A Comparison of the Fine Structure of Cultured MAC-21 and HeLa Cells*

Yusuke Fuse, Zane Price, and Charles M. Carpenter

(Nina Anderton Laboratory for Electron Microscopy, Division of Microbiologic Cytology, Department of Medical Microbiology and Immunology, School of Medicine, and the Division of Infectious Diseases, School of Public Health, University of California, Los Angeles, California; and Medical Research Programs, Veterans Administration Hospital, Long Beach, California)

SUMMARY

The fine structure of a cultured mucoid adenocarcinoma cell of human lung (MAC-21) is described and compared with the fine structure of the HeLa cell. The differences in fine structure between the two strains are primarily quantitative. The MAC-21 cell differed, however, in the following respects: a spindle-shaped cell with larger nucleus and increased nuclear membrane invaginations, and considerably larger amounts of perinuclear Golgi apparatus. The cytoplasm contained areas of low density, undefined by a limiting membrane, which are thought to be accumulations of mucin.

The two cell strains contained numerous multivesicular bodies, some with lamellae in various stages of development, suggesting that these organelles may be involved in the formation of the lipide-rich, myelinated structures observed in both HeLa and MAC-21 cells. The number of myelinated bodies was found to be inversely proportional to the pH of the medium.

One of the first successful attempts to establish a cell line from carcinomatous lung tissue of humans was that of Cailleau, who propagated, in continuous culture, cells from a mucoid adenocarcinoma (10). She described this cell line as epithelial in origin and designated it MAC-21. This report describes the fine structure of these cells and compares their micromorphology with that of HeLa cells. The HeLa cell was selected for comparative studies because of its epithelial origin and because a number of studies have been made of its morphology with both the optical and electron microscope. The majority of such studies, however, have been concerned with pathological effects involving virus infections (7, 21, 23, 29, 30). Only a limited number have been directed toward the normal fine structure of the HeLa cell (9, 18). Further data on the fine structure of the HeLa cell are presented, in addition to the description of the fine structure of the MAC-21 cell.

MATERIALS AND METHODS

HeLa cells were obtained from cultures that had been maintained in our laboratories for a number of years. MAC-21 cells were obtained from Dr. R. Cailleau and subsequently propagated in our laboratories.

The HeLa cells were grown in a culture medium consisting of 60 per cent Difco Yeast Extract Medium, 20 per cent Hyland Sherer Maintenance Solution, 10 per cent Difco Beef Heart Infusion Broth, and 10 per cent Hyland Calf Serum. The MAC-21 cells were grown in 90 per cent Hyland 199 and Hyland 10 per cent Calf Serum.

The cells were harvested after 4 and 7 days of growth, respectively, and prepared for electron microscopy in the following manner: phosphate-buffered osmium tetroxide at pH 7.4 was added to give a final concentration of 1 per cent OsO4 in each culture tube and left at 4°C for 30 minutes. This was subsequently replaced with 3 ml. of Hanks solution. The cells were then detached from the walls of the culture tubes by being scraped with a rubber policeman and pelleted by centrifugation at 1,000 r.p.m. for 10 minutes. The pellet was dehydrated for 20-minute periods in graded ethyl alcohols and embedded in epoxy resin following the technic of Luft (26). Sectioning was accomplished with the Porter-Blum microtome. The sections were stained with lead hydroxide as described by Watson (44).
Correlative optical microscopy was accomplished by fixing cover-slip cultures of cells, on days 1 through 8, after subculturing in absolute methanol, in 20 per cent formalin, or in osmic acid vapor. Methanol- and formalin-fixed cells were stained with hematoxylin and eosin or Giemsa's stain. Unfixed cells were simultaneously examined with phase and polarizing microscopes.Buffered formalin-fixed, paraffin-embedded sections of the MAC-21 cells were stained in Alcian blue for evidence of mucopolysaccharide.

