

Photoreceptor Transplantation in Late Stage Retinal Degeneration

Thomas A. Reh

Department of Biological Structure, University of Washington, Seattle, Washington, United States

Correspondence: Thomas A. Reh,
Department of Biological Structure,
University of Washington, Seattle,
WA 98195, USA;
tomreh@uw.edu.

Submitted: July 9, 2015
Accepted: July 14, 2015

Citation: Reh TA. Photoreceptor transplantation in late stage retinal degeneration. *Invest Ophthalmol Vis Sci.* 2016;57:ORSFg1-ORSFg7. DOI:10.1167/iovs.15-17659

The recent advances in cell-based therapies for the repair of the pigmented epithelium is providing additional impetus for the translation of photoreceptor transplantation to eventual clinical trials. The prospects for transplantation of photoreceptors as a potential therapy for the treatment of photoreceptor degeneration will depend on successfully addressing many critical issues in preclinical studies. Although most of the studies that have carried out transplants of photoreceptors have primarily used normal mice, there have been recent reports that have also shown some success following transplantation to mouse models of retinitis pigmentosa. However, while these results are promising, there are several key issues that require further investigation in order to better understand the optimum timing for transplantation, given the extensive remodeling of the retina that occurs in late stage disease.

Keywords: photoreceptor, retinal transplantation, remodeling

The potential for regenerative therapies for retinal disease is beginning to be realized. For diseases that involve the pigmented epithelium (RPE), for example, cell transplantation of embryonic stem cell-derived RPE cells has reached clinical trials (see other reviews in this volume). The successful development of cell-based therapies for the RPE provides additional impetus for the translation of photoreceptor transplantation to clinical trials as well. Although in principle, transplantation of photoreceptors could provide a therapy for a wide variety of inherited and acquired retinal diseases, retinitis pigmentosa (RP) has received the most attention. While RP may well be amenable to gene therapy approaches, the great diversity of different mutations that lead to RP (over 45 genes known and no single gene causing more than 10% of cases; Ref. 1 for recent review) further argues that a “one-size-fits-all” approach, such as transplantation of healthy rods, would make a good adjunct to gene therapy.² In addition, at very advanced stages of RP, rods and cones have largely degenerated, and conventional gene therapy would no longer be effective.

The prospects for transplantation of photoreceptors as a potential therapy for the treatment of photoreceptor degeneration will depend on successfully addressing many critical issues in preclinical studies. For transplantation to work in a disease like RP, there needs to be a reliable and relatively homogeneous source of the donor rod and/or cone photoreceptors.³ The host retina needs to be receptive to the transplanted cells, supportive of their migration to the outer nuclear layer (ONL; and not mislocalization to the inner retina), and the cells will need to make appropriate synaptic connections with the existing, hopefully normal, host circuit. In addition, the transplanted cells will face an existing ongoing disease process, and they need to be resistant to the negative by-stander effects of the surrounding degeneration and inflammation. These are only a few of the many considerations that need to be understood to design the best photoreceptor transplant clinical trial. In this review, these issues are considered in light of current studies and some suggestions for future research are proposed. The issue of cell source is

covered extensively in companion reviews in this Supplement; here the focus will be on the host environment. There have been quite a few reviews of the field of photoreceptor transplantation for the potential treatment of retinal degenerations, like RP, but few have focused on the issue of end-stage disease.⁴

Although most of the studies that have carried out transplants of photoreceptors have primarily used normal mice,³ there have been several more recent reports that have also shown some success following transplantation to mouse models of RP.⁵⁻⁷ Although, as will be discussed below, the results are promising, an important consideration is that RP is a very heterogeneous disease, both clinically and genetically. The age of onset and progression can be quite variable even in patients with the same mutation. Therefore, while mouse and rat models of RP have been extremely useful in understanding the cellular and molecular events associated with the onset and progression of the disease, it is important to be cautious when using them to predict outcomes from photoreceptor transplantation in humans.

Most of the known mutations that cause RP are in genes expressed by the rod photoreceptors. In addition, the loss of the rods can lead to the secondary death of the cone photoreceptors. In many cases, patients may have night blindness and tunnel vision for many years before the central vision is lost. However, in other cases, the cone loss can follow more rapidly. The degeneration of rods in RP appears to involve both caspase-dependent apoptotic mechanisms⁸ and calpain-dependent cell death pathways.⁹ The diversity of genetic defects that lead to rod death suggests a common pathway, but it is also possible that different mutations cause rod death via different mechanisms. For example, one of the more common rhodopsin mutations, Pro23His, stimulates the unfolded protein response, triggering apoptosis from persistent endoplasmic reticulum stress.¹⁰ Ultimately, as in other neural degenerations, glial cells undergo reactive changes, microglia are activated, and these and associated inflammatory processes likely contribute to the ongoing pathology.¹¹ Several hypotheses have been

