

The retinal pigment epithelium: a versatile partner in vision

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SUMMARY

The retinal pigment epithelium (RPE) is a monolayer of cuboidal cells that lies in close association with the rod and cone photoreceptors. This epithelium has diverse features, three of which are discussed in some detail in this review, namely the daily phagocytosis of rod and cone outer segment fragments that are shed from their distal ends; the uptake, processing, transport and release of vitamin A (retinol) and some of its visual cycle intermediates (retinoids); and some of the aspects of its apical and basolateral membrane polarity that are the reverse of most other epithelia.

Phagocytosis takes place at the apical surface via membrane receptor-mediated processes that are not yet well defined. Retinol uptake occurs at both the basolateral and apical surfaces by what appear to be separate receptor-mediated processes. The release of a crucial retinoid, 11-*cis* retinaldehyde (11-*cis* retinal), occurs solely across the apical membrane. Delivery of retinol across the basolateral membrane is mediated by a retinol binding protein (RBP) that is secreted by the liver as a complex with retinol (vitamin A). Within the cell, retinol and its derivatives are solubilized by intracellular retinoid binding proteins that are selective for

retinol (cellular retinol binding protein, CRBP) and 11-*cis* retinoids (cellular retinal binding protein, CRALBP). Release of 11-*cis* retinal across the apical membrane and re-uptake of retinol from the photoreceptors during the visual cycle is promoted by an intercellular retinoid binding protein (IRBP).

Na,K-ATPase, the membrane-integrated enzyme required to set up the ion gradients that drive other ion transporters, is largely localized to the apical membrane. This is the reverse of most epithelia. The RPE expresses the enveloped viral G protein and hemagglutinin on its basolateral and apical surface, respectively and does not appear to possess a general scheme for reversal of membrane protein polarity. Therefore possible alternative mechanisms for this reversal in Na,K-ATPase polarity are discussed. They include unique domains in the primary amino acid sequence of Na,K-ATPase subunits, cytoskeletal elements and components of the extracellular matrix. The precise mechanism remains unresolved.

Key words: retinal pigment epithelium, vitamin A, retinoids, phagocytosis, Na,K-ATPase

INTRODUCTION

The retinal pigment epithelium (RPE) is a multifunctional and indispensable component of the vertebrate retina. It is strategically placed between the large-bore capillary bed of the choroidal layer of the eye and the photoreceptors of the neurosensory retina. By virtue of this location, it forms part of the blood retinal barrier via its tight junctions (Peyman and Bok, 1972), the other barrier being at the level of retinal blood vessels. Additionally, the RPE is responsible for the net movement of ions and water in an apical to basal direction (retinal to choroidal), thereby maintaining the neurosensory retina in a proper state of dehydration and optical clarity. It absorbs stray light that would otherwise degrade the visual image and plays an adhesive role that aids in the attachment of the retina to the choroidal layer. Among the more fascinating aspects of RPE function are the uptake, processing and transport of retinoids that subserve vision, and the intermittent phagocytosis of detached distal portions of the rod and cone light-sensitive organelles

(outer segments), thereby subserving the renewal of each of these organelles by new membrane assembly at their proximal ends.

Early in the life of the individual the activities of the RPE begin. It continues to carry out these functions until it is lost through disease or death of the host. Under normal circumstances the RPE, once differentiated, does not renew itself by cell division. Thus it must remain viable and perform its array of functions throughout the lifetime of the individual, a period that can exceed 100 years in humans.

The RPE is remarkable not only in its versatility but also because it has a feature that makes it virtually unique among epithelia, at least in the view of those investigators who have an interest in epithelial cell polarity. Most of the surface Na,K-ATPase in the RPE is expressed on the apical membrane domain (Steinberg and Miller, 1973; Bok, 1982), a feature that is shared only by the epithelium of the choroid plexus (Quinton et al. 1973), a tissue with which it has some developmental kinship. What follows is a brief review of

several of the RPE functions that have held the attention of our laboratory.

PHAGOCYTOTIC PROPERTIES OF THE RETINAL PIGMENT EPITHELIUM

The outer segments of rods and cones are closely associated with the RPE via villous and pseudopodial attachments. These three cell types (rods, cones and RPE) interact in several ways, one of the most interesting of which involves a limb of the photoreceptor outer segment renewal process, namely RPE phagocytosis of distal portions of rod and cone outer segments (for a review, see Bok, 1985). The definitive evidence for this process relative to rod photoreceptors was gathered by tissue autoradiography following the injection of radioactive amino acids into animals. Radioactive membranous discs of the labeled rod outer segments were intermittently shed and then phagocytosed by the RPE following their displacement from the proximal to distal end of the outer segment by membrane assembly (Young and Bok, 1969). In some animals, such as the rat, this process exhibits a strong circadian rhythm (LaVail, 1976). Although the details of this interaction remain to be worked out, recent evidence from *Xenopus* suggests that the

circadian clock for this process resides in the photoreceptor cell itself (Cahill and Besharse, 1992). However the photoreceptor is not solely in control of disc shedding, since it has been observed that close approximation of photoreceptors and RPE is required before the process can take place (Williams and Fisher, 1987). Furthermore, the rat RPE also shows a circadian rhythm with respect to the processing of this phagocytic load. Lysosomal fusion can be triggered by both light and circadian mechanisms (Bosch et al., 1993). Following ingestion of an outer segment fragment (the fragment thereby becoming a phagosome), the lysosomal response occurs in two steps (Fig. 1A and B). First, small lysosomes fuse with the outer segment fragment to form phagolysosomes. Then larger lysosomes, formed by the fusion of smaller precursors, interact with the phagolysosomes through what appear to be bridge-like structures. This process was recently followed in detail by rapid freezing of rat RPE at various times before and after light onset, and monitoring of the fusion of lysosomes with cathepsin D antibodies (Bosch et al., 1993).

