

Acta Medica Okayama

Volume 40, *Issue* 6

1986

Article 4

DECEMBER 1986

Fluorescence and electron microscopic study of intracellular F-actin in concanavalin A-treated and cytochalasin B-treated Ehrlich ascites tumor cells.

Sadahiro Watanabe*

Yoshiki Takehara[†]

Yoshitaka Fujii[‡]

Eiji Okimasu**

Yasunori Moromizato^{††}

Junzo Sasaki^{‡‡}

*Okayama University,

[†]Okayama University,

[‡]Okayama University,

**Okayama University,

^{††}Okayama University,

^{‡‡}Okayama University,

Fluorescence and electron microscopic study of intracellular F-actin in concanavalin A-treated and cytochalasin B-treated Ehrlich ascites tumor cells.*

Sadahiro Watanabe, Yoshiki Takehara, Yoshitaka Fujii, Eiji Okimasu, Yasunori Moromizato, and Junzo Sasaki

Abstract

To investigate the involvement of actin filaments in concanavalin A (Con A)-induced capping and cytochalasin B (CB)-induced zeiotic knob migration, the distribution of F-actin was studied in Con A-treated and CB-treated Ehrlich ascites tumor cells (EATC) by fluorescence microscopy using heavy meromyosin conjugated with a fluorescent dye, N-(7-dimethylamino-4-methylcoumarinyl) maleimide, (DACM-HMM). In non-treated cells, the diffuse fluorescence of DACM-HMM was observed in the cytoplasm, particularly intensely under the plasma membrane and around the nucleus. In Con A- and CB-treated cells, the fluorescence was seen at Con A-induced-capped and CB-induced-knob-accumulated regions. This fluorescence was more intense in CB-treated cells. To study the actin filaments in these fluorescent regions more clearly, the soluble components of the cells were eliminated by treatment with Triton X-100 or saponin solution containing a low concentration of glutaraldehyde, and the detergent-treated and saponin-treated cells were observed under a transmission electron microscope. Concentrated actin filaments were observed directly beneath the Con A-induced capping area and CB-induced zeiotic knob-accumulation area. The area of concentrated actin filaments appeared to correspond to the electron dense area observed in the identical region in the cells fixed without detergent treatment. More actin filaments were observed in CB-treated cells than in Con A-treated ones.

KEYWORDS: DACM-HMM, Ehrlich ascites tumor cells, concanavalin A, cytochalasin B, actin, capping

*PMID: 3825593 [PubMed - indexed for MEDLINE]

Copyright (C) OKAYAMA UNIVERSITY MEDICAL SCHOOL

Fluorescence and Electron Microscopic Study of Intracellular F-Actin in Concanavalin A-treated and Cytochalasin B-treated Ehrlich Ascites Tumor Cells

Sadahiro Watanabe, Yoshiki Takehara, Yoshitaka Fujii, Eiji Okimasu, Yasunori Moromizato and Junzo Sasaki

Department of Anatomy, Okayama University Medical School, Okayama 700, Japan

To investigate the involvement of actin filaments in concanavalin A (Con A)-induced cap formation and cytochalasin B (CB)-induced zeiotic knob migration, the distribution of F-actin was studied in Con A-treated and CB-treated Ehrlich ascites tumor cells (EATC) by fluorescence microscopy using heavy meromyosin conjugated with a fluorescent dye, N-(7-dimethylamino-4-methylcoumarinyl)maleimide, (DACM-HMM). In non-treated cells, the diffuse fluorescence of DACM-HMM was observed in the cytoplasm, particularly intensely under the plasma membrane and around the nucleus. In Con A- and CB-treated cells, the fluorescence was seen at Con A-induced-capped and CB-induced-knob-accumulated regions. This fluorescence was more intense in CB-treated cells. To study the actin filaments in these fluorescent regions more clearly, the soluble components of the cells were eliminated by treatment with Triton X-100 or saponin solution containing a low concentration of glutaraldehyde, and the detergent-treated and saponin-treated cells were observed under a transmission electron microscope. Concentrated actin filaments were observed directly beneath the Con A-induced capping area and CB-induced zeiotic knob-accumulation area. The area of concentrated actin filaments appeared to correspond to the electron dense area observed in the identical region in the cells fixed without detergent treatment. More actin filaments were observed in CB-treated cells than in Con A-treated ones.

Key words : DACM-HMM, Ehrlich ascites tumor cells, concanavalin A, cytochalasin B, actin, capping

It has been proposed that intracellular microfilaments are linked to plasma membrane components and that they controll transmembranously the mobility of cell surface proteins (1). In fact, microfilaments attached to isolated membrane fractions has been observed electron microscopically (2, 3). Recently, on the mechanism of surface receptor redistribution, a few models have been proposed in which the role of the cytoskeleton is discussed (4-6). In a preceding paper, we reported the morphology of Con

A-induced receptor redistribution (cap formation) in EATC (7). This capping was accompanied by the grouping of microvilli (cell shape change) and by a slight concentration of microfilaments directly beneath the cap region. At a later stage, endocytosis of the Con A receptor complex was also observed. CB has been used to investigate the participation of actin filaments in Con A cap formation in EATC. CB had only a little effect on Con A-induced cap formation itself (8, 9), but caused drastic changes

on the cell surface. At an early stage, zeiotic knobs appeared around the cell surface, and at a later stage, they migrated to one pole of the cell resulting in an asymmetric shape similar to Con A-induced capping cells (8-12). In addition, when the EATC were treated with CB and Con A alternately, CB-induced knobs migrated to the capped region in Con A treated cells while Con A-induced grouping of microvilli occurred at the accumulated knob region in CB-treated cells (9). The same effect was achieved by substituting Con A with cationized ferritin (10, 11).

