

## Disc Morphogenesis in Vertebrate Photoreceptors

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**ABSTRACT** Electron microscopic examination of the bases of adult rod and cone outer segments (rhesus monkey, ground squirrel, and grey squirrel) has led to a new model of disc morphogenesis. In this model the disc surfaces and disc rims develop by separate mechanisms and from separate regions of the membrane of the inner face of the cilium. This membrane is alternately specified into regions that will form either the disc surfaces or the disc rims. The disc surfaces develop by an evagination or outpouching of the ciliary membrane. The two surfaces of an evagination, scleral and vitreal, each form one of the surfaces of adjacent discs. The disc rim is initially specified as a region of ciliary membrane between adjacent disc-surface evaginations. This region grows bilaterally around the circumferences of adjacent discs, *zippering together the apposed surfaces to form the rim and completed disc*. At the same time it seals the plasma-membrane edges of the evaginations, which have become detached from the surfaces. Incisures form in rod discs by infolding of the rim and surfaces together, and they begin to form before the rim is completed around the disc perimeter. When a number of new discs are developing simultaneously the ciliary membrane at the base of an outer segment consists of a stack of rim forming and surface forming growth points. This model provides, in addition, for the continuous renewal of outer-segment plasma membrane. It also establishes a developmental basis for the structural uniqueness of the disc rim. Finally, it indicates an evolutionary relationship between the discs of vertebrate visual cells and the membrane specializations of invertebrate visual cells.

In the developing vertebrate photoreceptor, a remarkable growth process takes place when the cilium becomes transformed into an outer segment. There is a tremendous increase in the surface area of the ciliary membrane, which is achieved by the packaging of the new membrane into a stack of closely spaced double-membrane discs. In the adult photoreceptor the disc forming process continues and is responsible for the renewal of the outer segment. In order to understand this process we can ask two questions: How are the constituents of the new membrane delivered to the outer segment from the inner segment? How is this membrane transformed into a disc? It is the second question, the question of disc morphogenesis, which we consider here.

The early observations of visual cells by electron microscopy provided the initial insights into the morphogenic question. In longitudinal sections through cone outer

segments Sjöstrand ('59) observed that the disc membranes were in continuity with the plasma membrane of the cell, and the membrane appeared *infolded*. The same was observed by others for the discs at the bases of rod outer segments (Moody and Robertson, '60; Cohen, '61a). This infolded appearance of the disc membrane in adult as well as in developing outer segments (Sjöstrand, '59; Tokuyasu and Yamada, '59; DeRobertis and Lasansky, '61) led to the hypothesis that disc formation occurred by a *process* of infolding of the plasma membrane (Sjöstrand, '59, '61; Moody and Robertson, '60; Yamada, '60; Okuda, '61; Cohen, '61a, b).

This idea received support from Nilsson's ('64) comprehensive study of visual cell development in the tadpole (*Rana pipiens*). He observed that the newly forming discs of both rods and cones were perpendicularly oriented with respect to the long axis of the cilium, even at the very early stages of development (Fig. 4 of Nilsson, '64). These discs were in con-

tinuity with each other and with the plasma membrane of the outer segment for one-half to two-thirds of their circumference and had the appearance of infoldings of the plasma membrane. Thus, Nilsson modeled disc formation as occurring by a process of infolding or invagination of the plasma membrane.

Disc morphogenesis also can be studied at the bases of *adult* visual cell outer segments. In fact, the continuity of the disc membrane with the plasma membrane at the bases of adult rods led to the suggestion that disc formation might continue in the adult (Cohen, '63; Nilsson, '64). It was definitively demonstrated in the autoradiographic studies of Young ('67) and Young and Bok ('69) that discs are continually generated at the bases of adult rod outer segments and are progressively displaced toward the tips of the outer segments, which are then shed and phagocytosed by the pigment epithelium.

An early suggestion that disc formation might continue in adult cones as well as in rods was made by Nilsson ('64), again, from the appearance of the discs at the bases of the outer segments. When it became clear from observations of cone disc shedding and pigment epithelial phagocytosis that mammalian cones also must be renewing their discs (see Anderson et al., '78), Anderson and Fisher ('76) examined the bases of adult squirrel cones and observed what appeared to be partially formed discs. Although the membrane there also appeared infolded, a re-examination led to the idea that new discs form by a process of outgrowth or evagination of the plasma membrane (Anderson et al., '78).

Eakin ('73) earlier had made the same interpretation for photoreceptor disc formation in the developing parietal eye of the Western fence lizard (*S. occidentalis*). These outer segments closely resemble those of the vertebrate lateral eye, and Eakin ('73) proposed that development of the discs occurred by a process of *evagination*. Based upon observations of the earliest stages of disc production in the developing visual cells of *Xenopus*, Kinney and Fisher ('78) recently proposed that discs form by repeated evagination of the plasma membrane of the connecting cilium.

These recent findings and a consideration of the implications of growth by evagination for an understanding of disc development led us to re-examine the question of disc morphogenesis in adult mammalian rods and cones. The adult cell, by comparison to the

developing cell offers distinct advantages for a study of this type. Developing outer segments are small, delicate, more difficult to fix, and are present at varying stages of development in any one retinal region (Nilsson, '64; Vogel, '78). It is especially difficult to align developing outer segments in the long axis of the cilium because of their small size, and because nearby cells vary widely in their orientation. In the adult, although the disc forming region at the base is more easily disrupted by tissue fixation and mechanical damage than is the remainder of the outer segment, it is possible to obtain well-aligned longitudinal sections through this region in both rods and cones.

This paper presents ultrastructural observations of newly forming discs in the basal region of mature mammalian rods and cones. These findings are most compatible with a model of disc morphogenesis in which the disc surfaces and disc rims develop by separate processes of growth from the plasma membrane of the cilium. The implications of this model will be discussed.

#### MATERIALS AND METHODS

Retinal photoreceptors in adult rhesus monkeys (*Macaca mullatta*), Eastern gray squirrels (*Sciurus carolinensis*), 13-lined ground squirrels (*Spermophilus tridecemlineatus*), and California ground squirrels (*Spermophilus beecheyi*) were examined by electron microscopy. All of the animals were fixed by intracardiac perfusion of a glutaraldehyde-formaldehyde mixture and post-fixed in buffered 2% osmium tetroxide. The fixation procedures have been published in detail elsewhere (Anderson and Fisher, '76, '79).

Thin sections (60-70 nm thick) were cut both parallel (longitudinal sections) and at right angles (tangential sections) to the long axes of the photoreceptor outer segments. Sections were placed on formvar coated slot grids for serial examination and stained with 1% aqueous uranyl acetate and Reynold's lead citrate.

#### RESULTS

The discs of a rod are arranged in a stack from the base of the outer segment (vitreous end) to the apex (scleral end) and are surrounded by the plasma membrane of the outer segment (Fig. 1). Each disc has two surfaces,

