Effects of Retinal Detachment on Rod Disc Membrane Assembly in Cultured Frog Retinas

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The authors compared rod outer segment (ROS) disc membrane assembly rates in detached and attached frog retinas to determine if there was a rapid impairment of membrane assembly in response to retinal detachment. Membrane assembly was quantified in vitro by incubating retinas in medium containing Lucifer yellow, which is entrapped by nascent discs. Video microscopy was used to detect incorporation of the dye. During the first 10 hr after separation of the retina from the retinal pigment epithelium (RPE), ROS–disc membrane assembly in isolated *Xenopus laevis* neural retinas continued at a near normal rate, 0.81 μm/10 hr, a 13% reduction ($P < .01$), compared with the 0.93 μm/10 hr observed in attached control retinas. The morphology of the OS appeared normal in most rod photoreceptors by transmission electron microscopy, although vesiculation of the most basal OS membranes was seen in a small population (25%) of rods. Approximately 90% of rod photoreceptors continued to assemble OS membranes for more than 10 hr after detachment, but by the end of 2 days, only 55% were still making new discs. The percentage of rods with normal basal OS membranes also decreased (to approximately 50%). Therefore, only 25% were assembling morphologically normal discs 2 days after detachment. In attached control regions, rod photoreceptors showed a comparatively minor response to culture conditions; assembly of morphologically normal discs continued for 2 days in about 85% and ceased in only 10%. These results indicate that the effects on disc membrane assembly of disrupting photoreceptor–RPE interaction in vitro initially are slight but become progressively severe with time. Invest Ophthalmol Vis Sci 32:2873–2881, 1991

Photoreceptors in the vertebrate retina normally are apposed to the retinal pigment epithelium (RPE); this juxtaposition is essential for normal rod outer segment (ROS) membrane turnover. Membrane turnover is accomplished by the shedding of disc membranes from the apical end of the ROS, phagocytosis of these membranes by the RPE, and the morphogenesis of new discs at the basal end.[1] Detachment of the neural retina in vitro can disrupt the shedding–phagocytosis portion of the renewal process.[2] The degree to which separation of the retina from the RPE effects ROS membrane assembly is less clear. Although ROS degeneration after retinal detachment in vivo is extensive, there is morphologic[3] and autoradiographic[4,5] evidence that assembly of new disc membranes continues. Additionally, biosynthesis and assembly of ROS membranes proceed for several hours in isolated

bovine[6] or frog[7-10] neural retinas. By contrast, Kaplan and coworkers reported that, in long-term culture (4 days), sustained assembly of normal disc membranes requires attachment of the frog retina to the RPE.[11]

We tried to determine if retinal detachment leads to quantitative and/or qualitative changes in ROS disc membrane assembly during the first 10 hr after separation of the frog retina from the RPE. We also examined the retina 2 days after detachment to determine the effects of a more prolonged period of detachment.

Materials and Methods

Isolation of Eyecups and Culture Conditions

Postmetamorphic *Xenopus laevis* (Charles Sullivan, Nashville, TN) were entrained for 1 month on a 12-hr alternating light–dark cycle. The frogs were handled according to the ARVO Resolution on the Use of Animals in Research. They were killed by decapitation in the latter part of the dark cycle under dim red safelights. Also under red safelights, their eyes were removed, and the anterior segments were dissected away to form eyecups.[12] These were maintained in Wolf and Quimby (GIBCO, Grand Island, NY) amphibian culture medium at 22–24°C. The medium was supplemented with $NaHCO_3$ (35 mM, final concentration)[12] and gassed with 95% O_2 and 5% CO_2.

The culture medium was changed at light onset and midway through the 10-hr incubations and at light onset and offset during the 2-day incubation.

Monitoring of Membrane Assembly

Nascent discs are open to the extracellular space.[13,14] As newly formed discs close, they entrap extracellular material, making it possible to label new discs with fluorescent dyes.[15-17] These discs then appear as a fluorescent band at the base of the OS. The height of this band is a measure of the quantity of disc membranes assembled during exposure to the dye.[18] We labeled nascent discs by culturing eyecups in culture medium containing 0.1% Lucifer yellow CH (LY; Sigma, St. Louis, MO). Beginning at the scheduled time of light onset, but remaining in the dark, eyecups were preincubated for 2 hr in LY-containing culture medium to allow for diffusion of the dye into the interphotoreceptor matrix and finally into the open discs.[18] The retinas then were removed completely from one eyecup from each frog by gently peeling them away from the RPE; contralateral retinas were left attached to the RPE as controls. Isolated retinas and intact eyecups were incubated separately in the continuous presence of LY for 10 hr in the light. The experiment was repeated three times, using three to six frogs per experiment.

In one experiment, some of the retinas were peeled only partially away from the RPE to compare directly detached and attached regions of the same retina. These eyecups were cultured for either 10 hr (6 eyecups) or for 2 days (3 eyecups). The detached regions remained separated from the RPE throughout the experiment. The 2-day incubation schedule consisted of (1) preincubation in LY for 2 hr in the dark, followed by partial detachment of retinas; (2) incubation in culture medium without LY for 46 hr on the normal lighting cycle; and (3) a second incubation in the presence of LY for 3 hr in the light.

