multifunction *O*-acyltransferase (MFAT) (Fig. 1) (7, 8).

Structure and function of selected visual cycle proteins

Rhodopsin structure and function

As mentioned above, rhodopsin consists of opsin and a covalently-bound retinal chromophore. It is a light-sensitive G protein–coupled receptor located in the lipid bilayer of outer segment disc membranes of rod cells. It has seven transmembrane α helices across the disc membrane. The photoreactive chromophore, 11-*cis*-retinal, is conjugated to a lysine residue of rhodopsin and oriented horizontally in the disc membrane to optimize interaction with photons. Isomerization of 11-*cis*-retinal into all-*trans*-retinal by light sets off a cascade of opsin conformational changes that leads to the formation of metarhodopsin II and activates the associated G proteins. This signal is transduced to a cGMP second messenger resulting in a change in the level of 5′GMP and the closure of cation channels (9, 10).

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[2] The abbreviations used are: GPCR, G protein–coupled receptor; RP, retinitis pigmentosa; iPS, induced pluripotent stem cell; LCA, Leber's congenital amaurosis; IRBP, interphotoreceptor retinoid-binding protein; DR, diabetic retinopathy; STGD, Stargardt disease; RPE, retinal pigment epithelium; rAAV, recombinant adeno-associated virus.

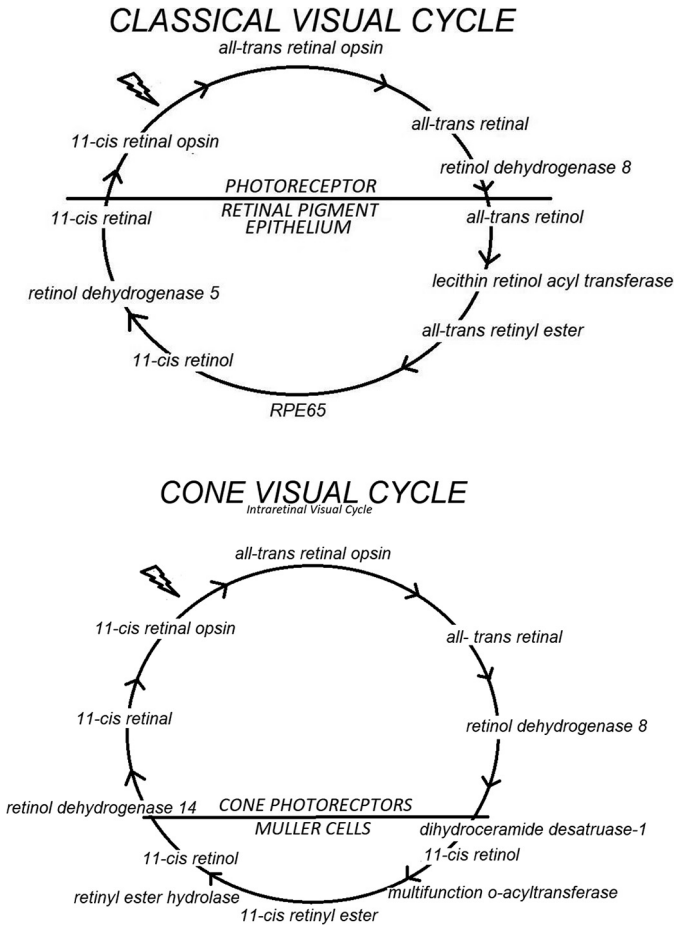

Figure 1. Classic (rod and cone photoreceptor) and the cone (cone photoreceptor) visual cycles.

RPE 65 structure and function

RPE65 is the known isomerohydrolase (isomerase I) in the classic rod and cone visual cycle. RPE65 is a member of the carotenoid oxygenase family and is expressed in the retinal pigment epithelium bound to endoplasmic reticulum. It is a Fe^{2+}-dependent isomerase enzyme that catalyzes the hydrolysis of all-*trans*-retinyl ester and the isomerization of all-*trans*-retinol to 11-*cis*-retinol (2). This is consistent with the observation in animal models where RPE65 KO resulted in a low level of 11-*cis*-retinol with an accumulation of retinyl esters in the eye. There are at least 60 known genetic mutations to RPE65 proteins that account for a variety of retinal and ocular diseases. Individuals afflicted with RPE65 gene mutations generally have early onset blindness (autosomal recessive LCA or RP).

IRBP structure and function

IRBP is secreted by rods and cones into the subretinal space where it constitutes the major protein component of the interphotoreceptor matrix and interacts with the cone "matrix sheath" (11). IRBP is composed of multiple modules (four in tetrapods and humans and two to three modules in teleosts) with each harboring ~300 amino acid residues (11).

