The Golgi Apparatus Remains Associated with Microtubule Organizing Centers during Myogenesis

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ABSTRACT In vitro myogenesis involves a dramatic reorganization of the microtubular network, characterized principally by the relocalization of microtubule nucleating sites at the surface of the nuclei in myotubes, in marked contrast with the classical pericentriolar localization observed in myoblasts (Tassin, A. M., B. Maro, and M. Bornens, 1985, *J. Cell Biol.*, 100:35–46). Since a spatial relationship between the Golgi apparatus and the centrosome is observed in most animal cells, we have decided to follow the fate of the Golgi apparatus during myogenesis by an immunocytochemical approach, using wheat germ agglutinin and an affinity-purified anti-galactosyltransferase.

We show that Golgi apparatus in myotubes displays a perinuclear distribution which is strikingly different from the polarized juxtanuclear organization observed in myoblasts. As a result, the Golgi apparatus in myotubes is situated close to the microtubule organizing center (MTOC), the *cis*-side being situated at a fixed distance from the nuclear envelope, a situation which suggests the existence of a structural association between the Golgi apparatus and the nuclear periphery. This is supported by experiments of microtubule depolymerization by nocodazole, in which a minimal effect was observed on Golgi apparatus localization in myotubes in contrast with the dramatic scattering observed in myoblasts.

In both cell types, electron microscopy reveals that microtubule disruption generates individual dictyosomes; this suggests that the connecting structures between dictyosomes are principally affected. This structural dependency of the Golgi apparatus upon microtubules is not apparently accompanied by a reverse dependency of MTOC structure or function upon Golgi apparatus activity. Golgi apparatus modification by monensin, as effective in myotubes as in myoblasts, is without apparent effect on MTOC localization or activity and on microtubule stability.

The main result of our study is to show that in a cell type where the MTOC is dissociated from centrioles and where antero-posterior polarity has disappeared, the association between the Golgi apparatus and the MTOC is maintained. The significance of such a tight association is discussed.

The Golgi apparatus (GA)[1] plays a key role in the processing and transport of secretory and lysosomal proteins as well as in the biogenesis and traffic of membranes. A specific role in the sorting of the proteins and their translocation to the correct destination is also attributed to Golgi elements (7, 29). It has often been suggested that cytoskeletal elements such as microfilaments and microtubules (Mt's) would be instrumental in the orderly membrane traffic (18). Association between lysosomes and Mt's has been demonstrated (4) and the morphological aspect of the GA is dramatically modified when Mt's are depolymerized by drugs or when the cellular tubulin is totally polymerized in an abnormal pattern by Taxol (6,

[1] *Abbreviations used in this paper*: GA, Golgi apparatus; GT, galactosyltransferase; MTOC, microtubule organizing center; Mt's, microtubules; WGA, wheat germ agglutinin.

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21, 22, 31, 33). However, these morphologically modified GA are functionally normal: protein processing, such as glycosylation, is not modified in cells treated with microtubule drugs (3, 23). In addition, vesicular transport supposed to mediate maturation of membrane and secretory proteins has been reconstituted in a cell-free system (25, 26). Concerning the role of Mt's in the secretion of specific secretory components, conflicting results have been reported (for a discussion of this aspect, see reference 23). It appears that a minimal effect in the distal steps of the secretion can be observed in some systems. By contrast, recent reports agree that Mt's play a role in the destination, relative to cell polarity, of plasma membrane proteins (20, 23). The microtubule network originates from the centrosome, constituted of the two centrioles and of the proper microtubule organizing material. As a rule, a spatial relationship between the GA and the centrosome is observed in animal cells. The position of the GA–centrosome complex on one side of the nucleus allows the definition of the cell axis (9, 12). Experiments demonstrating the coordinated reorientation of the GA and the centrosome led Kupfer et al. (13) to propose that the functional significance of the association between the GA and the centrosome was to maintain cell polarity by establishing a membrane traffic along a polarized microtubule network. This concept has been further elaborated in the light of more recent results (22). The only role of Mt's in the expression of cell polarity would be to compact the GA. This would be sufficient to create polarity in the insertion of new plasma membrane, i.e., to create a leading edge.

Myotubes produced by the fusion of competent myoblasts constitute a favorable experimental system for re-examining the relationship between Mt's, or microtubule nucleating structures, and the GA: myotubes do not migrate nor do they show any clear antero-posterior polarity. A dramatic reorganization of the microtubular network takes place at the stage of fusion: Mt's are no longer nucleated on centrosomes, but on the nuclear periphery where the pericentriolar material relocalizes (27). Mt's are also distributed evenly in the cell according to a parallel organization along the cell axis.

Using an immunocytochemical approach, we show here that the GA in myotubes displays a perinuclear distribution strikingly different from the polarized juxtanuclear organization observed in myoblasts, which corresponds to the microtubule organizing center (MTOC) distribution. Moreover, the stability of the GA with respect to Mt's depolymerization is also different in myoblasts and myotubes. In an attempt to define the functional significance of the MTOC–GA association, we studied the pattern of microtubular regrowth after depolymerization in myoblasts and myotubes treated with monensin. The GA is apparently in no way instrumental for polymerization of Mt's. We therefore conclude that the existence of an MTOC–GA association in cells that use different strategies for microtubule nucleation, from a centriole-associated MTOC on the one hand and a nucleus-associated MTOC on the other, argues for an as yet unknown role of Mt's, or tubulin, in the membrane traffic.

MATERIALS AND METHODS

Cells: Myotubes were produced in vitro as previously described (27).

Fixation: Cells were fixed (fixation I) in phosphate-buffered saline (150 mM NaCl, 10 mM Na/Na_2PO_4, pH 7.4) (PBS) that contained 3% formaldehyde for 30 min at 37°C, and then in methanol for 6 min at −20°C.

