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The Retinal Pigment Epithelium in Visual Function

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I. Introduction

The retinal pigment epithelium (RPE) is a monolayer of pigmented cells forming a part of the blood/retina barrier (72, 372, 492, 558). The apical membrane of the RPE faces the photoreceptor outer segments (Fig. 1). Long apical microvilli surround the light-sensitive outer segments establishing a complex of close structural interaction. With its basolateral membrane the RPE faces Bruch's membrane, which separates the RPE from fenestrated endothelium of the choriocapillaris (Fig. 1).

As a layer of pigmented cells the RPE absorbs the light energy focused by the lens on the retina (72, 86). The RPE transports ions, water, and metabolic end products from the subretinal space to the blood (144, 236, 369, 402, 558). The RPE takes up nutrients such as
glucose, retinol, and fatty acids from the blood and delivers these nutrients to photoreceptors. Importantly, retinal is constantly exchanged between photoreceptors and the RPE (30, 58, 596). Photoreceptors are unable to reisomerize all-trans-retinal, formed after photon absorption, back into 11-cis-retinal. To maintain the photoreceptor excitability, retinal is transported to the RPE reisomerized to 11-cis-retinal and transported back to photoreceptors. This process is known as the visual cycle of retinal. Furthermore, the voltage-dependent ion conductance of the apical membrane enables the RPE to stabilize ion composition in the subretinal space, which is essential for the maintenance of photoreceptor excitability (144, 558, 559). Another function in the maintenance of photoreceptor excitability is the phagocytosis of shed photoreceptor outer segments (72, 170, 187, 575). The photoreceptor outer segments are digested, and essential substances such as retinal are recycled and returned to photoreceptors to rebuild light-sensitive outer segments from the base of the photoreceptors. In addition, the RPE is able to secrete a variety of growth factors helping to maintain the structural integrity of choriocapillaris endothelium and photoreceptors. Furthermore, the secretory activity of the RPE plays an important role in establishing the immune privilege of the eye by secreting immunosuppressive factors (280, 581). With these complex different functions, the RPE is essential for visual function. A failure of any one of these functions can lead to degeneration of the retina, loss of visual function, and blindness. In the following sections these functions will be described in more detail.

II. EMBRYONIC ORIGIN OF THE RETINAL PIGMENT EPITHELIUM

The development and differentiation of RPE and photoreceptors reflects the unique relationship between both tissues (372, 373, 484, 487, 492). First, the RPE is essential for the proper development of the retina (260, 565). For this purpose, the RPE was found to secrete factors that promote photoreceptor survival and differentiation (3, 126, 306, 531–534). Second, the development of both tissues occurs in concert with developmental steps where the RPE depends on the specific stages in photoreceptor development, and vice versa (373, 484, 487, 492). In the case of a selective loss of the RPE, neuronal retina and photoreceptors are the most affected tissues in the eye (50, 51, 339). Third, the proper development of the retina appears to be dependent on the genes involved in the proper development of the melanogenesis pathway in RPE cells (286). Human albino eyes show abnormal fovea and chiasmatic projections (159, 361).

A. First Phase: Establishment of Two Layers

The development of both tissues can be divided into two main events. In the first stage, the structure of the two layers, the prospective RPE and prospective neuronal retina, is established. After invagination of the optic cup, cells of the neuroepithelium which, on one hand, are designated to become RPE cells and, on the other hand, cells which are determined to become the neuronal retina are building two layers opposed to each other (Fig. 2) and
separated by a thin remnant of lumen (487). The remnant of the lumen is filled by a new material, the interphotoreceptor matrix, the IPM (204, 206, 207). Before this stage, the RPE is a ciliated and pseudostratified epithelium (179). After the IPM is formed, the RPE starts to mature. At this stage both layers are still able to differentiate into RPE or neural retina if the IPM is disturbed (204, 206, 207, 224). Several factors were found to be essential for determination of RPE differentiation. The expression of the transcription factors OTX2 (homeodomain-containing transcription factor) and MITF (microphthalmia-associated transcription factor) appears to be critical initial steps of determination and differentiation of the RPE (384). In early development, before formation of the two layers, the region destined to form the anterior parts of the eye express cellular retinol binding protein (CRBP), cellular retinaldehyde binding protein (CRABP), and several enzymes in the retinal metabolism pathway. The embryonic retina anlage releases retinoic acid. RPE cells, in turn, express receptors for retinoic acid (RAR-β2), CRBP, and CRABP (see sect. vi for a more detailed description of these proteins) (396, 513, 515). In addition, it has been shown that retinoic acid alone can promote RPE differentiation (100, 145, 393, 396, 563), although an exchange of retinoic acid between the developing RPE and developing neural retina also seems to be of importance. This exchange and retinoic acid buffering are mediated by interphotoreceptor retinal binding protein (IRBP) (204). IRBP is present in the IPM from the onset of IPM formation, which is at embryonic day 19 in rodents, for example (110, 112, 156, 204, 206, 207, 562). IRBP is synthesized in cells destined to become photoreceptors and also those that will become RPE cells. Thus IRBP is produced long before it is needed in the visual cycle (see sect. vi) and must play an important role in development. This is supported by the finding that the IRBP concentration is much lower in the IPM of the developing eye than in the adult eye in which the visual cycle is fully functional. Onset of expression of the RPE specific protein of the visual cycle, RPE65, occurs in the same time frame as the synthesis of the IPM. RPE65 expression in the rat steadily increased from the first expression at embryonic day 18 to postnatal day 12, just before eye opening (364). Another important signaling pathway essential for the development of both tissues is the hedgehog signaling pathway (462, 623). Different hedgehog signaling pathways are active in the RPE and in the retina. As mentioned above, the selective differentiation of RPE cells mostly affects the development of photoreceptors and neuronal retina. However, the inactivation of the retina-specific hedgehog signaling leads to improper development of the retina (623). Thus the proper development of the retina is not solely dependent on the presence of the RPE.

The following developmental steps depend on the interaction of retina and RPE. As in the adult eye, the interface for this interaction is the IPM. The maturation of the RPE
begins with activation of the tyrosinase promoter, which marks the onset of melanogenesis (286). From that point, neuroectodermal cells begin to differentiate into the RPE. The RPE basement membrane and basement membrane of the endothelium form Bruch’s membrane. Over the whole time of embryonic development, Bruch’s membrane develops into a five-layered structure: the basement membrane of the RPE, the inner collagenous layer, the elastin layer, the outer collagenous layer, and the basement membrane of the choriocapillaris (195, 220, 257, 338, 381, 583). The formation of Bruch’s membrane starts near the RPE (448, 588), which is capable of synthesizing most of the extracellular matrix components present in Bruch’s membrane (102). The elastin layer is connected with the ciliary epithelium (318). During the development of the chick embryo, the expression of components present in Bruch’s membrane (102). The elastin could only be detected in the ciliary epithelium, which indicates that a part of Bruch’s membrane is possibly not only built by the RPE/choriocapillaris complex (606). By now the RPE is a pigmented epithelium showing almost complete apical to basolateral polarity (372) but with short microvilli and small basolateral membrane infoldings. At this stage ~75% of the RPE apical architecture has developed (372–374). The surface of the apical membrane is now three times greater than the one of the basolateral membrane. Na\(^+\)-K\(^+\)-ATPase, \(\alpha_v\beta_3\)-integrin (phagocytosis receptor), N-CAM-140 (a morphoregulator), and EMMPRIN (inducers of matrix metalloproteinase secretion) are now primarily localized in the apical membrane (265, 372, 374, 490, 493, 494, 582, 631). Later, the maturation of the apical surface is promoted by the presence of ezrin. Ezrin seems to be essential for the development of long apical microvilli (76, 78). In the developed RPE, ezrin and its associated proteins radixin and moesin might play a role in 11-cis-retinoid transport and, thus, in the visual cycle (see sect. vi). A key role is played by EBPs [ERM (ezrin, radixin, moesin)-binding phosphoprotein 50], which interacts with CRBP (75, 425). This polar distribution of these proteins is thought to be achieved by a suppression of basolateral sorting mechanisms. This may be due to masking of a basolateral targeting signal by its serine/threonine phosphorylation (92, 116). Furthermore, active apically directed transcytosis adds to the suppression of basolateral sorting mechanisms. This was shown using the influenza hemagglutinin (77). Another important factor for the establishment of the apical to basolateral polarity is the formation of tight junctions between RPE cells. Three stages of tight junction development have been described (34, 36, 314, 456, 481, 494, 634). In the early stage, key proteins for tight junction formation are expressed. Because these tight junction complexes are only rudimentary at this point, the RPE forms a leaky barrier at this stage. This corresponds with the stage of a partial apical to basolateral polarity. The tight junctional complexes define the apical and basolateral parts of the membrane because they decrease a free exchange of membrane proteins over the cell membrane (492). In this phase \(\gamma\)-tubulin was found in the apical membrane, whereas \(\alpha_1\beta_3\)-integrin is present in the basal membrane (494, 496). At the end of the early phase, defined as late early phase by Rizzolo (492), the Na\(^+\)-K\(^+\)-ATPase becomes apically polarized in the central region (493, 495) and apical microvilli start to elongate (89, 90). The second stage is designated the intermediate stage. During this stage tight junctions have constant tight junctional connections but develop a constant decrease in permeability. This is due to a major change in the isoform composition of tight junction proteins. Now tight junctions not only prevent free diffusion of membrane proteins over the cell membrane but also the underlying maturation of the cytoskeleton additionally defines the apical to basolateral polarity (492). In this phase a remodeling of the basolateral membranes occurs (490–492, 495). In the third or late stage of tight junction development, the composition of tight junction protein isoforms stabilizes and the tight junction permeability decreases to that characteristic of a tight epithelium. This is primarily due to an increase in the number and branching of tight junction strands. According to studies using chick RPE, mature tight junctions are composed of ZO-1, occludin, and the claudins 1, 2, 5, 12, and AL (481). The formation of tight junctions establishes the blood retina barrier and signifies coincident onset of epithelial transport. Thus, following completion of tight junctions, the RPE starts to express transporters like the glucose transporter, which is essential for transepithelial glucose transport (35).

B. Second Phase: Differentiation of RPE and Photoreceptors

With the differentiation properties acquired by the end of the first developmental phase, the RPE is prepared to interact with photoreceptors. Now primordial photoreceptors can start to differentiate (179). It is in this second developmental phase that the photoreceptors and RPE undergo the last differentiation events and become a functional unit. Primordial photoreceptors start to extend their outer segments. The RPE responds by elongating its apical microvilli into the subretinal space. The microvilli start to

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surround the growing outer segments of photoreceptors. By the end of differentiation, the RPE has developed two types of microvilli (84, 179): long microvilli (5–7 μm) that maximize the apical surface for epithelial transport (see sect. iv) and shorter microvilli that form photoreceptor sheaths for phagocytosis of photoreceptor outer segments (see sect. vii). Accompanying onset of microvilli growth at the apical membrane is the formation of deep basal infoldings in the basolateral membrane. Thus RPE and photoreceptors are interacting as they undergo their last maturation steps. This tight coordination of differentiation is also reflected in differential gene expression in these cells. For example, in the RPE, the expression of the visual cycle protein RGR opsin (591) and the putative Cl channel bestrophin is coordinated with the onset of the electrical activity in the retina including photoreceptors (33).

