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Reversible translocation of cytoplasmic actin into the nucleus caused by dimethyl sulfoxide

(cytoskeleton/fluorescence microscopy/microinjection)

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ABSTRACT **The addition of 10% dimethyl sulfoxide (Me_2SO) to PtK_2 and WI-38 cells caused stress fibers to disappear from the cytoplasm and numerous elongated inclusions to appear in the nucleus. When Me_2SO was removed, the stress fibers reformed and the nuclear inclusions disappeared. These nuclear inclusions reacted with fluorescent heavy meromyosin, phalloidin, and actin antibody. In the electron microscope, needle-like structures were seen to be composed of wavy filaments that bound heavy meromyosin. Antibodies against other components of stress fibers—tropomyosin, α-actinin, and myosin—did not react with the inclusions. When fluorescently labeled actin was microinjected into living PtK_2 and WI-38 cells, the fluorescent actin was incorporated into stress fibers. Subsequent exposure of the same cells to Me_2SO led to breakdown of the fluorescent stress fibers and the appearance of fluorescent inclusions in the nucleus. Removal of Me_2SO caused reversion to the normal interphase structure. These results indicate that under the influence of Me_2SO, dissolution of stress fiber releases actin in a form which allows it to diffuse into the nucleus where it then becomes organized into filamentous bundles.**

Evidence that actin is a constituent of the nuclei of a number of different cell types has been accumulating (1–7), although it is still not clear that its occurrence in nuclei is universal (8). The difficulty in identifying actin as an endogenous nuclear component is that the large amount of actin in the cytoplasm is a potential source of contamination of nuclear preparations. The finding by Weber and Osborn that the nucleus is surrounded by a "cage" of detergent-resistant microfilaments (9) emphasizes this problem.

Recently, Fukui and Katsumaru (10–12) have shown that under the influence of 10% Me_2SO, bundles of actin filaments will form *in situ* in nuclei of *Dictyostelium*, amoebae, and HeLa cells. These bundles were believed by these authors to arise from actin normally present in the nucleus. If true, this would indicate that the interphase nuclei of these cells contain a substantial amount of actin, which may play an important role in nuclear functions (10, 12). On the other hand, initial studies with Me_2SO and PtK_2 cells (13) showed that in treated cells, bundles of actin filaments appeared in the nucleus at the same time that actin-containing stress fibers disappeared from the cytoplasm. The present results, which combine immunofluorescence with antibodies against contractile proteins and microinjection of fluorescent actin into living cells, demonstrate that the same actin that participated in the cytoplasmic stress fibers moves into the nucleus under the influence of Me_2SO. There it reforms filamentous aggregates that clearly differ in composition from cytoplasmic stress fibers. This translocation of actin is rapid and fully reversible.

MATERIALS AND METHODS

Rat kangaroo cells (PtK_2) and WI-38 human fibroblasts were obtained from the American Type Culture Collection and were grown on glass cover slips in Falcon culture dishes under conditions as described (14, 15). Cells were treated with dimethyl sulfoxide (Me_2SO) by incubating them in normal medium to which spectrophotometrically pure Me_2SO was added to give a final concentration of 10% (vol/vol) Me_2SO. Tetramethylrhodamine-labeled actin was prepared and microinjected into living cells as detailed by Kreis *et al.* (15). For staining with fluorescent agents, cells were fixed in 3% (vol/vol) formaldehyde in standard saline (0.1 M KCl/0.01 M phosphate buffer/0.001 M $MgCl_2$, pH 7.0), rinsed for 5 min in standard saline, extracted for 5 min in 0.1% Triton-X 100 in standard saline, and washed with several changes of standard saline. Fluorescein-labeled heavy meromyosin was prepared as described (16) and used for 1 hr at 4°C to stain cells, which were then washed well with cold standard saline before being mounted in Elvanol on a glass slide. Cells were stained for 1 hr at room temperature with fluorescein-labeled phalloidin [obtained from Th. Wieland (17)]. Affinity-purified antibodies produced in rabbit against actin, α-actinin, tropomyosin, myosin, and tubulin [prepared as reported (18–20); unpublished data] and guinea pig antibody against prekeratin [obtained from W. W. Franke (21)] were used in indirect immunofluorescence staining. Cells were incubated with antibody for 45 min at 37°C, washed in several changes of standard saline for 10 min, and then stained for 45 min at 37°C either with fluorescein-labeled goat anti-rabbit antibody (Miles) or, in the case of anti-prekeratin-labeled cells, with fluorescein-labeled goat anti-guinea pig antibody. After being rinsed for 10 min in several changes of standard saline and briefly in distilled water, the cells were mounted in Elvanol on glass slides. Microscopic observation and photography were carried out as reported for microinjected (15) and stained (19) cells.

