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## Membrane Receptors and Transporters Involved in the Function and Transport of Vitamin A and Its Derivatives

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### Abstract

The eye is the human organ most sensitive to vitamin A deficiency because of vision's absolute and heavy dependence on vitamin A for light perception. Studies of the molecular basis of vision have provided important insights into the intricate mechanistic details of the function, transport and recycling of vitamin A and its derivatives (retinoid). This review focuses on retinoid-related membrane receptors and transporters. Three kinds of mammalian membrane receptors and transporters are discussed: opsins, best known as vitamin A-based light sensors in vision; ABCA4, an ATP-dependent transporter specializes in the transport of vitamin A derivative; and STRA6, a recently identified membrane receptor that mediates cellular uptake of vitamin A. The evolutionary driving forces for their existence and the wide spectrum of human diseases associated with these proteins are discussed. Lessons learned from the study of the visual system might be useful for understanding retinoid biology and retinoid-related diseases in other organ systems as well.

### Keywords

vitamin A; retinoid; STRA6; retinol binding protein; opsin; ABCA4

### 1. Introduction

Vitamins are essential organic molecules that an organism depends on for survival. An essential organic molecule becomes a vitamin if the organism cannot synthesize it and must obtain it from the diet. The principle diet of an organism (or its ancestor) may determine that organism's definition of vitamins. A well-known example is vitamin C, which is not a vitamin for many organisms such as mice [1], but vitamin C intake is essential for humans. Human ancestors likely had easy access to vitamin C-rich food and thus lost the ability (and the need) to synthesize vitamin C. A key feature of vitamin biology is that vitamin status is heavily influenced by the environment. In addition to diet, other environmental factors may also influence vitamin status. For example, human skin can produce vitamin D in the presence of UV light [2] (in this sense, vitamin D is not a true vitamin). Therefore, both light exposure and human skin color influence vitamin D status and diseases related to vitamin D deficiency [3]. Human intestinal microbes can produce vitamin K to alleviate its deficiency [4].

Vitamin A is arguably the most multifunctional vitamin in the human body and is essential for human survival at every point from embryogenesis to adulthood. The range of cellular activities it participates in is mind-boggling and is still being discovered. The molecular mechanism of vitamin A's physiological functions was first elucidated for vision [5, 6]. Since then, biological functions of vitamin A have been discovered in almost every vertebrate organ. Examples include its roles in reproduction, embryonic growth and

development, immune competence, maintenance of epithelial surfaces, and proper functioning of the adult brain [7–13]. The most well known example of vitamin A's large impact on human survival is the discovery that administration of vitamin A alone is sufficient to decrease childhood mortality by 20–70% in developing countries [14]. This finding has led to the development of an alternative vitamin A-rich diet to decrease childhood mortality [15]. Except for vision, which depends on the aldehyde form of vitamin A (retinal), most of the physiological functions of vitamin A can be ascribed to the acid form (retinoic acid) acting on nuclear hormone receptors that regulate gene transcription [16, 17]. New biological functions are continuously being discovered for vitamin A derivatives. For example, retinol, the alcohol form of vitamin A, also has distinct biological activities [18–23]. It was recently discovered that retinal inhibits adipogenesis [24, 25] and that retinoic acid regulates synaptic protein translation, in addition to its role in regulating gene transcription [26, 27]. Although many organisms (from unicellular organisms to vertebrates) use vitamin A aldehyde for light detection (vision or the equivalent of vision), the biological functions of vitamin A derivatives other than vitamin A aldehyde (e.g., retinoic acid) have only been well defined in vertebrates. Interestingly, retinoic acid was recently isolated from cyanobacteria, but its biological function has not been identified yet [28].

This review focuses on membrane receptors and transporters involved in the function and transport of vitamin A and its derivatives. Three kinds of membrane receptors and transporters involved in retinoid biology are discussed here: opsins, ABCA4 (ABCR) and STRA6. The opsins and ABCA4 are related to vitamin A aldehyde. Opsins use vitamin A aldehyde as the chromophore for light absorption, and ABCA4's function is to accelerate the elimination of vitamin A aldehyde in light-bleached photoreceptor cells. STRA6 is related to alcohol form of vitamin A, retinol, which is the major transport form of vitamin A in the blood.

## 2. Retinoid-Related Membrane Receptors and Transporters

### 2.1. Opsins: Vitamin A-based transmembrane light sensor

**2.1.1. Biological functions**—Visual pigments are the light-absorbing proteins in vision that use vitamin A-aldehyde as the chromophore. They belong to the seven-transmembrane domain G-protein coupled receptor family. Visual pigments in each organism are uniquely suited to the needs of each organism. Human has three cone visual pigments (the long-wave, medium wave and short wave pigments) responsible for bright light vision and trichromatic color vision [29–31] and one rod visual pigment rhodopsin responsible for dim light vision [32–35] (Figure 1). Mice do not have the long-wave cone pigment but have a medium wave cone pigment [36] and a UV cone pigment [37] responsible for dichromatic vision. The discovery of visual pigments has led to the discovery of many homologous proteins (opsins) that are expressed in rod and cone photoreceptor cells and/or other cell types. For example, chicken has a light-sensitive pinopsin in its pineal gland [38, 39]. In one extreme example of visual adaptation, a fish that has vision both above and below water (*Anableps anableps*) was recently found to have ten different opsins expressed in its eye [40]. Frog has melanopsin, which is an opsin expressed in its skin melanocytes, eye and brain [41]. Melanopsin is also expressed in light sensitive ganglion cells in the mammalian retina [42, 43] that, together with rod and cone photoreceptor cells, sense light for the circadian clock in the brain [44–47]. Vertebrate ancient (VA) opsin is an opsin expressed in the horizontal and amacrine cells of the retina and also in the brain of salmon [48, 49]. Parietopsin and pinopsin are expressed in the parietal eye of the lizard [50].

There are also opsins that are unlikely to sense light for light perception. Peropsin and retinal G protein-coupled receptor (RGR) are two opsin family members expressed in the retinal pigment epithelium (RPE) cell, a highly pigmented cell type that primarily functions

as the “caretaker” of photoreceptor cells in the eye [51, 52]. Peropsin is a plasma membrane protein, expressed specifically on the apical microvilli of the RPE cells (Figure 2) [53]. RGR is expressed on the intracellular membrane of the RPE cells [54] (Figure 2). Both peropsin and RGR share the signature lysine residue in the seventh transmembrane helix that is used by opsins to attach to the chromophore. RGR has been shown to have light-dependent isomerase activity [55, 56] and also regulates the primary retinoid isomerase in a light-independently manner [57]. RGR also mediates light-dependent translocation of retinyl esters [58]. Peropsin homologs have been identified in amphioxus [59] and spider [60]. These peropsins bind to all-*trans*-retinal as a chromophore and mediate light-dependent isomerization of the chromophore to the 11-*cis* form [59, 60]. Encephalopsin [61] and neuropsin [62] are two opsins that are expressed in the mammalian brain and other tissues. Although neuropsin's bird homolog is responsible for light sensitivity of the bird brain [63], the roles of these two opsins in the mammalian brain, which is not known to be photosensitive, are unknown. Given the fact that bovine rhodopsin can be functionally expressed in the mouse brain to sense light without exogenous retinal chromophore [64], these native opsins in the brain may have sufficient access to the retinal chromophore. The visual cycle to regenerate 11-*cis* retinal for vision is not known to be present in the brain. The likely endogenous retinal chromophore in the brain that can regenerate opsins is 9-*cis* retinal because 9-*cis* retinal can reconstitute functional rhodopsin [65, 66] and 9-*cis* retinoid is known to exist in vivo [67–69].