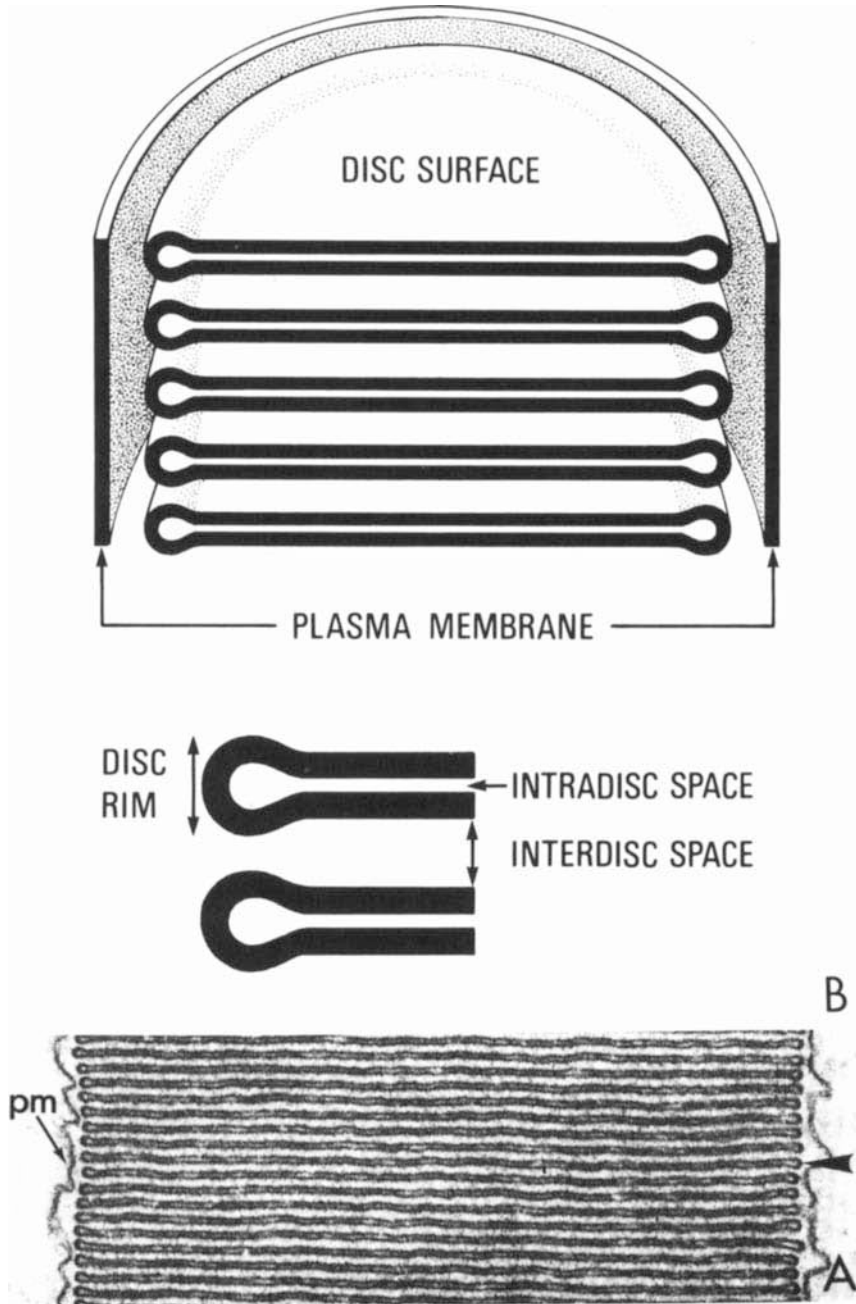


Fig. 1. Rod outer segment disc structure. A) Electron micrograph of a longitudinal section through a portion of a rod outer segment (rhesus monkey). pm, plasma membrane of the outer segment. arrowhead, the rim of a single disc.  $\times 75,600$ . B) Diagram of rod outer segment. The stack of mature discs is surrounded by the plasma membrane of the outer segment. Each disc has two surfaces which are joined at their perimeters by a rim. Inset: (After Hogan et al., '71). The disc surfaces enclose the intradisc space ( $20^\circ \text{A}$ ) and adjacent discs are separated from one another by the interdisc space ( $150^\circ \text{A}$ ). Diagram drawn by J. Weddell.

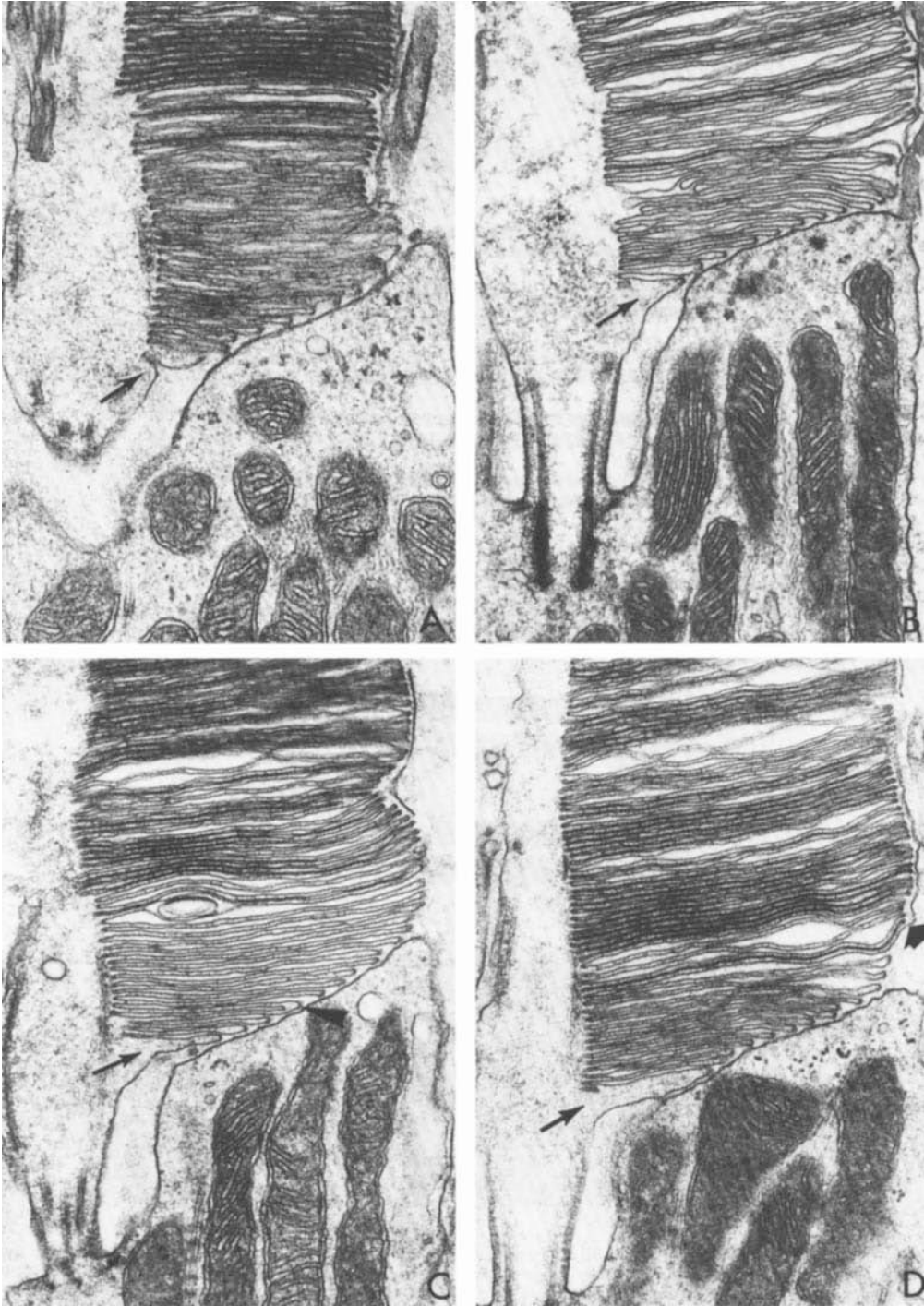


Fig. 2. Electron micrographs of longitudinal sections through the bases of four rod outer segments (rhesus monkey) arranged to suggest a developmental sequence for the most basal evagination (thin arrows) of each outer segment. The arrowhead in C shows an example of rim formation in an "immature" rod disc (see text, p. 510). The thick arrow in D indicates the first mature disc of the disc stack. A)  $\times 42,800$ . B)  $\times 35,700$ . C)  $\times 36,500$ . D)  $\times 36,500$ .