Recognition signals that subserve outer segment shedding and phagocytosis also remain unresolved (for a review, see Bok, 1988). It is believed that this process involves receptor-ligand interactions, but these membrane components have not been isolated.

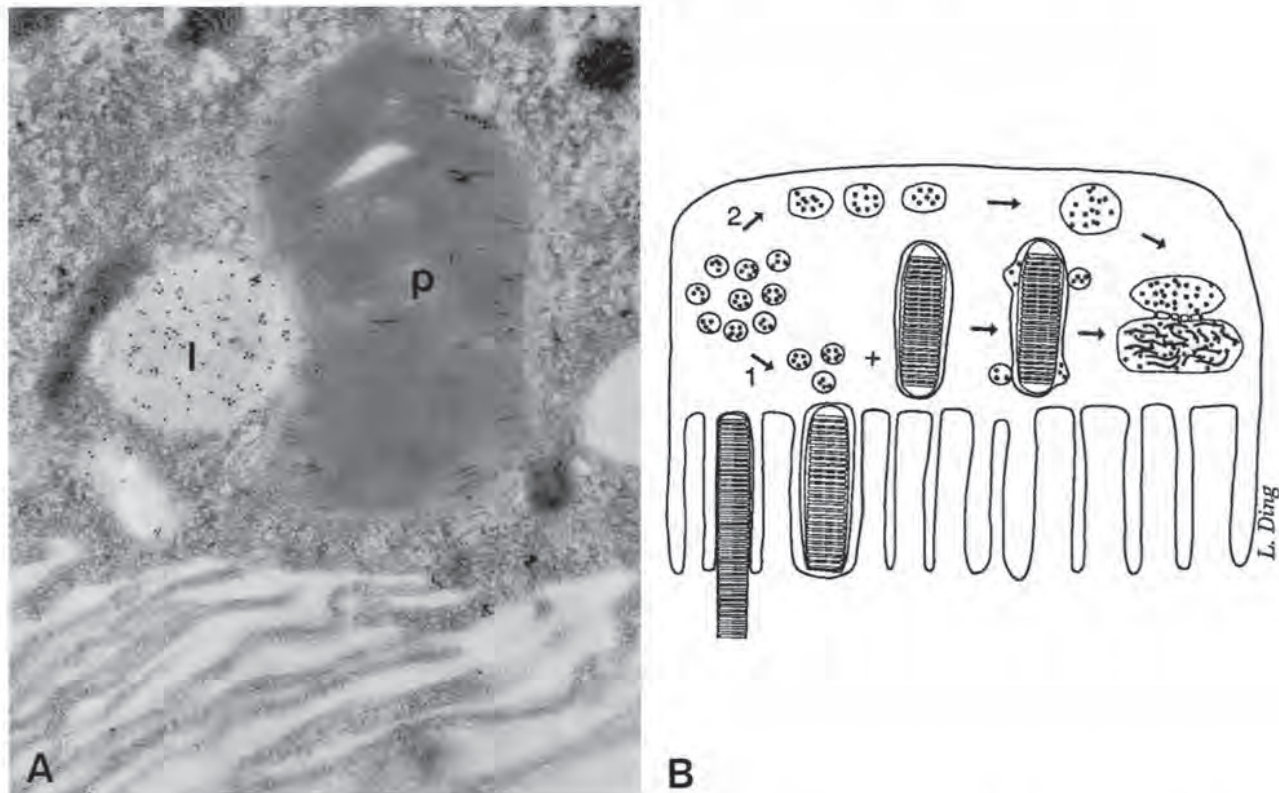


Fig. 1. (A) Rat RPE rapidly frozen and freeze dried prior to analysis by immunogold electron microscopy. The tissue was fixed 30 minutes after light onset. A large lysosome (l), stained for cathepsin D, lies adjacent to a phagolysosome (p), which was produced as a result of rod outer segment shedding. The apical microvilli of the RPE are observed at the bottom of the field. $\times 27,500$. (B) Diagram illustrating a summary of phagolysosome digestion. Digestion occurs in two steps, as observed from fixation and immunostaining of tissue from light-entrained animals at various times during the light-dark cycle. Initially, small lysosomes (1) fuse with ingested outer segment fragments. Additionally, small lysosomes fuse with one another to form larger lysosomes (2). In turn the larger lysosomes interact with phagolysosomes, possibly by exchanging material through bridge-like structures. (From Bosch et al., 1993, with permission.)

