CHANGES IN THE SURFACE MORPHOLOGY OF NORMAL AND TRANSFORMED MOUSE FIBROBLASTS AFTER DISTURBANCE OF ADHESIONS OF CELLS TO SUBSTRATE

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The surface morphology of normal embryonic mouse fibroblasts (MEF) and transformed fibroblasts of line L mice in culture during disturbance of cell-substrate adhesion by proteinases, EDTA, and urea was investigated with the scanning electron microscope. The morphology of rounding, induced in MEF and L cells by the above agents was almost independent of the type of agent. Rounding of MEF took place in three stages and was accompanied by considerable changes in the surface relief. The intermediate stage of formation of thick outgrowths was absent during rounding of the L cells and it took place without any change in surface relief. It is suggested that the differences discovered were connected with the weaker development of the lamelloplasm and bundles of microfilaments in the transformed cells compared with the normal cells.
KEY WORDS: *transformed cells; surface morphology; adhesions to substrate; lamelloplasm.*

Interaction between cells and different substrates plays an important role in their vital activity. The study of attachment of cells to the substrate and their spread over it in tissue culture has shown that this interaction is disturbed in many types of tumor cells [2] The opposite process — disturbance of the contact between normal and tumor cells and the substrate — has been inadequately studied.

In the investigation described below the dynamics of changes in the surface morphology of normal mouse embryonic fibroblasts (MEF) and transformed fibroblasts of L mice (L cells) was studied by scanning electron microscopy during experimental disturbance of cell-substrate adhesions.

EXPERIMENTAL METHOD

Secondary cultures of normal MEF and cultures of L cells [3, 5] were used. The cells were grown for 24 h (widely spaced cultures) in an atmosphere enriched with CO_2 on coverslips in medium 199 with 10% bovine serum and 100 units/ml monomycin. The cells were treated after five washings of the cultures with warm (37°C) Hanks' solution. Proteinase (trypsin, pronase, and papain) were added to the cells in Hanks' solution in a concentration of 100 μg/ml. EDTA was used in Bulbecco's solution without calcium and magnesium in a concentration of 200 μg/ml, and urea and sucrose were used in medium 199 without serum in a concentration of 1 M. All the solutions were at 37°C. Treated and control cells were fixed for scanning electron microscopy for not less than 30 min at 37°C with 2.5% glutaraldehyde solution in isotonic cacodylate buffer, pH 7.3-7.4 and postfixed for 1 h in 1% osmic acid in the same buffer. The cells were then dehydrated in ascending concentrations of acetone and dried from liquid CO_2 by the critical point transition method in a Bomar SPC-900/EX apparatus. The dried preparations were sprayed with silver and studied in the Stereoscan S-4 scanning electron microscope at an angle of 45° and with an accelerating voltage of 10 kV.

EXPERIMENTAL RESULTS

Normal fibroblasts in widely spaced culture were flattened and well spread out on the substrate. They had a well-developed lamelloplasm [3] and a smooth upper surface (Fig 1).

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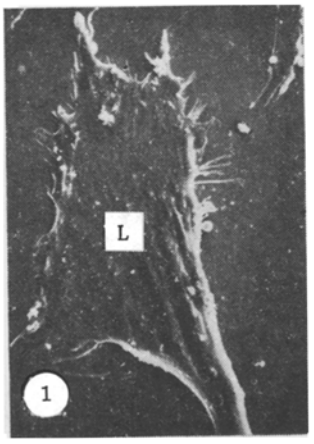

