Principles and Practice of Clinical Electrophysiology of Vision

Editors

John R. Heckenlively, M.D.
Professor of Ophthalmology
Jules Stein Eye Institute
Los Angeles, California

Geoffrey B. Arden, M.D., Ph.D.
Professor of Ophthalmology and Neurophysiology
Institute of Ophthalmology
Moorfields Eye Hospital
London, England

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Chiba University School of Medicine
Chiba, Japan

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Aston University
Birmingham, England

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Professor of Ophthalmology
University of Linköping
Linköping, Sweden

Richard G. Weleber, M.D.
Professor of Ophthalmology
University of Oregon Health Science Center
Portland, Oregon

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A Year Book Medical Publishers imprint of Mosby-Year Book, Inc.

Mosby-Year Book, Inc.
11830 Westline Industrial Drive
St. Louis, MO 63146

1 2 3 4 5 6 7 8 9 0 CL CL MV 95 94 93 92 91

Library of Congress Cataloging-in-Publication Data
Principles and practice of visual electrophysiology / [edited by] John R. Heckenlively, Geoffrey B. Arden.
p. cm.
Includes bibliographical references.
Includes index.
ISBN 0-8151-4290-0
1. Electroretinography. 2. Electrooculography. 3. Visual evoked response. I. Heckenlively, John R. II. Arden, Geoffrey B. (Geoffrey Bernard)
[DNLM: 1. Electrooculography. 2. Electrophysiology. 3. Electroretinography. 4. Evoked Potentials, Visual. 5. Vision Disorders—physiopathology. WW 270 P957]
RE79.E4P75 1991 91-13378
617.7 1547—dc20 CIP
DNLM/DLC
for Library of Congress

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The Photoreceptor–Retinal Pigmented Epithelium Interface

Gregory S. Hageman
Lincoln V. Johnson

This chapter deals with the interface between the photosensitive outer segments of photoreceptor cells and the retinal pigmented epithelium (RPE). At this interface, photoreceptor cells and cells of the RPE, both highly polarized, abut one another. The photoreceptor cells are responsible for converting light into electrical impulses through the process of transduction, a subject beyond the scope of this chapter; however, there are a number of excellent reviews that provide detailed descriptions of the process (see Chapter 7).[59, 110, 116] Photoreceptor cell outer segments, which contain the photosensitive visual pigments, are continually renewed through the addition of new membrane basally and concomitant shedding of old membrane from their apical tips. Shed outer segment membrane is ingested by the simple, cuboidal RPE cells, which are located directly adjacent to the photoreceptors and separate them from the choroidal vasculature. Interspersed between these two retinal layers is the interphotoreceptor matrix, a unique extracellular matrix that fills the "subretinal" space (Figs 6–1 and 6–3). The matrix is composed of molecules that appear to play a role in mediating biochemical and physical interactions among the retina, RPE, and choroidal vasculature. Thus the photoreceptor–RPE interface is an area of crucial importance to proper retinal function.

EMBRYOLOGICAL ORIGINS OF THE RETINA, RETINAL PIGMENTED EPITHELIUM, AND INTERPHOTORECEPTOR MATRIX

This section focuses primarily on human retinal development; references to other species are included where appropriate. A number of excellent reviews contain additional detail.[14, 24, 39, 65, 66, 79, 92, 106, 124, 127, 147] It should be noted that developmental stages of the human embryo have been defined on the basis of a variety of parameters including gestational time, crown-rump length, or heel length, and discrepancies in the time course of development are common in the literature. These discrepancies are complicated further by the fact that (1) the retina differentiates along a central-to-peripheral gradient, with an approximate 6-week lag,[71] (2) regional variations exist (e.g., the fovea), and (3) in some cases it is difficult to determine from which region of the developing retina published data have been derived.

Development of the Retina

The optic primordium and optic sulcus are evident within the neural fold of the diencephalon at

