A SCANNING ELECTRON-MICROSCOPE STUDY OF THE SURFACE FEATURES OF MAMMALIAN CELLS IN VITRO

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SUMMARY

The cell surface aspects and the density, distribution and morphology of surface cytoplasmic projections (microprocesses) of HeLa, BHK and baby mouse kidney cells, maintained under different culture conditions, have been compared using scanning electron microscopy. No significant differences were noted in the surface aspects of these cell strains. Comparison of the 3 cell strains, under identical culture conditions, showed variations from one strain to another in the density and morphology of the cell surface microprocesses which may reflect basic physiological differences between the strains. For a given cell strain, variations were observed in response to changes in culture conditions indicative that the presence of surface microprocesses is related to the physiological state of the cell.

INTRODUCTION

A feature shared by most cultured mammalian cells is the presence of surface cytoplasmic projections. These have been described using light microscopy (Lewis & Lewis, 1926; Gey, 1956; Taylor & Robbins, 1963; Cornell, 1969), and reported on using transmission electron microscopy either by direct visualization of intact or sectioned material (Porter, Claude & Fullam, 1945; Overman & Eiry, 1961; Overman, 1963; Taylor & Robbins, 1963; Cornell, 1969) or by the preparation of shadowed surface replicas (Cowan & Anderson, 1955; Easty & Mercer, 1960; Willoch, 1967; Fisher & Cooper, 1967; Follet & O'Neill, 1969; Follett & Goldman, 1970), and more recently using scanning electron microscopy (Dalen & Scheie, 1968, 1969; Follet & O'Neill, 1969; Hodges, 1970). A variety of terms have been given to the cytoplasmic processes extending from the surface of cultured cells, e.g. microvilli (Lewis & Lewis, 1926; Gey, 1956), microextensions, microspikes (Weiss, 1961; Taylor, 1966), filopodia (Trelstad, Hay & Revel, 1967) and retraction fibrils (Taylor & Robbins, 1963). The density and distribution of these surface cytoplasmic projections are variable in different cell types (Easty & Mercer, 1960; Overman & Eiry, 1961; Cooper & Fisher, 1968; Follet & O'Neill, 1969; Hodges, 1970) and appear to depend on the physiological state of the cells (Willoch, 1967; Cornell, 1969). In the present paper a comparison has been made, using the Nomarski interference-contrast and scanning electron-microscope techniques, of the cell surface aspects and the density, distribution and morphology of the surface cytoplasmic projections of HeLa, BHK and baby mouse kidney cells maintained under different culture conditions.
MATERIALS AND METHODS

The observations were made on cells from cultures of HeLa (obtained from Burroughs Wellcome Ltd. 1962), baby hamster kidney BHK21/C13 (Stoker & Macpherson, 1964) and CBM17 baby mouse kidney (Franks & Henzell, 1970) strains. The media used were (A) Waymouth's MB 752/1 medium (Waymouth, 1959) supplemented with 4% human serum (for cell stocks of HeLa, routinely transferred by syringing free the cells); (B) Waymouth's MB 752/1 medium containing 10% calf serum (for cell stocks of CBM17); (C) Eagle's E 4 medium (Dulbecco & Freeman, 1959) containing 10% calf serum (for cell stocks of BHK).

The CBM17 and BHK strains were routinely subcultured by trypsinization.

Aliquots of cell suspensions were taken from each of the stock cultures and grown under identical conditions in 225-ml Pyrex baby's feeding bottles for a total of 3 transfer generations. Cultures were gassed with 5% CO₂ in air (media A and B) and with 10% CO₂ in air (medium C) and kept at 36.5 °C.

For scanning electron microscopy, the cells were plated on 10- or 13-mm coverglasses (Chance) in 90-mm glass Petri dishes at a density of approximately 7 × 10^6 cells/cm². The cell cultures were fixed 48 h later in 2-5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.1. Processing of the cell cultures for examination in the scanning electron microscope was carried out as previously described (Hodges, 1970). The specimens were given an electrically conducting surface coating by vacuum evaporation of either gold/palladium alloy, gold, aluminium or chromium.

For light microscopy the coverslip preparations, after fixation and rinsing in buffer, were washed in 2 changes of distilled water, mounted directly in a drop of buffered glycerol and viewed in a Zeiss microscope using Nomarski's interference-contrast technique.

OBSERVATIONS

Light microscopy of the cell strains was carried out using Nomarski's interference-contrast system for comparison with the dehydrated scanning electron microscope preparations. No significant differences in morphology were noted (Figs. 1, 2) and features seen at the light-microscope level were recognizable by scanning electron microscopy but in greater detail (Fig. 3).

