4

Retinal Plasticity and Interactive Cellular Remodeling in Retinal Detachment and Reattachment

GEOFFREY P. LEWIS AND STEVEN K. FISHER

Neuroscience Research Institute, University of California Santa Barbara, Santa Barbara, CA, USA

Introduction

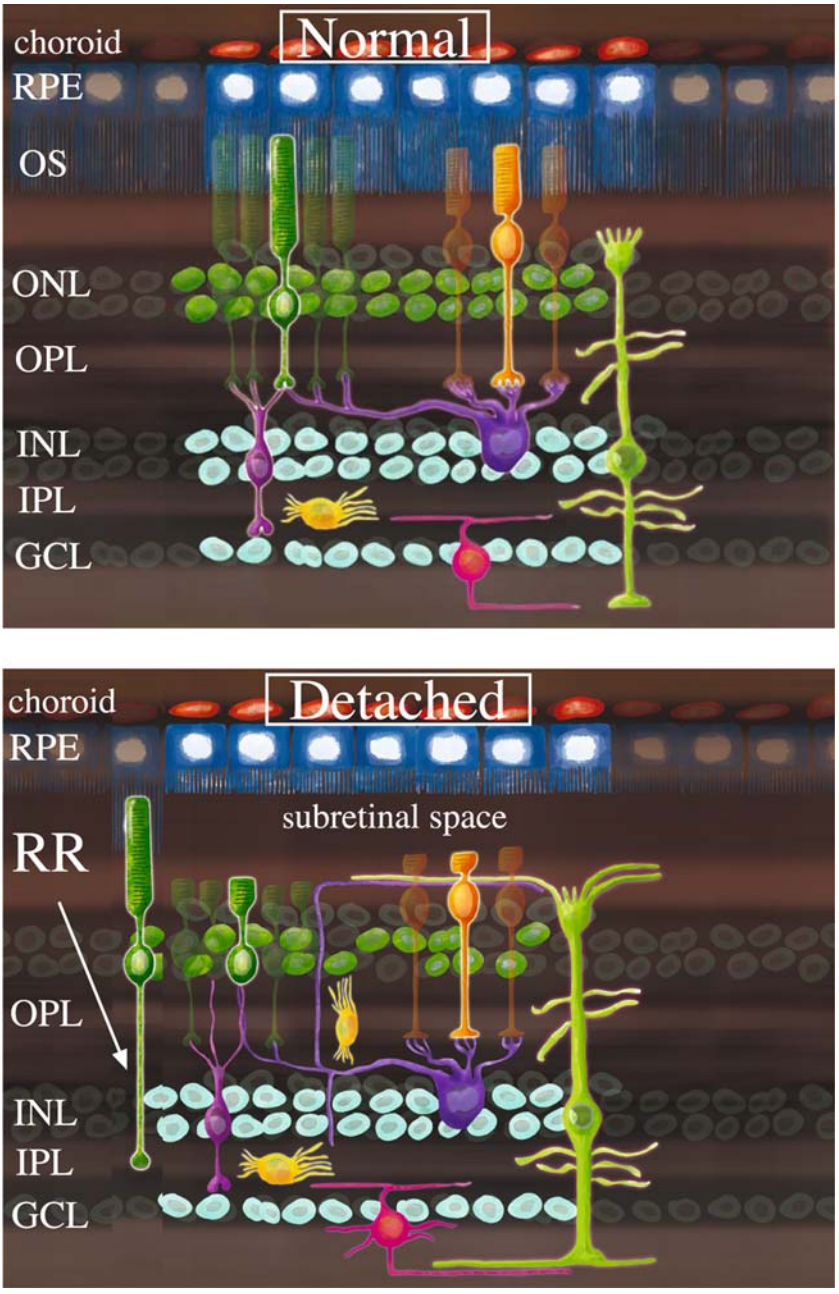
During development, the CNS demonstrates a remarkable ability to regenerate damaged neuronal processes. New processes are able to grow through lesioned areas and even re-establish connections to their targets (Saunders et al., 1995). This ability has traditionally been considered lost in the adult mammalian CNS. Indeed, molecules have been identified on some CNS glia that specifically inhibit axonal growth in the adult (Chen et al., 2000). Recent evidence, however, suggests that the adult CNS is in fact capable of a greater repertoire of responses to various forms of insult than previously thought, with many of these mediated through the up-regulation of growth associated proteins (see Emery et al., 2003 for review). Furthermore, there is considerable evidence that new neurons and glia are generated in specific regions of the adult brain as part of a normal renewal process (Gage, 2000). These responses are not limited to the brain and spinal cord. The adult mammalian retina also exhibits responses indicative of plasticity or remodeling as demonstrated by its ability to respond to enriched sensory environments (Pinaud et al., 2002) or injury (Coblentz et al., 2003) in up-regulating growth associated proteins, as well as generating new cells through cell division after injury (Fisher et al., 1991). Moreover, retinal neurons appear to have the ability to initiate the growth of neurites that can extend great lengths throughout all layers of the tissue. These responses appear to be relatively common to different species as well to different types of insult. Animal and human inherited degenerations, as well as various forms of trauma, all elicit some form of neuronal and glial remodeling in the adult retina (see Marc et al., 2003 for review).

In this chapter, retinal detachment and reattachment in the feline retina will be used as the primary form of injury that initiates dramatic remodeling

R. Pinaud, L. A. Tremere, P. De Weerd (Eds.), Plasticity in the Visual System: From Genes to Circuits, 55-78, ©2006 Springer Science + Business Media, Inc.

of both neurons and glia thus illustrating the plasticity retained in the adult retina. A benefit to this model is that the timing of both the injury and the recovery can be precisely controlled and the sequence of changes followed. Using this model we have observed that the growth of neuronal processes initiated by detachment occurs rapidly and in many cases does not appear to be a random process. Rather, much of the remodeling occurs as a result of morphological changes of other cell types. This "interactive cellular remodeling" begins with photoreceptors and continues to second and third order neurons as the detachment interval proceeds. We have proposed that the initiating factor of this remodeling is hypoxia of the outer retina (Lewis et al., 1999b). This results in programmed cell death of some photoreceptors (Cook et al., 1995) and programmed deconstruction of surviving photoreceptors (Mervin et al., 1999) which then sets in motion a specific sequence of remodeling events including both neurons and glia. Following retinal reattachment and a reperfusion of retinal oxygen levels, many of the glial and neuronal changes that begin as a result of detachment are halted and some are reversed. However, reattachment also elicits its own set plastic responses (Lewis et al., 2003) many of which are dependent on other cell types.

Retinal detachment is defined as the separation of the neural retina from the underlying retinal pigment epithelial (RPE) layer. At a cellular level, this results in a withdrawal of the rod and cone outer segments from the ensheathing microvillous processes of the RPE (Fig. 4.1, blue cells). Initially, this causes only minor structural damage to the outer segments but an immediate decline in vision. Moreover, this begins a cascade of cellular and molecular events in the retina that will continue as long as the retina remains detached. The first and most obvious structural change that can be observed is the degeneration of the photoreceptor outer segments. This portion of the cell becomes progressively truncated with time until there is eventually only an inner segment with a short connecting cilium present. In perhaps a remarkable example of neuronal regeneration, the highly degenerate outer segments can regenerate following retinal reattachment and most of them eventually appear morphologically normal. This, however, leads to an interesting dichotomy. On the one hand, the retina appears to be able recover from the loss of outer segments. On the other hand, visual deficits are not uncommon following successful reattachment surgery. Indeed it has been shown that only 20–60% of successful macular reattachments achieve a visual acuity of 20/50 or better (Burton et al., 1980; Tani et al., 1980; 1981; Ross, 2002). Moreover, it appears that the process of visual recovery is slow, in some cases continuing to change for months or years following reattachment (Liem et al., 1994; Ross, 2002). If the outer segments can recover relatively quickly (over a few weeks), what is it that accounts for the continued change in vision over time? We would like to propose here that changes in photoreceptors initiate downstream changes in many retinal cell types, resulting in a significant "rewiring" of the retina and ultimately leading to changes in vision. Recovery


Normal
choroid
RPE
OS
ONL
OPL
INL
IPL
GCL
Detached
choroid
RPE
subretinal space
RR
OPL
INL
IPL
GCL

itself would also require either physical rewiring or some other form of compensation for recovery of normal vision.

