MICROTUBULE AND MICROFILAMENT REARRANGEMENTS DURING CAPPING OF CONCANAVALIN A RECEPTORS ON CULTURED OVARIAN GRANULOSA CELLS

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ABSTRACT

Thin-section electron microscope analysis of rat and rabbit-cultured granulosa cells treated with concanavalin A (Con A) at 37°C revealed coordinated changes in the cytoplasmic disposition of microfilaments, thick filaments, and microtubules during cap formation and internalization of lectin-receptor complexes. Con A-receptor clustering is accompanied by an accumulation of subplasmalemmal microfilaments which assemble into a loosely woven ring as patches of receptor move centrally on the cell surface. Periodic densities appear in the microfilament ring which becomes reduced in diameter as patches coalesce to form a single central cap. Microtubules and thick filaments emerge associated with the capped membrane. Capping is followed by endocytosis of the con A-receptor complexes. During this process, the microfilament ring is displaced basally into the cytoplasm and endocytic vesicles are transported to the paranuclear Golgi complex along microtubules and thick filaments. Eventually, these vesicles aggregate near the cell center where they are embedded in a dense meshwork of thick filaments. Freeze-fracture analysis of Con A-capped granulosa cells revealed no alteration in the arrangement of peripheral intramembrane particles but large, smooth domains were conspicuous in the capped region of the plasma membrane. The data are discussed with reference to the participation of microtubules and microfilaments in the capping process.

Cytoplasmic microtubules (MT) and microfilaments (MF) have been implicated in a variety of membrane phenomena. For example, the selective inclusion of lectin receptors (17) and exclusion of transport carriers (31) from membrane internalized during phagocytosis is abolished by antimicrotubule agents such as colchicine or vinblastine. Evidence indicating that MT impart a restrictive influence on the translational move-ment of lectin receptors in the plasma membrane is derived from the observations that colchicine and other MT-disrupting agents promote the re-distribution of concanavalin A (Con A)-receptor complexes to form caps on several cell types (10, 18, 30, 35). In contrast, cytoplasmic MF appear to participate actively in the translocation of surface receptors because the drug cytochalasin B (CCB), which is thought to disorganize actin-like MF, is

capable of preventing or reversing cap formation on lymphocytes (8, 26) and tissue culture cells (29, 30, 34). The recent findings that actin (12) or tubulin (5) copurifies with isolated plasma membranes, coupled with morphological evidence of associations between MT (1) or MF (20) and the plasma membrane, are consistent with the hypothesis that these structures may regulate the translational mobility and/or topographical distribution of cell surface components (4, 9, 16, 27).

In this communication we describe the sequential changes in the distribution of Con A receptors on cultured ovarian granulosa cells which occur during the process of con A-induced cap formation. A corresponding sequence of rearrangements in the cytoplasmic disposition of MT, MF, and thick filaments (TF) in response to the binding of Con A and during the formation of surface caps is presented. The changes in distribution of these cytoskeletal elements are highly correlated with alterations in the distribution of membrane receptors for Con A as well as with attendant changes in cell shape.

MATERIALS AND METHODS

Culturing Conditions

Granulosa cells from sexually mature rats and Dutch Belted rabbits were obtained from large preovulatory ovarian follicles by slitting the follicle wall with an iris scalpel and gently scraping the epithelial cells free from the basement membrane as described previously (11). Six rat ovaries or two rabbit ovaries yielded enough cells to establish 30 culture dishes. The aggregates of cells released from follicles were passed through several changes of McCoy's 5A medium (modified, Grand Island Biological Co., Grand Island, N. Y.) to eliminate follicular fluid, and oocytes were removed with a micropipette. The remaining aggregates of granulosa cells were dissociated by passage through a graded series of micropipettes and transferred to a petri dish containing culture medium. The culture medium for rabbit cells consisted of McCoy's 5A medium (modified) supplemented with 1% L-glutamine (200 mM stock), 100 U/ml of penicillin, 5 μg/ml of streptomycin sulfate, and 15% serum obtained from adult female rabbits. Rodent granulosa cells were cultured in medium of similar composition, except that 15% fetal calf serum was used. Cultures were seeded by adding four microdrops of the pooled cells (containing a total of approx. 10^5 cells) to sterile 18-mm glass cover slips contained in 35 × 10 mm tissue culture dishes (Falcon Plastics, Division of BioQuest, Oxnard, Calif.). The culture dishes were placed in the incubator for 1 h to allow the cells to attach to the substrate, and then 2.5 ml of medium was added to each

dish. Cultures were incubated at 37°C in a humidified atmosphere (95% air, 5% CO_2), and the medium was changed every 2 days. All experiments were conducted on 5-day cultures except those which were examined 2 days subsequent to Con A treatment.

Experimental Design and Labeling Conditions

LIGHT MICROSCOPY: Cultures were rinsed three times in phosphate-buffered saline (PBS) and preincubated in PBS for 30 min at 37°C or 4°C. The cells were exposed to Con A (Sigma Chemical Co., St. Louis, Mo., grade IV) in PBS (100 μg/ml) for 10 min at 4°C or 37°C, washed twice with PBS, incubated with horseradish peroxidase (200 μg/ml) (Sigma Chemical Co., type VI) in PBS for 10 min at 4°C or 37°C, and washed two additional times with PBS before incubation at 37°C for 0, 10, 20, or 45 min. In some experiments, cultures were fixed with 1% paraformaldehyde in PBS before Con A and horseradish peroxidase labeling. In other experiments, 0.05 M α-D-methylglucopyranoside (α-MG) was included in the postlabel medium for 30 min. Cultures were fixed for 30 min with 1% glutaraldehyde in PBS after the designated incubation periods followed by three rinses in PBS. Peroxidase activity was demonstrated by reacting cultures for 15 min with diaminobenzidine (0.5 mg/ml) (Sigma Chemical Co.) in 0.1 M Tris-buffer (pH 7.4) containing 0.01% H_2O_2 (19). Some cultures were treated with peroxidase alone and processed as indicated to test for nonspecific adsorption of the marker enzyme to the cell surface. After two washes in PBS, phase and bright-field photographs were taken of cultures fixed at various times and the samples were subsequently processed for electron microscopy. Cell viability was assayed by dye exclusion with trypan blue. Greater than 80% viability was routinely observed 3 h after Con A treatment. Cultures examined 2 days after experimental manipulation showed a marked increase in cell density.

ELECTRON MICROSCOPY: Hemocyanin was also employed as a marker molecule for Con A-binding sites (CABS) on surface replica and thin-section preparations of granulosa cells. In these experiments, cultures were exposed to Con A (100 μg/ml) at 4°C or 37°C, followed by incubation with keyhole limpet hemocyanin (see reference 23) (500 μg/ml) for 10 min at 4°C or 37°C. The cells were processed through fixation as indicated above. Again, prefixed and α-MG treated cultures were processed as controls. Samples were postfixed in 1% OsO_4 (aqueous) for 15 min, dehydrated through a series of cold, graded ethanols to amyl acetate, and dried with a hot air stream for the preparation of surface replicas (28). Replicas were made by selecting densely populated areas of the cover slip under a dissecting microscope and placing cover slip fragments on the specimen stage of a Balzers apparatus (Balzers AG, Balzers, Liechtenstein). Shadowing with platinum and carbon was performed at a temperature of −115°C and a vacuum pressure of 10^{-6}

torr. Replicas were removed from the glass by immersion in hydrofluoric acid, rinsed in distilled water, and cleaned in Clorox. After mounting on uncoated or Formvar-coated copper grids, the replicas were examined in a Philips 200 electron microscope.

Thin-section analyses were performed on 5-day cultures of rat or rabbit ovarian granulosa cells treated with Con A and labeled with horseradish peroxidase or hemocyanin and fixed as described above. Samples were subsequently washed in buffer, osmicated in 1% aqueous OsO_4 for 15 min, dehydrated through a series of cold, graded ethanols, and embedded in a mixture of Epon and Araldite (2). Monolayers were infiltrated with the embedding mixture in tissue culture dishes, and individual cell colonies were subsequently cut out and remounted on blank Epon blocks. Thin sections were cut with a diamond knife, with the cells oriented perpendicular or parallel to the substrate. Sections were collected on uncoated 300-mesh grids and stained with uranyl acetate (33) and lead (25).