The coordinated maturation requires the adaption of RPE cells to different functional properties of the retina. Differentiated RPE cells in the macula are smaller with 14 μm in diameter and a height of 12 μm than cells in the periphery which have a diameter of 60 μm and which show a variable height (380, 580, 665). Together with a higher melanin content (625), the different cell morphology in the macula leads to organization of melanosomes, which is essential for a more efficient light absorption (85). This is believed to be the reason for the observation that the RPE in the macula appears darker (85, 86). For interaction with photoreceptors, RPE cells develop sheaths that closely surround photoreceptor outer segments (31, 555, 560). Because cones and rods are surrounded by different types of sheaths, the RPE cells in the macula have a different apical architecture (555, 560). Due to the higher number of photoreceptors per RPE cell in the macula, macular RPE cells (143, 194, 544) are adapted to a higher turnover rate of shed photoreceptor outer segments (505) (see sect. vi). For example, macular RPE cells display higher enzyme activities that are required for degradation processes (87, 93, 249).

The RPE is essential for the development of retinal structures partly because of its capability to secrete a variety of growth factors. Growth factors secreted by the RPE function in both endothelial cell differentiation and photoreceptor differentiation (3, 48, 94, 282, 306). This secretion of growth factors is maintained in the adult eye and helps to stabilize the structural integrity of the retina (described in detail in sect. viii).

III. ABSORPTION OF LIGHT AND PROTECTION AGAINST PHOTO-OXIDATION

A. The RPE Is Able to Absorb Light Energy Focused by the Lens Onto the Retina

The RPE increases optical quality by forming a dark pigmented wall cover of the inner bulbus, which aids in absorption of scattered light. In addition, RPE pigmentation is essential for maintenance of visual function. Light enters the eye via the pupil and is focused onto the macula lutea by the lens. This concentrates light energy onto the retina. The outer retina is also exposed to an oxygen-rich environment. The blood perfusion of the choriocapillaris is 1,400 ml · min⁻¹ · 100 g tissue⁻¹, which is even higher than the perfusion in the kidney (17, 19). Venous blood from the choriocapillaris shows a 90% O₂ saturation, indicating that there is a negligible O₂ extraction during the passage through the choriocapillaris (16–19). By comparison, venous blood from retinal vessels shows a O₂ saturation of 45% (16–19). The retina is thought to float on the choriocapillaris, which appears to function as a bed of blood-filled vessels. This combination is ideal to allow photo-oxidation and subsequent oxidative damage. This photo-oxidative activity is increased by the load of reactive oxygen species produced by phagocytosis of shed photoreceptor outer segments (see sect. vii) (399). As elegantly reviewed by Boulton and Dayhaw-Barker (86), the RPE has three lines of defense against this damage and toxins. The first line is absorption and filtering of light. For this purpose, the RPE contains a complex composition of various pigments that are specialized to different wavelengths and the special wavelength-dependent risks (44–46). General light absorption occurs via melanin in melanosomes (85). This is supplemented by additional light absorption by photoreceptors. Photoreceptors contain as the most important pigments the carotenoids lutein and zeaxanthin (74, 244, 245, 332, 435). These pigments form a kind of biological sunglasses that absorb blue light (44–46). Blue light appears to be most dangerous for RPE cells in the adult eye because it permits the photo-oxidation of lipofuscin components to cell toxic substances (52, 53, 352, 503, 504, 547, 549, 550, 552–554). The exposure of the adult retina to ultraviolet light is rather low because the lens absorbs most of the ultraviolet light. However, melanosomes and blue light absorbing pigments are only responsible for the absorption of ~60% of light energy (84, 85). This implies the presence of other pigments that have not been described yet. One of these pigments might be lipofuscin, which accumulates in the RPE during life (140, 625). As a light-absorbing pigment lipofuscin might first be beneficial for visual function. In the older eye, lipofuscin concentration seems to reach a toxic concentration for the RPE. The second line of defense is made by antioxidants. As enzymatic antioxidants, the RPE contains high amounts of superoxide dismutase (185, 399, 428, 447) and catalase (399, 594). As nonenzymatic antioxidants, the RPE accumulates carotenoids, such as lutein and zeaxanthin (45, 46), ascorbate, α-tocopherol, and β-carotene (45, 429). This is supplemented by glutathione and melanin, which itself can function as an antioxidant. The third line of
defense is the cell’s physiological ability to repair damaged DNA, lipids, and proteins.

B. Increasing Imbalance of Protective and Toxic Factors With Aging Leads to Retinal Degeneration

A reduced capability to absorb light energy is an important factor in the cascade of events leading to age-related macular degeneration (AMD), the most common cause for blindness in industrialized countries (20, 45, 52, 84, 86, 261, 311, 659). An increase in oxidative stress due to a reduction in protective mechanisms or an increase in number and concentration of active photo-oxidative reaction species are believed to contribute to the pathogenesis of AMD (84, 86, 87). One starting point is the accumulation of lipofuscin in the RPE (139–141). The onset of this chain of events is based in age-dependent changes of the RPE. This includes a reduction in the cell density of RPE cells while the epithelial layer remains intact (143, 226, 415). The reduction in cell density itself may result from apoptosis, which is caused by accumulation of toxic substances. This is enhanced by an age-related decrease in one of the most important antioxidants, α-tocopherol (186). Additional important age-related alterations are changes in pigmentation. These changes include age-dependent reduction of melanosomes as well as an increase in the number of lipofuscin granules (165, 166, 521, 625). New types of pigmented organelles can also be detected. These are melanolysosomes, which are a sign of melanin degradation and melanolipofuscin granules, which are a result of accumulation of lipofuscin in melanosomes (165). The increase in the amount of reactive oxygen species destabilizes intracellular membrane compartments such as lysosomes and mitochondria. The resulting decrease in metabolic efficiency produces more lipofuscin and reactive oxygen species. It has been reported that oxidative stress can be seen as the accumulation of advanced-glycation end products (AGEs) in Bruch’s membrane (243). These AGEs may also play an important role in the induction of choroidal neovascularization (CNV). The developing neovascular tissue contains high amounts of AGE and active receptors for AGEs (RAGE) expressed on RPE cells (242). RPE cells are able to secrete vascular defense is the cell’s physiological ability to repair damaged DNA, lipids, and proteins.

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be a consequence of incomplete degradation of metabolic end products from both photoreceptors and RPE. However, a more detailed analysis of the protein composition of Drusen led to alternative theories of Drusen formation (128, 226, 519). In one theory, the formation of Drusen begins with loss of RPE cells that are removed by an inflammatory event. The resulting gap in the epithelial barrier is actively closed by adjacent RPE that secretes a new extracellular matrix (226). The major matrix component is vitronectin (228). The theory is supported by detection of active dendritic cells and active components of the complement system in Drusen (414, 415). The hydrophobic material and lipoproteins could be the debris left over by incomplete degradation of cells. The end stages of the disease are either geographic atrophy (GA), a loss of RPE and photoreceptors over large areas, or CNV with subsequent intraocular bleeding and formation of a disciform scar (20, 99, 659).

IV. TRANSEPITHELIAL TRANSPORT

A. The RPE Transports Nutrients and Ions Between Photoceptors and the Choriocapillaris

1. Transport from subretinal space to blood

There is a large amount of water produced in the retina derived primarily as a consequence of the large metabolic turnover in neurons and photoreceptors. Furthermore, intraocular pressure leads to a movement of water from the vitreous body into the retina (236, 366, 369). This establishes the need for constant removal of water from the retina. Water in the inner retina is transported by Müller cells (411, 418), and water in the subretinal space is eliminated by the RPE. Furthermore, the water transport is required for close structural interaction of the retina with its supportive tissues in establishment of an adhesive force between RPE and the retina (308, 366, 370).

The RPE transports ions and water from the subretinal space or apical side to the blood or basolateral side (Fig. 3A) (268). Therefore, the RPE has the structural properties of an ion transporting epithelium. Tight junctions establish a barrier between the subretinal space and choriocapillaris (34, 313, 315, 434, 492). The paracellular resistance is 10 times higher than the transepithelial resistance, classifying the RPE as a tight epithelium (404, 405). Furthermore, the RPE has an apical to basolateral polarity by structure, organization of organelles, and distribution of the membrane proteins (72, 107, 136, 189, 219, 290, 372–374, 477, 490–494). On the apical side, the RPE extends long microvilli, and on the basolateral side, the membrane is thrown into deep foldings. The majority of mitochondria are located near the basolateral side (372).

The Na⁺–K⁺-ATPase, which is located in the apical membrane, provides the energy for transepithelial transport (218, 372, 374, 450, 490, 491, 493–495, 631). This localization is achieved during embryonic development from the first expression of the Na⁺–K⁺-ATPase and likely results from suppression of basolateral sorting mechanisms (see sect. II). Constant elimination of water from the subretinal space produces an adhesion force between retina and RPE that is lost by inhibition of Na⁺–K⁺-ATPase by ouabain (181). The transport of water is mainly driven by a transport of Cl⁻ and K⁺ (71, 83, 144, 180–182, 190, 191, 236, 264, 272, 298, 301, 325, 333, 363, 366, 369, 402–404, 426, 477, 478, 511, 558, 603, 629, 632). The Cl⁻ transport results in an apical positive transepithelial potential of ~5–15 mV (404, 405).