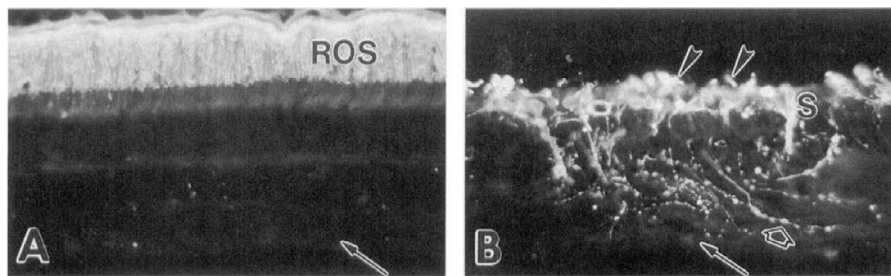


FIGURE 1. Example of rod photoreceptor sprouting in a human RP patient. (A) Normal retinal structure and rhodopsin+ rod morphology. (B) RP patient shows extensive rhodopsin+ rod neurites (arrows) penetrating to the inner retina. GCL, ganglion cell layer; INL, inner nuclear layer; OPL, outer plexiform layer. Reprinted with permission from Li ZY, Kljavin IJ, Milam AH. Rod photoreceptor neurite sprouting in retinitis pigmentosa. *J Neurosci.* 1995;15:5429-5438. © 1995 Society for Neuroscience.

advanced to explain the secondary cone loss that follows rod degeneration in RP. One of the proposed mechanisms, that rods provide a soluble factor that supports cone survival, has received support from the work of Leveillard et al.¹² The rod-derived cone viability factor (RdCVF) that the authors discovered several years ago has recently been shown to act through a receptor, Basigin-1, to stimulate glucose uptake in cones.¹³ Many of the changes in the retina that occur in end-stage RP appear to be triggered by cone degeneration (see below), and further research to prevent or delay secondary cone degeneration has important implications for rod photoreceptor transplant studies.

THE STATE OF THE RETINA IN ADVANCED RP

As noted above, the degeneration of rods occurs prior to cone degeneration in RP. For many years, it was thought that the pathology was confined to the loss of the outer retina, though one of the hallmarks of RP is the migration of the pigmented epithelial cells to associate with the retinal vasculature. In the past 30 years, a more detailed view of the changes in the inner retina has shown the striking changes that accompany outer retinal degeneration in RP. Some of the first evidence for extensive remodeling of the retina in RP came from studies by Li et al.¹⁴ beginning in the mid-90s. Using donor retinas from patients aged 24 to 91 years, they found that in regions of significant photoreceptor loss, there was a dramatic sprouting of rod axons into the inner nuclear layer, the inner plexiform layer, and even through the ganglion cell layer to the inner limiting membrane (Fig. 1). The rod neurite sprouting was most pronounced in regions of the retina where the rod cell death was the most extensive. When the rod layer was reduced to a single cell layer, for example, the neurite sprouting was exuberant. These neurites frequently had SV-2 and synaptophysin varicosities at their terminals but did not appear to be making ectopic synapses at the electron microscopic level, but rather the neurites were usually associated with Müller glial processes. Cone photoreceptors also displayed some aberrant axonal sprouting into the inner nuclear layer to the inner plexiform layer. Farris et al.¹⁵ followed up on these initial findings and further demonstrated that GABA+ amacrine cells and calbindin+ horizontal cells exhibited similar neurite sprouting. The neurites from these cells were also associated with Müller glial processes and also contacted the rod neurite sprouts. The authors caution that future attempts at retinal repair might be complicated by the remodeling of the existing circuitry, though they also were encouraged that the photoreceptor sprouting was not observed in the macula. The phenomenon of rod neurite sprouting is not observed in mouse models of RP; however, it does occur in rhodopsin transgenic pigs and cats with retinal dysplasia.

Although mouse models of photoreceptor degeneration do not show the aberrant sprouting phenomena reported in RP patients, there is evidence for extensive remodeling of the remaining cells. In the rd1 retina, for example, bipolar cell and horizontal cell dendrites begin to retract as the rods degenerate.¹⁶ More extensive changes in the inner retina occur in most mouse models of inherited photoreceptor degeneration. Marc et al.¹⁶ have found that cone loss in particular triggers Müller glial activation and hypertrophy that leads to columns of glial cells with extensive overlapping processes to form a seal at the outer limiting membrane.^{16,17} Along with the glial hypertrophy comes some inner retinal neuron death and extensive remodeling of the remaining neurons (Fig. 2). Ectopic fascicles of neurites travel through the retina, likely on the glial surfaces, and give rise to “microneuromas” composed of GABA+ and glycinergic amacrine processes with bipolar axons. The retina becomes extensively rewired, and it is likely that normal signal processing will be significantly impaired. In addition to the mouse models, the phenomenon of extensive cellular remodeling occurs in human RP retinas and in RCS and P23H rats as well. Similar changes also occur after light-induced photoreceptor degeneration in rats, and interestingly the zonal pattern of remodeling has some similarities with late-staged age-related macular degeneration. These results have led the authors to conclude that regardless of the cause of the retinal damage, if there is a significant loss of photoreceptors, and particularly cone photoreceptors, the remaining cells of the retina respond by extensive remodeling of their position and connections.

