# Injury and Repair: Retinal Remodeling

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## Key Points

- Introduce retinal remodeling as a concept.
- Describe progression of retinal remodeling as phases.
- Describe specific retinal remodeling events as well as mechanisms of remodeling.
- Discuss implications for therapeutics.

## Glossary

**glial seal** a compaction of the distal microvillar processes of mature Müller cells and their stabilization by intermediate junctions to form a barrier to diffusion and cellular movement.

**microneuromas** New, anomalous complexes of synapses by mature neurons

**migration** the ability of a cell to relocate to a new position in a tissue. It is often thought that mature neurons cannot migrate without "de-differentiating," but mature retinal neurons can migrate both intra- and extra-retinally without losing their characteristic molecular profiles.
**neuritogenesis** the activation of new dendrites and axons from neurons; typically associated with developing cells, it is vigorously demonstrated by mature neurons in remodeling retinas.
**proteinopathy** a chronic accumulation of misfolded or agglomerated proteins that occurs in cells late in retinal degeneration.
**reprogramming** a major change in the gene expression profile of a cell, typically the expression of genes not characteristic of homeostasis
**self-signaling** the ability of retinal networks to generate their own excitatory activity in the absence of photoreceptor drive.


## Abstract

Retinal remodeling is a collection of molecular and cellular revisions triggered by retinitis pigmentosa (RP), Usher syndrome, age-related macular degeneration (AMD), and likely glaucoma. These revisions include neuronal rewiring and neurotransmitter receptor reprogramming; neuritogenesis and synaptogenesis (chemical and electrical); self-signaling; neuronal migration; neuronal death; glial hypertrophy; altered glial gene expression; vascular remodeling; retinal pigmented epithelium (RPE) invasion and hyperpigmentation, and in the terminal stages, extensive proteinopathies. Metabolic and circuit revisions begin as soon as photoreceptor stress is initiated, and accelerate upon complete local photoreceptor loss. Retinal remodeling impacts the timing and potential outcomes of gene therapy, survival factor treatments, stem or progenitor cell implantation, retinal transplantation, and bionic implants.


## Introduction

Retinal remodeling is a collection of molecular and cellular revisions triggered by primary inherited degenerative diseases such as retinitis pigmentosa (RP), Usher syndrome; secondary degenerative diseases with mixed environmental/genetic risks, such as AMD and glaucoma; and acquired retinal defects such as prolonged retinal detachment and light-induced retinal damage. These revisions include anomalous neuronal rewiring (targeting canonically inappropriate cells) and reprogramming (expressing canonically inappropriate genes/proteins or repressing characteristic genes/proteins); *de novo* neuritogenesis and synaptogenesis; spontaneous and corruptive self-signaling; bipolar cell dendrite truncation; supernumerary axon generation; neuronal migration along hypertrophic Müller cell columns; neuronal death; altered glial molecular profiles; vascular remodeling; and retinal pigmented epithelium (RPE) invasion, vascular occlusion and hyperpigmentation (Gargini et al., 2007; Jones et al., 2003; Li et al., 1995; Marc et al., 2003, 2007, 2008; Pfeiffer et al., 2019, 2020). Some revisions (e.g., reprogramming) begin as soon as photoreceptor stress is initiated, while others (e.g., synaptogenesis) manifest during photoreceptor loss (Marc et al., 2007). Importantly, local survival of even heavily altered cones appears to somewhat stabilize late-stage remodeling, apparently by providing a compartment for bipolar cell dendrites. Remodeling impacts the timing and potential outcomes of gene therapy, survival factor treatments, stem or progenitor cell implantation, retinal transplantation, and bionic implants.

Remodeling was likely missed in the wider literature for many years as rodent models were the standard for retinal degeneration research. Most rodent models do not live longer than 2 years and only the most aggressive models of retinal degeneration progress into obvious histological retinal remodeling within the first year. However, most researchers did not wait long enough after degeneration of the photoreceptors to continue to examine retinal histology and assumptions were made as to the refractory nature of the neural retina post photoreceptor degeneration. It was not until the detailed imaging studies of Ann Milam (U Penn) in the mid-1990s that the nature and scope of remodeling in human RP became evident (Li et al., 1995). More recently, Enrica Strettoi (CNRS Pisa Italy) and Bryan W. Jones (U Utah) (Jones et al., 2003) independently demonstrated that remodeling was also characteristic of animal models of human inherited retinal degenerations. Though remodeling was not initially given much credence despite strong homology to central nervous system (CNS) degenerative disorders, it is now gaining understanding as a serious denouement of retinal disease, but might open the door to potentially become a new model system for understanding CNS neurodegenerative disorders.