The Na⁺–K⁺-ATPase establishes a gradient for sodium from the extracellular space to the intracellular space. At the apical membrane this gradient facilitates uptake of HCO₃⁻ via the Na⁺–HCO₃⁻ cotransporter and uptake of K⁺ and Cl⁻ via the Na⁺–K⁺–2Cl⁻ cotransporter (5, 236, 267, 290, 295, 297, 298, 327, 346). Accumulation of Cl⁻ results in a high intracellular Cl⁻ activity of ~20–60 mM (290, 325, 402, 477, 632). The intracellular Cl⁻ activity is increased by coupling Cl⁻ transport to HCO₃⁻ transport for regulation of intracellular pH. For this purpose, a basolateral Cl⁻/HCO₃⁻ exchanger extrudes HCO₃⁻ from the cell and increases the intracellular Cl⁻ concentration (150, 151, 295, 346, 347). Cl⁻ leaves the cell via Ca²⁺-dependent Cl channels (248, 475, 574, 577, 585, 607) and via ClC-2 channels (80, 248, 287, 629). In addition, immunohistochemical analysis shows the expression of cystic fibrosis transmembrane conductance regulator (CFTR) in the RPE, which might provide an additional efflux pathway for Cl⁻ over the basolateral membrane (71, 401, 482, 629, 635). CFTR is known to function as a Cl channel activated by protein kinase A (PKA) (2). The observation of a cAMP-dependent increase in transepithelial Cl⁻ transport indicates the functional role of CFTR in the RPE (71, 401). In the regulation of intracellular pH, the activity of the Cl⁻/HCO₃⁻ exchanger and the activity of the Cl channels are combined resulting in a cycling of Cl⁻ over the basolateral cell membrane. In addition, the Cl⁻/HCO₃⁻ exchanger also decreases the efficiency of extrusion of Cl⁻ from the cytosol and, thus, the efficiency of transepithelial Cl⁻ transport. Intracellular acidification results in a decrease in the transport activity of the Cl⁻/HCO₃⁻ exchanger (150, 347). Thus intracellular acidification results in an increase in epithelial Cl⁻ transport, which might be important in elimination of water from the retina during increased metabolic activity. This is of importance because increased metabolic activity may cause intra- and extracellular edema in response to increased formation of lactic acid as well as increased uptake of glucose (80, 369).
K⁺ entering the RPE on the apical site by Na⁺-K⁺-ATPase and Na⁺-K⁺-2Cl⁻ cotransporter can leave the cell through the basolateral or the apical membrane via K⁺ channels. Normally, the basolateral K⁺ conductance is higher than the apical K⁺ conductance establishing the basis for the net transepithelial K⁺ transport from the subretinal space to the choroidal site (64, 189, 213, 250, 290, 328, 405, 477). Depending on changes in the K⁺ concentration in the subretinal space, this transport direction can be changed (see below) (328). The apical K⁺ conductance is primarily provided by inward rectifier K⁺ channels of the Kir 7.1 subtype (274, 276, 323, 528, 536, 571, 576, 593, 649). These K⁺ channels are located in the apical microvilli of the RPE (323, 536). Two main functions have been attributed to these inward rectifier K⁺ channels. The close colocalization of Kir 7.1 and Na⁺-K⁺-ATPase suggests that these act synergistically. Kir 7.1 promotes a cycling of K⁺ through the apical membrane (214, 215, 250, 273, 275, 276, 323, 436–438, 528, 536). The resulting decrease in intracellular K⁺ concentration together with decreasing gradient of K⁺ against which the Na⁺-K⁺-ATPase is transporting facilitates the Na⁺ transport out of the cell through the apical membrane. In this way, the Kir 7.1 increases the efficiency of Na⁺-K⁺-ATPase for transporting Na⁺ across the apical membrane. As mentioned above, the transepithelial transport of ions is linked to pH regulation. The inward rectifier K⁺ channels appear to be dependent on extra- and intracellular pH (657). Acidification results in an activation of these K⁺ channels and therefore facilitates Na⁺ transport by Na⁺-K⁺-ATPase, thereby transepithelial transport. With these properties, Kir channels can help the transepithelial transport adapt to increases in metabolic activity and pH regulation required for transport of lactic acid. A second function of Kir 7.1 is to react in response to changes in the subretinal K⁺ concentration, which will be described in detail in section V (276, 528). Channels responsible for efflux of K⁺ over the basolateral membrane have not been clearly identified. Candidates include the large-conductance Ca²⁺-dependent K⁺ channel (508, 592) or the M-type K⁺ channel (587). These K⁺ channels can both provide a K⁺ conductance over a broad voltage range, and
they are coupled to second messenger signaling pathways making them good candidates for function in transepithelial transport of K⁺.

Epithelial transport of Cl⁻ and K⁺ drives epithelial transport of water. The transport rate of water was estimated between 1.4 and 11 μl · cm⁻² · h⁻¹ (123, 152, 271, 602, 604, 640). Because the RPE is a tight epithelium, water cannot pass through the paracellular transport route. Thus movement of water occurs mainly by transcellular pathways. In a recent publication, expression of aquaporin-1 was detected in the RPE. Furthermore, it was shown that the transepithelial transport of water is facilitated by the functional presence of aquaporin-1 (238, 556).

In the RPE's role to support photoreceptor function, it is responsible for elimination of metabolic end products from the photoreceptors (237, 302, 330, 660). The most important metabolic end product seems to be lactic acid for which a subretinal concentration of 19 mM has been reported (4). Photoreceptor outer segments are known to produce lactic acid and might represent the major source for lactic acid (263, 637). A smaller part of lactic acid might come from inner segments, which react to transient changes in illumination with increases in lactate production (21, 67, 620). The transport of lactic acid by the RPE (Fig. 3B) requires an efficient regulation of intracellular pH (452). Lactic acid is removed from the subretinal space by the lactate-H⁺ cotransporter MCT1 (54, 345, 467) and the Na⁺-dependent transporter for organic acids (302). Protons are delivered to the subretinal space by the apically located Na⁺/H⁺ exchanger (150, 295, 658). Thus uptake of lactic acid by MCT1 is by tertiary active transport. Lactic acid is extruded through the basolateral membrane from the intracellular space to the choroid by MCT3 (467, 650) and the Na⁺/lactate exchanger (302). The subretinal pH as well as intracellular pH of the RPE are regulated by transepithelial transport of HCO₃⁻ (295, 296, 346). The HCO₃⁻ cotransporter transports HCO₃⁻ into the cell (267, 290, 295, 301, 326, 327, 329, 346). This is an electrogenic cotransporter which transports 1 Na⁺ with 2 HCO₃⁻ (326, 327, 329). Thus the transport direction is dependent on the apical transepithelial potential and intracellular HCO₃⁻ concentration. This enables the HCO₃⁻ transport system to regulate the transport direction in response to pH changes. At high intracellular and subretinal pH, HCO₃⁻ is taken into the RPE cells by the Na⁺/HCO₃⁻ cotransporter in the apical membranes and leaves the cell through the basolateral membrane in exchange with Cl⁻ mediated by the Cl⁻/HCO₃⁻ exchanger (144). This results in a subretinal to choroid directed HCO₃⁻ transport. At low intracellular and subretinal pH, new driving forces for HCO₃⁻ are established. In this case HCO₃⁻ is taken up by the Cl⁻/HCO₃⁻ exchanger in the basolateral membrane and leaves the cell through the Na⁺-HCO₃⁻ cotransporter in the apical membrane resulting in a HCO₃⁻ transport from the choroid to the subretinal space. The coupling of transepithelial HCO₃⁻ transport with pH regulation and Cl⁻ transport is explained above.

There is a small amount of transepithelial Na⁺ transport by the RPE, and the pathway controlling this is not fully understood. Na⁺ is extruded from the cell through the apical membrane via Na⁺-K⁺-ATPase, but it enters the cell primarily through the Na⁺-2Cl⁻-K⁺ cotransporter. Therefore, most of the transported Na⁺ recycles through the apical membrane. However, for the small amount of transepithelial transported Na⁺ which leaves the RPE cell through the basolateral membranes, the basolateral transport mechanism is not clear. This transmembrane transport is coupled to other metabolites and has to occur against a Na⁺ gradient. A Na⁺-coupled lactic acid transporter and an additional Na⁺-HCO₃⁻ cotransporter have been proposed to mediate this function in bovine RPE (301, 302). Using the Na⁺/HCO₃⁻ cotransporter seems unlikely because the other HCO₃⁻ transport mechanisms in the basolateral membrane should not support the establishment of a large enough HCO₃⁻-dependent driving force to transport Na⁺ against its concentration gradient.

RPE-mediated removal of water from the retina and the subretinal space is coordinated with the neuronal retina. The regulation of water and ion transport by neuropeptide Y and serotonin imply such a coordination. Neuropeptide Y stimulates both transport of Cl⁻ and water via stimulation of Ca²⁺-dependent Cl⁻ channels (23). Serotonin increases transepithelial potential, which mainly results from transport of Cl⁻ from the subretinal space to the choroid (91, 269–272, 424, 449). Furthermore, the stimulating effect of epinephrine on fluid absorption suggests that there is a sympathetic influence (49, 289, 478, 511). At least purinergic stimulation of RPE cells results in activation of several types of ion channels as well as in an enhancement of fluid absorption (363, 406, 508).

2. Transport from blood to the photoreceptors

In one direction, the RPE transports electrolytes and water from the subretinal space to the choroid, and in the other direction, the RPE transports glucose and other nutrients from the blood to the photoreceptors. To transport glucose, the RPE contains high amounts of glucose transporters in both the apical and the basolateral membranes. Both GLUT1 and GLUT3 are highly expressed in the RPE (35, 54, 582). GLUT3 mediates the basic glucose transport while GLUT1 is responsible for inducible glucose transport in response to mitogens or oncogenes and can, thus, adapt glucose transport to different metabolic demands.

Another important function of the RPE is the transport of retinol to ensure the supply of retinal to the photoreceptors. The bulk of the retinal is exchanged be-
between RPE and photoreceptors during the visual cycle in which all-trans-retinol is taken up from photoreceptors, isomerized to 11-cis-retinal, and redelivered to photoreceptors (30). The vitamin A (all-trans-retinol) uptake from the bloodstream through the basolateral membrane constitutes a smaller, additional supply to this process. The uptake of vitamin A occurs in a receptor-mediated process with recognition by a serum retinol-binding protein/transthyretin (RBP/TTR) complex (466, 476, 615). Within the RPE cell, all-trans-retinol binds to CRBP (513–515) and enters the isomerization and oxidation steps of the visual cycle (30). The subsequent reactions are described in detail in section vi. Once vitamin A is converted to 11-cis-retinal, it is transported to the photoreceptors where it binds to opsin and can serve in its function to initiate the phototransduction cascade (30). The direction of transepithelial transport of vitamin A from the blood to the photoreceptors is achieved by binding retinal to specific binding proteins that ensure absence of free retinol inside the cell (254). In addition, the rapid transfer through the visual cycle maintains a constant gradient of retinol from inside the RPE cell and the bloodstream and drives the directed transport (392, 512).

The space between the RPE and photoreceptors, the IPM, forms an important interface for interaction between RPE and photoreceptors. The IPM mediates adhesion between the RPE and photoreceptor layer, phagocytosis by the RPE (described in more detail in section vii), and nutrient exchange between RPE and the photoreceptors (204, 224). The IPM is a complex structure containing IRPB, growth factors such as basic fibroblast growth factor (bFGF), hyaluronan and hyaluronan binding proteoglycans, sulfated glycosaminoglycans, and matrix metalloproteases (1, 225, 253, 258, 259, 320). The IPM changes its structure between light and dark (610). In spite of its importance, the exchange of retinal and retinol between RPE and the photoreceptors is not understood (204). A very promising candidate to mediate exchange of retinal and retinol was IRBP (72, 107, 111, 113, 114, 206, 207, 254, 532, 562). IRBP functions to solubilize retinal and retinol, which are otherwise insoluble in water and mediates targeting of these compounds and defines the transport direction (107, 108, 130, 445, 446, 457, 458, 518). This role for IRBP is further supported by the observation that IRBP is not only present in the IPM but also in endosomes of the RPE (131). The transport direction is then defined by the rapid turnover of IRBP between the IPM and the RPE. However, this model was recently challenged by investigation of IRBP−/− mice (453). These mice still have an intact visual cycle and a normal regeneration of retinal that can be interpreted in one of two ways: either there is an efficient alternative retinoid transport pathway between RPE and photoreceptors which can compensate for the loss of the IRBP-dependent pathway, or IRBP has a function other than transport of retinal and retinol between RPE and photoreceptors. Even though this alternative function has not been established, detailed structure/binding correlation studies support its existence. IRBP isolated from dark-adapted retinas carries larger quantities of 11-cis-retinal and 11-cis-retinol than from light-adapted retinas (348). This suggests that IRBP has a 11-cis-retinal buffering function and that it protects this essential compound from oxidative damage. With this buffering function, photoreceptors could take up 11-cis-retinal from a pool and would therefore not be dependent on the numerous reactions of the visual cycle pathway to supply 11-cis-retinal, which would be much slower. This would result in faster regeneration of rhodopsin after the onset of light.

