

Fate of Microtubule-organizing Centers during Myogenesis In Vitro

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ABSTRACT Microtubule organization and nucleation were studied during in vitro human myogenesis by immunocytology that used monoclonal and polyclonal antitubulin antibodies and a rabbit nonimmune serum that reacts with human centrosomes. In myoblasts, we observed a classical microtubule network centered on juxtannuclear centrosomes.

Myotubes possessed numerous microtubules organized in parallel without any apparent nucleation centers. Centrosomes in these cells were not associated one to each nucleus but were often clustered in the vicinity of nuclei groups. They were significantly smaller than those of the mononucleated cells. The periphery of each nucleus in myotubes was labeled with the serum that labels centrosomes suggesting a profound reorganization of microtubule-nucleating material. Regrowth experiments after Nocodazole treatment established that microtubules were growing from the periphery of the nuclei. The redistribution of nucleating material was shown to take place early after myoblast fusion. Such a phenomenon appears to be specific to myogenic differentiation in that artificially induced polykaryons behaved differently: the centrosomes aggregated to form only one or a few giant nucleating centers and the nuclei did not participate directly in the nucleation of microtubules. The significance of these results is discussed in relation to the possible role of the centrosome in establishing cell polarity.

Differentiation of myoblasts involves their withdrawal from the cell cycle, the onset of synthesis of specific proteins, the fusion of cell membranes, and the intermixing of their cytoplasm (9, 31, 33, 40, 46). A dramatic degree of reorganization of numerous cells is involved, with the consequent formation of a single functional unit. Such a unit must be achieved through the reorganization of any individual compartment, in parallel with a dramatic modification of the constituents of the cytoskeleton. During this process, mutual interactions between myofibrils and intermediate filaments are well documented (23), whereas more scattered information is available for microtubules.

The study of myofibrillogenesis of insect striated muscles led Auber (5) to propose that microtubules would both create an asymmetry of the cell shape and realize a scaffold for myofibrils. The effect of colchicine on myogenesis in vivo was studied in the regenerating tail of the frog tadpole (43). Multinucleate myosacs containing randomly oriented myofibrils were obtained, leading Warren (43) to propose a role of microtubules in the maintenance of cell shape, but the same

author provided evidence against the generality of this conclusion. Several reports recently have emphasized the potential role of microtubules in the myofibrillar organization (4, 16, 41). Myofibril-depleted myotubes were cultured in taxol or Colcemid-containing media (41). Taxol treatment led to the generation of pseudosarcomeres in which actin filaments were lacking, whereas myosin filaments and microtubules appeared to interdigitate in a specific pattern. Myofibrils, however, assembled in the presence of Colcemid though these were very disorganized.

One of the remarkable features of the microtubule network in most animal cells is its organization from a unique center close to the nucleus, the centrosome. The latter is constituted, in most animal cells, of a pair of centrioles surrounded by an ill-defined material which is the real microtubule organizing center (MTOC).¹ Nothing is known about the fate of centrosomes during myogenesis, but, in an ultrastructural study on

¹ *Abbreviations used in this paper:* MTOC, microtubule-organizing center; PEG, polyethylene glycol.

microtubule organization in tadpole regenerating muscles, Warren (44) concluded that centrosomes were no longer acting as MTOCs.

We have undertaken an immunofluorescence study of microtubule organization during human *in vitro* myogenesis. Particular attention was devoted to the sites of microtubules growth through the use of two approaches. In the first, nascent microtubules were decorated with antitubulin antibodies in cells previously treated with depolymerizing agents, whereas in the second, MTOCs were localized with a nonimmune rabbit serum (No. 0013) that reacts with human centrosomes (29). This serum stains the pericentriolar material as judged by an ultrastructural study that uses immunoperoxidase techniques (30, 36), and the labeling changes significantly during the cell cycle in growing cells with maximum size being observed at the metaphasic spindle poles. The amount of labeling material rapidly decreases at telophase. In mitotic cells blocked by taxol, serum 0013 labels the center of each of the numerous small asters produced by the drug away from centrioles and kinetochores (36). From these observations, we concluded that the antigen(s) recognized by serum 0013 were likely to be involved in the control of microtubule nucleation. The characterization of this (these) antigen(s) will be reported elsewhere.

We report here the results obtained on human myotubes that have been retained in culture from 1 to 8 d. Microtubule organization is not centered on the centrosomes that are no longer associated with nuclei on a one-to-one basis. The microtubules grow from the periphery of the nuclei, where the nucleating material, previously associated with myoblast centrioles, relocates. This redistribution is realized early after fusion. It is apparently specific of myogenic syncytia.

MATERIALS AND METHODS

Cells: Normal muscles were obtained from patients undergoing surgical operations. The biopsy specimens were collected in a sterile container containing culture medium and stored at 4°C. The specimens were dissociated by the technique of Yasin et al. (49). The cells were plated on 75-cm² flasks (50–100 mg of muscle per flask) in a growth medium containing 60 parts Ham's F10, 30 parts Dulbecco's modified Eagle's medium containing 50 U/ml penicillin and 50 µg/ml streptomycin, 10 parts fetal calf serum, two parts embryo extract, and 2 mM L-glutamine. The next day the medium was removed, the flasks were washed, and cells were re-fed with growth medium. Every 2 or 3 d, the cells were fed with growth medium. When cells were almost confluent, subcultures were performed after trypsinization. At the second or third subculture 2,000 cells were plated on 14-mm round coverslips precoated with gelatin. The next day the cells were re-fed with growth medium and then every 2 or 3 d. When cells were aligned and began to fuse, they were fed with Dulbecco's modified Eagle's medium, containing 50 U/ml penicillin and 50 µg/ml streptomycin supplemented with 4 mM L-glutamine, 10 parts horse serum, and two parts embryo extract, and then fed every 3 d.

HeLa cells were used to produce artificial polykaryons. Cells were cultured on glass coverslips until they reached a semiconfluent state. Culture medium (Eagle's minimal essential medium containing 10% fetal calf serum) was removed, and the coverslips were immersed for 2–3 min in 50% polyethylene glycol 4000 (PEG) (wt/wt) in saline medium containing 5% DMSO. Culture medium was then slowly added in order to dilute the PEG to 5%. After 5 min at room temperature, cells were placed in fresh medium at 37°C for a period of 10–15 min followed by a final change of medium. To cultivate polykaryons for several days, it was necessary to prevent the growth of unfused cells. This was achieved by adding mitomycin C in the medium at 0.1–0.3 µg/ml.

Fixation: Cells were fixed (fixation I) in PBS (150 mM NaCl, 10 mM Na₂HPO₄, pH 7.4) containing 3% formaldehyde for 30 min at 37°C, then in methanol for 6 min at –20°C. Cells were finally extracted with 0.25% Triton X-100 in PBS for 2 min at 20°C.

Alternatively (fixation II), living cells were washed for 5 s at room temperature with an extraction buffer at pH 6.9 containing 45 mM PIPES, 45 mM HEPES, 10 mM EGTA, 5 mM MgCl₂, and 1 mM phenylmethylsulfonyl

fluoride (27). They were then lysed in extraction buffer supplemented with 1% Triton X-100 for 30 s, washed in extraction buffer, fixed in methanol at –20°C for 6 min, and washed in 150 mM NaCl, 10 mM Tris buffer, pH 7.4.

Immunocytochemistry: Immunocytochemical labeling of microtubules was accomplished using purified polyclonal sheep-antitubulin antibodies (18) or monoclonal anti- α - and β -tubulin antibodies (Amersham France SA, Les Ulis). Nucleation sites of microtubules were specifically labeled with a nonimmune rabbit anticentrosome serum 0013 (29). The second antibodies were respectively rabbit anti-sheep, goat anti-mouse or sheep anti-rabbit immunoglobulins labeled either with fluorescein, Texas Red, or peroxidase. The antibodies were diluted in PBS containing 0.1% Tween 20 (PBS/Tween) and 3% bovine serum albumin. All the washing steps were performed in PBS-Tween.