**OBSERVATIONS**

*Optical microscopy.*—HeLa cells and MAC-21 cells could be readily differentiated by examination of invaginations and some cytoplasmic processes or microvilli. The MAC-21 has a more regular contour with fewer invaginations but a larger number of microvilli (Fig. 3). The size and number of microvilli of both cell strains apparently depend to some extent on the age of the individual cells. Generally, the cell membrane becomes increasingly irregular, and there is a reduction in the number of microvilli as the cell degenerates. Desmosomes as reported by Bruni (9) were present in HeLa cells but were not observed in the MAC-21 cells. The cytoplasm of the two cell strains, with one or two exceptions, is similar, containing the classical organelles, membrane systems, and inclusions.

**TABLE 1**

<table>
<thead>
<tr>
<th>MAC-21 Cell</th>
<th>HeLa Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.08 X 4.65</td>
<td>5.08 X 5.74</td>
</tr>
<tr>
<td>22.70 X 7.14</td>
<td>11.9 X 7.91</td>
</tr>
<tr>
<td>1.2 per cent</td>
<td>3.6 per cent</td>
</tr>
<tr>
<td>0.177</td>
<td>0.201</td>
</tr>
</tbody>
</table>

One of the most conspicuous differences between the two types of cells was the development of many Golgi complexes surrounding the nucleus of the MAC-21 cell (Fig. 4). The Golgi complexes in this cell tend to be spherical, with a center diameter of approximately 2 μ. The Golgi granules commonly seen in many animal cells were not observed. The Golgi complexes in the HeLa cell were generally fewer in number, but otherwise their architecture was similar to that of the MAC-21 cells. The canaluli of the Golgi system in the degenerating cells of both strains were swollen and vacuolated and contained a matrix of low density.

The endoplasmic reticulum was more pronounced in the MAC-21 cells, and there was a larger amount of the rough type scattered throughout the cytoplasm but with denser accumulations near the cell periphery. The HeLa cell, on the other hand, lacked the pronounced anastomosing net-
work of canaliculi observed in the MAC-21 cell. The number of ribosomes in both cell strains appeared to be proportional to the development of the granular endoplasmic reticulum. The smooth variety was evenly distributed throughout the cytoplasm of both cells.

The mitochondria of the two cell strains exhibited the typical configuration of these organelles. Those of the MAC-21 cells, however, tended to be more elongated (Fig. 5) than those in the HeLa cell. The mitochondria of the two cell types were, in general, arranged in three layers according to size. The larger were located in the perinuclear region of the cytoplasm, with a layer of smaller ones in the peripheral area. The central region of the cytoplasm contained those of intermediate size. Many mitochondria in 4-day-old cultures contained varying amounts of amorphous, electron-dense, material. The deposition of this substance increased each day thereafter.

Another definitive difference between the HeLa cell and the MAC-21 cell was the presence of tonofibrils in the peripheral cytoplasm of many HeLa cells (Fig. 6). These were seen only rarely in the MAC-21 cells. Multivesicular bodies were routinely observed in the peripheral cytoplasm of both cell types (Fig. 7). These have been reported previously for the HeLa cell (9) and have been observed in a variety of other cells, both normal and neoplastic, and in cells infected with virus (19, 25, 31, 38). These organelles measured from 300 to 600 m in diameter, with a surrounding membrane 50–60 A thick. They contained many small vesicles whose diameter ranged from 10 to 50 m. The membrane of this organelle was occasionally incomplete, and a number of vesicles were seen scattered throughout the surrounding cytoplasm.

This phenomenon indicates that the surrounding membrane of this organelle may break down with the subsequent liberation of the small vesicles into the cytoplasm. No evidence of a central body or nucleoid, as reported by Sotelo (38), was observed. Multivesicular bodies were seen with what appeared to be lamellae forming inside (Fig. 8). This phenomenon suggests the possibility that the concentration of material within the vesicle occasionally reached a concentration gradient where lamellae formation could occur. These observations were rare, however, and at present there is insufficient evidence to indicate that they are involved in the formation of lipide-rich bodies.