Interphase cells of BHK and HeLa strains and polygonal and fibroblast-like cell types of CBM17 strain (see Hodges, 1970) were flattened on the glass substratum and 3 main cytoplasmic regions - nuclear, juxtanuclear and peripheral zones - could be distinguished (Figs. 3–6). Cells from the 3 strains showed a relatively smooth-textured nuclear region with large protrusions corresponding to nucleoli, and smaller and more irregular protrusions possibly representing heterochromatin.

Numerous depressions of variable depth, probably indicative of underlying nuclear pores, were located irregularly round the edge of the nuclear zone and at the base of the nucleoli. The juxtanuclear region had a rough-textured surface probably due to the cisternae of the underlying endoplasmic reticulum and Golgi complex. Localized accumulations of spherical or ovoid bodies, approximately 0.5–0.6 μm in diameter and possibly corresponding to lysosomes, were frequently seen in the juxtanuclear zone of the BHK cells (Fig. 4). These bodies were occasionally seen in HeLa cells and more rarely in CBM17 fibroblast-like cells. The rough-textured juxtanuclear cytoplasmic zone merged into a smoother-textured peripheral cytoplasmic region which was extensive in HeLa cells (Fig. 3) and in the polar regions of some BHK cells but relatively restricted in both types of CBM17 cells.
Table 1. Effect of culture conditions on the relative density and distribution of cytoplasmic processes on cell surfaces

<table>
<thead>
<tr>
<th>Medium</th>
<th>Cell strain</th>
<th>Upper cell surface</th>
<th>Lateral cell surface</th>
<th>Density of processes</th>
<th>Length of processes, μm†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Nuclear region</td>
<td>Juxtanuclear region</td>
<td>Peripheral region</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>HeLa*</td>
<td>Some</td>
<td>Numerous</td>
<td>Numerous</td>
<td>+ + +, very numerous</td>
</tr>
<tr>
<td></td>
<td>CBM17-type 2†</td>
<td>Rare</td>
<td>Numerous</td>
<td>Few to numerous</td>
<td>+ ++(+), very numerous</td>
</tr>
<tr>
<td></td>
<td>BHK</td>
<td>Some to few</td>
<td>Some</td>
<td>None to some</td>
<td>+ ++, numerous</td>
</tr>
<tr>
<td>B</td>
<td>HeLa</td>
<td>Some</td>
<td>Numerous</td>
<td>Numerous</td>
<td>+ ++(+), very numerous</td>
</tr>
<tr>
<td></td>
<td>CBM17-type 1§</td>
<td>Numerous</td>
<td>Very numerous</td>
<td>Few to very numerous</td>
<td>+, numerous</td>
</tr>
<tr>
<td></td>
<td>CBM17-type 2†</td>
<td>Rare</td>
<td>Numerous</td>
<td>Few to numerous</td>
<td>+ ++, numerous</td>
</tr>
<tr>
<td></td>
<td>BHK</td>
<td>Few</td>
<td>Some</td>
<td>Some</td>
<td>+ ++(+), numerous</td>
</tr>
<tr>
<td>C</td>
<td>HeLa</td>
<td>Few</td>
<td>Some</td>
<td>Some</td>
<td>+ +, relatively numerous</td>
</tr>
<tr>
<td></td>
<td>CBM17-type 2†</td>
<td>Rare</td>
<td>Numerous</td>
<td>Few to some</td>
<td>+ ++, very numerous</td>
</tr>
<tr>
<td></td>
<td>BHK*</td>
<td>None to some</td>
<td>None to some</td>
<td>None to some</td>
<td>+, some</td>
</tr>
</tbody>
</table>

* Medium used for cell stocks; †average value for 2 experiments (20 cells counted); ‡ fibroblast-like cell; § polygonal-type cell.
Cytoplasmic processes (or microprocesses), varying in distribution and size were present over the upper free surface and around the lateral edges of interphase cells. A survey showed variations in the relative distribution, density, and length of these microprocesses related to the conditions of culture (Table 1). The upper surface projections, when present, were commonly found collapsed on to the cell surface and were generally in the form of elongate unbranched or occasionally bifurcate processes with tapered or slightly dilated tips (Figs. 7, 8). Comparison of the laterally situated microprocesses of cells grown under similar culture conditions revealed some morphological differences between the different cell types. Changes in culture conditions were found to influence the morphology of the microprocesses of certain of the cell types. Slender, tapering, straight or slightly contorted microprocesses of varying lengths showing relatively frequent bifurcations into unequal branches extended from the edges of the CBM17 type 2 cells maintained in Waymouth's 752/1 media in a gas phase containing 5% carbon dioxide (Fig. 9). Shorter, blunter microprocesses were also seen, showing occasional dilations which were usually located at the tip. Maintenance of the CBM17 cells in Eagle's E4 medium and in a 10% carbon dioxide atmosphere resulted in a relatively increased density of the microprocesses and these were more contorted in form and frequently bifurcated.

