MICROTUBULES IN CONE MYOID ELONGATION IN THE TELEOST RETINA

ROBERT H. WARREN and BETH BURNSIDE

From the Bermuda Biological Station, St. George, Bermuda. Dr. Warren's present address is the Department of Anatomy, University of Miami School of Medicine, Miami, Florida 33152, and Dr. Burnside's present address is the Department of Physiology-Anatomy, University of California at Berkeley, Berkeley, California 94720.

ABSTRACT

The myoids of retinal cone cells of the blue-striped grunt (*Haemulon sciurus*) undergo significant elongation during dark adaptation of the retina. Longitudinally oriented microtubules are present in myoids both before and after elongation. Injection of colchicine into the vitreous of the eye in vivo disrupts the microtubules in the myoids and prevents dark-adaptive myoid elongation. Counts of microtubules in transverse sections along the lengths of elongating myoids show that there is a uniform decrease in the number of microtubules at any one point along the myoid as the myoid elongates. The magnitude of the decrease is proportional to the extent of the elongation. The product of the mean myoid microtubule number (determined from counts at progressive intervals along the myoid) and the myoid length remains essentially constant during myoid elongation, indicating that the total quantity of microtubules in the myoid does not increase with elongation. Serial section tracings of the microtubules along the myoids suggest that individual microtubules do not extend the length of the myoid and that the myoid microtubular apparatus consists of bundles of overlapping shorter microtubules. We propose that elongation of the myoid is accompanied by sliding redistribution of microtubules along the length of the myoid, and that the sliding may be generated by interactions between microtubules in regions where they closely overlap in bundles. We find no evidence for the involvement of discrete, electron-dense microtubular organizing centers in myoid elongation.

KEY WORDS microtubules · retinal cone · colchicine · cell shape

Microtubules are now generally attributed a role in development and maintenance of cell shape (4, 16). They possess an intrinsic structural rigidity; they regularly appear in elongating cells or cell processes oriented parallel to the axis of elongation; and their disruption with microtubule inhibitors such as colchicine generally abolishes cell anisometry or prevents cell shape change (4, 5, 23).

Although cell elongation is an intrinsic part of most morphogenetic processes and also plays a role in the normal activities of many differentiated cells, we understand very little about the mechanism by which elongation is achieved. That microtubules are necessary is now generally assumed, but it is not at all clear whether they provide the motive force for elongation. We feel that a pri-

mary step in understanding the role of microtubules in cell elongation is to define more precisely the organization of cytoplasmic microtubules in elongating cells and to characterize the changes in this organization which take place as the cell elongates.

In recent studies (5, 23), we have investigated this problem in cells undergoing elongation during differentiation. In the present study we examine microtubule distribution in a cell which undergoes a reversible elongation and contraction as part of its daily activity.

The cone photoreceptor cells of the teleost retina provide a favorable system for the study of microtubules during cellular elongation. In teleosts, the photoreceptive outer segments of the rods and cones are situated at the end of a cellular stalk, termed the "myoid," which changes in length during dark or light adaptation of the retina (2). In a light-adapted retina, the cone myoids are contracted, and the rod myoids are elongated. Thus, the cones are positioned relatively closer to the incoming light, and the more light-sensitive rods are located further away from the light and buried in a layer of light-absorbing, protective pigment. During dark adaptation, there is a gradual reversal in relative positions of the rod and cone outer segments as the cone myoids elongate and the rod myoids contract. The existence of microtubules in rod and cone myoids has been reported in a preliminary study of the catfish retina (1), and in various other fish and amphibians (2).

In our present study, we have taken advantage of the fact that cone myoid elongation can be initiated at a precise time by placing a fish in the dark. One then has a large population of cells whose myoids are elongating in synchrony and in which the degree of elongation can be measured. The regular packing of the cone cells in a discrete layer greatly facilitates the electron microscope sampling of selected regions of the cells at progressive stages of myoid elongation. It has thus been feasible to analyze the organization of the microtubular apparatus in the cone in detail and to correlate changes in distribution of the microtubules with myoid elongation. With colchicine studies, we have been able to show that the presence of the microtubular apparatus is required for myoid elongation to occur. Our observations also suggest that the microtubular apparatus consists of relatively short microtubules which overlap along the length of the myoid and that these short microtubules are displaced axially as the myoid elongates.

