

Construction of the Endoplasmic Reticulum

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Abstract. To study the construction of the ER, we used the microtubule-disrupting drug nocodazole to induce the complete breakdown of ER structure in living cells followed by recovery in drug-free medium, which regenerates the ER network within 15 min. Using the fluorescent dye 3,3'-dihexyloxycarbocyanine iodide to visualize the ER, we have directly observed the network construction process in living cells. In these experiments, the ER network was constructed through an iterative process of extension, branching, and intersection of new ER tubules driven by the ER motility previously described as tubule branching. We have tested the cytoskeletal requirements of this process. We find that newly formed ER tubules are aligned with single microtubules but not actin fibers or vimentin intermediate filaments. Microtubule polymerization preceded the extension of ER tubules and, in experiments with a variety of different drugs, appeared to be a necessary condition for the ER network formation.

Furthermore, perturbations of the pattern of microtubule polymerization with microtubule-specific drugs caused exactly correlated perturbations of the pattern of ER construction. Induction of abnormally short, nonintersecting microtubules with 20 μ M taxol prevented the ER network formation; ER tubules only extended along the few microtubules contacting the aggregated ER membranes. This requirement for a continuous network of intersecting microtubules indicates that ER network formation takes place through the branching and movement of ER membranes along microtubules. Cytochalasin B had no apparent effect on the construction of the ER network during recovery, despite apparently complete disruption of actin fibers as stained by phalloidin. Blockage of protein synthesis and disorganization of intermediate filaments with cycloheximide pretreatment also failed to perturb ER construction.

THE ER was first discovered and defined, in terms of its striking morphology, as an extensive network of interconnected membrane tubules spread throughout the endoplasm (Porter et al., 1945; for review see Palade, 1956). Although subsequent work has shown that elements of ER can also form sheet-like cisternae (Palade, 1956), this reticular structure is so pervasive as the principal morphology of the ER membranes in many cell types that it still serves well as the primary feature by which the ER is defined as an organelle. However, despite extraordinary advances in understanding of the ER's biochemical activities (for review see Fawcett, 1981), the basis for forming its reticular structure remains poorly understood, largely because of the difficulties of visualizing ER development in live cells. The recent discoveries of vital stains for the ER (Terasaki et al., 1984; for review see Terasaki, 1989) have thus opened an especially interesting opportunity to study the construction of the ER and to understand the cell biological processes of its formation.

Recently, three separate groups have hypothesized a similar mechanism for construction of the ER: i.e., branching and intersection of ER tubules to form a network (Dabora

and Sheetz, 1988; Lee and Chen, 1988; Vale and Hotani, 1988). Although these three studies pursued quite different approaches, they arrived at similar hypotheses, proposing that such motility might be driven by microtubules and could produce the ER's characteristic reticular structure. Several unresolved issues remained as critical tests of this hypothesis. First, previous studies of ER motility *in vivo* showed no evidence of its involvement in ER construction nor of its cytoskeletal basis (Lee and Chen, 1988). The *in vitro* studies and morphological and biochemical indications of ER-microtubule interactions (Terasaki et al., 1986; for a summary of reports of ER-microtubule interactions see Lee and Chen, 1988) suggested involvement of microtubule motility; however, a recent study of ER motility in characean alga reported movement along actin fibers (Kachar and Reese, 1988). We have therefore sought to test directly in mammalian cells the respective roles of actin filaments and microtubules in ER motility. A further question about the possible mechanism of ER construction has been raised by striking differences in *in vitro* models of this process: in one, stationary microtubules served as tracks for the movement and branching of membranes (Dabora and Sheetz, 1988); in another, membranes moved and branched by adhesion to sliding microtubules (Vale and Hotani, 1988). This basic difference implies quite different models of ER construction for the two studies,

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raising the question of which is more relevant to ER construction as it occurs *in vivo*.

To address these questions, we have examined the time course and cytoskeletal requirements of ER construction *in vivo* during recovery from treatment with the microtubule disruptant, nocodazole. Disruption of microtubules causes gradual collapse of the ER, forming an aggregate of membranes around the nucleus; upon transfer to drug-free medium, however, epithelial cells reconstruct full ER networks within 15 min (Terasaki et al., 1986), providing a convenient model for observing the mechanism of network formation. Using this system, we demonstrate that construction of the ER takes place via ER motility, through the branching and intersection of ER tubules on a previously formed network of microtubules, and does not appear to involve filamentous actin or vimentin intermediate filaments.

Materials and Methods

Cell Culture

CV-1 cells (African green monkey kidney epithelial cell line) were obtained from the American Type Tissue Culture Collection (Rockville, MD) and grown at low density on 12-mm square glass coverslips (Bradford Scientific, Epping, NH) or etched coverslips (Bellco Glass, Inc., Vineland, NJ) in DME (Gibco Laboratories, Grand Island, NY) supplemented with 10% calf serum (M. A. Bioproducts, Walkersville, MD) at 37°C with 5% CO₂ and 100% humidity.

Live-Cell Visualization of Nocodazole Recovery

CV-1 cells treated for 6 h with 1 µg/ml nocodazole (Janssen Life Sciences Products, Piscataway, NJ) were stained for 5 min in culture medium containing 2.5 µg/ml 3,3'-dihexyloxycarbocyanine iodide and 1 µg/ml nocodazole, rinsed 1 min in drug-free medium, and mounted for microscopy in drug-free medium using a silicon rubber chamber (Chen, 1989; Terasaki, 1989). Video recording was performed on a microscope (Photomicroscope III; Carl Zeiss, Inc., Thornwood, NY) with a Neofluar lens (100×), 100-W xenon lamp, video camera (C2400-08; Hamatsu Photonics, Hamatsu City, Japan), video recorder (Super-VHS, JVC, Osaka City, Japan), and filter system as described previously (Terasaki et al., 1986; Lee and Chen, 1988). All operations were performed at 37°C.

Nocodazole Treatment and Recovery

For nocodazole treatment, cells were incubated overnight in culture medium supplemented with 10% calf serum and 1 µg/ml nocodazole (Janssen Life Sciences Products). Cells were allowed to recover by rinsing and incubating them in culture medium with 10% calf serum for 30 min or as indicated in the text. All incubations were performed at 37°C in a cell culture incubator.

To test the effects of drugs on cells recovering from nocodazole, cells that were in nocodazole overnight were treated with the drugs described below for specified times in the continuous presence of nocodazole followed by "recovery" without nocodazole but in the presence of the drugs for additional specified times. *p*-Trifluoromethoxyphenylhydrazine (5 µM) was for 30 min with and 15 min without nocodazole; nigericin (5 µg/ml) was for 30 min with and 15 min without; cytochalasin B (0.5 µg/ml) was for 2 h with and 30 min without; cycloheximide (20 µg/ml) was for 6 h with and 15 min without; vanadate (10 µM) was for 1 h with and 1 h without; taxol (20 µM) was for 1 h with and 1 h without; acrylamide (5 mM) was for 4 h with and 30 min without; vinblastine (5 µg/ml) was for 1 h with and 1 h without.