**2.1.2. Evolutionary roles**—Light is not only the ultimate energy source of most living organisms on earth, it is also used throughout evolution as an information source. One definition of “light” is the region of the electromagnetic spectrum to which photoreceptor cells can respond (that's why X-rays are not considered “light”). The use of vitamin A-based chromophore for light detection is extremely ancient and widespread throughout evolution. More than 300 prokaryotic and eukaryotic seven-transmembrane domain proteins are known to use vitamin-A aldehyde as the chromophore for light absorption [70]. Although there are other chromophores in biology [71], two unique properties of vitamin A aldehyde likely explain why it is the most popular chromophore for photoreception in evolution. It combines readily with proteins through its aldehyde end and can create photopigments with absorption maxima in the range of the peak wavelengths of sunlight reaching earth surface. In addition, its long chain of conjugated double bonds allows drastic light-induced geometrical isomerization, which is the basis of initiation of signal transduction. Interestingly, all multicellular organisms that depend on retinal chromophore for light perception use the 11-*cis* form, despite the existence of other forms (e.g., 9-*cis*, 13-*cis* and all-*trans* forms). Most vertebrate visual pigments use 11-*cis* retinal, but some fishes and amphibians use 11-*cis* 3,4-dehydroretinal as a second chromophore [72, 73]. Invertebrates use 11-*cis* 3-hydroxylretinal, 11-*cis* 4-hydroxylretinal, 11-*cis*, 3,4-hydroxylretinal and 11-*cis* retinal as chromophores [74, 75]. In contrast, all unicellular organisms use all-*trans* retinal as the chromophore [70].

The evolutionary driving forces for the emergence of vitamin A-based light sensors include not only light detection but also differentiation of different wavelengths of light (color vision). By conjugating covalently to opsins, retinal's absorption maximum can be red-shifted away from the UV range. In addition to avoiding damage from UV light, the red-shift allows the retinal chromophore to have different absorption maxima that are influenced by the opsin proteins [36, 76–79]. Diversification of absorption maxima of visual pigments is required for color vision. The emergence of a new color vision sensation can be driven by the emergence of new visual pigments. It has been demonstrated in animal models that expressing a new cone visual pigment alone is sufficient to drive the new sensation in color space [80–82]. In addition to rod and cone photoreceptor cells in the eye, opsin expression has made other cell types in the eye [41, 42, 58, 83, 84] and extraocular tissues, such as skin and brain, light-sensitive [38, 39, 41, 50, 63].

In addition to light detection, opsins may have other functions. Vertebrate rhodopsin was recently found to function as a lipid flipase [85]. This will be discussed later in the context of ABCA4. Rhodopsin in *Drosophila* larvae, but not adults, was recently found to function as a thermosensor in addition to its role as a light sensor [86]. Because vitamin A is required for this activity, this is the first time a vitamin A-based protein has been linked to thermosensation. Since the human skin, not the eye, is known to be important in thermo sensation, opsin expression elsewhere in the body may be relevant for the task (Figure 3).

**2.1.3. Human diseases**—Mutations in cone visual pigments cause color blindness [30, 31, 87]. Red/green color blindness is in fact the most common single-locus genetic disorder in humans. Rhodopsin mutations are implicated in two distinct human diseases: retinitis pigmentosa [88–92], which is a progressive form of retinal degeneration that primarily targets the rod photoreceptor cells, and congenital night blindness [93–96], a non-progressive form of blindness that affects rod vision. Mutations in RGR have also been associated with retinitis pigmentosa [97]. Human diseases associated with other opsins (e.g., melanopsin and peropsin) have not yet been identified.

## 2.2. ABCA4 (ABCR): ATP-dependent transport of light-bleached chromophore

**2.2.1. Biological function**—ABCA4 (ABCR) is the only known member of the large ATP-binding cassette (ABC) transporter family that specializes in the transport of vitamin A derivatives [98, 99]. ABCA4 is the only gene associated with recessive Stargardt macular dystrophy, the most common form of early-onset macular degeneration [100]. ABCA4 was also independently identified biochemically as the photoreceptor Rim protein [101, 102]. ABC transporters exist from bacteria to human and are known to transport a wide variety of molecules such as sugars, peptides, metals, lipids, and drugs across membranes. CFTR, a member of the ABC transporter family, even functions as a chloride channel. ABCA4 is most homologous to ABCA1, the Tangier disease protein involved in cholesterol efflux [103, 104], but ABCA4 is unlikely involved in cholesterol transport. What might ABCA4 transport?

Based on the phenotypes of ABCA4 knockout mice [105] and biochemical properties of purified and reconstituted ABCA4 from photoreceptor cells [106–108], it was hypothesized that ABCA4 functions to transport vitamin A aldehyde (retinaldehyde) released from bleached rhodopsin from the disc membrane so that it can be reduced by retinol dehydrogenase in the cytoplasm.

This hypothesis has been confirmed by overwhelming independent evidence. Traditional transport assays that depend on the separation of substrates from liposomes do not apply to retinaldehyde because of its tight association with membranes (more technical challenges discussed below). Since ABC transporters' substrates are known to stimulate their ATP hydrolysis (as their transport consumes ATP), analyzing ATP hydrolysis of purified and reconstituted transporter is another potential method to find the substrate. ABCA4 was purified from bovine eyes and reconstituted into liposomes. Retinaldehyde and a large number of compounds including different retinoids were tested for their ability to stimulate the ATP hydrolysis of ABCA4 [106]. Indeed, retinaldehyde is one of few compounds that stimulate ABCA4's ATP hydrolysis and is the only compound that behaves as expected of a substrate, with a simple Michaelis-Menten behavior indicative of a single-saturable binding site and an interaction with ABCA4 at a rate-limiting step in the ATP hydrolysis cycle [106]. Although ABCA4 does not distinguish different isomers of retinaldehyde [106], its predominant substrate is likely all-trans retinal, not 11-cis retinal, given the rapid binding of 11-cis retinal to apo-opsin. Phenotypes of ABCA4 knockout mice provided independent strong support for the role of ABCA4 in clearing all-trans retinal from disc membranes

[105]. ABCA4 knockout mice show delayed dark adaptation, consistent with delayed clearance of all-trans retinal, which likely increases the noise of photoreceptor cells by activating rhodopsin [109, 110]. Interestingly, phosphatidylethanolamine (PE) and the adduct formed between retinal and PE, N-retinylidene-PE are significantly elevated in ABCA4 knockout mice [105]. It was proposed that ABCA4 functions as an N-retinylidene-PE flippase. Solid phase binding assay demonstrated that N-retinylidene-PE, not free retinal, is the preferred substrate for ABCA4 [111, 112]. This is consistent with the propensity of retinaldehyde to form Schiff base with primary amines and the finding that retinaldehyde stimulation of ABCA4's ATP hydrolysis depends on the presence of PE and is absent in phosphatidylcholine vesicles [106, 107]. N-retinylidene-PE binds to ABCA4 at a 1:1 ratio and can be quantitatively released by addition of ATP [111].

