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CILIARY ROOTLETS IN PRIMATE RODS AND CONES.(U)
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While ciliary rootlets have been reported in the inner segments of rods, little was known of their presence in the remainder of rhesus monkey rods and their presence in cones. In this study, we have observed striated rootlets in rhesus monkey rods and cones. The striated rootlet system in some rods and cones appears to form direct communication between the outer segments and the synapses. In addition, there are many complex associations, interconnections, and bridges between rootlet filaments and organelles of the photoreceptors.

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CILIARY ROOTLETS IN PRIMATE RODS AND CONES

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JUNE 1982

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Ciliary Rootlets in Primate Rods and Cones- Schuschereba and Zwick

In conducting the research described in this report, the investigation adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care, Institute of Laboratory Animal Resources, National Research Council.

Jm O Marshall Jr 7 Jun 82
(Signature and date)

CILIARY ROOTLETS IN PRIMATE RODS AND CONES

-Schuschereba and Zwick

ABSTRACT

While ciliary rootlets have been reported in the inner segments of rods, little is known about their presence in the remainder of rhesus monkey rods and their presence in cones. In this study, we have observed striated rootlets in rhesus monkey rods and cones. The striated rootlet system in some rods and cones appears to form a direct communication between the outer segments and the synapses. In addition, there are many complex associations, interconnections, and bridges between rootlet filaments and organelles of the photoreceptors. Complex architecture of the rootlet, its associations, and variable occurrence within photoreceptors suggests that it has dynamic functions. It is well known that mammalian photoreceptors do not contract; however, they can align their outer segments with the exit pupil. The striated rootlet may subserve the alignment function in both rods and cones.

CILIARY ROOTLETS IN PRIMATE RODS AND CONES

--Schuschereba and Zwick

Ciliary rootlets in the inner segments of human and primate photoreceptors are reported (1,2) and are similar to the roots and rootlets in many other organisms and cell types (3). Photoreceptor rootlets are composed of 60-70 Å diameter filaments which usually form several filament bundles that contain dense 600-800 Å periodic striations along their length. The rootlet bundles within photoreceptors seem to originate near the cilium basal body and course through the inner segment toward the nucleus. Where and how rootlets terminate are unclear, except in the guinea pig where they have been observed intermittently throughout the length of the photoreceptor and are believed to end in the spherule (4). The functional significance of rootlets is also unclear; however, Pitelka (3) reviewed studies which suggest that they may subserve contraction, stimulus conduction, and anchorage functions.

Although mammalian photoreceptors are not known to contract in response to either light or dark adaptation, some minute cellular movements may be possible since the actomyosin system has recently been demonstrated in photoreceptor inner segments (5,6). Studies (7,8) over the last decade have reported that photoreceptors must maintain a special alignment to maximize their quantum catch. Furthermore, speculation suggests that the alignment process is both minute and active (8,9).

The present study was undertaken to determine if the rootlet was present the full length of primate rods and cones, and if primate rootlets structurally serve as an alignment system for photoreceptors.

MATERIALS AND METHODS

A normal eye from a light-adapted rhesus monkey (*Macaca mulatta*, 3 yr old and weighing 3 kg) was used in this study. The animal was anesthetized with sodium pentobarbital (10 mg/kg intravenously), the eye was enucleated, dissected through the equator, and immersed in fixative (4C, 3% glutaraldehyde/2% paraformaldehyde in 0.1M phosphate buffer, pH7.4). Further dissection was carried out in fixative under a dissecting microscope, until the macula and 1 mm^2 pieces of paramacular tissue were isolated. After 4-hr fixation (4C), tissues were washed, en bloc stained with aqueous 0.5% uranyl acetate, dehydrated in graded ethanols, and embedded in an Epon-Araldite (1:1) mixture.

Longitudinal and transverse sections were cut thick and thin; Thick sections were stained with methylene blue - Azure II and 0.2%

basic fuschin (10). Thin sections were stained with 2% uranyl acetate in 50% ethanol and in lead citrate, then were examined and photographed with a Philips 201 electron microscope at 60 KV.

RESULTS

Longitudinal and transverse sections of both rhesus retinal rods and cones show striated filaments arranged in bundles, mainly running parallel and occasionally tangential to the major axis of the photoreceptor inner segment. In rods and cones, the rootlet bundle striations were in lateral registry throughout the ellipsoid region even though separated by mitochondria. The striated filaments were seen intermittently throughout the full length (ellipsoid to spherule) of some rods, and mainly in the ellipsoid and pedicle regions of some cones. Wavy filaments were similarly distributed in both photoreceptors and contained peak-peak undulations corresponding to the rootlet striations.