HeLa cells grown in Waymouth's 752/1 media and a 5 or 10% carbon dioxide-air gas phase were characterized by very filamentous, frequently bifurcated, lateral microprocesses (Fig. 10). A striking difference in the morphology of the microprocesses was seen when the HeLa cells were maintained in Eagle's E4 medium in a 10% carbon dioxide gas phase. Under these conditions of culture the microprocesses, whilst relatively numerous, were much shorter and were generally straight, with only occasional bifurcations (Fig. 11). Lateral microprocesses of BHK cells were in general tapering, straight or occasionally bifurcated (Fig. 12), and under the different conditions of culture no difference in morphology was seen though there were variations in the relative density of the microprocesses present (see Table 1).

At the prophase stage of mitotic division CBM17, BHK and HeLa cells (maintained under cell stock conditions) showed partial and irregular retraction of the cell cytoplasm from the underlying substratum and the main cytoplasmic regions could not be easily distinguished (Figs. 13-16). Lateral contact with the substratum was maintained by 2 types of cytoplasmic extensions: by slender contorted microprocesses, the relative density of which appeared to be increased at this stage of mitosis in cells from the 3 strains (Figs. 13, 14); and by long, straight, generally unbranched processes, much fewer in number and which are similar to the 'retraction fibrils' described by Taylor & Robbins (1963) and to the 'long microextensions' of Dalen & Scheie (1968) (Fig. 15). These retraction fibrils were most commonly observed in the CBM17 fibroblast-like cells. Upper surface microprocesses were present but only over the still-flattened regions of the cells, whilst towards the rounded-up region of the cell progressively pronounced foldings of the cytoplasmic membrane were apparent (Figs. 13-16). Cytoplasmic blebs, occasionally seen in the rounded-up regions of CBM17 fibroblast-like cells, were a frequent occurrence in BHK cells (Fig. 18). It was not possible to distinguish between cells in metaphase, anaphase or early telophase.
BHK, CBM17 and HeLa cells at these stages of division were rounded, showed deep foldings of the cytoplasmic membrane (Fig. 17), with numerous cytoplasmic blebs present in BHK cells, and were attached to the substratum by relatively broad cytoplasmic processes. Fine lateral or upper-surface microprocesses were not in evidence in these cells. Cells in late telophase showed the presence of a cleavage furrow and separation of the 2 daughter cells. A bulbous body (?mid-body) was occasionally observed located along the telophase bridge (Fig. 19). Deep foldings of the cytoplasmic membrane were apparent in the still-rounded regions of both daughter cells whilst the presence of slender upper surface microprocesses was restricted to the areas of the cell showing a more flattened configuration (Fig. 20). The cell membrane in the furrow region showed a very smooth surface and horizontally oriented ridges suggestive of stress lines were in evidence.

DISCUSSION

Many of the morphological features of cells seen by scanning electron microscopy correspond to similar structures observed in the living or fixed cell at the light-microscope level (Boyde, Grainger & James, 1969; Hodges, 1970). It would therefore appear from these observations and from the present study that the preparative techniques, which at present involve complete cellular dehydration and in vacuo examination of specimens, do not introduce an inordinate amount of cell distortion. However, as discussed by Boyde et al. (1969), whilst retraction in the horizontal plane is probably restricted by the close relationship which exists between cell and underlying substrate, more substantial shrinkage might be expected in the vertical plane. Thus cell organelles, such as the microprocesses extending from the upper free surface of the cell and presumably possessing a relatively low dry mass, would be expected to present a collapsed appearance as, in fact, was observed in this study. Although the spatial relationship of these microprocesses is not maintained by the present preparative techniques, their relative density and distribution can be assessed. Examination of the 3 different cell strains reveals that the presence and number of microprocesses on the upper, free surface of the interphase cell is not a constant feature. Thus, numerous microprocesses are found on the surface of HeLa cells in accord with previous findings (Overman & Eiry, 1961; Fisher & Cooper, 1967; Pugh-Humphreys & Sinclair, 1970) and on CBM17 polygonal cells, whilst relatively few are present on CBM17 fibroblast-like cells and very few on BHK cells. The apparently greater frequency and overall distribution of microprocesses on the surface of the epithelial-like cells suggests a highly dynamic cell surface which may reflect an inherent property of cells specialized for absorption or resorption. Time-lapse cinemicrography has provided evidence of the essentially transient nature of the surface cytoplasmic processes of cultured mammalian cells, remodelling probably in response to shifts in physiological conditions (Taylor & Robbins, 1963). This is in contrast to the apparently stable nature of the microvilli of luminal epithelia in vivo and suggests the reassessment of the use of the term of microvilli for processes seen in cells in vitro as already discussed by Taylor (1966) and Dalen & Scheie (1969).
A comparison of the 3 cell strains under identical culture conditions shows differences in the frequency and morphology of the laterally situated microprocesses which could reflect an intrinsic property of the cell strain. However, for a single cell strain variations in density and morphology of the microprocesses are observed in response to changes in culture conditions. There is evidence that reduced glucose concentration in the medium may decrease the density of cytoplasmic processes of HeLa cells (Willoch, 1967) whilst changes in the serum supplement of the culture medium can be accompanied by changes in the density and distribution of the processes on mouse embryo cells (Cornell, 1969). These different observations strongly suggest a connexion between the physiological state of the cell and the presence of microprocesses on its surface. At mitosis, these slender microprocesses are invariably absent from the upper free surface of the BHK, HeLa and CBM cell strains. Instead, the cell membrane assumes a deeply folded configuration as the cell rounds up for division, suggestive of a mechanism for the conservation of membrane. The present observations on the BHK cell strain differ somewhat from the findings of Follett & Goldman (1970), who reported, as well as the folded configuration of the cell membrane, the presence of cytoplasmic processes on the upper surface of the dividing cells. The surface of the dividing cell is also frequently marked by small cytoplasmic blebs (Mazia, 1961) and there is evidence that a ribonucleoprotein (Boss, 1955) or changes in localized concentrations of adenosine triphosphate (Lettre, Albrecht & Lettre, 1951) may be responsible for these transient cytoplasmic protrusions. In the present study, cytoplasmic blebs are most frequently seen in the dividing cells of the BHK strain, possibly reflecting a physiological characteristic of this strain.

