ABCA4, a member of the family of ATP binding cassette (ABC) proteins found in rod and cone photoreceptors, has been implicated in the transport of retinoid compounds across the outer segment disk membrane following the photoactivation of rhodopsin. Mutations in the ABCA4 gene are responsible for Stargardt macular dystrophy and related retinal degenerative diseases that cause a loss in vision. To identify the retinoid substrate that interacts with ABCA4, we have isolated ABCA4 from rod outer segment disk membranes on an immunopurification matrix and analyzed retinoid compounds that bind to ABCA4 using high performance liquid chromatography and radiolabeling methods. When all-trans-retinal was added to ABCA4 in the presence of phosphatidylethanolamine, 0.9 mol of N-retinylidene-phosphatidylethanolamine and 0.3 mol of all-trans-retinal were bound per mol of ABCA4 with an apparent $K_d$ of 2–5 μM. ATP and GTP released these retinoids from ABCA4, whereas ADP, GDP, and nonhydrolyzable derivatives, adenosine 5′-[(β,γ-imido)triphosphate and guanosine 5′-[(β,γ-imido)triphosphate, were ineffective. One mole of N-retinyl-phosphatidylethanolamine, the reduced form of N-retinylidene-phosphatidylethanolamine, bound per mol of ABCA4, whereas 0.3 mol of all-trans-retinal were bound in the absence of phosphatidylethanolamine. No binding of all-trans-retinol to ABCA4 was observed. Our results indicate that ABCA4 preferentially binds N-retinylidene-phosphatidylethanolamine with high affinity in the absence of ATP. Our studies further suggest that ATP binding and hydrolysis induces a protein conformational change that causes N-retinylidene-phosphatidylethanolamine to dissociate from ABCA4.

ABCA4, also known as ABCR or the rim protein, is a member of the ABCA subfamily of ATP binding cassette (ABC) transporters (1–3). It is localized along the rims and incisures of rod and cone photoreceptor outer segment disk membranes where it is thought to play a role in the visual cycle (3–5). Mutations in the gene encoding ABCA4 are responsible for a variety of autosomal recessive retinal degenerative diseases that cause a severe loss in vision. These include Stargardt macular dystrophy, fundus flavimaculatus, cone-rod dystrophy, and retinitis pigmentosa (1, 6–9). Mutations in ABCA4 have also been suggested to predispose individuals to age-related macular degeneration (10).

ABCA4 is a 250-kDa glycoprotein that is organized as two tandemly arranged halves each containing a transmembrane segment followed by a large extracellular domain, a multipassing membrane domain, and a nucleotide binding domain (3, 11). Biochemical studies have implicated ABCA4 in the movement of retinoids across the disk membrane. The ATPase activity of immunoaffinity purified ABCA4 is increased up to 4-fold by the addition of 11-cis or all-trans-retinal, but not other retinoids (12–14). Abca4 knockout mice show a light-dependent increase in all-trans-retinal, N-retinylidene-PE, and PE in photoreceptor outer segments, and a progressive accumulation of the directinal pyridinium compound, A2E, in retinal pigment epithelial cells in the form of lipofuscin deposits (15–17). Like many individuals with Stargardt disease, abca4 knockout mice also show a delayed recovery of dark adaptation, a process thought to arise from the accumulation of all-trans-retinal in disk membranes following the photobleaching of rhodopsin (18). Together, these studies suggest that ABCA4 facilitates the removal of all-trans-retinal derivatives from disk membranes following photoreabsorption. This prevents the formation of toxic directinal pyridinium side products in disk membranes that otherwise would progressively accumulate in retinal pigment epithelial cells as a result of phagocytosis of outer segment (12, 15, 17, 19–21).

Although these studies suggest that ABCA4 mediates the removal of retinoids from disk membranes, to date there is no direct evidence that ABCA4 functions as a retinoid transporter. Furthermore, the identity of the retinoid substrate that interacts with ABCA4 remains to be determined. The aldehyde group of all-trans-retinal is known to react with the primary amine of PE to form an equilibrium mixture of the Schiff-base adduct, N-retinylidene-PE, and free all-trans-retinal (13, 22, 23). Accordingly, it has been proposed that ABCA4 may act as a flippase to translocate N-retinylidene-PE from the lumen to the cytoplasmic side of the disk membrane (12, 15). A number of ABC transporters including multidrug-resistant proteins encoded by the human ABCA4 gene. The functions of ABCA4 include the transport of cholesterol, sphingolipids, and retinoids across the disk membrane.
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coded by the mdr2 and mdr3 genes in mice (24, 25) and MsbA in Escherichia coli (26) have been reported to actively flip phospholipids across the lipid bilayer. Alternatively, ABCA4 could actively extrude all-trans-retinol from the disk membranes analogous to the ATP-dependent efflux of hydrophobic compounds from membranes by P-glycoprotein (27).