Cells were grown for electron microscopy on 60-mm Falcon culture dishes and fixed and embedded in the dish (22). To identify actin ultrastructurally, heavy meromyosin (3 mg/ml in standard saline) was added for 1–2 hr at 4°C to dishes of cells that had been fixed for 5 min at −20°C in ethanol and then washed with standard saline at 4°C. After unbound heavy meromyosin was removed by rinsing with standard saline, the cells were fixed in 1% glutaraldehyde buffered with 0.1M sodium phosphate (pH 7.0) to which 0.2% tannic acid was added (23). The samples were then processed in the same way as control cells.

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Abbreviation: Me_2SO, dimethyl sulfoxide.

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RESULTS

Stress fibers are especially prominent in flat tissue culture cells of epithelial or fibroblastic origin, such as PtK_2 cells, which grow in interconnected sheets with well-developed junctions (Fig. 1*A*), and in WI-38 cells (not shown). Exposure of such cells to 10% Me_2SO for 30 min caused a dramatic effect on the morphology: in PtK_2 cells, the stress fibers disappeared and the cells pulled away from their neighbours (Fig. 1*B*). WI-38 cells

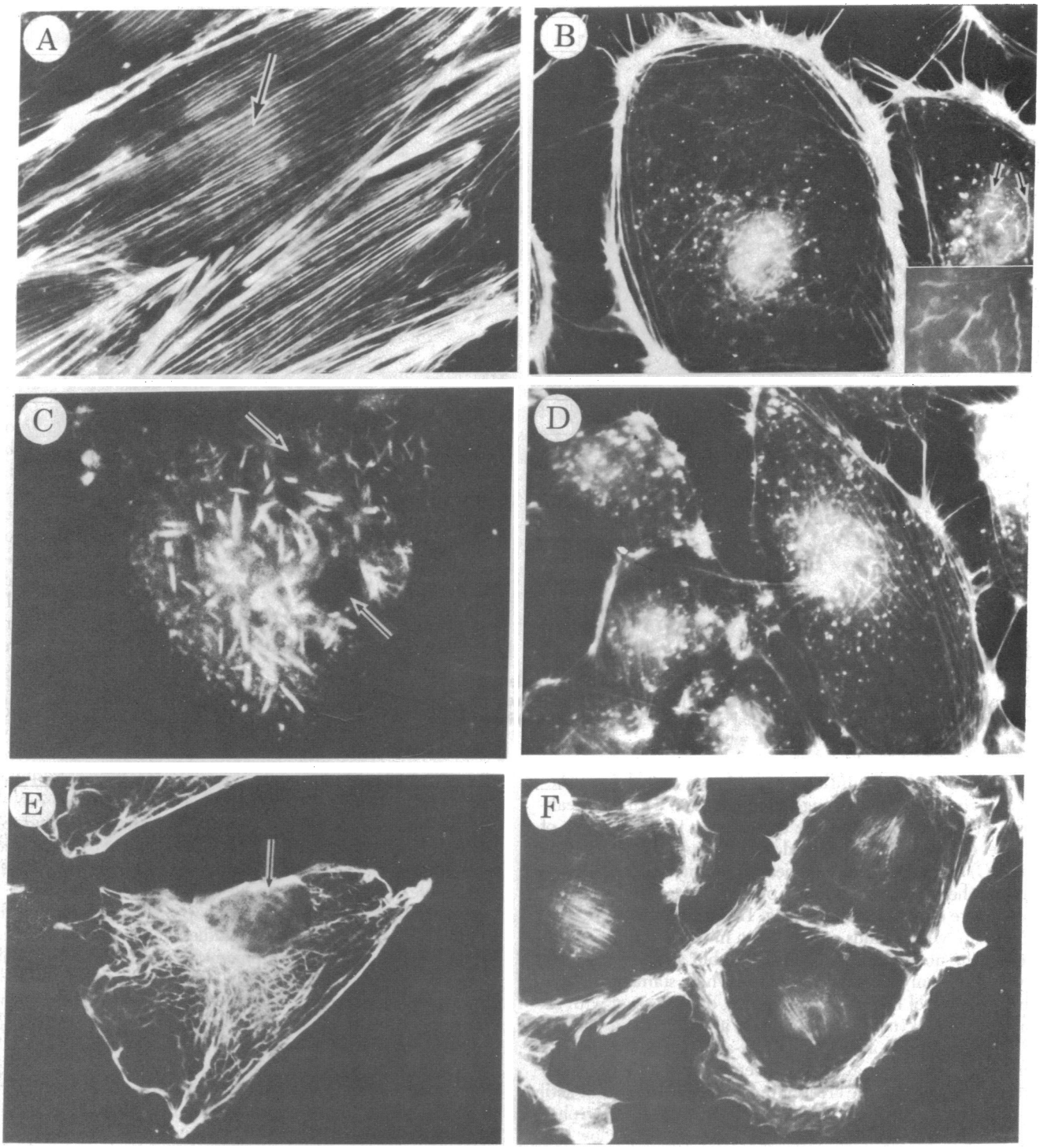

