Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 5686–5689, June 1994
Cell Biology

Disruption of the Golgi apparatus by brefeldin A blocks cell polarization and inhibits directed cell migration

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Communicated by Israel Gelfand, March 14, 1994 (received for review January 5, 1994)

ABSTRACT **The role of the Golgi apparatus in the motile activity of fibroblasts was examined with brefeldin A (BFA), which disrupts the Golgi apparatus in a variety of cells. Upon incubation with BFA, Swiss mouse 3T3 fibroblasts lost their typical polarized morphology, in which the leading edge is characterized by intensive lamellipodia formation. BFA affected cell asymmetry as demonstrated by a decrease in the morphometric indices, dispersion, and elongation. After BFA treatment, cells showed little protrusional activity and did not form a dense actin network at the leading edge, and consequently the rate of cell migration into an experimental wound was significantly reduced. In addition, BFA prevented an increase in pseudopodial activity and prevented the formation of long processes induced by phorbol 12-myristate 13-acetate. The effects of BFA on cell shape and protrusional activity were quantitatively similar to those observed with the microtubule-disrupting agent nocodazole, although BFA had no effect on microtubule integrity. These results suggest that the integrity of both the Golgi apparatus and microtubules is necessary for the generation and maintenance of fibroblast asymmetry, which is a prerequisite for directed cell migration.**

Directed migration of a variety of cell types depends on the polarized distribution of protrusions (pseudopods) at the cell periphery (1). For example, in directionally migrating fibroblasts, most pseudopodial activity is located at the leading edge of the cell, whereas no protrusional activity is observed at the sides and trailing edge, resulting in the typical fan-like appearance of fibroblasts. The asymmetric distribution of protrusional activity is a general characteristic of directional motility and/or directional growth. Fish keratocytes, which are highly motile, display intensive pseudopodial activity at the edge of the cell which is oriented in the direction of movement (2), and in neurons, protrusional activity is restricted to the growth cone (3). In all of these cases, pseudopodial activity requires local polymerization and crosslinking of actin filaments at the leading edge (4).

Polarization of pseudopodial activity also depends on other cytoskeletal elements. Vasiliev, Gelfand, and collaborators (5–7) showed that in cultured fibroblasts, disruption of microtubules by drugs such as colchicine or nocodazole resulted in the loss of asymmetry of cell shape and a decrease in directed cell migration. Two potential mechanisms can explain the role of microtubules in maintaining cell asymmetry. First, microtubules could play a structural role by mechanically restricting the formation of pseudopods at the sides and trailing edge of the cell. Second, since microtubules provide the framework upon which the molecular motors kinesin and dynein vectorially transport organelles and vesicles (8), they may be involved in transport of the cell components necessary for formation of the leading edge. The observation that microinjection of fibroblasts with anti-kinesin antibodies results in loss of cell polarity (5, 9) supports the latter possibility.

We have examined the role of the Golgi apparatus in the motile activity of cells. For this, we used brefeldin A (BFA) (10–12), which disrupts the Golgi apparatus, resulting in subsequent inhibition of vesicle transport to the cell surface without affecting microtubule organization (13). We report that disruption of the Golgi apparatus leads to changes in cell morphology and motility that are indistinguishable from those caused by disruption of microtubules. These results suggest that the generation and maintenance of fibroblast asymmetry, which is a prerequisite for directed migration, depends on maintaining both the supply of Golgi apparatus-derived vesicles and the integrity of microtubule-based vesicle transport.

MATERIALS AND METHODS

Cell Culture. Swiss mouse 3T3 cells were cultured in Dulbecco's modified Eagle's medium containing 10% calf serum and maintained in a water-saturated atmosphere of 7% CO_2. Cells were dissociated with trypsin/EDTA and plated at $\approx 5 \times 10^3$ per cm^2 on glass coverslips. The effect of BFA on cell shape was measured 1 or 2 days after plating; BFA was prepared as a stock solution (10 mg/ml) in dimethyl sulfoxide.