Electron Microscopy

Osmicated tissues were used to evaluate ROS disc morphology. Tissues were fixed immediately in 1% glutaraldehyde and 1% paraformaldehyde in 0.1 M sodium cacodylate, pH 7.3, for 2 hr, rinsed in cacodylate buffer, and then fixed in cacodylate-buffered 1% OsO_4 for 1 hr. Next the tissues were rinsed in water, en bloc stained in 1% uranyl acetate in water for 30 min, and rinsed in water again. After this they were dehydrated in acidified 2,2-dimethoxypropane[19] for 10 min and embedded in Spurr's resin.[20] Thin sections were cut and stained with uranyl acetate and lead citrate. Isolated retinas and eyecups from one of the 10-hr experiments were processed in this manner.

Freshly isolated retinas served as controls for possible effects of the isolation procedure. Three retinas from each treatment and 15–20 rod photoreceptors per retina were used to calculate the percentage of rods with abnormal basal discs.

Light Microscopy

Nonosmicated tissues were used for LY-band measurement and immunofluorescence. The tissues were fixed immediately in 0.1% glutaraldehyde and 4% paraformaldehyde in 0.1 M sodium cacodylate, pH 7.3, for 30 min at 4°C, and then in cacodylate-buffered 4% paraformaldehyde overnight at 4°C. Tissues were then reduced in three changes of $NaBH_4$ (0.5 mg/ml) to decrease autofluorescence before being dehydrated in ethanol and embedded in Spurr's resin.[20] We collected 0.5-μm thick sections of retina for LY-band measurement. The LY labeling was visualized by epifluorescence microscopy with a fluorescein excitation filter. To assess the morphology of labeled ROS discs in retinas from which LY-band measurements were obtained, 0.25-μm thick sections were mounted on copper grids and initially examined by epifluorescence; these sections then were stained with uranyl acetate and lead citrate and viewed by transmission electron microscopy (TEM). The percentage of rods with abnormal basal discs was calculated for comparison with osmicated tissues.

Immunofluorescence

To establish the presence of opsin in the ROS discs formed after retinal detachment, opsin and LY were colocalized on sections of tissues cultured in LY-containing medium for 10 hr and processed for light microscopy. We collected 4-μm thick sections, and etched them with sodium ethoxide[21] before incubation with the antiopsin antibody. Monoclonal antibody 15.18 to turtle opsin,[22] a gift from Vijay Sarthy, was used and diluted 1:500 (ascites fluid) with phosphate-buffered saline and 0.5% bovine serum albumin, pH 7.3. The mouse antibody was recognized by a rhodaminated, affinity-purified goat anti-mouse immunoglobulin G antibody (Cappel/Organon Teknika, West Chester, PA), diluted 1:200. Omission of the primary antibody served as a control for nonspecific binding of the secondary antibody. The sections were incubated in the primary antibody solution overnight, rinsed in buffer, incubated in the secondary antibody solution for 3 hr, rinsed in buffer, and mounted in 5% n-propyl gallate in glycerol to preserve fluorescence intensity. The secondary antibody was visualized by epifluorescence microscopy with a rhodamine excitation filter, and LY in the same section was visualized with a fluorescein excitation filter.

Image Enhancement and Measurement of LY Band

A silicon-intensified target camera (DAGE 66; DAGE-MTI, Michigan City, IN) was used to capture images of sectioned material for subsequent measurement (10-hr experiments),[18] using a MegaVision 1024XM image processor (Santa Barbara, CA) or for analysis (2-day experiments) using a Hamamatsu DV 3000 image processor (Waltham, MA). Images were enhanced by contrast stretching and digital filtration.

For the 10-hr experiments, LY-band height was measured midway across the width of the ROS of 50–70 longitudinally oriented rod photoreceptors per retina or per attached or detached region. Data from three to six retinas per treatment and from replicate experiments, if done, were pooled to obtain average LY-band height values, analyze variance, and calculate the t value for comparisons using the Student's t-test. For the 2-day experiment, an average of 75 rods per attached or detached region from three retinas were scored as described in Table 1. The percentage of rods with abnormal basal OS morphology also was calculated.

Results

Rod Photoreceptor Morphology

After 10 hr in culture, the morphology of retinas separated from the RPE was comparable with that of attached control retinas when the retinas were viewed by epifluorescence, although there was more swelling in the inner nuclear layer and the inner and outer plexiform layers (Fig. 1A). The band of LY fluorescence at the base of the OS appeared normal (rectangular and uniformly intense) in most rod photoreceptors (Figs. 1A, 2A). When viewed by TEM, most of the rods in isolated retinas had normal morphology (Figs. 2B, 3A–B). The only obvious structural defect was vesiculation of the most basal OS membranes (Fig. 3C); this occurred in 25% of rods and occasionally was accompanied by deformation of adjacent discs. By comparison, aberrant basal OS membranes rarely were seen in attached or freshly isolated control retinas. Vesiculated or deformed basal discs were observed with equal frequency in retinas postfixed in osmium and those that were not osmicated. When fluorescence and TEM images of the same section were compared, a normal LY band usually corresponded to an intact stack of basal discs (Figs. 2A–B). LY was retained in the basal discs in isolated retinas rinsed in culture medium without LY for 10 min before fixation, indicating that the newly formed discs had closed completely. When sections were labeled with antiopsin, the LY-containing discs and the more distal disc membranes were opsin positive (Fig. 4).