The precise function of the upper surface microprocesses of cultured cells still remains undefined though these processes obviously increase the surface area of the cell and presumably enhance the transfer of nutrients and synthesized materials. Similarly, the purpose of the microprocesses along the lateral edges and on the under surface of the cells remains speculative though probably they are implicated in the function of locomotion and of cellular attachments, contacts between cells being made first in the region of the fine cytoplasmic processes (see Ambrose, 1968). Alterations in the frequency, shape and distribution of surface microprocesses in response to environmental conditions seems likely to be correlated with modifications in membrane synthetic processes, whilst variations observed between different cell strains in the density and morphology of the microprocesses presumably reflect intrinsic differences in the membrane synthetic processes and physiological requirements of these cell strains.

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Surface features of mammalian cells

REFERENCES


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Fig. 1. HeLa cell culture. Dehydrated preparation viewed by scanning electron microscopy. Al coated. × 260.

Fig. 2. HeLa cell culture. Wet preparation viewed by the Nomarski interference-contrast technique. × 300.

Fig. 3. Interphase cells of HeLa strain, showing 3 main cytoplasmic regions. $j$, juxtanuclear zone; $n$, nuclear zone; $p$, peripheral zone. Au/Pd coated. × 580.

Fig. 4. BHK21/C13 cell culture. Accumulation of spherical bodies in juxtanuclear zone ($j$). Au coated. × 590.

Fig. 5. CBM17 polygonal-type cells. Au/Pd coated. × 1425.

Fig. 6. CBM17 fibroblast-like cells. Au/Pd coated. × 1850.
Fig. 7. CBM17 polygonal-type cell showing upper surface cytoplasmic processes collapsed on to cell surface. Au/Pd coated. $\times 14400$.

Fig. 8. BHK21/C13 cell showing bifurcate microprocesses on upper cell surface. Au coated. $\times 17600$.

Fig. 9. CBM17 fibroblast-like cell showing lateral microprocesses. Au/Pd coated. $\times 9500$.

Fig. 10. HeLa cell in MB 752/1 medium showing slender filamentous lateral microprocesses. Au coated. $\times 5400$.

Fig. 11. HeLa cell in E4 medium showing short, straight microprocesses. Au coated. $\times 9200$.

Fig. 12. BHK cell showing straight lateral microprocesses. Au coated. $\times 10000$. 
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Figs. 13, 14. HeLa cells at prophase showing partial and irregular retraction of cell cytoplasm from substratum. Au/Pd coated. $\times 5400$. 
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Figs. 15, 16. CBM17 fibroblast-like cells showing microprocesses on upper surface of interphase cell (a) and on partially retracted region of prophase cell (b), and a retraction fibril (r). Au/Pd coated. Fig. 15, x 5500; Fig. 16, x 11000.

Fig. 17. CBM17 cell at metaphase-anaphase showing foldings of cytoplasmic membrane (fc). Au/Pd coated. x 22000.
Fig. 18. BHK cell showing cytoplasmic blebs (cb). Au coated. × 8500.

Fig. 19. CBM17 fibroblast-like cell showing bulbous body (? mid-body) (m) along the telophase bridge. Cr coated. × 8200.

Fig. 20. HeLa cell at telophase. Au/Pd coated. × 4600.