Cytoplasmic topography of focal contacts

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Abstract To investigate the structure of focal contacts, the cytoplasmic faces of fibroblast membranes were examined in solution by scanning force and immunofluorescence microscopy. Focal contacts were identified in scanning force topographs by correlation with fluorescence images. Finer details were resolved in topographs of the focal contacts than in fluorescence micrographs. Increased separation of ventral plasma membranes from the substrate correlated with the duration of cell culture. The cytoplasmic projections of the focal contacts also increased with the cell culture period. These changes accompanied lateral spreading of fibroblasts during a period of several hours after seeding cells in culture medium.

Key words: Actin; Cell adhesion; Immunofluorescence; Plasma membrane; Scanning force microscopy; Vinculin

1. Introduction

Focal contacts, sites of attachment between adherent cells and the extracellular matrix, are protein assemblies through which a cell senses and attaches to its surroundings. The transmembrane components are the integrins which serve as receptors for extracellular matrix proteins such as fibronectin, collagen, and laminin [1–3]. Integrins also bind cytoplasmic proteins, such as talin and α-actinin, which appear to be involved in the connection of actin stress fibers to the membrane in focal contacts [4]. Other proteins such as focal adhesion kinase, $pp60^{c-src}$, tensin, and paxillin seem to contribute to the signaling events in these structures [5].

A few details about the ultrastructure of focal adhesions are known: In previous studies, the measured or calculated distance between the substratum and the plasmalemma at focal contacts ranged from 10 to 50 nm [6–8]. In thin sections of focal contacts viewed with electron microscopy after immunocytochemistry, vinculin was found to lie closer to the plasmalemma than α-actinin [8]. Another investigation, performed using quick-freeze, deep-etch, rotary-replication of specimens for scanning electron microscopy, revealed many lateral associations of stress fibers with the membrane, and focal contacts appeared to involve aggregates that were immunolabeled for vinculin and talin along the final few microns of stress fibers [9].

Additional information about the structure of focal contacts has now been obtained by scanning force microscopy. A typical scanning force micrograph is a color-encoded topograph. It is a digital recording of the separation necessary to maintain a constant force between a spring-mounted stylus and a sample surface throughout a serial, two-dimensional scan of the surface. With a low contact force and a sharp stylus, the resulting image may reveal molecular topography. In images of dried cytoskeletal preparations, a fine web of intracellular filaments and organelles was observed [10]. However, an advantage of this microscopy with respect to other high-resolution microscopies is that specimens need not be dehydrated or exposed to solvents. The technique has proved suitable for imaging on a molecular scale the disassembly of purified clathrin complexes in solution [11]. On a larger scale, scanning force microscopy on the surface of cells in solution has been used to visualize cytoskeletal reorganization and the movements of granules [12–14] or to measure physical properties of cells [15].

In this investigation, images of the cytoplasmic face of plasmalemma maintained in physiologically buffered solutions were recorded using scanning force and immunofluorescence microscopy to determine the intracellular topography of focal contact-associated proteins. Fine details were revealed in focal contacts positively identified by the correlation of scanning force and immunofluorescence micrographs. Measurements from the topographs showed that both the separation of ventral plasma membranes from the substrate and the cytoplasmic extension of focal contacts increased with the duration of the cell culture.

2. Materials and methods

2.1. Culture of 10-day-old chicken embryo fibroblasts

Fibroblasts taken from 10-day-old chicken embryos [16] were cultured at 37°C, 5% CO_2 in Dulbecco's modified Eagle's medium (Biowhittaker, Verniers, Belgium) with 5% fetal calf serum (Irvine Scientific, Santa Ana, CA), 2 mM glutamine (HyClone, Cramlington, The Netherlands), 100 U/ml penicillin and 100 μg/ml streptomycin (Biochrom KG, Berlin, Germany). Cells were passaged within 1–2 days after confluency and discarded after the fourth passage.

2.2. Preparation of ventral plasma membranes

15 mm diameter coverslips (Knittel Glaser, Germany) were boiled in 0.25 N HCl for 20 min and rinsed with MilliQ+ (Millipore, Bedford, MA) purified water followed by ethanol, and stored in MilliQ+ purified water. The coverslips were rinsed in medium and placed in the wells of a 12-well culture plate (Costar, Cambridge, MA). Some coverslips were first incubated overnight on 50–70 μl droplets of 20 μg/ml fibronectin (Collaborative Research, Bedford, MA) before cell seeding.

A confluent dish of chicken embryo fibroblasts was washed with phosphate-buffered saline (PBS), trypsinized, and the cells were then pelleted and resuspended in fresh medium twice. 1/60 to 1/10 of the resuspended cells (higher concentrations were used for shorter cultures) were added to coverslips and the volume was adjusted to 2 ml. The culture plates were incubated for 1.5–15 h at 37°C in 5% CO_2. Each coverslip was rinsed quickly with ice-cold MilliQ+ purified water and allowed to rest for 1 min to osmotically rupture the cells. Then a stream of ice-cold water from a wash bottle was squirted against each coverslip to shear away the dorsal membrane of the adherent cells. 1–2 ml of 3% formaldehyde in PBS also containing 0.1 mM $CaCl_2$ and 0.1 mM $MgCl_2$ was added to each well to fix