Microtubule Depolymerization: Microtubules were depolymerized by Nocodazole. Cells were cultured in 5×10^{-6} or 10^{-6} M Nocodazole for 2 h and then fixed either immediately or after 30 s to 5 min recovery in medium alone at 37°C.

RESULTS

Myogenic Cell Culture

Human myogenic cells in growth medium proliferated to confluency and became aligned for fusion. In differentiation medium, extensive fusion took place. Myotubes rapidly (2 d) possessed >10 nuclei, flattened and enlarged in length and width. At 8 d huge myotubes, often Y-shaped, contained hundreds of nuclei, either clustered, particularly in branching parts, or in rows at the center or at the periphery of the myotubes. These myotubes displayed characteristic myofibrils when observed by phase-contrast microscopy. In most cases, they did not seem to be in register (Fig. 1, *d–e*). In contrast with the rat system, the human myotubes produced *in vitro* never showed spontaneous contraction in our work.

Microtubule Pattern

Most of our study was performed on 8-d-old myotubes, which possessed considerable numbers of microtubules compared with the mononucleated cells present in the same culture (Fig. 1, *a–c*). For an enlarged view of microtubule pattern in mononucleated cells, see Fig. 2*b*. These microtubules were both extremely elongated and aligned in a parallel arrangement corresponding to myotube and myofibrils axis. Mononucleated cells, whether myoblasts or fibroblasts, displayed a typical organization of the microtubule network and presented a primary cilium. Usually, the presence of the latter allowed the MTOC to be identified unambiguously (Fig. 2*d*).

MTOC Localization

The use of the anti-human centrosome serum 0013 allowed the localization of centrosomes in mononucleated cells (Fig. 2). They were in a juxtannuclear position and usually at the center of the microtubule aster. After drug-induced depolymerization, microtubules regrew from these sites (Fig. 2, *c–d*). After treatment with the same serum, myotubes presented the following features: (*a*) individual centrosomes were scattered throughout the cytoplasm, and were often located in the vicinity of nuclear groups, but not on an exact one-to-one basis (Fig. 3, *a* and *b*); (*b*) although a precise centrosome count could not be achieved in complete myotubes, the number of identifiable centrosomes appeared to be significantly lower than the total number of nuclei that was present in the same region of a myotube; and (*c*) peripheral labeling of nuclei was clearly detected, a phenomenon that was not observed in mononucleated cells (Fig. 3, *b–d*, Fig. 4, *a, c*, and *e*). A close observation of centrosomes showed that they were

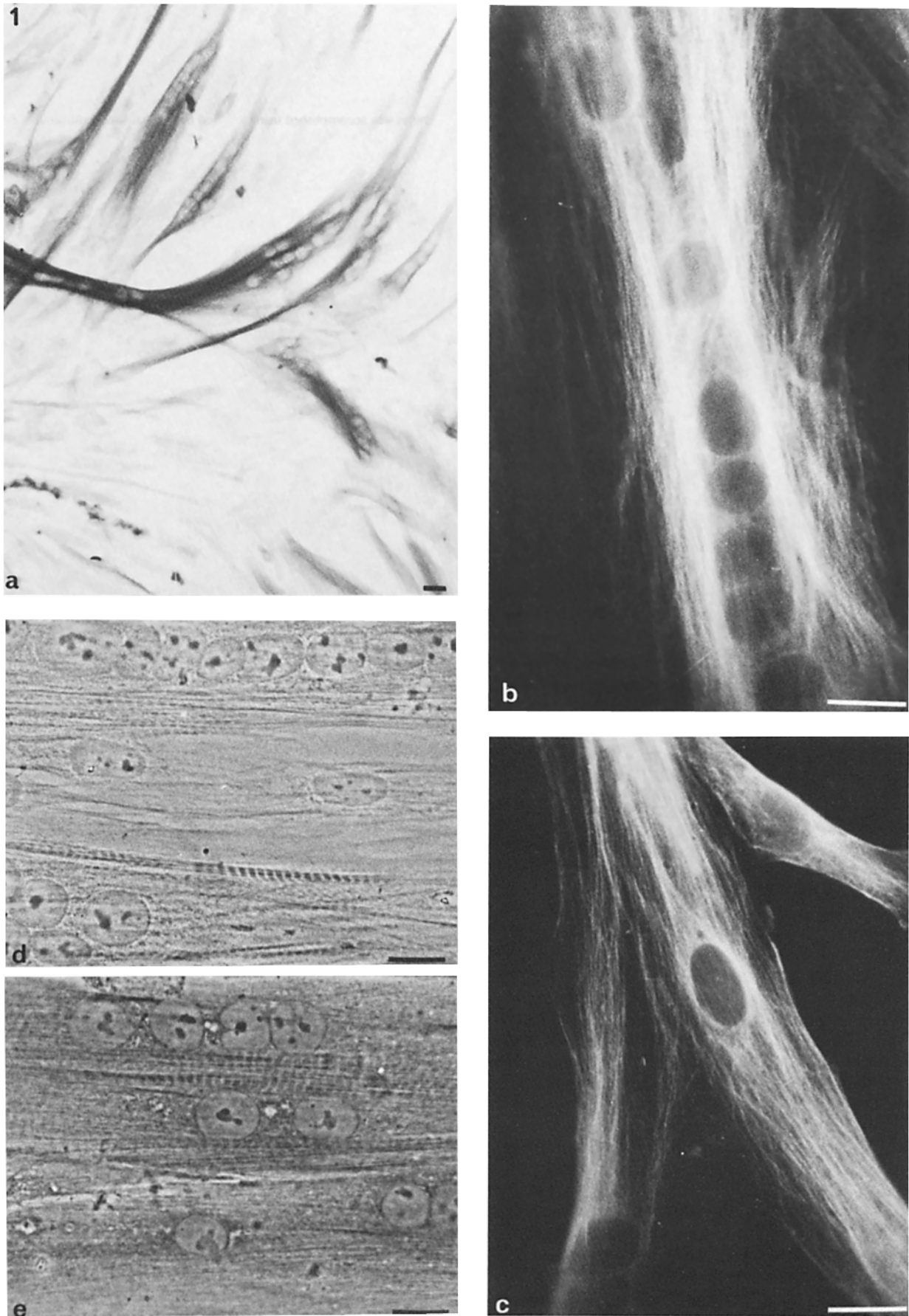


FIGURE 1 Microtubule organization in 8-d-old myotubes as revealed by purified antitubulin antibodies. (a) Low-magnification micrograph of a culture after immunoperoxidase staining. Note the heavy staining of myotubes compared with mononucleated cells. $\times 400$. (b and c) Details of myotubes after immunofluorescent staining. Note the abundance of microtubules close to the nuclei. $\times 1,500$. (d and e) Myotubes observed by phase-contrast microscopy. Myofibrils are visible. They do not appear to be in a precise register. Bars, $10 \mu\text{m}$. $\times 1,000$.

