Possible role of nucleus–membrane interaction in capping of surface membrane receptors

(cap formation/ cytochalasin B/enucleation/cytoplasts/karyoplasts)

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ABSTRACT Interaction of multivalent ligands and cell surface receptors can induce redistribution of these receptors to form patches and caps. In this study, we have investigated the role of nucleus–membrane interaction in the capping of membrane components. Mouse L cells and leukemia EL4 cells were enucleated with the aid of cytochalasin B, yielding cytoplasts and karyoplasts. Capping of surface receptors was induced by allo- and hetero-immune sera followed by fluorescein-conjugated anti-IGG serum, or by the plant lectin concanavalin A. Capping could easily be induced in intact cells, but virtually no capping was detected in the nucleus-free cytoplasm. Interestingly, karyoplasts, which possess cell-membrane components but very little cytoplasm, could be easily induced to cap their surface antigens. Hence, cap formation of membrane components seems not to be an autonomous membrane process. The data suggest that interaction of surface membranes and inner cell components associated with the nucleus is involved in the movement of surface membrane receptors.

Singer and Nicolson (1) have proposed that membrane components are free to diffuse in the membrane lipid matrix. Rapid intermixing of surface antigens of hybridized cells (2), and lateral diffusion of membrane proteins (3), as well as aggregation of surface components into "patches" and "caps" (4-7), support the fluid mosaic theory of membrane structure (8). Nonrandom distribution of membrane components towards one pole of the cell (cap formation), generated by interaction with exogenous multivalent ligands such as lectins or antibodies, can be induced in many types of cells, but a few exceptions have been noted (4, 9). This inability of some cells to cap may be due to a rigid membrane structure, to a restricting cytoskeletal system (9-11), or to the lack of an active nucleus, as may be the case in erythrocytes. Nicolson and Painter (12) have suggested that the spectrin complex, which is associated with human erythrocyte membrane, controls transcellular mobility of membrane proteins. The role of a transmembranous system, composed of membrane determinants and intracellular fibrillar structures, in the modulation of cell surface activities has recently been discussed by Edelman (13), de Petris (14), and Nicolson (15). To evaluate the contribution of intracellular components to the capping process, we have studied the redistribution of surface antigens of cells enucleated with cytochalasin B. Cytochalasin B, a metabolite from Helminthosporium dematioides, has been shown to induce nuclear extrusion of mammalian cells, resulting in nucleus-free cytoplasmic cells and in karyoplasts (micrincells) (16, 17). By examining cap formation in the membranes of intact cells, cytoplasts, and karyoplasts, which are similar but surround different intracellular systems, information on intracellular control of capping can be obtained. The evidence presented in this paper indicates that capping of plasma membrane receptors by antibodies or lectins is not an autonomous membrane process and suggests that the nucleus or a nucleus-associated structure plays a role in cap formation.

MATERIALS AND METHODS

Animals and Cells. C57BL/6 (H-2b), AKR/J (H-2k), BALB/c (H-2d), and C3H/eB (H-2k) male mice (histocompatibility-2 antigen types in parentheses) and New Zealand rabbits were provided by the Weizmann Institute animal breeding center. Leukemia EL4 of C57BL/6 mice, obtained from J. Wunderlich, National Cancer Institute, Bethesda, Md., was maintained in ascites form by weekly transfer of 25 X 10^6 cells to C57BL/6 male mice. Mouse L cells, obtained from the laboratory of M. Revel of this Institute, were grown in vitro as monolayers in Waymouth's medium or in Dulbecco's modified Eagle's medium (DEM) supplemented with 10% heat-inactivated fetal calf serum (FCS) (Gibco) and antibiotics.

Immunizations and Reagents. Rabbit antisera to mouse cells was prepared by two biweekly intravenous injections, each of 1 X 10^6 EL4 cells, and bleeding 1 week after the second injection. BALB/c anti-EL4 (anti-H-2d) hyperimmune serum was prepared by 6 weekly intraperitoneal injections, each of 25 X 10^6 EL4 cells, and bleeding 1 week after the last injection. AKR/J anti-C3H/eB serum (anti-θ C3H) was prepared by 9 weekly intraperitoneal injections, each of 10 X 10^6 C3H/eB thymocytes. C57 anti-C3H/eB (anti-H-2k) hyperimmune serum was prepared by 6 weekly intraperitoneal injections each of 1 X 10^6 C3H/eB spleen cells. The fluorescein isothiocyanate (FITC)-labeled reagents goat anti-mouse 7S IgG (GAMG-FI) and goat anti-rabbit 7S IgG (GARG-FI) were obtained from Meloy, Springfield, Va. Guinea pig serum was used as a complement source. Phosphate-buffered saline (PBS) supplemented with 10% fetal calf serum (PBS-FCS) was used to dilute antiserum. Concanavalin-A-FITC (Con-A-FI) was obtained from Miles-Yeda, Rehovot, Israel, and colchicine from Ciba. Cytochalasin B (CB) was obtained from Aldrich, Milwaukee, Wisc., and a stock solution (2 mg/ml) was made in dimethyl sulfoxide.