In this study, we have developed a solid-phase assay to identify retinoid compounds that bind to ABCA4 in the presence of phospholipid as a important step in understanding mechanisms of action of this ABC transporter. Using this assay in conjunction with HPLC and radiolabeling methods, we show here that ABCA4 preferentially binds N-retinylidene-PE in the absence of adenine or guanine nucleotide triphosphates. ATP and GTP, but not nonhydrolysable nucleotide analogues, release N-retinylidene-PE from ABCA4 suggesting that N-retinylidene-PE is the substrate that binds to and is actively transported by ABCA4.

Materials and Methods

Reagents—All-trans-retinal, all-trans-retinol, soybean phospholipids, and CHAPS were purchased from Avanti Polar Lipids. Radio-labeled sodium borohydride (NaB\textsubscript{3}H\textsubscript{4}) was obtained from Amersham Biosciences or PerkinElmer Life Sciences and Complete Protease Inhibitor was from Roche Applied Sciences. All organic solvents (chloroform, hexane, and methanol) were HPLC grade and water was distilled and deionized. Protonated N-retinylidene-PE (\(\lambda_{max} 450 \text{ nm; } \epsilon 31,300 \text{ m}^{-1} \text{ cm}^{-1}\)), the Schiff base conjugate of all-trans-retinal and DOPE, and its NaB\textsubscript{3}H\textsubscript{4} reduced conjugate, N-retinyl-PE (\(\lambda_{max} 330 \text{ nm; } \epsilon 37,000 \text{ m}^{-1} \text{ cm}^{-1}\)), were prepared and purified as previously described (13).

All-trans-retinal and all-trans-retinol were radiolabeled as described by Garwin and Saari (28). \(^{3}H\text{I}l\text{all-trans-retinal was used to prepare }^{3}H\text{I}l\text{retinylidene-PE, as described previously (13).}

ROS and the Rim 3F4 Monoclonal Antibody—ROS were isolated on a continuous sucrose gradient from previously frozen, dark-adapted bovine retinas under dim red light (29) and stored in the dark at a protein concentration of 8–10 mg/ml in 20% sucrose, 0.01 M Tris-Cl, pH 7.5, 0.1 M NaCl, 18 mM CHAPS, 1 mM dithiothreitol, 3 mM MgCl\textsubscript{2}, and 1 mM dithiothreitol Inhibitor was from Garwin and Saari (28). Rim 3F4 monoclonal antibody, directed against an epitope near the C terminus of ABCA4, was purified from 30 ml of solubilized ROS, but without solubilized ROS and without retinoid substrate. No detectable retinoid compounds were extracted from the immunoaffinity matrix in these control samples.

Analysis of Retinoid Bound to ABCA4 Using a Radiolabel Assay—The assay required less than one-tenth the amount of ROS and Rim 3F4-Sepharose. Assays were carried out in triplicate for each point determination. Approximately 60 \(\mu\)l of gravity packed Rim 3F4-Sepharose 2B beads were added to the inner unit of an Amicon Ultrafuge MC 0.45-\(\mu\)m centrifugal filter device. The matrix was washed thoroughly with column buffer by centrifugation. Approximately, 0.6 ml of dark-adapted, CHAPS-solubilized ROS (prepared as described above) was added to the Rim 3F4-Sepharose 2B beads. The filter device was sealed with parafilm, wrapped in aluminum foil, and placed on a rotating wheel at 4 °C for 60 min. The beads were then washed 6 times with 0.5 ml of column buffer by centrifugation to remove unbound protein.