While the cause of the rod sprouting and cellular remodeling in the degenerating retina is not known, it is likely that these changes reflect changes in cell adhesion molecules and extracellular matrix, since these classes of molecules are known to be critical in cell migration and neurite growth. In addition to the changes in retinal neurons and glia, the RPE migration into the neural retina along the vasculature to produce the well-known bone-spicules characteristic of advanced RP also suggests changes in retinal extracellular matrix. Although changes in extracellular matrix (ECM) have been extensively characterized in the RPE and choroid in studies of AMD, there is much less known about the changes in retinal ECM that accompany degeneration of photoreceptors. Several matrix metalloproteases (MMPs) and their endogenous inhibitors (TIMPs) are upregulated in the rd1 mice, and in particular, MMP2 and TIMP2 are associated with Müller glial processes.¹⁷ Proteoglycans are a major class of ECM molecules in the retina, and they play many important roles in development and tissue maintenance¹⁸; chondroitin sulfate proteoglycan (CSPG) in particular increases in mouse models of photoreceptor degeneration¹⁹ and may be involved in the remodeling processes. However, in most assays, CSPG has

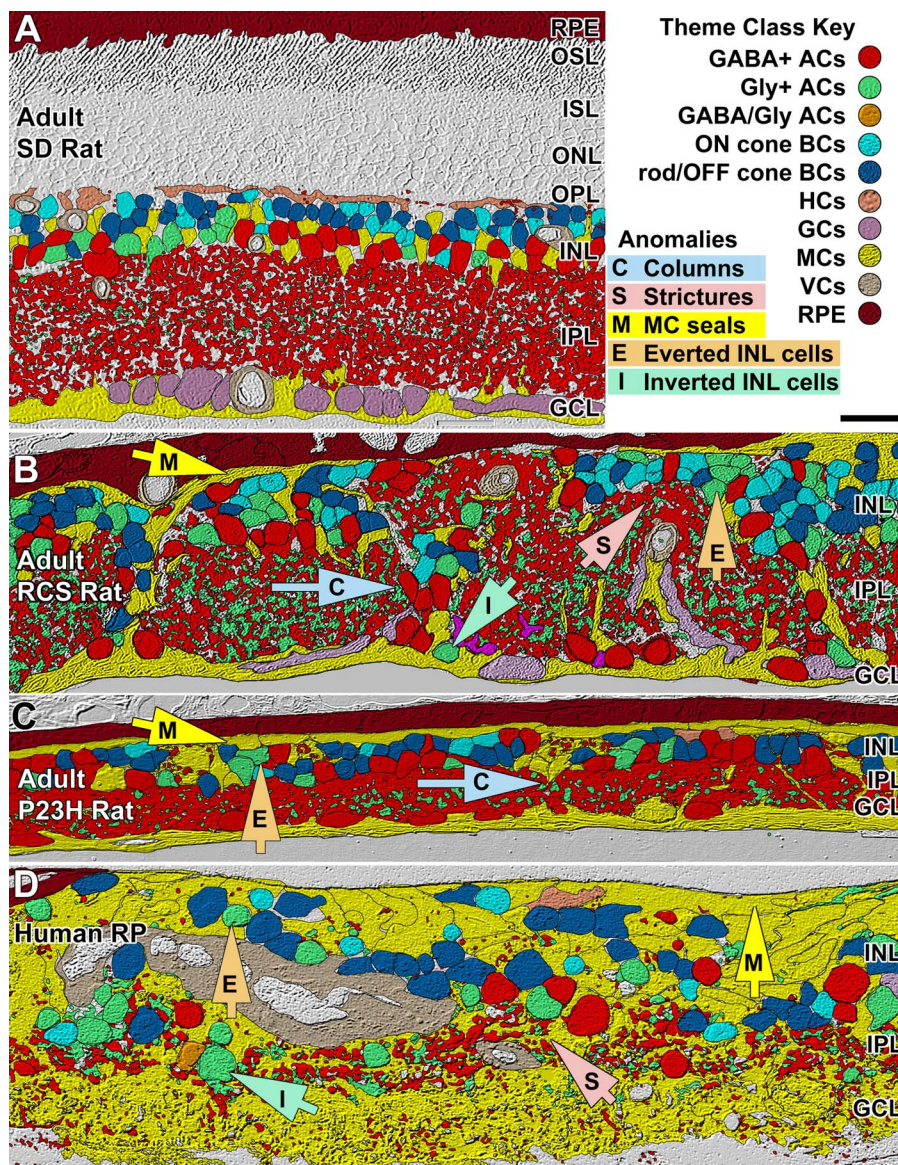


FIGURE 2. Thin sections of retina at late stages of retinal degeneration show remodeling of inner retina. (A) Normal P700 Sprague-Dawley (SD) rat, (B) P900 RCS albino rat, (C) P372 P23H line 1 transgenic rat, and (D) human RP. Remodeling disrupts lamination of the inner retina in the RCS rat, the P23H rat, and the human RP retina at late stages of degeneration. Glial hypertrophy (yellow) is also present in all cases, and the Müller processes at both the inner and outer limiting membrane are extensive. Reprinted with permission from Jones BW, Watt CB, Frederick JM, et al. Retinal remodeling triggered by photoreceptor degenerations. *J Comp Neurol.* 2003;464:1-16. © 2003 Wiley-Liss, Inc.

been shown to be inhibitory to axonal growth, and so the increases of CSPG on Müller glia would be expected to reduce neurite sprouting, not stimulate it. Additional research into the molecular changes in the retina that lead to the extensive remodeling of cells and circuits is critically needed to develop approaches to slow or prevent these changes.