Delivery of docosahexaenoic acid to photoreceptors is a third kind of transport of importance for visual function (41). Membranes of neurons and photoreceptors as well as photoreceptor disk membranes are selectively built from phospholipids that are highly enriched with docosahexaenoic acid, a 22:6ω3 fatty acid (26, 29, 178, 566, 621, 633). This compound cannot be synthesized by neuronal tissue. Thus neuronal tissue is dependent on the delivery of 22:6ω3. New photoreceptor outer segments that are rebuilt from the base, the inner segment of photoreceptors, selectively require 22:6ω3 fatty acid (26, 29, 566, 621, 633). This compound is synthesized from the precursor, linolenic acid, in the liver and transported in the blood bound to plasma lipoprotein (26, 29, 566, 621, 633). The RPE preferentially takes up docosahexaenoic acid in a concentration-dependent manner (41–43, 208, 209). In the RPE this fatty acid is incorporated into glycerolipids in de novo synthesis reactions for storage and connection into the recycling pathways during the phagocytosis process (see sect. vii)(499).

B. Increases in Epithelial Transport Help to Treat Edema: Reductions in Epithelial Transport Cause Retinal Degeneration

In diabetic retinopathy or in some forms of inherited macular degeneration, a clinically important complication is the formation of macular edema (369). Macular edema is most likely caused by damage to the blood/retina barrier, which is formed by the RPE and the endothelium of retinal vessels. In the case of diabetic retinopathy, the site of damage is in the endothelium of retinal vessels. Macular edema is successfully treated by administration of inhibitors of carbonic anhydrase (119, 176, 369, 561, 639, 641). This results in a reduction of intracellular pH and intracellular HCO₃⁻ concentration (180, 293, 371, 404, 604, 648). As a result, transport activity of the Cl⁻/HCO₃⁻ exchanger is reduced, and the uptake of Cl⁻ via the basolateral membrane is reduced. This increases the efficiency of Cl⁻ release through the basolateral membrane and
enhances transepithelial Cl\textsuperscript{−} transport. Epithelial transport of Cl\textsuperscript{−} drives the transport of water and eliminates the fluid of the edema.

Epithelial transport of Cl\textsuperscript{−} was found to be essential for visual function. This was shown in a transgenic mouse model with a disrupted gene for Cl\textsuperscript{−}C-2 Cl channels (80, 287). The resulting phenotype has a retinal degeneration comparable to retinitis pigmentosa. The disease is caused by a lack of epithelial transport of Cl\textsuperscript{−}, the RPE shows no transepithelial potential. It is likely that this results in the inability to extrude lactic acid from the photoreceptor side leading to subsequent metabolic stress and loss of photoreceptors (80). However, the exact mechanism for the retinal degeneration is not fully understood and needs further evaluation. A second example of retinal degeneration associated with defective Cl\textsuperscript{−} transport is Best’s vitelliform macular degeneration (12, 32, 98, 157, 198, 210, 358, 375, 377, 378, 416, 464, 470, 567, 624). In this disease the retinal degeneration is caused by degeneration of RPE (198). This results in the formation of a bull’s eye shaped lesion that resembles an egg yolk. The shape of the lesion gave rise to the name of this disease. The bull’s eye lesion primarily contains extracellular fluid, suggesting there is a reduction in epithelial Cl\textsuperscript{−} transport (624). The leading symptom for diagnosis of Best’s vitelliform macular degeneration is a reduction in the light peak-to-dark ratio in the patient’s electro-oculogram (EOG) (198). The light peak results from the activation of basolateral Cl\textsuperscript{−} conductance (188, 190, 191, 193). Light-dependent stimulation of the retina leads to release of a light peak substance from the inner retina or photoreceptors (188). The light peak substance diffuses to the RPE and leads to activation of basolateral Cl\textsuperscript{−} channels via activation of intracellular second messenger pathways (192, 193). Because it is likely that the underlying second messenger pathway results in an increase in intracellular Ca\textsuperscript{2+}, the basolateral Cl\textsuperscript{−} conductance is provided by Ca\textsuperscript{2+}-dependent Cl channels. The as yet unidentified light peak substance is very likely ATP, which is known to activate the inositol 1,3,4-trisphosphate (InsP\textsubscript{3})/Ca\textsuperscript{2+} second messenger system. Furthermore, an increase in intracellular InsP\textsubscript{3} increases the concentration of cytosolic free Ca\textsuperscript{2+} and subsequently Ca\textsuperscript{2+}-dependent Cl channels (573, 577). Thus the reduction of the light peak in patients’ EOG points to a reduction in epithelial Cl\textsuperscript{−} transport, which might be the cause for the pathology of the disease. The gene responsible for Best’s vitelliform macular degeneration has been isolated and identified as VMD2 gene (378, 464). The VMD2 gene product is named bestrophin (32, 98, 157, 358, 378, 416, 464, 567). Recent data suggest on several lines of evidence that bestrophin itself represents a new family of Ca\textsuperscript{2+}-dependent Cl channels (175, 473–475, 585, 605). This elegantly explains the cause for the light peak reduction in the patient’s EOG. Heterologous expression studies showed for 15 different bestrophin mutations a loss of Cl channel function (585). Thus reduction in the light-peak amplitude in the patients’s EOG results from reduction in basolateral Cl\textsuperscript{−} conductance. However, studies searching for more bestrophin mutations described patients with bestrophin mutations that show normal light peaks or an onset of the light-peak reduction that occurs later than the onset of macular dystrophy (12, 157, 319, 358, 525, 617). In addition, in a recently published rat model for Best’s disease, overexpression of wild-type bestrophin did not change light-peak amplitude but desensitized luminance response while two investigated mutant bestrophins could generate the expected decrease in light-peak amplitude (376). These observations cannot be easily explained by the theory that Best’s disease is caused by a lack of Cl channel function.

Another example of Cl\textsuperscript{−} transport alteration in the RPE that might result in disease is cystic fibrosis (287, 482). Patients with cystic fibrosis have reduced amplitudes of the fast oscillation in the EOG (71, 629). Like the light peak, the fast oscillation represents a signal in the EOG that is generated by activation of Cl channels in the basolateral membrane of the RPE. The expression of CFTR has been established in RPE cells. Furthermore, part of the transepithelial transported Cl\textsuperscript{−} is dependent on cAMP-activated Cl conductance (269–272, 321, 325, 342, 401, 403, 422). Thus patients with cystic fibrosis have additional reduction in the transepithelial Cl\textsuperscript{−} transport by the RPE. However, patients with cystic fibrosis do not develop retinal degeneration perhaps because there is a compensatory activation of other Cl channels, such as the Ca\textsuperscript{2+}-dependent Cl channel, in the RPE.

Alteration of transepithelial transport may function in the pathogenesis of age-related macular degeneration. As described above, the development of Drusen is required for diagnosis of this disease (20). In 20–25% of patients, Drusen become large and confluent (311) and establish large diffusion barriers between blood vessels and the RPE. This may lead to development of areas with reduced supply of oxygen and glucose, mimicking hypoxia (183, 360). These regions of hypoxic stress may cause degeneration of adjacent photoreceptors and subsequent loss of vision in areas of Drusen. A further consequence of metabolic stress might be the induction of choroidal neovascularization, the most severe complication in the etiology of age-related macular degeneration (311). Hypoxia reduces the secretion of pigment epithelium-derived growth factor (PEDF) by the RPE (134). PEDF is a potent antiangiogenic factor. The development of choroidal neovascularization can lead to intraocular bleeding, which is the main cause for vision loss (20). This theory is supported by the observation that choroidal neovascular membranes contain high amounts of advanced glycation end products that are generated by reactive oxygen species during reduced metabolism (242).
Finally, reduction in the delivery of retinal to photoreceptors was found to induce a form of photoreceptor dystrophy (526). This rare disease was described as a symptom of the retinol binding protein deficiency syndrome. Here, patients show mutations in the retinol binding protein 4, \( RBP4 \), gene. This protein appears to be essential for uptake of retinol into the RPE cells. Because of the reduced retinol uptake, patients have no detectable rod function in dark adaption or in their scotopic ERG. However, these patients do not have a very severe degenerative phenotype, suggesting that there must be an alternative tissue source for vitamin A.

V. SPATIAL BUFFERING OF IONS IN THE SUBRETNAL SPACE

A. Spatial Buffering of Ions in the Subretinal Space Maintains Excitability of Photoreceptors

The RPE not only stabilizes the ion homeostasis in the subretinal space by transepithelial transport of ions, but it is also able to compensate for fast occurring changes in the ion composition in the subretinal space (558). This function is comparable to the ability of glia cells for spatial buffering of ions. In fact, a major part of the spatial buffering is enabled by Müller glia cells (427, 486, 644). Another part is controlled by the RPE. Stimulation of photoreceptors by light reduces the dark current, which consists of an influx of Na\(^+\) via open cGMP-gated cation channels in the outer segments which is counterbalanced by an efflux of K\(^+\) via K\(^+\) channels in the inner segment (39). The reduction of the K\(^+\) efflux results in a decrease of the subretinal K\(^+\) concentration from \(-5\) to 2 mM (144, 558, 559). This leads to a change in the equilibrium potential for K\(^+\), which in turn disturbs the phototransduction cascade in the outer segments of the photoreceptors. The RPE compensates for these changes in the following manner: the decrease in the subretinal K\(^+\) concentration hyperpolarizes the apical membrane of the RPE (436–438), which causes activation of inward rectifier K\(^+\) channels (276, 528, 536, 649, 657). These inward rectifier channels respond with a mild rectification and an increase in activity in response to a decrease in the extracellular K\(^+\) concentration (276). The activation of inward rectifier K\(^+\) channels then causes a change in the ratio of apical and basolateral K\(^+\) conductance (328). Normally, the basolateral K\(^+\) conductance is higher than the apical conductance which results in directed epithelial transport of K\(^+\) from the subretinal space to the choroidal site (328). The rise in apical K\(^+\) conductance increases the portion of K\(^+\) that is transported back to the subretinal space. As a result, K\(^+\) is secreted to the subretinal space and compensates for the light-induced decrease of subretinal K\(^+\). Application of K\(^+\) channel blocker can prevent the hyperpolarization-induced decrease in intracellular K\(^+\) (215, 273). The compensation for the light-induced decrease in subretinal K\(^+\) is supported by the Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter that appears to work in the early phase of apical membrane hyperpolarization in the opposite direction, leading to an additional K\(^+\) outflow of the cell (64, 65). In addition, the decrease in subretinal K\(^+\) concentration changes the Cl\(^-\) transport (64, 151, 213, 214). The decrease in subretinal K\(^+\) concentration decreases the uptake of Cl\(^-\) via the Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter in the late phase of apical membrane hyperpolarization. The subsequent decrease in intracellular Cl\(^-\) activity changes the equilibrium potential for Cl\(^-\) through the basolateral membrane which has a high Cl\(^-\) conductance (64, 65). This results in a hyperpolarization of the basolateral membrane and decreases the driving force for Cl\(^-\) to leave the cell via the basolateral membrane. These compensatory mechanisms can be monitored in the electroretinogram (ERG) as the c-wave and delayed hyperpolarization (214, 436, 558). The c-wave results from the hyperpolarization of the apical RPE cell membrane, and the delayed hyperpolarization results from hyperpolarization of the basolateral membrane. Light-dependent activation of photoreceptors closes cation channels in the outer segments and thus increases the subretinal Na\(^+\) concentration, which is compensated for by the Na\(^+\)/H\(^+\) exchanger and by the Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter in the apical membranes of the RPE. The subsequent decrease in the subretinal Na\(^+\) concentration after closing of cation channels during the transition from light to darkness is compensated for by Na\(^+\)-K\(^+\)-ATPase in the photoreceptor’s inner segments and by the Na\(^+\)-K\(^+\)-ATPase of the RPE (255). It is believed that this might be why the Na\(^+\)-K\(^+\)-ATPase is localized to the apical membrane of the RPE.