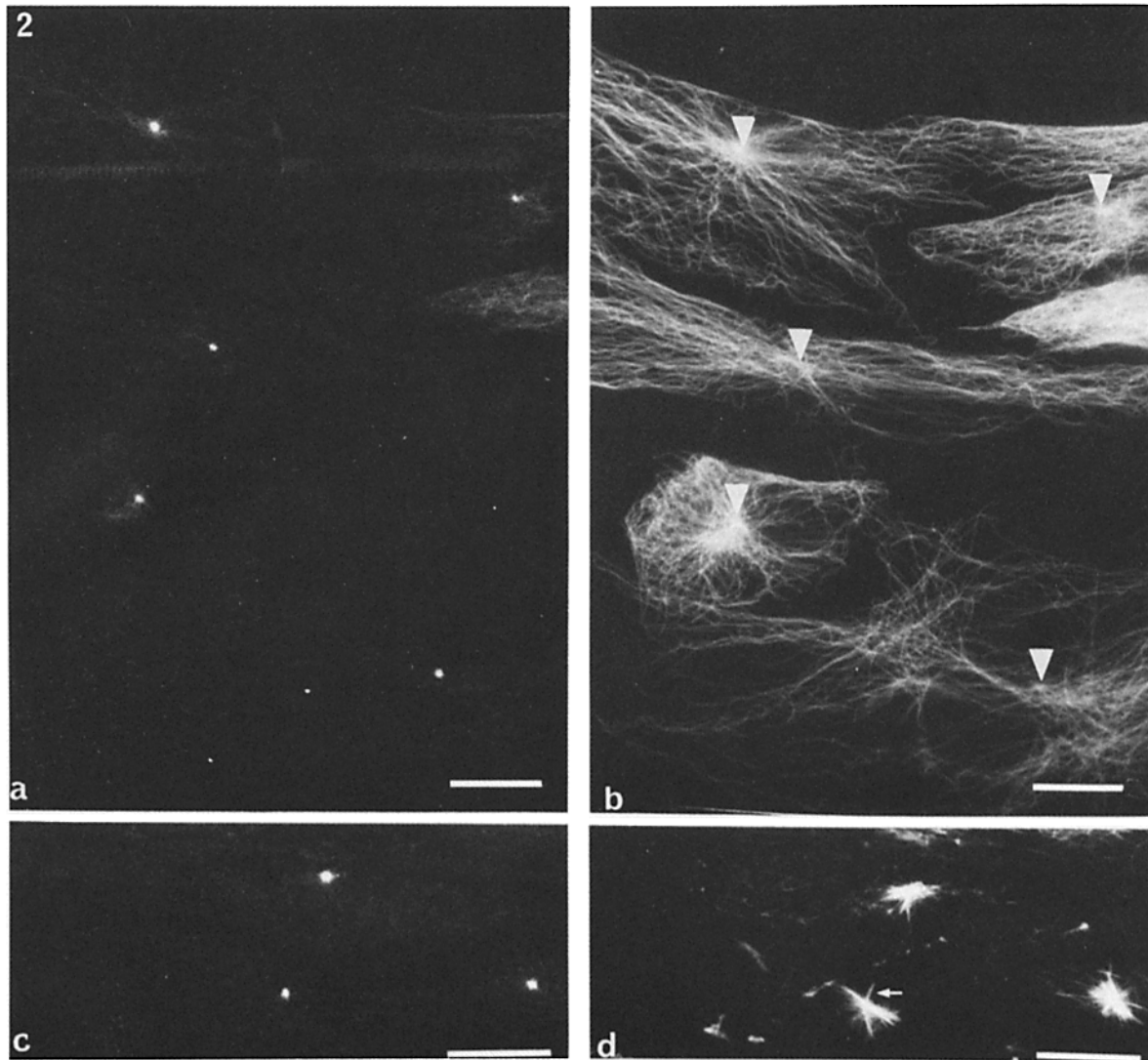


FIGURE 2 Mononucleated cells stained by double immunofluorescence. (a and b) Control cells. (c and d) Microtubule regrowth 1 min after Nocodazole treatment. (a and c) Decoration of centrosomes with serum 0013. (b) Microtubule pattern. Centrosomes are localized at the center of microtubule asters (arrowheads). (d) Microtubules regrow from centrosomes. The arrow points to a primary cilium. Bars, 10 μm . (a and b) $\times 1,200$; (c and d) $\times 1,400$.

clearly different from those present in the mononucleated cells. The labeling was particularly weak, and frequently the two centriolar cylinders could be distinguished (Fig. 4a).

Pattern of Microtubule Regrowth in Myotubes

To investigate the possible significance of the redistribution of pericentriolar material, we depolymerized microtubules by exposing them to Nocodazole (10^{-6} to 5×10^{-6} M) for 2 h (15) and let them regrow for various periods of time. This study was monitored by double-immunofluorescence techniques. After treatment with 5×10^{-6} M Nocodazole, virtually complete depolymerization of microtubules occurred both in myotubes and mononucleated cells. Only centrioles, often bearing a primary cilium, could be seen both in mononucleated cells and myotubes (Fig. 4b). Serum 0013 decorated the periphery of nuclei and the dot at the basis of some primary cilia.

When microtubules were allowed to regrow for 2–5 min after withdrawal of the drug, two patterns were observed that appeared to be related to the cell type. In mononucleated cells, microtubules regrew from a single site that was located close to the nucleus (Fig. 4d). A totally different picture was

observed in myotubes where microtubules sprouted from the periphery of the nuclei (Fig. 4, d and f). This picture was observed when nuclei were aggregated together in groups, or were present in rows, and was also seen when nuclei were located at some distance from each other. After a longer period of time, the picture was rather more difficult to analyze, because the normal parallel arrangement of microtubules was rapidly reestablished. Individual nucleating sites, close to nuclei, were also observed in some regions within myotubes. They corresponded to serum 0013-positive structures, whether individual centrioles or pairs of centrioles. To emphasize the specificity of the microtubule growth pattern in myotubes, Fig. 5b shows an optical field devoid of myotubes, where the asterlike growth of microtubule observed in mononucleated cells is clearly illustrated. The “sunlike” growth of microtubules observed in myotubes (Fig. 5a) was never seen.

Initiation of MTOC Redistribution during Myogenesis

In an attempt to estimate the time of redistribution of MTOC from a pericentriolar localization in myoblasts to a perinuclear localization in myotubes, we performed double-

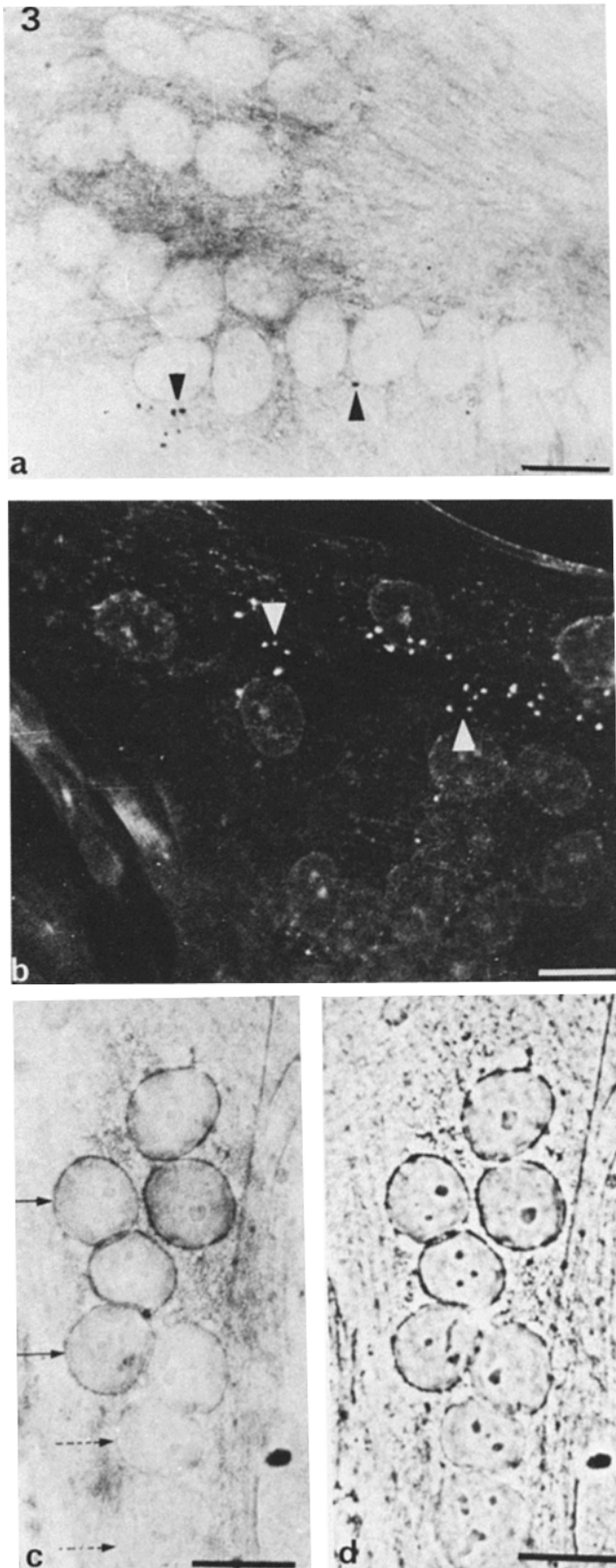


FIGURE 3 Immunoperoxidase (a, c, and d) and immunofluorescence (b) of myotubes with serum 0013. (a and b) Two aspects of centrosomes (arrowheads) in myotubes that show that they no longer associate individually to nuclei. In a, a few centrosomes can be observed beside numerous nuclei. In b, centrosomes are numerous and scattered in the myotube. (b-d) The periphery of nuclei are specifically stained in myotubes (arrows). Some nuclei remain unstained (dotted arrows) as shown in c and reinforced by phase contrast in d. Bars, 10 μ m. (a) \times 1,200; (b) \times 1,000; (c and d) \times 1,400.