Years of daily phagocytosis by the RPE are thought eventually to take their toll in some individuals. Over time, lipofuscin accumulates in the aging RPE until, in some cases, the cells are virtually engorged with this material and function is almost certainly compromised (for a review, see Young, 1987). Some investigators believe that this aging process is a causative factor in age-related macular degeneration, a leading cause of retinal blindness in countries with large senior populations. The macular photoreceptors, which include both rods and cones, then die as a secondary response to an aged and incompetent RPE. Currently, there is considerable interest in developing methods for the transplantation of healthy RPE into these individuals (for a review, see Bok et al., 1993). Transplantation of the RPE is feasible because it is capable of leaving its postmitotic state when placed in culture or the appropriate microenvironment. Indeed, healthy RPE has been transplanted successfully into the retinas of rats that carry a mutation that impairs the phagocytic function of the RPE and leads to photoreceptor death (Bok and Hall, 1971; Mullen and LaVail, 1976). RPE transplantation rescues the adjacent photoreceptors from this fate (Li and Turner, 1988).

UPTAKE PROCESSING, TRANSPORT AND RELEASE OF RETINOIDS BY THE RPE

One of the highly-specialized functions of the RPE is its role in the visual or retinoid cycle (Fig. 2). This involves the repeated movement of vitamin A (retinol) and some of its derivatives (retinoids) back and forth between the rods and cones (photoreceptors) and the RPE (Dowling, 1960). The purpose of this cycle is to regenerate 11-*cis* retinaldehyde, the retinoid that serves as the chromophore for the visual pigments in rod and cone outer segments and whose photon triggered isomerization to the all-*trans* form is the first step in phototransduction. The RPE is the cellular site in which regeneration of 11-*cis*-retinaldehyde (11-*cis*-retinal) takes place (Bernstein et al., 1987). Therefore, all-*trans* retinol, the product of photoisomerization and reduction in the photoreceptor outer segment, must leave the photoreceptors, traverse the extracellular space between the photoreceptors and RPE and undergo reisomerization to 11-*cis* retinal and oxidation prior to returning to the photoreceptors across the extracellular space. This, in summary, is the visual cycle.

During the visual cycle, the retina is conservative with respect to the reutilization of its endogenous retinoids, as indicated by the fact that they are so tightly sequestered there once they are taken up for the circulation (Dowling, 1964). The delivery of retinol from the blood to the retina and its recycling within the retina involve a cohort of binding proteins that solubilize and protect retinol and its derivatives from damage (Fig. 2). Retinol is a lipid-soluble molecule and its solubility in water is in the nanomolar range. Its concentration in the blood, however, is micromolar. This increase in solubility is achieved by plasma retinol binding protein (RBP), with which it is secreted by the liver (Kanai et al., 1968). RBP circulates as a complex with transthyretin, a thyroxine-binding protein, and delivers its cargo of retinol to the RPE by a receptor-mediated process, the precise molecular mechanisms of which remain unresolved (Heller,

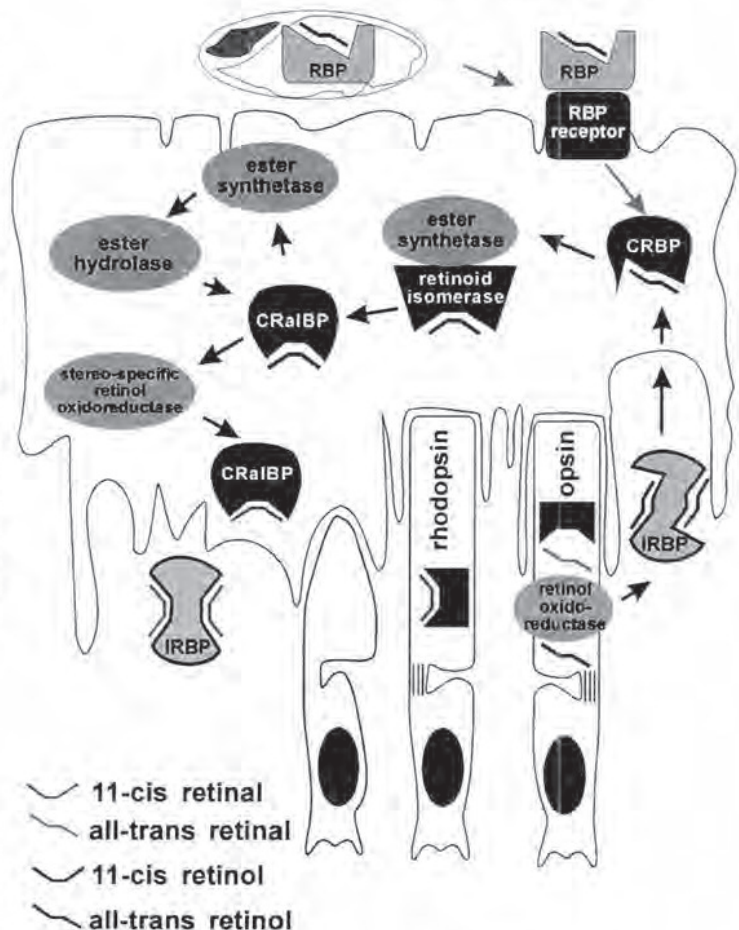


Fig. 2. Diagram of the visual cycle. This complicated process involves a group of extracellular and intracellular retinoid binding proteins that solubilize and protect the retinoids and serve as substrate carriers for enzymes. The cycle also involves rod and cone photopigments that initiate phototransduction, plus enzymes that convert retinol into its various visual cycle intermediates, the final product being the photopigment chromophore, 11-*cis* retinaldehyde (11-*cis* retinal). Basolateral uptake and apical uptake and release of retinol are mediated by separate binding proteins. See text for further details.

