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—— BRIEF NOTE ——

CATIONIZED FERRITIN-INDUCED CAP FORMATION AND THE EFFECT OF CYTOCHALASIN B

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Abstract. Cytochalasin B (CB) was added to Ehrlich ascites tumor cells previously labelled with cationized ferritin (CF) to study the linkage of surface molecules with the intracellular cytoskeletal structure. CF gathered to one pole of the cell and caused a protrusion consisting of the grouping of microvilli in the cell (cap formation) as observed in Concanavalin A-treated cells. Zeiotic knobs formed by CB around CF-treated cells were CF-free on the surface and they gathered to the CF-induced capping area after further incubation, suggesting a close relationship between surface molecules and the microfilaments which were action sites of cytochalasins.

Key words : cytochalasin B, Ehrlich ascites tumor cells, capping, zeiosis, cationized ferritin.

Redistribution of cell surface molecules is often accompanied by the change in the cytoskeletal system. The close relationship between surface molecules and intracellular microfilaments was shown by electron and fluorescent microscopy (1-3). Cytochalasin B (CB), one of the metabolites in mould, *Helminthosporium dematioideum*, has been used as a probe for many cellular activities (4). The drug causes zeiotic knobs to from on the cell surface (5). In the preceding paper, we reported that the effect of CB on Ehrlich ascites tumor cells was separated into two steps: zeiotic knob formation and their migration to one pole of the cell (6). In the present study, the relationship between the surface molecules and the site of action of CB is studied using cells previously labelled with cationized ferritin.

Materials and methods. Ehrlich ascites tumor cells were harvested from the abdomen of Swiss mice 6-10 days after inoculation. Cells were washed with Dulbecco's phosphate buffer saline (7) by repeated centrifugation to remove contaminated red blood cells and suspended in the same solution. Cationized ferritin (CF) and cytochalasin B (CB) were purchased from Sigma Co. and CB was dissolved in dimethyl sulfoxide at 1 mg/ml.

Cells (2×10^6/ml) were preincubated at 37 ℃ for 10 min and incubated

with CF (50 μg/ml) for 15 min, then washed by centrifugation to remove excess CF and further incubated with CB (10 μg/ml) for 10 min. The reaction was stopped by the addition of an equal volume of 5% glutaraldehyde to the incubation medium. These cells were post-fixed with 1% Osmium tetraoxide in 0.1 M phosphate buffer (pH 7.4) and centrifuged in agar (8). Cell pellets were dehydrated in a graded acetone series and embedded in Epon. Thin sections (60-70 nm thick) were stained with uranyl acetate and lead citrate and examined in a JEOL 100CX electron microscope.

Results and discussion. When CF was added to fixed cells, CF particles bound uniformly on cell surface including microvilli membrane (Fig. 1), but when cells were incubated with CF at 37℃ for 15 min before fixation, CF gathered to one pole of the cell and induced a cap formation (Fig. 2). The cap area was larger than that of the Con A-cap and some cells had CF aggregates around the whole cell surface except on microvilli membrane. This cap formation was concomitant with the grouping of microvilli. In the cytoplasm, vacuoles containing CF granules were scattered under a cap. These findings were essentially identical to Con A-induced capping (2). Histone and other polycations induced phagocytosis of iron colloid added simultaneously (9, 10). Poly L-lysine also induced capping in lymphocytes (11) and macrophages (12). These results suggest that CF causes cross linking of the anionic sites on cell surface and induce capping. In the mitotic cells, CF did not induce capping and CF particles were observed around the cell surface as small clusters (Fig. 3). When the cells having a CF-induced cap were treated with CB (10 μg/ml) at 37℃ for 10 min, zeiotic knobs without CF granules were found near the CF-induced cap area (Fig. 4). As previously described, zeiotic knobs induced by CB first occurred around the cell surface and later migrated to one pole of the cell (6). The knob formation and migration by CB was also confirmed in CF-treated cells and in Con A-treated cells by the time course study. Knobs formed on Con A-treated cells were used to obtain the membrane fraction without Con A receptors (13).

The process of Con A-induced cap formation and CB-induced knob migration were morphologically similar (manuscript in preparation). Both processes were accompanied by microvilli changes and were inhibited under conditions of low temperature and in the presence of sodium azide. Recently, the distribution of F-actin was investigated in CF-, Con A- and CB-treated cells with a fluorescent material, N-(7-dimethylamino-4-methylcoumarinyl) maleimide (DACM)- heavy meromyosin (HMM) conjugate which specifically binds to F-actin. Fluorescent masses were seen most clearly in the cytoplasm under gathered zeiotic knobs of CB-treated cells. In Con A-treated cells, co-capping of actin with Con A receptors was observed as reported in other cell types (3). In CF-treated cells, fluorescence of the actin mass was weak and rare. These results suggest a close relationship between the degree of actin co-capping and the mode of cell surface

protrusion. Zeiotic knobs migrated even after ligand-induced capping and they always gathered in the capped area. This suggests that microfilaments used for ligand-induced capping may not be utilized for CB-induced knob migration although the two types of microfilaments are closely linked with each other. The present results suggest a close association between surface molecules and intracellular cytoskeletal components.

