Repair of the degenerate retina by photoreceptor transplantation

Amanda C. Barbera, Claire Hipperta, Yanai Durana, Emma L. Westa, James W. B. Bainbridge, Katherine Warre-Cornisha, Ulrich F. O. Luhmann, Jorn Lakowskib, Jane C. Sowden, Robin R. Alia,c,1, and Rachael A. Pearsonb,1

aDepartment of Genetics, University College London Institute of Ophthalmology, London EC1V 9EL, United Kingdom; and bDevelopmental Biology Unit and Molecular Immunology Unit, University College London Institute of Child Health, London WC1N 1EH, United Kingdom

Edited by Eric A. Pierce, Massachusetts Eye and Ear Infirmary, Boston, MA, and accepted by the Editorial Board November 14, 2012 (received for review August 6, 2012)

Despite different aetiologies, age-related macular degeneration and most inherited retinal disorders culminate in the same final common pathway, the loss of photoreceptors. There are few treatments and none reverse the loss of vision. Photoreceptor replacement by transplantation is proposed as a broad treatment strategy applicable to all degenerations. Recently, we demonstrated restoration of vision following rod-photoreceptor transplantation into a mouse model of stationary night-blindness, raising the critical question of whether photoreceptor replacement is equally effective in different types and stages of degeneration. We present a comprehensive assessment of rod-photoreceptor transplantation across six murine models of inherited photoreceptor degeneration. Transplantation is feasible in all models examined but disease type has a major impact on outcome, as assessed both by the morphology and number of integrated rod-photoreceptors. Integration can increase (Prph2+/+Δβ07), decrease (Crb1fl/fl, Gnat1−/−, Rhino−/−), or remain constant (PDE6β+/++/−, Prph2fl/fl), with disease progression, depending upon the gene defect, with no correlation with severity. Robust integration is possible even in late-stage disease. Glial scarring and outer limiting membrane integrity, features that change with degeneration, significantly affect transplanted photoreceptor integration. Combined breakdown of these barriers markedly increases integration in a model with an intact outer limiting membrane, strong gliotic response, and otherwise poor transplantation outcome (Rhino−/−), leading to an eightfold increase in integration and restoration of visual function. Thus, it is possible to achieve robust integration across a broad range of inherited retinopathies. Moreover, transplantation outcome can be improved by administering appropriate, tailored manipulations of the recipient environment.

Results

We chose six clinically relevant murine models of inherited retinal disease that represent a range of degeneration speeds: four models of Retinitis pigmentosa (RP) (Prph2+/+Δβ07, Prph2+/−Δβ07, Prph2−/−Δβ07, PDE6β+/++/−), a model of Lebers congenital amaurosis (Crb1fl/fl), and a model of stationary night-blindness (Gnat1−/−). Each model undergoes progressive loss of photoreceptors over a period ranging from ~10% loss over 12 mo (Gnat1−/−) to near complete loss of rods within 3 wk (PDE6β+/−) (Table S1).


The authors declare no conflict of interest.

This article is a PNAS Direct Submission. E.A.P. is a guest editor invited by the Editorial Board. Freely available online through the PNAS open access option.

1To whom correspondence may be addressed. E-mail: rachael.pearson@ucl.ac.uk or r.ali@ucl.ac.uk.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1212677110/-/DCSupplemental.
Transplanted Rod-Photoreceptors Integrate with Different Efficiency in Different Models of RP. We first examined the number of transplanted Nrl.GFP−/− rod-photoreceptor precursors integrating into each of the models at a time when the recipient retina is mature (6–8 wk) and compared those to age-matched wild-type controls. At 3 wk posttransplantation, integration into adult Gnat1−/−, Prph2rd2/rd2, and PDE6βfl/fl recipients was similar to wild-type (Fig. 1A; left axis, gray box plot). Conversely, integration was significantly higher in the Crb1rd8/rd8 and significantly lower into the Rhodopsin−/− mouse. The differences in disease severity mean that at 6–8 wk of age the recipients were at very different stages of their degeneration (Fig. 1B, right axis, black plots, and Table S1), yet we found that even models at mid- (Crb1rd8/rd8, Prph2rd2/rd2) and late- (PDE6βfl/fl) stage degeneration showed levels of integration comparable to wild-type.

