Taxol Induces Postmitotic Myoblasts to Assemble Interdigitating Microtubule-Myosin Arrays that Exclude Actin Filaments

PARKER B. ANTIN, SUZANNE FORRY-SCHAUDIES, THEA M. FRIEDMAN, S. J. TAPSCOTT, and HOWARD HOLTZER

Department of Anatomy, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104

ABSTRACT   Taxol has the following effects on myogenic cultures: (a) it blocks cell replication of presumptive myoblasts and fibroblasts. (b) It induces the aggregation of microtubules into sheets or massive cables in presumptive myoblasts and fibroblasts, but not in postmitotic, mononucleated myoblasts. (c) It induces normally elongated postmitotic myoblasts to form stubby, star-shaped cells. (d) It reversibly blocks the fusion of the star-shaped myoblasts into multinucleated myotubes. (e) It augments the number of microtubules in postmitotic myoblasts, and these are assembled into interdigitating arrays of microtubules and myosin filaments. (f) Actin filaments are largely excluded from these interdigitating microtubule-myosin complexes. (g) The myosin filaments in the interdigitating microtubule-myosin arrays are aligned laterally, forming A-bands ~1.5 μm long.

Depolymerization of microtubules by Colcemid or related drugs results in changes in cell shape accompanied by changes in the distribution and function of the cytoskeletal and contractile proteins (3, 5–7, 15, 25, 31, 36). These changes can be seen most clearly in developing muscle where microtubules, intermediate-sized filaments, and striated myofibrils are all oriented parallel to the longitudinal axis of the mononucleated, postmitotic myoblasts or multinucleated myotubes. After exposure to Colcemid, the normally elongated postmitotic mononucleated myoblasts retract and assume a rounded morphology (19, 24, 42); similarly, multinucleated myotubes retract and form "multinucleated myosacs" (2, 29). In these cells the alignment of myofibrils is greatly distorted and the intermediate filaments are aggregated into immense cables (10, 19, 25).

In contrast to Colcemid, taxol promotes tubulin polymerization and stabilizes microtubules both in vitro and in cultured fibroblasts (32, 33). To explore further the contribution of microtubules to myofibril formation, we cultured myogenic cells in the presence of taxol. We report here that the response of presumptive myoblasts and fibroblasts to taxol differs considerably from that of postmitotic mononucleated myoblasts. Taxol not only blocks fusion of postmitotic myoblasts into multinucleated myotubes but also converts such myoblasts into star-shaped cells. These star-shaped mononucleated myoblasts assemble orderly arrays of interdigitating microtubule-myosin

complexes. Actin filaments are greatly reduced in number in taxol-treated myoblasts and often are totally excluded from the interdigitating microtubule-myosin arrays. Although actin filaments are depleted in these interdigitating microtubule-myosin arrays, the myosin filaments align laterally and are ~1.5 μm in length.

MATERIALS AND METHODS

Cell Culture

Primary cultures of mononucleated cells were prepared from breast muscles of 11-d chick embryos (2, 10). The cells were plated onto collagen-coated cover slips in 35-mm petri dishes at 1.5 × 10⁶ cells/ml. Taxol (Natural Products Branch, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, Md.) in dimethyl sulfoxide was added to the medium (final concentration, 10 μM) when the cultures were established, and the cells remained in the drug until fixed on day 4. In a second set of experiments, taxol was added to day-3 cultures and the cells were reared in the drug for another 3 d. There was no detectable difference in the effect of taxol on the mononucleated, postmitotic myoblasts in these two sets of experiments. The response of multinucleated myotubes to taxol will be detailed elsewhere.

Fluorescence and Electron Microscopy

Striated myofibrils were visualized by use of a fluorescein-conjugated, affinity-purified, rabbit anti–light meromyosin (anti-LMM). In Ouchterlony tests this anti-LMM forms a single band with adult skeletal LMM or with the whole myosin molecule, whereas it forms a spur with cardiac myosin (11, 14). It does

not precipitate myosins from presumptive myoblasts, fibroblasts, smooth muscles, nerve cells, or other nonmuscle cells. The anti-LMM localizes exclusively along the lateral edges of the A-band in both skeletal and cardiac myofibrils (14, 18). To detect nascent Z-bands, an affinity-purified anti-α-actinin was used; this was a kind gift of Dr. S. Craig, Medical School, The Johns Hopkins University, Baltimore, Md. Its properties are described in Craig and Pardo (9). The distribution of microtubules was followed by the use of an antitubulin kindly provided by Dr. B. R. Brinkley, Medical School, Baylor University, Houston, Tex. Its properties are described in reference 5.

To visualize the localization of the anti-LMM and the antitubulin in the same myoblasts, we double-stained the cells as described in Bennett et al. (4) and Tapscott et al. (39). After primary staining with antitubulin, the cells were treated concurrently with rhodamine-conjugated rabbit anti-sheep IgG and with directly conjugated fluorescein anti-LMM.

In some experiments it is desirable to distinguish presumptive myoblasts and/ or fibroblasts that do not bind anti-LMM from postmitotic myoblasts that do bind anti-LMM. To this end, control cells were triple-stained. First, they were stained with the fluorescein-conjugated anti-LMM. After extensive washing with 0.5 M Tris/0.5% Triton X-100, the cells were incubated for 1.5 h with rhodamine-coupled goat anti-rabbit IgG. Lastly, the cells were stained with bisbenzimide (1 μg/ml in phosphate-buffered saline), which stains all nuclei green (39). This procedure allows localization of the anti-LMM in both the fluorescein and rhodamine channels and the nuclear bisbenzimide stain in only the fluorescein channel of the fluorescence microscope. Thus, even after inspection of the green bisbenzimide-stained nuclei for a period that bleached the fluorescein on the anti-LMM, this antibody could still be localized because it was also tagged with rhodamine (see Fig. 1 a and b).

For EM, cells were prepared as previously described (40).

RESULTS

Effects of Taxol on Postmitotic, Mononucleated Myoblasts

FLUORESCENCE MICROSCOPY: Schiff and Horwitz (33) report that taxol blocks fibroblasts in G_2 or M. Similarly, cell multiplication in myogenic cultures is inhibited by taxol. This inhibition of cell replication and/or survival can be appreciated by comparing the density of a day-4 control culture (Fig. 1 a) with that seen in a comparable taxol-treated culture (Fig. 2). In another series of experiments, cultures were stained with bisbenzimide. Ten randomly selected microscopic fields from control cultures contained 2,196 nuclei; a similar number of microscopic fields from taxol-treated cultures contained 215 nuclei.