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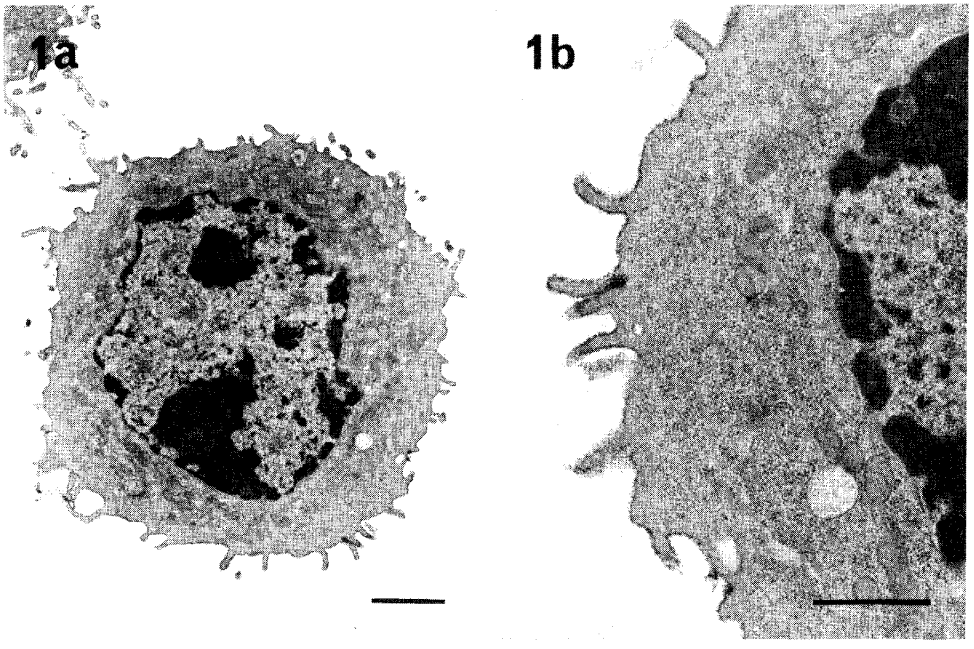
LEGENDS FOR FIGURES

Fig. 1. (a) Ehrlich ascites tumor cells without any treatments. Cells are incubated at 37 ℃ for 15 min, fixed with glutaraldehyde solution and then labelled with cationized ferritin (CF). Ferritin grains are distributed diffusely around the cell surface. Bar 2 μm. (b) High magnification of (a). Bar 1 μm.

Fig. 2. Ehrlich ascites tumor cells having CF-induced cap. Cells are incubated with CF at 37 ℃ for 15 min, then fixed. CF gathers to one pole of the cell concomitantly with the grouping of microvilli (cap formation). Bar 1 μm.

Fig. 3. (a) Ehrlich ascites tumor cells in mitotic phase. Cells are treated with CF before fixation. Cap formation is not observed in mitotic cells. CF grains are found diffusely on cell surface. Bar 2 μm. (b) High magnification of (a). Bar 1 μm.