Another distinctive feature of the MAC-21 cell was the presence of mucin accumulation in the MAC-21 cell.
strain was the presence of areas of low density in the peripheral cytoplasm of many of the cells (Fig. 9). This area was devoid of a limiting membrane and of all cytoplasmic organelles. It consisted primarily of an amorphous matrix containing a scattering of granules approximately 160–250 Å in diameter. These low density areas were not found in HeLa cells. Connection of these areas with the Golgi complex or with endoplasmic reticulum could not be established.

Needle-like paracrystals have been reported to occur frequently in cytoplasmic inclusions of HeLa cells (18). This observation was confirmed for the HeLa cells, and the crystals were also observed in the MAC-21 cells. They range in width from 25 to 30 mμ, and in length from 0.2 to 1.0 μ. Paracrystals were observed in mitochondria and lipide-rich bodies (Fig. 10) but were not observed free in the cytoplasm, except as shown in Figure 11, where they had developed to such a length that they penetrated the surrounding membrane. Such crystals have been observed in cells derived from guinea pig spleen (unpublished data) as well as the MAC-21 and HeLa cell strains. The crystals are more commonly seen in cells which show evidence of degeneration. Nine-day cultures contained many more paracrystals than 4-day cultures. The paracrystals probably represent accumulations of salts or antibiotics from the media. The crystals could not be detected in the polarizing microscope, because they were below resolution limits.

A large number of dark granules, approximately 1 μ in diameter, were observed in the cytoplasm of both MAC-21 and HeLa cells by bright-field and phase microscopy (Fig. 3). They were similar to the particles identified by Mackenzie as being lipide in nature (27). The number of granules increased in inverse proportion to the pH of the medium, following subculture, but decreased immediately after replacement of medium (Chart 2). These granules apparently correspond to the lipide-rich particles observed with the electron microscope. Examples of these organelles are presented in Figure 12. Their sizes range from 0.2 to 1.2 μ, and they are limited by a single membrane. Al-

![](chart.png)

**Chart 2.**—pH of medium, cell growth, and number of lipide-rich bodies as related to the age of the subculture. The number of lipide-rich bodies varies inversely with pH.

though the majority of these organelles contained several concentric, laminated ring systems, some had only a single system. An amorphous matrix, small vesicles, or a combination of both, and occasionally needle-like paracrystals were usually observed between lamellae (Fig. 11). The fine structure of these organelles was similar to that of particles identified by a number of investigators (13, 28, 35, 39, 40) as myelin figures rich in lipoprotein.

The nuclear envelope of both the HeLa and MAC-21 cell was identical with that of other cells previously described (1, 2, 20, 43, 45, 46). However, the nuclear envelope of the MAC-21 was considerably more irregular than that of the HeLa cell, and nuclear pores were observed more frequently in the membrane of the MAC-21 cells.
than in the HeLa. Considerable deviations in the 60 A spacing between the lamellae of the nuclear membrane occurred in the degenerating cells of both types.

Grimestone (22) has suggested from his study on the flagellate *Trichonema* that a connection exists between the outer membrane of the nucleus and the parabasal body or Golgi complex of the flagellate. Edwards (16) described a connection between the outer membrane of the nucleus and the rough endoplasmic reticulum in cells from a bronchogenic carcinoma. Evidence of connections between the outer nuclear membrane and cytoplasmic membrane systems was apparent in both HeLa and MAC-21 cells (Fig. 4), but the culmination of these connections could not be determined.

The nucleoli of the two cell types appeared to be no different from those previously reported for other normal or neoplastic cells (5, 6, 8, 42).

