Independent Alterations in Cell Shape and Intramembranous Particle Topography Induced by Cytochalasin B and Colchicine in Normal and Transformed Cells

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SUMMARY

Native differences in cell shape and plasma membrane organization in contact-inhibited and transformed cells and the effects of cytochalasin B and colchicine on these cells have been examined by scanning electron microscopy and freeze fracture-electron microscopy. Confluent BALB/c 3T3 cells show a flat, polygonal shape with limited cell overlapping, some microvilli, and plasma membranes with an aggregated distribution of intramembranous particles. Simian virus 40-transformed BALB/c 3T3 cells, by contrast, have a pleomorphic, bipolar spindle shape, extensive cell overlapping, more numerous surface projections, and a random distribution of intramembranous particles.

Treatment of 3T3 and SV3T3 cells with 10^-6 M colchicine produced changes in cell shape and induced intramembranous particle aggregation in SV3T3 cells but did not significantly affect the freeze fracture morphology of 3T3 plasma membranes. Treatment of 3T3 and SV3T3 cells with cytochalasin B (1 µg/ml) also produced marked changes in cell shape and induced intramembranous particle disaggregation in 3T3 cells, but it did not affect intramembranous particle distribution in SV3T3 cells. Lower doses of colchicine (10^-8 M) or cytochalasin B (1 to 50 ng) modulated intramembranous particle distribution in transformed and normal 3T3 cells, respectively, without seriously affecting cell shape. These results are interpreted to suggest that modulation of cell shape or cell surface topography and intramembranous particle distribution are separable phenomena.

INTRODUCTION

Normal and transformed cells have a variety of structural and functional differences in the plasma membrane. Transformed cells show increased lectin agglutination (2) and enhanced ligand-induced receptor redistribution using ferritin- or fluorescein-conjugated concanavalin A (4, 14). However, recent studies suggest no association between concanavalin A-receptor redistribution, agglutination, and density-dependent inhibition of growth (22). The composition of membrane proteins and lipids also differs between normal and transformed cells. In general, transformed cells contain less complex glycolipids (8), decreased quantities of a 220,000-dalton glycoprotein (18), and decreased quantities of actin-like, membrane-associated proteins (24). Differences in intrinsic membrane function or organization can be inferred from transport studies (10) and by our studies showing differences in the distribution of protein-associated intramembranous particles in normal and transformed cells (21). Intramembranous particles have a clustered distribution in contacted 3T3 cells and are more uniformly distributed in SV3T3 cells, in mitotic 3T3 cells, and in non-contacted 3T3 cells (6, 21). We have utilized the membrane-active drugs vinblastine and colchicine, which also affect microtubule polymerization, and cytochalasin B, which, among other things, affects microfilaments. These agents have been used to help define the mechanisms controlling intramembranous particle topography and to determine whether this is correlated with cell shape in the 3T3-SV3T3 system.

Scanning electron microscopic studies have examined the surface topography of normal and transformed cells of the 3T3 and other cell lines (11, 17). In the studies using 3T3 cells, Porter et al. (17) showed that the contact-inhibited cells form a flat monolayer and suggested the similarity of these cells to endothelial cells. The 3T3 cells transformed by various means demonstrate a more pleomorphic and fusiform shape and, depending on the clone, may show significantly more surface modifications in the form of membrane ruffles, microvilli, or blebs (17). In another study, nontumorigenic C3H mouse embryo cells were shown to be rather flat, polygonal cells without many surface modifications during interphase (11). These C3H cells transformed by chemical carcinogens became more pleomorphic and developed more surface modifications, as with the virally transformed 3T3 cells (11).

In this paper, parallel freeze fracture and scanning electron microscopy studies are presented to examine the relationship between intrinsic membrane structure and general cell shape.

MATERIALS AND METHODS

BALB/c 3T3 (clone A31) and simian virus 40-transformed BALB/c 3T3 mouse embryo cells (SV3T3) (clone A31, sub-
clone 6), a gift of Dr. George Todaro, were grown in Dulbecco’s minimal essential medium (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 10% calf serum (Flow Laboratories, Rockville, Md.) as previously described (6). Transformed SV3T3 and contact-inhibited 3T3 cells were grown to a density of between $5 \times 10^4$ to $1 \times 10^6$ cells/sq cm and 2 to 3 $\times 10^3$ cells/sq cm, respectively, prior to the addition of membrane-active drugs. 3T3 and SV3T3 cells were then treated with medium alone or with medium plus colchicine ($10^{-8}$ to $10^{-6}$ M) (Sigma Chemical Co., St. Louis, Mo.) or cytochalasin B (1 ng to 5 $\mu$g/ml) (Aldrich Chemical Co., Milwaukee, Wis.) for 1 to 4 hr at 37°C.

