The 220-kDa Rim Protein of Retinal Rod Outer Segments Is a Member of the ABC Transporter Superfamily*

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Outer segments of mammalian rod photoreceptor cells contain an abundantly expressed membrane protein that migrates with an apparent molecular mass of 220 kDa by SDS-gel electrophoresis. We have purified the bovine protein by immunoaffinity chromatography, determined its primary structure by cDNA cloning and direct peptide sequence analysis, and mapped its distribution in photoreceptors by immunocytochemical and biochemical methods. The full-length cDNA encodes a 2280-amino acid protein (calculated molecular mass of 257 kDa) consisting of two structurally related, tandem arranged halves. Each half consists of a hydrophobic domain containing six putative transmembrane segments followed by an ATP-binding cassette. A data base homology search showed that the rod outer segment 220-kDa protein is 40–50% identical in amino acid sequence to the ABC1 and ABC2 proteins cloned from a mouse macrophage cell line. Photoaffinity labeling with 8-azido-ATP and nucleotide inhibition studies confirmed that both ATP and GTP bind to this protein with similar affinities. Concanavalin A labeling and endoglycosidase H digestion indicated that the rod outer segment protein contains at least one carbohydrate chain. Immunocytochemical and biochemical studies have revealed that the 220-kDa glycoprotein is distributed along the rim region and incisures of rod outer segment disc membranes. From these studies we conclude that the 220-kDa glycoprotein of bovine rod outer segment disc membranes or Rim ABC protein is a new member of the superfamily of ABC transporters and is the mammalian homolog of the frog photoreceptor rim protein.

Outer segments of rod and cone photoreceptor cells are highly specialized subcellular structures that serve as the site for phototransduction. The rod outer segment (ROS) consists of a highly ordered axial array of over 500 discs surrounded by a separate plasma membrane. Each disc is made up of two closely spaced lamellar membranes circumscribed by a hairpin loop or rim region (1). The continuous disc membrane encloses a compartment called the disc lumen or intradiscal space. The perimeter of the disc is interrupted by one or more incisures that penetrate toward the center of the disc. Filamentous structures extend from the rim region of the discs to adjacent discs and to the plasma membrane (2–4).

The protein composition of ROS disc membranes differs from that of the plasma membrane (5). Although both membranes contain rhodopsin as the major membrane protein, the cGMP-gated channel (6), the Na/Ca-K exchanger (7), and the GLUT-1 glucose transporter (8) are predominantly, if not exclusively, present in the ROS plasma membrane. Guanylate cyclase (9), the peripherin/rds-rom-1 complex (10–12) and a high molecular mass (220–290 kDa) glycoprotein (5, 13) are present in disc membranes. The peripherin/rds-rom-1 complex in mammalian ROS and the 290-kDa glycoprotein or rim protein of frog ROS each constitute 3–4% of the total ROS membrane protein and are localized to the rim region and incisures of rod and cone disc membranes (10–15). The frog rim protein has been reported to undergo a light-activated phosphorylation reaction (16); however, the role of this reaction in photoreceptor cell function remains to be determined. An abundant 220-kDa membrane glycoprotein is also present in mammalian ROS disc membranes (17, 18). Photoaffinity labeling studies indicate that this protein specifically binds ATP and GTP (19). Although the distribution of the 220-kDa protein in mammalian ROS disc membranes has not yet been determined, it is generally thought that this protein may be the homolog of the frog 290-kDa rim protein.

The function of the frog rim protein is not currently known. Its large size and distribution along the rim region of ROS discs, however, has led to the suggestion that it may constitute the filament-like structures that link adjacent discs together and/or connect the discs to the plasma membrane (2, 4). As a first step in defining the role of the rim protein in ROS structure and function and its possible involvement in retinal degenerative diseases, we have cloned and sequenced the cDNA for the bovine 220-kDa protein and studied its molecular properties and cellular distribution. In this paper we report that the bovine 220-kDa glycoprotein is a novel member of the ATP-binding cassette (ABC) transporter superfamily. Like the frog rim protein, it is localized to the rim region of bovine ROS disc membranes.