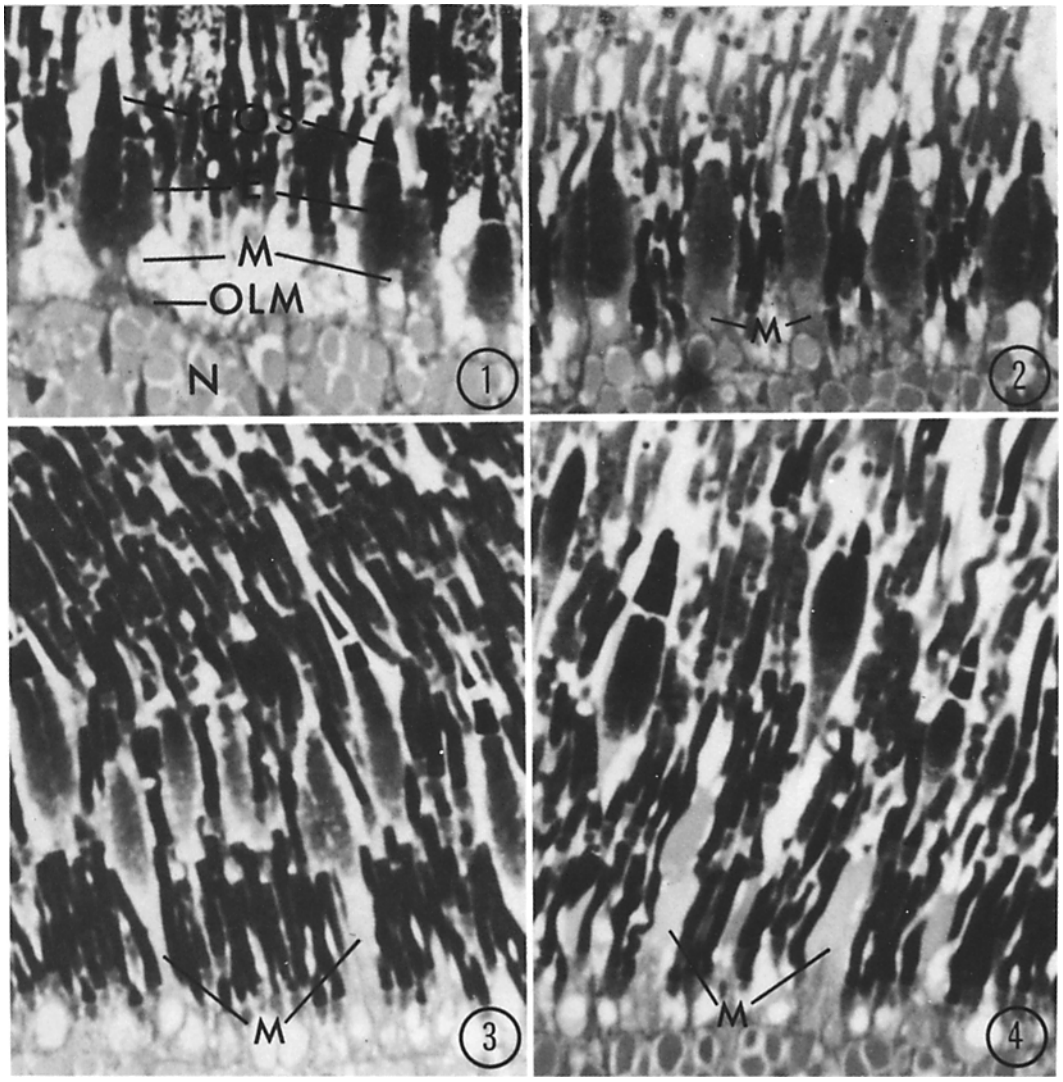
MATERIALS AND METHODS

Live blue-striped grunts (*Haemulon sciurus*) were taken from shallow reefs in the vicinity of the Bermuda Biological Station and kept in running seawater tanks in the station. Light-adapted specimens were exposed to normal fluorescent room light for at least 2 h (approx. ~80 foot-candles). For dark adaptation, the fish were transferred to a running seawater tank in a totally dark room. Fixation of dark-adapted retinas was carried out as rapidly as possible under a dim red light. To fix a retina, the eye was enucleated, and the front half of the eye including the lens was dissected away. The posterior half of the eye with the retina *in situ* was immersed in fixative at room temperature. The initial fixative contained 3% glutaraldehyde and 2% sucrose, buffered in 0.07 M Na-cacodylate (pH 7.3). The buffer was diluted from a 0.2 M stock solution containing 0.06% $CaCl_2$ and 0.0022 M KCl. After 2 h of initial fixation, the tissues were rinsed for 0.5 h in the same buffer containing 4% sucrose. Postfixation was carried out for 1 h with 1% OsO_4 in the 0.07 M Na-cacodylate buffer. Tissues were dehydrated in alcohol and propylene oxide and embedded in Epon.

Intraocular injections of colchicine were directed into the vitreous of the eyes of light-adapted fish 7.5–12.5 cm long. An injection of 25 μl of teleost Ringer's containing 8 mg/ml (2×10^{-2} M) of colchicine (Sigma Chemical Co., St. Louis, Mo.) proved effective in blocking cone myoid elongation. By measuring eye volume, we estimate that the colchicine should be diluted at least 16-fold in the vitreous, thus producing a final concentration of approx. 1.25×10^{-3} M in the vitreous. We cannot, however, predict the colchicine concentration at the level of retinal cone cells. After taking into consideration the possibility that some of the injected colchicine may be lost due to turnover or flushing in the vitreous, and that the colchicine must penetrate through many cell layers before reaching the cone myoids behind the outer limiting membrane at the back of the retina, it seems likely that the effective concentration of colchicine at the target cell is considerably less. After the injection, the fish were kept in the light for 0.5 h to allow diffusion of the colchicine into the retina. They were then placed in the dark. After 2 h of darkness, both eyes were removed and fixed. Uninjected eyes and eyes injected only with teleost Ringer's served as controls.

RESULTS

In the light-adapted grunt retina, the cone ellipsoids lie close to the outer limiting membrane (Fig. 1) and the contracted myoids appear as short, broad trunks. When the fish is transferred to total darkness, the cone myoids begin to elongate within 10 min (Figs. 2–5). The maximum length achieved by the cone myoids after 2 h is



FIGURES 1–4 Light micrographs of thick sections normal to grunt retinas showing progressive stages of cone myoid elongation with dark adaptation. *COS*, cone outer segment; *E*, ellipsoid of cone; *M*, myoid; *OLM*, outer limiting membrane; and *N*, nuclei of rods and cones.

FIGURE 1 Light-adapted retina. Cone myoids are contracted, and the ellipsoids are about 5 μm from the outer limiting membrane. × 1,250.

FIGURE 2 20 min of dark adaptation; myoids = 8 μm. × 1,250.

FIGURE 3 40 min of dark adaptation; myoids = 22 μm. × 1,250.

FIGURE 4 Full dark adaptation after 120 min; myoids = 28 μm. × 1,250.

determined by the intensity and duration of previous light exposure (2). In this study, the maximum length achieved was 28 μm for the grunt retinas. With elongation, the diameter of the myoid decreases considerably.