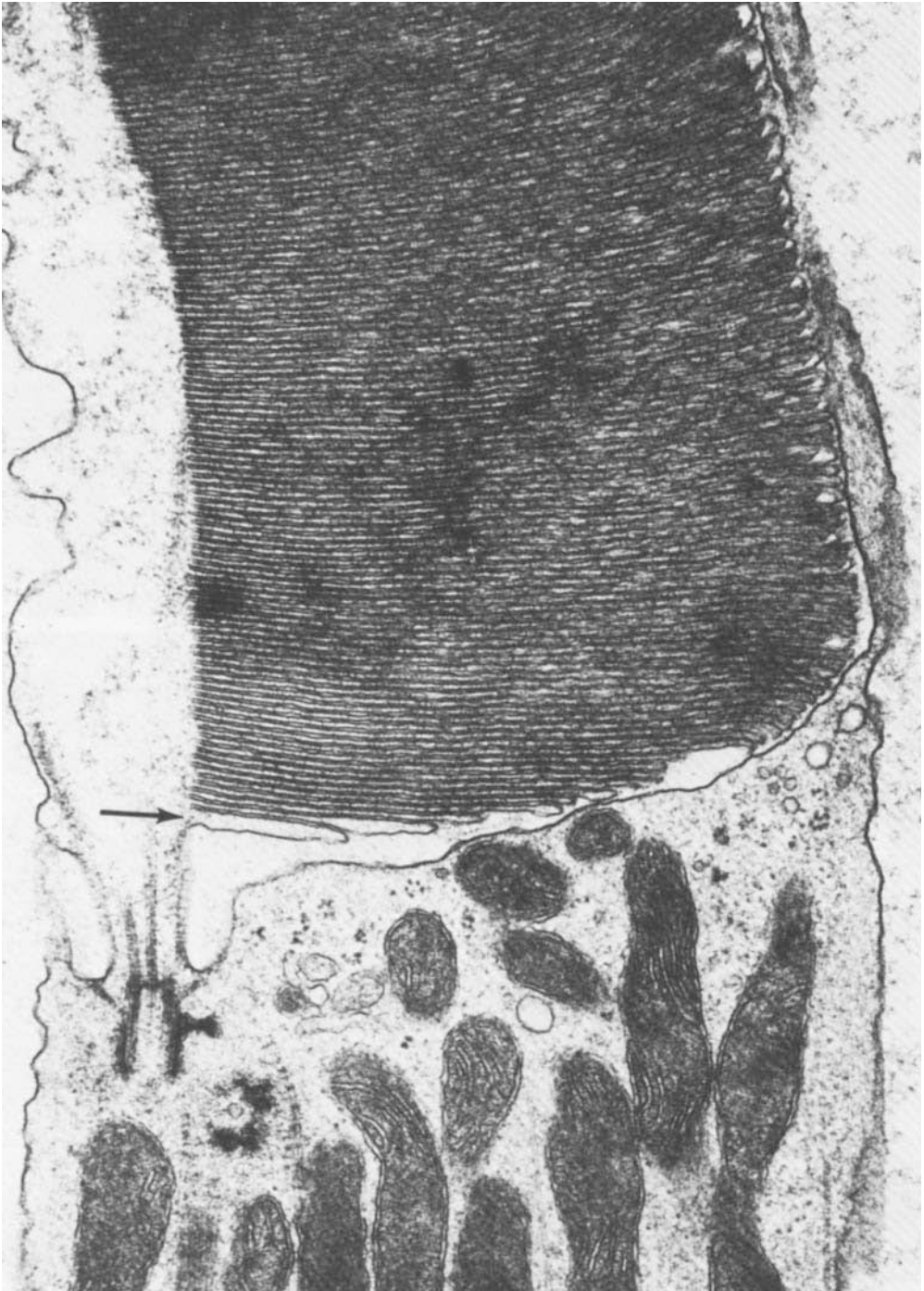


Fig. 3. Electron micrograph of a longitudinal section through the base of a cone outer segment (rhesus monkey). The arrow points to the most basal evagination; above this the evaginations progressively increase in diameter until they reach the diameter of the mature discs of the stack.  $\times 39,000$ .

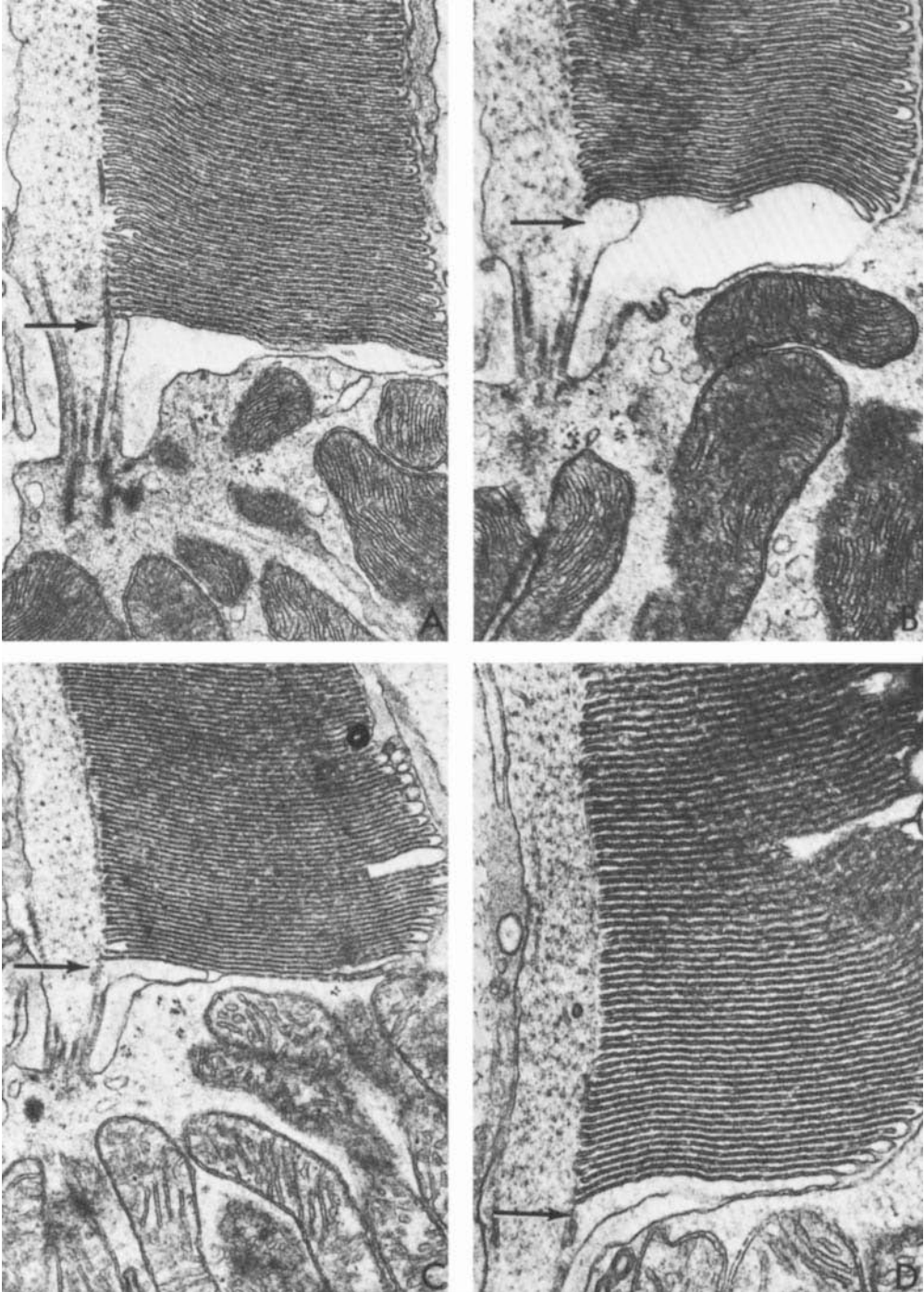


Fig. 4. Electron micrographs of longitudinal sections through the bases of four cone outer segments (ground squirrel) arranged to suggest a developmental sequence for the most basal evagination (arrows). The evagination is just visible in A as a slight bulge at the inner face of the cilium directly vitreal to a notch in the ciliary membrane. From B to D the most basal evagination progressively increases in diameter. It is filled with ciliary matrix and also becomes progressively thinner in width from B to D. Rims on some of the discs can be seen in each section. A)  $\times 30,400$ . B)  $\times 35,200$ . C)  $\times 25,600$ . D)  $\times 35,200$ .

vitreal and scleral, and a rim that joins the perimeters of these surfaces. The disc surfaces enclose the *intradisc* space, while adjacent discs are separated from each other by the *interdisc* space. In rods each mature disc is a self-contained structure, which is completely detached from the plasma membrane. In cones each mature disc appears to retain an attachment to the plasma membrane through which the intradisc space is open to the extracellular space around the outer segment.

#### *Bases of outer segments*

The morphology of the outer segment changes abruptly at its base. In rods it is possible to identify the first mature disc of the stack. By definition this is a disc that has grown to full size and appears to have separated completely from the plasma membrane (Fig. 2D, thick arrow). Vitreal to this disc there are a series of folds or steps in the plasma membrane that progressively increase in diameter in the vitreal to scleral direction (Fig. 2C, D). These folds are the newly forming discs, which usually have been interpreted as developing by an infolding or invagination of the plasma membrane.