Approximately 10% of the surviving cells in the taxol-treated cultures are broad, highly spread "fibroblastic" cells without distinct processes. Their nuclei are very flat. These cells do not bind anti-LMM and are probably presumptive myoblasts and fibroblasts (8, 20, 23, 24). The remaining survivors in taxol (90%) are mononucleated "stubby" cells, most frequently with two to six tapering processes. These processes confer a distinct star shape on most of these cells (Fig. 4 a-f). Immunofluorescent staining with anti-LMM and anti-α-actinin reveals that the arms of the star-shaped cells contain individual A-bands ~1.5μm in length. These dimensions are indistinguishable from those of A-bands in control myotubes (Figs. 1 a and b and 3) and mature myofibrils (21, 23). The orientation, number, and width of the individual myofibrils vary. Most commonly, those myofibrils that occupy the interior of the myoblast tend to be straight, though frequently overlapping with other myofibrils, whereas others follow the contours of the cell surface. Often a circular striated myofibril girdles the nucleus.

Myogenic and nonmyogenic cells treated with taxol for 4 d bind the antitubulin, showing areas of greater or lesser intensity. What is particularly germane to the focus of this paper is the spatial relationship between microtubules and striated myofibrils. Accordingly, taxol-treated postmitotic myoblasts were double-stained with anti-LMM and antitubulin. As illustrated

in Figs. 5 a and b, 6 a and b, and 7 a and b, the same myofibril that appears striated with labelled anti-LMM often appears as a continuous intensely-labeled stripe with antitubulin. It is interesting that even after the cell polarity is disrupted with taxol the microtubules and myofibrils maintain a coextensive distribution.

The star-shaped, postmitotic myoblasts are the equivalent of the elongated, postmitotic myoblasts found in vivo and in all normal cultures (Fig. 3; references 21-23). Many of these normal postmitotic myoblasts fuse within several hours after their terminal mitosis to form multinucleated myotubes (18-20, 23, 24). Taxol not only dramatically alters the morphology of the postmitotic myoblasts but also promptly blocks fusion. Both of these effects are reversible. When medium with taxol is replaced by normal medium, the myoblasts lose their star-shaped configuration, elongate greatly, and fuse to form thin multinucleated myotubes in spite of their low density (Fig. 8).

EM: In normal myoblasts or myotubes, microtubules are not particularly prominent. They tend to orient parallel to, and between, individual myofibrils. Occasionally, a microtubule crosses over or under a myofibril (16, 25, 26, 41), but there is no obvious structural relationship between these two components. Microtubules, along with longitudinally oriented 10-nm filaments (4, 18, 25), appear to be rigorously excluded from the paracrystalline domain of mature or assembling thick and thin filaments. Even in insects in which the ratio of surrounding microtubules to interdigitating thick and thin filaments is high, microtubules do not interdigitate with either myosin or actin filaments (1).

EM sections revealed that taxol induces a striking augmentation in numbers of microtubules and a remarkable interdigitation of microtubules and myosin filaments that is, we believe, unprecedented in either normal or abnormal muscle. To determine the ultrastructural relationship between microtubules and myosin filaments, we prepared longitudinal and cross sections. Particular attention was directed to sectioning those striated myofibrils that subtended the cell membrane.