Analysis of Cell Shape and Motility. Dispersion and elongation indices of cell outlines were calculated according to Dunn and Brown (14). Cells were labeled with tetramethylrhodamine isothiocyanate (TRITC)-conjugated phalloidin and microscopic images were captured with a charge-coupled-device camera. Cell outlines were identified and analyzed with software provided by Z. Kam, Department of Chemical Immunology, Weizmann Institute of Science. Approximately 40–50 cells were analyzed for each experimental point.

A confluent cell monolayer obtained ≈1 week after plating was used for experiments on cell migration into an experimental wound. Experimental wounds were made in the middle of the coverslip by using the plunger of a disposable Eppendorf syringe. Video microscopy of cell migration was performed with a Plan Neofluar ×100/1.3 n.a. objective of a Zeiss Axiophot microscope, equipped with Nomarski optics, a JVC video camera, and a Panasonic video cassette recorder.

In experiments examining the effect of BFA pretreatment on process formation, cells were incubated with BFA for 18 hr prior to addition of phorbol 12-myristate 13-acetate (PMA, 100 ng/ml) for various times; BFA was present throughout the incubation with PMA. To quantify the effect of BFA on the formation of long-processes, cells were labeled with

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Abbreviations: BFA, brefeldin A; PMA, phorbol 12-myristate 13-acetate; TRITC, tetramethylrhodamine isothiocyanate; C_6-NBD-ceramide, *N*-[*N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)-ε-aminohexanoyl]-D-*erythro*-sphingosine.

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TRITC-conjugated phalloidin to visualize cell outlines and with 4′,6-diamidino-2-phenylindole (DAPI) to visualize the nucleus. A cell was considered to have a long process ("tail"; see Fig. 5*C*) if the length of the process was >10 times the diameter of the nucleus.

Immunofluorescence and Fluorescence Microscopy. Microtubule distribution was examined by using a monoclonal anti-α-tubulin antibody (clone DM1A, Sigma) and actin distribution was examined by using TRITC-conjugated phalloidin. The Golgi apparatus was visualized by *N*-[*N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)-ε-aminohexanoyl]-D-*erythro*-sphingosine. (C_6-NBD-ceramide) (15) after fixation in 3% paraformaldehyde.

RESULTS

BFA and Cell Morphology. Initial experiments demonstrated that incubation with low concentrations of BFA (0.2–1 μM) resulted in dispersion of the Golgi apparatus, as visualized by C_6-NBD-ceramide, in Swiss 3T3 fibroblasts. After several hours of incubation with BFA, cells lost their typical fan-like shape with prominent lamellipodial activity at the leading edge (Fig. 1*A*) and became discoid with a random distribution of small lamellipodia over the entire cell periphery (Fig. 1*C*). In control cells, the Golgi apparatus was prominently labeled with C_6-NBD-ceramide (Fig. 1*B*) and, in many cells, was positioned forward of the nucleus in the direction of the cell edge which displayed pseudopodial activity (16). Disruption of the Golgi apparatus by BFA (Fig. 1 *B* and *D*) occurred within <1 hr of incubation; by this stage not all cells had lost their polarized morphology (data not shown). However, no discernible Golgi apparatus was observed in discoid cells after BFA treatment (Fig. 1*D*). These results indicate that disruption of the Golgi apparatus by BFA preceded changes in cell morphology.

The effects of BFA on cell morphology were compared with those induced by the microtubule-disrupting agent nocodazole and quantified by using morphometric indices of dispersion and elongation. These indices reflect the degree of cell asymmetry (14). Dispersion and elongation indices decreased during incubation with either 0.5 μM BFA or 10 μM nocodazole (Fig. 2), indicating loss of polarization of cell shape. Asymmetry was restored after removal of BFA or nocodazole (Fig. 2), demonstrating that the effects of these two drugs were reversible. The loss of asymmetry was not due to "rounding" of the cells (i.e., their partial detachment from the substratum), since BFA-treated cells, which occupied a large area of the substrate, also had a low dispersion index (data not shown) and, moreover, little change was observed in the average projected area of the cell. Thus, after 8 hr, the area occupied by BFA-treated cells was 1382 ± 104 μm^2, that occupied by nocodazole-treated cells was 1265 ± 88 μm^2, and that occupied by control cells was 1539 ± 114 μm^2. Prolonged incubation with a high concentration of BFA (5 μM) led to a more pronounced decrease in cell area and ultimately to complete rounding of the cells.