DRUG TREATMENTS: Triplicate 5-day cultures of rabbit granulosa cells were washed three times in phosphate-buffered saline (PBS) and incubated at 37°C for 30 min in dimethylsulfoxide (DMSO, 0.5%, Fisher Scientific Co., Pittsburgh, Pa.), cytochalasin B (CCB, Aldrich Chemical Co., Milwaukee, Wis., 5 µg/ml), dinitrophenol ($\times$ 10^{-3} M; Sigma Chemical Co.), sodium fluoride and sodium azide (respectively, 4 $\times$ 10^{-3} M, 2 $\times$ 10^{-2} M; Sigma Chemical Co.) or PBS alone (control), or for 60 min in colchicine (10^{-5} M, Sigma Chemical Co.) or vinblastine sulfate (10^{-5} M, Eli Lilly and Co., Indianapolis, Ind.). The cultures were subsequently labeled with Con A followed by hemocyanin as previously described, and incubated in PBS containing the drugs at the same concentrations for 30 min at 37°C.

FREEZE-FRACTURE ELECTRON MICROSCOPY: Cultures were prepared for freeze-fracture analysis of Con A capping by processing the cells as indicated above but treated only with Con A (100 µg/ml) for 10 min at 37°C. After a 20-min incubation at 37°C and fixation in glutaraldehyde, cells were cryoprotected by incubation in 25% glycerol in 0.1 M cacodylate buffer for 1 h, removed from the glass substrate with a rubber policeman, and frozen onto paper discs with liquid Freon 22 (Virginia Chemicals, Inc., Portsmouth, Mass.). Fracturing and shadowing were performed in a Balzers apparatus as described above. All micrographs of replicas are arranged with the direction of shadow from bottom to top.

RESULTS

Surface Distribution of Con A during Capping

Granulosa cell cultures are typically comprised of small colonies (10–30 cells) of epithelioid cells which have broad areas of contact between cells.

Cytoplasmic organelles are randomly distributed in the cytoplasm as observed under phase optics (Fig. 1 A), and stress fibers are occasionally seen. Treatment of 5-day cultures with peroxidase alone at 37°C results in a small amount of spotty binding (not shown) which we attribute to pinocytosis, but this staining is far less than that observed after Con A exposure and occurs randomly on the cells. Prefixation of granulosa cells before Con A and horseradish peroxidase treatment results in a dispersed distribution of reaction product (Fig. 1 B). The same pattern of CABS is observed on cultures fixed immediately after labeling.

On cells exposed to Con A at 37°C for 10 min, large aggregates of reaction product are found near the cell center (Fig 4 B) which are internalized in numerous vesicles by 45 min after Con A treatment (Figure 5 B). As receptors are cleared toward the cell center (10–20 min), a dense ring is apparent in capping granulosa cells under phase optics (Fig. 2 A). This ring decreases in diameter as receptor is gradually displaced inwardly (Fig. 3 A) and ultimately becomes obscured by both the overlying peroxidase-stained cap and by the rounded shape of the cell (Fig. 4 A). During Con A capping, retraction fibers to adjacent cells appear at the cell margin and progressively assume a more centrally located position (Figs. 3 A and 4 A). In surface replicas of cells capped at 37°C, retraction fibers are observed to terminate at the boundary of caps seen as a closely packed aggregate of hemocyanin molecules (Fig. 6). Incubation of cells with the competing hapten sugar, α-MG, for 30 min subsequent to Con A treatment removes essentially all peroxidase or hemocyanin detectable at all time intervals except 45 min, by which time CABS internalization had occurred.

Kinetics of Capping and Drug Effects

The temperature at which the labeling with Con A followed by hemocyanin was carried out was found, in surface replica preparations, to influence both the rate of receptor movement (Table I) and the final surface disposition of Con A caps. Processing of cells at 37°C resulted typically in a maximal capping response (80% capped) by 20 min after labeling. As indicated above, caps formed at 37°C are located over the cell center (Figs. 4 A and 6). Cultures labeled at 4°C and maintained at this temperature display a dispersed distribution of CABS. Cells labeled at 4°C and subsequently warmed to 37°C demonstrate a delayed capping

response with maximal numbers of caps appearing 45 min after processing (Table I). In addition, caps formed after cold labeling tend to form at the cell periphery.

The effects of various drugs on the capping process have been examined. As many studies have already indicated (15, 32), inhibition of energy metabolism with dinitrophenol or a combina-
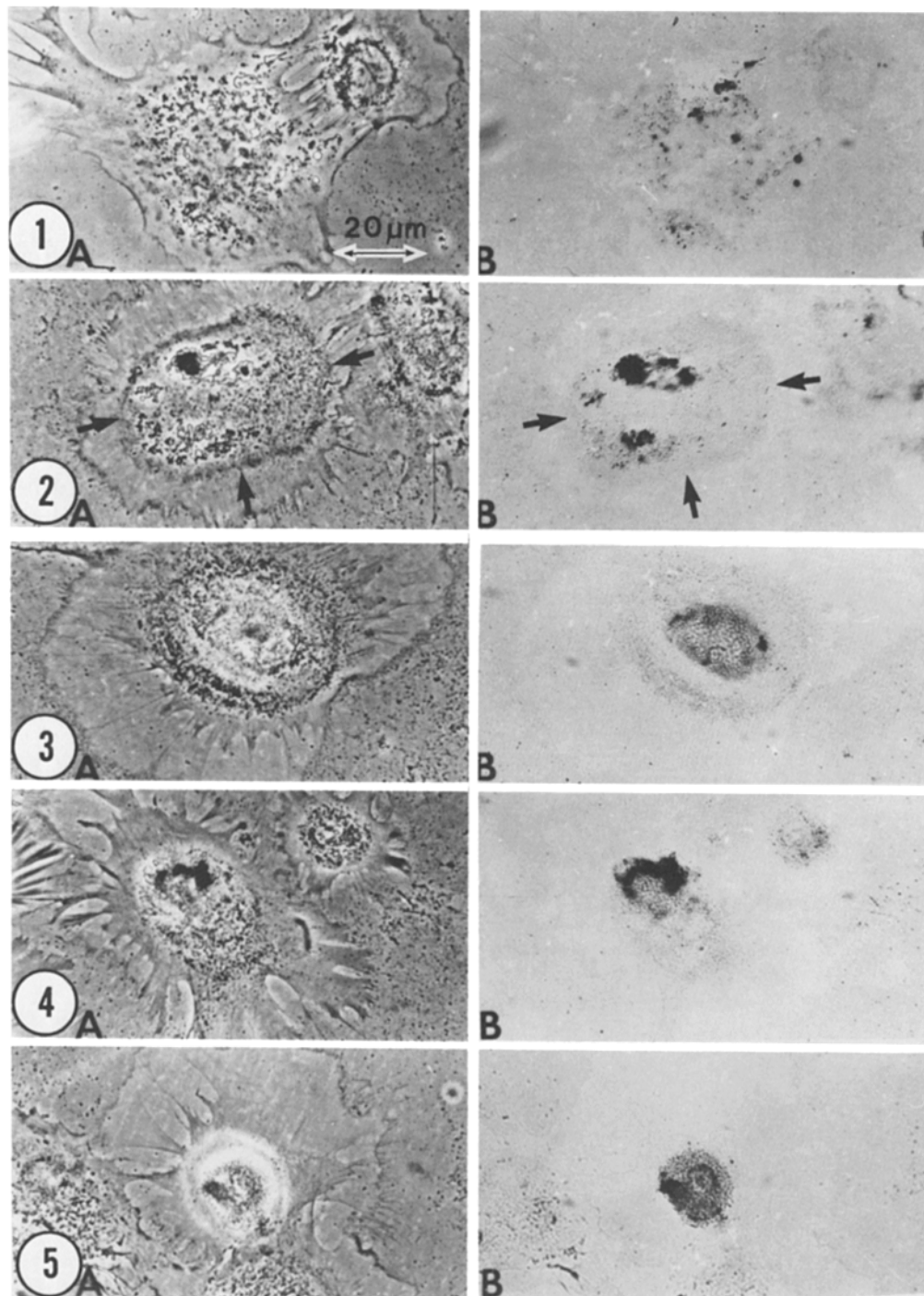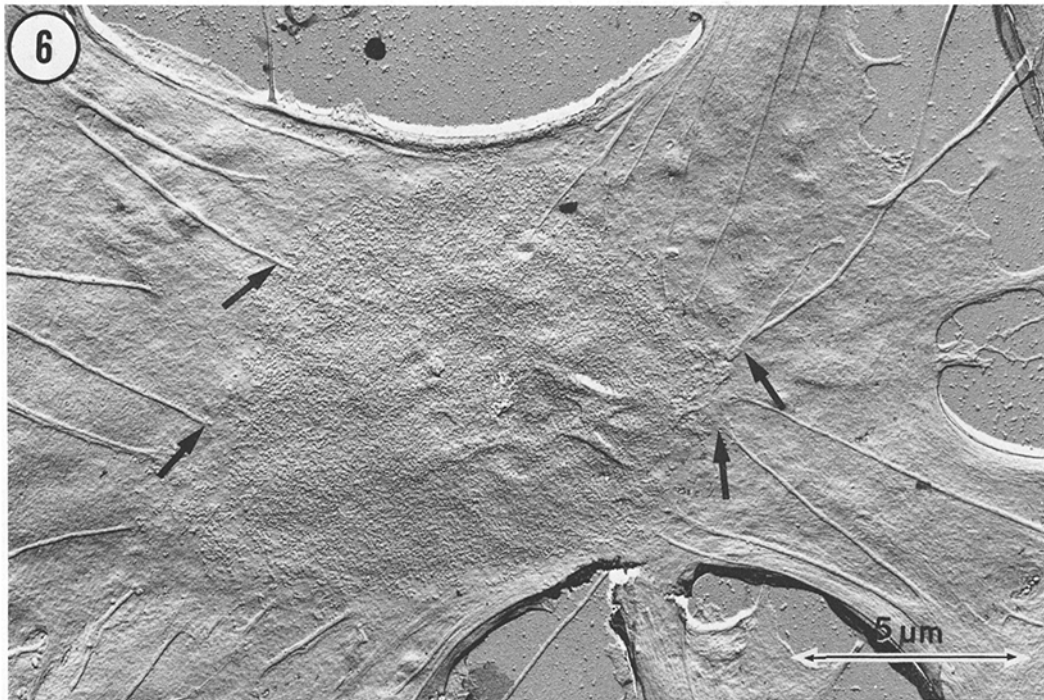
FIGURE 6 A capped granulosa cell is depicted from a sample fixed 20 min after labeling at 37°C; the cap assumes a central disposition on the cell surface and is delineated at its boundaries by retraction fibers (arrows) emanating from neighboring cells.

