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THE RETINAL PIGMENT EPITHELIUM APICAL MICROVILLI AND RETINAL FUNCTION

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1. INTRODUCTION

The RPE performs highly specialized, unique functions essential for homeostasis of the neural retina. These include phagocytosis of photoreceptors shed outer segments, directional transport of nutrients into and removal of waste products from photoreceptor cells and visual pigment transport and regeneration. All of these functions involve the RPE apical microvilli.[1–4]

The RPE is a low cuboidal epithelium containing very long sheet-like apical microvilli that project into the interphotoreceptor matrix. The microvilli interact with the tips of the rod and cone photoreceptor outer segments extending from the outer retinal surface. The cone-RPE association is much less studied however, as many as 30–40 microvilli can be associated with a single cone. These vary in length with only a few reaching the outer segment. The RPE apical microvilli ensheath the outer segments of photoreceptor cells, extending for as long as half the outer segment.[5] A single RPE microvillous may completely surround the outer segment or multiple microvilli can encircle each other while surrounding the photoreceptor outer segments. Intracellular organelles are mostly absent from the cone-ensheathing microvilli while they are very abundant in the microvilli ensheathing the rod outer segments.

The RPE basal surface is highly infolded and interacts with the underlying Bruch's membrane, [1] an acellular layer separating the RPE from the choriocapillaris. The polarized organization of the RPE is essential for the vectorial transport of different molecules between the choriocapillaris and the neural retina and vice-versa. A unique characteristic of the RPE is the "reversed polarity" of select proteins such as the Na,K-ATPase pump, EMMPRIN and the adhesion molecule N-CAM. These proteins are found at the apical surface of the RPE, rather than at the basolateral surface as in other epithelia.[6–8]

2. RPE MICROVILLI STRUCTURE

The RPE microvillar structure has not been extensively studied. However, available information indicates that RPE microvilli possess an internal core bundle of densely packed actin filaments.[9] Myosin VIIa has been detected at the base of apical processes[10] while villin, fimbrin and myosin I have not been detected.[11,12] The entire length of the RPE microvilli has been shown to contain ezrin and EBP50.[11,13–15] The mouse RPE microvilli-enriched fraction, described below, contained several cytoskeletal components, among them various types of actin and tubulin, β-spectrin, ezrin, moesin, EBP50, and profilin.

A more complete definition of the protein composition of the RPE apical microvilli should provide insights into other biochemical processes occurring at this critical interface that are important for the support and maintenance of vision.

3. RPE MICROVILLI PROTEINS AND FUNCTION

Recently, we have improved a method to isolate RPE apical microvilli. The procedure relies on the binding of N-acetylglucosamine and sialic acid-containing glycoconjugates present in abundance on the RPE apical surface[16] to the WGA lectin conjugated to agarose beads. Mass interactions of the surface glycoconjugates with the immobilized lectin on the bead allow for the detachment of the RPE microvilli upon physical removal of the WGA beads. The RPE isolated microvilli are resolved by SDS-PAGE, in gel digested with trypsin, and peptides extracted and analyzed by mass spectrometry.[3,17] This procedure was done in mice eyecups with the RPE exposed and it has resulted in the identification of over 283 proteins, distributed over functional categories such as retinoid-metabolizing, cytoskeletal, enzymes, extracellular matrix components, membrane proteins and transporters, among others. A summary of selected proteins identified by this method is presented in Table 72.1 and has been recently described.[3,17]

The beads with the isolated RPE microvilli on their surface can be used for immunolabeling experiments, morphological (light and electron microscopy) as well as in biochemical experiments. In Figure 72.1 beads with isolated mouse RPE were fixed in 4% paraformaldehyde, permeabilized in triton X100, reacted with both a rabbit antibody to (A) protein kinase A regulatory subunit II (PKA_{RII}) and (B) a mouse antibody to protein kinase A regulatory subunit I (PKA_{RI}). Parallel samples were processed for transmission electron microscopy and RPE microvilli are observed on the surface of the agarose beads (C and D).

Examples of proteins identified in the RPE microvilli by both mass spectrometry and other methods include Na,K-ATPase, Glut-1, monocarboxylate transporter, carbonic anhydrase, basigin, and the chloride intracellular channel 6.[17] The cone and rod-associated matrix, present on top of the RPE apical surface, are firmly attached to the RPE apical surface. This is one of the reasons for the mass spectrometric identification of several novel extracellular matrix components such as fibromodulin, lumican, undulin 1, and neuroglycan C in the RPE isolated microvilli. The proteomic method therefore provides an unbiased account of proteins present in the RPE apical microvilli.