Alternatively (fixation II), living cells were washed for 5 s at room temperature with a stabilizing buffer at pH 6.9 that contained 45 mM PIPES, 45 mM HEPES, 10 mM EGTA, 5 mM $MgCl_2$, 1 mM phenylmethylsulfonyl fluoride (15). (PIPES, HEPES, and EGTA were obtained from Sigma Chemical Co., St. Louis, MO.) They were then lysed in the same buffer supplemented with 0.5% Brij 58 for 30 s, washed, 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 monoclonal anti α- and β-tubulin antibodies (Amersham France SA, Les Ulis). Nucleation sites of microtubules were specifically labeled with the non-immune rabbit anti-centrosome serum 0013 (17, 27). GA was labeled either with wheat germ agglutinin-rhodamine (WGA-Rho) (Reactifs IBF, France; reference 30) or with affinity-purified antibodies against human β-galactosyltransferase produced in rabbit (1, 24).

The second antibodies were, respectively, goat anti-mouse, or sheep anti-rabbit immunoglobulins labeled either with fluorescein isothiocyanate (Cappel Laboratories, Cochranville, PA), Texas red (Amersham France, SA, Les Ulis), or peroxidase (Institut Pasteur Production). The antibodies were diluted in PBS containing 0.1% Tween 20 and 3% bovine serum albumin. All washing steps were performed in PBS-Tween.

Drug Treatment: Mt's were depolymerized by nocodazole (Janssen Pharmaceuticals, Beerse, Belgium). Cells were cultivated with 5.10^{-6} M nocodazole for 2 h and then fixed, or they were allowed to recover without nocodazole for 30 s to 2 h at 37°C. The GA was disorganized using monensin (sodium salt, Sigma Chemical Co.) at 14 μg/ml for 2 h (28). Cells were immediately fixed. Alternatively, cells were treated for 1 h with monensin at 14 μg/ml, then with monensin and nocodazole (5.10^{-6} M) for 2 h and then fixed, or allowed to recover without nocodazole, in maintaining the concentration of monensin for 1 min to 2 h at 37°C before fixation.

Electron Microscopy: The immunoperoxidase labeling was performed essentially as described by Louvard et al. (14) with the following modifications. Myoblasts and myotubes grown on cover slips were washed with PBS. The cells were then fixed with 2% (wt/vol) paraformaldehyde, 0.05% (vol/vol) glutaraldehyde in PBS for 30 min at room temperature. The glutaraldehyde was then quenched twice with 50 mM NH_4Cl in PBS for 15 min, followed by a rinsing in PBS that contained 0.05% (wt/vol) saponin and 0.2% (wt/vol) gelatin for 30 min. The cells were then incubated at room temperature either (*a*) for 2 h with monospecific anti-β-galactosyltransferase antibodies, diluted in PBS that contained 0.05% saponin and 0.2% gelatin, followed by Fab fragments of anti-rabbit IgG conjugated to horseradish peroxidase (Institut Pasteur Production, France), or (*b*) for 1.5 h with 50 μg/ml WGA-peroxidase in the same buffer (Miles Yeda). After washing in the presence of saponin, cells were refixed with 5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.3, washed in the cacodylate buffer, incubated in the medium described by Graham and Karnovsky (8) for 45 min in the dark, fixed in 2% (wt/vol) OsO_4 in the cacodylate buffer, dehydrated in ethanol, and embedded in Epon. Sections parallel to the plane of the monolayer were examined with a Philips 201 electron microscope.

For morphologic observation, cells were fixed directly with 5% glutaraldehyde in the 0.1 M cacodylate buffer for 1 h, washed with the same buffer, postfixed with 2% OsO_4 in the cacodylate buffer, dehydrated, and embedded. The sections were stained with alcoholic uranyl acetate and lead citrate.

RESULTS

Morphology of the GA in Myotubes

Eight days after fusion, two cell types were present in culture: mononucleated cells (fibroblasts or unfused myoblasts) and huge, often Y-shaped myotubes, which contain hundreds of nuclei either clustered or in rows, and loosely organized myofibrils (27).

Depending on the cell type, the GA displayed different organizations. In mononucleated cells, the GA assumed a juxtanuclear position and manifested various aspects from a compact to an extended form (Fig. 1, *a* and *b*; see also Fig. 4*a*).

In myotubes, GA appeared closely associated with the periphery of most of the nuclei (Fig. 1, *a* and *b*; see also Fig. 4, *c* and *e*). Individual nuclei in myotubes could be surrounded by Golgi elements not connected with other Golgi structures elsewhere in the cell, whereas groups of nuclei often appeared

