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Retinal Pigment Epithelium Polarity in Health and Blinding Diseases

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Abstract

The polarized phenotype of the retinal pigment epithelium is critical for the outer retina-blood barrier and support of photoreceptors and underlying choroid, and its disruption plays a central role in degenerative retinopathies. Although the mechanisms of polarization remain mostly unknown, they are fundamental for homeostasis of the outer retina. Recent research is revealing a growing picture of interconnected tissues in the outer retina, with the retinal pigment epithelium at the center. This review discusses how elements of epithelial polarity relate to emerging apical interactions with the neural retina, basolateral cross-talk with the underlying Bruch's membrane and choriocapillaris, and tight junction biology. An integrated view of outer retina physiology is likely to provide insights into the pathogenesis of blinding diseases.

Keywords

apical- basolateral polarity; tight junction; outer retina- blood barrier; tissue cross- talk; choroid

Introduction

The retinal pigment epithelium (RPE) is located strategically between the photoreceptors and the choroidal vasculature (Figure 1A), the only blood supply for the outer retina [1]. As many epithelia, the RPE is polarized into apical and basolateral plasma membrane domains separated by tight junctions. However, many plasma membrane proteins display a reversed polarity compared with extraocular epithelia, e.g. Na/K-ATPase [2], Integrin αvβ5 [3], NCAM [4], CD147 [5], CAR [6] and Monocarboxylate transporters [7]. The cellular mechanisms that determine RPE polarity are poorly understood, but they involve sorting and transit of proteins through endosomal compartments (Reviewed in refs [8,9]). Characterizing these mechanisms is a critical need in retina physiology since the polarized organization of the RPE mediates important support functions to the neural retina and maintains access to

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the choroidal blood supply [1,9]. Basolateral RPE interactions with a laminar extracellular matrix called Bruch's membrane and the choriocapillaris constitutes the outer-retina blood barrier (ORBB), which regulates exchange of substances between the neural retina and the circulation [8,10]. Here we will discuss an emerging picture of cross-talk mechanisms between the neural retina and the ORBB, and also within the components of the ORBB itself (Figure 1B). We will consider how the three defining characteristics of the epithelial phenotype: tight junctions, apical plasma membrane, and basolateral domain including the Bruch's membrane, relate to maintenance of the outer retina as a functional unit.

Tight Junctions

The RPE tight junctions constitute a key component of the ORBB since they restrict the passage of substances between the choroid and the outer retina. Recent work highlighted a crucial role of the tight junction protein Claudin-19 in RPE trans-epithelial permeability, retinal differentiation and disease [11]. Disruption of RPE cell-cell contacts was recently found to stimulate production of Vascular endothelial growth factor (VEGF) in cell culture [12], a secreted pro-angiogenic factor that we will discuss later. This is part of an emerging concept that associates intact tight junctions with VEGF secretion, as several groups have proposed strategies that target RPE tight junctions to minimize the detrimental effects of VEGF during diabetic retinopathy and choroidal neovascularization [13–15].

Given the importance of tight junctions for maintenance of the epithelial phenotype, recent interest has been directed towards novel factors that increase tight junction permeability like C-reactive protein [16] and tissue factor [17]; or stimulate tight junction formation like somatostatin [13], nicotinamide [18], lysophosphatidic acid [19] and HIWI2-mediated activation of Akt [20]. Most of these recent studies were performed in RPE cell lines, therefore their translational relevance in native RPE still remains to be determined. A special mechanism of tight junction formation is mediated by interactions with the basement membrane [21]. Loss of RPE tight junctions could lead to epithelial-mesenchymal transition and recent reports have addressed the possible contributions of this process to proliferative retinopathy [22,23], diabetic retinopathy [24] and age-related macular degeneration (AMD) [25].

Apical Domain

RPE barrier functions rely on directional solute and fluid transport through polarized epithelial transporters and channels. This activity is the foundation of many RPE support functions to the photoreceptors, since otherwise they would be isolated from the circulation. These support functions are summarized in Figure 2 and reviewed elsewhere [1]. They explain the close relationship between the RPE apical membrane and photoreceptors.