To test perturbation of recovery by low concentrations of nocodazole, cells treated overnight with nocodazole were transferred to culture medium containing 10% calf serum and nocodazole (200, 140, 80, 50, 30, or 10 ng/ml) for 2 h, fixed, and stained. For taxol perturbations of recovery, cells treated overnight with nocodazole were incubated 1 h in medium with 1 µg/ml nocodazole and taxol (20, 10, 5, 2, 1, or 0.2 µM) and transferred for 1 h to medium with taxol alone (at the same concentration). All incubations were performed at 37°C in a cell culture incubator.

Fixation and ER Staining

ER staining of fixed cells was performed using a variation of the procedure of Terasaki et al. (1984) altered to reduce autofluorescence background. Cells grown on etched coverslips were fixed by immersion in 0.025% glutaraldehyde in sucrose-cacodylate buffer (0.1 M sucrose, 0.1 M sodium cacodylate, pH 7.4) for exactly 30 s followed by 3 min in 3.7% formaldehyde in the same buffer. After a 2-min rinse in sucrose-cacodylate buffer, cells were stained 30 s in 2.5 µg/ml 3,3'-dihexyloxycarbocyanine iodide in sucrose-cacodylate, rinsed briefly, and mounted for microscopy with a silicon rubber chamber containing sucrose-cacodylate buffer supplemented with 1 mg/ml phenylenediamine. Cells were photographed on a microscope (Axiophot; Carl Zeiss, Inc.) using a Neofluar (100×) lens and T-MAX 400 film (Eastman Kodak Co., Rochester, NY) exposed and developed at exposure index 1,600. The location of each photographed cell on the etched coverslips was recorded. Cells were then processed immediately for visualization of the cytoskeleton.

Fluorescent Staining of Microfilament Bundles

Cells were permeabilized 5 min in Penman's cytoskeletal extraction buffer (1% Triton X-100, 300 mM sucrose, 10 mM Pipes, 2.3 mM MgCl₂, 100 mM KCl) at 4°C, washed for 5 min in PBS twice, and incubated 30 min at 37°C and 100% humidity with 40 µl nitrobenzoxadiazole-phalloidin (Molecular Probes Inc., Eugene, OR) prepared at the recommended working dilution in PBS. After two 5-min washes in PBS, the coverslips were mounted on a silicon rubber chamber in PBS containing 1 mg/ml phenylenediamine and photographed on a microscope (Axiophot; Carl Zeiss, Inc.) as described above.

Immunofluorescent Stainings of Microtubules or Intermediate Filaments

For staining of microtubules, coverslips were incubated 5 min in dithio-bis-succinimidyl propionate (Pierce Chemical Co., Rockford, IL) in PBS, rinsed twice in PBS, incubated for 5 min in 1% Triton X-100 in microtubule-stabilizing buffer (1 mM EGTA, 4% polyethylene glycol 8,000, 100 mM Pipes, pH 6.9), rinsed twice in microtubule-stabilizing buffer, and fixed 5 min in 100% methanol at -20°C. For staining of vimentin intermediate filaments, cells were fixed 5 min in 100% methanol at -20°C, rinsed briefly in distilled water, washed twice in PBS, and incubated 1 h at 37°C with either mouse monoclonal anti- α -tubulin or rabbit anti-vimentin serum (Chen et al., 1985). After removal of the primary antibody and two washes in PBS, cells were incubated for 1 h at 37°C with either rhodamine-conjugated goat anti-mouse IgG, or rhodamine-conjugated goat anti-rabbit IgG (Cooper Biomedical, Inc., Malvern, PA). The coverslips were then washed in PBS, rinsed briefly in distilled water, and mounted in glycerol gelatin (Sigma Chemical Co., St. Louis, MO) containing 0.2% *n*-propylgallate (Sigma Chemical Co.). Microscopy was performed on a microscope (Axiophot; Carl Zeiss, Inc.) as described above.

Results

We used a high-sensitivity video camera to observe directly the dynamics of ER network construction in live cells recovering from the effects of nocodazole. In these recordings, the reticular structure formed over a period of 20 min through a process of successive tubule extension, branching, and interconnection (Fig. 1). After 2 min of recovery, new ER tubules began to extend outwards from the nuclear region; over the next 4 min, they advanced towards the cell periphery forming long (10–20 µm), unbranched, radially extending tubules. Behind this front of tubular extension, small, tangentially oriented tubules began at 4–6 min to branch and interconnect, producing a narrow reticulating zone. After 7 min of recovery, the primary tubules of the outer, extending zone began to branch; after this time, the growing ER network typically appeared divided into an extending zone, characterized by long, unbranched, radially extending tubules, and a reticulating zone, where the ER tu-