FIG. 1. (*A*) Part of a sheet of PtK_2 cells in control medium; stained with actin antibody. Stress fibers are prominent. The arrow indicates the nuclear area of one cell in the sheet. (×500.) (*B*) PtK_2 cells stained with actin antibody after 30 min in 10% Me_2SO. Cells have retracted from one another but are still connected by fine actin-containing cytoplasmic strands. Stress fibers are greatly diminished and nuclear rods have appeared. Close examination of the nuclear actin bundles reveals some which are wavy (arrows). (×500.) The insert shows wavy bundles at higher magnification (×1400.) (*C*) Higher magnification of another nucleus of a PtK_2 cell treated with Me_2SO for 30 min and stained with actin antibody. This nucleus has a particularly high number of bundles that are excluded from the nucleoli (arrows). (×750.) (*D*) PtK_2 cells stained with fluorescent phalloidin after 30 min in 10% Me_2SO. (×500.) (*E*) PtK_2 cells stained with prekeratin antibody after 30 min in 10% Me_2SO. The prekeratin antibody does not stain the nuclear inclusions (arrow points to nucleus) that are present under these conditions. (×750.) (*F*) PtK_2 cells stained with actin antibody after a 30 min recovery from Me_2SO treatment. Inclusions are no longer present in the nuclei, and cells have begun to reestablish contacts with one another. Stress fibers reform first at the periphery, but also can be seen faintly in the center of the cells. (×500.).

