Morphological Components Associated With Frog Cone Outer Segment Disc Margins

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The two margin morphologies of frog retinal cone outer segment (COS) discs were examined by thin sectioning, freeze-fracture, and deep-etch, rotary shadowing techniques. The disc margin adjacent to the connecting cilium, which morphologically resembles the terminal loop of rod outer segment (ROS) discs, exhibited a distinctive staining density tightly apposed to the membrane surface facing the lumen. This density was crescent-shaped in longitudinal sections, and a continuous band in cross-sections. Associated with the disc margin opposite the cilium, two additional extracellular structures were observed: a globular staining density located at the outer edge of the membrane loop forming the margin, and filaments axially interconnecting adjacent margins. The globular densities and filaments were spaced at regular intervals along the margin. Where these margins were adjacent to calycal processes, the globular densities appeared to span the extracellular gap and interconnect the membranes of the COS discs and the calycal process. Distinctive intramembrane particles were observed along both margins by freeze-fracture. The distribution of the globular and filamentous elements suggests that they may have a role in maintaining the radial dimensions and axial spacing of the associated disc margin by forming an extracellular framework. Invest Ophthalmol Vis Sci 28:646–657, 1987

...and maintained at regular intervals, the disc repeat being slightly, but consistently, greater than that of ROS discs. Also, when exposed to hypotonic media prior to fixation, COSs become spherical in shape, accompanied by disruption of the planar membrane regions. The disc margins, however, appear to maintain nearly normal morphology and axial spacing. Lastly, while rarely mentioned in the literature but typically observed in published micrographs, COS margins exhibit net axial alignment (see Steinberg et al10 for a discussion of this feature and its significance). The COS disc margins are regularly and predictably positioned axially with respect to one another, giving the COS a smooth and well-defined shape. These observations would seem to indicate that COS disc margins have associated component(s) that provide axial stabilization and limit disc diameter.

Mature frog COS disc membranes, however, exhibit several structural features which sharply distinguish their pattern of organization from that found in ROS discs. In contrast to the fairly uniform diameter of ROS discs, the conical shape of the COS is associated with a progressive decrease in frog COS disc diameter from 2–4 μm at the base, to about 1 μm near the distal tip. In cross section, COS discs are oval to nearly circular in outline, and lack incisures. Mature ROS discs, on the other hand, are deeply indented at regular intervals by incisures. Finally, and perhaps most significantly, mature COS discs remain continuous both with adjacent discs and with the outer segment plasmalemma for about half their circumference, in

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contrast to mature ROS discs which are isolated from each other and the overlying plasmalemma. The retention of plasmalemma continuity for a portion of the perimeter results in COS discs having two margin morphologies of opposite membrane curvature and lamellar connectivity. The COS disc margin adjacent to the connecting cilium joins the two membranes of a single disc in a highly curved structural specialization, which morphologically resembles the ROS disc terminal loop, and is separated from the overlying COS plasmalemma. Opposite the cilium, a second margin type is formed by the divergence of the two membranes of a disk to join with membranes of adjacent COS discs in membrane loops. For convenience, in this article we will refer to these two margins as the "closed" and "open" margins, respectively. At an intermediate position around the circumference of the COS disc, the closed and open margins are continuous, and continuous with the COS plasmalemma, at a topological saddle point (see ref. 13 for illustrative diagrams).

These differences in margin structure are likely to be reflected as differences in the components responsible for stabilization and axial alignment of the disc margins. The stabilization of the two distinct COS disc margin morphologies would seem to require a minimum of two structural complexes.

In this study we demonstrate several morphological components associated with the two COS disc margin types. The morphology, location, and organization of these disc margin components suggest that they may be involved in maintaining the structural organization of these regions. Portions of this work were presented in preliminary form.

