Unphosphorylated Gelsolin Is Localized in Regions of Cell-Substratum Contact or Attachment in Rous Sarcoma Virus–transformed Rat Cells

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ABSTRACT   Regions associated with cell-substratum contact or attachment in Rous sarcoma virus (RSV)-transformed rat fibroblasts (RR1022 cells) were identified by reflection-interference microscopy. Electron microscopy of such regions revealed the presence of discrete membrane-associated structures composed of a paracrystalline lattice of hexagons and pentagons to which actin filaments appear to be attached. Staining of actin by biotin-labeled heavy meromyosin showed that transformed cells, unlike normal fibroblasts, lack prominent actin fibers, and that, instead, much of the fluorescence is concentrated in loci corresponding to locations of transient association between the cell and the substratum. In stationary cells, such loci were found in rosette formation, predominantly in the region beneath the nucleus. In cells engaged in active movement, such as during migration into a wound, the actin-containing spots were concentrated in the region of the leading edge. A similar pattern of staining was observed with antibody to gelsolin, a 91,000-dalton Ca^{2+}-dependent actin filament-shortening protein. Since the action of gelsolin on actin is reversible and dependent on physiologically relevant changes in calcium concentration, the localization of gelsolin, together with actin-bundling proteins such as α-actinin, in the regions containing many small microfilament bundles on the ventral side of cytoplasm suggests that gelsolin may be a component of the mechanism for the disassembly and assembly of actin during the dissolution and reformation of structures for cell-substratum contact during cell locomotion. Regulation of gelsolin activity was not dependent on protein phosphorylation, as shown by lack of ^{32}P-incorporation into gelsolin in either transformed or normal fibroblasts.

Eucaryotic cells in culture adhere to the substratum through discrete membrane regions. Such foci were first recognized by Curtis (12), Cornell (10), and Abercrombie et al. (1) and were regarded as cell feet. With the aid of reflection-interference microscopy (RIM)[1] these foci, now designated adhesion plaques, have been shown to be localized at the termini of actin-containing stress fibers and to occupy the plane of cell-substratum interaction (5, 7, 9, 12, 41). However, such well defined plaque structures are not commonly observed in Rous sarcoma virus (RSV)-transformed fibroblasts which lack large actin fibers (43). Instead, a collection of actin-containing spots

is observed in regions of cell-substratum contact (13, 38, 39). These structures are identified as grayish areas when observed by RIM, in contrast to the black images of adhesion plaques, found at the termini of large actin fibers. Recently, a number of proteins that interact with actin have been localized within the adhesion plaques as well as the cell-substratum contact points by immunofluorescence microscopy (7, 15–17, 30). These include vinculin and α-actinin which are actin "bundling" proteins that promote formation of paracrystalline or fibrous actin structures, respectively, in vitro (8, 14, 24, 32, 42). On the basis of interactions observed in vitro, it has been suggested that these proteins may also organize actin filaments within the structures associated with cell attachment to the substratum (7, 17, 24).

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[1] Abbreviations used in this paper: RIM, reflection-interference microscopy; RSV, Rous sarcoma virus; PBS, phosphate-buffered saline.

In the present study, we examined by electron microscopy the structures at the ventral side of the cell surface, which appear to be associated with cell-substratum contact or attachment in a line of rat sarcoma cells transformed by the Schmidt-Ruppin strain of RSV. RSV-transformed rat cells display numerous prominent actin-containing spots (13, 38). These structures appear to be composed of a paracrystalline lattice of hexagons and pentagons to which microfilaments attach. We demonstrate by immunofluorescence staining the association of another actin-binding protein, gelsolin, with the actin-containing spots. Gelsolin is a 91,000-dalton protein that is found in a large variety of cells (46) and that binds to and shortens actin in the presence of Ca^{2+} concentrations $>10^{-7}$ M (32, 34). Gelsolin is the only protein identified thus far in regions involved in cell-substratum contact or attachment, which can reversibly disassemble actin filaments, and whose regulation is dependent on changes in Ca^{2+} concentration within the physiological range. The identification of such a "destructive" component for actin filaments within cell-substratum contact areas, together with "constructive" components, suggests a mechanism for the rapid and reversible formation and dissolution of structures transiently involved in cell-substratum contacts and attachments during cell locomotion.