Surgical Procedures

Retinal detachments in our laboratory are created by first removing the lens and vitreous and then infusing a solution of sodium hyaluronate (Healon; 0.25%), a natural component of the vitreous and interphotoreceptor matrix, in a balanced salt solution between the retina and RPE via a glass micropipette (Lewis et al., 1999a). The Healon is used to prevent the retina from spontaneously reattaching. The pipette, which is secured to a micromanipulator, is attached to tubing that connects to an infusion pump. This allows precise control of the position and size of the detachment. The pipette, which creates a small hole as it passes through the retina, produces what we believe models rhegmatogenous detachments. In humans, a rhegmatogenous detachment refers to the fact that a hole or tear is present in the retina. As a result, fluid passes through the break in the neural retina to the subretinal space, thus creating the detachment. This is an important distinction since retinal detachments that occur without the presence of a hole, instead developing as a result of fluid accumulating under the retina (in what is termed a "serous" detachment), often show very few signs of photoreceptor degeneration or visual loss. The reason for the differences in the responses is unclear, however, it has been suggested that the composition of the subretinal fluid remains relatively normal because it does not mix with fluid from the vitreous, perhaps retaining factors in the subretinal space that the preserve the retina. Although in this animal model there is no large tear in the retina as there often is in human rhegmatogenous detachments, the retinal changes documented so far in

FIGURE 4.1. Diagram of the morphology of retinal cell types in the normal and detached retina, illustrating the remodeling that occurs following detachment. Retinal detachment occurs between the RPE (blue) and the photoreceptor OS. This initiates the degeneration and shortening of rod (dark green) and cone (yellow) OS as well as a retraction of rod axon terminals to their cell body into the ONL. Rod bipolar cell dendrites (light purple) and neurites from the axon terminals of horizontal cells (dark purple) extend adjacent to the retracted rod terminals. Horizontal cell processes continue to grow wildly into the subretinal space. Ganglion cell bodies (red) in the GCL sprout neurites. Microglia (yellow) in the IPL become activated and migrate towards the degenerating photoreceptors. Müller cells (light green), which span the width of the retina, hypertrophy, extend processes into the subretinal space, and act as substrates for the growth of horizontal cells and ganglion cells. Retinal reattachment (RR) stimulates the growth of rod axons into the inner retina (arrow) as well as the growth of Müller cells onto the vitreal surface of the retina that then serves as a substrate for the growth of ganglion cell neurites. (GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; ONL, outer nuclear layer; OS, outer segments; RPE, retinal pigment epithelium.)

human detachments are essentially identical to those observed in the feline retinas (Chang et al., 1996; Sethi et al., 2005).

Experimentally, retinal reattachments are created by pneumatic retinopexy. That is, the fluid is removed from the vitreous chamber, allowing fluid to flow out of the subretinal space through the hole and the retina to settle back down on the RPE. The eye is then flushed with 20% sulfur hexafluoride (mixed with filtered room air) that acts as a tamponade to keep the retina adjacent to the RPE until the 2 layers become physiologically attached.

Retinal Detachment and Reattachment

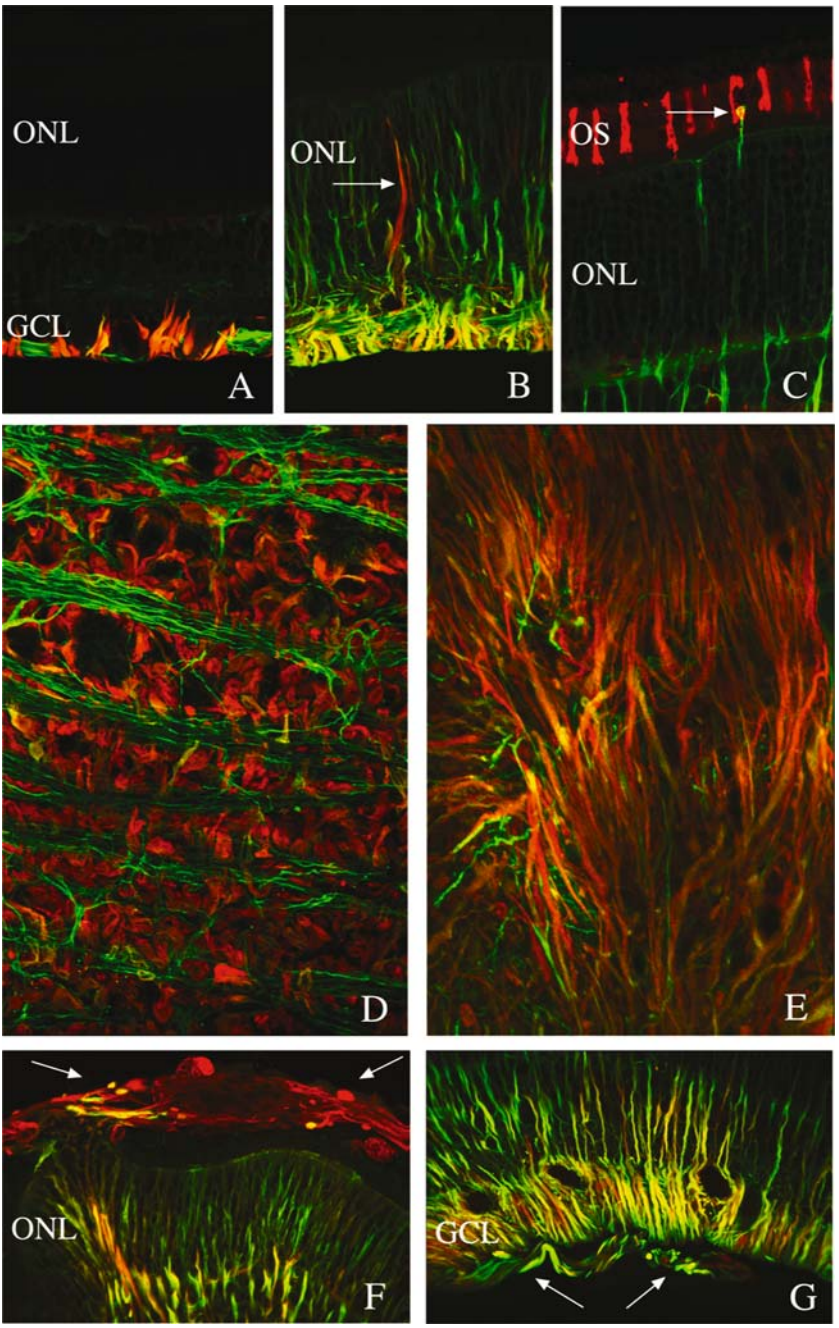
The retina is a highly organized tissue consisting of 3 nuclear layers and 2 synaptic layers (Fig. 4.1A). Because it is so highly structured, any significant change from its normal architecture is easily noted by conventional histological procedures. As early as 1968 investigators used light and electron microscopy to reveal that detachment induced a number of changes in photoreceptors including outer segment degeneration, the loss of mitochondria, and the disorganization of organelles involved in protein synthesis and trafficking (Machemer, 1968; Kroll and Machemer, 1969a, 1969b; Anderson et al., 1983). It wasn't until the introduction of immunocytochemistry, however, that it became apparent that cells within the adult retina do not simply degenerate over time but are actually capable of significant cellular remodeling. While evidence suggests that the photoreceptors are the first neuronal cell type to respond to detachment, Müller cells also respond rapidly to detachment and since they appear to be intimately involved in all levels of the neuronal remodeling, they will be introduced first.

Müller cells

Müller cells are radial glial cells that span the entire width of the retina (Fig. 4.1A, light green cell). The inner most portion of the cell lies adjacent to the vitreous and possesses a club shaped "endfoot" while the cell body lies in the central part of the retina, in the inner nuclear layer (INL). The outer portion of the cell consists of numerous branches that weave around photoreceptors and ends with a tuft of microvilli that interdigitate between the photoreceptor inner segments. Because of this they are uniquely positioned to detect changes in photoreceptors or the interphotoreceptor matrix that occurs as a result of detachment. These polarized cells contribute to the 2 boundaries of the retina. Their endfeet and basement membrane (the "inner limiting membrane", ILM) form the vitreal border of the neural retina while at the photoreceptor border, adherens junctions between the Müller cells and photoreceptors make up the "outer limiting membrane" (OLM). For many years the exact function of Müller cells was enigmatic and they were generally described as playing only a supportive role. It is now becoming increasingly evident that they are intimately involved in many essential functions

including glutamate recycling, potassium regulation and pH balance in the retina (see Sarthy and Ripps, 2001 for review).