1975; Heller and Bok, 1976). Putative receptors for RBP exist on the basolateral membrane of the RPE (Bok and Heller, 1976). Autoradiographic studies involving ^{125}I -labeling of RBP strongly suggest that the protein does not enter the RPE cells during retinol delivery. Following the entry of retinol into the cell, its solubilization continues and its processing commences. Studies from a variety of laboratories combine to suggest the following series of events (Fig. 2) that ultimately lead to the release of the finished product, 11-*cis* retinaldehyde, from the apical membrane of the RPE, across the extracellular matrix and into the photoreceptors (for a review, see Saari, 1990).

Within the RPE, retinol is bound to cellular retinol-binding protein (CRBP, Bok et al., 1984), a protein that has no amino acid sequence homology with RBP but belongs instead to a family of fatty acid binding proteins (Sundelin et al. 1985). The bound retinol is esterified primarily to

palmitic acid and the resulting retinyl ester serves as both the storage form of retinol in the RPE and the substrate for retinoid isomerase (Barry et al., 1989), a membrane-bound enzyme that converts the ester to 11-*cis*-retinol (Bernstein et al., 1987). This retinoid isomer is bound by yet another intracellular binding protein named cellular retinal binding protein (CRALBP, Futterman et al., 1977). It presents the retinoid to an oxidoreductase (Saari and Bredberg, 1982), which converts it to the final product, 11-*cis* retinaldehyde. The cellular release of 11-*cis*-retinal then takes place across the apical membrane of the RPE. The release process is promoted by a large extracellular retinoid binding glycoprotein (Adler and Klusznik, 1982) called interphotoreceptor retinoid binding protein (IRBP, Lai et al., 1982; Liou et al., 1982). The mechanism for this release appears to involve a receptor-mediated process by virtue of its specificity for IRBP. In tissue culture (Carlson and Bok, 1992) and in amphibian eyecups (Okajima et al., 1989) that have had the neurosensory retina removed, release of 11-*cis*-retinal is up to 5-fold higher in the presence of IRBP than it is when other proteins are substituted for it, including CRALBP, which has a much higher affinity for 11-*cis*-retinal than IRBP. IRBP is thought to mediate the delivery of 11-*cis*-retinal to the photoreceptors and the return of all-*trans*-retinol back to the RPE following photobleaching. Again, the mechanisms for this have not been resolved (Saari, 1990). Nonetheless, the efficiency of delivery of all-*trans*-retinol across the apical membrane appears to be at its highest under experimental conditions when the delivery is via IRBP as opposed to non-specific carrier proteins such as bovine serum albumin (Okajima et al., 1989).

Individuals who suffer from a rare autosomal recessive syndrome called Refsum's disease have a deficiency in phytanic acid α hydroxylase, which leads to the accumulation of a branched fatty acid called phytanic acid (Klenk

and Kahlke, 1963; Eldjarn et al., 1966). In addition to neurologic disorders, these patients suffer from night blindness and retinitis pigmentosa due to the loss of their RPE and photoreceptors. There are two hypotheses that suggest a molecular mechanism for this cell degeneration. One hypothesis favors a metabolic basis and suggests that phytanic acid competes with palmitic acid for the esterification of retinol, thereby competing with retinyl palmitate in the visual cycle (Levy, 1970). The molecular distortion hypothesis speculates that phytanic acid, due to its side chains, perturbs the integrity of lipid bilayers and leads to a general decline in cell function, particularly in regions of high membrane density such as myelin and photoreceptor outer segments (Steinberg, 1978) or perhaps by causing lysosomal membranes to become leaky.

Recently, we were able to model Refsum's disease in RPE cell cultures grown on porous supports and to test the antimetabolite hypothesis (Bernstein et al., 1992). Human and bovine RPE cells were exposed to levels of phytanic acid (200 μ M) comparable to those measured in human patients. The cells were then analyzed morphologically and by high performance liquid chromatography in order to assess their ability to process retinoids. We were able to show that their ability to esterify retinol and to isomerize all-*trans* retinoids to 11-*cis* retinoids remained intact, in spite of the synthesis of a large amount of retinyl phytanate. This provides evidence against the hypothesis that phytanic acid inhibits retinol processing by the RPE and favors the distortion hypothesis.