The light-induced changes in ion transport do not only maintain ion homeostasis in the subretinal space. The changes in the transport direction also imply light-dependent changes in water transport (268). This effect is based on the fact that the activity of the apical Na\(^+\)-HCO\(_3\)\(^-\) cotransporter is dependent on the membrane potential (326). Light-induced hyperpolarization of the apical membrane results in a decrease of its transport activity, which subsequently leads to intracellular acidification (301, 344, 346). This increases Cl\(^-\) efflux through the basolateral membrane (see sect. iv) and results in an increase in Cl\(^-\) and water transport from subretinal space to choroid. The light-induced increase in water absorption seems to be of importance to control subretinal space volume during changes in illumination. With the use of measurements of the concentration of tetramethylammonium (TMA\(^-\)) ions by a TMA\(^-\)-sensitive electrode, it was shown that illumination leads to transient volume increases in the retina.
that were most pronounced in the region between outer nuclear layer and subretinal space (266, 341). It is likely that changes in the extracellular volume of the inner retina are compensated for by Müller cells, whereas the RPE compensates for the changes in the subretinal space. In the dark, the apical membrane of the RPE is depolarized. Now the activity of the Na\(^+\)-HCO\(_3\) \]^+\)-cotransporter rises causing intracellular alkalinization (267, 346). In consequence, less Cl\(^-\) leaves the cell through the basolateral membrane. This reduces fluid absorption in the dark (301). Studies on epithelial transport indicate a more severe consequence (65, 603). A decreased efflux of Cl\(^-\) out of RPE cells increases intracellular Cl\(^-\) concentration providing a driving force for Cl\(^-\) to leave the cell through the apical membrane. Indeed, an apical Cl\(^-\) secretion was observed when K\(^+\) concentration at the apical site was increased from 2 to 5 mM (151, 347). Because apical Cl channels have not yet been described, the Cl\(^-\) efflux mechanism through the apical membrane is unclear. However, the postulated Cl\(^-\) secretion in the dark should result in a fluid secretion in the dark into the subretinal space. Indeed, a corresponding fluid movement from the basolateral side to the apical side has been observed in transepithelial transport studies but is not proven in vivo. The physiological significance of a fluid secretion into the subretinal space in the dark is not clear. However, the secretion likely occurs only for a short time until the increase in subretinal K\(^+\) concentration has been compensated and the apical membrane is no longer depolarized.

B. Spatial Buffering Gives Rise to Wave Forms in the ERG: Monitoring of Metabolic Status

To date, changes in the ability of the RPE to mediate spatial ion buffering have not been linked to degenerative disease of either the photoreceptors or the RPE. However, the action of spatial buffering can be monitored in recordings of the ERG. Changes in signals that arise at the RPE can give information about diseases caused by alterations of RPE function. These alterations can be attributed to changed metabolic activity of the RPE (133, 291, 349–351, 558). The c-wave of the ERG arises from an increase in the apical K\(^+\) conductance in the RPE and correlates with the function of K\(^+\) buffering in the subretinal space that is reduced under hypoxic conditions (349, 351). However, many of these changes arise in the interaction between RPE and neuronal retina and reveal the tight interactive function of both tissues. The current detailed picture of the energy metabolism-associated events leading to retinal degenerations has been drawn primarily from methods investigating RPE/retina interactions in the ERG.

VI. VISUAL CYCLE OR RETINOID CYCLE

A. Exchange of Retinal Between Photoreceptors and RPE: Isomerization and Reisomerization Between 11-cis and all-trans

Photoreceptors lack cis-trans isomerase function for retinal and are unable to regenerate all-trans-retinal into 11-cis-retinal after transduction of light energy into electrical impulses (30). Therefore, retinal is converted by two major metabolic pathways. One pathway includes binding of retinal to opsin as 11-cis-retinal and release from opsin as all-trans-retinal during recovery of rhodopsin after light absorption. The second pathway serves for the regeneration or better reisomerization of all-trans-retinal into 11-cis-retinal (Fig. 4). The reisomerization occurs in the RPE. Thus retinal entering the second pathway is cycled between photoreceptors and the RPE to ensure constant excitability of photoreceptors. This shows how closely photoreceptors and RPE interact in the process of vision. However, recent studies have described a second pathway of retinal reisomerization (28, 386). It appears that cones can regenerate a part of their photoisomerized retinal in a second visual cycle occurring in Müller cells. The metabolic pathways of the visual cycle are not completely understood. Most of the understanding comes from studies on mechanisms of retinal degenerations. Excellent reviews on this topic have been published by Thompson and Gal (596, 597) and by Besch et al. (58). Much in the following description of the metabolic pathway of retinal regeneration will need to be further verified by extensive future studies.

Light transduction is initiated by absorption of light by rhodopsin which is composed of a seven transmembrane domain G-coupled receptor protein, opsin, and the chromophore 11-cis-retinal (247). Absorption of light changes the conformation of 11-cis-retinal into all-trans-retinal. The conformational change of retinal results in formation of active rhodopsin, meta-rhodopsin, which in turn is able to activate transducin in the next step in the phototransduction cascade (256, 444). The active state of rhodopsin is terminated by phosphorylation by rhodopsin receptor kinase and subsequent binding with arrestin (39, 40). Inactivated rhodopsin releases all-trans-retinal and binds 11-cis-retinal again in several subsequent steps so that rhodopsin can again be activated by absorption of light. Accumulating all-trans-retinal leaves the intradiscal space facilitated by the activity of an ATP-binding cassette protein (ABCR) (584, 628) known as ABCA4 (9–11, 13–15, 340, 537). It functions in rods and cones as ATP-dependent flippase for N-retinylidene-phosphatidylethanolamine (N-retinylidene-PE). This requires the reaction of all-trans-retinal with phosphatidylethanolamine (352). After hydrolysis, N-retinylidene-PE is released to the cy-
tosol of the photoreceptors as all-trans-retinal. Here, it is reduced to all-trans-retinol by a membrane-bound retinol dehydrogenase that requires NADPH (221, 483). The all-trans-retinol dehydrogenase (RDH) belongs to the family of membrane-bound short-chain alcohol dehydrogenases (SCAD or short chain acetyl-CoA dehydrogenases). All-trans-retinol is then translocated to the RPE on a carrier protein that may be IRBP (204, 206). The exact mechanism for uptake into the RPE is not known but might be a trapping of all-trans-retinol inside the RPE by formation of insoluble fatty acid retinyl ester. Inside the RPE all-trans-retinol is bound to CRBP (513, 514, 516). The isomerization is initiated by an esterification step which includes transfer of an acyl group to retinol catalyzed by lecithin:retinol transferase (LRAT) (387, 407, 507, 601, 666). The isomerization requires in addition the participation of a chaperone. This chaperone, RPE65 (retinal pigment epithelium-specific protein 65 kDa), was first isolated as a RPE-specific protein (239–241, 432, 485). Studies of RPE65 loss of function implicate its role in the retinol isomerization cycle (217, 278, 292, 357, 485, 527, 598, 614). In recent publications it has been suggested that RPE65 functions as a retinylester binding protein (124, 200–203, 284, 385, 645) and as a chaperone for all-trans-retinylesters (202, 645). Furthermore, RPE65 can adapt the visual cycle on the different retinoid requirements in dark and light (202, 645). A membrane-bound form mRPE65 is triply palmitoylated, has high affinity to all-trans-retinylesters, and is a palmitoyl donor for the acylgroup transfer by LRAT (645). This form ensures 11-cis-retinal production during light. The soluble form, sRPE65, is not palmitoylated and has high affinity to vitamin A.

FIG. 4. Visual cycle. Light transduction starts with photon absorption by rhodopsin. The process of light absorption underlies the stereochemical change of 11-cis-retinal into all-trans-retinal. All-trans-retinal is metabolized into all-trans-retinol and transported to the RPE. In the RPE retinol reisomerized to 11-cis-retinal and then redelivered to the photoreceptors. The regeneration seems to follow two pathways. One occurs through an as yet unidentified enzyme thought to function as a isomerohydrolase. The second pathway involves a light-dependent pathway using the RPE-retinal G protein-coupled receptor which isomerizes all-trans-retinal to 11-cis-retinal following light absorption. IRBP, interstitial retinal binding protein; CRBP, cellular retinol binding protein; CRALBP, cellular retinaldehyde binding protein; atRDH, all-trans-retinol dehydrogenase; 11cRDH, 11-cis-retinol dehydrogenase; LRAT, lecithin retinol acyltransferase; RPE65, RPE specific protein with molecular mass of 65 kDa; RGR, RPE-retinal G protein-coupled receptor; RBP/TTR, retinol-binding protein/transthyretin complex. [From Thompson and Gal (597), with permission from Elsevier.]
This form is responsible for a reduced 11-cis-retinal production in the dark. The palmitoyl switch between these two RPE65 forms is controlled by the activity of LRAT and the concentration of 11-cis-retinol in the RPE (645). With these characteristics RPE65 function is a rate-limiting step for the visual cycle. The enzyme that catalyzes the isomerization of all-trans-retinyl ester to 11-cis-retinol is called isomerohydrolase and has not yet been identified (137). Isomerohydrolase uses the energy from ester hydrolysis in a reaction that might occur in two separate reaction steps. In the first step hydrolysis of retinylester occurs via the retinylester hydrolase function of the enzyme complex. In the second step isomerization occurs through a carbocation intermediate. The next step in the regeneration process of retinal is the oxidation of 11-cis-retinol to 11-cis-retinal. This NAD and NADP-requiring reaction step is catalyzed by 11-cis-retinol dehydrogenase or RDH5, which belongs to the family of short-chain dehydrogenases (146, 285, 538). This reaction is supported by a cellular retinyaldehyde-binding protein (CRALBP) that accelerates the enzymatic reaction (517). Immunoprecipitation studies suggest that RDH5, RPE65, LRAT, and CRALBP form complexes in which CRALBP positions retinol for optimal enzymatic turnover (63, 120, 121, 538). This pathway to regenerate retinal is supplemented by an alternative pathway involving the activity of RPE-retinal G protein-coupled receptor (RGR) (120, 121, 246, 288, 535, 591). RGR converts all-trans-retinal into 11-cis-retinal, in an inverted light-induced rhodopsin isomerization reaction (121). The substrate for RGR is produced from all-trans-retinol of the retinylester pool. A retinol dehydrogenase, presumably not RDH5, produces all-trans-retinal, which will be subsequently transformed into 11-cis-retinal by RGR using light energy. Analysis of RGR knock-out mice (see below) suggests that the RGR-dependent, alternative pathway serves to adapt the retinal turnover to changes in ambient light (120). Thus two pathways are responsible to regenerate 11-cis-retinal. For clearer presentation, these pathways will be considered separately and referred to as the RDH5 or the RGR pathway. It is not clear if a RGR-dependent pathway can be separated from a RDH5-dependent pathway in normal individuals. The data from studies of knock-out mice indicate some independence because of the compensatory effects (120, 147). RGR functions to maintain constant levels of 11-cis-retinal independent of different levels of ambient light (302). The RDH5 pathway is important for the fluctuating acute turnover of retinal during the process of vision. Thus the loss of function of RGR reduces and destabilizes the overall levels of 11-cis-retinal and leads to photoreceptor degeneration. In contrast, loss of function of RDH5 results in a delayed recovery from light exposure and, thus, to night blindness. The RGR pathway is important to maintain the 11-cis-retinal levels after the onset of light, whereas the RDH5 pathway regulates 11-cis-retinal for recovery from light exposure. The RGR knock-out mice cannot maintain 11-cis-retinal levels in ambient light. Exposure of Rgr-/- mice leads to a drop in the levels of 11-cis-retinal and rhodopsin (120). In addition, the RGR pathway can better compensate for loss of function in the RDH5 pathway versus the RDH5 pathway, which incompletely compensates for loss of function of RGR. That there is the capacity for a partial compensation can be seen in the fact that Rgr-/- mice show a rather late onset (9 mo) of retinal degeneration (120) compared with other animal models for retinal degeneration (222).