It should be noted that the majority of the cones in the grunt retina occurs in pairs as "twin cones." In these laterally adherent pairs, both of the cones are of approximately the same length and shape. All observations in this paper refer to twin cones,

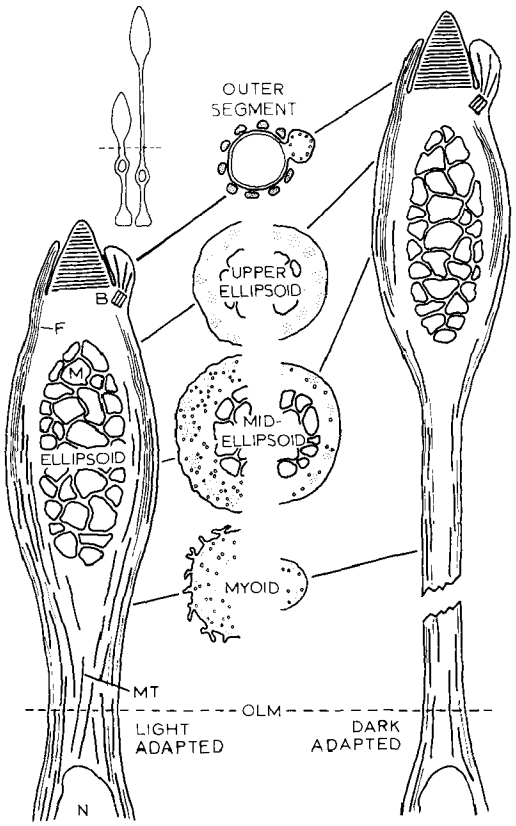


FIGURE 5 Diagrammatic summary of relative changes in length of the cones (*inset*, top left) and in distribution of the microtubules during the cone myoid elongation (left and right). In light-adapted cones (left), microtubules (thick lines, labeled *MT*) are numerous from the level of the nucleus to the mid-ellipsoid. In elongate, dark-adapted cones (right), the number of microtubules has decreased uniformly at all levels between nucleus and mid-ellipsoid. Microfilament organization is indicated by the thin lines, labeled *F*. *B*, basal body of the cilium; *F*, 6 nm filaments; *M*, mitochondria of ellipsoid; *OLM*, outer limiting membrane; *MT*, microtubules; and *N*, nucleus.

although we have noted that there are no significant internal differences between twin and single cones in the grunt retina.

Distribution of Microtubules in Light-Adapted Cones

Longitudinally oriented microtubules are observed at all levels of cone cells, except in the upper ellipsoid region (Fig. 5). Microtubules are particularly numerous in myoid and lower ellipsoid levels alongside the mitochondrial cluster (Fig. 6), but terminate at about mid-ellipsoid level. Above the mid-ellipsoids, the only microtubules usually observed are those of the cilium in the accessory outer segment. Cytoplasmic microtubules are observed only rarely in the vicinity of the ciliary basal body in the upper ellipsoid.

Microtubules in contracted cones are often closely associated with one another, particularly in the rim of cytoplasm around the mitochondria in the lower ellipsoid (Fig. 6). Although many microtubules lie within 60 nm of one another, there is little evidence of bridges interconnecting the microtubules. Many of the microtubules in the ellipsoid are closely grouped around bundles of 6-nm filaments. The filament bundles arise at the apex of the ellipsoid from the microvillar processes ringing the outer segment and course downward into the lower ellipsoid. The organization of these filament bundles is described in the preceding paper (6).

Changes in Microtubular Organization with Myoid Elongation

The overall distribution of microtubules in elongating cones is similar to the pattern seen in light-adapted cones. Longitudinally oriented microtubules are present at all levels of cones except in the upper ellipsoid. There is, however, a progressive and significant decrease in numbers of microtubules appearing in transverse sections as the cones elongate. This decrease is observed both in ellipsoids (Figs. 6 and 7) and in myoids (Figs. 8 and 9), although the elongation occurs only in the myoid.

The decrease in microtubule numbers along the dark-adapting cones has been determined from counts of microtubules appearing in transverse sections of cones (Table I). Looking specifically at the myoids, there is an inverse relationship between myoid length and the number of microtubules observed in transverse sections of myoids at progressive lengths (Fig. 10). For example, after 40 min of dark adaptation, there is an approximate fourfold increase in myoid length corresponding to a fourfold decrease in microtubule number in the myoids. This inverse relationship suggests that the total length of polymerized microtubules might not change significantly as the myoids elongate. To test this, we multiplied the average number of microtubules in transverse sections of myoids by the lengths of the myoids (Table I). Although these values increase slightly as the myoids elongate, there is no indication of a significant change in quantity of microtubules in