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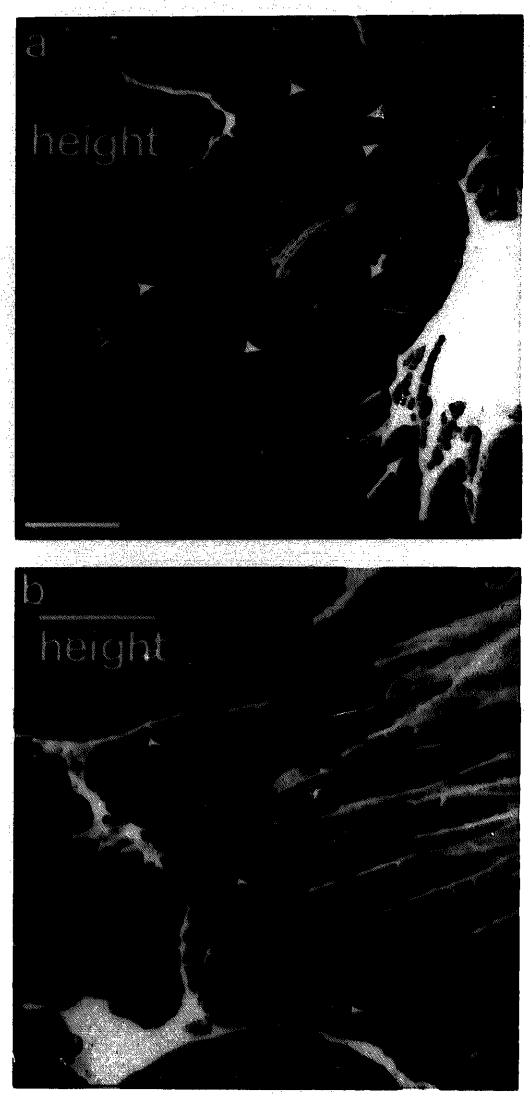

Fig. 1. (a) A scanning force micrograph of a ventral plasma membrane from a cell cultured 3 h on fibronectin-coated glass. Small holes in the membrane (arrowheads) were visible as well as an intact cell (arrow). (b) A higher magnification, scanning force micrograph of the left-hand edge of the same ventral plasma membrane revealed numerous small particles (arrowheads). Scale bars: (a) 10 μm, (b) 4 μm. Color-encoded height ranges: (a) 800 nm, (b) 200 nm.

proteins during 20 min of incubation at room temperature. The coverslips were rinsed in PBS containing 0.05% NaN_3 and mounted on slides for phase microscopy.

2.3. Phase microscopy

The coverslips were viewed in phase contrast at 400× through an Axiophot microscope (Zeiss, Oberkochen, Germany) and approx. 200 μm diameter circles were scribed around intact ventral plasma membranes, using an eccentric diamond scribe inserted in the microscope nosepiece.

2.4. Scanning force microscopy

Marked coverslips were mounted for examination in a NanoScope III MultiMode Scanning Force Microscope (Digital Instruments, Santa Barbara, CA) resting in a vibration-damped, optical microscope (Nikon, Japan). 80–200× magnification permitted positioning of a force-sensing, cantilever stylus within a scribed circle. Pyramidal, silicon nitride styli integrated in cantilevers with spring constants of approx. 0.06 N/m (Digital Instruments specifications) were used to trace in continuous contact mode the topography of the ventral plasma membranes in PBS with sodium azide. The repulsive, contact force was typically 0.3 nN. The feedback gains ranged from 0.3 to 5 at scan speeds of approx. 500 μm/s at low magnification or 15–40 μm/s at high magnification. Relative separation of the surface and the stylus, constant force or height mode, was recorded along with the cantilever deflection, error, at higher magnification.

2.5. Fluorescence microscopy

Ventral plasma membranes were fluorescently labeled to reveal actin and vinculin. The coverslips were removed from the scanning force microscope mounts, treated with 0.2% Triton X-100 (to permeabilize intact cells), incubated with 0.2% gelatin in PBS, and then incubated for 1 h with fluorescein-conjugated phalloidin and a monoclonal antibody against vinculin from chicken (Sigma, St. Louis, MO). Subsequently, the coverslips were washed and incubated with sheep anti-mouse antibodies conjugated with rhodamine (Boehinger Mannheim, Germany) for 30 min. The coverslips were washed in PBS, mounted on slides, and the encircled membranes were located using phase contrast before observation using fluorescence at 1000×.

2.6. Image processing and analysis

Images were processed using the Nanoscope III software version 3.20. To remove offsets in the slow scan direction, the average height was subtracted from each line along the fast scan axis. When necessary, a first-or second-order plane was subtracted to eliminate tilt or bow typical of scans of 10 μm or more. In most images, the contrast was improved by subtracting the error image from the height image. Student's *t*-test was used to statistically verify differences between average heights measured at 1.5 h and successive time points.

3. Results

3.1. Complete topographic profiles of complex membrane surfaces

To study the structure of the cytoplasmic components of focal adhesions, fibroblasts were cultured on glass coverslips and then ruptured by osmotic lysis and sheared using a stream of water. Vigorous squirting tore the membrane on the upper cell surface (dorsal plasma membrane) away from the membrane in contact with the substrate (ventral plasma membrane) [16]. Most of the intracellular organelles were also sheared away in the stream of water. Scanning force and optical microscopy were performed on the ventral plasma membrane fragments that remained attached to the substrate.