Materials and Methods

Isolation of Retinas

Northern grass frogs (Rana pipiens pipiens) were obtained from Nasco (Fort Atkinson, WI) and maintained in 22°C incubators on a 14L:10D photoperiod. Retinas were removed from dark-adapted frogs under dim red illumination and placed in frog Ringer. Pigment epithelium-free regions of the retinas were isolated and used for these studies. Subsequent manipulations of the retinas were performed under normal room illumination.

Thin Section Studies

Retinal tissue was prepared using two protocols. In the first, retinas were conventionally fixed with 2% glutaraldehyde in 0.1 M Na-cacodylate buffer, pH 7.4, for 1–2 hr, rinsed in buffer, and then post-fixed with 1% OsO4 in the same buffer for 1 hr. In the second procedure, the fixation protocol was modified to take advantage of the enhanced staining of cellular components when tannic acid is added to the primary fixative. In this procedure, retinas were fixed 2–6 hr with 2.5% glutaraldehyde in 0.1 M Na-cacodylate buffer, pH 7.4, containing 0.2% tannic acid (Mallinkrodt [St. Louis, MO] AR# 1764; the pH re-adjusted after addition of the tannic acid). The fixed tissue was then rinsed and post-fixed with 1% OsO4 in the same buffer for 5–60 min. Following post-fixation, all specimens were rinsed in distilled water, stained en bloc with 1% aqueous uranyl acetate, and placed in the dark for 1 hr. The tissue pieces were then rinsed in distilled water, dehydrated in ethanol followed by propylene oxide, and embedded in Araldite 506 or Epon 812 (Pelco, Tustin, CA) resins.

Silver-grey to dark grey thin sections were cut with a Dupont diamond knife on an LKB Ultratome III (LKB Instruments, Gaithersburg, MD) or Reichert Ultracut E (AO/Reichert, Buffalo, NY) ultramicrotome. Epon sections were stained with 7.5% aqueous uranyl acetate followed by Sato’s lead stain. Araldite sections were stained with 2% potassium permanganate followed by Sato’s lead stain.

Liquid Helium-Copper Block Freezing

Pieces of retina were fixed for 15–60 min with 1% glutaraldehyde in 0.1 M Na-cacodylate buffer, pH 7.4, rinsed in buffer, and then exposed to 1% OsO4 in 0.1 M Na-cacodylate, pH 7.4 until the ROSs darkened slightly (approx. 30–60 sec). The retinas were then rinsed in buffer, and the ROSs gently dislodged with a fine camel hair brush, leaving the COSs attached to the retina. Pieces of retina trimmed to 2 mm X 3 mm were transferred, COSs up, to copper freeze-fracture planchettes (Balzers BUO 12 056-T, Balzers, Nashua, NH). Just prior to freezing, the pieces of retina were briefly rinsed with distilled water to remove salts, and then quick-frozen by the method of Heuser (liquid helium-cooled copper block), using the device of Escaig. Frozen samples were stored under liquid nitrogen until fractured.

Freeze-Fracture

Replicas were obtained using a Balzers BA 360M freeze-fracture/etch unit. At a specimen temperature of −180°C and a vacuum of 10−7 torr, the frozen retina was fractured within the first 10 μm with a razor blade mounted in a liquid nitrogen-cooled microtome. Following fracture, the specimens were immediately shadowed with 1–2 nm of platinum/carbon from 45°, followed by carbon evaporation (5–10 nm thickness) from approx. 80°. Replicas were cleaned in bleach, rinsed in distilled water, and mounted on Formvar-coated 75 mesh grids (Pelco, Tustin, CA).
Fig. 1. (a) Longitudinal section through the apical inner segment and basal outer segment of a single cone cell near the ciliary axis (an indistinguishable image is also given by the principal member of a double cone, data not shown) showing typical morphologies of the closed (CL) and open (O) margins. Glutaraldehyde fixation. Bar = 1 μm. (b) Higher magnification of the closed margins outlined in 1a. Note the crescent-shaped staining densities within the lumen (arrowheads). Bar = 50 nm. (c) Higher magnification of the open margins outlined in 1a. Associated with the membrane loops are globular densities protruding into the extracellular space (arrowheads), and axially-oriented filamentous components interconnecting adjacent loops (arrows). Bar = 50 nm.
Deep-Etching and Rotary-Shadowing