The response of EATC to some drugs such as Con A and CB is very rapid, and the morphological changes accompanying these responses seem to be closely correlated to the intracellular rearrangement of cytoskeletal components (for a review see reference 6). However, it seems that differences may exist in the effect of these drugs on different cell types. Therefore, the present study was designed to investigate the intracellular cytoskeletal changes during Con A-induced cap formation and CB-induced zeiotic knob migration in EATC. For this purpose, the distribution of F-actin within the cells was studied by fluorescence microscopy using DACM-HMM, and then studied by electron microscopy in which the F-actin was directly observed using cytoskeletons prepared by treatment with the detergents.

Materials and Methods

Cells. Ehrlich ascites tumor cells (EATC) harvested from the abdomen of Swiss mice 6-10 days after ascites cell inoculation (7) were washed three times with Dulbecco's phosphate-buffered saline (PBS).

Drugs. Concanavalin A was extracted and purified from jack bean powder (13). N-(7-dimethylamino-4-methylcoumarinyl) maleimide (DACM) was purchased from Wako Pure Chemical Industries,

Ltd., and cytochalasin B (CB) was purchased from Nakarai Chemical Co. Other chemicals were all of analytical grade.

Preparation of DACM-HMM. Tryptic myosin fragments, heavy meromyosin (HMM), were prepared from rabbit skeletal muscle according to the methods of Yagi and Yazawa (14) and were conjugated with DACM according to the method of Namihisa *et al.* (15).

Fluorescence microscopy. Fluorescent probing of the cells was performed principally according to the method of Namihisa *et al.* (15). Cells (2×10^6 cells/ml) were preincubated at 37°C for 15 min in PBS and incubated with Con A (10 µg/ml) or cytochalasin B (10 µg/ml) at 37°C for 15 min (7). After the incubation, a few drops of cell suspension were placed on a slide glass for 30 sec and air dried. The slide glass was fixed in absolute acetone at 0°C for 5 min, then washed with a washing solution (0.1 M KCl, 5 mM MgCl₂, 6 mM sodium phosphate buffer, pH 7.4) and stained with a few drops of DACM-HMM (2-4 mg/ml) for 1-3 h at room temperature in a moist chamber. Cells were washed with the washing solution for more than 3 h in an ice bath. As a control, DACM-bovine serum albumin (DACM-BSA) or DACM was used instead of DACM-HMM. The cells were observed with a fluorescence microscope (Olympus BH-RFL) with a V filter (450 nm) as an excitation filter and a cutting filter which eliminates waves below 475 nm. Photos were taken with Kodak Tri-X film.

Scanning electron microscopy. As described previously (8), Con A- or CB-treated cells were fixed with 2.5% glutaraldehyde solution and placed on a poly-L-lysine coated slide glass. The cells were dehydrated in a graded ethanol series, immersed in isoamyl acetate and dried by the critical point drying method. The specimens were coated with Pt-Pd and viewed under a scanning electron microscope (Hitachi HHS-2R) operated at 20 kv.

Transmission electron microscopy. Cells were fixed with 2.5% glutaraldehyde solution, postfixed with 1% OsO₄, dehydrated in an acetone series, and embedded in Spurr's low viscosity embedding medium. Thin sections were stained with uranyl acetate and lead citrate, and observed under a transmission electron microscope (JEM 100-CX) operated at 80 kv.

Treatment of Triton X-100 or saponin. Con A-treated and CB-treated cells were harvested by centrifugation, and resuspended (2×10^6 cells/ml) in 0.2% Triton X-100 or 0.2% saponin containing 0.1% glutaraldehyde in PBS. The concentrations of Triton X-100 and saponin were determined by the Trypan blue dye exclusion test. EATC in 0.1% or less of Triton excluded the dye. In order to preserve the cell morphology as much as possible, the lowest concentration of the detergent for preparing cytoskeletal shells was determined to be 0.2%, and mild fixation with 0.1% GA was also done at the same time. After incubation at 37°C for 5 min, cells were further fixed with 2.5% glutaraldehyde containing 0.1% tannic acid and postfixed with 1% OsO_4 . Thin sections were obtained as described above. For HMM staining, HMM was added to the cell suspension and incubated for 1 h before fixation with 2.5% glutaraldehyde.

Results

The surface of EATC, which is normally covered with evenly distributed long and thin microvilli, changed characteristically

after Con A and CB treatments as reported previously (8). Con A induced the grouping of some of the microvilli to one pole of the cell where the Con A receptors were capped (Fig. 1a). CB caused zeiotic knob formation on the cell surface. These knobs migrated to one pole of the cell resulting in the formation of a protrusion consisting of many zeiotic knobs as the incubation time increased (Fig. 1b). Remaining short microvilli were seen in the same area as zeiotic knobs, and the other hemisphere of the cell was completely devoid of microvilli (Fig. 1b).