There is an essential incompatibility between this interpretation and the appearance

of the folds. In any single longitudinal section the recent history of disc development in that outer segment can be traced. As predicted by the evidence that growth proceeds in the vitreal to scleral direction (Young, '67), the folds progressively increase in diameter in this direction. Yet, with rare exceptions, the ciliary edges of all folds, even the smallest ones, are vertically aligned with each other and with those of the mature discs (Fig. 2-4). This means that the folds are already fully invaginated from their inception, and growth of the discs cannot occur by a deepening of invaginations.

This indicates that the principal direction for the expansion of new discs must be outwards or away from the cilium, and the bases of individual outer segments are most compatible with this view. The region of partially formed discs is seen, then, to consist of a series of outpouchings or evaginations of the plasma membrane of the inner or centric face of the cilium—i.e., the surface of the cilium facing an extension of the geometric axis of the outer segment (Fig. 2C, D). These evaginations progressively expand in diameter as they are displaced sclerally. They also tend to become thinner (Fig. 2C, D) (Matsusaka, '74).

Longitudinal sections from different rod outer segments can be arranged, as in Figure 2, to suggest a developmental sequence. This

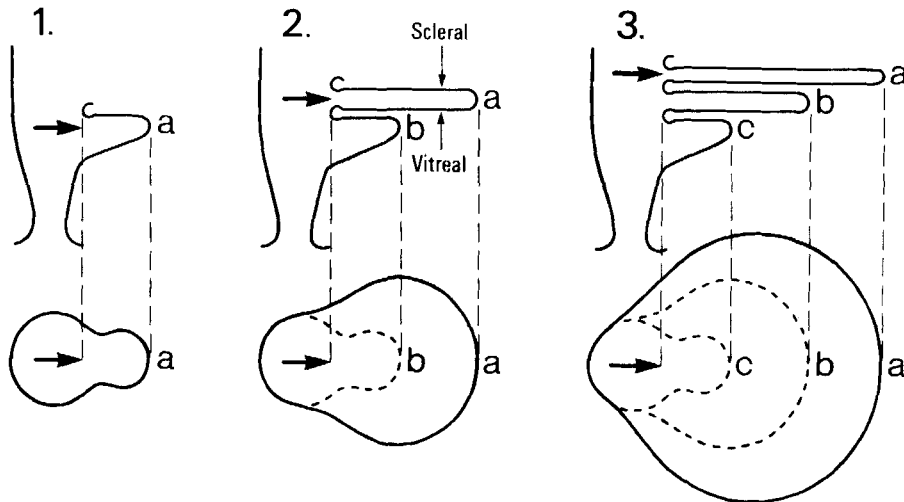


Fig. 5. Diagram of disc surface formation. The upper drawings (1-3) show the early developmental stages of three evaginations at the base of an outer segment as they appear in longitudinal section, while the lower drawings represent their appearances in tangential section. The sequence traces the development of three evaginations a, b, c. Each evagination develops as an outgrowth from the membrane of the inner face of the cilium. It consists of scleral and vitreal surfaces and is filled with cytoplasm. As an evagination expands in diameter it becomes progressively thinner. The successive addition of newer evaginations displaces the older evaginations sclerally. In tangential section each evagination appears as a circular bud that progressively expands in diameter. Diagram drawn by J. Weddell.

series traces the development of a single evagination, the newest one at the base of each outer segment (Fig. 2A-D, arrows). The evagination is barely visible in A as a small bulge at the inner face of the cilium (arrow), immediately vitreal to a small notch in the membrane (see below). In B-D the newest evagination appears as an outpouching of the membrane, which is filled with ciliary cytoplasm (or matrix) (arrows). As the evagination increases in diameter, growing away from the cilium, it also tends to become thinner.

The bases of cone outer segments (Fig. 3) are similar in appearance to those of rods. There is a region of partially forming discs at the base, but the transition to the stack of mature discs is less abrupt than in rods. This is because all or most of the cone discs remain attached to the plasma membrane (Cohen, '61b, '68; Anderson et al., '78). The transition to mature discs can be interpreted as the region of the stack where the discs have reached their maximum diameter. As in rods, it is possible to identify the partially formed discs as a series of evaginations of plasma membrane. Because the evaginations seem to adhere more closely to each other in cones, the *intradisc* space of each new disc can be observed to originate *between* adjacent evaginations.

Figure 4 traces the development of the newest evagination (arrows) in a series of longitudinal sections through four cone outer segments. The first evagination tends to be markedly broader than those scleral to it, and the thinning of the evaginations as they increase in diameter is much more pronounced in these micrographs than in the examples that we were able to find for rods.

#### *Development of disc surfaces*

A model for disc surface development by evagination is diagrammed in Figure 5. The upper drawings (1-3) show the early developmental stages of three evaginations at the base of an outer segment, as they appear in longitudinal section. Disc development begins at the very base of the outer segment in the region of the ciliary stalk. The membrane of the ciliary inner face pushes outward, forming a pouch or evagination that has scleral and vitreal surfaces, and is filled with cytoplasm. Successive evaginations are added, in turn, and the older evaginations are sclerally displaced. Each evagination becomes thinner as it expands in diameter. The lower drawing represent these structures as they might ap-

pear in tangential sections. It is assumed that they are generally circular and begin as small buds at the inner face of the cilium, which gradually grow larger in circumference. The relative size of the bud in relation to the cilium and the way it enlarges are only suggested.

These evaginations are the origin only of the *disc surfaces*. Each evagination will contribute one of its surfaces to each of two adjacent discs. In Figure 5 (3), for example, a disc will be formed between evaginations *a* and *b*. Evagination *a* provides the scleral surface of the disc, while evagination *b* provides the vitreal surface. The space between these membranes will be the *intradisc* space of the newly formed disc. Note that the *interdisc* spaces scleral and vitreal to this disc will be provided by the cytoplasm, respectively, of evaginations *a* and *b*. Thus, the progressive thinning of the evagination as it increases in diameter and moves sclerally appears to be a necessary step in the formation of the *interdisc* space.

#### *Development of disc rims*

To complete a disc it is necessary to join the two surfaces together at their perimeters. Rim formation also is initiated at the inner face of the cilium. It begins there as a small region of continuity between the vitreal surface of the evagination that has just formed and the scleral surface of the newest evagination. This region appears in longitudinal section as a notch on the inner face of the ciliary stalk (Fig. 2A, 4A). At that point on the ciliary inner face the membrane forms a "hairpin" turn, which joins the apposed disc surfaces at the ciliary arc of their perimeters. The mechanism of formation of this turn or loop and the timing of its appearance relative to that of the evagination that has just formed are not clear. It could be interpreted as occurring by an infolding of the membrane, but this is probably not the case because the loop is only 250 Å in diameter and aligned from its inception with the loops scleral to it; i.e., there is no evidence that this region migrates or infolds further towards the ciliary axis.

Figure 6 shows, in serial sections, the form of the newest evagination at the base of a monkey cone outer segment. Although the newest evagination is absent in *a*, there is a notch just scleral to the region in which it will appear in *b* and *c*. The form of the evagination, as suggested by its appearance in *b* and *c*, is that of a narrow bud. The notch in *a* is probably located at the lateral extent of the rim, which has formed just scleral to the bud in *c*.