There are several interesting puzzles concerning ABCA4's biochemical function. First, ABCA1, the closest homolog of ABCA4, functions in cholesterol efflux from cells [103, 104]. ABCA1 has also recently been shown to be involved in retinol efflux in intestinal cells during intestinal absorption of vitamin A [113]. Surprisingly, ABCA4 functions in the opposite transport direction as ABCA1 (influx not efflux).

Second, due to technical challenges and the fact that retinal diffuses through membranes by itself, ABCA4's transport activity has not been directly demonstrated using purified ABCA4 (despite the efforts of several groups). Retinal's ability to diffuse through membranes without ABCA4's help has been confirmed by three studies that detected little or no role of ABCA4 in all-trans retinal clearance or all-trans retinol formation after light bleaching [105, 114, 115]. Although the fraction of retinal transported to the cytoplasmic side of the disc membrane by ABCA4 may be very small, this fraction is still detrimental in the long term if not removed by ABCA4; this will be discussed later in ABCA4-associated human diseases.

Third, it was recently found using a novel lipid flippase assay that rhodopsin functions as a lipid flippase with high efficiency but little specificity for lipid head groups [85]. Given its high abundance in rod outer segment (90% of total protein) and its extreme high efficiency as a lipid flippase [85], it is hard to imagine why the much lower-abundance ABCA4 is needed. Since rhodopsin's lipid flippase activity does not depend on ATP, it would be interesting to add ATP to observe ABCA4's activity in the same outer segment preparation that demonstrated the rapid flippase activity of rhodopsin [85].

Fourth, ABCA4 is expressed in other tissues, which are not known to have high flux of retinaldehyde like the photoreceptor cells after light bleaching (Figure 3). For example, *in situ* hybridization and Western blot analysis showed that ABCA4 is expressed in the choroid plexus [116], which is part of the blood brain barrier. What is the function of ABCA4 in extraocular tissues? It was previously observed that both retinol and retinoic acid (but not retinoid analog  $\alpha$ -ionone) can stimulate the ATP hydrolysis of ABCA4, although they are not as potent as retinaldehyde [106]. In addition to a retinoid transporter/lipid flippase, another possibility is that in other tissues, ABCA4 functions similarly to ABCA1, its closest homolog that functions in cholesterol and retinol efflux from cells [103, 104, 113].

**2.2.2. Evolutionary roles**—The evolutionary driving force for the emergence of a protein may be best understood by comparing a system that depends on it and a similar system that does not depend on it. Why does vertebrate vision depend on ABCA4, while invertebrate vision does not? The ultimate reason may originate from a basic difference in the visual pigments. Invertebrate visual pigments do not release their chromophore after light absorption and can revert the chromophore back to the original state by subsequent light absorption. Therefore, invertebrate visual pigments are called bistable pigments [117]. Invertebrate opsins can also function as light-dependent isomerase to convert retinal from



the all-trans form to 11-cis form [118]. In contrast, vertebrate visual pigments release the retinal chromophore after light bleaching. The consequence of this release (or instability after light bleaching) is that there is a need to regenerate the released chromophore after every photoisomerization reaction. For vertebrate visual pigments, the released chromophore goes through a series of complex enzymatic reactions and transport events called the visual cycle before it is converted back to the 11-cis retinal chromophore [119–122]. Although it has generally been assumed that bistable pigments do not need a visual cycle to regenerate, it was recently discovered that *Drosophila* does have enzymes involved in the regeneration of visual chromophore [123]. Unlike vertebrate rhodopsins, *Drosophila* rhodopsin can be internalized and degraded in a light-dependent manner. Although a bistable pigment does not need chromophore regeneration after photoisomerization, *Drosophila* regenerates its chromophore released from degraded rhodopsin, not bleached rhodopsin [123]. This regeneration is important during nutritional deficiency.

Why does vertebrate vision use the kind of pigments that require much higher maintenance, with a complex regeneration cycle after every photoisomerization event? Vertebrates do have bistable opsins, such as parapinopsin in the lamprey pineal gland [124] and melanopsin in the vertebrate retina [125]. Like invertebrate opsins, melanopsin can even form a functional pigment with all-trans retinal due to its intrinsic isomerase activity [125, 126]. What are the advantages of the complex regeneration cycle for vertebrate visual pigments? The first likely advantage is that they can be regenerated in complete darkness, unlike bistable pigment, which depends on light to regenerate. A photoreceptor cell with a visual pigment that can be regenerated in complete darkness can function better and continuously in darkness at night. Invertebrates can also regenerate their pigment in the dark in the initial formation of the bistable pigment [127]. The second likely advantage is spectral discrimination. Although both vertebrates and invertebrates have color vision, a bistable pigment that has two absorption maxima (one for activation and one for regeneration) may be less precise than a pigment that has only one major absorption maximum for distinguishing different wavelength of lights, which is the basis of color vision.

Given its essential role in vision, the vertebrate visual cycle has been the subject of intense investigation. Most, but not all, components of the vertebrate visual cycle have been identified [119]. ABCA4 is part of the vertebrate visual cycle. However, ABCA4 was an unexpected and unknown link in the visual cycle because there was no previous biochemical or physiological evidence for the existence of an ATP-dependent transporter for vitamin A-derivative in vision. Specifically, there was no previous evidence that retinol dehydrogenase needs the assistance from a transporter to provide its all-trans retinal substrate. As mentioned above, even after the discovery of ABCA4, kinetic studies of all-trans retinal clearance or all-trans retinol formation after light bleaching using ABCA4 knockout mice have demonstrated that the vast majority of all-trans retinal released from rhodopsin does not need ABCA4's help to pass through disc membranes to reach retinol dehydrogenase [105, 114, 115]. Consistently, it has been extremely challenging to demonstrate transport or flippase activity using purified ABCA4. What is the evolutionary driving force for the existence of a transporter that is seemingly doing so little biochemically?

Compared to bistable pigments, vertebrate visual pigments have a serious downside. Because the chromophore is released after every photoisomerization event, rhodopsin still keeps on releasing retinal chromophore even in daylight when rod photoreceptors are saturated and not functional for visual perception. Despite the fact that retinal can diffuse through membranes by itself, the high flux of retinal in the vertebrae photoreceptor cells during daytime can constantly generate such a high amount of all-trans retinal that it needs ABCA4's help to completely pass through membrane so that it can be reduced to the less toxic retinol form. All-trans retinal can activate rhodopsin independently of light [109, 110].

Without ABCA4, the accumulated retinal increases the noise of photoreceptor cells and delay dark adaptation. ABCA4's ability to accelerate dark adaptation [105, 128] is obviously of immediate value to vertebrate survival. Dark adaptation makes it possible for vision to quickly adjust to much dimmer background light levels [129, 130]. For example, faster dark adaptation can make a life and death difference for a caveman entering a dark cave with a bear inside. Another likely driving force is ABCA4's contribution to reduced all-trans retinal toxicity due to the large amount of all-trans retinal generated by photoreceptor cells in daylight [131, 132].