EXPERIMENTAL PROCEDURES

Materials—A λZAPII oligo(dT)-primed retinal cDNA library was a generous gift of W. Baehr, and the pcDNAII random-primed cDNA library was made from bovine retinal poly(A) RNA. Monoclonal antibodies PMc 1D1 and PMs 4B2 against the α- and β-subunits of the bovine cGMP-gated channel, respectively, and PM 2D9 against the Na/Ca-K exchanger have been reported (6, 20, 21).

Preparation of ROS and Disc Membranes—ROS were isolated under dim red light from freshly dissected or previously frozen retinas by continuous sucrose gradient centrifugation; ROS membranes were iso-
lated by hypotonic lysis of ROS as described previously (5). The ROS membranes resuspended in 0.01 M Tris-HCl, pH 7.4, at a protein concentration of 8–10 mg/ml were either used immediately or stored at −70 °C in light-tight vials. Disc membranes were separated from ricin-gold-dextran labeled plasma membranes by mild trypsin digestion and subsequent sucrose gradient centrifugation (5).

Preparation and Purification of Monoclonal and Polyclonal Antibodies—Monoclonal antibody Rim 3F4 against the bovine 220-kDa disc rim protein was generated from a BALB/c mouse that had been repeatedly immunized with a partially purified preparation of the 220-kDa protein by standard procedures (22). The immunogen was prepared by solubilizing SDS-stained center disk of bovine retinas at 4 °C in light-tight vials. Disc membranes were separated from ricin-gold-dextran labeled plasma membranes by mild trypsin digestion and subsequent sucrose gradient centrifugation (5).

Immunofluorescence Purification of the 220-kDa Rim Protein—The Rim 3F4 antibody was coupled to CNBr-activated Sepharose 2B at a concentration of 2 mg of protein/ml of packed beads (20). The 220-kDa rim protein was purified by incubating 1 mg of ROS membranes, solubilized in 0.5 ml of 18 mM CHAPS or 1% Triton X-100 in 0.02 mM Tris-HCl, pH 7.4, 5 mM MgCl₂, with 0.2 ml of Rim 3F4-Sepharose 2B beads at 4 °C for 1 h. The unbound fraction was isolated by low speed centrifugation through a Superfuge filter unit (Millipore Corp.); the beads were then washed twice with 0.5 ml of solubilization buffer. Finally, the bound protein was released from the beads with 0.1 ml of solubilization buffer containing 0.1 mg/ml 3F4 peptide.

Endoglycosidase Hf Treatment and 8-Azido-[35S]ATP Labeling of the 220-kDa Rim Protein—For endoglycosidase treatment, 100 μl of ROS membranes solubilized in 10 mM CHAPS, 0.02 mM Tris-HCl, pH 7.4, 5 mM MgCl₂ was treated with endoglycosidase Hf (New England Biolabs) according to the manufacturer’s instructions and subjected to SDS-polyacrylamide gel electrophoresis and Western blotting.

The 220-kDa rim protein was photoaffinity labeled with 8-azido-[35S]ATP and automated fluorescent sequencing (Licor, Inc.). Northern Blot Analysis—For Northern blots, 10 μg of total RNA from bovine retinas were hybridized at 65 °C for 2 h in Rapid-hyb buffer (Amersham) with 32P-labeled probes synthesized by random-priming (Pharmacia T7Quick Prime Kit). Probes were derived from an oligo(dT)-primed bovine retinal cDNA library. Overlapping clones were isolated and sequenced in both directions.

Peptide Sequencing and Amino Acid Analysis—For N-terminal peptide sequencing and amino acid analysis, 100–200 μg of the immunoaffinity-purified 220-kDa protein protein was carried out as described previously (5). For Western blotting, proteins were electrophoretically transferred onto Immobilon membranes using the buffer system of Towbin et al. (29). The membranes were labeled with the Rim 3F4 or PrimT1 antibody for 1 h at room temperature followed by sheep anti-mouse or donkey anti-rabbit IgG-Fab'₂ (Jackson ImmunoResearch) conjugated to horseradish peroxidase. Alternatively, membranes were treated with monoclonal 3F4 antibody conjugated to horseradish peroxidase (Sigma). Immunolabeling for Light and Electron Microscopy—For light microscopy, cryosections of bovine retina were labeled with Rim 3F4 or PrimT1 antibodies followed by goat anti-mouse IgG or goat anti-rabbit IgG conjugated to gold-dextran (8). For electron microscopy, glutaraldehyde-fixed, LR-White resin embedded bovine retina sections were labeled with the purified RimT1 antibody overnight and subsequently labeled with 10-nm diameter goat anti-rabbit IgG gold (12).