Polarized RPE solute transport

Many RPE support functions rely on the plasma membrane distribution of transporters. Recent research has advanced our understanding of key membrane transporters in the RPE. For instance, glucose is transported from the choroid to the photoreceptors via GLUT1 [26–28]. The photoreceptors utilize this glucose to produce lactate, which is eliminated and

absorbed by the RPE via apical MCT1 transporters [7] (Figure 2). Recently, Kanow et al. [29] showed that RPE cells favor lactate as a nutrient over glucose, which is instead preferred by the photoreceptors. In addition, the RPE also utilizes proline to produce citrate and other metabolites, which are secreted apically for photoreceptor utilization [30]. Importantly, dietary proline protected the RPE from oxidative damage [31]. This emerging retinal ecosystem of coordinated nutrient transport and utilization provides the framework for membrane distribution of RPE transporters (Figure 2).

Fluid transport by the RPE maintains the low volume of the apical subretinal space and it has been proposed to depend on KCl transport [1,8]. Bestrophin is a basolateral Cl^- channel affected in retinal degenerative diseases (recently reviewed [32]). Recently, Bestrophin has been shown to be regulated by ATP binding to a site mutated in patients [33], and also possibly by trafficking to the plasma membrane [34,35], although the latter remains to be confirmed in RPE. K^+ is transported via cation channels, like the newly recognized Anoctamin 4 in the RPE [36]. Further characterization of polarized ion transport will undoubtedly contribute to our full understanding of directional fluid transport by the RPE.

Apical receptor-mediated RPE interactions with photoreceptors

A major support function of the RPE is circadian clearing of shed photoreceptor outer segments by phagocytosis. This requires binding of milk fat globule–EGF 8 (MFG-E8) to RPE apical αvβ5 Integrin receptors [3,37] (Figure 2), and internalization via activity of focal adhesion kinase (FAK) and Mer tyrosine kinase (MerTK) [38–40]. Recently, it was found that apical Plexin-B1 serves as a receptor for Semaphorin-4D, inhibiting outer segment internalization, therefore contributing to the circadian pattern [41]. The Plexin-B1/Semaphorin-4D pair may be important for retina homeostasis as knockout mice have higher phagosome content [41].

Phagocytosed outer segments are a source of cholesterol that needs to be eliminated from the RPE (Figure 2). Recent work has shown that cholesterol efflux occurs via apical and basolateral ABCA1 transporters [42]. This bidirectional efflux is relevant because cholesterol accumulates in basolateral (drusen) and apical (subretinal drusenoid) deposits, which have been independently associated with macular degeneration [43–45]. However, the bidirectional efflux could be subject to differential regulation since cholesterol loading stimulates efflux preferentially towards the apical side [46]. Defects in RPE phagocytosis have been linked to photoreceptor degeneration [47], and further characterization of the RPE endocytic pathway may shed light into the mechanisms of retinal disease [48,49]. An important step is to identify the physiological roles of additional endocytic receptors, for instance the apical receptor Megalin is necessary for retina structure and visual function as described in recently generated RPE-specific Megalin knockout mice [50].

RPE primary cilium

Early studies suggested that the primary cilium disappeared in mature RPE [51]. However, this observation may have been due to artefacts in the histological preparation [9]. The functional role of the RPE primary cilium has recently been confirmed by two groups. Buskin et al. [52] found polarity and functionality defects in RPE cells obtained from

retinitis pigmentosa patients carrying mutations in mRNA splicing factors (PRPF) that control cilia genes. May-Simera et al. [53] showed that disruption of ciliary proteins in cultured RPE cells causes abnormal RPE phenotype, possibly by interfering with PKCδ, a mediator of apico-basal polarity. The authors also observed abnormal morphology in RPE cells obtained from patients harboring a mutation in CEP290 and mice knockout for Bbs8, two ciliary proteins illustrated in Figure 2.