Fluorescence Microscopy. Fluorescence was observed in a Zeiss reflected-light microscope equipped with a high-pressure mercury lamp (HBO-200), exciter filter 1 (BG-12) and barrier filters 44 and 50. Photographs were taken with a 55C Zeiss camera and Kodak Tri-X (400 ASA) film. Exposure time for fluorescence was 1–2 min.

Incorporation of Radiochemicals. [Methyl-3H]thymidine (1 mCi/ml) was obtained from the Nuclear Research Center, Negev, Israel; [3H]leucine (1 Ci/mmol) and Na_2^35ClO_3 (1 mCi/ml) were obtained from the Radiochemical Centre, Amersham, England. Four X 10^6 cytoplasts, karyoplasts, or
intact cells in 0.5 ml of Dulbecco's modified Eagle's medium (leucine-free) + 10% FCS were labeled with 5 μCi of [3H]leucine or 1 μCi of [methyl-3H]thymidine at 37°C in a CO2/air incubator for 2–7 hr. Incorporation of radioactivity into tri-chloroacetic-acid-insoluble material was determined in a Packard liquid scintillation counter. To determine Na14CrO4 incorporation, 10 to 30 × 106 cells were incubated in 1 ml of PBS-FCS containing 300 μCi of Na14CrO4 for 1 hr at 37°C, and then washed twice. Radioactivity was assayed in a Packard crystal scintillation counter.

**Enucleation.** EL4 and L cells were enucleated in the presence of cytochalasin B (10 μg/ml) according to the method of Wigler and Weinstein (18). Enucleated cells (cytoplasts) and enucleated particles (karyoplasts) were collected and allowed to regenerate for 60 min. Of 100 × 106 cells subjected to enucleation, up to 60 × 106 cytoplasts and at least 40 × 106 karyoplasts were recovered in a typical experiment. In a few experiments with L cells, the enucleation procedure of Prescott and Kirkpatrick (19) was followed. A summary of results obtained in over 20 different enucleation experiments performed with EL4 and L cells is given in Table 1. EL4 cytoplasts (Cyt-EL4) and L cytoplasts (Cyt-L) as well as EL4 or L cell karyoplasts (Kar-EL4, Kar-L) are smaller than the corresponding enucleated cells. All particles exhibit dye (trypan blue) exclusion ability, incorporate and retain Na14CrO4, a property that has been shown to be an indicator for cell membrane integrity [see Berke and Amos (20)]. The inability of cytoplasts and karyoplasts to incorporate significant amounts of [methyl-3H]thymidine into acid-insoluble material (DNA) is compatible with the extremely high degree of enucleation achieved in this study. Incorporation of [3H]leucine into acid-insoluble material (protein) was 23% in Cyt-L and 3.3% in Cyt-EL4, as compared to the corresponding intact cells. Both Cyt-EL4 and Cyt-L could be lysed by specific antiserum in the presence of complement, and their susceptibility to immune lysis was very close to that of intact cells, indicating a similar density of cell surface antigens. Adherent Kar-L attach to plastic surfaces over a limited surface area of the cell and spread out only very slightly. The results

**Mobility of cytoplast surface receptors**

To detect plasma membrane antigens, 4 × 106 cytoplasts or nucleated cells were incubated with 0.1 ml of antiserum, anti-H-2a, anti-H-2b, anti-β2, or rabbit anti-mouse, diluted in PBS-FCS, for 30 min on ice and then washed twice with PBS-FCS by centrifugation. Antibody-coated cells were then treated with fluorescein-conjugated goat anti-mouse globulin (GAMG-FI) or with conjugated goat anti-rabbit globulin (GARG-FI), diluted 1:10 in PBS-FCS, for 30 min on ice, washed once in the cold, and examined for cell-bound fluorescence. Ring type distribution of fluorescence on EL4, L cells, Cyt-EL4, and Cyt-L was detected with all antisera used. The staining was specific, as no fluorescence was observed in cells that had not been reacted with the appropriate antiserum prior to the addition of the fluorescence reagent. A similar degree of fluorescent staining and of susceptibility to lysis by antibody and complement (unpublished results) was observed for cytoplasts and intact cells, including similar densities of membrane antigens.