Replicas of deep-etched, rotary-shadowed COSs were obtained using a Balzers BA 360M freeze-etch unit. Samples were fractured as indicated above, at a specimen temperature of -95°C. The fracture surface was etched 3–5 min at -95°C, and then rotary-shadowed with platinum/carbon at an angle of 24°. The replica was strengthened by carbon evaporation from approximately 80°. Replicas were cleaned and recovered as indicated above.

Microscopy and Measurements

Thin sections and replicas were examined using a Philips EM 301 or a Philips EM 420 (Mahwah, NJ) electron microscope at 80 keV, equipped with a liquid nitrogen-cooled anticontamination device. In addition, the Philips EM 420 was equipped with a ±60° tilt stage which permitted optimum specimen orientation. Electron optical magnification was calibrated with a 21,600 lines/cm grating replica.

Freeze-fracture micrographs have been mounted so that the shadowing direction is from below. Terminology for fracture surfaces follows that recommended by Branton et al. Micrographs of deep-etch, rotary-shadowed replicas were printed in reverse contrast, following the recommendation of Heuser. Measurements of COS disc margin-associated components are presented as mean ± SD.

All procedures involving animals complied with the ARVO Resolution on the Use of Animals in Research.

General COS Morphology

The retina of the Northern grass frog contains three different types of cone cells, the single cones and the principal and accessory members of double cones. The photoreceptors of the Northern grass frog were described by Nilsson, who found the outer segments of single and double cones to be morphologically identical. Our observations support those of Nilsson; the overall morphology, as well as the disc margin structures described below, were identical among all three cone outer segment types. In this paper, the specific cone outer segment type is identified in the figure legends, while the results are discussed as features of frog cone outer segments in general. In the case of freeze-fractured and freeze-etched COS discs, the outer segments of single cones and principal members of double cones could not be clearly distinguished on the basis of the amount of the photoreceptor and surrounding structures exposed by etching.

Results

Overall COS Morphology

A comparison of the appearance of frog COSs fixed with glutaraldehyde alone, and glutaraldehyde containing tannic acid, is presented in Figures 1 and 2, respectively. The gross morphology of the COSs was not altered by the presence of tannic acid (Figs. 1a, 2a), but examination of the membranes of the disc margins at higher magnification (Figs. 1b–c, 2b–c) indicated better delineation of membrane-associated components in the presence of tannic acid. The axial alignment of the closed and open margins is readily apparent at this magnification, with open margins possessing a noticeably greater degree of axial alignment than closed margins.

COS Disc Closed Margin

In longitudinal thin sections, distinctive crescent-shaped staining densities were seen within the closed margin loops, tightly apposed to the membrane surface facing the lumen (Figs. 1b, 2c, 3a–c). These internal densities measured 13.0 ± 1.2 nm in length (in the axial direction) and 6.1 ± 0.7 nm in width (n = 198) at their midpoint. While the staining of these densities was significantly enhanced with tannic acid (Figs. 2c, 3a–c), similar densities could be detected in retinas fixed with glutaraldehyde alone (Fig. 1b). When viewed with a ±60° tilt stage, the densities were most clearly visualized when the viewing axis was oriented tangential to the disc edge. Image overlap effects were reduced or eliminated with proper orientation, providing support that these densities were not superposition artifacts of the membrane.