**DISCUSSION**

The differences in fine structure between the HeLa and MAC-21 cells were not as great as was expected in view of their different origin. The differences were primarily quantitative, and are probably related to cell function as shown by a number of reports on undifferentiated malignant cells (3, 12, 16, 17, 19). A number of these variations may also be associated with the artificial environment in which the cells were maintained.

The secretory nature of the bronchogenic adenocarcinoma cell suggested that the MAC-21 cells might have a highly developed Golgi apparatus, and this was confirmed. The more highly developed Golgi apparatus of the MAC-21 cell was, in fact, one of the major morphologic characteristics differentiating it from the HeLa cell. Although there was some loss of polarity in the neoplastic cell from which the MAC-21 line was derived, a complete loss was noted in the cultured MAC-21 cell.

The mitochondria of the two cells were similar. Variations in size, shape, and cristae arrangement were observed, but such variations are commonly observed in both normal and neoplastic cells (4, 24, 32, 37). A marked increase in matrix density occurred in many mitochondria during the initial 4 days of growth in both cell types and continued to a lesser extent until subculture. This correlated with an increase in the cytoplasmic lipide particles, which in turn was inversely proportional to the pH of the medium (Chart 2). This increase in density may be the result of a change in mitochondrial membrane permeability and with a retention of some substance as yet unidentified (38).

The nuclear structure of the two cells was similar, although the membrane showed considerable irregularity with variations in thickness and number of pores. This is in accordance with other findings on normal and tumor cells (16). The roughness of the nuclear envelope of the MAC-21 cell as compared with the HeLa cell may be related to the secretory function of the mother cell and its correspondingly more active metabolism. The speed of nuclear rotation in the two cells was not compared, but the mechanical stresses of such rotation would conceivably have an effect on nuclear membrane invaginations. The outer nuclear membrane and the cytoplasmic membrane systems appeared to be connected in both HeLa and MAC-21 cells. The large numbers of Golgi complexes lying in close proximity to the sacculations of the nuclear membrane of the MAC-21 cells suggested the possibility that the Golgi complex may originate or at least have a connection with the outer laminae of the nuclear membrane.

The cytoplasm of both HeLa and MAC-21 cells contained a fibrillar component which Bruni (9), in his study of the HeLa cell, has suggested is a precursor of keratin. Squamous cells of the cervix and columnar cells of the bronchus occasionally produce keratin and, under various pathological conditions, may become excessively keratinized.

The origin and significance of the multivesicular bodies in both cell strains are obscure. Fogh and Edwards (19) speculate that these are associated with the Golgi complex. The larger vesicles closely resemble virus-like particles observed in certain animal tumors (4, 15). Multivesicular bodies have, however, been observed in normal cells (38), and the suggestion has been made that these are a normal constituent related to the growth cycle. The fact that lamellae are occasionally detected within the multivesicular bodies suggests that these entities may be involved in the formation of myelin figures.

The MAC-21 line probably originated from a mucin-secreting goblet cell, and the large areas of low density in the cytoplasm may represent accumulations of either mucin or glycogen or a combination of both. The polysaccharide of mucin has a low affinity for both osmium and lead hydroxide which could account for the relatively low electron density of these areas (36). The dense granules, on the other hand, were osmiophilic and stained with lead hydroxide. This, in conjunction with their size, indicated that they were probably glycogen or ribonucleoprotein (35). Alcian blue stain was nonspecific; the entire cytoplasm stained blue.

The dense bodies in the cytoplasm of the HeLa and MAC-21 cells may be phospholipide-rich absorption droplets, as suggested by Stoeckenius (39,
40), Revel (35), and Miller (28). These particles also resembled the lysosomes of deDuve (13) and the phagosomes of Strauss (41). The composition of the medium influenced the number of myelin figures present but was probably not the only factor involved, since it has been shown that the addition of heat-killed Brucella cells to a culture of HeLa cells and the addition of brucellergin to cultures of guinea pig spleen can also cause an increase in myelin figures (unpublished data). The lamellar form of the dense bodies predominated, but amorphous types were often observed. Both forms have a single limiting membrane and both appear to contain lipide material. It has been suggested (33) that both the amorphous and myelinated forms originate from mitochondria and that the amorphous structure may represent an intermediate step in the formation of myelin figures. The presence of multivesicular bodies containing lamellae in various stages of development would suggest that such might be the case. Mitochondria involved in the formation of lamellar structures have been observed in kidney proximal tubule cells of mice previously given injections of tetanus toxin (unpublished data).