Specimens for freeze fracture were routinely prepared in tissue culture medium alone or following exposure to 15% glycerol-phosphate-buffered saline with calcium and magnesium (pH 7.4) for 15 min at room temperature. Selected specimens were prepared in 10% sucrose-phosphate-buffered saline or following prefixation in 1% paraformaldehyde with similar results. Following treatment with the various drugs, cells were gently scraped, frozen, and fractured as described (6). Approximately equal numbers of inner and outer fracture faces were examined of at least 30 cells for each sample with the percentage of fracture faces having particle aggregates determined as previously described (6).

For scanning electron microscopy, cells were initially seeded onto 60-mm Falcon tissue culture plates into which had been placed sterile, 10-mm round glass coverslips (Bellco Glass, Inc., Vineland, N. J.) and were allowed to grow for at least 3 days. Plates were then washed 3 times with serum-free medium and treated with similar concentrations of colchicine or cytochalasin B in serum-free medium, as in the freeze fracture studies. Cells were then fixed in 0.1 M 3% glutaraldehyde-cacodylate buffer (pH 7.4) (Fischer Scientific Co., Chicago, Ill.) for 30 min at 37°C, then overnight at 4°C. Specimens were stepwise dehydrated in ethanol (0 to 100%)–Freon 13 (0 to 100%) and critical point-dried in a Denton vacuum apparatus and viewed with a Kent Cambridge Model 4S stereoscan microscope at an accelerating voltage of 20 kV.

RESULTS

Contact-inhibited 3T3 cells have a clustered distribution of PMP* (Fig. 1), whereas a more uniform distribution of PMP is observed in SV3T3 cells (Fig. 2) when prepared with or without cryoprotectants or with prior fixation in 1% formaldehyde or paraformaldehyde (5). 3T3 cells examined by scanning electron microscopy illustrate a very flat, almost polygonal shape with limited cell overlapping and some microvilli (Fig. 3) and appear almost as endothelial cells. SV3T3 clone 6 cells are observed to be more pleomorphic with a bipolar spindle shape, extensive cell overlapping, and more pronounced membrane modifications including microvilli and blebs (Fig. 4).

Treatment of SV3T3 cells with colchicine induces a dose- and time-dependent aggregation of PMP. Control SV3T3 cells show a random distribution of PMP, whereas 60 and 90% of fracture faces have aggregates of particles after a 4-hr exposure to $10^{-8}$ and $10^{-6}$ M colchicine, respectively (Table 1). The aggregation of particles induced by colchicine in transformed cells occurs at $10^{-6}$ M, which also perturbs gross cell morphology, visualized as a retraction and a slight rounding up of cells, a less spindle-shaped appearance, and the appearance of increased membrane modifications, including microvilli and blebs (Fig. 5). This may result from the disruption of microtubules, although this is not entirely clear presently. However, treatment of SV3T3 cells with $10^{-6}$ M colchicine induces PMP clustering without inducing a significant perturbation in gross cell shape or surface topography, and the cells appear essentially as control SV3T3 cells.

Treatment of contact-inhibited 3T3 cells with colchicine ($10^{-9}$ to $10^{-6}$ M) resulted in no significant alteration in the percentage of fracture faces with particle aggregates. However, $10^{-6}$ M colchicine did produce changes in the shape of 3T3 cells as evidenced by some slight retraction of cells from one another and the presence of increased numbers of microvilli that appeared predominantly located over the central part of the cells (Fig. 6). Treatment of 3T3 cells with $10^{-5}$ M colchicine failed to significantly affect PMP distribution (Table 1) or the scanning electron microscopic appearance of cells.

In this study, contact-inhibited 3T3 cells have clusters of PMP on approximately 70% of their fracture faces (Table 1). Treatment of these cells with cytochalasin B at doses ranging from 1 to 50 ng/ml produced disaggregation of particle clusters within 1 hr, after which time only 20 to 30% of the fracture faces showed particle aggregation (Table 1). These dosages of cytochalasin B were observed to have differential effects on the scanning morphology of 3T3 cells. Cytochalasin B at 1 to 50 ng/ml has no profound effect on 3T3 morphology, except for a minimal retraction of cells from one another, yet these concentrations induced significant disaggregation of PMP in 3T3 cells. Interestingly, cytochalasin B at 5 $\mu$g/ml induced significant changes in cellular morphology visualized by the appearance of fine fibers radiating from the cell periphery, which may represent sites of cellular attachments to the substrate (Fig. 7), and, occasionally, centrally located membrane blebs were observed. Paradoxically, in 3T3 cells, ng concentrations disaggregated particles, and 5 $\mu$g/ml did not.