RESULTS

Monoclonal and Polyclonal Antibodies to the 220-kDa ROS Membrane Protein—Monoclonal antibody Rim 3F4 and polyclonal antibody PrimT1 were used as probes to clone, characterize, and localize the major 220-kDa protein of bovine ROS.
membranes. The specificity of these antibodies is shown by Western blotting in Fig. 1. Both antibodies labeled a single protein having an apparent molecular weight of 220,000 in both ROS membranes and the flow-through (unbound fraction) obtained when Triton X-100 solubilized ROS membranes were passed through a DEAE anion exchange column. The labeled protein co-migrated with the prominent Coomassie Blue-stained 220-kDa protein present in both samples. In contrast, monoclonal antibodies PMe 2D9 against the 230-kDa Na/Ca-K exchanger (21) and PMs 4B2 against the 240-kDa β-subunit of the cGMP-gated channel (PMs 4B2) for comparison. The 220-kDa protein was present in both fractions; the exchanger and channel were only present in the ROS membrane fraction.

Cloning and Primary Structure of the 220-kDa Protein—The 220-kDa protein was cloned by initially screening an oligo(dT)-primed bovine retinal cDNA expression library with the PrimT1 antibody. Of the six cDNA clones isolated in the initial screen, the longest clone (λ2) was 1.8 kb in length and had a polyadenylation signal and a poly(A) tail. Rescreening of the oligo(dT)-primed library and a random primed retinal cDNA library with oligonucleotide probes resulted in the isolation of several overlapping clones (Fig. 2). Upstream clone (p114) contained an ATG start codon with an adjacent upstream Kozak consensus sequence (31). The nucleotide sequence immediately downstream from this ATG coded for an amino acid sequence that was identical to that determined by direct N-terminal sequencing of the isolated 220-kDa protein (see below).

The overlapping clones contained an open reading frame of 6843 base pairs that encodes a protein of 2280 amino acids having a calculated molecular mass of 257,000 daltons (Fig. 3). The predicted amino acid sequence consists of two structurally related, tandemly arranged halves: each half of the protein has a cluster of hydrophobic segments followed by a conserved ATP-binding cassette (32). Hydropathy profiles (33) predict at least 12 transmembrane segments, six in each half of the protein. Fourteen consensus sequences for N-linked glycosylation are present (Fig. 3).

Data base homology searches indicate that the ROS 220-kDa protein is a member of the ABC transporter superfamily. Overall, it is 40–50% identical in amino acid sequence to the ABC1 and ABC2 proteins from a mouse macrophage cell line (34) and 37% identical to ABC-C, a putative transporter recently cloned from a human medullary thyroid carcinoma cell line (35). The ABC-binding domains of these proteins display an even higher degree of identity (>60%), particularly within the two short stretches of the Walker motifs and the active transport signature located upstream of the Walker B pattern (32). The ROS 220-kDa protein is more distantly related to other well studied members of the ABC transporter superfamily such as the cystic fibrosis transregulator and the multi-drug resistance protein (MDR1 P-glycoprotein), showing a sequence identity of 20–22% overall and 20–26% for the ABC domains. A 43% sequence identity is observed between the N- and C-terminal ABC domains of the bovine ROS 220-kDa protein, a value similar to that between the two cassettes within ABC1 and MDR1 P-glycoprotein. However, a high degree of conservation is found for the C-terminal ABC domain from different mammalian ROS 220-kDa proteins; for example, the bovine and human proteins are 94% identical in this region.

Northern Blot Analysis of Retinal RNA—The size of the mRNA transcripts for the 220-kDa protein was estimated by Northern blotting. As shown in Fig. 4, an EcoRI/SacI fragment from clone p41 (see Fig. 2) hybridized to transcripts of 9.5 and 6.8 kb in length. An upstream fragment from clone p55 gave a similar labeling pattern (data not shown).