## Retinal Remodeling

Remodeling kinetics are largely independent of the source of photoreceptor deafferentation, occurs in distinct phases, and has specific implications for retinal circuitry (**Figs. 1** and **2**). In phase 1, neuronal and glial cells react to photoreceptor stress signals prior to photoreceptor death. In phase 2, neurons, glia and microglia (Gupta et al., 2003) interact in the processes of photoreceptor death, outer nuclear layer decimation and formation of the glial seal, encapsulating the remnant retina. In phase 3, neural cells

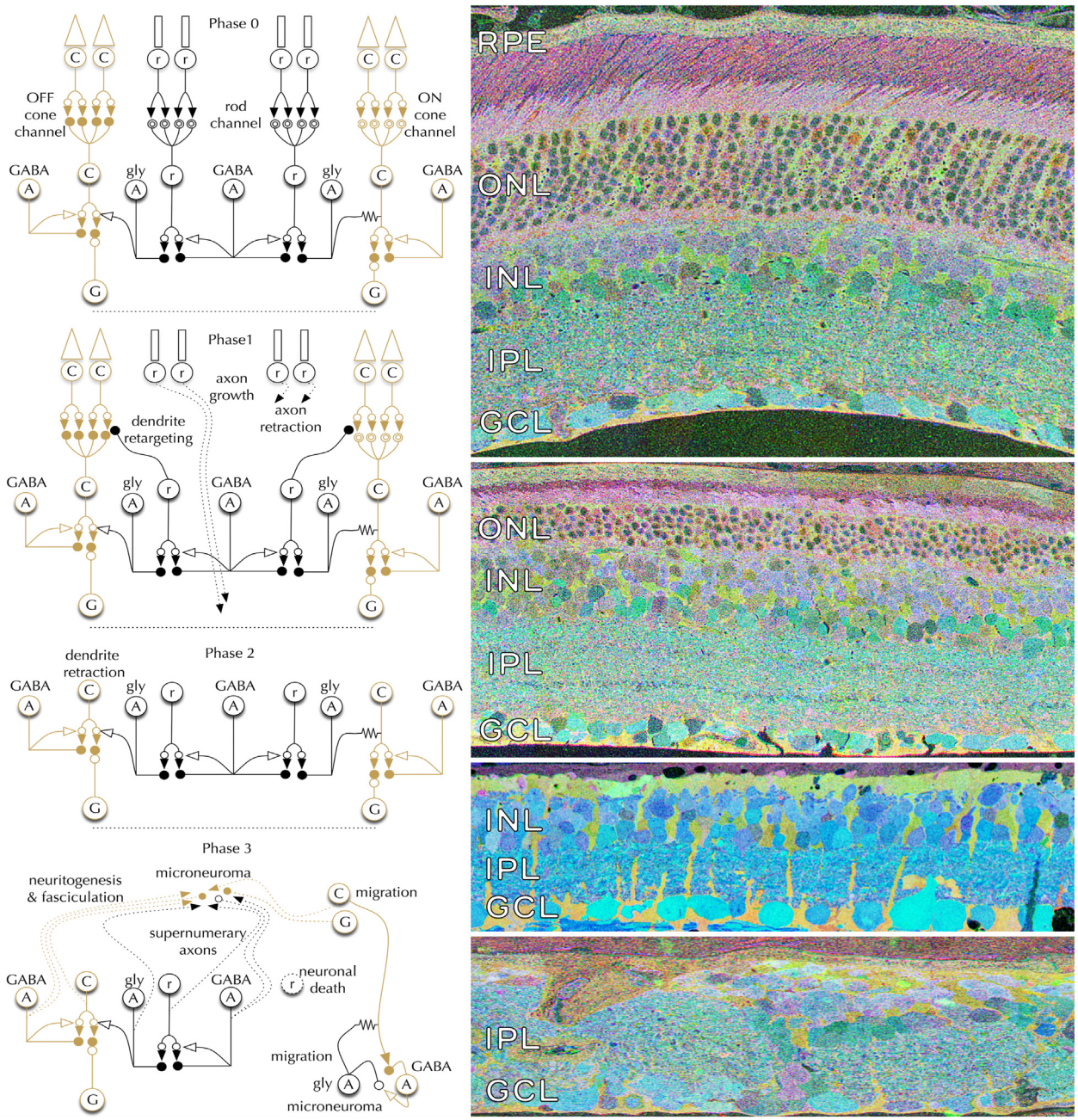


**Fig. 1** Remodeling phases in the mammalian retina. Example wiring diagram is on the left with exemplar histological sections with spatial metabolomics profiles of taurine, glutamine, glutamate assigned to red, green, and blue color channels on the right. Phase 0 is the normal retina prior to the onset of acquired or inherited defect stress. The mammalian retina uses separate cone (C) and (r) channels prior to converging on retinal ganglion cells (G). Cone bipolar cell receive cone input with either ionotropic sign-conserving glutamate receptors (solid circle) or metabotropic sign-inverting glutamate receptors (double circle), and drive amacrine cells (A) and ganglion cells which decode signals with sign-conserving glutamate receptors. Rod bipolar cells receive rod input with metabotropic sign-inverting glutamate receptors and then drive glycinergic rod amacrine cells, which fan their signals out to ON cone channels via gap junctions (resistor symbol) and OFF cone channels via glycine release decoded by inhibitory receptors (open circles).In phase 1, rod photoreceptors reprogram to either bypass rod bipolar cells with new axons or retract their synapses. Rod bipolar cells both retract dendrites and retarget some to adjacent cone pedicles. In phase 2, photoreceptors are lost and all bipolar cells lose their dendrites. In phase 3 extensive rewiring, neuritogenesis, microneuroma formation, cell migration and neuronal death occur.

