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Microtubules and Actin Filaments in Teleost Visual Cone Elongation and Contraction

Beth Burnside

Department of Physiology-Anatomy, University of California at Berkeley, Berkeley, California, and The Bermuda Biological Station, St. George's West, Bermuda

Teleost retinal cones contract in light and elongate in darkness. This paper describes the disposition of microtubules and cytoplasmic filaments in cone cells of 2 species of fish (Haemulon sciurus and Lutjanus griseus). In Haemulon, the neck-like "myoid" region of the cone changes in length from 5 μ to 75 μ. Maximal observed rates of elongation and contraction are comparable to that of chromosome movement in mitosis (2–3 μ/min). Microtubules presumably participate in cone elongation, since numerous longitudinal microtubules are present in the myoid region, and colchicine blocks dark-induced elongation.

Myoid shortening, on the other hand, appears to be an active contractile process. Disruption of microtubules in dark-adapted cones does not produce myoid shortening in the absence of light, and light-induced myoid shortening is blocked by cytochalasin-B. Cone cells possess longitudinally-oriented thin filaments which bind myosin subfragment-1 to form arrowhead complexes typical of muscle actin. Myoid thin filaments are clearly observed in negatively stained preparations of isolated cones which have been disrupted with detergent after attachment to grids. These myoid filaments are not, however, generally preserved by conventional fixation, though bundles of thin filaments are preserved in other regions of the cell. Thus, actin filaments are poorly retained by fixation in precisely the region of the cone cell where contraction occurs. Cone cells also possess longitudinally-oriented thick filaments 130–160Å in diameter. That these thick filaments may be myosin is suggested by the presence of side-arms with approximately 150 Å periodicity. The linear organization of the contractile apparatus of the retinal cone cell makes this cell a promising model for morphological characterization of the disposition of actin and myosin filaments during contraction in a nonmuscle cell.

Key words: microtubules, actin, photoreceptor, motility

INTRODUCTION

Cell shape change entails localized contraction, elongation, or some combination of these motile processes. Today it is generally accepted that cytoplasmic contraction results from activities of the cell's actomyosin (1), and that elongation is produced by microtubules (2). Numerous investigators are attacking the biochemical problems of structure, assembly, and regulation of the molecules responsible for these fundamental aspects of motility. Though much has been learned about cytoplasmic actins, myosins, and tubulins, we still know almost nothing about the structural mechanisms whereby microtubules and

actomyosin systems exert their effects in nonmuscle cells. As the long history of muscle research makes clear, we must understand the morphological details of these phenomena before we can develop accurate and meaningful structural and functional models for motility in living nonmuscle cells.

Since actin and myosin are both found in nonmuscle cells, it is generally assumed that cytoplasmic contraction resembles in some way the sliding-filament mechanism described in muscle. According to this model, actin and myosin filaments actively slide past one another; this sliding is driven by cyclic, mechanochemical activities of myosin bridges (3, 4). The actual structural organization of the contractile apparatus in nonmuscle cells, however, is not at all clear. Nonmuscle cells contain extremely high ratios of actin: myosin compared to muscle [as much as 33:1 for Physarum, compared to 2:3 for skeletal muscle by weight (5)] , and thick filaments resembling myosin filaments of muscle are rarely observed in nonmuscle cells without special treatment. For these reasons our understanding of actin and myosin filament interactions in nonmuscle cells has been very limited. We do not even know that myosin must be in the filamentous form to participate in contraction (1). It is not clear where actin is attached for transmission of the tension developed by hypothetical actin-myosin interactions, though growing evidence suggests that actin is membrane-associated (6, 7).

In part, these limitations in our understanding of the structural basis of nonmuscle contractility result from the complexity of contractile phenomena observed in nonmuscle cells. I have attempted to reduce these complexities by using the teleost retinal cone as a model system to study the structural bases of cell shape determination. The teleost retinal cone cell undergoes remarkably simple, linear elongation and contraction in response to changes in light conditions. Thus, cell shape changes may be quantitated as linear changes in cell length, and the linear organization of the cell's contractile apparatus makes it more amenable to structural studies.

In teleost eyes, accommodation to differing light intensities is accomplished not by pupillary control as in mammals, but rather by means of 2 cooperative mechanical adjustments (called photomechanical movements) within the retina itself: 1) elongation and contraction of photoreceptors, and 2) migration of pigment in the retinal pigmented epithelium (RPE) (8). In the light, cones contract, drawing their photopigment-bearing outer segments toward the incoming light; meanwhile, rods elongate and thereby thrust their more light-sensitive outer segments into the advancing pigment of the RPE. In darkness, these movements are reversed. In retinal pigmented epithelium, light induces movement of pigment granules into long finger-like processes which extend alongside the photoreceptor outer segments; in darkness, pigment granules withdraw to the perinuclear region, thus exposing the rod outer segments. Cone movements were chosen for study because cones are much larger and less fragile than rods and because their movements are more uniform.

This paper describes cone elongation and contraction in the blue-striped grunt (Haemulon sciurus) and the grey snapper (Lutjanus griseus). Rates of elongation and contraction, effects of microtubule inhibitors and cytochalasin-B, and the distribution of cytoplasmic filaments in the cone cell are considered. Another paper, by Warren and Burnside (9, 10), describes changes in distribution of microtubules during elongation in the grunt; these observations will only be summarized here.

A preliminary report of this work has been presented in abstract form (11).

METHODS

Animals

Two species of fish from the inshore waters of the Bermuda Islands were used for these studies: the grey snapper (Lutjanus griseus) and the blue-striped grunt (Haemulon sciurus). Although any fish which feeds both in day and evening exhibits photomechanical movements, blue-striped grunts exhibit an extreme cone length change and thus were used for rate and inhibitor studies. The long, delicate myoids of their cones, however, made isolation of single cones difficult; thus, for such studies, the grey snapper was used. Fish were maintained in running seawater tanks at the Bermuda Biological Station. Light-cycling was either ambient (fish in tanks near windows) or artificially imposed (fish in darkroom exposed to 14 hr with an incandescent 40 watt bulb and 10 hr darkness). In order to minimize possible circadian effects on photomechanical movements, fish were maintained on the artificial cycle for at least 1 week before use in experiments; fish were not exposed to continuous darkness.

Rate Studies

Since photomechanical movements are influenced by circadian rhythms, light intensity, and other external factors (8, 12), conditions were specifically chosen to maximize rates of cone contraction and elongation. For contraction rate studies, fish were taken from darkness at the end of the dark period of their entrained cycle and exposed to 100 footcandles of mixed indirect sunlight and fluorescent lights. For elongation rate studies, fish maintained for 14 hr at reduced light intensities (less than 50 footcandles) were exposed to darkness at the end of their entrained light cycle. Fish were sacrificed at prescribed intervals and their eyes prepared for electron microscopy.

Electron Microscopy

After removal of the cornea, iris, and lens, the posterior eyecup was immersed intact in a fixative consisting of 2% glutaraldehyde (Fisher): 2% paraformaldehyde in either 0.1 M cacodylate buffer (pH 7.5), or in standard salt solution (SSS: 0.1 M KCl; 5 mM $MgCl_2$; 6 mM phosphate buffer, pH 7.0). Fixation in SSS yielded better preservation of filaments than any other of numerous fixatives tried, although overall tissue preservation with SSS was somewhat inferior to that in cacodylate. Retinas were fixed from 1.5–24 hr then postfixed in 2% OsO_4 in SSS or 0.1 M cacodylate (pH 7.0) for 2 hr, dehydrated in graded alcohols, and embedded in epon (13). Grey to silver sections obtained on a Porter Blum MT-2 ultramicrotome were stained with uranyl acetate and lead citrate (14), and examined on a Philips 201, or a JEOL 100-B electron microscope. Measurements of filament diameters were obtained by comparison to a diffraction grating photographed and printed in parallel with cross-sectioned filaments in thin sections. Periodicities along longitudinally-sectioned thick filaments were obtained in similar fashion. Estimates are derived from at least 50 measurements and are expressed as mean ± standard deviation.