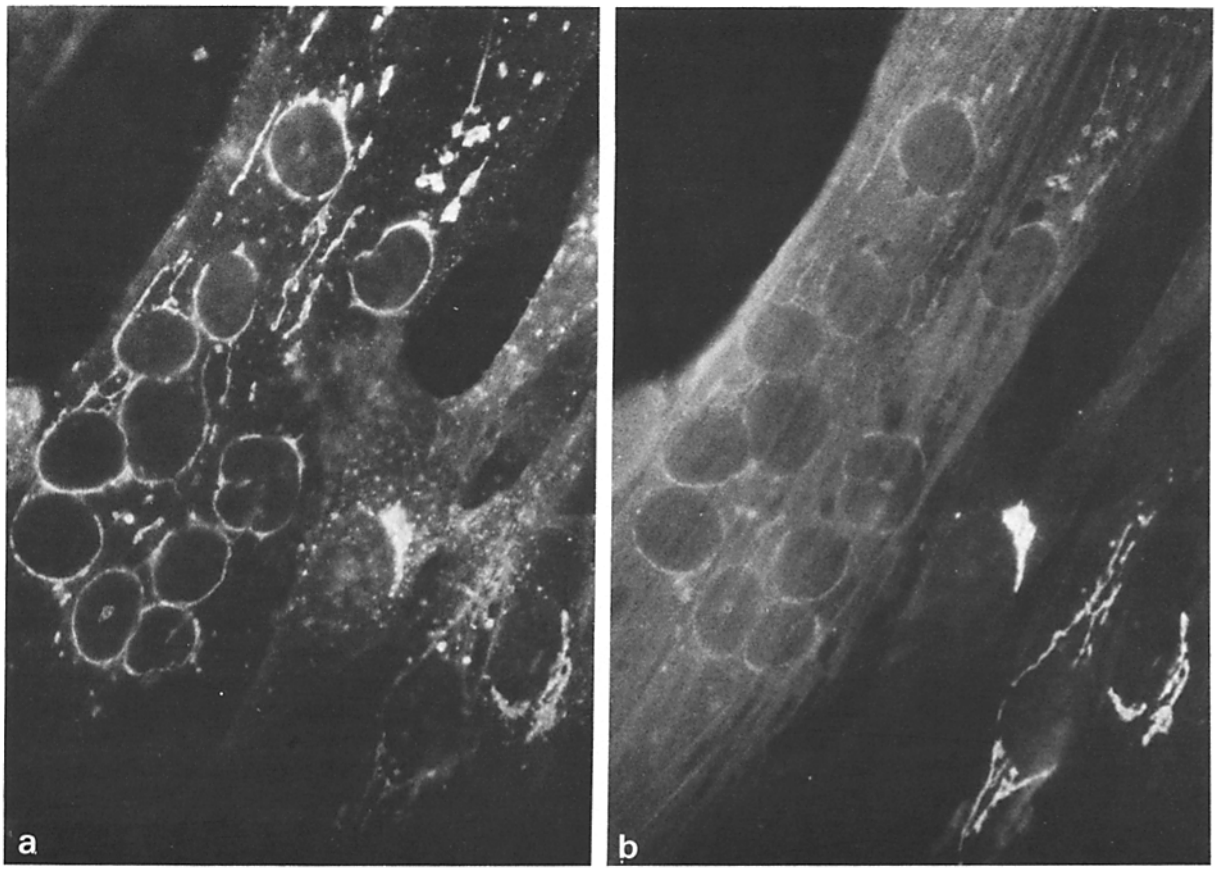

FIGURE 1 GA as revealed by double staining with WGA (*a*) and by anti-GT (*b*) in three mononucleated cells and one myotube. Compare the perinuclear labeling in the myotube to the juxtanuclear GA in mononucleated cells. WGA and GT stainings are basically identical. Some differences can however be observed. The anti-GT labeling observed in mononucleated cells (lower right corner) appears more specific than the lectin one which also stains surface glycoproteins. In 8-d-old myotubes, a homogeneous cytoplasmic labeling is observed with anti-GT and not with WGA, which apparently correlates a weak perinuclear labeling. × 1,200.

wrapped in huge Golgi systems which could extend away from the nuclei. In addition, 8-d-old myotubes often presented a uniform staining with anti-galactosyltransferase (anti-GT) which could indicate a maturation effect involving the expression of GT not associated with the GA. We can rule out the possibility that this labeling corresponds to the plasma membrane, since it was significantly decreased when the permeabilization with methanol which followed formaldehyde fixation (fixation I) was omitted. It was almost suppressed by the Brij-58 extraction (fixation II).

The perinuclear distribution of the GA in myotubes corresponded at the ultrastructural level to cisternae aligned along the nuclear surface, at a constant distance from the nuclear envelope of ~0.1 to 0.2 μm (Fig. 2, *a–c*). The dictyosomes constituted a continuum around the nuclei. Staining for GT (Fig. 2*c*) allowed us to assess the polarity of such a perinuclear GA. The *trans* side, or maturing face, was distal with respect to the nucleus. Extension of the GA within the cytoplasm also appeared in continuity with the perinuclear GA (not shown). In contrast with WGA, no pericellular labeling was observed.

In mononucleated cells, the GA appeared to be organized in a complex way. The trans side, as assessed by anti-GT, was usually facing centrioles (Fig. 3). The mononucleated pattern of the GA was observed as long as the myoblasts were not fused. Even myoblasts capable of fusion, as shown by the typical alignment which immediately precedes fusion, still possessed the pattern typical of mononucleated cells (data not shown). In contrast, early myotubes, possessing only three to five nuclei, already presented perinuclear GA (data not shown).

Spatial Relationship between the GA and the MTOC

The change in GA organization from a polarized juxtanuclear localization in myoblasts to a perinuclear distribution in myotubes may be anticipated as long as the association between the GA and the MTOC is maintained during myogenesis (27). We attempted to define this co-distribution more precisely, using a double immunofluorescence technique. In mononucleated cells, centrosomes were always to be found within the space covered by the GA (Fig. 4*b*), from a central location to a peripheral one. This is consistent with the ultrastructural data (Fig. 3).