**2.2.3. Human diseases**—Human diseases associated with ABCA4 are surprisingly diverse (Figure 1). ABCA4 was first discovered as responsible for Stargardt macular dystrophy, the most common form of early-onset macular degeneration [100]. ABCA4 has also been implicated in retinitis pigmentosa [133–135] and cone rod dystrophy [135–137]. Genetic studies have linked both ABCA4 [138] and its closest homolog ABCA1 [139, 140] to age-related macular degeneration, the leading cause of blindness in the elderly. Diseased-associated ABCA4 variants have been functionally analyzed [141, 142]. It is generally hypothesized that the degree of loss of ABCA4 function determines the disease association (Stargardt disease being the mildest, cone rod dystrophy a more severe form, and retinitis pigmentosa the most severe form).

ABCA4 is the only gene known to be responsible for autosomal recessive Stargardt disease, which is characterized by the loss of central vision, progressive atrophy of the RPE, appearance of orange-yellow flecks around the macula, and dark adaptation defects [128, 143]. Histopathologically, it is characterized by massive accumulation of lipofuscin-like material in the RPE. Stargardt disease is traumatic because of the suddenness in the loss of central vision, normally around the teenage years. What's responsible for the sudden loss of central vision? The key to this question might be the composition of the lipofuscin. Based on the Stargardt disease phenotypes and the localization of ABCA4 in the photoreceptor outer segment, it was hypothesized that the lipofuscin that accumulates in Stargardt disease patients might be N-retinylidene-N-retinylethanolamine (A2E) [105, 106, 144], which is the Schiff base reaction product of two retinaldehyde molecules and ethanolamine and was first identified as a major component of the fluorophore associated with aging human eyes [145, 146]. This hypothesis is tightly linked to the proposed function of ABCR in eliminating all-trans retinal released by bleached opsins, as described above. A2E formation is indeed dramatically higher in ABCA4 knockout mice than the wild-type [105]. A2E has also been detected in Stargardt disease donor eyes [147]. Visualization of A2E in Stargardt patients using fundus autofluorescence imaging provided a potential clue as to why Stargardt disease is manifested as macular degeneration [148, 149], even though ABCA4 is expressed in both rod and cone photoreceptor cells [144, 150]. A2E formation due to the loss of ABCA4 is another strong piece of evidence supporting its role in eliminating all-trans retinal, the precursor to A2E.

Malfunction of ABCA4 leads to the accumulation of all-trans retinal and N-retinylidene-PE in the photoreceptor outer segment [105]. Further condensation of all-trans retinal with N-retinylidene-PE forms the precursors of A2E [146, 147]. A detailed biogenic pathway for A2E formation has been proposed [146, 147]. Although A2E originates from the photoreceptor outer segments, A2E accumulates in RPE cells through a process of daily phagocytosis of the photoreceptor outer segments by the RPE cells [151, 152]. A2E is structurally so unique that no lysosomal enzymes can degrade it. As a result, it gradually accumulates in the RPE cells and this accumulation is irreversible because RPE cells cannot get rid of it [147]. This gradual accumulation of A2E, a toxin, explains why it takes many years for Stargardt patients to lose their sight and the suddenness of that loss. A2E mediates its toxicity as a photooxidizer [153] and a detergent to destabilize membrane [153–155].

A2E also impairs the phagocytic activity of the RPE cells [156] and inhibits retinoid isomerase activity in the RPE cells as an additional means of toxicity [157]. A2E can induce complement activation on RPE cells [158, 159]. Increased complement activation and complement dysregulation due to A2E accumulation in RPE cells have recently been demonstrated using ABCA4 knockout mice [160]. Given the origin of 2E from vitamin A, A2E may be regarded as a new form of vitamin A-related toxicity: a vitamin A derivative that the cell cannot get rid of. A2E (and its related compounds) is the first and only known vitamin A derivative that has only toxic effects and no beneficial functions. In addition to A2E, it was recently found that all-trans retinal accumulation alone can be sufficiently toxic in the retina independently of A2E formation [131, 132].

One hallmark of A2E-related phenotypes is that they can be highly influenced by environmental factors, such as light and retinoid levels. It was first discovered using ABCA4 knockout mice that A2E does not form without light [147]. A2E formation is also influenced by retinoid levels. Vitamin A supplementation worsens A2E related phenotypes [161], while vitamin A deficiency lessens the phenotypes [162]. Based on these findings, two general strategies have been developed to reduce A2E levels: reduction of light exposure and deceleration of the visual cycle. Both strategies have worked effectively in animal models [147, 162, 163]. Other small-molecule based therapies [164] and gene therapy [165, 166] have also been explored to treat ABCA4-associated diseases. Since one of the ultimate reasons that A2E accumulates in RPE is that it cannot be degraded, another treatment strategy is to develop an enzyme that can degrade A2E. It has been demonstrated that A2E can indeed be degraded by an exogenous enzyme (horseradish peroxidase) [167]. Another potential strategy is to grow bacteria on synthesized A2E [146, 168] as the sole carbon source to select a “superbug” that evolves a means to degrade A2E.

### 2.3. STRA6: cellular uptake of vitamin A from the blood

**2.3.1. Biological function**—STRA6 was originally identified in cancer cells as a retinoic acid-stimulated gene of unknown function [169, 170]. Through an unbiased strategy of biochemical purification and mass spectrometry, it was identified as the membrane receptor for plasma retinol binding protein (RBP) [171]. RBP is the principle vitamin A carrier protein in the blood [172–178]. STRA6 binds to RBP with high affinity and mediates cellular uptake of vitamin A from the vitamin A/RBP complex (holo-RBP) [171]. Consistent with its role in vitamin A uptake, increasing STRA6 expression in cancer cells by retinoic acid stimulation enhances cellular vitamin A uptake from holo-RBP. Conversely, suppressing STRA6 expression in cancer cells or RPE cells suppresses cellular vitamin A uptake [171], and knocking down STRA6 expression leads to decreased retinoid uptake in the eye [179]. Generation and functional analysis of more than 900 STRA6 mutants have led to the identification of an RBP binding domain in STRA6 [180]. Mutations in any of the three essential residues in this domain are sufficient to abolish the RBP binding and vitamin A uptake activity of STRA6 [180].

STRA6 is expressed in cells or tissues known to depend on vitamin A for proper function. For example, STRA6 is highly expressed in the RPE cells and is specifically localized to the basolateral membrane of the RPE cells [171], the exact cellular location expected of a protein involved in taking up vitamin A from the choroidal blood [181] (Figure 2). Its expression in the immune system is consistent with the role of vitamin A in immune regulation and in preventing infectious diseases [9, 182, 183]. STRA6 is highly expressed in the placenta, which is essential for fetal absorption of vitamin A for embryonic development [178]. STRA6's expression in the brain is consistent with known neuronal functions of vitamin A [10, 11, 184] including recently discovered roles in controlling neuron generation [185] and synaptic protein translation [26, 27]. In general, STRA6 localizes primarily but



not exclusively to blood/organ barriers such as blood/retina barrier, blood/brain barrier and maternal/fetal barrier [169, 171]. Both human and animal studies have shown that loss of STRA6 function can lead to severe defects in multiple organs [179, 186, 187]. Human diseases associated with STRA6 mutations will be discussed in detail below.