Occasionally, images suggestive of axially-oriented filamentous densities, similar in appearance to the linear interdisc densities between the terminal loops of ROS discs, were observed between closed margin loops (arrows, Figs. 1b, 3a–c). Unlike the case with ROS discs, these filamentous densities were poorly defined, and...
Fig. 3. a–c Examples of typical closed margin morphologies in the different cone types and different regions along the COS observed by glutaraldehyde-tannic acid fixation. (a) Closed margins of an accessory COS in a region where the margin is in close apposition to the COS plasmalemma (compare with Fig. 2c, where equivalent margins are adjacent to the ciliary cytoplasm). (b) Single COS in a region similar to that in 3a. (c) Principal COS in the region of the ciliary cytoplasm. Bar = 50 nm. (d–f) Examples of open margin morphologies in different cone types and regions not associated with calycal processes. (d) Single COS. (e) Principal COS. (f) Accessory COS. Bar = 50 nm.
were not found in axial alignments for more than 2–3 closed margins. Their lateral position with respect to the edge of the closed margin was also more variable than that observed for the linear interdisc densities of ROS disc terminal loops.5

In cross-section, the closed margin density appears as a continuous densely staining band 5.8 ± 0.9 nm wide (n = 128) on the lumenal side of the disc margin membrane profile (Figs. 4, 5). The band was easily distinguished from the ciliary cytoplasm and the planar region of the disc membrane on the basis of its staining properties. The staining intensity of the band was significantly greater than that of the cytoplasm or the planar disc membrane. The band also stained more uniformly compared with the mottled staining pattern of the cytoplasm and planar disc membrane (Fig. 5). Sections 20–25 nm in thickness, measured by the section-fold method,20 indicate that the densely staining band represents the cross-section of a single closed margin, rather than the superposition of two or more margins, as might be expected with thicker sections (eg 60 nm). The disc repeat period measured in longitudinal sections was about 33 nm, which agrees with Nilsson.8

COS Disc Open Margin

Along the open margins of each disc, two structures distinctly different from the closed margin densities were observed. Protruding from the outermost edge of the open margin loops in longitudinal sections, were globular staining densities measuring 10.0 ± 2.4 nm (n = 25) parallel to the plane of the disc and 6.0 ± 1.2 nm (n = 21) in axial dimension (Figs. 1c, 2b, 3d–f; arrowheads). Along those regions of the open margin adjacent to calycal processes, the globular densities filled the space between the open margin membrane and the plasmalemma of the calycal process (Figs. 2b, arrowheads; 4, arrows). In cross-sections, these globular densities were observed along the entire open disc margin (Fig. 6), spaced at 20.8 ± 4.5 nm intervals (n = 17). While the general shape of these densities was globular, filamentous profiles were occasionally observed, particularly where the margins have pulled away from the plasmalemma of the calycal process. In these regions of the open margin, the globular density remained attached to the open margin membrane loop and the calycal process membrane, while the density appears to have been deformed (Fig. 2b, large arrowheads). In other areas (Fig. 2b, small arrowheads), and along the open margin not associated with calycal processes (Fig. 3d–f, arrowheads), the densities were uniform in both size and shape.

In addition to the globular densities projecting from the edges of the open margin membrane loops, axially-oriented filaments 2.2 ± 0.7 nm in diameter and 15.9 ± 2.1 nm in length (n = 17), interconnected adjacent loops (Figs. 1c, 2b, 3d–f; arrows). Tilt series of these filaments indicate that they are not image overlap effects of the membrane profiles. Where slight shrinkage occurred during tissue processing, these filaments were often bent or kinked midway along their length.

 Freeze-Fractured COSs

Fractured COS discs revealed alternating particle-rich and particle-poor lamellar fracture faces very similar to those typical of ROS discs.27,28 In COS disc cross-fractures, the closed margin displays a row of 8–10 nm particles on the PF face and a relative absence of particles in the EF face of this region (Fig. 7). These closed margin particles were qualitatively different from the background texture of the PF face. In some instances, these large particles exhibit a solid, cord-like appearance (Fig. 8, arrowheads). Along the open margin, a row of 8–9 nm diameter particles was observed on the EF face (Fig. 9).