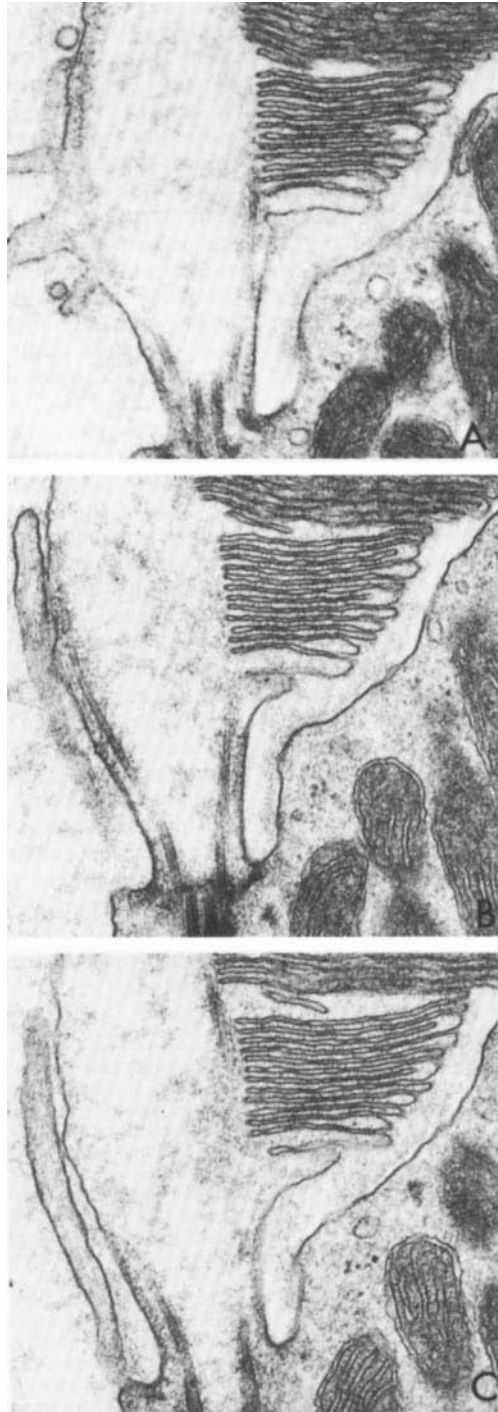


Fig. 6. Electron micrographs of serial longitudinal sections through the base of a cone outer segment (rhesus monkey). The newest evagination appears as a small bud in b and c. This evagination is absent in a, but there is a notch in the ciliary membrane just scleral to where the bud appears in b and c.  $\times 46,600$ .

This rim is not perfectly aligned in *c* with the more scleral evaginations, but the notch in *a* is so aligned; in addition, the basal 13 rims are not perfectly aligned in *b* and *c* with those more scleral, but they are almost so aligned in *a*.<sup>1</sup>

The continuation of the rim around the perimeters of adjacent disc-surface evaginations also can be observed in longitudinal sections. When a section passes through the rim, the disc surfaces appear in continuity and there is an envelope of plasma membrane nearby (Fig. 6C). The presence of the rim in any one longitudinal section indicates that the rim has formed in the region of that section but not that the rim is complete around the entire circumference of the disc. Since the plasma membrane envelope always is present when there is a rim, both the rim and the closure of the plasma membrane must occur simultaneously.

Tangential sections are better suited to illustrate the relationship between the newly forming rim and plasma membrane and the extent of rim formation around the perimeter of the disc. These are more difficult to obtain than longitudinal sections, since the plane of section must pass perpendicular to the cilium through the very narrow rim region (Cohen, '70). Figure 7 shows a tangential section of this type through the base of a monkey cone. The newly formed rim and plasma membrane appear on one side of the disc and are in continuity at the growth point (arrow). This is illustrated, as well, for monkey rods in Figure 8, where the micrographs also have been arranged to indicate a developmental sequence in the vitreal to scleral direction (A-D). In the most vitreal section (A) the newly formed rim and plasma membrane appear in continuity on both sides of the disc near the cilium (arrows). In B rim formation has progressed about half-way around the disc, bilaterally, while in C the process appears complete since the entire disc is enclosed by the newly formed plasma membrane.

Rods and cones differ in the timing of rim formation around the perimeter of the disc. In rods it is rare to observe rims between the growing evaginations at the base of the outer segment in longitudinal sections. Often, only the first mature disc in the outer segment exhibits a rim (large arrow, Fig. 2D); a rare exception of rim formation at the base appears in Figure 2C (arrowhead). Since the tendency, however, was to select sections that passed through the ciliary axis, only a small region of the circumference of the disc was sampled.

Off-axis sections were also examined, therefore, but it was still rare to find rims between the growing evaginations in rods. This is exactly opposite to what would be predicted if growth occurred by invagination. In that case the rim is the leading edge of the invagination, and therefore rims at the basal region of growing discs should have been common in rods. Thus, it appears that in rods the major portion of the rim forms only when the evaginations have grown almost to their full diameter. Since it is difficult to observe rims in the partially formed discs, even those of near complete diameter, we suggest that rim formation occupies a relatively brief period of time.

In cones, rim formation begins earlier than in rods. Rims can be observed, for example, in Figure 6C, between the small evaginations at the base of the outer segment. It is also common in any one section to observe individual discs and clusters of discs with rims, alternating with discs that do not have rims (Fig. 4). In cones, therefore, rim formation appears to occur concurrently with the growth of disc surfaces.<sup>2</sup> It has been shown previously for cones that rim formation must be somewhat asymmetrical since the disc openings along the outer segment tend not to be aligned (Anderson et al., '78). Moreover, these openings tend to become smaller from the base to the apex of the outer segment, indicating that rim formation continues along the length of the outer segment (Anderson et al., '78). In cones rim formation, although beginning earlier in disc development than in rods, is probably a slower process that may never be completed.

<sup>1</sup>Very small lateral displacements of one or more rims may appear at the ciliary membrane of the inner face. These can be explained by irregularities in the vertical alignment of the outer segment and by minor obliquities in the section. Most importantly, since the rim is circular in shape, the likelihood increases as disc diameter decreases that the knife will pass through different points on the perimeter of the rim. For the smallest discs at the very base, any displacement of the knife on the perimeter of the rim will appear in longitudinal sections as a lateral displacement.

<sup>2</sup>The partially formed rod discs at the base often appear more disrupted than in cones. The evaginations tend to separate and lose their perpendicular orientation with respect to the cilium. A possible explanation is that the absence of rims between the growing disc evaginations in rods allows them to separate during tissue preparation; while they tend to hold together in cones because of the presence of rims between the partially formed discs. (This is not to imply that the rim is the only factor responsible for the adherence between the disc surfaces.)

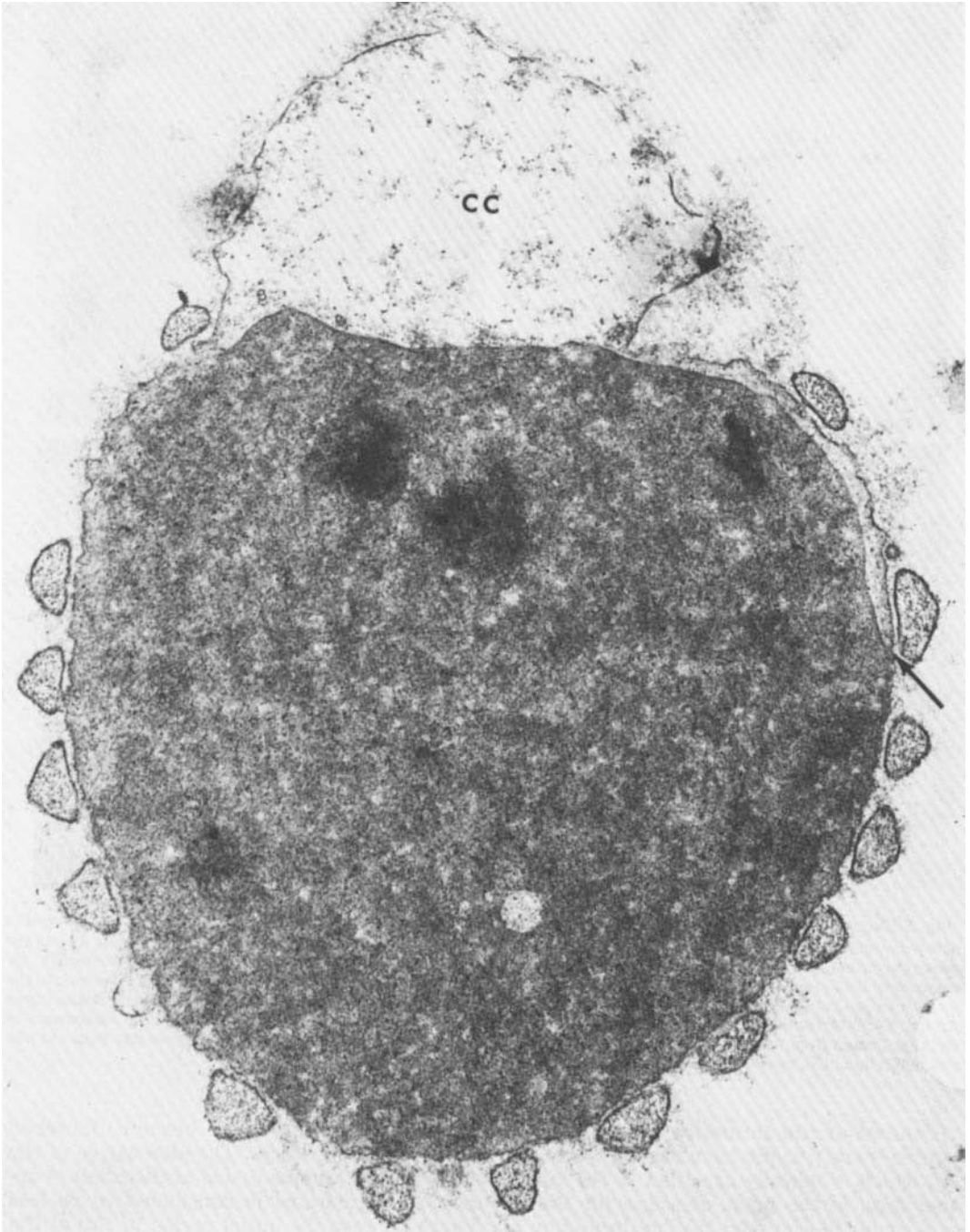


Fig. 7. Electron micrograph of a tangential section through a disc near the base of a cone outer segment (rhesus monkey). The newly formed rim and plasma membrane appear in continuity on one side of the disc at the growth point (arrow). Calycal processes surround the outer segment. CC, connecting cilium X 44,000.

