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ATP-binding cassette transporter ABCA4: Molecular properties and role in vision and macular degeneration

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Abstract ABCA4, also known as ABCR or the rim protein, is a member of the ABCA subfamily of ATP binding cassette (ABC) transporters expressed in vertebrate rod and cone photoreceptor cells and localized to outer segment disk membranes. ABCA4 is organized in two tandem halves, each consisting of a transmembrane segment followed successively by a large exocytoplasmic domain, a multispanning membrane domain, and a nucleotide-binding domain. Over 400 mutations in ABCA4 have been linked to Stargardt macular degeneration and related retinal degenerative diseases that cause severe vision loss in affected individuals. Direct binding studies and ATPase activation measurements have identified *N*-retinylidene-phosphatidylethanolamine, a product generated from the photobleaching of rhodopsin, as the substrate for ABCA4. Mice deficient in ABCA4 accumulate phosphatidylethanolamine, all-*trans* retinal, and *N*-retinylidene-phosphatidylethanolamine in photoreceptors and the diretinal pyridinium compound A2E in retinal pigment epithelial cells. On the basis of these studies, ABCA4 is proposed to actively transport or flip *N*-retinylidene-phosphatidylethanolamine from the lumen to the cytoplasmic side of disc membranes following the photobleaching of rhodopsin. This transport activity insures that retinoids do not accumulate in disc membranes. Disease-linked mutations in ABCA4 that result in diminished transport activity lead to an accumulation of all-*trans* retinal and *N*-retinylidene-PE in disc membranes which react to produce A2E precursors. A2E progressively accumulates as lipofuscin deposits in retinal pigment epithelial cells as a result of phagocytosis of outer segment discs. A2E and photo-oxidation products cause RPE cell death and consequently photoreceptor degeneration resulting in a loss in vision in individuals with Stargardt macular degeneration and other retinal degenerative diseases associated with mutations in ABCA4.

Keywords Transporter · Photoreceptors · Stargardt macular degeneration · Disease mechanisms · ABC transporters

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Introduction

ATP binding cassette (ABC) transporters comprise a superfamily of proteins found in virtually all living organisms (Higgins 1992). They typically function in the movement of a wide variety of compounds across cell membranes including amino acids, peptides, ions, metabolites, vitamins, fatty acid derivatives, steroids, organic anions, phospholipids, drugs and other compounds. ABC transporters consist of two membrane domains that provide a pathway for the translocation of a substrate across the membrane and two ATP-binding cassettes or nucleotide binding domains that bind and hydrolyzed ATP, thereby supplying the energy for substrate transport.

Eukaryotic ABC transporters typically exist as either full or half transporters. Full transporters consist of a single polypeptide chain harboring two membrane domains and two nucleotide binding domains. Half transporters are homodimers or heterodimers in which each polypeptide

chain contains a membrane domain and nucleotide binding domain (Higgins 1992; Borst and Elferink 2002). The detailed mechanism by which ABC transporters translocate substrates across membranes is not known, although it is generally thought that the energy derived from the binding and hydrolysis of ATP is coupled to the transport of a substrate across cell membranes through a series of allosteric protein conformational changes that alter the interactions between the two nucleotide binding domains and between nucleotide binding domains and the membrane domains (Higgins and Linton 2004).

The human genome is known to contain at least 48 genes that encode ABC transporters (Dean and Annilo 2005; Dean and Allikmets 2001). These transporters have been organized into seven subfamilies (ABCA-ABCG) based on the amino acid sequence and organization of their nucleotide binding domains. Two subfamilies ABCE and ABCF, however, contain nucleotide binding domains but not membrane domains (Oswald et al. 2006). Members of these two subfamilies do not function as transporters, but instead are involved in the regulation of protein biosynthesis.

The ABCA subfamily, consisting of 12 members, has been the focus of many recent studies since several of its members have been implicated in severe inherited diseases linked to defects in lipid transport (Borst et al. 2000; Kaminski et al. 2006). ABCA transporters are full transporters with a similar domain organization and an overall sequence identity ranging from 30 to over 70%. Mutations in ABCA1 are known to cause Tangier disease, an autosomal recessive disorder resulting in low levels of circulating high density lipoproteins and the accumulation of cholesterol and cholesterol esters in peripheral tissues and cells including macrophage foam cells (Brooks-Wilson et al. 1999; Oram 2002). Mutations in ABCA12 are responsible for harlequin and lamellar ichthyosis, diseases of the skin generally resulting from defective lipid transport (Lefevre et al. 2003; Akiyama et al. 2005), and mutations in ABCA3 have been linked to respiratory distress syndrome arising from defective lipid transport associated with lung surfactant metabolism (Ban et al. 2007; Shulenin et al. 2004).

ABCA4 is another key member of the ABCA subfamily that has been associated with inherited retinal degenerative diseases causing severe vision loss (Allikmets 2000). ABCA4, also known as the rim protein or ABCR, was first cloned independently in 1997 as an abundant high molecular weight glycoprotein expressed in retinal photoreceptor cells (Illing et al. 1997; Azarian and Travis 1997) and as a retinal specific protein encoded by the gene linked to Stargardt macular degeneration (Allikmets et al. 1997a). Since this time, ABCA4 has been extensively studied at a genetic, biochemical and cell biology level. Molecular genetics studies have revealed that mutations in ABCA4 are responsible for a spectrum of retinal degenerative diseases including Stargardt macular degeneration, autosomal cone-rod dystrophy, retinitis pigmentosa and age-related macular degeneration (Allikmets 2000; Rozet et al. 1998, 1999; Rivera et al. 2000). Biochemical studies on the purified protein and characterization of *abca4* knockout mice have provided novel insight into the structural and functional properties of ABCA4 and its role in photoreceptor cell biology and the pathogenesis of Stargardt disease as briefly reviewed here.

Tissue and cellular localization

Unlike most ABCA transporters, ABCA4 has a restricted tissue distribution. *ABCA4* gene expression is observed in the retina, but not in other tissues by Northern blot analysis (Allikmets et al. 1997a). The retina is a highly organized, striated layer of cells responsible for the initial steps in vision. It consists of 5 major neuronal cell types: rod and cone photoreceptors, bipolar cells, horizontal cells, amacrine cells and ganglion cells. In situ hybridization studies have localized *ABCA4* expression specifically to the photoreceptor cell layer of the retina (Allikmets et al. 1997a). Although ABCA4 is primarily expressed in retinal photoreceptors, a recent report documents *ABCA4* mRNA and protein expression in the choroid plexus of rat brain suggesting that this transporter may also function outside the retina (Bhongsatiern et al. 2005).

Rod and cone photoreceptors are highly specialized neurons which mediate the initial steps in vision. These differentiated cells consist of an outer segment which is joined to the inner segment by a thin connecting cilium (Fig. 1a). The inner segment houses the mitochondria, endoplasmic reticulum, Golgi, and other subcellular organelles. The cell body harboring the nucleus lies below the inner segment and is joined to the synaptic region responsible for the transmission of electrical signals to secondary neurons of the retina.

