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Focus on Molecules: *ABCA4* (*ABCR*) – An Import-Directed Photoreceptor Retinoid Flipase

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ABCA4; *ABCR*; juvenile macular degeneration; age-related macular degeneration

1. Structure

Previously known as the photoreceptor rim protein (RmP), human *ABCA4* (*ABCR*) (NP000341.2) is a large (2,273 aa, 220 kD), retina-specific adenosine triphosphate (ATP)-binding cassette transporter (ABC). The proposed structure of *ABCR*, which is based on hydropathy plots and its homology to other ABCs, consists of two six transmembrane domains (TMD), two large glycosylated extracellular domains (ECD1, 602 aa, ECD2, 289 aa), and two conserved intracellular nucleotide binding domains (NBD1, NBD2, ~140 aa each) (Fig. 1A) (see Sun and Nathans, 2001; Molday 2007). When inserted into the membrane, the TMDs of *ABCR* are predicted to form a gated, barrel/hyperboloid structure that is permeable to retinoid ligands and controls channel access to extracellular/intracellular binding sites. In general, the TMDs of the ABC protein family are not highly conserved, an observation that is consistent with the diverse transport functions of this protein family. When ATP binds to the NBDs, *ABCR* undergoes a series of conformational changes that bring the NBDs together and rotates and tilts the TMDs thereby modulating ligand binding to the gated channel. The current proposed alternating access-release model for retinoid transport by *ABCR* is based on recent structural analyses of bacterial importer/exporter ABCs (Fig. 1B). The model proposes that retinoid transport occurs as a result of alternating exposure of external and internal TMD ligand binding site(s) located along the permeation channel that are gated by coupled structural changes in NBDs that themselves are modulated by the binding and hydrolysis of ATP.

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2. Function

The results of comparisons of mammalian *ABCR* to other ABCs, biochemical and cellular localization studies of *ABCR*, and analyses of *ABCR* knockout mice suggest that mammalian *ABCR* functions as an inward-directed retinoid flipase. Retinoid substrates imported by *ABCR* from the extracellular or intradiscal (rod) membrane surfaces to the cytoplasmic membrane surface are all-trans retinaldehyde (**ATR**) and N-retinyl-phosphatidylethanolamine (**NR-PE**), a covalent Schiff base adduct (-C=N-) of ATR with PE. Once transported to the cytoplasmic surface, ATR is reduced to vitamin A (**VA**) by *trans*-retinol dehydrogenase (*RDH*) and then transferred to the retinal pigment epithelium (**RPE**) where it is converted to 11-*cis*-retinal.

The only biochemical assay that has been developed for *ABCR* function is enhanced ATP hydrolysis that occurs when *ABCR*, reconstituted (with PE) in membranes, is exposed to candidate ligands. No assay currently exists that measures actual substrate transfer across membranes. In support for an *ABCR* flipase function, certain retinoids stimulate ATPase activity, which occurs by simple Michaelis-Menten uncompetitive activation kinetics, as expected for a transporter with a single class of binding sites. Preferred ligands are protonated NR-PE ($K_d \sim 4 \mu\text{M}$) and ATR. Only NBD2 exchanges ATP with solution, while NBD1 has adenosine diphosphate (**ADP**) tightly bound.

ABCR is localized to outer segment disk edges of rods and cones. *ABCR* expression relative to rhodopsin is approximately 1:120 (1×10^6 copies/photoreceptor, $\sim 10^3$ copies/disk). Hydrophobic ATR formation is directly proportional to visual pigment bleaching, and upon release from opsins ATR can appear on both membrane surfaces to spontaneously and reversibly react with PE ($\text{NR-PE} \rightleftharpoons \text{ATR} + \text{PE}$). PE is more abundant in rod disk than plasma membranes, and is differentially distributed on cytoplasmic (>75%) and extracellular (<25%) surfaces. The ATR fraction that distributes to the cytoplasmic membrane surface is reduced to VA by *RDH*. VA esterification in RPE can pull the equilibrium to NR-PE hydrolysis by mass action. Additional kinetic barriers emerge for the fraction of ATR solubilized on the extracellular/intradiscal (rods) membrane surfaces. While some may diffuse directly to the cytoplasmic leaflet, another fraction reacts with PE to become protonated NR-PE at physiological pH (pK_a 6.0, unprotected Schiff base) and is trapped inside the disk as a charged species. *ABCR* could facilitate transfer of this population to the cytoplasmic surface.