Microtubule Disruption in Dark-Adapted, Isolated Retinas

Dark-adapted grunt retinas were dissected in dim red light (to avoid light adaptation), and placed into closed tubes containing incubation medium [L-15 culture medium (Gibco) adjusted to a final NaCl concentration of 1.7 M; hereafter referred to as modified L-15].

The medium was pregassed with 100% oxygen and retained in closed tubes throughout the experiment. Retinas so cultured were exposed to 3 different experimental conditions: a) 10,000 lb/in^2 hydrostatic pressure in a chamber designed and operated by E. D. Salmon (15), b) 10^{-3} M colchicine (Sigma), and c) low temperature (4°C). A fourth preparation was maintained in modified L-15 alone at ambient temperature (24°C) for control. Retinas were maintained at the designated conditions for 40 min and then immediately immersion-fixed for electron microscopy at room temperature. The experiment was carried out in darkness.

Cytochalasin-B Injections

Cytochalasin-B (Aldrich) was dissolved in 5% dimethyl sulfoxide (DMSO) in modified Hank's Balanced Salt Solution (adjusted to 1.7 M NaCl). Blue-striped grunts (8 cm long) received intraocular injections of this solution calculated to produce a final CB concentration of 5 μg/ml or 1 μg/ml in the right eye, and control DMSO or buffer injections in the left eye. Four experimental groups of 2 fish each were employed for each CB concentration: 1) light-adapted fish left in light; 2) light-adapted fish transferred to dark; 3) dark-adapted fish left in dark; and 4) dark-adapted fish transferred to light. For groups 2 and 4, fish were left 1.5 hr after injection before transfer to opposite light conditions to allow penetration of the drug. Eyes were fixed 3.5 hr after injection. To test recovery from CB effects, groups 2 and 4 were repeated with the modification that 24 hr was allowed after injection before transfer to opposite light conditions. Eyes were fixed 2 hr after transfer.

Myosin Subfragment-1 Binding Studies

These studies were carried out as described in a previous paper (16).

Retinal Dissociation and Enrichment for Cones

The dark-adapted retina, along with attached vitreous, was removed from the posterior eyecup in dim red light to avoid light adaptation. Explanted retinas were incubated in the dark for 15–20 min on a rotary shaker in dissociation medium [Eagle's Basal Medium (BME), adjusted to 99g/l NaCl, 5 mM EGTA, 10 mg/ml glucose, 20 units/ml Penicillin-Streptomycin mix (Gibco), and 1 mg/ml papain (Sigma)]. Retinas were then washed gently 3 times with culture medium similar to the dissociation medium except that 0.1 mg/ml deoxyribonuclease (Sigma) is included instead of papain. The retina was resuspended in culture medium and sheared gently by vortexing. The resulting supernatant medium, which contains predominately rods and cones, was then layered onto a step gradient of 20% and 40% sucrose, and spun at low speed in a desktop centrifuge. Cone cells collect at the 20%:40% sucrose interface. Differential centrifugation in culture medium with low speeds also suffices to enrich for cones, though more rod contamination is present. All procedures are carried out at 22°C up to layering onto cold sucrose; centrifugation and subsequent handling are carried out in the cold.

Negatively-Stained Preparations

Cone suspensions prepared as above were placed in a large drop on carbon-coated formvar grids which had been made hydrophilic by glow discharge. Cells were allowed to settle for 2 min onto the grids, then washed dropwise with 0.1–1% Triton-X-100 in SSS with 1 mM DTT. After further washing dropwise with SSS containing DTT, the grids were negatively stained with 1% aqueous uranyl acetate.

RESULTS

Morphology of the Teleost Retinal Cones

The teleost retinal cone is a long, slender, highly polarized cell (Fig. 1). At its distal end is the photoreceptive outer segment, while at its proximal end is the synaptic specialization (pedicle) which transmits sensed changes in light conditions to the more proximal retinal neurons. At midlength, cone cells are attached by junctional complexes to one another and to adjacent rods via supportive Mueller cells; this dense junctional layer is

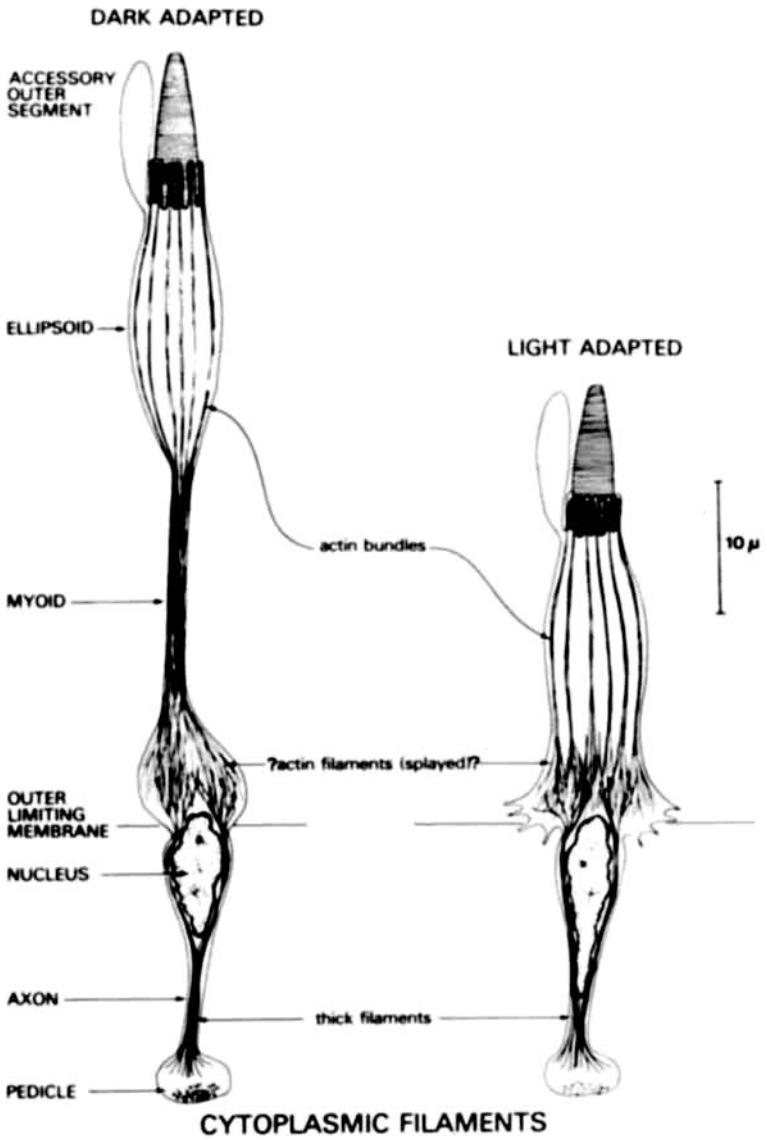

Fig. 1. Schematic illustration of dark- and light-adapted grey snapper cones illustrating the distribution of cytoplasmic filaments described in text. Filament organization in the perinuclear region is yet uncertain because these filaments are not well preserved by fixation procedures. Note that the change in cell length is mediated entirely by the myoid portion of the cone.

called the outer-limiting membrane (OLM) and provides a prominent landmark in the retina.

Distal to the OLM are the ellipsoid and the myoid portions of the cone. The ellipsoid is an expanded, mitochondrion-packed region just below the outer segment; the myoid is a neck-like stalk connecting the ellipsoid to the OLM. The photomechanical elongation and contraction of the cone cell are mediated almost exclusively by the myoid; it forms a long slender stalk in the dark and contracts to form a broad short connection between the ellipsoid and OLM in the light (Fig. 1). During contraction the surface membrane of the myoid is apparently thrown into numerous longitudinally-oriented flutes which protrude between the adjacent rods. The cone nucleus lies just below the OLM. Between the nucleus and the synaptic pedicle is a slender connection called the "axon."