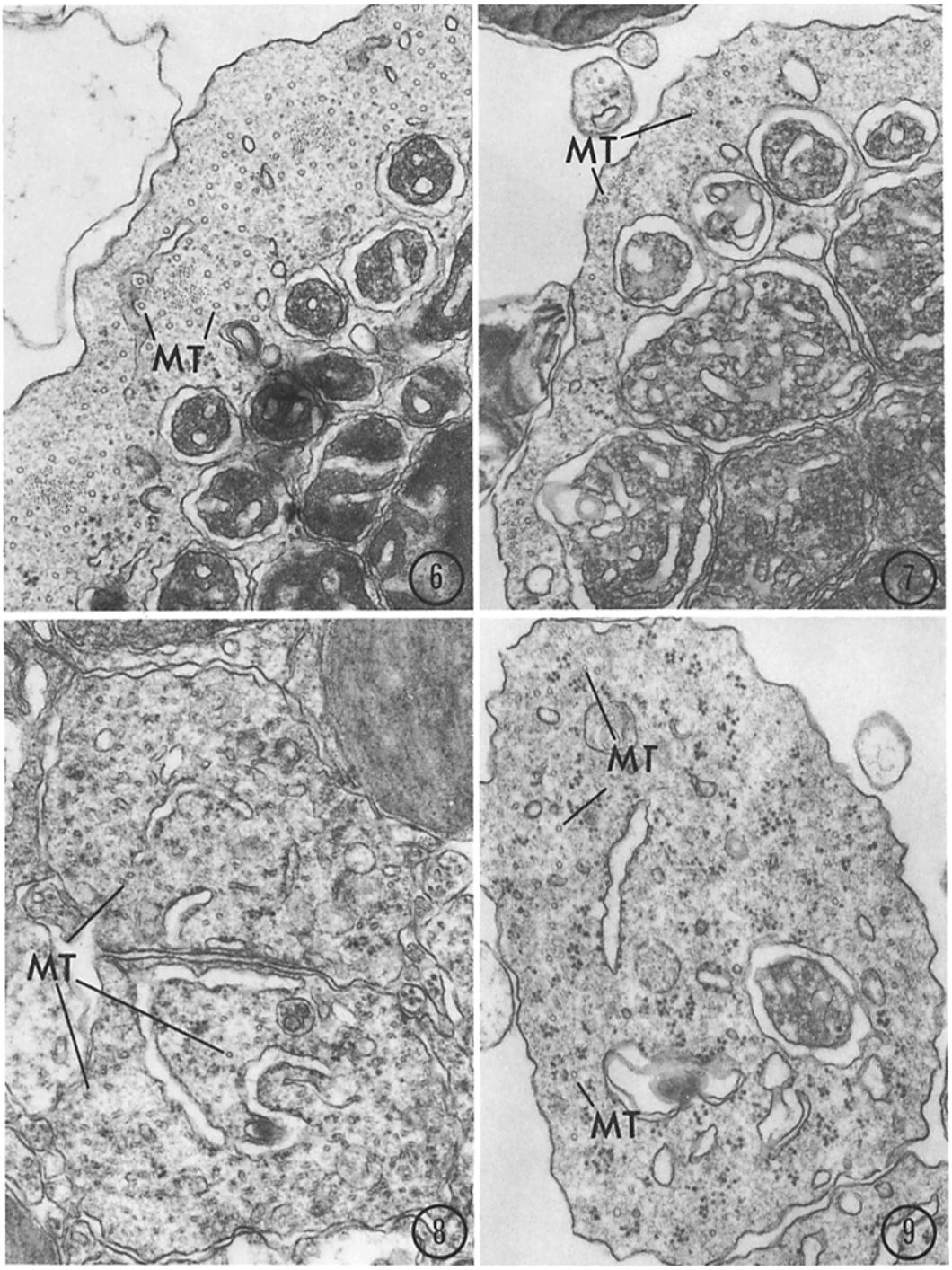


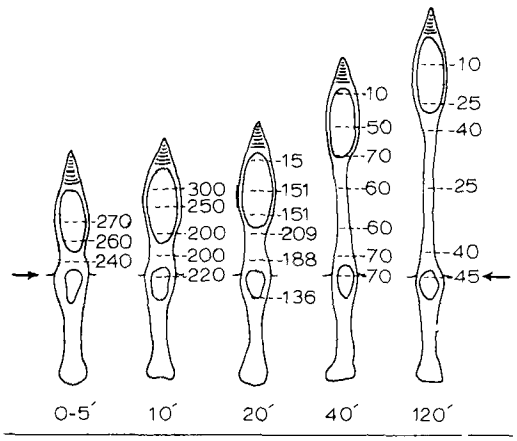
FIGURE 6 Transverse section of light-adapted cone at lower ellipsoid level. Numerous microtubules (*MT*) are grouped around bundles of 6-nm filaments. × 32,000.

FIGURE 7 Transverse section of cone lower ellipsoid after 120 min of dark adaptation. Only a few microtubules (*MT*) are found in the peripheral rim of cone cytoplasm. × 32,000.

FIGURE 8 Transverse section of cone myoid after 40 min of dark adaptation. Microtubules (*MT*) are closely packed in the myoid. × 43,000.

FIGURE 9 Transverse section of cone myoid after 120 min of dark adaptation. Microtubules (*MT*) are scattered around the periphery of the myoid. × 43,000.

TABLE I



A. Length of time in dark (min)	0-5	10	20	40	120
B. Myoid length (μm)	5	6	8	22	28
C. $\bar{X}$ microtubules in myoid	250	206	171	66	35
D. ($\bar{X}$) × (myoid length)	1.250	1.239	1,368	1.452	980

Microtubules were counted in transverse sections of cones at different levels down the cone and at progressive stages of dark adaptation. The numbers at different levels in the drawings are means of counts from 1 to 3 transverse sections taken at one level from similar cones. $\bar{X}$ (rows C and D) is the mean number of microtubules from all levels along the myoids from lower ellipsoid to outer limiting membrane. The numbers in row D are an estimation of total length of polymerized microtubules in the myoid. (Arrows indicate level of outer limiting membrane.)

the myoids through 40 min of elongation. After 120 min, however, we observed a decrease in total microtubule length of about 25% in comparison to the mean value at previous stages.

The extent of lateral microtubular association at progressive stages of myoid elongation has been estimated by counting the percentages of microtubules that lie within a distance of 60 nm of one another (edge-to-edge) in transverse section (see table II). It is apparent, on inspection, that cone myoid microtubules are distributed in clusters in the cone myoids. The maximum observed spacing between microtubules within these clusters is 60 nm. Therefore we chose this separation distance as representative of the maximum distance within which microtubules could conceivably interact. While the myoids are increasing in length, the percentages of microtubules lying within 60 nm of one another remain essentially constant. In fully elongated myoids, the percentage of microtubular association is quite variable.

Microtubule Lengths in Light- and Dark-Adapted Cones

The continuities of individual microtubules were traced in serial sections in order to answer two questions: (*a*) Do microtubules terminate along the lengths of myoids? and (*b*) If terminations do appear, are they associated with any structural features that could be characterized as "microtubular organizing centers" (MTOC's)? Individual microtubules were traced in three sets of

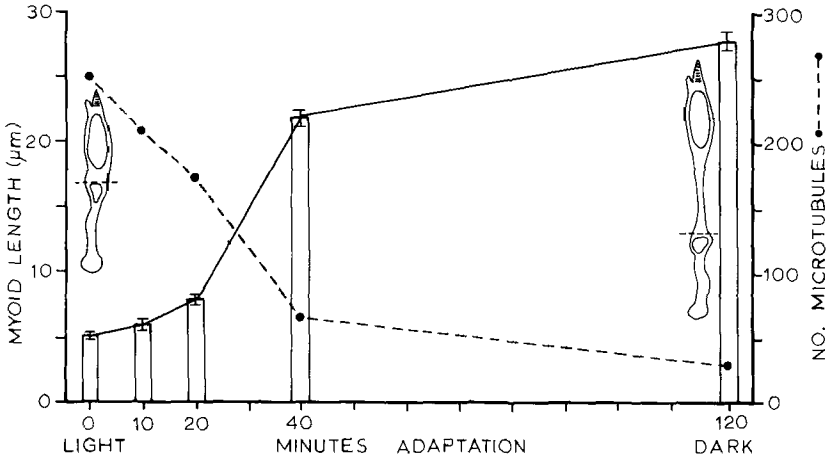


FIGURE 10 The inverse symmetrical relationship between numbers of microtubules seen in transverse sections of myoids (——) and myoid length (– – –) is shown for increasing myoid length with dark adaptation from left to right.