To induce redistribution of membrane antigens, the antibody-reacted cells were transferred from ice to 37°C and examined for patch and cap formation. The results show cap formation in whole cells, up to 50% capped cells in 60 min (Fig. 1B), but virtually no capping in cytoplasts (Fig. 1C). Clear patch formation was evident in cytoplasts and in intact cells that did not cap, as well as in intact cells that had been kept at 4°C (Fig. 1A). Prolonged incubation (up to 4 hr) of cytoplasts that had previously been reacted with antibody did not result in cap formation but rather in internalization of the label. Similar results were obtained, irrespective of the specific antiserum employed in the first antibody layer and of the fluorescent anti-globulin reagent used for the second antibody layer. In another set of experiments, it was found that treatment of L cells with Con-A-Fl, 80 μg/ml for 20 min at 25°C and then for 60 min at 37°C, to perturb membrane receptors, resulted in cap formation in 40% of intact cells but only in 6% of the cytoplasts.

**Capping of karyoplast surface receptors**

Karyoplasts, cytoplasts, and intact EL4 and L cells were treated either indirectly with fluorescent antiserum or directly with Con-A-Fl. To induce redistribution of membrane receptors and cap formation, treated cells were transferred from ice to 37°C. Examination of the fluorescence showed clear cap formation of the karyoplast surface receptors (Fig. 1D). The degree of capping of surface antigens observed in karyoplasts was very similar to that found in intact cells (Table 2). Treatment with Con-A-Fl resulted in capping in 26% of the Kar-L cells, as compared to only 6% observed in Cyt-L.

**Effects of cytochalasin B and colchicine**

Since cytochalasin B (CB) had been shown to influence redistribution of surface components (14), it was necessary to exclude the possibility that the lack of capping in cytoplasts was due to treatment of the cells with CB during enucleation. Control EL4 and L cells were treated with CB, 10 μg/ml for 60 min, then washed and allowed to regenerate for 60 min in the absence of CB. CB-treated cells were then treated with antiserum to induce
cap formation. As can be seen in experiment c of Table 3, pre-
treatment of cells with CB had no significant influence on their
capacity to undergo capping with antisera. Hence, the pre-
liminary exposure of cells to CB during enucleation could not
in itself account for the lack of cap formation found in cyto-
plasts.

Colchicine has been found to enhance cap formation in a few
systems where capping could not be demonstrated (9–11, 14, 21). Therefore, the influence of colchicine on capping of surface
receptors of cytoplasts and whole cells was tested. It was found
(Table 3) that colchicine did not promote cap formation in
cytoplasts and that in higher concentrations the drug inhibited
cap formation in intact cells and in karyoplasts. Similar results
were obtained whether the cells were treated with specific
antisera or Con-A-Fl. Hence, it appeared that the lack of cap
formation in cytoplasts could not be attributed to a colchicine-
sensitive structure(s) that restricted redistribution of sur-
face receptors.

**DISCUSSION**

Antibody- or lectin-induced redistribution of membrane recep-
tors toward one pole of the cell (capping) is an energy-
dependent process which has been demonstrated in many cell
types (for review see ref. 4). A role for cytoskeletal organelles
such as microtubules and microfilaments in the redistribution
of cell-surface determinants has been suggested (8, 13, 14, 21).
Cytoskeletal microtubules and microfilaments have been im-
plied, because drugs that disrupt microtubules and micro-
filaments have been shown to influence redistribution of
membrane components. Supporting the hypothesis of cyto-
skeletal control of membrane receptor mobility are the recent
findings of Poste et al., who reported that local anesthetics in-
fluence mobility of membrane receptors via their effects upon
cytoskeletal structures (22).

The present study provides a new approach to investigating

<table>
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<th>Experiment</th>
<th>Cells</th>
<th>Pretreatment</th>
<th>Caps (%)</th>
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<tr>
<td>a*</td>
<td>EL4</td>
<td>Colchicine, 25 μM</td>
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<tr>
<td></td>
<td>EL4</td>
<td>Colchicine, 1 μM</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>L</td>
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<td>50</td>
</tr>
<tr>
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<td>L</td>
<td>—</td>
<td>5–10</td>
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<tr>
<td>b†</td>
<td>L</td>
<td>Colchicine, 100 μM</td>
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<tr>
<td></td>
<td>L</td>
<td>—</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Kar-L</td>
<td>Colchicine, 100 μM</td>
<td>5–10</td>
</tr>
<tr>
<td></td>
<td>Kar-L</td>
<td>—</td>
<td>26</td>
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<tr>
<td></td>
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<td></td>
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</tr>
<tr>
<td>c‡</td>
<td>EL4</td>
<td>CB, 10 μg/ml</td>
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<td>46</td>
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<td></td>
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<tr>
<td></td>
<td>L</td>
<td>—</td>
<td>34</td>
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* EL4 whole cells and cytoplasts pretreated with colchicine were
  reacted with an anti-H-2b serum and then with GAMG-Fl (as
detailed in Results). Percent capping was determined after 90
min at 37°.
† L whole cells, karyoplasts and cytoplasts pretreated with colchi-
cine were incubated with concanavalin-A-Fl (80 μg/ml) for 20
min at room temperature, washed, and allowed to cap for 60 min
at 37°.
‡ EL4 and L cells pretreated with CB were reacted with an anti-H-
2b and an anti-H-2k serum, respectively, and then with GAMG-
Fl. Percent capping was determined after 90 min at 37°.