Three observations were made in myotubes. (*a*) Perinuclear GA co-distributed almost perfectly with the MTOC (Fig. 4, *c–f*), although when the MTOC was evenly distributed around nuclei, in some cases the GA aligned on the same nuclei was interrupted (Fig. 4, *c–d*). (*b*) Scattered centrioles, detected by MTOC-reacting serum 0013, even having lost most of their associated MTOC (27), were not linked with Golgi stacks

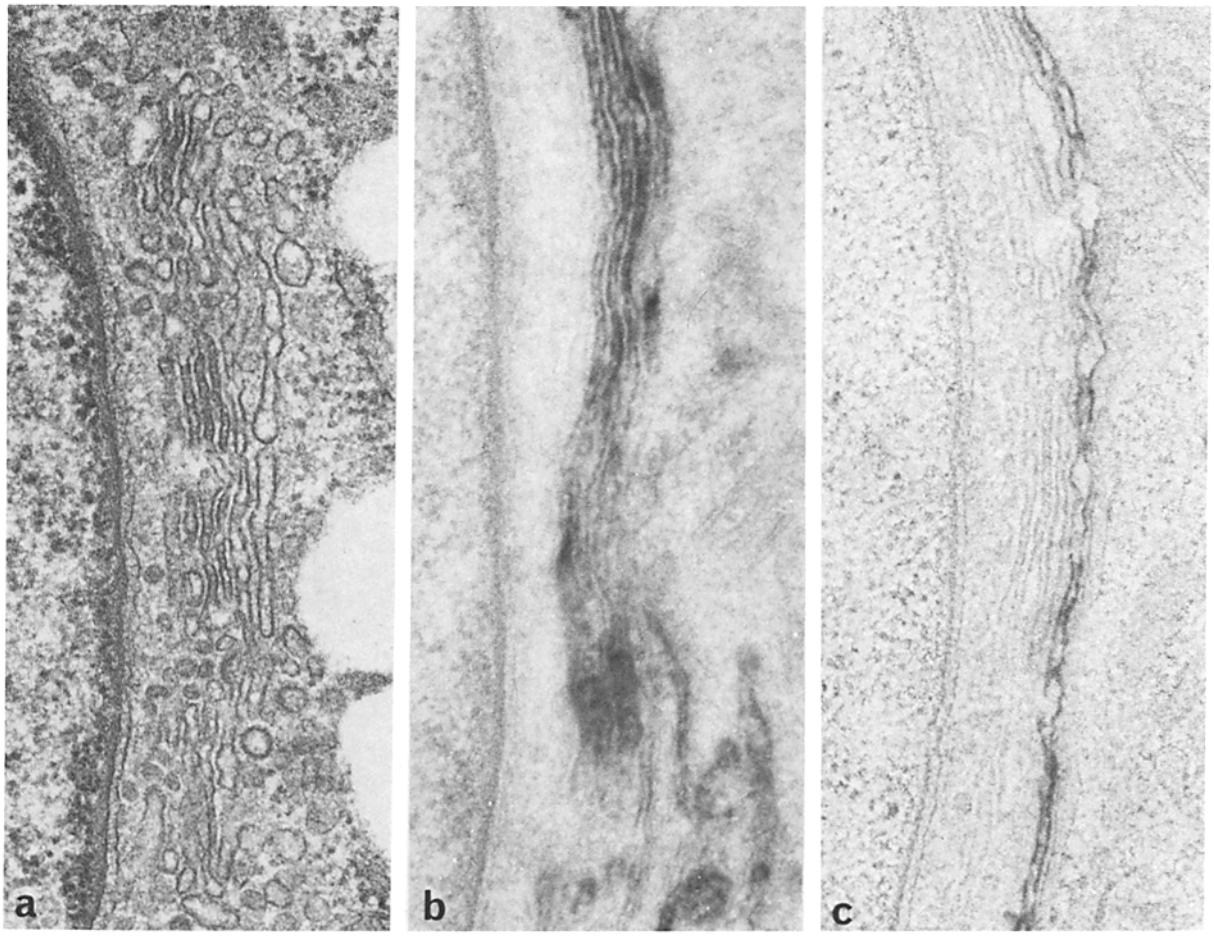

FIGURE 2 Detail of the perinuclear GA of myotubes. (*a*) Transmission electron microscopy reveals between 5 and 7 cisternae in a stack. (*b*) WGA staining. The four distal cisternae are labeled. (*c*) Anti-GT staining. The more distal cisternae is apparently labeled at the membrane itself. Compare to Fig. 3. × 50,000.

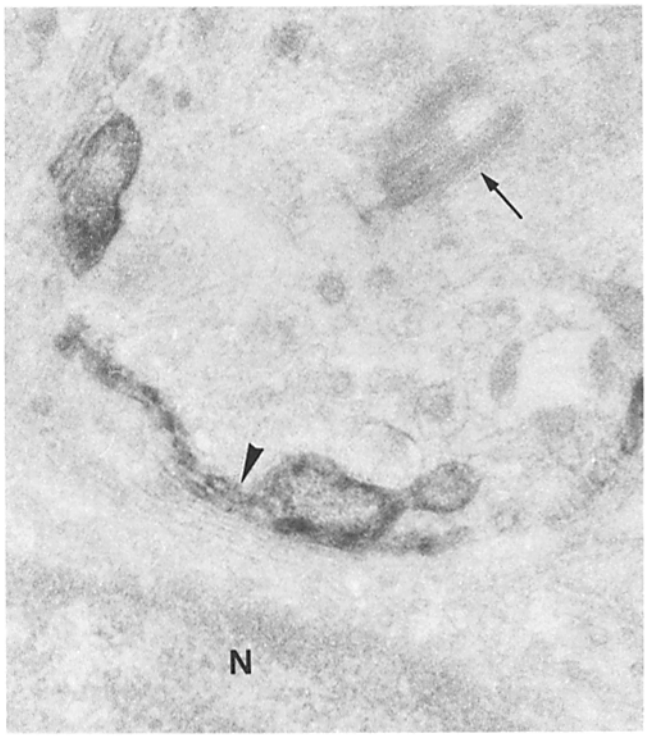

FIGURE 3 Ultrastructural aspect of GA in a mononucleated cell as revealed by anti-GT labeling. The arrow points to the centrosome. The arrowhead points to the *trans* side of the Golgi stacks. × 46,000.