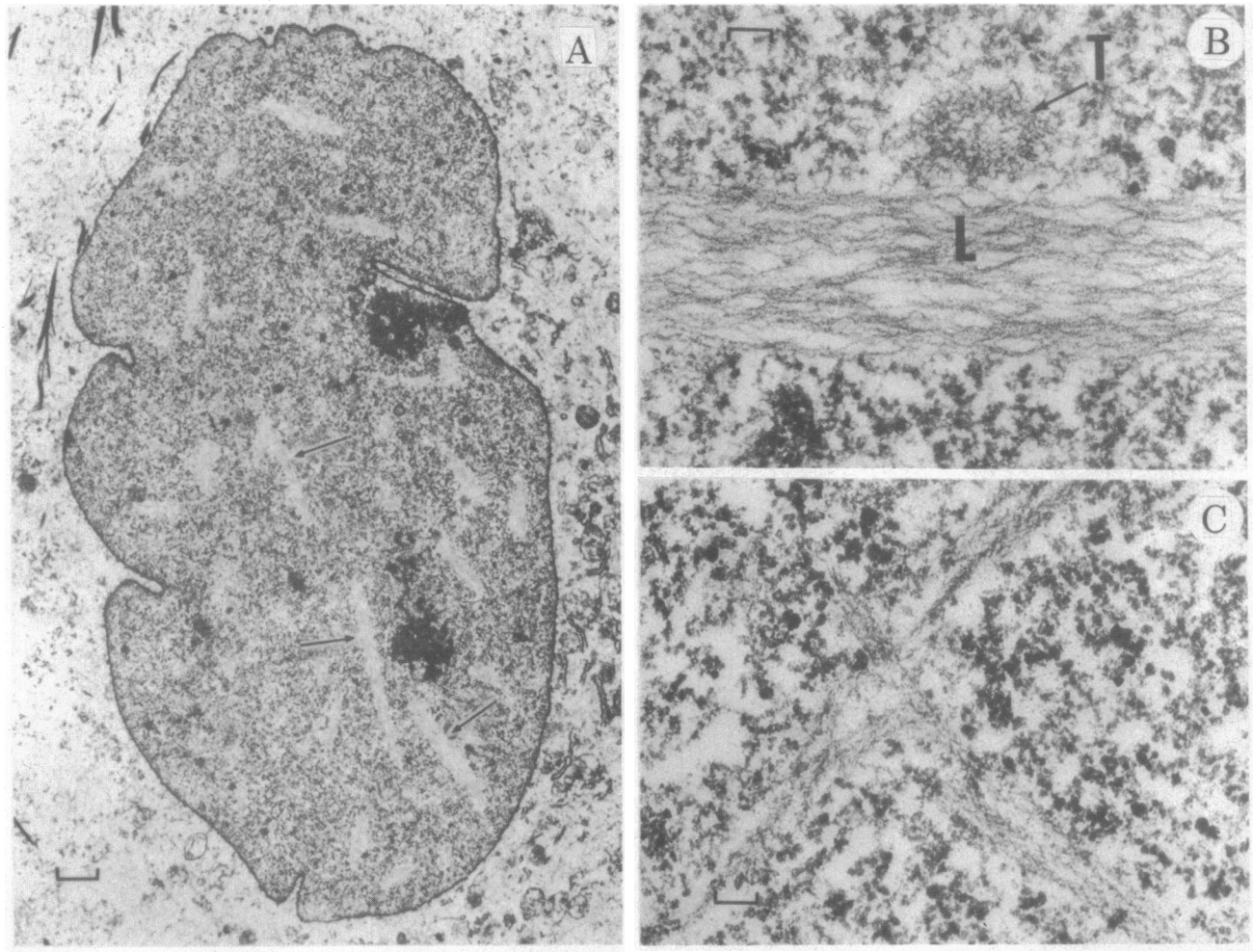

FIG. 2. (*A*) Low magnification electron micrograph of a PtK_2 nucleus in a cell treated for 30 min with Me_2SO. The arrows point to three of the many nuclear inclusions. (Scale = 1 μm.) (*B*) Nuclear bundles sectioned transversely (T) and longitudinally (L). Note the wavy nature of component filaments in the longitudinal bundle. (Scale = 0.1 μm.) (*C*) Nuclear bundles stained with heavy meromyosin. Filaments are straight and decorated with arrowheads. (Scale = 0.1 μm.)