Peptide Sequencing and Amino Acid Composition of the 220-kDa Protein—Peptide sequences of the 220-kDa ROS protein were obtained to confirm that the cDNA clones code for the protein sequence of the major 220-kDa protein of bovine ROS membranes. The N-terminal 18 amino acids of the immunoaffinity purified 220-kDa protein was found to be identical to a sequence derived from clone p114 and immediately downstream from a methionine (Fig. 3). Mild trypsin digestion of the 220-kDa protein in ROS membranes followed by purification on a Rim 3F4 antibody affinity column resulted in two large peptide fragments having apparent molecular masses of 120 and 115 kDa (see Fig. 8). The N-terminal sequence of the 120-kDa fragment was identical to the N-terminal sequence of the full-length 220-kDa, whereas the sequence of the 115-kDa fragment was identical to a sequence starting at position 1311, close to the middle of the 220-kDa protein (Fig. 3). These results indicate that mild trypsin digestion cuts the 220-kDa...
protein essentially in half, producing tryptic fragments corresponding to two homologous domains. Trypsin digestion has also been reported to split MDR1 P-glycoprotein into two homologous halves (36).

In addition, nine smaller peptide fragments were derived from trypsin and/or endoproteinase Lys-C digestion of the purified, detergent-solubilized 220-kDa protein. The N-terminal sequences of these peptides were identical to sequences found within the 220-kDa protein sequence deduced from cDNA cloning (Fig. 3). The sequence of peptide number 21 was of particular interest. Although 36 of the 37 amino acids of this peptide correspond to positions 1564–1600 of the full-length 220-kDa protein sequence, the amino acid at position 23 of the peptide sequence (position 1586 of the 220-kDa sequence) could not be identified. Since this residue is an Asn that is part of a N-linked glycosylation consensus sequence, it is likely that this residue is covalently modified with an oligosaccharide chain, and therefore, not identifiable by sequencing.

Further evidence that the immunoaffinity purified 220-kDa protein is the same as the protein encoded by the cloned cDNA was obtained from amino acid analysis. In general, the experimentally determined amino acid composition of the purified 220-kDa protein is within 15% of the amino acid composition determined from the deduced protein sequence (data not shown).

![ABC Transporter of Retinal Rod Outer Segments](http://www.jbc.org/)

**Fig. 3.** Amino acid sequence of the 220-kDa bovine ABC transporter of ROS. The two putative ATP-binding cassettes (double underline) and hydrophobic segments (single underline) predicted to be potential transmembrane segments by hydropathy plots are indicated. N-terminal peptide sequences derived from the 220-kDa protein or proteolytic fragments of the 220-kDa protein are indicated by a single overtype line. The 115- and 120-kDa fragments were obtained by trypsin digestion of the 220-kDa protein in ROS membranes. Peptides 4 and 5 were obtained from tryptic digestion of the purified, detergent solubilized 220-kDa protein, while peptide 25 was isolated from endoproteinase Lys-C digestion on the 120-kDa tryptic fragment. All other peptides were obtained by endoproteinase Lys-C digestion of the 115-kDa fragment. The 11-amino acid epitope for monoclonal antibody Rim 3F4 is also indicated. Consensus sequences for N-glycosylation are denoted with an asterisk (*)

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fraction and the bound fraction (lane a) Coomassie Blue-stained 220-kDa protein present in the ROS membrane. The electrophoretically transferred to Immobilon membranes and labeled with 8% polyacrylamide gel and either stained with Coomassie Blue (endoglycosidase-treated). Western blots were labeled with the Rim 3F4 antibody (lane b), detergent-solubilized ROS membranes (lane a) and immunoaffinity purified preparations (lane c). The unbound fraction was collected and the bound fraction was subsequently eluted with the 3F4 peptide. The Coomassie Blue-stained 220-kDa protein present in the ROS membrane fraction (lane a) and the bound fraction (lane c) was labeled by the Rim 3F4 antibody and concanavalin A; oligomers of rhodopsin were also labeled with concanavalin A in ROS membranes (lane a) and in the unbound fraction from the affinity column (lane b). The immunoaffinity purified 220-kDa protein fraction, however, appears to be largely free of rhodopsin.