immunofluorescence experiments on myoblasts that were competent for fusion, as judged by the characteristic pattern of cellular alignment, and on young myotubes (12–20 h after fusion). Typical alignments of myoblasts competent for fusion are shown in Fig. 6. In this experiment, a microtubule regrowth after 30 s was followed by antitubulin staining. A unique site of growth was observed in each cell (Fig. 6b), corresponding to the centrosome (Fig. 6a). No nuclear profile could be observed with either type of antibody. Microtubules in early myotubes were already organized in roughly parallel arrays along the axis of the cell (Fig. 7, b and d), in contrast to the pattern in mononucleated cells. Two observations followed labeling with serum 0013: (a) the periphery of some nuclei was labeled weakly (two out of three in Fig. 7c, for instance); (b) centrioles remained close to the nuclei, while often appearing separate from each other in the same pair (Fig. 7, a and c). When we looked for early microtubule regrowth in young myotubes, we could observe a perinuclear regrowth corresponding to the MTOC localization (Fig. 8).

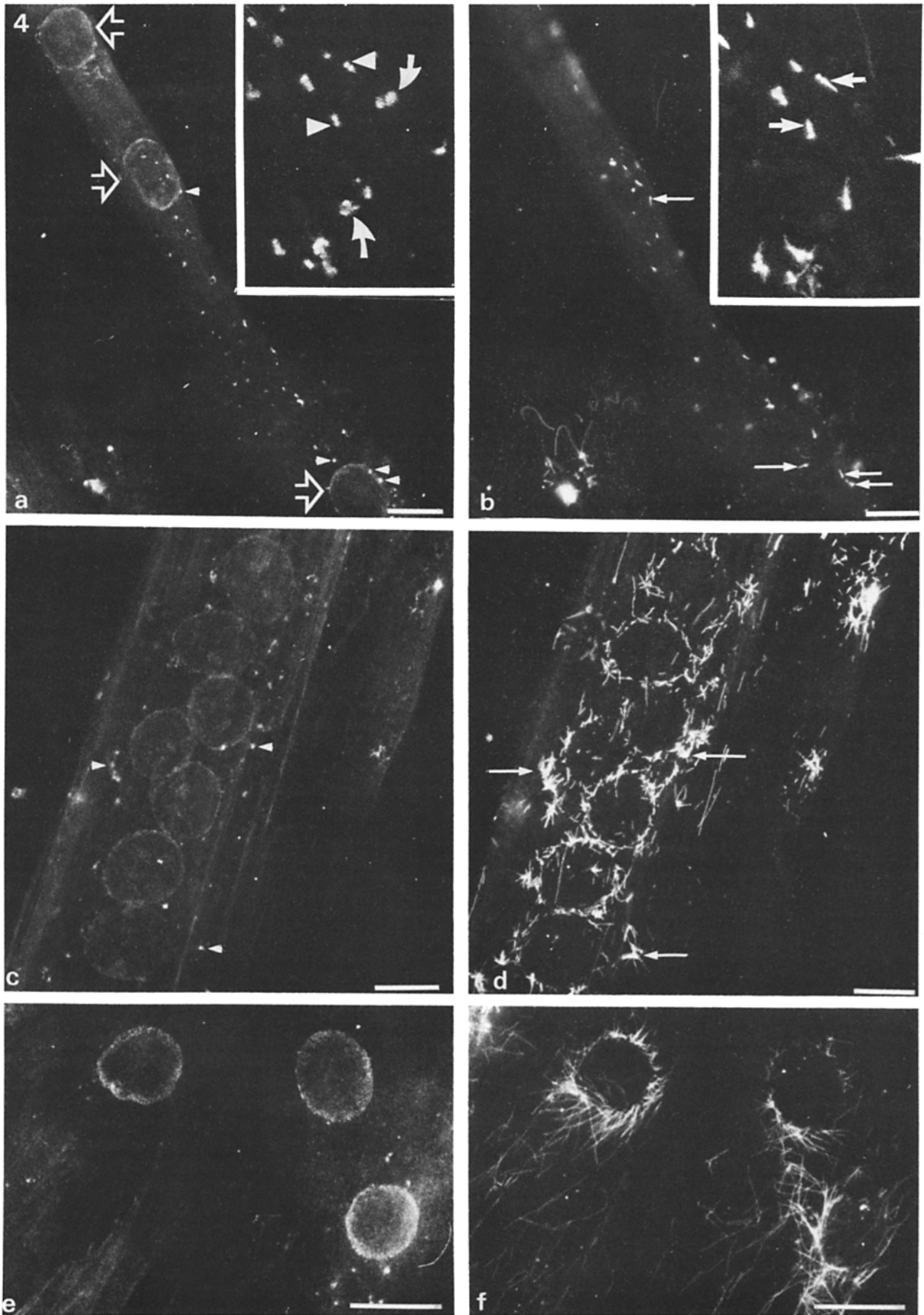
MTOC Localization and Microtubule Pattern in Artificial Syncytia

One of the most important questions raised by the observation of MTOC redistribution in myotubes is the following: Is it specific to the muscle system or is it bound to cellular reorganization when cell fusion occurs? In an attempt to answer this question, we produced polykaryons from cells unrelated to the muscle system, i.e., epithelial HeLa cells. We used PEG 4000 as a fusion agent. 24 h after fusion, cells were processed for double-immunofluorescence staining using antitubulin and serum 0013. Microtubules were abundant and their pattern was difficult to analyze (Fig. 9, b and e). It was clearly in contrast, however, to the parallel microtubules observed in myotubes. MTOC staining revealed most often one or few enlarged and heavily labeled dots, which apparently corresponded to tight clusters of centrosomes (Fig. 9, c and f). Small dots of the size of centrosomes in individual cells were also observed scattered into nuclei clusters.

PEG-induced polykaryons could be kept in culture for several days if the growth of unfused cells was prevented. After 5 d, the centrosome pattern in polykaryons was basically unchanged. In particular, the periphery of nuclei was unstained by serum 0013. In large polykaryons, however, one got the impression that the former single giant centrosome observed 24 h after fusion had been reorganized into several areas of identical size, evenly distributed within the cytoplasm (data not shown).

