Molecular Vision **1999; 5:16** <http://www.molvis.org/molvis/v5/p16>
Received 27 January 1999 | Accepted 10 August 1999 | Published 11 August 1999

Lactose promotes organized photoreceptor outer segment assembly and preserves expression of photoreceptor proteins in retinal degeneration

Monica M. Jablonski, Amira Wohabrebbi, Cynthia S. Ervin

Retinal Degeneration Research Center, Department of Ophthalmology, The University of Tennessee-Memphis, Memphis, TN

Purpose: We have previously shown that lactose promotes the proper assembly of photoreceptor outer segments in the absence of the retinal pigment epithelium (RPE). The purpose of this study was to determine if the difference between organized and disorganized membranes was a variation in the amounts of two structural proteins, opsin and *rds*/peripherin.
Methods: Eye rudiments were dissected from *Xenopus laevis* embryos and the RPE was removed prior to culturing in the following media: Niu-Twitty medium; Niu-Twitty with mannose; Niu-Twitty with lactose. Controls included retinas that matured in vitro with an adherent RPE. Photoreceptor ultrastructure was evaluated with emphasis on outer segment membrane organization. The relative amounts of opsin and *rds*/peripherin, two outer segment-specific proteins, were determined, as were their immunolabeling patterns.
Results: In control retinas, outer segments were composed of stacked, flattened membranous saccules. Opsin labeling of rod outer segments was very dense, indicative of normally organized disc membranes, and *rds*/peripherin labeling was heavy at the outer segment disc periphery and incisures. In the absence of the RPE, a whorl-like profile of outer segments is present in what would be the sub-retinal space. Opsin immunolabeling was patchy and disorganized. Immunolabeling of *rds*/peripherin was present, but in a disorderly array. Mannose showed no protective effect. In contrast, lactose promoted the formation of organized outer segments and allowed for near normal expression of both photoreceptor markers. In retinas with disorganized outer segments, the expression of opsin is downregulated while the expression of *rds*/peripherin is maintained or upregulated.
Conclusions: Lactose protects against the retinal degeneration induced by RPE removal by preserving the outer segment structure and the photoreceptor immunolabeling patterns. It also maintains constant the relative amounts of opsin and *rds*/peripherin. It is possible that in degenerating retinas, photoreceptors upregulate *rds*/peripherin expression in attempt to provide additional support for the proper folding of nascent membranes, however this is insufficient to permit organization of the photoreceptor outer segments. Our results suggest that rescue-effect of lactose is mediated by a non-*rds*/peripherin related mechanism.

The photoreceptor (PR) outer segment (OS) is highly structured and is comprised of stacked, flattened membranous saccules in perfect register. This formation is thermodynamically unfavorable [1], suggesting that molecular forces may be needed maintain this conformation. It has been demonstrated in several laboratories that reduced levels of opsin and *rds*/peripherin are associated with altered OS structure and subsequent PR degeneration. In the rhodopsin knockout, mice lacking both opsin alleles fail to form rod OS and PR degenerate rapidly, while those mice with a single copy of the opsin gene elaborate OS with a disrupted morphology and have a slow PR degeneration [2,3]. These data suggest that in addition to its role in the phototransduction cascade, opsin may also play an as of yet undefined structural role [2,3].

The structural relevance of *rds*/peripherin has been much more widely evaluated. Experimental data suggest the existence of an adhesive force at the OS disc periphery, and support indirectly that *rds*/peripherin is responsible for exerting this stabilizing effect [4]. An insertion in the *rds*/peripherin gene produces a truncated protein that, in the *rds*/*rds* homozygous mouse, precludes the normal development of outer segments by impairing disc folding, leading to a slowly progressive degeneration of both rod and cone PRs [5]. The *rds*/+ heterozygote mouse has a much milder form of the disease in which OS are formed, yet they are disorganized, with swollen and vacuolated discs [6]. This phenotype is most likely due to haploinsufficiency, rather than a dominant negative effect [5]. Mutations in the orthologous human gene, peripherin/RDS, cause a wide spectrum of retinal manifestations that are inherited in an autosomal dominant fashion. Histopathology confirms the presence of shortened and disorganized OS [7], in close analogy to what has been observed in *rds*/+ mice [6].

Disruption of OS structure can also be caused by retinal pigment epithelium (RPE) dysfunction or loss. The importance of an intact and fully functional RPE on PR structure and survival has been known for many years. Retinal detachment studies indicate that separation of the neural retina from the overlying RPE layer results in rapid cone and rod OS degeneration and the degree of recovery of cell morphology and function are negatively correlated with the duration of the detachment [8,9]. In the Royal College of Surgeons rat, it has been shown that a defect in the RPE results in PR degeneration

Correspondence to: Monica M. Jablonski, Ph.D., The University of Tennessee-Memphis, Department of Ophthalmology, 956 Court Avenue, Memphis, TN, 38163; Phone: (901) 448-7572, FAX: (901) 448-7193; email: mjablonski@mail.eye.utmem.edu

unless growth factors are injected into the subretinal space [10] or an RPE transplant is performed [11]. Additionally, mutations in RPE-specific genes have been documented in human forms of retinal degenerations (e.g., RPE65 gene [12,13], cellular retinoid binding protein (CRALBP) [14], bestrophin [15], and PEDF [16-18]).