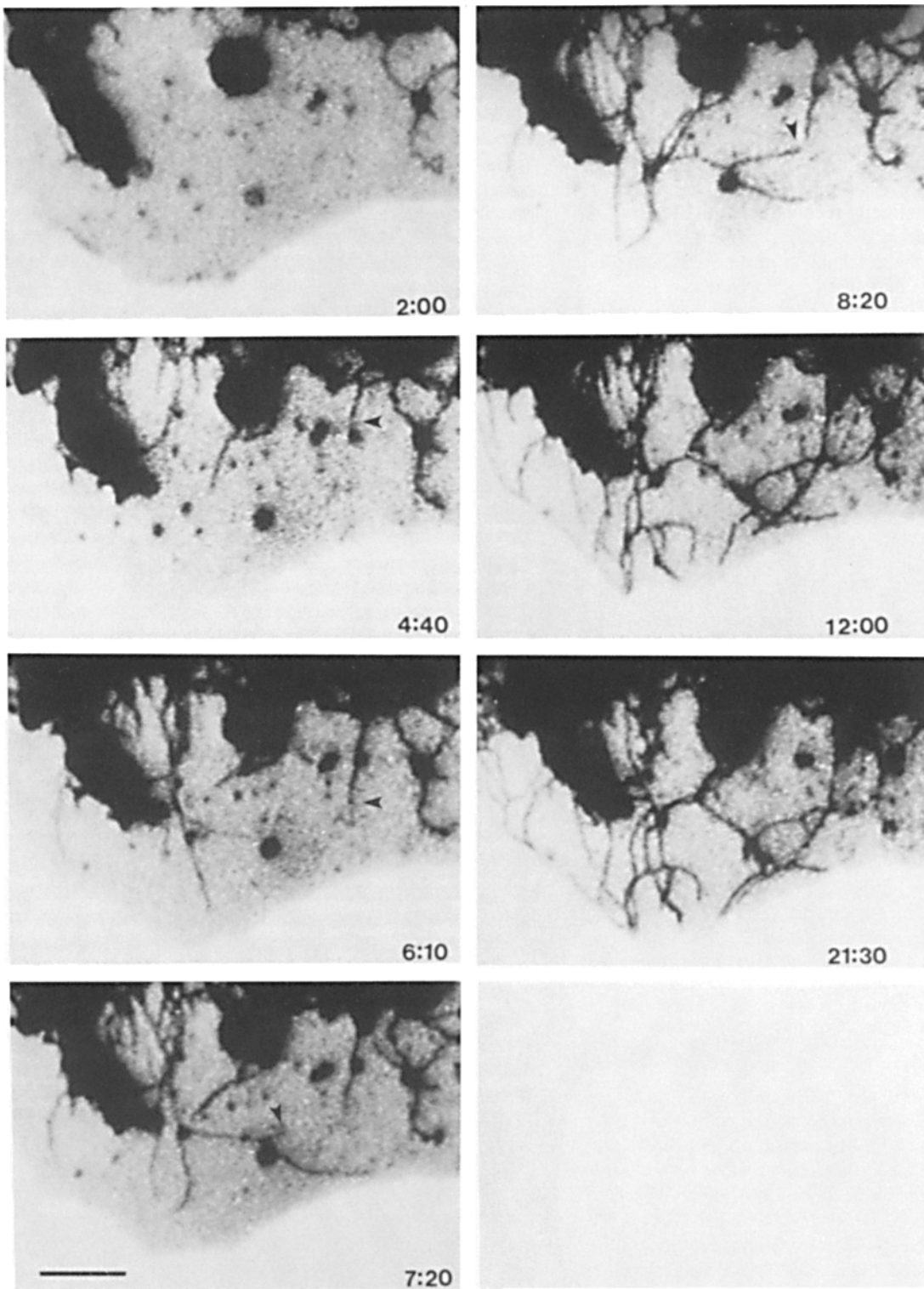


Figure 1. Construction of the ER during nocodazole recovery. A living CV-1 cell stained with 3,3'-dihexyloxycarbocyanine iodide examined by fluorescence microscopy during nocodazole recovery; the time (minutes:seconds) after transfer to drug-free medium is indicated on each photograph. The ER network was formed by first extending long, unbranched tubules away from the perinuclear region; these tubules then formed branches that intersected to produce a reticulum. Several tubules were caught in mid-course of extension in consecutive time points (*arrowheads*). Bar, 5 μ m.

bules branched tangentially and interconnected. Further branchings and intersections of branchings over the next 14 min generated an interconnected ER network. No tubules developed dissociated from other ER

tubules were formed by branching off the existing ER through a process of linear extension (Fig. 2) that corresponded in all observed characteristics to the tubule branching motility described previously from observations of in-

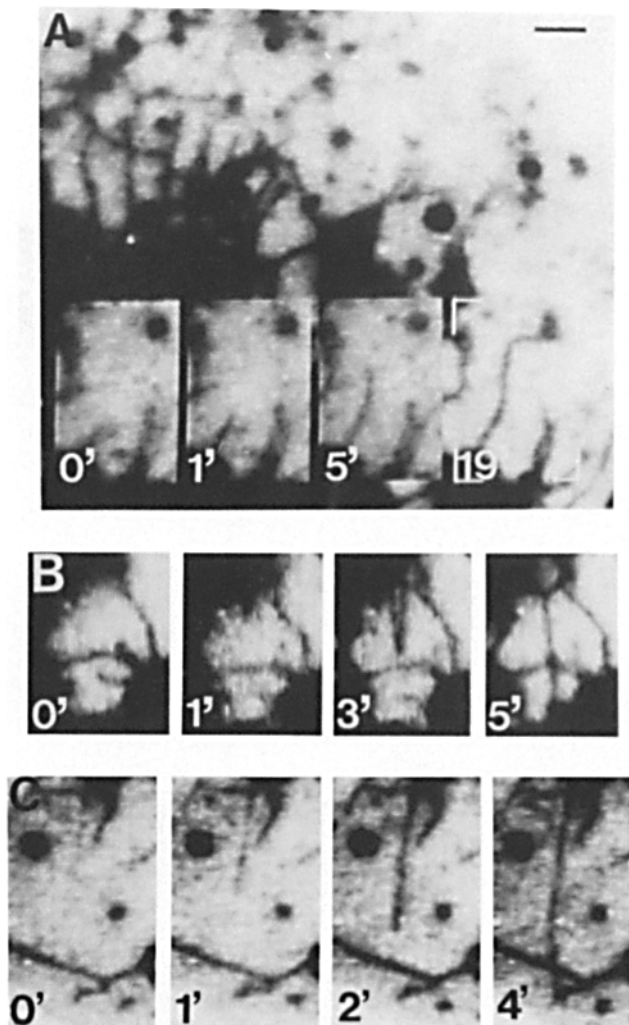


Figure 2. Formation of new ER tubules. (A) New tubules in the extending zone of a CV-1 cell. The last of the four frames is given as a full field to show the surrounding structure. (B and C) Tubule extension in the reticulating zone. Because of frequent pauses and reversals of direction, the overall speed appears slower than the instantaneous speed of the motion. Times on the photographs are given in seconds. Bar, 2 μm .

terphase ER networks (Lee and Chen, 1988). The tubule branching motility observed in recovering cells was similar to that of normal ER in its linearity of motion, duration, speed, frequent reversals of direction following the same line, and generation of triple junctions and polygonal reticulum. No other mechanism of forming new ER tubules was observed in any of the experiments; furthermore, ring closure and sliding (Lee and Chen, 1988) were the only other ER movements observed and did not contribute towards the extension of the ER network (data not shown). Thus, the tubule branching motility appeared to be the basis of ER network construction. To test whether these results might be artifacts of dye staining and observation during recovery, we examined cells fixed with glutaraldehyde at different times during recovery from nocodazole (data not shown). Cells at these time points, unstained until after fixation, showed an identical sequence and rate of linear extension, branching, and intersection for ER network formation as cells dyed before fixation.