These surface changes, which were accompanied by intracellular cytoskeletal changes, were studied by fluorescence and electron microscopy. Specific binding of DACM-HMM to intracellular actin was confirmed by the fact that DACM-BSA or DACM alone did not stain the cells. In the non-treated cells, DACM-HMM were observed around the nucleus and under the plasma membrane, so that the contour of the nucleus was clearly observed in the central

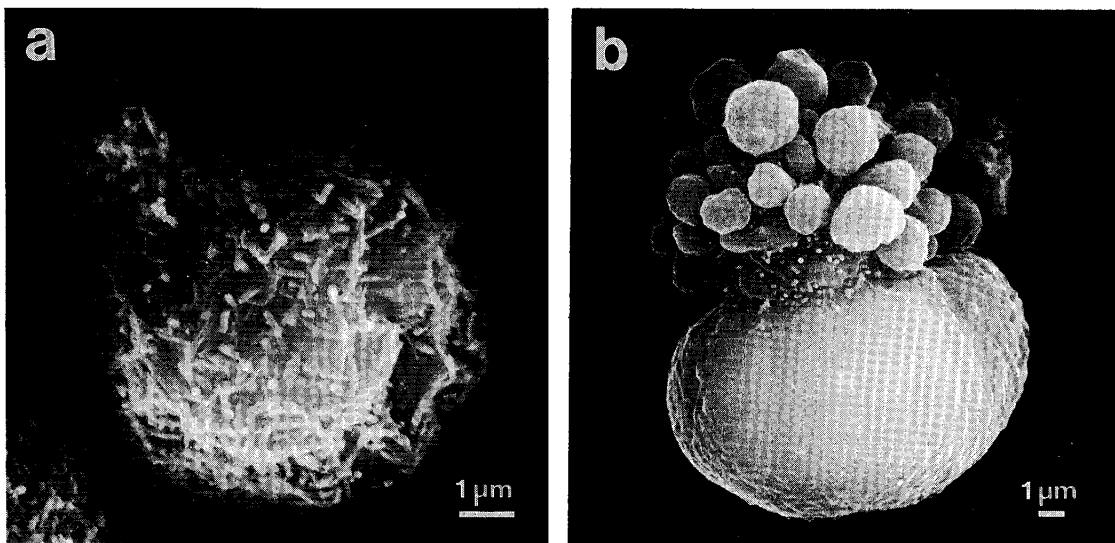


Fig. 1 Scanning electron micrographs of Ehrlich ascites tumor cells (EATC). (a) Cell treated with Con A (10 $\mu\text{g}/\text{ml}$) at 37°C for 15 min. (c) Cell treated with CB (10 $\mu\text{g}/\text{ml}$) at 37°C for 15 min.

region of the cell (Fig. 2a). Fine filaments encircling the nucleus were observed by transmission electron microscopy (Fig. 3a). A few filaments were observed irregularly under the plasma membrane. In Con A-treated cells, the ring pattern of DACM-HMM, which was observed around the nucleus of the control cells, was diminished, and condensation of DACM-HMM was observed at one pole of the capped cells (Fig. 2b, c). The difference in the shape of caps among these cells depended on the difference in the position of the cap on the cell and the degree of completion of the capping process. By transmission electron microscopy, a protrusion consisting of microvilli appeared at one pole of the cells (Fig. 3b). A slightly electron dense area was observed directly beneath the grouped microvilli (Fig. 3c). In CB-treated cells, bright fluorescence of DACM-HMM was observed in most cells at the protrusion of zeiotic knobs (Fig. 2d). By transmission electron microscopy, an electron dense area was observed at the base of grouped zeiotic knobs (Fig. 3d). Ribosomes were completely excluded from this area and filamentous structures were observed in some of these areas.

To investigate further the intracellular change of actin filaments, soluble components in Con A- or CB-treated cells were

Fig. 3 Transmission electron micrographs of EATC without detergent treatment. (a) Perinuclear region of control cells. The many fine filaments which run along the nucleus (N) coincide with the fluorescent ring of DACM-HMM shown in Fig. 2a. (b) Con A-induced capped region. Many microvilli group to form a protrusion. At higher magnification of this region (c), a slightly dense amorphous region (arrows) was observed. (d) CB-induced zeiotic knob grouped region. A slightly dense amorphous region (arrows) was observed under the protrusion of zeiotic knobs.

removed by treatment with Triton X-100 or saponin solution containing a low concentration of glutaraldehyde (GA). For the preparation of cytoskeletal shells of EATC, we first attempted to treat the cells with a low concentration of Triton or saponin alone, but this preparation was not so good for observing the overall cytoskeletal structure because of severe cell damage such as disappearance of the cell contour and leakage of cytoskeletal filaments themselves. Therefore, addition of a low concentration of GA to the detergent solution was tested, and better morphology of the cytoskeletal shell was found to be preserved by treating the cells with 0.1% GA and 0.2% Triton or saponin. The cytoskeletal shells thus obtained were fixed with 2.5% GA containing 0.1% tannic acid. Some preparations were stained with HMM before the fixation with 2.5% GA and 0.1% tannic acid in order to