A number of studies in the past have shown that vitamin A uptake mediated by the RBP receptor does not depend on endocytosis of RBP [181, 188–195]. Consistently, STRA6 has nine transmembrane domains [196], unlike endocytosis receptors which often are single-transmembrane domain proteins. STRA6-mediated vitamin A uptake does not depend on endocytosis of RBP [171]. STRA6's mechanism is highly specific. It mediates retinol uptake only if retinol is bound to RBP, and does not mediate retinol uptake if retinol is bound to BSA or to  $\alpha$ -lactoglobulin, a milk retinol binding protein [171].

STRA6 is not homologous to any membrane receptors, transporters or channels of known function, and its sequence provides no clue as to its substrate uptake mechanism. Because STRA6-mediated vitamin A uptake does not depend on cellular energy [171], its substrate uptake mechanism is also not primary or secondary active transport. Because STRA6's substrate, retinol, is not free, has no charge, and can only be provided one molecule at a time by RBP, STRA6's mechanism is also distinct from channels or facilitated transporters, which are driven by an electrochemical gradient of the free substrate. STRA6's mechanism is further complicated by the high affinity interaction between retinol and RBP, its dependence on both RBP binding to deliver retinol and RBP dissociation for the next RBP to bind, and the involvement of intracellular proteins [171]. Therefore, STRA6's mechanism cannot be explained by a known cellular uptake or membrane transport mechanism.

As a new type of multitransmembrane domain protein that has just been studied functionally for a few years, it is not surprising that there are a lot of questions regarding STRA6. The following lists some questions about STRA6's vitamin A uptake mechanism:

**2.3.1.1:** We [171] and other investigators [179, 197] have found that LRAT stimulates STRA6's vitamin A uptake activity. What is the nature STRA6's vitamin A uptake activity without LRAT? If STRA6 can take up vitamin A without LRAT, why does LRAT stimulate its activity? If STRA6 cannot take up vitamin A (by itself) without LRAT, is STRA6 only a receptor for RBP and does it play any role in vitamin A uptake other than being a receptor for RBP? STRA6 is not an enzyme that can convert retinol to retinyl esters. What is the role of STRA6 in STRA6/LRAT-mediated retinyl ester accumulation? LRAT is localized intracellularly in the ER [198] and cannot physically interact with extracellular RBP.

**2.3.1.2:** Is LRAT the only protein that can stimulate STRA6's vitamin A uptake activity? If LRAT stimulates retinol uptake because of its ability to store vitamin A, do the cell membranes, which have larger capacity to store retinol, also stimulate retinol uptake? It has been proposed in many previously published hypothetical models of RBP receptor mechanism that cellular retinol binding protein (CRBP) may play an important role in RBP receptor-mediate retinol uptake? This hypothesis is reasonable given the known ability of CRBP-I to supply retinol for LRAT [199, 200]. There are several CRBPs. Can all CRBPs stimulate STRA6's retinol uptake activity? What's the difference between CRBP and LRAT in stimulating vitamin A uptake by STRA6? Why can LRAT stimulate STRA6's vitamin A uptake activity independently of CRBP?

**2.3.1.3:** Although LRAT can stimulate STRA6's retinol uptake activity, it was discovered previously that STRA6 and LRAT cannot take up retinylamine from the retinylamine/RBP complex [197] or retinoic acid from the retinoic acid/RBP complex [179]. These experiments demonstrated that LRAT is not a general stimulator of STRA6's activity. It is

known from previous studies [179, 197, 201, 202] that RBP can bind other retinoids in addition to retinol, although retinol is its natural ligand [172, 173]. Is it still possible for STRA6 to take up other retinoids bound to RBP? Answer to this question can also provide clues to STRA6's mechanism.

**2.3.1.4:** Without knowing STRA6's mechanism, LRAT's stimulatory effect on STRA6 as observed by us [171] and other investigators [179, 197] suggests that LRAT may “drive” vitamin A uptake into the cell. However, it has been demonstrated that the reverse reaction of vitamin A loading into extracellular apo-RBP still happen in the presence of LRAT [179]. If LRAT drives cellular vitamin A uptake, how does it permit cellular vitamin A loss? One hypothesis to explain retinol loss instead of uptake in the presence of LRAT is that retinyl ester hydrolase [203] present in certain cell types can prevent LRAT from enhancing STRA6's retinol uptake activity to make it possible for partial retinol loss to occur.

**2.3.1.5:** There are a few interesting questions regarding retinol loading into apo-RBP, since this is a possible mechanism for cells to lose retinoid store. If STRA6 encounters both holo-RBP and apo-RBP, does it mediate cellular vitamin A uptake or cellular vitamin A loss? Does the reverse reaction of vitamin A loading into apo-RBP happen in human blood, which contains mostly holo-RBP and a small fraction of apo-RBP [204] because of the active removal of apo-RBP through kidney filtration? If STRA6 mediates cellular vitamin A loss when it is exposed to the blood, how does it function as the RBP receptor to mediate cellular vitamin A uptake?

**2.3.1.6:** Scavenger receptor class B, type I (SR-BI) is the receptor for HDL that mediates cellular cholesterol uptake [205]. Mechanistically, STRA6 is perhaps most similar to SR-BI because both membrane proteins take up molecules bound to extracellular carrier proteins (RBP or HDL) without endocytosis. Is STRA6's mechanism the same as SR-BI's mechanism? STAR6 is completely unrelated to SR-BI at the sequence level. STRA6 has 9 transmembrane domains [196], while SR-BI has two [206, 207].

Current research aims to address above questions on STRA6's vitamin A uptake mechanism (unpublished results).

**2.3.2. Evolutionary roles—**The RBP/STRA6 system of vitamin A delivery is by no means the only possible mechanism for cells or tissues to obtain vitamin A. In some sense, it is surprising that STRA6 (or RBP) exists because there are theoretically much simpler mechanisms to deliver vitamin A or retinoid. First, retinoids clearly have the ability to diffuse systemically by themselves. For example, retinoid-based drugs can be targeted to any organ by random diffusion. Retinoid can “hitchhike” to abundant serum proteins like serum albumin, which binds many small molecules promiscuously, to be transported in the blood. In contrast, each RBP can only bind and deliver one vitamin A molecule and seems to exist primarily for this purpose. Surprisingly, virtually all vitamin A in the blood is bound to RBP under physiological conditions, not to the much more abundant serum albumin. Second, for RBP, why does it need a specific receptor like STRA6 instead of leaking retinol randomly into tissues? Due to its hydrophobicity, retinoid has the ability to pass through membranes without any assistance from a protein.