Fig. 9, a longitudinal section just beneath the membrane of a star-shaped myoblast, illustrates five consecutive sarcomeres of an early assembling myofibril. This taxol-treated myoblast contains thick filaments that are ~1.5 μm long but that have not yet achieved their rigorous lateral alignment, two characteristics common also to immature myofibrils in normal myoblasts (23, 25). Variability in the dimensions of I-bands and the amount of electron-opaque Z-band material is also characteristic of nascent myofibrils in both control and taxol-treated cells. Similarly, the future I-band in both control and taxol-treated cells is associated with elements of the sarcoplasmic reticulum (Figs. 9 and 10). However, in taxol-treated myoblasts, the I-band frequently consists largely of microtubules instead of morphologically recognizable thin filaments (Fig. 9, inset). Microtubules extending >7 μm in length and spanning at least three consecutive sarcomeres are often seen in such cells. Even at the relatively low magnification of Fig. 10, the microtubules can be observed intermingling with loosely packed, thick filaments that make up the forming A-bands. That, in fact, microtubules do interdigitate with myosin filaments and that, in these arrays, there are no morphologically identifiable thin filaments is shown in Fig. 11. Tilted thin sections of the kind illustrated in Fig. 11 viewed stereoscopically not only confirm the absence of thin filaments in these developing A-bands but reveal considerable detail regarding the myosin-microtubule and the myosin-myosin linkages. These structural details will
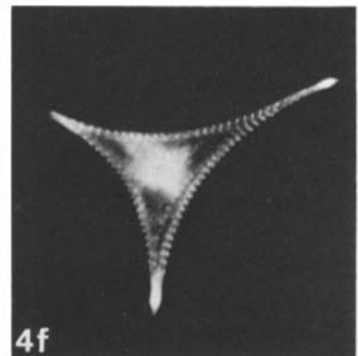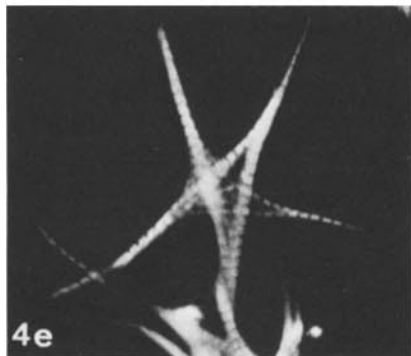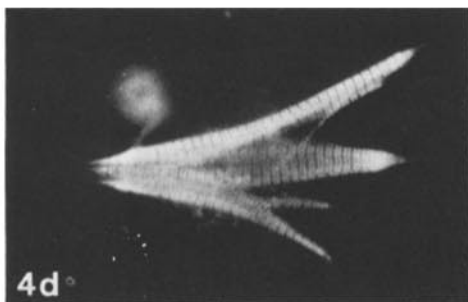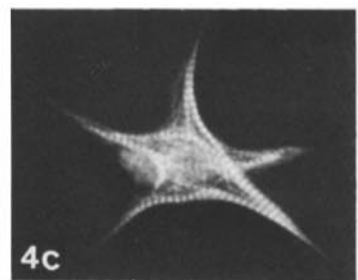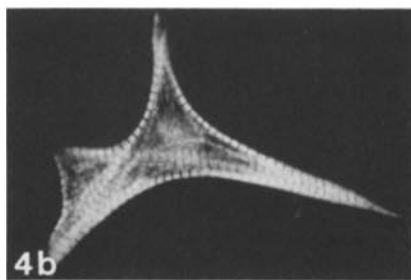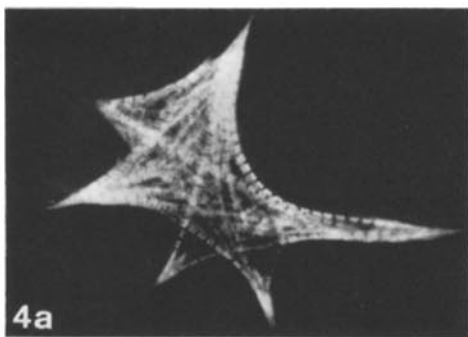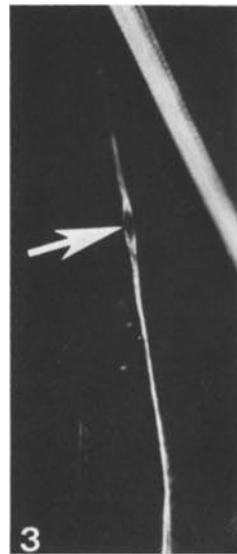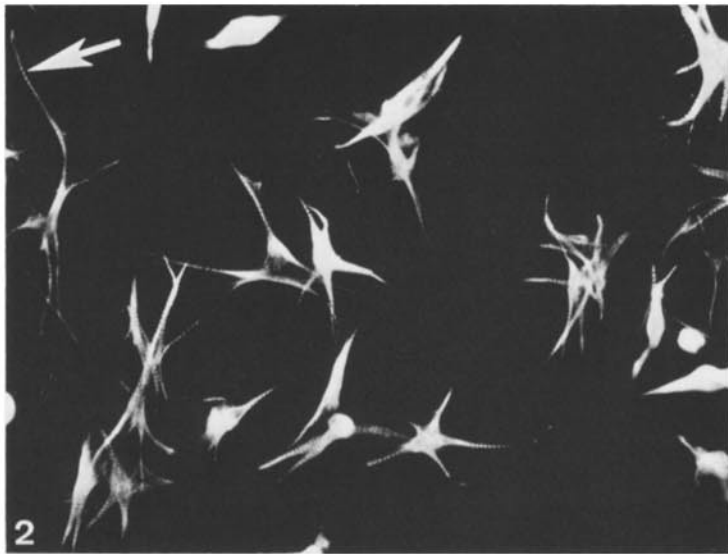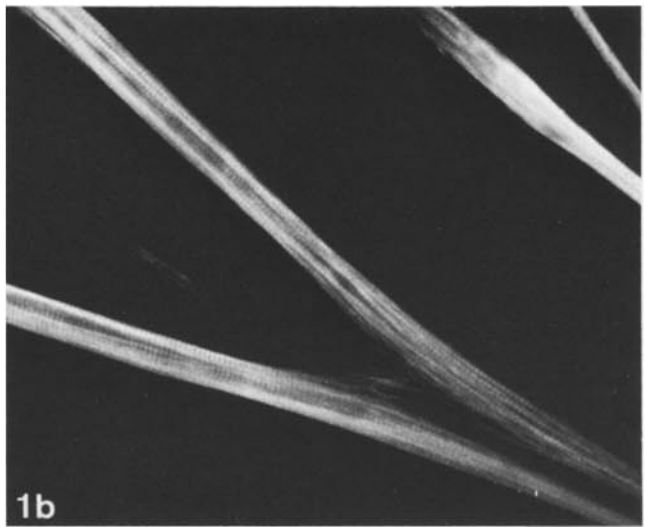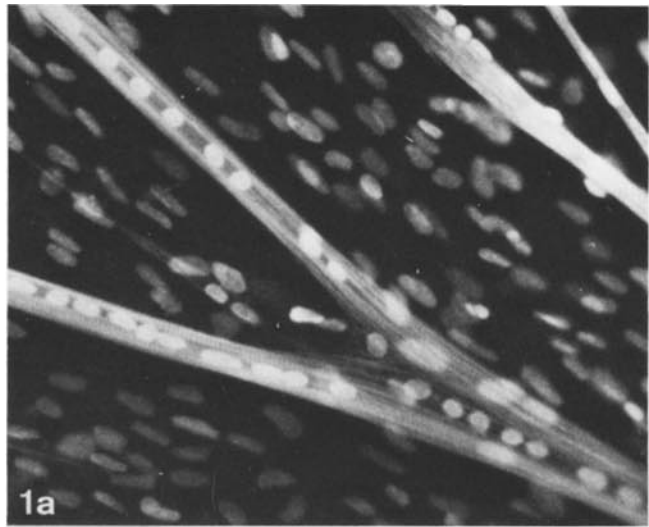
be described elsewhere (Somlyo, Somlyo, and Holtzer, manuscript in preparation).

A more critical demonstration of the interdigitation of microtubules and thick filaments, as well as the absence of morphologically recognizable actin filaments in such complexes, is shown in cross sections of taxol-treated, star-shaped cells (cf. Fig. 12, a cross section through a control 4-d myoblast, with Figs. 13 and 14). The most obvious features of these micrographs are the great diminution in recognizable thin filaments that would be expected to associate with thick filaments, and the remarkable parallel arrays of interdigitating microtubule-myosin complexes. The most frequent arrangements of the microtubule-myosin complexes in cross section are: (a) A central microtubule with an electron-lucent halo, surrounded by five equidistant myosin filaments, or (b) two microtubules, with center-to-center spacings of 60–80 nm surrounded by six to eight equidistant myosin filaments. Absolute numbers of microtubules, myosin filaments, and actin filaments in Figs. 12 and 13 are of interest. The numbers of myosin:actin: microtubules for Fig. 12 are 120:250:2, and for Fig. 13 are 108: 2(?):64. The question mark regarding the number of actin filaments in Fig. 13 refers to the fact that we cannot be certain that these filaments are actin rather than 10-nm filaments. To obtain a more reliable, though admittedly still crude estimate of filament ratios, we prepared a photographic montage ~7 μm^2 from a random section through a star-shaped cell. Only those filaments that were cut in cross section were counted. In this case the ratio of myosin:actin:microtubules is 733:88:513. The difficulty in distinguishing actin filaments from 10-nm filaments in cross section necessitated scoring both of these filament types as actin. Therefore, the figure for actin filaments may be inflated. In regions of the star-shaped cells where the density of the microtubules is more normal, small clusters of myofibrils are observed to consist of a more normal complement of interdigitating thick and thin filaments. A rigorous correlation between fluorescence and EM micrographs is dif-

ficult. Whether a series of "sarcomeres" form in the total absence of all thin filaments remains to be determined.