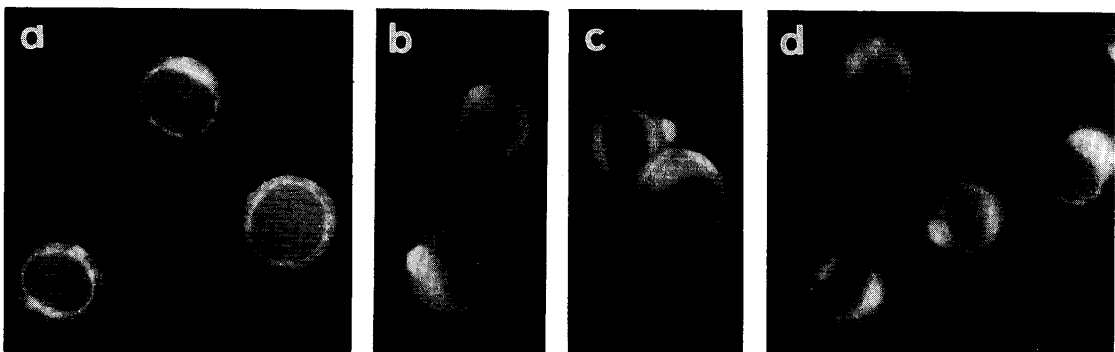
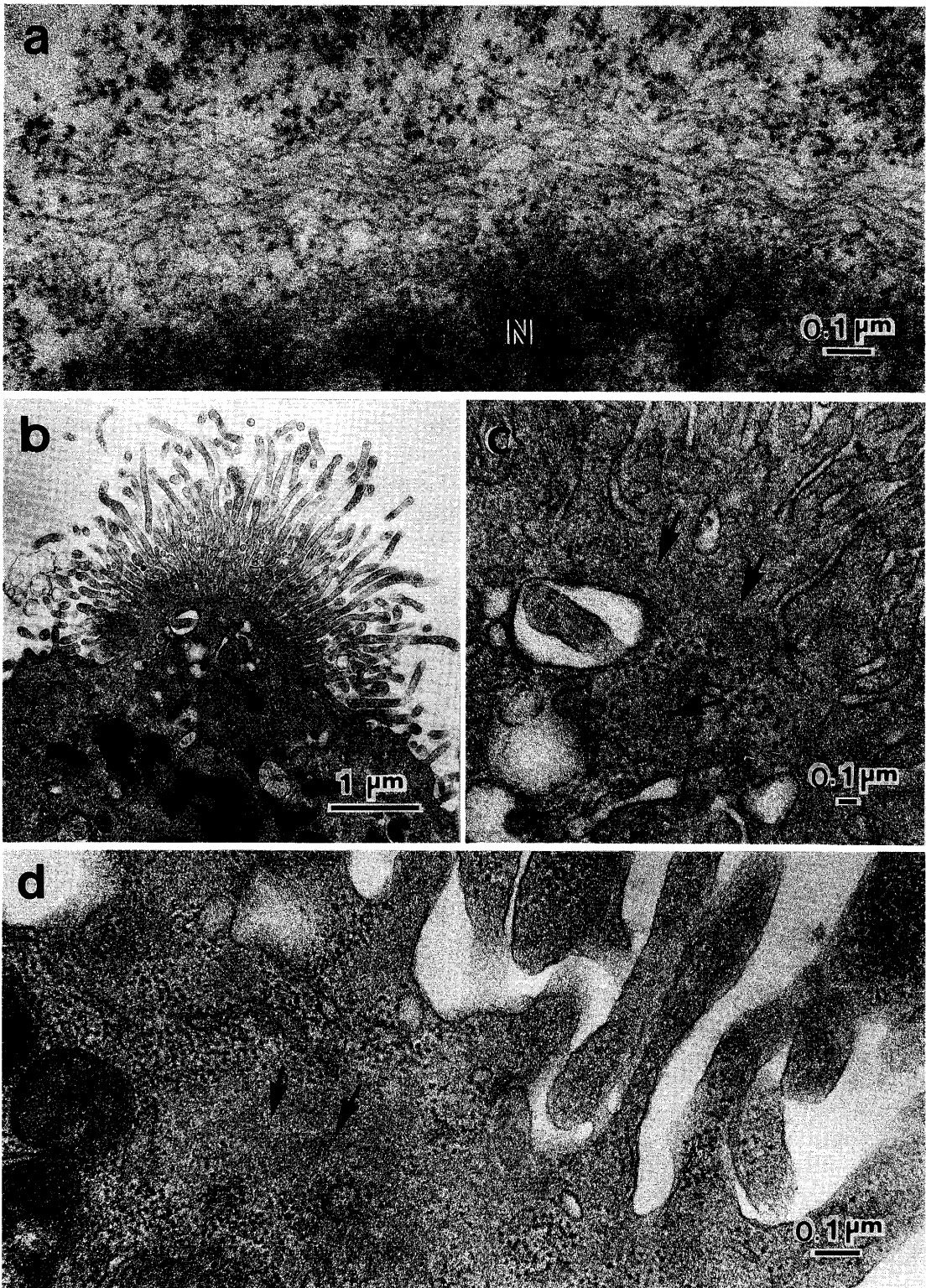