rounded up under the influence of Me_2SO (not shown). Accompanying this shape change in conjunction with the breakdown of stress fibers was the appearance in the nucleus of bundles of filaments that stained positively with actin antibody (Fig. 1*C*) and with fluorescent heavy meromyosin (not shown). Most of these nuclear bundles appeared as tapered rods, but many were curved in outline (Fig. 1*B*). Fluorescently labeled phalloidin, which binds only to actin in the F-form (17), also stained the nuclear bundles (Fig. 1*D*); but antibodies against myosin, tropomyosin, and α-actinin, which stained the stress fibers of control PtK_2 and WI-38 cells, did not react with the nuclear bundles (not shown) but gave a weak, diffuse staining of the cytoplasm. In addition, actin, α-actinin, and tropomyosin remained associated at high concentrations with the membranes, especially in the areas of the former junctions (for actin staining, see Fig. 1 *B* and *D*). The nuclei in untreated interphase cells never had bundles of actin, but there was often a low level of stain associated with the nuclear area (Fig. 1*A*).

Although stress fibers were almost completely disrupted by Me_2SO, the microtubule and prekeratin (Fig. 1*E*) filament networks were maintained. Neither tubulin nor prekeratin antibodies stained the nuclear bundles (Fig. 1*E*). Ten to 30 min after Me_2SO removal, the nuclear bundles disappeared and actin cables reappeared, first in large fibers associated with the reforming junctional areas and in short fibers in the central part of the cell (Fig. 1*F*). Forty-five min after removal of the drug, the cells displayed an extensive network of well-organized stress fibers that stained with antibodies against contractile protein in the same way as occurred in control cells, indicating that these fibers contained again the full complement of cytoskeletal proteins. At that time the cells were morphologically indistinguishable from cells prior to Me_2SO treatment (Fig. 1*A*).

Ultrastructural observations clearly demonstrated the intranuclear localization of the bundles (Fig. 2*A*) and indicated the wavy nature of the component filaments (Fig. 2*B*). When exposed to heavy meromyosin, the filaments were always straight in appearance with arrowhead decoration characteristic of actin filaments (Fig. 2*C*). Opposite polarity of arrowheads of adjacent filaments was observed within a single bundle.

To determine whether Me_2SO caused the same actin originally present in the stress fibers to move into the nucleus and form bundles, fluorescent actin was microinjected into living cells and its fate observed in the same cells before and after Me_2SO treatment (Fig. 3 *A–E*). Individual cells were microinjected, and the same cell was observed in normal medium [in which the stress fibers became fluorescent (Fig. 3 *A* and *C*)] and then in medium containing 10% Me_2SO [in which the nuclear bundles appeared and stress fibers were greatly diminished in the cytoplasm (Fig. 3 *B* and *D*)]. Finally, 90 min after the same cell had been returned to normal medium, the fluorescent actin bundles had disappeared from the nucleus (Fig. 3*E*) and stress fibers reformed. These cells, which were exposed