tion of sodium fluoride-sodium azide prevents CABS patching and capping but not receptor clustering. Capping is also inhibited by cytochalasin B treatment but, as reported earlier (1), the antimicrotubule agents colchicine or vinblastine do not inhibit CABS cap formation on cultured granulosa cells. Interestingly, caps formed in the presence of colchicine or vinblastine are located at the cell periphery and thus mimic the effect of cold treatment indicated above.

Cytoskeletal Alterations during Capping

The dorsal surface of cultured granulosa cells is regularly contoured, although occasional microvilli are found at the lateral borders between cells. Prefixation with paraformaldehyde followed by

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FIGURES 1-5 Phase (A) and corresponding bright field (B) images of cultured rabbit granulosa cells at various times after con A-peroxidase treatment at 37°C. All micrographs, × 600.

FIGURE 1 Distribution of CABS on granulosa cell prefixed with paraformaldehyde; reaction product (black) is diffusely distributed over the cell surface.

FIGURE 2 CABS aggregate by 10 min to form large patches near the cell center (B). Note that CABS are circumscribed by a dense cytoplasmic ring which excludes organelles from the cell periphery (arrows).

FIGURE 3 By 20 min after labeling, CABS are localized to the cell center (B); retraction fibers emanate from the cell margin to the edge of the cap (A).

FIGURE 4 A central cap (20-min sample) occupies a large dorsal evagination of the cell surface.

FIGURE 5 Prominent cell rounding is observed by 45 min postlabeling, and cellular organelles have accumulated in the cell center (A); CABS are internalized in numerous peroxidase-reactive vesicles confined to the cell center (B).

Distribution of CABS on Ovarian Granulosa Cells

Temperature of Label-ing	Time after Labeling	Distribution			
		Random	Clustered	Patched	Capped
	min	%	%	%	%
37°C	0	30	60	8	2
"	10	5	32	45	18
"	20	0	8	12	80
"	45	0	4	7	89
4°C	0	44	47	9	0
"	10	21	44	30	5
"	20	0	12	25	63
"	45	0	5	11	84*

Cells were processed for making surface replicas, and triplicate samples from each time-interval were examined. 100 cells per time-period were counted and classified as random (diffusely distributed CABS), clustered (10-30 hemocyanin molecules), patched (40-80 hemocyanin molecules), or capped (single large aggregate).
* Prior exposure to 4°C resulted in the formation of multiple (2-4) caps per cell which were peripherally located on the cell surface.

Con A-peroxidase treatment or fixation after labeling at 4°C results in the deposition of a continuous layer of reaction product on the cell surface (Fig. 7a). If α-methyl mannoside is present after Con A-peroxidase or Con A-hemocyanin treatment, binding of marker molecules is reversed and CABS are not detected (Fig. 7b).

In untreated cells, a thin (20-40 nm) layer of MF subtends the plasma membrane, and occasional MT and TF randomly course through the cytoplasm. Treatment with Con A-hemocyanin at 37°C followed by immediate fixation results in the formation of Con A-receptor clusters separated by unlabeled portions of the membrane (Fig. 7c). Accompanying the rapid clustering of Con A-receptors is a thickening of the layer of submembranous MF which now occupies a cortical zone measuring 40-80 nm in width. As Con A-receptor is cleared from peripheral parts of the cell and small patches of CABS form, more MF aggregate immediately beneath surface patches (Fig. 7d); MT and TF are unchanged in their cytoplasmic disposition. The MF aggregates subtending CABS patches are comprised of uniformly oriented 6-8 nm filaments.

Between 10 and 20 min after labeling at 37°C, several cytoplasmic changes occur during patch formation. The cortical MF system becomes more extensive and, in sections of cells oriented perpendicular to the substrate, two distinct layers of MF appear beneath surface Con A patches. These layers are of comparable width (50-60 nm), are comprised of MF measuring 6 nm in diameter,

and are arranged at right angles to each other (Fig. 8a). Small groups of MT appear subjacent to the innermost MF layer which are oriented in the same direction as the overlying MF. Dense areas are commonly observed within the MF network. The patched membrane at this time remains evenly contoured. On the basis of earlier phase microscope observations and thin-section data, it is apparent that the most superficial assembly of MF comprises a ringlike structure that circumscribes and subtends CABS patches being moved centrally on the cell surface. MT at this stage radiate from the paranuclear Golgi apparatus of the cell.

From 20 to 40 min after labeling at 37°C, large numbers of granulosa cells form central caps which are rapidly internalized. The formation of the cap and internalization of Con A receptors are difficult to dissociate from each other during this time interval because of the asynchronous behavior of the cells comprising a given population. We have thus arbitrarily subdivided these events largely on the basis of changes in membrane structure that indicate the onset of active endocytosis.