Fig. 2 Fluorescence micrographs of DACM-HMM impregnated EATC. (a) Control cells. (b, c) Cells treated with Con A as in Fig. 1a. (d) Cells treated with CB as in Fig. 1b. ($\times 330$)



confirm the existence of F-actin. Even after these treatments, Con A-induced capped areas and CB-induced knob migration areas could be detected easily by transmission electron microscopy, because the characteristic protrusion remained fairly intact (Fig. 4a and Fig. 5a). At the base of these protrusions, fine filaments were clearly observed (Fig. 4b and Fig. 5b), while perinuclear and submembranous filaments, which were observed in control cells, were almost disappeared. These results correspond with the results obtained by fluorescence microscopy shown in Fig. 2. The intracellular features of zeiotic knob grouped areas and Con A cap areas were somewhat different between Triton-treated and non-treated cells. Many filaments, which were not so obvious in non-treated cells, appeared in Triton-treated cells. These filaments were typical in the case of CB-treated cells as shown in Fig. 3d and Fig. 5b. These filaments were shown to be F-actin by the decoration of filaments by HMM (Fig. 4c, d and Fig. 5c, d). As can be seen in Fig. 5b, more filaments gathered in CB-treated cells than in Con A-treated cells (Fig. 4b). Actin filaments were also seen in the knobs themselves.

Discussion

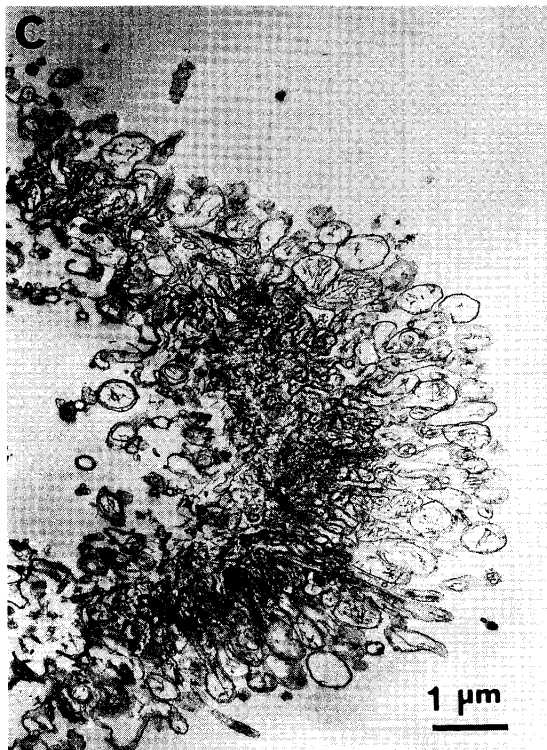
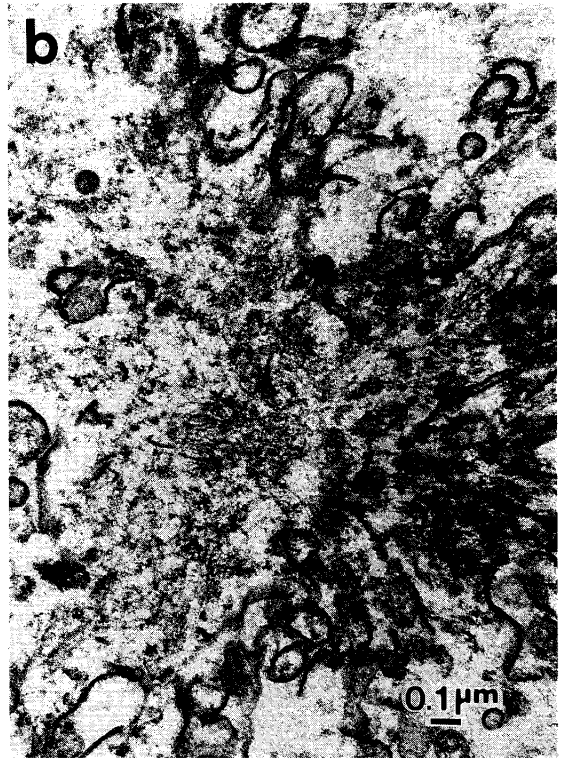
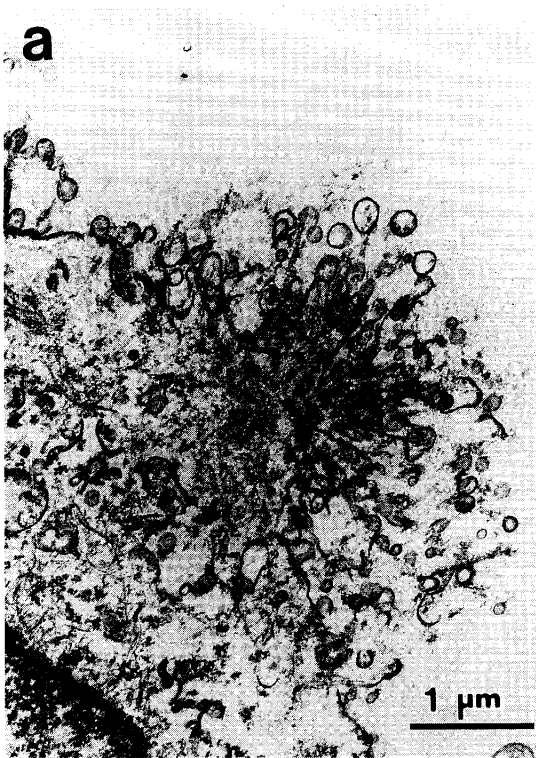
As reported previously, Con A-treated and CB-treated EATC show a similar surface morphology (cell asymmetry); Con A cap and grouped zeiotic knobs (7-9, 11). It is generally believed that these surface changes are accompanied by microfilament reorganization within the cells. There are many reports concerning the cytoskeletal changes of Con A treated cells (for a review, see references 5, 6). The present study is focused on the relationship between the Con A capping and CB-induced knob grouping, and cytoskeletal changes of Con A-treated