One of the most striking aspects of the topographs of ventral plasma membranes was the complete view of the complex weave of components that resulted. Fig. 1 is an example of the thorough view of ventral plasma membrane structure provided by scanning force microscopy. In Fig. 1a, a ventral plasma membrane lay beside an unsheared cell (arrow). The height of the intact cell exceeded the vertical scan range of the microscope and thus saturated the color-encoded height scale. Fine details of the surface of the intact cell were not visible while the nearby ventral plasma membrane had clearly distinguished stress fibers that radiated from the center. The terminations of the stress fibers were clearly depicted in a higher magnification image, Fig. 1b. In addition to high contrast detection of focal adhesion sites and stress fibers, small holes in the membrane (Fig. 1a, arrowheads) and small particles of approximately 100 nm diameter (Fig. 1b, arrowheads) were observed. These features were not detected in immunofluorescence or phase contrast micrographs of the membrane (data not shown).

In another example, a ventral plasma membrane exhibited a pair of prominent actin bundles (Fig. 2c,d; arrows) which appeared to connect directly to focal contacts (Fig. 2b,d; arrowheads). Actin in the fibers and vinculin in the focal contacts was identified using immunofluorescence microscopy,

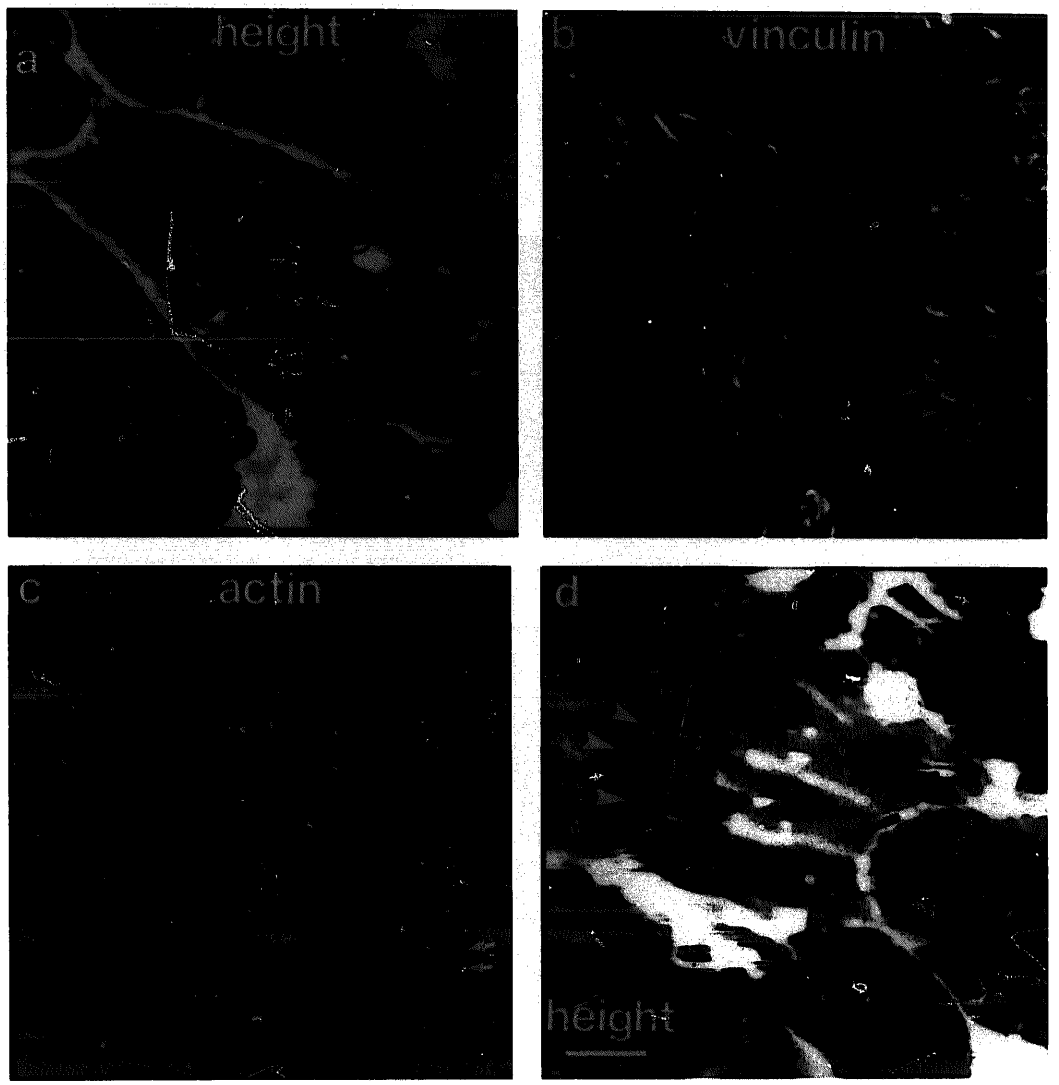

Fig. 2. (a) A scanning force micrograph of a ventral plasma membrane from a cell cultured 15 h on glass. Some damage to the membrane (dark outline) was evident. (b) Vinculin or (c) actin labeling of the same ventral plasma membrane by immunofluorescence. Vinculin-labeled focal adhesions (arrowheads) and actin-labeled stress fibers (arrows) are indicated. (d) A higher magnification scanning force micrograph of the boxed area in a showing the focal adhesions (arrowheads) and stress fibers (arrows) indicated in b and c. The rectangular box is an example of a region for which parallel cross-sections were averaged to measure focal contact heights (see Fig. 5a). Scale bars: (a–c) 10 μm, (d) 2 μm. Color-encoded height ranges: (a) 450 nm, (d) 350 nm.

but the precise alignment at these junctions was succinctly displayed in the topograph (Fig. 2d). A damaged area of this ventral plasma membrane was visible in scanning force micrographs (Fig. 2a; dark outline). Ventral plasma membranes of which the protein structures had survived the shearing process, but the lipid bilayer had been partially disrupted were often observed. Such damage was difficult to detect using either fluorescence or phase contrast microscopy.