Following retinal detachment the highly polarized morphology of Müller cells is rapidly altered as they undergo a number of biochemical and structural changes (see Fisher and Lewis, 2005 for review). These changes can be traced to events that occur within minutes of the retina becoming detached. Indeed, it has been shown that the extracellular signal–regulated kinase (ERK) is phosphorylated within 15 minutes of creating the detachment, and immunoreactivity for the immediate-early response genes, cFos and cJun, increases within 2 hours in these cells (Geller et al., 2001). Moreover, the FGF receptor, previously identified in Müller cells (Yamamoto et al., 1996), is phosophorylated with 15 minutes of detachment and dephosphorylated by 2 hours (Geller et al., 2001). Perhaps as a result of these early events Müller cells, normally quiescent, soon begin to proliferate. While the peak number of cells dividing occurs 3 days after detachment the Müller cells continue divide as long as the retina remains detached (Fisher et al., 1991; Geller et al., 1995). The details of the mitotic events in these cells are unknown, however, the presence of large rounded mitotic figures near the outer retinal border suggests that they must lose their complex morphology as they dedifferentiate and re-differentiate as part of the cell cycle. Morphological remodeling of Müller cells can be observed as early as one day after detachment (Lewis et al., 1994; 1995). At this time, they begin to hypertrophy with their main trunk and lateral branches expanding in size. Also at this time they greatly alter their expression for many proteins with glutamine synthetase, carbonic anhydrase II, and cellular retinaldehyde binding protein decreasing in expression (Lewis et al., 1989) and two intermediate filament proteins, glial fibrillary acidic protein (GFAP) and vimentin dramatically increasing. Expression of the latter are particularly useful to mark these cells as they remodel. In the normal feline retina these proteins are mainly restricted to the endfoot of the Müller cell (Fig. 4.2A). Following detachment these proteins rapidly increase their expression so that within a few days they fill the entire cytoplasm of the cell (Fig. 4.2B). Even though both GFAP and vimentin are upregulated, the balance of the two shifts over the course of the detachment time (Lewis and Fisher, 2003). In the normal retina, vimentin is the predominant protein in the endfoot with GFAP being less abundant. Following detachment there is a shift to more GFAP expression as the endfeet rapidly take on a more elongated and branched appearance compared to that in normal retina. This is readily seen in whole mount preparations where the endfeet are transformed from their clubbed appearance to long processes extending laterally along the inner border of the retina (Figs. 4.2D, 4.2E). The remodeling of the endfeet then progresses to a significant expansion of the entire cell with GFAP becoming the predominant intermediate filament protein. Interestingly, a sub-population of Müller cells express predominantly vimentin in the outer retina and it are these cells that extend beyond their normal boundary at the OLM into the subretinal space (Fig. 4.2B, arrow).


ONL
GCL
A
ONL
B
OS
ONL
C
D
E
ONL
F
GCL
G

The growth of these cells into the subretinal space, however, is not random. Indeed, those cells that initially extend past the OLM invariably do so adjacent to cones (Fig. 4.2C, arrow) (Lewis et al., 2000). Once this growth is initiated the Müller cells continue to expand over the surface of photoreceptors creating what is termed a glial scar (Fig. 4.2F, arrows). The consequences of the presence of the glial scar are several-fold. First, photoreceptor outer segments cannot regenerate and reform a connection with the RPE if this scar is present (Anderson et al., 1983). Second, photoreceptor cell bodies appear to be carried into this scar as Müller cells hypertrophy, thereby depleting the retina of photoreceptors. Third, the scar appears to act as a substrate for the growth of aberrant neuronal processes originating from horizontal and ganglion cells (discussed below; Fisher and Lewis, 2003).

Retinal reattachment appears to greatly slow much of the intraretinal hypertrophy of the Müller cells as well as their growth into the subretinal space (Lewis et al., 2002; 2003). It does not, however, completely halt their reactivity since cell division can still be observed a month after retinal reattachment, albeit at low levels. In addition, reattachment appears to be the stimulus in redirecting the growth of Müller cells from the subretinal space towards the vitreal surface of the retina; Müller cell growth on the vitreal surface is never observed in the detached retinas. As was the case with the growth of Müller cells into the subretinal space, the growth onto the vitreal surface also can have devastating consequences for vision. These cells can

FIGURE 4.2. Laser scanning confocal images demonstrating the remodeling of Müller cells after detachment and reattachment. (**A,B**) Sections are labeled with antibodies to the intermediate filament proteins vimentin (red) and glial fibrillary acidic protein (GFAP, green). (**A**) In the normal retina vimentin is the predominant intermediate filament in the Müller cell endfeet in the GCL while GFAP is less prevalent. (Anti-GFAP labeling also occurs in the astrocytes in the GCL.) (**B**) Following a 7-day detachment both proteins are upregulated although GFAP is expressed more widespread. A few cells express predominantly vimentin (arrow) and it are these cells that will grow into the subretinal space. (**C**) The initial growth of Müller cells (GFAP, green) into the subretinal space (arrow) occurs adjacent cone photoreceptor OS (labeled with peanut agglutinin, red). (**D,E**) Retinal flat-mounts focused at the level of the GCL and labeled with anti-vimentin (red) and anti-GFAP illustrating the remodeling of glia. (**D**) In normal retina the endfeet (mostly red) appear club-like while astrocyte processes (green) are organized in parallel arrays. (**E**) Following a 7-day detachment the endfeet hypertrophy and grow laterally across the retina while the astrocyte processes become highly disorganized. (**F,G**) Sections of detached retina illustrating the differential expression of intermediate filaments in sub-**(F)** and epiretinal (**G**) membranes. (**F**) The Müller cells that grow into the subretinal space following detachment express predominantly vimentin (red, arrows). (**G**) Those that grow onto the vitreal surface of the retina following reattachment express predominantly GFAP (green, arrows). (GCL, ganglion cell layer; ONL, outer nuclear layer; OS, outer segments).

form significant membranes, termed "epiretinal membranes" that contract and cause a re-detachment of the retina. In addition, we have observed, both in feline and human samples, that epiretinal membranes can act as substrates for the growth of ganglion cell neurites (discussed below). The mechanism that redirects the growth from the subretinal to the vitreal surface is not known, however, intermediate filaments once again appear to be involved. The Müller cells that first penetrate the inner limiting membrane and enter the vitreous cavity express predominantly GFAP over vimentin; just the opposite to the expression profile for those growing into the subretinal space (Fig. 4.2G, arrows). Even as the membranes mature and become quite large, GFAP predominates on the leading edge (Lewis and Fisher, 2003). From these examples it is clear that Müller cells are highly involved in the interactive cellular remodeling that occurs as a result of detachment; they are interacting with cone photoreceptors in the formation of subretinal glial membranes and they are interacting with second and third ordering neurons in creating a substrate for neurite sprouting and growth.

Astrocytes

Astrocytes, the only other glial cell type in the retina, also undergo cellular remodeling after detachment. In the normal retina they are located within the ganglion cell layer, their processes intimately associated with the axons of the ganglion cells (Figs. 4.2A, 4.2D). Following detachment they follow the same proliferation time scale as Müller cells, peaking at day 3 (Fisher et al., 1991; Geller et al., 1995) but cell division continues at low levels even after retinal reattachment (Lewis et al., 2003). It is difficult to examine the change in intermediate filament levels on sections because the large increase in intermediate filaments in Müller cells overwhelms that of the astrocytes. When viewed in a wholemount preparation, however, it is clear that these cells dramatically change their morphology in response to detachment. In normal retina astrocyte processes label more heavily with anti-GFAP than the Müller cell endfeet, which express predominantly vimentin (Fig. 4.2D). These GFAP labeled processes can be seen extending in organized parallel arrays as they follow bundles of ganglion cell axons. Following a detachment interval of just 3 days these processes begin to appear disorganized and by day 7, they extend in what appears to be a random orientation (Fig. 4.2E). In addition, following retinal reattachment, astrocytes migrate out of the retina, perhaps using Müller cells as a substrate, and contribute to the composition of epiretinal membranes (data not shown).