Evolution came up with the specific vitamin A delivery system (from liver store to RBP to STRA6 on target cells or tissues) for targeted delivery of vitamin A to achieve high specificity and efficiency and to prepare for deficiency. An analogy for the liver/RBP/STRA6 system of vitamin A delivery is water delivery to a house. In this analogy, the liver is the water reservoir and treatment plant. RBP is essential in mobilizing the liver-stored vitamin A [208] and serves as a buffer to maintain stable vitamin A concentration in the

blood [175]. The buffering function is important to avoid the adverse effects of both low and high retinoid on the growth and function of diverse organs. Without this buffering function, blood retinoid level would fluctuate dramatically depending on dietary intake. STRA6 binds to RBP with high specificity and affinity [171] and is analogous to the water taps in each house that control water intake. Without water taps that serve to control the outflow, water flowing out randomly would be both wasteful and cause damage. If a nonspecific mechanism were always available (rain or flooding), this specific delivery system would not be essential for survival. However, most, if not all, animals living in natural environments lack continuous access to vitamin A (some don't eat anything for months at a time). In addition to surviving deficiency, the specific mechanism also is much less wasteful and minimizes the damage and side-effects caused by nonspecific mechanism (rain or flooding).

Cellular signaling through retinoids occurs in a precise spatiotemporal manner, and random distribution of retinoids can have toxic effects. The short and long-term toxic side effects caused by random retinoid diffusion are illustrated by toxicity of retinoid drugs such as Accutane that diffuse randomly and are delivered independently of the RBP/STRA6 system [209–211]. Experiments done more than 30 years ago in an animal model [212] and a study of human patients with hypervitaminosis A [213] have shown that more toxicity is associated with vitamin A delivery independent of RBP. An excessive dose of vitamin A is toxic only when the level of vitamin A in the circulation is so high that it is presented to cells in a form other than bound to RBP, such as in retinyl esters [172]. An increase of 10% in retinyl ester is regarded as a sign of vitamin A overload. Toxicity associated with excessive retinoid has also been recently demonstrated using knockout mouse models [131, 132]. Toxicity of excessive endogenous free retinal has also been demonstrated in the *Drosophila* visual system [214].

STRA6 is a relatively recent “invention” by evolution. Like ABCA4, STRA6 only exists in vertebrates, not in lower organisms. The likely reason to explain the “invention” of STRA6 for vertebrates is that vertebrates use vitamin A for other biological activities such as regulating gene transcription in addition to light sensing. Invertebrates do not need RBP or STRA6 perhaps because precise delivery of vitamin A is unnecessarily complex and costly (in the sense that one RBP only delivers one vitamin A molecule). Random diffusion of retinoid would have much less toxicity if retinoids were not capable of regulating the transcription of hundreds of genes (as in the case of vertebrates). Although invertebrates don't need RBP or STRA6 for vitamin A delivery, they have developed specific mechanism for dietary carotenoids transport. Interestingly the uptake mechanism of  $\beta$ -carotene is shared between the invertebrate and the vertebrate and is mediated by the SR-BI family of receptors [113, 215–218]. One possible explanation is that  $\beta$ -carotene is not as membrane permeable as retinol and requires assistance to efficiently pass through cell membranes.

**2.3.3. Human diseases**—Consistent with the diverse and critical functions of vitamin A, STRA6 mutations cause a wide spectrum of pathological phenotypes including the absence of eyes (anophthalmia), mental retardation, congenital heart defects, lung hyperplasia, intrauterine growth retardation and embryonic lethality [186, 187, 219–224]. STRA6 is the first example of a retinoid signaling pathway gene whose mutations cause developmental abnormalities in humans [13]. The severe phenotypes caused by STRA6 mutations suggested that there might be less redundancy in vitamin A transport mechanisms than other aspects of retinoid signaling in humans. Redundancy is necessary to ensure that backup mechanisms take over when one mechanism fails. However, the great variability in phenotypes associated STRA6 mutations, as discussed below, suggests that there is redundancy in vitamin A transport as well. Human mutations identified in STRA6 belong to two categories: large deletions and point mutations. All human disease-associated point mutations that have been analyzed abolish STRA6 cell-surface expression, RBP binding and

vitamin A uptake activity [180]. Consistently, retinoid analysis showed that knockdown of STRA6 in zebrafish led to decreased tissue retinoid uptake [179].

One hallmark of STRA6 mutations in humans is the tremendous phenotypic variability of pathological phenotypes ranging from embryonic lethality to “mild” eye-specific phenotype, although the eye phenotype is shared by all patients surviving to birth [186, 187, 219–224] (Figure 1). In fact, this is perhaps one of the most extreme examples of phenotypic variability ever known to be caused by mutations of one single human gene. The variability of STRA6 mutation phenotypes in humans is likely caused by variable degrees in the loss of STRA6 function (genetic factor) and the variability in vitamin A intake of the affected individuals (environmental factor). It's well established that vitamin A intake alone (either insufficient or excessive) is sufficient to cause severe developmental defects without any genetic contribution. RBP/STRA6 independent mechanisms of retinoid delivery (e.g., serum albumin bound retinoid independent of liver storage if retinoid intake is sufficiently large) may take over in the absence of RBP or STRA6. But as shown by the extreme severe phenotypes such as embryonic lethality, such a random mechanism often does not work. Without the RBP/STRA6-mediated specific delivery system, an individual is at the “mercy” of the environmental factor by completely depending on constant but not excessive dietary vitamin A intake. As discussed in the above section, problems associated with random mechanisms are what make it necessary to evolve RBP/STRA6 in the first place.

Although currently there is no evidence for its existence, another interesting possibility to account for phenotypic variability are genetic modifiers. For examples, genetic modifiers have been identified to explain the phenotypic variability of cystic fibrosis [225, 226], another human disease that affects the lung (STRA6 mutation can lead to lung hypoplasia). If variability in dietary intake of vitamin A is wholly responsible for the phenotypic variability, constant supplement of the appropriate amount of vitamin A could potentially lessen the severity of the disease. As illustrated by ABCA4, a disease heavily influenced by an environmental factor can be targeted for treatment by changing the environmental factor, which is usually easier to modify than genetic factors.

It is well known that different tissues are very different in their vulnerability to vitamin A deficiency and excess. It is notable that the eye is the organ most sensitive to STRA6 mutations. This is consistent with the fact that the eye is the organ that most depends on vitamin A for both adult physiological function [119, 120, 227] and for embryonic development [228] and therefore is most sensitive to vitamin A deficiency.

### 3. General lessons learned from the visual cycle

Because of the dependence of human beings on vision, our high sensitivity to vision defects, and vision's absolute dependence on vitamin A, vision research has offered a wealth of information of the mechanisms of vitamin A related proteins, despite the fact that both the photoreceptor cells and the RPE cell are extremely rare cell types in the body as a whole. All three kinds of membrane proteins described here are involved in the visual cycle, which is perhaps the best understood retinoid transport and recycling system. Mechanistic studies of the visual cycle have generated some likely general principles on retinoid transport, storage and usage:

#### 3.1. Storage vs. usage

A cell that needs vitamin A for physiological function is likely not the cell that takes up vitamin A from the blood. This is clearly the case for vision (Figure 2). The RPE cell is known to express STRA6, which takes up vitamin A from the blood, and to store a large quantity of vitamin A as retinyl esters, but ultimately the cell type that needs vitamin A is

photoreceptor cell. This principle may also apply to the brain. Blood-brain barriers like the choroid plexus, meninges, and a subset of astrocytes express STRA6 at high levels. It was recently demonstrated that the meninges are the source of retinoic acid for corticogenesis [185]. Despite the many known physiological functions of retinoic acid on neuron [10, 11], STRA6 is not known to be expressed in brain neurons, which, like photoreceptor cells in the retina, are not directly exposed to the blood. There might exist a local delivery system equivalent to the visual cycle to deliver retinoid from cells at the blood brain barrier to neurons.