respond to deafferentation and non-neural cells form new cytoarchitectures in the remnant retina. The speed of these phases depends on the nature of the degeneration (Fig. 1). Aggressive primary rod, cone-rod or cone dystrophies, as well as RPE phagocytosis defects can rapidly transit phases 1 and 2 to extensive phase 3 remodeling. Slower photoreceptor degenerations (e.g., autosomal dominant RP models of rhodopsin mutations) can lead to extended periods of cone survival, delaying the onset of phase 3.

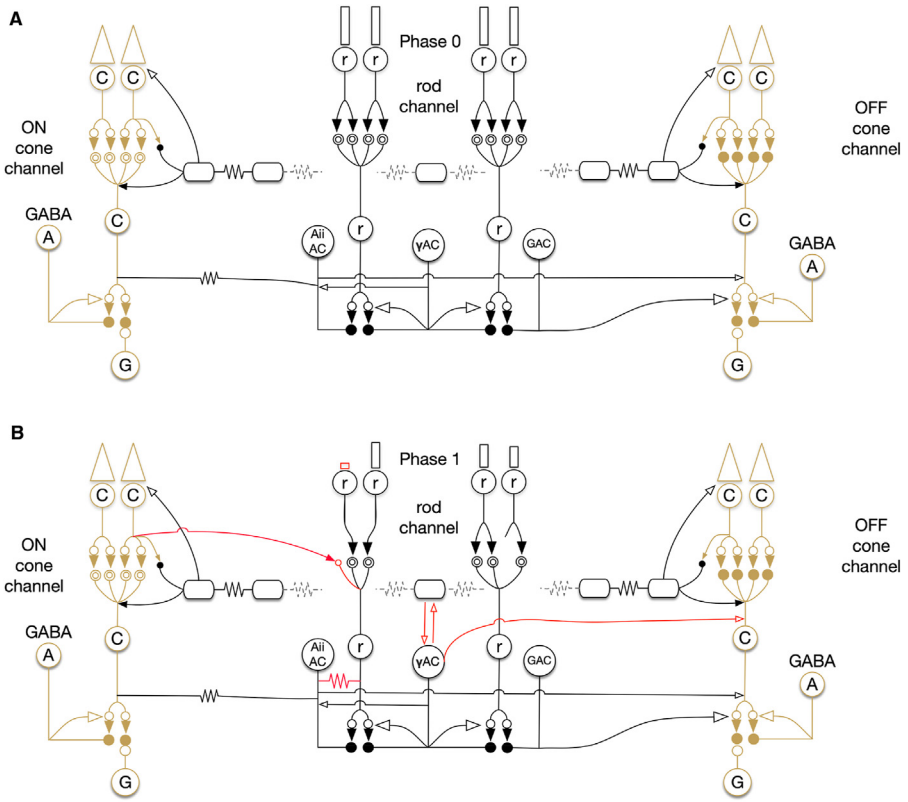


**Fig. 2** Altered network found in RPC1, an early retinal degeneration connectome where rod photoreceptors are still abundant (A and B) Phase 0 (Healthy) bipolar cell network (A) Schematic network diagram highlighting connectivity evaluated in RPC1. Solid arrows indicate glutamatergic synapses. Open arrows indicate inhibitory synapses (GABA or glycine). Solid circles indicate ionotropic glutamate receptors, while mGluR6 is indicated by the double open circles. Gap junctions are indicated by zig-zag lines. Red lines indicate aberrant connectivities observed in the RPC1 volume. (B) Phase 1 Schematic diagram of remodeled network found in RPC1. Notation is the same as used in A with the addition of red arrows highlighting the corrupted network components.

In rodent models, the faster overall kinetics are partly due to the small eye and the likely constancy of cell-cell interaction areas. Greater loss of peripheral retina is often tolerated in humans as long as the macula is spared.