TABLE II

Paraxial Association of Microtubules in Myoids and Lower Ellipsoids

Stage	No. cells examined	No. microtubules counted	Mean percent paired		
			%	*SD*	*SE*
0–5 min DA	2	411	76	–	–
10 " "	4	375	72	3.7	1.9
20 " "	2	376	73	–	–
40 " "	5	351	72	13	5.7
120 " "	30	700	range 40–70	–	–

* DA: dark adapted.

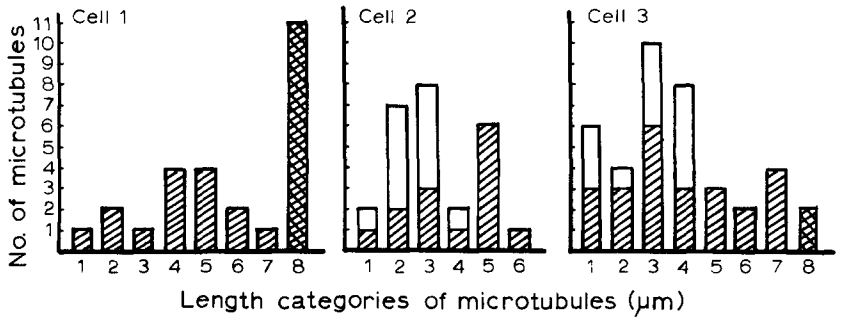


FIGURE 11 The bars represent numbers of microtubules of a given length range that were traced through serial sections of the lower ellipsoid of a light-adapted cone (Cell *1*) and myoids of two cones after 120 min of dark adaptation (Cells *2* and *3*). Each bar represents the number of observed microtubules that were of length (in micrometers) equal to or less than the number below the bar but greater in length than the number below the bar to the left. Within each bar, clear areas indicate microtubules with both ends visible in the serial sections, slanted lines indicate microtubules with only one end seen in serial section, and cross-hatching indicates microtubules with no ends visible, i.e., the microtubule extended entirely through the set of sections. The population of microtubules traced in the elongate myoids appears to contain a higher proportion of shorter microtubules.

serial sections (80–100 nm thick) from three separate cells. The first set of sections covered a distance of 7 μm along the lower ellipsoid level of a light-adapted cone, and the second two sets covered distances of 7 μm and 6.5 μm, respectively, along the upper myoid of dark-adapted cones. In all of these serial sections, the continuities of microtubules were traced in regions where the microtubules remained normal to the plane of section throughout the set of sections. The length of all microtubules traced are illustrated by the bar histograms in Fig. 11.

Microtubule length is highly variable in both light- and dark-adapted cones. In a light-adapted cone (Cell *1*, Fig. 11), 11 of the 26 microtubules traced extended the entire 7 μm length of the lower ellipsoid segment, while the remaining 15 microtubules terminated at some level within the segment. Of 65 microtubules traced in two dark-adapted cones (Cell *2* and *3*, Fig. 11), only 4 extended the entire length of the 6.5–7 μm-long segments of the upper myoids, 34 terminated within the segment, and 27 microtubules began and ended within the segment. Of the latter microtubules, many were less than 2 μm in length.

Microtuble terminations appear to be scattered randomly along the lengths of the segments of the light- and dark-adapted cones. Most microtubules terminate abruptly in the cytoplasm, and there are no obvious relationships of microtubules to electron-dense MTOC's. Several terminations of microtubules may be observed in Figs. 12 and 13 in short sets of serial sections taken from the cells *2* and *3*. It has been noted that microtubules are often closely associated with endoplasmic reticulum along their length, and vesicles of the endoplasmic reticulum may appear in close proximity to microtubular terminations (Fig. 13*f*). To the reader who may experience some difficulty in tracing continuities or terminations of microtubules in the short sets of serial sections presented in Figs. 11–13 due to the shifting positions of the