### 3.2. Protein bound form vs. free form

Despite the fact that retinoids other than retinyl esters predominantly exist as protein bound forms (e.g., RBP or CRBP), free retinoid does exist under physiological conditions, at least transiently. For example, free retinol is generated by reduction of retinal following light bleaching of rhodopsin at the beginning of the visual cycle. The retinol intermediate of the visual cycle, which eventually moves out of the photoreceptor cell into the RPE cell, was first discovered about 40 years ago [229]. This transient form of free retinol can be visualized in the photoreceptor cell following light bleaching using sensitive fluorescence microscopy [230, 231]. Although the retinal chromophore is supposed to be covalently bound to opsins, biochemical and electrophysiological studies have demonstrated that the free retinal chromophore gets exchanged between opsin molecules [232, 233]. How protein-mediated transport and free diffusion is balanced in each organ or cell context need to be further studied. For example, is the morphogenic gradient of retinoic acid during embryonic development in its free form, the protein bound form (CRABP), or both?

### 3.3. Environmental factors and human diseases

Retinoid related diseases can be substantially influenced by environmental factors. Both ABCA4 and STRA6-related human diseases can be influenced by environmental factors. In the case of ABCA4, environmental factors such as light can have a huge impact. Without light, the vitamin A-related toxin A2E does not even form in animal model of Stargardt disease, despite the knockout of the gene [147]. Loss of ABCA4 phenotype is also influenced by retinoid levels [162]. This lesson makes it necessary to control environmental factors that influence overall or tissue-specific retinoid levels, especially diet [234], in designing experiments and to understand human disease phenotypes. Phenotypes associated with loss of STRA6 are also highly variable in humans, ranging from embryonic lethality to the “mild” phenotype of anophthalmia [186, 187, 219–223]. As discussed above, environmental factors such as dietary intake of vitamin A likely contribute to the high phenotypic variability.

### 3.4. Seemingly small contributions can be critical

Despite the overwhelming amount of independent experimental evidence for the biochemical function of ABCA4 in transporting alltrans retinal for its reduction to all-trans retinol, little or no change in either the clearance of alltrans retinal or the production of all-trans retinol was observed in ABCA4 knockout mice [105, 114, 115]. These in vivo experiments demonstrated that ABCA4 contributes little to retinal transport compared to what retinal can do by itself. These surprising findings illustrate an important lesson that a protein might only affect retinoid transport slightly, but this activity can be sufficiently important to justify its existence. Human retinal diseases associated with ABCA4 mutations demonstrate that loss of such a seemingly “slight” contribution has serious consequences (e.g., delayed dark adaptation and toxic A2E accumulation).



### 3.5. Passive diffusion vs. active or facilitated transport

Despite its ability to diffuse through membrane, retinoid transporters do exist to facilitate its transport. In addition to ABCA4's function described above, ABCA4's closest homolog, ABCA1, is involved in retinol efflux in small intestine cells [235]. SR-BI, which was first identified as a HDL receptor mediating cholesterol uptake [205], has been shown to mediate beta-carotene uptake in the small intestine [113, 218]. As mentioned above, SR-BI homologs in *Drosophila* also mediate carotenoid uptake [215–217].

### 3.6. Need vs. cost

For a biological need (e.g., higher sensitivity or specificity), evolution may come up with a seemingly costly and complex mechanism instead of using a theoretically simpler mechanism. One example in retinoid biology is the vertebrate visual pigment, which is unstable after light absorption and needs to be regenerated after every photoisomerization event. It is amazing to realize that as we see the world, every photon we detect depends on the regeneration of the bleached chromophore through the complex visual cycle consisting of several retinoid-related enzymes, transport proteins and binding proteins. In bright light when rod vision is saturated and not useful, the continuous regeneration of rhodopsin through this complex cycle may even be considered “wasteful”. As discussed above, the choice of this unstable pigment may be advantageous because the pigment can be continuously regenerated in complete darkness and may have better resolution in spectral sensitivity. Another example in retinoid biology is the delivery of vitamin A. Although retinoid can diffuse systemically, evolution came up with a specific vitamin A delivery protein in the blood (RBP) and its receptor (STRA6) for vitamin A uptake. Instead of employing the highly abundant serum albumin, which is capable of binding to vitamin A, virtually all vitamin A in the blood is bound to RBP. Although this choice is seemingly costly because each RBP can only bind one vitamin A molecule, it has the advantages discussed above.

### 3.7. There exist vitamin A derivatives that have only toxicity and no beneficial function

A2E and related compounds are the only known examples. Most, if not all, other known vitamin A derivatives (e.g., alcohol, aldehyde or ester forms) have beneficial biological functions. The biological functions of less common vitamin A derivatives such as retinoylation of proteins [236] are still not well understood.

## 4. Unsolved questions

Despite what we have learned, there are still numerous interesting questions that remain unanswered. Addressing these questions will lead to better understanding of retinoid biology and retinoid-related human diseases and potentially lead to the development of new therapies for human diseases. In addition to questions raised in previous sections, other examples are listed below:

- 4.1. How does the liver sense RBP concentration in the blood to maintain its stable concentration under physiological conditions to prevent excessive secretion or insufficient secretion? This question also applies to the adipose tissue, another major site of vitamin A storage. Excessive secretion of RBP has been linked to insulin resistance [237]. Is there a membrane receptor that senses blood RBP concentration to control its secretion?
- 4.2. Under physiological conditions, the blood maintains micromolar concentrations of holo-RBP. STRA6 can efficiently take up vitamin A from nanomolar holo-RBP concentrations. If STRA6 takes up vitamin A from the blood continuously, a STRA6-expressing cell would become bloated with vitamin A in days if not

months. What are the mechanisms that control cellular vitamin A uptake by STRA6 to prevent excessive uptake?

- 4.3. STRA6 was first identified as a retinoic acid-stimulated gene in cancer cells [169, 170, 238] Certain cancer cells have more than 100 fold higher STRA6 expression levels than normal cells [170]. What is the role of STRA6 in cancer cells? Retinoid can both enhance and suppress cell growth. Is STRA6's role positive or negative for cancer cells? This knowledge may be useful in targeting cancer cells.
- 4.4. Are there other receptors and transporters for retinoid? There is strong evidence for the existence of a receptor for interphotoreceptor retinoid binding protein on RPE cells that mediates the export of 11-cis retinal produced by the RPE [239, 240]. The identity of this receptor is still unknown.
- 4.5. There exists a homolog of STRA6, the RBP receptor What is the function of the STRA6 homolog [241]? Is it also involved RBP binding or retinoid uptake?
- 4.6. Humans also have an orphan GPCR that is rapidly induced by retinoic acid [242]. Is it involved in retinoid biology?