To further complicate our understanding of critical factors influencing PR health or degeneneration, a large body of work suggests that carbohydrates also are necessary for proper OS formation and organization. We have previously demonstrated that, in the absence of the RPE, OS membrane ultrastructure can be modified by adding specific sugars to the culture medium. OS membranes elaborated in the absence of the RPE, yet in the presence of permissive sugars (e.g., galactose and lactose), assemble into stacked flattened membranous saccules, very similar to those that have been maintained in the presence of the RPE. Conversely, retinas exposed to non-permissive sugars (e.g., glucose and mannose) elaborated disordered OS [19,20]. Data from other laboratories support our contention that carbohydrates are necessary for OS formation and organization. It has been demonstrated that in adult *Xenopus* retinas tunicamycin-induced inhibition of N-linked oligosaccharides causes nascent membranes to form tubulo-vesicular opsin-containing profiles [21-23]. A recent study in transgenic mice lacking the glycosylation site(s) of opsin corroborates the hypothesis that proper glycosylation of this protein is necessary for normal OS morphogenesis [24]. Taken together, these data suggest that the disorganization of OS membrane saccules may be due to underglycosylation of proteins within the retina. Given the documented abundance of glycoproteins, glycolipids, glycosaminoglycans, and lectins in the retina/RPE complex it may be that sugars play a role in cellular adhesion or a more downstream role in cell signaling involving the regulation of gene transcription/translation.

While data from several laboratories suggest that opsin and *rds*/peripherin are involved in the proper folding of OS membranous discs, the effect of RPE dysfunction or removal upon these proteins has not yet been explored. In addition, it is unknown whether lactose-mediated rescue alters the expression of these proteins. The purpose of the present study was to combine our previously documented alterations in PR architecture with the characterization of opsin and *rds*/peripherin under four experimental conditions: healthy retinas, degenerating retinas, non-protected retinas exposed to mannose, and retinas that have been protected from degeneration with lactose. We evaluated the immunolocalization patterns and quantified the protein levels. The hypothesis tested was that a alteration in the amounts of opsin and *rds*/peripherin would be detected in retinas undergoing degeneration, while the rescuing effect of lactose may be due to a stabilizing effect upon protein expression.

METHODS

Culture of Developing Retinas: The culture preparation used in these experiments has been previously described [19,20,25-27]. *Xenopus laevis* embryos were obtained through induced breeding of adult *Xenopus* by injection of human chorionic gonadotropin (Sigma Chemical Co., St. Louis, MO). Embryos were staged by external morphological criteria [28] after which time eye rudiments from stage 33/34 embryos were isolated. At stage 33/34, outer segments are just beginning to be elaborated, therefore all membranous disc material is produced in vitro [19].

Eye rudiments without a closely apposed RPE were placed into Niu-Twitty (NT) media alone [29], NT containing 5 mM mannose (Sigma Chemical Co., St. Louis, MO) or 5 mM lactose (Eastman Kodak Company, Rochester, NY). Controls included eye rudiments allowed to mature in vitro in the presence of an apposed RPE in NT medium alone. Cultures were maintained at 23 °C for 3 days under cyclic lighting conditions, at which time the eyes have reached approximately stage 42 of the in vivo developmental scale [19,30]. Prior to removal of the eye rudiments, embryos were maintained under identical lighting conditions. The handling of animals was in accordance with the Declaration of Helsinki and The Guiding Principles in the Care and Use of Animals (DHEW Publication NIH 80-23).

Morphological Assessment and Immunocytochemistry: Prior to harvesting for morphological or biochemical analysis, each rudiment was grossly examined under a dissecting microscope for rudiment integrity and smoothness of the neuroepithelial surface to ensure that all rudiments were undamaged and intact. Any rudiments that exhibited an uneven surface or had many loose cells associated with it were discarded.

For structural analysis, eyes were fixed on ice in Tucker fix (2% glutaraldehyde and 1% osmium tetroxide), dehydrated, embedded in Araldite/EMbed812 (Electron Microscopy Sciences, Fort Washington, PA). One micron thick sections were placed on microscope slides, stained with toluidine blue O and examined on a Nikon Microphot fx microscope. Images were captured using a Dage-MTI video camera (Michigan City, IN) and NIH image version 1.61 image analysis system. For immunocytochemical localization analysis, eyes were fixed for 2 h in Davidson fixative (32% ethanol, 2% formalin, 11% acetic acid), dehydrated, embedded in Unicryl (Electron Microscopy Sciences, Fort Washington, PA), cut at one micron thickness and collected on microscope slides. The sections were incubated in 5% goat serum (Vector Laboratories, Burlingame, CA) in phosphate buffered saline (PBS), rinsed in PBS and incubated overnight in primary antibody. The following antibodies were used: B630N anti-opsin, 1:2000 dilution [31] which we have previously demonstrated to immunolabel the principal rods [19]; and xrds38 antiserum, 1:4000 [32,33], which we have previously shown to label both rods and cones [33]. Gold-conjugated secondary antibodies followed by silver enhancement were applied to the tissue, as described by the manufacturer (Electron Microscopy Services, Fort Washington, PA).

Retinal sections were viewed on a Nikon Eclipse E400 microscope equipped with Sensys Color Camera (Photometrics, Tucson AZ) and images were collected using MetaMorph Imaging System software (Universal Imaging Corporation, West Chester, PA). Two images were collected of each retinal section: a brightfield image which shows the morphology of the tissue; and another image taken with epipolarized light which shows only the immunolabeling pat-

tern. The epipolarized image was color enhanced and merged with the brightfield image so that the specific immunolabeling patterns could be easily distinguished.

Quantitation of outer segment proteins: Three sets of 15-20 eyes were collected, ground and solubilized with sodium cholate detergent (Sigma Chemical Co., St. Louis, MO). Extracted proteins were applied in duplicate to Hybond-P membrane (Amersham Pharmacia Biotech, Buckinghamshire, England) using a slot blot apparatus (Biorad, Hercules, CA). Blots were incubated in primary antibody overnight at 4 °C (anti-opsin; B630N [31] at 1:10,000 dilution and xrds38 antiserum [32,33] at 1:10,000). The ECF Western blotting kit (Amersham Pharmacia Biotech, Buckinghamshire, England) was used according to manufacturer's protocols. Blots were scanned on a Storm 860 (Molecular Dynamics, Sunydale, CA) and data were quantified using ImageQuant software version 1.1 (Molecular Dynamics, Sunydale, CA). For each of the three repetitions, data were normalized to values obtained for eyes maintained with an intact RPE. The student's T-test was employed to determine significant differences between eyes that were maintained with an intact RPE and any other culture condition.