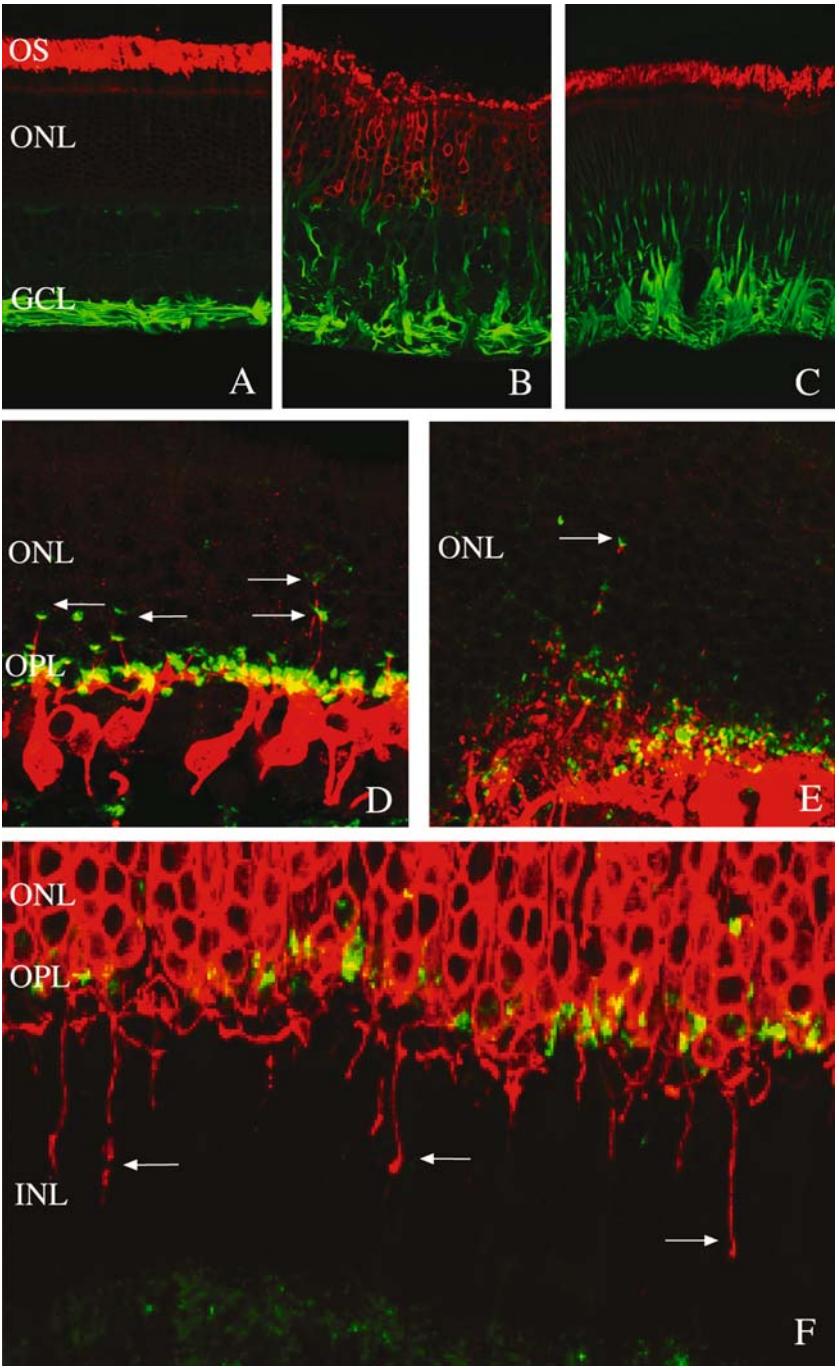
Photoreceptors

The photoreceptor cell is highly polarized with an outer segment at the distal end (which contains the molecular machinery for phototransduction) an inner segment, cell body, axon and synaptic terminal (Fig. 4.1A, dark green = rod cell & yellow = cone cell). These cells, considered the "first order neurons" of the

retina since the visual transduction signal begins here and is transferred via 2nd and 3rd order neurons to the brain, are the first neuronal cell type in the retina to respond to detachment, with virtually every region of the cell undergoing change. Both rods and cones show evidence of plasticity but with striking differences in their response; rods very actively remodel while cones appear to become almost quiescent (Rex et al., 2002). This difference is evident structurally but even more so when examining the expression of their respective opsins, the photopigments responsible for capturing light. In the normal retina these proteins are restricted to the outer segment (Fig. 4.3A, rod opsin) but beginning within a few days after detachment, concomitant with outer segment degeneration, immunolabeling shows that the entire rod cell plasma membrane contains relatively high levels of rod opsin (Fig. 4.3B). Cones show a similar response initially but within a few days of detachment most stop expressing their opsins altogether. This pattern of protein expression continues as long as the retina remains detached. Following retinal reattachment, as the outer segments regenerate, the expression of cone opsin returns and rod opsin once again is restricted to the outer segments (Fig. 4.3C) (Lewis et al., 2003).

Antibodies to synaptic proteins can be used to visualize the synaptic terminal morphology of both rods and cones. The results indicate that both appear to change but in different ways. Early in detachment, the synaptic terminals of many rods begin to retract from the outer plexiform layer where they connect to second order neurons (the rod bipolar and horizontal cells) (Erickson et al., 1983; Lewis et al., 1998). The end result is that rod terminals, often with shortened synaptic ribbons, become located directly adjacent to their nucleus, appearing much like they do in the developing retina (Fig. 4.3D). The cone terminals, however, do not retract. They do change their morphology, with their multiple invaginations having a more shallow appearance or being lost completely, when compared to normal retina (Lewis et al., 2003).

Another remarkable display of cellular plasticity is observed in rods following retinal reattachment (Lewis et al., 2003). In retinas detached for 3 days and reattached for 28, the distribution of rod opsin, that at one time outlined the entire cell, usually appears relatively normal and is restricted to the regenerated outer segment (Fig. 4.3C). In some focal regions across the retina, however, the recovery appears to be incomplete; outer segments are shorter and opsin levels remain elevated in the plasma membrane. In these focal regions, rod axons often can be observed extending well beyond their normal target in the outer plexiform layer deep into the inner retina (Fig. 4.3F, arrows). The retraction and growth of rod axons appears to be a recapitulation of development; the retracted rod terminals appear structurally as if they were from an early period of development and the overgrowth of some rod axons appear similar to those observed deep in the inner layers of the normal developing ferret retina (Johnson et al., 1999). As of now there is no evidence that these terminals form synaptic connections in either case.


OS
ONL
GCL
A
B
C
ONL
OPL
D
ONL
E
ONL
OPL
INL
F

We also find no evidence of cone axon growth and this may be related to the fact that these terminals do not retract following detachment.

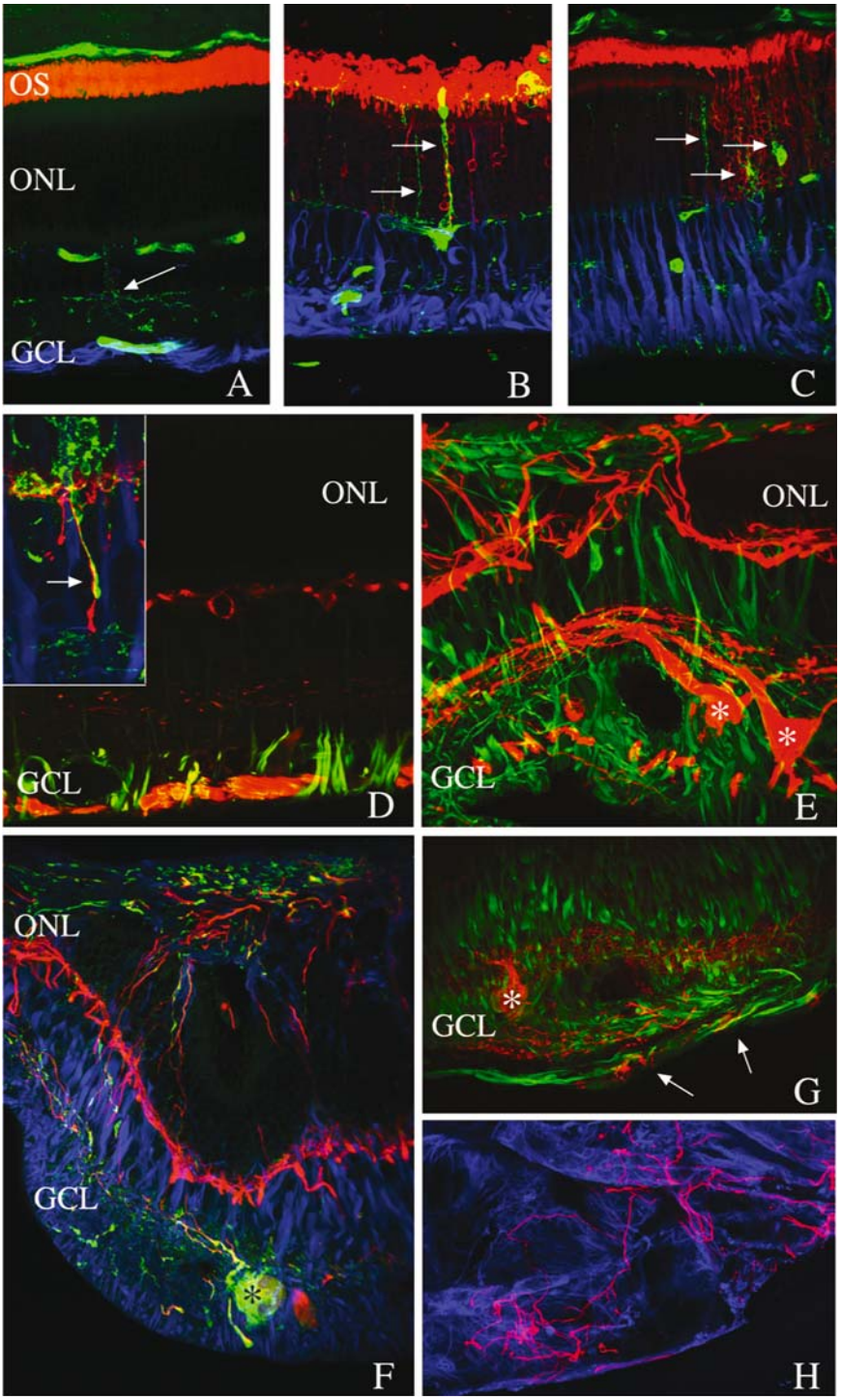
The factor(s) that initiates the re-growth of rod axons is not known but may be related to a physical interaction of photoreceptors with the RPE apical surface since we do not observe it in the detached retina. As mentioned above, over much of the reattached retina the outer segments and their interface with the RPE are indistinguishable from normal retina. However, in the abrupt patchy regions where rod and cone outer segment regeneration is incomplete, the cone-ensheathing apical RPE apical processes are either short or missing indicating that a truly normal physical interaction of RPE and outer segments had not been re-established (Lewis et al., 2003). Fortuitously, in these patchy regions, there is continued redistribution of rod opsin in the cell bodies and axons allowing for visualization of the aberrant rod axon growth beyond their normal targets. Without the elevated opsin levels the rod extensions would not have been observed. Thus we can't rule out the possibility that axon extension occurs in these other areas. Rod axon extensions have also been found in the human retinal degenerations, retinitis pigmentosa (RP) and age related macular degeneration (AMD) (Li et al., 1995; Fariss et al., 2000; Gupta et al., 2003), and in human detached retinas with "complex detachments" (i.e. the retinas had been reattached at least once before the tissue samples were obtained) (Sethi et al., 2005). In all of these examples the interaction between the RPE and outer segments may have been compromised. The molecular signals that stimulate the growth of rod axons have not been extensively studied but recent evidence indicates that regulation of Ca2+ influx may be involved since L-type channel antagonists inhibited rod axon outgrowth from salamander photoreceptors in culture