MATERIALS AND METHODS

Cells: Cells of the HB subclone, isolated in our laboratory, are descendents of the RR1022 cell line from the American Type Culture Collection (Rockville, MD). RR1022 cells are derived from an in vivo sarcoma induced in an Amsterdam rat by infection with the Schmidt-Ruppin strain (subgroup D) of RSV (3, 22, 36). The HB subclone consists of transformed cells as indicated by the 95–98% colony-forming efficiency in soft agar. Immunoprecipitation with tumor-bearing rabbit serum indicates the presence of a high amount of the transformation gene product (pp60^{src}) in these cells (Garber, E., personal communication).

Normal rat cells derived from rat kidney (rat 1), obtained from the American Type Culture Collection (Rockville, MD), exhibited contact inhibition of growth in monolayer cultures. Previous experiments using immunoprecipitation with monospecific antisera have shown that rat 1 cells contain no detectable amounts of pp60^{src} (Garber, E., personal communication).

Both the wild-type RR1022 cells and cells of the HB clone were grown in reinforced Eagle's medium (4) containing 10% heat-inactivated calf serum. All cultures were incubated in Falcon plastic petri dishes in a humidified atmosphere of 5% CO_2 in air at 37°C.

Antibodies: Purified α-actinin was isolated and rabbit antiserum against it prepared as previously described (14, 30). Actin-containing microfilaments were visualized with the aid of fluorescein-conjugated biotin and avidin-labeled heavy meromyosin (20). Goat antiserum to gelsolin was prepared as reported previously (46). Cell-bound rabbit or goat IgG against a specific protein was detected with fluorescein-conjugated goat IgG directed against rabbit globulin or rhodamin-conjugated rabbit IgG directed against goat globulin (Antibodies Inc., Davis, CA), respectively. To reduce nonspecific binding, all secondary antisera were preabsorbed with formaldehyde-fixed acetone-extracted HB cells for 45 min at 37°C with constant agitation, after which the cellular elements were removed by centrifugation at 10,000 rpm for 10 min.

Indirect Immunofluorescence Staining and Photomicroscopy: Cells grown on round glass coverslips (Corning No. 1, 18 mm in diameter) were rinsed with warm phosphate-buffered saline (PBS) (pH 7.2) and fixed in 3.7% formaldehyde for 20 min at room temperature. The formaldehyde solution was then aspirated and the cells were rinsed with PBS before permeabilization with acetone at −20°C. After acetone treatment, the cells were again rinsed with PBS. Antiserum (50 µl) against the specific protein of interest was placed on the cell side of the coverslip and the sample incubated for 20 min at room temperature. After three rinses with PBS, cell-bound IgG was detected after further incubation for 20 min with fluorescein-conjugated goat IgG directed against rabbit globulin or rhodamin-conjugated rabbit antigoat IgG. The amounts of the primary and secondary antisera were standardized in each experiment with respect to the concentrations of IgG applied on each of the coverslips. After three rinses, the cells on coverslips were either mounted with buffered glycerol on microscopic slides or stained with fluorescent heavy

meromyosin, which binds to the actin subunits of microfilaments, and then mounted. Preparations of coverslip samples were then examined under phase-contrast and epifluorescence illumination using a Zeiss UV microscope equipped with HBO-200 W mercury lamp. Photographs on Kodak Tri-X or Ektachrome ASA 400 film were taken using a 63× Ph 3/NA 1.4 oil immersion objective for both phase-contrast and fluorescence microscopy.

RIM: Coverslip cultures for immunofluorescence staining were also examined by reflection interference microscopy using a combination of an antiflex neofluar Zeiss 63× objective and Zeiss polarizer, analyzer, and VG9 green filters.

Electron Microscopy: Monolayer cultures were fixed with 1% glutaraldehyde in PBS at room temperature for 30 min. For preservation of the fine structure of actin filaments, the procedure reported by Maupin-Szamier and Pollard (35) was adapted for our sample preparation. After in situ rinsing with PBS, the fixed samples were postfixed in 1% OsO_4, 0.1 M Na^+ phosphate, pH 6, for 20 min on ice. The cells were rinsed again with PBS and then dehydrated in a graded series of concentrations of ethanol in water and embedded in Epon 812 (34). The embedded samples were removed from the culture dish by mechanical force and then remounted on other Epon blocks with the bottom of the monolayer cultures facing the diamond knife. The immediate plane of the cell-substratum region was thin sectioned and 20 serial sections, each 750–800 Å thick, were obtained. The sections were collected on copper grids, stained with saturated uranyl acetate for 30 min at 60°C and with lead citrate for 5 min at 22°C (33, 37). The grids were viewed in a Philips 300 electron microscope operating at 80 kv.