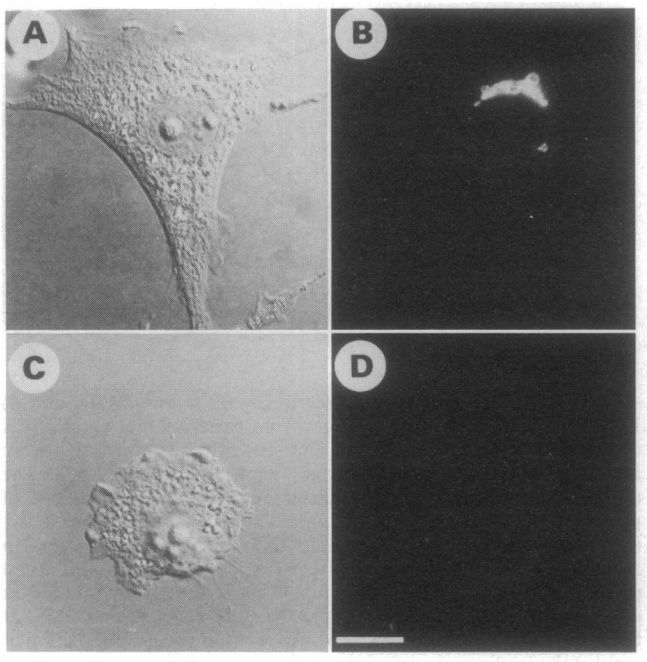

FIG. 1. Effect of BFA on cell morphology and on the integrity of the Golgi apparatus. (*Left*) Nomarski images of control (*A*) and BFA-treated (*C*) cells. (*Right*) C_6-NBD-ceramide labeling of the Golgi apparatus of control (*B*) and BFA-treated (*D*) cells. (Bar = 20 μm.)

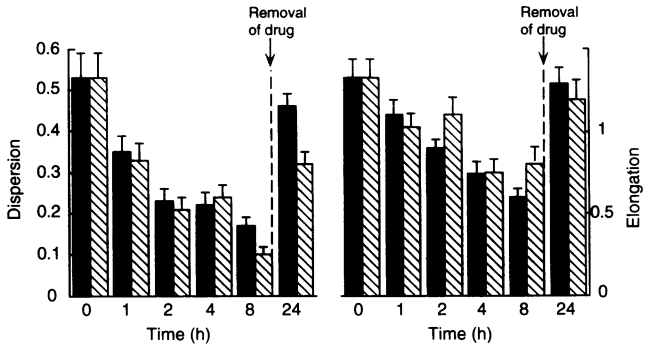

FIG. 2. Quantification of the time course of the morphological effects induced by BFA and nocodazole. Filled bars, 0.5 μM BFA; hatched bars, 10 μM nocodazole. Values represent means ± SEM.

BFA and the Cytoskeleton. BFA had no apparent effect on the radial distribution of microtubules (Fig. 3). In contrast, significant changes in the actin cytoskeleton were observed after BFA treatment (Fig. 4). In control cells, actin was organized into two types of structures: (*i*) actin-rich regions located directly beneath the membrane at the leading edge and (*ii*) parallel bundles of actin filaments oriented along the long axis of the cell (Fig. 4*A*). After BFA treatment, a less pronounced accumulation of actin-rich areas at the cell periphery (Fig. 4 *B–D*) was always observed, but the distribution of actin bundles varied, being organized either circumferentially (Fig. 4*B*) or radially (Fig. 4*C*) or stretching from adhesion points without obvious orientation to each other (Fig. 4*D*). The effects of nocodazole on actin reorganization (data not shown) were identical to those of BFA.