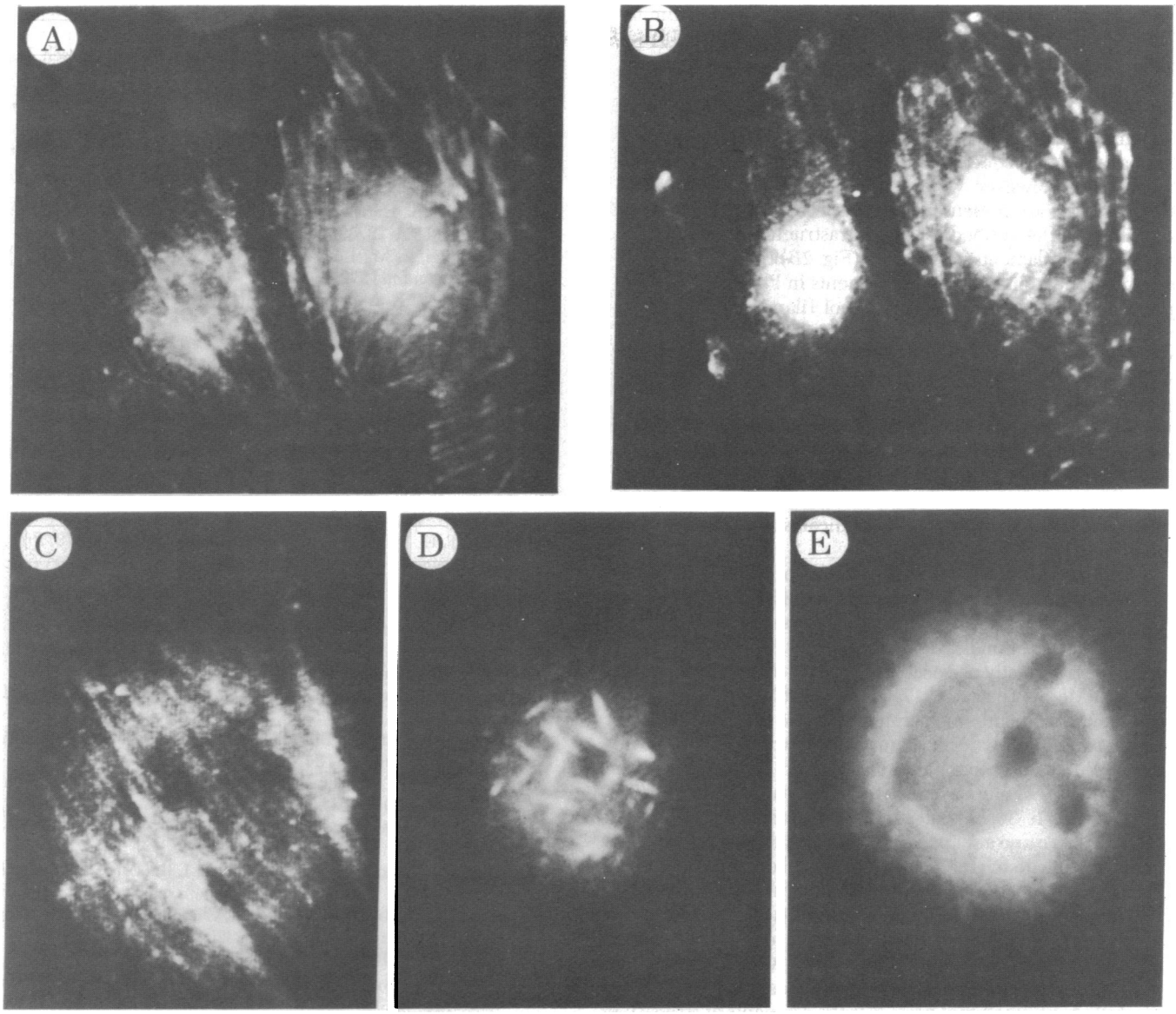

FIG. 3. (*A*) Living PtK_2 cells 1 hr after injection with fluorescently labeled actin. (×600.) (*B*) The same cells 30 min after exposure to 10% Me_2SO. Actin is concentrated primarily in the nucleus. (×600.) (*C*) Higher magnification of the nuclear area in the control cell on the left in Fig. 3*A*. (×1500.) (*D*) Higher magnification of the nucleus of the cell on the left in Fig. 3*B*, to emphasize the nuclear inclusions. (×1500.) (*E*) The same nucleus as in Fig. 3*D*, 90 min after Me_2SO has been removed. Nuclear inclusions are absent. The irregular shape of the nucleus is not an effect of Me_2SO treatment but frequently is observed in large PtK_2 cells. See, for example, the cell on the right in Fig. 3*A*. (×1500.)

to UV light periodically after microinjection, took considerably longer (90–120 min) to fully recover from the treatment with Me_2SO than the cells used for immunofluorescence.