 Deep-Etched COSs

Figure 10 shows an accessory COS and principal cone inner segment prepared by the deep-etch, rotary-shadowing technique. The true surfaces of the open margins and the calycal processes have been exposed below the plane of the fracture, which is indicated by asterisks. Along the open margins, thin, axially-oriented filaments appeared to interconnect the extracellular surfaces of adjacent margin edges (Figs. 11, 12).
filaments are seen along the entire length of the open margin, occurring up to the saddle point marking the junction between the COS plasmalemma, open, and closed margins (Fig. 11, arrowheads). The filaments were 2.6 ± 0.4 nm in width (including Pt/C coat; n = 20), 21.4 ± 1.2 nm in length (n = 37) and spaced at 20.4 ± 5.4 nm intervals (n = 25). No significant difference in dimensions and spacing were observed for filaments between discs near the base and the tip of the COS. Tangential views of the open margin indicate that the filaments were set in from the open margin by approximately 12 nm (Fig. 12). Where the margins were separated due to swelling, filaments up to 40 nm in length were observed.

The open margin filaments are probably not artifacts deposited onto the surface of the deep-etched cells, because they display a nonrandom axial orientation, appear to be set in from the edge of the open margin rather than lying or draping across the edges, and are not present on the calycal processes or COS plasmalemma. Like the filaments interconnecting adjacent ROS disc terminal loop regions,⁷ they appear to be real structures having well-defined shape and location.

Discussion

The distinctive and stable morphology of COSs argues for the presence of an underlying structural framework. In the ROS, this stabilization has been attributed, in part, to the filamentous cytoplasmic connections that have been observed between adjacent disc terminal loops.⁷ The results of this study indicate that similar structural elements may exist in association with COS discs. It was observed that the margins of COS discs exhibit distinctive morphological elements: 1) crescent-shaped densities within the lumen of the closed margin, 2) axially-oriented filaments interconnecting the edges of adjacent open margins, 3) globular densities localized to the edges of the open margin, and 4) freeze-fracture particles associated with both margins. To the best of our knowledge, this is the first demonstration of such components in COSs.

The COS closed disc margin joins the two planar membranes of a single disc in a highly curved structural specialization, which bears a striking resemblance to ROS disc terminal loops. The generation and maintenance of the distinctive membrane curvature is con-
ceptually difficult to assign solely to the phospholipid component of the membranes there. The shape and location of the closed margin luminal component observed in this study suggests that it could act to mold and/or stabilize the membrane curvature of this margin into the characteristic ROS disc terminal loop-like configuration. Additionally, the continuous appearance in cross section suggests that it could provide a lateral structural framework for the closed margin.

The clear visualization of the distinctive crescent-shaped COS disc closed margin densities was facilitated by optimizing the orientation of the membrane profiles during observation. Although these densities could be observed in COSs prepared by conventional fixation procedures, the addition of tannic acid to the primary fixative had the effect of increasing the staining intensity, and thus the delineation, of these closed margin densities. This is most likely due to the tannic acid-mediated mordanting effect on osmicated tissue with subsequent lead staining. The addition of tannic acid did not alter the overall morphology of the COS disc margins, nor did it completely fill the disc lumen like a negative stain. It also appears unlikely that the closed margin densities are the result of anomalous staining by the inner surfaces of regions of high membrane curvature, since the comparably curved region...
of the open margin membrane loops (i.e., the cytoplasmic surface) were devoid of any similar staining density.

We have observed suggestive evidence for cytoplasmic connections between the closed margins, similar to those observed between terminal loops of ROS discs. Unlike the connections observed in ROSs, however, those observed in COSs are less clearly defined and more variable in location. Whether our failure to routinely observe them is due to their infrequency, or is due to their being obscured by the cytoplasmic content in this region, is uncertain. However, the presence of some kind of component interconnecting the cytoplasmic surfaces of closed margins is suggested by their regular axial spacing.