FIGURE 4.3. (**A,B,C**) Laser scanning confocal images of sections labeled with anti-rod opsin (red) and anti-GFAP (green) illustrating the degeneration and regeneration of rods as well as the hypertrophy of Müller cells. In normal retina rod opsin is restricted to the OS and GFAP is found only in Müller cell endfeet and astrocyte processes. (**B**) Following a 3-day detachment the outer segments are truncated, rod opsin is redistributed to the rod cell bodies in the ONL, and GFAP increases in Müller cells. (**C**) Following 3 days of detachment and 28 days of reattachment, rod outer segments regenerate and have a near normal morphology, rod opsin is no longer present in the rod cell bodies and the increase in GFAP is halted. (**D,E**) Confocal images of 7-day detachments demonstrating that neurite outgrowth from rod bipolar cells (**D**, red, anti-protein kinase C) and horizontal cells (**E**, red, anti-calretinin) is initially associated with the retraction of rod synaptic terminals (green, anti-synaptophysin, arrows). (**F**) Confocal image showing rod axon growth into the inner retina observed following retinal reattachment (red, anti-rod opsin, arrows). Anti-synaptophysin labeling (green) is observed in the terminals of rods and cones, both in their normal location in the OPL and on occasion, in the terminals of the axon extensions. (GCL, ganglion cell layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; OS, outer segments).

(Zhang and Townes-Anderson, 2002). The only correlation we have observed that may be relevant is an apparent association of these rod processes with activated microglia.

Microglia

Microglial cells are in fact not true glia but derived from bone marrow precursor cells that enter the retina at early stages of development from the optic nerve and lie "dormant", usually in the inner and outer plexiform layers of the adult retina. Following trauma and disease however, they are stimulated to assume macrophage-like functions, migrating into the damaged area and phagocytosing debris. Microglia have previously been shown to become activated in various forms of retinal degeneration including the RCS rat (Thanos et al, 1992; Roque et al., 1996), light damaged mice (Ng et al., 2001; Harada et al., 2002), glaucoma (Naskar et al., 2002; Lam et al., 2003), laser photocoagulation (Humphrey et al., 1996), and human RP (Gupta et al., 2003). It has been proposed that stressed or dying photoreceptors attract microglia which then eventually kill the photoreceptors since inhibition of their activation quantitatively reduces photoreceptor degeneration (Thanos et al., 1995). Indeed, microglia are known to secrete molecules that kill neurons such as pro-inflammatory cytokines, proteases, tumor necrosis factor, and nitric oxide (Lee et al., 2002). It is not known if microglia kill photoreceptor cells following retinal detachment, but they do become activated and migrate throughout the retina, eventually becoming prominent among the degenerating photoreceptors (Figs. 4.4A, 4.4B) (Lewis et al. 2005). They begin their migration towards the outer retina within a day of detachment and continue to be present at higher levels as long as the retina remains detached. When the retina is reattached after 3 days of detachment (a time when significant numbers of microglia are present in the outer retina) and examined a month later the microglial cell response appears, for the most part, to be reversed (Fig. 4.4C, left half of image). However, in focal regions of the retina where photoreceptor regeneration appears to lag behind, the number of microglia remains high, at a level similar to that observed during detachment (Fig. 4.4C, right half of image). At this time it is uncertain if the microglia in the disrupted areas are simply phagocytosing debris from dying photoreceptors or if they are actually contributing to photoreceptor cell death. Data from Harada et al. (2002) using light to induce photoreceptor degeneration, suggest that microglia derived neurotrophic factors can also modulate trophic factor expression in Müller cells. The result of such a microglial-Müller cell interaction could be beneficial or harmful at the level of individual photoreceptors depending on the type and balance of factors released. Because microglia may also control, at some level, the responses of Müller cells, they may also be key to modifying the glial responses that occur after detachment. Controlling this balance of survival and death factors may turn out


OS
ONL
GCL
A
B
C
ONL
GCL
D
ONL
GCL
E
ONL
GCL
F
GCL
G
H

to be an important step in preventing photoreceptor degeneration. If, however, microglia are releasing some type of "cell killing" factor after detachment, its effects are highly specific in the feline retina because only photoreceptors appear to die in this model.

We have observed a structural interaction between microglia and the remodeling of rod axons as they extend deep into the inner retina following retinal reattachment. Fine microglia cell processes can often be observed directly adjacent to the elongated rod axons (Fig. 4.4D, inset). The fact that microglia become activated upon detachment prior to the outgrowth of rod axons might suggest that its their presence that stimulates rod axon outgrowth, if they are involved at all. This hypothesis may be testable by using agents that will inhibit the microglia response (Thanos et al., 1995).

FIGURE 4.4. (**A,B,C**) Laser scanning confocal images illustrating the activation of microglia (green; isolectin B4) following detachment. (Sections are also labeled with anti-rod opsin, red, and anti-GFAP, blue, to follow the responses of photoreceptors and Müller cells, respectively.) (**A**) In the normal retina microglia are located in the IPL (arrow). (Note: blood vessels are also labeled with the lectin used to label microglia and appear green.) (**B**) Following a 3-day detachment microglia migrate into the ONL (arrows). (**C**, left half of image) When the retina is reattached for 28 days following a 3-day detachment the activation of microglia is for the most part reversed. (**C**, right half of the image) In focal regions where there is poor photoreceptor recovery as illustrated by increased opsin redistribution (red) in the ONL, there is continued activation of microglia (arrows). (**D**, **inset**) At higher magnification in regions of poor photoreceptor recovery in the reattached retinas, microglia (green) are often observed in association with rod axons (red) that extend into the inner retina. (**D,E**) Confocal sections labeled with anti-neurofilament protein (red) and anti-GFAP (green) illustrating the upregulation of neurofilament protein that occurs after detachment. (**D**) In normal retina anti-neurofilament protein (red) labels ganglion cell axons in the GCL and horizontal cells; anti-GFAP (green) labels only the endfeet of Müller cells. (**E**) Following 28 days of detachment ganglion cell bodies upregulate neurofilament protein (asterisks) and horizontal cells sprout neurites that grow along the processes of reactive Müller cells into the subretinal space. (**F,G,H**) Confocal images illustrating the growth of neurites from ganglion cells. (**F**) Following 28 days of detachment ganglion cell bodies upregulate GAP 43 (green, asterisk) and sprout processes that grow throughout the retina and into the subretinal space adjacent to reactive Müller cells (blue, anti-GFAP). These processes are distinct from the horizontal cell processes labeled with anti-neurofilament (red) also present adjacent to Müller cells in the subretinal space. (**G**) Anti-GAP 43 labeled ganglion cell neurites (red) can also be found in epiretinal membranes (green, anti-GFAP, arrows) in retinas reattached for 28 days following a 3-day detachment. Note the ganglion cell body also labeled with anti-GAP 43 (asterisk). (**H**) Ganglion cell neurites (red, anti-neurofilament) are also observed growing in epiretinal membranes removed from human patients. These membranes consist largely of Müller cells (blue, anti-GFAP). (GCL, ganglion cell layer; ONL, outer nuclear layer; OS, outer segments).

Bipolar and Horizontal cells

Second order neurons (Fig. 4.1, purple cells) begin to remodel in apparent response to the changes initiated by the photoreceptors in a classic example of interactive cellular remodeling. As the rod axon terminals retract towards their cell body, the rod bipolar dendrites and neurites from what appear to be the horizontal cell axon terminals (Lewis et al., 1998; Linberg et al., 2004) grow into the outer nuclear layer, remaining adjacent to these retracted terminals (Figs. 4.3D, 4.3E, arrows). It is not known if the processes of the second order neurons are actually physically connected to the rod terminals as the remodeling occurs or if they first become disconnected and then reconnect to their "lost" terminal at some later time. Evidence from culture systems indicates that neurite growth can be initiated by applying mechanical tension to the cell (Lamoureux et al., 2002). This suggests that if rod terminals remain connected to the processes of second order neurons, physical retraction may exert sufficient mechanical tension on the second order neurons to stimulate neurite growth. Interestingly, rod bipolar dendrites that have grown into the outer nuclear layer are almost always found adjacent to a retracted rod terminal even in very degenerate retinas. This is unlike the response of the horizontal cells that seem to have an additional growth phase. Once the horizontal cell outgrowth starts after detachment, some processes can grow wildly throughout the retina and even into the subretinal space (Figs. 4.4D, 4.4E, 4.4F). The structural evidence points to a tantalizing interaction between the Müller cells and these aberrant horizontal cell processes. These longer processes are invariably found adjacent to reactive Müller cells (based on increased intermediate filament labeling) (Figs. 4.4E, 4.4F). As the Müller cells hypertrophy throughout the retina and into the subretinal space, horizontal cells appear to follow, using these processes as a scaffold or substrate for growth. Indeed, horizontal cell neurites can be observed extending great distances in the subretinal space in association with a Müller cell "scar". While the molecular signals that attract the horizontal cells are unknown, interestingly the bipolar cells do not appear to use these cues in their course of remodeling. This type of interactive remodeling, where neurons appear to grow adjacent to reactive Müller cells has also been observed in other forms of retinal degeneration (see Marc et al., 2003 for review) indicating that this phenomenon is not unique to retinal detachment.