Unidentified filaments were also detectable under and around the ventral plasma membranes. In both Fig. 3a (15 h culture) and Fig. 3c (3 h culture) the perimeters of the membranes were obvious and many fibers were found crossing the membranes. Fluorescent, actin labeling was delimited by the membrane perimeters and numerous parallel stress fibers were visible on the membrane of a cell cultured overnight (Fig. 3b) or for 3 h (Fig. 3d). On both membranes there were also filaments that lay transverse to the predominant orientation (arrows in Fig. 3a,c,e,f). In the fluorescence image (Fig. 3b), actin labeling did not reveal the transverse filaments crossing the membrane of Fig. 3a (also Fig. 3f), while the transverse fibers in Fig. 3c (also Fig. 3e) were clearly labeled in Fig. 3d. The unlabeled, transverse filaments were continuous with filaments that extended beyond the perimeter of the membrane (Fig. 3a), but they did not colocalize with anti-vinculin labeling (data not shown), as reported previously for cable-like extracellular structures lying under ventral plasma membranes [8].

The areas surrounding ventral plasma membranes differed greatly according to the duration of the cell culture. After an overnight culture (Fig. 3a) the substrate was strewn with filaments that were not labeled by fluorescent phalloidin (Fig. 3b) and therefore did not appear to be actin. Instead for ventral plasma membranes prepared after brief cultures (Fig. 3c) the surrounding substrate was free of such filaments.