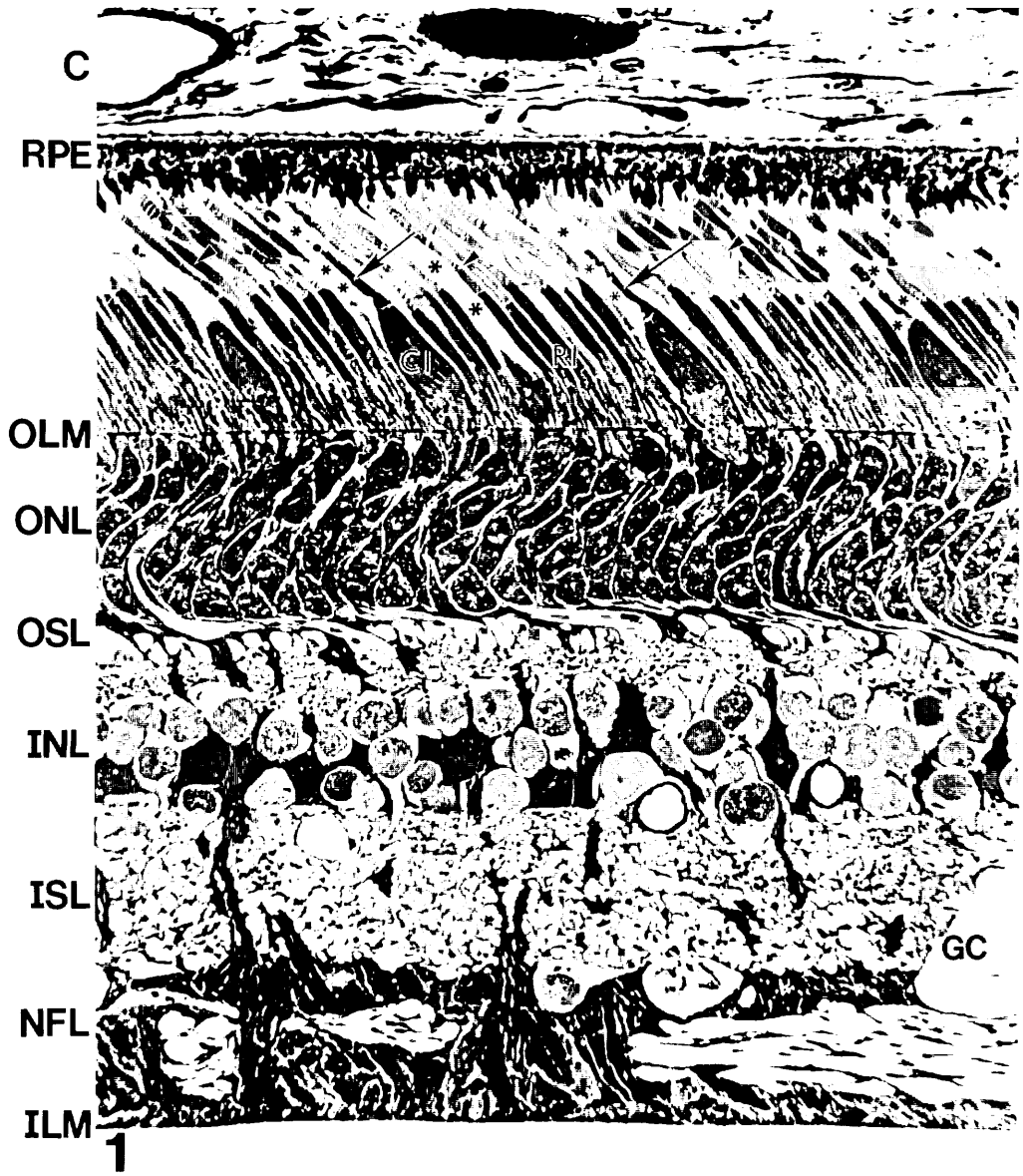

FIG 6–1.
Light micrograph of a section of central retina from a monkey eye depicts the relationship between the choroid *(C)*, retinal pigmented epithelium *(RPE)*, interphotoreceptor matrix *(asterisks)*, and neural retina. The neural retina is composed of a defined number of cell types arranged in a precise lamellar configuration. The apical surface of the neural retina contains highly polarized photoreceptor cells that abut the apical surface of the retinal pigmented epithelium. Interspersed between the apicies of these two retinal layers is the interphotoreceptor matrix *(asterisks)*, a unique extracellular matrix that fills the subretinal space. Two types of photoreceptor cells can be identified morphologically. Cone photoreceptor inner segments *(CI)* are large in diameter, and the outer segments *(arrows)* are broader basally and tapered toward their apical tips. In contrast, rod photoreceptor inner *(RI)* and outer *(arrowheads)* segments retain a relatively uniform diameter that is smaller than that of cone photoreceptors, (*OLM* = outer limiting membrane; *ONL* = outer nuclear layer (contains photoreceptor cell nuclei); *OSL* = outer synaptic layer; *INL* = inner nuclear layer [contains nuclei from Müller, amacrine, bipolar, and horizontal cells]; *ISL* = inner synaptic layer; *GC* = ganglion cell; *NFL* = nerve fiber layer; *ILM* = inner limiting membrane).

about 22 days of gestation. The retina develops subsequently as an evagination from this region at approximately 25 days (2.6 mm) of gestation. This outpocketing enlarges to form the primary optic vesicle, which remains attached to the diencephalon by the optic stalk. At this stage, the cavity of the optic vesicle (future subretinal space) remains in communication with the ventricle of the brain through the optic stalk. The neural epithelium of the optic vesicle is a columnar epithelium containing an abundance of mitotically active cells.

During the fourth week of gestation (4.5 mm), the optic vesicle invaginates upon itself, and this results in the formation of the optic cup, a structure consisting of two neuroectodermally derived epithelial cell layers with their ventricular surfaces directly apposed. The cavity of the optic vesicle is all but obliterated during this time and remains only as a potential space, termed the *subretinal* or *interphotoreceptor space.* Although the molecular events that lead to invagination are not fully understood, recent evidence suggests that calcium[63] and extracellular matrix components[146] may be involved.

Although both layers of the retina differentiate from a continuous neural epithelium, their subsequent differentiation at both the cellular and molecular levels is quite diverse. The outermost layer of this neuroepithelium remains a single cellular layer and becomes the RPE. The innermost layer, the presumptive neural retina, thickens rapidly and becomes stratified; by 4 weeks (4 to 4.5 mm) of gestation, the neural retina is approximately 0.1 mm thick and consists of eight to nine distinct rows of cells. Both epithelial layers extend peripherally to form the ciliary body epithelium and posterior aspect of the iris. During the invagination process, the choroidal fissure, through which blood vessels pass into the interior of the eye, is formed along the ventral portion of the optic stalk.

During cellular differentiation of the neural retina, undifferentiated neuroblasts, which make up the entire thickness of the retina from the ventricular to the vitreal surfaces, typically lose their attachment to the vitreal surface and migrate to the ventricular surface where mitosis occurs. Following cell division, daughter cells migrate toward the vitreal surface and ultimately reestablish connections with it. This process is repeated at each round of cell division, eventually resulting in the differentiation of a stratified neural epithelium. The glial or Müller cells can be distinguished at 4 weeks of gestation. By 5 weeks of gestation (5 to 7 mm) the nerve fiber layer is visible in the central retina (although this layer is lacking in the macula, even at birth[39]), as are ganglion cells.[114] The layer of Chievitz, a transient fiber layer that separates the retinoblast layer into two nucleated layers, also forms during the fifth week of gestation.[92, 127]

By 7 to 8 weeks (20 to 23 mm) the inner neuroblast layer separates into two layers of nuclei that consist of potential ganglion cells (inner layer) and amacrine and Müller cells (outer layer). The ganglion cells give rise to nerve fibers that course toward the future optic nerve and form the nerve fiber layer. The inner limiting membrane also is clearly evident by this stage. During the ninth and tenth weeks of gestation (40 to 50 mm), photoreceptor, horizontal, and bipolar cells begin to differentiate within the outer neuroblast layer.[50, 66, 143] Horizontal and bipolar cells migrate into the layer of Chievitz and become separated from photoreceptor cells by the outer plexiform layer.[66] Amacrine and Müller cell bodies intermingle with those of horizontal and bipolar cells, and the transient layer of Chievitz is thereby obliterated; however, it persists in the macular region until birth. At this same time extensive junctional complexes, including gap junctions, macula adherens, zonula adherens, and zonula occludens, can be observed between cells of the neural retina and pigmented epithelium.[50, 66] Between 12 and 15 weeks of gestation, cellular proliferation in the outer neuroblast layer ceases[127] except in the macular region.[104] The development of the macular region slows and begins to lag behind the development of the extramacular regions at this time. By 7 months of gestation, all layers except the macular region, which is not completely developed until 16 weeks postpartum, have assumed adult arrangement and proportion.