Endoglycosidase H digestion was carried out to further evaluate the extent to which the 220-kDa protein is glycosylated. As shown in Fig. 5B, only a slight increase in mobility of the 220-kDa protein was observed after treatment with endoglycosidase H. These results indicate that the 220-kDa protein contains at least one N-linked oligosaccharide chain, but it is unlikely to be highly glycosylated. Photoaffinity Labeling of the 220-kDa Protein with 8-Azido-(α-32P)ATP—Photoaffinity labeling was carried out to determine if the putative ATP-binding domains of the 220-kDa protein bind nucleotides. As shown in Fig. 6A, 8-azido-(α-32P)ATP covalently labeled the 220-kDa protein in both ROS membranes (lane a) and immunoaffinity purified preparations (lane c). Monomeric and oligomeric forms of rhodopsin were also labeled with concanavalin A in ROS membranes (lane a) and in the unbound fraction from the affinity column (lane b). The immunoaffinity purified 220-kDa protein fraction, however, appears to be largely free of rhodopsin.

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The 220-kDa Protein in Bovine Rod Outer Segments—The 220-kDa protein could be efficiently purified from detergent-solubilized ROS by immunoaffinity chromatography. As shown in Fig. 5A, the 220-kDa protein was quantitatively adsorbed to a Rim 3F4-Sepharose column and eluted with an 11 amino acid peptide corresponding to the Rim 3F4 epitope localized near the C terminus of the protein (see Fig. 3). No other prominent proteins in the immunoaffinity purified sample were detected on SDS-polyacrylamide gels stained with Coomassie Blue.

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220-kDa protein is an ABC transporter capable of binding both ATP and GTP.

Cellular and Subcellular Distribution of the 220-kDa Protein in Retina—The distribution of the 220-kDa protein in retina tissue was examined using immunocytochemical labeling methods. The Rim 3F4 antibody labeled the outer segments of rod, but not cone, photoreceptor cells in bovine retinal cryosections (Fig. 7A). Weak staining was observed in the inner segment layer, but no staining was detected in other retinal cells. A similar pattern of labeling was observed for rat retina and when the PrimT1 polyclonal antibody was used as primary antibody (data not shown). Labeling was specific since addition of excess 3F4 peptide inhibited Rim 3F4 antibody labeling (Fig. 7B).

The subcellular distribution of the 220-kDa protein in ROS was studied using immunogold labeling methods for electron microscopy. When LR White resin embedded retinal sections were labeled with the PrimT1 antibody, immunogold particles were found to be distributed along the periphery and incisures of the ROS where the rim region of the discs are in close proximity to the plasma membrane (Fig. 7, C and D). This labeling pattern is similar to that described by Papamaster et al. (13) for a 290-kDa rim protein in frog outer segments. The Rim 3F4 monoclonal antibody did not label these sections presumably due to inaccessibility of the 3F4 epitope in resin embedded samples.

Since postembedding immunogold labeling studies could not unambiguously establish whether the 220-kDa protein was localized to the rim region of disc membranes or the plasma membrane, disc membranes were isolated from trypsin-treated ROS (5) for Western blot analysis. As shown in Fig. 8, the Rim 3F4 monoclonal antibody intensely labeled the 220-kDa protein in ROS membranes and its 115-kDa tryptic fragment in highly purified disc membranes. A less intensely labeled 120-kDa fragment was also observed in the disc membrane fraction; this fragment is most likely derived from trypsin cleavage at a secondary site within the 220-kDa. Absence of plasma membrane contamination in the disc fraction was verified in labeling studies employing a plasma membrane specific cGMP-gated channel antibody (6). As shown in Fig. 8, the antichannel antibody PMc 1D1 labeled the 63-kDa α-subunit of the cGMP-gated channel in ROS membranes, but no labeling of the trypsinized channel subunit (6) was observed confirming its absence in the disc fraction. The immunocytochemical and biochemical studies taken together indicate that the bovine 220-kDa protein is preferentially, if not exclusively, localized to the rim and incisures of ROS discs.