Rates of Elongation and Contraction

Maximum observed rates of myoid elongation and contraction are illustrated in Fig. 2. Elongation rate (2.5 μ/min) and contraction rate (3 μ/min) are remarkably similar under the conditions used in these experiments.

Inhibitor Studies

Intraocularly injected colchicine blocks dark-induced cone elongation (9, 10) when administered in concentrations which disrupt myoid microtubules. Colchicine does not block light-induced contraction or induce movements in the absence of light changes.

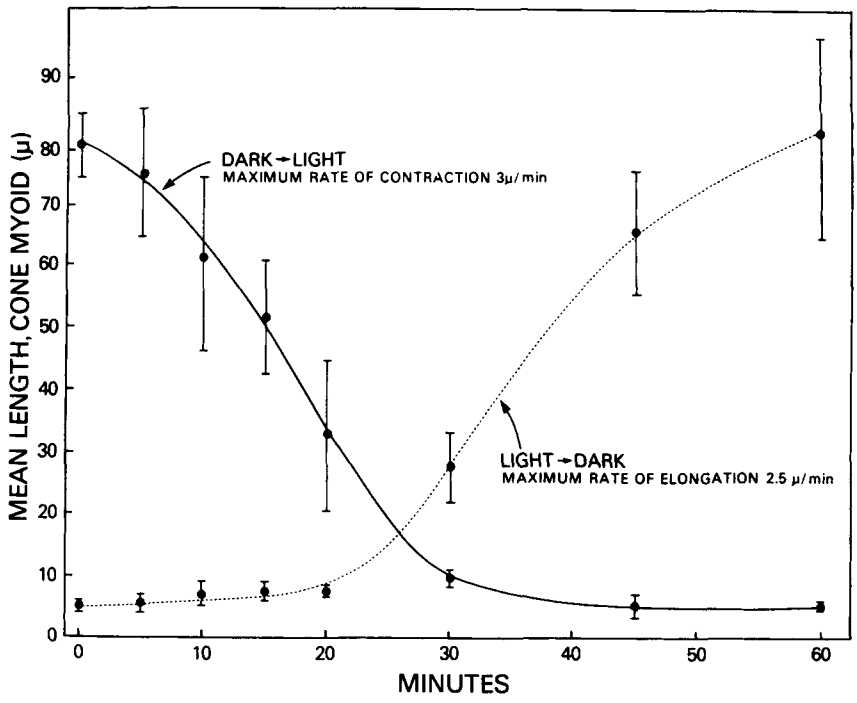

Fig. 2. Rates of elongation and contraction of Haemulon cone myoids after changing light conditions. (Standard deviations for points are indicated; n = 10 for each point.)

Disruption of microtubules in dark-adapted retinas does not produce shortening (collapse) of previously elongated cone myoids in the absence of light, either in colchicine-injected dark-adapted eyes in situ (9, 10), or in isolated cultured dark-adapted retinas treated with cold, high hydrostatic pressure, or colchicine (Fig. 3). Myoid microtubules are present in control cultured retinas but absent in high hydrostatic pressure- and colchicine-treated retinas, and greatly reduced in numbers in the cold-treated retinas. Coupled with the disappearance of microtubules in high-pressure-treated retinal cones is the appearance of electron-dense tactoids in the myoid region (not shown).

Intraocularly injected cytochalasin-B (at a calculated final concentration in the eye of 5 μg/ml) reversibly blocks light-induced cone contraction (Fig. 4). It does not, however, interfere with dark-induced elongation nor produce elongation or contraction in the absence of changes in light conditions. Normal light-induced cone contractions are again observed by 24 hr after CB treatment at 5 μg/ml (Fig. 4). Intraocular CB concentrations of 1 μg/ml have no effect on cone movements.

Cytoplasmic Filaments

Thin Filaments. Teleost retinal cones contain numerous longitudinally-oriented thin filaments. These filaments appear to be slightly thicker than muscle actin filaments, measuring 78 ± 8 Å in diameter by the method described in Materials and Methods. The accuracy implied by such precise measurements is spurious because of the uncertainty produced by measuring an 80 Å filament diameter in a section 500–600 Å in thickness,

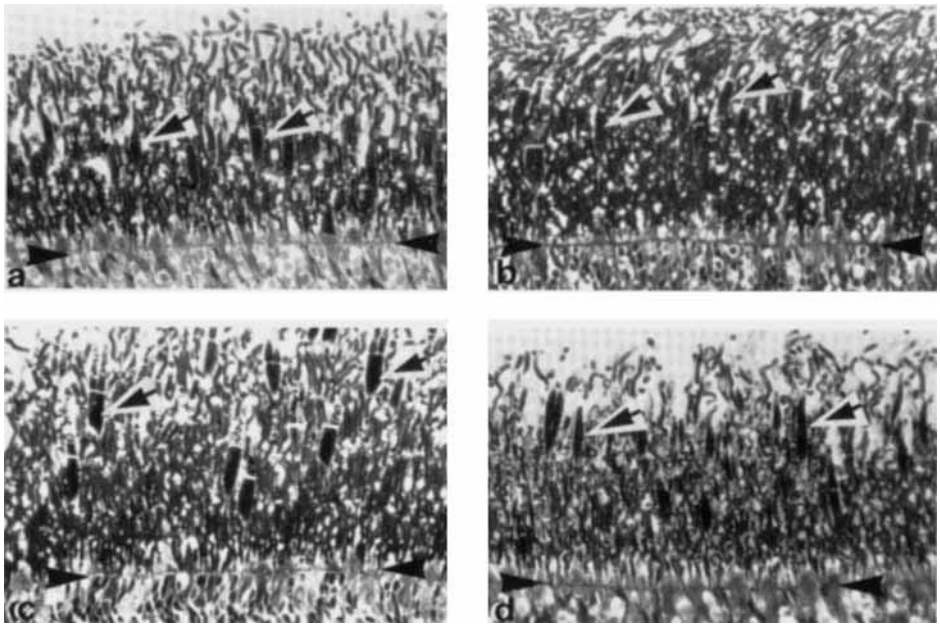

Fig. 3. Disruption of myoid microtubules in isolated dark adapted retinas does not produce cone shortening in the absence of light. a) control retina, b) retina subjected to cold (4°C), c) retina subjected to 10,000 lb/in^2 hydrostatic pressure, d) retina subjected to 10^{-3}M colchicine. Note that all cones (black and white arrows on ellipsoids) have remained elongated. Compare to contracted cones in Fig. 4e. Horizontal black arrows indicate the outer limiting membrane (× 625).