IRBP has long been thought to play a role in the canonical visual cycle involving retinoid exchange between rods and RPE (12–14). However, understanding IRBP's function in this process has proved to be more challenging than initially anticipated (15–18). Recently, experimental evidence has implicated IRBP as having an important function in the cone visual cycle, involving retinoid exchange between cones and Müller glial cells (19–27).

The mechanism(s) involved in IRBP's role(s) in the cone visual cycle are only beginning to be elucidated. We recently reported that IRBP binds to the cone outer segment and Müller cell microvilli pericellular matrices (11). Such association could target and/or facilitate delivery/uptake of its retinol ligands. Furthermore, we reported that IRBP has free radical scavenging activity (28) and can protect all-*trans*- and 11-*cis*-retinol from photodegradation (29).

Gene mutations and retinal diseases

Retinal diseases from gene mutations

Based on all listings in the RetNet, there are 321 known gene mutations in human chromosomes 1–22, X, and mitochondria that result in retinal diseases. They consist of mutations in a wide range of proteins from ocular components such as rhodopsin to complexes I, II, and IV of the mitochondrial electron transfer chain. Table 1 provides a summary of all 321 gene mutations with an example of a mutated protein in each chromosome.

Selected retinal diseases from gene mutations in visual cycle proteins

RP—This retinal disease is classified as a rod-cone dystrophy, meaning that rods are affected first and then the cones. Typically, patients initially notice difficulties with night vision, trouble adapting to dim illumination, or trouble with side vision in dim light. This can begin in childhood, but some patients may not notice it until in their teens or twenties. Most patients will have symptoms by the time they reach age 30. Eventually, only a small area of central vision remains, and this central vision may be maintained for years with 25% of patients maintaining vision well enough to be able to read for most of their lives (30). Of course, this means that 75% of patients will eventually lose enough central vision, in addition to their already lost peripheral vision, that they are unable to read.

Clinically, a classic "bone-spicule" pattern appears in the mid-peripheral retina; the retinal arterioles are narrowed, and the optic nerve has a pallor. Visual fields early on may exhibit what is described as a ring scotoma, but later the scotoma extends both directions to include all but the central vision. Electroretinography signal, a measurement of visual response to light, may be severely depressed even before retinal changes are visible.

RP is the most common (1 in 3000) genetically inherited retinal disease and can be either an autosomal recessive, autosomal dominant, or X-linked disorder of the eye that can evolve from mutations in more than 50 different genes. As many as half of the RP cases have no other affected family members and are designated as sporadic. Some of these cases designated as sporadic may turn out to have been recessive, but many may be due to new mutations occurring in the germline cells that lead to RP. The inherited genetic mutation causes a progressive loss of rod photoreceptor cells followed by loss of cone photorecep-

Table 1

Genes and mapped loci causing retinal diseases

The data were accessed at https://sph.uth.edu/retnet/disease.htm on March 28, 2018. Please note that the JBC is not responsible for the long-term archiving and maintenance of this site or any other third party hosted site.

Chromosome	Mutations	Example of a disease with the mutated protein and gene mutation ID
1	28	Recessive Leber's congenital amaurosis; RPE65; candidate gene for LCA
2	22	Recessive retinitis pigmentosa; zinc finger protein; linkage mapping
3	17	Dominant retinitis pigmentosa; rhodopsin; linkage mapping
4	21	Recessive retinitis pigmentosa; LRAT; candidate gene
5	9	Dominant Wagner disease; versican; linkage mapping
6	20	Age-related macular degeneration; complement component 2; association study
7	9	Dominant tritanopia; blue cone opsin; candidate gene
8	12	Recessive Jobert syndrome; centrosome--spindle pole protein; whole-exome sequencing
9	11	Age-related macular degeneration; Toll-like receptor 4; link mapping; association study
10	22	Recessive retinitis pigmentosa; IRBP; homozygosity mapping; candidate gene
11	19	Recessive Usher syndrome; myosin VIIA; linkage mapping
12	13	Recessive fundus albipunctatus; RDH5; candidate gene
13	5	Somatic retinoblastoma; RB1; deletion mapping; candidate gene
14	13	Recessive Leber's congenital amaurosis; RDH 12; homozygosity mapping; linkage mapping
15	9	Recessive Usher syndrome; calcium- and integrin-binding protein; linkage mapping
16	16	Recessive Leber's congenital amaurosis; clusterin-associated protein 1; whole exome sequence
17	16	Dominant retinitis pigmentosa; carbonic anhydrase IV; linkage mapping
18	3	Recessive retinal dystrophy; α1-laminin; homozygosity mapping
19	10	Age-related macular degeneration; complement component 3; association study
20	8	Recessive retinitis pigmentosa; Kizuna centrosomal protein; whole-exome sequencing
21	2	Recessive cone-red dystrophy; chromosome 21 open reading frame 2; homozygosity Ma
22	5	Dominant Sorsby's fundus dystrophy; tissue inhibitor of MP3; linkage mapping
X	24	Protanopia; red cone opsin; candidate gene
Mitochondria	7	Leber's hereditary optic neuropathy; complex I, II or V; sequencing
Total	321	