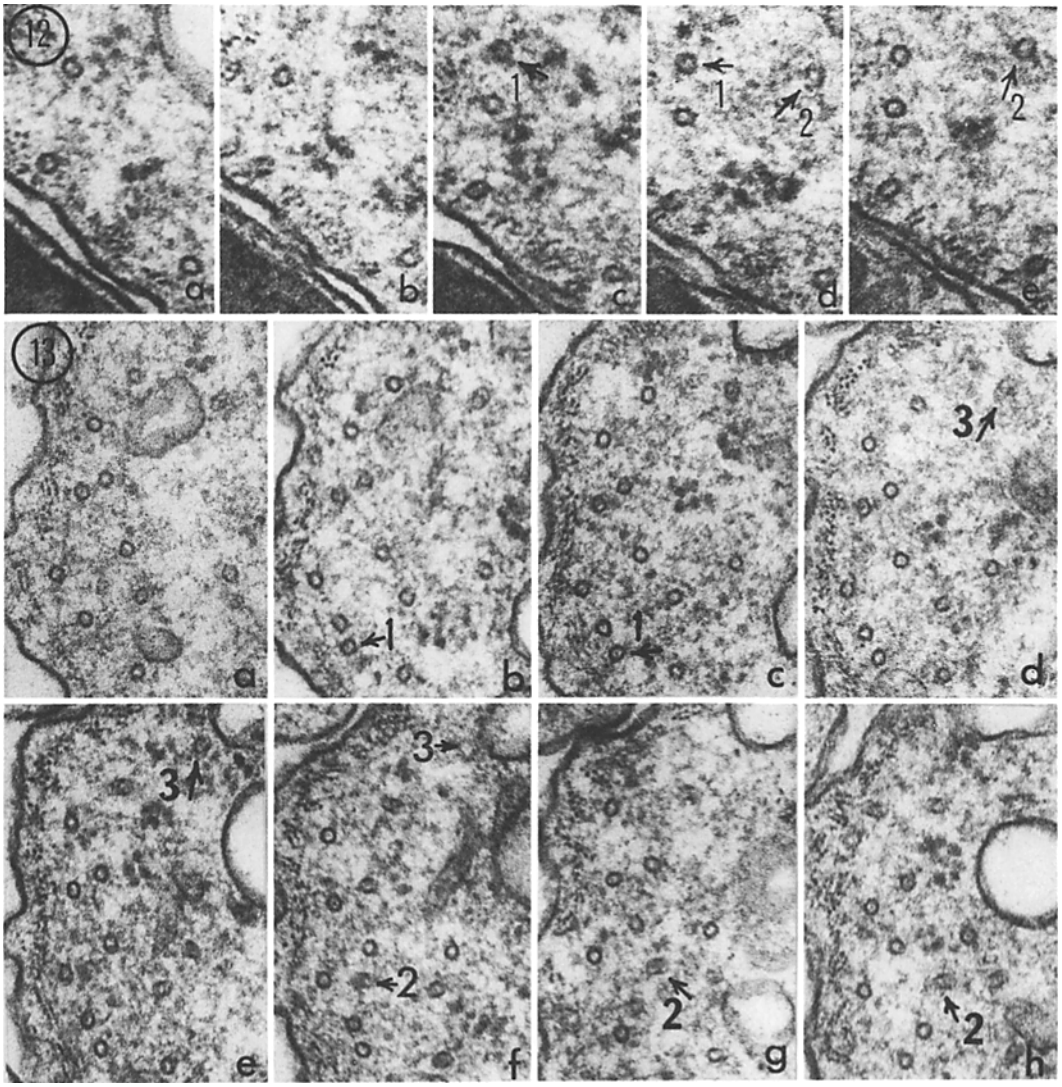


FIGURE 12 (a–e) Five consecutive serial transverse sections from the myoid of a fully dark-adapted cone. New microtubules appear in sections *c* and *d* (arrows *1* and *2*, respectively). The ends of the new microtubules are not associated with any obvious structures in the cytoplasm. × 105,000.

FIGURE 13 (a–h) Eight consecutive serial transverse sections of fully dark-adapted cone myoids. Two new microtubules (arrows *1* and *2*) appear in sections *b* and *f*, while one microtubule terminates in section *f* (arrow *3*). A vesicle of endoplasmic reticulum appears in close proximity to the microtubular termination in section *f*. × 89,700.

microtubules, we emphasize that all interpretations are based on careful tracing, comparison, and re-checking of all microtubules in the field through the entire set of sections. Thus, for any "termination," we can say with confidence that the microtubule considered does not subsequently reappear within the set of serial sections.

Effect of Colchicine upon Myoid Elongation

Colchicine injection was effective in blocking cone myoid elongation in fish that were placed in total darkness for 2 h (Fig. 14). In control eyes that were uninjected (Fig. 15), or were injected