(Fig. 4, *e–f*). (*c*) Golgi stacks extending away from the nuclei were not associated with any detectable serum 0013–positive material.

We have so far been unsuccessful in our attempts to define the relative ultrastructural localization of the MTOC and the GA, using an immunoperoxidase approach. However, in control condition as well as in microtubule regrowth experiments (Figure 5), transmission EM showed Mt's in the space between the GA and the nucleus, which suggests that the MTOC was situated within the space between nucleus and the GA or at the surface of the nucleus.

Sensibility of Myotube GA to Microtubule Depolymerization

In the light of the dramatic effect of microtubular pattern modification on GA morphology in mononucleated cells (6, 21, 31), we tested this effect on the GA in myotubes. When Mt's were depolymerized by $5 \cdot 10^{-6}$ M nocodazole over a 2-h period, the perinuclear GA was only slightly modified in myotubes, contrasting with the profound fragmentation observed in mononucleated cells in the same culture (Fig. 6, *a–b*). The perinuclear labeling with WGA or with anti-GT was less continuous, but remained associated with the nuclear periphery. The Golgi stacks not associated with nuclei were apparently modified to a greater extent. They appeared more compacted than they were in control myotubes.

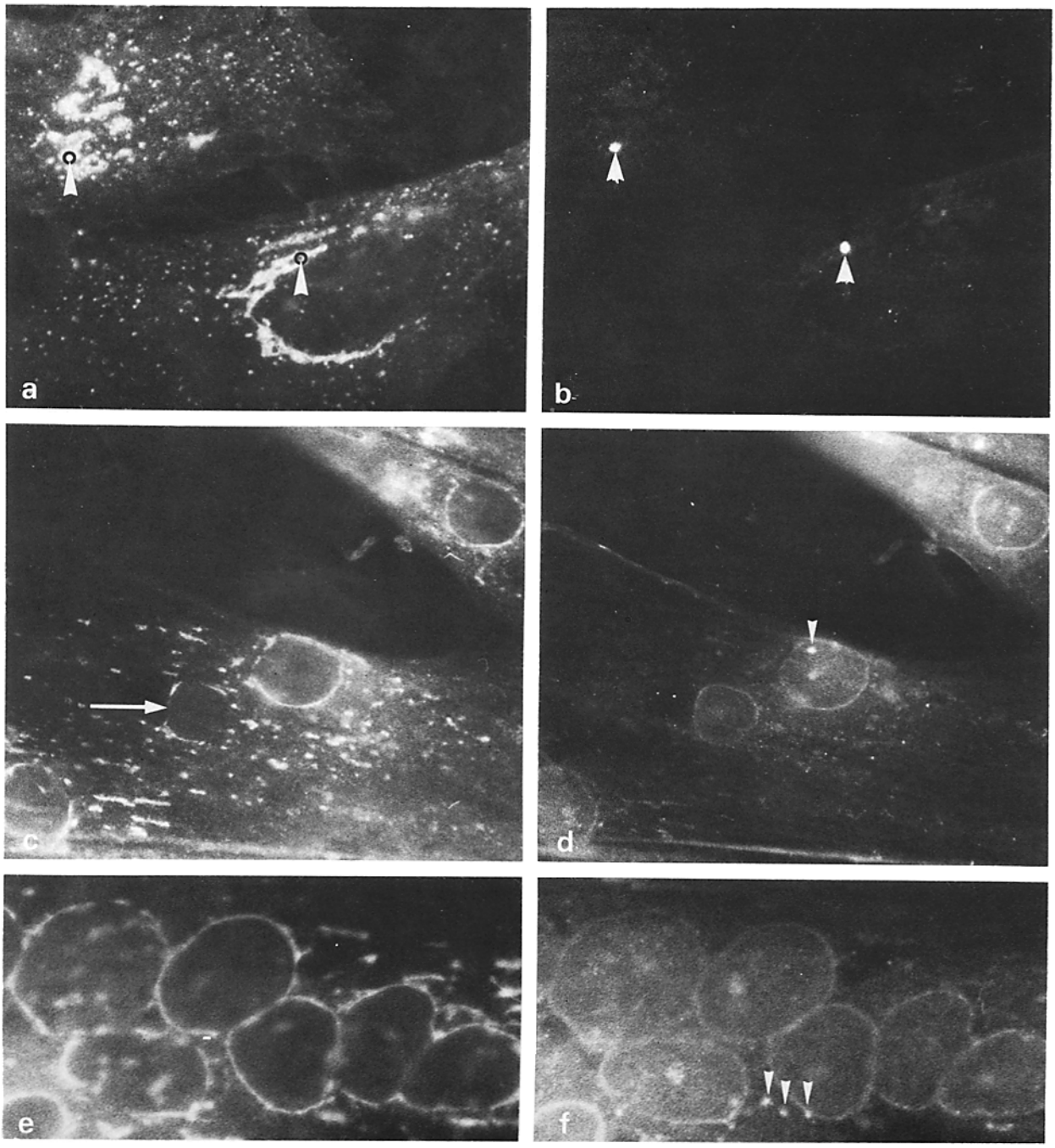

FIGURE 4 GA and MTOC localizations. Double-labeling with WGA (*a*, *c*, and *e*) and with serum 0013 (*b*, *d*, and *f*) in mononucleated cells (*a–b*) and in myotubes (*c–f*). (*a–b*) Centrosomes are localized within the GA. The black circle with a white arrowhead indicates the centrosome within the GA. (*c–f*) GA and MTOC co-distribute in myotubes. This co-distribution is not always perfect. The arrow in *c* points to a nucleus which is only partially labeled with WGA and evenly labeled with serum 0013. Golgi elements not associated with nucleus are unstained with serum 0013. Centrioles (arrowheads in *d* and *f*) are not associated with Golgi elements. (*a*, *b*, *e*, and *f*) × 2,400; (*c* and *d*) × 1,200.