Table 2. Capping of surface antigens of karyoplasts,
cytoplasts, and intact cells

<table>
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<th></th>
<th>L Cells</th>
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<tr>
<td>Karyoplasts</td>
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<td>32</td>
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<tr>
<td>Intact cells</td>
<td>38</td>
<td>39</td>
</tr>
<tr>
<td>Cytoplasts</td>
<td>9†</td>
<td>10</td>
</tr>
</tbody>
</table>

* Cells (2 x 10⁶) were incubated for 30 min on ice with 0.1 ml of
  specific antiserum (anti-H-2b, anti-H-2k, or anti-th) diluted
  with PBS-FCS, washed twice with 2 ml of cold PBS-FCS, and then
  incubated for 30 min on ice with GAMG-Fl or GARG-Fl diluted
  1:10 with PBS-FCS, washed once, and resuspended in 0.1 ml of
  PBS-FCS. To form caps cells were incubated for 2 hr at 37°.
† Most cap-forming cells were contaminating karyoplasts (see
  Table 1).
the contributions of inner cell components to the redistribution and movement of cell-surface determinants. We have shown that removal of a nucleus from a cell abolishes the capping ability of the resulting cytoplast. Whereas this finding alone is insufficient for one to conclude that nucleus–membrane interactions play a role in cap formation, the capping of karyoplast surface receptors supports the hypothesis that the nucleus or a nucleus-associated structure plays, directly or indirectly, a role in cap formation.

Carter (16) has shown that CB enucleates cells. Enucleation is enhanced if a centrifugal force is applied in the presence of CB (17, 18). The mechanism of enucleation is not entirely clear (23), but it is believed that CB reversibly disassembles the cortical actin-like microfilaments, leading to a reduction in the viscosity of the cortical layer of the cytoplasm and to enucleation. Ultrastructural and biochemical studies (16–19, 23, 24) of cytoplasts and karyoplasts reveal similar characteristics as well as clear structural and functional differences. Plasma membrane, cytoplasm, ribosomes, mitochondria, and endoplasmic reticulum are found in both cytoplasts and karyoplasts. The cytoplast, unlike the karyoplast, contains centrioles, microtubules and microfilaments, and the Golgi complex (24). The two cells differ in activity. Firm adherence, spreading, and motility have been detected in cytoplasts (25); karyoplasts, on the other hand, lack any motility or spreading capacity. The presentation of membrane antigens on cytoplasts appears similar to that of intact cells, for both cell types react similarly with allo- and heteroantibody and with Con A, as detected by immunofluorescence. In addition, cytoplasts and intact cells could be lysed to the same extent by antibody and complement (unpublished results). Sethi and Brandis (26) have induced a high degree of tumor-specific immunity in mice by injecting enucleated cells, demonstrating that cell-surface antigens are not affected drastically by the enucleation.

A clear separation of the nucleus from the centrosphere and microtubules is obtained in enucleation (24, 25). Since the cytoplast is capable of adhering, moving, and spreading in a manner comparable to intact cells, it is conceivable that it has a functional cytoskeletal mechanism. The karyoplast seems to be devoid of any contractile mechanism (24), although a recent demonstration of spreading and dividing karyoplasts indicates a cytoskeletal activity (27). Our findings of cap formation in karyoplasts but not in cytoplasts (Fig. 1 and Table 2) suggest that the submembranous cytoskeletal structures that are involved in motile activities of the cell surface are insufficient for executing cap formation independently. Osborn and Weber (28) and Brinkley et al. (29) have recently shown that microtubules appear to grow from a tubulin-containing cylindrical structure located on the perinuclear space. Since this nucleus-associated organizing structure has been detected in enucleated cells (28), the possibility that it plays a direct role in controlling redistribution of membrane components seems unlikely. Thus, we would like to suggest that the nucleus or a nucleus-associated structure, probably via a cytoskeletal or contractile system (13), is the intracellular organelle that controls the redistribution of surface membrane antigens. Another explanation is that a short-lived protein or RNA molecule must be present before capping can occur, and removal of the nucleus results in its decay without replacement.

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