### Table 1. Membrane assembly during a 2-day period of detachment

| | Percentage of ROS with | | |
Retina	One band (no assembly)	One band plus (>10 hr of assembly)	Two bands (2 days of assembly)
#1 Det	12	46	42
#1 Att	1	12	87
#2 Det	10	30	60
#2 Att	6	3	91
#3 Det	8	30	62
#3 Att	3	3	94

Rod photoreceptors in detached (Det) and attached (Att) regions of the same retina were counted in one of three categories, according to the amount of OS disc membrane assembled during the 2 days following partial detachment of the retina. The categories are defined as follows. *1 band:* ROS with a single LY band that had not been noticeably displaced upward. These rods did not assemble a measurable amount of OS disc membrane after the time of the detachment. *1 band plus:* ROS in which the initial LY band had been displaced by subsequent disc formation, but a second band was not present (Fig. 5B). Although these rods assembled greater than 10 hours' worth of OS disc membrane, they were no longer assembling discs when the second LY pulse was given. *2 bands:* ROS with an upper LY band from the LY preincubation and a lower band from the second LY pulse (Fig. 5A,B). These rods were still assembling OS disc membrane 2 days after the time of detachment.

When eyecups with partially detached retinas were maintained in culture for 2 days, the overall appearance of the retinas by epifluorescence resembled that of retinas from the 10-hr experiments, but two differences in rod photoreceptor morphology were evident. First, rod inner and outer segments in detached regions were elongated, and second, basal ROS membranes, especially in detached regions, were disorganized more frequently. About 10% of the rods in attached control regions had abnormal basal OS morphology compared with about 50% of the rods in detached regions. A range of morphologies was observed, from normal (Figs. 5A–B) to severely disorganized. In the latter, basal membranes appeared whorled or vesiculated in fluorescent images (Fig. 5C). Comparison of fluorescent and TEM images of the same section (not shown) confirmed the assessment of basal OS morphology made with epifluorescence. Only the most proximal discs, if any, became labeled with LY during the second pulse (Figs. 5B–C), indicating that most of the discs formed after detachment closed completely.

Membrane Assembly Rates

In the 10-hr experiment, a band of LY fluorescence was present at the base of the OS of all except the most peripheral approximately half dozen rod photoreceptors. Any LY bands that appeared abnormal were not measured. Because the appearance of the LY band was usually an accurate reflection of basal OS morphology, most LY-band measurements represented
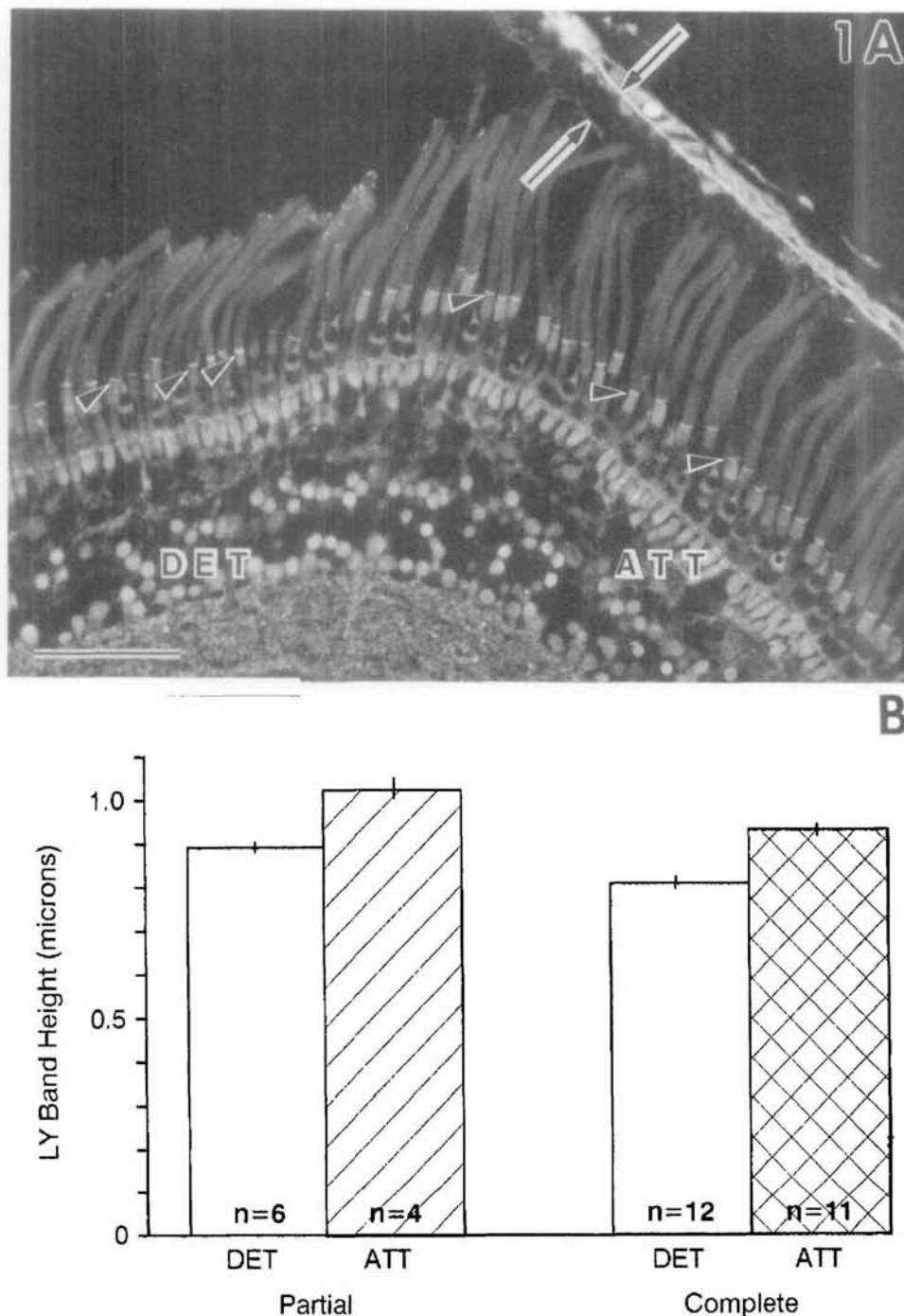
Fig. 1. Ten hours of membrane assembly in the absence or presence of the RPE. (A) Fluorescence image of a partially detached retina (4-μm thick section). In the detached region (DET), ROS are not in contact with the RPE, while in the attached control region (ATT), the ROS and the RPE remain juxtaposed. The morphologies of the detached and the attached regions are comparable. Arrows point to the basal and apical surfaces of the RPE. Arrowheads point to bands of LY fluorescence, which mark ROS discs assembled during a 10-hr period of detachment. The slight fluorescence of the photoreceptor ellipsoids is autofluorescence. Scale bar = 50 μm. (B) ROS disc membrane assembly during a 10-hr period of detachment was quantified by measuring the height of LY bands. In this histogram, average membrane assembly rates for partially detached (single experiment) or completely detached (three replicate experiments) retinas are shown compared to control rates. In the case of either partial or complete detachment, a 13% reduction in rate ($P < .01$) was observed. n = number of retinas used, DET = detached retina, ATT = attached retina. Error bars represent ±1 SEM.