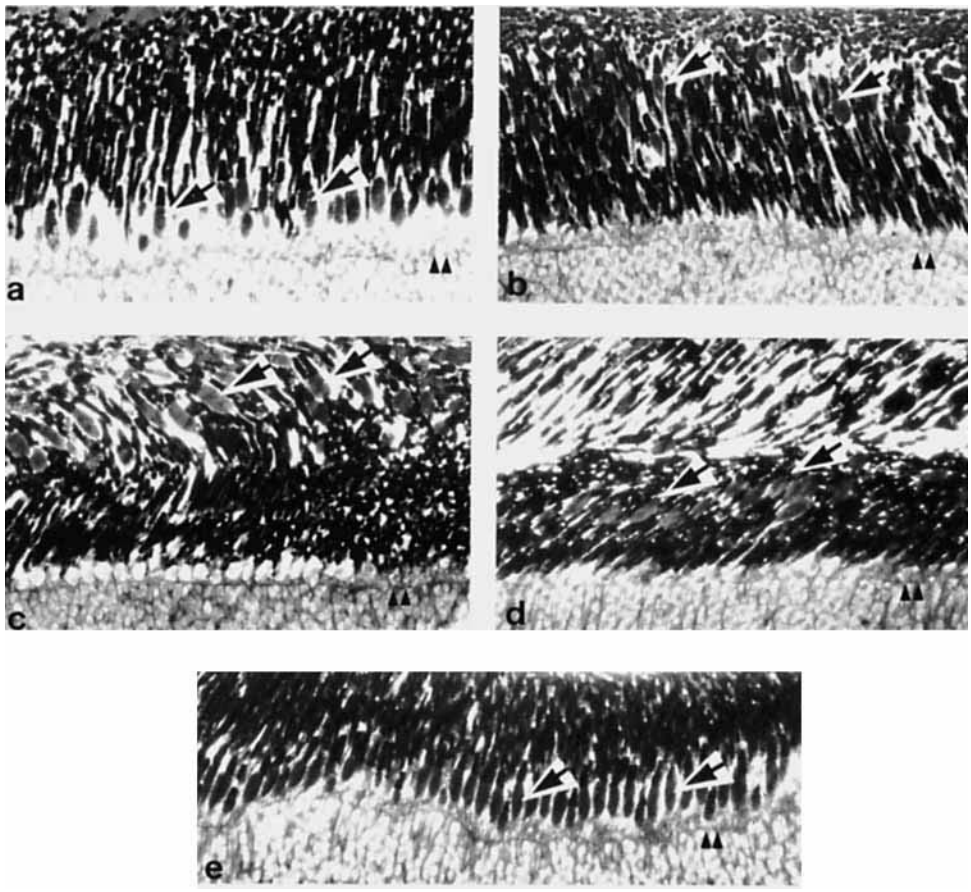

Fig. 4. Cytochalasin-B inhibits light-induced cone contraction. Retinas from eyes subjected to intraocular CB injection, 5μg/ml final concentration: a) light-adapted fish left in light; cones remain short; b) light-adapted fish transferred to dark; cones elongate normally; c) dark-adapted fish transferred to light; cones fail to contract; d) dark-adapted fish left in dark; cones remain elongated; e) dark-adapted fish left in dark 24 hr after CB injection and then transferred to light; cones contract normally. Black and white arrows indicate cone ellipsoids; double arrows indicate outer limiting membrane (× 625).

and by variable effects of fixation and preparation. I give these values primarily to illustrate the range of variability observed in filament diameter and periodicity measurements below.

The distribution of thin filaments within the cone cell is illustrated schematically in Fig. 1. Filament bundles (approximately 0.1 μ in diameter) originate from dense tips of microvillus-like projections which form a chalice around the base of the outer segment (Fig. 1). These discrete filament bundles course downward through the peripheral cytoplasm of the ellipsoid, gradually coalescing into larger and larger bundles until in the myoid they form a single loose skin of filaments. The distribution of thin filaments in the perinuclear and axonal regions of the cone are not yet clear because of technical difficulties to be described below.

Filament bundles containing 60–300 filaments are readily preserved in the ellipsoid region by conventional fixation (Figs. 5a, 6a). Though arranged primarily in discrete bundles, the filaments do not display marked lattice-like packing arrangements.

Filament-to-filament distance is relatively variable and averages 106 ± 20 Å. When retinas are glycerinated before fixation, ellipsoid filaments are more clearly preserved and are more closely packed in the bundles (Figs. 5b, 6b). The thin filaments of the ellipsoid bundles bind myosin subfragment-1 to yield arrowhead configurations typical of muscle actin (Figs. 5c, 6c). In all cases where decoration of ellipsoid filaments was observed, the bundles of filaments were splayed into loose arrays (Figs. 5c, 6c).

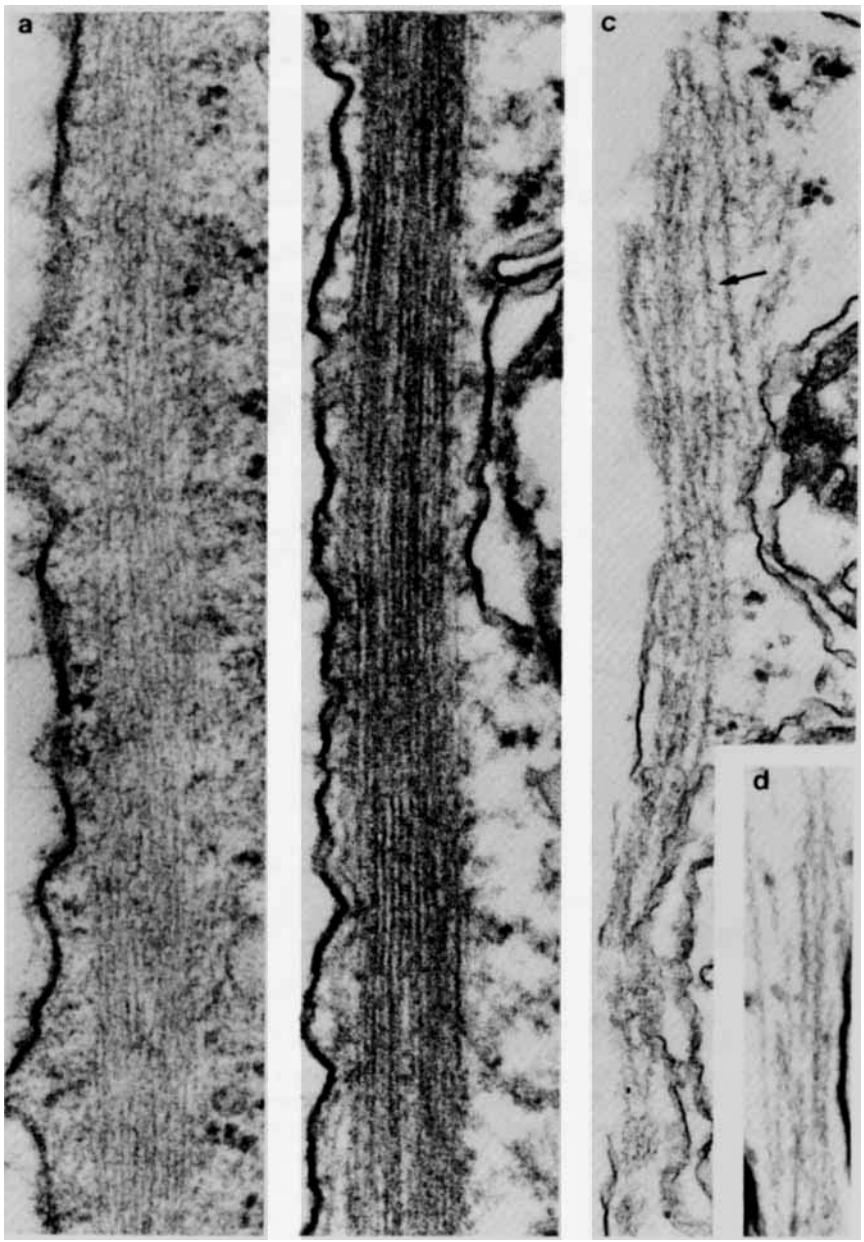

Fig. 5. Longitudinal sections of filament bundles (fb) in the ellipsoid region of the teleost cone (a) after conventional fixation, (b) after glycerination, and (c,d) after incubation with myosin subfragment-1. Some arrowhead configurations are indicated with arrows (a,b × 72,000, c,d × 64,000).