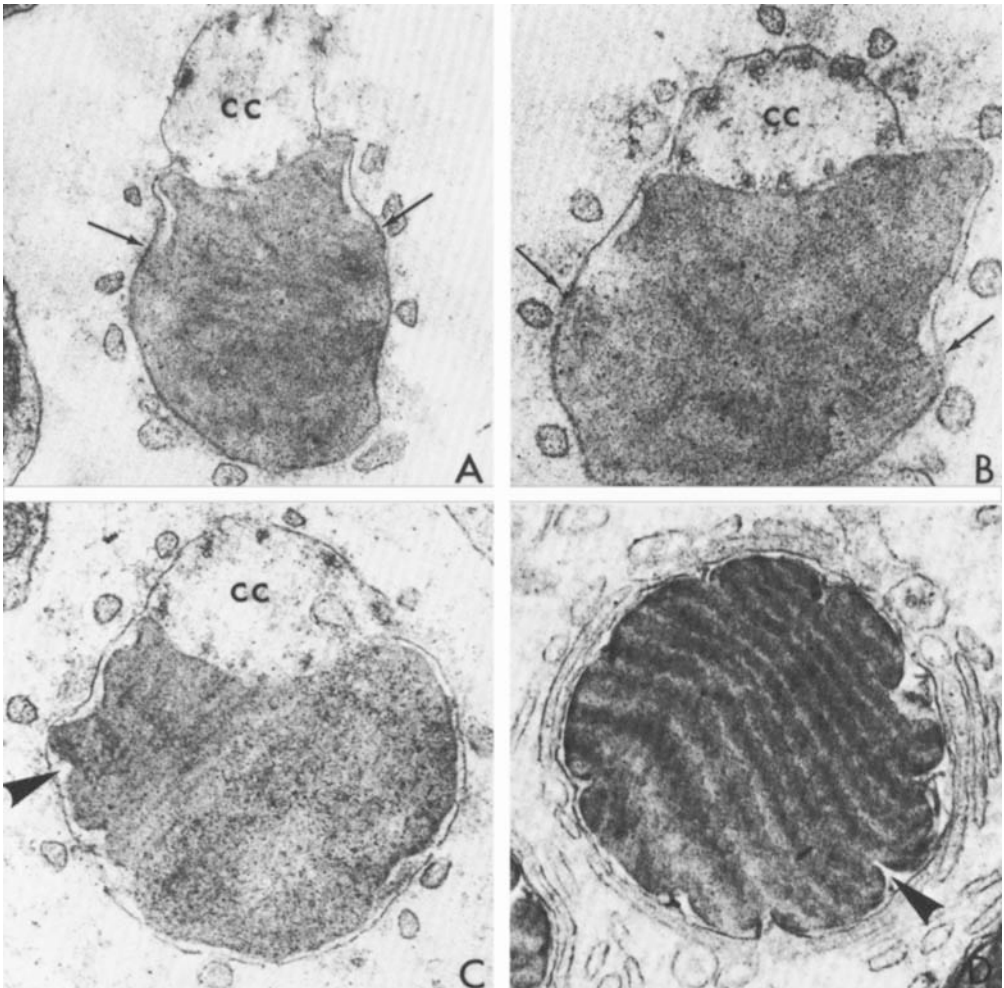


Fig. 8. Electron micrographs of tangential sections through the bases of rod outer segments (rhesus monkey) arranged to show a developmental sequence in the vitreal to scleral direction (A-D). In A the newly formed rim and plasma membrane appear in continuity near the cilium on both sides of the disc (arrows). In B rim formation has progressed bilaterally, approximately half-way around the disc. In C the rim and plasma membrane are completely formed but the incisesures into the disc interior are only beginning to form (arrowhead). D shows a mature disc near the tip of the outer segment. The incisesures into the disc interior (arrowhead) give the disc a scalloped appearance. The outer segments in A-C are surrounded by calycal processes from the inner segments, while in D the outer segment is surrounded by apical processes from the pigment epithelium. CC, connecting cilium. A)  $\times 35,000$ . B)  $\times 48,000$ . C)  $\times 31,000$ . D)  $\times 38,000$ .

A model of rim formation is presented in Figures 9 and 10. We have assumed that the rim, which is initially specified at the ciliary inner face, is the point of origin for the remainder of the rim; and that its formation in rods is bilaterally symmetrical. The continuation of the rim around the disc perimeter is visualized as a *growth event* that zippers together the disc surfaces and simultaneously seals the plasma-membrane edges of the

evaginations that have become detached from the disc surfaces. The alternative of rim formation by simple fusion of the edges of apposed evaginations is considered to be less likely because the initial region of rim at the ciliary inner face is not formed by fusion. Also, in cones, rims begin to form between the small evaginations and, therefore, the rim must continue to grow as the disc surfaces expand in diameter.

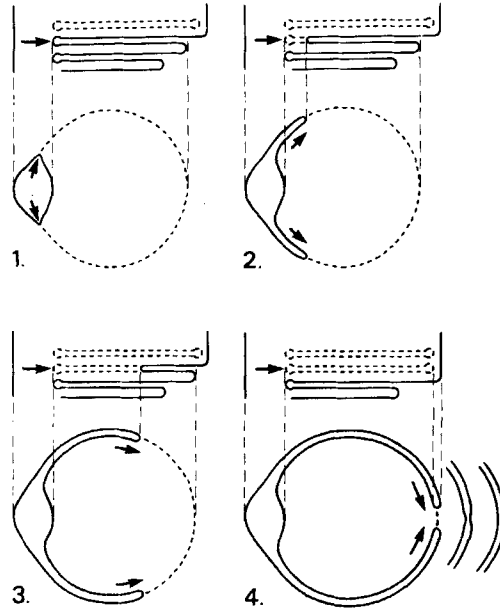


Fig. 9. Diagram of rim formation in rods. The diagram shows the formation of a rim between adjacent disc surface evaginations in longitudinal sections (upper drawings, 1-4) and in tangential sections (lower drawings, 1-4). The rim originates from the region of ciliary membrane between adjacent evaginations (arrows, upper drawings, 1-4). Its growth around the perimeters of the evaginations is assumed to be bilaterally symmetrical in rods. The growing region of ciliary membrane is folded at the growth point (arrows, lower drawings, 1-4) so that the inner portion is the rim, while the outer portion is the new plasma membrane. During rim formation the disc surfaces become detached from the plasma-membrane edges of the evaginations. These edges are then sealed by the new plasma membrane. Diagram by J. Weddell.

Figure 9 diagrams rim formation around the perimeter of a rod disc that has almost reached its full diameter. A bilaterally symmetrical growth of ciliary membrane joins the disc surfaces. Since the rim forming region of membrane is continuous with the plasma membrane of the cilium, its growth around the disc perimeter produces a fold at each of the two growth points. During rim formation the disc surfaces become detached from the plasma-membrane edges of the evaginations, and the outer membrane of the fold seals the plasma membrane around its circumference. The growing membranes finally meet at the disc perimeter opposite the cilium (Fig. 9, 4) and final closure is by fusion. Note that the space between the two membranes of the fold becomes the space that exists in the outer segment between the disc rim and the plasma membrane.