Wound-healing Experiment: We employed the monolayer wounding technique to investigate the morphology of the ventral side of the cell surface in cells that have lost contact with their neighbors and have been provided a free border. HB cells were allowed to grow to confluency and then an area, 2 mm in diameter, was removed from the monolayer with a sharp razor blade. The cultures were washed several times with the growth medium to remove cell debris. Approximately 6 h later, the cells had recovered from the mechanical shock of the wounding and had started to spread and migrate into the wound. Such cultures were then processed for various light and electron microscopic studies.

Protein Phosphorylation and SDS PAGE Analysis: Cells were grown in 60-mm tissue culture plates in reinforced Eagle's MEM containing 10% calf serum. Cells were labeled with [³H]leucine (500 µCi/ml, 40–60 Ci/mmol), or carrier-free ³²PO₄ (250 µCi/ml) (New England Nuclear, Boston, MA) in medium lacking leucine or phosphate, respectively. The labeling medium was supplemented with 10% dialyzed calf serum and 10% complete medium. Cells were labeled in a total volume of 2.0 ml of medium for 14 h at 37°C. For immunoprecipitation, the cells were washed with cold PBS and scraped with a rubber policeman into the following buffer: 20 mM Tris–maleate, pH 8.0, 100 mM NaCl, 10 mM KCl, 1 mM EDTA, 1 mM dithiothreitol, 1% (vol/vol) Trasylol, 10% glycerol, 1% Triton X-100, 0.5% sodium deoxycholate, and 1 mM phenylmethylsulfonylfluoride. The cell lysate was centrifuged at 15,000 g for 5 min in an Eppendorf microfuge placed in a cold room (4°C). The supernatant fraction was removed and divided into two equal samples for immunoprecipitation with 10 µl of goat anti-gelsolin serum or 10 µl of preimmune goat serum. The samples were incubated on ice for 5 h, after which 25 µl of rabbit-antigoat-IgG serum was added. After an additional period of 75 min on ice, 90 µl of a 1:3 slurry of protein A-Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ) was added. The mixtures were incubated for 45 min at 4°C with frequent vortexing. Then the immune complexes, which had adsorbed onto the sepharose beads, were washed four times with buffer. The immune complexes were solubilized in SDS PAGE sample buffer and were analyzed by SDS PAGE and fluorography (27, 28).

RESULTS

Morphology and Localization of Structures Associated with Cell-Substratum Attachment

Monolayer cultures of the HB clone of RR1022 cells exhibit a considerable degree of homogeneity in regard to cell shape and cytoskeletal organization. The micrographs shown here are representative of 80–90% of the cell population. Immunofluorescence microscopy with fluorescent heavy meromyosin shows that HB cells do not possess large actin fibers in the cytoplasm. Rather, most of the actin-containing filaments are organized in arrays of short fibers scattered throughout the diffusely staining cytoplasm. Examination of the same cell specimen by fluorescence and RIM shows that the most

prominent structures that contain actin are spots (Fig. 1 a) located mainly in the plane of close contact regions between the cell and the substratum, which appear gray (Fig. 1 b). The actin fibers extending to the cell periphery terminate in regions of adhesion plaques that appear as black elongated structures distinct from the close contact regions.