## Phase 1

The first evidence that remodeling precedes photoreceptor death was the demonstration that human rods harboring a rhodopsin defect were able to form new fascicles of axons, bypass their normal bipolar cell targets and project into the ganglion cell (ganglion cell) layer prior to apoptotic stress and death. Rodent and rabbit models degenerations show the same ability as human retinas. Furthermore, rodents with autosomal recessive RP, such as the *Pdeb6*$^{rd1}$ mouse (the rd1 mouse) show truncated bipolar cell dendritic arbors and horizontal cell axonal fields long before photoreceptor death. Müller cell stress signals and protective alterations in neuronal glutamate receptor expression in light-induced retinal degeneration (LIRD) albino rodents are activated within hours of light-stress onset and long before photoreceptor death. Newer data demonstrates that more occult changes occur during phase 1 remodeling including the formation of new gap junctions between rod bipolar cells and Aii amacrine cells as well as between OFF-cone bipolar cells and Aii amacrine cells. These findings may explain the clinical sequelae of slow adaptation between photopic and scotopic environments.

## Phase 2

Degenerations transition to extensive cell-autonomous and/or bystander photoreceptor death. The nuclear layer (ONL) is dismantled with the involvement of activated microglia and hypertrophic Müller cells. The details are poorly understood and may vary according to gene defect. For example, dominant mutations that impair rhodopsin trafficking may activate endoplasmic reticulum (ER) stress in several ways: anomalous protein multimerization, inhibition of proteasome cycling, and activation of the unfolded protein response. This results in a slow dismantling of the ONL by sporadic apoptosis. Conversely, mutations of transduction pathways that trigger death calcium-dependent apoptosis are faster and more coherent. In either case photoreceptor apoptosis and migroglial-initiated bystander cytolysis may create debris zones that must be cleared. The mechanisms of such clearance are

unknown. Phase 2 ends with the entombment of the remnant neural retina by a thick seal of distal Müller cell processes similar to the normal outer limiting membrane, with extensive adherens junctions between the processes. Though often termed a glial scar, there is no evidence that the seal involves astrocyte proliferation as in CNS glial scars. There are occasional breaks in the seal that are associated with phase 3 RPE and choroidal vascular invasion of the neural retina and neuronal escape into the choroid.

### Phase 2+

In some degenerations, the death of rods is slow and seems to trigger variable bystander killing, leaving clusters of deconstructed cones with apparently functional synaptic contacts. This results in patches of retina suspended in late phase 2 (phase 2+) in a sea of phase 3 retina. The extent to which these preserve vision is unknown, but they definitely provide evidence that even marginal rescue of cones is a critical step if late phase therapies are to be viable.

### Phase 3

After loss of all photoreceptors, the stability of neuronal connectivity in the retina becomes progressively compromised via at least nine distinct processes (see below) including molecular reprogramming, individual cell rewiring, large-scale neuritogenesis, synaptogenesis and microneuroma formation, spontaneous self-signaling, neuronal migration to ectopic foci, progressive neuronal death, Müller cell remodeling and altered gene expression, as well as RPE and vascular remodeling and migration. In severe degenerations, including human late stage RP and geographic atrophy, the revision of the retina can be so severe that no visual function could ever be restored. In other cases, the neural retina survives but is likely to be so altered that upstream strategies such as subretinal implants, stem/progenitor cell transplants, or even fetal retinal transplants will likely not successfully deliver form vision. Further, there is increasing evidence that bionic implants and transplants do not stabililze phase 3 remodeling and may accelerate it.

### Phase 3+/4

Retinas continue to progressively remodel long after rod and cone photoreceptors have been lost. Topology of the inner retina continues to revise and cells continue to die. Interestingly, proliferation of proteinopathies including extensive ubiquitin deposition, synucleins, and tau-proteins begin to be expressed in the remaining neural retinal neurons while enzymatic activity in Müller glia, notably glutamine synthetase completely collapses in its expression.

### Progressive Retinal Neurodegeneration

Late phase 4 remodeling resembles progressive neurodegeneration, and may in fact represent a good model for CNS neurodegenerative diseases with all of the hallmarks associated with those diseases being represented in retina including proteinopathies, alterations in protein expression like gap junctions, and collapse of enzymatic pathways such as glutamine synthetase in Müller glia.