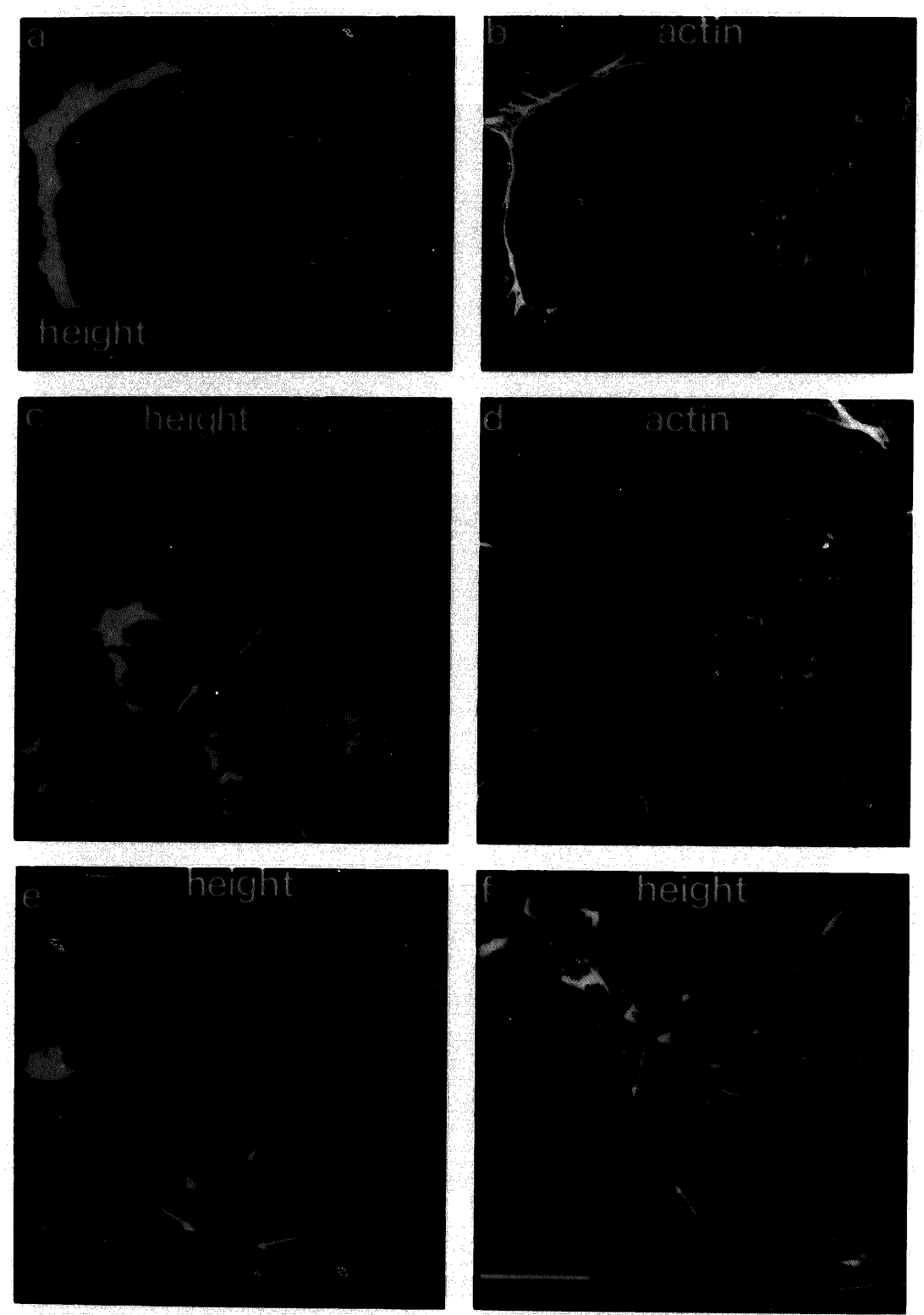

Fig. 3. (a) A scanning force micrograph of a ventral plasma membrane from a cell cultured 15 h on glass and (b) immunofluorescent, actin labeling of the same ventral plasma membrane. Arrows indicate filaments oriented transversely with respect to the majority of filaments. (c) A scanning force micrograph of a ventral plasma membrane from a cell cultured 3 h on fibronectin-coated glass and (d) immunofluorescent, actin labeling of the same ventral plasma membrane. (e,f) Higher magnification scanning force micrographs of the transverse filaments indicated in c and a (respectively). Scale bars: (a–d) 20 μm, (e) 5 μm, (f) 10 μm. Color-encoded height ranges: (a) 500 nm, (c,e) 250 nm, (f) 400 nm.

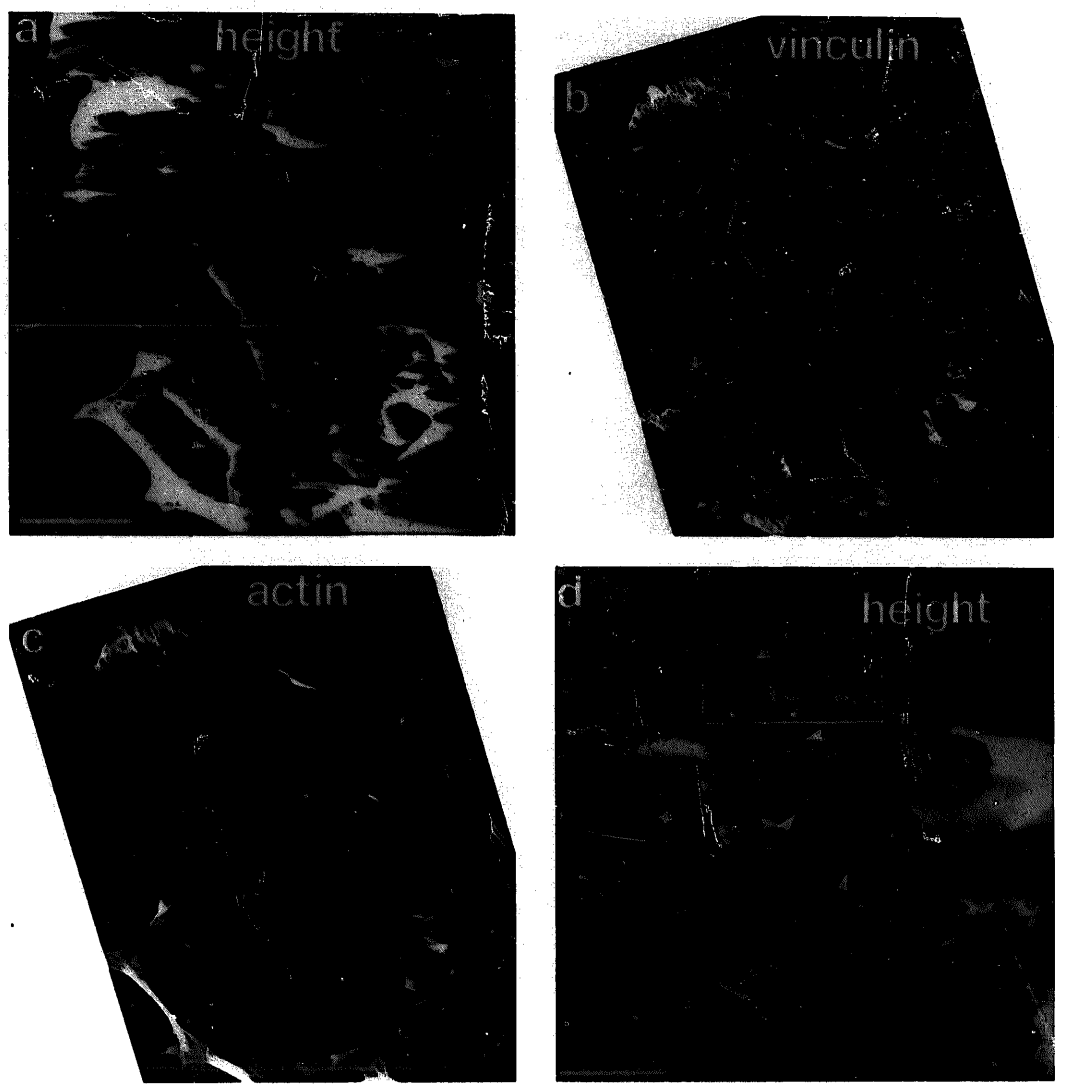

Fig. 4. (a) A scanning force micrograph of a ventral plasma membrane from a cell cultured 3 h on fibronectin-coated glass. Immunofluorescent labeling of (b) vinculin and (c) actin on the same ventral plasma membrane. (d) A higher magnification scanning force micrograph of the focal contact in the boxed area in a. Arrows indicate 60 nm actin-containing fibrils. The rectangular box is an example of a region for which a pixel height histogram was calculated to measure relative heights between focal contacts, the plasmalemma, and the substrate (see Fig. 5b). Scale bars: (a–c) 20 μm, (d) 2 μm. Color-encoded height ranges: (a,d) 200 nm.