At the ultrastructural level, individual stacks of perinuclear GA of nocodazole-treated myotubes gave the impression of being separated from each other and slightly modified in shape, but still associated individually with the nucleus (Fig. 5 *b*). In mononucleated cells, individual stacks of cisternae were spread into the cytoplasm. They often seemed to keep a quasi-normal organization (data not shown).

Sensibility of the Myotube GA to Monensin

The monovalent cation ionophore monensin has been shown to have a specific effect on the GA (28). After 2 h in 14 μg/ml monensin, a dramatic swelling of the Golgi stacks was produced in both mononucleated cells and myotubes. This could be assessed by phase-contrast microscopy and by specific labeling (Fig. 6, *c* and *d*). Labeling with anti-GT of monensin-treated cells contrasted with the same labeling in nocodazole-treated cells (cf. Fig. 6, *a–c*). It was weak and concerned only some vesicles among unlabeled ones.

Microtubular Pattern, MTOC Distribution, and MTOC Activity in Monensin-treated Cells

We have investigated the effect of the disturbance of the

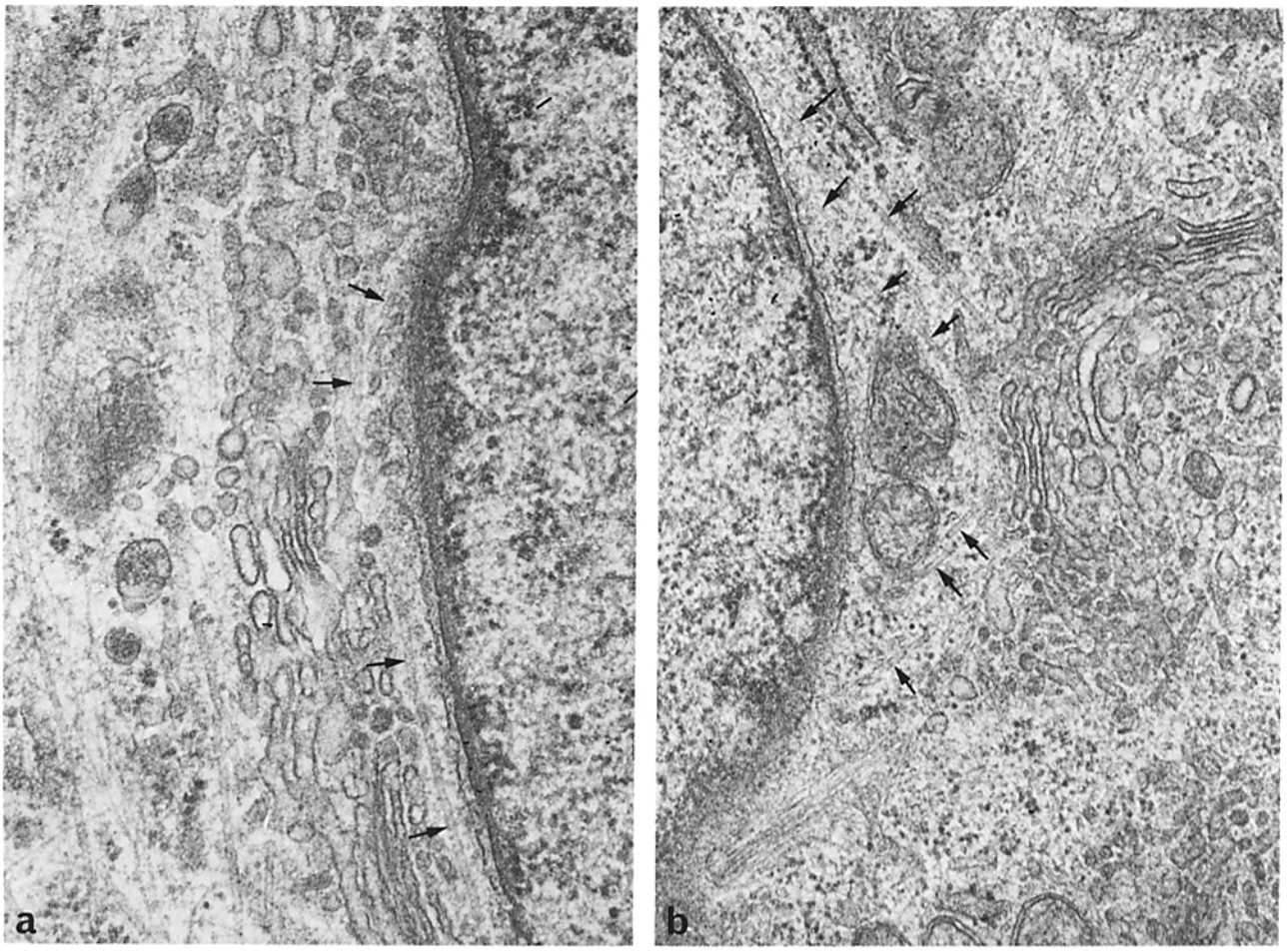

FIGURE 5 Microtubules (arrows) are present within the space between GA and nucleus in control myotubes (a) as well as in myotubes allowed to recover for 5 min from a 2-h treatment with 5.10^{-6} M nocodazole (*b*). (a) × 50,000; (*b*) × 40,000.

GA function by monensin on the microtubular network. We compared the microtubular pattern and the MTOC distribution in cells treated with monensin and in control cells. We also looked at regrowth of Mt's after depolymerization with nocodazole when monensin was present in the medium. The microtubule network was apparently normal in the presence of monensin over a 2-h period. Sustained treatment (15 h) apparently did not decrease the number of Mt's. The pattern was slightly modified concomitantly with cell shape changes imposed by the monensin-induced vesicular state of the cytoplasm (data not shown).