RODS

Filament bundles make major connections to the basal bodies in the outer ellipsoid regions. Smaller bundles in the ellipsoid region formed connections between the plasma membrane of the outer ellipsoid region and mitochondria and between major rootlet bundles and the plasma membrane. Occasionally, non-striated filaments were seen bridging the microtubules and each other. In addition, there was convergence of striated and wavy filaments. Periodic densities similar to rootlet bundle densities (Figure 1) were seen at the calyx base. Occasionally rootlet bundles that extended into calyces, connected with the larger and more central photoreceptor rootlet bundles. In rods, rootlet filaments coursed and sometimes ended near the outer limiting membrane but we saw no direct connections. Rootlet bundles also coursed axially within the inner receptor fiber, past the outer limiting membrane and around the nucleus. Segments of rootlet appeared in the outer plexiform layer and in rod spherules (Figure 2). In spherules, the filaments were closely associated with mitochondria and smooth endoplasmic reticulum and suggested that they merged with the synaptic membranes (Figure 2 Inset). The synaptic membranes of the spherule occasionally contained periodic densities that corresponded to the interval between densities on the merging rootlet bundle.

In cross-sections of rods, rootlet bundles coursed toward mitochondria, perpendicular to the receptor axis. Striated filaments in cross-section decreased from the outer ellipsoid to the inner myoid regions (300 vs 15), with a corresponding decrease of wavy filaments.

CONES

The striated rootlet bundle had similar structure, connections,

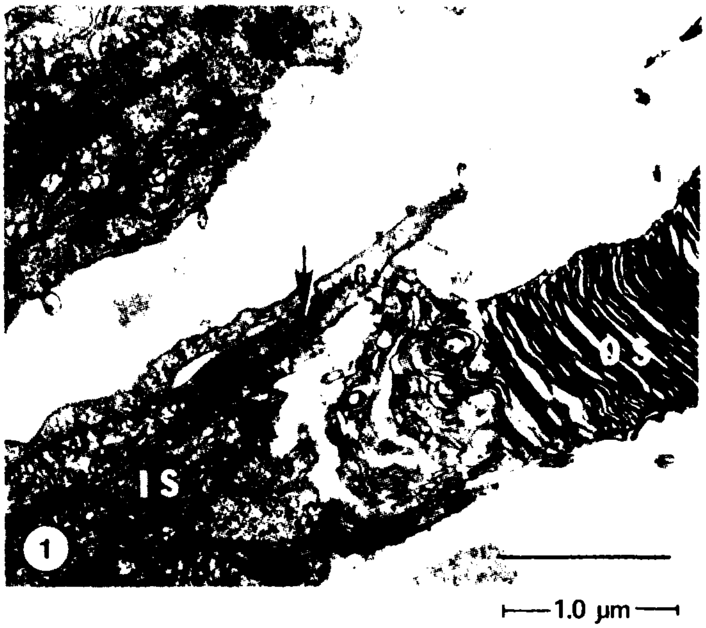

Figure 1. Electron micrograph of rhesus monkey rod. A rootlet (arrow) extends from the inner segment (IS) into the calyx (C). Outer segment (OS).