\(^{3}H\text{Iall-trans-retinal (5–10} \times 10^{10} \text{ dpm per 0.5 ml of reaction mixture at a final all-trans-retinol concentration of 10} \mu\text{M or as otherwise indicated} \text{ was added to the ABCA4-Rim 3F4-Sepharose. The specific activity of the }^{3}H\text{Iall-trans-retinal ranged from 500 to 5000 dpm/\mumol. The filtration device was sealed as above and rotated at 4 or 22 °C for 30 min. Duplicate } 5-\mu\text{M samples were removed to determine the specific activity of the labeled sample by scintillation counting. The inner device was placed in a disposable culture test tube (13 × 100 mm) and centrifuged for 30 s in a clinical centrifuge. The inner device was then inserted into a second test tube and washed twice with 0.5 ml each of column buffer by centrifugation. For scintillation counting, 25 \(\mu\)l of the combined eluate was removed. The washings were repeated 2 more times to remove the unbound labeled all-trans-retinal. The last wash typically showed only background counts. Each inner filtration device containing the immobilized ABCA4 with bound labeled retinoid was placed in a 1.0 × 100-mm culture test tube. The \(^{3}H\text{Iall-trans-retinal was extracted from the matrix with 0.5 ml of ice-cold methanol for 5 min on ice followed by centrifugation. A second extraction with 0.5 ml of ice-cold methanol was performed and the two methanol extractions were pooled. The methanol extraction procedure was repeated 2 more times. The counts of the methanol extractions of each sample were determined by liquid scintillation counting. Each value is the average of two separate experiments. In general, the procedures were carried out under dim light to prevent any photoreaction of the retinoids. Controls were treated in the same way except that the immunoaffinity matrix was not treated with solubilized ROS, i.e. had no added ABCA4. The counts in the controls, typically less than 10% of the test sample, were subtracted to determine the amount of specifically bound retinoid.

Displacement of N-Retinylidene-PE by N-Retinyl-PE—The extent to
which N-retinyl-PE can displace N-retinylidene-PE bound to ABCA4 was determined as follows. [3H]N-retinylidene-PE at a concentration of 10 μM was bound to immobilized ABCA4 in the presence of phospholipids as described above. The immunoaffinity matrix was suspended in 0.5 ml of column buffer containing various concentrations of N-retinylidene-PE. After 30 min, the matrix was washed and the bound labeled retinoid was subsequently extracted with organic solvent and quantified as outlined above. Similar experiments were carried out to determine the effect of all-trans-retinal on bound [3H]N-retinyl-PE.

Nucleotide-dependent Release of Retinoids from ABCA4—Immobilized ABCA4 containing bound retinoid was suspended in 0.5 ml of column buffer. ATP or other nucleotides was added to obtain the indicated final nucleotide concentration. The filtration device was rotated for 15 min at 4 or 22 °C, after which the beads were washed four times with 0.5-ml column buffer prior to extraction of the bound retinoid as described above.

RESULTS

Isolation of ABCA4 on a Rim 3F4-Sepharose Affinity Column—An immunoaffinity matrix consisting of the Rim 3F4 anti-ABCA4 monoclonal antibody coupled to Sepharose 2B was used to isolate ABCA4 from bovine ROS (3, 30). Fig. 1 shows a Coomassie Blue-stained gel and a Western blot of the ROS starting material (lane 1) and the fraction that selectively binds to the immunoaffinity matrix (lane 2). The bound fraction contained one major stained protein that migrated with an apparent molecular mass greater than 220 kDa. The Western blot labeled with the Rim 3F4 antibody confirmed that this major protein is ABCA4.

Retinoid Binding to ABCA4 as Measured by HPLC—All-trans-retinal is known to react with PE to form an equilibrium mixture of N-retinylidene-PE and free all-trans-retinal (13, 22). To determine which retinoid preferentially binds to ABCA4, all-trans-retinal was added to CHAPS-solubilized, immobilized ABCA4 in the presence of a mixture of DOPE, DOPC, and DOPS phospholipids. After removal of unbound material, the bound retinoid substrate was extracted with organic solvents and analyzed by HPLC. Fig. 2, A and B, shows an HPLC trace of the extracted retinoids measured at 450 and 368 nm, respectively. The major peaks were identified as protonated N-retinylidene-PE and free all-trans-retinal on the basis of their retention time (in relation to standards) and absorption spectra.

Quantitative analysis indicated that over 1 mol of total retinoid bound per mol of ABCA4 with N-retinylidene-PE comprising over 75% of the retinoid and all-trans-retinal the remaining portion (Table I). Addition of ATP released essentially all the bound N-retinylidene-PE and all-trans-retinal from ABCA4 (Table I). In the presence of DOPE alone, 0.33 mol of all-trans-retinal was extracted per mol of ABCA4 (Fig. 2C and Table I).