## Remodeling Events

### Reprogramming

Reprogramming is a shift, possibly reversible, in the gene expression of cells to anomalous molecular and anatomic states. This is best known for Müller cells in retinal stress where intermediate filament expression is elevated, but is also becoming evident for retinal neurons as well, especially in terms of glutamate receptor expression. Receptor reprogramming can begin in phase 1−2 when rods lose the ability to directly signal rod bipolar cells. In the *Pde6b*$^{rd1}$ mouse, expression, localization and function of the mGluR6 receptor essential for proper ON pathway encoding decreases. In both mouse and human RP models, rod ON bipolar cells appear to transiently up-regulate expression of ionotropic glutamate receptors (iGluRs). In the LIRD mouse model, rapid upregulation of protective GluR2 subunits occurs with 24 h after initiation of photoreceptor stress. There is also evidence of GABA receptor redistribution in ON bipolar cells of the *Pdeb6*$^{rd1}$ mouse. Further the expression of dendrites in all bipolar cells appears to be completely suppressed after rod and cone loss and supernumerary axons are formed. While the mechanisms initiating these changes are not yet known, they demonstrate that mature retinal neurons can change their architectures and receptor expressions, while glia can change their architectures and metabolic profiles. More reprogramming events are certain to be found.

### Rewiring

Rewiring is the switching of dendritic or axonal targeting. The first known instance of this was the escape of rod synaptic terminals from bipolar cell dendrites to form long axonal fascicles (Fig. 1), but with no established targets; cones behave similarly in some cases. In other forms of retinal degeneration, photoreceptors retract their synaptic terminals, which may associated with alterations in the balance of cyclic adenosine and guanosine monophosphate production. The numerous reports of bipolar cell sprouting in many model degenerations, as well as aging mice likely reflect simple extension of the dendrites still attached to retracting rod synaptic

terminals and not true plasticity. More concretely, bipolar cells bereft of rod input transiently retarget dendrites to cone terminals and, though they do not make structurally appropriate ribbon-associated synapses, they do express anomalous iGluRs. This is consistent with the disappearance of the electroretinogram b-wave despite the fact that no rod bipolar cells die early in phase 2, and with observations that ON center ganglion cell responses disappear long before those of OFF center ganglion cells.

### Neuritogenesis

Neuritogenesis is the large scale evolution of new processes by amacrine cells, bipolar cells and ganglion cells in phase 3. The mechanism of activation is unknown, but it encompasses all cell classes, suggesting a global signaling process and a pan-neural response. Many new fascicles course just distal to the glial seal and contain mixed neurites in patterns never observed in normal retina. In other regions, especially near invading RPE processes, large tracts of tiny new neurites with only one or two microtubules form homogeneous fascicles. The tracts can traverse several hundred microns, suggesting significant alteration in the spatial patterning of circuits.

### Synaptogenesis and Microneuromas

A logical extension of neuritogenesis is synaptogenesis. The extent to which new synapses are made in the inner plexiform layer of phase 3 remodeling retina is not clear, but in regions of Müller cell hypertrophy, surrounding migration columns (see below), in the ganglion cell layer and especially in the remnant distal retina, numerous new amacrine cell, bipolar cell and ganglion cell connections are made. The most distinctive zones are microneuromas, which range from 10−100 μm in width and contain abundant conventional and ribbon synapses. The mechanisms that stimulate new synapse formation are also unknown but seem closely associated with RPE processes. As RPE cells are known to release several growth factors, it is plausible that they are the activators of synaptogenesis. The wiring within microneuromas seems chaotic and, by serial section reconstruction and modeling, such networks appear to be resonant, which is incompatible with visual processing.

### Novel Gap Junction Formation

In the normal retina, ON-cone bipolar cells make gap junctions with Aii amacrine cells bridging the scotopic pathways with the photopic pathways, and expanding the range at which the visual system operates. However, in early phase 1 of retinal degeneration, rod bipolar cells and OFF-cone bipolar cells begin to make gap junctions with Aii amacrine cells in the same stratifications that they normally make ribbon synaptic connections.

### Self-Signaling

A number of observations suggest that retinal degenerations lead to spontaneous self-signaling in phase 3 retina. Photopsias (scintillating illusions common in RP) are initiated in the retina and the generator mechanisms can remain quiescent for decades in the absence of vision, only to be reactivated by experimental transocular currents. Spontaneous, erratic retinal waves of depolarization occur in rodent models of RP after light-driven responses are lost. Physiological measures show that rodent amacrine cells and ganglion cells are glutamate-activated in phase 3. ganglion cell activation in the phase 3 *Pdeb6*$^{rd1}$ mouse is clearly glutamatergic and not intrinsic. This means that bipolar cells must be voltage modulated in some way. However, most remodeled bipolar cells lack functional glutamate receptors and they must be depolarized by another mechanism. One plausible mechanism is periodic amacrine cell membrane potential fluctuations, leading to modulation of bipolar cell anion currents and modulation in bipolar cell glutamate release. Some isolated amacrine cells have shown endogenous oscillations in $K^+$ channel conductance, alternately hyperpolarizing and depolarizing them, presumably modulating GABA (or glycine) release. The isolation of amacrine cells from normal visual drive in retinal degenerations may unmask this intrinsic capacity. Once self-signaling is initiated, resonant networks created by rewiring or microneuroma formation can rapidly generate periodic activity. Such networks may be inimical to restoration. Alternatively, resonant signaling may spread via gap junction mediated mechanisms as evidence in the literature documents that blocking of gap junctions eliminates the spontaneous activity and that rod and OFF cone bipolar cells which do not normally express gap junctions with Aii amacrine cells begin forming gap junctions.