Neural retina-RPE interaction regulates choroid development

An underdeveloped area of research is how the neural retina influences the choroidal vasculature, despite this being potentially critical to maintain a blood supply to the outer retina. A remarkable study by Goto et al. [54] described a pathway involving retinoic acids produced via Aldehyde Dehydrogenase 1 A1 (Aldh1a1) in the neural retina, from where they reach the RPE and activate the transcription factor Sox9, which controls VEGF expression and choroid development (Figure 2). In agreement with this, another study found that the transcriptional relationship Pax6-Sox9 controls coordinated RPE and choroid vasculature development [55]. It was also found that Pax6-Sox9 control expression of other factors in addition to VEGF that could be secreted by the RPE towards the choroid. These recent reports reveal a growing picture of cell-cell communication between tissues at the outer retina. An important question is the nature of these pathways in adult tissue and their contributions to retinopathies.

Basolateral Domain

An important aspect of RPE polarity is the ability to basolaterally secrete components of the Bruch's membrane and choroidal trophic factors (Figure 3). The Bruch's membrane is a five-layer sheet rich in elastin and collagen [56]. The outermost layer is the RPE basement membrane, composed of collagen-IV, laminin, fibronectin and glycosaminoglycans [57]. Each of these components has a distinct effect in RPE adhesion [58] and barrier properties [59]. The RPE secretes VEGF basolaterally [60], a growth factor for the choriocapillaris supplying the outer retina. Diffusion of substances to and from the choriocapillaris and across the Bruch's membrane is central to ORBB function.

Bruch's membrane

The composition of the Bruch's membrane depends on synthesis and degradation of ECM components [57], which change with age [61] and during AMD [62]. This impairs RPE adhesion [63,64] and is relevant for RPE-based therapies [65]. A Bruch's membrane-modifying factor of recent interest is Fibulin-3 (*EFEMP1*), an ECM-remodeling protein secreted by the RPE (Figure 3) and associated with macular degeneration [66]. In AMD eyes, Fibulin-3 accumulates between the RPE and Bruch's membrane [66,67]. The role of Fibulin-3 in the Bruch's membrane is not well understood. However, it is likely to regulate matrix-metalloproteases (MMP), its regulators (TIMP) and proteoglycans as shown recently in mice carrying a Fibulin-3 mutation that causes macular degeneration and impaired diffusion across the Bruch's membrane [68]. Polarized secretion of MMPs and TIMPs may be of great relevance in ECM-remodeling occurring during RPE wound-healing, which is associated with PVR [69].

Several recent studies have focused on Bruch's membrane-mediated RPE signaling. For instance, the complement pathway, essential for RPE defense functions, can be activated by modifications in the ECM [70–72]. Such alterations could be post-translational modifications induced by oxidative stress, e.g. nitration and advanced glycation end products [72–74], which accumulate during retinal disease. The recent emphasis in these areas can benefit stem cell and RPE transplantation therapies, which require correct differentiation of RPE cells on the adequate substrate [75,76]. It has also been shown that aged Bruch's membrane impairs RPE cell adhesion and function [63].

The close association between the RPE and the choroid vasculature can also modify the Bruch's membrane. It was recently shown that when co-cultured with endothelial cells, an RPE cell line increases ECM deposition and expression of ECM genes [77]. It is unknown how this compare to native Bruch's membrane since this study used cell lines and the endothelial cells were not of choroidal origin. There is also evidence that the choroid endothelium can directly modify the Bruch's membrane. We recently observed a switch in expression profile of ECM genes in mice choroid during early postnatal development [21]. This was associated with secretion of collagen cross-linker lysyl oxidases by endothelial cells, which increased the assembly of ECM collagen bundles in co-cultures with primary RPE cells (Figure 3). Importantly, this EC-mediated modification of the ECM enhanced the barrier properties of the RPE [21]. These findings illustrate the ability of endothelial cells to influence the RPE phenotype and the ORBB.