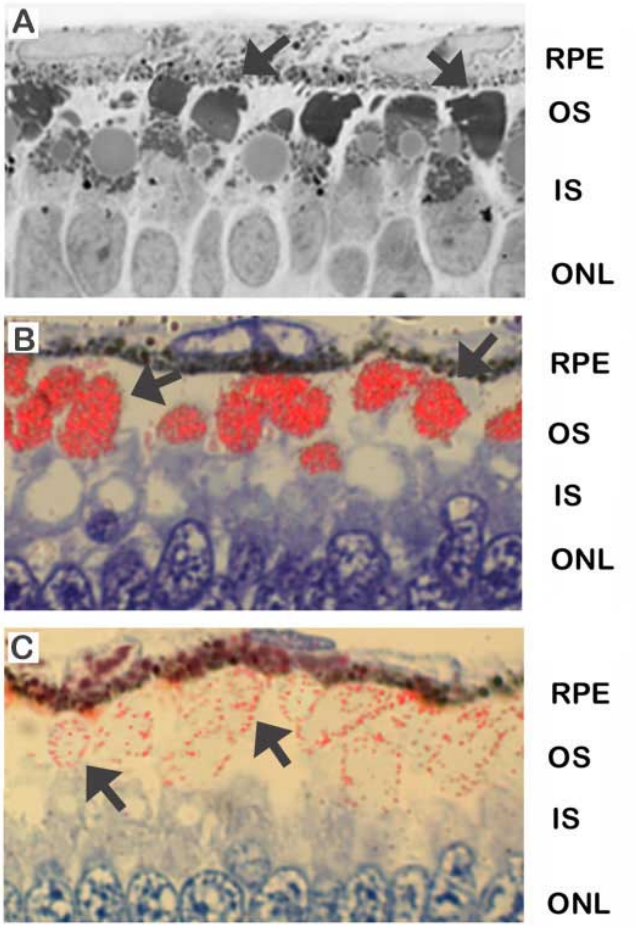

Figure 1. Morphology and immunolocalization patterns of retinas maintained for 3 days in vitro in the presence of an intact retina-RPE complex. **A**. In the presence of the RPE, photoreceptor outer segments are composed of an orderly array of stacked discs with no evidence of membranous whorls (arrows). **B**. Anti-opsin antibody immunolabels heavily in a linear pattern the outer segments of these retinas, indicative of organized discs. **C**. *rds*/peripherin is detected at the outer segment disc periphery and in a striated pattern over the outer segment, likely at the incisures (arrows).

RESULTS

Morphology and immunolocalization patterns in control retinas (3-day in vitro culture in the presence of the RPE): In control retinas that were allowed to mature in vitro with a normally apposed RPE (Figure 1A), the OS are highly organized and tightly stacked, yielding individual profiles that are in line with individual PR inner segments. No loose whorls of membrane are seen. An amorphous material can be discerned in the interphotoreceptor space between the OS and the RPE. The opsin labeling is very heavy over the OS of the majority of photoreceptors (Figure 1B). The labeling is very dense and linear indicating organized stacked OS membranes. The immunolabeling patterns of *rds*/peripherin reflected the presence of this glycoprotein at the disc periphery and incisures

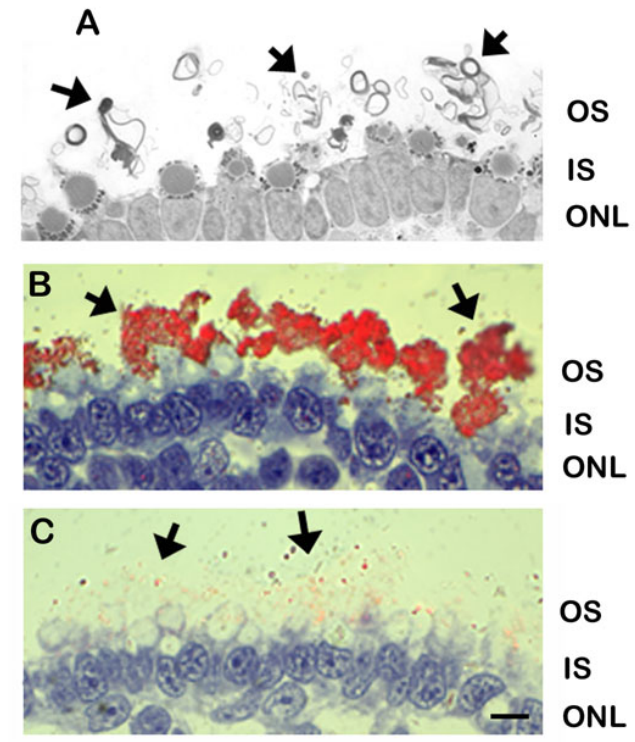

Figure 2. Morphology and immunolocalization patterns in degenerating retinas induced by RPE removal. **A**. In the absence of the RPE, photoreceptor outer segments are elaborated as whorl-like structures, forming a membranous mat at the outer retinal surface (arrows). Outer segments are not localized to individual inner segments, as seen in control conditions (see Figure 1A). **B**. The opsin immunolabeling pattern is also indicative of this altered conformation of outer segment membranes. Individual opsin-positive outer segment profiles are not distinguishable. Rather, an irregularly shaped semi-continuous band of immunoreactivity is present (arrows). **C**. The pattern of *rds*/peripherin labeling was very irregular and much of it is out of the plane of focus, reflecting the disordered array of outer segments (arrows). Bar = 10 μm.

depicted in the stripe-like patterns over the OS layer (Figure 1C).