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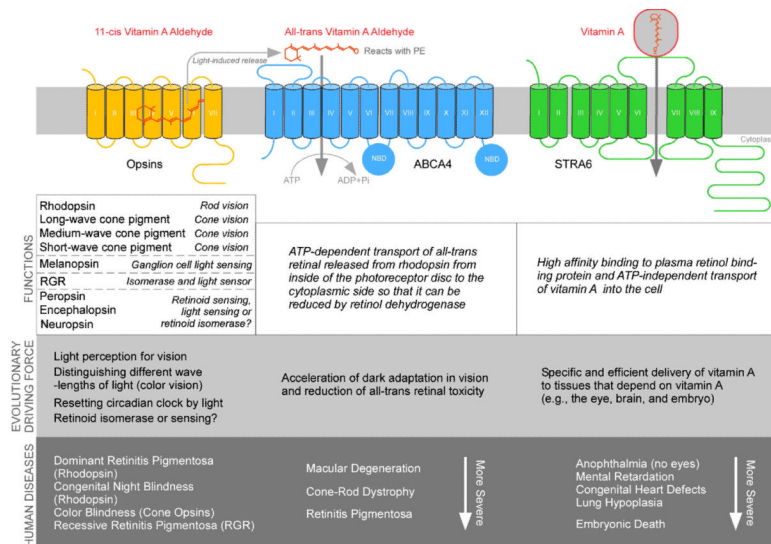


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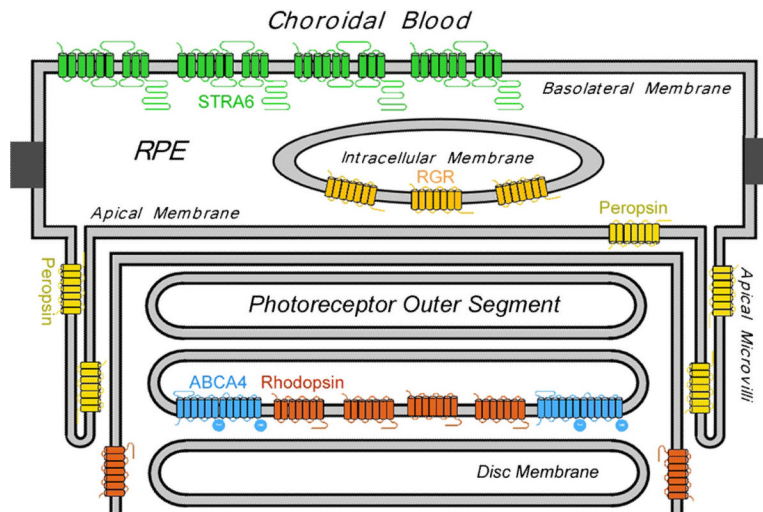
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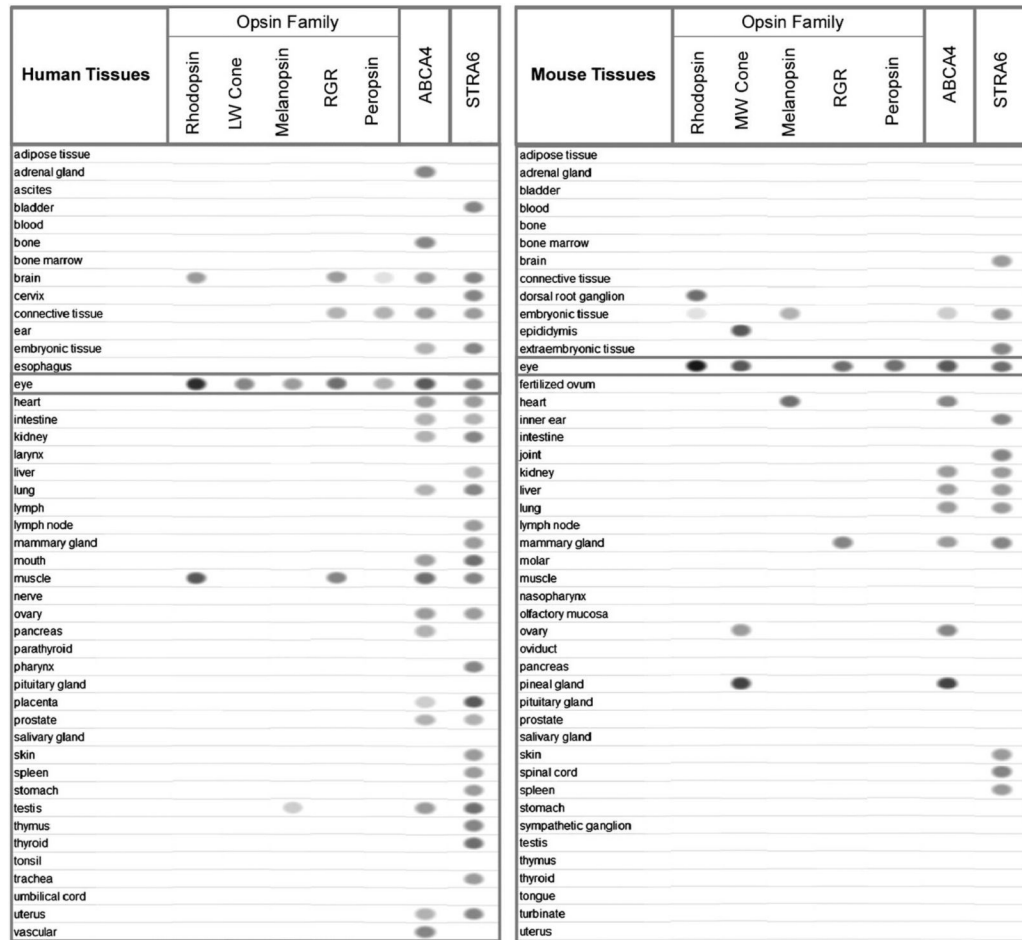
**Figure 1.** Human membrane receptors and transporters discussed in this review that mediate the function and transport of vitamin A and its derivatives. The transmembrane topologies of these proteins are depicted in the upper picture. Rhodopsin is depicted in the picture as an example for opsins. Physiological functions, likely evolutionary driving forces, and human diseases associated with these proteins are presented in the lower picture.





**Figure 2.**

Retinoid-related membrane receptors and transporters in the RPE and photoreceptor outer segment are localized to highly specific cellular structures. Rhodopsin is localized to both the disc membrane and the plasma membrane of the photoreceptor outer segment. ABCA4 is localized only to the disc membrane of the outer segment. It is unclear why rhodopsin is expressed on the plasma membrane where there is no ABCA4. Peropsin is localized to the apical microvilli of the RPE cell surface. RGR is localized to the internal membrane of the RPE cells. STRA6 is localized to the basolateral membrane of the RPE cells.



**Figure 3.**

Distribution of retinoid-related membrane receptors and transporters in human and mouse tissues. The intensity of the ovals indicates the abundance of each message as suggested by EST (Expressed Sequence Tag) counts recorded by NCBI's EST Profile Viewer. The absence of the oval for a particular tissue means no detectable expression. Abundant expression from rare cell types, such as the RPE cells, may be underrepresented in this analysis.