the assembly of structurally normal discs. The rate of disc membrane assembly was reduced slightly in the detached retina during the 10 hr after separation from the RPE. The average membrane assembly rate in completely isolated retinas was 13% lower than the average rate in attached control retinas, 0.81 μm/10 hr compared with 0.93 μm/10 hr. Similarly, when detached (0.89 μm/10 hr) and attached (1.02 μm/10 hr) regions of partially detached retinas were compared, a 13% reduction in rate was obtained (Fig. 1B). The

reduction in rate was statistically significant ($P < .01$). The rates calculated for the attached retina were comparable with those measured by Vaughan and co-workers[18] using this assay. In TEM images of sections of the nonosmicated retinas used for LY-band measurement, a proximal disc stack 1 μm in height contained approximately 30 discs. Hence, on average, rods in completely isolated retinas assembled 24 new discs in 10 hr. Those in attached control retinas assembled 28 new discs in 10 hr.
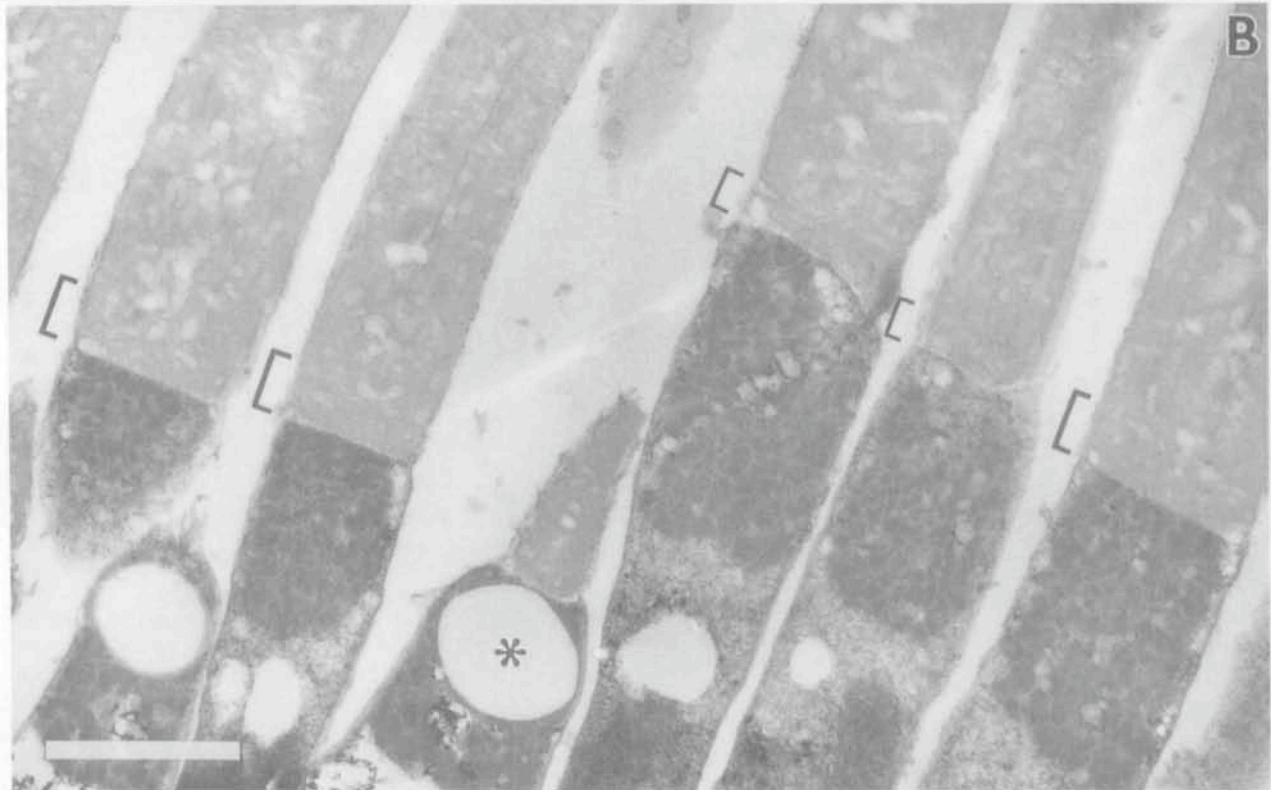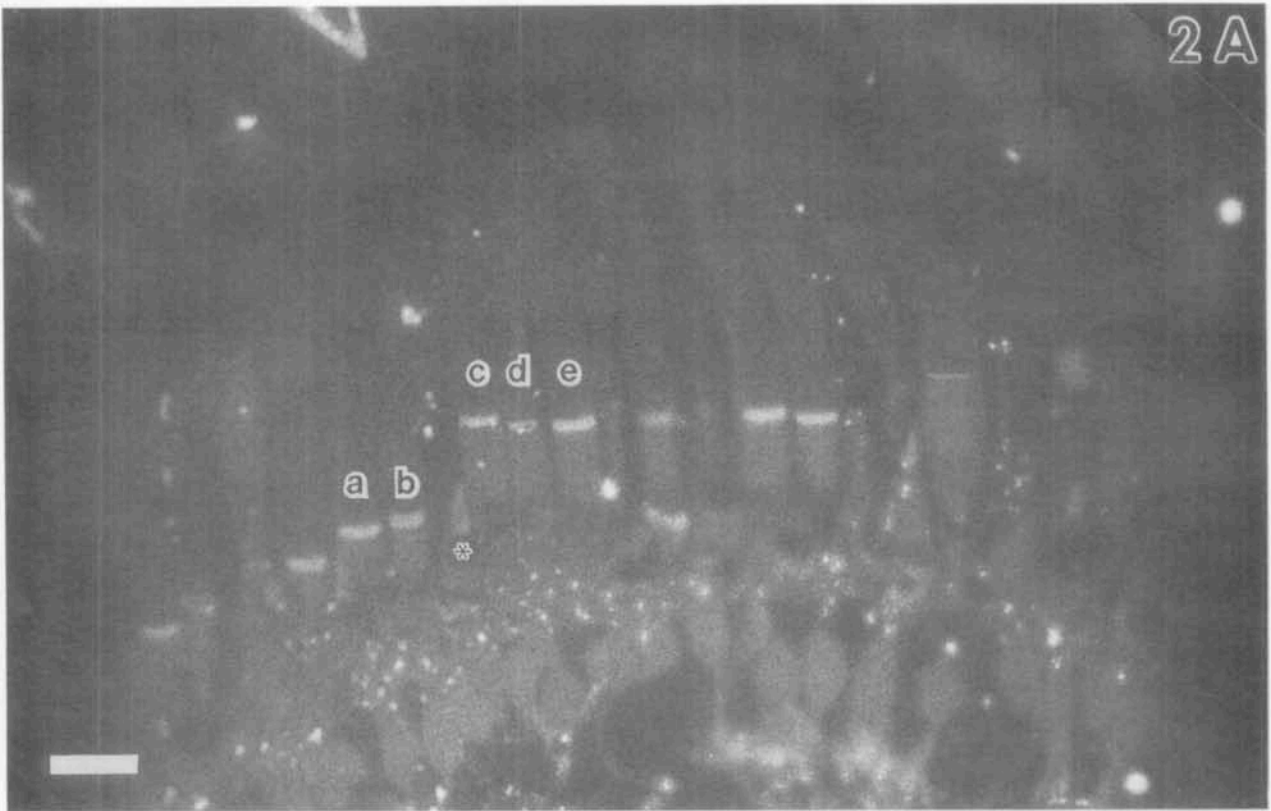
Fig. 2. Morphology of LY-labeled discs in tissue processed for LY-band measurement. (A) Fluorescence image showing LY bands at the base of several ROS in an isolated retina. After 10 hr, the height of the LY band was 1.5 μm in the rod marked a, and 1.6 μm, 1.3 μm, 1.1 μm, and 