The Effects of Taxol on Presumptive Myoblasts and Fibroblasts

The effect of taxol on the overall morphology of replicating presumptive myoblasts and fibroblasts is very different from its effect on postmitotic myoblasts. It does not induce these cells—or heart cells or cartilage cells—to become stubby or star-shaped. Although no effort has been made to quantitate microtubules per cell in presumptive myoblasts and fibroblasts, most sections of these taxol-treated cells display many more microtubules than do untreated cells. The distribution of microtubules in these cells falls into three major classes, all of which differ qualitatively from those found in postmitotic myoblasts: (a) They appear as parallel microtubules that are aligned along the cytoplasmic face of some, but not all, endoplasmic reticulum (ER) cisternae. Views of such cisternae in which the microtubules are cut in cross section reveal a precise spacing, with respect to both cisternal membranes and adjacent tubules (Fig. 16; see also reference 33). Such arrays of microtubules have not been observed in the star-shaped myoblasts. (b) They form several tightly packed cables that may consist of 200–300 microtubules (Fig. 15). Morphologically, the bridges that link the microtubules in these cables appear similar to those in axons that link microtubules to neighboring 10-nm neurofilaments (35). No cell organelle, including 10-nm filaments, ribosomes, mitochondria, ER, etc., has yet been observed within the paracrystalline domain of these cables of microtubules. Cables of microtubules have not been observed in star-shaped myoblasts. (c) Loose clusters of 50–200 microtubules also form parallel arrays. However, the distances between individual microtubules in these loose clusters is >80 nm. No obvious morphological connections are detected between individual microtubules. Frequently, 10-nm filaments

---

FIGURE 1 (a) A day-4 culture triple-stained with (i) fluorescein-labeled rabbit anti-LMM, followed by (ii) incubation with rhodamine-labeled goat anti-rabbit IgG, and (iii) treatment with bisbenzimide to stain nuclei green. The localization of the anti-LMM to the lateral edges of the A-bands is partially obscured owing to the low magnification and to the fluorescent haze of the green bisbenzimide-stained nuclei. The flat, oval nuclei are characteristic of both replicating presumptive myoblasts and fibroblasts. X 330. (b) The same microscopic field as illustrated in a, viewed to reveal the rhodamine-labeled anti-LMM only. Note that the specificity of the anti-LMM is such that it is not bound to either the microfilaments subtending the cell membranes or to the cytoplasmic myosin in the presumptive myoblasts or fibroblasts. X 330.

FIGURE 2 A day-4 culture grown continuously in taxol and stained with fluorescein-labeled anti-LMM only. Staining with bisbenzimide was deliberately omitted, because the fluorescent haze associated with the stained nuclei obscures visualization of the striated myofibrils in these small myoblasts. Inspection under the phase-contrast and fluorescence microscopes reveals that in this particular microscopic field there is not a single cell that does not bind the anti-LMM in a striated fashion—i.e., there are no presumptive myoblasts or fibroblasts in this microscopic field. In the thicker regions of some cells the striations are not in the plane of focus, hence the apparent diffuse fluorescence. The striated myofibrils extend into the finest of processes (arrow) in these irregular, star-shaped, postmitotic myoblasts. Note the failure of the juxtaposed mononucleated myoblasts to fuse to form multinucleated myotubes. X 260.

FIGURE 3 A fluorescence micrograph of a control day-4 culture illustrating a typical elongated, postmitotic, mononucleated myoblast stained with anti-LMM only. Under higher magnification and with appropriate focusing, A-bands can be recognized throughout the length of the cell. Arrow points to a single nucleus. Such mononucleated, postmitotic myoblasts exhibiting a single myofibril may exceed 600 μm in length. X 360.
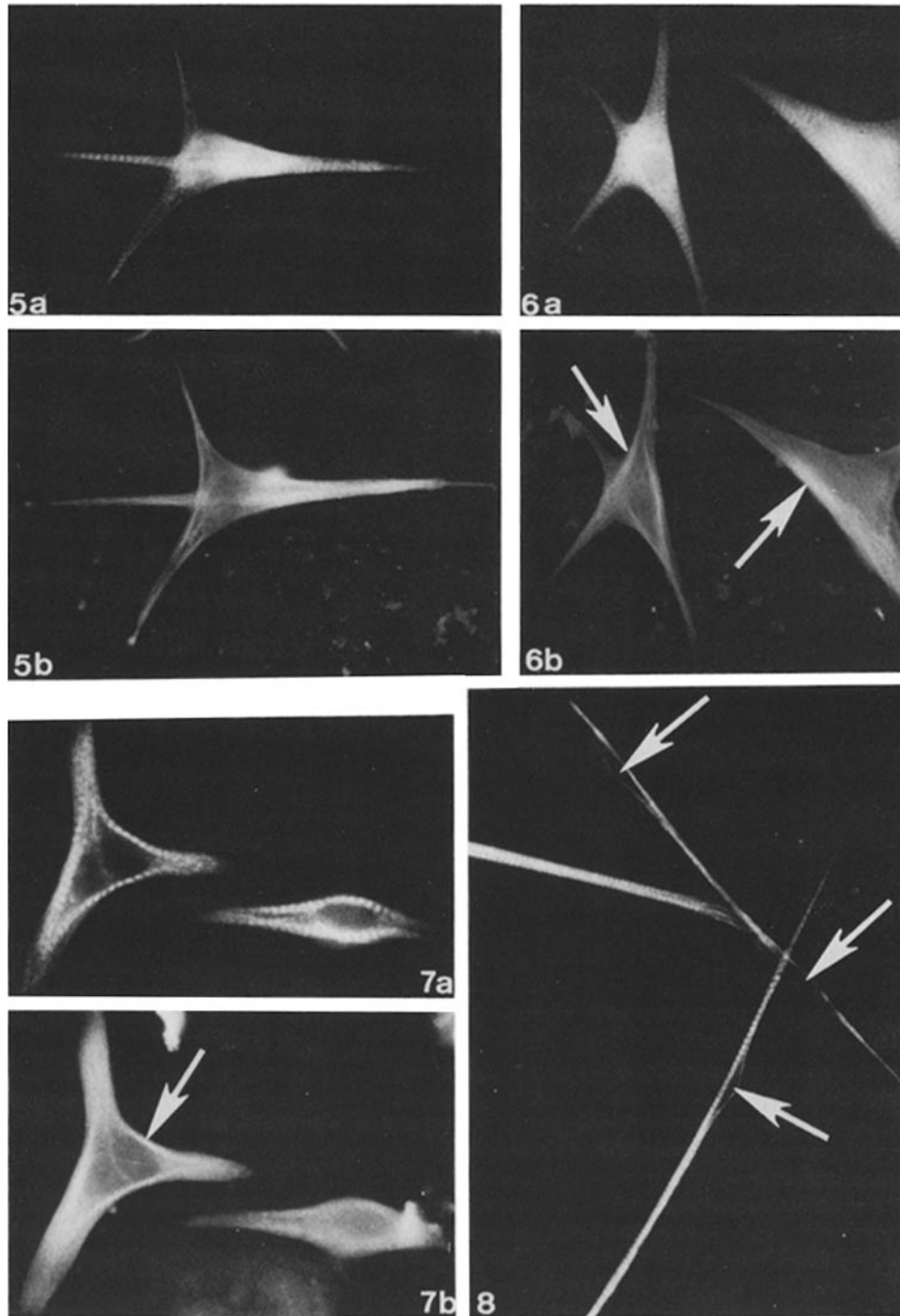
FIGURE 4 Fluorescence micrographs of individual postmitotic, star-shaped myoblasts reared in taxol for 4 d. The myoblasts in a–e are stained with labeled anti-LMM. The myoblast in f is stained with anti-α-actinin, to reveal the Z-band. At these higher magnifications, the individuality of the myofibrils and the size of the A-bands are more apparent. Many myofibrils subtend the cell membrane, faithfully outlining the overall cell morphology. (a, d, and e) X 625; (b, c, and f) X 510.