As part of this study, the binding of all-trans-retinol and N-retinyl-PE, the reduced form of N-retinylidene-PE, was studied. As shown in Fig. 2D and Table I, all-trans-retinol did not bind to ABCA4 even when 100 μM substrate was used in this study. In contrast, essentially 1 mol of N-retinyl-PE bound per mol of ABCA4 (Fig. 2E and Table I).

Several controls were carried out to assess the specificity of retinoid binding. In the absence of added substrate, no retinoids were detected by HPLC indicating that ABCA4 from dark-adapted ROS does not contain endogenously bound retinoid. Furthermore, no retinoid compounds were detected when all-trans-retinal was added to the Rim 3F4-Sepharose 2B matrix lacking ABCA4. This indicates that all-trans-retinal and N-retinylidene-PE do not bind to the immunoaffinity support.

Binding of N-Retinylidene-PE and All-Trans-Retinal to ABCA4—The affinity of ABCA4 for N-retinylidene-PE and all-trans-retinal was studied by determining the amount of retinoid bound as a function of increasing all-trans-retinal concentration. N-Retinylidene-PE and all-trans-retinal display conventional saturation binding curves with half-maximum binding occurring at all-trans-retinal concentrations of 2.1 and 1.8 μM, respectively (Fig. 3). Maximum binding approached 0.90 mol for N-retinylidene-PE compared with 0.27 mol for all-trans-retinal, values that are in general agreement with quantitative measurements at a single concentration of all-trans-retinal in Table I.

Binding of Radilabeled Retinoid to ABCA4—The large quantities of ROS and Rim 3F4-Sepharose required for HPLC analysis prompted us to develop a more sensitive radiolabel retinoid binding assay. In this procedure, [3H]all-trans-retinal was added to immobilized ABCA4 in the presence of a DOPE/DOPC/DOPS phospholipid mixture and the total amount of labeled retinoid bound to ABCA4 was measured after organic solvent extraction by scintillation counting. We first examined the binding of labeled retinoid by ABCA4 as a function of all-trans-retinal concentration. The binding curve and Scatchard analysis are shown in Fig. 4, A and B. The apparent dissociation constant for retinoid binding was estimated to be 5.4 μM, a value in close agreement with that estimated by the HPLC method. A linear relationship was observed by Scatchard analysis.

To determine whether the binding studies were carried out under equilibrium conditions, the time course for association and dissociation of retinoid from ABCA4 was investigated. Fig. 5A shows that ABCA4 bound a maximum amount of retinoid within the first minute at 4 °C. Once bound, no significant loss in retinoid occurred over a 60-min period (Fig. 5B).

Effect of Nucleotides on Retinoid Binding to ABCA4—As shown in Table I, the addition of ATP released bound N-retinylidene-PE and all-trans-retinal from ABCA4. We studied the effect of other adenine nucleotides using the radiolabel assay. Whereas ATP released essentially all the labeled retinoid (>95%) from ABCA4 at both 4 and 22 °C, ADP and AMP-PNP had relatively little effect (Table II). At 4 °C, 85% of the retinoid remained bound to ABCA4 in the presence of ADP and 79% in the presence of AMP-PNP. At 22 °C, 63% of the retinoid remained bound to ABCA4 in the presence of AMP-PNP. ATPases are known to hydrolyze AMP-PNP at a slow rate (31). The loss in retinoid observed at 22 °C and to a lesser extent at 4 °C may result from the partial hydrolysis of AMP-PNP by ABCA4 under these conditions.
Because guanine nucleotides have been reported to be present at high concentrations in ROS (32), we examined the effect of the guanine nucleotides, GTP, GDP, and GMP-PNP, on the release of [3H]retinoid from ABCA4. GTP, like ATP, released essentially all the retinoid, whereas GDP and the nonhydrolyzable GTP derivative, GMP-PNP, had little effect (Table II).