RPE/choroid cross-talk

Our findings that endothelial cells promote RPE barrier properties [21], together with a recent similar report [77], are part of an emerging notion of endothelia as instructive niches for regeneration and homeostasis of surrounding tissues [78,79]. The signals originating in the choroid can modify the Bruch's membrane as discussed, and we found that this activates basolateral β1-integrin receptors in RPE cells [21] (Figure 3). Lysyl oxidases are secreted factors that we found modified the Bruch's membrane and preserved vision [21]. However, additional choroidal factors could also target the RPE directly. Future work will need to identify novel secreted factors in choroid-RPE cross talk.

One of the best characterized RPE secreted factors is VEGF, which is released basolaterally (Figure 3) and maintains the choriocapillaris [60,80,81]. RPE-specific knockout of VEGF in adult mice resulted in dramatic ablation of the choriocapillaris and deficient vision [80]. Recent work has shown that VEGF inhibition results in decreased complement regulators, including complement factor H through a mechanism that involves VEGF receptor 2, PKC-alpha and CREB signaling [82]. These results suggest that VEGF protects the choroid vasculature through both trophic and complement regulatory functions. The ramifications of VEGF signaling in the choroid are expected to continue to dominate the field since it has important implications in therapy of neovascular (wet) AMD. However, additional regulators of angiogenesis secreted by the RPE may represent alternatives to anti-VEGF treatment. It was recently reported that Galectin-1 (Figure 3) is increased in RPE in a mouse model of choroidal neovascularization and it promotes choroid angiogenesis and fibrosis [83]. It was recently proposed that exosomes secreted by RPE cells in culture (Figure 3) could promote

angiogenesis via a VEGF-independent pathway [84]. However, in vivo confirmation is still pending. A word of caution, a separate study using the same RPE and endothelial cell lines established a negative relationship in angiogenesis [77], while it is known that RPE promotes angiogenesis in native choroid [85].

Concluding remarks

Despite the importance of RPE polarization to retina physiology, how this polarity is achieved is poorly understood. Part of this phenotype may be explained by the absence of the clathrin adaptor protein AP-1B, which is characteristic of epithelial cells [6]. However, this small aspect of RPE polarity still doesn't explain the complex phenotype of this epithelium. Recently, we used quantitative surface proteomics to uncover extended roles of clathrin adaptors in epithelial polarity [86]. Similar approaches applied to RPE cells could shed light on the mechanisms of RPE polarity [87], and the growing body of interactions between the RPE and neighboring tissues. A holistic picture of the connections among the cell types in the outer retina will be fundamental in our understanding of retinopathies like macular degeneration, proliferative and diabetic retinopathies.