The organization of the open margins of COS discs is distinct from that of the closed margins. The margin is defined by the membrane loops formed by the divergence of the two membranes of a single disc to join with the membranes of adjacent discs. In this region, the COS disc does not have a terminating margin, but contributes its membranes to sites of direct continuity with adjacent discs. We observed two structures associated with this margin that are different in morphology from the closed margin densities.

The axially-oriented filaments seen adjacent to the edge of the open margin in both thin section and deep-etch, rotary-shadowing preparations appear to interconnect adjacent discs. Their position and distribution suggest that they may be the structural elements responsible for the resistance of the open margin to disruption by osmotic stress noted by Cohen. In ROSs, filamentous connections between adjacent terminal loops of adjacent discs have been demonstrated by freeze-substitution, and deep-etch, rotary-shadowing techniques, and have also been suggested to account for the mechanical stability of the terminal loops. The filaments found along the COS disc open margins, however, are fundamentally different from the filaments observed in ROS discs. In COSs, the filaments are extracellular structures, while in ROSs they are cytoplasmic. Nevertheless, they may serve an analogous function.

The globular densities located at the edges of the open margin membrane loops also appear to be integral components of the margin, rather than interphotore-
ceptor matrix components. These structures are well-defined in orientation and dimensions, and are localized to the outermost edge of the membrane loop along the entire length of the open margin. In cross-sections of COSs, they appear as discrete structures, regularly spaced along the margin edge. Interestingly, these globular densities are spaced at intervals corresponding to the lateral spacing of the axially-oriented thin filaments. Although there is no direct evidence that the globular and filamentous elements are components of a single morphological complex, the similar lateral spacing of both elements is suggestive of such an association.

The distinctive freeze-fracture particles associated with both the open and closed margin indicate the presence of substantial intra- and/or transmembranous elements in these regions. One interpretation is that these freeze-fracture particles represent the intramembranous protein segments of transmembrane proteins along the margins. A transmembrane component of the margin-associated elements would provide one means to axially interconnect extracellular and cytoplasmic structural elements for axial stabilization of these regions (see below).

The position and overall distribution of the open margin elements suggests that they are grossly organized into a two-dimensional structural framework or lattice, and play a role in defining the lateral extent and axial alignment of the open margins. Several features of the open margin suggest the presence of such a structural network. For example, the open margin is in direct continuity with the COS plasmalemma, and as a result is subject to expansion by the movement of new membrane components from the plasmalemma. Yet, the open margin has a smooth and very regular outline in planar view, and is well-aligned with adjacent open margins. Unconstrained membrane bilayers would be likely to display irregular outlines and alignment under these circumstances, if their perimeters were not controlled in some manner. The calycal processes seem unlikely candidates for this role, because there are large stretches of open margin between adjacent calycal processes in which the perimeter would not be constrained. Yet, the open margin does not bulge outward between adjacent calycal processes. The simplest explanation for these discrepancies is the existence of specific structural components associated with the open margins that define and maintain the perimeter. We suggest that the filamentous and globular elements we have observed along the open margin are portions of these structural elements.

There are specific constraints, however, for the long-range organization of an open margin structural complex, imposed by the conical geometry of the COS. Locally, such components could exert effective short-range interactions, thereby generating the lateral and axial spacings observed. However, such a pattern of organization cannot be a long-range feature of an open margin complex. If it were, the open margin region of the COS would define a limiting cylindrical surface, rather than the observed conical frustrum.

The restrictions inferred above for a fixed conical geometry are further complicated by the dynamic aspects of COS disc membrane turnover. COS discs undergo basal synthesis and apical shedding similar to those of ROS discs.30-36 During this process, the discs also decrease in size as they are displaced distally. Because it is the length of the open margin that disproportionately decreases in the apical direction,8 the number of complexes that can fit along this margin at successive disc locations must also decrease, if their average lateral spacing is to be maintained. There are two potential mechanisms whereby the surface lattice and a conical geometry can be accommodated. In the first, open margin complexes are in equilibrium with a pool of complexes distributed throughout the lamellar domain of the disk. In this case there would be a continual removal and reinsertion of complexes on a random basis along the open margin. The net occupancy in this type of dynamic equilibrium would be such as to maintain a sufficient number of connections to preserve the conical geometry, while being able to accommodate progressive decreases in margin length. Alternatively, the open margin complexes are sequentially removed in the vicinity of the saddle points as the margin decreases in length. The individual components could diffuse back into the COS plasmalemma and/or disc lamellar region.