RPE POLARITY: THE ISSUE OF Na,K-ATPASE TRAFFICKING AND POLARITY OF VIRAL BUDDING

As mentioned earlier, the RPE shares a unique feature with

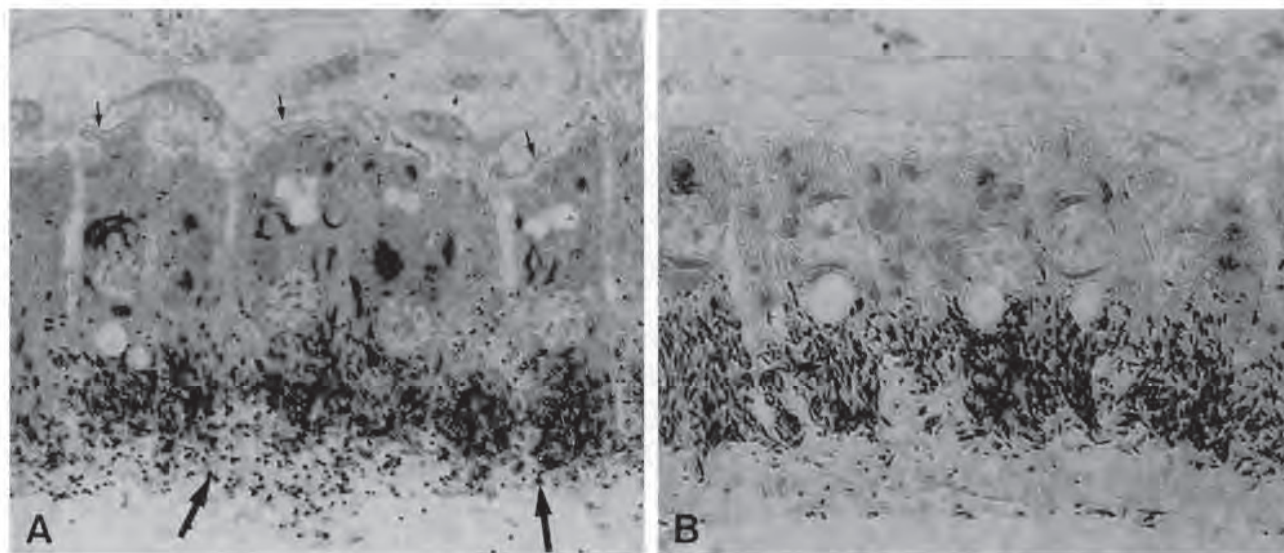


Fig. 3. Light microscopic autoradiograms of apical binding of ^3H -ouabain by frog RPE. The tissue was freeze dried and fixed with osmium tetroxide vapors after incubation in this specific marker for Na,K-ATPase. (A) ^3H -ouabain binding, as indicated by the location of silver grains, is confined primarily to the apical microvilli (large arrows) with no detectable binding on the basolateral surface (small arrows). (B) Apical binding of ^3H -ouabain was significantly reduced when a 3-fold excess of non-radioactive ouabain was present in the incubation medium. $\times 940$. (From Bok, 1982, with permission.)