DEVELOPMENT OF PHOTORECEPTOR CELLS

Photoreceptor cells are probably specified within the outer layers of the neural retina as early as 10 gestational weeks (40 to 50 mm),[149] but they are difficult to identify. By 12 weeks (83 mm) of gestation, however, cone photoreceptors are easily identified by their relatively large, slightly oval configuration, lightly stained or electron lucent cytoplasm, large juxtanuclear accumulation of smooth endoplasmic reticulum, and a single cilium.[66] Rod photoreceptors, which have distinct, dense nuclei, can be identified conclusively by 15 weeks (120 mm) of gesta-

tion.[66] At 18 weeks (156 mm) of gestation, a single layer of large, pale-staining cone photoreceptor cell bodies is visible in the outermost portion of the neural retina. The smaller rod photoreceptors comprise the remainder of the outer nuclear layer. Some synapses are established by cone photoreceptor pedicles by 12 weeks of gestation; however, synapses are not observed in association with rod photoreceptor cells until approximately 18 weeks of gestation. By 24 weeks, both types of photoreceptor cells are well polarized and have distinct inner segments that extend approximately 2 μm beyond the outer limiting membrane.[71] Rudimentary cone outer segments, which begin to develop at 16 weeks, are numerous and filled with whorls of tubular structures at this stage.[71] The majority of cone but not rod photoreceptor cell outer segments have stacks of disc membranes by 24 weeks of gestation. In contrast, rod photoreceptor cells contain a mixture of uniform and randomly oriented discs even at 28 weeks[71] and do not resemble adult outer segments until approximately 36 weeks.[149]

Development of the Fovea

Although it has been recognized for some years that development of the human fovea lags behind that of the central retina, recent studies have provided detailed information regarding its development.[59, 155] The fovea can be identified at approximately 22 weeks of gestation by the existence of a photoreceptor layer that contains only cones and by the presence of an unusually thick layer of ganglion cells. Following birth, the fovea continues to develop, a process that is characterized by deepening of the foveal depression, narrowing of the rod-free zone (foveola), and maturation and elongation of foveolar cone photoreceptor cells, including the differentiation of outer segments and development of basal axosomal processes that constitute Henle's fiber layer. The fovea is not fully differentiated until the third or fourth postnatal year (Fig 6–2).

Development of the Retinal Pigmented Epithelium

At 5 to 6 weeks (15 to 20 mm) of gestation the presumptive retinal pigmented epithelium exists as a pseudostratified layer of columnar epithelial cells that have a dense cytoplasm, oval nuclei, and the first detectable pigment granules.[66] Mitoses are numerous and are located primarily in the farthest ventricular portion of this epithelium. By 7 weeks (20 mm) of gestation, basal and lateral infoldings of RPE cell plasma membrane and apical microvilli can be observed.[100] In addition, distinct "terminal bars" consisting of zonula occludens and zonula adherens, are evident.[100] By 8 weeks (27 to 31 mm) of gestation the RPE is established as a simple cuboidal epithelium. A close apposition between RPE and neural retinal cells is attained following invagination of the optic vesicle. Intercellular junctions, including both gap junctions and zonula adherens junctions, are present between these two cell layers at this time.[50]

Development of the Interphotoreceptor Space

The interphotoreceptor space is the extracellular matrix-filled remnant of the central cavity of the embryonic optic vesicle. It is within this interphotoreceptor space that important interactions between RPE cells and photoreceptor cells of the neural retina take place. Little information exists in humans pertaining to the development of the interphotoreceptor space or its contents, collectively referred to as the interphotoreceptor matrix. A.T. Johnson and coworkers,[71] however, have demonstrated that interstitial retinol-binding protein, a major component of the adult interphotoreceptor matrix, is first detectable in human retinas at approximately 20 weeks of gestation, a time that corresponds to photoreceptor cell outer segment differentiation. Between 15 and 18 weeks of gestation, intercellular junctions that form between RPE and neural retinal cells earlier in development gradually disappear, and an obvious interphotoreceptor space filled with a detectable flocculent material is visible at the tips of photoreceptor cell inner segments. By 24 weeks, the interphotoreceptor space widens, and distinct domains of flocculent interphotoreceptor matrix that are termed *cone matrix sheaths* are selectively associated with cone photoreceptor cell inner and outer segments. Chondroitin 6-sulfate, a major component of cone matrix sheaths,[55] is first present between 17 and 18 weeks of gestation and is solely associated with cone outer segments. Peanut agglutinin (PNA)-binding glycoconjugates, additional major structural components of cone matrix sheaths,[54, 55, 72, 73] are present within the interphotoreceptor space at the time of its earliest formation. By 17 to 18 weeks of gestation, interphotoreceptor matrix–containing, peanut agglutinin–binding constituents, are visible as concentrated accumulations that are primarily associated with cone photoreceptor cells. It should be pointed out that the expression of these two major