course between, and generally parallel to, these dispersed microtubules.

DISCUSSION

These experiments with taxol confirm our earlier findings that postmitotic myoblasts do not have to achieve a critical density to fuse, to initiate the synthesis of those contractile proteins that are unique to striated muscle (8, 12, 13, 27, 43), or to assemble those proteins into striated myofibrils (19–21, 24, 28). How taxol reversibly blocks fusion is unknown. It has been suggested that of the 3 major events required for fusion—i.e. (1) generation of fusion-competent postmitotic myoblasts, (2)

FIGURES 5-7   Fluorescence micrographs of six star-shaped myoblasts double-stained with anti-LMM and antitubulin. The myoblasts in Figs. 5 a, 6 a, and 7 a demonstrate the localization of the anti-LMM, whereas Figs. 5 b, 6 b, and 7 b demonstrate the localization of the antitubulin in these same cells. Note the coincidence of the striations localized by the anti-LMM and the intense fluorescent band revealed by the antitubulin (arrows). (Figs. 5 and 6) × 520; (Fig. 7) × 660.

FIGURE 8   A fluorescence micrograph illustrating the reversibility of the taxol effect. Postmitotic myoblasts that had become star-shaped and that had been blocked from fusing during 4 d in taxol became greatly elongated and fused to form multinucleated myotubes consisting of two to five nuclei when grown in normal medium for 6 d. The thin myotube at the left contained four nuclei outside the microscopic field. The myotube at the bottom contained two nuclei in addition to the one illustrated. Arrows point to nuclei. There are no nonmyogenic cells in this microscopic field as determined by phase microscopy. × 340.

cell-cell recognition of migrating, polarized myoblasts, and (3) melding of juxtaposed membranes of aligned postmitotic myoblasts—the latter two require extensive movements of myoblasts along each other's surfaces (20–24). Although myoblasts in taxol display occasional pseudopodial activity, translatory movements are inhibited (our unpublished observations; see also reference 30). The reversible effects of taxol on primary myogenic cells render it unlikely that (a) a specific diffusible factor that is a function of cell density is indispensable for fusion and (b) postmitotic mononucleated myoblasts reared at
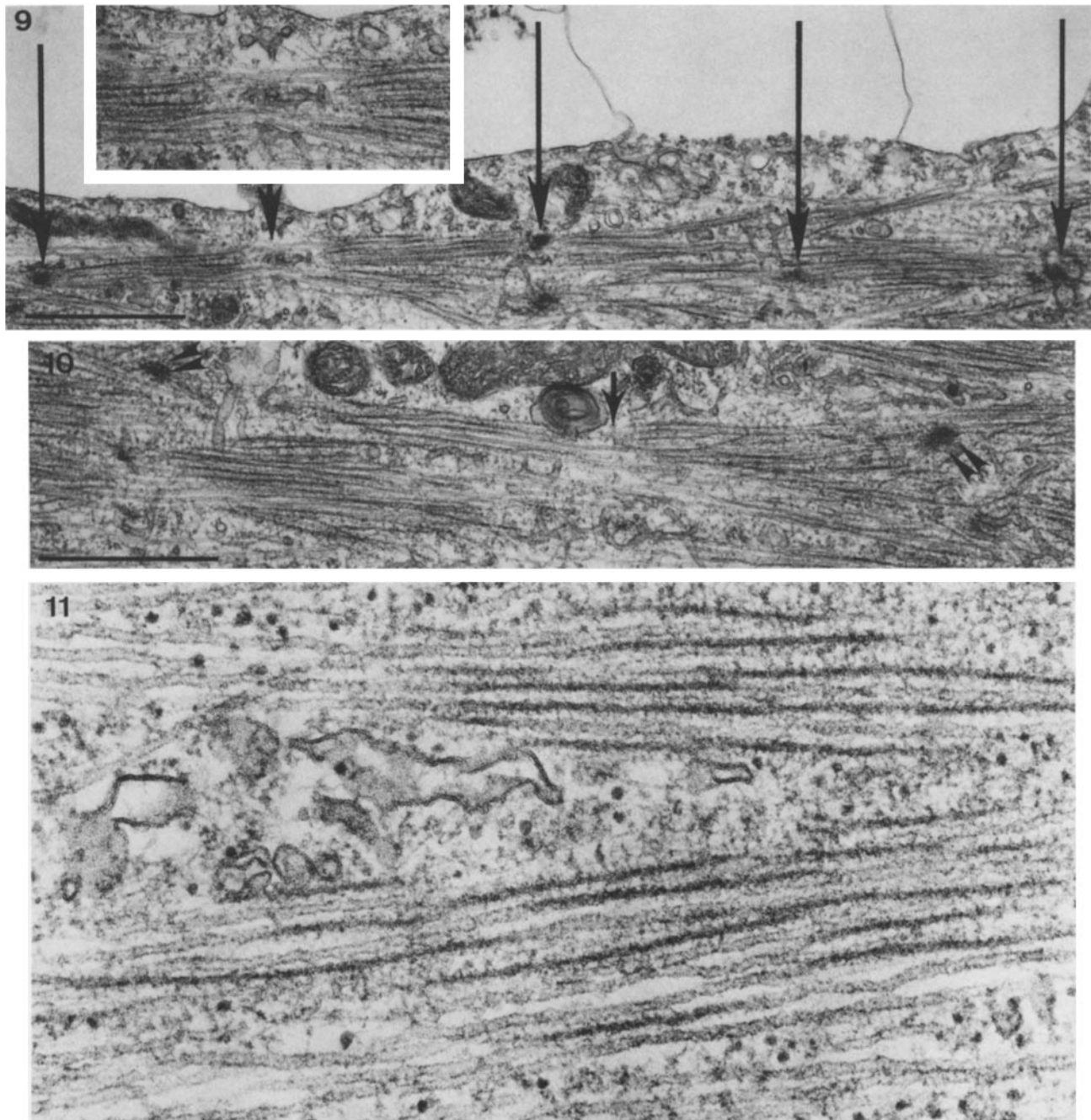