The transport of 11-cis-retinal back to photoreceptors occurs through binding to IRBP in the subretinal space. How 11-cis-retinal leaves the RPE and enters photoreceptor cells is not understood. With this last step of retinal regeneration, retinal reenters the other metabolic pathways by binding to opsins and serves in its function for light transduction.

B. Inherited Retinal Degenerations Are Caused by Mutations in a Variety of Genes of the Visual Cycle

Reduction in the activity of the visual cycle is responsible for several types of retinal and RPE degenerations. To date the cause of many retinal degenerations has been identified as gene defects leading to reduced function of a broad range of proteins involved in the visual cycle reaction cascade.

Mutations in the ABCA4 gene can lead to a variety of diseases (9–11, 13, 14, 57, 340, 383, 416, 537, 539, 540, 584, 661–663). These mutations lead to different degrees of reduced function. Reduced ABCA4 protein function decreases the efficiency of all-trans-retinal elimination from the intradiscal space decreasing amounts of retinal that can enter the visual cycle to be isomerized back to 11-cis-retinal. Interestingly, the degree of functional loss of ABCA4 protein leads to different types of retinal degeneration (11). A total loss of function causes retinitis pigmentosa, a photoreceptor degeneration which is characterized by a loss of vision from the periphery to the center of vision due to rod photoreceptor followed by cone photoreceptor cell death and accumulation of pigment granules in the fundus (11, 383). A different type of photoreceptor degeneration, collectively referred to as cone-rod dystrophies, are caused by mutations causing a severe reduction in the transport activity versus a total loss of function (540). The most common disease that results from mutations in ABCA4 is Stargardt disease or fundus flavimaculatus, inherited forms of macular degeneration, and loss of vision occurring early in life (10, 14, 15, 27, 68, 141, 210, 390, 416, 539, 661–663). This disease can be regarded as a RPE disease because it starts with the
degeneration of the RPE. Thus mutations in a gene expressed in photoreceptors can result in degeneration of the RPE. In addition, ABCA4 mutations have been associated with age-related macular degeneration (9, 13, 57, 210, 416). How loss of function in the ABCA4 transporter protein leads to retinal degeneration is best understood in the case of Stargardt disease. The reduced ABCA4 function causes accumulation of all-trans-retinal and N-retinylidene-PE (see above) in the disks of the photoreceptor outer segments (480). Photoreceptor outer segments are shed from the photoreceptors in a circadian regulated process and are phagocytosed by the RPE constituting another important function of the RPE for the maintenance of visual function (see sect. vii). The phagocytosed outer segments undergo enzymatic digestion within the RPE. In the case of Stargardt disease, the RPE is confronted with photoreceptor outer segments that contain abnormally high levels of all-trans-retinal and N-retinylidene-PE. As a consequence, during enzymatic digestion within phagosomes N-retinylidene-N-retinylethanolamine (A2E) is generated (197, 388, 479). A2E is a major fluorophore of lipofuscin and has several toxic effects on the RPE that are described in section iii. Thus, with reduced function of ABCR, the RPE is poisoned by A2E. The resulting loss of function of the RPE and the possible loss of RPE cells secondarily leads to photoreceptor degeneration and subsequent loss of vision (548, 552, 611). As mentioned above, some forms of age-related macular degeneration have been associated with mutations of ABCR gene (9, 11). Also in age-related macular degeneration, accumulation of A2E appears to be a hallmark for the induction of the disease (52, 530). Here, ABCR show only a small loss of function leading to a slower poisoning of RPE cells.

Within the RPE, retinal and retinol are transported by binding to retinol binding proteins. CRALBP mutations have been described that lead to an early-onset retinal dystrophy known as retinitis punctata albescens, which is characterized by the appearance of uniform white dots in the fundus picture (391, 408, 545). In these patients a delayed dark adaption and elevated dark adaption thresholds leading in the beginning to night blindness show the importance of CRALBP in the visual cycle, since these mutations lead to a decreased efficiency to regenerate retinal (205).

As mentioned earlier, mutations in the 11-cis-retinal dehydrogenase gene RDH5 cause less severe phenotypes. RDH5 mutations lead to decreased protein stability, mislocalization in the cytosol, and decreased enzyme activity (343). The patients suffer from fundus albipunctatus, a congenital night blindness caused by a delayed rod-cone pigment regeneration after strong bleaches and white dots in the fundus picture (199, 367, 368). In contrast, mutations in the RGR gene cause the much more severe retinitis pigmentosa (410, 622). The differences in the phenotype resulting from RGR mutations and from RDH5 mutations show again the relative functional importance of the two retinal regenerating pathways.

A severe phenotype, retinal dystrophy, also arises from mutations in the RPE65 gene (167, 200, 217, 239–241, 357, 359, 409, 460, 461, 541, 598, 600, 614, 622, 643). As described above, RPE65 function is rate limiting for the visual cycle by enhancing or decreasing 11-cis-retinal production in dependence of the illumination (202, 645). A lack of RPE65 leads to abnormally low levels of chromophore and increased levels of phosphorylated opsin (612). The disturbance of retinal turnover also results in an accumulation of lipofuscin in RPE cells (292). Lipofuscin appears to play an important role in the etiology of age-related macular degeneration by destabilizing RPE cells and increasing their susceptibility to photo-oxidative damage (202). Mice with a disrupted RPE65 gene show dramatically reduced light- and dark-adapted ERGs before onset of photoreceptor degeneration (485, 527, 612, 613). The remaining visual function comes from rod photoreceptors (527). This residual activity suggests that there is either an additional, not yet described pathway, to regenerate all-trans-retinal or that there could be an incomplete compensation by RGR-dependent and/or RDH5-dependent pathways. Because RGR can directly isomerize all-trans-retinal into 11-cis-retinal, it is likely that this pathway is responsible for the remaining retinal regeneration. However, in RPE65−/− mice, without the retinyl ester binding protein RPE65, all-trans-retinal must reach the RPE directly and not via the ABCR-mediated pathway. This very likely occurs during phagocytosis of photoreceptor outer segments, which is described in more detail in the next section. In humans, mutations of the RPE65 gene lead to retinal dystrophies including Leber’s congenital amaurosis (142, 167, 217, 240, 241, 357, 359, 409, 460, 461, 541, 506, 508, 600, 622, 643). These diseases are early onset showing the importance of RPE65 in the visual cycle. In animals, such as the RPE65 transgenic mouse or the naturally occurring retinal dystrophy in Briard dogs, mutations in the RPE65 gene result in retinitis pigmentosa. However, RPE65 mutations represent examples of diseases caused by a gene expressed in the RPE leading to disease which initially affects photoreceptors.

A comparable phenotype to the one of RPE65 mutations results from mutations in the LRAT gene, the gene encoding lecithin:retinol transferase (597, 599). Patients with LRAT mutations have an early-onset, severe retinal dystrophy. The visual field becomes restricted to tiny islands. LRAT mutations include changes that cause amino acid changes resulting in loss of function or truncated proteins due to missense mutations. The net effect is the accumulation of toxic levels of vitamin A in RPE cells. This can be explained by the fact that LRAT is responsible for the palmityl switch of RPE65 to the form with high affinity to vitamin A. In patients with LRAT...
VII. PHAGOCYTOSIS

A. Photoreceptor Outer Segment Renewal: Phagocytosis of Shed Photoreceptor Membranes by the RPE

Photoreceptors contain high amounts of photosensitive molecules. As discussed in section VI, photoreceptors are exposed to intense levels of light. This leads to accumulation of photo-damaged proteins and lipids. In addition, retinal itself can generate photo-oxidative radicals. Thus, during each day, the concentration of light-induced toxic substances increases inside the photoreceptors (45). The light transduction by photoreceptors is dependent on the proper function and structure of proteins, retinal, and membranes. Therefore, to maintain the excitability of photoreceptors, the photoreceptor outer segments (POS) undergo a constant renewal process (72, 73, 232, 430, 558, 652, 654–656). In this renewal process POS are newly built from the base of outer segments, at the cilium. The tips of the POS that contain the highest concentration of radicals, photo-damaged proteins, and lipids are shed from the photoreceptors. Through coordinated POS tip shedding and formation of new POS, a constant length of the POS is maintained. Shed POS are phagocytosed by the RPE. In the RPE, shed POS are digested and important molecules, such as retinal or docosahexaenoic acid, are redelivered to photoreceptors in a manner comparable to the visual cycle (66, 72). For recycling, docosahexaenoic acid is removed from phospholipids and redelivered to photoreceptors as a fatty acid (26, 41, 42, 208, 209, 499). Retinal undergoes the RPE-specific part of visual cycle and is redelivered as 11-cis-retinal to photoreceptors. Here again, RPE and photoreceptors are closely interacting partners. This interaction is essential for maintaining the structural integrity of photoreceptors and, thus, visual function. Furthermore, the process of disk shedding and phagocytosis must be tightly coordinated between both the RPE and the photoreceptors. A failure in this regulation would result in POS that are either too long or too short. The process of POS shedding and phagocytosis is under circadian control (334–337, 439, 651). In the process of phagocytosis every RPE cell is facing an average ranging between 20 and 45 photoreceptors in Rhesus monkeys (544, 653). In humans, the ratio was estimated in the fovea with 23 photoreceptors per RPE cell average over 9 decades (194). The turnover rate for one entire photoreceptor outer segment is 10 days (653).