1.4 μm in rods b, c, d, and e, respectively. This image is of a 0.25-μm thick section mounted on a copper, hexagonal mesh grid; the grid mesh shows as dark bars. Scale bar = 10 μm. After being viewed by epifluorescence, the section was stained for TEM. (B) The TEM image of the rods marked a–e in (A). A cone (asterisk) serves as a landmark. Basal ROS discs appear normal. The height of each LY band measured in (A) is denoted by a bracket. Scale bar = 5 μm. OS = outer segment, IS = inner segment.
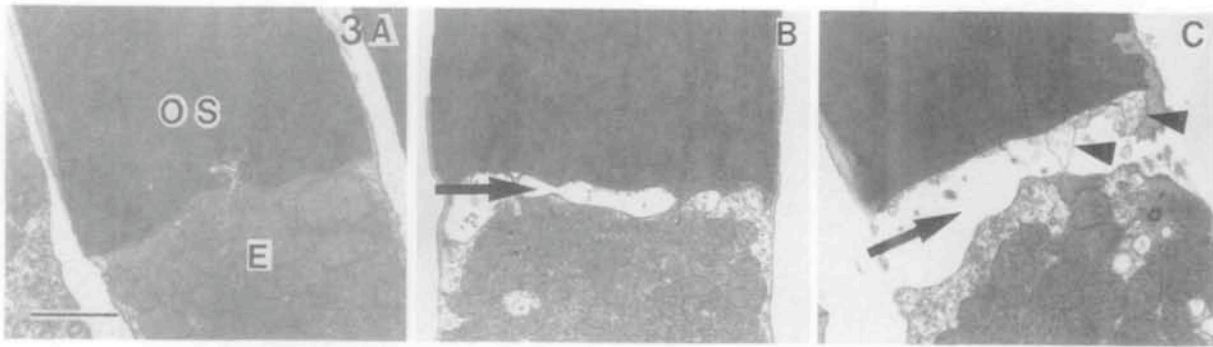
Fig. 3. Basal ROS disc morphology after a 10-hr period of detachment. (**A**) A ROS in an attached control retina post-fixed in osmium tetroxide contains closely spaced, intact discs. Rods in attached control retinas showed no gap between the inner and outer segments. Rods in freshly isolated retinas (not shown) were distinguishable from those in attached retinas only by the presence of a small cleft between the inner and outer segments. Similarly, rods in retinas detached for 10 hr show a cleft (arrows) of varying width between the inner and outer segment (**B, C**). Discs, including the most proximal, appeared structurally normal, as in (**B**), in 70% to 75% of rods. Vesicles (arrowheads) at the base of the OS, as in C, were the most common morphologic defect seen. Scale bar = 2 μm. OS = outer segment, E = ellipsoid.