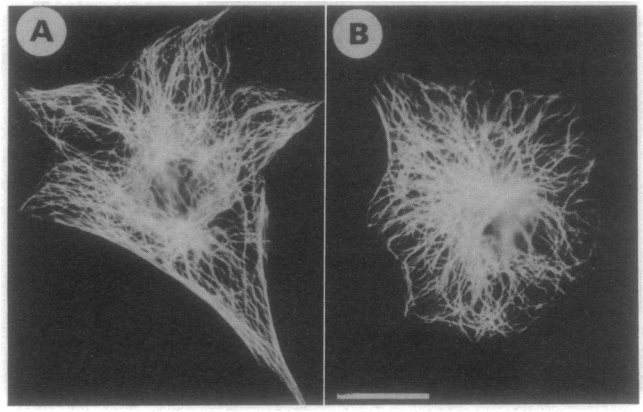

FIG. 3. Distribution of microtubules in control cells (*A*) and cells treated with BFA (0.5 μM) for 18 hr (*B*). BFA treatment has no effect on the radial distribution of microtubules. (Bar = 20 μm.)

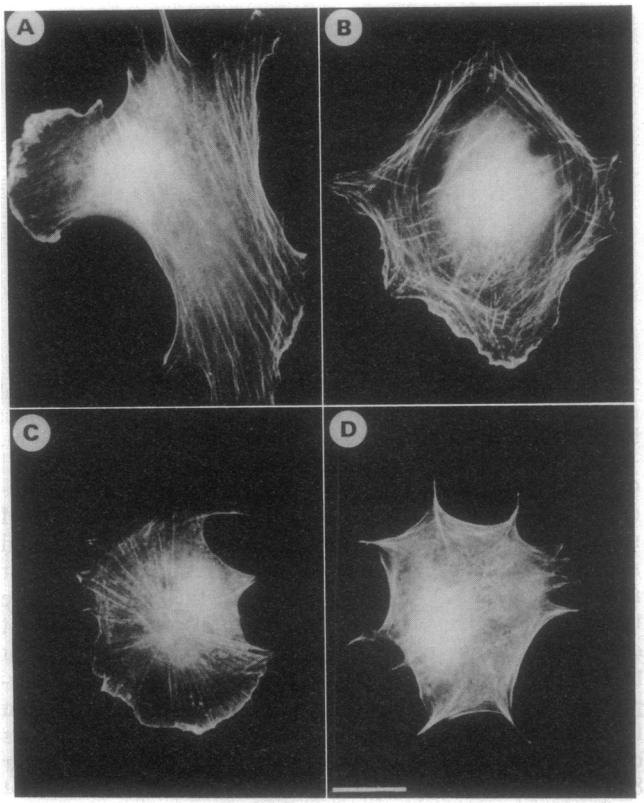

FIG. 4. BFA causes reorganization of the actin cytoskeleton. (*A*) Control cells. (*B–D*) Cells treated with BFA (0.5 μM) for 18 hr. Note the changes in the organization of actin bundles and the loss of polarized distribution of actin-rich lamellipodia after BFA treatment. (Bar = 20 μm.)

BFA Inhibits Protrusional Activity and Cell Motility. Two experimental paradigms were used to examine the effects of BFA on pseudopodial activity. First, it is known that the pseudopodial activity of migrating fibroblasts can be enhanced by activation of protein kinase C. Thus, phorbol esters induce changes in morphology and in organization of the actin cytoskeleton (17, 18), resulting in accumulation of actin at the leading edge, destruction of large actin bundles, and formation of long processes (Fig. 5*A*). Pretreatment with BFA prevented the PMA-induced formation of long processes (Fig. 5 *B* and *C*), similar to the effect of pretreatment with Colcemid (19).