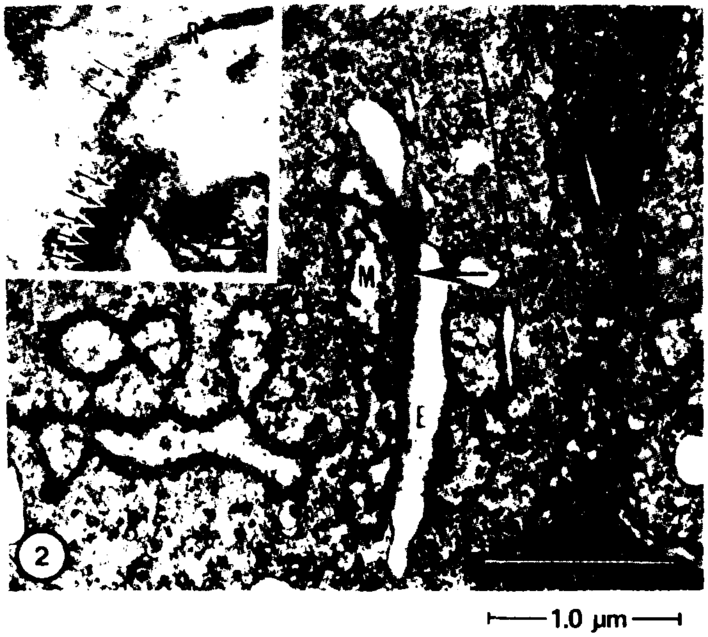

Figure 2. Electron micrograph of the rhesus monkey rod synapse, the spherule (S). A rootlet (arrow) is flanked by a mitochondrion (M) and endoplasmic reticulum (E). Inset: Rootlet filaments (R) with periodic densities appear to course toward the dense synaptic membranes (S) of the spherule. Densities (arrows) on the synaptic membranes (S) have about the same interval as the densities (arrows) on the rootlet (R).

and associations described in rods, but were observed less frequently. Rootlet filaments also occurred at calycal bases but, unlike rods, points of apparent termination were near dense granules (Figure 3). Other observations suggested that wavy filaments joined the calyx core filaments at the calyx base and that rootlet bundles made perpendicular insertions, 90 degrees off the main cell axis, to different rootlet bundles.

Major bundles, in the ellipsoid region that connected with the basal body, coursed axially between the mitochondria for a short distance (about 1 um) before they lost their striations. In addition, some specimens revealed weakly striated filaments continuing an intramitochondrial course toward the inner-ellipsoid/outer-myoid regions. The major difference between rod and cone rootlets was that no cross-sectional or longitudinal profiles of the filaments, to this date, have been observed in the cone myoid, outer nuclear layer and inner receptor fiber (outer plexiform layer) regions. However, striated rootlets (Figure 4 and inset) and wavy filaments appeared again in the cone pedicle.

In cross-section of cone inner segments, the rootlet bundle was much smaller (10-20 filaments/bundle) than in rods. Rootlet filaments appeared to splay into groups of 5-12 as they coursed deeper between the central core of inner segment mitochondria. As the filaments approached the myoid region their cross-sectional profiles disappeared, but microtubules surrounded the more peripheral mitochondria and continued toward the synapse.

DISCUSSION

The existence of rootlet filaments in both proximal and distal regions of rods and cones suggests that there may be rootlets throughout the length of some photoreceptors (Figure 5). Both striated and non-striated filaments may make up the full length filament course in photoreceptors. Therefore, a communication line is highly suggested from the outer segments to the synaptic contacts, via the outer segment microtubules and rootlet filaments. Dynamic functions of the rootlet are suggested by the fact that rootlet filaments have a complex intraphotoreceptor architecture and appear to insert or bridge, or both, into many intraphotoreceptor membranes, including basal bodies (microtubule organizing and kinetic centers). Dynamic functions of the photoreceptor rootlet may include the tethering of organelles to the basal body to facilitate interaction of organelles and their products (3). This may include the interaction of both chemical and physical events in the outer segment, inner segment and synapse.

Striations at the calyx base in rods and cones suggests that calycal filaments communicate with the striated filaments. The

Figure 3. Electron micrograph of rhesus monkey cone. A rootlet (r) extends from the inner segment (IS) into the calyx (c). Rootlets appear to terminate near the dense granules (arrow) at the base of the calyx.

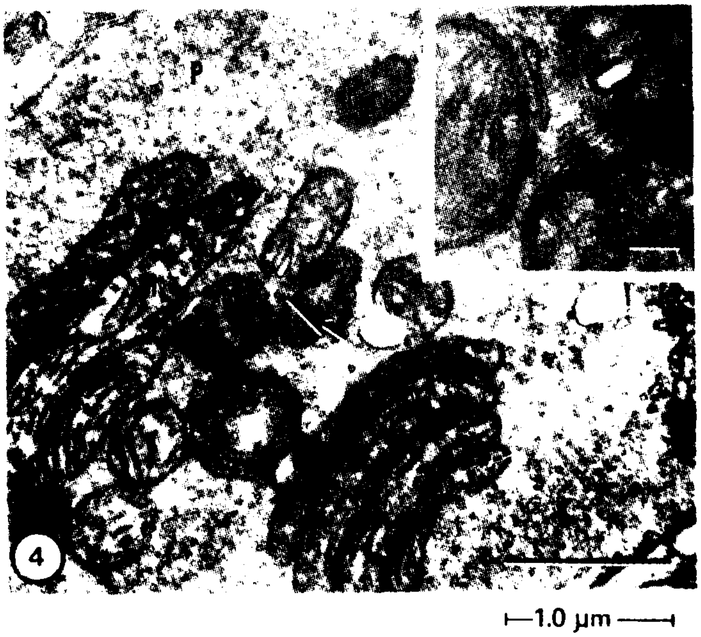

Figure 4. Electron micrograph of rhesus cone synapse, the pedicle (P). A rootlet (arrow) is surrounded by mitochondria (M). Inset: Higher magnification of same rootlet.