These events are also illustrated in the three-dimensional drawings of Figure 10. The smaller drawings (left) view them from outside the outer segment, while the interrupted lines

indicate the plane of longitudinal section for each of the larger drawings (right). To better visualize the rim forming region, it is presented in tangential section at the scleral end of each outer segment. In A the rim region of the ciliary inner face has not yet begun to grow. In B the rim and plasma membrane have partially formed but have not yet reached the plane of section. In C the plane of section is through a region where the rim has formed.

#### *Incisure formation*

Vertebrate rod discs have clefts or incisures, and we were interested in the timing of incisure formation relative to the formation of the disc surfaces and the rims. In particular, we wondered if the disc-surface evaginations contained incisures, in which case the rim, while forming, would follow the pattern already laid down in the disc surface. This possibility did not seem likely, however, because it would produce incisures in the plasma membrane, and these have never been

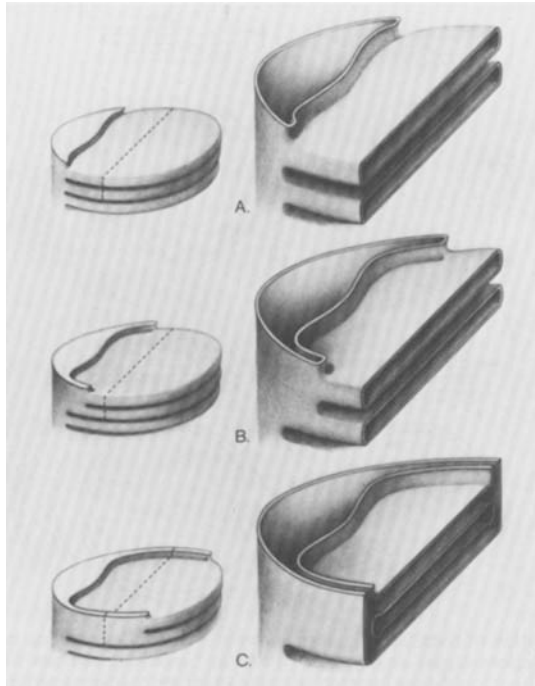


Fig. 10. Three-dimensional drawing of rim formation. The development of the rim progresses from A to C. The small drawings on the left indicate the plane of longitudinal section (interrupted lines) for the larger drawings on the right. Rim formation also is shown in tangential section at the scleral end of each outer segment. In A and B the rim has not yet reached the plane of longitudinal section. The new plasma membrane can be seen in the surface view to have sealed the edges of adjacent evaginations. The growth of the rim cannot yet be seen in the longitudinal section but can be seen in the tangential section. In C rim formation extends beyond the plane of longitudinal section and the rim of the new disc can be seen in longitudinal section. Drawings by J. Weddell.

observed. Since the incisures are quite shallow in monkey and squirrel rods, we looked for them in off-axis sections through the partially formed discs at the base. As expected, incisures were never observed in the disc-surface evaginations but only after the rim had formed. Thus, incisure formation must occur by an infolding of the rim and disc surfaces together. Incisure formation occurs, however, before rim formation is complete around the entire perimeter of the disc, as shown in Figure 11. Closure of the disc, therefore, is not a prerequisite for incisure formation. This is also known from the ultrastructure of cat and monkey cone discs, which have a single incisure, but which are probably open at some point on their circumference (Steinberg and Wood, '75, Anderson and Fisher, '79).

## DISCUSSION

### *Comparison of invagination and evagination*

The model of disc formation proposed here now can be compared with the earlier model of disc formation by invagination (Nilsson, '64). Although in both models disc development begins at the very base of the outer segment and proceeds sclerally, the two models propose different mechanisms for the formation of the discs. In the invagination model the disc surfaces and disc rim form a continuous structure from the start of disc development. Development is initiated by an invagination of the outer segment plasma membrane, with the rim forming the leading edge of the growing disc. As the disc is displaced sclerally, the entire disc, rim and surface, grow toward the

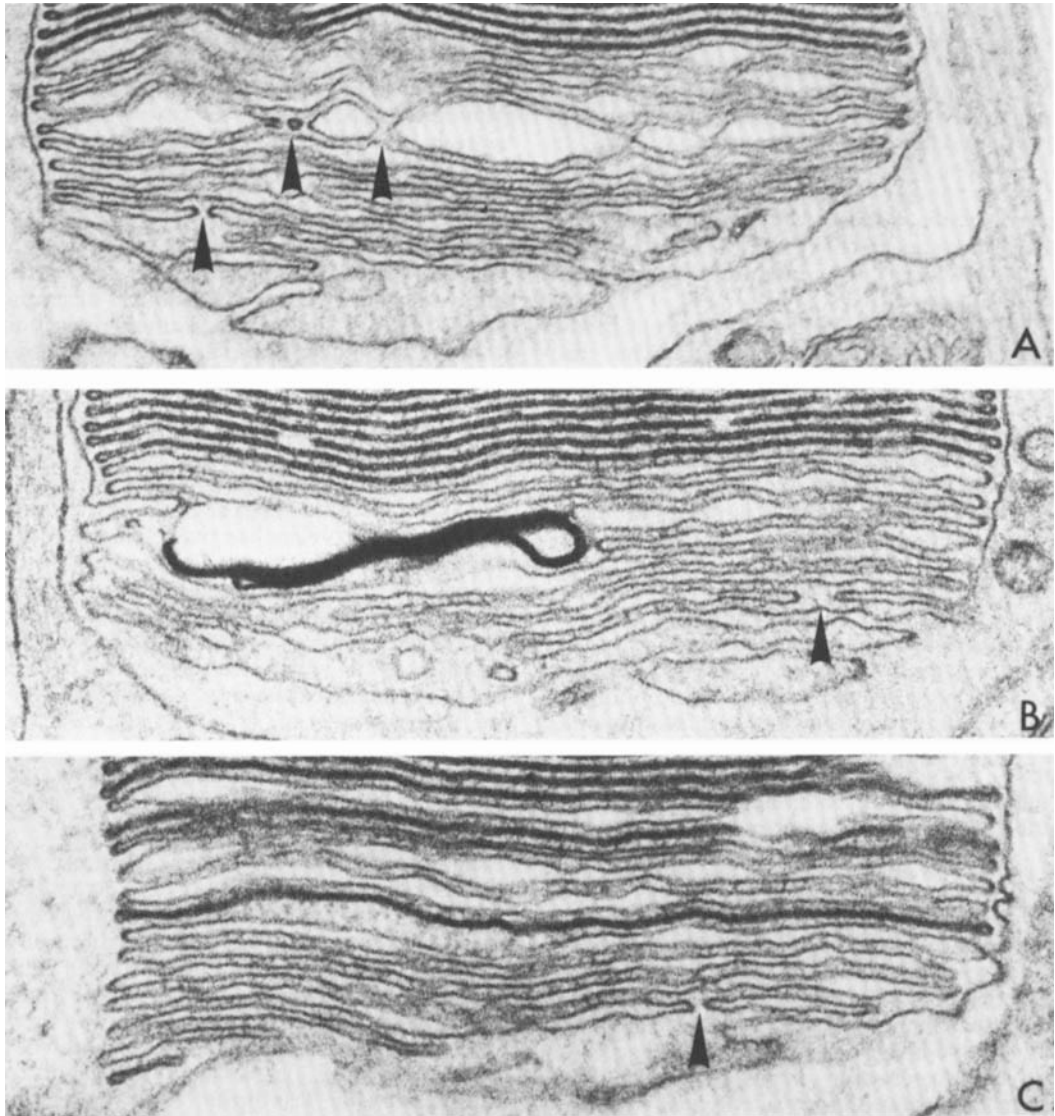


Fig. 11. Electron micrographs through the bases of three rod outer segments (ground squirrel). The sections are "off-axis" in relation to the ciliary stalk. They have been selected to show examples of incisure formation (arrowheads) in newly forming discs before the rim has completed its growth around the perimeter of the disc. A)  $\times 81,000$ . B)  $\times 62,500$ . C)  $\times 84,000$ .

ciliary axis. The attachment point of the disc to the plasma membrane gradually narrows and the disc eventually "pinches off" from the plasma membrane.