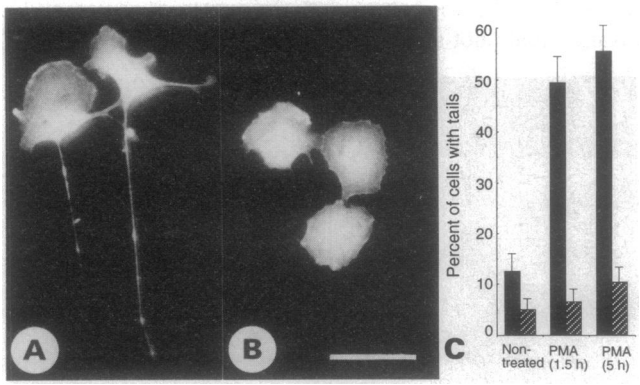

FIG. 5. BFA prevents the ability of PMA to stimulate lamellipodial activity and formation of long processes. After PMA treatment (1.5 hr), Swiss 3T3 cells extend long processes (*A*), but preincubation (18 hr) with BFA (1 μM) (*B*) prevents this effect. (Bar = 50 μm.) (*C*) Quantification of the effect of pretreatment with BFA (hatched bars) on PMA-induced formation of long processes. Filled bars, control cells.

Second, since fibroblast motility depends on pseudopodial activity, we examined whether BFA-treated cells were able to migrate into an experimental wound. The leading edge of cells at the border of an experimental wound is characterized by extension and retraction of broad and flat lamellipodia (Fig. 6*A*), with ruffles containing a dense actin network (Fig. 6*C*). This protrusional and retractional activity results in directed cell migration (Fig. 6*E*). After BFA treatment, cells showed little protrusional and retractional activity (Fig. 6 *B*

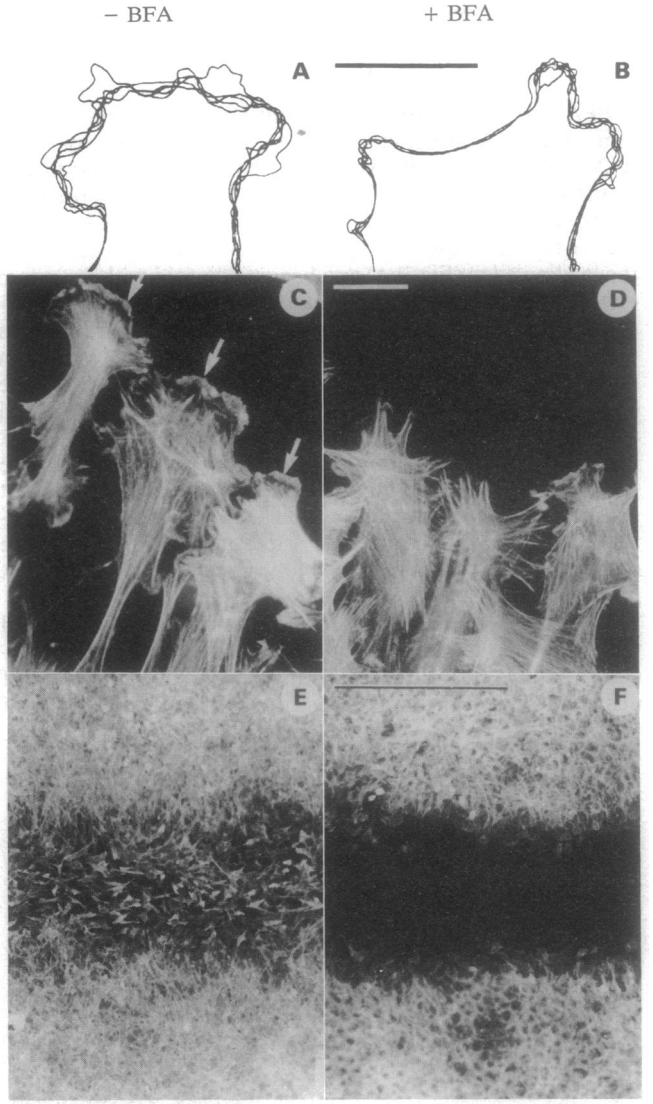

FIG. 6. Effect of BFA on directional cell migration. (*A* and *B*) Lamellipodial activity at the edge of an experimental wound was examined by video microscopy. Outlines of the leading edge were traced from five successive images captured every 25 sec. The difference in superimposed outlines demonstrates the extent of protrusional and retractional activity. The amplitude of these activities is much higher in control cells than in BFA-treated cells. In addition, pseudopodial activity is restricted to a small area of the cell periphery in BFA-treated cells but is more evenly distributed in control cells. (Bar = 20 μm.) (*C* and *D*) Actin distribution in cells at the edge of an experimental wound. Arrows indicate accumulation of actin at the leading edge of control cells. After BFA treatment, there are few actin-rich regions at the cell periphery, and actin is concentrated mainly in stress fibers. (Bar = 20 μm.) (*E* and *F*) An experimental wound of 0.5 mm is completely filled with cells in control cultures 8 hr after wounding but devoid of cells after BFA treatment. (Bar = 500 μm.)