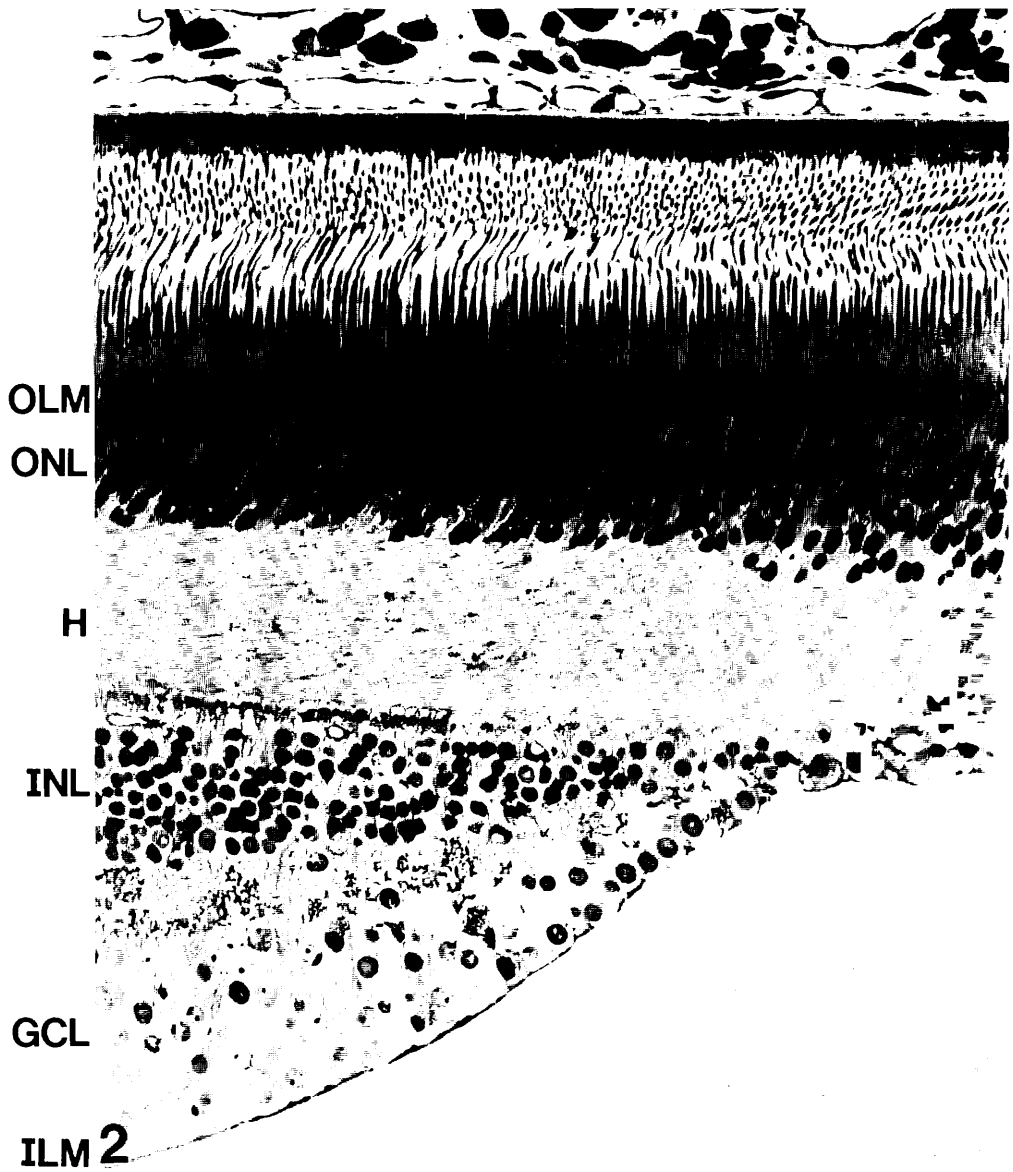

FIG 6–2.
Light micrograph of a section of a fovea of a monkey retina. In contrast to other regions of the neural retina, inner and outer segments of foveal cones are of a narrower diameter and appear more rodlike (compared with cones in Fig 6–1). In the central fovea, only cone photoreceptor cell bodies are present within the outer nuclear layer *(ONL)*. Henle's layer *(H)* consists of cone photoreceptor cell axons (*OLM* = outer limiting membrane; *INL* = inner nuclear layer; *GCL* = ganglion cell layer; *ILM* = inner limiting membrane).

cone matrix sheath–associated constituents occurs at the time when rudimentary, outer segments first differentiate, approximately 10 weeks prior to the appearance of definitive photoreceptor disc membranes. Possibly cone matrix sheath–associated constituents may be necessary for the subsequent differentiation and survival of photoreceptor cell outer segments.

RETINA-RETINAL PIGMENTED EPITHELIUM-INTERPHOTORECEPTOR MATRIX: MORPHOLOGY, COMPOSITION, AND FUNCTION

As detailed above (see the previous section) the neural retina develops as a stratified epithelium, one basal surface bordering the vitreous cavity and the

other apical surface in close association with the RPE. The cellular composition and organization of a mature retina is described in Chapter 5. Of most interest to this chapter is the scleral surface of the neural retina (Figs 6–1 to 6–3). Microvillous extensions of Müller cells form junctional complexes with adjacent photoreceptor inner segments to seal the interphotoreceptor space lying between the neural retina and the RPE. The interphotoreceptor space is filled by a specialized extracellular matrix termed the interphotoreceptor matrix (Figs 6–1 and 6–3). The apical surfaces of retinal pigmented epithelial cells contain numerous microvilli and are specialized for the phagocytosis of shed packets of photoreceptor outer segment membranes, one of a number of RPE cell activities that contribute to photoreceptor cell function.