Morphology and immunolocalization patterns in degenerating retinas (absence of the RPE and also with the non-permissive sugar, mannose): In retinas that matured in the absence of the RPE, PR-OS membrane structure was markedly disorganized, with little evidence of normal disc stacking (Figure 2A). A whorl-like arrangement of OS membranes was present, rather than compact individual OS structures as seen in Figure 1A. The same array of OS abnormalities was observed when retinas were cultured in the presence of mannose (Figure 3A), a sugar we have previously shown to be non-permissive in allowing OS organization in the absence of the RPE [20,25]. In these degenerating retinas the inner segment structure was also missing from some photoreceptors, however, the outer nuclear layer appeared to be intact (Figure 2A and Figure 3A).

In parallel to these structural changes, the immunolabeling and localization patterns were dramatically altered in retinas undergoing degeneration induced by RPE removal. Opsin immunolabeling appeared reduced and altered in conformation (Figure 2B). Individual OS profiles could not be distinguished. Rather heavy patches of label alternating with complete lack of label and still other areas of a non-uniform irregularly shaped profiles of immunoreactivity were present distal to the inner segments, similar to the OS contours seen in Figure 2A. Some areas of immunolabelling are out of the plane of focus, reflecting in three dimensions the whorls of membrane rather than tight cylinders of OS evident in the presence of the RPE. Opsin trafficking appears to be normal in that no opsin label is detected in other areas of the cell including the synaptic terminus. Also reflecting the altered OS conformation, *rds*/peripherin labeling was present in a random pattern in degenerating retinas (Figure 2C and Figure 3C), rather than in the orderly linear pattern notable in control retinas (see Figure 1C). Identical labeling patterns were observed in retinas that matured in the presence of mannose (Figure 3B and Figure 3C).

Morphology and immunolocalization patterns in retinas cultured in the absence of the RPE and the permissive sugar, lactose: In the absence of the RPE, lactose promoted the formation of well-organized OS segment membranes (Figure 4A), and allowed for normal expression of both PR-specific pro-

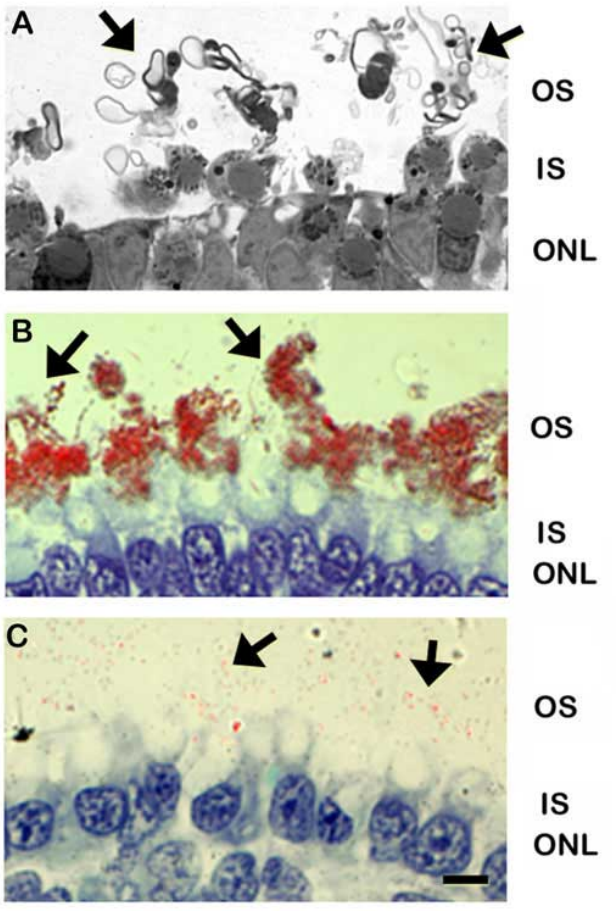

Figure 3. Retinas are not protected from degeneration by mannose as evidenced by aberrant ultrastructure and immunolabeling patterns. **A**. The morphology of the outer segment membranes is very similar to degenerating retinas with no sugar added. The ultrastructure indicates that membranous whorls lacking an orderly array are present. In addition, many inner segments are missing. **B**. The immunolabeling pattern of opsin reflects also the disorganization of the outer segments. **C**. Similarly, the *rds*/peripherin labeling pattern lacks the linear pattern noted in control retinas. Bar = 10 µm.