DISCUSSION

The process of cell fusion that leads to the human myotube *in vitro* appears to be accompanied by a dramatic and unprecedented redistribution of the microtubule-nucleating material, from the pericentriolar area to the nuclear periphery. This has been assessed in two ways: (a) by decorating specifically the periphery of nuclei of myotubes with a rabbit serum that stains pericentriolar material (29) while only labeling the centrosome of mononucleated cells present in the same culture, and (b) by demonstrating microtubule regrowth from the nuclear periphery in myotubes in contrast to the asterlike regrowth from the centrosomes in mononucleated cells. How this redistribution is effected is still unknown. One possible mechanism is the dissociation of the microtubule-nucleating material from the myoblastic centrioles and its translocation to the periphery of nuclei. Alternatively, this material can be



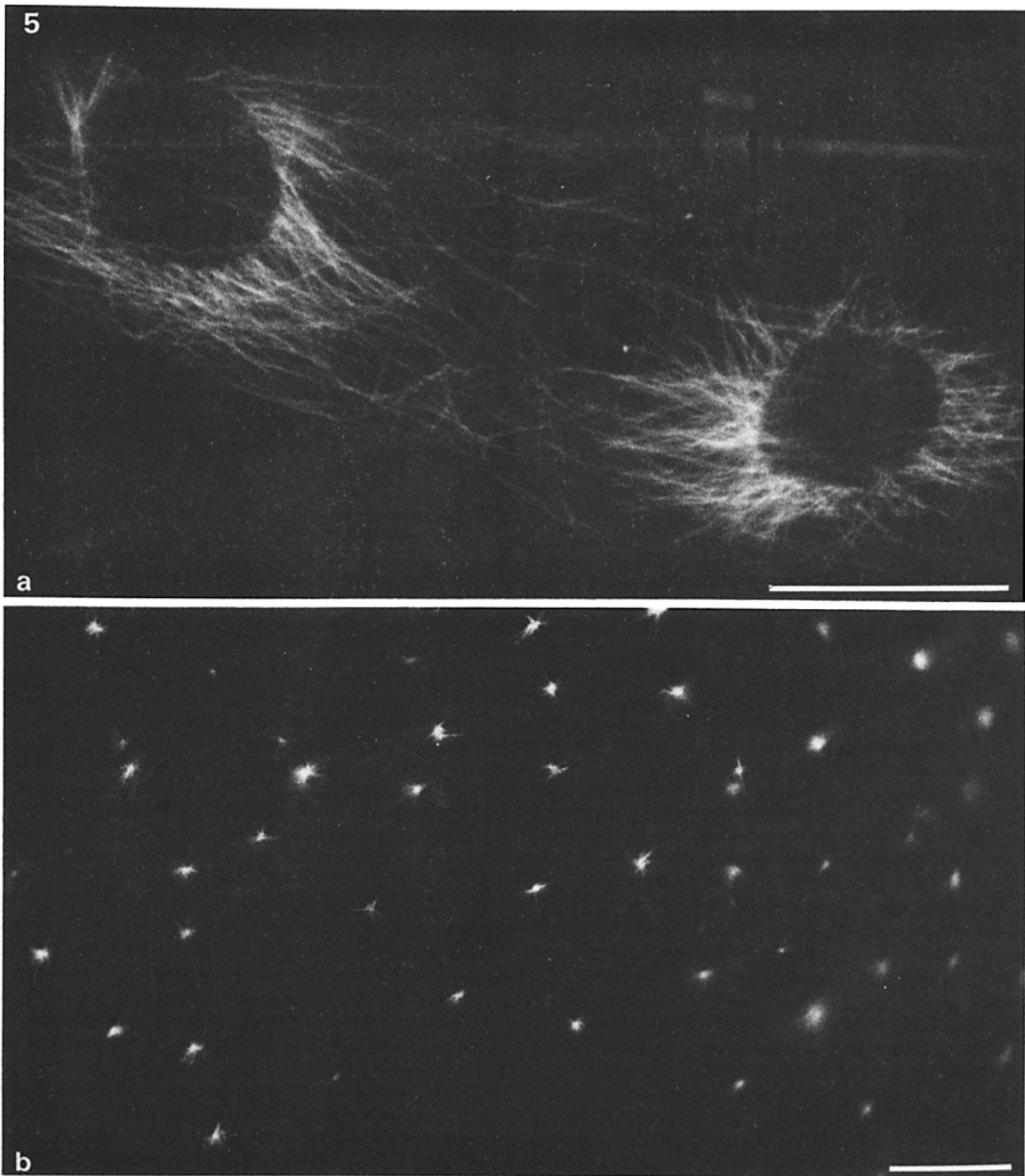


FIGURE 5 Comparison of microtubule regrowth from nuclei and centrosomes. (a) A "sunlike" pattern is shown around nuclei in myotubes. (b) Field without myotubes. Only asterlike structures are observed corresponding to centrosomes of mononucleated cells. Nuclear profiles can not be distinguished. Bars, 10 μm . (a) $\times 4,000$; (b) $\times 2,000$.

FIGURE 4 Microtubule regrowth after depolymerization by Nocodazole. Double labeling with serum 0013 (a, c, and e) and antitubulin antibodies (b, d, and f) after various periods of regrowth. (a and b) Aspect of myotubes after Nocodazole treatment. Microtubules are totally depolymerized. Only primary cilia (arrows) can be decorated with antitubulin (b). These primary cilia are associated with centrioles (arrowheads in a) as demonstrated with higher magnification (insets) where individual centrioles in a pair can be distinguished. Note that some serum 0013-positive material is not associated apparently with tubulin-positive structures (curved arrows). Nuclei are decorated with serum 0013 in myotubes (opened arrows) whereas the nucleus of the mononucleated cell (in the lower left corner) is unlabeled. (c and d) 1 min regrowth. In c, note that the perinuclear staining is observed only in myotubes. Compare with the two mononucleated cells (in the upper right corner). Some centrioles are observed in the myotubes. In d, microtubules regrow from the nuclear periphery in the myotube and from individual sites (arrows) corresponding to centriolar profiles (arrowheads in c). Typical asterlike regrowth from the centrosome of mononucleated cells can be observed. (e and f) 2 min regrowth. (e) Detail of a large myotube. The perinuclear staining with serum 0013 is particularly heavy. Centrioles are scattered around the lower right nucleus. Bars, 10 μm . (a and b) $\times 1,000$; (c and d) $\times 1,200$; (e and f) $\times 1,700$.

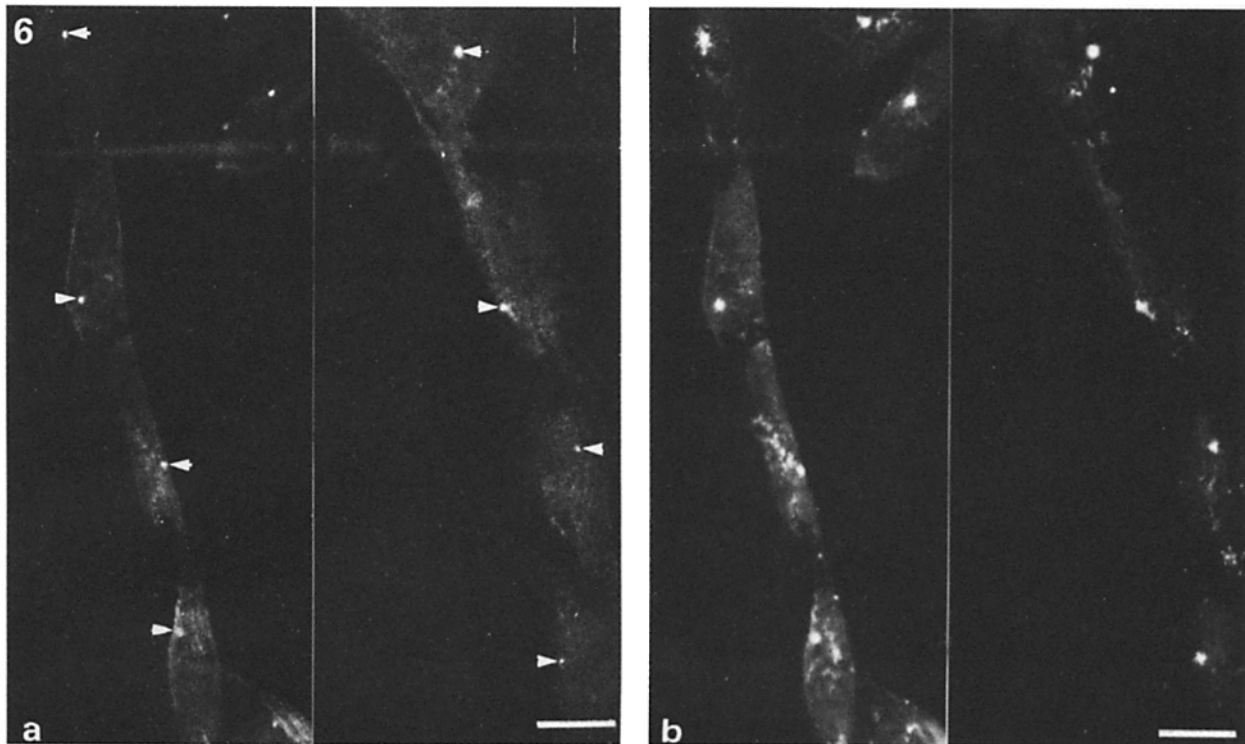


FIGURE 6 Microtubules regrowth in myoblasts competent for fusion. Cells are aligned in a characteristic pattern. (a) Centrosome staining (arrowheads). (b) Microtubule staining 30 s after removal of Nocodazole. No nuclear profile can be observed with either serum. Microtubule regrowth is only observed at the centrosomes. Bars, 10 μm . $\times 1,000$.