Ability of Transplanted Rod-Photoreceptors to Assume Normal Morphology Is Significantly Affected by the Recipient Environment. Photoreceptor survival and function are critically dependent upon the correct formation and maintenance of synapses and inner/outer segments; both are prerequisites for effective photoreceptor transplantation therapy. The ability of endogenous rods to elaborate segments differs dramatically (Fig. S1); those in the Gnat1−/− mouse form long segments similar to wild-type, but those formed by rods in the PDE6βfl/fl models, if present, are extremely short. Such structural pathologies are likely to present very different recipient environments that may affect the maturation of transplanted cells. We therefore examined the ability of transplanted rod precursors to form segments and synapses within the different degenerating retinæ (Fig. 2 and Table S2).

![Fig. 1. Photoreceptor integration is dependent upon recipient disease type.](A, Left axis, box plots) number of integrated rods 3 wk after transplantation into 6- to 8-wk-old models of retinal degeneration, compared with wild-type controls. n = number of eyes. Black bars: statistical significance (ANOVA with Tukey's correction). Right axis (black dots), recipient ONL thickness at 6–8 wk (n = 3 per model). Black asterisks: statistical significance. (B) Representative images of integrated cells in each model. (Scale bar, 25 μm.) Dotted line denotes boundary of ONL/INL, dashed line denotes boundary of ONL. ONL, outer nuclear layer; INL, inner nuclear layer.

We assessed both the number of integrated rods with segments and their morphological quality (Fig. 2A). In all models we found the integrated wild-type rods showed correct expression of the protein missing in the disease model (Fig. 2C). However, overall, the morphology and frequency of segment formation by integrated rods correlated with the ability of the endogenous donor photoreceptors to form segments. Over 70% of rods integrated within the ONL of wild-type, Gnat1−/−, and Crb1rd8/rd8 recipients developed segments and adopted typical rod-like morphologies with long segments (Fig. 2C, i–iii) like those of the endogenous rods in these models. In contrast, only a fifth of integrated rods found within the Rhodopsin−/− recipient ONL developed segments (Fig. 2A) and these were short (Fig. 2C, vii). At 3 wk of age, the ONL of the PDE6βfl/fl retina is reduced to a single layer of cones; despite this, significant numbers of rods were found within the remaining ONL. Gross morphology was markedly different to normal rods, with enlarged cell bodies and multiple processes (Fig. 2C, viii), but some developed projections oriented toward the retinal pigment epithelium that colocalized with β-phosphodiesterase (PDE) (Fig. 2A–C, viii), indicative of photoreceptor segment morphogenesis. Comparison in Figure 3 between wild-type, Prph2rd2/rd2, and PDE6βfl/fl models shows that those formed by cells transplanted into Pph2rd2/rd2 recipients were shorter than those formed in wild-type recipients.

Spindle presynaptic-like structures, typical of rod photoreceptors, were formed by over 65% of integrated rods in wild-type, Gnat1−/−, and Crb1rd8/rd8 recipients (Fig. 2B and D, i–iii). Significantly fewer presynaptic-like structures were observed following transplantation in Pph2rd2/rd2, and Rhodopsin−/− recipients (Fig. 2B and D, iv and vi). Most severely affected were cells transplanted into PDE6βfl/fl recipients, where only intact integrated rods possessed processes that terminated in bouton-like structures (Fig. 2B and D, vi). A qualitative assessment of all models indicated that these structures typically colocalized with the ribbon synapse protein RIBEYE (Fig. 2D). Thus, the cytoarchitecture of the recipient retina influences the ability of transplanted rod precursors to assume mature rod-photoreceptor morphotype, although all environments tested were able to support segment and synapse formation to some degree.

Disease Progression Has a Major Impact on Transplanted Photoreceptor Integration Efficiency. We next sought to determine how disease progression affects transplanted rod precursor integration and how long the degenerative recipient retina remains permissive to transplantation. Cells were transplanted into each model at three stages of degeneration: early, mid, and late (Fig. 3A and Table S1). The number of transplanted rod precursors integrating into wild-type recipients remained constant across all timepoints examined (Fig. 3A, i). Conversely, integration efficiency decreased in the Gnat1−/− model as disease progressed (Fig. 3A, ii) and was already markedly lower in the Rhodopsin−/− model than in any other model and continued to decline steeply over time (Fig. 3A, iv). Integration into the Crb1rd8/rd8 mouse presented a bimodal pattern, first increasing then decreasing sharply when transplanted into late-stage recipients (Fig. 3A, iii). Unexpectedly, integration significantly increased with disease in the Pph2rd2/rd2 model (Fig. 3A, iv) and remained constant in Pph2rd2/rd2 and PDE6βfl/fl, despite significant endogenous photoreceptor loss. Thus, very different trends in integration were observed in the different models of retinal degeneration as disease progressed (Fig. 3A, blue lines). These data suggest that the recipient microenvironment plays a major role in determining photoreceptor transplantation success and that different factors may be important in each model. We next examined aspects of the microenvironment of each of the disease models, specifically disease severity, OLM integrity, and glial scarring, to try to identify factors that could account for the differences observed in integration efficiency.