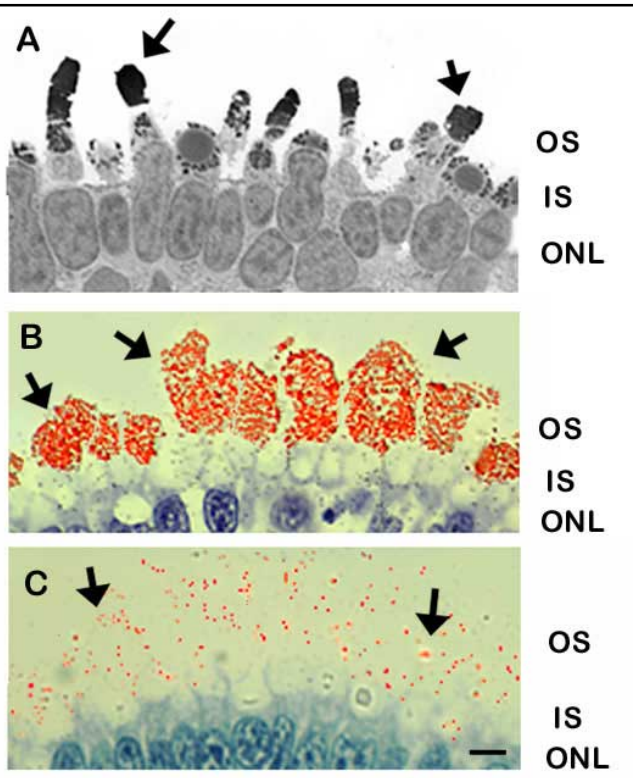

Figure 4. Retinal rescue mediated by lactose: morphology and immunolocalization patterns. **A**. Lactose permitted organization of the photoreceptor outer segments in the absence of the RPE. Individual outer segment profiles in a stacked orderly array are present (arrows). **B**. Opsin immunopositive labeling is present in a pattern similar to that noted under control conditions in which the RPE was present (arrows). **C**. *rds*/peripherin was localized to the periphery of the outer segment (arrows). Bar = 10 µm.

teins (Figure 4B,C). The structure of the OS in lactose-protected retinas (Figure 4A) resembled very closely the organization of membranes under control conditions (Figure 1A). Individual OS were in line with inner segments with no whorl-like arrays, as those seen in degenerating retinas (Figure 2A and Figure 3A). In protected retinas, opsin labeling was very similar to those of control retinas (Figure 4B). Individual OS profiles with linear labeling patterns were evident, consistent with the organized OS membrane disc lamellae observed morphologically. Similar to control conditions, *rds*/peripherin labeling was found in linear arrays over the OS periphery (Figure 4C).

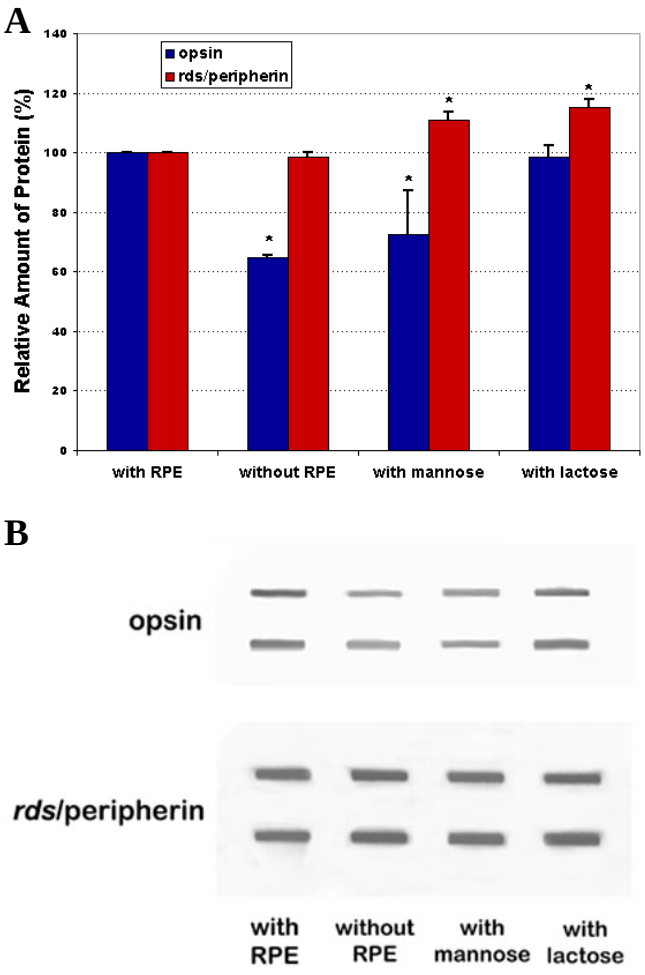

Figure 5. Levels of *rds*/peripherin are maintained in degenerating retinas. **A**. Relative amounts of opsin and *rds*/peripherin in control, degenerating and rescued retinas. Values are normalized to the amounts of each protein in control retinas with an attached RPE. In the absence of the RPE, photoreceptors downregulate expression of opsin, while maintaining constant the level of *rds*/peripherin. Mannose does not increase the opsin expression, however, it does cause an elevation in *rds*/peripherin levels. Lactose prevents the downregulation of opsin induced by RPE removal while simultaneously stimulating an upregulation of *rds*/peripherin. * = $p < 0.05$ compared to healthy retinas maintained with an adherent RPE. **B**. Representative immunoblot illustrating the differences in opsin and *rds*/peripherin protein expression under the various experimental conditions.

Quantitation of opsin and rds*/peripherin:* In eyes undergoing degeneration induced by RPE removal, there is a significant decrease in opsin, with no change in the level of *rds*/peripherin. A similar pattern is noted in retinas maintained in the presence of mannose: opsin expression is downregulated with a slight elevation in level of *rds*/peripherin. Only in retinas rescued by lactose was the level of opsin maintained compared to controls with an intact retina-RPE complex. In the lactose-rescued eyes, an increase in the amount of *rds*/peripherin also was measured relative to controls (Figure 5).

DISCUSSION

Our results indicate that RPE-deprived retinas undergo PR degeneration demonstrated by a derangement of OS membrane structure, altered immunolocalization patterns of opsin and *rds*/peripherin and loss of inner segments. These degenerating retinas also downregulate opsin expression, while maintaining constant *rds*/peripherin levels. Mannose, a non-permissive sugar [20,25], improved neither OS morphology, nor immunolocalization patterns. It also lacked a positive effect upon the expression of opsin. Lactose, a permissive sugar [20,25], exerted a neuroprotective action on the retinal degeneration induced by RPE removal as evidenced by the preservation of the expression and localization patterns of opsin and *rds*/peripherin, as well as both outer and inner segment structure. In addition, it maintained constant the relative amount of opsin while increasing *rds*/peripherin compared to control non-degenerating retinas with an adherent RPE.