In the 2-day experiment, the presence of two LY bands (an upper band from the LY preincubation and a lower band from the final LY incubation) or the presence of a single LY band that had been displaced distally marked the OS membranes assembled after partial detachment of the retina. No LY band could be detected in 5% or less of the ROS in both attached and detached regions; these ROS were not included in calculations. A total of 9% of the rod photoreceptors in detached areas did not assemble OS disc membranes after detachment; these had a single LY band at the base of the OS. Percentages for individual eye-
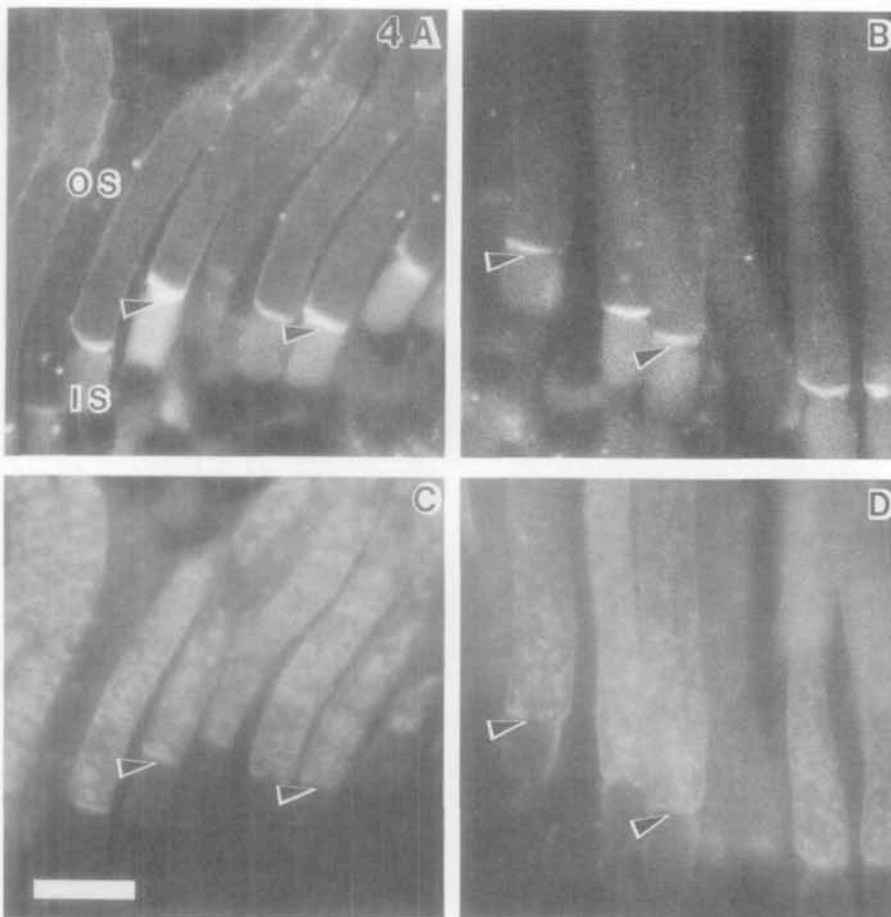
Fig. 4. Colocalization of opsin and LY band. New discs in an attached (**A**) and a detached retina, (**B**) which have entrapped LY during 10 hr in culture, are seen as a fluorescent band when viewed with a fluorescein excitation filter. When the same fields are viewed with a rhodamine excitation filter (**C, D**), ROS show fluorescent opsin labeling. The bottom of the LY band (arrowheads) coincides with the bottom border of the ROS opsin labeling (arrowheads). Four-micron-thick sections of Spurr's-embedded tissue were etched with sodium ethoxide prior to incubation in antibody. The patchy appearance of the antibody labeling is due to the use of etched sections. Scale bar = 10 μm. OS = outer segment, IS = inner segment.
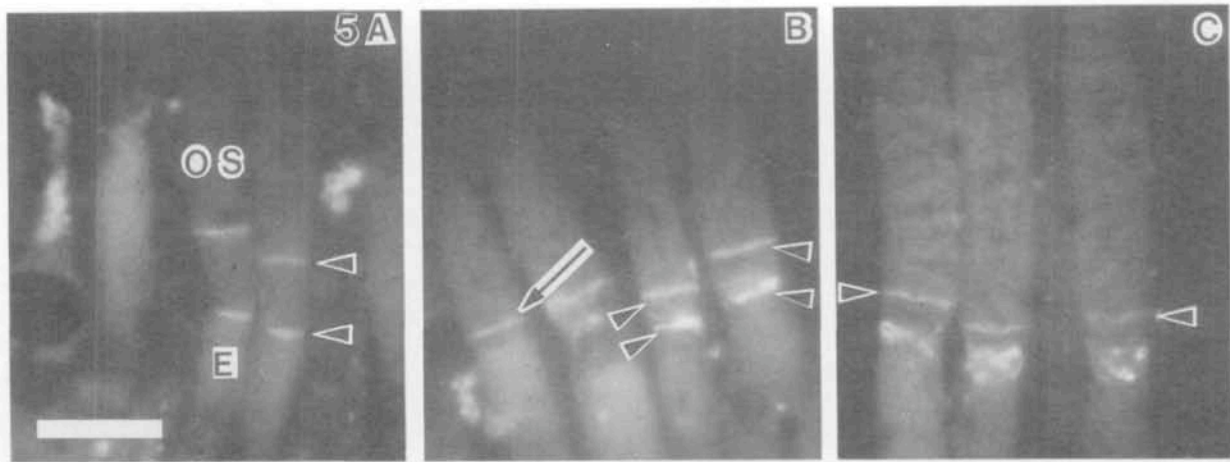
Fig. 5. Two days of membrane assembly in partially detached retinas. Two pulses of LY were used to monitor disc formation in 2-day experiments: one just prior to detachment and one at the end of 2 days. Video-enhanced fluorescence images of attached control regions (A) and detached regions (B, C) are compared. (A) ROS have two LY bands (arrowheads) and normal basal morphology. (B) Two ROS have two distinct LY bands (arrowheads). The bases of these ROS appear to be intact. One ROS has only an initial LY band (arrow), which appears to have been displaced by subsequent disc membrane assembly. (C) The lower LY bands (arrows) of these ROS are disorganized; however, OS membranes above the upper LY bands (arrowheads) appear to be intact. Scale bar = 10 μm. OS = outer segment, E = ellipsoid.