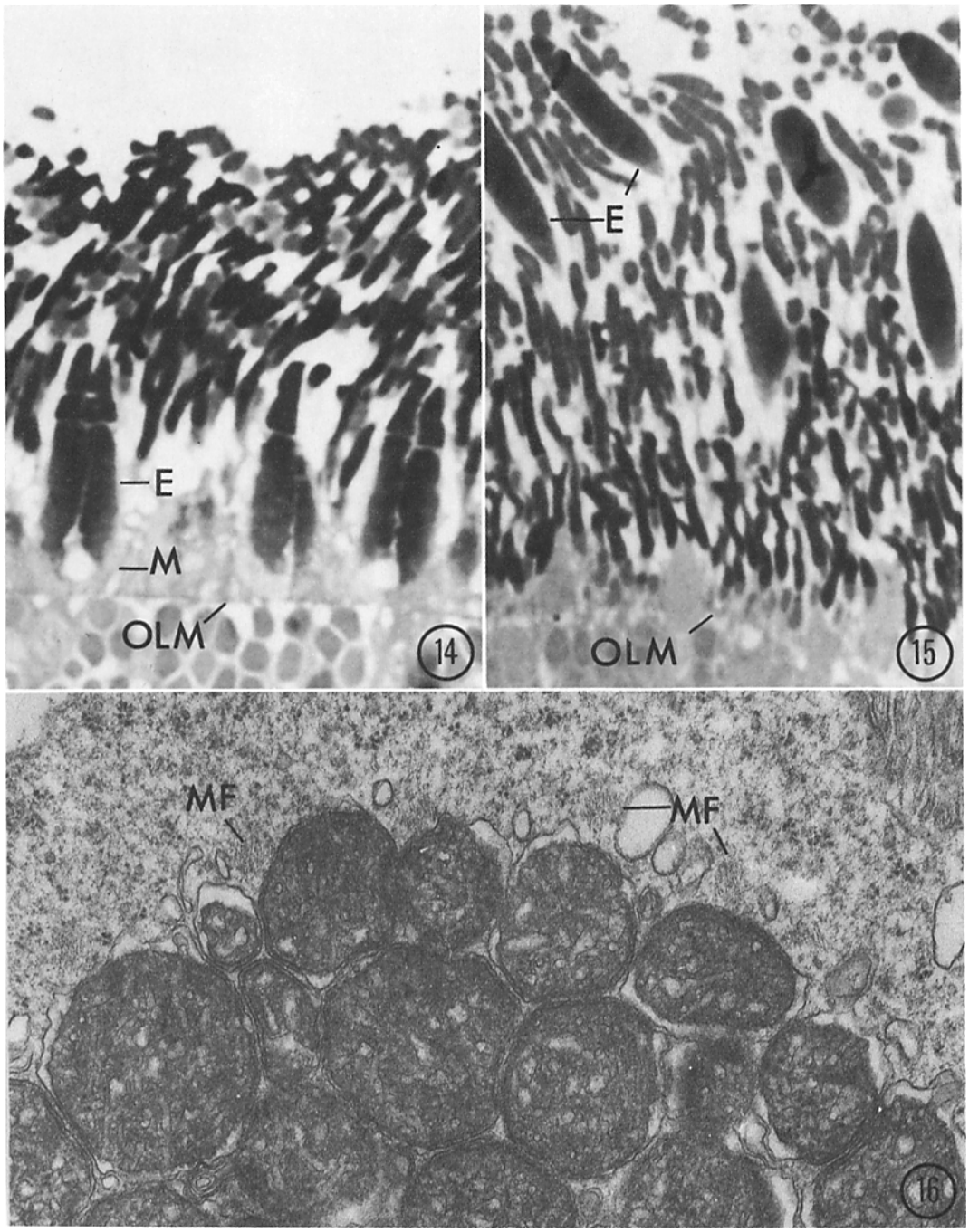


FIGURE 14 Retina after colchine injection into the eye and 2 h of darkness. Cone myoids (*M*) have failed to elongate. *E*, ellipsoid; and *OLM*, outer limiting membrane. × 1,250.

FIGURE 15 Dark-adapted control eye from the same fish as the colchicine-treated eye in Fig. 19. Cone myoids have elongated. *E*, ellipsoid; and *OLM*, outer limiting membrane. × 1,250.

FIGURE 16 Transverse section of the lower ellipsoid of a cone from a retina 2.5 h after injection of 25 μl of 2×10^{-2} M colchicine in teleost Ringer's into the eye. Microtubules are absent. × 39,600.

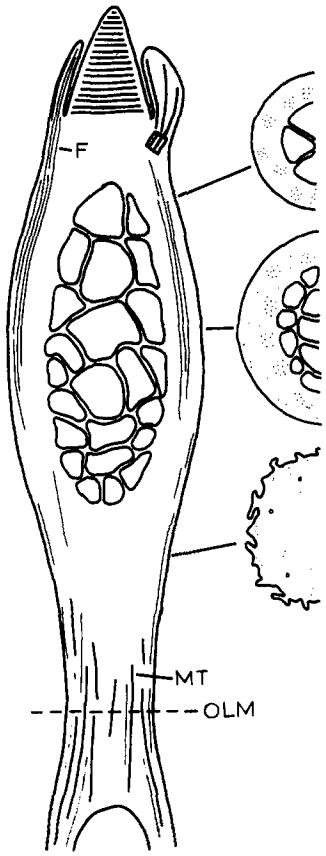


FIGURE 17 Representation of microtubule distribution in a cone 2.5 h after injection of colchicine into the posterior chamber of the eye. Microtubules (*MT*) are absent from the lower ellipsoid and most of the myoid but are still present in the cone below the outer limiting membrane (*OLM*). 6 nm filament bundles (*F*) appear in normal distribution.

with teleost Ringer's alone, the myoids elongated normally during the period of dark adaptation. Colchicine appears selectively to depolymerize microtubules in the upper myoid and ellipsoid as these regions are essentially devoid of microtubules in electron micrographs (Fig. 16), while in the region of the cone below the outer limiting membrane the microtubules are still present. Other organelles in the cones appear to be distributed normally after colchicine treatment. The resultant microtubule distribution in cone cells after colchicine treatment is summarized in Fig. 17.

DISCUSSION

The presence of microtubules in teleost cone myoids has been demonstrated by Ali (2) and by Adomian and Sjöstrand (1). In this study, we describe the changes in microtubule organization associated with cone elongation in the retina of the blue-striped grunt.

Our assumption that microtubules play a critical role in myoid elongation rests in large part upon the observation that colchicine disrupts the myoid microtubules and blocks elongation. While the binding of colchicine to tubulin is well established, there is evidence that colchicine can bind to other cellular constituents, such as the plasma membrane at higher dosages (20). Since our calculations indicate an initially high concentration of colchicine in the vitreous of the eye immediately after injection, it is conceivable that some nonspecific action of colchicine on the cone cells could block elongation. Although this possibility cannot be ruled out, we feel that the likelihood is minimal. In the first place, the concentration of colchicine at the cone cells is probably much less than that calculated for the vitreous immediately after injection. We would expect some of the colchicine to be washed from the eye by the aqueous humor that bathes the vitreous before flowing out of the canals of Schlemm. The residual colchicine must diffuse through the vitreous and through the multiple cell layers of the retina overlying the cone myoids. The effective dose at this level is almost certainly much lower in comparison to the injection site. Of several dosages used, all lower doses failed to disrupt microtubules and also failed to block elongation. In other experiments, we found, moreover, that cone *contraction* occurred normally even in the presence of sufficient colchicine to disrupt myoid microtubules. It therefore seems unlikely that colchicine exerts its effect by an indiscriminate poisoning of the cell.

We believe that an essential role of microtubules in cone myoid elongations is to provide structural or cytoskeletal support to the myoid. The microtubules may also, as will be considered in more detail below, participate actively in producing the elongation. For either function, we propose that the overall structural integrity of the microtubular apparatus depends upon lateral interactions between microtubules. This follows from our observation that microtubules do not extend the full length of the elongating myoid, but rather overlap in a regular manner all along the length of the myoid. A similar condition exists in elongating skeletal muscle myoblast where it has also been shown that microtubules do not extend the full length of the cell, but rather overlap in staggered fashion (23). Although the lateral asso-

ciations of microtubules with one another in both the systems is striking, the nature of the interaction between the microtubules remains undefined since regular bridges interconnecting the microtubules have not been observed in either system. Presumably, the substances involved in these microtubular interactions are morphologically indistinct in thin section after routine preparative methods. Microtubules in the myoids often do have a clear zone of exclusion around them, as has been reported for cytoplasmic microtubules in many other systems.

From the analysis of numbers of microtubules appearing in transverse sections of cones during elongation, we find that axial microtubules decrease in number along the myoids as the myoids elongate. This decrease in microtubular number along the myoids is inversely related to the increase in myoid length so that the total cumulative length of microtubules remains approximately constant in the myoids during their elongation. In the discussion that follows, we will consider the implications of the above observations regarding two aspects of microtubular function in cone myoids: (*a*) The mechanism of microtubular involvement in the elongation; and (*b*) the control of microtubular organization.