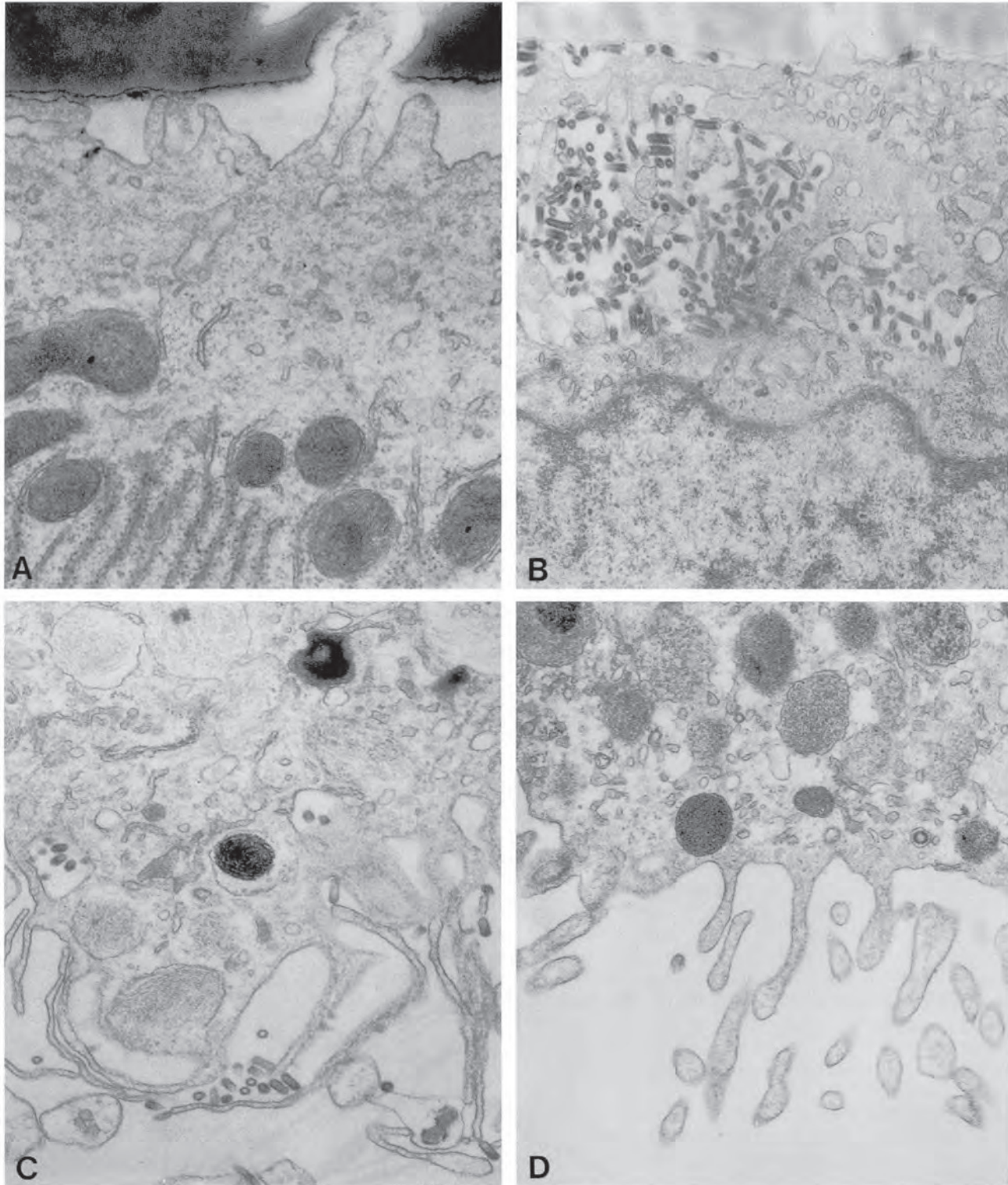


Fig. 4. Polarized budding of vesicular stomatitis virus (VSV) and influenza virus from human RPE cultured from a 2-year-old donor. (A and C) Basolateral and apical surfaces following influenza virus infection. (B and D) basolateral and apical surfaces following VSV infection. The orientation of the micrographs is with the base of the RPE up, in order to be consistent with Figs 1-3. (A-C), $\times 19,500$; (D), $\times 26,000$.

the epithelium of the choroid plexus, namely predominant expression of its Na,K-ATPase on the apical membrane (Fig. 3) rather than the basolateral expression observed in

most other epithelia (Steinberg and Miller 1973; Bok, 1982). Due to this reversal in polarity for Na,K-ATPase, we wondered whether the RPE might possess general mem-

brane protein trafficking mechanisms that are the reverse of that in the kidney. To explore this possibility, we infected cultured human and bovine RPE with enveloped viruses whose progeny bud from specific membrane domains (Rodriguez-Boulan and Sabatini, 1978), as dictated by the sorting of their envelope glycoproteins (Rodriguez-Boulan and Pendergast, 1980). Influenza hemagglutinin (HA) is sorted to the apical membrane in kidney (MDCK) and other polarized epithelial cells, whereas vesicular stomatitis (VSV) glycoprotein (G-protein) is targeted to the basolateral membrane. Cultured human (Fig. 4) and bovine RPE cells exhibited the same pattern of viral budding as has been observed in other polarized epithelia (Bok et al., 1992). Therefore, as far as viral envelope glycoproteins are concerned, the RPE does not appear to have a generalized sorting mechanism that is unique.

We must therefore search for alternative mechanisms in order to gain an understanding of Na,K-ATPase trafficking in the RPE. Hammerton et al. (1991) have shown that MDCK cells insert their Na,K-ATPase into both the basolateral and apical membranes, and then achieve polarity by inactivating and then removing the apical proteins. The RPE may do the reverse by inactivation and removal of Na,K-ATPase from the basolateral membrane.

There is a growing impression, based on experimental evidence, that Na,K-ATPase polarity might be dictated by cytoskeletal elements. Fodrin and ankyrin also show a reversal of polarity in the RPE. Gunderson et al. (1991) have shown that these proteins are polarized to the apical domain. Furthermore, Rizzolo and Heiges (1991) have found that apical polarization of the RPE does not occur until day 11 in the chick embryo, a time when the developing photoreceptors begin to elaborate their extracellular matrix. The RPE is peculiar in the sense that both its basolateral and apical membranes are in contact with highly organized extracellular matrices, the basal lamina on the basal side and an intricate system of proteoglycan sheaths surrounding individual photoreceptor outer segments on the apical side (Johnson et al., 1986; Sameshima et al. 1987). Thus interactions between cytoskeletal elements, Na,K-ATPase and extracellular matrix components could be involved in dictating the polarity of this enzyme.

Finally, we should not dismiss the possibility that information for trafficking could reside in the amino acid sequence of the Na,K-ATPase α or β subunits. Protein (McGrail and Sweadner, 1986) and mRNA analysis (Gundersen et al., 1991) suggest that the α isoform is the same in the kidney and RPE, namely α_1 . As in the kidney, the β_1 isoform has also been detected with antibodies and by mRNA analysis (Gundersen et al., 1991; Rizzolo and Heiges, 1991), although the abundance of β_1 does not appear to be 1:1 with α_1 . Thus it is conceivable that the RPE contains a unique β isoform that dictates polarity.

The mRNA isoform classification of RPE ATPase subunits that has been performed to date has been limited to northern blot analysis. This is a relatively low resolution method, which cannot detect subtle differences in size or nucleotide sequence. Thus we must remain receptive to the possibility that, in spite of apparent isoform identity among α and β subunits in RPE and kidney, minor sequence differences that result in small changes at the protein level

could determine polarity. Brewer and Roth (1991) have shown that a single amino acid change from cysteine to tyrosine in the cytoplasmic domain of influenza HA changes its expression from the apical to the basolateral membrane.

A FEW PARTING POINTS

The RPE presents an interesting target for investigations into its diverse functions in the healthy state as well as for issues involving the process of aging and genetic disorders. Whereas the RPE has not been included in the mainstream investigations of the majority of those interested in epithelial polarity, I hope that this short review has made a case for the versatility of this epithelium and some of the unique features of its polarity. Recent advances in cell culture, particularly in the case of human, bovine and chicken RPE, have made it possible to explore more readily this interesting tissue under a variety of controlled conditions.