The *ABCR*^{-/-} knockout mouse has delayed dark adaptation but normal final rod threshold relative to controls (Weng et al., 1999). This suggests both *ABCR* dependent and independent (e.g. bulk transmembrane diffusion) pathways that remove ATR/NR-PE from extracellular membranes. After strong bleaches there is significant accumulation of ATR and NR-PE in outer segments, and decreases of VA and VA esters. Levels of the toxic cationic *bis*-pyridinium salt, N-retinylidene-N-retinyl-ethanolamine (**A2E**), increase with age but no retinal degeneration ensues. A2E forms from reaction of two molecules of ATR with PE. These findings support a role for *ABCR* to remove ATR/NR-PE from the extracellular photoreceptor surfaces during bleach recovery, and to suppress accumulation of A2E. No A2E accumulates in mice raised in darkness because visual pigment bleaching and turnover (visual retinoid cycle) is required to generate the ATR substrate needed for toxic A2E formation. The double knockout (*ABCR*^{-/-} and rod *RDH* (*RDH8*^{-/-})) severely constrains ATR clearance from rods. This causes large and rapid accumulations of both A2E and the toxic retinal dimer-PE conjugate (**Ret-Di-PE**) (another product of combining two ATR molecules and PE), formation of drusen, and RPE and photoreceptor degeneration and neovascularization. This model presents a full spectrum of human dry and wet age-related macular degeneration (**AMD**) (Maeda et al., 2008). The phenotype is suppressed by

darkness and an RPE65 inhibitor, which indicates that cellular pathology ultimately originates from visual pigment bleaching, ATR formation, and retinoid visual cycle turnover. In the short mouse lifetime emergence of a lipofuscin-dependent atrophic and neovascular retinal degeneration required complete “system” bottleneck to stall conversion of ATR to VA.

3. Disease Involvement

Stargardt macular dystrophy (**STGD**) is a hereditary juvenile macular degeneration (frequency 1/10,000) that emerges in the first two decades of life. STGD is clinically associated with yellow-white flecks at the RPE level causing focal, progressive macular RPE atrophy and associated photoreceptor loss. STGD is characterized by reductions in visual acuity and color vision, paracentral visual field changes, cone ERG changes, delayed dark adaptation late in disease, and substantial accumulation of autofluorescent RPE lipofuscin. Yellow lesions are due to clusters of RPE cells engorged with fluorescent lipofuscin pigments that contain toxic A2E/Ret-Di. The large human *ABCR* gene is mapped to 1p13-p21 (ID: 601691), and covers a span of approximately 130 kilo base pairs with fifty exons (processed mRNA: 7326 nt; open reading frame: 6819 nt). Known variants/mutations (>400) cause autosomal recessive STGD (*STGD1*, Mendelian Inheritance in Man (**MIM**): 248200), a later onset milder variant called fundus flavimaculatus (*FF*, MIM ID: 248200), recessive cone-rod dystrophy (*CORD3*, MIM: 604116), recessive retinitis pigmentosa (*RP19*, MIM: 601718), and contribute to AMD risk (*ARMD2*, MIM: 153800). *ABCR* mutations create a wide phenotypic spectrum (mild: *ARMD2* → *STGD1/FF* → *CORD3* → *RP19*: severe). In HEK293 cells expression of human *ABCR* variants leads to varied expression levels, ATP binding, and ATR- stimulated ATPase activity relative to normal. Broad clinical phenotypic heterogeneity (time of onset, clinical severity) is ultimately dependent upon the nature of the specific mutations involved. Mutations can affect the amount of remaining wild type *ABCR* activity, or cause toxic or dominant negative effects due to mutant proteins with abnormal folds.

ABCR may benefit vision on short and long time scales. Transducin is activated when opsin binds ATR noncovalently, at least *in vitro*, to elevate rod threshold by physiological “dark light”. Removal of ATR/NR-PE by *ABCR* appears important to normal bleach recovery kinetics and to mitigate persistent opsin signaling that drives photoreceptor death. *ABCR* could mitigate long term effects by mobilizing ATR to suppress irreversible addition of a second ATR molecule to NR-PE to form dihydro-N-retinylidene-N-retinyl-phosphatidylethanolamine (A2PE-H2). A2PE-H2 traps ATR into visually useless ligands, accumulates in outer segments, and is further oxidized to N-retinylidene-N-retinyl-phosphatidyl-ethanolamine (A2-PE). Upon disk shedding and phagocytosis of outer segment material by RPE cells, A2-PE is hydrolyzed in the RPE phagolysosome to form toxic A2E. A2E cannot be metabolized and accumulates as a major component of fluorescent RPE lipofuscin. Accumulating ATR also drives formation of toxic Ret-Di-PE complexes that, with A2E, exert cumulative or threshold toxicity at the primary RPE level with secondary photoreceptor demise in juvenile and age-related macular degenerations.