The remodeling of the retina that occurs with advanced RP may in part be due to inflammatory processes that accompany neural cell loss. Microglial activation occurs in response to neural degeneration throughout the central nervous system, and while these cells can be beneficial, acting as scavengers to phagocytose the degenerating rods, they can also play a more active role in the disease pathogenesis.²⁰ Inhibition of microglial activation using minocycline, for example, significantly reduces rod apoptosis in rd10 mice,¹¹ possibly by preventing their phagocytosis of rods prior to their death. Moreover, microglia are known to regulate synapse forma-

tion,²¹ and if this mechanism is more active, it might promote the microneuroma formation observed in late stage degeneration. In principle, reducing inflammation and promoting cone survival, potentially with RdCVF or similar approaches, might prevent the extreme remodeling of the retina as RP progresses and make it more receptive for transplanted cells.

TRANSPLANTATION OF PHOTORECEPTORS

Transplantation of photoreceptors as dissociated cells or in small aggregates into the subretinal space of normal mice causes a small percentage of the transplanted cells to migrate into the host retina ONL, though the majority of the cells remain in the subretinal space.²²⁻²⁴ Photoreceptor cell bodies migrate into the ONL and send axonal processes to the outer plexiform layer to make synapses with the host bipolar cells. The incorporated cells resemble normal rods morphologically,

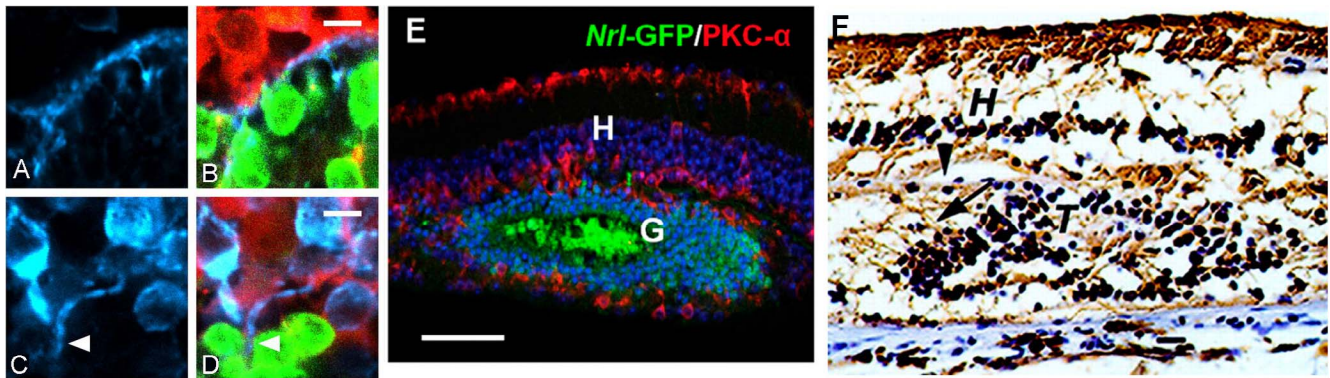


FIGURE 3. Examples of transplantation to late-stage degenerated retina. (A–D) Integration of GFP+ mouse cells transplanted to rd1 host. (A, B) Synaptophysin (blue) expression was appropriately located between host INL marked by DsRed (red) expression and the donor-derived ONL marked by GFP (green). (C, D) Host bipolar cells, marked with PKC α (blue), extended processes (arrowheads) into the graft. Scale bars: 5 μ m for A–H. Parts A–D reprinted with permission from Singh MS, Charbel Issa P, Butler R, et al. Reversal of end-stage retinal degeneration and restoration of visual function by photoreceptor transplantation. *Proc Natl Acad Sci U S A*. 2013;110:1101–1106. (E) Example of graft of a retinal structure derived from Nrl-GFP miPSCs showing structured ONL directly contacting host INL. Reprinted with permission from Assawachananont J, Mandai M, Okamoto S, et al. Transplantation of embryonic and induced pluripotent stem cell-derived 3D retinal sheets into retinal degenerative mice. *Stem Cell Reports*. 2014;2:662–674, an open access article under the CC BY-NC-ND license. © 2014 The Authors. (F) Fetal human sheet transplant to the subretinal space of a 94-year-old patient with AMD after 3 years. Graft–host interface membrane indicated by arrowheads. GFAP, Positive brown staining is present in the graft. Radial glial fibers are observed (arrow). H, host; T, transplant. Scale bar: 20 μ m. Reprinted with permission from del Cerro M, Humayun MS, Sadda SR, et al. Histologic correlation of human neural retinal transplantation. *Invest Ophthalmol Vis Sci*. 2000;41:3142–3148. © 2000 ARVO.

make outer segments, and express levels of rod-specific proteins similar to the host neighboring rods. This phenomenon occurs best when the donor rods are from neonatal mice, but fully mature, adult mouse rods are capable of the same level of migration and differentiation when transplanted.²⁵ Cone photoreceptors can also be transplanted, though the numbers of cells that integrate are low. The rods (or cones) behave similarly whether derived from mouse retina, or from embryonic stem cells or induced pluripotent cells.^{26,27} Several studies have also documented varying degrees of function from the transplanted cells.^{5,6,26,28,29}

Can Transplanted Rods Migrate Appropriately and Mature in a Degenerating Retina?