In a confluent culture in which cells are in contact with neighboring cells on all sides, the actin-containing spots are commonly seen in rosette formation, located primarily in the area immediately beneath the nucleus. This localization appears to reflect the attachment of stationary cells to substratum. However, in a sparse culture, the discrete actin-contain-
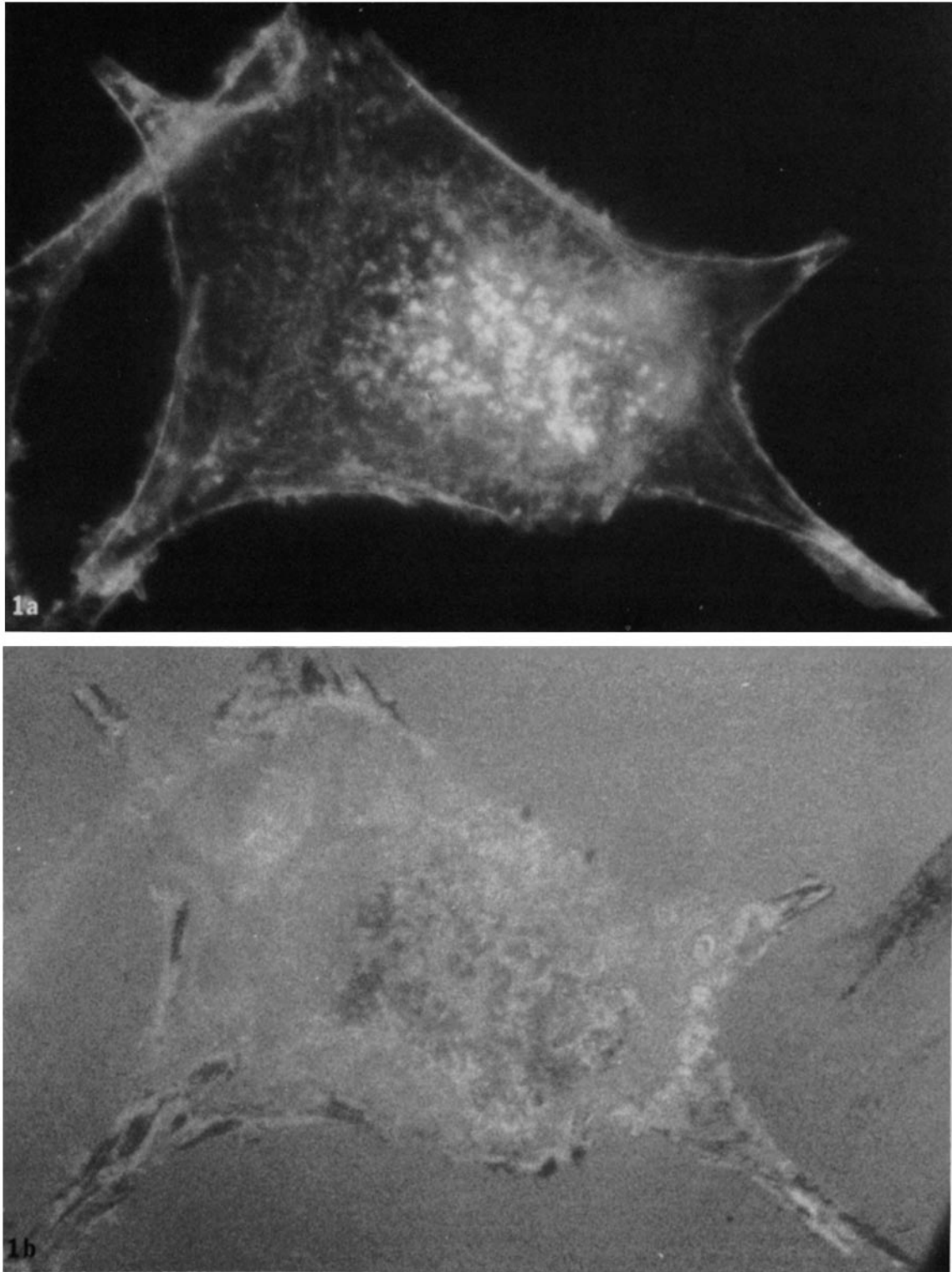
FIGURE 1 Localization, in clone HB of RSV-transformed RR1022 cells of (a) actin by fluorescence microscopy, and (b) cell-substratum attachment regions by RIM (b). Cells were stained with fluorescein-conjugated biotin and avidin-labeled heavy meromyosin. In 80-90% of the cells in culture actin was mainly distributed in spots or dots (a), which were located in the plane adjacent to cell attachment to the substratum (b). × 2,000.