3.2. High-resolution topography of focal contacts

When fibroblasts were cultured for 1.5–3 h, they formed large focal adhesions on their peripheries [16]. The ventral plasma membranes prepared from these brief cultures were often free of overlying stress fibers. The ventral plasma membrane shown in Fig. 4a had broad focal contacts revealed by fluorescent labeling of vinculin in Fig. 4b. In a high magnification scanning force micrograph of the focal contact (Fig. 4d), fine parallel fibers as small as 60 nm in width were revealed (arrowheads). These fibers were probably very thin bundles of actin filaments associated with vinculin and other focal contact proteins, since this focal contact was also lightly stained for actin (Fig. 4c). Such fine fibers were not resolved in the fluorescence micrograph at 1000×.

3.3. Growth of focal contacts

From the topographic data, the relative heights between focal contacts, plasmalemma, and substrate were measured in two ways. In images with little noise, two cursors were placed arbitrarily along an average cross-section and the vertical distance between them was recorded. For example, Fig. 5a depicts an average cross section calculated from a series of parallel cross-sections lying in the box of Fig. 2d. The vertical distance between the cursors, which corresponded to the relative height of a focal contact above the plasma membrane, was 34.1 nm. Instead in noisy images, a histogram of the number of pixel heights was determined for all pixels within a region. For stacked planar features, i.e. membranes, such a histogram has a modal distributon with noise contributing to the breadth of the peaks. Fig. 5b shows such a histogram for the region of Fig. 4d bounded by the rectangular box. Three peaks were obvious and the relative height of the middle peak, the cytoplasmic face of the plasma membrane, with respect to the lowest peak, the substrate, was 25.9 nm.

57 measurements of the distance from the substrate to the cytoplasmic (upper) face of the membrane were made using