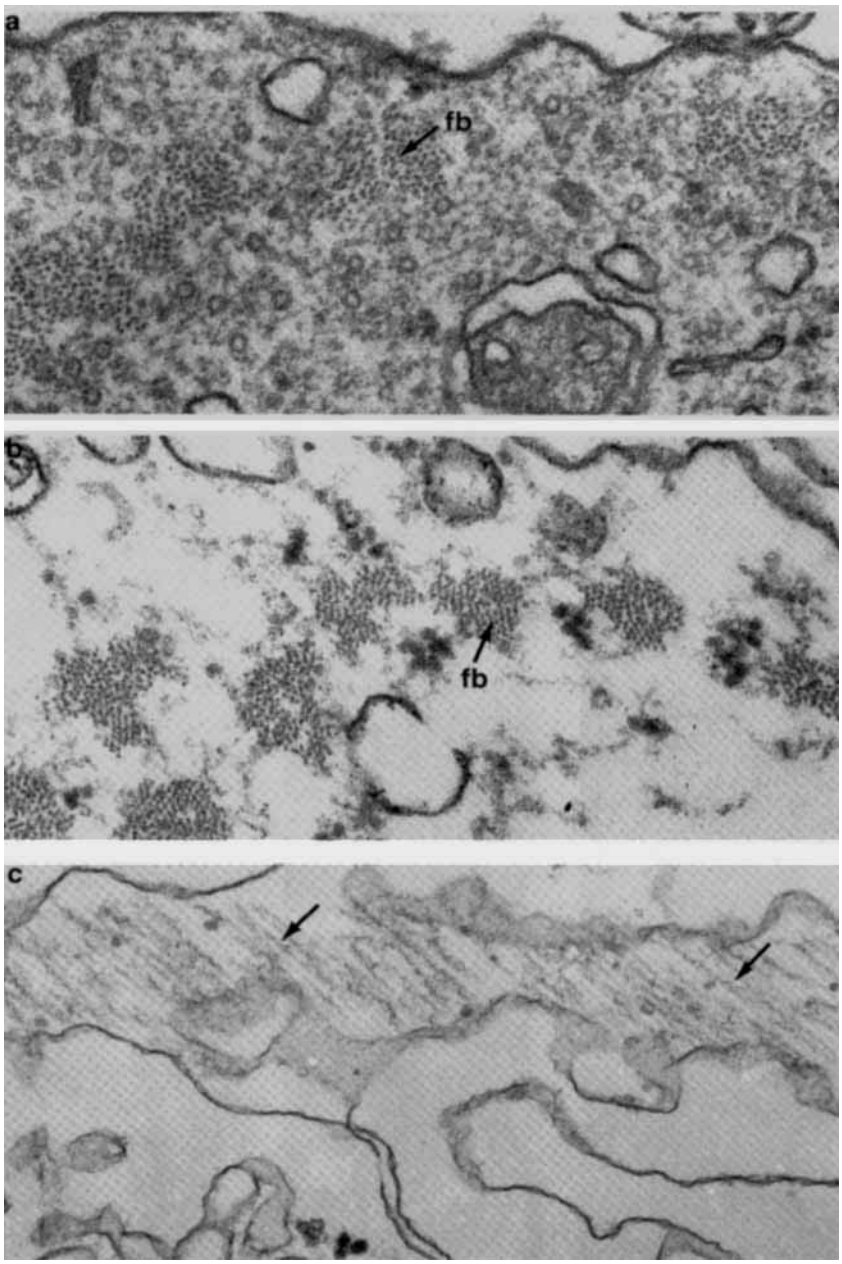

Fig. 6. Transverse sections of filament bundles (fb) in the ellipsoid region of the teleost cone (a) after conventional fixation, (b) after glycerination, and (c) after glycerination and incubation with myosin subfragment-1. Arrows indicate arrowhead configurations (× 72,000).

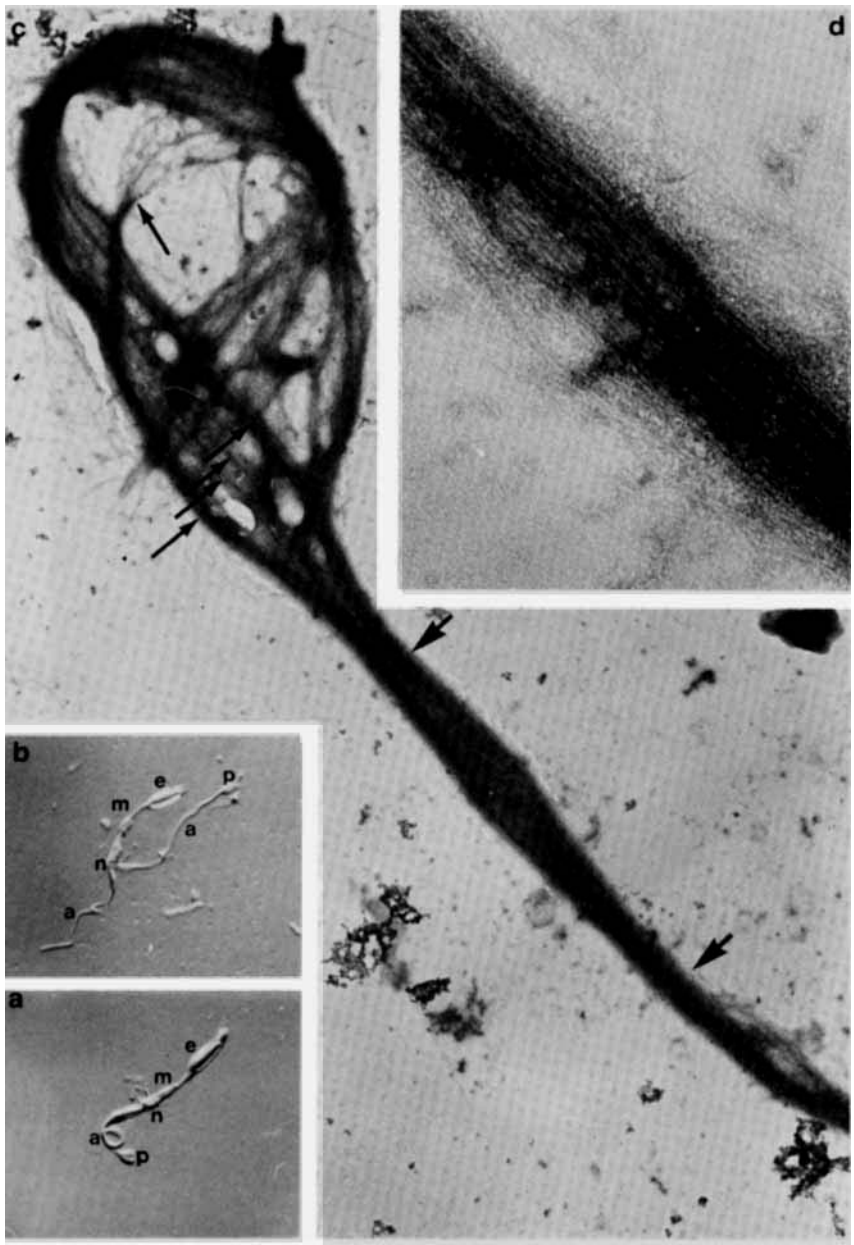

Fig. 7. Isolated cones from dissociated retinas (a,b) attached to grids and washed with Triton-X-100 (c,d). The actin filament "skeleton" of the cell remains attached to the grid after negative staining with uranyl acetate. The individual bundles of the ellipsoid retion (e) have been folded around during washing (small arrows). These bundles fuse to form a large skein of filaments in the myoid region (large arrows). Myoid filaments are illustrated at higher magnification in (d). The parts of the cone are designated on the Nomarski micrographs of the isolated cones (a and b): e = ellipsoid, m = myoid, n = nucleus, a = axon, p = pedicle. Figure 7b is a twin cone; 2 axons are visible (a, b × 400; b × 24,000, c × 120,000).