RPE apical microvilli play important roles in retinal attachment. Ensheathment of the outer segment tips by apical projections may contribute to adhesion by providing frictional or electrostatic interactions.[18] Any disruption of the relationship between cone and rod photoreceptors and the RPE will result in pathological consequences. A retinal detachment, for example, is a separation of the photoreceptor outer segments from its apical RPE microvilli. After clinical reattachment, return of normal vision depends, upon the restoration of a functional relationship between proteins present in the RPE apical surface and the photoreceptors outer segments.

Alterations in the proteins present in the RPE apical microvilli will likely impair vision as a consequence of disrupting the structural and functional nurturing of the photoreceptors by the RPE. Some of the RPE apical proteins identified in the RPE microvilli fraction have already been shown to be involved in retinal degenerations. The list of RPE apical proteins involved in retinal diseases is likely to grow as we learn more about the RPE proteome.

An interaction between cellular retinaldehyde-binding protein (CRALBP) and ERM-binding phosphoprotein 50 (EBP50) in RPE microsomes was recently described.[15] Our proteomic analyses was highly enriched in several retinoid processing proteins such as cellular retinaldehyde-binding protein, 11-*cis*-retinol dehydrogenase, cellular retinol-binding protein 1, interphotoreceptor retinoid-binding protein, EBP50, and ezrin. These results support the existence of a visual cycle protein complex in the RPE apical microvilli.[3] Several forms of

retinitis pigmentosa are known to be caused by mutations in visual-cycle protein genes such as RPE65, CRALBP, IRBP.[19,20]

Macular edema resulting from pathologies such as uveitis, postoperative period following cataract extraction,[21] retinitis pigmentosa,[22] serpiginous choroiditis[23] and epiretinal membranes,[24] has been widely treated with carbonic anhydrase inhibitors.[25] Polarized distribution of carbonic anhydrase activity in the RPE apical surface has been reported. Carbonic anhydrase XIV was one of the proteins we identified by proteomic analysis in isolated RPE microvilli.[17]

Aging studies have shown a decrease in both the number and the length of epithelial microvilli, and a declined function of plasma membrane enzymes and receptors.[26–29] Specifically, a decrease in the activity of some of the enzymes detected in RPE microvilli like Na,K-ATPase, LDH, glutathione S-transferase, phosphoglycerate kinase, adenylate kinase[30] and catalase has been established in various epithelia.[31–33] Future studies involving these and other proteins may help to improve our understanding of aging diseases such as macular degeneration.

Most recently we have pursued proteomic analysis of rat RPE microvilli. One of the proteins consistently found in rat RPE microvilli is ceruloplasmin. The localization of ceruloplasmin in the RPE has been previously described.[34,35] Retinal degeneration has been reported in patients with the autosomal recessive disease called aceruloplasminemia, a deficiency in ceruloplasmin.[36]

4. CONCLUSIONS

Progress is being made in characterization of the RPE and its apical microvilli. The years to come will bring further definition of key proteins and pathways present in RPE microvilli as well as a better understanding of their function in vision.