degraded while a *de novo* synthesis takes place that addresses products to new targets. We have studied the timing of the MTOC redistribution in order to decide whether it took place before or after fusion. If it were before fusion, the MTOC redistribution around nuclei could be related to the recent observation that postmitotic myoblasts respond to taxol treatment in a different manner from mitotic myoblasts, producing star-shaped cells (16). Our data show opposite results: myoblasts undergo no modification of microtubule regrowth as long as fusion does not occur. In contrast, early myotubes containing two to four nuclei already possess redistributed nucleating material as judged by direct labeling of the MTOC or by microtubule regrowth. Two observations are noteworthy in these young myotubes: (a) the redistribution of MTOC concerns only a part of the surface of some of the nuclei, (b) the centrioles are still located close to the nuclei, but often present a structural modification, in which individual centrioles of a pair are separated from each other. This feature is reminiscent of the splitting phenomenon described by Sherline and Mascardo (38) at the early stage of cell stimulation by growth factors. A more dramatic splitting of the centrosome has been observed by Schliwa et al. (37) during neutrophil activation. In the latter case, a fragmentation of the MTOC was demonstrated. Similar events could occur during myogenesis. The splitting of centrioles observed in young myotubes could be also accompanied by their dissociation from the MTOC, the latter being directly translocated to the nuclear periphery. In this line, it has been demonstrated clearly that the juxtannuclear centrosome is physically associated with the nucleus in many types of cell (6, 21, 26, 32). We have recently obtained evidence that the pericentriolar material, which is labeled by serum 0013, participates in this association in human lymphoblastic KE 37 cells (28). Local

association of MTOC with nuclear periphery would therefore exist in mononucleated cells.

The restricted staining of the nuclear periphery observed in young myotubes could be the reflect of this fact. In older myotubes, centrosomes are no longer associated one-by-one to nuclei. They form small or large clusters lying in the vicinity of nuclei. Our rough estimation on the number of centriolar profiles, compared with nuclear profiles, indicates a decrease in the number of centrioles. We can eliminate that this is due to superimposition of centriolar profiles, in that centrioles clusters are not tightly packed. Moreover, the absence of most pericentriolar material often allows the resolution of individual centrioles. Because centrioles are only exceptionally observed in skeletal myofibers, while they are present in satellite cells (24) and in myocardial cells (12), we favor the hypothesis of a progressive elimination of centrioles during myotube formation. An electron microscopy study is being undertaken to produce more evidence on this aspect. If it were to be confirmed, this would constitute a new illustration of the fact that, as a rule, centrioles cannot be elsewhere in a cell than within the MTOC material. Only drug-treated cells might show dislodged centrioles, away from the MTOC material (14, 36, 47, 48).

To decide whether the behavior of MTOC material that we observed in myotubes was a specific process of myogenesis, we produced artificially induced polykaryons, using human cells unrelated to muscle. A totally different picture was observed. Centrosomes aggregated in one or a few large MTOC inside or at the edge of nuclei clusters, as judged by specific labeling. These MTOCs apparently contained all of the serum 0013-positive material. In particular, no nuclear profile could be decorated with this serum. These MTOCs are so tightly packed that it is not possible, at the light microscopy

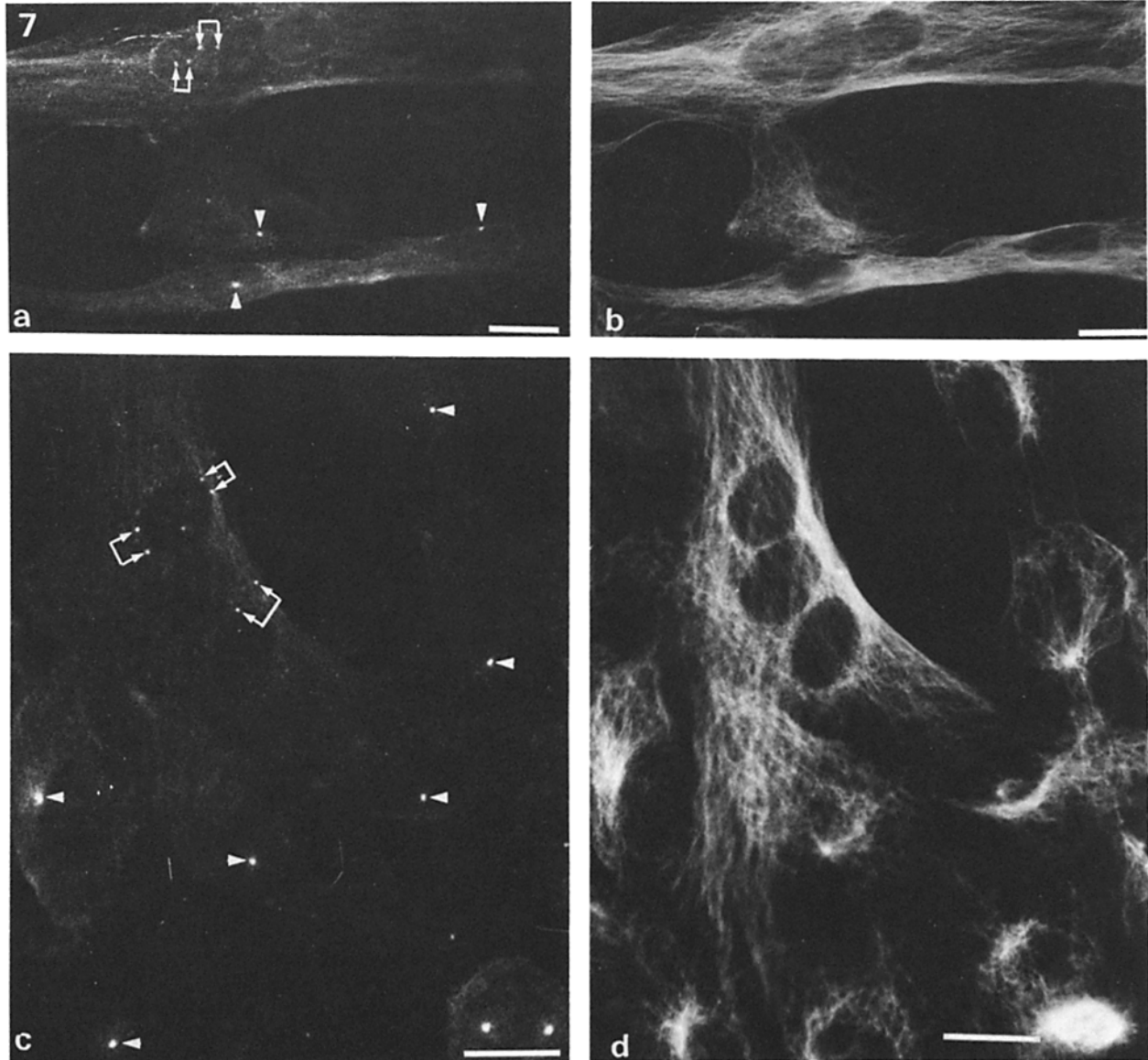


FIGURE 7 Microtubule pattern and MTOC distribution in early myotubes (24 h after fusion). (a and c) Centrosome labeling. Note that centrosomes are not associated individually with nuclei, and that they appear to be split (double arrows). Compare the split centrioles with the size of centrosome in mononucleated cells (arrowheads). Nuclei are labeled, although incompletely (two nuclei out of three in c). (b and d) Microtubule pattern. It shows by now the parallel arrangement observed in older myotubes, whereas the mononucleated cells present the typical microtubule network organized about centrosome (arrowheads in paired figures). A cell undergoing mitosis can be seen in the lower right corner in c and d. Bars, 10 μm . (a and b) $\times 1,000$; (c and d) $\times 1,400$.