Three prominent changes occur as cap formation is completed. The capped plasma membrane initially becomes scalloped in contour (Fig. 8b) and, subsequently, becomes thrown into numerous folds and plications (Fig. 9a). During this alteration in surface morphology, the submembranous MF, which have assumed the form of a circular band, are displaced from the plasma membrane and numerous MT appear in association
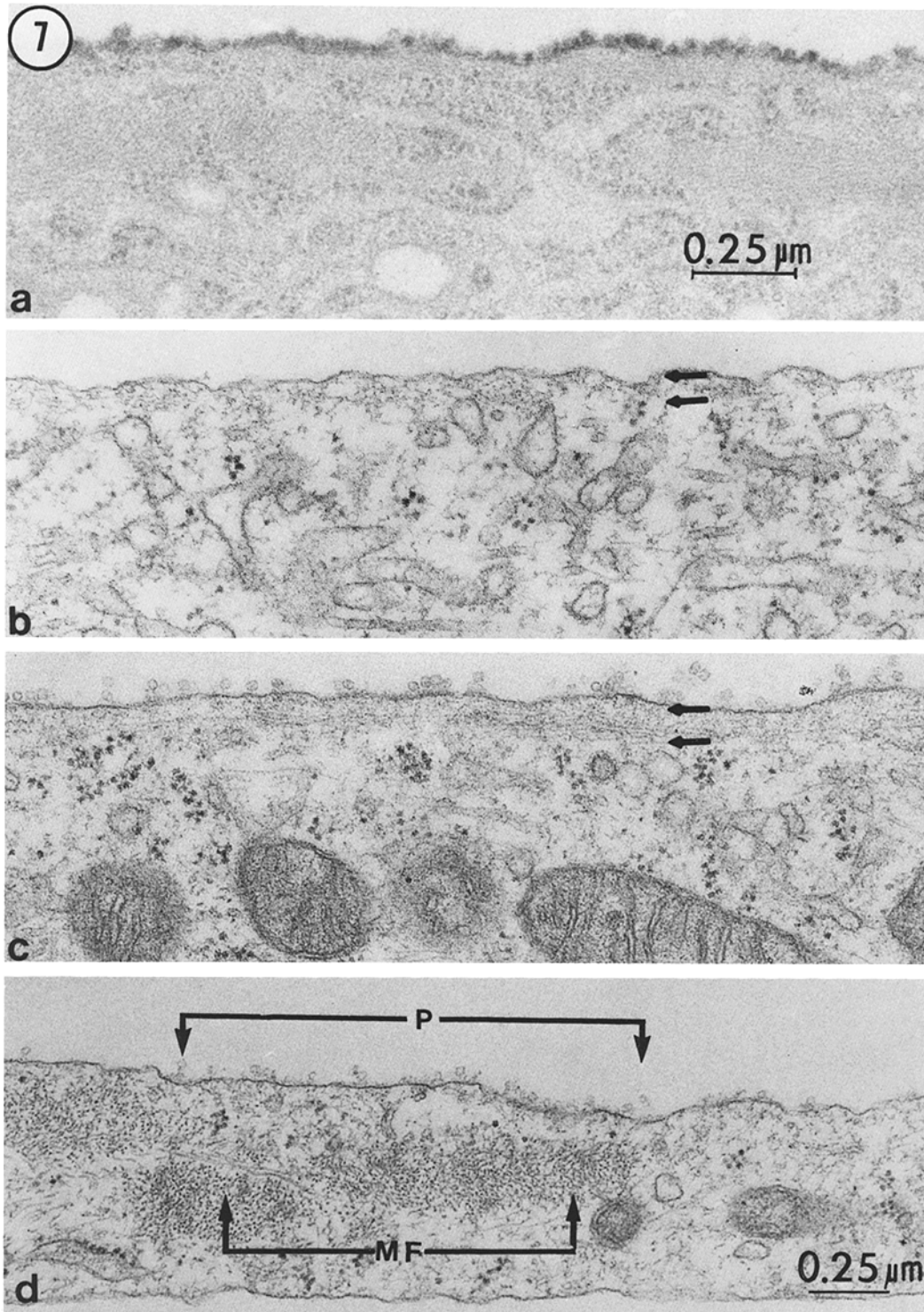
FIGURE 7 (a) Con A-peroxidase-labeled granulosa cell processed at 4°C and fixed immediately. A continuous granular staining is observed. (b) Portion of the dorsal surface and cytoplasm of rabbit granulosa cell treated with con A and hemocyanin at 4°C and incubated for 30 min in the presence of α-methylmannose. Note the absence of hemocyanin binding and the delicate network of subplasmalemmal microfilaments (arrows). (c) Clusters of CABS on the surface of a granulosa cell fixed after labeling with Con A and hemocyanin at 37°C. A thickening of submembranous microfilaments (arrows) is noticeable beneath hemocyanin clusters. (d) Patch of CABS (P) removed from cell margin is illustrated; note cross-sectioned profile of underlying MF band (MF). (a–c) × 63,000; (d) × 49,000.
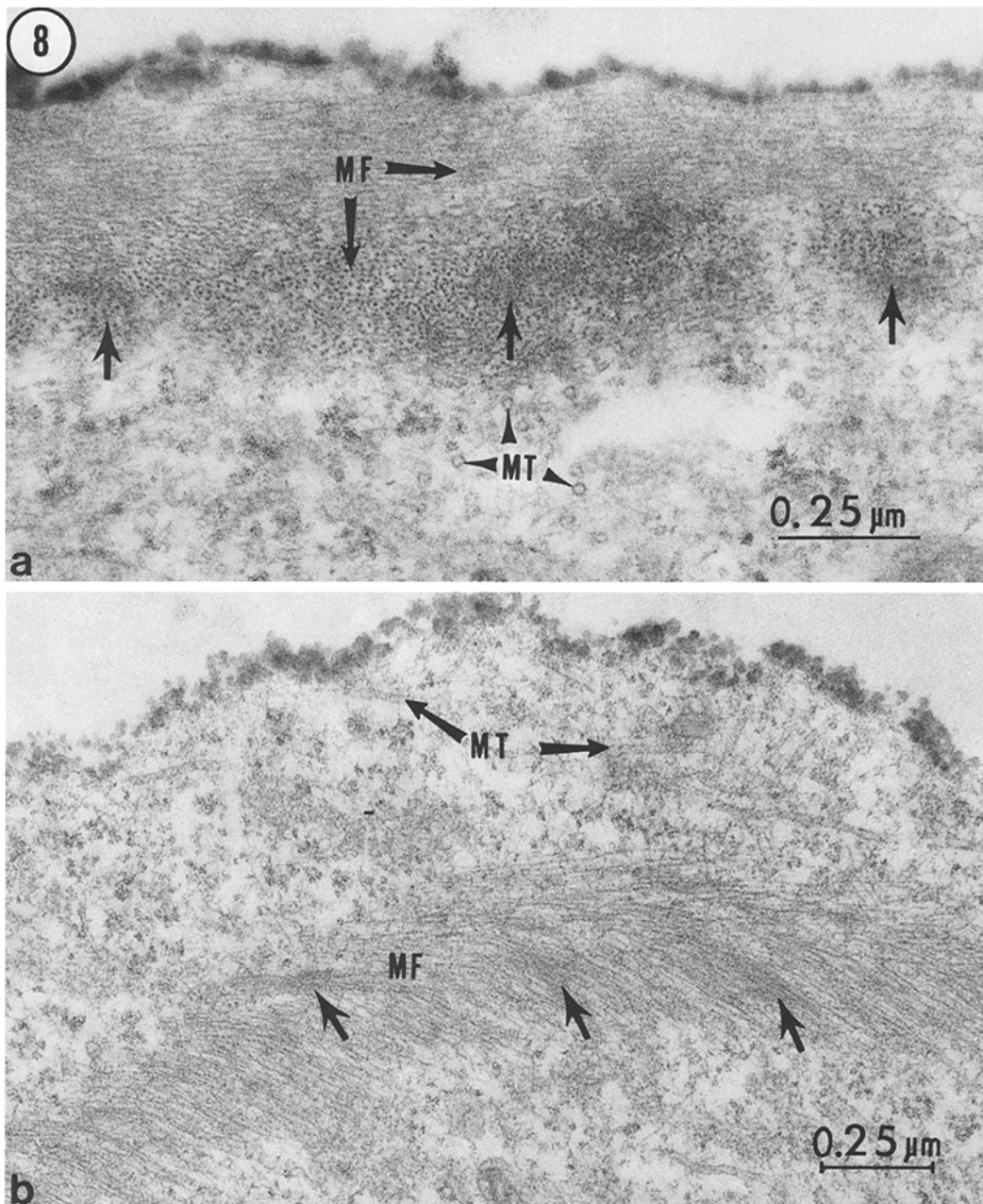
FIGURE 8 (a) Transmission electron micrograph of CABS patch formed 20 min after labeling with Con A-peroxidase at 37°C. Section is perpendicular to the plane of the membrane and reveals thick microfilament band (MF) comprised of two filament bundles coursing at right angles to each other. (b) Early cap formed 20 min after labeling, illustrating inward displacement of microfilament band (MF) from the capped membrane which now presents an irregular contour. Microtubules (MT) appear beneath the capped membrane. (a) × 78,900; (b) × 62,800.