Displacement of N-Retinylidene-PE by N-Retinyl-PE—To determine whether N-retinylidene-PE and N-retinyl-PE bind to
Binding of N-retinylidene-PE and all-trans-retinal as a function of added all-trans-retinal concentration using the HPLC method. All-trans-retinal was added to ABCA4 in the presence of DOPE, DOPC, and DOPS phospholipids at the indicated concentration. Bound retinoid was extracted with organic solvent and the amount of N-retinylidene-PE (○) and all-trans-retinal (●) was determined by HPLC. Data are the average of two determinations with error bars indicating the range of measurements from the average value. The curves (solid line) were fitted as a simple binding curve with $K_d$ of 2.1 μmol/mol N-retinylidene-PE and $K_d$ of 1.8 for all-trans-retinal.

Table 1

| Retinoid added     | Phospholipid | ATP | Retinoid bound | Quantity |
|--------------------|--------------|-----|----------------|----------|
| All-trans retinal (50 μm) | PL           | 0   | N-Retinylidene-PE | 0.74 ± 0.13 (n = 3) |
| All-trans-retinal (50 μm) | PL           | 50  | N-Retinylidene-PE | 0.002 ± 0.001 (n = 2) |
| All-trans-retinal (50 μm) | PC           | 0   | N-Retinylidene-PE | 0.0 (n = 1) |
| All-trans-retinol (50 μm) | PL           | 0   | All-trans-retinal | 0.33     |
| All-trans-retinol (100 μm) | PC           | 0   | All-trans-retinol | 0.0 (n = 2) |
| All-trans-retinyl-PE (33 μM) | PL           | 0   | N-Retinyl-PE     | 1.07 ± 0.04 (n = 2) |

* Phospholipid (PL) refers to 45% DOPE, 45% DOPC; 10% DOPS. PC refers to 100% DOPC; n is the number of experiments. Values shown are ± S.D. for three separate experiments; Values ± range from average for two separate experiments.

Discussion

ABC transporters comprise one of the largest families of membrane transporters (33). To date 49 genes in the human genome are known to encode ABC transporters (nutrigene.4t.com/humanabc.htm). Mutations in a significant number of these genes cause a variety of severe diseases including cystic fibrosis, Tangier’s disease, familial intrahepatic cholestasis, hyperinsulinemic hypoglycemia, adrenoleukodystrophy, Zellweger syndrome, and sitosterolemia (34). Despite the importance of these membrane proteins in cell function, the physiological substrate is known for only a small number of ABC transporters. This is particularly true for the ABCA subfamily. In the case of ABCA4, previous ATPase assays and studies on abca4 knockout mice suggest that ABCA4 functions as a retinoid transporter, but the identity of this substrate and the mechanism of transport were not determined (12, 15).

Using both HPLC and radiolabeling techniques, we have examined the binding of various retinoids by immunoaffinity purified ABCA4 as an essential step in determining the function of ABCA4 in photoreceptor cells. When all-trans-retinal was added to ABCA4 in the presence of PE, N-retinylidene-PE bound to ABCA4 with high affinity at a level approaching 1 mol/mol of ABCA4. Binding was highly specific because N-retinylidene-PE did not bind to the immunoaffinity matrix in the absence of ABCA4.

In addition to N-retinylidene-PE, a smaller amount of all-trans-retinal (∼0.3 mol/mol of ABCA4) was extracted from immobilized ABCA4 when all-trans-retinal was added to solubilized ABCA4 in the presence of PE. This bound all-trans-retinal could arise from the partial dissociation of N-retinylidene-PE into all-trans-retinal and PE within the binding pocket. In this case 1 mol of ABCA4 binds 1 mol of retinoid, distributed between N-retinylidene-PE and all-trans-retinal at a ratio of about 3:1. Another possibility is that all-trans-retinal...
binds to a second site present in a subset of ABCA4 proteins. Alternatively, our preparations could contain another protein that is associated with ABCA4 and binds all-trans-retinal. However, such a protein would have to exhibit high affinity binding and ATP-dependent release of all-trans-retinal, similar to that observed for the interaction of N-retinylidene-PE with ABCA4. Additional studies are needed to resolve this issue.

The binding of N-retinylidene-PE to detergent-solubilized ABCA4 is strong with half-maximum binding occurring in the low micromolar range. This is consistent with the concentration of retinoid needed to stimulate the ATPase activity of ABCA4 (12, 13). The association of N-retinylidene-PE with ABCA4 is rapid, such that maximum binding is achieved within the first minute after the addition of all-trans-retinal. Furthermore, binding is stable at 4 °C with no observable dissociation of N-retinylidene-PE over a period of 1 h.