Ganglion cells

Ganglion cells (Fig. 4.1, red cell), the third order neuron and the retinal cell type responsible for relaying the visual information to higher centers in the brain, also undergo significant structural and molecular remodeling as a result of detachment. The first evidence that ganglion cells respond to detachment was the observation that GAP 43 is upregulated in a subpopulation of these cells (Figs. 4.4F, 4.4G; Coblentz et al., 2003). During development,

as new processes are being elaborated in the retina, GAP 43 is expressed at high levels in ganglion cells. As the animal matures this protein is lost almost entirely from the cell, with the only anti-GAP 43 labeling occurring in 2 bands in the inner plexiform layer. Upregulation of the protein is first detected by immunocytochemistry in some ganglion cell bodies at 3 days of detachment with the number of labeled cells increasing at later time-points. As with GAP 43, the 70 and 200 kDa polypeptide forms of neurofilament protein are not detectable in the cell bodies of ganglion cells until the retina is detached at least 3 days (Fig. 4.4E). The cells showing an increase in GAP 43 and neurofilament protein expression also undergo significant structural remodeling by elaborating fine neurites from their cell body (Coblentz et al., 2003). Since these antibodies do not label cell bodies and branches from their main dendritic trunks in non-detached retina, the morphology of these cells cannot be directly compared to ganglion cells in the normal retina. However, when compared to data from studies using Golgi impregnation or dye injection, it becomes apparent that these cells have a morphology unlike any described previously for feline ganglion cells. The most compelling morphological data for this remodeling comes from the many angular neurites on the basal surface of the cell body and the very long processes that extend into the outer retina, the subretinal space or into the vitreous (discussed below).

At this point, the mechanisms underlying ganglion cell remodeling is unknown. There is no evidence that the cells directly connecting to ganglion cells (the cone bipolar cells and amacrine cells) actively remodel following detachment. Amacrine cells, however, have been shown to sprout new processes in the human genetic retinal degeneration, RP (Fariss et al., 2000), so it is possible that a similar response is occurring after detachment and it has yet to be observed. Although there is no evidence that Müller cells induce neurite sprouting from ganglion cells, there is evidence for an interaction between Müller cells and neurites growing from ganglion cells. Ganglion cell processes can be observed extending adjacent to reactive Müller cells through the entire width of the retina and into the subretinal space as well as in association with Müller cells that have grown into the vitreous cavity (Figs. 4.4F, 4.4G). This is apparently not an uncommon phenomenon since neurites from ganglion cells can be found in both sub- and epiretinal membranes removed from human patients at the time of reattachment surgery (Fig. 4.4H, epiretinal membrane).

Mechanisms of Retinal Remodeling

Retinal detachment results in remodeling of at least 6 cell types in the feline retina and a diagram of these events is shown in Figure 4.1B. The remodeling illustrated throughout this chapter may be interpreted as a cascade of cellular interactions that are initiated by the separation of neural retina and RPE. The specific mechanisms that begin the process, however, are unknown and most likely involve multiple factors acting in concert. For example,

detachment results in the disruption of the interphotoreceptor matrix and the release of endogenous growth factors which may initiate some of the events that follow (Hageman et al., 1991). One of these components is basic fibroblast growth factor (bFGF; FGF II). When injected into a normal eye bFGF can induce similar remodeling events in Müller cells to those observed after retinal detachment (Lewis et al., 1992) and neurite outgrowth is greatly accentuated by intraocular administration of bFGF when the retina is detached (G. Lewis, unpublished data). In addition, bFGF has been observed in the subretinal fluid taken from human patients at the time of reattachment surgery (La Heij et al., 2001) and the bFGF receptor becomes phosphorylated in the retina within minutes after production of a detachment (Geller et al., 2001). These data suggest a significant role for bFGF in the remodeling of retinal neurons and glia that occurs after detachment. Finally, bFGF (and other neurotrophins) can also protect photoreceptors from degeneration (La Vail et al. 1992; Lewis et al., 1999a) thus increasing a likely role for factors of this type in retinal remodeling. Intraocular delivery of factors that modulate the expression or action of bFGF could be used to further elucidate its role in retinal detachment.

The evidence that many of the remodeling events are "downstream" from photoreceptor degeneration stems from experiments using hyperoxia to treat detachment. When experimental animals were placed immediately into a hyperoxic environment (70%) following the production of a retinal detachment and examined on day 3 (the peak of photoreceptor cell death and non-neuronal cell proliferation) there was a significant preservation of photoreceptor structure, a reduction of photoreceptor cell death, and less Müller cell reactivity (Mervin et al., 1999; Lewis et al., 1999b). Hyperoxia also maintained the overall structure of the interphotoreceptor matrix and apparently prevented the dispersal of bFGF from its stores in that location, thus potentially explaining the lack of Müller cell proliferation and hypertrophy and giving further evidence for the role of bFGF in retinal detachment. In experiments where hyperoxia was delayed until a day after the production of a detachment and retinas examined 6 days later, much of the remodeling of neurons that would normally occur at this time (rod terminal retraction, neurite sprouting from 2nd and 3rd order neurons) was prevented (Lewis et al., 2004). This again supports the hypothesis that preservation of photoreceptors prevents downstream changes in other neuronal cell types.

Retinal remodeling has now been observed in many forms of induced and inherited degenerations and this topic was extensively reviewed in a recent paper by Marc et al. (2003). As pointed out in that paper remodeling is observed in RP, AMD, rd and Crx -/- mice, RCS rats, Abyssinian cats, light damage, glaucoma, ischemia, drug toxicity, and even aged animals. What becomes apparent from this list is that remodeling can occur quickly or occur over the course of decades. A common denominator, however, appears to be the compromising of photoreceptors. Following trauma such as detachment, many of the photoreceptor cell changes (terminal retraction, outer segment

deconstruction, death) occur quickly, within the first few days and the remodeling occurs rapidly as well. In the inherited degenerations, photoreceptor changes often occur over the course of years and likewise the neuronal remodeling also appears to occur over years seemingly without subsiding. Another common factor is that in almost all cases, whether it is a result of induced or inherited degeneration, the neuronal remodeling is accompanied by glial remodeling, and based on immunocytochemical observations, there is often an interaction between these two cell types. It is fairly easy to envision remodeling as a cascade of events starting with photoreceptors and proceeding to 2nd and 3rd order neurons. As indicated by Marc et al. (2003) remodeling is most extreme when photoreceptor loss is essentially complete, resulting in "deafferentation" of the inner retina. Müller cells and other non-neuronal cells may react more independently from photoreceptor cell death since Müller cell events are among the earliest identified after detachment (Geller et al., 2001).

Conclusion

Understanding retinal detachment has assumed broader relevance in recent years because short-term retinal detachment occurs as part of experimental therapies in use or proposed for retinal degenerative diseases (e.g., RP, AMD) including retina–RPE transplantation (Bok 1993; Del Cerro et al., 1997), macular translocation (de Juan et al., 1998; Eckardt et al., 1999), or the introduction of trophic factors or vectors for transfection of retinal cells (Lewin et al., 1998). Thus, it is essential that we gain an understanding of the consequences of inducing a detachment if we are going to optimize the regenerative capacity of the retina. A critical theme that continues to return since first introduced in 1986 (Anderson et al.), is that reattachment does not simply return the retina to its pre-detachment state. Many of the changes induced by detachment such as photoreceptor cell death are permanent and reattachment will have no effect on these cells. Other changes, however, such as outer segment growth, protein distribution, rod terminal growth and glial hypertrophy *are* affected by reattachment and indicate that the retina has the ability to re-remodel during its recovery phase. This remodeling, however, does not always result in normal retinal morphology; the RPE/photoreceptor interface often appears structurally abnormal, rod axons over-grow, missing their normal targets, and Müller cells re-direct their growth from the subretinal space into the vitreous cavity often forming epiretinal membranes. Outer segment regeneration, therefore, appears to be only part of the story in regards to recovery of vision; re-establishing the RPE/photoreceptor interface, synaptic circuitry and normal expression of Müller cell proteins will most likely all be significant components of the recovery process.