### Migration

Neuronal migration is a large-scale remodeling event. In most instances migration is closely associated with both hypertrophy of Müller cells and anomalous vascular tangles (see below). All forms of cell mixing occur: collections of amacrine cells and bipolar cells can become displaced to the ganglion cell layer. Conversely, amacrine and even ganglion cells can migrate to the distal margin of the remnant retina. One fundamental question is whether these cells remain functional. Ultrastructurally, cells in migration columns such as ganglion cells seem to have processes extending both distally and proximally much like neuroepithelial cells in development. But after migration, the original orthotopic processes seem to be retracted. Migrated cells seem heavily connected to microneuromas. A more serious form of migration (emigration) occurs in when RPE cells and the basement membrane are focally ablated and the distal seal can surge into the choroid. Large tracts of Müller cells and neurons can emigrate, decimating

the remaining neural structures. This is especially evident in LIRD and suggests that it may play a role in loss of vision in severe non-vascular forms of AMD (Marc et al., 2008) such as geographic atrophy.

### Cell Death

There is little evidence of glial death in remodeling, but the proportions and numbers of survivor neurons change significantly. bipolar cells form the largest cohort of neurons (other than photoreceptors) in normal retina, but amacrine cells always predominate in phase 3 suggesting that bipolar cell death is far more common than amacrine cell or ganglion cell death. This may be due to the fact that bipolar cells are the only retinal cells that lose all of their glutamatergic input. Neurons require a basal level of $Ca^{++}$ influx to maintain homeostatic gene expression and, via self-signaling, amacrine cells and ganglion cells clearly possess the critical glutamatergic input required to provide both transmitter-gated $Ca^{++}$ flux and voltage-gated $Ca^{++}$ channel activation. bipolar cells clearly lack that input. As subjects age, amacrine cells and ganglion cells also decrease in number. In any event, loss of neurons has strong implications for all therapeutic interventions, including epiretinal implants.

### Müller Cell Remodeling

Müller cells make up nearly 50% of the mass of the peripheral primate retina and are one of the major drivers of remodeling. While there has been little analysis of phase 1 Müller cell function in inherited retinal degenerations, there is abundant evidence that they respond to rapid, coherent photoreceptor stress initiated by LIRD within hours by increasing intermediate filament expression, displaying distal process hypertrophy in the outer nuclear layer, and increasing arginine expression (a marker of increased protein synthesis). In phase 2, Müller cells play a lead role in forming a seal between the remnant RPE and/or choroid. The transport characteristics of that seal are unknown, but its formation is paralleled by a massive increase in Müller cell glutamine levels. Müller cells normally export glutamine from Müller cells to surrounding neurons. This transport is voltage-sensitive and, like many Na-coupled transporters, allows increasing export with depolarization. Depolarization of Müller cells is closely coupled to light-activated events and the loss of photoreceptors may play some role in progressive hyperpolarization of Müller cells and retention of gluamine. But as the increase in Müller cell glutamine is temporally linked to the formation of the distal seal, the mechanism of glutamine retention appears more complex than just constraining Müller cell voltage.

### RPE Remodeling

In classical RP, invading RPE cells are one of the hallmarks of advanced disease. After formation of the Müller cell seal, certain RPE cells and sometimes choriocapillaris endothelia are able to penetrate into the neural retina, forming large complexes of hypertrophic Müller cells, RPE with altered melanosomes encapsulating invading and remnant retinal capillaries, clusters of new neurites and columns of migrating neurons. RPE cells seem to be the foci of large-scale morphologic derangements in the survivor retina, but whether they are an intiator or responder is not clear. The RPE remains partially intact for long periods in many retinal degenerations, including the Royal College of Surgeons (RCS) rat *mertk*$^{-/-}$ defect, but the RPE layer can become broken by patches of invading RPE cells and apical processes. In the RCS rat, apical REP processes can extend to the ganglion cell layer.

### Vascular Remodeling

New capillaries invade the neural retina in phase 3, emanating from both vitreal and choroidal sources. Little is known of either the fundamental transport properties of these new vessels (e.g., whether they are fenestrated or not) but both molecular and genetic profiling shows that neural retina in RP is metabolically deprived. New imaging data as well as electron microscopy suggest that the new vessels are too attenuated and too heavily invested by hypertrophic Müller cells and RPE to allow proper perfusion of the retina. These anomalous foci may trigger neuronal migration. The stimuli for vascular remodeling remain unknown, but VEGF secretion by invading RPE cells is one plausible source.