Both opsin [34,35] and *rds*/peripherin [36] are intramembranous glycoproteins with distinct functions and expression patterns in the PR-OS. The apoprotein, opsin, is the most abundant protein of the PR-OS, comprising approximately 80-90% of the rod OS protein [35]. Opsin is localized on the lamellar portion of OS discs and the surrounding plasma membrane. Although opsin coupled with its chromophore initiates the phototransduction cascade, evidence suggests that it also plays structural role that is yet undefined [2,3,37]. The data presented herein provide further evidence that a decrease in the amount of opsin (Figure 5) is associated with a degenerative retinal condition.

rds/Peripherin is localized exclusively at the disc rims and incisures of both rod and cone outer segments [31,38-40]. It is postulated that *rds*/peripherin belongs to a group of adhesion molecules that stabilize the folding of the OS disc into its proper conformation [36,41,42]. We have recently isolated and characterized three *Xenopus* homologs, termed xrds, of mammalian *rds*/peripherin. In both photoreceptor types, xrds is hypothesized to form heterotetrameric or higher-order *rds*/peripherin complexes that stabilize disc rims by bridging the intradiscal space in the terminal loop region of OS discs. In the *rds*/+ heterozygote mouse, in which an insertion in one allele of the *rds*/peripherin gene produces a truncated protein, outer segments are formed, but they are disorganized, with swollen and vacuolated discs [6], similar to what we have shown in the retinal degeneration induced by RPE removal (Figure 2A).

The disorderly array of OS membranes in our degenerating retinas cannot be ascribed to haploinsufficiency of *rds*/

peripherin because the levels of this protein are maintained or slightly elevated, despite a downregulation of opsin (Figure 5A). This results in a relative decrease in the amount of opsin while maintaining the amount of *rds*/peripherin, suggesting that these two glycoproteins are differentially regulated and that this form of degeneration has minimal effect upon *rds*/peripherin transcription or translation. Another possible explanation is that in response to the downregulation of opsin, photoreceptors upregulate the relative amount of *rds*/peripherin in attempt to assist the nascent membranes into an organized, properly folded array. Our results suggest that an increase in the amount of *rds*/peripherin relative to opsin is insufficient to explain the rescue of photoreceptors from degeneration in this model and that the lactose-mediated rescue must occur by a yet unknown mechanism.

Although many of the permissive sugars (e.g., lactose and galactose), as well as many of the non-permissive sugars we previously tested (e.g., mannose and glucose) can readily participate in glycolysis, they differ in their respective abilities to exert an organizational effect upon OS assembly [20,25]. This demonstration provides evidence that the structural manifestations provided by lactose and the other permissive sugars we previously described are not accounted for by providing an energy source via the glycolytic pathway. It is possible, however, that mannose and other non-permissive sugars are unable to fulfill a particular functional role or participate in glycosylation in the same manner as lactose and other permissive sugars. It has been documented that opsin is transiently hyperglycosylated with galactose during disc morphogenesis and that it is removed upon assembly of nascent discs [37]. If indeed, galactose is temporarily required for proper membrane assembly, it is plausible that in our system, exogenous lactose participates in this assembly process, after which time it is removed and secreted into the media. Mannose, the predominant sugar residue of native opsin [43], may have no role in the assembly, but rather serve a separate function. For this reason, exogenous mannose may not stimulate proper assembly. In addition, although both mannose-binding and lactose-binding lectins [44-52] are present in the retina-RPE complex, the downstream effects of sugar-lectin binding are unknown.

In summary, our results indicate that removal of the RPE dramatically altered PR structure, as well as the immunolabeling patterns and relative amounts of opsin and *rds*/peripherin. A decreased opsin content is associated with the degenerating phenotype, while an elevation in *rds*/peripherin is not sufficient to permit outer segments to fold with the proper structural conformation. Lactose exerted a neuroprotective action on the retinal degeneration induced by RPE removal by preserving the expression and localization patterns of both PR-specific proteins. This effect was not accounted for by nonspecific glycosylation or by providing an energy source, since mannose did not exert a rescuing effect. These data suggest that lactose-mediated rescue of PR degeneration occurs via a non-*rds*/peripherin related mechanism. Other possible modes of rescue are currently being evaluated.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Alessandro Iannaccone, M.D. for his insights into the clinical relevance of our experimental findings and for his critical review of this manuscript. We also acknowledge Andre DeBellevuè and Mike Davis from Advanced Scientific Inc., Meraux, LA for their assistance with collecting the images presented in this manuscript. The following have generously provided antibodies for our use: Dr. Paul Hargrave, University of Florida, Gainesville, FL and Dr. Gabriel Travis, University of Texas Southwestern. This study was supported by a National Eye Institute grant EY10853 (MMJ), a pre-doctoral fellowship from the Center for Neuroscience at the University of Tennessee (AW), an unrestricted departmental grant from Research to Prevent Blindness (RPB), New York. MMJ is an RPB William and Mary Greve Special Scholar.