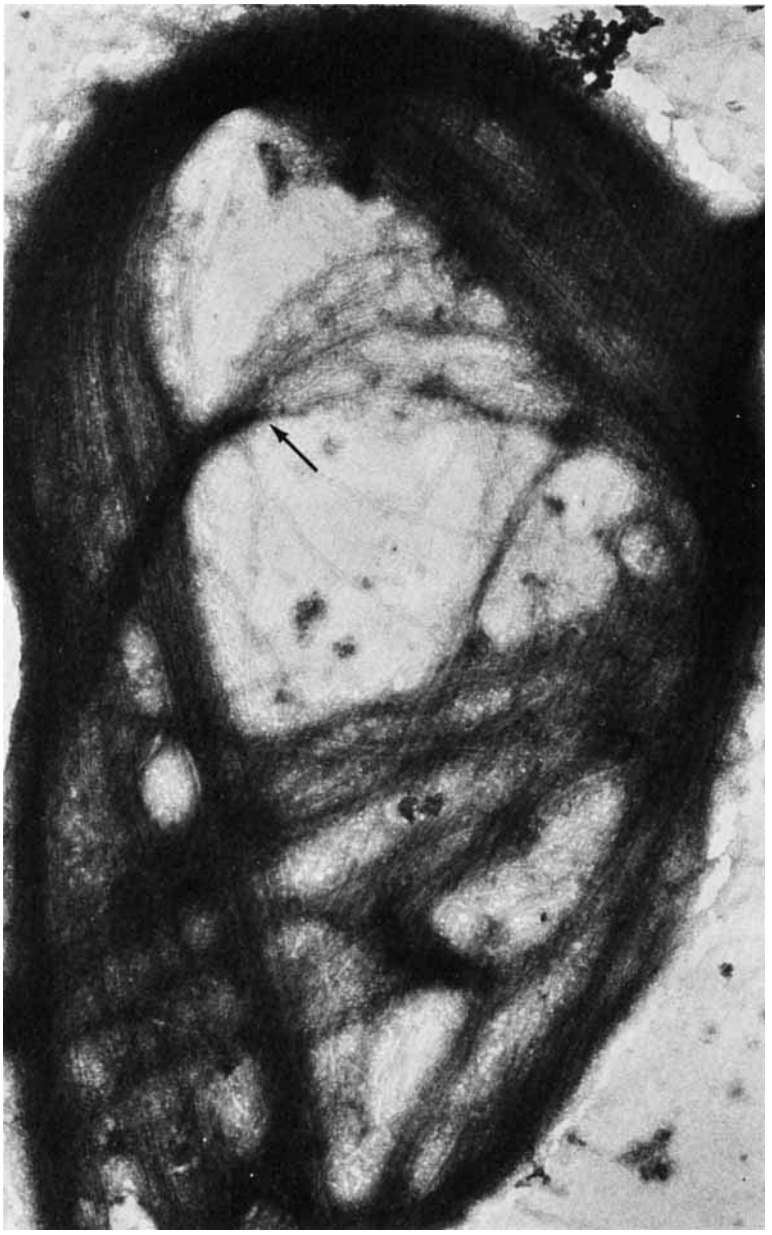

Fig. 8. Ellipsoid actin bundles from cone cell illustrated in Fig. 7. Note region where bundle splays into individual filaments (arrow). Bundles are approximately 0.1μ in diameter as observed in sections (× 72,000).

Myoid thin filaments are not generally preserved in conventional fixation, but they are clearly visible in negatively stained preparations of detergent-disrupted cones (Figs. 7, 8). The presence of myoid thin filaments in rare fixed preparations corroborates the negative-stain observation. In preparations of detergent-disrupted cones, portions of 0.1–0.3 μ diameter ellipsoid bundles were frequently observed while occasionally the

entire length of the ellipsoid and myoid were retained (Fig. 7). In these cases it was clear that the filaments of the discrete ellipsoid bundles were continuous with those of the myoid (Figs. 7, 8). The clarity of the myoid filaments in negatively-stained preparations contrasts with the variability of their preservation in thin-sectioned myoids. The same variability in fixation is observed in perinuclear and axonal thin filaments; in these regions the large nucleus has, so far, interfered with attempts to discern filament distribution by negative staining.

In detergent-treated preparations of isolated cones, both ellipsoid filament bundles and myoid thin filaments decorate with myosin subfragment-1 to form arrowhead complexes typical of muscle actin (Fig. 9).

"Thick" Filaments. Longitudinally-oriented filaments averaging 140 Å in diameter (140 ± 16 Å) are present between the nucleus and pedicle of the cone cells (Fig. 10). These filaments tend to associate laterally into ribbon-like structures in many preparations, though in the best preserved retinas they occur as more discrete entities (Fig. 10a). In many fixations no thick filaments are seen. The tendency to form lateral associations into ribbons does not appear to vary with state of adaptation but rather with fixation. Near the nucleus the large central bundle of thick filaments may splay into many smaller bundles which pass alongside the nucleus to approach the outer limiting membrane. Only extremely rarely were thick filaments seen in the myoid above the outer limiting membrane.

In longitudinal sections of thick filaments, periodic side-arms are sometimes visible (Fig. 10c). Measurements of 150 examples of these repeating distances yielded an average periodicity of 154 Å (± 15 Å).

Intermediate Filaments. Intermediate filaments (101 ± 11 Å) in diameter are occasionally observed in the axonal region of the cone cell between the nucleus and pedicle (Fig. 10b). Interestingly, 100 Å filaments and the thicker 140 Å filaments have never been observed in the same cone cell. The intermediate filaments are generally seen in cone cells of retinas which appear to be less well-fixed by conventional criteria (state of mitochondria, extraction, swelling, etc.).

DISCUSSION

Teleost retinal cones elongate in the dark and contract in the light; these cells contain longitudinally-oriented microtubules (Fig. 11) and thick and thin filaments (Fig. 1). Since elongation is colchicine-sensitive (9, 10), we assume that microtubules play a critical role in the elongation process. Cone shortening, on the other hand, appears to be an active contractile process, rather than merely a collapse produced by disassembling cytoskeletal microtubules. Short, light-adapted cones contain more myoid microtubules in cross-section than elongated cones (9, 10); experimental disruption of microtubules in elongated, dark-adapted cones with microtubule inhibitors does not produce shortening of cones; and intraocularly injected cytochalasin-B (approximately 5 μg/ml) blocks cone contraction in vivo. The mechanism by which CB interferes with contraction is as unclear in the present system as it is in other systems in which CB has been shown to interfere with cytoplasmic contractile machinery (17, 18, 19).

Rates of elongation and contraction in teleost cones are similar and relatively slow (2–3 μ/min). Ali (8,12) has shown that it is the rate, rather than the extent, of elongation or contraction that is affected by external conditions such as light intensity, previous light experience, or possible diurnal rhythms. The rates presented here were obtained