The photoreceptor outer segment functions in the detection of light and its conversion into an electrical signal in a process known as phototransduction. In rod cells, it consists of a plasma membrane that encloses stack of over 1,000 flattened, closed discs packed with the photoreceptor protein rhodopsin (Fig. 1a). Cone outer segments have a similar organization although the disc and plasma membrane form a continuous folded membrane system. Immunofluorescence labeling studies have localized ABCA4 to the outer segments of rod and cone photoreceptors (Molday et al. 2000; Sun and Nathans 1997). ABCA4 has been further localized to the rims and incisures of discs by immunoelectron microscopy (Fig. 1b)

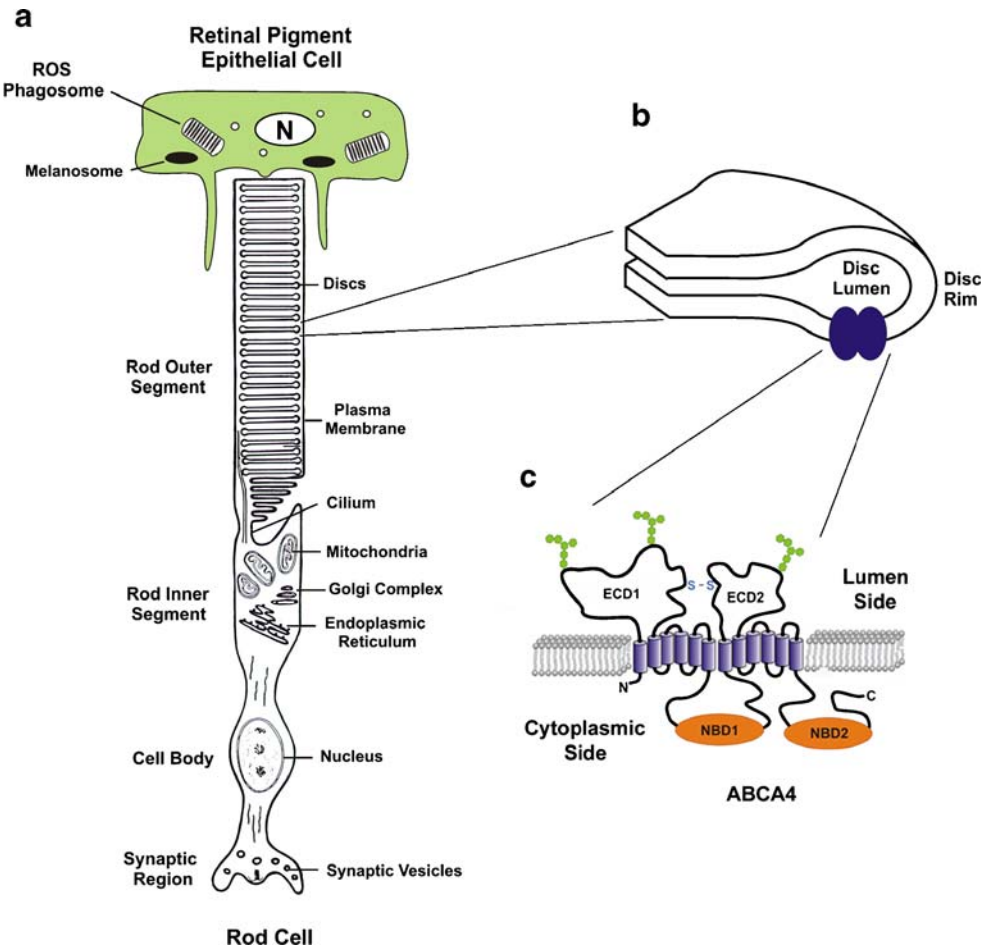

Fig. 1 Diagrams representing the rod photoreceptor and retinal pigment epithelial (RPE) cells, disc rim region, and the structural features of ABCA4. **a** The rod photoreceptor consists of an outer segment, a thin connecting cilium, an inner segment, cell body and synaptic region. The outer segment is composed of a stack of discs surrounded by a separate plasma membrane. The RPE cell adjacent to the photoreceptor cells provides nutrients to the photoreceptor cells as well as functions in the visual cycle and phagocytosis of outer segments as part of the disc renewal process. **b** Enlarged diagram of the rim region of a disc where ABCA4 resides. **c** A topological model for ABCA4 showing the exocytoplasmic domains (ECD), multi-spanning membrane domains, and the nucleotide binding domains (NBD) in both the N and C half of full transporter. N-linked oligosaccharide chains are shown with hexagons in ECD1 and ECD2

(Illing et al. 1997; Papermaster et al. 1978, 1982). The finding that ABCA4 is localized to disc membranes together with genetic studies showing that mutations in ABCA4 are responsible for Stargardt macular degeneration implicate ABCA4 in an important photoreceptor function required for long term cell survival.

Structural features

Human ABCA4 is a large single polypeptide of 2,273 amino acids organized as two structurally related tandem-arranged halves (Fig. 1c) (Illing et al. 1997; Allikmets et al. 1997a; Nasonkin et al. 1998). Topological models generated from computer algorithms indicate that each half contains a single hydrophobic transmembrane segment (H1) followed in succession by a large exocytoplasmic (extracellular or lumen) domain (ECD), a multiple spanning domain (MSD) and a nucleotide binding domain (NBD) (Illing et al. 1997; Bungert et al. 2001). The 24 amino acid N-terminal segment contains numerous positively charged lysine and arginine residues and therefore is predicted to reside on the cytoplasmic side of the membrane. Both ECD1 having 602 amino acids and ECD2 with 289 amino acids contain multiple N-linked glycosylation sites consistent with the location of these large domains on the lumen side of the disc membrane (Bungert et al. 2001). These domains also contain numerous conserved cysteine residues predicted to form intrachain disulfide bonds. In the case of ABCA1 these extracellular domains bind apolipoproteins and in particular apolipoprotein A-1, an interaction that is thought to play a key role in cholesterol efflux (Chroni et al. 2004; Wang et al. 2000, 2001). In ABCA4, no protein–protein interactions involving these domains have been reported to date.

Both NBD1 and NBD2 contain approximately 140 amino acids and share a 35% identity in amino acid sequence. As in other ABC transporters, both NBDs contain an active signature motif, also known as the C loop, as well as Walker A and Walker B motifs. The identity of the transmembrane segments within the MSDs of ABCA4 has not been resolved experimentally. Various computer programs predict 5–6 membrane spanning segments in each MSD (Illing et al. 1997). The requirement to place the ECDs on the lumen side of the disc membrane and the NBDs on the cytoplasmic side, however, argues for 5 transmembrane segments in both MSD1 (H2–H6) and MSD2 (H8–H12). In addition ABCA4 contains a stretch of

170 amino acids downstream from NBD2. This C-terminal region is highly conserved between different vertebrates suggesting that it plays an important structural and/or regulatory role. Indeed, genetic screening has identified patients with autosomal recessive cone-rod dystrophy having a deletion mutation that removes the last 24 amino acids including a conserved VFVNFA motif (Fitzgerald et al. 2004; Stenirri et al. 2006).

Analysis of ABCA4 from a variety of vertebrates has revealed a high degree of amino acid sequence conservation (Yatsenko et al. 2005). Human ABCA4 is 88–89% identical in sequence to bovine and mouse ABCA4 and 66% identical to *Xenopus laevis*. As expected, the highest degree of conservation is found within the predicted transmembrane segments and the NBDs. Comparative analysis of ABCA4 from various species support an evolutionary model in which in which the full-length ABCA4 transporter evolved from the fusion of two distinct ABCA half-transporter progenitors (Yatsenko et al. 2005). Within the ABCA subfamily ABCA4 is most closely related to ABCA1 sharing a 50% identity and 60% similarity in amino acid sequence and displaying a similar membrane topological organization (Bungert et al. 2001; Fitzgerald et al. 2002; Takahashi et al. 2006).