Double immunofluorescence with WGA and MTOC-reacting serum 0013 after 2 h or 15 h of monensin treatment did not show any detectable modification of the MTOC distribution in both myotubes and mononucleated cells (data not shown). Regrowth of Mt's was studied for 0 to 2 h after a 2-h nocodazole treatment. We did not observe any significant difference in the pattern nor in the timing of regrowth (data not shown).

DISCUSSION

We have demonstrated in this work that in vitro myogenesis involves a dramatic modification of the GA. The GA, in myoblasts as in most cell types in culture, shows a juxtanuclear localization near the centrosome (1, 14, 24). In myotubes, it constitutes a continuum around individual nuclei or groups of nuclei. A remarkable feature of this perinuclear GA was its contiguity with the nuclear envelope, suggesting the existence of links between both organelles. Judged by the established *trans* localization of galactosyltransferase (24), the polarity of the Golgi stacks is such that the *cis* side is close to the nucleus. This polarity, although anticipated, raises geometrical difficulties for vesicle transit between endoplasmic reticulum and the *cis* face. We have shown previously (27) that myogenesis also involved redistribution of the MTOC material from the centrosome to the nuclear periphery. Both events take place at the same time, just after fusion. Therefore, GA and MTOC redistribution during myogenesis appear to be correlated. This could suggest that the GA and the MTOC are associated. Double immunofluorescence of the GA and the MTOC has indeed confirmed the co-redistribution of both organelles.

The use of immunoperoxidase on ultrathin sections did not, however, allow us to localize the MTOC, which may be discrete, as indicated by previous work (27). Presence of Mt's in the space between the nucleus and the GA during regrowth experiments suggests, but does not prove, that the MTOC is on the nuclear side. Arguments for an association between the nucleus and the MTOC do exist (see the discussion in reference 27). The perinuclear distribution of the GA in myotubes, respecting a precise gap with the nucleus, suggests the existence of some structure linking the GA to the nuclear membrane. This is supported by experiments of microtubular depolymerization by nocodazole, in which a minimal effect was observed on GA organization, which strongly contrasted