and *D*), and consequently the rate of cell migration into the experimental wound was significantly reduced (Fig. 6*F*). The effects of BFA on cell motility were similar to those observed with microtubule-depolymerizing agents (20).

DISCUSSION

In the current report, we demonstrate that incubation of Swiss 3T3 fibroblasts with low concentrations of BFA (0.2–1 μM) leads to loss of polarization of cell shape and pseudopodial activity. As a result, pronounced reorganization of the actin cytoskeleton occurs, and consequently cells are unable to form long processes and to migrate into an experimental wound. The effects of BFA on cell motility are strikingly similar to those obtained using microtubule-disrupting agents (5–7, 19, 20) (see also Fig. 2).

The necessity of a centralized microtubule system in maintaining cell polarity appears to depend on cell type (21). In fibroblasts, disruption of microtubules by drugs such as colchicine or nocodazole results in loss of asymmetry of cell shape and a decrease in directed cell migration (5–7). In addition, blocking vesicle transport along microtubules with anti-kinesin antibodies (9) results in loss of cell polarity.

Even though the effects of BFA on cell morphology and motility are almost identical to those observed with microtubule-disrupting agents, the cellular target of action of these drugs is completely different. Thus, we observed no effect of BFA on microtubule integrity, consistent with published studies (13), and preliminary observations from video microscopy suggest that no change occurs in the motility of mitochondria, supporting the idea that BFA has no effect on microtubule-based motor activity. In contrast, it is now well established that BFA interacts with the cellular machinery responsible for vesicle budding (22, 23), disrupting both the integrity of organelles of the central vacuolar system, and the transport of vesicles between these organelles (10–12). In particular, BFA disrupts the Golgi apparatus and inhibits vesicle transport from distal Golgi apparatus compartments to the cell surface (24). The similarities in the effects on cell organization and motility of BFA, of microtubule-disrupting agents, and of inhibition of the molecular motor kinesin (9, 25) may therefore result from disruption of the supply of intracellular vesicles containing the components necessary to form and maintain leading-edge activity. Thus, BFA affects the availability of vesicles, inhibition of the activity of microtubule motors affects the transport of vesicles, and disruption of microtubules affects the molecular framework upon which vesicles are transported.

It has been proposed that the polarized delivery of vesicles derived from the Golgi apparatus and their subsequent insertion at the plasma membrane provide a basis for polarized lamellipodial activity at the leading edge (26). This suggestion was based on experiments in which the newly synthesized G protein of vesicular stomatitis virus was observed to be inserted initially at the leading edge of motile fibroblasts (27, 28). However, direct proof of this proposal has been lacking due to the unavailability of experimental data directly characterizing the role of the Golgi apparatus and vesicle transport in the regulation of cell shape and motility. Our current data support this proposal by demonstrating that disruption of the Golgi apparatus inhibits lamellipodial activity; similarly, cultured gonocytes (29) are unable to develop processes after BFA treatment, and axonal growth is inhibited in cultured hippocampal neurons (30). Together, these results demonstrate multiple ways of regulating pseudopodial activity and cell migration, either by modifying the cytoskeletal components or the molecular motors involved in vesicle transport or by modifying the supply of the vesicles themselves. Modifying and regulating the cellular machinery that is responsible for vesicle fusion and/or budding may provide an alternative means for controlling cell motility and cytoskeletal structure.

We thank Benny Geiger for support and encouragement, Natalie Prigozhina for help in measurement of cell shape, and Zvi Kam for providing software. This work was supported by the Raschi Foundation (to A.D.B.). A.H.F. is the incumbent of the Recanati Career Development Chair in Cancer Research.

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