with shallow furrows of the membrane (Fig. 8b). This is especially prominent at the edges of the cap, whereas in more central regions of the cap the deep surface infoldings impinge upon the subtending MF bundle (Fig. 9a) which gradually shifts to a deeper location in the cytoplasm as endocytosis
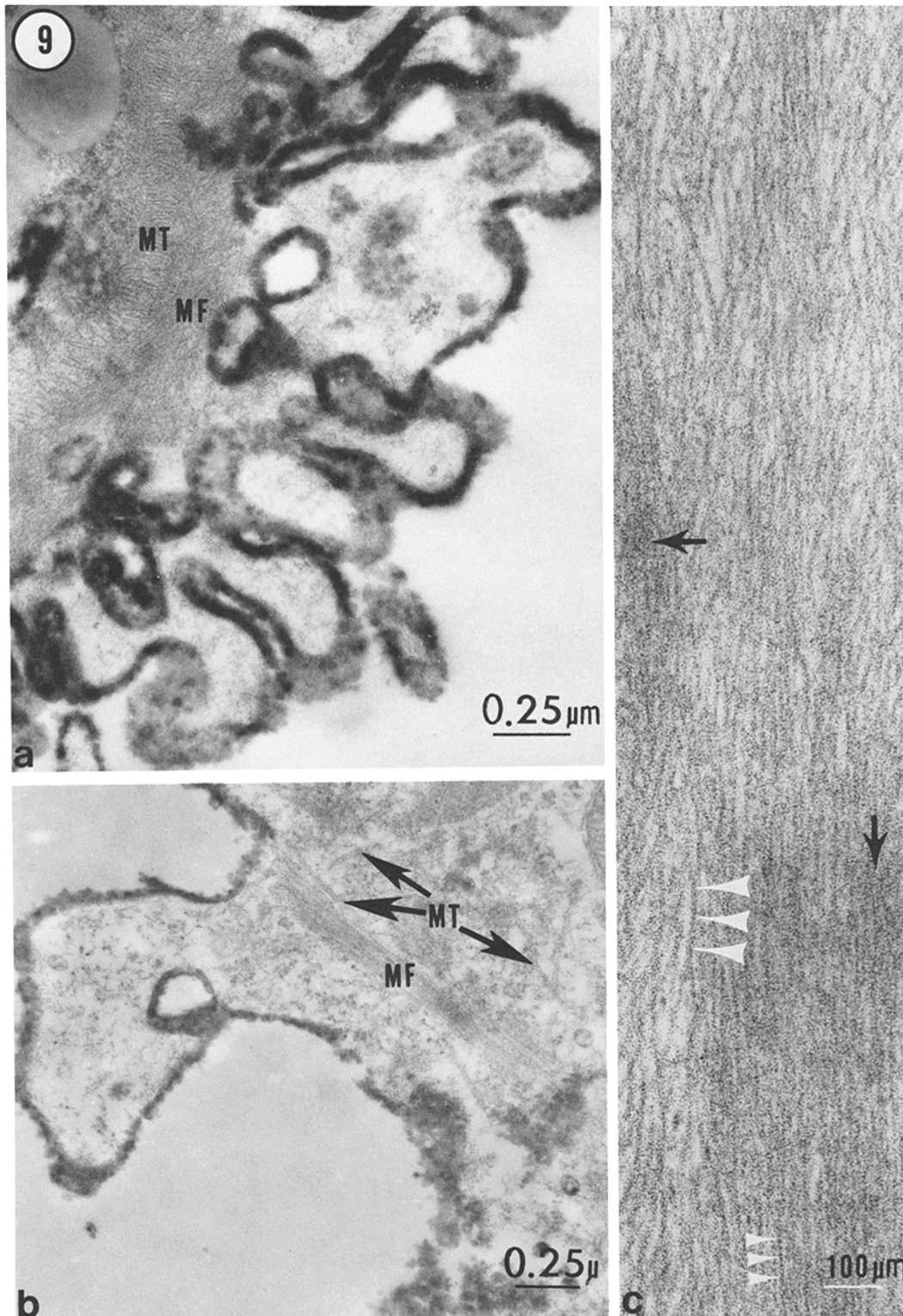
FIGURE 9 (a) Portion of capped granulosa cell showing extensive membrane plications and infoldings found typically at the onset of receptor internalization. Microfilaments (MF) and microtubules (MT) subtend the cap. (b) Microfilament bundle (MF) is evident in the cytoplasm conjoining peroxidase-labeled surface invagination (lower) and outpocketing (upper). Microtubules (MT) surround the MF bundle. (c) High magnification image of MF bundle on the right and network of intermediate filaments on the left. Densities within the MF bundle are apparent (arrows). (a) × 46,900; (b) × 40,800; (c) × 94,000.

proceeds. Bulbous evaginations and infoldings of capped membrane are often connected by short groups of MF (Fig. 9b). This area is rich in MT. Densities within MF bundles are amorphous and the constituent 6 nm MF remain arranged in parallel arrays (Fig. 9c).

Between 45 and 60 min after labeling, Con A is removed from the plasma membrane by endocytosis. The formation of endocytotic vesicles is attended by cell rounding and a change in the microtubular system of the cell. MT radiating from the Golgi area of the cell are abundant beneath the cap and closely approximate the capped region of the plasma membrane (Fig. 10). Direct physical contact between MT and the plasma membrane is rarely observed. The alignment of endocytotic vesicles containing CABS along extensive, more deeply situated MT is commonly observed in this region of the cell (Fig. 10, inset). Concomitant with the emergence of MT is the appearance of TF, 10 nm in diameter, which populate the cytoplasm underlying the cap.

In sections parallel to the substrate and just beneath the cap (Fig. 11a), the deeply situated MF ring is observed to encompass the area of active membrane internalization. This region of the cytoplasm contains predominantly MT and TF which laterally associate with each other via short extensions or branches from TF (Fig. 11b). Endocytotic vesicles appear to be preferentially arranged along TF-MT complexes.

The parallel arrays of MF comprising the circular band demarcate the boundary between nonendocytotic and endocytotic portions of the cytoplasm harboring the MT and TF. Fig. 9c illustrates the relationship between MF and TF aggregates in longitudinal section at this interface. Cross-sectional profiles of MF (Fig. 12b) and TF (Fig. 12c) from the same cell at the same magnification show the MF to be 6 nm in diameter and the TF to be 10-12 nm in diameter. MF are further distinguished from TF by being regularly arranged and closely packed. Within 60 min of Con A treatment at 37°C, capped CABS have been removed from the cell surface in the form of vesicles which aggregate in a juxtanuclear position (Fig. 12a). TF are interspersed among the vesicular aggregates, and dense accumulations of MF are observed surrounding the vesicular aggregates.