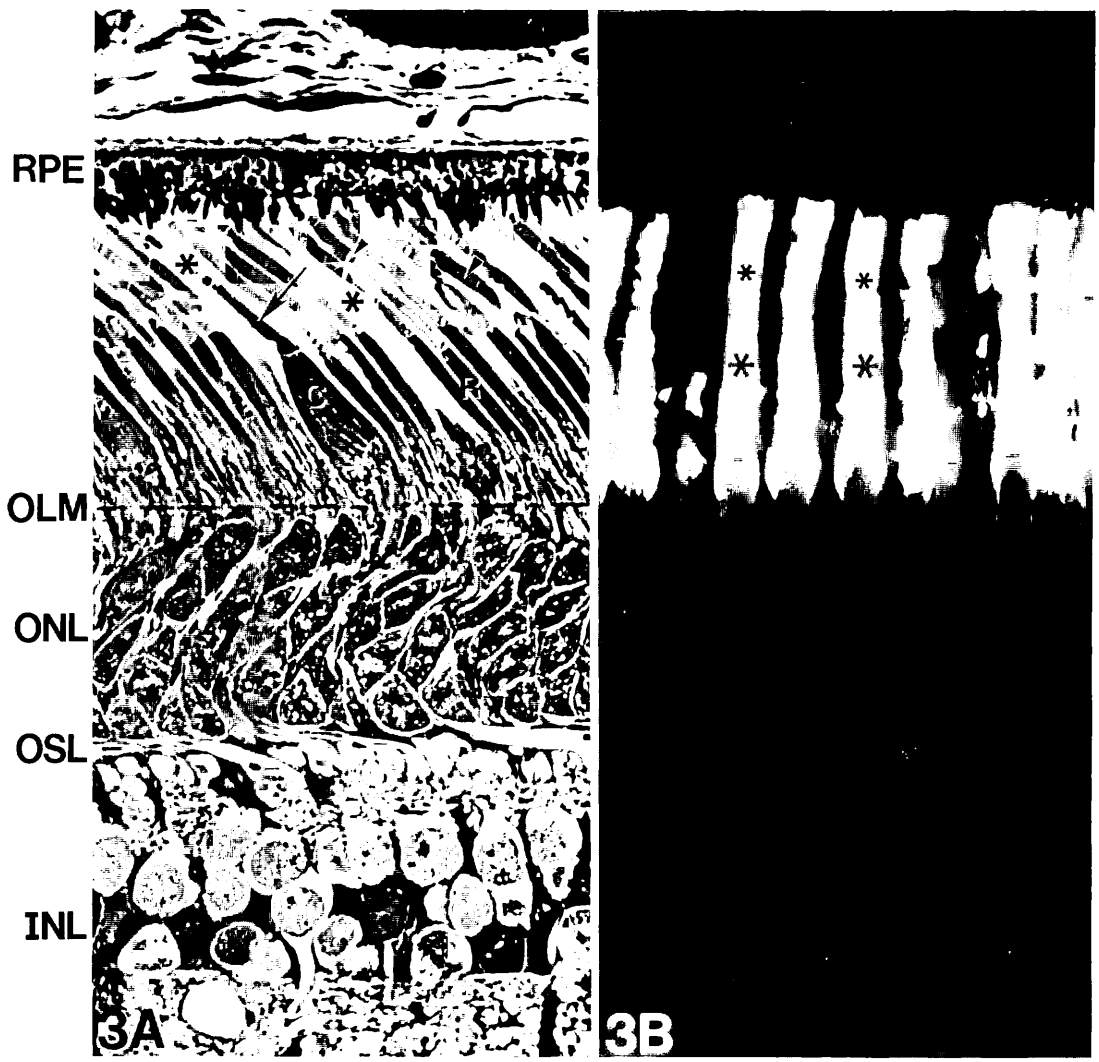

FIG 6–3.
Light micrograph of a region of the section depicted in Figure 6–1 **(A)** and a fluorescence light micrograph **(B)** of a section of monkey retina that shows the distribution of peanut agglutinin–binding molecules in monkey retina. Peanut agglutinin–binding molecules in monkey and human retinas are specifically localized to domains of cone photoreceptor cell–associated interphotoreceptor matrix that have been termed cone matrix sheaths *(asterisks).* Chondroitin 6-sulfate containing proteoglycan and peanut agglutinin–binding glycoconjugates are major constituents of cone matrix sheaths (*C* = cone photoreceptor cell; *R* = rod photoreceptor cell; *arrow,* cone outer segment; *arrowhead,* rod outer segment; *RPE* = retinal pigmented epithelium; *OLM* = outer limiting membrane; *ONL* = outer nuclear layer; *OSL* = outer synaptic layer; *INL* = inner nuclear layer).

RPE Cell Cytology and Function

The RPE has roles important to the maintenance of retinal, especially photoreceptor, cell function and homeostasis.[32]

The polygonal cells of the RPE form a simple (one cell layer thick) cuboidal epithelium with their basal surfaces attached to a basement membrane, which is part of a collagen-rich layer of extracellular matrix known as Bruch's membrane. Bruch's membrane separates the retinal pigmented epithelium from its primary vascular supply, the choroidal capillaries, which are the major source of nutrients for the outer retina; numerous basal infoldings of retinal pigmented epithelial cell plasma membranes facilitate nutrient and waste product exchange. The best characterized of the transport functions is retinol, which complexes with opsin in photoreceptor cell outer segments and is absolutely necessary in the process of phototransduction. The RPE mediates the transport of retinol from the choroidal vasculature to the interphotoreceptor space by utilizing a number of retinoid-binding proteins as carriers.[17, 18]

Laterally, RPE cell membranes are joined by intermediate (adhering) junctions, and between adjacent cells continuous bands of tight junctions prevent paracellular flow of large molecules to and from the subretinal space, thus contributing to the blood-retinal barrier (tightly sealed retinal vasculature also contributes significantly to this barrier).

Apically, RPE cells have numerous microvilli that project into the interphotoreceptor space and are closely associated with photoreceptor cell outer segments. This association facilitates another major function of the RPE cells, the phagocytosis and digestion of shed photoreceptor outer segment membrane produced by ongoing renewal of photoreceptor outer segments; phagosomes involved in the degradation of phagocytosed membrane are typical components of RPE cytoplasm. The dynamic relationship that exists between the RPE and photoreceptors during outer segment membrane turnover is well established.[20] The molecular mechanisms that regulate shedding and subsequent phagocytosis by the RPE have not been elucidated, although a receptor-mediated process involving both photoreceptors and retinal pigmented epithelium has been hypothesized.[15] Studies by McLaughlin and coworkers[95–97] have demonstrated a loss of certain lectin receptors in shed, unphagocytosed disc packets in the Royal College of Surgeons (RCS) rat (an animal that has a defect in the ability of the RPE to ingest shed disc), and this suggests that phagocytosis may involve a cell surface signal from the photoreceptor to the RPE. In other studies, RPE and outer segment membrane–associated molecules have been identified and are being characterized as potential participants in receptor-mediated recognition and/or phagocytosis. The sequence of morphological events that occurs during shedding and ingestion of outer segments has been thoroughly investigated in monkey and human retinas by transmission electron microscopy.[64, 129, 130, 151] Cone photoreceptors are also known to shed their discs in a diurnal rhythm, the majority shedding their membranes at night, although species variations have been reported.

The apical surface membranes of RPE cells are also rich in Na^+-K^+ adenosine triphosphatase (ATPase) molecules that mediate ion fluxes and influence the transport of other molecules into and out of the subretinal space.[107] Abundant cytoplasmic pigment (melanin) granules are also present in retinal pigmented epithelial cells; these are also important to retinal function and serve to absorb scattered light.

Additionally, RPE cells are known to synthesize and secrete a number of proteins, glycoproteins, and proteoglycans that are part of the interphotoreceptor matrix.[4, 5, 14, 41, 131] The extent to which any of these interphotoreceptor matrix components are important to structural or functional interactions between retinal photoreceptors and the RPE is largely unknown. However, several recent studies suggest that as yet undefined factors secreted by RPE cells may be important in influencing retinal differentiation.[91, 128, 137] Additionally, it has been suggested that proteoglycans in the interphotoreceptor matrix, at least some of which are likely to be products of the RPE, may be important in retina–RPE adhesion.[56, 58]

Photoreceptor Cell Cytology and Function

The highly polarized photoreceptor cells form the outermost layer of the neural retina (see Fig 6–3,A). Their cell bodies form the outer nuclear layer; their axonal processes extend basally to synapse with bipolar and horizontal cells in the outer synaptic layer (see Chapter 5). The scleral portions of photoreceptor cells, or outer segments (see Fig 6–3,A), are modified ciliary structures formed by elaborations of plasma membrane containing high concentrations of photosensitive, integral membrane molecules. The most abundant protein of rod outer segment disc membranes is the rod photopigment rhodopsin, a glycoprotein with a molecular weight of approxi-

mately 42 kilodaltons (kD) that is present with a packing density of approximately 30,000 molecules per μm^2. The carboxy terminus of rhodopsin is located in the interdiscal space, whereas the amino terminus projects into the intradiscal space. Another recently discovered outer segment membrane glycoprotein is the "rim" protein,[121] which has a molecular weight of 240 to 290 kD and is located along the edges of outer segment discs and incisures. Other proteins that have been identified in association with outer segment membranes include peripherin (33-kD dimer), glyceraldehyde-3-P-dehydrogenase (38 kD), a cyclic guanosine monophosphate gated channel protein (63 kD), and a spectrinlike protein (240 kD) (Molday, unpublished observations). Other molecular constituents located within photoreceptor outer segments participate in phototransduction (see Chapter 7). The adjacent inner segments, which contain mitochondria and the metabolic synthetic machinery responsible for the biosynthesis and transport of molecules for both the outer segment and axonal portions of the cell, extend into the interphotoreceptor space and are surrounded by the interphotoreceptor matrix. The photoreceptor inner segment membranes form junctional complexes with the surrounding glial elements of the retina, the Müller cells. These junctional complexes establish what has been termed the *outer limiting membrane* (see Fig 6–1), a region thought to act as a molecular sieve[28] to partially seal the interphotoreceptor space from the neural retina.