Microtubules and ER Construction

To test the possible role of microtubules in this process, we have examined the order of precedence of microtubule vs. ER reconstruction by fixing cells at 1-min intervals during nocodazole recovery and double staining for ER and microtubules (Fig. 3). These experiments showed that microtubules polymerized first and that ER tubules began to form and extend once microtubules were already present. 2 min after transfer to drug-free medium, many small microtubule fragments were visible throughout the cytoplasm and, by 4 min, had already formed a network of long (20–30 μm) microtubules extending to the cell periphery. No tubular ER was observed until 3–4 min and, at 4 min, was still confined to the region around the nucleus, although in some cases recovery was slower. In cells treated with 1 $\mu\text{g}/\text{ml}$ nocodazole for 3–4 d, transfer to drug-free medium for 1 h resulted in normal recovery of microtubule structure but no reconstruction of the ER (data not shown). These results indicate that microtubules do not have a structural dependence on ER formation and do polymerize independently of ER reconstruction. By contrast, the ER construction appeared to depend on microtubule polymerization; ER tubules were never observed to form in the absence of microtubules in any of our experiments. As previously reported (Terasaki et al., 1986), newly formed ER tubules were found to be aligned with single microtubules at all stages of recovery (Fig. 3). To test further the dependence of ER on microtubules, a variety of drugs were tested for their effect on microtubule and ER formation during nocodazole recovery (Table I). In all cases, drugs that perturbed or prevented microtubule polymerization affected ER recovery identically; under no circumstances did ER reform without microtubules.

Perturbation of Microtubule Recovery

To characterize the ER's structural dependence on microtubules, we have examined the effects of perturbations in microtubule recovery on construction of the ER. By transferring nocodazole-treated cells not into drug-free medium but into medium containing low concentrations of nocodazole (ranging from 0.2 to 0.01 $\mu\text{g}/\text{ml}$) for 2 h, we have generated a range of different densities of microtubule recovery, ranging from none to near normal (Fig. 4). In these experiments, the density of ER recovery was found to be precisely correlated with the density of microtubule recovery, and ER tubules

Table I. Effects of Drugs on the Recovery of Microtubules and ER

Drug treatment	Microtubule recovery	ER recovery
<i>p</i> -Trifluoromethoxyphenylhydrazone	+++	+++
Nigericin	+++	+++
Cytochalasin B	+++	+++
Cycloheximide	+++	+++
Vanadate	+++	+++
Taxol	+++*	+
Acrylamide	+	+
Vinblastine	–	–
Nocodazole	–	–

* Abnormally short microtubules.

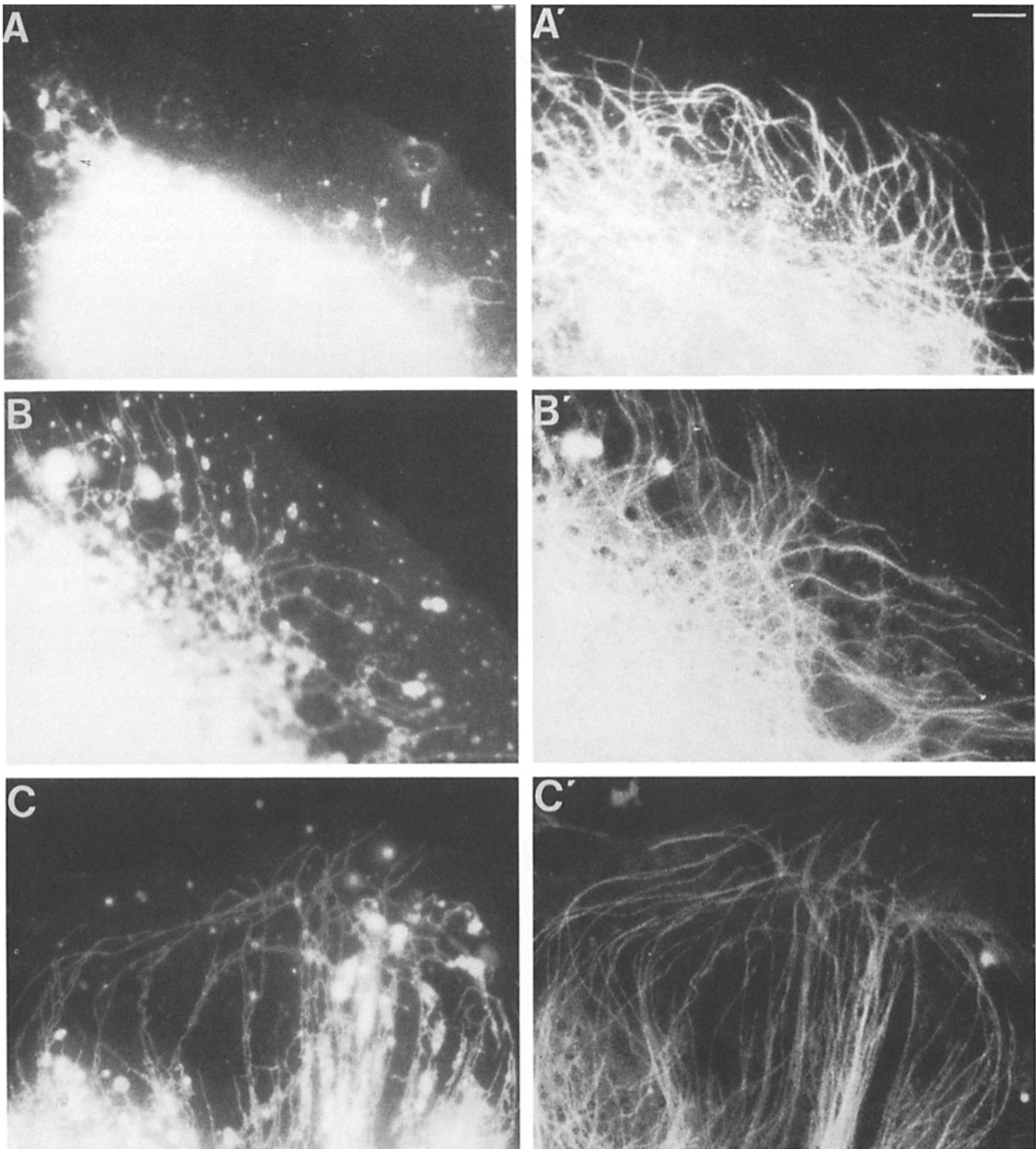


Figure 3. Order of ER and microtubule formation during nocodazole recovery. ER (A–C) and microtubules (A'–C') in CV-1 cells at successive stages of recovery. The microtubules polymerized first, before extension of the ER (A and A'). From 4–8 min, the ER extended outwards from the perinuclear region and was aligned with microtubules (B and B'). After 10–15 min of recovery, the ER formed an interconnecting network extending to the cell periphery and was still aligned with microtubules (C and C'). Bar, 5 μ m.

were found to be exactly aligned with single microtubules. Thus, ER tubule extension appeared to have a direct, localized requirement for the presence of a microtubule. Treatment with 5 mM acrylamide for 4 h before recovery had a similar effect of perturbing the density and pattern of microtubule and ER recovery; again, the ER tubules were found to be aligned with single microtubules (data not shown). We

also tested the effects of transfer to 20 μ M taxol on ER recovery (Fig. 5). Although this treatment increased the density of microtubule polymerization, inducing formation of numerous, abnormally short microtubules, it significantly reduced the extent of ER recovery. Specifically, the ER failed to form in areas where the microtubules were present as isolated, nonintersecting filaments and extended only into regions