Functional properties

Photoreceptor specific expression of ABCA4 and its localization to rod and cone outer segment disc membranes led to the initial suggestion that ABCA4 may transport a substrate that is critical for photoreceptor function or survival (Illing et al. 1997; Allikmets et al. 1997b; Sun et al. 1999). Retinal, the chromophore of rhodopsin and cone opsin, was considered as a prime candidate. This was investigated experimentally by determining if selected retinal derivatives stimulate the ATPase activity of ABCA4 in line with studies showing that substrates transported by P-glycoprotein activate its ATPase activity (Shapiro and Ling 1994). For these measurements ABCA4 was isolated from bovine rod outer segments by immunoaffinity chromatography using the monoclonal antibody Rim 3F4 (Illing et al. 1997; Sun et al. 1999; Ahn et al. 2000). ABCA4 captured on the immunoaffinity resin was subsequently eluted with the competing 3F4 peptide and reconstituted into lipid vesicles composed of brain phospholipids. Purified and reconstituted ABCA4 was found to have a low basal ATPase activity (Sun et al. 1999; Ahn et al. 2000). Addition of 11-*cis* or all-*trans* retinal resulted in a 3–5 fold stimulation in ATPase activity. This effect was specific for retinal, since other retinoids including retinoic acid, retinol, retinyl esters and unrelated drugs had little effect on the basal ATPase activity. The basal and retinal stimulated ATPase activity was dependent on the lipid composition of the liposomes (Ahn et al. 2000). The highest basal and retinal stimulated ATPase activity was observed when ABCA4 was reconstituted with rod outer segment lipids that have a high (>40%) content of phosphatidylethanolamine (PE) (Fliesler and Anderson 1983). This elevated activity could be reproduced by the addition of synthetic PE to brain lipid mixtures having a relatively low PE content (Ahn et al. 2000). The importance of PE was further demonstrated by the finding that ABCA4 reconstituted in vesicles lacking PE was devoid of ATPase activity (Sun et al. 1999; Ahn et al. 2000).

Aldehydes react with primary amines to form Schiff base adducts. In the case of photoreceptors, all-*trans* retinal released from photoactivated rhodopsin reacts with PE in the disc membrane to form the Schiff base adduct, *N*-retinylidene-PE (Anderson and Maude 1970; Poincelot et al. 1969)(see Fig. 3b). This reaction also occurs upon the addition of all-*trans* retinal to lipid vesicles containing PE resulting in an equilibrium mixture of all-*trans* retinal and PE with *N*-retinylidene-PE (Ahn et al. 2000). Hence, either all-*trans* retinal or *N*-retinylidene-PE could serve as the true substrate for ABCA4. This was investigated by identifying the retinoid compound that bound to ABCA4 by HPLC analysis (Beharry et al. 2004). *N*-retinylidene-PE selectively bound to ABCA4 with high affinity (K_d~4 μM) when all-trans retinal was added to the immobilized protein in the presence of PE. Addition of either ATP or GTP, but not ADP or GDP, effectively dissociated *N*-retinylidene-PE from ABCA4. *N*-retinyl-PE, the product generated by reduction of *N*-retinylidene-PE with sodium borohydride, competed with *N*-retinylidene-PE for binding to ABCA4 suggesting that this derivative binds to the same site as *N*-retinylidene-PE (Beharry et al. 2004). Although these studies provide strong evidence that *N*-retinylidene-PE is the true substrate for ABCA4, to date ATP-dependent transport of *N*-retinylidene-PE across the lipid bilayer has not been measured. Thus, it remains to be confirmed experimentally that ABCA4 actively transports or flips *N*-retinylidene-PE across membranes.

The binding properties of the NBDs within ABCA4 were studied by photoaffinity labeling techniques (Ahn et al. 2003). When ABCA4 in ROS membranes was treated with 8-azido-ATP and subsequently cleaved into half molecules with trypsin, only NBD2 contained labeled ATP. Similarly, NBD2, but not NBD1, was labeled with 8-azido-ATP or 8-azido-ADP when both the N and C halves were co-expressed and co-assembled in COS-1 cells as a functional complex. Finally, the nucleotide content of purified ABCA4 was examined (Ahn et al. 2003). Native ABCA4 contained one tightly bound ADP that could not be exchanged with excess ADP or GDP, presumably in NBD1. Together, these studies indicate that only NBD2 in the C-

half of ABCA4 binds and hydrolyzes ATP (Ahn et al. 2003). NBD1 with a tightly bound ADP most likely plays a crucial, noncatalytic role through its interaction with NBD2 and MSDs. In a separate series of studies, both NBD1 and NBD2 when expressed individually in bacterial cells hydrolyze ATP (Biswas and Biswas 2000; Biswas 2001), suggesting that the tight binding of ADP to NBD1 is a consequence of its interaction with the MSDs and/or NBD2 with in the functional transporter.

These studies, together with mechanistic studies of other ABC transporters (Higgins and Linton 2004), suggest a possible mechanism for ABCA4 mediated transport of *N*-retinylidene-PE (Fig. 2a). In the initial step, ABCA4 binds *N*-retinylidene-PE in the absence of ATP. The subsequent binding of ATP to NBD2 induces a protein conformational change that enables the two NBDs to interact. This interaction is coupled to a conformational change in the MSDs which effectively translocates *N*-retinylidene-PE from its high affinity site on the lumen side of the disc membrane to a low affinity site on the cytoplasmic side. ATP hydrolysis serves to disengage the NBDs resulting in the dissociation of *N*-retinylidene-PE from the transporter. ABCA4 returns to its initial state upon the dissociation of ADP from NBD2.

ABCA4 knockout mice

In a different although complementary approach, Travis and colleagues examined the role of ABCA4 in photoreceptors by generating and characterizing *abca4* knockout mice (Weng et al. 1999; Mata et al. 2000, 2001). The retina of