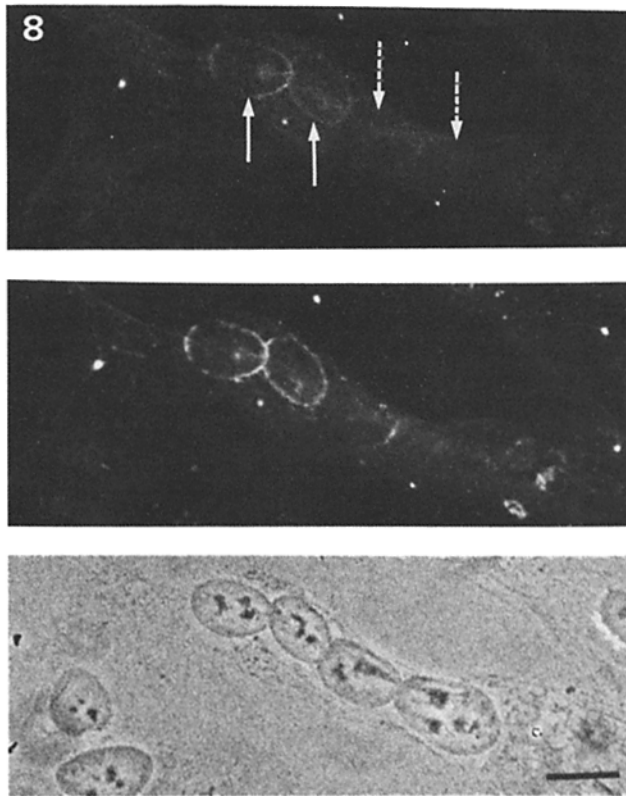


FIGURE 8 MTOC pattern and microtubule regrowth in early myotubes (24 h after fusion). (a) Centrosome staining. (b) Microtubules staining after 1-min regrowth. (c) Phase-contrast micrograph. In the myotube two nuclei (arrow) out of four (dotted arrow) are labeled with serum 0013. Microtubule regrowth precisely corresponds to the areas that are positive with serum 0013. Bars, 10 μ m. \times 1,000.

level, to observe if centrioles are present. However, a virus-induced polykaryons have been produced by Wang et al. (42) from baby hamster kidney 21 cells. They observed essentially the same behavior of the MTOCs that we observed in PEG-induced polykaryons. They performed an electron microscopy study that demonstrates that the fused MTOCs contained centrioles. Centrosomes appear therefore capable of aggregating in a unique or quasi-unique nucleating structure in artificially induced polykaryons. The mechanism of this aggregation has been shown to involve microtubules (42). The stability of such fused MTOCs is also dependent on microtubules integrity: studying binucleated cells, Watt et al. (45) have shown that mild depolymerization of microtubules (by cold) maintains a unique MTOC, whereas depolymerizing drugs (Colcemid, griseofulvin, vinblastin) separate the former centrosomes of parental cells. Centrosome aggregation have been observed also in mononucleated cells such as murine neuroblastoma cells NIE 115, which possess numerous centrioles capable of acting as a unique MTOC in particular circumstances (39).