The functional implication of the remodeling that occurs in retinal detachment and other retinal degenerations is not known. It's quite possible that the extent of remodeling observed after an event such as short-term detachment

does not dramatically affect vision. On the other hand, continued long-term neuronal remodeling as part of the recovery process could explain the continued change in visual acuity that has been reported to occur for years after successful reattachment surgery. Strategies to improve visual recovery after detachment may be to provide a retinal environment that optimizes photoreceptor survival and prevents Müller cell (and possibly microglial cell) activation. The strategies that have shown some success experimentally include delivering drugs intraocularly that accelerate the physical process of reattachment (Nour et al., 2003), administering trophic factors (Lewis et al., 1999a) or hyperoxia (Lewis et al., 1999b; 2004) to prevent photoreceptor cell death, or delivering pharmacological agents to prevent the activation of microglia (Thanos et al., 1995). If proven to be effective at improving recovery of vision, strategies such as these could become an important adjunct to retinal detachment surgery.

Acknowledgements: The authors would like to thank Drs. Charanjit Sethi, David Charteris and Robert Avery for providing human retinal specimens as well as for their scientific and clinical input. We would also like to thank Ken Linberg, Kevin Talaga, and Katrina Carter for their technical assistance and informative discussions.

References

D. H. Anderson, W. H. Stern, S. K. Fisher, P. A. Erickson, and G. A. Borgula, Retinal detachment in the cat: the pigment epithelial-photoreceptor interface, *Invest. Ophthalmol. Vis. Sci.* **24**, 906–926 (1983).

D. Bok, Retinal transplantation and gene therapy: Present realities and future possibilities, *Invest. Ophthalmol. Vis. Sci.* **34**, 473–6 (1993).

T.C. Burton, Recovery of visual acuity after retinal detachment involving the macula, *Trans. Am. Ophthalmol. Soc.* **80**, 475–97 (1982).

M. S. Chen, A. B. Huber, M. E. van der Haar, M. Frank, L. Schnell, A. A. Spillmann, F. Christ, and M. E. Schwab, Nogo-A is a myelin-associated neurite outgrowth inhibitor and an antigen for monoclonal antibody IN-1. *Nature* **403**, 434–439 (2000).

Y. Chu, M. F. Humphrey, and I. J. Constable, Horizontal cells of the normal and dystrophic rat retina: a wholemount study using immunolabeling for the 28 kDa calcium binding protein, *Exp. Eye Res.* **57**, 141–148 (1993).

F. E. Coblentz, M. J. Radeke, G. P. Lewis, and S. K. Fisher, Evidence that ganglion cells react to retinal detachment, *Exp. Eye Res.* **76**, 333–342 (2003).

B. Cook, G. P. Lewis, S. K. Fisher, and R. Adler, Apoptotic photoreceptor degeneration in experimental retinal detachment. *Invest. Ophthalmol. Vis. Sci.* **36**, 990–996 (1995).

E. de Juan, A. Loewenstein, N. M. Bressler, and J. Alexander, Translocation of the retina for management of subfoveal choroidal neovascularisation II: a preliminary report in humans, *Am. J. Ophthalmol.* **125**, 635–646 (1998).

M. Del Cerro, E. S. Lazar, and D. Diloreto, The first decade of continuous progress in retinal transplantation, *Micros. Res. Tech.* **36**, 130–141 (1997).

C. J. Chang, W. W. Lai, D. P. Edward, and M. O. Tso, Apoptotic photoreceptor cell death after traumatic retinal detachment in humans, *Arch Ophthalmol*. **113**, 880–886 (1995).
D. L. Emery, N. C. Royo, I. Fischer, K. E. Saatman, and T. K. Mcintosh, Plasticity following injury to the adult central nervous system: is recapitulation of a developmental state worth promoting? J. *Neurotrauma* **20**, 1271–1292 (2003).
P. Erickson, S. Fisher, D. Anderson, W. Stern, and G. Borgula, Retinal detachment in the cat: the outer nuclear and outer plexiform layers, *Invest. Ophthalmol. Vis. Sci.* **24**, 927–942 (1983).
C. Eckardt, U. Eckardt, and H. D. Conrad, Macular rotation with and without counter rotation of the globe in patients with age-related macular degeneration, *Graefe's Arch. Clin. Exp. Ophthalmol.* **237**, 313–25 (1999).
R. N. Fariss, Z. Y. Li, and A. H. Milam, Abnormalities in rod photoreceptors, amacrine cells, and horizontal cells in humans with retinitis pigmentosa, *Am. J. Ophthalmol.* **129**, 215–223 (2000).
S. K. Fisher, P. A. Erickson, G. P. Lewis, and D. H. Anderson, Intraretinal proliferation induced by retinal detachment. *Invest. Ophthalmol. Vis. Sci.* **32**, 1739–1748 (1991).
S. K. Fisher, and G. P. Lewis, Müller cell and neuronal remodeling in retinal detachment and reattachment and their potential consequences for visual recovery, *Vis. Res.* **43**, 887–897(2003).
S. K. Fisher and G. P. Lewis, Cellular effects of detachment on the neural retina and the retinal pigment epithelium, in *Retina*, edited by S. J. Ryan and C. P. Wilkinson (Mosby, St. Louis, 2004) Vol. 3. "Surgical Retina" (2005, in press).
F. H. Gage, Mammalian neural stem cells, *Science* **287**, 1433–1438 (2000).
S. F. Geller, G. P. Lewis, D. H. Anderson, and S. K. Fisher, S.K. Use of the MIB-1 antibody for detecting proliferating cells in the retina, *Invest. Ophthalmol. Vis. Sci.* **36**, 737–744 (1995).
S. F. Geller, G. P. Lewis, and S. K. Fisher, FGFR1, signaling, and AP-1 expression following retinal detachment: reactive Müller and RPE cells, *Invest. Ophthalmol. Vis. Sci.* **42**, 1363–1369 (2001).
N. Gupta, K. E. Brown, and A. H. Milam, Activated microglia in human retinitis pigmentosa, late-onset retinal degeneration, and age-related macular degeneration, Exp. Eye Res. 76, 463–471 (2003).
G. S. Hageman, M. A. Kirchoff-Rempe, G. P. Lewis, S. K. Fisher, and D. H. Anderson, Sequestration of basic fibroblast growth factor in the primate retinal interphotoreceptor matrix. *Proc. Nat. Acad. Sci.* **88**, 6706–6710 (1991).
T. Harada, C. Harada, S. Kohsaka, E. Wada, K. Yoshida, S. Ohno, H. Mamada, K. Tanaka, L. F. Parada, and K. Wada, Microglia-Müller glia cell interactions control neurotrophic factor production during light-induced retinal degeneration. J. *Neurosci.* **22**, 9228–9236 (2002).
E. C. La Heij, M. P. H. Van de Waarenburg, H. G. T. Blaauwgeers, A. G. H. Kessels, J. de Vente, A. T. A. Liem, H. Steinbusch, and F. Hendrikse, Levels of basic fibroblast growth factor, glutamine synthetase, and interleukin-6 in subretinal fluid from patients with retinal detachment, *Am. J. Ophthalmol*. **132**, 544–550 (2001).
M. F. Humphrey and S. R. Moore, Microglial responses to focal lesions of the rabbit retina: correlation with neural and macroglial reactions, *Glia* **16**, 325–341 (1996).
P. T. Johnson, R. R. Williams, K. Cusato, and B. E. Reese, Rods and cones project to the inner plexiform layer during development, J. *Comp. Neurol*. **414**, 1–12 (1999).