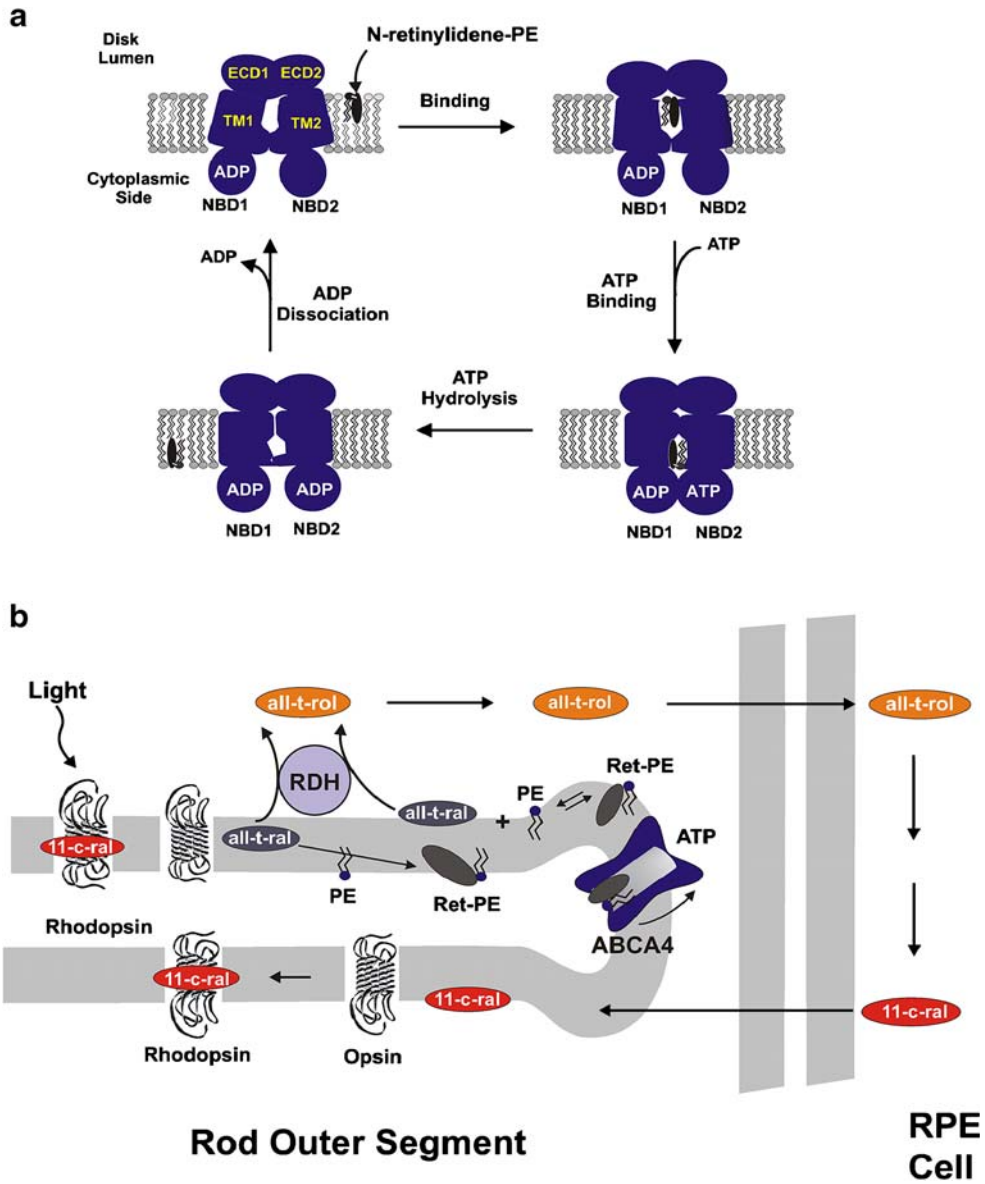

Fig. 2 Transport of *N*-retinylidene-PE across membranes by ABCA4 and its role in the visual cycle. **a** A possible mechanism by which ABCA4 transports *N*-retinylidene-PE across the disk membrane. *N*-retinylidene-PE on the lumen side of the disk binds to a high affinity site in ABCA4. ATP binds to NBD2 resulting in a conformational change which promotes a strong interaction of the two NBDs and movement of *N*-retinylidene-PE from a high affinity site on the lumen to a lower affinity site on the cytoplasmic side. ATP hydrolysis disengages the NBDs enabling the *N*-retinylidene-PE to dissociate from ABCA4. In the final step, ADP dissociation from NBD2 returns ABCA4 to its initial state. **b** ABCA4 is proposes to transport *N*-retinylidene-PE trapped on the lumen side of the disk membrane to the cyctoplasmic side where it can dissociate into all-*trans* retinal and phosphatidylethanolamine (PE). All-*trans* retinal can then be reduced by all-*trans* retinol dehydrogenase (RDH) and recycled back to 11-cis retinal via the visual cycle in the retinal pigment epithelial (RPE) cells. All-t-ral—all-*trans* retinal; Ret-PE—*N*-retinylidene-PE; 11-c-ral—11-*cis* retinal; all-t-rol—all-*trans* retinol

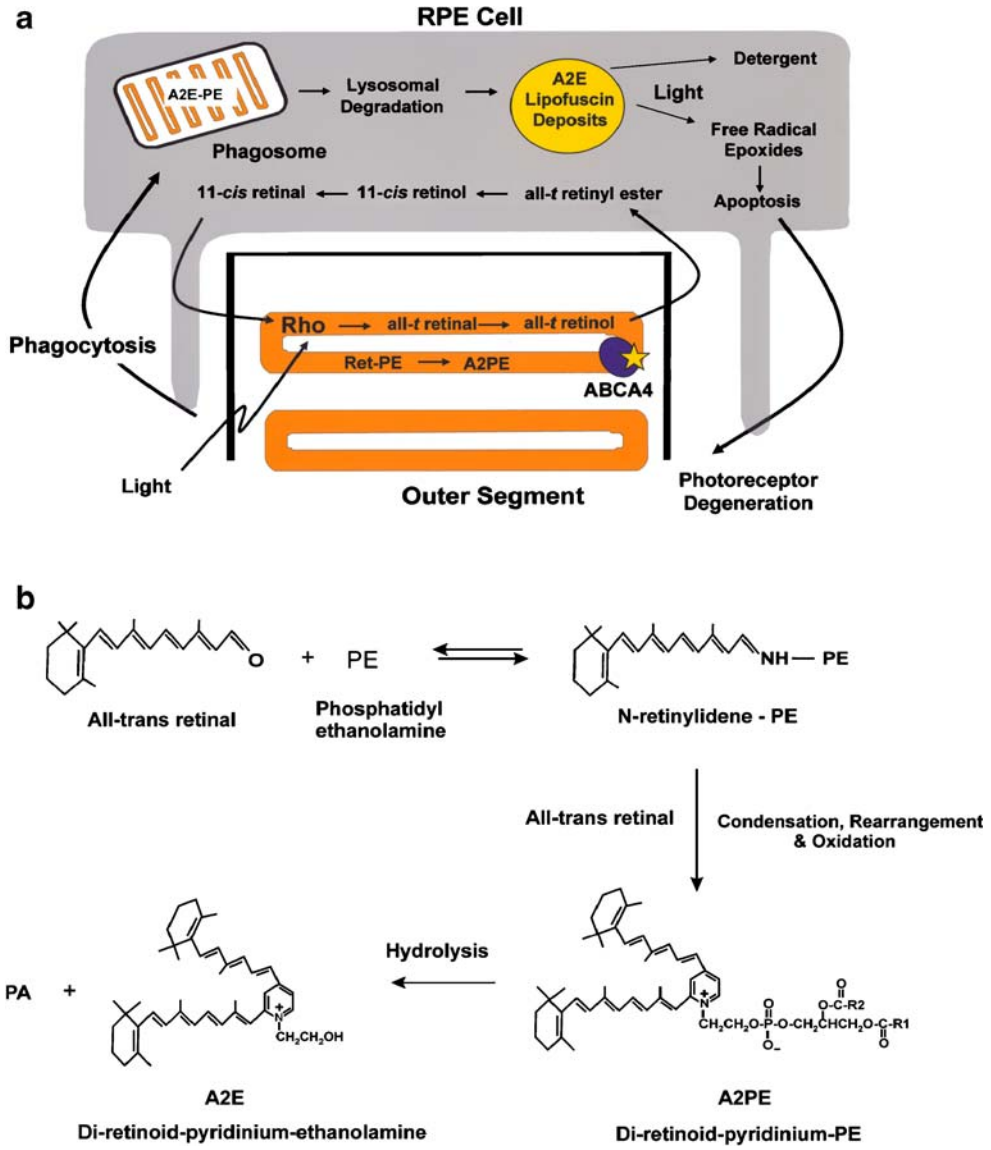

Fig. 3 Role of ABCA4 in Stargardt Macular Degeneration. **a** Diagram showing the effect of mutations in ABCA4 on the processing of all-*trans* retinal following the photobleaching of rhodopsin. All-*trans* retinal released from rhodopsin can be recycled to 11-cis retinal for the regeneration of rhodopsin through the visual cycle. However, a fraction of all-*trans* retinal will react with phosphatidylethanolamine (PE) on the lumen side of the disk. Loss in transport activity due to disease-associated mutations, result in the accumulation of *N*-retinylidene-PE on the lumen side of the disk membrane. *N*-retinylidiene-PE can react with another molecular of all-*trans* retinal to produce the diretinoid pyridinium compound A2PE. Upon phagocytosis of outer segment by the retinal pigment epithelial (RPE) cells, the components of the outer segment are metabolized. However, although A2PE can be hydrolyzed to A2E, it can not be degraded further. Accordingly, A2E will progressively accumulate in RPE cells as lipofuscin deposits. A2E and photo-oxidized products are toxic resulting in apoptosis of RPE cells and consequently photoreceptor degeneration and a loss in vision. **b** Chemical reactions of all-*trans* retinal and phosphatidylethanoloamine (PE) leading to the formation of the diretinoid compounds A2PE generated in photoreceptor outer segments and A2E produced by hydrolysis of A2PE in retinal pigment epithelial cells