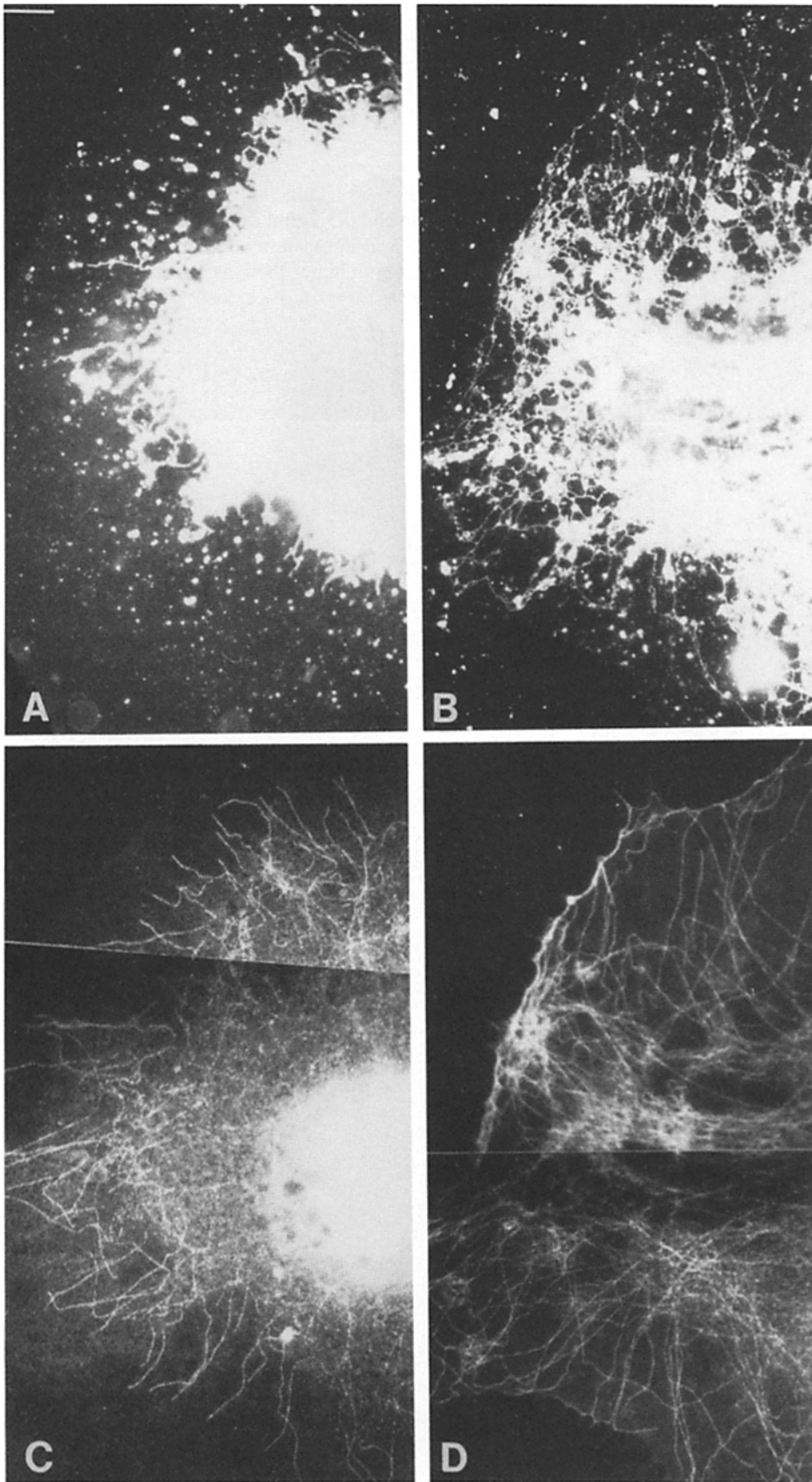


Figure 4. Effect of low concentrations of nocodazole on ER construction. CV-1 cells treated with 1 $\mu\text{g/ml}$ nocodazole, allowed to recover 2 h in low concentrations of the drug, and stained for ER (*A* and *B*) and microtubules (*C* and *D*). (*A* and *C*) 200 ng/ml nocodazole. A low density of microtubules formed, extending only partly towards the cell periphery; similarly, only a few tubules of ER formed, which only partly extended towards the cell periphery. (*B* and *D*) 50 ng/ml nocodazole. Microtubules polymerized throughout the cytoplasm, and the ER formed as regions of single tubules, in areas where there were few microtubules, and as a loosely reticulated network, in areas where intersecting microtubules were present as a network. Bar, 10 μm .

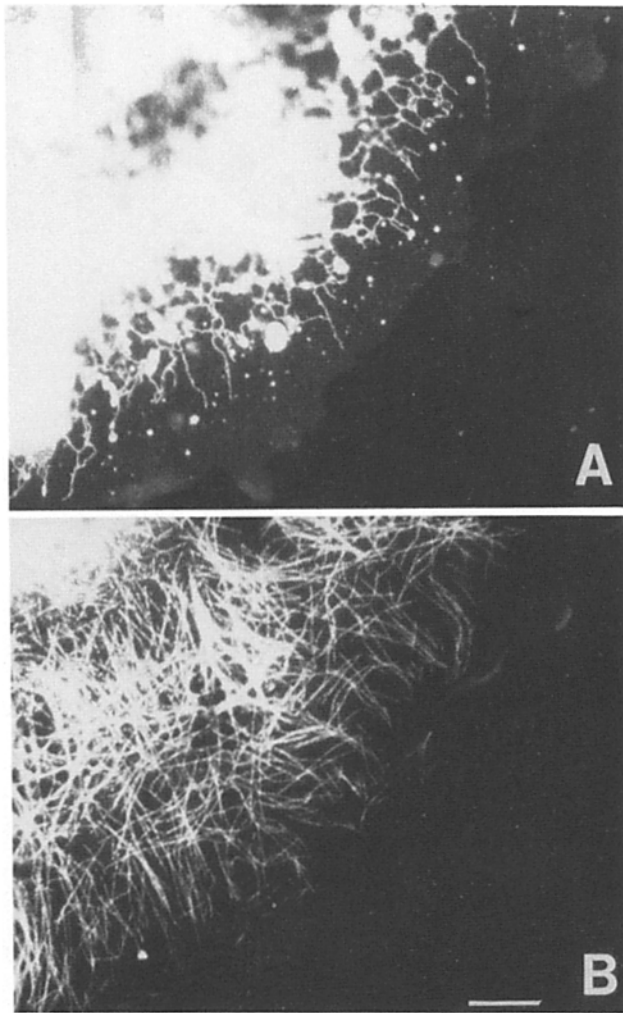


Figure 5. Effect of taxol on ER construction. Nocodazole-treated CV-1 were allowed to recover 1 h in medium containing 20 μ M taxol and stained for ER (A) and microtubules (B). Bar, 5 μ m.

where the microtubules formed a continuous network. Thus, the simple presence of polymerized microtubules was insufficient to cause ER network formation; microtubules appeared to support ER tubule extension only when the individual microtubules intersected and contacted the ER, providing a framework on which the ER could extend.