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REFERENCES

- Adler, A. J. and Klusznik, K. M. (1982). Proteins and glycoproteins of the bovine interphotoreceptor matrix: composition and fractionation. *Exp. Eye Res.* **34**, 423-434.
- Barry, R. J., Canada, F. J. and Rando, R. R. (1989). Solubilization and partial purification of retinyl ester synthetase and retinoid isomerase from bovine ocular pigment epithelium. *J. Biol. Chem.* **264**, 9231-9238.
- Bernstein, P. S., Law, W. C. and Rando, R. (1987). Isomerization of all-trans retinoids to 11-cis retinoids in vitro. *Proc. Nat. Acad. Sci. USA* **84**, 1849-1853.
- Bernstein, P. S., Lloyd, M. B., O'Day, W. T. and Bok, D. (1992). Effect of phytanic acid on cultured retinal pigment epithelium: an in vitro model for Refsum's disease. *Exp. Eye Res.* **55**, 869-878.
- Bok, D. and Heller, J. (1976). Transport of retinol from the blood to the retina: an autoradiographic study of the pigment epithelial cell surface receptor for plasma retinol-binding protein. *Exp. Eye Res.* **22**, 395-402.
- Bok, D. (1982). Autoradiographic studies on the polarity of plasma membrane receptors in retinal pigment epithelial cells. In *The Structure of the Eye* (ed. J. G. Hollyfield), pp. 247-256. New York: Elsevier North Holland.
- Bok, D. (1985). Retinal photoreceptor-pigment epithelium interactions. *Invest. Ophthalmol. Vis. Sci.* **26**, 1659-1694.
- Bok, D. (1988). Retinal photoreceptor disc shedding and pigment epithelium phagocytosis. In *Retinal Diseases: Medical and Surgical Management, Vol 1* (ed. S. J. Ryan, T. E. Ogden and A. P. Schachat), pp. 69-81. St. Louis: C. V. Mosby Co.
- Bok, D. and Hall, M. O. (1971). The role of the pigment epithelium in the etiology of inherited retinal dystrophy in the rat. *J. Cell Biol.* **49**, 664-682.
- Bok, D., Hageman, G. S. and Steinberg, R. H. (1993). Repair and replacement to restore sight. Report from panel on photoreceptor/retinal pigment epithelium. *Arch. Ophthalmol.* **111**, 463-471.
- Bok, D., O'Day, W. and Rodriguez-Boulan, E. (1992). Polarized budding of vesicular stomatitis and influenza virus from cultured human and bovine retinal pigment epithelium. *Exp. Eye Res.* **55**, 853-860.
- Bok, D., Ong, D. E. and Chytil, F. (1984). Immunocytochemical localization of cellular retinol binding protein in the rat retina. *Invest. Ophthalmol. Vis. Sci.* **25**, 1-7.
- Bosch, E., Horwitz, J. and Bok, D. (1993). Phagocytosis of outer segments by retinal pigment epithelium: phagosome-lysosome interaction. *J. Histochem. Cytochem.* **41**, 253-263.
- Brewer, C. and Roth, M. G. (1991). A single amino acid change in the