both homozygous and heterozygous mice displayed a normal in appearance with well-preserved photoreceptor cells and no apparent disorganization of the outer segments often associated with photoreceptor degeneration (Weng et al. 1999; Mata et al. 2001). However, ultrastructural studies revealed the presence of lipofuscin deposits within the retinal pigment epithelium (RPE), a layer of cells adjacent to rod and cone photoreceptors (Fig. 1a). Electrophysiological recordings of *abca4* knockout mice were normal with the exception of delayed dark adaptation in rod photoreceptors (Weng et al. 1999). The most striking observation was elevated levels of protonated *N*-retinylidene-PE, all-*trans* retinal and PE in the retina, and the diretinal pyridinium compound A2E in RPE cells of homozygous and heterozygous *abca4* knockout mice exposed to continuous or cyclic lighting conditions (Weng et al. 1999; Mata

et al. 2001; Radu et al. 2004). These studies indicate that ABCA4 is not required for normal outer segment structure or morphogenesis nor is it directly involved in phototransduction. Instead, these studies implicate ABCA4 in the removal of all-*trans* retinal and *N*-retinylidene-PE from disc membranes following the photobleaching of rhodopsin (Weng et al. 1999).

Role of ABCA4 in the visual cycle

In rod cells, phototransduction is initiated when light activates rhodopsin in disc membranes by converting 11-*cis* retinal to its all-*trans* isomer. This leads to activation of the visual cascade culminating in a decrease in cGMP, a closure of cGMP-gated channels in the plasma membrane, and a hyperpolarization of the cell (Lamb and Pugh 2006; Arshavsky et al. 2002). Following photoexcitation, the rod cell returns to its dark state through a series of reactions involving inactivation of rhodopsin and other components of the visual cascade, resynthesis of cGMP, and regeneration of rhodopsin. Similar photoexcitation and recovery mechanisms take place in cone outer segments although in some cases the proteins involved are encoded by a different although related set of genes.

All-*trans* retinal generated from the photoexcitation of rhodopsin has to be converted back to 11-*cis* retinal for the regeneration of rhodopsin. This occurs through a series of enzyme catalyzed reactions occurring in both the photoreceptors and RPE cells and collectively known as the visual cycle or retinoid cycle (Saari 2000; Lamb and Pugh 2004). Briefly, following the photoexcitation, all-*trans* retinal dissociates from rhodopsin and is subsequently reduced to all-*trans* retinol by retinol dehydrogenase (RDH) on the cytoplasmic surface of disc membranes using NADPH as a reducing agent (Fig. 2b). All-*trans* retinol is routed from the photoreceptors to the adjacent RPE cells where it is converted to all-*trans* retinylester by the enzyme lecithin:retinol acetyltransferase (LRAT) and subsequently isomerized to 11-*cis* retinol by the isomerohydrolase RPE65 (Jin et al. 2005; Moiseyev et al. 2005). 11-*cis* retinol is oxidized to 11-*cis* retinal by 11-*cis* retinal dehydrogenase and this retinoid is transported back to the rod photoreceptor where it recombines with opsin in disc membranes to regenerate rhodopsin (McBee et al. 2001).

What is the role of ABCA4 in this process? Analysis of *abca4* knockout mice together with the biochemical studies of purified ABCA4 has led to a conceptual model for the role of ABCA4 as a retinoid transporter in the visual cycle (Fig. 2b). In this model, all-*trans* retinal which dissociates from opsin following the photobleaching of rhodopsin, reacts with PE to form an equilibrium mixture of *N*-retinylidene-PE and free all-*trans* retinal. Most of the all-*trans* retinal diffuses to the cytoplasmic surface of disc membranes where it is reduced to all-*trans* retinol by all-*trans* retinol dehydrogenase (RDH) and is converted to 11-*cis* retinal via the visual cycle as discussed above. However, a significant fraction of the all-*trans* retinal diffuses to the lumen side of the disc membrane where it reacts with PE to form *N*-retinylidene-PE. This compound, possibly in its protonated state, is trapped on the lumen side of the disc membrane. ABCA4 is envisioned to bind and translocate or flip *N*-retinylidene-PE from the lumen to the cytoplasmic side of the disc membrane utilizing ATP hydrolysis as an energy source (Fig. 2a and b). Once *N*-retinylidene-PE reaches the cytoplasmic side of the disc membrane it dissociates into all-*trans* retinal and PE. All-*trans* retinal is reduced by RDH to all-*trans* retinol for processing through the visual cycle. Thus, ABCA4 insures that all of the all-*trans* retinal produced from the photobleaching of rhodopsin in rods is made accessible to RDH for reduction to all-*trans* retinol thereby preventing the accumulation of retinoids in the disc membrane (Sun et al. 1999; Weng et al. 1999). ABCA4 is likely to play a similar role in cone photoreceptors (Molday et al. 2000).

ABCA4 and Stargardt macular degeneration

Stargardt macular degeneration is a relatively common autosomal recessive disorder affecting as many as 1 in 10,000 individuals (Allikmets et al. 1997a; Gelisken and De Laey 1985; Stargardt 1909). It is characterized by a loss in central vision in the first or second decade of life, the presence of yellow lipofuscin deposits in the central retina at the level of the RPE cells, progressive bilateral atrophy of rod and cone photoreceptors and underlying RPE cells in the macular region of the retina, and a delay in dark adaptation (Weleber 1994; Fishman et al. 1991; Cremers et al. 1998). Over 400 mutations in ABCA4 have been linked to Stargardt disease, most of which are missense mutations (Allikmets 2000; Rozet et al. 1998, 1999; Lewis et al. 1999; Maugeri et al. 1999; Webster et al. 2001). In addition to Stargardt disease, mutations in ABCA4 have been linked to a number of other related, but more severe, retinal degenerative diseases including autosomal recessive cone-rod dystrophy and retinitis pigmentosa (Cremers et al. 1998; Maugeri et al. 2000; Martinez-Mir et al. 1998). Genetic analysis has also suggested an association of heterozygous ABCA4 alleles with age-related macular degeneration (Allikmets et al. 1997b).

Missense and deletion mutations in ABCA4 associated with Stargardt macular degeneration are distributed throughout the protein (Allikmets 2000; Lewis et al. 1999). The effect of these disease-causing mutations has been examined by studying protein expression levels and

the ability of the mutated proteins to bind to and hydrolyze ATP (Sun et al. 2000). A number of mutants expressed at very low levels, presumably due protein misfolding and rapid degradation in the endoplasmic reticulum. Some mutants expressed at levels comparable to wild-type ABCA4. These proteins exhibited either low basal and retinal activated ATPase activity or normal basal activity but little or no retinal activated activity (Sun et al. 2000). The differential activity associated with the various disease-causing mutations may explain in part the variable phenotype associated with Stargardt disease (Maugeri et al. 1999; Shroyer et al. 1999). More recently, the effect of mutations in the trafficking of ABCA4 to outer segments has been studied in transgenic *Xenopus laevis* (Wiszniewski et al. 2005). A number of mutants were retained in the inner segment of the photoreceptors presumably due to protein misfolding and retention in the endoplasmic reticulum. This suggests that mislocalization as well as protein misfolding and diminished function is responsible for the disease.