calycal filaments resemble the core filaments of intestinal epithelium microvilli (11), cochlear hair cells (12), and goldfish photoreceptor calyces (13), all of which are involved in some form of movement. Although no striated filaments have been reported in any of these processes, a cross-linking of filaments to form stable bundles has been proposed for goldfish photoreceptors (13). Considering this possibility, our findings suggest both goldfish and mammalian ellipsoid regions may be more similar in structure, and perhaps function, than previously thought; yet there is no known contraction in mammalian photoreceptors. Since there is evidence that calycal filaments of both rhesus rods and cones interact with the striated rootlet near the outer ellipsoid, this interaction maybe contractile and may involve the inner and outer segments and the calyx. Furthermore, the wavy filaments suggest an intermediate form of the striated filaments.

It is of interest that the largest filament bundle is in the outer ellipsoid region of rods (300+ filaments), near the large energy reserves of the mitochondria. In the rod myoid, fewer striated filaments (<50) are near glycogen particles. The reduction and absence of filament numbers in the rod and cone myoids, respectively, is perhaps due to bundle splaying and membrane insertions. Both the difference in filament numbers and energy sources for the striated filaments between the ellipsoid and myoid regions may also reflect different functional needs for the rootlet in the two photoreceptors.

Existence of rootlets in the synaptic regions, in addition to suggesting a communication line to the outer segments, may also regulate impulse conduction due to the contacts with synaptic membranes. Perhaps rootlets can act as a negative feedback circuit between the synapse, inner segment organelles, and the outer segment photo-labile membranes.

Previously, myosin was thought not to exist in mammalian inner segments; however, immunofluorescence studies have correlated the occurrence of actomyosin with that of striated rootlets (5) and more recently myosin was localized on the striations of rootlets (6). Other studies (14) document that an ATPase is localized on rootlet striations. The proximities of mitochondria and endoplasmic reticulum to the rootlets corresponds to the proximities of mitochondria and the sarcoplasmic reticulum to the actin and myosin filaments in skeletal muscle. Increasing evidence, therefore, suggests that the rootlet may have functions similar to muscle. If this analogy is correct, the endoplasmic reticulum of the inner segment could subserve contraction by regulating calcium ions, and the mitochondria could provide ATP for an energy source (Figure 6). Assuming that the actin -like filaments of the rootlet are involved in contraction, two points can be made regarding contraction: 1) due to the complex network of filament connections, it would not be unreasonable to speculate that contraction of sets of microfilaments produce a complex response that