The formation of new discs at the base of the COS presents a different problem. New complexes must be formed between the first mature COS disc and the underlying growing disc to propagate the stabilizing framework. For a disc-shedding event, all connections between the affected discs (the basalmost of the shed packet and the apical remaining disc) must be broken. There may be subtle differences in the micro-environment near the COS tip that permit the localized breakdown of this stabilizing framework to release a packet of discs. Unfortunately, we are not able to make predictions about the rearrangements of these margin components by comparison to ROS disc shedding, because the mechanism by which the disc rim filaments are severed during shedding is also unknown. Given the current understanding of disc renewal mechanisms, the observation of open margin lattices in a setting of conical geometry implies that such processes exist for margin assembly and disassembly.

The molecular nature of the morphological elements we have detected along the margins of COS discs is not known, but the recent findings of Papermaster et al97
raise an interesting possibility. Using EM immunocytochemical techniques, they demonstrated specific labeling along the COS disc margin, and diffuse labeling within the planar region of the COS disc using antibodies to the large intrinsic membrane protein previously localized to the ROS disc terminal loop.38 Their results suggest that COS discs and ROS discs in the frog share a common perimeter-associated component. The closed margins have many structural features in common with ROS terminal loops, as indicated previously, and are the likely sites of antibody localization in COSs observed by Papermaster et al.37 The resolution of their bovine serum albumin embedded COSs however, is not sufficient to make accurate comparisons with the margin structures observed in this study.

The relationship between the globular densities and the calycal process plasmalemma is also noteworthy. Along those portions of the open margin adjacent to a calycal process, the globular densities appear to span the extracellular space and interconnect the two membranes. This behavior suggests an attachment function, but the purpose of this is not clear at present.

A summary of our observations of the two COS disc margins, and proposals for the structural organization of these regions, are presented in Figure 13. The upper half of the figure schematically depicts the morphological elements associated with the closed and open margins observed in this study. It would seem, however, that the overall axial stability of COS margins would require the presence of additional components to interconnect and support the elements that have been observed. In the lower half of Figure 13, we have integrated such additional components into our representation of the two margins. In the case of the closed margin (lower left), filamentous cytoplasmic elements have been added to interconnect the crescent-shaped lumenal densities (via the observed intramembrane particles). The cytoplasmic filaments have been positioned at a location comparable to the connections observed in ROSs,5-7 based on the close similarity of the COS closed margin and the ROS terminal loop; the transmembrane elements have been placed at an intermediate position to interconnect the lumenal and cytoplasmic elements. Along the open margin (lower right), we have indicated two possibilities for interaction between the globular and filamentous components (13 A, B). In one case (A), we have permitted the globular and filamentous components to be separate elements, while in the other (B), they are part of a single complex that is able to interact with similar complexes in adjacent open margins. While these additional components of closed and open margin complexes were not observed or differentiated in this study, the structural and mechanical properties of COS margins suggest their presence.

Note added in proof: In a recent quantitative EM autoradiographic study of squirrel cones, Anderson et al (1986)39 found an initial localization of a fucosylated component at the base of the COSs which became completely randomized in distribution only after several hours. They suggested on the basis of these results that some mechanism existed to restrict rapid longitudinal diffusion of COS membrane proteins, and that this mechanism might be situated at the disc margins. The COS disc margin-associated components observed in this study are ideally situated to act as margin-associated barriers to axial diffusion of membrane proteins in COSs.

Key words: retina, cone photoreceptor, cone outer segment, disc membranes, disc margin

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