Loss or diminished function of ABCA4 as an *N*-retinylidiene-PE transporter can explain most of the characteristic features exhibited by patients with Stargardt macular degeneration and *abca4* knockout mice. As discussed above, all-*trans* retinal dissociates from opsin following the photobleaching of rhodopsin in rods or cone opsin in cones. The fraction of all-*trans* retinal that reacts with PE on the lumen side of the disc membrane is inaccessible to RDH. The inability of mutant ABCA4 to effectively transport or flip *N*-retinylidene-PE from the lumen to the cytoplasmic side of the disc membrane leads to an accumulation of *N*-retinylidene-PE and all-*trans* retinal in photoreceptor disk membranes (Fig. 3a). Excess all-*trans* retinal can reassociate with opsin to form a retinal-opsin complex that activates the visual cascade, although less efficiently than photoactivated rhodopsin (Buczylko et al. 1996; Surya and Knox 1998). This low level of activity can contribute to background noise and the observed delay in the recovery of rod cells to their dark state as found in patients with Stargardt disease and *abca4* knockout mice (Weng et al. 1999; Mata et al. 2001; Fishman et al. 1991; Parish et al. 1998; Eldred and Lasky 1993; Mata et al. 2000). More importantly, *N*-retinylidene-PE and all-*trans* retinal in the disc membranes can react to form the diretinal pyridinium compound A2PE through chemical condensation, rearrangements and oxidation (Fig. 3b) (Mata et al. 2000; Parish et al. 1998; Eldred and Lasky 1993; Ben-Shabat et al. 2002).

Photoreceptor outer segments are continually being renewed. Packets of aged disc membranes are shed from the distal end of the outer segments and phagocytized by adjacent RPE cells, while new disc membrane is added at the proximal end of the outer segments. The outer segment is completely renewed over a 10 day period. Normally, the aged disc membranes are completely digested by the lysosomal system of the RPE cells. However, although A2PE formed in outer segment discs can be hydrolyzed to A2E and phosphatidic acid in the phagolysosomsal compartment, RPE do not have enzymes capable of further degrading A2E. As a result A2E and related side products progressively accumulate in RPE cells as fluorescent lipofuscin deposits observed in individuals with Stargardt diseases and in *abca4* knockout mice (Fig. 3a) (Ben-Shabat et al. 2002; Delori et al. 1995; Bui et al. 2006; Jang et al. 2005).

A2E has a negative effect on RPE function and survival. It can act as a detergent compromising the membrane integrity of the subcellular organelles and an inhibitor of normal RPE degradative functions (Eldred and Lasky 1993; Holz et al. 1999). Importantly, in the presence of oxygen and light A2E can be converted into free radical epoxides which are capable of killing RPE cells (Sparrow et al. 2000; Sparrow and Boulton 2005). The death of RPE cells will result in photoreceptor degeneration and a loss in vision since RPE cells are crucial for photoreceptor cell survival. In particular, RPE cells provide nutrients for photoreceptor cell survival, function in the visual cycle to regeneration rhodopsin, and ingest and degrade aged outer segment discs as part of the outer segment renewal process. Most recently, photo-oxidation products of A2E have been reported to activate the complement system suggesting that A2E and related compounds may serve as a trigger for age-related macular degeneration (Zhou et al. 2006).

Concluding remarks

Over the past 10 years significant progress has been made in characterizing the structural and functional properties of ABCA4 and defining its role in the visual cycle and Stargardt macular degeneration. Like other ABCA family members, ABCA4 is implicated in lipid transport across the cell membrane and more specifically the transport of *N*-retinylidene-PE, the lipid produced as a bi-product of the bleaching of photopigments in rod and cone photoreceptors. Interestingly, the direction of transport proposed in the current model is from the lumen to the cytoplasmic side of the disk membrane. This is in the opposite direction suggested for ABCA1 and other eukaryotic ABC transporters such as P-glycoprotein which transport substrates from the cytoplasmic to the extracellular or lumen side of membranes. Further studies are needed to confirm that ABCA4 is an “inward” flippase.

Although many structural properties of ABCA4 have been characterized, a complete high resolution structure remains to be determined. This will be important in further defining the mechanism of transport. Another important

unresolved issue is the regulation of ABCA4. Is its activity as a transporter regulated through posttranslational modifications, ligand binding, or protein-protein interactions? Finally, another active area of research is the development of therapeutic approaches to slow or eliminate RPE and photoreceptor degeneration in individuals with Stargardt disease and related diseases linked to mutations in ABCA4. Several approaches are under investigation in animal models for Stargardt disease. These include the use of retinoid analogues to slow the visual cycle thereby preventing the production of A2E compounds (Radu et al. 2003) and the development and application of gene therapy to deliver the normal ABCA4 gene to photoreceptors of *abca4* knockout mice.

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References

Ahn J, Wong JT, Molday RS (2000) The effect of lipid environment and retinoids on the ATPase activity of ABCR, the photoreceptor ABC transporter responsible for Stargardt macular dystrophy. J Biol Chem 275:20399–20405

Ahn J, Beharry S, Molday LL, Molday RS (2003) Functional interaction between the two halves of the photoreceptor-specific ATP binding cassette protein ABCR (ABCA4). Evidence for a non-exchangeable ADP in the first nucleotide binding domain. J Biol Chem 278:39600–39608

Akiyama M, Sugiyama-Nakagiri Y, Sakai K, McMillan JR, Goto M, Arita K, Tsuji-Abe Y, Tabata N, Matsuoka K, Sasaki R, Sawamura D, Shimizu H (2005) Mutations in lipid transporter ABCA12 in harlequin ichthyosis and functional recovery by corrective gene transfer. J Clin Invest 115:1777–1784

Allikmets R (2000) Simple and complex ABCR: genetic predisposition to retinal disease. Am J Hum Genet 67:793–799

Allikmets R, Singh N, Sun H, Shroyer NF, Hutchinson A, Chidambaram A, Gerrard B, Baird L, Stauffer D, Peiffer A, Rattner A, Smallwood P, Li Y, Anderson KL, Lewis RA, Nathans J, Leppert M, Dean M, Lupski JR (1997a) A photoreceptor cell-specific ATP-binding transporter gene (ABCR) is mutated in recessive Stargardt macular dystrophy. Nat Genet 15:236–246

Allikmets R, Shroyer NF, Singh N, Seddon JM, Lewis RA, Bernstein PS, Peiffer A, Zabriskie NA, Li Y, Hutchinson A, Dean M, Lupski JR, Leppert M (1997b) Mutation of the Stargardt disease gene (ABCR) in age-related macular degeneration. Science 277:1805–1807

Anderson RE, Maude MB (1970) Phospholipids of bovine outer segments. Biochemistry 9:3624–3628

Arshavsky VY, Lamb TD, Pugh EN Jr (2002) G proteins and phototransduction. Annu Rev Physiol 64:153–187

Azarian SM, Travis GH (1997) The photoreceptor rim protein is an ABC transporter encoded by the gene for recessive Stargardt's disease (ABCR). FEBS Lett 409:247–252

Ban N, Matsumura Y, Sakai H, Takanezawa Y, Sasaki M, Arai H, Inagaki N (2007) ABCA3 as a lipid transporter in pulmonary surfactant biogenesis. J Biol Chem 282:9628–9634

Beharry S, Zhong M, Molday RS (2004) N-retinylidene-phosphatidylethanolamine is the preferred retinoid substrate for the photoreceptor-specific ABC transporter ABCA4 (ABCR). J Biol Chem 279:53972–53979

Ben-Shabat S, Parish CA, Vollmer HR, Itagaki Y, Fishkin N, Nakanishi K, Sparrow JR (2002) Biosynthetic studies of A2E, a major fluorophore of retinal pigment epithelial lipofuscin. J Biol Chem 277:7183–7190

Bhongsatiern J, Ohtsuki S, Tachikawa M, Hori S, Terasaki T (2005) Retinal-specific ATP-binding cassette transporter (ABCR/ABCA4) is expressed at the choroid plexus in rat brain. J Neurochem 92:1277–1280

Biswas EE (2001) Nucleotide binding domain 1 of the human retinal ABC transporter functions as a general ribonucleotides. Biochemistry 40:8181–8187