Barber et al.

PNAS | January 2, 2013 | vol. 110 | no. 1 | 355
OVL Integrity and Glial Scarring Affect Photoreceptor Transplantation Success. It has been reported that both OVL integrity (10, 15) and glial scarring (13), particularly CSPG deposition (11, 14, 19), can affect transplantation into the retina. We analyzed changes in both factors between early- and late-stage degeneration in each of the models using immunohistochemistry (Fig. 3 B and C), Western blot (Fig. S1 B and C), and ultrastructural analysis (Fig. S1 B and C) to ascertain if either factor influences the ability of transplanted photoreceptors to integrate.

In wild-type recipients, integration remained constant with age (Fig. 3 A, i and D, i, black trend line). As expected in the absence of degeneration, no glial scarring was observed (Fig. 3 B, i and D, i, green trend line, and Fig. S1 A, i); GFAP expression was minimal and CSPGs were sparsely distributed throughout the IPM at all stages examined (Fig. 3 B, i). Similarly, there were no changes in OVL integrity (Fig. 3 C, i and D, i, red trend line): ZO-1 expression appeared as a continuous unbroken line (Fig. 3 C, i) and at the ultrastructural level, neatily aligned adherens junctions of normal appearance were observed between Muller glial and photoreceptors (Fig. S1 B, i). In Gnat1−/− recipients, despite undergoing only mild degeneration, the ONL decreased modestly but significantly (Fig. 3 A, ii and D, ii). The OVL remained intact throughout (Fig. 3 C, ii and D, ii, and Fig. S1 B, ii) and there was little change in CSPG deposition (Fig. 3 B, ii). However, GFAP, which may be inhibitory to integration (13), increased by the latest stage examined (Fig. 3 B, ii and D, ii, and Fig. S1 A, ii). Integration also decreased with degeneration in Rho−/− recipients (Fig. 3 A, vi and D, vi), although the initial levels were much lower and the subsequent decline more pronounced. Despite rapid degeneration, OVL integrity was maintained even in late-stage degeneration [in contrast to previous reports (16)] (Fig. 3 C, vi and Fig. S1 B, vi). However, degeneration is associated with a strong glial response, including significant up-regulation of GFAP (Fig. 3 B, vi and Fig. S1 A, vi) and CSPG condensation at the edge of the ONL. A bimodal pattern of integration was observed in Crb1Δ307 recipients (Fig. 3 A, iii and D, iii) (see also ref. 10), whereby increasing disruption of the OVL [permitting increased integration (10, 15)] appears to be offset by a delayed but significant increase in glial scarring. CRB1 is an essential component of the OLM adherens junctional complex (17). Accordingly, significant disruptions in OVL integrity were observed (Fig. 3 C, iii and Fig. S1 B, iii and C, iii). GFAP expression was limited in Crb1Δ307 in early degeneration but increased significantly by late-stage (Fig. 3 B, iii, and Fig. S1 A, iii) together with moderate CSPG condensation (Fig. 3 B, iii). Strikingly, integration into the Prph2Δ307 recipient increased with disease progression (Fig. 3 A, iv and D, iv). In this model, the OVL undergoes some remodeling where cell death was apparent (Fig. 3 C, iv, and Fig. S1 B, asterisks, and C, iv), although this did not change with degeneration. However, there was a very marked reduction in glial scarring: extensive GFAP expression was observed throughout the retina in early degeneration, but decreased, particularly within the OVL, by late degeneration (Fig. 3 B, iv, and Fig. S1 A, iv). CSPG expression also decreased (Fig. 3 B, iv). Integration efficiency was similar in the Prph2Δ307 recipient regardless of the stage at which cells transplanted (Fig. 3 A, v and D, v). Some disorganization of the OVL was observed, although this was similar at both early- and late-stage degeneration (Fig. 3 D, v). Interestingly, despite an increase in GFAP expression (Fig. 3 B, v and Fig. S1 A, v), CSPGs at the outer edge of the OVL decreased in end stage disease (Fig. 3 B, v). In PDE6βΔ3/Δ3 recipients, integration efficiency was surprisingly unaffected by disease progression (Fig. 3 A, vii and D, vii). This model demonstrated significant glial scarring (Fig. 3 B, vii, and Fig. S1 A, vii), yet also underwent an increase in disturbances in OVL degeneration. Taken together, these data show that neither the recipient ONL cytoarchitecture, nor the rate of endogenous photoreceptor loss, are limiting factors for transplanted rod precursor integration.