A substance permitting the communication between photoreceptor and RPE that controls phagocytic activity has not been identified. In addition, initiation and intracellular regulation of phagocytosis can be studied in isolated cells by challenging RPE cell cultures with isolated POS (171, 229, 235, 251, 417). Thus it seems that the presence of shed POS is sufficient to initiate phagocytosis. Thus the basis of coordination between POS shedding and phagocytosis is the presence of POS. The initial step in phagocytosis (Fig. 5) is the specific binding of POS at the apical membrane of the RPE (229). In the next step, the recognition of POS binding is transferred to the intracellular space by activation of a second messenger cascade, which in turn activates the ingestion of bound POS (229). CD36, MerTK, and integrin receptors have all been described as regulators of POS phagocytosis. The macrophage scavenger receptor CD36 was found to regulate the rate of POS internalization (174, 509, 510, 551). A recent study suggests an interaction between CD36 and the toll-like receptor TLR4 adapting the metabolism of RPE cells to handle ingested POS (305). The receptor tyrosine kinase c-mer (MerTK) transduces the specific binding of POS to the RPE into activation of the second messenger cascade that initializes internalization of POS (118, 135, 154, 190, 233, 235, 251). Binding of POS requires the activation of \( \alpha_\beta_5 \)-integrin receptors (170, 171, 173, 174, 400, 664). MerTK and \( \alpha_\beta_5 \)-integrin receptors interact in the initialization of phagocytosis (170). An essential role of MerTK is evident (148, 168, 616). Cells lacking the MerTK receptor are able to bind POS but are not able to ingest them (118). It is noteworthy, however, that al-

![Fig. 5. Phagocytosis. The molecules involved in the initiation of photoreceptor outer segment phagocytosis are shown. The initiation starts with binding of photoreceptor outer membranes. The event of binding is transduced into an intracellular signal, a rise in intracellular inositol 1,4,5-trisphosphate (InsP3), which in turn leads to ingestion of the bound photoreceptor outer segment membranes. Binding is likely mediated by integrins, and signal transduction occurs through the receptor tyrosine kinase MerTK. Ingestion involves the macrophage receptor CD36. Coordinate signal transduction occurs through integrin and MerTK interaction through the focal adhesion kinase, CD96, macrophage phagocytosis receptor; FAK, focal adhesion kinase; Gas6, growth-arrest-specific protein 6; MerTK, receptor tyrosine kinase c-mer; PLC, phospholipase C; POS, photoreceptor outer segment.](https://physrev.physiology.org)
though RPE cells lacking MerTK cannot ingest POS, these cells are capable of nonspecific phagocytosis (154, 251). In RPE cells with intact MerTK, the binding of POS leads to an increase in intracellular InsP₃, which does not occur in RPE cells with disrupted MerTK receptor tyrosine kinase (251). The lack of InsP₃ activation in cells with disrupted MerTK is responsible for the inability to ingest POS. Thus MerTK is essential for transfer of this signal into the intracellular space. The binding of POS to RPE cells seems to be mediated by αᵥβ₅-integrin receptors (170, 171, 174, 400). Antibody-induced inhibition of αᵥβ₅-integrin receptors dramatically reduced binding and as a consequence ingestion of POS (171). In a recent publication, Finnemann (170) could show that activation of αᵥβ₅-integrin receptor is not only essential for POS binding but also plays a role in activation of MerTK. Thus both the receptors MerTK and αᵥβ₅-integrin interact. This interaction is mediated by activation of focal adhesion kinase (FAK). In this model, POS binding to αᵥβ₅-integrin receptor activates FAK, in turn phosphorylates MerTK, and then activates the subsequent second messenger cascade. If both receptor types are involved in binding and recognition of POS, then additional proteins are also required for this process. αᵥ-Integrin receptor predominantly recognizes vitronectin (125). Finneman et al. (171) found that in an in vitro POS phagocytosis assay that vitronectin itself did not change POS phagocytosis. The group concluded that other surface proteins on POS that are capable of binding to αᵥβ₅-integrin receptor mediate this process. The specific binding partner of MerTK is growth-arrest-specific protein 6 (Gas6). Hall and co-workers (234, 235) could demonstrate that Gas6 is required for proper phagocytosis of POS. However, the interaction of phagocytosis receptors, intermediate proteins, and POS has not been entirely elucidated.

As mentioned above, the binding and recognition of POS activates the second messenger InsP₃ (251). The activation of this second messenger cascade results in an increase in the number of phosphorylated proteins (252). Initiation of phagocytosis requires tyrosine phosphorylation (170). An increase in intracellular InsP₃ also causes a subsequent increase in intracellular free Ca²⁺. Both in concert seem to regulate phagocytosis, whereas an increase in InsP₃ alone activates the ingestion of POS (251, 498). The increase in intracellular free Ca²⁺ coupled to subsequent activation of protein kinase C may act as a shut-off signal for phagocytosis (231). While the InsP₃/Ca²⁺ second messenger system functions in initiation of phagocytosis, the cAMP second messenger system is important in the regulation of phagocytosis (153, 230). An increase in intracellular cAMP reduces phagocytic activity. This is physiologically achieved by β-adrenergic stimulation and by stimulation of adenosine A₂ receptors (49, 212, 230). Both the InsP₃/Ca²⁺ and the cAMP second messenger systems can interact. The group of Osborne and co-workers (423, 424, 449) demonstrated coregulation by both systems by serotonin via protein kinase C. Interestingly, bFGF (or FGF-2) can stimulate POS phagocytosis. This might result from a bFGF-dependent reduction in the cAMP production in RPE cells. Nash and Osborne (423) showed that bFGF decreases the forskolin-induced cAMP production. This effect is even more remarkable because bFGF can also increase POS phagocytosis activity in RPE cells lacking MerTK (395). However, because bFGF has no effect on cAMP production in RPE cells lacking MerTK (423), the rescue effect of bFGF is mediated by another pathway.

It has been proposed that the rate of phagocytosis is dependent on the rate at which apical POS phagocytosis receptor containing cell membrane cycles between the extracellular and intracellular space (79). This theory was derived from the measurement of overlapping binding and ingestion kinetics (229). Kinetic studies have shown that inhibition of ingestion reduces binding of POS, and vice versa.

There is also a circadian regulation of POS phagocytosis (60, 177, 335, 337, 469). The major burst of rod outer segment phagocytosis takes place with the onset of light (61). However, extensive studies on the circadian regulation of photoreceptor outer segment shedding and phagocytosis revealed a more complex pattern with differences between cones and rods (595) and with differences among different species (59). The circadian control is dependent on a circadian clock in the retina which (95–97) is known to regulate adaptive changes in the retina (211). How this circadian clock controls the onset of POS phagocytosis is also not entirely understood (430). An important transmitter to coordinate phagocytosis and the circadian clock seems to be dopamine (62, 469). The negative regulation of phagocytosis by increasing cAMP levels and the presence of dopamine receptors linked to adenylyl cyclase activation suggest that circadian regulation may be mediated through the dopamine/melatonin system (59, 95–97, 430, 431, 433, 442, 488, 489). In a recent paper an additional role of integrin receptors was shown (421). Mice lacking αᵥβ₅-integrin lack a synchronized phagocytosis of shed POS with no burst of phagocytosis after onset of light (421).

### B. Failure of Photoreceptor Outer Segment Phagocytosis Leads to Retinal Degeneration

Inability of the RPE to phagocytose POS causes an autosomal recessive form of retinitis pigmentosa (148, 154, 187, 413, 420, 600, 616, 622). This was first described in an animal model for retinitis pigmentosa, the Royal College of Surgeons or RCS rat (73, 88, 118, 154, 190, 355, 413, 575). The genetic defect in this animal results in synthesis of a truncated, dysfunctional MerTK protein and,
thus ultimately, to loss of POS phagocytosis receptor function (135). Furthermore, biochemical studies have shown that the truncated MerTk protein cannot be directed to the cell membrane. The lack of MerTK blocks POS phagocytosis and therefore leads to subsequent loss of photoreceptors (118, 148, 154, 199, 335). As proof that the defect in the RCS rat is truly due to lack of MerTK, the transfection of RCS rat RPE with normal MerTK rescues the loss of function (616). Comparable mutations in MerTK have also been identified in humans with autosomal recessive retinitis pigmentosa (187, 600).

A defect in RPE POS phagocytosis may also cause retinal degeneration in Usher type 1B patients (196). Usher syndrome is a congenital concomitant and progressive loss of hearing and vision (6, 161, 294, 304, 463). The gene product of the Usher type 1B gene is myosin VIIa. Defects in myosin VIIa function were initially thought to affect photoreceptor outer segment structure through disturbed vesicular transport outer segment components along the cilium. However, recent in vitro analysis of RPE cells isolated from mice lacking myosin VIIa showed that these RPE cells display a reduced ability to ingest bound POS due to disturbed vesicle trafficking and fusion between phagosomes and lysosomes. However, the capability per se to bind and ingest POS is in myosin VIIa−/− mice comparable to that of wild-type mice. Thus the intracellular machinery to handle ingested POS is compromised in RPE cells from myosin VIIa-deficient mice and that the primary defect is in the RPE instead of the photoreceptors (196).

POS phagocytosis might also contribute to the onset of age-related macular degeneration (84). As described above, POS are shed because they accumulate high amounts of oxidized lipids and proteins which therefore end up within the RPE after phagocytosis. In addition, the RPE itself is exposed to high amounts of oxygen radicals due to exposure to high light energy in combination with the high oxygen concentration in the choriocapillaris and must, thus, compensate for very high photo-oxidative activity (see sect. iii). Thus POS phagocytosis could be considered as an additional burden on the RPE by oxidative stress that might over time ultimately poison the RPE. An important “early” event of AMD is the loss of RPE cells likely due to oxidative damage (143, 226). In eyes without eye disease, the RPE cell density remains stable until the ninth decade of life (194). As a result, the remaining RPE cells face a higher number of photoreceptors shedding more outer segments increasing their oxidative stress even further, eventually snowballing and causing large regions of RPE atrophy (58, 84, 86). In addition, there are observations suggesting that this process might also be one of the reasons that AMD starts in the macula because a higher lysosomal activity has been detected in RPE cells of the macula compared with RPE cells from the periphery, suggesting there is a higher rate of phagocytosis in the macula (86, 87, 93). This could result from a higher rate of photon absorption by photoreceptors in macula compared with the periphery (58, 546). Furthermore, the predominant photoreceptors in the macula, cones, have a higher energy demand and production than rods (21, 127, 316, 317, 459, 468, 522, 642), which are the predominant photoreceptors in the periphery. The increased metabolic activity supports the need for increased phagocytic activity in this region. Finally, there are also reports of a higher number of photoreceptors per RPE cell in the macula (143). However, this has not been supported by other studies (194).