We have followed a similar approach in examining the possible involvement of intermediate filaments and actin in the construction of the ER. First, we double stained cells fixed at different times during recovery to look for structural correlations between these structures and extension of the ER (Fig. 6). These experiments showed no clear evidence of correlations between ER construction and either vimentin intermediate filaments or actin fibers. During recovery from nocodazole, vimentin filaments, like ER, gradually extended outwards from the nuclear region along the line of newly formed microtubules. However, vimentin intermediate filaments appeared to advance more slowly than the ER, not extending to the outer regions of the cell until after 15–20 min of recovery. ER tubules were commonly observed extending beyond the region of vimentin filament staining in recovering

cells. Actin structures appeared unaffected by nocodazole treatment and remained unchanged throughout these experiments. No apparent correlation between actin fibers and growing ER tubules was observed.

To test the role of actin filaments in the construction of the ER, we have examined the effect of cytochalasin B on cells recovering from nocodazole. Cells treated overnight with 1 μ g/ml nocodazole were incubated for 2 h in medium with 1 μ g/ml nocodazole and 0.5 μ g/ml cytochalasin B and allowed to recover for 30 min in medium containing cytochalasin B only. Fixation and staining of these cells for ER, actin, and microtubules showed that the ER network formed normally despite the absence of actin fibers as visualized by phalloidin staining (Fig. 7). ER network construction was completely normal in speed and morphology in such cells, indicating that actin is not involved in the process of ER construction.

To assess the involvement of intermediate filaments in the construction of the ER, we have examined the effects of drugs disrupting intermediate filaments on cells recovering from nocodazole. Cycloheximide has been shown to cause disorganization of intermediate filaments (Sharpe et al., 1980), although as an inhibitor of protein synthesis it has many other cell biological effects. Treatment with 20 μ g/ml cycloheximide for 6 h before recovery from nocodazole did not significantly effect ER network construction, despite extensive inhibition of vimentin bundle development as visualized by immunofluorescence staining (Fig. 8). In some cells, this treatment resulted in a lower density of ER formation, probably because of other effects of the 6-h protein synthesis block. However, the ER reformed normally in many cells with equally low intermediate filament formation. In such intermediate filament-disrupted cells, ER construction appeared normal in speed and morphology, and it thus seems unlikely that intermediate filaments are involved in the ER network formation process.

Discussion

These results demonstrate how a complex organelle structure can be formed through cytoskeletal motility, indicating a new role for interphase microtubules in the construction of ER, as previously hypothesized (Dabora and Sheetz, 1988; Lee and Chen, 1988; Vale and Hotani, 1988). Our results are consistent with *in vitro* studies of microtubule motility-driven membrane network formation (Dabora and Sheetz, 1988; Vale and Hotani, 1988) and may help indicate which of these two *in vitro* systems more accurately reflects ER construction in living cells. They differ in their observations of how the motility was generated: binding of membranes to sliding microtubules (Vale and Hotani, 1988) vs. movement of membranes along stationary microtubules (Dabora and Sheetz, 1988). Our results showing microtubule extension before ER construction during nocodazole recovery appear to favor the latter model. Specifically, the sliding filament model implies simultaneous advance of the microtubules and ER since the membranes move by binding to moving microtubules. In contrast, our results suggest formation of the microtubule network before construction of the ER and movement of the ER along the track of already formed microtubules. The observation that nonintersecting microtubules cannot support ER extension favors this view. If the ER were formed by binding