### Proteinopathy

Retinas undergoing remodeling also begin to demonstrate substantial accumulation of pathological proteins including increased ubiquitin deposition as a measure of protein degradation and accumulation found within ganglion cells initially underneath stressed photoreceptors in early retinal degeneration. By later stages in retinal degeneration, all neuronal classes begin to express ubiquitin deposition. Abnormal protein accumulations are common in retinal degenerative disease with amyloid-beta being found in AMD retinas, though they accumulate in the RPE with age. Other retinal degenerative diseases including RP are associated with an increase in alpha-synuclein (α-syn), a protein who's mutated form in common in Parkinson's disease. In early retinal degeneration, phosphorylated α-syns are found in horizontal cells along with slight increases in the inner retinal neurons. This mirrors observations that horizontal cells are among the first neurons to respond and sprout in retinal degenerative diseases. By mid-stage retinal degeneration, α-syns become uniformly increased in ganglion cells and other inner retinal neurons. Phosphorylated forms of α-syn also begin to aggregate within neurons and in aggregates representing cellular debris associated with retinal cell death.

## Impact of Remodeling on Therapeutics

The potential reversibility of remodeling events varies. For example, phase 1 and 2 changes in reprogramming of gene expression and neurite switching are reminiscent of normal plastic behavior and might respond to the appropriate therapeutic signals. However, phase 3 changes such as rewiring, neuritogenesis and microneuroma formation, migration, and neuronal cell death are not reversible by any known means. Intermediate phenomena such as Müller cell, RPE and vascular remodeling are similarly challenging. Human RP patients show the full spectrum of remodeling defects, so most therapies are impacted by a narrowing therapeutic window.

### Primary Gene Therapy

All prospective primary gene therapies (those targeting known gene defects) depend on photoreceptor survival. However, it is clear that photoreceptor deconstruction starts long before photoreceptor death. The recent successes with gene therapy for replacing deficient RPE65 in that variant of Leber Congenital Amurosis (LCA) is not likely relevant to primary rod or cone gene defects, nor to defects associated with the accumulation of genotoxic and cytotoxic debris in the subretinal space (e.g., MERTK defects). In human rod dystrophies, it is clear that mutations impacting rhodopsin trafficking also lead to changes in inner segment function and photoreceptor architecture. Rod photoreceptor rewiring in human RP is unlikely to be reversible regardless of the success in replacing defective phototransduction genes. So far, gene therapy successes in animal models are largely restricted to prenatal or early postnatal genetic interventions. With the exception of "soft" diseases such as LCA and stationary night blindnesses that don't trigger photoreceptor deconstruction, most retinal degenerations clearly have only a tiny window for gene therapy. Primary gene therapies are currently restricted to phase 1.

### Survival Factor Therapy

One strategy to retard retinal degeneration involves the use of survival factors such as neurotrophins (e.g., ciliary neurotrophic factor, CNTF) that slow photoreceptor apoptosis. These strategies were validated for slower models of adRP (the rat P23H and S344ter rhodopsin transgenic models). Some argue that the structural preservation afforded by CNTF in these models does not reflect a parallel functional rescue. There is evidence that early postnatal CNTF infusion in other rodent models of RP negatively alters photoreceptor gene expression profiles, activates Müller cell stress signaling and alters inner retinal organization. Simple survival factor therapy without a known cellular and molecular target is not likely to generalize well across human RP types. At present, survival factors offer little prospect of reversing or retarding remodeling. Survival factor therapies are restricted to phase 1 and early phase 2.

### Stem/Neuroprogenitor Cell Therapy

Several groups have shown that isolated stem/progenitor cells, particularly murine postnatal day 5 rod neuroprogenitor cells, have the potential to intercalate in the outer nuclear layer and possibly repopulate the retina. The efficiency of such intercalation is low and it is not likely these cells will survive in phase 3 retinas. Penetration of the Müller cell seal is unlikely and several groups have failed to get significant numbers of exogenous photoreceptors to extend synapses through it. Direct injection into the retina is likely to activate microglial killing and, in any event, isolates any surviving photoreceptors from the remnant RPE. For photoreceptor progenitor cells to be successful in rescuing vision they must also lead to cone survival and reconnect with existing neurons before morphologic remodeling begins. This excludes most current RP patients. Stem/neuroprogenitor cell therapies are current restricted to phase 1 and early phase 2.