- cytoplasmic domain alters the polarized delivery of influenza virus hemagglutinin. *J. Cell Biol.* **114**, 413-21.
- Cahill, G. M. and Besharse, J. C.** (1992). Light-sensitive melatonin synthesis by *Xenopus* photoreceptors after destruction of the inner retina. *Vis Neurosci.* (in press).
- Carlson, A. and Bok, D.** (1992). Promotion of the release of 11-*cis* retinal from cultured retinal pigment epithelium by interphotoreceptor retinoid-binding protein. *Biochemistry* **31**, 9056-9062.
- Dowling, J. E.** (1960). Chemistry of visual adaptation in the rat. *Nature* **188**, 114-118.
- Dowling, J. E.** (1964). Nutritional and inherited blindness in the rat. *Exp. Eye Res.* **3**, 348-356.
- Eldjarn, L., Stokke, O. and Try, K.** (1966). α -oxidation of branched chain fatty acids in man and its failure in patients with Refsum's disease showing phytanic acid accumulation. *Scand. J. Clin. Lab. Invest.* **18**, 694-695.
- Futterman, S., Saari, J. C. and Blair, S.** (1977). Occurrence of a binding protein for 11-*cis* retinol in retina. *J. Biol. Chem.* **252**, 3267-3271.
- Gundersen, D., Orlowski, J. and Rodriguez-Boulan, E.** (1991). Apical polarity of Na,K-ATPase in retinal pigment epithelium is linked to a reversal of the ankyrin-fodrin submembrane cytoskeleton. *J. Cell Biol.* **112**, 863-872.
- Hammerton, R. W., Krzeminski, K. A., Mays, R. W., Ryan, T. A., Wollner, D. A. and Nelson, J. A.** (1991). Mechanism for regulating cell surface distribution of Na,K-ATPase in polarized epithelial cells. *Science* **254**, 847-850.
- Heller, J.** (1975). Interactions of plasma retinol binding protein with its receptor. Specific binding of bovine and human retinol binding protein to pigment epithelium cells from bovine eyes. *J. Biol. Chem.* **250**, 3613-3619.
- Heller, J. and Bok, D.** (1976). A specific receptor for retinol binding protein as detected by the binding of human and bovine retinol binding protein to pigment epithelial cells. *Amer. J. Ophthalmol.* **81**, 93-97.
- Johnson, L. V., Hageman, G. S. and Blanks, J. C.** (1986). Interphotoreceptor matrix domains ensheath vertebrate cone photoreceptor cells. *Invest. Ophthalmol. Vis. Sci.* **27**, 129-135.
- Kanai, M., Raz, A. and Goodman, D. S.** (1968). Retinol binding protein: the transport protein for vitamin A in human plasma. *J. Clin. Invest.* **47**, 2025-2044.
- Klenk, E. and Kahlke, W.** (1963). Über das Vorkommen der 3,7,11,15-tetramethyl-hexadecansäure (phytansäure) in den cholesterinestern und anderen Lipoidfraktionen der Organe bei einem Krankheitsfall unbekannter Genese (Verdacht auf Heredopathia atactica polyneuritiformis (Refsum Syndrome)). *Hoppe Seyler's Z. Physiol. Chem.* **333**, 133-139.
- Lai, Y-L., Wiggert, B., Liu, Y-P. and Chader, G. J.** (1982). Interphotoreceptor retinol-binding proteins: possible transport vehicles between compartments of the retina. *Nature* **298**, 848-849.
- LaVail, M. M.** (1976). Rod outer segment disk shedding in the rat retina. Relationship to cyclic lighting. *Science* **194**, 1071-1074.
- Levy, I. S.** (1970). Refsum's syndrome. *Trans Ophthalmol. Soc. UK* **90**, 181-186.
- Li, L. and Turner, J. E.** (1988). Inherited retinal dystrophy in the RCS rat: prevention of photoreceptor degeneration by pigment epithelial cell transplantation. *Exp. Eye Res.* **47**, 911-916.
- Liou, G. I., Bridges, C. D. B. and Fong, S.-L.** (1982). Vitamin A transport between retina and pigment epithelium - an interstitial protein carrying endogenous retinol (interstitial retinol binding protein). *Vision Res.* **22**, 1457-1468.
- McGrail, K. and Sweadner, K.** (1986). Immunofluorescent localization of two different Na,-ATPase in the rat retina and in identified dissociated retinal cells. *J. Neurosci.* **6**, 1272-1283.
- Mullen, R. J. and LaVail, M. M.** (1976). Inherited retinal dystrophy: primary defect in pigment epithelium determined with experimental rat chimeras. *Science* **192**, 799-801.
- Okajima, T.-I. L., Pepperberg, D. R., Ripps, H., Wiggert, B. and Chader, G. J.** (1989). Interphotoreceptor retinoid binding protein: role in delivery of retinol to the pigment epithelium. *Exp. Eye Res.* **49**, 629-644.
- Peyman, G. A. and Bok, D.** (1972). Peroxidase diffusion in the normal and laser-coagulated primate retina. *Invest. Ophthalmol.* **11**, 35-45.
- Quinton, P. M., Wright, E. M. and Tormey, J. McD.** (1973). Localization of sodium pumps in the choroid plexus epithelium. *J. Cell Biol.* **58**, 724-730.
- Rizzolo, L. J. and Heiges, M.** (1991). The polarity of the retinal pigment epithelium is developmentally regulated. *Exp. Eye Res.* **53**, 549-553.
- Rodriguez-Boulan, E. and Pendergast, M.** (1980). Polarized distribution of viral envelope proteins in plasma membrane of infected epithelial cells. *Cell* **20**, 45-54.
- Rodriguez-Boulan, E. and Sabatini, D.** (1978). Asymmetric budding of viruses in epithelial monolayers; a model system for study of epithelial polarity. *Proc. Nat. Acad. Sci. USA* **75**, 5071-5075.
- Saari, J. C.** (1990). Enzymes and proteins of the mammalian visual cycle. In *Progress in Retinal Research* (ed. N. Osborne and G. Chader), pp. 363-382. Oxford: Pergamon Press.
- Saari, J. D. and Bredberg, L.** (1982). Enzymatic reduction of 11-*cis* retinol bound to cellular retinal-binding protein. *Biochem. Biophys. Acta.* **715**, 266-272.
- Sameshima, M., Uehara, E. and Ohba, N.** (1987). Specialization of the interphotoreceptor matrices around cone and rod photoreceptor cells in the monkey retina as revealed by lectin cytochemistry. *Exp. Eye Res.* **45**, 751-762.
- Steinberg, D.** (1978). Phytanic acid storage disease: Refsum's syndrome. In *The Metabolic Basis of Inherited Disease*, 4th edn. (ed. J. B. Stanbury, J. B. Wyngarden and D. S. Fredrickson), pp. 688-706. New York; McGraw-Hill.
- Steinberg, R. H. and Miller, S.** (1973). Aspects of electrolyte transport in frog pigment epithelium. *Exp. Eye Res.* **16**, 36-52.
- Sundelin, J., Anundi, H., Tragardh, L., Ericksson, U., Lind, P., Ronne, H., Peterson, P. A. and Rask, L.** (1985). The primary structure of rat liver cellular retinol-binding-protein. *J. Biol. Chem.* **260**, 6488-6493.
- Williams, D. S. and Fisher, S. K.** (1987). Prevention of rod disk shedding by detachment from the retinal pigment epithelium. *Invest. Ophthalmol. Vis. Sci.* **28**, 184-187.
- Young, R. W.** (1987). Pathophysiology of age-related macular degeneration. *Surv. Ophthalmol.* **31**, 291-306.
- Young, R. W. and Bok, D.** (1969). Participation of the retinal pigment epithelium in the rod outer segment renewal process. *J. Cell Biol.* **42**, 392-402.