Although these studies have sparked a new interest in transplantation as a potential therapy for retinal degeneration, there are some outstanding issues that require additional investigation to determine the feasibility of the approach. For example, while many rods migrate into normal retinas, a majority of the transplanted cells remain in the subretinal space. These rods can actually undergo a great degree of differentiation, and even elongate outer segments with stacked discs³⁰; however, they also prevent the normal retina from contact with the RPE and induce apoptotic cell death in the photoreceptors subjacent to the transplant, similar to that observed in a retinal detachment. Attempts to improve rod photoreceptor migration into the ONL, by disrupting the outer limiting membrane and reducing CSPGs,^{5,31} have increased the number by 2- to 4-fold; however, even in the best cases, when 100,000 rods are transplanted, roughly 60% to 70% are more detrimental than beneficial. On the other hand, in some models of degeneration, like *Crx*^{-/-}, *Crb1*^{rd8/rd8}, and *Prph2*, the migration of transplanted rod photoreceptor cells into the ONL appears to be more extensive than that which occurs in the wild type.⁵ Nevertheless, the glial seal, characteristic of end-stage disease, is likely to form a formidable barrier to integration and leave a large fraction of the rods in the subretinal space.

Although most investigators transplant immature rods, due to their robust integration and survival properties, the immature cells will differentiate in diseased host retinas, at various stages of degeneration. For example, Homma et al.³² transplanted Nrl-GFP rods and iPSC-derived Nrl-GFP rods to *Crx*^{-/-} mice, and determined their functional maturation by directly recording electrical activity of rods in slice culture preparations of retina. The transplanted cells acquire rhodopsin 1 and HCN-1 expression within 2 weeks and respond to high-K with an increase in CA⁺². Similar results in rod differentiation after transplant to rd1 mice were observed by others.⁶

Will Transplanted Photoreceptors Make Appropriate Connections in a Degenerated Retina?

A second important consideration concerns the ability of rods to migrate into the ONL in severely degenerated retina. In an extensive study of several different mouse models of retinal degeneration, Barber et al.⁵ reported no correlation between ONL thickness and cell migration, though the different models differed substantially in the degree of migration observed. As long as there is an ONL to migrate into, the rods will do so, even if it is much thinner due to degeneration. However, when there is no ONL left, for example in the 10- to 12-week-old rd1 mice, Singh et al.⁶ found that the rods form a mass in the subretinal space. More than half of the rods are polarized toward the RPE; similarly to the cells that remain in the subretinal space in normal mice, the rods in the reconstituted ONL extend short outer segments; however, while some transplanted cells make connections with the host bipolar cells (Fig. 3), how frequently this occurs is not quantified in the study. The degree of connectivity is going to be of importance when considering the translation of these findings to end-stage RP in humans; at 2 to 3 months of age, the rd1 mice do not yet show the extensive remodeling of advanced RP, likely because they still retain some cone photoreceptors, and it remains to be determined whether photoreceptor transplantation can be successful in truly end-stage disease, where extensive glial

hypertrophy is likely to form a significant barrier to rod and cone axons.

Can Sufficient Numbers of Photoreceptors Be Transplanted to Have a Functional Benefit?

A third issue is whether sufficient photoreceptor cells can be transplanted to have a beneficial effect. Here the data are more encouraging. In the few studies that have assessed function after retinal cell transplantation, there is a good correlation between the number of photoreceptors that successfully migrate into the host retina, and the likelihood that one sees a positive response in a functional assay. Yet even a relatively small number of rods can provide a measurable response; using scotopic head tracking, Barber et al.⁵ were able to detect a difference in contrast sensitivity when 30,000 rods migrated into the retina of $Rho^{-/-}$ mice, but not when 10,000 rods migrated into the retina, even though the normal mouse retina has more than 3,000,000 rods. A similar correlation was observed in the number of integrated Nrl^{+} rods and ERG B-wave amplitude after transplantation of retinal cells derived from human ESCs into $Crx^{-/-}$ mice. Moreover, Singh et al.⁶ found functional improvement in the pupillary light response, a behavioral light avoidance test, and visually evoked cerebral cortical blood flow after transplantation of rod photoreceptors to rd1 mouse retinas, even when nearly all the cells remain in the subretinal space.

Will Transplanted Rods Survive Long-Term in a Degenerated Retina?

A fourth issue is whether the transplanted rods will survive for long periods of time in a somewhat toxic environment. Several results suggest some optimism on this point. Whereas transplants to wild type retinas show little change in the number of integrated rods over 9 months, transplanted rods in several RP mutant models, $Gnat1^{-/-}$, $Crb1^{-/-}$, and $Rho^{-/-}$, do not survive as well when analyzed 9 months after transplantation.⁵ Interestingly, two models of $Prph2$ show no decline in the number of integrated rods over time, and thus a more detailed analysis of these mutants may provide some clues as to what factors are important for extended transplant survival. Both Barber et al.⁵ and Singh et al.⁶ transplanted rods to the rd1 retina, but rod integration and/or survival was only followed for 3 weeks in Barber et al.,⁵ so it is difficult to compare with the other mutants. Singh et al.⁶ transplanted cells after nearly all host rods had degenerated, and the authors analyzed the retinas for transplanted cells up to 80 days later. Within about 3 weeks after the transplant, about half the mice no longer had detectable transplanted rods. However, approximately 33% of the mice still had surviving cells after 12 weeks following the transplantation, though the number of cells was not quantified. None of the behavioral analyses were carried out with the long-term surviving animals; in Barber et al.,⁵ the $Rho^{-/-}$ mice were analyzed 3 to 4 weeks after transplantation, and in Singh et al.,⁶ 2 weeks after transplantation. Similarly, $Crx^{-/-}$ were analyzed for the presence of an ERG within 3 weeks of transplant by Lamba et al.²⁵ Thus, it remains an open question whether rod transplantation can restore visual responses in a sustainable way, but at least for some mutant models, like $Prph2$, the data are encouraging that the number of transplanted rods does not appear to decline over many months.