Fig. 1

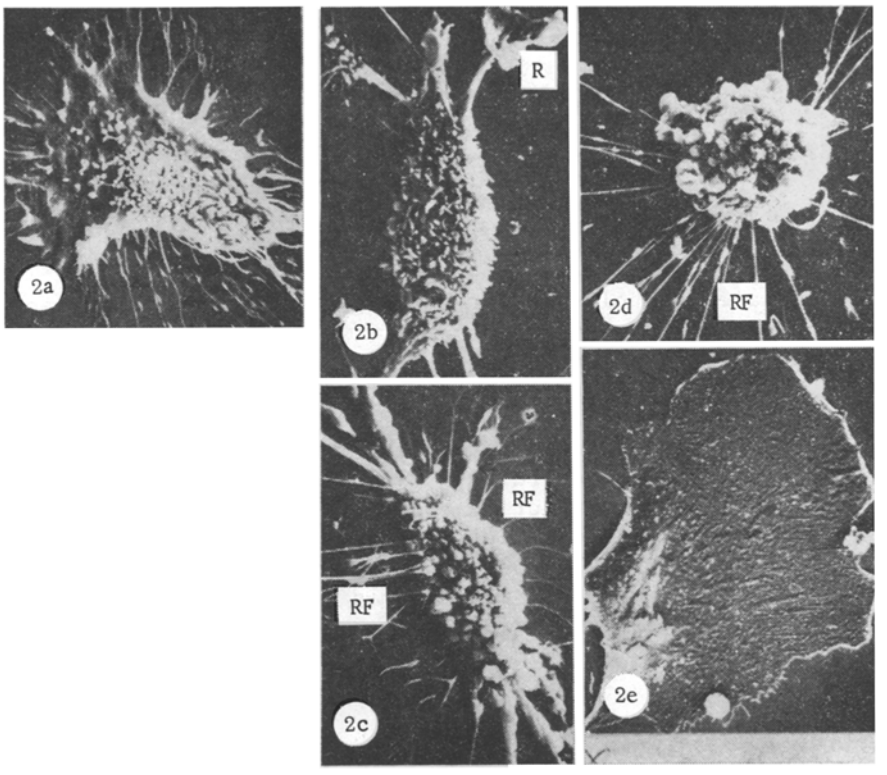

Fig. 2

Fig. 1. Normal embryonic fibroblast. L) lamelloplasm, 870×.

Fig. 2. Stages of rounding of MEF. a) stage of compression (5 min in urea); arrows indicate retraction fibrils (1400×). b) Stage of thick outgrouths (20 min in EDTA); cell covered by microvilli; outgrowths marked by arrows. R) Ruffle at end of outgrowth (1800×). c) Stage of thick outgrowth (10 min in urea); cell covered with vesicles; arrows indicate outgrowths. FR) Retraction fibrils (1600×). d) Sphere stage (15 min in urea); well covered with vesicles. RF) Retraction fibrils with thickenings (1700×). e) Cell after 2 h in sucrose: no difference from control (900×).

Under the influence of proteinases, EDTA, and urea adhesion of the cells to the substrate was disturbed, and this was shown as compression of the cells and their successive rounding and separation from the substrate. All agents used acted more or less similarly. The process of rounding could be divided in all cases into three stages: compression of the cells, the formation of thick outgrowths, and the formation of a sphere with retraction fibrils. At the compression stage the morphology of the cells differed from that of the control: The cells became less spread out, microvilli, ruffles, or vesicles appeared on the surface of many of them, and retraction fibrils [4, 9], leaving the cell body or the lamelloplasm, were formed (Fig. 2a). Sometimes the lamelloplasm was reduced but was still preserved. At the stage of formation of thick outgrowths most cells were highly compressed and often had lost their lamelloplasm. The cell surface was covered with vesicles or folds and there were few retraction fibrils. Most cells gave off a few long (up to 60 μ), thick (about 1.2 μ) processes. They lay on the surface of the substrate or were attached to it by their distal end, forming lamellar areas of vertical ruffles at the site of attachment (Fig. 2b). Under the influence of urea this stage was ill defined and had some special features: The cells formed many retraction fibrils and the morphology of the processes differed from that in cells treated with EDTA or proteinases (Fig. 2c). At the stage of a sphere with retraction fibrils the cells were completely rounded. The numerous retraction fibrils which they gave off (up to 50 μ long and about 0.25 μ thick) usually branched at the site of attachment to the substrate into thinner filaments about 0.17 μ thick, which extended over the surface of the substrate. In the cells treated with urea the retraction fibrils often had characteristic oval thickenings measuring about 1 μ (Fig. 2d). The surface of the cells at this stage was usually covered by numerous vesicles about 0.9 μ in diameter (Fig. 2d). The