tor cells. One form of autosomal dominant RP is associated with a missense mutation, A346P, located in the rhodopsin gene. This mutation has been found to interfere with normal regeneration of photoreceptors. Mutations resulting in a truncated rhodopsin protein have been associated with autosomal recessive disease. The loss of these photoreceptor cells results in poor night and peripheral vision, and later central vision, which can and will ultimately lead to blindness (31, 32). An RPE65 mutation accounts for ~2% of RP (32, 33).

LCA—This is an inherited autosomal recessive retinopathy. People born with LCA have greatly reduced vision at birth, although their retinas appear to be normal fundoscopically. Nystagmus is common with the eyes showing difficulty tracking. Sufferers are found to rub their eyes frequently stimulating their retinas to produce light-like impulses called pressure phospenes. Electoretinograms reveal very little retinal function. By the time patients reach puberty the retinal arterioles are constricted, and pigmentary changes of the retinal pigment epithelium occur similar to those with RP. Although the best corrected vision with glasses or contact lenses is limited to finger counting or light perception, it can remain fairly stable throughout early adulthood (34). A co-morbidity is often keratoconus. Speculation exists whether this stems from the associated eye rubbing or whether it is due to the genetics of LCA (35). LCA2 is the form of LCA linked to a mutation in the RPE65 gene (36). RPE65 is the isomerase responsible for converting all-*trans*-retinal to 11-*cis*-retinal, which is essential for proper functioning of the visual cycle (1). An RPE65 mutation results in the accumulation of all-*trans*-retinyl esters and the reduction of rhodopsin in the rod photoreceptor outer segment. The reduction of rhodopsin leads to major retinal abnormalities and dysfunction at birth (37, 38). The autosomal recessive mutations in RPE65 account for ~6–16% of LCA instances (33, 39).

Stargardt disease (STGD)—This is the most common inherited (1:10,000) juvenile macular condition. Clinically, yellow flecks of lipofuscin pigment are found in the macula (40). Disease progression occurs at different rates among individuals, but usually when the vision decreases to 20/40, it descends rapidly toward a final vision of 20/200 to 20/400 (34). The patient retains peripheral vision because only the central vision is impaired. The loss of central vision stems from atrophy of the macular retinal pigment epithelium and neuroepithelium (40). STGD is a recessive hereditary condition (41). The causality of STGD disease is generally a mutation in the ABCA4 gene that codes for a transmembrane protein that moves all-*trans*-retinal from inside the photoreceptor disc into the cytoplasm where it is converted to retinol in the visual cycle (42). A multitude of STGD causative ABCA4 mutations have been described (43). The lack of ABCA4 protein function leads to a toxic accumulation of all-*trans*-retinal, which ultimately causes the death of photoreceptor cells. Currently, there is no treatment for Stargardt disease (44, 45).

Diabetic retinopathy (DR)—This represents the leading cause of blindness in working age adults. It is a reaction to the hyperglycemia associated with both type 1 and type 2 diabetes, but other factors such as lipid levels, blood pressure, and genetics also play a role (43, 46). Diabetic retinopathy begins with damage to retinal capillaries noted clinically as small dots of hemorrhage and microaneurysms and loss of retinal neurons. When the damage to the vasculature reaches a stage where oxygen supply and carbon dioxide removal are sufficiently reduced, the hypoxic retina responds with the development of new blood vessels. Unfortunately, it is this abnormal blood vessel development, referred to as proliferative diabetic retinopathy, that results in retinal detachment and possible blindness.

Currently, the treatments for diabetic retinopathy are only applicable at the later stages of the disease process (46). The pan-retinal photocoagulation entails a series of laser burns on the retina, which destroy some retinal elements thus decreasing oxygen demand while increasing oxygen flow from the under-

lying choroid. While preserving sight, the patient is left with reduced peripheral and night vision. More recently, anti-vascular endothelial growth factor injections have been used, resulting in constructive atrophy of aberrant vasculature and apparent stabilization of the diabetic retina, but many questions remain about the long-term impact of this treatment on the retina. Although control of the systemic factors in diabetes helps to prevent or delay the development of retinopathy, there is no regimen available to specifically treat or prevent disease development. Many agents, including inflammation, advanced glycation end products, protein kinase C, and oxidants, are thought to play a role in the induction of diabetic retinopathy. It has also been noted that patients with diabetes have reduced levels of IRBP, which have been linked to the progression of DR (43). Garcia-Ramirez *et al.* (58) found that the elevated levels of glucose and of the cytokines TNFα and IL-1β, associated with diabetes, lead to reduced IRBP expression. Recently, Malechka *et al.* (47) documented attenuated levels of 11-*cis*-retinal, IRBP, and rhodopsin in diabetic rats. How the lower amount of IRBP in patients may contribute toward the development of diabetic retinopathy is yet unclear (48).