Will the Rods Help to Keep the Cones Alive?

As noted above, there is evidence for an interaction between rods and cones. Specifically, the rods are known to secrete a soluble factor, RdCVF, which promotes cone glucose uptake

via stimulation of the Basigin receptor. In principle, then, the transplantation of rods prior to substantial cone degeneration could promote their survival and function, and this could be an additional benefit to rod transplantation in RP. Mohand-Said et al.³³ demonstrated that sufficient number of rods could indeed be transplanted into the subretinal space of rd1 mice at a time when 30% of the cones had already degenerated (5 weeks) and a small, but significant, positive effect on cone survival was observed 2 weeks later. However, more recently, Singh et al.⁶ did not find such a benefit from rod transplantation into the rd1 mouse. They compared host cone morphology and L/M opsin levels (quantified from retinal sections through the entire retina) 2 weeks after transplantation and found no differences between the transplant and sham operated mice. This difference may have to do with differences in the timing of the transplant, since the earlier study did their transplants at 7 weeks, while Singh et al.⁶ used 10- to 12-week-old mice; perhaps when the degeneration of the cones has advanced too far, transplanted rods can no longer prevent their continued loss.

Will Rods Transplanted Earlier in the Disease Progression Slow the Course of the Degeneration?

Another potential benefit from rod transplantation into degenerating retina might be that the healthy young rods might slow the degeneration of the diseased host rods. Many of the proposed mechanisms of rod degeneration in RP could be non-cell-autonomous. If this is the case, a sufficient number of donor rods could slow the overall rate of loss in those rods that still remain in the host at the time of the transplant. At the present, there is not much evidence for this possibility. However, this may be due in part to the fact that the transplantation procedure itself leads to some retinal damage in small mouse eyes, and particularly in regions where large numbers of rods remain in the subretinal space, there is usually an increase in rod loss subjacent to the transplant even in normal mice. Thus, for these technical difficulties, there are no studies that have shown transplants of rods can reduce or prevent host rod loss. This is in contrast to the large number of studies showing that many different cell types transplanted to the subretinal space of RCS rats can slow rod loss. It is not clear what underlies this difference in the response of the rat and the mouse, but additional studies, potentially using other rod degeneration models in rats might shed some light on this puzzle. Nevertheless, it may be that a very large number of rods would need to be transplanted to prevent host rod degeneration; chimeric mice with roughly 50% wild type rods and 50% mutant rods still undergo rod degeneration of both mutant and wild type rods, though the rate for both is slower than if the retina were composed only of mutant cells.³⁴

What About Retinal Sheet Transplants?

As discussed in the preceding section, once the ONL has degenerated, it may be difficult for the dissociated cells to reconstitute a new layer. This has led some investigators to propose "retinal sheet" transplants. Fetal retinal sheets have been used in studies dating over 30 years.³⁵ Transplants of mouse, rat, and even embryonic human retina have been made into experimental animals.³⁶ In addition, embryonic human retinal sheets have been transplanted to RP patients and shown to provide some visual improvement.³⁷ In the animal models, the subretinal transplanted retinal sheets develop quite well and can form normal laminated structures; however, while connections can be observed between the graft and host, and some functional improvement can be demonstrated,³⁸⁻⁴⁰ in many cases the transplanted retina and the host are not well

integrated. In one noteworthy case, a 94-year-old man with AMD received a fetal human retinal transplant died of unrelated causes 3 years after the transplantation.⁴¹ Analysis of his eye showed that the transplanted cells remained in the subretinal space without immunosuppression. While the transplanted retina was clearly separate from the host, and there was evidence of a glial barrier between the host and graft, there were some regions of closer apposition and potential cellular integration (Fig. 3).

With the development of methods to produce laminated retinal structures *in vitro*,⁴²⁻⁴⁴ there is a renewed interest in the potential of using some type of retinal sheet transplant for RP. Assawachananont et al.⁷ derived laminated structured retina tissue from Rx-GFP ESCs and allowed them to differentiate for up to 3 weeks *in vitro* in the presence of retinoic acid and taurine to encourage rod photoreceptor development. They then transplanted 2- to 3-week-old retinal sheets into the subretinal space of 6- to 8-week-old rd1 mice and analyzed them after 2 weeks to 3 months. The transplanted ESC-derived retinal sheets differentiated into laminated, quite mature retina in some cases, particularly when retinal sheet cultures that were less than 3 weeks old were transplanted. Transplants from older cultures led to less well-organized sheets, and in some cases they found primarily clusters of photoreceptors remaining from these transplants. Retina from embryonic stages tends to form rosettes in the subretinal space, when transplanted either as dissociated cells or as sheets, and so the older cultures might have some advantages; however, there is as yet no functional evidence that the retinal structures derived from ESCs or iPSCs will restore light responses or behavioral improvement to the rd1 retina.