ABC transporters typically utilize energy derived from ATP hydrolysis to transport substrates across membranes (33). Therefore, we explored the effect of hydrolyzable and nonhydrolyzable nucleotides on bound N-retinylidene-PE with ABCA4. In contrast the nonhydrolyzable nucleotide triphosphate analogues, AMP-PNP and GMP-PNP, and nucleotide diphosphates, ADP and GDP, were largely ineffective, even at very high concentrations. These experiments strongly suggest that ATP or GTP binding and hydrolysis is required to release N-retinylidene-PE from ABCA4. ATP or GTP hydrolysis provides the energy needed to induce the protein conformational change that converts ABCA4 from its high affinity retinoid binding state to its low affinity state, thereby releasing bound N-retinylidene-PE. Although transport, itself, is not measured in this study, it is likely that such a conformational change induced by ATP hydrolysis may occur as part of the mechanism by which N-retinylidene-PE is transported across the disk membranes.

The binding of all-trans-retinol and N-retinyl-PE to ABCA4 was also investigated to further assess the specificity of ABCA4 for retinoids. All-trans-retinol did not bind to detergent-solubilized ABCA4 even at high concentrations, a result that is consistent with the inability of this compound to stimulate the ATPase activity of detergent-solubilized ABCA4 (13). On the other hand, N-retinyl-PE was bound by ABCA4 in stoichiometric amounts. Because N-retinyl-PE displaced N-retinylidene-PE from ABCA4, it is likely that these retinoids bind to
The same site on ABCA4. However, N-retinyl-PE displays different properties than N-retinylidene-PE. N-Retinyl-PE does not activate the ATPase activity of ABCA4 (13). Furthermore,
ATP and AMP-PNP both release N-retinyl-PE from ABCA4 at 22 °C, but not at 4 °C. This suggests that the simple binding of these nucleotide triphosphates to ABCA4 is sufficient to dissociate this retinoid from ABCA4 at the higher temperature.

Previous enzymatic studies have shown that ABCA4 exhibits basal and retinal stimulated ATPase activity that generates a double reciprocal plot consisting of parallel lines when the rate of ATP hydrolysis is measured as a function of ATP concentration (12, 13). This behavior has been interpreted to reflect an “uncompetitive” mode of activation or a double displacement mechanism (35). In this kinetic mechanism, ATP is proposed to first interact with ABCA4 to form a modified intermediate complex, but not free ABCA4, binds and hydrolyzes in NBD1 to generate a site accessible for the binding of all-trans-retinal (or N-retinylidene-PE). On the basis of these and mutagenesis studies, a complex model for ATP-dependent substrate transport has been proposed in which ATP is first bound and “partially” hydrolyzed in NBD1 to generate a site accessible for the binding of all-trans-retinal (or N-retinylidene-PE). This is followed by the transport of the retinoid and the binding and hydrolysis of a second molecule of ATP at NBD2 (35) to complete the cycle. Our studies question the validity of this proposed mechanism. Specifically, our results clearly show that ABCA4 binds N-retinylidene-PE with high affinity in the absence of ATP. Furthermore, previous studies have indicated that ATP binding and hydrolysis only occurs at NBD2 (36).

Our data suggest a mechanism in which the substrate, N-retinylidene-PE, first binds to a high affinity site in ABCA4, presumably within the membrane domain. Subsequently, ATP is bound and hydrolyzed in NBD2 inducing a protein conformational change that converts the high affinity retinoid binding site to a low affinity site. This causes the release of retinoid from this site as part of the transport mechanism. The conformational change induced by ATP hydrolysis may involve the dimerization of NBD1 and NBD2 as proposed for P-glycoprotein (37). Our finding that N-retinylidene-PE binds to ABCA4 in the absence of ATP is consistent with earlier studies showing that drugs bind to P-glycoprotein in the absence of ATP binding and hydrolysis (38).

The methodology used in this study has applications for studying substrate binding to other membrane transporters. It may be particularly useful in resolving the uncertainties related to the putative function of ABCA1 as a mediator of cholesterol and phospholipid transport (39).

In summary we provide the first direct biochemical evidence for the binding of N-retinylidene-PE to ABCA4. This result, together with the finding that ATP binding and hydrolysis promotes the dissociation of N-retinylidene-PE from ABCA4, provides strong support for the role of ABCA4 in the transport of N-retinylidene-PE across the photoreceptor outer segment disk membranes.

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