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* of special interest

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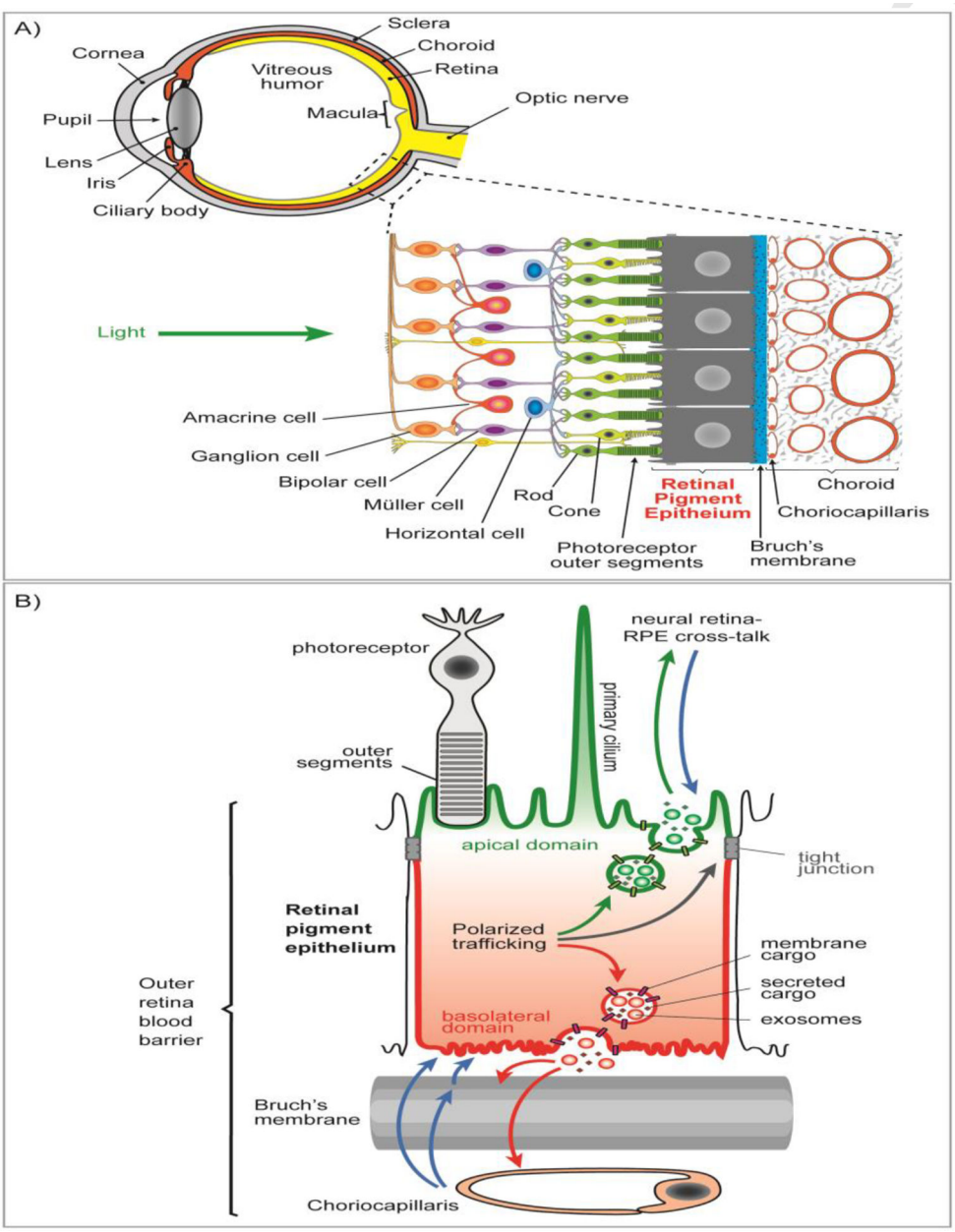

Figure 1: Retinal pigment epithelium in relation to photoreceptors and choroid.

A) Localization of the retinal pigment epithelium in the eye at the interface between the neural retina and choroid. **B)** Polarized trafficking of secreted factors, intracellular proteins, organelles and membrane receptors and transporters dictates the identity of the apical (green), basolateral (red) plasma membrane domains and tight junctions. Apical trafficking determines interactions with photoreceptors at the neural retina. Basolateral trafficking mediates interactions with the Bruch's membrane and choroidal vasculature. Green and red arrows represent pathways acting at the apical and basolateral domains respectively. Blue arrows represent cross-talk pathways originating at the neural retina or choroid. The polarized organization of the retinal pigment epithelium permits the formation of the outer retina-blood barrier, also composed by the Bruch's membrane and the choroidal vasculature.