DISCUSSION

Although the high molecular weight rim protein had been shown to be a major membrane protein of ROS almost 20 years ago (13), no detailed studies have been reported on its structural properties since this time. In this study we have purified the bovine 220-kDa rim protein, mapped its distribution in ROS, and determined its primary structure by cDNA cloning and direct peptide sequencing.
THE LARGE SIZE OF THE RIM PROTEIN RELATIVE TO MOST OTHER ABC TRANSPORTERS

The function of the rim protein is not currently known. On the basis of its large size and location along the disc rims, it had been postulated that the rim protein might be directly involved in the active extrusion of drugs from mammalian cells (41), and cystic fibrosis transregulator, which functions as a chloride channel and has been linked to cystic fibrosis (42).

Fig. 8. Localization of the 220-kDa protein to the disc membranes of ROS. Disc membranes were separated from the ricin-gold-dextran-labeled plasma membrane after mild trypsin treatment by sucrose gradient centrifugation (5). Untreated ROS membranes (lane a) and trypsin-treated disc membranes (lane b) were subjected to SDS-polyacrylamide gel electrophoresis. Gels were stained with Coomassie Blue (CB) and Western blots were labeled with the Rim 3F4 antibody against the 220-kDa protein and the PMc 1D1 antibody against the α-subunit of the cGMP-gated channel. The 220-kDa protein is cleaved by trypsin to 120- and 115-kDa fragments as visualized by Coomassie Blue staining; the latter is intensely stained with the Rim 3F4 antibody in isolated disc membranes. Absence of PMc 1D1 labeling in the disc fraction confirms that the disc fraction is not contaminated with plasma membrane from ROS as previously reported (6).

The bovine ROS 220-kDa glycoprotein has been localized to the rim region and incisures of bovine ROS disc membranes by a combination of immunogold labeling and biochemical techniques. On this basis we conclude that this protein is the mammalian homolog of the 290-kDa frog rim protein first reported by Papermaster et al. (13). However, a notable difference in labeling of photoreceptors is apparent. Whereas a polyclonal antibody to the frog rim protein labels both frog rod and cone outer segments (15), antibodies against the bovine rod protein used in the present study labels only outer segments of rod photoreceptors. It appears that either mammalian cone photoreceptors do not express a rim protein, or more likely, the cone rim protein is immunochecemically different from the rod rim protein and may be encoded by a separate gene. Alternatively, the rim protein in cones may be less abundantly expressed or less accessible such that cone labeling is not detected using the reagents employed in this study.

The Rim 3F4 monoclonal antibody has been used to purify the rim protein from Triton X-100 and CHAPS solubilized ROS in a single step. Analysis of the purified protein by SDS-gel electrophoresis indicates that no other intensely stained proteins are present in this preparation. Furthermore, the amino acid composition of the purified rim protein is in close agreement with the composition calculated from its sequence. On the basis of these results, it is likely that the rim protein is composed of a single type of subunit and, at least in detergent solution, is not tightly associated with other proteins in stoichiometric amounts. It remains to be determined if the rim protein exists as a single polypeptide or as a protein complex consisting of several identical subunits.

Primary structural analysis indicates that the rim protein is a member of the superfamily of ABC transporters. Like other eukaryotic ABC transporters (37), the rod rim protein consists of two structurally related halves, each half consisting of a hydrophobic domain with multiple predicted membrane spanning segments followed by a highly conserved ATP-binding cassette. The presence of nucleotide binding folds is consistent with earlier photoaffinity labeling studies (19) and studies carried out here, indicating that the rim protein specifically binds both ATP and GTP. The large size of the rim protein (2264 amino acids; calculated $M_r = 257,000$) makes this protein one of the largest ABC transporters reported to date.