FIGURE 9 A longitudinal section just beneath the sarcolemma of a star-shaped myoblast demonstrating five consecutive sarcomeres. The five vertical arrows indicate the very early formation of the electron-opaque Z-band. The future I-bands are demarcated by a relatively well developed sarcoplasmic reticulum, which is also characteristic of normal developing as well as mature I-bands. The inset illustrates at least nine microtubules spanning on I-band; these same microtubules laterally interdigitate with the flanking A-bands. No thin filaments could be observed in this particular I-band. Bar, 1.0 μM. × 25,000. Inset, × 46,500.

FIGURE 10 Note the interweaving of the microtubules among the thick filaments forming the two A-bands that occupy the center of the micrograph. The double arrowheads point to the early-forming, irregular Z-bands. Such irregular masses of electron-opaque material are also characteristic of the early Z-bands during the early stages of formation of normal myofibrils. The single arrow in center of micrograph points to the I-band region, rich in microtubules but wanting in thin filaments. Bar, 1 μM. × 29,000.

FIGURE 11 A thin section illustrating the interdigitating microtubule-myosin arrays. Numerous linkages connect myosin filaments to adjacent myosin filaments and to adjacent microtubules. We are indebted to Dr. A. V. Somlyo for this micrograph. Bar, 0.1 μM. × 96,000.

low density have the option to readily reenter the cell cycle. These findings are contrary to a large number of previous reports.

Little is known of the factors that orient microtubules. In nerve fibers and in the mitotic apparatus, they tend to orient parallel to one another, as in normal postmitotic myoblasts.
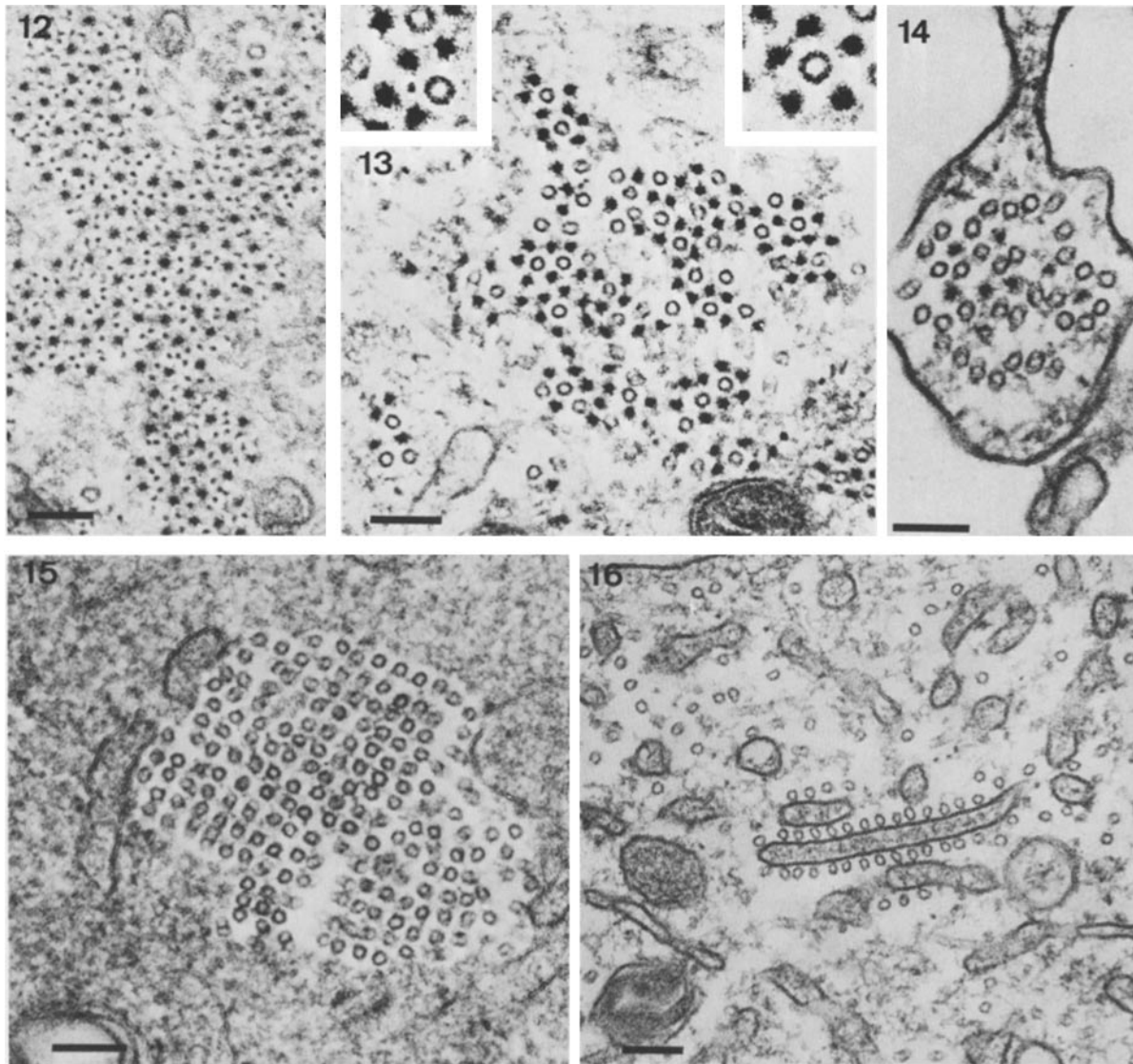

FIGURE 12 A cross section through a day-4 normal myogenic cell. In this emerging myofibril there are ~120 myosin filaments interdigitating with ~250 actin filaments. There are only two microtubules in this field (upper right and lower left), and both are excluded from the domain of the myofibril. Bar, 0.1 μM. × 90,000.

FIGURE 13 A cross section through a single, parallel-oriented cluster of interdigitating arrays of microtubules and myosin filaments from a taxol-treated, star-shaped cell. Actin filaments are not evident. The myosin filaments are frequently triangular and fine processes often bridge adjacent thick filaments and, less frequently, adjacent microtubules. The inset on the right illustrates the common arrangement of five equidistant myosin filaments surrounding one microtubule. The inset on the left illustrates what is either a single actin or a 10-nm filament inserted among three thick filaments and one microtubule. Bar, 0.1 μM. × 99,000. Insets, × 192,000.