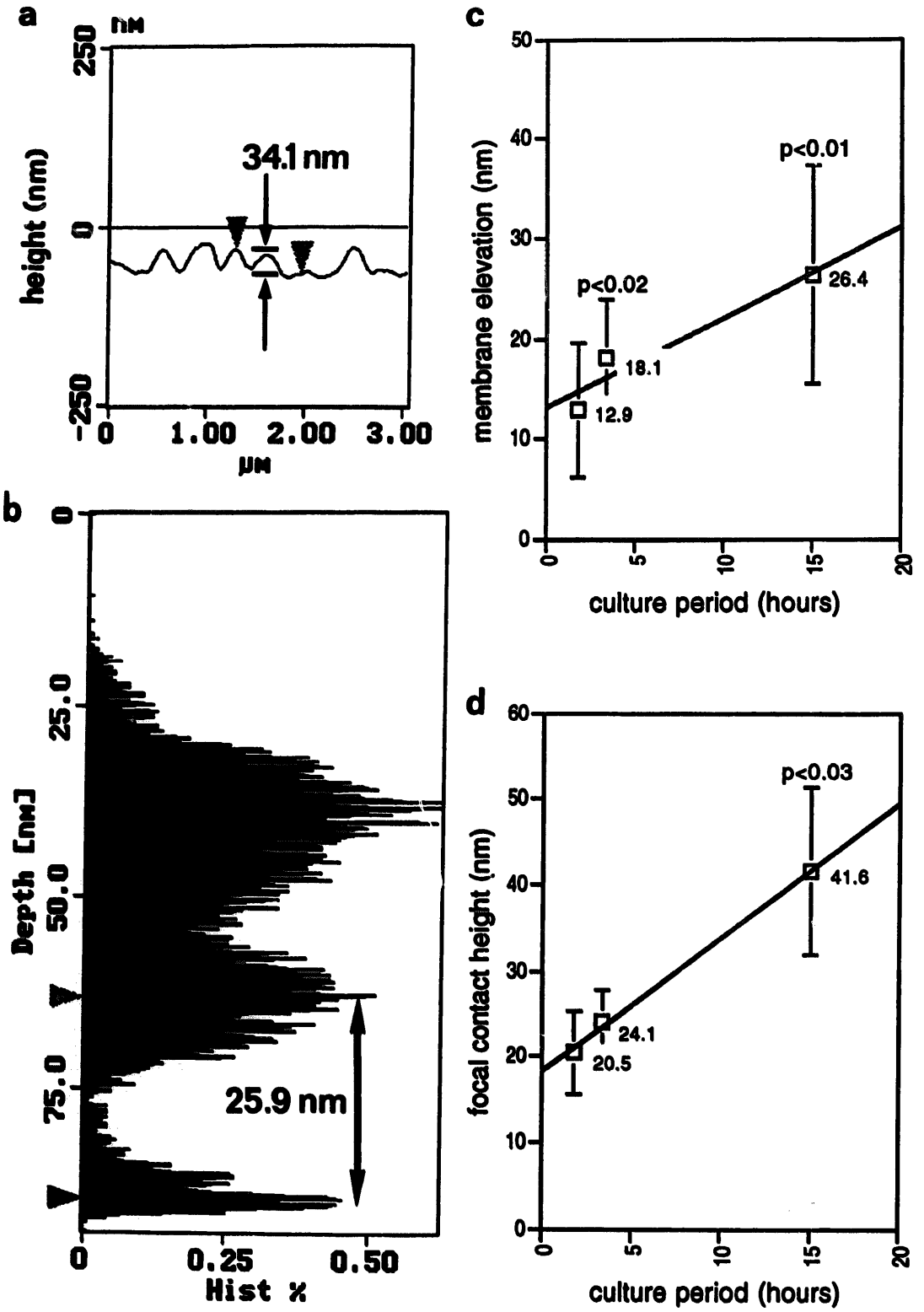

Fig. 5. (a) An example of a average cross-section calculated for parallel cross-sections in the box in Fig. 2d. The low points correspond to the cytoplasmic face of the plasmalemma and the five bumps to vinculin-labeled structures. Cursors were arbitrarily placed along the curve to measure a focal contact height of 34.1 nm. (b) An example of a pixel height histogram calculated for the area in the rectangular box of Fig. 4d. Three distinct levels were apparent in the area and resulted in a trimodal histogram. Cursors were modally placed on the histogram to measure the middle peak, the cytoplasmic face of the membrane, 25.9 nm above the lowest peak, the substrate. (c) Separation of ventral plasma membrane from the substrate versus the duration of cell culture. The distance between the cytoplasmic (upper) face of the ventral plasma membrane and the substrate was measured in areas free of overlying structures. (d) Cytoplasmic profiles of focal contacts on ventral plasma membranes versus the duration of cell culture. The distance between the cytoplasmic (upper) face of the ventral plasma membrane and the peak of a focal contact was measured.

cross-sections and height histograms. The measurements ranged from 6.1 to 43.6 nm in areas free of fibers and focal adhesions and increased with the duration of the cell culture (Fig. 5c). This increase may have been related to the fibrous residue that accumulated on the substrate during overnight cultures (see section 4). The focal contacts also grew more prominent on the cytoplasmic face of the membrane as a function of the cell culture period (Fig. 5d). The focal adhesions considered in Fig. 5d had little or no actin labeling, so the bulk of actin-containing stress fibers did not significantly contribute to the measurement.

Topographic profiles permitted easy measurement of focal contact heights with respect to either the membrane (Fig. 5d) or the substrate, but the separation between the membrane and the substrate under focal contacts could only be estimated. Therefore, the minimum measured thickness of the membrane, 6.1 nm, and the focal contact height above the membrane were subtracted from the focal contact height above the substrate. The remainder, the separation between the membrane and the substrate under the focal contacts, averaged 11.7 ± 9.0 nm.

4. Discussion

4.1. *Exposed, supported specimens aid scanning force microscopy*

Scanning force microscopy of cell membranes is more revealing in the periphery of adherent cells that is flattened against the substrate [10,14,17]. Accordingly, the preparation of ventral plasma membranes was useful in two respects: (1) Cytoplasmic structures were traced more accurately by eliminating overlying dorsal plasma membrane and cytoplasmic components. In the future such preparations will be useful, because the ventral plasma membranes are completely accessible to manipulation by mechanical or biochemical methods. It should be possible to monitor the structural changes resulting from signal transduction events at the cytoplasmic face of the plasma membrane. (2) Rigidly supporting ventral plasma membranes on a glass substrate improves resolution. The resolution that can be expected for topographs of biological, 'soft' samples has been estimated from indentation versus force curves for gelatin films in mixtures of propanol and water [18]. The indentation curve for cells fixed with glutaraldehyde [15] is most similar to that of gelatin in 50% propanol which was calculated to have an elastic modulus of 0.2 MPa. From [18] one can estimate the resolution of topographs of fixed cell membranes to be approx. 90 nm. The slightly higher resolution in Fig. 4 may reflect the additional support of the hard glass substrate as opposed to fluid, partially fixed cytoplasm.

Fixation of the ventral membranes was used to permit stable scanning in contact mode. As a result, the resolution of the specimens may have improved since the elastic modulus of cell surfaces increases with fixation [15] as it does for gelatin films in solutions of increasing alcohol percentages [18].

4.2. *Topography provides a complete picture of complex intracellular structures*

Immunocytochemistry is used to reveal very selectively antigenic structures. This method enhances the contrast of labeled structures, but their organization with respect to adjacent, unlabeled components cannot be determined. Instead, in scanning force microscopy, the height of any membrane component that is significantly different from its surroundings is contrasted and quantitated. In topographs, but not in fluorescence or phase contrast micrographs, fine details such as 100 nm diameter particles (Fig. 1), holes in the lipid bilayer (Figs. 1 and 2), and unlabeled filaments (Fig. 3) were revealed in addition to fibers and focal contacts.

Focal contacts were always visible in scanning force micrographs unless overlaid by bulky stress fibers. On ventral plasma membranes prepared from cells cultured for 1.5–3 h, unencumbered focal contacts measuring several microns in length were common on the periphery. These focal adhesions exhibited fine fibrillar structures probably related to actin networks associated with stress fiber terminals. Such fine fibrillar structure (Fig. 4) was not revealed in fluorescence images of the same membranes labeled for actin and vinculin.

The fine structure depicted in the topographs could have been better analyzed if molecular labeling methods suitable for scanning force microscopy had been available. Such methods are being developed [19] and will be valuable as further improvements in scanning force microscopy, such as resonance scan modes which transmit less shearing force to the sample, are employed to reveal even finer details of membranes maintained in solution. In addition, such techniques would eliminate the need to relocate features in a complementary microscope.