Two types of photoreceptor cells, rods and cones, can be identified cytologically (see Figs 6–1 and 6–3,A) (see Chapter 5). Subclasses of cone photoreceptors have been identified, each possessing different spectral sensitivities, and corresponding differences in the molecular nature of the photosensitive pigments concentrated in their outer segments.[102] The outer segments of rods and cones differ structurally; rod photoreceptor outer segments retain a relatively uniform diameter from apex to base, while cone photoreceptor outer segments are broader basally and taper toward their tips. In both cases, photoreceptor outer segments are formed by extensive folding of the photoreceptor cell membrane; in rods these "disc membranes" are pinched off and enclosed by the cell membrane.[20] In contrast to the case for rods, however, the structural relationship between vertebrate cone photoreceptor outer segment disc membranes and their enveloping plasma membrane remains uncertain, especially in primates. Conventional ultrastructural studies of nonmammalian species suggest that the majority of cone disc membranes remain continuous with the plasma membrane, and thus the intradiscal spaces are open to the interphotoreceptor space.[33, 34, 40, 105, 125] It has generally been assumed that most if not all of the discs in mammalian cones are also continuous with the plasma membrane, but many of the connections appear to be extremely small.[9, 10, 20, 21, 29] In several species, open intradiscal spaces are more easily visualized in the proximal than in the distal portions of cone outer segments.[33] Recent ultrastructural studies of monkey and human cone photoreceptors have identified novel regions of outer segments, termed *cone notches*, that demarcate a site of abrupt transition between cone photoreceptor discs that are open to the interphotoreceptor space and those that appear isolated. These results suggest that at least at some levels the gross organization of primate cone photoreceptor cell outer segment membranes may be more similar to that of rod photoreceptor cells. A number of investigators have shown that the fluorochrome Procion yellow selectively associates with cone outer segments in a variety of species, including primates.[36, 37, 82, 83] These investigators have suggested that this staining may represent dye infiltration into open cone discs, although cone-specific binding of Procion yellow may be a result of preferential insult to cone membranes rather than a result of penetration into patent cone discs.

There also appear to be differences in the mechanism of membrane renewal in the outer segments of rod and cone photoreceptor cells. Rod cell outer segment discs are added basally and migrate as intact units toward the apical tip of the outer segment, where they are ultimately shed. This continuing assembly at the proximal end of the photoreceptor outer segment is balanced by continuing shedding of the distal tip of the outer segment such that the overall length of the outer segment remains constant. In contrast, cone photoreceptor cell outer segments renew more randomly and show no selective incorporation of amino acids into the basal region of the photoreceptor cell outer segment.[153, 154] For both photoreceptor cell types, however, RPE cells appear to be responsible for the phagocytosis of shed outer segment membrane and clearance of the interphotoreceptor space. Cone photoreceptor cell inner segments are generally larger than those of rod photoreceptor cells and are densely packed with mitochondria. Cone photoreceptor cell bodies typically occupy the outermost layer (nearest the sclera) of the outer nuclear layer, with the remainder of the outer nuclear layer being composed of rod cell bodies.

Although rod and cone photoreceptor cells exhibit differences in their overall structure, function, and

susceptibility to degeneration in various diseases, relatively little is known concerning the biochemical bases for these differences, especially with respect to cones. Our lack of knowledge pertaining to the molecular composition of cone photoreceptors is probably due to an inability to isolate these cells since they represent only a small percentage of the total population of photoreceptors in most species and since, until recently, few cone photoreceptor cell-specific probes have been available to aid investigators in this purification.

Significant new knowledge about the biochemical and morphological uniqueness of cone photoreceptor cells and their surrounding environment is emerging. For example, new information on compositional differences between rod and cone photoreceptor cells, including differences in the α-subunit of transducin,[53, 89] cyclic guanosine monophosphate phosphodiesterase,[70] neurotransmitters and amino acid metabolism,[23, 42, 81, 121] cytochrome oxidase activity, vitamin D–dependent calcium-binding protein,[122, 144] disc rim protein,[108, 109] bovine serum albumin–binding molecules, and carbonic anhydrase, have been documented. In addition, cone photoreceptors have been shown to accumulate selectively various sugars, including fucose by goldfish cone photoreceptor cells,[25, 26] galactose by bovine cone photoreceptor cells,[77] and 2-deoxyglucose by dark-adapted primate cone photoreceptor cells.[126] Additional differences in the molecular composition of cone photoreceptor cells have been elucidated by monoclonal antibodies. Lemmon[88] and Szél and coworkers[133–136] have generated monoclonal antibodies that specifically label cone outer segments in a variety of species, and Bunt-Milam and coworkers have generated an antibody that binds to the outer segments of certain subclasses of cone photoreceptor cells in a number of species. Similarly, we have generated a monoclonal antibody that selectively labels cone but not rod photoreceptor cell plasma and disc membranes in pig, monkey, and human retinas. These probes should provide powerful tools with which to continue to establish the molecular bases for differences between rod and cone photoreceptor cells. More recently, molecular biological techniques have begun to provide some insights into compositional differences between rod and cone photoreceptor cells.[80, 94, 102, 103, 113, 141]

Müller Cell Cytology and Function

Müller cells are the primary glial elements of the retina. Unlike neurons of the retina, Müller cells span almost the entire width of the retina and extend radially from the inner limiting membrane at the vitreal surface to just beyond the level of the outer limiting membrane where they form junctional complexes with adjacent photoreceptor cells; their nuclei are located within the inner nuclear layer. The scleral surfaces of Müller cells border the interphotoreceptor space and extend numerous microvillous processes into it. Specific membrane-associated transport systems sequestered in these apical cell membranes are likely to be involved in controlling to some extent the composition of the interphotoreceptor matrix.[116] Müller cells may also participate in retinal carbohydrate metabolism by serving as a source of stored nutrients in the form of glycogen,[104] in the degradation of neurotransmitter levels,[120] and in the regulation of extracellular glutamine levels.[115] Maintenance of appropriate potassium levels in the retina by the active pumping of potassium ions into the vitreous also appears to be a major Müller cell function.[104]