DISCUSSION

Whether actin and other contractile proteins play any role in the structure and function of the nucleus is as yet unknown. The recent experiments of Rungger *et al.* (24), who microinjected actin antibody into *Xenopus* oocyte nuclei and prevented chromosome condensation, suggest an important role for nuclear actin in the precise distribution of genetic material during proliferation. Despite biochemical work that indicates that actin is a genuine constituent of the nuclei of several cell types (1–7), it has not been found in purified nuclear preparations of other cells, for example, mouse hepatocytes (8). Experiments with microinjected proteins have shown that actin readily diffuses from the cytoplasm into the nucleus in amoebae (3, 25) and *Xenopus* oocytes (1). These findings are in agreement with earlier observations on *Physarum polycephalum*, that actin is transported into the nucleus during the late G-2 phase (1).

Fukui and Katsumaru (10–12) have found that Me_2SO causes aggregates of actin filaments to form in the nuclei of *Dictyostelium*, amoebae, and HeLa cells *in situ*. They have assumed that the actin comprising the aggregates derives from a nuclear actin that is different in its properties from cytoplasmic actin (10–12). Preliminary work with Me_2SO-treated PtK_2 cells using fluorescent heavy meromyosin as an indicator of actin localization showed that stress fiber dissolution accompanied nuclear bundle formation (13). This suggested to us that the actin in the nuclear bundles arose from the breakdown of cytoplasmic stress fibers. The microinjection experiments reported in this paper confirm this view. We cannot eliminate the possibility that part of the actin in the nuclear bundles existed in the nucleus prior to Me_2SO treatment and joined with the cytoplasmic actin to form nuclear aggregates. Since the nuclear membrane remained intact during Me_2SO-treatment, as judged on an ul-

trastructural level, the actin must have been altered from its fibrous form in the stress fiber to a soluble form able to diffuse rapidly across the nuclear membrane and then to repolymerize into filaments.

Fluorescent antibody staining shows that other contractile proteins that are associated with actin in stress fibers—myosin, tropomyosin, and α-actinin—do not concomitantly move into the nucleus. However, we cannot rule out the possibility that these proteins are present in the nuclear bundles in a form inaccessible to the antibodies. The ultrastructural image of the nuclear actin filaments in PtK_2 cells (Fig. 2*B*) confirms that they are different from stress fiber filaments in PtK_2 cells (26; unpublished observations). Both types of filaments look similar when decorated with heavy meromyosin (Fig. 2*C*; 27), but when undecorated, the nuclear filaments are in wavy segments, whereas undecorated microfilaments of stress fibers are long and straight. It may be that the lack of tropomyosin on the nuclear filaments causes them to be partially degraded during osmium fixation (28), but even in the fluorescence microscope, rods can be seen that have an overall wavy outline (Fig. 1*B*). However, the binding of heavy meromyosin and phalloidin to these filaments (Figs. 2*C* and *D*) indicates that they are composed of F-actin.

Me_2SO is known to have many effects on cells (29), including the enhancement of membrane permeability (30). However, this can certainly not be the primary cause for the formation of actin filament bundles inside the nucleus, because the nuclear envelope is perfectly permeable to proteins of the size of G-actin without Me_2SO treatment (7, 31). Even if Me_2SO were to increase nuclear membrane permeability, it is not clear why actin would subsequently form filamentous bundles only in the nucleus. With respect to the rapid breakdown of stress fibers, the effect of Me_2SO can be compared with that of proteases (32), cytochalasin-B (33, 34) and agents that transform cells (35). All these agents cause a shape change—a rounding-up of cells—in conjunction with the breakdown of stress fibers, but only Me_2SO treatment leads to the formation of nuclear actin filaments.

The rapid reversal of the reported effects shows that the cells are not damaged. Within 10–30 min after removal of the drug, PtK_2 cells flattened out and the junctions were formed at precisely the previous location. The reappearance of stress fibers started at the junctional sites, as if the remnants of stress-fiber elements that resisted Me_2SO treatment could serve as nucleation centers. With respect to the rapid and reversible breakdown of stress fibers, treatment with Me_2SO mimics the normal events observed during the cell cycle (14, 36, 37). However, this cannot be said for the nucleus, because in higher eukaryotes the nuclear membrane breaks down at the same time (during prophase) as do the stress fibers, and a distinction between nuclear and cytoplasmic actin is no longer possible.

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