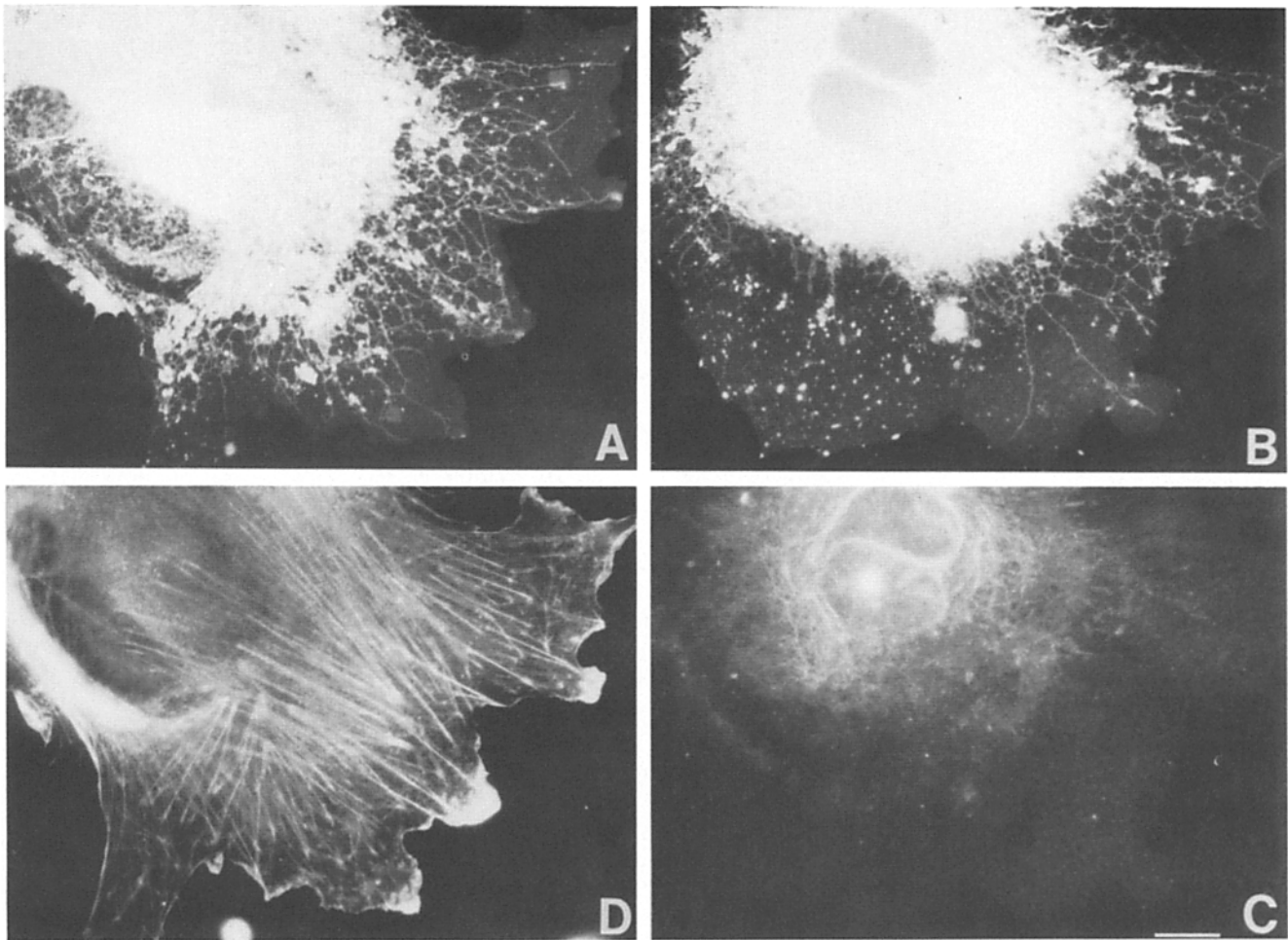


Figure 6. Comparison of actin and intermediate filament structure with ER construction. Nocodazole-treated CV-1 were incubated 6 min in drug-free medium, fixed, and double stained for ER (*A* and *B*) and either vimentin (*C*) or actin (*D*). Bar, 10 μ m.

to sliding microtubules, it could extend into regions of nonintersecting microtubules, as was in fact observed in the experiments of Vale and Hotani (1988).

The concordance of these *in vitro* and *in vivo* studies suggests that such biochemical systems could be used to characterize the molecules involved in ER-microtubule interactions and motility. It is clear that factors other than microtubules are important for ER construction. When cells treated with nocodazole for 3–4 d were transferred to drug-free medium, the microtubules reformed normally, but the ER remained aggregated in the perinuclear region and did not form a tubular network. It thus appears that other factors are required for ER extension that are gradually lost during long-term nocodazole treatment. It may well be that depolymerization of microtubules blocks new synthesis of proteins involved in ER-microtubule interactions, either through changes in gene expression, disruption of microtubule-based intracellular transport, or even the disruption of ER structure itself. It is possible that cell homogenates from cells treated 3–4 d could be used for *in vitro* studies to identify these factors by using fractionated normal cell homogenates to rescue the network formation activity.

During nocodazole recovery, the microtubules thus appear to act as a dynamic framework for construction of the ER,

on which the ER membranes extend and interconnect to form a reticulum. What relevance does this result have to the extension of the ER during cell spreading? Normally, microtubules gradually extend into advancing regions of spreading cells after the extension of actin fibers (Rinnerthaler et al., 1988) and appear to support extension of the ER into these regions; preventing such microtubule formation, by plating cells into medium containing nocodazole, blocks extension of the ER (Terasaki et al., 1986). Nocodazole recovery, by contrast, produces rapid polymerization of microtubules not only in peripheral, extending regions but throughout the entire cell and, correspondingly, produces rapid extension of the ER throughout the entire cell. From this point of view, we propose that nocodazole recovery in effect mimics the normal process and consequences of microtubule extension during cell spreading or movement but in a rapid, cell-wide manner. Nocodazole recovery of other cell lines tested (MCF-7, ptK-2, and 64F-3) showed a similar time course and mechanism of ER construction (Lee, C., and L. B. Chen, unpublished results). Furthermore, initial studies of ER extension in spreading cells (untreated with nocodazole and after trypsinization and replating) have obtained similar results, including the appearance of extending and reticulating zones during ER construction, alignment of extending ER tubules with