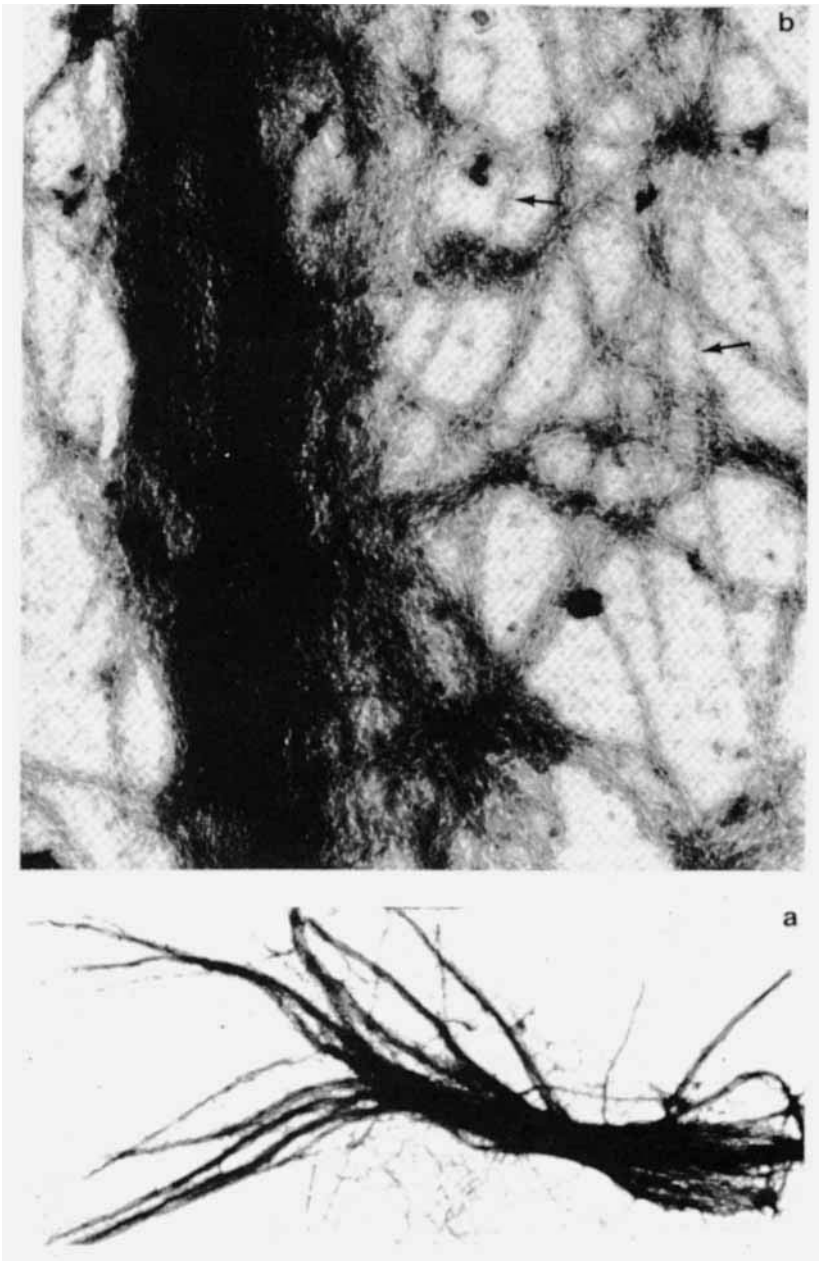

Fig. 9. Filament bundles from isolated cones which have been incubated with myosin subfragment-1 after Triton-X wash. The "skeleton" of filaments is retained in the grid (a) as in Fig. 7. Filaments of the bundles have decorated with S-1 to form typical arrowhead complexes (b) (see arrows) (a × 8,800; b × 68,800).

under conditions selected to optimize these conditions and were the maximal rates observed in this laboratory. Similar rates have been observed in other fish (1–2 μ/min) (12). These rates are comparable to the rate of prometaphase congression and anaphase movement of chromosomes in the mitotic spindle (0.25–2.0 μ/min) (20), but are significantly

slower than most other examples of cytoplasmic motility, such as particle movements in marine eggs (up to 5 μ/sec), melanocyte granule movement (up to 5 μ/sec), or fast transport in nerve axons (1–5 μ/sec) (21, 22).

Microtubules

Changes in microtubule distribution in teleost cones during elongation have been described in detail by Warren and Burnside (9, 10). The number of axial microtubules observed in cross-sections of the elongating myoid decreases approximately 10-fold from the contracted state to the elongated state. This observation is consistent with observed changes in distributions of microtubules in other elongating cells (23, 24), and suggests that cell elongation is not generated merely by elongation of a preexisting set of microtubules; rather, this observation is consistent with the hypothesis that sliding of microtubules contributes to elongation.

Microtubules are associated with cell elongation because they are almost always found oriented parallel to the long axis of elongating cells or projections, and because their disruption with colchicine or other mitotic inhibitors generally blocks elongation (2). The actual mechanism, however, by which microtubules contribute to elongation is still a mystery. Two proposed mechanisms are most commonly considered: 1) that microtubules themselves elongate by adding on more subunits, and thereby push on the ends of the cell (25); and 2) that adjacent microtubules actively slide past one another to decrease overlap, opposite ends thus pushing against the cell (26). Such sliding has been shown to occur between adjacent microtubules of the trypsinized flagellar axonemes (27), and between ribbons of microtubules in the ciliated protozoan Stentor (28). However, correlation of cytoplasmic microtubule sliding with elongation in metazoan cells has proved more difficult to document (2, 24, 29).

Several studies have described changes in microtubule distribution during elongation (9, 10, 23, 24, 30). Each of these studies has found that the number of microtubules observed in cross-sections of elongating cells decreases dramatically as the cell elongates. In fact, in neural plate cells (23, 30) and teleost cones (9, 10) the number of microtubules in cross-section decreases in proportion to the increase in length, thus suggesting that the total cumulative length of microtubules in the cell is conserved. These observations argue against elongation by polymerization of new subunits onto preexisting microtubules, at least in its simplest form; rather some microtubules would have to be elongated at the expense of others. Other evidence against the simplistic model comes from the observation that the cell's microtubular apparatus is composed of overlapping arrays of short microtubules in teleost cones (9, 10) and myoblasts (24), and that 70–75% of the microtubules in teleost cones lie close enough to neighboring microtubules to interact by means of dynein-like intertubular bridges (9, 10, 29). Nevertheless we are able to rule out unequivocally only the simplistic model that a set of microtubules extending the full length of the cell gets longer and thereby elongates the cell. Needless to say many other permutations on this mechanism can be imagined.

Thin and Thick Filaments

My present understanding of the filament distribution in cones is illustrated in Fig. 1. Longitudinally-oriented thin filaments bind myosin subfragment-1 and thus presumably contain actin. The accuracy of the thin filament distribution illustrated in Fig. 1 in the region near the outer-limiting membrane (note question marks) is limited by the lability of thin filaments in this region to EM fixation and by the interference of the bulky nucleus with my ability to see negatively stained filaments in this region.