CONCLUSIONS AND FUTURE DIRECTIONS

The further development of the photoreceptor transplant field will depend on many considerations, including the best cell source, the state of the donor photoreceptor at the time of transplantation, and the production of enriched or purified rods and cones. In addition, future developments in imaging technology should provide the means to better characterize the state of the retina at the time of transplantation. The studies reviewed above suggest that there are many critical variables that will need to be considered to design clinical trials for photoreceptor transplantation that will have the best chance of success. The potential for transplanted rods to provide a survival benefit to the remaining host rods and cones is still possible, but the few studies that have addressed this issue have not consistently supported the possibility. By contrast, the evidence that photoreceptor transplant can restore some light responses and visual behavioral responses, even to late stage degenerated retina, is promising. Sheet transplants or dissociated cells behave somewhat similarly in the subretinal space of late staged degenerated retina; in both cases, they remain in the subretinal space and at least some of the photoreceptors can be appropriately directed toward the pigmented epithelium. In both types of transplants, there is some evidence for synaptic connections between the host and graft, though methods to improve the degree of cellular integration are warranted. Results from the few human patients who have been followed after fetal retinal transplants suggests that immunosuppression may not be necessary for photoreceptor transplants, and that long-term survival of cells in the subretinal space can occur in humans. The degree to which the Müller glial hypertrophy and retinal reorganization that occurs following cone degeneration contributes to the very limited visual improvement observed in the human patients who have received transplants needs further evalua-

tion. Whether photoreceptor transplants can prevent inner retinal remodeling remains a critical question for this field, and likely the future of visual improvement from photoreceptor transplantation in end-stage RP depends on the answer.

Acknowledgments

The author thanks Deepak Lamba for his thoughts and Olivia Bermingham-McDonogh for her generally insightful criticisms.

Supported by Foundation Fighting Blindness, National Eye Institute (NIH), and National Institute of General Medical Sciences (NIGMS).

Disclosure: **T.A. Reh**, Acucela (R)

References

- Wert KJ, Lin JH, Tsang SH. General pathophysiology in retinal degeneration. *Dev Ophthalmol*. 2014;53:33-43.
- Rossmiller B, Mao H, Lewin AS. Gene therapy in animal models of autosomal dominant retinitis pigmentosa. *Mol Vis*. 2012;18:2479-2496.
- Jayakody SA, Gonzalez-Cordero A, Ali RR, Pearson RA. Cellular strategies for retinal repair by photoreceptor replacement. *Prog Retin Eye Res*. 2015;46:31-66.
- Pearson RA, Hippert C, Graca AB, Barber AC. Photoreceptor replacement therapy: challenges presented by the diseased recipient retinal environment. *Vis Neurosci*. 2014;31:333-344.
- Barber AC, Hippert C, Duran Y, et al. Repair of the degenerate retina by photoreceptor transplantation. *Proc Natl Acad Sci U S A*. 2013;110:354-359.
- Singh MS, Charbel Issa P, Butler R, et al. Reversal of end-stage retinal degeneration and restoration of visual function by photoreceptor transplantation. *Proc Natl Acad Sci U S A*. 2013;110:1101-1106.
- Assawachananont J, Mandai M, Okamoto S, et al. Transplantation of embryonic and induced pluripotent stem cell-derived 3D retinal sheets into retinal degenerative mice. *Stem Cell Reports*. 2014;2:662-674.
- Cottet S, Schorderet DF. Mechanisms of apoptosis in retinitis pigmentosa. *Curr Mol Med*. 2009;9:375-383.
- Sancho-Pelluz J, Arango-Gonzalez B, Kustermann S, et al. Photoreceptor cell death mechanisms in inherited retinal degeneration. *Mol Neurobiol*. 2008;38:253-269.
- Chiang WC, Kroeger H, Sakami S, et al. Robust endoplasmic reticulum-associated degradation of rhodopsin precedes retinal degeneration. *Mol Neurobiol*. 2014;52:679-695.
- Peng B, Xiao J, Wang K, So KF, Tipoe GL, Lin B. Suppression of microglial activation is neuroprotective in a mouse model of human retinitis pigmentosa. *J Neurosci*. 2014;34:8139-8150.
- Leveillard T, Mohand-Said S, Lorentz O, et al. Identification and characterization of rod-derived cone viability factor. *Nat Genet*. 2004;36:755-759.
- Ait-Ali N, Fridlich R, Millet-Puel G, et al. Rod-derived cone viability factor promotes cone survival by stimulating aerobic glycolysis. *Cell*. 2015;161:817-832.
- Li ZY, Kljavin IJ, Milam AH. Rod photoreceptor neurite sprouting in retinitis pigmentosa. *J Neurosci*. 1995;15:5429-5438.
- Fariss RN, Li ZY, Milam AH. Abnormalities in rod photoreceptors, amacrine cells, and horizontal cells in human retinas with retinitis pigmentosa. *Am J Ophthalmol*. 2000;129(2):215-223.
- Marc RE, Jones BW, Watt CB, Strettoi E. Neural remodeling in retinal degeneration. *Prog Retin Eye Res*. 2003;22:607-655.
- Jones BW, Watt CB, Frederick JM, et al. Retinal remodeling triggered by photoreceptor degenerations. *J Comp Neurol*. 2003;464:1-16.