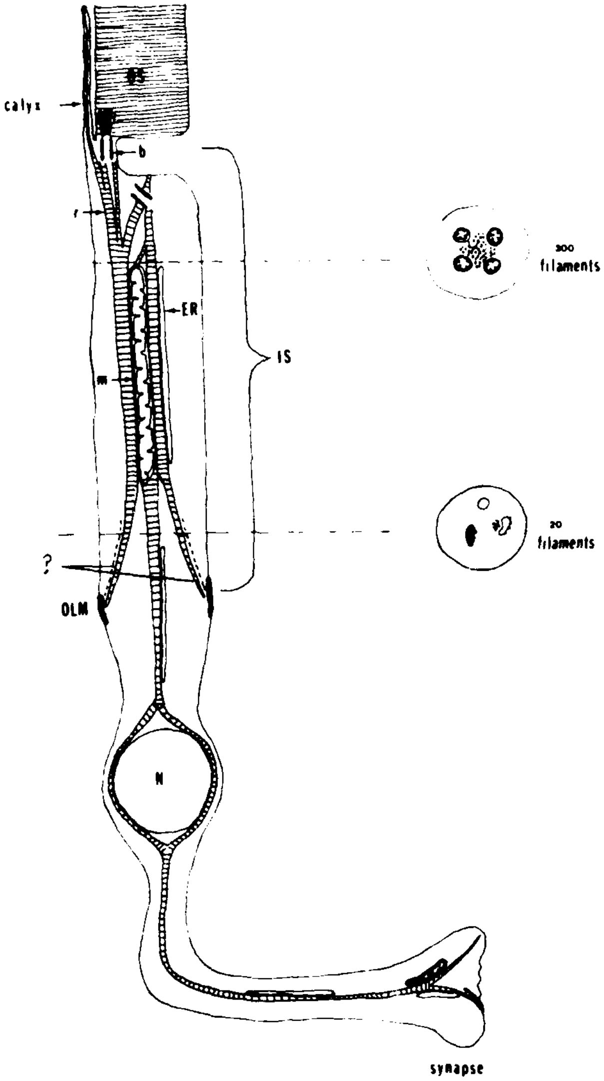

Figure 5. Schematic of rhesus monkey photoreceptor. A basal body (b), connecting the inner (IS) and outer segments (OS), has rootlets (r) originating from it and extending into the inner segment and microtubules originating from it and extending into the outer segment. Rootlets extend into the base of the calyx and appear to communicate with the calyx core filaments. Rootlets also attach to the secondary basal body, the plasma membrane and mitochondria (m). Within the inner segment and synapse, the rootlet is flanked by mitochondria and endoplasmic reticulum (ER). Cross-sections through the inner segment near the outer segment reveal about 300 filaments. Cross-sections near the outer limiting membrane (OLM) show about 20 filaments. The reduction in filament numbers near the OLM suggests that some of the filaments are terminating in the outer portion of the inner segment. Termination maybe occurring via filament splaying or membrane insertion. A small group of filaments also progresses through the central part of the photoreceptor past the OLM, around the nucleus (N), and terminates in the synaptic regions of the cell, near dense membranes.

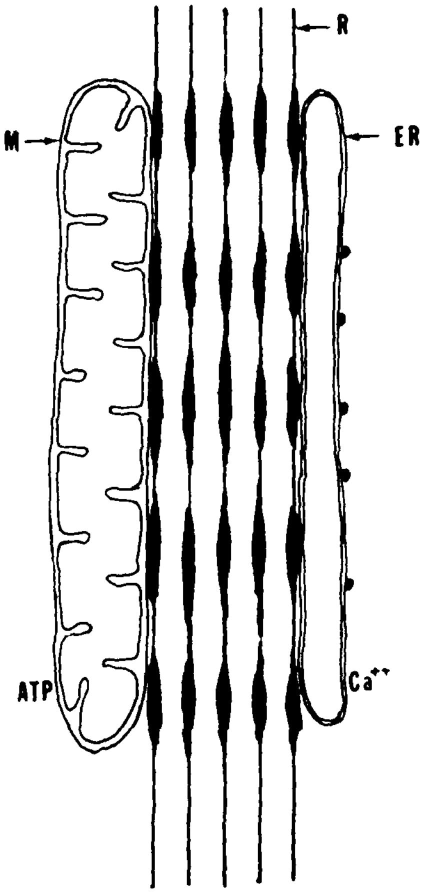

Figure 6. Contraction model for the rootlet. The mechanism whereby filament bundles contract and relax, if at all, is unknown, however, the structural requirements for contraction appear to be present in photoreceptors. This is evident by the fact that rootlet filaments (R) are flanked by mitochondria (M) and endoplasmic reticulum (ER). The contraction model is analogous to the contraction model for skeletal muscle where actin - myosin filaments are flanked by mitochondria which provide ATP as the energy source and the sarcoplasmic reticulum, which regulates calcium ions for muscular contraction. In this model the mitochondria provide ATP as the energy source while the ER is the calcium ion regulator.

could maintain an optimum outer segment alignment toward the exit pupil; and 2) due to the large number of filaments located in the outer ellipsoid, it is expected that the greatest influence of contraction would be on the outer regions of the photoreceptor.

Enoch et al (8) presented data supporting photoreceptor alignment toward the exit pupil; this alignment need only involve a minute radial (pivoting) movement. The involvement of rootlets in orienting cilia is reported (15), and an analogous structure exists in a phototropic phycomycete (16). Since the primate photoreceptor rootlet system appears to contain the necessary structural components for contraction, this system could subserve photoreceptor alignment toward the exit pupil.

CONCLUSIONS

Both rods and cones contain complex striated rootlet structures and associations that may represent intraphotoreceptor communication between the outer segments and synapses. These structures, although the mechanics are unknown, could control alignment by contraction and relaxation of sets of these filaments.

RECOMMENDATION

Additional research is needed to determine how the dynamics of photoreceptor rootlets are involved in light damage effects under low irradiance conditions. Alteration of rootlet processes may represent another system through which light damage to the retina can be manifested.

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