Freeze-Fracture Observations

Replicas of rabbit granulosa cell cultures treated with Con A for 10 min, washed, and incubated for 20 min revealed no dramatic alteration in the density or distribution of E- or P-face (6) particles (Fig. 13a) when compared to untreated controls (not shown). The identification of capped granulosa cells on freeze-fracture replicas was based on (a) changes in cell shape (rounding) known to occur by 30 min and (b) images showing both the cytoplasmic organization of cross-fractured capped cells and in-plane fractures of the capped membrane. The circular band of MF subtending the cap served as a useful landmark for identifying the capped portion of granulosa cells. Numerous particle-rich endocytotic vesicles were observed within the microfilament band. Fractures through the membrane superficial to this structure reveal the presence of circular smooth-membrane domains free of intramembrane particles on both the E- and P-faces of the capped plasma membrane (Fig. 13b). These areas are similar in texture to the membranous component of Con A caps on Entamoeba histolytica described by Pinto da Silva et al. (19).

DISCUSSION

The data presented in this paper demonstrate that the capping of CABS on cultured granulosa cells is accompanied by alterations in the cytoplasmic disposition of MT, MF, and TF. These changes are summarized in Fig. 14. As Con A-receptor clusters are cleared from the cell margin, a thickening and aggregation of submembranous MF located immediately beneath the surface receptors is observed (Fig. 14A). During the central displacement of CABS which results in cap formation, the subtending MF assemble into a continuous ring which appears to contract towards the cell center (Fig. 14B). MT radiating from the cell center are subjacent and antiparallel to the MF ring initially; but as the cell rounds up during the capping process, MT are observed to course through the MT ring and terminate beneath the capped membrane (Fig. 14C). CABS are internalized within smooth-surfaced vesicles subsequent to cap formation, and frequent associations between vesicles and MT or TF are observed in this area of the cell (Fig. 14D). The endocytotic vesicles containing CABS are transported to the juxtanuclear Golgi apparatus where they accumulate and become enveloped by a dense network of MF and TF.

Although these results clearly illustrate that rearrangements in the organization of the cytoskeletal system occur as a consequence of Con A-induced capping, the data do not permit an accu-
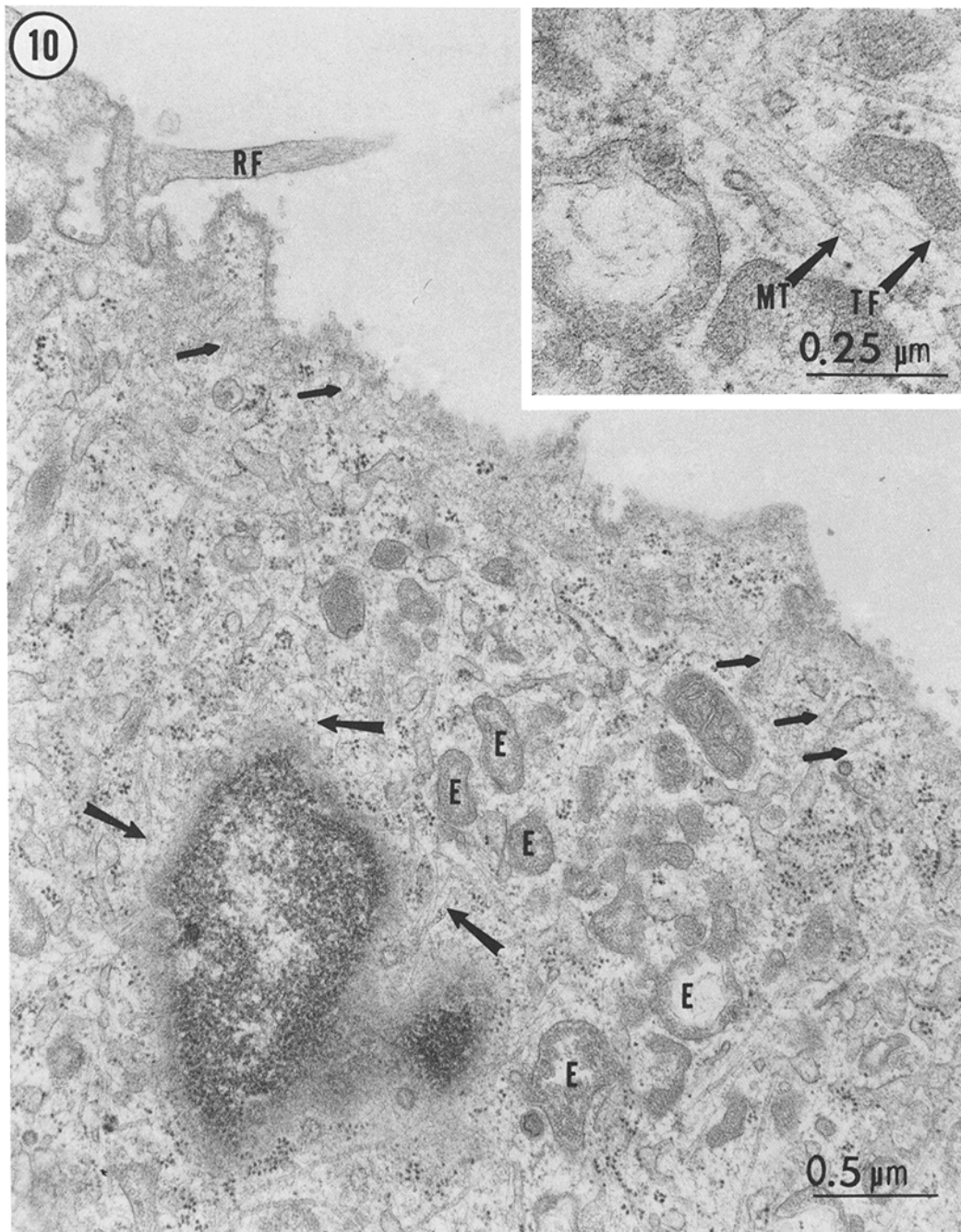
FIGURE 10 Rabbit granulosa cell labeled with Con A-hemocyanin at 37°C and fixed 45 min later; the section is roughly parallel to the plane of the membrane and reveals the nucleus (lower left) and numerous endocytotic vesicles (*E*) containing hemocyanin molecules. Note the presence of a retraction fiber (*RF*) at one edge of the cap and microtubules associated with the capped membrane (small arrows) and nuclear envelope (large arrows). *Inset*: Hemocyanin-laden endocytotic vesicles are seen coursing along microtubules (*MT*) and thick filaments (*TF*). Fig. 4, × 34,500; *inset*, × 78,500.
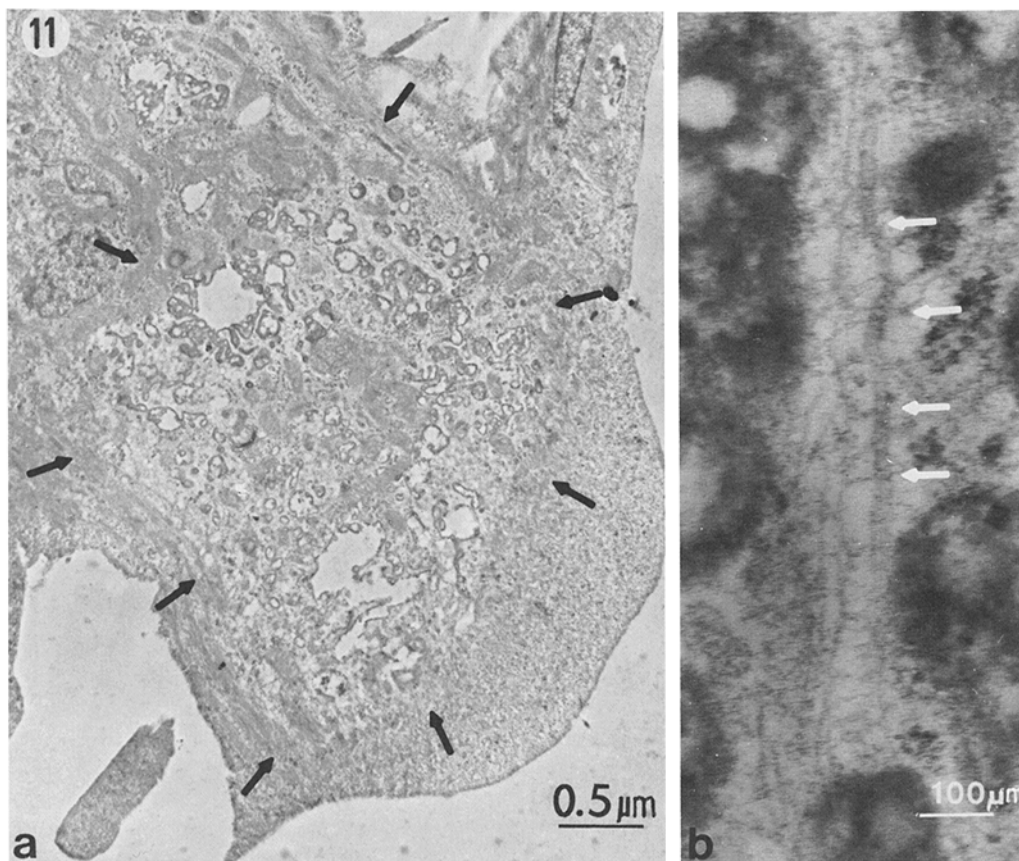
FIGURE 11 (a) Low-magnification electron micrograph of a rat granulosa cell fixed 45 min after Con A-peroxidase treatment at 37°C. Peroxidase-labeled vesicles and surface infoldings are confined to a paranuclear position and circumscribed by a dense filamentous band (arrows). (b) Enlargement of endocytotic area depicted in (a) showing intermediate filament extensions interacting with a microtubule (arrows). (a) × 11,000; (b) × 98,000.