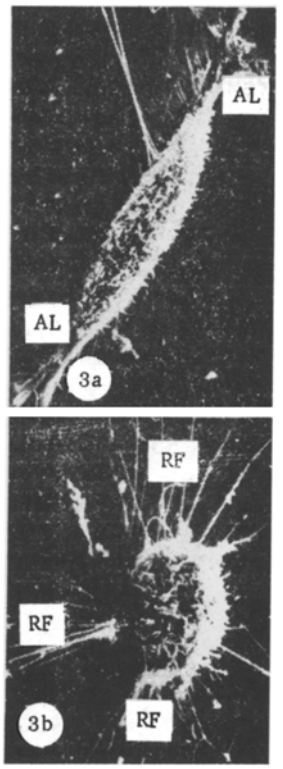

Fig. 3. Rounding of L cells. a) Control cell covered with microvilli. AL) Areas of lamelloplasm (850×). b) Round cell covered with microvilli (5 min in trypsin). RF) retraction fibrils (1600×).

rounding action of urea discovered in these experiments was not due to hypertonicity of the medium, for incubation of the cells in 1 M sucrose solution did not change their morphology significantly (Fig. 2e). All the substances used were reversible in their action: Eighteen h after transfer of the rounded cells to the growth medium they were indistinguishable from the controls.

Control L fibroblasts in widely spaced culture were fusiform or polygonal in shape, they were poorly spread out over the substrate, and they formed a poorly developed lamelloplasm (Fig. 3a). The cell surface was covered by numerous microvilli about 1.6 μ long. Treatment of the L cells with substances disturbing adhesion led to their rounding and separation from the substrate. When proteinases and urea were used the L cells rounded completely sooner than the MEF (5 and 5 min compared with 30-45 and 15 min respectively), whereas when EDTA was used, they did so after about the same time (30-45 min). Proteinases, EDTA, and urea caused compression and rounding of the cells and disappearance of the lamelloplasm. The stage of formation of thick outgrowths was absent. Large L cells formed retraction fibrils similar to MEF and stayed covered with microvilli (Fig. 3b). The action of urea on the L cells, just as on MEF, was not due to hypertonicity of the medium. All the substances used were reversible in their action.

The results thus show that the mechanisms of adhesion of transformed cells to the substrate are different (or modified) from those in normal cells. For instance, after disturbance of adhesions, rounding of MEF took place in three evidently consecutive stages, and their surface relief under these circumstances was significantly changed. During rounding of L cells the intermediate stage of formation of thick outgrowths typical of MEF was absent and the surface relief of the cells remained covered with microvilli. It was shown previously that the lamelloplasm in transformed fibroblasts is more weakly developed than normally and that the submembranous bundles of contractile microfilaments are absent [3, 7, 8]. This phenomenon is evidently connected with reduction or absence of firm contact between the cells and substrate, associated with bundles and formed mainly in the region of the lamelloplasm [1]. Since the thick processes evidently connect the cell body to the sites of firm contact, absence of the latter leads to disappearance of the stage of outgrowth during rounding. The lack of change in the surface relief of the L cells during

rounding may be the result of reduction of the bundles of microfilaments and also of the formation of new or lengthening of the old microvilli. Rounding of the flattened, normal fibroblasts also takes place almost without any change in its surface area [6], and this must lead to the appearance of surface projections (vesicles etc.) on a spherical cell. The L cells are initially highly compressed, and their rounding can therefore take place without the formation of additional surface projections. Further investigations will show whether the differences discovered are typical of other types of transformed cells also.

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