VIII. SECRETION

A. The RPE Secretes a Variety of Growth Factors

As already noted, RPE and photoreceptor development are interdependent. The mechanisms controlling this may not be limited solely to the presence of corresponding surface proteins and their interaction with complimentary receptors, including integrins (373, 394, 492). This can also be controlled by growth factors that can act over a longer distance. The RPE is known to produce and to secrete a variety of growth factors (589) as well as factors that are essential for maintenance of the structural integrity of retina (104, 557) and choriocapillaris (638), e.g., different types of tissue inhibitor of matrix metallo-protease (TIMP) (8, 138, 155, 451, 471, 472, 506, 646). Thus the RPE makes factors that support survival of photoreceptors and ensure a structural basis for optimal circulation and supply of nutrients. The RPE is able to secrete fibroblast growth factors (FGF-1, FGF-2, and FGF-5) (81, 82, 115, 126, 149, 225, 281, 283, 303, 309, 310, 564, 619), transforming growth factor-β (TGF-β) (303, 324, 389, 590), insulin-like growth factor-I (IGF-I) (382, 542), ciliary neurotrophic factor (CNTF) (106, 619), platelet-derived growth factor (PDGF) (101, 103), VEGF (3, 322, 356, 360, 419, 638), lens epithelium-derived growth factor (LEDGF) (7), members of the interleukin family (280, 581, 630), and pigment epithelium-derived factor (PEDF) (134, 306, 440, 441, 557).

In the healthy eye, the RPE secretes PEDF (134, 306, 557), which helps to maintain the retinal as well as the choriocapillaris structure in two ways. PEDF was described as a neuroprotective factor because it appeared to protect neurons against glutamate-induced or hypoxia-induced apoptosis (105, 441, 557). In addition, PEDF was shown to function as an antiangiogenic factor that inhibits endothelial cell proliferation and stabilizes the endothelium of the choriocapillaris (134, 306, 440). These effects on vascularization also play an important role in the embryonic development of the eye (48, 282). Another vasoactive factor made in the RPE is VEGF, which is secreted
in low concentrations by the RPE in the healthy eye (3, 356, 638) where it prevents endothelial cell apoptosis and is essential for an intact endothelium of the choriocapillaris (94). VEGF also acts as a permeability factor stabilizing the fenestrations of the endothelium (497). The secretions of TIMP-1 and TIMP-3 are additional factors that stabilize the endothelium as well as the extracellular matrix (8, 24, 138, 155, 162, 451, 471, 472, 506, 646). In a healthy eye, PEDF and VEGF are secreted at opposite sides of the RPE; PEDF to the apical side where it acts on neurons and photoreceptors and the majority of VEGF is secreted to the basal side where it acts on the choroidal endothelium (47, 69). Growth factor secretion changes in response to damage or injury, which stimulates the RPE to also secrete neuroprotective factors including bFGF or CNTF (70, 106, 627). This is thought to protect photoreceptors from light-induced damage. The group of Reme and co-workers (216, 223, 278) has demonstrated that following a range of intensified light exposures photoreceptors started to show signs of apoptosis that were fully reversible. This may be due to increased secretion and de novo synthesis of different neuroprotective factors by the RPE such as PEDF, CNTF, and bFGF, which are known to be protective against light damage (105, 619).

As the part of the epithelia that separates the inner eye from the bloodstream, the RPE functions in establishing immune privilege of the eye (280, 581, 630). There are several lines of evidence that point to an immunosuppressive function of the RPE. RPE cells can suppress activation of CD4+ and CD8+ T cells (164) as well as inducing apoptosis in the Jurkat T-cell line (163). Because in vivo the RPE is physically separated from the bloodstream by Bruch’s membrane, these immunosuppressive effects must be due to secretion of soluble factors and not by direct interaction with blood cells (160, 164, 280, 353). However, the cross talk between the blood cells and the RPE is not fully understood.

The mechanisms involved in the regulation of secretion by the RPE are comparable to other known secretory tissues (Fig. 6). For example, RPE cells express L-type Ca2+ channels of the neuroendocrine subtype, which corresponds with the α1D or CaV1.3 subunit of Ca2+ channels (397, 500, 501, 568, 577–579, 608, 609). The same subtype of L-type Ca2+ channels is responsible for controlling the secretory activity of, for example, beta islet cells of the pancreas (37, 38, 117, 647). In vitro studies revealed that VEGF secretion by RPE cells is dependent on the activity of L-type channels (569). Direct stimulation of L-type channels by the L-type channel opener BAY K 8644, a substance which directly binds to the channel, increases VEGF secretion while the inhibition of L-type channels by nifedipine reduces VEGF secretion (569). L-type channels belong to the group of high-voltage activated Ca2+ channels. RPE cells express a splicing variant of the CaV1.3 subunit which has not been published for other tissues (636). The electrophysiological properties of this splice variant permit these L-type channel to function in epithelial cells by being activated at lower voltages. Normally, RPE cells do not undergo changes in their membrane potential more positive than ~30 mV (192, 405, 571, 609, 626). To function under these conditions, L-type channels in the RPE show very negative activation thresholds (398, 501, 608, 609). Another unusual feature of the RPE L-type channels is that their voltage dependence is regulated by tyrosine kinase (398, 501, 568, 570), which allows a mode of activation that differs from that in excitable cells. Unlike L-type channels in excitable cells, L-type channels in RPE cells are not activated by changes in the membrane voltage. These channels are activated by tyrosine kinase-dependent shifts in their voltage-dependent activa-

![Image of the retina and choroid](image_url)
tion towards more negative potentials, towards the rest-
ing potential of RPE cells (398). The receptor tyrosine kinase, bFGF receptor FGFR-2, coprecipitates with L-type channels and leads to their activation (501). The same properties have been observed for the cystosolic subtype of tyrosine kinase, pp60c-src (568). The activation by pp60c-src tyrosine kinase links these Ca²⁺ channels to the InsP₃/Ca²⁺ second messenger system (398). Thus L-type channels in the RPE are ideal targets for growth factor-dependent signaling, implicating an influence of growth factors on the secretory activity of the RPE. In fact, bFGF was found to stimulate both L-type channels and VEGF secretion (501, 569).

To allow rapid changes in intracellular Ca²⁺ ho-
meostasis, L-type channels are under the control of outwardly rectifying K⁺ channels (274, 277, 571, 572, 587, 593, 626). These K⁺ channels were identified as K⁺ channels mainly composed of Kv1.3 subunits (572). The outwardly rectifying channels activate at the same potentials as L-type channels and thereby limit activation of L-type channels by stabilizing the resting potential (572). In addition, outwardly rectifying K⁺ channels are also stimulated by tyrosine kinase (572). Thus concomitant with each stimulation of L-type channels by activation of ty-
rosine kinase there is also stimulation outwardly rectify-
ing K⁺ channels. Because outwardly rectifying K⁺ channels activate more slowly than the L-type channels, an influx of Ca²⁺ through L-type channels can occur until it is terminated by hyperpolarization from K⁺ channel activation. This regulation prevents an overload of Ca²⁺ in the cell after L-type channel activation. In addition, intracellular Ca²⁺ homeostasis is controlled by transport mol-
ecules that eliminate Ca²⁺ from the intracellular space. The RPE expresses the cardiac subtype of Na⁺/Ca²⁺ ex-
changer (NCX-1), which shows a transport stoichiometry of 3Na⁺ to 1Ca²⁺ (169, 354, 365). Expression of this Na⁺/Ca²⁺ exchanger in RPE cells gives these cells a comparable transport efficiency to cells like myocardial cells, which are dependent on fast and efficient termina-
tion of Ca²⁺ signals. RPE cells also express Ca²⁺-ATPase that functions as a very efficient transport molecule to terminate rises in intracellular free Ca²⁺ (299). In summary, the combination of specific Ca²⁺ channels, a Na⁺/ Ca²⁺ exchanger, and the Ca²⁺-ATPase enables RPE cells to generate fast and very distinctive intracellular Ca²⁺ signals as second messenger.

B. Changes in the Secretory Activity Are Associated With Proliferative Diseases in the Retina

The healthy ability of the RPE to secrete growth factors can protect photoreceptors from damage, but dys-
regulated growth factor secretion can also be involved in the pathogenesis of retinal diseases (99, 183, 261, 307, 465, 589, 638, 659). These forms of retinal degenerations are characterized by proliferation of cells. One important disease with a proliferative component is age-related macular degeneration. The most severe complication in age-
related macular degeneration is the development of choroidal neovascularization (CNV) (20, 22, 99, 132, 183, 184). Although the initiation of this neovascularization is not understood, several mechanisms have been implicated including local hypoxia, wound healing processes subse-
quent to RPE cell loss, and inflammatory processes (99, 132, 226, 300, 412). To date, analysis of CNV membranes has identified several different types of growth factors within the CNV membranes (184, 279, 331, 618). In each case the major angiogenic factor causing the development of choroidal neovascularization is VEGF. Many studies have shown that the major source of VEGF seems to be RPE cells (3, 183, 312, 356, 360, 412, 419, 638). For exam-
ple, RPE cells in neovascular tissues obtained from pa-
tients with age-related macular degeneration secreted VEGF at a higher rate compared with RPE cells from eyes without neovascularization (569). The increased rate of RPE-derived VEGF secretion was due to intracellular sig-
naling mediated by increased activity of L-type Ca²⁺ chan-
nels (569). Many extracellular stimuli have been proposed to induce increased VEGF secretion. These include growth factors such as IGF-I (331, 502, 542) or tumor necrosis factor-α (TNF-α) (443), the intregrin ligand vitro-
nectin (158, 227, 228, 412), and AGEs, which act via stimulation of the receptor for AGE's RAGE (242, 243, 360). Because IGF-I is produced by photoreceptors in healthy eyes but by RPE cells and photoreceptors in eyes with age-related macular degeneration, it can contribute to a signaling pathway in which photoreceptors can stimu-
ulate VEGF secretion by RPE cells (502, 542, 543).

IX. SUMMARY

Initially only regarded as the dark cover of the inner wall of the bulbus, today’s understanding of the RPE shows it is essential for visual function providing multiple functions that support normal photoreceptor function. This role was unraveled in studies on normal RPE func-
tion as well as in studies of retinal degeneration. The view of the RPE has been refined to the point that RPE and photoreceptors are together considered a functional unit. This is supported by studies of diseases resulting from defects in genes normally expressed in photoreceptors that lead to degeneration of the RPE, such as ABCR. Or vice versa, studies showed that defects of genes ex-
pressed in the RPE results primarily in degeneration of photoreceptors, such as MerTK or RPE65. This is due to the synergistic interaction of these tissues. Integrated interaction includes, for example, the visual cycle in
which retinal cycles between photoreceptors and the RPE. In both tissues, the structure of retinal is changed by light transduction: in photoreceptors from 11-cis- to all-trans-retinal and in the RPE from all-trans- to 11-cis-retinal. Another example is the phagocytosis of photoreceptor outer segments. The length of photoreceptor outer segments and, thus, optimal photoreceptor structure for light perception, is a product of the tight and coordinate interaction of both tissues.

This emerging appreciation of the RPE and the photoreceptors as a single functional unit should improve our ability to elucidate the pathogenic mechanisms and facilitate development of therapies for different forms of degenerative diseases leading to blindness.

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