4. Future Studies

To advance *ABCR* knowledge a cellular assay is critically needed to measure actual transmembrane retinoid transport. Many important questions about *ABCR* structure, function, and system integration remain. What is the atomic structure under various conditions of NR-PE and ATP binding, what TMD residues constitute ligand binding sites, and what is the coupling mechanism of ATP binding/hydrolysis to conformational changes? What fraction of ATR is released from opsin on extracellular/intracellular membrane

leaflets, does *ABCR* interface with recently proposed ATR exit sites on bleached rhodopsin, what fraction of the extracellular pool is mobilized by *ABCR*, and how are these pools modulated by bleaching level? What are the kinetics of *ABCR* performance? Can inhibitors and activators be identified (e.g. A2-PEH2)? Does *ABCR* interact with *tRDH* on the cytoplasmic surface, or with proteins in the disk rim or lumen, or subretinal space (e.g. IRBP)? Does phosphorylation, or other modulation, regulate function? What molecular and “system” problems underlie the diversity of clinical *ABCR* phenotypes?

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ABBREVIATIONS

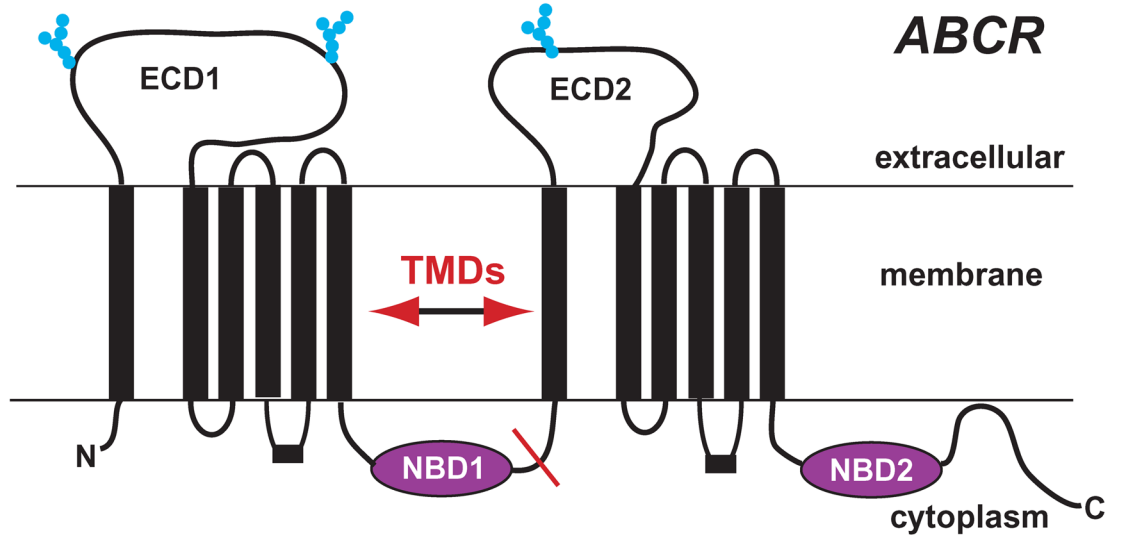
| | |
|--------------------|---|
| A2E | N-retinylidene-N-retinyl-ethanolamine |
| ABC | ATP-binding cassette |
| <i>ABCR</i> | ATP-binding cassette retinoid protein, subfamily A, number 4 (<i>ABCA4</i>) |
| ADP | adenosine diphosphate |
| AMD | age-related macular degeneration |
| ATP | adenosine triphosphate |
| ATR | all- <i>trans</i> -retinal |
| MIM | Mendelian Inheritance in Man |
| NBD | nucleotide binding domain |
| NR-PE | N-retinylidene-phosphatidyl-ethanolamine |
| PE | phosphatidyl-ethanolamine |
| Ret-Di | retinal dimer |
| RPE | retinal pigment epithelium |
| STGD | Stargardt macular dystrophy |
| TMD | transmembrane domain |
| <i>tRDH</i> | <i>trans</i> -retinol dehydrogenase |
| VA | vitamin A |