Fig. 4 Transmission electron micrographs of cytoskeletal shells of Con A-treated EATC. Con A-treated cells were treated with either Triton X-100 (a, b) or saponin (c, d) containing 0.1% GA as described in Methods. (a) The Con A-induced capped region was easily identified by the existence of a protrusion which consisted of grouped microvilli. (b) Higher magnification of the capped region. Filamentous structures were observed under the cap region. (c) HMM decorated Con A capping. (d) Higher magnification of (c). Actin filaments with arrowhead structures (HMM) were seen under the cap.

and CB-treated EATC were observed using DACM-HMM and detergents.

In our experience, cells adhering either to a substratum or to other cells seemed to be more resistant to detergent than free cells suspended in medium. If free cells were treated with detergent alone (for example, 0.2% Triton X-100) without fixative, the overall structures of the cells were disrupted and the orientation of cytoskeletal components was disordered. This may be due to the lack of an intercellular or cell-substratum adhesion site. On the other hand, the cytoskeletal system of cells which are grown on a substratum seems to be remarkably resistant to detergents, and the cytoskeleton of these cells can easily be observed by treatment with detergent alone (16). The treatment with the mixture of detergent and low concentration of GA overcomes these problems for cytoskeletal shell preparation of free cells. The same treatment was used for the immunoelectron microscopic preparations, in which the incorporation of antibody into the cells was required before the fixation (17, 18). We think that this technique will be widely used especially for the observation of the free cell cytoskeleton.

By this technique, some interesting features of intracellular microfilaments were observed. Intracellular microfilaments were gathered under the Con A cap and CB-induced zeiotic knob migration area. This area corresponded to the electron dense