Current models of most eukaryotic ATP transporters have 12 transmembrane segments, six in each hydrophobic domain, and both the ATP-binding cassettes and the N and C terminus localized on the cytoplasmic side of the membrane (37). At least 12 membrane spanning segments (see Fig. 3) are predicted for the rim protein from hydrophathy plots (33). The rim protein is glycosylated like other well characterized ABC transporters. However, the number of N-linked oligosaccharide chains is likely to be small since digestion with endoglycosidase H has only a minor effect of the mobility of the rim protein on SDS gels. Although the site(s) of glycosylation has yet to be determined directly, the inability to detect Asn-1586 in peptide number 21 by direct peptide sequencing strongly suggests that this residue, which is part of a consensus sequence for N-linked glycosylation, is covalently bound to an oligosaccharide chain. Therefore, one predicts that this segment resides on the intradiscal or lumen side of the disc membrane. On the basis of these studies, we propose a working model for the membrane organization of the Rim ABC protein as shown in Fig. 9. A novel aspect of this model is the presence of a large intradiscal loop between each of the first two membrane spanning segments for each half of the protein. Such segments are not predicted in models for other ABC transporters such as P-glycoprotein and cystic fibrosis transregulator and contribute to the unusually large size of the Rim ABC protein relative to most other ABC transporters. Relatively large intradiscal segments are also found in the peripherin/rds and rom-1 subunits and have been suggested to be involved in the formation and stabilization of the highly curved rim region of the disc membranes (11, 38).

The rod rim protein is most closely related to ABC1 and ABC2 transporters of macrophages (34) as shown in the dendrogram comparing the C-terminal nucleotide-binding domains of representative ABC transporters (Fig. 10). Other related proteins include an ABC-C protein recently cloned from a human medulary thyroid carcinoma cell line (35), the NodI protein involved in nodulation of plant roots by Rhizobium (39), and the YLH4 putative transporter from Caenohabditis elegans (40). The rim protein is more distantly related to MDR1 P-glycoprotein, involved in the active extrusion of drugs from cells (41), and cystic fibrosis transregulator, which functions as a chloride channel and has been linked to cystic fibrosis (42).

The function of the rim protein is not currently known. On the basis of its large size and location along the disc rims, it had...
To our knowledge, the Rim ABC protein is the first ABC transporter. The relationship of the C-terminal ATP-binding cassettes of the proteins was calculated by the unweighted pair group method with arithmetic mean (PC Gene, Inteligenetics, Inc.). Distances along the horizontal axis are proportional to the differences between the sequences. The following were compared: Rim ABC, bovine ROS 220-kDa glycoprotein; YLH4, putative ABC transporter in ced–7 locus of C. elegans; NODI, involved in root nodulation by Rhizobium (39); TAP1, mouse antigen-processing-associated transporter (51); MDR1, human multi-drug resistance transporter (41); HLYB, hemolysin exporter in E. coli (52); CFTR, human cystic fibrosis transmembrane conductance regulator (42); ABC1 and ABC2, from a mouse macropage cell line (34); and ABC-C, from a human medullary thyroid carcinoma cell line (35).

been suggested that this protein may constitute the filamentous structures that link adjacent discs together and/or connects discs to the plasma membrane (2, 4). The finding that the Rim ABC protein is a member of the ABC transporter superfamily makes this less likely. However, it could play a role in anchoring such filaments to the disc rim membrane.

In a number of cases, ABC transporters have been shown to be involved in the active transport of a variety of hydrophobic molecules across membranes including drugs (43), lipids (44), metabolites (45), and peptides (46, 47). The mouse MDR2 P-glycoprotein and its human counterpart (MDR3) have also been reported to function as a flippase promoting the transfer of phosphatidylcholine from the inner to the outer leaflet of the plasma membranes of hepatocytes (48). It is possible that the Rim ABC protein may also function in the transport of specific metabolites (45), and peptides (46, 47). The mouse MDR2 P-glycoprotein and its human counterpart (MDR3) have also been reported to function as a flippase promoting the transfer of phosphatidylcholine from the inner to the outer leaflet of the plasma membranes of hepatocytes (48). It is possible that the Rim ABC protein may also function in the transport of specific metabolites (45), and peptides (46, 47).

Some ABC transporters have been reported to be involved in morphological processes. The ABC1 protein of mouse macrophages, and on the basis of its size and location, it appears to be the mammalian homolog of the frog rim protein. To our knowledge, the Rim ABC protein is the first ABC transporter found in vertebrate retina.
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