Table 1

<table>
<thead>
<tr>
<th>Conditions</th>
<th>3T3</th>
<th>SV3T3</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>70</td>
<td>10</td>
</tr>
<tr>
<td>Cytochalasin B (5 $\mu$g/ml; 1 hr)</td>
<td>55</td>
<td>10</td>
</tr>
<tr>
<td>Cytochalasin B (1 $\mu$g/ml; 1 hr)</td>
<td>35</td>
<td>10</td>
</tr>
<tr>
<td>Cytochalasin B (50 ng/ml; 1 hr)</td>
<td>30</td>
<td>15</td>
</tr>
<tr>
<td>Cytochalasin B (1 ng/ml; 1 hr)</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Colchicine ($10^{-4}$ M; 1 hr)</td>
<td>80</td>
<td>15</td>
</tr>
<tr>
<td>Colchicine ($10^{-5}$ M; 1 hr)</td>
<td>80</td>
<td>15</td>
</tr>
<tr>
<td>Colchicine ($10^{-4}$ M; 4 hr)</td>
<td>90</td>
<td>90</td>
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</tbody>
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*The abbreviation used is: PMP, plasma membrane intramembranous particles.
TREATMENT OF SV3T3 CELLS WITH CYTOCHALASIN B (5 µg/ml)

Treatment of SV3T3 cells with cytochalasin B (5 µg/ml) did not affect particle distribution under the conditions used (Table 1) but did significantly affect cell shape in these cells, which tended to be even more spindle-shaped and elongated and demonstrated retraction fiber formation (Fig. 8), as seen in 3T3 cells treated with cytochalasin B (5 µg/ml). Treatment of transformed cells with ng concentrations of cytochalasin B did not affect intrinsic membrane freeze fracture appearance nor did it significantly affect scanning electron microscopic appearance.

DISCUSSION

The results of this study and our previous studies show that the distribution of PMP differs in the 3T3 and SV3T3 cells (6, 21). Particles are randomly distributed in transformed 3T3, in noncontacted 3T3, in mitotic 3T3, and in 3T3 cells infected with a temperature-sensitive mutant SV40 grown at the restrictive temperature (6, 7, 21). Cell contact-associated aggregation of particles is observed in 3T3 cells and in 3T3 cells infected with a temperature-sensitive mutant grown at the restrictive temperature (6, 7, 21).

The results of this study also show that colchicine induces a dose- and time-dependent aggregation of plasma membrane particles in SV3T3 cells at concentrations that appear to alter gross cell morphology (10⁻⁴ M) and at concentrations that do not significantly affect cell shape (10⁻⁹ M). In this study, it was also observed that cytochalasin B induces disaggregation of particles in contact-inhibited 3T3 cells within 1 hr of treatment at doses ranging from 1 to 50 ng/ml, but we observe that cytochalasin B causes changes in cell shape only at concentrations above 0.5 µg/ml. This is significant, since early work of Porter (15), among others, suggested that microtubules and, possibly, microfilaments form a cytoskeleton which maintains cell shape. Studies have shown that dibutyryl cyclic 3',5'-AMP will produce a "reverse transformation" of Chinese hamster ovary cells which is blocked by Colcemid (9). Later studies by Porter et al. (16) combining scanning and transmission electron microscopy more clearly examined this reverse transformation and suggested the association of increased microtubule formation following treatment with dibutyryl cyclic 3',5'-AMP and the reverse transformation. Of further significance are membrane studies which have suggested the role of microtubules and, in certain cases, microfilaments in regulating certain membrane transport activities (1) and membrane-receptor dynamics for lectins such as concanavalin A (3, 25). This influence has been based primarily on the utilization of drugs such as colchicine or vinblastine. Other studies have suggested a parallelism between intrinsic membrane structure (6) and lectin-induced cell agglutination (5) in mitotic cells which generally round up and change from their normal spread-out interphase morphology.

It was observed in these freeze fracture and scanning electron microscopic studies that, at certain concentrations, cytochalasin B and vinblastine will affect intrinsic membrane structure and perturb cell shape and surface morphology. In addition, at somewhat lower concentra-

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Fig. 1. Plasma membrane fracture face of a 3T3 cell demonstrating aggregates of PMP, associated with significant bare membrane surface. × 35,000.

Fig. 2. Plasma membrane fracture face of an SV3T3 cell demonstrating a uniform distribution of PMP. × 41,000.

Fig. 3. Scanning electron micrograph of high-density 3T3 cells, growing as a monolayer. × 500.

Fig. 4. Scanning electron micrograph of an SV3T3 cell demonstrating a more spindle-shaped appearance and significant cell-cell overlapping. × 1,000.
Fig. 5. Scanning electron micrograph of SV3T3 cells treated with 10⁻⁴ M colchicine for 4 hr, demonstrating some cell retraction and more pronounced membrane modifications such as blebs and microvilli. × 1,000.

Fig. 6. Scanning electron micrograph of high-density 3T3 cells treated with 10⁻⁴ M colchicine for 4 hr. × 500.

Fig. 7. Scanning electron micrograph of high-density 3T3 cells treated with cytochalasin B (5 μg/ml) for 1 hr, demonstrating a centripetal retraction of cells from the substrate with retraction fiber formation. × 500.

Fig. 8. Scanning electron micrograph of SV3T3 cells treated with cytochalasin B (5 μg/ml) for 1 hr. × 1,000.
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