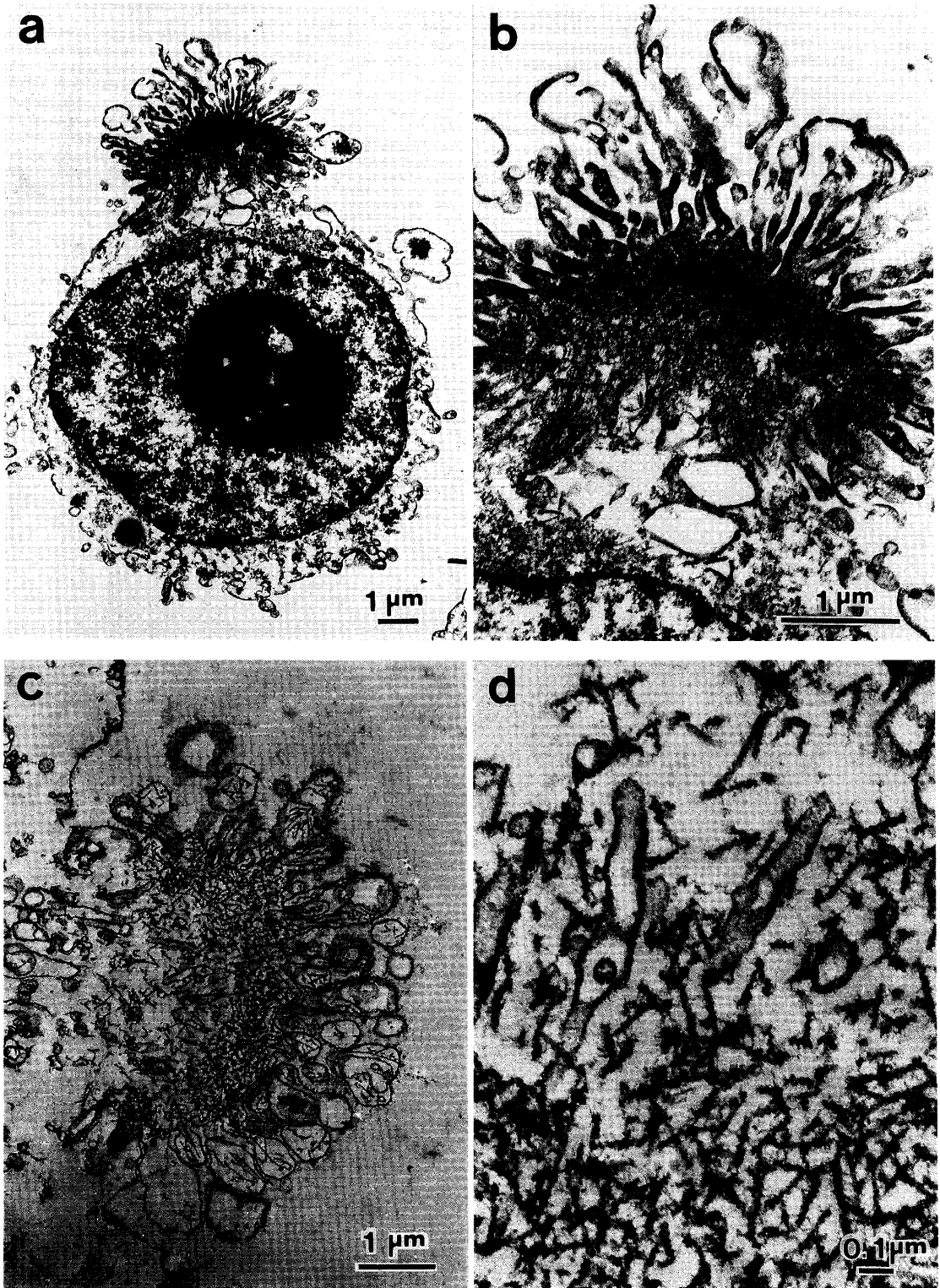


Fig. 5 Transmission electron micrographs of cytoskeletal shells of CB-treated EATC. CB-treated cells were treated with either Triton X-100 (a, b) or saponin (c, d) containing 0.1% GA as in Fig. 4. Micrographs (c) and (d) show the results of HMM treatment. Many more filaments were observed under the CB-induced zeiotic knob grouped region than the Con A-induced capped region (Fig. 4a, b).

area which was observed in the cells fixed without detergent treatment. We considered this electron dense area to be composed mainly of actin, which could be seen in its filamentous form after removal of the surrounding cytoplasmic materials by detergent treatment. In addition, from the observation of the cytoskeletal shell, it was demonstrated that more actin filaments accumulated directly beneath the CB-induced knob grouped area than beneath the Con A cap. The number of accumulated filaments may correlate closely with the degree of microvilli accumulation; the microvilli of CB-treated cells accumulated completely in the area of the grouped zeiotic knobs (Fig. 1c), while some of those of Con A-treated cells remained distributed over the cell surface. We postulated that the microfilaments in the microvilli were connected to the cytoplasmic microfilaments and that the accumulation of microvilli on the cell surface was associated with the accumulation of microfilaments in the cytoplasm.

On the other hand, it is generally believed that CB binds to the barbed end of F-actin and inhibits addition of monomeric actin to this end, and thus decreasing the total F-actin content (19-23). Therefore, it was expected that actin filament content in EATC should have been decreased after CB treatment. It was also expected that the actin filament content in Con A-treated cells should be higher than that of CB-treated cells, because actin polymerization during Con A-induced capping was observed (24). Our present morphological data, however, showed results opposite to these expecta-

tions.

There are two proposed mechanisms of Con A receptor capping, one in which participation of microfilaments is direct (4, 6) and one in which it is indirect (5). Oliver *et al.* proposed a model in which microfilament-receptor linkages were not directly involved in propelling the receptors (5). This model involves initially the recruitment of microfilaments which produce a characteristic (asymmetric) change in cell shape and the resulting microfilament-membrane interaction (a kind of asymmetric tension) acting inward and along the cell surface leads to the generation of wave which in turn leads to receptor movement into protuberance, the region of accumulated microfilaments (surfboard mechanism). On the other hand, Bourguignon proposed the presence of X protein within the plasma membrane which linked surface receptors to microfilaments, and thus the cytoskeleton has a direct role in receptor redistribution (6). The mechanism of CB-induced knob grouping and the effect of CB on Con A receptor capping have not been clarified yet. Sundqvist *et al.* showed that CB caused blebs and co-capping of actin and membrane components in the transformed cells (25), whereas, Glenn *et al.* reported that CB did not cause the redistribution of membrane components (Con A receptors) (26). Mori *et al.* found that CB treatment itself did not cause the redistribution of Con A receptors in EATC (9).

In spite of this controversy, we consider that these two processes, Con A cap formation and CB knob migration, are closely linked, because, when CB-treated cells with knobs grouped at one pole were incubated with Con A, the Con A-receptor complex also gathered to the base of these knobs, and conversely, when cells already having a Con A cap were treated with CB, zeiotic knobs gathered at the Con A cap area (9,

10). We have two speculations about these phenomena. One is that microfilaments used for Con A capping may be different from those used for CB-knob migration. The other is that the cells gained polarity by the first treatment with Con A or CB, and this polarity caused the second migration of CB-knobs or the Con A-receptor complex to this polarized region. This polarity may be closely correlated to the "asymmetry" proposed by Oliver *et al.*(5). Their model does not resolve the mechanisms of all events occurred in EATC completely, however, it explains how Con A receptors redistribute into regions of existing microfilament accumulation. So, we are now interested in "the surfboard mechanism" proposed by them.

Acknowledgments. The authors wish to express our gratitude to Prof. Nagayasu Ohtsuka for his continued support throughout the course of this study. We are indebted to the late Dr. Tetsuji Kaneshige, Mr. Hiroaki Kaneshige and Mr. Juni Aoki, Central Laboratory of Okayama Chuo Hospital, for the use of the scanning electron microscope.