rate definition of the roles subserved by these cytoplasmic structures in the regulation of receptor mobility. To date, the evidence implicating cytoskeleton components in the regulation of receptor movement has been based on the effects of a variety of drugs thought to selectively impair the function of MT or MF (7, 8, 10, 18, 23, 24, 32, 36). The information obtained in this study should aid in partially explaining the mechanism by which cytoplasmic structures, notably MT and MF, influence the topography and movement of cell surface receptors.

There is general agreement that the contractile proteins actin and myosin are widely distributed among animal cells and that these cytoplasmic constituents participate in many cellular activities (21). Filamentous actin has been identified ultra-structurally as thin filaments or MF by their ability to form distinctive arrowhead complexes after treatment with heavy meromyosin (14). Pollard and Korn (20) have presented morphological evidence indicating that MF terminate on the inner surface of the plasma membrane, and recent biochemical analyses have shown actin to be associated with membrane fractions isolated from 3T3 fibroblasts and HeLa cells (12). While an association between MF and membranes is implicit from observations of this kind, little direct evidence is available implicating MF in the regulation of cell surface receptor movements. Our observations on the rearrangement of MF during Con A capping clearly indicate that these structures participate in the translocation of surface receptors. In addition, the inhibition of capping by drugs which interfere
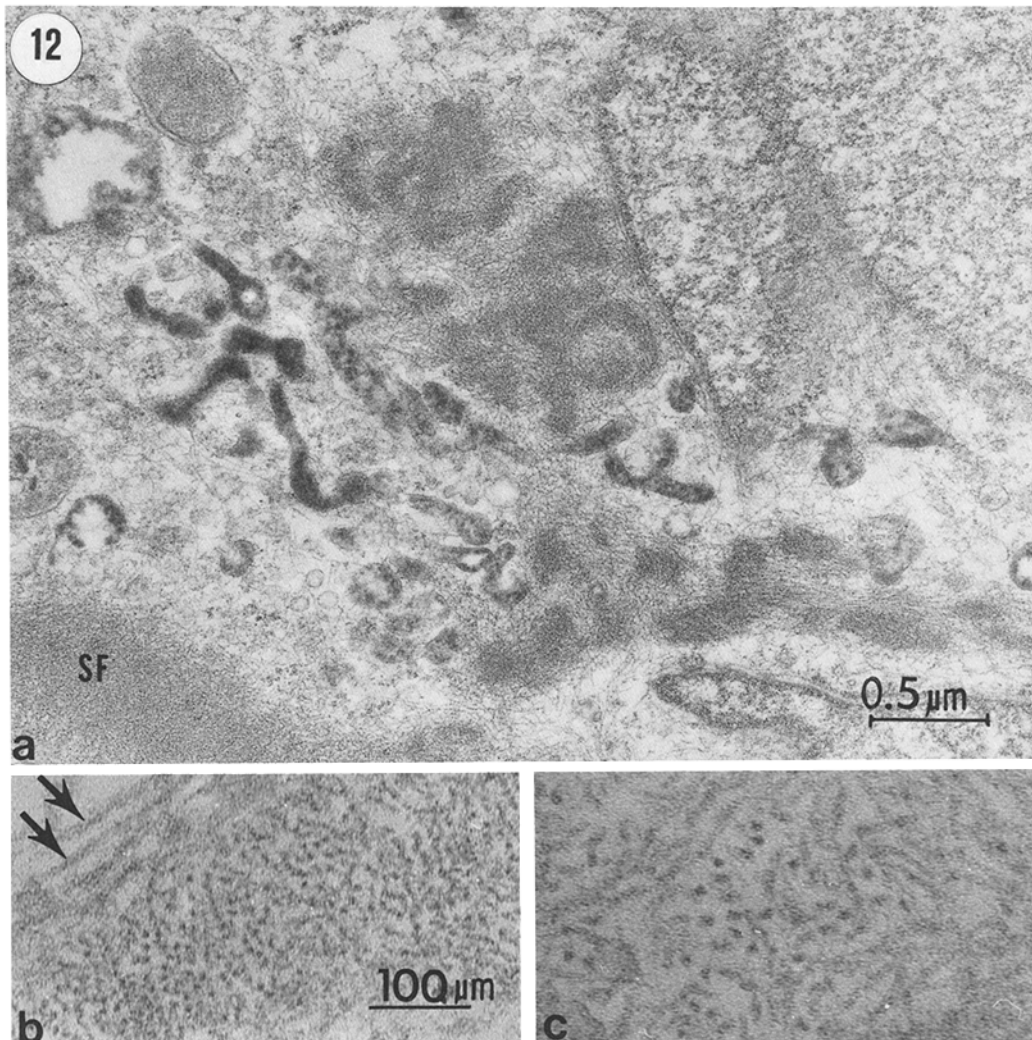
FIGURE 12 (a) Paranuclear region of rat granulosa cell treated with Con A-peroxidase at 37°C and fixed 60 min later. A stress fiber (SF) composed of microfilaments is situated along the basal surface of the cell. Receptor-laden vesicles are concentrated near the nucleus and embedded in remnants of the microfilament band. (b) High-magnification micrograph of cross-sectioned bundle of microfilaments (6 nm in diameter); arrows indicate longitudinal profiles of intermediate filaments (10 nm in diameter). (c) Micrograph of same magnification as (b) from the same field showing greater diameter of intermediate filaments in cross-sectional view. (a) × 31,400; (b and c) × 135,000.

with energy metabolism or MF function, such as cytochalasin B or local anesthetics as shown in this study and elsewhere (7, 8, 22, 23, 24, 30, 32), lends additional support to the notion that MF are actively involved in the movement of surface receptors during capping. The factors governing the association of MF with the membrane and the contractile properties of MF during capping are, however, poorly understood.