Fig. 2. Morphology of integrated rods is influenced by recipient retinal environment. (A and B) Percentage of integrated rods that develop outer segments (A) and presynaptic-like structures (B) (n = 3 or more per model; ANOVA with Tukey’s correction). (C and D) Typical morphology, outer-segment length (C) and presynaptic-bouton formation (D) of integrated cells. (C) Integrated cells expressed the rod-specific transcription factor Nrl (green), rod α-transducin (C, ii), peripherin-2 (C, v), rhodopsin (C, vi), or p-PDE (C, vii) (red), as appropriate; such markers are absent in the respective endogenous photoreceptors. (D) Most arrowheads but not all colocalized with RIBEYE (red). Dotted line in vii denotes ONL/INL boundary. (Scale bar, 25 μm.)

Rod-Photoreceptor Transplantation Success Is Independent of ONL Thickness and Rate of Degeneration. Measurement of ONL thickness at each stage highlighted the different rates of endogenous photoreceptor loss in each model (Fig. S2A), but there was no correlation between the rate of degeneration and integration efficiency. For example, in the Gnat1−/− degeneration is largely stationary after 3 mo of age (Fig. S2A, black squares), yet integration declines over time (Fig. 3 A, i and D, i). Conversely, a rapid rate of degeneration in PDE6βΔ3/Δ3 recipients (Fig. S2A, white squares) was accompanied by little change in integration (Fig. 3 A, vii and D, vii). It has previously been suggested that changes in recipient ONL cell density may influence transplanted cell outcome (18). However, few changes in cell density were observed either between early- and late-stage degeneration or between models (Fig. S2B) and none correlated with the different trends in integration. Finally, we examined whether there is a threshold or minimum ONL thickness that is required for integration success. The mean ONL thickness of each model at each degenerative stage was plotted against the corresponding mean integration (Fig. S3); we found no correlation highlighting the finding that successful photoreceptor transplantation can be achieved even in a thinned ONL. Notably, integration above or similar to wild-type was observed in some (Prph2Δ3/Δ3, Prph2Δ307, PDE6βΔ3/Δ3), but not all models at late-stage degeneration.
integrity in late degeneration (Fig. 3 C, vii, and Fig. S1 B, vii and C, vii). Of note, ultrastructural analysis revealed that although adherens junctions were present, they were larger in size and fewer in number than in wild-type and the majority were formed between Müller glial cells, rather than Müller glia and photoreceptors, indicating a significant degree of remodeling.
These data demonstrate that despite sharing the same final common pathway of photoreceptor loss, different models and stages of retinal degeneration present very different microenvironments through which donor cells must migrate, leading to strikingly different outcomes in photoreceptor transplantation efficacy. Specifically, the extent of glial scarring and changes to OLM integrity appear important in determining transplantation outcome in different types of retinal degeneration.


The observed correlations between glial scarring, OLM integrity, and transplant efficacy indicated it may be possible to improve integration by administering a tailored modulation of the microenvironment for a given disease type and treatment timepoint. To determine whether glial scarring and OLM integrity are indeed responsible for impeding integration in the degenerating recipient retina, we manipulated these factors pharmacologically and assessed their impact on transplanted rod-photoreceptor integration. We chose the Rho−/− mouse, the model with the poorest transplantation outcome, which also presented with an intact OLM together with a strong glial response.