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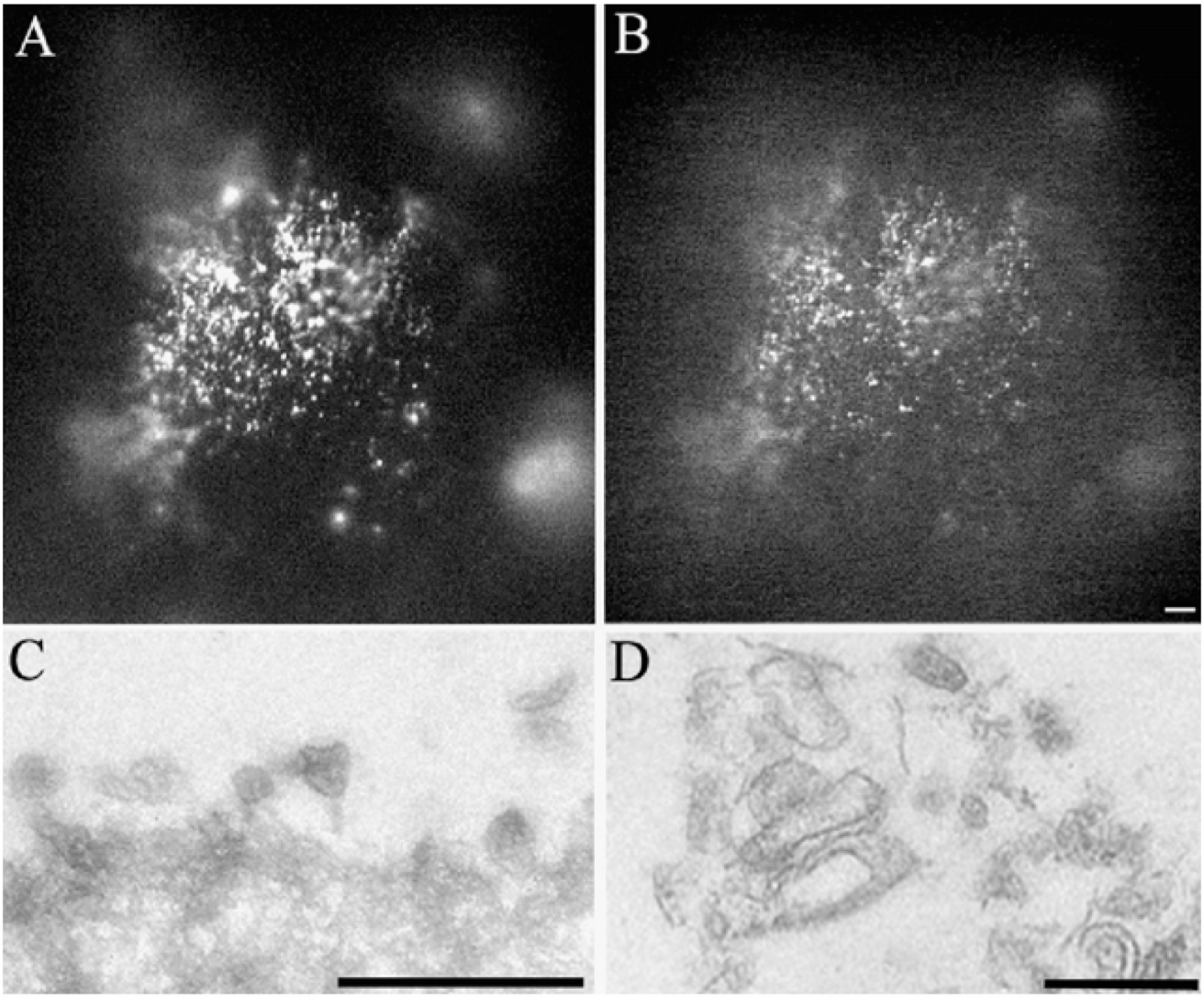

Figure 72.1. Morphological analysis of isolated WGA-beads with mouse RPE microvilli on their surface

WGA beads scraped off the mouse eyecups were reacted with antibodies to protein kinase A regulatory subunit alpha II (PKA_{RII}) (A) and protein kinase A regulatory subunit I (PKA_{RI}) (B). Alternatively, the isolated beads were fixed in 2.5% glutaraldehyde, and processed for transmission electron microscopy (TEM). Low (C) and high (D) magnification of these beads revealed extensive surface areas covered by the RPE microvilli. Bars = 100μm (A, B), 1μm (C) and 0.5μm (D).

Table 72.1

Selected proteins identified on WGA-beads after incubation with apical RPE.

Proteins	Accession Number[a]	Peptide Matches	Frequency[b]
Annexin A2	P07356	2	1
Annexin A5	P48036	3	1
Basigin	P18572	6	3
Carbonic anhydrase XIV	Q9WVT6	2	1
Chloride intracellular channel 6	Q96NY7	2	3
Cellular retinaldehyde-binding protein (CRALBP)	Q9Z275	6	3
ERM-binding phosphoprotein (EBP50)	Q9JJ19	1	1
Ezrin	P26040	4	2
Fibromodulin	P50608	4	2
Glucose transporter type 1 (Glut-1)	*P17809*	3	3
Interphotoreceptor retinoid-binding protein (IRBP)	P49194	5	3
L-lactate dehydrogenase A chain (LDH)	P06151	3	1
Lumican	P51885	9	3
Malate dehydrogenase	P14152	2	2
Membrane-associated adenylate kinase	Q9R0Y4	2	1
Monocarboxylate transporter 1	*AAC13720*	3	3
Neuroglycan C	Q9QY32	1	1
Retinol dehydrogenase, 11-cis (RDH5)	Q27979	3	3
Sodium/potassium-transporting ATPase alpha-1	P06685	6	3
Undulin 1	A40970	7	2
Vitronectin receptor a subunit (integrin αv)	P43406	3	2

[a] Swiss Protein database and NCBI (in italics) accession numbers are shown.

[b] Results from three independent experiments.