Biswas EE, Biswas SB (2000) The C-terminal nucleotide binding domain of the human retinal ABCR protein is an adenosine triphosphatase. Biochemistry 39:15879–15886

Borst P, Elferink RO (2002) Mammalian ABC transporters in health and disease. Annu Rev Biochem 71:537–592

Borst P, Zelcer N, van Helvoort A (2000) ABC transporters in lipid transport. Biochim Biophys Acta 1486:128–144

Brooks-Wilson A, Marcil M, Clee SM, Zhang LH, Roomp K, van Dam M, Yu L, Brewer C, Collins JA, Molhuizen HO et al (1999) Mutations in ABCI in Tangier disease and familial high-density lipoprotein deficiency. Nat Genet 22:336–345

Buczylko J, Saari JC, Crouch RK, Palczewski K (1996) Mechanisms of opsin activation. J Biol Chem 271:20621–20630

Bui TV, Han Y, Radu RA, Travis GH, Mata NL (2006) Characterization of native retinal fluorophores involved in biosynthesis of A2E and lipofuscin-associated retinopathies. J Biol Chem 281:18112–18119

Bungert S, Molday LL, Molday RS (2001) Membrane topology of the ATP binding cassette transporter ABCR and its relationship to ABCl and related ABCA transporters: identification of N-linked glycosylations sites. J Biol Chem 276:23539–23546

Chroni A, Liu T, Fitzgerald ML, Freeman MW, Zannis VI (2004) Cross-linking and lipid efflux properties of apoA-I mutants suggest direct association between apoA-I helices and ABCA1. Biochemistry 43:2126–2139

Cremers FP, van de Pol DJ, van Driel M, den Hollander AI, van Haren FJ, Knoers NV, Tijmes N, Bergen AA, Rohrschneider K, Blankenagel A et al (1998) Autosomal recessive retinitis pigmentosa and cone-rod dystrophy caused by splice site mutations in the Stargardt's disease gene ABCR. Hum Mol Genet 7:355–362

Dean M, Allikmets R (2001) Complete characterization of the human ABC gene family. J Bioenerg Biomembr 33:475–479

Dean M, Annilo T (2005) Evolution of the ATP-binding cassette (ABC) transporter superfamily in vertebrates. Annu Rev Genomics Hum Genet 6:123–142

Delori FC, Staurenghi G, Arend O, Dorey CK, Goger DG, Weiter JJ (1995) In vivo measurement of lipofuscin in Stargardt's disease–Fundus flavimaculatus. Invest Ophthalmol Vis Sci 36:2327–2331

Eldred GE, Lasky MR (1993) Retinal age pigments generated by self-assembling lysosomotropic detergents. Nature 361:724–726

Fishman GA, Farbman JS, Alexander KR (1991) Delayed rod dark adaptation in patients with Stargardt's disease. Ophthalmology 98:957–962

Fitzgerald ML, Morris AL, Rhee JS, Andersson LP, Mendez AJ, Freeman MW (2002) Naturally occurring mutations in the largest extracellular loops of ABCAI can disrupt its direct interaction with apolipoprotein A-I. J Biol Chem 277:33178–33187

Fitzgerald ML, Okuhira K, Short GF 3rd, Manning JJ, Bell SA, Freeman MW (2004) ATP-binding cassette transporter Al contains a novel C-terminal VFVNFA motif that is required for its cholesterol efflux and ApoA-I binding activities. J Biol Chem 279:48477–48485

Fliesler SJ, Anderson RE (1983) Chemistry and metabolism of lipids in the vertebrate retina. Prog Lipid Res 22:79–131

Gelisken O, De Laey JJ (1985) A clinical review of Stargardt's disease and/or fundus flavimaculatus with follow-up. Int Ophthalmol 8:225–235

Higgins CF (1992) ABC transporters: from microorganisms to man. Annu Rev Cell Biol 8:67–113

Higgins CF, Linton KJ (2004) The ATP switch model for ABC transporters. Nat Struct Mol Biol 11:918–926

Holz FG, Schutt F, Kopitz J, Eldred GE, Kruse FE, Volcker HE, Cantz M (1999) Inhibition of lysosomal degradative functions in RPE cells by a retinoid component of lipofuscin. Invest Ophthalmol Vis Sci 40:737–743

Illing M, Molday LL, Molday RS (1997) The 220-kDa rim protein of retinal rod outer segments is a member of the ABC transporter superfamily. J Biol Chem 272:10303–10310

Jang YP, Matsuda H, Itagaki Y, Nakanishi K, Sparrow JR (2005) Characterization of peroxy-A2E and furan-A2E photooxidation products and detection in human and mouse retinal pigment epithelial cell lipofuscin. J Biol Chem 280:39732–39739

Jin M, Li S, Moghrabi WN, Sun H, Travis GH (2005) Rpe65 is the retinoid isomerase in bovine retinal pigment epithelium. Cell 122:449–459

Kaminski WE, Piehler A, Wenzel JJ (2006) ABC a-subfamily transporters: structure, function and disease. Biochim Biophys Acta 1762:510–524

Lamb TD, Pugh EN Jr (2004) Dark adaptation and the retinoid cycle of vision. Prog Retin Eye Res 23:307–380

Lamb TD, Pugh EN Jr (2006) Phototransduction, dark adaptation, and rhodopsin regeneration the proctor lecture. Invest Ophthalmol Vis Sci 47:5137–5152

Lefevre C, Audebert S, Jobard F, Bouadjar B, Lakhdar H, Boughdene-Stambouli O, Blanchet-Bardon C, Heilig R, Foglio M, Weissenbach J et al (2003) Mutations in the transporter ABCA12 are associated with lamellar ichthyosis type2. Hum Mol Genet 12:2369–2378

Lewis RA, Shroyer NF, Singh N, Allikmets R, Hutchinson A, Li Y, Lupski JR, Leppert M, Dean M (1999) Genotype/Phenotype analysis of a photoreceptor-specific ATP-binding cassette transporter gene, ABCR, in Stargardt disease. Am J Hum Genet 64: 422–434

Martinez-Mir A, Paloma E, Allikmets R, Ayuso C, del Rio T, Dean M, Vilageliu L, Gonzalez-Duarte R, Balcells S (1998) Retinitis pigmentosa caused by a homozygous mutation in the Stargardt disease gene ABCR. Nat Genet 18:11–12

Mata NL, Weng J, Travis GH (2000) Biosynthesis of a major lipofuscin fluorophore in mice and humans with ABCR-mediated retinal and macular deseneration. Proc Natl Acad Sci U S A 97:7154–7159

Mata NL, Tzekov RT, Liu X, Weng J, Birch DG, Travis GH (2001) Delayed dark-adaptation and lipofuscin accumulation in abcr+/- mice: implications for involvement of ABCR in age-related macular degeneration. Invest Ophthalmol Vis Sci 42:1685–1690

Maugeri A, Klevering BJ, Rohrschneider K, Blankenagel A, Brunner HG, Deutman AF, Hoyng CB, Cremers FP (2000) Mutations in the ABCA4 (ABCR) gene are the major cause of autosomal recessive cone-Rod dystrophy. Am J Hum Genet 67:960–966

Maugeri A, van Driel MA, van de Pol DJ, Klevering BJ, van Haren FJ, Tijmes N, Bergen AA, Rohrschneider K, Blankenagel A, Pinckers AJ et al (1999) The 2588G–>C mutation in the ABCR gene is a mild frequent founder mutation in the Western European population and allows the classification of ABCR mutations in patients with Stargardt disease. Am J Hum Genet 64:1024–1035