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A



B

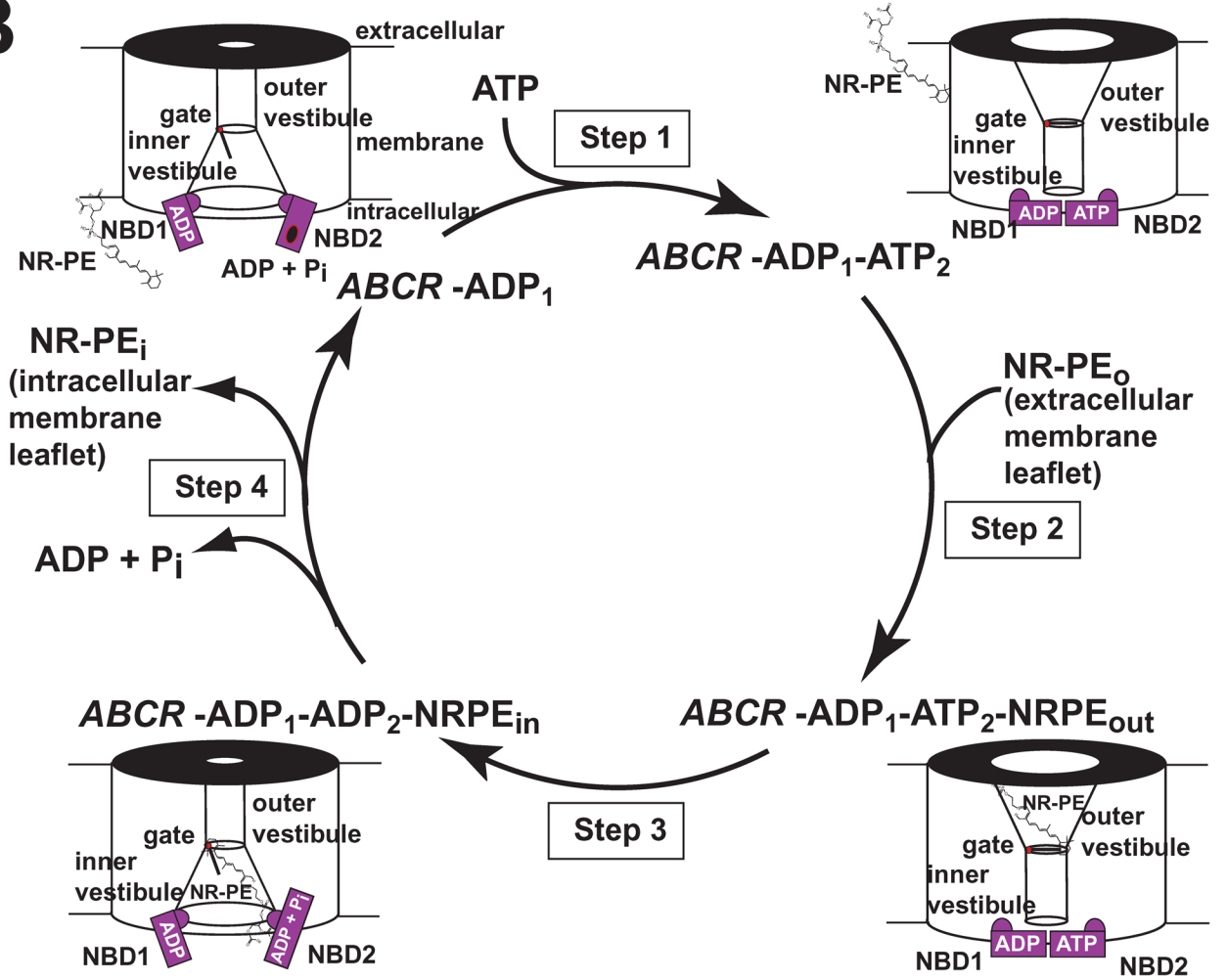


Figure 1.

ABCR Structure and Function. **(A)** Secondary structure schematic is shown (adapted from Bungert et al. 2001, *J Biol. Chem.* 276: 23539-23546). Red line shows the approximate half point. **(B)** *ABCR* modeled as a retinoid *flipase* that transports retinoids (NRPE, ATR) from photoreceptor extracellular to cytoplasmic surfaces. An *alternating access-release model* for *ABCR* has the following steps (modeled schematically): 1) binding of adenosine-triphosphate (ATP) to NBD2 which promotes spatial NBD1/NBD2 interaction, and coupled kinetic transition of TMDs to expose outer vestibule high affinity binding site, 2) binding of NR-PE/ATR on extracellular side of gate, 3) ATP hydrolysis promotes gate opening and movement of NR-PE/ATR along the TMD channel to a low affinity binding site on intracellular side of gate, and 4) and release of adenosine diphosphate (ADP) and phosphate (Pi) to promote separation of NBDs and release of bound ligand.