ing spots often form rows extending from the subnuclear region to the area of active membrane ruffling or into the membrane ruffle itself (Fig. 2a and b). Examination of the same cell specimen by both fluorescent microscopy and RIM shows that the actin-containing spots are located in regions where the ventral surface of the pseudopods makes close contact with the substratum (Fig. 2, c and d). The spots are usually positioned in a formation indicative of the direction of membrane ruffling and cell spreading. As described below for wounded cultures, the attachment-associated structures are always concentrated at the leading edge of cells migrating into the wound.
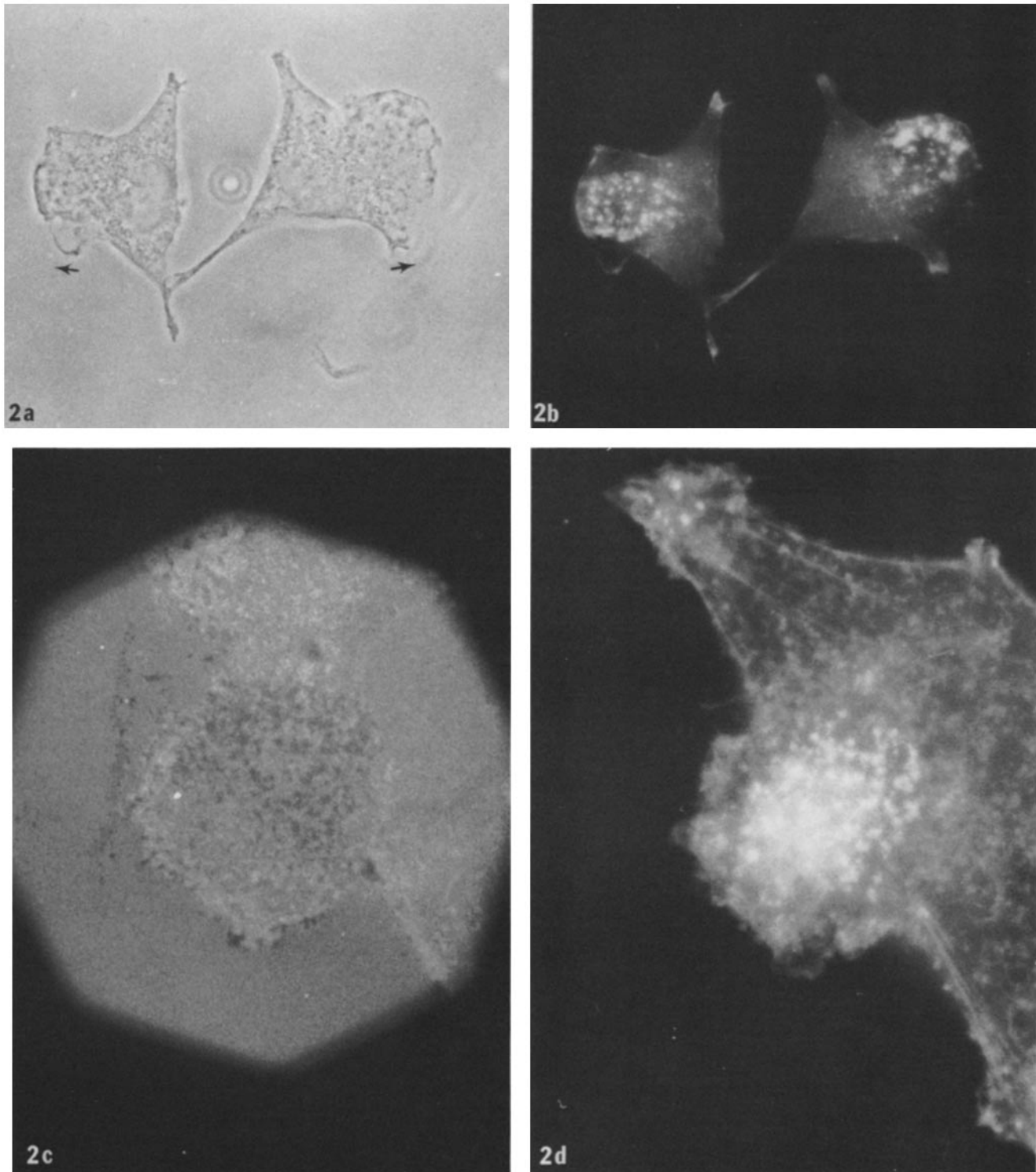