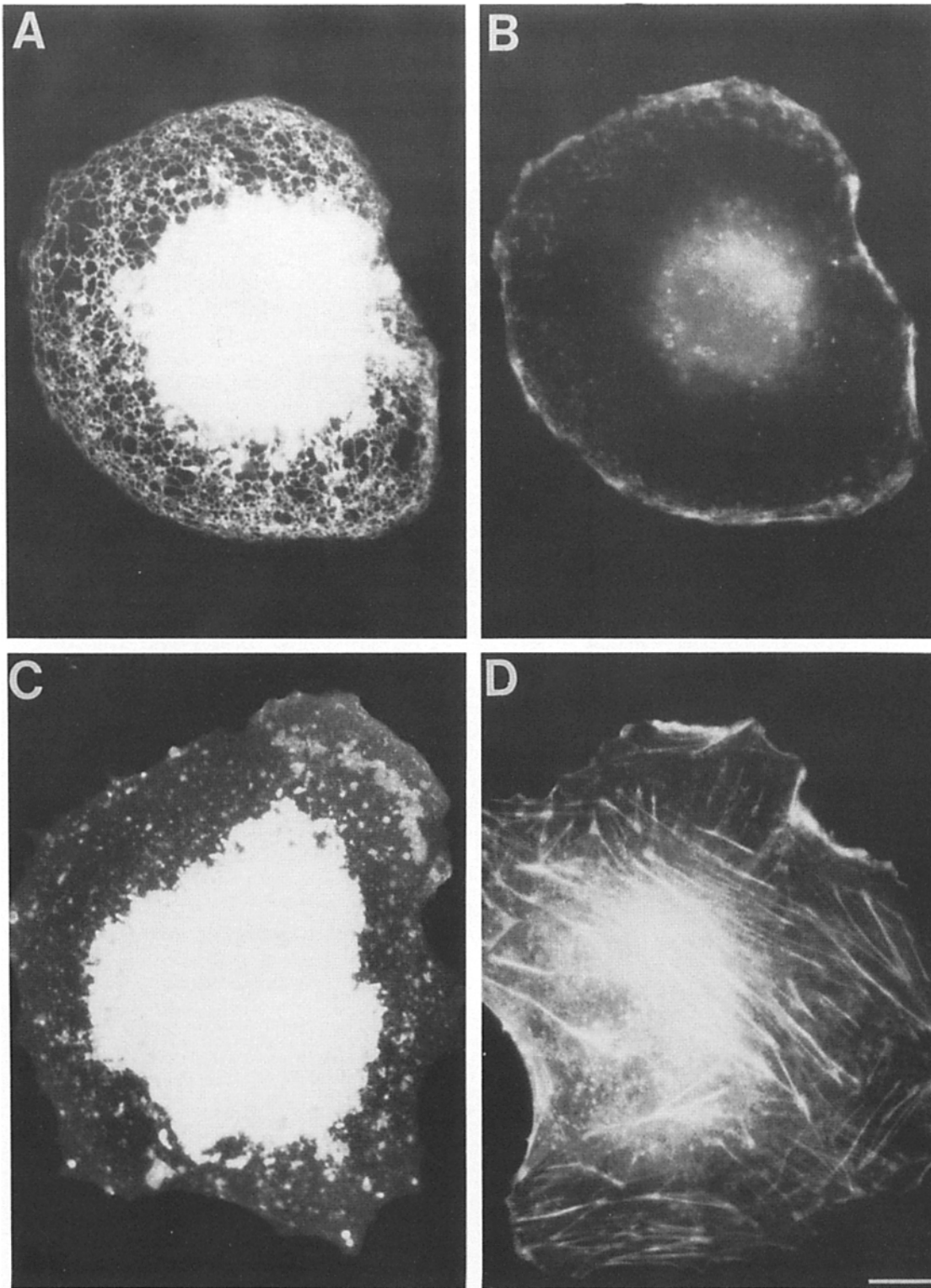


Figure 7. Effect of cytochalasin B on ER construction. (A) Nocodazole-treated CV-1 pretreated 2 h in 0.5 $\mu\text{g/ml}$ cytochalasin B, allowed to recover 15 min in cytochalasin-containing medium, and then fixed and stained for ER. (B) Phalloidin staining of A. (C) Nocodazole-treated CV-1 fixed and stained for ER. (D) Nocodazole-treated CV-1 allowed to recover 15 min in drug-free medium, then fixed, stained for ER, and restained with phalloidin. B and D were stained, photographed, and printed identically to facilitate comparison. Bar, 15 μm .

single microtubules, and many striking morphological similarities to ER construction during nocodazole recovery (Lee, C., M. Ferguson, and L. B. Chen, unpublished results).

These observations support a paradigm for establishing

specific organelle structures and localization by control of organelle-cytoskeleton interactions. Microtubules, in particular, appear to be involved in the distribution of not only ER but also mitochondria, lysosomes, and the Golgi apparatus

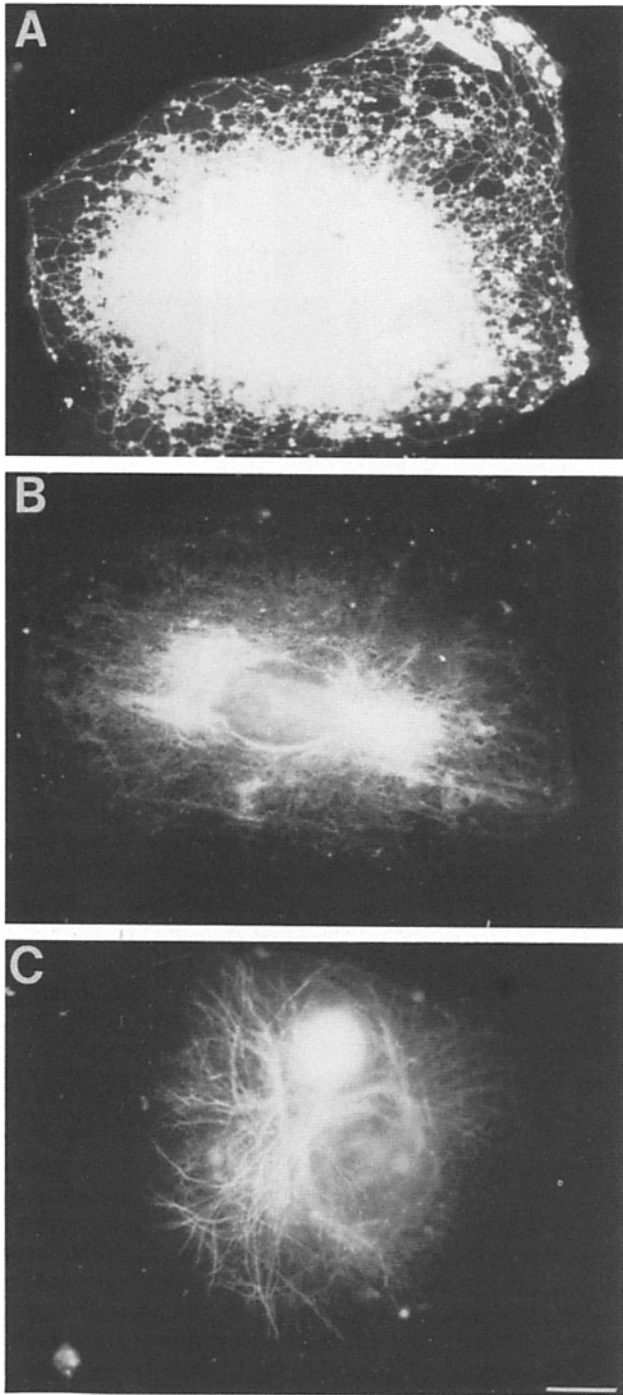


Figure 8. Effect of cycloheximide on ER construction. (A) Nocodazole-treated CV-1 cells pretreated 6 h in 20 μ g/ml cycloheximide, allowed to recover 15 min in cycloheximide-containing medium, and then fixed and stained for ER. (B) Vimentin staining of A. (C) Nocodazole-treated CV-1 allowed to recover 15 min in drug-free medium, then fixed, stained for ER, and restained with anti-vimentin antibody. Bar, 15 μ m.

(Goldman, 1971). All of these organelles have been observed in some cases to form tubular networks similar to the ER (Louvard et al., 1982; Johnson et al., 1980; Swanson et al., 1987; Lin and Queally, 1982; Kobayashi and Pagano, 1988) and to undergo microtubule-associated motility (Heggeness et al., 1978; Wang and Goldman, 1978; Summerhayes et al.,

1983; Phaire-Washington et al., 1980; Ho et al., 1987); mitochondria have been directly observed to form networks through branching and intersection (Chen, 1988), and this motility has been found to be microtubule dependent (Summerhayes et al., 1983). Observation of organelle development by live staining during nocodazole recovery may be useful for studying the role of microtubules in the organization of these structures. In cells treated overnight with nocodazole, all phase-contrast-visible membrane organelles are aggregated in the region around the nucleus; however, within 2–4 h after transfer to drug-free medium, the intracellular distribution of organelles appears normal (Lee, C., and L. B. Chen, unpublished results). Study of the time course and sensitivities of this organizational process might shed light on the apparent involvement of microtubules in generating the strikingly different distributions of ER, mitochondria, lysosomes, and Golgi apparatus. In particular, it may be interesting to test whether microtubule heterogeneities, reflecting posttranslational modifications of tubulin such as detyrosination and acetylation (Gunderson et al., 1984; Bulinski et al., 1988), play a role in the establishment of these differing distributions. Finally, the prospect of identifying the molecules involved in regulating microtubule-motility driven ER construction is particularly exciting since it offers a new opportunity for biochemical characterization of the systems that specify and regulate internal cell architecture. In vitro studies of such molecules, including cytoskeletal- and organelle-bound recognition proteins, motors, and proteins that regulate their interactions, could provide a rigorous foundation for understanding the structural control in cells and the spectacular diversity and specificity of organelle organizations in different cell types.

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