We used siRNAs targeted against ZO-1 to provide a reversible disruption of the OLM, a strategy we have previously shown to increase photoreceptor integration (10). Rho−/− and wild-type mice received either siRNA targeting ZO-1, a proven nontargeting control siRNA, or no injection 48 h before transplantation of Nrl.GFP+ve-rod-precursors to the same region. The number of integrated rods was markedly increased in ZO-1 siRNA-treated Rho−/− retinae compared with eyes receiving nontargeting siRNA or no pretreatment (4.2-fold and 2.3-fold increases, respectively) (Fig. 4A). Although the overall number of cells integrating into the wild-type mouse was higher than in the Rho−/−, a similar pattern was observed (4.4-fold and 2.2-fold increases, respectively). We next used chondroitinase ABC (ChABC) to enzymatically digest CSPGs (11, 14, 19) and combined this with transplantation of Nrl.GFP+ve-rod-precursors in Rho−/− and wild-type recipients. In Rho−/− mice, ChABC treatment led to a highly significant increase in integration compared with controls (eighthfold increase) (Fig. 4 A, i). In wild-type recipients, where there is no gliosis and CSPG expression is diffuse, the effect although present was less marked (2.5-fold increase) (Fig. 4 A, ii). We next examined the impact of combining the two manipulations. Wild-type and Rho−/− recipients received ZO-1 siRNA 48 h before coadministration of Nrl.GFP+ve-rod-precursors and ChABC. Combined treatment led to significant increases in transplanted cell integration in both wild-type and Rho−/− retinae (Fig. 4A, and Fig. S4). Thus, both the OLM and CSPG deposition impede the integration of transplanted photoreceptors into the degenerating retina but integration can be improved by targeted disruption of these barriers.

Finally, we sought to determine if the increase in integration achieved using combined treatment was sufficient to restore visual function, as assessed by optokinetic head-tracking behavior in Rho−/− mice (Fig. 4B, and SI Materials and Methods) (5, 20). Seven of the Rho−/− mice receiving combined treatment did so only in one eye and either no injection or cells only in the contralateral eye, before being tested 3–4 wk posttransplantation. No consistent head-tracking behavior was observed upon presentation of scotopic stimuli to control eyes. In contrast, head-tracking was seen following stimulation of six of seven eyes receiving the combined treatment. Histological assessment revealed a positive correlation between improvement in contrast sensitivity and the number of integrated rods (Fig. 4B), as shown previously when testing optomotor head-tracking responses in transplanted Gnat1−/− mice (5). Of note, greater numbers of integrated cells were required in Rho−/− recipients to generate contrast sensitivities equivalent to those recorded in Gnat1−/− recipients.

Discussion

Photoreceptor transplantation has the potential to restore vision following retinal degeneration (2, 5). While it could potentially be applied to a wide range of retinal degenerations, there have been no systematic assessments of efficacy across a spectrum of retinal dystrophies. Here we show that it is possible to achieve robust integration even in severely degenerate retinae and in a variety of murine models with very different aetiologies. In contrast to what might be expected, neither the rate nor extent of degeneration affected photoreceptor integration, indicating that photoreceptor replacement therapy may be an effective therapeutic strategy for severe retinal degeneration. Indeed, a more complex pattern was observed where integration increased, decreased, or remained constant with disease progression, and opposing trends were observed even in models with similar rates of degeneration. We show that the aetiology specific to each gene defect impacts both on the number and the morphology of the integrated rods within a given disease model. We also demonstrate that two characteristics of retinal degeneration, glial scarring and changes in OLM integrity, significantly affect transplantation outcome. Broadly, integration decreases in those models in which OLM integrity is maintained, but in which gliosis increases with disease progression (Gnat1−/−; Rho−/−). Integration remains constant in models in which the OLM is disrupted, but gliosis increases (Pde6b−/−/−/−). Finally, integration increases in the model in which the OLM undergoes remodeling and gliosis decreases with disease progression (Prph2+/−Aβ40). Importantly, it is possible to manipulate these barriers and increase transplanted photoreceptor integration to levels sufficient to restore visual function.

There are notable differences in the pattern of gliosis in the different models; GFAP+ve fibers were seen extending throughout
the ONL in those models in which integration efficiency declined with degeneration (Gnat1−/−; Rho−/−), but the most poorly performing model (Rho−/−) also displayed significant CSPG deposition. Conversely, in the Peph2Δ1Δ2/Δ1Δ2, in which integration efficiency remained similar at different stages of degeneration, CSPG deposition decreased over time. In the Peph2Δ1Δ2/Δ1Δ2 model, we observed a striking decrease in GFAP expression in the ONL, although such regional changes were not reflected in the global changes in GFAP expression. Thus, the specific characteristics of the glial response may be as important as its absolute magnitude.