Microtubules and Cellular Elongation

Although microtubules are often associated with cellular elongation, the mechanism of microtubular participation is not at all clear. It is not known whether microtubules actually provide the motive force for elongation or whether they merely act as stabilizing cytoskeletal elements that consolidate an elongation produced by some other organelles. The observation that colchicine inhibits elongation is compatible with either interpretation.

It has been proposed that elongation of the mitotic spindle and movement of chromosomes could be produced in part by growth of microtubules through subunit addition (12) such that a pushing force would arise in the cell. We feel that this type of model for active microtubular involvement in myoid elongation is not consistent with our observations that (*a*) the total quantity of polymerized microtubules does not appear to increase in the myoid and (*b*) the myoid microtubular apparatus is composed of relatively short, overlapping microtubular units. This model cannot be completely ruled out; but if it does occur, it would seem that a rather fine control of microtubular polymerization and depolymerization in different regions of the myoid microtubular apparatus would be required such that the numbers of microtubules at different levels of the myoid would remain constant, as we have observed.

Our observations are consistent with the interpretation that microtubules slide over one another in the myoid as it elongates. Interestingly enough, our data also suggest that the microtubules in the lower cone ellipsoid are redistributed although the ellipsoid itself does not elongate. We cannot account for this, although it seems possible that some of the ellipsoid microtubules could be shifted gradually into the myoid as it elongates. Active displacement of microtubules by sliding is known to occur in the highly organized axonemes of cilia and flagella (19, 21) and in certain protozoan axostyles (3, 11, 13). Although these sliding movements are normally very restricted in vivo, it has been shown that dramatic sliding displacement can occur in the flagellar axoneme when restaining linkers are digested by trypsin (21). The concept of active microtubular sliding in myoid elongation is attractive in its apparent simplicity, although more direct evidence pertaining to the nature of the microtubular interaction in the myoid is certainly required. We would like to propose, as a hypothesis for future work, that the cytoplasmic bundles of microtubules associated with cell elongation in the myoid are "primitive" correlates of the ciliary axoneme or protozoan axostyle in the same way that cytoplasmic actin filament bundles of nonmuscle cells are thought to correlate with the myofibril in muscle cells.

It is, of course, possible that microtubular sliding, whether active or passive, does not contribute to the actual mechanism of elongation. Thus, the apparent sliding redistribution of microtubules during myoid elongation could indicate that the microtubules are passively pulled out by some other forces in the cell. On the other hand, active sliding apart of microtubules could simply redistribute microtubules as supporting elements along the lengthening myoids and play no part in causing myoid elongation. These possibilities lead to the consideration of other systems in the cell that might contribute to the production of elongation.

In the accompanying paper by Burnside (6), it has been shown that the cones contain both thick and thin filaments. The thin (actin) filaments in the myoids and ellipsoids are organized into prominent bundles that are closely associated with the microtubules. While we cannot determine at present whether an actomyosin-based system contrib-

utes to the production of myoid elongation, the close association of the microtubules and the actin filament bundles is interesting in light of the recent suggestions that microtubules and actin may be associated in some other systems, particularly in the mitotic spindle apparatus (7, 9, 18). It should be noted, however, that if actomyosin plays a role in mitosis, its function would most likely involve contraction (i.e., between chromosome and pole), rather than an elongation.

In the final analysis, determination of the mechanism of myoid elongation will require closer study of both the microtubular and actomyosin systems in the cells, their interactions, and their control mechanisms. Isolation of intact cone cells and of myoid microtubular and microfilamentous organelles and artificial reactivation of elongation may produce much useful information concerning these questions. In terms of microtubule-microtubule and microtubule-actin interactions, it will be necessary to characterize the elements that are associated with the microtubules in vivo. Recent work on microtubule-associated proteins isolated from nerve axons (8) gives promise that microtubular linking elements may be isolated and characterized from other systems.

Control of Microtubular Organization

There are many unanswered questions regarding the control of microtubular function during myoid elongation. If, for example, microtubules slide apart during elongation, what controls the polarity of the sliding at the level of interaction between individual microtubules such that the entire microtubular apparatus elongates? If there is local polymerization or depolymerization of microtubules, do these events occur simultaneously in different regions of the cone and what controls local conditions in the cytoplasm so that one or the other event is favored (in this regard, see reference 22)? Current research on the cytoplasmic control of microtubular organization and function focuses on three general aspects of this problem: The nature of the tubulin dimer-$\leftrightarrow$polymer equilibrium; the contribution of divalent cations, primarily Ca^{++} to this equilibrium; and the role of MTOC's (14, 22, 24). In the remainder of this discussion, we will comment upon structural observations in the present work that pertain to the role of MTOC's.

In many cells, electron-dense MTOC's appear in regions of the cytoplasm from which microtubules originate, as at the poles of the mitotic spindle or in the cell center of an interphase cell. Typically, a MTOC is amorphous and electron dense, although it may contain organized structure such as a centriole (15). At present, there are relatively little data concerning MTOC involvement in microtubular organization during cell elongation. In a study of myogenic cell elongation (23), it appeared as if the cytoplasmic microtubules were loosely associated with a centriole during the early phases of cellular elongation but lost their association with the cell center as the cell elongated. The free ends of these microtubules were scattered randomly along the length of the cell, and no obvious electron-dense MTOC material was observed at the ends. In teleost cone cells, a centriolar basal body is located at the apex of the ellipsoid, but it appears to have little or no structural interaction with the microtubular apparatus of the myoid. Nothing resembling a centriole or a MTOC has been observed in any other region of the cone, although relatively fewer sections of cones below the outer limiting membrane have been examined. As in the elongating myogenic cells, the ends of microtubules are randomly distributed and are not associated with obvious electron-dense MTOC's. The association of vesicles of endoplasmic reticulum or other membrane with the ends of microtubules has been noted in both myogenic cells and cone myoids and other systems (10, 17, 23, 25), and the possible role of such membrane elements in microtubular organization merits further investigation. On the basis of the present evidence, however, it seems reasonable to conclude that obvious, discrete dense MTOC's are not required for myoid elongation.

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