This study of the fine structure of two cell types failed to demonstrate any component or variation of cellular components that was specifically characteristic of neoplastic cells. This is in agreement with Dmochowski’s extensive review (14) of the literature involving the electron microscopy of viruses and tumors.

REFERENCES


30. Morgan, C.; Hovde, C.; Rose, H.; and Moore, D. Struc-

Fig. 1.—Phase-contrast photomicrograph of a monolayer of HeLa cells 4 days following subculture. The cell membrane of each cell is usually in contact with adjacent cells. Nuclei and lipide-rich particles are prominent. X 1300.

Fig. 2.—H. & E.-stained monolayer of MAC-21 cells 4 days following subculture. The cells are widely spaced and exhibit the typical spindle shape. Lipide-rich particles are not evident with routine stains. X 1500.

Fig. 3.—Bright-field photomicrograph of MAC-21 cells fixed with osmium vapor 4 days following subculture. Lipide-rich particles with a relatively constant size are prominent. Large microvilli can also be distinguished. X 1300.

Fig. 4.—An abundance of perinuclear Golgi material (G) is a conspicuous characteristic of the MAC-21 cell. The arrow points out a possible connection of the outer nuclear membrane with a Golgi complex. X 30,000.
FIG. 5.—Cytoplasm of the MAC-21 cell. Mitochondria tend to be filamentous, with the longer ones adjacent to the nucleus. Normal cristae are evident. Rough endoplasmic reticulum can be seen scattered throughout the cytoplasm. A lipide-rich particle containing lamella and vesicles is adjacent to the nuclear membrane. X33,000.

FIG. 6.—Tonefibrils, possibly a precursor of Keratin, 100–150 A in width were occasionally found in the peripheral cytoplasm of the HeLa cell. They were seldom detected in the cytoplasm of the MAC-21 cell. X45,000.

FIG. 7.—A multivesicular body in the cytoplasm of the MAC-21 cell. These are regularly observed in the cytoplasm of both cell strains. The many small vesicles of varying diameter are surrounded by a single membrane. Occasionally the surrounding membrane appears to be incomplete, and a number of the vesicles are to be observed scattered throughout the surrounding cytoplasm. X80,000.

FIG. 8.—Multivesicular bodies (arrows) in the cytoplasm of a MAC-21 cell, containing lamellae in the process of formation. X30,000.
Fig. 9.—An area devoid of cytoplasmic organelles and without a limiting membrane is occasionally found in the cytoplasm of the MAC-21 cell. It consists of an amorphous matrix containing a scattering of granules approximately 160–250 Å in diameter. These regions probably represent accumulations of mucopolysaccharide. ×15,000.

Fig. 10.—Paracrystals of unknown origin are sometimes found in various cytoplasmic organelles. These crystals have a relatively constant width, ranging between 25 and 30 μm. The length, however, is variable. These crystals have been found within lipide-rich granules and not infrequently mitochondria. ×40,000.

Fig. 11.—A paracrystal that has developed to such a length that it has penetrated the organelle membrane and extended into the surrounding cytoplasm. This phenomenon is apparently rare. ×75,000.

Fig. 12.—Lipide-rich particles in the peripheral cytoplasm of a MAC-21 cell 7 days following subculture. These organelles have a single limiting membrane. Most of the particles have a fine structure consisting of several concentric rings. There is considerable variation in ring spacing and the number of ring systems. ×30,000.
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