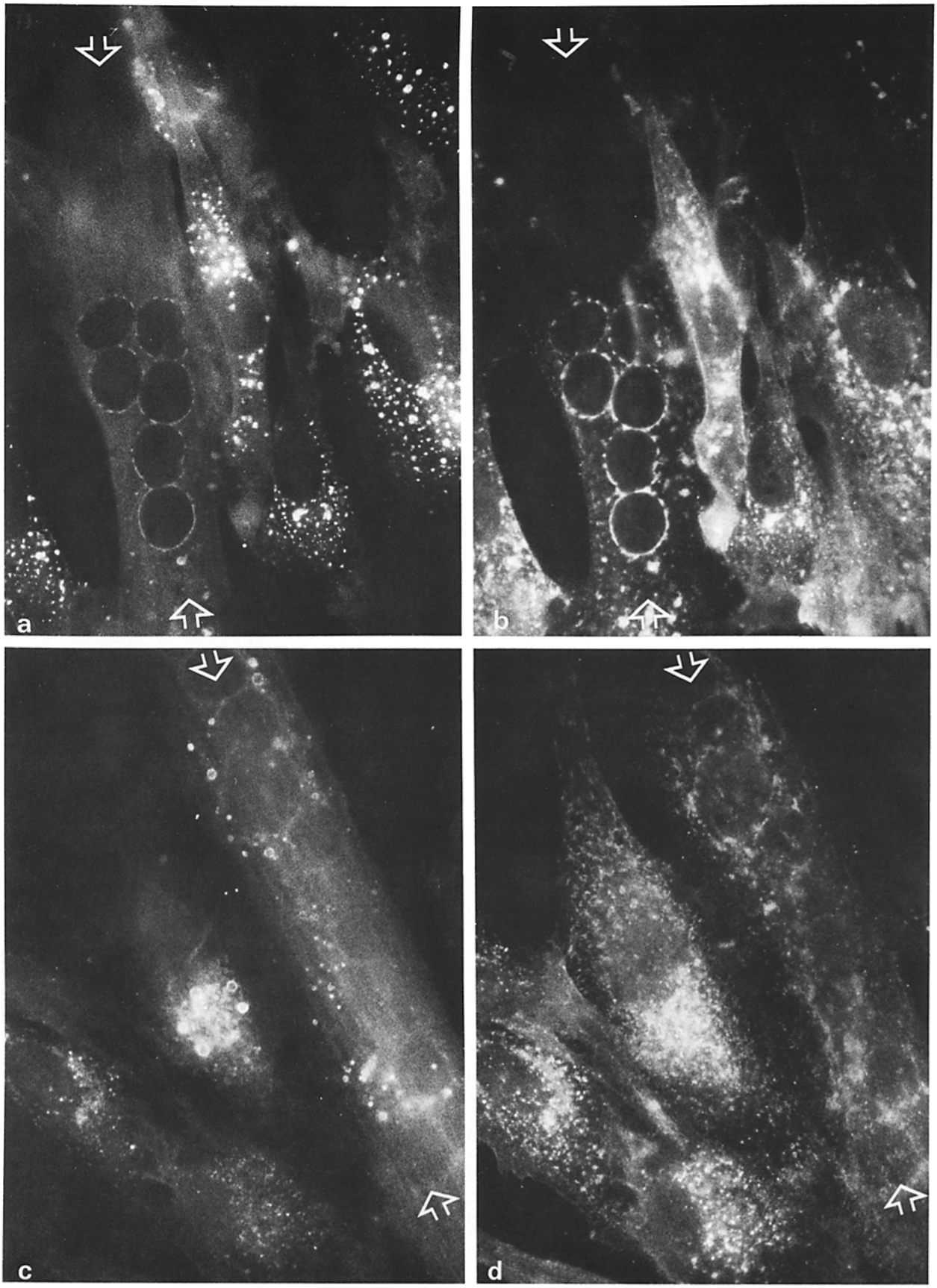

FIGURE 6 Effect of drug treatment on GA followed by double-labeling with anti-GT (*a* and *c*) and with WGA (*b* and *d*). (*a* and *b*) Aspect of GA after 5.10^{-6} M nocodazole treatment for 2 h. In mononucleated cells (right side of each picture) GA appears scattered into the cytoplasm. In the myotube (open arrows), GA maintains a perinuclear pattern but appears fragmented. (*c* and *d*) Aspect of GA after 14 μg/ml monensin during 2 h. In mononucleated cells (lower left corner) as well as in the myotube (open arrows), GA appears dramatically swelled. Individual vesicles can easily be distinguished with anti-GT staining (*c*). × 1,000.

with the effect observed in mononucleated cells. The GA remained associated with the nuclei, presenting, however, a fragmented aspect which corresponded at the ultrastructural level to dictyosomes, individually associated with the nucleus (see Fig. 5 *b*).

Rogalski et al. (22) have recently speculated on the possibility that the fragmentation of the GA in mononucleated cells treated with microtubule disrupting drugs does not in fact constitute a fragmentation but "a lateral unfolding of the structure, at the intersaccular connecting regions without extensive structural or functional impairment of the stack of saccules themselves." Our own results also support the view that microtubule disruption principally affects the connecting structures between cisternae of adjacent Golgi stacks in myoblasts as well as in myotubes. But rather than an "unfolding" or "extension," our data indicate a disruption of the connecting structures since in the case of the myotubes, in which cisternae stacks remain in situ, one should be able to detect a structural modification of them. No such thing has been observed.

The interaction between Mt's and the GA is not clear at the present time. From existing literature, we can retain three apparently established facts: (*a*) the tyrosin residue of α-tubulin is involved in the association (injection of anti-α-tubulin, reacting specifically with the tyrosylated form, does disorganize the GA) (34); (*b*) the stability of Mt's can be dramatically modified by microinjection of a nonhydrolysable analogue of GTP, without causing GA disruption (32); and (*c*) Golgi vesicles interact with one end of Mt's in Taxol-treated cells (22). Rogalski and Singer (22) have speculated on the possibility that lateral interaction between GA vesicles and Mt's could exist together with a preferential interaction of the GA with the minus end of Mt's. This would explain both the co-localization of the centrosome and the GA as well as the effect of microtubule disrupting drugs. So far, the experimental evidence is lacking.

The GA–MTOC association has often been interpreted as implying a functional dependency of the GA with respect to Mt's, although experimental data do not clearly support this view (see the introduction). The reverse is also possible, i.e., the GA could modulate, through unknown processes (pH, Ca^{++},...), the activity of the MTOC. To investigate this aspect, we treated cells with monensin, which has an effect on the GA that is well known although not clearly understood (28, 29). Some effect on the microtubular pattern could have been anticipated since the two ionophores monensin and FCCP have both been shown to inhibit the intracellular transport of Alphavirus membrane glycoproteins (10) and since FCCP is known to induce a rapid and complete disruption of Mt's in vivo (15, 16). We were unable to detect any significant effect on the Mt's network, nor did we observe anything concerning MTOC redistribution or regrowth of Mt's. As a matter of fact, regrowth of Mt's on centrosomes of mononucleated cells recovering from nocodazole treatment was always very active when the GA was still fragmented and located at the periphery of the cell (data not shown). We disagree in this respect with a paper by Couchman and Rees (5).

We are therefore left with a situation in which disruption of Mt's apparently has a minimal effect on GA-associated functions such as secretion, and in which GA disruption has no detectable effect on distribution and nucleation of Mt's. And yet, two facts strongly suggest some kind of functional link. (*a*) A clear effect of disruption of Mt's on GA morphology exists, probably at the connection between cisternae of adjacent dictyosomes, in mononucleated cells (reference 22, and this work) and in myotubes where the GA has a particular organization. (*b*) A highly correlated redistribution of the MTOC and the GA takes place during cell differentiation leading to myogenesis, together with the disappearance of regular centrosomes as active MTOC. It is too early to interpret the significance of these facts. Perhaps, rather than viewing Mt's as the factors in this association, it is more appropriate to think that the equilibrium between tubulin and Mt's is the important parameter for GA integrity. It has been reported that vinblastin treatment induced GA fragmentation only when paracrystals are formed. With short treatment, cells have normal GA and no Mt's (33). Information from other works points to molecular tubulin as an important factor in membrane traffic; i.e., the presence of tubulin in coated vesicles (11, 19).

Finally, myogenesis apparently constitutes a possible illustration of the "organizing" or "morphogenetic" role of the centriolar apparatus itself, consistently suspected by early cytologists (35) and yet never proved nor disproved (see reference 2). When the centriolar apparatus is present, it appears to mobilize the MTOC material, which in turn, through an unknown mechanism in which Mt's or tubulin molecules play a role, organizes the GA in its immediate vicinity. As a result, cell polarity is defined. When the centriolar apparatus dissociates from the nucleus, the MTOC relocalizes homogeneously at the surface of the nucleus as does the GA, which apparently acquires additional links with the nucleus. In consequence, a stable GA pattern is established, facing any domain of the plasma membrane of the myotube. Cell polarity is no longer evident. Although oversimplified, the view maintaining the binding of the centrosome to both the nucleus and the GA as a crucial element for the expression of cell polarity requires further experimentation.

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