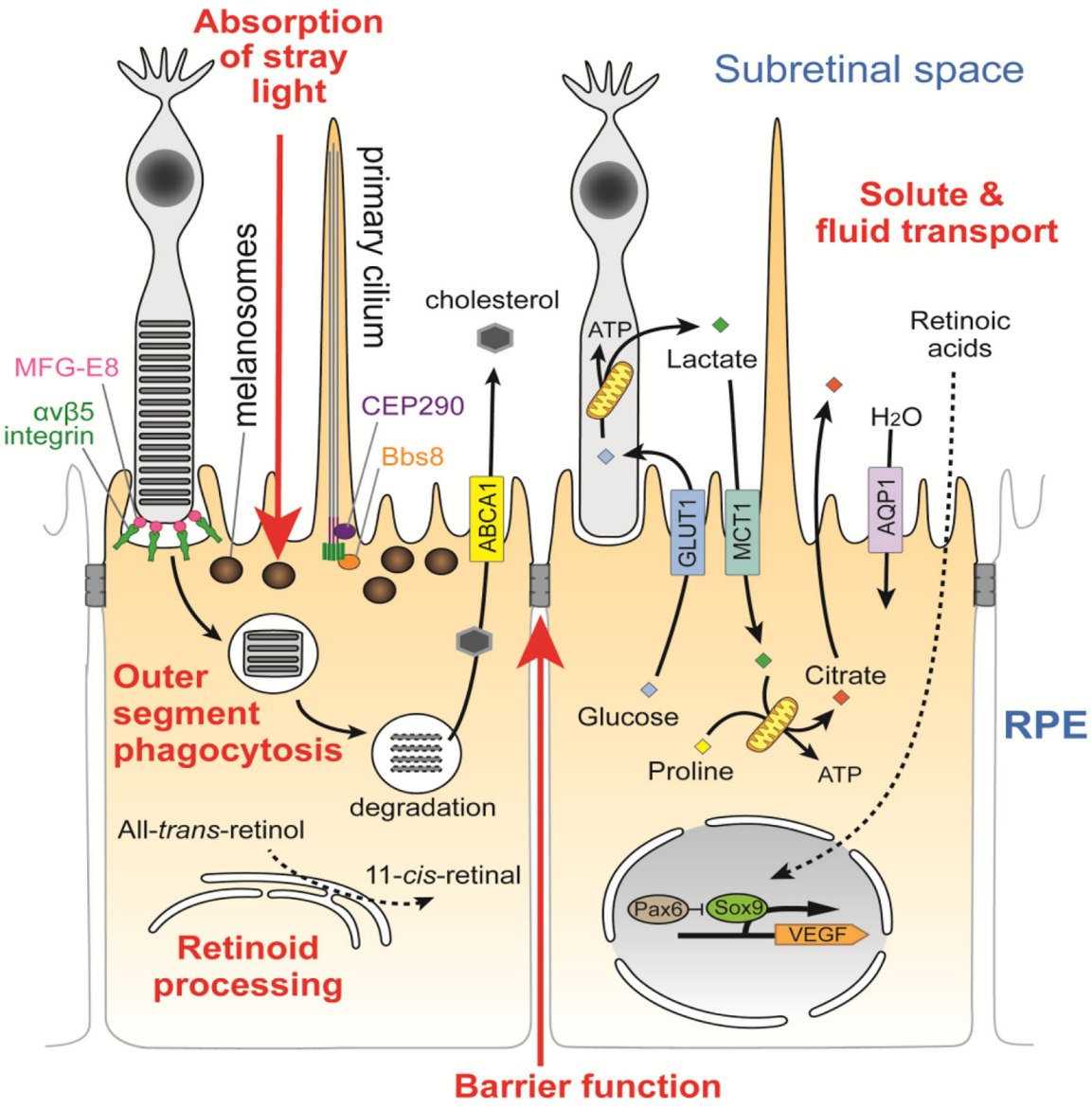

Figure 2: Key interactions at the apical domain of the retinal pigment epithelium.

The retinal pigment epithelium provides many photoreceptor-support functions (indicated in red font) which require interactions through the apical domain. Only the processes discussed in this review that contribute to these functions are represented here in detail. Dotted lines represent pathways that may contain intermediate steps. Abbreviations used: RPE, retinal pigment epithelium; MFG-E8, milk fat globule–EGF 8; ABCA1, ATP binding cassette subfamily A member 1; GLUT1, Glucose Transporter Type-1; MCT1, Monocarboxylate Transporter-1; AQP1, aquaporin-1; VEGF, Vascular Endothelial Growth Factor; Pax6, Paired Box 6; Sox9, SRY (Sex-Determining Region Y)-Box 9, CEP290, Centrosomal Protein 290; Bbs8, Bardet-Biedl Syndrome 8.