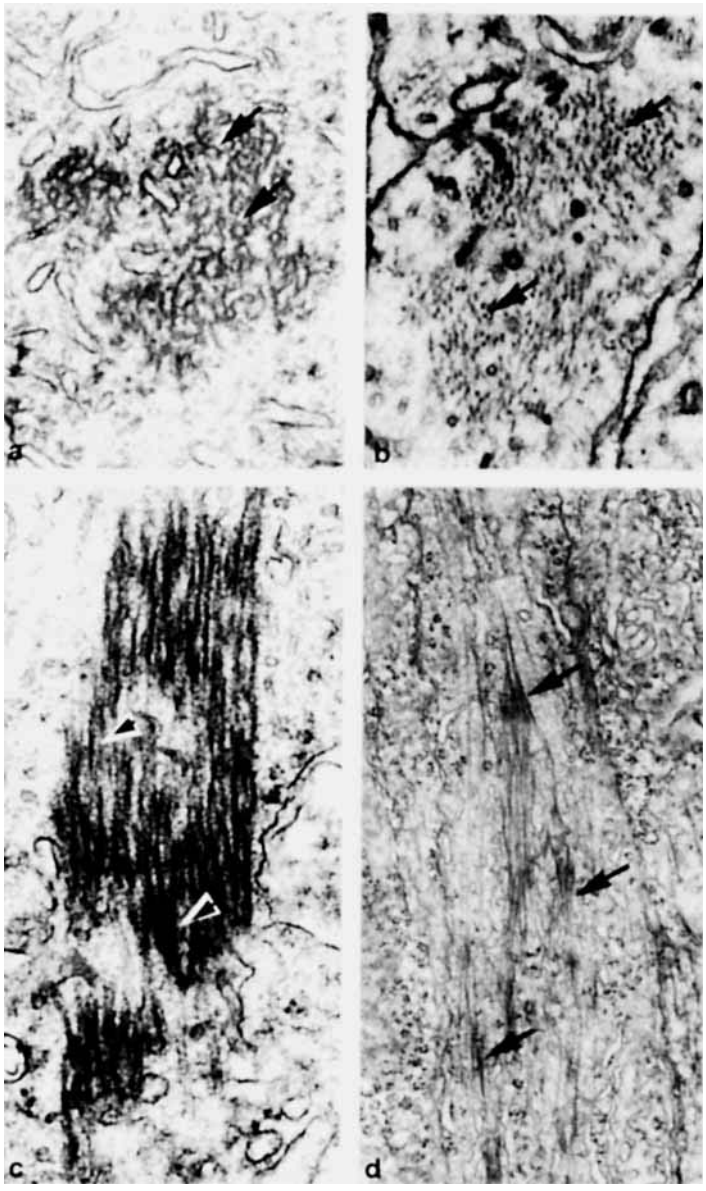

Fig. 10. Thick filaments, approximately 140 Å in diameter, illustrated in cross-section (a) (arrows) and longitudinal sections (c,d) (arrows) of the cone axon region. Intermediate (100 Å) filaments (b) (arrows) are also sometimes seen in this region. Note periodicities along thick filaments in c (arrows); this repeat is approximately 150 Å (a,c × 60,000, b × 64,000; d × 18,400).

Thin filament bundles of the ellipsoid (where no contraction occurs) are well preserved by fixation and negative staining. The myoid filaments are clearly visualized in negative-stain preparations of isolated detergent-disrupted cones, but are rarely preserved in conventional fixation. This discrepancy in preservation of myoid thin filaments between negative-stain and fixation procedures in precisely the region of the cell where

contraction occurs raises some questions about our current understanding of thin filament distributions in nonmuscle cells. In conventionally fixed cells the thin filaments we see are not necessarily representative of the in vivo distribution of polymerized actin. Some filaments may not be actin while, on the other hand, some actin is almost certainly lost. Pollard (31) has shown that muscle f-actin, without associated tropomyosin, is not preserved by conventional aldehyde:osmium fixation procedures. Thus, bundles of filaments which are well preserved (such as stress fibers in cultured cells) may not represent the full complement of polymerized actin present in the living state. In teleost cones

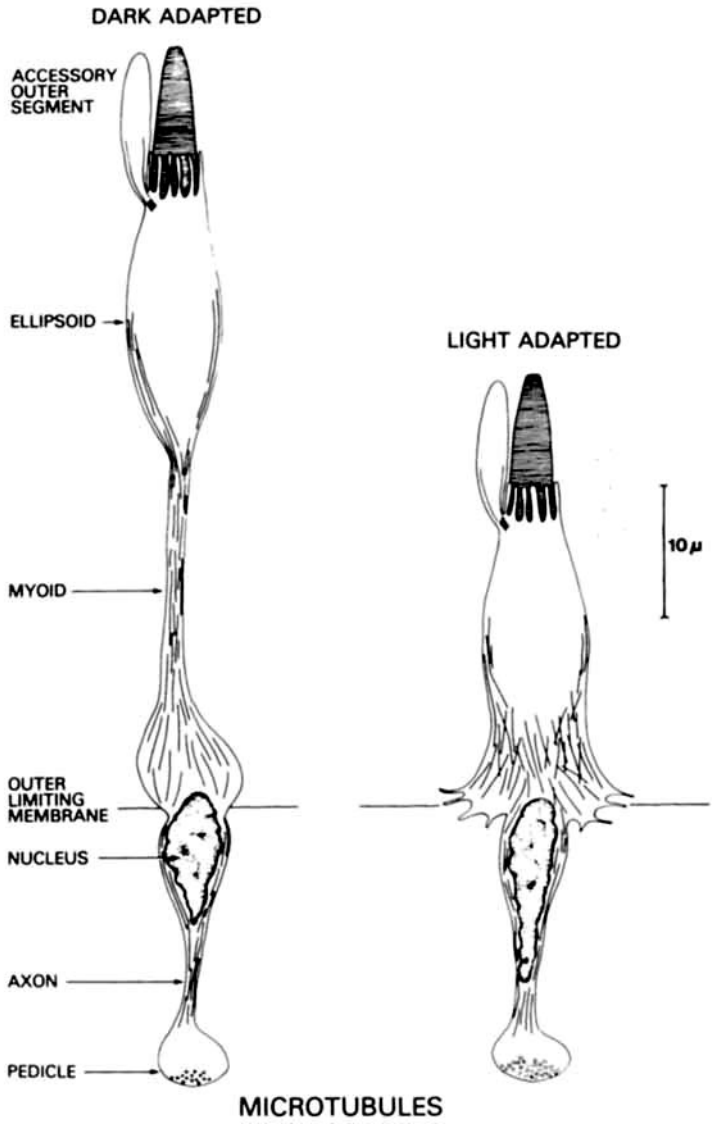

Fig. 11. Schematic drawing of light- and dark-adapted grey snapper cones illustrating distribution of cytoplasmic microtubules.

where contractile and noncontractile regions of the cell are topographically distinguishable, typical filament bundles (resembling stress fibers) are preserved only in the noncontractile part of the cell. In the contractile region no filaments are preserved by conventional fixation, although filaments are known to be present in this region by negative staining of detergent-treated cells. These observations raise questions about whether filaments observed in most cell types after conventional fixation are really "contractile" rather than, say, cytoskeletal in function (cf. 32). In cases where oriented filaments are appropriately arranged to account for observed cellular contractions [e.g., cytokinesis, cell shape determination (17, 33)] individual filaments are often poorly resolved after fixation. Resolution of these fixation problems will be critical to our ability to characterize filament distribution changes during cytoplasmic contractions. The teleost cone provides a useful tool for a search for an actin-preserving fixative because the contractile portion of the cell can be specifically examined while undergoing contraction.

Thick filaments, approximately 140 Å in diameter, are found in the cone cell between the nucleus and the synaptic pedicle (Fig. 1). These filaments are clearly distinguishable from 100 Å neurofilaments seen in nearby nerve processes of the outer plexiform layer both by their morphology and by their lability to some fixations. The composition and function of these thick filaments is not yet clear, but their size and the presence of side-arms with approximately 150 Å periodicity in the best preserved examples suggest they might be myosin. In several fixations, the cone's thick filaments appeared to be laterally aggregated into ribbon-like structures reminiscent of those observed after conventional fixation of vertebrate smooth muscle (34, 35, 36). Since these ribbons are observed in smooth muscle only after immersion fixation in glutaraldehyde but not in freeze substitution or perfusion fixation, it has been generally assumed that the aggregation of filaments into ribbons is an artifact of the slow fixation (37). Immersion fixation was also used for the cone preparation described here. Anti-myosin antibody localization studies are in progress to ascertain whether the cone's thick filaments are in fact myosin.

CONCLUDING REMARKS

The linear organization of the teleost retinal cone makes it a promising model for elucidating the structural bases of elongation and contraction. We know that microtubules are necessary for cone elongation, and we know, in some detail, what changes in microtubule distribution accompany the elongation process (10). I am presently attempting to produce motile models of teleost cones in which physiological parameters of the elongation process may be further delineated.

We know that cone shortening appears to be an active contractile process, and we know that cones possess thick and thin filaments which are appropriately oriented to participate in contraction. We do not yet know unequivocally, however, that these particular filaments represent the cell's contractile apparatus, nor, if they do, how they produce contraction. Currently a search is underway for a fixation procedure which will allow more precise documentation of changes in filament distribution during contraction. The discovery of a preparatory procedure which faithfully retains the cell's actomyosin system is a critical step in developing our concepts of the structural basis of cell contractility. The unique orientation and distribution of filaments in the cone cell makes it particularly amenable to such a fixation search.

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