cups are shown in Table 1. We found 36% of the rods in detached areas produced substantial amounts of disc membrane after detachment but stopped assembly before the second LY pulse was given. Although the vertical displacement of the LY bands was not measured, the height of the stacks of new discs appeared greater than that observed after 10 hr in culture (Fig. 5B); no second LY band was present. We found 55% of the rods in detached regions, compared with 91% of the rods in attached control regions, were still assembling discs at the end of the 2-day period, ie, they were labeled with two well-defined LY bands. If we only consider the assembly of morphologically normal discs, these percentages fall to 25% in detached regions and 83% in attached regions. The distance between the top of the upper and the bottom of the lower LY band was 3.3 μm in detached areas and 4.2 μm in attached areas on average (only 10 rods per detached or attached area from 2 retinas were measured). The reduction in average membrane assembly rate in detached regions was estimated to be 20% for rod photoreceptors that continued to make normal discs.

Discussion

Photoreceptor OS progressively degenerate and do not regrow after detachment of the neural retina in vivo,[3] even though assembly of new OS membranes is observed several weeks after detachment.[4,5] It is important, therefore, to know if and how soon membrane assembly is impaired after separation of the retina from the RPE. Beginning with O'Brien and coworkers in 1972,[6] several studies showed that biosynthesis and assembly of ROS membranes are maintained for several hours in isolated neural retinas. Incorporation of radiolabeled leucine into ROS rhodopsin and subcellular fractions followed the same time course observed in attached retinas in vivo.[6-8] Electron microscopic autoradiography after incorporation of radiolabeled precursors revealed that the discs assembled were normal.[7-10] Thus, in the short term, attachment of the neural retina to the RPE is not required for assembly of morphologically normal disc membranes to occur. These studies, however, did not determine whether there are quantitative changes in membrane assembly after retinal detachment.

We directly compared membrane assembly rates (obtained from LY-band height measurements) in detached and attached frog retinas in culture. Our measurements showed that there was a slight (13%), but statistically significant ($P < .01$), reduction in membrane assembly within the first 10 hr of retinal detachment. We believe that the importance of this result lies in the demonstration that membrane assembly can occur at a near-normal rate in the detached retina. In the short term, therefore, the rate of membrane assembly was not changed greatly.