REFERENCES

1. Corless JM, Fetter RD, Costello MJ. Structural features of the terminal loop region of frog retinal rod outer segment disk membranes: I. Organization of lipid components. J Comp Neurol 1987; 257:1-8.
2. Lem J, Krasnoperova NV, Calvert PD, Kosaras B, Cameron DA, Nicolo M, Makino CL, Sidman RL. Morphological, physiological, and biochemical changes in rhodopsin knockout mice. Proc Natl Acad Sci U S A 1999; 96:736-41.
3. Humphries MM, Rancourt D, Farrar GJ, Kenna P, Hazel M, Bush RA, Sieving PA, Sheils DM, McNally N, Creighton P, Erven A, Boros A, Gulya K, Capecchi MR, Humphries P. Retinopathy induced in mice by targeted disruption of the rhodopsin gene. Nat Genet 1997; 15:216-9.
4. Falk G, Fatt P. Changes in the structure of the disks of retinal rods in hypotonic solutions. J Cell Sci 1973; 13:787-97.
5. Ma J, Norton JC, Allen AC, Burns JB, Hasel KW, Burns JL, Sutcliffe JG, Travis GH. Retinal degeneration slow (rds) in mouse results from simple insertion of a haplotype-specific element into protein-coding exon II. Genomics 1995; 28:212-9.
6. Hawkins RK, Jansen HG, Sanyal S. Development and degeneration of retina in rds mutant mice: photoreceptor abnormalities in the heterozygotes. Exp Eye Res 1985; 41:701-20.
7. Flannery JG, Farber DB, Bird AC, Bok D. Degenerative changes in a retina affected with autosomal dominant retinitis pigmentosa. Invest Ophthalmol Vis Sci 1989; 30:191-211.
8. Guerin CJ, Anderson DH, Fariss RN, Fisher SK. Retinal reattachment of the primate macula. Photoreceptor recovery after short-term detachment. Invest Ophthalmol Vis Sci 1989; 30:1708-25.
9. Guerin CJ, Lewis GP, Fisher SK, Anderson DH. Recovery of photoreceptor outer segment length and analysis of membrane assembly rates in regenerating primate photoreceptor outer segments. Invest Ophthalmol Vis Sci 1993; 34:175-83.
10. Faktorovich EG, Steinberg RH, Yasumura D, Matthes MT, LaVail MM. Photoreceptor degeneration in inherited retinal dystrophy delayed by basic fibroblast growth factor. Nature 1990; 347:83-6.
11. Li L, Turner JE. Optimal conditions for long-term photoreceptor cell rescue in RCS rats: the necessity for healthy RPE transplants. Exp Eye Res 1991; 52:669-79.
12. Gu SM, Thompson DA, Srikumari CR, Lorenz B, Finckh U, Nicoletti A, Murthy KR, Rathmann M, Kumaramanickavel G,

Denton MJ, Gal A. Mutations in RPE65 cause autosomal recessive childhood-onset severe retinal dystrophy. Nat Genet 1997; 17:194-7.