18. Inatani M, Tanihara H. Proteoglycans in retina. *Prog Retin Eye Res.* 2002;21:429-447.
19. Hippert C, Graca AB, Barber AC, et al. Müller glia activation in response to inherited retinal degeneration is highly varied and disease-specific. *PLoS One.* 2015;10:e0120415.
20. Brown GC, Neher JJ. Microglial phagocytosis of live neurons. *Nat Rev Neurosci.* 2014;15:209-216.
21. Bilimoria PM, Stevens B. Microglia function during brain development: new insights from animal models. *Brain Res.* 2015;1617:7-17.
22. MacLaren RE, Pearson RA, MacLaren RE, et al. Retinal repair by transplantation of photoreceptor precursors. *Nature.* 2006;444:203-207.
23. West EL, Pearson RA, MacLaren RE, Sowden JC, Ali RR. Cell transplantation strategies for retinal repair. *Prog Brain Res.* 2009;175:3-21.
24. Bartsch U, Oriyakhel W, Kenna PF, et al. Retinal cells integrate into the outer nuclear layer and differentiate into mature photoreceptors after subretinal transplantation into adult mice. *Exp Eye Res.* 2008;86:691-700.
25. Gust J, Reh TA. Adult donor rod photoreceptors integrate into the mature mouse retina. *Invest Ophthalmol Vis Sci.* 2011;52:5266-5272.
26. Lamba DA, Gust J, Reh TA. Transplantation of human embryonic stem cell-derived photoreceptors restores some visual function in Crx-deficient mice. *Cell Stem Cell.* 2009;4:73-79.
27. Gonzalez-Cordero A, West EL, Pearson RA, et al. Photoreceptor precursors derived from three-dimensional embryonic stem cell cultures integrate and mature within adult degenerate retina. *Nat Biotechnol.* 2013;31:741-747.
28. Santos-Ferreira T, Postel K, Stutzki H, et al. Daylight vision repair by cell transplantation. *Stem Cells.* 2015;33:79-90.
29. Pearson RA, Barber AC, Rizzi M, et al. Restoration of vision after transplantation of photoreceptors. *Nature.* 2012;485:99-103.
30. Eberle D, Kurth T, Santos-Ferreira T, Wilson J, Corbeil D, Ader M. Outer segment formation of transplanted photoreceptor precursor cells. *PLoS One.* 2012;7:e46305.
31. Suzuki T, Akimoto M, Imai H, et al. Chondroitinase ABC treatment enhances synaptogenesis between transplant and host neurons in model of retinal degeneration. *Cell Transplant.* 2007;16:493-503.
32. Homma K, Okamoto S, Mandai M, et al. Developing rods transplanted into the degenerating retina of Crx-knockout mice exhibit neural activity similar to native photoreceptors. *Stem Cells.* 2013;31:1149-1159.
33. Mohand-Said S, Kicks D, Simonutti M, et al. Photoreceptor transplants increase host cone survival in the retinal degeneration (rd) mouse. *Ophthalmic Res.* 1997;29:290-297.
34. Huang PC, Gaitan AE, Hao Y, Petters RM, Wong F. Cellular interactions implicated in the mechanism of photoreceptor degeneration in transgenic mice expressing a mutant rhodopsin gene. *Proc Natl Acad Sci U S A.* 1993;90:8484-8488.
35. Aramant RB, Seiler MJ. Progress in retinal sheet transplantation. *Prog Retin Eye Res.* 2004;23:475-494.
36. Seiler MJ, Araman RB. Cell replacement and visual restoration by retinal sheet transplants. *Prog Retin Eye Res.* 2012;31:661-687.
37. Radtke ND, Aramant RB, Seiler M, Petry HM. Preliminary report: indications of improved visual function after retinal sheet transplantation in retinitis pigmentosa patients. *Am J Ophthalmol.* 1999;128:384-387.
38. Seiler MJ, Aramant RB, Seeliger MW, Bragadottir R, Mahoney M, Narfstrom K. Functional and structural assessment of retinal sheet allograft transplantation in feline hereditary retinal degeneration. *Vet Ophthalmol.* 2009;12:158-169.
39. Peng Q, Thomas BB, Aramant RB, Chen Z, Sadda SR, Seiler MJ. Structure and function of embryonic rat retinal sheet transplants. *Curr Eye Res.* 2007;32:781-789.
40. Thomas BB, Seiler MJ, Sadda SR, Aramant RB. Superior colliculus responses to light: preserved by transplantation in a slow degeneration rat model. *Exp Eye Res.* 2004;79:29-39.
41. del Cerro M, Humayun MS, Sadda SR, et al. Histologic correlation of human neural retinal transplantation. *Invest Ophthalmol Vis Sci.* 2000;41:3142-3148.
42. Lamba DA, Reh TA. Microarray characterization of human embryonic stem cell-derived retinal cultures. *Invest Ophthalmol Vis Sci.* 2011;52:4897-4906.
43. Phillips MJ, Wallace KA, Dickerson SJ, et al. Blood-derived human iPS cells generate optic vesicle-like structures with the capacity to form retinal laminae and develop synapses. *Invest Ophthalmol Vis Sci.* 2012;53:2007-2019.
44. Nakano T, Ando S, Takata N, et al. Self-formation of optic cups and storable stratified neural retina from human ESCs. *Cell Stem Cell.* 2012;10:771-785.