Strategies to advance the cure of blinding afflictions

Pharmacological approach

Some have recommended the use of vitamin A and fish oils to help reduce progression of RP in adults, but its effectiveness remains controversial (30, 49, 50). Although the use of retinoid isomers and visual cycle inhibitors has been shown to be effective in research studies (9), no pharmaceutical treatments are available or currently approved for retinal diseases such as LCA or STGD. Targets for pharmaceuticals such as β-cyclodextrins are being investigated in an effort to enhance photoreceptor survival in individuals suffering from these maladies (43). Systemic treatments for DR are the intensive control of blood glucose, blood pressure, and lipid levels. Minocycline has been suggested as a possible treatment to prevent the development of DR due to its anti-inflammatory effects and the fact that it crosses the blood–brain barrier enabling it to reach the target inflammatory diabetic processes in the retina, but studies have not yet been completed to explore this possibility (48).

Gene therapy approach

Gene therapy represents one of the experimental strategies for the prevention and treatment of maladies associated with RPE65 mutation. Bainbridge *et al.* (51) used an rAAV vector to subretinally deliver human RPE65 cDNA under the control of the RPE65 native promoter. One of the three patients in the trial "showed evidence of improvement in retinal function by microperimetry, dark-adapted perimetry, and visual mobility" (51). Maguire *et al.* (52) subretinally injected rAAV harboring human RPE65 cDNA under the control of the chicken β-actin promoter. All three young adults in this trial "showed evidence of improvement in retinal function based on testing of visual acuity and pupillometry (pupillary light reflex)." Following treatment, the pupillary response to light was three times greater than the baseline. Visual acuity improved, and the visual field was enlarged 2 weeks after treatment" (52). A third group also tested rAAV-mediated delivery of human RPE65 cDNA with the RPE65 native promoter. Cideciyan *et al.* (53) also reported that one out of three of their trial subjects showed evidence of improved retinal function, including dark adaptation, increased light sensitivity, and visual field expansion (54).

December, 2017, marked the first approval by the Food and Drug Administration of an injection-based gene-delivery regimen to treat an eye disease. Voretigene neparovovec-ryzl (Luxturna) was approved to treat homozygous dysfunctional RPE65 retinal conditions such as LCA. Voretigene neparvovec-ryzl (Luxturna) uses an adeno-associated vector to deliver a complete copy of the RPE65 gene to treat retinal conditions such as LCA (55).

Other approaches

Stem cell therapy is being tested for the treatment of blinding disorders. Muniz *et al.* (5) utilized human pluripotent stem cells *in vitro* and derived functional RPE capable of all-*trans*-retinol uptake from the conditioned culture medium, processing it into 11-*cis*-retinal for secretion. This study provided the proof of principle of using pluripotent stem cells (iPS) from a patient to generate iPS-RPE for intraocular or retinal injection. Assawachananont *et al.* (56) were able to subretinally transplant both embryonic and induced pluripotent stem cell–derived three-dimensional retinal sheets into mice with advanced retinal degeneration and showed that the transplanted tissue developed an outer nuclear layer along with complete inner and outer photoreceptor segments. Thus, the authors of this study provided the "proof of concept" for transplantation therapy in the treatment of retinal degenerative diseases (56).

More recently, Kashani *et al.* (57) reported evidence for improved visual function in a small group of patients with non-neovascular age-related macular degeneration after implanting a sheet of RPE cells under the degenerated macula. None of the four patients receiving the implant have shown any further vision loss, and one patient demonstrated visual improvement by being able to view 17 more letters of the alphabet compared with before the treatment (57).

Discussion

Tremendous efforts are being expended in devising strategies to prevent and cure maladies of the eye. Basic science has laid the requisite foundation in this regard, and different laboratories are testing therapeutic strategies in cell and animal models, although a few groups have been conducting early clinical trials. Basic scientists, clinicians, and physicians from different countries are able to share research results and information freely over cross-sections of populations and to different countries around the globe. Given these favorable circumstances, one can be optimistic that a cure for the blinding disorders is forthcoming.

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