We have shown, however, that the region of disc formation at the bases of adult mammalian rods and cones conforms more closely

in ultrastructural appearance to an evagination model. A number of observations suggested this mechanism: (1) The edges or rims of the smallest discs at the inner face of the cilium were aligned vertically with the stack of mature discs. (2) The increase in diameter of the forming discs was in a direction away from

the cilium. (3) Partially formed rod discs tended to have only a small arc of rim at the inner face of the cilium. (If development occurred by infolding, a larger area of rim circumference should have formed). (4) The rim was initially specified as a membrane continuity at the inner face of the cilium between adjacent disc-surface evaginations. (5) Finally, the micrographs showed evaginations of the ciliary membrane that were filled with ciliary matrix and which became thinner as they were displaced sclerally.

The principal features of the present model can be summarized as follows: The disc develops from the plasma membrane of the cilium at its inner or centric face, and growth is directed outward or away from the cilium. The disc surfaces and disc rims are *alternately* specified at the inner face of the cilium at separate regions—the disc surfaces as membrane evaginations and the disc rims as hair-pin loops of membrane that maintain membrane continuity between adjacent evaginations. The initial specification and the continued growth of the surfaces and rims appear to occur by separate processes. The disc surfaces grow by expansion and thinning of the evaginations as they are displaced sclerally. Rim formation, which begins at the inner face of the cilium, progresses bilaterally from that point around the circumferences of adjacent disc-surface evaginations.

#### *Some implications of the model*

##### Plasma membrane of the outer segment

In rods the discs are completely enclosed in the plasma membrane of the outer segment. In cones the enclosure is probably not complete since many, and perhaps all, discs retain a small opening to the extracellular space. In both photoreceptor types, however, the plasma membrane is continually lost when the tips of the outer segments are shed and phagocytosed by the pigment epithelium. The current model provides for the *continuous renewal* of the outer-segment plasma membrane during the process of disc formation. The edge of each disc-surface evagination (not to be confused with the *rim* of the disc) represents newly generated plasma membrane. During rim formation the disc surfaces become detached from the plasma membrane of the adjacent evaginations and these plasma-membrane edges become joined. Thus, each time an evagination forms and each time that two evaginations seal at their edges, new plasma membrane is added to the outer seg-

ment. In cones, since this membrane remains in continuity with the discs, it must be displaced sclerally as each disc is so displaced. (Molecular replacement of the plasma membrane may still occur as the disc is displaced sclerally.) In rods it is not clear if this occurs once the disc completely detaches from the plasma membrane.

##### Disc diameter

In the stack of mature rod and cone discs there occasionally are individual discs or groups of discs that vary in diameter from the others of the stack. The reason for the failure of all discs to reach the same diameter is not known. The proposed model, however, provides for the possibility of variations in disc size. The disc evagination as it expands away from the cilium must grow out to a uniform diameter if all of the discs are to be of the same size. It is easy to visualize that changes in the rate of delivery of new membrane components or, perhaps, in the rate of specification of new discs might influence disc diameter. [Such rate changes may occur as a function of phase of the dark/light cycle, which has been shown to be a key determinant of the rate of new disc addition. (Behsharse et al., '77).] The alignment of these discs would be uniform at the ciliary side, but would vary at the opposite side.

##### Structural uniqueness of the rim

The present view of disc formation may provide a developmental basis for a structural difference between the rims, and the disc surface. That disc rims may differ structurally was already suggested by Sjöstrand ('49) from their distinct ultrastructural appearance. Additional evidence for a difference was their relatively greater resistance to osmotic stress (DeRobertis and Lasansky, '61) and to solubilization by Tris buffer (Falk and Fatt, '69) compared to the surfaces. This possibility has recently received strong support from the findings of Papermaster et al. ('78) showing that a large protein of molecular weight about 290,000 daltons (Papermaster et al., '75, '76) is confined to the disc rims in frog rods. It has received additional support from the recent freeze-fracture observation of a particulate structure that is confined to the edge of the disc (Sjöstrand and Kreman, '78).

This structural distinctiveness of the rim is incorporated into the present model by an assembly process that produces either rim or surface membrane in a sequence where they are alternately specified. It is almost as if the



rim, because of a more "rigid" structure, represents a region of membrane that remains fixed and will not evaginate.

#### Growth points

Each origin at the cilium of a disc-surface evagination is a growth point, as are the rim forming regions between these evaginations. Since more than one evagination grows at once, new membrane is simultaneously added to each of a number of growing evaginations. There is thus a *stack of growth points* at the base of the outer segment.

The question of whether new membrane components reach the outer segment through the cytoplasm via the membrane or by both routes has not yet been answered. If the pathway is via the membrane of the outer segment and ciliary stalk, as recent evidence suggests (Papermaster et al., '75), then the flow of components would continue to each separate growth point. Since each disc-surface evagination is in continuity with the plasma membrane of the cell, the new components would reach the growing disc via this route.

As rim formation proceeds, the area of disc-surface membrane that is in continuity with the plasma membrane gradually decreases. In rods, when rim formation is completed the disc is no longer attached to the plasma membrane. The only route for membrane component addition or loss is then through the cytoplasm of the outer segment; and there is evidence from the autoradiographic studies of Bok and Young ('72) that membrane renewal occurs through this route.

The evidence so far indicates that most, if not all, cone discs remain attached to the plasma membrane as they are displaced to the apex of the outer segment. Therefore, although renewal may occur via a cytoplasmic pathway, a membrane pathway is open along the entire outer segment.

If disc formation occurs via the membrane route, then this route also may be essential for subsequent changes in disc *structure*. In cones the discs undergo substantial changes in diameter along the entire outer segment. After the initial expansion of the disc at the base of the outer segment to its maximum diameter, it then undergoes, in many cones, a progressive reduction in diameter as it is displaced sclerally. This change in disc form is at the basis of the taper of cone outer segments. In selected cat cones, for example, where the taper is not very obvious, disc diameter still decreases by as much as 15%

from the base to the apex of the cone (Steinberg, unpublished observation). At the same time, another change in disc structure occurs in cat cones, a progressive deepening of the disc's single incisure (Steinberg and Wood, '75). Thus, cone discs must be capable of shrinking as they ascend the outer segment, and the loss of membrane components may occur through the attachment point.

#### *Relationship to invertebrate visual cells*

Eakin ('63) has divided the light-sensitive organelles of animals into two types, ciliary and rhabdomeric. Both can be found among the invertebrates, but it is the ciliary type that most closely resembles the visual cells of vertebrates; and it is likely that a ciliary line of evolution extends from the invertebrates to the vertebrates (Eakin, '65). A survey of the structure of these invertebrate ciliated visual cells demonstrates that the membrane specializations of the ciliary shaft are outgrowths. The sensory cells in the ocelli of hydromedusae and sea stars, for example, have microvilli projecting from the ciliary shaft (Eakin, '72). In other forms the extensions from the ciliary shaft are flattened into lamellae (Eakin, '72) as in the apical region of a ctenophore, *Pleurobrachia pileus* (Horridge, '64). In *Amphioxus* the ciliated ependymal cells, which are presumed photosensitive, also have lamellae-like appendages (Eakin, '72).

These primitive cells differ considerably in morphology from vertebrate rods and cones; there are missing links in the evolutionary pathway. Yet, the formation of photoreceptor membrane by an outgrowth from the ciliary shaft is clearly present in these primitive cells. A major difference from vertebrate cells is the absence of disc formation. Could it be that the lamellae of invertebrate cells are the homologues of disc-surface evaginations, and the mechanism for joining the evaginations together is absent?

In conclusion, a new model of disc development in vertebrate photoreceptors has been presented. This model emphasizes that disc development occurs by essentially the same mechanisms in rods and cones. It proposes a key role for the membrane of the inner face of the cilium. The model indicates that disc development occurs by separate processes of membrane growth for the disc surfaces and the disc rim, the major features being that the surfaces and rim are alternately specified at the inner face of the cilium, that the surfaces develop by evagination of ciliary membrane,

while the rim initially develops by the specification of a "hairpin" loop of membrane between adjacent evaginations, a region of ciliary membrane that does not evaginate. This region grows out bilaterally around the circumferences of adjacent evaginations, zippering them together to form the disc rims, and at the same time sealing the detached plasma-membrane edges of the evaginations. The region at the inner face of ciliary membrane at the base of an outer segment consists then of a stack of growth points, alternately specified into rim and surface, and simultaneously forming a series of new discs. Finally, development of the disc surfaces by evagination in vertebrate visual cells resembles the mechanism of membrane specialization in ciliated invertebrate visual cells.

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