It should be emphasized that the changes in MF organization noted in this study are a consequence of Con A binding to the cell surface. To what extent ligand-receptor interactions of this kind alter the cytoplasmic or membrane-associated calcium levels of the cell is not resolved, but recent data point to an essential role for calcium in the capping process. Schreiner and Unanue (26) have shown that exposure of anti-Ig capped lympho-
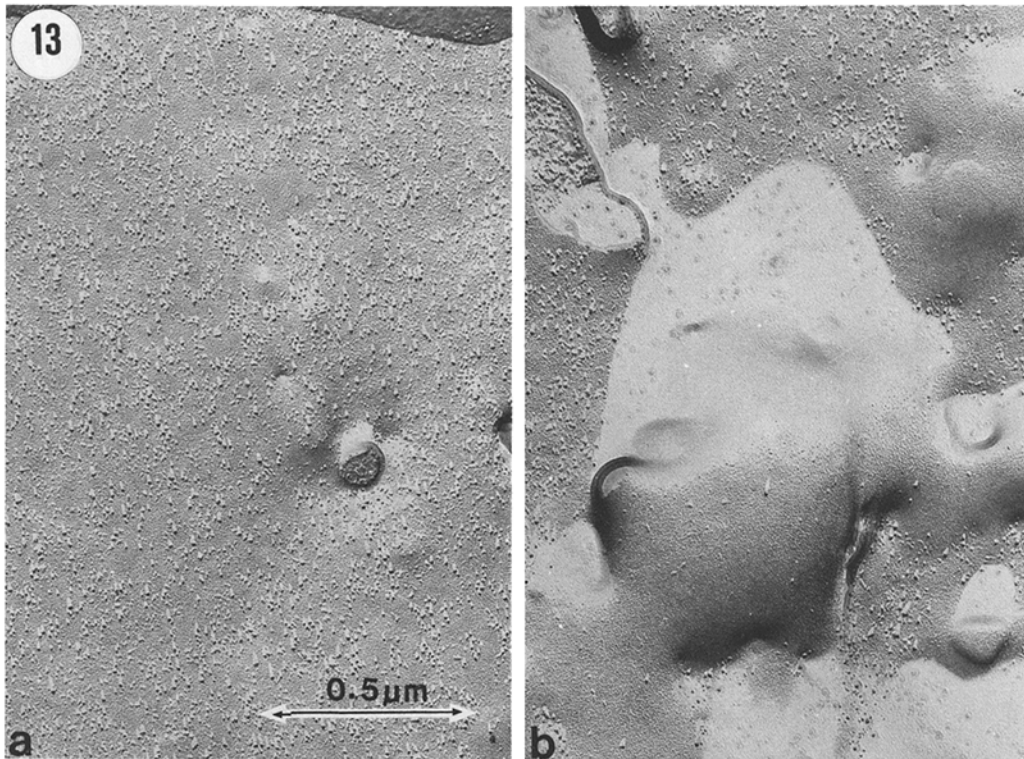
FIGURE 13   Freeze-fracture replica of capped rabbit granulosa cell illustrating the P-face structure of noncapped (a) and capped (b) areas of the membrane. Note the presence of large, smooth membrane domains in the capped membrane. (a and b) × 52,000.

cytes to the divalent cation ionophore A23187 results in the disruption of surface caps. While their suggestion that the contraction of cortical MF mediates both the formation and maintenance of surface caps is deserving of further attention, it will be of equal importance to establish both the nature and extent of MF attachment to membrane receptors induced to form caps. In the present study, few direct connections between MF and the membrane were observed during the capping process. Since the CABS are aggregated into large patches, it would seem unlikely that each receptor is linked to a single actin filament. More likely, perhaps, is the idea that domains of CABS patches are fastened by relatively few connections to the MF ring subtending the membrane. This structure may then contract centrally and draw CABS patches to the cell center where intermixing would result in the formation of a single cap.

While our data suggest that MF may actively regulate Con A receptor movements during capping, the role of MT in this process is more diffi-

cult to resolve. Capping experiments conducted on cultures exposed to cold or the antimicrotubule agents colchicine or vinblastine show that the disruption of cytoplasmic MT does not impair the ability of granulosa cells to cap. However, under these conditions caps assemble at peripheral locations on the cell surface, suggesting that MT have an orienting or directing influence on the flow of receptor complexes. A similar role for MT has been invoked by Berlin et al. (4), Oliver et al. (17), and Ukena and Berlin (31) who demonstrated that the ability of cells to selectively include or exclude various surface components from membrane internalized during phagocytosis was abolished by colchicine. Unfortunately, very little is known about the nature of the association between MT and membranes under physiological conditions, although recent evidence has been obtained to support the idea that these components may interact under experimental situations (3).

It is significant that Con A capping on certain cell types requires the drug-induced disruption of
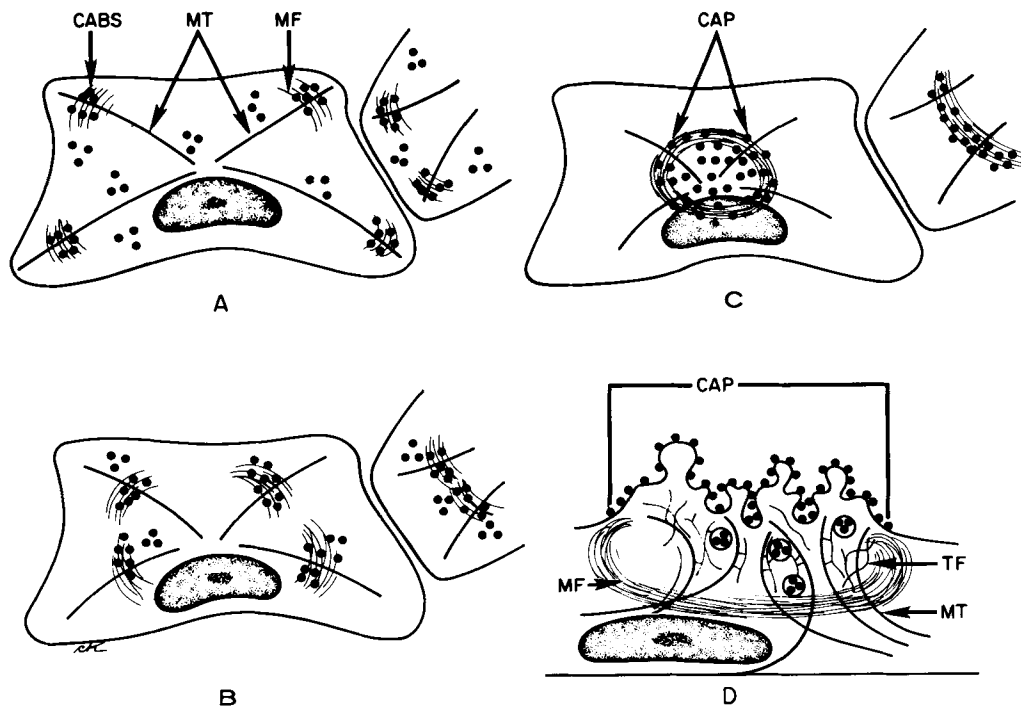
FIGURE 14   Diagram summarizing the changes in the distribution of cytoskeletal elements during Con A cap formation on ovarian granulosa cells. The topographical distribution of Con A-binding sites (black circles) on the cell surface during clustering (A), patching (B), and capping (C) has been superimposed upon the MT and MF network at comparable stages. Groups of MF (A) are depicted as becoming organized into a circular structure (B) which ultimately subtends the surface cap (C). MT are envisioned to orient the MF during this process and are themselves drawn inward during this process. Fig. 14 D represents a lateral view of the capped area during endocytosis of receptor complexes. MT and TF coexist within the MF ring and frequently interconnect endocytotic vesicles in transit to the juxtanuclear area of the cytoplasm.

MT (10, 18), whereas in our studies cap formation occurs spontaneously in response to Con A treatment. Although the mechanism underlying the different mobile behavior of Con A receptors on various cell types is not understood, it is apparent that Con A binding to the surface of the human polymorphonuclear leukocyte induces MT assembly (13). Thus, a better understanding of the mechanism whereby MT restrict receptor movements will depend in part on the elucidation of the cytoplasmic changes that occur as a consequence of lectin binding.

In summary, a controlled series of molecular rearrangements in membrane and cytoplasmic structure accompanies the Con A-induced formation and internalization of caps on cultured ovarian granulosa cells. The data provide morphological support for the idea that cytoplasmic MT and MF influence the mobility and topography of lec-

tin receptors associated with the cell surface (4, 9, 16, 27). It is clear that MF play an active role in the redistribution of surface receptors during capping and, further, that MT have an orienting influence on the direction of MF-mediated movements of the receptors. It is suggested that the modulation of cell surface receptor mobility by cytoskeletal elements is based upon coordinated structural and functional interactions between MT and MF and, perhaps, TF.

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