FIGURE 14 A cross section through a fine process of a taxol-treated, star-shaped myoblast. Seven myosin filaments are surrounded by ~35 microtubules. In a comparable section through a normal myoblast, seven myosin filaments would interdigitate with 15–20 actin filaments, while the microtubules would be entirely outside of the domain of the growing myofibril. Bar, 0.1 μM. × 104,000.

FIGURE 15 A cross section through a presumptive myoblast or fibroblast illustrating the immense cables of microtubules that taxol induces in these types of cells. No cell organelles are present within the domain of such cables. The cables course irregularly through the cytoplasm for distances of >5 μm. An electron-lucent halo immediately surrounds each microtubule within the cable as well as the entire cable. Such cables have not been observed in postmitotic myoblasts. Bar, 0.1 μM. × 102,000.

FIGURE 16 A cross section through a presumptive myoblast or fibroblast illustrating a sheet of parallel-arrayed microtubules covering a portion of the cisterna of the rough endoplasmic reticulum (RER). These associations between microtubules and some RER membranes extend >3 μm. The center-to-center spacing of these microtubules ranges from 40 to 60 nm. Microtubules covering the RER do not appear linked, although occasionally a fine process appears to connect them to the membrane of the RER. Complexes of microtubules and RER have not been observed in postmitotic myoblasts. Bar, 0.1 μm. × 82,500.

This contrasts with their radial orientation in asters and their complex network in other types of cells (30, 37, 38). Fibroblasts, epithelial cells, and "uninduced" neuroblastoma cells—all relatively isometric—display 5–10 initiation centers distributed around the nucleus (37). However, when a neuroblastoma cell is induced to form a process, the multiple initiation centers coalesce and funnel the microtubules into an axonlike process. In taxol-treated postmitotic myoblasts, clusters of 10–100 interdigitating microtubules and myosin filaments are oriented parallel to one another; nevertheless, within 300–400 nm two or three clusters of similar size may be oriented at different angles. If microtubules "polarize" a cell or a cytoplasmic domain, how can two types of filaments within one domain be parallel to one another, while several hundred nanometers away similar filaments are incorporated into a domain with a different orientation? Alternatively, are microtubules and myosin filaments oriented by yet unknown cellular factors? In this regard the behavior of the initiation centers in normal vs. taxol-treated myoblasts will be worth following.

The formation of arrays of interdigitating microtubules and myosin filaments raises many questions. For example, in the establishment of microtubule-myosin arrays, what conditions preclude the assembly and/or maintenance of actin filaments? If indeed actin monomers fail to polymerize into filaments, will this alter the rates of monomer synthesis and degradation? If taxol, directly or indirectly, inhibits actin synthesis, will the number of actin filaments diminish over time in taxol, as a consequence of normal turnover? In this context the finding that there is a considerable pool of G-actin in presumptive myoblasts and fibroblasts but not in postmitotic myoblasts is intriguing (our unpublished observations). The same questions addressed to the synthesis, degradation, and polymerization of actin must also be addressed to tropomyosin and the troponins. Then, too, how will the turnover of the myosins associated with microtubules compare with that of the myosins associated with actin? Lastly, is it possible that, in taxol, actin monomers in some manner have been incorporated into the microtubules?

Finding that myosin filaments interact with microtubules raises the issue of the biological significance of such complexes. Hayashi et al. (17) and Shimo-Oka et al. (34) have recently reported that: (a) myosin and tubulin coprecipitate at low ionic strength, (b) myosin filaments whose cross-bridges were decorated by tubulin aggregate side by side, and (c) 1 mol of myosin binds 2 mol of tubulin dimer. These investigators suggest that such interactions between myosin and tubulin might be involved in many aspects of cell motility or cytoplasmic flow.

Virtually nothing is known of the way in which sarcomeres are assembled (19, 21–24). When the very early myofibril consists of but three to five thick and ~10–20 interdigitating thin filaments, the thick filaments are 1.5 µm in length and form the "nucleus" of the future A-bands. These early myofibrils are surrounded by many microtubules. Growth in diameter of these incipiently striated myofibrils appears to involve the peripheral deposition of alternating thick and thin filaments. Growth in length appears to involve the addition of new thick filaments at the ends of the myofibrils (21, 22, 29). The data in this paper raise the possibility that microtubules may perform a heretofore unsuspected role in the assembly of sarcomeres—possibly by providing a scaffold or a substrate along which myosin monomers polymerize and/or delivering the nascent thick filaments either to the periphery or end of a growing myofibril.