### Retinal Transplantation

The team of Seiler and Aramant pioneered the effort to insert sheets of fetal retina into the subretinal space of degenerating retina. They have successfully demonstrated long-term photoreceptor survival and some visual driving in rats. Remodeling remains a major barrier in several ways. The extent of transplant-to-host neurite intermingling is small and the glial seal predominates. Further, the transplanted retina begins to remodel and appears even more susceptible to alteration than the host. This suggests that remodeling signals emanate from the survivor retina. Certainly after phase 3 bipolar cell dendrite truncation in the host retina, a semi-intact fetal retina cannot recapitulate normal circuitry by tandem connections. It is likely that most transplant-to-host connectivity is AC → AC driven and probably functionally random. Even so, such networks can generate light driven behavior. Though lacking the spatiotemporal precision normal ganglion cells, transplants have the potential for much higher sensitivity, range and resolution than bionic mechanisms. Retinal transplantation therapies are largely restricted to phases 1−2, but may function if connection can traverse the glial seal in phase 3 retinas.

### Secondary Gene Therapies: Photosensitive Proteins

A recent development of import is the ability to induce expression of photosensitive proteins in survivor neurons by viral or molecular transfection, generating light responses directly in neurons. Several groups have shown that it is possible to express channelrhodopsin 2 (ChR2) in retinal bipolar cells, and elicit ChR2-bipolar cell-driven photoresponses in ganglion cells and light-dark preferences in behavioral search. This is an important advance and represents the potential to convert blind retinas into navigational systems without surgical intervention and ancillary equipment. The challenge is to demonstrate that this is possible in phase 3 profoundly blind patients, as these are the most obvious candidates for therapy. This technique has, once again, only been established in phase 2 or earlier models. Further, as with transplantation, there is no evidence that such secondary therapies will be resistant to remodeling, prevent cell death, or overcome signal corruption. A significant amount of basic research remains to be done, but despite promise, secondary gene therapies still seem limited to phases 1−2 although they may function in phase 3, even if randomly targeted.

### Bionic Implants

The most successful schemes to restore vision to the profoundly blind from aggressive phase 3 retinal degenerations are epiretinal bionic implants. Surgically placed near the ganglion cell layer, epiretinal implants provide direct current stimulation of retinal ganglion cells or nearby circuitry to activate patches of visual sensation. The argument that such stimulation conflates ON and OFF responses seems irrelevant as several implanted patients can now successfully navigate with such devices. It is clear that the severe remodeling, especially neuronal death, limits candidacy. All implants, be they epiretinal, intraretinal or subretinal seem to induce further glial and neuronal remodeling. Severe remodeling, bipolar cell death and microneuroma formation will more severely impact subretinal models.

### The Importance of Cone Rescue

In every model, the survival of cones seems to delay the onset of phase 3, holding the retina in a phase 2+ state indefinitely. The effect is local and small patches of cones preserve connected bipolar cell dendrite structure even when surrounding bipolar cells have lost all dendrites and glutamate receptors. The preservation persists even when cones are severely deconstructed, lacking outer segments and visual pigment expression, and significantly reduced in size. This suggests that cone contact alone, perhaps through synaptic integrins, may be sufficient to preserve bipolar cell function. Expression array studies suggest that cone deconstruction may be accelerated by metabolic deprivation. While preserving cones will not rescue vision, it will permit holding the survivor retina in suspended animation, making an array of interventions such as stem/progenitor cell transplantation, fetal retinal sheet transplantation and secondary gene therapy viable for adults suffering from advanced retinal degenerations. Revisiting survival factor research with a focus on cones may be the critical advance needed for all intervention methods.

## Summary

Retinal remodeling and plasticity as a phenomenon is a universal response to neuronal deafferentation caused by photoreceptor degeneration. This is true for primary inherited retinal degenerative diseases like RP and Usher syndrome, as well as secondary retinal degenerative diseases like AMD. The changes in retina in response to photoreceptor cell loss include abnormal neuronal rewiring and neuronal reprogramming, new neurite growth from neurons, neuronal migration, cell death, altered metabolic profiles in glia, as well as vascular alterations and RPE invasion of the neural retina. As long as cone photoreceptors survive, more substantial changes to retinal circuitry and topology are held off as cone input appears to stabilize the neural retina.

Retinal remodeling occurs in phases with evidence of neural programming and wiring changes occurring prior to photoreceptor cell death. The phased revision of retina is progressive and exists on a continuum from phase 1 with changes in photoreceptors and early responses by Müller glia. Phase 2 includes photoreceptor cell death, and the beginnings of Müller cell hypertrophy and glial seal formation as well as new gap junction formation from rod and OFF cone bipolar cells to Aii amacrine cells. Phase 3 includes significant changes with neuronal sprouting, and synaptic connectivity, cellular migration, cell death in the inner retina and the deposition of pathological proteins. Late stage 3 into 4 demonstrates continued remodeling and neurodegeneration as well as significant deposition of pathological proteins including ubiquitin and synucleins.

The implications of retinal remodeling for various vision rescue therapies are substantial and understanding the biology of remodeling in the context of retinal degeneration will facilitate better outcomes for vision rescue strategies.

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