Myotubes differ from other cells in that each centrosome

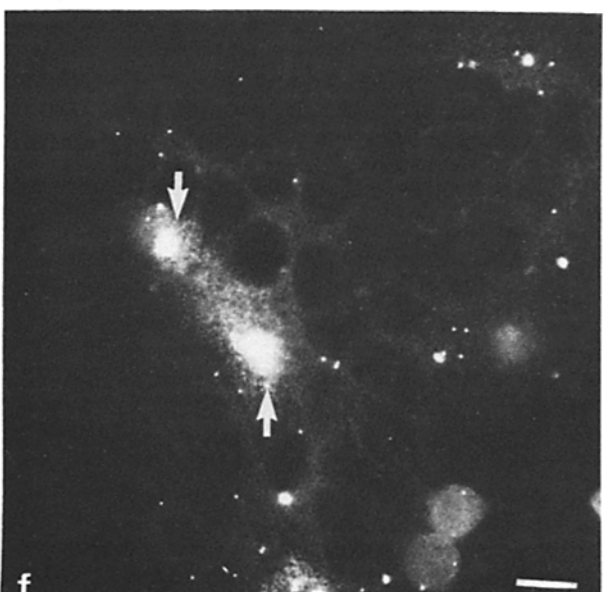
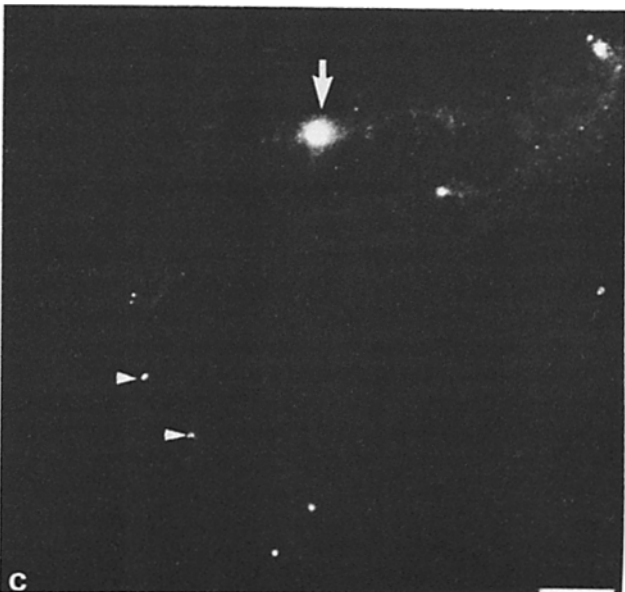
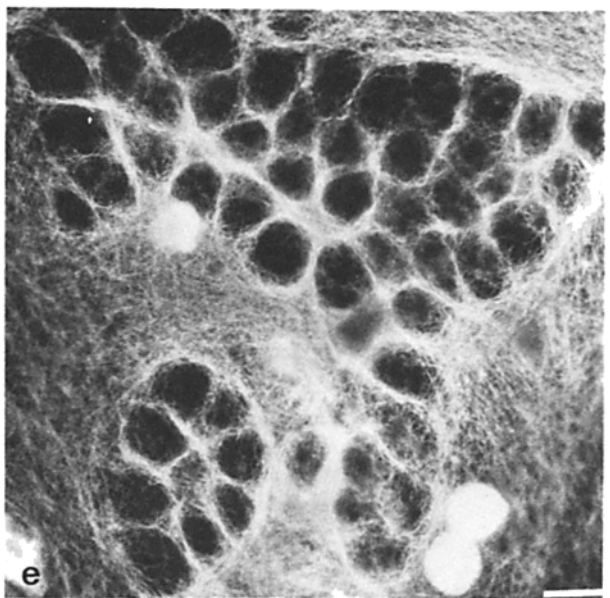
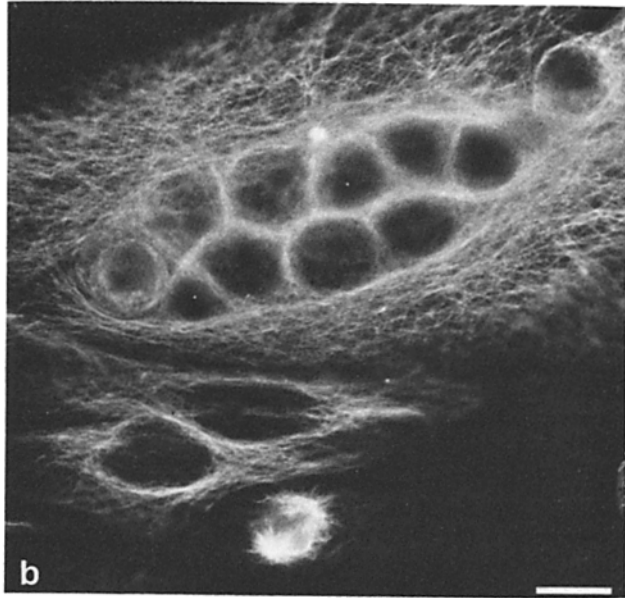
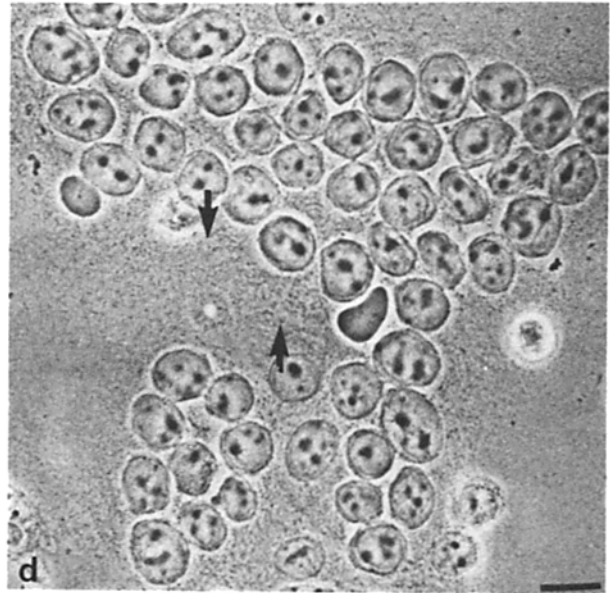
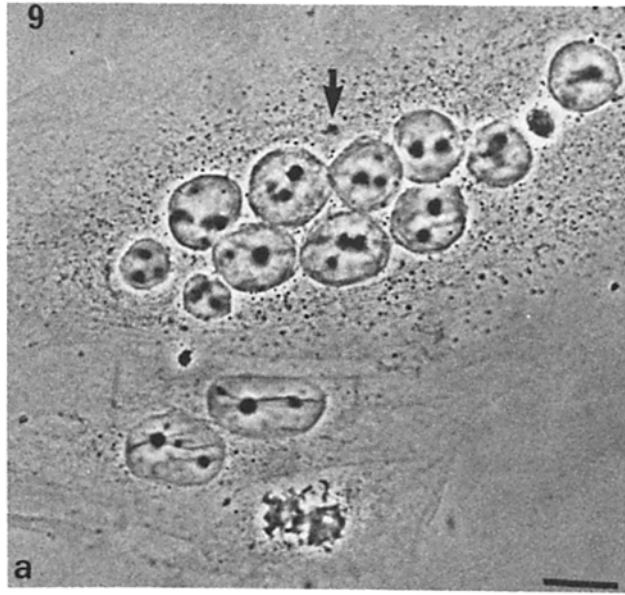
does not aggregate with the others as a whole. Rather, the components of individual centrosomes dissociate from each other: the MTOC relocates elsewhere (around nuclei) whereas centrioles gather in loose clusters. We have suggested above that the latter were progressively eliminated. If this is true, myogenesis appears as a favorable model to raise the question of the functions of centrioles in cells. Except for its participation as a template of cilia and flagella, nothing is known concerning centrioles functions in cells. Microtubules have been shown to control cell shape and cell polarity during locomotion (1, 25, 37). Membrane ruffling, for example, seems to be more pronounced in the domains of the cell surface that are close to microtubule asters (37). It has been suggested that centrioles themselves could act as a directional device (2, 3, 7). Experimental evidence for and against this proposal has recently been published (10, 19). Myotubes do not locomote nor do they show any sign of anteroposterior polarity, the structural unit of myofibrils, the sarcomere, being symmetrical about the M band.

The fact that the MTOC relocated preferentially at the surface of the nuclei is the remarkable feature established by the present work. We have mentioned above the possibility that in mononucleated cells, the MTOC establishes an association with a restricted domain of the nuclear surface. In most eucaryotic cells that do not possess centrioles, but a centriolar equivalent, the latter, as a rule, is associated with the nucleus (13). It can even be located within the nuclear envelope (13, 34). A tight association between the nuclear periphery and MTOC appears to have been maintained during evolution. It has even been suggested that centrioles might be generated at the nuclear membrane (17, 20). In plant cells, which constitutively lack centrioles, microtubules seem to be directly connected to the nuclear envelope through dense amorphous material (22).

From these data, the MTOC could be seen as capable of interacting with two organelles in the cell, the nucleus and the centrioles, with a greater affinity for the latter. When present, centrioles could mobilize MTOC material on their surface, i.e., on a very restricted area close to the nucleus. As a consequence, the microtubule pattern would be itself restricted to an angular sector, establishing in this way the cell polarity. When centrioles are absent, the MTOC material redistributes evenly on the second organelle, the nucleus. As a consequence, the microtubule patterns would be distributed evenly in the cellular space, at least at their origin. We tentatively illustrate this proposal in Fig. 10.

Finally, the abundance of microtubules in young myotubes is noteworthy. It contrasts with the paucity of microtubules in myofiber observed *in vivo*, with the exception of cardiac muscle in which, interestingly, microtubules are abundant around nuclei (35). Preliminary attempts to localize microtubules and MTOC on semithin frozen sections of muscle tissue have revealed a typical microtubule pattern and centrosome staining only in satellite cells. We were unable to detect MTOC around nuclei and elsewhere in myofibers. Although

FIGURE 9 PEG-induced polykaryons from HeLa cells. (a and d) Phase-contrast micrograph of polykaryons (arrows correspond to the preferential localization of serum 0013-positive material). (b and e) Microtubule network are revealed by antitubulin antibodies. There is no obvious favored orientation of the microtubules. (c and f) Centrosome labeling with serum 0013. The label is concentrated in one or two enlarged and heavily stained area. Compare the size of centrosomes in mononucleated cells (arrowheads in c). Some individual centrosomes are scattered in cytoplasm. Note that nuclei are not decorated with serum 0013, as in myotubes (see Figs. 3, b-d, 4, a, c, and e). Bars, 10 μ m. (a-c) \times 1,000; (d-f) \times 800.



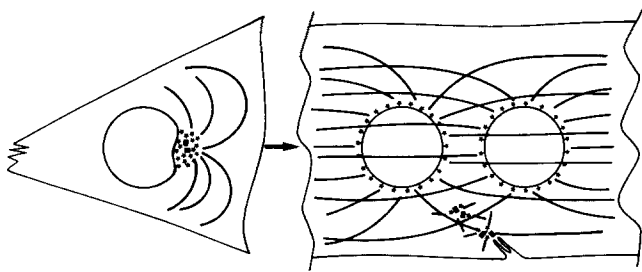


FIGURE 10 Diagrammatic interpretation of the transition in microtubule organization from myoblast to myotube: the dissociation of each centrosome from its respective nucleus involves also the dissociation of centrioles (black rectangles) from the nucleating material (small stars). The latter remains associated with the nucleus and redistribute around it. As a consequence, cell polarity is lost.

preliminary, this result could suggest that fully differentiated myofibers are no longer capable of nucleating microtubules. The abundance of microtubules in young myotubes is remaining the situation in growing and in regenerating muscle (11). This strengthens the hypothesis previously suggested by several authors (4, 5, 16, 41) that microtubules possess an organizational role during myofibrillogenesis, as also they probably do in any other cell types (8).

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