Integration efficiency is typically higher in models of degeneration in which OLM integrity is compromised than in those in which it remains intact. Of all the models studied, the Cbri−/− model, which has a profoundly disrupted OP (10, 17), had the highest levels of integration. Of note, we observed significant differences in OLM adhesions junction composition in the different models. In wild-type mice, these junctions form between photoreceptors and Müller glia. However, in the Peph2Δ1Δ2/Δ1Δ2, and PDE6βΔ13/Δ13 models, many formed directly between Müller glia, indicating significant OLM remodeling. This was supported by the presence of photoreceptors mislocalized within the IPM. In these models, integration efficiency increased or remained constant, presumably because of, at least in part, the continued changes in OLM integrity. Surprisingly, despite significant endogenous rod cell death in the Rho−/− mouse, the adhesions junctions retain the typical photoreceptor–Müller glia association.

Although important, it is unlikely that OLM integrity and glial scar formation solely govern transplantation outcome within the degenerate retina; many more factors are likely to be involved. Here, our assessment of glial scarring focuses primarily on the up-regulation of GFAP and deposition of CSPG, and the application of ChABC leads to the breakdown of only some CSPGs. However, gliosis has many attributes, including Müller cell hypertrophy and proliferation and microglia accumulation. The biological roles of all these changes are unclear and their impact on cell transplantation has not been addressed. Others have shown that CSPG degradation, either by ChABC (11, 14, 19) or by endogenous enzymes (21), enhances the integration of transplanted cells. However, there are conflicting reports of the role of GFAP: one study reported an increase in transplanted cell integration in the GFAP−/−Vim−/− mouse (13), suggesting that GFAP might be inhibitory to migration; others have reported enhanced integration around sites of GFAP up-regulation (22).

Further work is needed to ascertain the exact role of intermediate filament assembly in transplantation outcome.

Recently, we have shown that it is possible to restore vision in the Gnat1−/− mouse by rod-photoreceptor transplantation (5). In this model, the retinal cytoarchitecture is well preserved and the integrating cells displayed morphologies almost indistinguishable from wild-type rods. However, as we have shown here, transplanted photoreceptor morphologies are profoundly affected by the altered cytoarchitecture. Although the numbers of cells integrating within the PDE6βΔ1Δ1 and Gnat1−/− models were similar, in PDE6βΔ1Δ1 mice integrated photoreceptors often had multiple processes, with few synaptic-like structures or segments. Similarly, photoreceptors integrated within the Rho−/− mouse developed only short outer segments (present study and refs. 2 and 14). The failure to elaborate outer segments does not necessarily prevent a photoreceptor from functioning: outer segments fail to form in the Peph2Δ1Δ2/Δ1Δ2 mouse, yet these animals retain some residual function for several weeks postbirth (23). However, it is likely that a greater number of integrated cells will be required to restore visual function in these recipients than in recipients with more normal outer-segment morphology. Accordingly, although we observed restoration of optokinetic headtracking in some Rho−/− mice following transplantation combined with OLM disruption and CSPG degradation, more integrated cells were required to generate contrast thresholds equivalent to those we reported recently for Gnat1−/− recipients (5). This finding highlights the need to find additional ways to achieve high levels of integration in advanced degeneration.

Materials and Methods

Full methods are available in SI Materials and Methods. Single subretinal transplantsations of 200,000 live P6–8 Nrl.GFP−/−–rod-photoreceptors were given to wild-type (C57BL/6J) mice and models of inherited retinal degeneration at early-, mid-, and late-stages of degeneration (Results). Cell integration was assessed 3–4 wk posttransplantation. Gliosis was manipulated using ChABC (19) and OLM integrity was manipulated using siRNAs directed against ZO-1 (10). Optomotor responses were recorded as previously described (5). See Table S3 for immunohistochemistry.

ACKNOWLEDGMENTS. We thank A. Eddaoudi for technical assistance. This work was supported by grants from the British Retinitis Pigmentosa Society (GR566), Wellcome Trust (082217; 086128), Royal Society (RG080398), and Medical Research Council UK (G0300034; G0901550 mr/004553/3). R.A.P. is a Royal Society University Research Fellow; J.C.S. is supported by Great Ormond Street Hospital Children’s Charity, and R.R.A. is partially funded by the Department of Health’s National Institute for Health Research Biomedical Research Centre, Moorfields Eye Hospital, and the Milti Trust.