4.3. *Interpreting height profiles of ventral plasma membranes*

The distance between the plasmalemma and the substrate at focal contact sites averaged 11.7 ± 9.0 nm. Calculations of this separation from variable-angle total internal reflection fluorescence microscopy measurements averaged 24±13 nm [20]. In a study made using tandem scanning confocal microscopy, calculated separations between the plasmalemma and the substrate ranged from 10 to 50 nm and increased radially from the center of focal contacts [7]. This separation was also measured in electron micrographs of vertically sectioned, adherent cells and was found to range from 10 to 20 nm [6,8]. In comparison to these data, the measurements from the topographs were computationally simple, were not compromised by dehydration artifacts, and indicated very close contact between the plasma membrane and the substrate at focal contacts.

In areas without focal contacts, the separation of the membrane from the substrate increased with the duration of cell culture. Changes in this separation have been previously related to a shift from a high percentage of focal and 'close' contacts in 6–12 h cultures to more widely separated contacts in 24–36 h cultures [8]. These wider 'extracellular matrix' contacts appeared to involve fibronectin containing 'cables' at the extracellular face of the ventral plasma membrane which elevated the cell 100 nm or more above the substrate. The present topographic measurements (Fig. 5) are low in comparison. However, the broader separations measured using transmission electron microscopy might have been skewed by alteration of the structures during the dehydration necessary for the preparation of thin sections of adherent cells [6]. Alternatively, the height measurements made using scanning force micrographs might have been low due to compression of the sample by the stylus [15].

As the plasma membrane became elevated above the substrate, the cytoplasmic extension of vinculin-labeled struc-

tures, that are usually defined as focal contacts, also increased (Fig. 5). However, Chen and Singer have reported that extracellular matrix contacts also contain vinculin [8]. They argued that since adhesion of cells to the substrate was strongest on the periphery and adhesive strength was greatest in focal contacts, vinculin-labeling sites in the interior of a ventral plasma membrane were likely to have been extracellular matrix contacts. While they measured a 60 nm thick electron dense cytoplasmic zone associated with focal contacts, they did not find similar densities associated with extracellular matrix contacts. In the present study, the membrane adjacent to vinculin-labeled features did not become more separated from the substrate with time, so a similar transition from focal to extracellular matrix contacts is not evident. The cytoplasmic extension observed in vinculin-containing sites appears to reflect that of focal contacts.

4.4. Conclusions

In order to take advantage of the high resolving power of the scanning force microscope, a specimen should be immobilized on a flat, rigid support. Slightly better than predicted resolution resulted when ventral plasma membranes were fixed, but not dehydrated, and supported on glass for scanning force microscopy of focal contacts. Arrays of actin-containing fibers as little as 60 nm in width were found associated with vinculin in large focal contacts at the peripheries of membranes isolated from fibroblasts cultured for 1.5 to 3 h. In fibroblasts incubated for 15 h, the ventral plasma membrane became elevated from the substrate and focal contacts extended into the cytoplasm.

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References

[1] Hynes, R.O. (1992) Cell 69, 11–25.
[2] Otey C.A., Pavalko, F.M. and Burridge, K. (1990) J. Cell Biol. 111, 721–729.
[3] Tapley, P., Horwitz, A., Buck, C., Burridge, K., Duggan, K. and Rohrschneider, L. (1989) Oncogene 4, 325–333.
[4] Horwitz, A., Duggan, K., Buck, C., Beckerle, K. and Burridge, K. (1985) Nature 320, 531–533.
[5] Schaller, M.D. and Parsons, J.T. (1993) Trends Cell Biol. 3, 258–262.
[6] Bereiter-Hahn, J., Fox, C.H. and Thorell, B. (1979) J. Cell Biol. 82, 767–779.
[7] Davies, P.F., Robotewsky, A. and Griem, M.L. (1994) J. Clin. Invest. 91, 2640–2652.
[8] Chen, W. and Singer, S.J. (1982) J. Cell Biol. 95, 205–222.
[9] Samuelsson, S.J., Luther, P.W., Pumplin, D.W. and Bloch, R. J. (1994) J. Cell Biol. 122, 485–496.
[10] Pietrasanta, L.I., Schaper, A. and Jovin, T.M. (1994) J. Cell Sci. 107, 2427–2437.
[11] Wagner, P., Kernen, P., Hegner, M., Ungewickell, E., Semenza, G. (1994) FEBS Lett. 356, 267–271.
[12] Fritz, M., Radmacher, M. and Gaub, H.E. (1994) Biophys. J. 66, 1328–1334.
[13] Spudich, A. and Braunstein, D. (1995) Proc. Natl. Acad. Sci. USA 92, 6976–6980.
[14] Schoenberger, C. and Hoh, J. (1994) Biophys. J. 67, 929–936.
[15] Hoh, J. and Schoenberger, C. (1994) J. Cell Sci. 107, 1105–1114.
[16] Cattelino, A., Longhi, R. and De Curtis, I. (1995) J. Cell Sci. 108, 3067–3078.
[17] Henderson, E., Haydon, P.G. and Sakaguchi, D.S. (1992) Science 257, 1944–1946.
[18] Radmacher, M., Fritz, M., and Hansma, P.K. (1995) Biophys. J. 69, 264–270.
[19] Eppell, S.J., Simmons, S.R., Albrecht, R.M. and Marchant, R. E. (1994) Biophys. J. 68, 671–680.
[20] Burmeister, J.S., Truskey, G.A. and Reichert, W.M. (1994) J. Microsc. 173, 39–51.