REFERENCES

1. Auber, J. 1969. La myofibrillogenèse du muscle strié. I. Insectes. *J. Microsc. (Paris).* 8: 197–232.
2. Bischoff, R., and H. Holtzer. 1967. The effect of mitotic inhibitors on myogenesis in vitro. *J. Cell Biol.* 36:111–127.
3. Bennett, G. S., S. A. Fellini, J. M. Croop, J. J. Otto, J. Bryan, and H. Holtzer. 1978. Differences among 100Å filament subunits from different cell types. *Proc. Natl. Acad. Sci. U. S. A.* 75:4364–4368.
4. Bennett, G. S., S. A. Fellini, Y. Toyama, and H. Holtzer. 1979. Redistribution of intermediate filament subunits during skeletal myogenesis and maturation in vitro. *J. Cell Biol.* 82:577–584.
5. Brinkley, B., S. Fistel, J. Marcum, and R. Pardue. 1980. Microtubules in cultured cells, indirect immunofluorescent staining with tubulin antibody. *Int. Rev. Cytol.* 63:59–95.
6. Brinkley, B. R., G. M. Fuller, and D. P. Highfield. 1976. Tubulin antibodies as probes for microtubules in dividing and non-dividing mammalian cells. In *Cell Motility.* R. Goldman, T. Pollard, and J. Rosenbaum, editors. Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y. 435–445.
7. Bryan, J. 1974. Biochemical properties of microtubules. *Fed. Proc.* 33:152–157.
8. Chi, J. C., S. A. Fellini, and H. Holtzer. 1975. Differences among myosins synthesized in non-myogenic cells, presumptive myoblasts and myoblasts. *Proc. Natl. Acad. Sci. U. S. A.* 73:4999–5003.
9. Craig, S. W., and J. V. Pardo. 1979. Alpha-actinin localization in the junctional complex of intestinal epithelial cells. *J. Cell Biol.* 80:203–210.
10. Croop, J. M., and H. Holtzer. 1975. Response of fibrogenic and myogenic cells to cytochalasin B and to Colcemid. I. Light microscopic observations. *J. Cell Biol.* 65:271–285.
11. Croop, J. M., Y. Toyama, A. Dlugosz, and H. Holtzer. 1980. Selective effects of phorbol 12-myristate 13-acetate on myofibrils and 10 nm filaments. *Proc. Natl. Acad. Sci. U. S. A.* 77:5273–5277.
12. Dienstman, S. R., and H. Holtzer. 1977. Skeletal myogenesis: control of proliferation in a normal cell lineage. *Exp. Cell Res.* 107:355–364.
13. Emerson, C. P., and S. K. Beckner. 1975. Activation of myosin synthesis in fusing and mononucleated myoblasts. *J. Mol. Biol.* 93:431–447.
14. Fellini, S. A., and H. Holtzer. 1976. The localization of skeletal light meromyosin in cells of myogenic cultures. *Differentiation.* 6:71–74.
15. Franke, W. W., E. Schmid, M. Osborn, and K. Weber. 1978. Different intermediate-sized filaments distinguished by immunofluorescence microscopy. *Proc. Natl. Acad. Sci. U. S. A.* 75:5034–5038.
16. Goldstein, M. A., and M. L. Entman. 1979. Microtubules in mammalian heart cells. *J. Cell Biol.* 80:183–195.
17. Hayashi, M., K. Ohnishi, and K. Hayashi. 1980. Dense precipitate of brain tubulin with skeletal muscle myosin. *J. Biochem. (Tokyo).* 87:1347–1355.
18. Holtzer, H., G. S. Bennett, S. J. Tapscott, J. M. Croop, A. Dlugosz, and Y. Toyama. 1981. Changes in intermediate-sized filaments during myogenesis and neurogenesis. In *International Cell Biol, 1980–81.* H. Schweiger, editor. Springer-Verlag, New York. 293–305.
19. Holtzer, H., J. Croop, S. Dienstman, H. Ishikawa, and A. Somlyo. 1975. The effects of cytochalasin-B and colcemid on myogenic cultures. *Proc. Natl. Acad. Sci. U. S. A.* 72:513–517.
20. Holtzer, H., J. Croop, Y. Toyama, G. Bennett, S. Fellini, and C. West. 1980. Differences in differentiation programs between presumptive myoblasts and their daughters, the definitive myoblast and myotubes. In *Plasticity of Muscle.* D. Pette, editor. W. de Gruyter and Co., New York. 133–146.
21. Holtzer, H., J. Marshall, and H. Fink. 1957. An analysis of myogenesis by the use of fluorescent antimyosin. *J. Cell Biol.* 3:705–723.
22. Holtzer, H., and J. Sanger. 1971. Myogenesis: old views rethought. *Excerpta Med. Int. Congr. Ser.* 240:122–133.
23. Holtzer, H., J. Sanger, H. Ishikawa, and K. Strahs. 1973. Selected topics in skeletal myogenesis. *Cold Spring Harbor Symp. Quant. Biol.* 37:549–566.
24. Holtzer, H., K. Strahs, A. Somlyo, and H. Ishikawa. 1975. Thick and thin filaments in postmitotic mononucleated myoblasts. *Science (Wash. D.C.).* 188:943–945.
25. Ishikawa, H., R. Bischoff, and H. Holtzer. 1968. Mitosis and intermediate-sized filaments in developing skeletal muscle. *J. Cell Biol.* 38:538–555.
26. Ishikawa, H., R. Bischoff, and H. Holtzer. 1969. The formation of arrowhead complexes with heavy meromyosin in a variety of cell types. *J. Cell Biol.* 73:312–330.
27. Kligman, D., and M. Nameroff. 1980. Analysis of the myogenic lineage in chick embryos. I. Studies on the terminal cell division. Exp. Cell Res. 125:201–210.
28. Moss, P. S., and R. C. Strohman. 1976. Myosin synthesis by fusion-arrested chick embryo myoblasts in cell culture. *Dev. Biol.* 48:431–437.
29. Okazaki, K., and H. Holtzer. 1965. An analysis of myogenesis in vitro using fluorescein-labelled antimyosin. *J. Cytochem. Histochem.* 13:726–739.
30. Osborn, M., and K. Weber. 1977. The display of microtubules in transformed cells. *Cell.* 12:561–571.
31. Ruff, E. 1980. The control of microtubule assembly in vivo. *Int. Rev. Cytol.* 59:2–83.
32. Schiff, P. B., J. Fant, S. B. Horwitz. 1979. Promotion of microtubule assembly in vitro by taxol. *Nature (Lond.).* 277:665–667.
33. Schiff, P. B., and S. B. Horwitz. 1980. Taxol stabilizes microtubules in mouse fibroblast cells. *Proc. Natl. Acad. Sci. U. S. A.* 77:1561–1565.
34. Shimo-Oka, T., M. Hayashi, and Y. Satanabe. 1980. Tubulin-myosin interaction. Some properties of binding between tubulin and myosin. *Biochemistry.* 21:4921–4926.
35. Smith, D. S., U. Jarlfors, and H. Beranek. 1970. The organization of synaptic axoplasm in the lamprey (*Petromyzon marinus*) central nervous system. *J. Cell Biol.* 46:199–219.
36. Soifer, D., editor. 1975. Biology of cytoplasmic microtubules. *Ann. N. Y. Acad. Sci.* 253 pp.
37. Spiegelman, B. M., M. A. Lopata, and M. W. Kirschner. 1979. Multiple sites for the initiation of microtubule assembly in mammalian cells. *Cell.* 16:239–252.
38. Spiegelman, B. M., M. A. Lopata, and M. W. Kirschner. 1979. Aggregation of microtubule initiation sites preceding neurite outgrowth in mouse neuroblastoma cells. *Cell.* 16:253–263.
39. Tapscott, S. J., G. S. Bennett, Y. Toyama, and H. Holtzer. 1981. Intermediate filament proteins in the developing chick spinal cord. *Dev. Biol.* In press.

40. Toyama, Y., C. West, and H. Holtzer. 1979. Differential response of myofibrils and 10-nm-filaments to a co-carcinogen. *Am. J. Anat.* 156:131-146.
41. Van Winkle, W. B., and A. Schwartz. 1978. Morphological and biochemical correlates of skeletal muscle contractility in the cat. I. Histochemical and electron microscopic studies. *J. Cell. Physiol.* 97:99-120.
42. Warren, R. H. 1968. The effect of colchicine on myogenesis *in vivo* in *Rana pipiens* and *Rhodnius prolixus* (Hemiptera). *J. Cell Biol.* 39:544-555.
43. Yeoh, G. C. T., and H. Holtzer. 1977. The effect of cell density, conditioned medium and cytosine arabinoside on myogenesis in primary and secondary cultures. *Exp. Cell Res.* 104:63-78.