Interphotoreceptor Matrix Structure and Function

As described above (see the section on embryological origins), the interphotoreceptor matrix is likely to play a major role in maintaining retinal function by mediating biochemical interactions between the retina, RPE, and choroidal vasculature. Ultrastructural studies of the interphotoreceptor matrix have confirmed the presence of amorphous extracellular substance within the interphotoreceptor space in a variety of species, including monkeys and humans.[48, 117] Thick cuffs of amorphous material are observed to encapsulate most cone photoreceptor cell outer segments, in contrast to the finely granular material interspersed between adjacent rod photoreceptors.[48, 72]

Early investigations identified the presence of anionic, carbohydrate-containing molecules in the interphotoreceptor matrix of a variety of species including monkeys and humans[45–47, 49, 56, 74, 84, 90, 117, 123, 143, 156], observed susceptibility of some of these interphotoreceptor matrix components to specific enzyme treatments indicated that both glycoproteins and glycosaminoglycans are constituents. More recent studies employing lectin histochemistry have provided substantial additional information as to the nature of carbohydrate-containing molecules within the interphotoreceptor matrix.[16, 54, 73, 76, 78, 97, 118, 138–140, 148] One of the most striking contributions of these lectin-based studies has been the identification of microdomains of interphotoreceptor matrix glycoconjugates. These

studies have demonstrated that interphotoreceptor matrix components are heterogeneously distributed and that the heterogeneities fall into two basic patterns, those showing apical-basal differences and those showing photoreceptor cell type–specific differences in composition. Wheat germ agglutinin–binding glycoconjugates in monkeys and humans are present within the interphotoreceptor matrix surrounding rod photoreceptors and are virtually absent in the interphotoreceptor matrix surrounding cone photoreceptor cells.[56, 118] Additional evidence for compartmentalization of some molecules contained in the interphotoreceptor matrix has been provided by investigations of the distribution of PNA-binding molecules. PNA-binding molecules in monkey and human retinas are specifically localized to domains of cone photoreceptor cell–associated interphotoreceptor matrix. The existence of cone matrix sheaths in human retinas has been confirmed by histochemical staining with a cationic copper phthalocyanin dye, cuprolinic blue.[54, 68, 72, 73, 76, 143]

The majority of studies directed toward biochemical characterization of the interphotoreceptor matrix have concentrated on its soluble rather than insoluble components.[14, 61, 62] Recently, however, investigations have focused on characterizing the aqueous, insoluble components of the interphotoreceptor matrix (Fig 6–4,A). In higher vertebrate species, including monkeys and humans, these components appear to constitute a significant portion of the interphotoreceptor matrix as compared with soluble constituents.

The soluble fraction of the interphotoreceptor matrix from bovine eyes consists predominantly of protein and glycoprotein (98%) with some glycosaminoglycan (2%). The most prominent proteins identified by sodium dodecyl sulfate–polyacrylamide gel electrophoresis include bands of 47 kD and 140 kD.[4, 5] Similar proteins have been identified in the interphotoreceptor matrix of human retinas.[1] The major soluble glycoprotein (140 kD) of the interphotoreceptor matrix is an interstitial retinol-binding protein.[27, 30, 110] In addition, a number of other soluble interphotoreceptor matrix proteins and glycoproteins have been identified; these include mucinlike glycoproteins,[2] a variety of enzymes,[3] a cyclic guanosine monophosphate–phosphodiesterase,[11] soluble antigen,[145] trophic factors (Adler and Hewitt, unpublished data), and a variety of serum-containing proteins, including immunoglobulins and albumin.[6, 56] In addition, small–molecular weight, soluble glycosaminoglycans have been identified. These may be degradation products of larger interphotoreceptor proteoglycans.

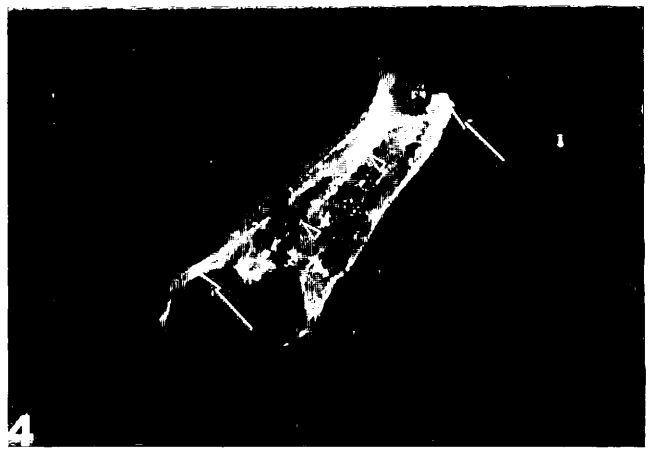

FIG 6–4.
Fluorescence light micrograph depicting an isolated cone matrix sheath exposed to fluorescein-conjugated PNA. Cone matrix sheaths examined in this manner show a distinct substructure with numerous longitudinally orientated fibers extending the entire length of the sheath *(arrowheads)*. These longitudinal structures appear to be interconnected by a finer anastomosing fibrous network. In addition, longitudinal fibers appear to insert into distinct fibrous rings of similar dimension at both the proximal and distal ends of the sheath *(arrows)*.

More recent biochemical and immunocytochemical studies have confirmed that a large proportion of the interphotoreceptor matrix is composed of aqueous-insoluble glycoconjugates. These include proteoglycans which contain chondroitin 4-sulfate and chondroitin 6-sulfate.[55, 111] Chondroitin 4-sulfate is distributed uniformly throughout the matrix, whereas chondroitin 6-sulfate proteoglycan is associated specifically with cone matrix sheaths,[55] and may be a component of a larger proteoglycan intercalated within the cone photoreceptor cell plasma membrane. Based on high-performance liquid-size exclusion chromatography, the major constituent of cone matrix sheaths is resolved as a peak approximately 800 kD, which suggests that cone matrix sheaths are composed of extremely high molecule weight proteoglycans or proteoglycan aggregates. In addition to chondroitin 6-sulfate glycosaminoglycan, cone matrix sheath proteoglycans contain a significant quantity of O-linked oligosaccharides that bind PNA.