A. J. Kroll, and R. Machemer, Experimental retinal detachment in the owl monkey. V. Electron microscopy of reattached retina, *Am. J. Ophthalmol.* **67**, 117–130 (1969a).
A. J. Kroll, and R. Machemer, Experimental retinal detachment and reattachment in the rhesus monkey, *Am. J. Ophthalmol.* **68**, 58–77 (1969b).
S. Kusaka, A. Toshino, Y. Ohashi, and E. Sakaue, Long-term visual recovery after scleral buckling for macula-off retinal detachments, *Jap. J. Ophthalmol.* **42**, 218–222 (1998).
T. T. Lam, J. M. Kwong, and M. O. Tso, Early glial responses after acute elevated intraocular pressure in rats, *Invest. Ophthalmol. Vis. Sci.* **44**, 638–645 (2003).
P. Lamoureux, G. Ruthel, R. E. Buxbaum, and S. R. Heidemann, Mechanical tension can specify axonal fate in hippocampal neurons, J. *Cell Biol.* **159**, 499–508 (2002).
M. M. LaVail, K. Unoki, D. Yasumura, M. T. Matthes, G. D. Yancopoulos, and R. H. Steinberg, Multiple factors, cytokines, and neurotrophins rescue photoreceptors from the damaging effects of constant light, *Proc. Natl. Acad. Sci. USA* **89**, 11249–11253 (1992).
Y. B. Lee, A. Nagai, A., and S. U. Kim, Cyotkines, chemokines and cytokine receptors in human microglia, J. *Neurosci. Res.* **69**, 94–103 (2002).
S. Lewin, K. A. Drenser, W. W. Hauswirth, S. Nishikawa, D. Yasamura, J. G. Flannery, and M. M. LaVail, Ribozyme rescue of photoreceptor cells in a transgenic rat model of autosomal dominant reinitis pigmentosa. *Nature Medicine* **4**, 967–971 (1998).
G. P. Lewis, P. A. Erickson, C. J. Guerin, D. H. Anderson, and S. K. Fisher, Changes in the expression of specific Müller cell proteins during long-term retinal detachment, *Exp. Eye Res*. **49**, 93–111 (1989).
G. P. Lewis, P. A. Erickson, C. J. Guerin, D. H. Anderson, and S. K. Fisher, Basic fibroblast growth factor: A potential regulator of proliferation and intermediate filament expression in the retina, J. *Neurosci.* **12**, 3968–3978 (1992).
G. P. Lewis, C. J. Guerin, D. H. Anderson, B. Matsumoto, and S. K. Fisher, Rapid changes in the expression of glial cell proteins caused by experimental retinal detachment, *Am. J. Ophthalmol.* **118**, 368–376 (1994).
G. P. Lewis, B. Matsumoto, and S. K. Fisher, Changes in the organization of cytoskeletal proteins during retinal degeneration induced by retinal detachment *Invest. Ophthalmol. Vis. Sci.* **36**, 2404–2416, (1995).
G. P. Lewis, K. A. Linberg, and S. K. Fisher, Neurite outgrowth from bipolar and horizontal cells following experimental retinal detachment, *Invest. Ophthalmol. Vis. Sci*. **39**, 424–434 (1998).
G. P. Lewis, K. A. Linberg, S. F. Geller, C. J. Guerin, and S. K. Fisher, Effects of the neurotrophin BDNF in an experimental model of retinal detachment. *Invest. Ophthalmol. Vis. Sci.* **40**, 1530–1544 (1999a).
G. P. Lewis, K. Mervin, K. Valter, J. Maslim, P. J. Kappel, J. Stone, and S. K. Fisher, Limiting the proliferation and reactivity of retinal Müller cells during detachment: the value of oxygen supplementation, *Am. J. Ophthalmol.* **128**, 165–172 (1999b).
G. P. Lewis and S. K. Fisher, Müller cell outgrowth following experimental detachment: association with cone photoreceptors, *Invest. Ophthalmol. Vis. Sci.* **41**, 1542–1545 (2000).
G. P. Lewis, C. S. Sethi, D. G. Charteris, W. P. Leitner, K. A. Linberg, and S. K. Fisher, The ability of rapid retinal reattachment to stop or reverse the cellular and molecular events initiated by detachment, *Invest Ophthalmol. Vis. Sci.* **43**, 2412–2420 (2002).
G. P. Lewis, C. S. Sethi, K. A. Linberg, D. G. Charteris, and S. K. Fisher, Experimental retinal reattachment: A new perspective, *Molec. Neurobiol.* **28**, 159–175 (2003).

G. P. Lewis and S. K. Fisher, Upregulation of GFAP in response to retinal injury: Its potential role in glial remodeling and a comparison to vimentin expression, in *Int. Rev. Cytol. A Survey of Cell Biology,* edited by K. W. Jeon (Elsevier, Academic Press, San Diego CA, 2003), Vol. 230, pp. 263–290.

G. P. Lewis, C. S. Sethi, K. M. Carter, D. G. Charteris, and S. K. Fisher, Microglial cell activation following retinal detachment (RD): A comparison between species, *Molecular Vision,* 11,491–500 (2005).

G. P. Lewis, K. C. Talaga, K. A. Linberg, R. L. Avery, and S. K. Fisher, The efficacy of delayed oxygen therapy in the treatment of experimental retinal detachment. *Am. J. Ophthalmol.* 137,1085–1095 (2004)..

Z. Y. Li, I. J. Kljavin, and A. H. Milam, Rod photoreceptor neurite sprouting in retinitis pigmentosa, J. *Neurosci.* **15**, 5429–5438 (1995).

A. T. A. Liem, J. E. E. Keunen, G. J. Meel, and D. Norrern, Serial foveal densitometry and visual function after retinal detachment surgery with macular involvement. *Ophthalmol*. **101**, 1945–1952 (1994).

K. A. Linberg, G. P. Lewis, K. M. Carter, and S. K. Fisher, Immunocytochemical evidence that B-type horizontal cell axon terminals remodel in response to retinal detachment in the cat, *Invest. Ophthalmol. Vis. Sci.* **45**, ARVO E-Abstract 4604 (2004).

R. Machemer, Experimental retinal detachment in the owl monkey: IV, The reattached retina, *Am. J. Ophthalmol.* **66**, 1075–1091 (1968).

K. Mervin, K. Valter, J. Maslim, G. P. Lewis, S. K. Fisher, and J. Stone, J., Limiting the death and deconstruction during retinal detachment: the value of oxygen supplementation, *Am. J. Ophthalmol.* **128**, 155–164 (1999).

R. E. Marc, B. W. Jones, C. B. Watt, and E. Strettoi, Neural remodeling in retinal degeneration, in *Progress in Retinal and Eye Research,* (Pergamon Press, 2003), Vol. 22, pp. 607–655.

R. Naskar, M. Wissing, and S. Thanos, Detection of early neuron degeneration and accompanying microglial responses in the retina of a rat model of glaucoma, 43, 2962–2968 (2002).

T. F. Ng and J. W. Streilein, Light induced migration of retinal microglia into the subretinal space, *Invest. Ophthalmol. Vis. Sci.* **42**, 3301–3310 (2001).

M. Nour, A. B. Quiambao, W. M. Peterson, M. R. Al-Ubaidi, and M. I. Naash, P2Y(2) receptor agonist INS37217 enhances functional recovery after detachment caused by subretinal injection in normal and rds mice, *Invest. Ophthalmol. Vis. Sc.* **44**, 4505–4514 (2003).

S. J. Park, I. B. Kim, K. R. Choi, J. I. Moon, S. J. Oh, J. W. Chung, and M. H. Chun, Reorganization of horizontal cell processes in the developing FVB/N mouse retina, *Cell Tissue Res.* **306**, 341–346 (2001).

T. S. Rex, R. N. Fariss, G. P. Lewis, K. A. Linberg, I. Sokal, and S. K. Fisher, A survey of molecular expression by photoreceptors after experimental retinal detachment, *Invest. Ophthalmol. Vis. Sci.* **43**, 1234–1247 (2002).

R. S. Roque, C. J. Imperial, and R. B. Caldwell, Microglial cells invade the outer retina as photoreceptors degenerate in Royal College of Surgeons Rats, *Invest. Ophthalmol. Vis. Sci.* **37**, 196–203 (1996).

W. H. Ross, Visual recovery after macula-off retinal detachment, *Eye* **16**, 440–446 (2002).

V. Sarthy and H. Ripps, The Retinal Müller cell, in *Perspectives in Vision Research,* edited by C. Blakemore (Plenum Press, New York, 2001).

N. R. Saunders, A. Deal, G. W. Knott, Z. M. Varga, and J. G. Nicholls, Repair and recovery following spinal cord injury in a neonatal marsupial (*Monodelphis domestica*). *Clin. Exp. Pharmacol. Physiol.* **22**, 518–526 (1995).

C. S. Sethi, G. P. Lewis, S. K. Fisher, W. P. Leitner, D. L. Mann, P. J. Luthert, and D. G. Charteris, Glial remodeling and neuronal plasticity in human retinal detachment with proliferative vitreoretinopathy, *Invest. Ophthalmol. Vis. Sci.* **46**, 329–342 (2005).

E. Strettoi, V. Pignatelli, C. Rossi, V. Porciatti, and B. Falsini, Remodeling of second-order neurons in the retina of rd/rd mutant mice, *Vis. Res.* **43**, 867–877 (2003).

P. Tani, D. M. Robertson, and A. Langworthy, Rhegmatogenous retinal detachment without macular involvement treated with scleral buckling. *Am. J. Ophthalmol.* ***90***, 503–508 (1980).

P. Tani, D. M. Robertson, and A. Langworthy, Prognosis for central vision and anatomic reattachment in rhegmatogenous retinal detachment with macula detached. *Am. J. Ophthalmol.* **92**, 611–20 (1981).

S. Thanos, Sick photoreceptors attract activated microglia from the ganglion cell layer: a model to study the inflammatory cascades in rats with inherited retinal dystrophy, *Brain Res.* **14**, 21–28 (1992).

S. Thanos, W. Richter, and H. J. Thiel, The protective role of microglia-inhibiting factor in the retina suffering of hereditary photoreceptor degeneration, in *New strategies in prevention and therapy. Cell and tissue protection in ophthalmology,* edited by U. Pleyer, K. Schmidt, and H. J. Thiel (1995), pp. 195–203.

C. Yamamoto, N. Ogata, M. Matsushima et al., Gene expressions of basic fibroblast growth factor and its receptor in healing of rat retina after laser photocoagulation, *Jpn. J. Ophthalmol.* **40**, 480–490 (1996).

N. Zhang and E. Townes-Anderson, Regulation of structural plasticity by different channel types, *J. Neurosci*. **22**, 7065–7079 (2002).