References

- Nicolson GL: Transmembrane control of the receptors of normal and tumor cells. *Biochim Biophys Acta* (1976) **457**, 57-108.
- Condeelis J: Isolation of concanavalin A caps during various stages of formation and their association with actin and myosin. *J Cell Biol* (1979) **80**, 751-758.
- Phillips MJ, Oda M, Yousef IM and Funatsu K: Effect of cytochalasin B on membrane-associated microfilaments in a cell-free system. *J Cell Biol* (1981) **91**, 524-527.
- Loor F: Cell surface-cell cortex transmembranous interactions with special reference to lymphocyte functions; in *Cytoskeletal Elements and Plasma Membrane Organization*, Poste G and Nicolson GL eds, Elsevier/North-Holland Biomedical Press, (1981) pp 253-335.
- Oliver JM and Berlin RD: Mechanism that regulate the structural and functional architecture of cell surface. *Internat Rev Cytol* (1982) **74**, 55-94.
- Bourguignon LYW and Bourguignon GJ: Capping and cytoskeleton. *Int Rev Cytol* (1984) **87**, 195-224.
- Sasaki J, Kanda S, Otsuka N, Nakamoto S and Mori M: Morphology of cap formation in Ehrlich ascites tumor cells induced by concanavalin A. *Cell Struct Funct* (1979) **4**, 1-10.
- Sasaki J, Imanaka M, Watanabe S, Otsuka N, Nakamoto S and Mori M: A scanning electron microscopic study of the two step effect of cytochalasin B on Ehrlich ascites tumor cells. *Acta Med Okayama* (1981) **35**, 197-204.
- Mori M, Nakamoto S, Kirizuka K, Sadahira Y, Awai M, Seno S, Sasaki J and Utsumi K: "Pseudocap" formation in Ehrlich ascites tumor cells induced by cytochalasin B. *Acta Med Okayama* (1982) **36**, 483-486.
- Sasaki J, Imanaka M, Watanabe S, Mori M, Nakamoto S, Kirizuka K and Otsuka N: Cationized ferritin-induced cap formation and the effect of cytochalasin B. *Acta Med Okayama* (1982) **36**, 307-312.
- Imanaka M: Cytochalasin B-induced morphological changes in Ehrlich ascites tumor cells. *Okayama Igakukai Zasshi* (1983) **95**, 41-59 (in Japanese).
- Nakamoto S: Movement of plasma membrane proteins of Ehrlich ascites tumor cells in relation to cap formation induced by concanavalin A. *Acta Med Okayama* (1981) **35**, 221-234.
- Agrawal BBL and Goldstein IJ: Protein-carbohydrate interaction. VI. Isolation of concanavalin A by specific adsorption on cross-linked dextran gels. *Biochim Biophys Acta* (1967) **147**, 262-271.
- Yagi K and Yazawa Y: Isolation and characterization of a subfragment of myosin A. *J Biochem* (1966) **60**, 450-457.
- Namihisa T, Yamura K, Saifuku K, Imanari H, Kuroda H, Kanaoka Y, Okamoto Y and Sekine T: Fluorescent staining of microfilaments with heavy meromyosin labeled with N-(7-dimethylamino-4-methylcoumarinyl)maleimide. *J Histochem Cytochem* (1980) **28**, 335-338.
- Osborn M and Weber K: The detergent-resistant cytoskeleton of tissue culture cells includes the nucleus and the microfilament bundles. *Exp Cell Res* (1977) **106**, 339-349.
- Jones JR, Goldman AE, Yang H-Y and Goldman RD: The organizational fate of intermediate filament networks in two epithelial cell types during mitosis. *J Cell Biol* (1985) **100**, 93-102.
- Yang H-Y, Lieska N, Goldman AE and Goldman RD: A 300,000-mol-wt intermediate filament-associated protein in baby hamster kidney (BHK-21) cells. *J Cell Biol* (1985) **100**, 620-631.
- Hartwig JH and Stossel TP: Interaction of actin, myosin and actin-binding protein of rabbit pulmonary macrophages. III. Effect of cytochalasin B. *J Cell Biol* (1976) **71**, 295-303.
- Lin DC and Lin S: Actin polymerization induced by a motility-related high affinity cytochalasin binding complex from human erythrocyte membrane. *Proc*

- Natl Acad Sci USA (1979) **76**, 2345-2349.
21. Lin DC, Tobin KD, Grumet M and Lin S: Cytochalasins inhibit nuclei-induced actin polymerization by blocking filament elongation. *J Cell Biol* (1980) **84**, 455-460.
22. Brown SS and Spudich JA: Mechanism of action of cytochalasin: Evidence that it binds to actin filament ends. *J Cell Biol* (1981) **88**, 487-491.
23. MacLean-Fletcher S and Pollard TD: Mechanism of action of cytochalasin B on actin. *Cell* (1980) **20**, 329-341.
24. Rao KM and Varani J: Actin polymerization induced by chemotactic peptide and concanavalin A in rat neutrophils. *J Immunol* (1982) **129**, 1605-1607.
25. Sundqvist KG and Ehrnst A: Cytoskeletal control of surface membrane mobility. *Nature* (1976) **264**, 226-231.
26. Glenney JR Jr, Hixon DC and Walborg EF Jr: Inhibition of concanavalin A induced agglutination of Novikoff tumor cells by cytochalasins and metabolic inhibitors. *Exp Cell Res* (1979) **118**, 353-364.

Received: April 15, 1986

Accepted: October 1, 1986

Reprint request to:

Sadahiro Watanabe

Department of Anatomy

Okayama University Medical School

2-5-1 Shikata-cho

Okayama 700, Japan