Relatively little is known about the function of most of the interphotoreceptor matrix constituents. Perhaps the only interphotoreceptor matrix molecules that have been characterized with respect to function are interstitial retinol-binding proteins and vitamin A. Preliminary studies in a number of laboratories[67, 79, 128, 167, 168] suggest that cone matrix

sheath-associated constituents may indeed participate in retinal adhesion, since cone matrix sheaths retain their cellular attachments and become extremely elongated in experiments in which the retina is gently peeled from the pigmented epithelium immediately following enucleation. Intravitreal injection of xylosides (compounds that disrupt proteoglycan synthesis) results in cone matrix sheath disruption and localized retinal detachments (Lazarus and Hageman, 1989). Intravitreal or subretinal injections of chondroitinase or neuraminidase reduce adhesion by as much as 80% without affecting retinal function or histology.

It appears that rod photoreceptor cells are the primary cells involved in the synthesis of interstitial retinol-binding protein and its subsequent secretion into the interphotoreceptor matrix.[51, 52, 67, 116] It has also been demonstrated that a number of interphotoreceptor matrix-containing constituents originate from the systemic vasculature and are transported into the interphotoreceptor space by the retinal pigmented epithelium.[61]

PATHOLOGIES AFFECTING THE RPE-PHOTORECEPTOR-INTERPHOTORECEPTOR MATRIX COMPLEX

Pathologies affecting the RPE-photoreceptor-interphotoreceptor matrix interface have been reported in association with human disease and animal models. Such pathologies may be the direct result of abnormalities in either RPE or photoreceptor cells. Because of their close structural and functional relationships (see the previous section on structure and function of the interface), an abnormality in one of these cell types might be expected to influence the viability of the other. It can be speculated that this phenomenon would most often involve a primary abnormality in retinal pigmented epithelial cells that secondarily affects photoreceptor cells because of the numerous functions crucial to photoreceptor homeostasis that are performed by retinal pigmented epithelial cells. Such is the case for the RCS rat (see a later section), which exhibits a retinal pigmented epithelium-based pathology that indirectly results in photoreceptor cell death. Conversely, in a number of mutant mouse strains (see the later section on retinal-degenerative mice) the primary abnormalities are in photoreceptor cells themselves.

Our understanding of abnormalities affecting the RPE-photoreceptor interface comes largely from studies of animals, while less is known concerning the cellular bases of human retinal pathologies (see Chapters 42 and 43). Since a number of comprehensive reviews pertaining to animals exhibiting retinal degeneration have been published,[31] only a few specific examples are described below.

RPE-Based Pathologies

RCS Rat

The best characterized of retinal pigmented epithelium-based pathologies is that exhibited by the RCS rat.[19, 22, 38, 98] This mutant strain of rat has a defect that affects the ability of retinal pigmented epithelial cells to phagocytose shed photoreceptor cell outer segment membrane. Photoreceptor cells develop apparently normally until about 18 days postnatally, but degenerate thereafter. As a result, the interphotoreceptor (subretinal) space becomes filled with membranous debris.[60, 98] The recognition and binding of photoreceptor cell outer segments at the apical surfaces of RCS retinal pigmented epithelial cells may be normal, the defect specifically affecting phagocytosis. It has recently been shown [42a] that rod death can be prevented by various "sham operations" on the retina and especially by subretinal or intraretinal injection of basal fibroblast growth factor (bFGF). This is a constituent of the normal interphotoreceptor matrix (personal observations) and therefore the lack of specific trophic factors, derived from RPE, leads to the death of rods in the RCS rat.

Mucopolysaccharidosis VI

Feline mucopolysaccharidosis VI (MPS VI) is an inherited disease affecting the lysosomal enzyme arylsulfatase B. Animals with this enzymatic defect exhibit large intracellular accumulations of dermatan sulfate owing to their inability to degrade this glycosaminoglycan.[58] This abnormality is systemically widespread but especially notable in cells of the retinal pigmented epithelium, which because of their highly phagocytic nature accumulate numerous membrane-bound inclusions containing poorly degraded glycosaminoglycans.[8] These inclusions are present at the time of birth of affected animals, increase in size and number with time, and ultimately result in massive hypertrophy of the retinal pigmented epithelium. This hypertrophy disrupts the normal orientation of adjacent photoreceptor outer segments but apparently does not result in photoreceptor cell death.[8] Retinal pigmented epithelial cells from cats with MPS VI thus appear to be capable of continued phagocytosis of shed photoreceptor cell outer segment membrane and physiological support of photoreceptor cells in spite of the fact that they

have an important enzymatic defect and are severely hypertrophied.

Photoreceptor Cell–Based Pathologies

Retinal-Degenerative Mice

A number of mutant mouse strains exhibiting inherited photoreceptor cell degeneration have been described. The best characterized of these are the *rd* (retinal degeneration[85, 86]), *rds* (retinal degeneration slow[119, 142]), and *pcd* (Purkinje cell degeneration[84, 99]). Each of these mutants exhibits degeneration and death of photoreceptor cells, but with differing time courses. For example, almost all photoreceptor cells degenerate in homozygous *rd* mice by 2 months postnatally, while some viable photoreceptors remain in *rds* and *pcd* retinas as late as 1 year postnatally. In each of these mutants, the defect appears to be expressed in photoreceptor cells and leads directly to their death and degeneration. Specific biochemical defects have not been identified for any of these mutants; however, the *rd* strain develops abnormally high accumulations of cyclic guanosine monophosphate,[44] the result of an abnormality in the α subunit of the specific rod. Phosphodiesterase, which hydrolyses cyclic guanosine monophosphate in the matrix is greatly altered, but there are no known secondary changes in the RPE.[43, 44]

Progressive Rod-Cone Degeneration

Miniature poodles exhibit an inherited disease, termed progressive rod-cone degeneration, that directly affects both rod and cone photoreceptor cells. This is a late-onset disease occurring after photoreceptor cells have fully differentiated in apparently normal fashion.[7] Later, individual rod outer segments become disordered, and die. Similar changes occur in cone outer segments, but slightly later than in rods. Ultimately, photoreceptor cell outer segments are completely lost, and degeneration of photoreceptor cell inner segments and cell bodies occurs. Late in the disease process, cells of the retinal pigmented epithelium are observed to become hypertrophied and may invade the remaining neural retina.

Acknowledgments

Portions of the research described herein were supported by grants from the National Eye Institute to G.S.H. (EY06463) and to L.V.J. (EY04741); by an unrestricted grant from Research to Prevent Blindness, Inc. (to the Bethesda Eye Institute); and by the Bethesda Eye Institute Resident Research Fund.

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