Fig. 4. Ehrlich ascites tumor cells having both CF-induced cap and CB-induced zeiotic knobs. Cells are incubated with CF at 37 ℃ for 15 min, washed and further incubated with CB at 37 ℃ for 10 min. Knobs formed by CB are found to migrate to CF-induced cap area. Bar 2 μm.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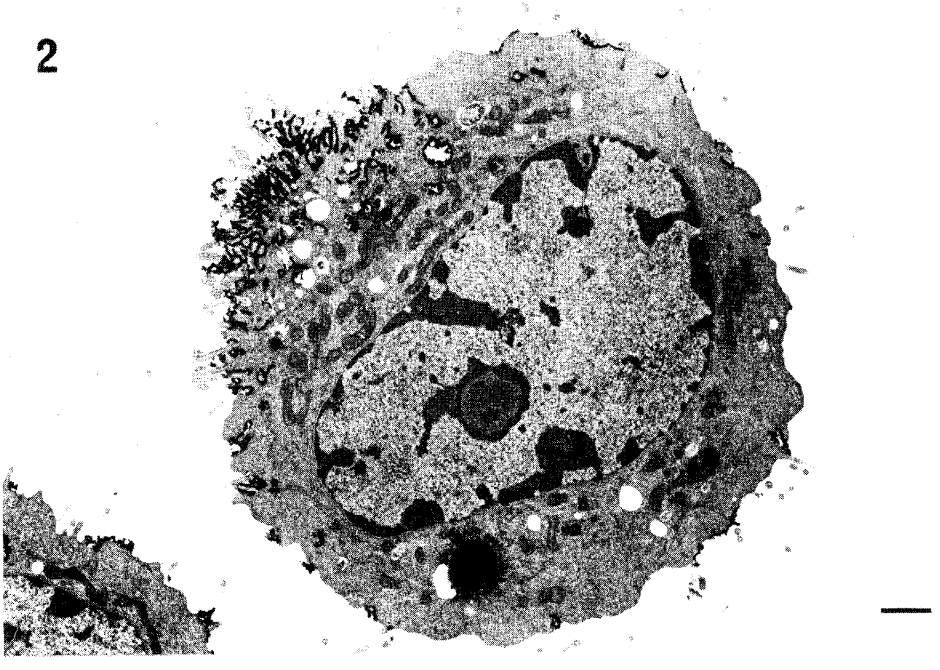
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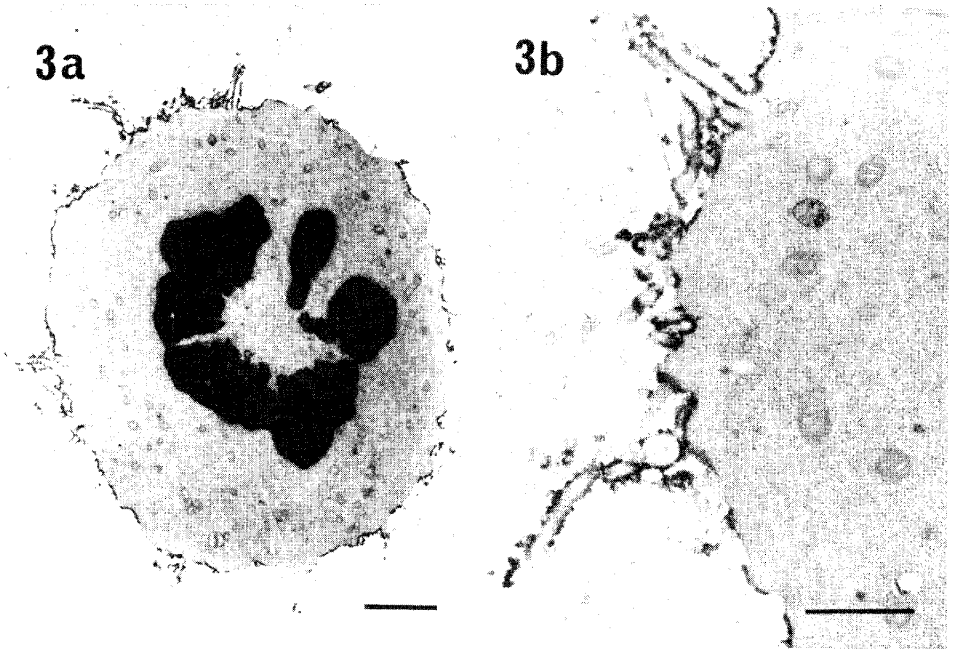
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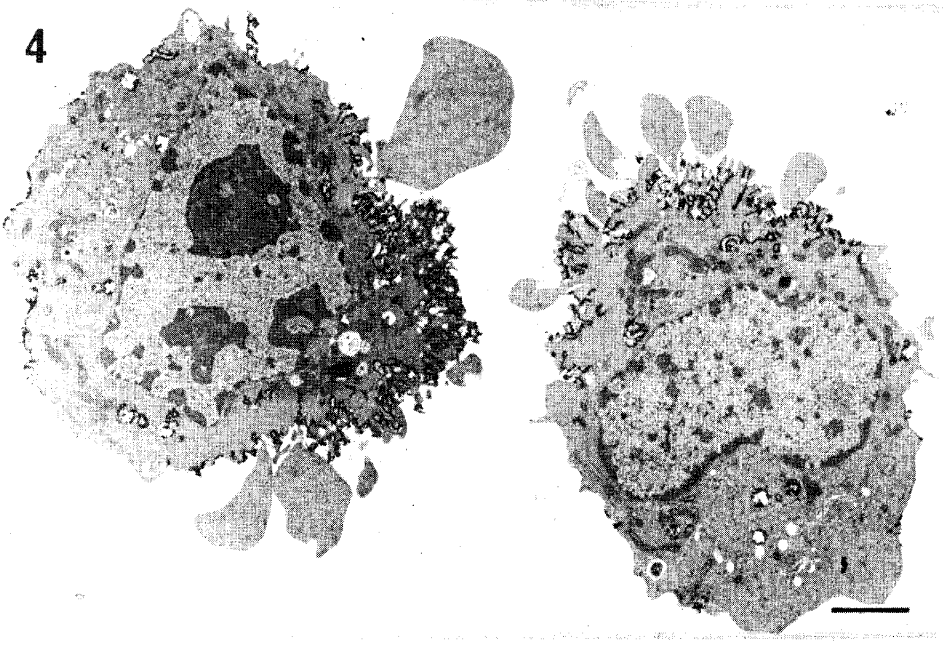
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