McBee JK, Palczewski K, Baehr W, Pepperberg DR (2001) Confronting complexity: the interlink of phototransduction and retinoid metabolism in the vertebrate retina. Prog Retin Eye Res 20:469–529

Moiseyev G, Chen Y, Takahashi Y, Wu BX, Ma JX (2005) RPE65 is the isomerohydrolase in the retinoid visual cycle. Proc Natl Acad Sci U S A 102:12413–12418

Molday LL, Rabin AR, Molday RS (2000) ABCR expression in foveal cone photoreceptors and its role in Stargardt macular dystrophy. Nat Genet 25:257–258

Nasonkin I, Illing M, Koehler MR, Schmid M, Molday RS, Weber BH (1998) Mapping of the rod photoreceptor ABC transporler (ABCR) to 1p2l -p22.1 and identification of novel mutations in Stargardt's disease. Hum Genet 102:21–26

Oram JF (2002) ATP-binding cassette transporter Al and cholesterol trafficking. Curr Opin Lipidol 13:373–381

Oswald C, Holland IB, Schmitt L (2006) The motor domains of ABC-transporters. What can structures tell us? Naunyn Schmiedebergs Arch Pharmacol 372:385–399

Papermaster DS, Reilly P, Schneider BG (1982) Cone lamellae and red and green rod outer segment disks contain a large intrinsic membrane protein on their margins: an ultrastructural immuno-cytochemical study of fiog retinas. Vision Res 22:1417–1428

Papermaster DS, Schneider BG, Zorn MA, Kraehenbuhl JP (1978) lmmunocytochemical localization of a large intrinsic membrane protein to the incisures and margins of frog rod outer segment disks. J Cell Biol 78:415–425

Parish CA, Hashimoto M, Nakanishi K, Dillon J, Sparrow J (1998) Isolation and one-step preparation of A2E and iso-A2E, fluorophores from human retinal pigment epithelium. Proc Natl Acad Sci U S A 95:14609–14613

Poincelot RP, Millar PG, Kimbel RL Jr, Abrahamson EW (1969) Lipid to protein chromophore transfer in the photolysis of visual pigments. Nature 221:256–257

Radu RA, Mata NL, Bagla A, Travis GH (2004) Light exposure stimulates formation of A2E oxiranes in a mouse model of Starsardt's macular deseneration. Proc Natl Acad Sci U S A 101:5928–5933

Radu RA, Mata NL, Nusinowitz S, Liu X, Sieving PA, Travis GH (2003) Treatment with isotretinoin inhibits lipofuscin accumulation in a mouse model of recessive Stargardt's macular degeneration. Proc Natl Acad Sci U S A 100:4742–4747

Rivera A, White K, Stohr H, Steiner K, Hemmrich N, Grimm T, Jurklies B, Lorenz B, Scholl HP, Apfelstedt-Sylla E, Weber BH (2000) A comprehensive survey of sequence variation in the ABCA4 (ABCR) gene in Stargardt disease and age-related macular degeneration. Am J Hum Genet 67:800–813

Rozet JM, Gerber S, Souied E, Ducroq D, Perrault I, Ghazi I, Soubrane G, Coscas G, Dufier JL, Munnich A, Kaplan J (1999) The ABCR gene: a major disease gene in macular and peripheral retinal degenerations with onset from early childhood to the elderly. Mol Genet Metab 68:310–315

Rozet JM, Gerber S, Souied E, Perrault I, Chatelin S, Ghazi I, Leowski C, Dufier JL, Munnich A, Kaplan J (1998) Spectrum of ABCR gene mutations in autosomal recessive macular dystrophies. Eur J Hum Genet 6:291–295

Saari JC (2000) Biochemistry of visual pigment regeneration: the Friedenwald lecture. Invest Ophthalmol Vis Sci 41:337–348

Shapiro AB, Ling V (1994) ATPase activity of purified and reconstituted P-glycoprotein from Chinese hamster ovary cells. J Biol Chem 269:3745–3754

Shroyer NF, Lewis RA, Allikmets R, Singh N, Dean M, Leppert M, Lupski JR (1999) The rod photoreceptor ATP-binding cassette transporter gene, ABCR, and retinal disease: from monogenic to multifactorial. Vision Res 39:2537–2544

Shulenin S, Nogee LM, Annilo T, Wert SE, Whitsett JA, Dean M (2004) ABCA3 gene mutations in newborns with fatal surfactant deficiency. N Engl J Med 350:1296–1303

Sparrow JR, Boulton M (2005) RPE lipofuscin and its role in retinal pathobiology. Exp Eye Res 80:595–606

Sparrow JR, Nakanishi K, Parish CA (2000) The lipofuscin fluorophore A2E mediates blue light-induced damage to retinal pigmented epithelial cells. Invest Ophthalmol Vis Sci 41:1981–1989

Stargardt K (1909) Uber familiare, progressive degeenration under makulagegend des augen. Albrecht von Graefes Arch Ophthalmol 71:534–550

Stenirri S, Battistella S, Fermo I, Manitto MP, Martina E, Brancato R, Ferrari M, Cremonesi L (2006) De novo deletion removes a conserved motif in the C-terminus of ABCA4 and results in cone-rod dystrophy. Clin Chem Lab Med 44:533–537

Sun H, Molday RS, Nathans J (1999) Retinal stimulates ATP hydrolysis by purified and reconstituted ABCR, the photoreceptor-specific ATP-binding cassette transporter responsible for Stargardt disease. J Biol Chem 274:8269–8281

Sun H, Nathans J (1997) Stargardt's ABCR is localized to the disc membrane of retinal rod outer segments. Nat Genet 17: 15–16

Sun H, Smallwood PM, Nathans J (2000) Biochemical defects in ABCR protein variants associated with human retinopathies. Nat Genet 26:242–246

Surya A, Knox BE (1998) Enhancement of opsin activity by all-trans-retinal. Exp Eye Res 66:599–603

Takahashi K, Kimura Y, Kioka N, Matsuo M, Ueda K (2006) Purification and ATPase activity of human ABCA1. J Biol Chem 281:10760–10768

Wang N, Silver DL, Costet P, Tall AR (2000) Specific binding of ApoA-I, enhanced cholesterol efflux, and altered plasma membrane morphology in cells expressing ABCI. J Biol Chem 275:33053–33058

Wang N, Silver DL, Thiele C, Tall AR (2001) ATP-binding cassette transporter Al (ABCAI) functions as a cholesterol efflux regulatory protein. J Biol Chem 276:23742–23747

Webster AR, Heon E, Lotery AJ, Vandenburgh K, Casavant TL, Oh KT, Beck G, Fishman GA, Lam BL, Levin A et al (2001) An analysis of allelic variation in the ABCA4 gene. Invest Ophthalmol Vis Sci 42:1179–1189

Weleber RG (1994) Stargardt's macular dystrophy. Arch Ophthalmol 112:752–754

Weng J, Mata NL, Azarian SM, Tzekov RT, Birch DG, Travis GH (1999) Insights into the function of rim protein in photoreceptors and etiology of Stargardt's Disease from the phenotype in *abcr* knockout mice. Cell 98:13–23

Wiszniewski W, Zaremba CM, Yatsenko AN, Jamrich M, Wensel TG, Lewis RA, Lupski JR (2005) ABCA4 mutations causing mislocalization are found frequently in patients with severe retinal dystrophies. Hum Mol Genet 14:2769–2778

Yatsenko AN, Wiszniewski W, Zaremba CM, Jamrich M, Lupski JR (2005) Evolution of ABCA4 proteins in vertebrates. J Mol Evol 60:72–80

Zhou J, Jang YP, Kim SR, Sparrow JR (2006) Complement activation by photooxidation products of A2E, a lipofuscin constituent of the retinal pigment epithelium. Proc Natl Acad Sci U S A 103: 16182–16187