Qualitatively, we found that discs assembled during the first 10 hr after detachment were normal by three criteria. First, new discs, comprising the region defined by the LY band, appear morphologically normal when observed by TEM in all but 25% of rods (in these, only the most basal few discs were disorganized). This result agreed with previous studies showing normal ROS disc morphology in isolated retinas.[7-10] Second, newly formed discs were closed completely, as evidenced by retention of entrapped LY

after rinsing in plain medium. Third, new discs contained the visual pigment apoprotein, opsin. Others[7,23] showed that opsin, the major protein constituent of ROS disc membranes, continues to be incorporated into ROS membranes in isolated retinas. We confirmed these results using immunofluorescence. Although no quantitation was done, the labeling intensity of basal discs in the detached retinas was not obviously different from that in the attached retinas (Figs. 4C–D). Whatever defects may exist in disc membranes formed after detachment, opsin content is probably normal. Even after long-term retinal detachment (30 days) in the cat, newly synthesized protein is transported to the truncated OS, and the OS membranes label with antibodies to opsin.[5]

It did not matter whether retinas were isolated completely from the RPE or only partially separated from it; membrane assembly was reduced by the same amount, and ROS morphology was indistinguishable. This lack of difference does not exclude the presence of diffusable factors from the RPE that either normally provide trophic support for the photoreceptors or, alternatively, hasten degeneration after detachment. We did not test for such factors in our study.

When abnormal OS membranes were visible as deformed LY bands, these bands were not measured and therefore did not contribute to membrane assembly values. In some ROS, however, membrane vesiculation was not detected by epifluorescence, even though vesicles were labeled with LY. In such cases, the LY-band height was not an accurate measure of membrane renewal because height values were inflated. As a result, the membrane assembly rate for the detached retina may be slightly lower than calculated. The error is small, however, because vesicles constitute only a small portion of the total height of the corresponding LY band and epifluorescence detects disorganization in most ROS.

Although the effects of retinal detachment on membrane assembly are initially slight, they become more severe with time. Most rods (91%) in the detached retina continued to assemble new discs for more than 10 hr, as shown by displacement of the LY band from the first dye pulse a significant distance from the base of the OS. By 2 days after detachment, however, only 55% of the rod photoreceptors are still assembling disc membranes. Moreover, the percentage of ROS with disorganized basal membranes increased from 25% 10 hr postdetachment to approximately 50%. This means that, of the 55% of rods still making new discs, one half were assembling discs that appeared morphologically normal. Over a 2-day period, the response to retinal detachment was such that the number of rods assembling normal discs declined, but membrane assembly in these rods continued at a

near-normal rate. The membrane disorganization seen after 2 days (Fig. 5C) was similar to that observed by Kaplan and coworkers in long-term detachments in vitro.[11] The small amount of vesiculation observed in basal OS membranes after 10 hr may be the first indication of abnormal membrane assembly. The results of our 2-day experiment showed a gradual impairment of membrane assembly with more rods affected as the retina was detached for increasing periods of time. Kaplan and coworkers also proposed that attachment of the retina to the RPE was thought to be necessary for normal membrane assembly to be sustained over the long term.[11]

Membrane assembly also was impaired, although to a lesser extent, in attached control regions after 2 days in culture. We found 15% fewer rods assembling morphologically normal OS disc membranes after 2 days in culture than after 10 hr. Therefore, a small part of the impairment of membrane assembly in detached regions represented a general response to prolonged in vitro conditions, but the greater part was the result of separation from the RPE.

Whereas the percentage of ROS with aberrant basal membrane morphology increased considerably from 10 hr to 2 days postdetachment, disorganization of ROS membranes was limited to the most proximal discs, even after 2 days. The morphology of ROS was normal above the upper LY band (Figs. 5B–C). When vesicles were present at the base of the OS, they were filled with LY in some rods but not in others, possibly indicating a difference in their origin (eg, vesiculation of closed discs or evaginating plasma membrane). The disorganization of new disc membranes observed in detached retinas was probably a reflection of some difference in the membranes made after detachment; it was observed rarely in freshly isolated and attached control retinas. A biochemical difference in the new ROS membranes or an alteration in some aspect of disc morphogenesis may prevent the formation of stable discs and/or their organization and maintenance in an orderly stack. Such a defect has been proposed.[3] However, it cannot be the sole cause of the OS degeneration seen after mammalian retinal detachments in vivo because degeneration is so rapid[3,24] that disruption of preexisting membranes must also occur. In addition, the most common description of OS degeneration after detachment is one of disorganization occurring first at the distal end of the OS and gradually progressing toward the connecting cilium. The loss of disc membrane stability or proper organization could, however, account for the failure of OS to regrow when the neural retina remains separated from the RPE.

We conclude that a progressive impairment of ROS disc assembly occurs after the retina is detached from

the RPE in vitro. The change from near-normal rates of membrane assembly and near-normal disc morphology is not a rapid response. Data from our 2-day experiment indicate that most rod photoreceptors continue normal disc assembly for more than 10 hr after detachment. Thus, membrane assembly is not controlled tightly by photoreceptor–RPE interaction. Using the frog isolated retina may help us understand the role of the photoreceptor–RPE interface in regulating the OS renewal process[2] and, hence, more clearly understand the OS degeneration that occurs as a result of detachment in vivo.

Key words: membrane renewal, photoreceptor, in vitro, outer segment, video microscopy

Acknowledgments

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