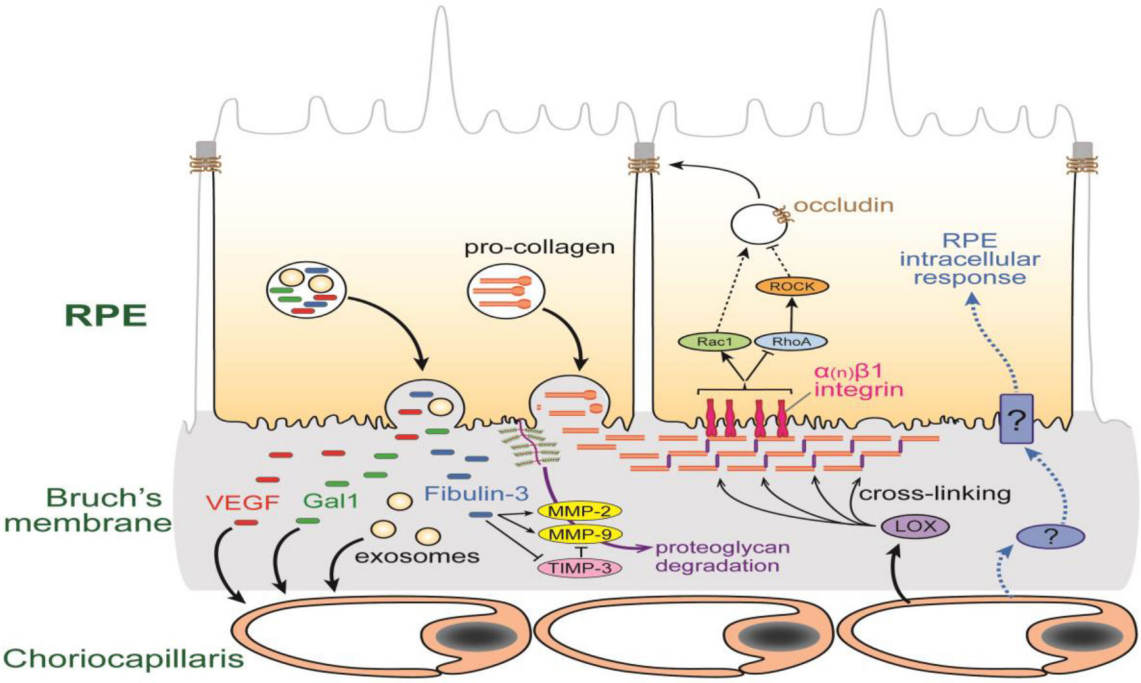

Figure 3: Basolateral retinal pigment epithelium interactions with the Bruch's membrane and choriocapillaris.

Basolateral targeting of membrane receptors, secreted factors and extracellular matrix components dictates the key interactions at the outer retina-blood barrier discussed in this review and represented here. The pathway represented by the blue arrows indicates a hypothetical mechanism of direct communication between the choroid endothelium and the retinal pigment epithelium involving unidentified (?) secreted endothelial factors and epithelial receptors. Dotted lines represent pathways that may involve intermediate steps. Abbreviations used: RPE, retinal pigment epithelium; VEGF, Vascular Endothelial Growth Factor; Gal1, Galectin-1; MMP, matrix metalloproteinase; TIMP-3, Tissue Inhibitor of Metalloproteinases 3; RhoA, Ras Homolog Family Member A; ROCK, Rho Associated Coiled-Coil Containing Protein Kinase; Rac1, Rac family small GTPase 1; LOX, Lysyl oxidases; $\alpha_{(n)}\beta 1$ represents integrin receptors formed by association of a β1 subunit and an α subunit.