13. Marlhens F, Bareil C, Griffoin JM, Zrenner E, Amalric P, Eliaou C, Liu SY, Harris E, Redmond TM, Arnaud B, Claustres M, Hamel CP. Mutations in RPE65 cause Leber's congenital amaurosis. Nat Genet 1997; 17:139-41.
14. Maw MA, Kennedy B, Knight A, Bridges R, Rothe KE, Mani EJ, Mukkadan JK, Nancarrow D, Crabb JW, Denton MJ. Mutation of the gene encoding cellular retinaldehyde-binding protein in autosomal recessive retinitis pigmentosa. Nat Genet 1997; 17:198-200.
15. Petrukhin K, Koisti MJ, Bakall B, Li W, Xie G, Marknell T, Sandgren O, Forsman K, Holmgren G, Andreasson S, Vujic M, Bergen AA, McGarty-Dugan V, Figueroa D, Austin CP, Metzker ML, Caskey CT, Wadelius C. Identification of the gene responsible for Best macular dystrophy. Nat Genet 1998; 19:241-7.
16. Goliath R, Tombran-Tink J, Rodriquez IR, Chader G, Ramesar R, Greenberg J. The gene for PEDF, a retinal growth factor, is a prime candidate for retinitis pigmentosa and is tightly linked to the RP13 locus on chromosome 17p13.3. Mol Vis 1996; 2:5 .
17. Tombran-Tink J, Pawar H, Swaroop A, Rodriguez I, Chader GJ. Localization of the gene for pigment epithelium-derived factor (PEDF) to chromosome 17p13.1 and expression in cultured human retinoblastoma cells. Genomics 1994; 19:266-72.
18. Koenekoop R, Pina AL, Loyer M, Davidson J, Robitaille J, Maumenee I, Tombran-Tink J. Four polymorphic variations in the PEDF gene identified during the mutation screening of patients with Leber congenital amaurosis. Mol Vis 1999; 5:10 .
19. Stiemke MM, Landers RA, al-Ubaidi MR, Rayborn ME, Hollyfield JG. Photoreceptor outer segment development in Xenopus laevis: influence of the pigment epithelium. Dev Biol 1994; 162:169-80.
20. Stiemke MM, Hollyfield JG. Effect of sugars on photoreceptor outer segment assembly. In: Anderson RE, LaVail MM, Hollyfield JG, editors. Degenerative Diseases of the Retina. New York: Plenum Publishing; 1995. p. 129-37.
21. Fliesler SJ, Rayborn ME, Hollyfield JG. Membrane morphogenesis in retinal rod outer segments: inhibition by tunicamycin. J Cell Biol 1985; 100:574-87.
22. Defoe DM, Besharse JC, Fliesler SJ. Tunicamycin-induced dysgenesis of retinal rod outer segment membranes. II. Quantitative freeze-fracture analysis. Invest Ophthalmol Vis Sci 1986; 27:1595-601.
23. Ulshafer RJ, Allen CB, Fliesler SJ. Tunicamycin-induced dysgenesis of retinal rod outer segment membranes. I. A scanning electron microscopy study. Invest Ophthalmol Vis Sci 1986; 27:1587-94.
24. Quiambao AB, Fliesler SJ, Peachey NS, Krasnoperova N, Lem J, Al-Ubaidi MR. Opsin glycosylation is necessary for normal outer segment morphogenesis and/or maintenance. Invest Ophthalmol Vis Sci 1999; 40:S212.
25. Stiemke MM, Hollyfield JG. Outer segment disc membrane assembly in the absence of the pigment epithelium: the effect of exogenous sugars. Brain Res Dev Brain Res 1994; 80:285-9.
26. Lolley RN, Farber DB, Rayborn ME, Hollyfield JG. Cyclic GMP accumulation causes degeneration of photoreceptor cells: simulation of an inherited disease. Science 1977; 196:664-6.
27. Hollyfield JG, Witkovsky P. Pigmented retinal epithelium involvement in photoreceptor development and function. J Exp Zool 1974; 189:357-78.
28. Nieuwkoop PD, Faber J, editors. Normal table of Xenopus laevis (Daudin); a systematical and chronological survey of the development from the fertilized egg till the end of metamorphosis. Amsterdam: North Holland; 1956.
29. Jacobson AG. Amphibian cell culture, organ culture, and tissue dissociation. In: Wilt FH, Wessells NK, editors. Methods in developmental biology. New York: Crowell; 1967. p. 531-42.
30. Hollyfield JG, Rayborn ME. Photoreceptor outer segment development: light and dark regulate the rate of membrane addition and loss. Invest Ophthalmol Vis Sci l979; 18:117-32.
31. Rohlich P, Adamus G, McDowell JH, Hargrave PA. Binding pattern of anti-rhodopsin monoclonal antibodies to photoreceptor cells: an immunocytochemical study. Exp Eye Res 1989; 49:999-1013.
32. Kancherla V, Kedzierski W, Travis GH, Jablonski MM. Abnormal formation of outer segments in Xenopus laevis eye rudiments cultured with rds antisense oligonucleotides. In: Hollyfield JG, Anderson RE, LaVail MM, editors. Retinal degenerative diseases and experimental therapy. New York: Plenum. In press 1999.
33. Kedzierski W, Moghrabi WN, Allen AC, Jablonski-Stiemke MM, Azarian SM, Bok D, Travis GH. Three homologs of rds/peripherin in Xenopus laevis photoreceptors that exhibit covalent and non-covalent interactions. J Cell Sci 1996; 109:2551-60.
34. Daemen FJ. Vertebrate rod outer segment membranes. Biochim Biophys Acta 1973; 300:255-88.
35. Papermaster DS, Dreyer WJ. Rhodopsin content in the outer segment membranes of bovine and frog retinal rods. Biochemistry 1974; 13:2438-44.
36. Travis GH, Sutcliffe JG, Bok D. The retinal degeneration slow (rds) gene product is a photoreceptor disc membrane-associated glycoprotein. Neuron 1991; 6:61-70.
37. Smith SB, St Jules RS, O'Brien PJ. Transient hyperglycosylation of rhodopsin with galactose. Exp Eye Res 1991; 53:525-37.
38. Molday RS, Hicks D, Molday L. Peripherin. A rim-specific membrane protein of rod outer segment discs. Invest Ophthalmol Vis Sci 1987; 28:50-61.
39. Connell G, Boscom R, Molday L, Reid D, McInnes RR, Molday RS. Photoreceptor peripherin is the normal product of the gene responsible for retinal degeneration in the rds mouse. Proc Natl Acad Sci U S A 1991; 88:723-6.
40. Arikawa K, Molday LL, Molday RS, Williams DS. Localization of peripherin/rds in the disk membranes of cone and rod photoreceptors: relationship to disk membrane morphogenesis and retinal degeneration. J Cell Biol 1992; 116:659-67.
41. Bascom RA, Manara S, Collins L, Molday RS, Kalnins VI, McInnes RR. Cloning of the cDNA for a novel photoreceptor membrane protein (rom-1) identifies a disk rim protein family implicated in human retinopathies. Neuron 1992; 8:1171-84.
42. Bhatia PK, Travis GH. Rds/peripherin and rom-1: A new class of adhesion molecules. Invest Ophthalmol Vis Sci 1994; 35:1834.
43. Plantner JJ, Kean EL. Carbohydrate composition of bovine rhodopsin. J Biol Chem 1976; 251:1548-52.
44. Bridges CD. Lectin receptors of rods and cones. Visualization by fluorescent label. Invest Ophthalmol Vis Sci 1981; 20:8-16.
45. Bridges CDB, Fong SL. Lectins as probes of glycoprotein and glycolipid oligosaccharides in rods and cones. Neurochemistry 1980; 1:255-67.
46. Rayborn ME, Gao H, Myers KM, Landers RA, Hollyfield JG. Rod photoreceptors in a variety of mammalian species are surrounded by stable interphotoreceptor matrix domains. Invest Ophthalmol Vis Sci 1990; 31[Suppl]:153.
47. Lahiri D, Hollyfield JG. Development of WGA-binding domains in the IPM of Xenopus laevis embryos. Invest Ophthalmol Vis Sci 1992; 33:815.
48. Johnson LV, Hageman GS. Enzymatic characterization of peanut agglutinin-binding components in the retinal interphotoreceptor matrix. Exp Eye Res 1987; 44:553-65.

49. Hageman GS, Johnson LV. Structure, composition and function of the retinal interphotoreceptor matrix. Prog Retin Eye Res 1991; 10:207-49.
50. Blanks JC, Johnson LV. Specific binding of peanut lectin to a class of retinal photoreceptor cells. A species comparison. Invest Ophthal Vis Sci 1984; 25:546-57.
51. Castagna LF, Landa CA. Isolation and characterization of a soluble lactose-binding lectin from postnatal chicken retina. J Neurosci Res 1994; 37:750-8.
52. Eisenbarth GS, Ruffolo RR Jr, Walsh FS, Nirenberg M. Lactose sensitive lectin of chick retina and spinal cord. Biochem Biophys Res Commun 1978; 83:1246-52.