FIGURE 2   Localization of actin in locomoting HB cells. (a and b) Actin localization by fluorescence staining (b) can be related to the leading ruffling edge and pseudopod regions observed by phase-contrast microscopy in a. Arrows indicate the direction of locomotion. (c and d) Corresponding images seen by fluorescence (d) and reflection interference optics (c) reveal that the actin-containing spots present in the forming pseudopod are localized adjacent to the plane of close contacts between the plasma membrane and the substratum. (a and b) × 1,200. (c and d) × 2,000.
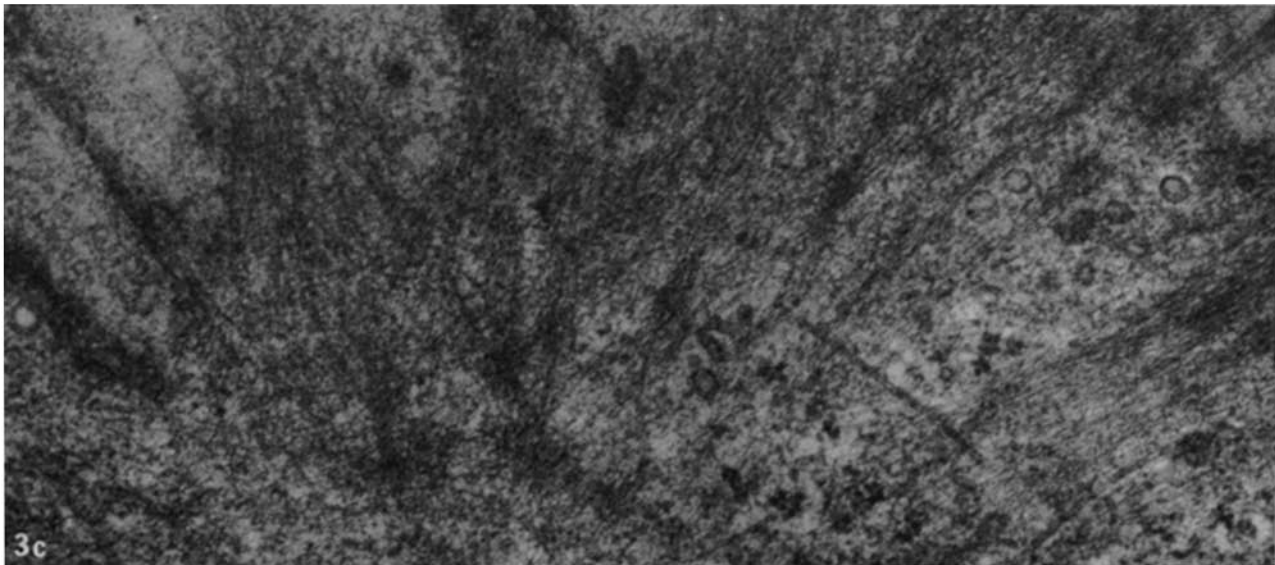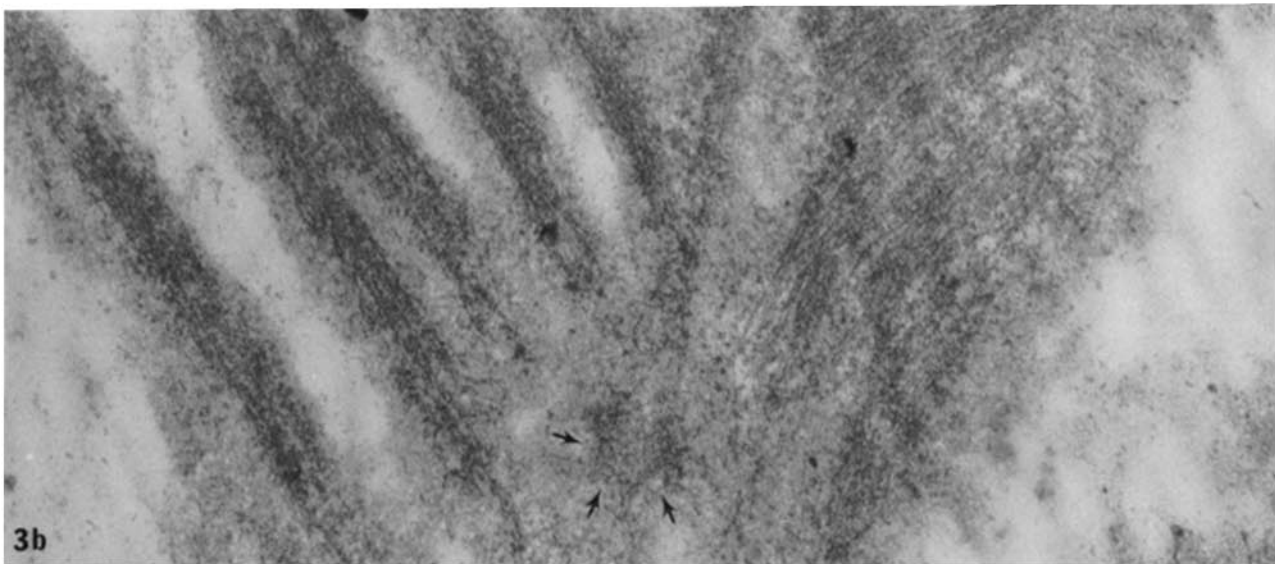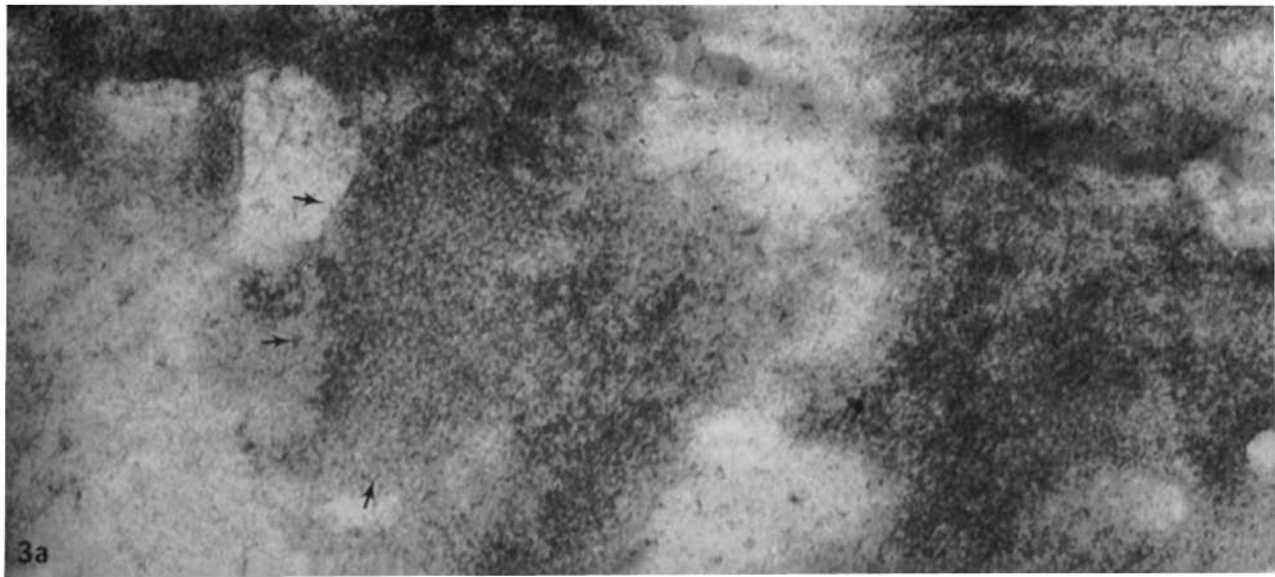
FIGURE 3 Structure of cell-substratum attachment regions observed by electron microscopy of serial sections cut parallel to the substratum through the plane of HB cell attachment. Arrows indicate corresponding regions of an attachment site observed at different levels from the substratum. (a) Densely packed hexagonal or pentagonal structures, which appear to form a paracrystalline lattice (arrows), are observed in the first or second sections (0–1,400 Å) from the substratum. × 200,000. (b) Bundles of microfilaments can be seen radiating from lattice regions in the third section (in the region of 1,400–2,800 Å) from the substratum. × 80,000. (c) Numerous microfilaments and other cellular organelles such as microtubules, vesicles, and polysomes are seen in sections that are ~4,000–6,000 Å from the substratum. × 80,000.
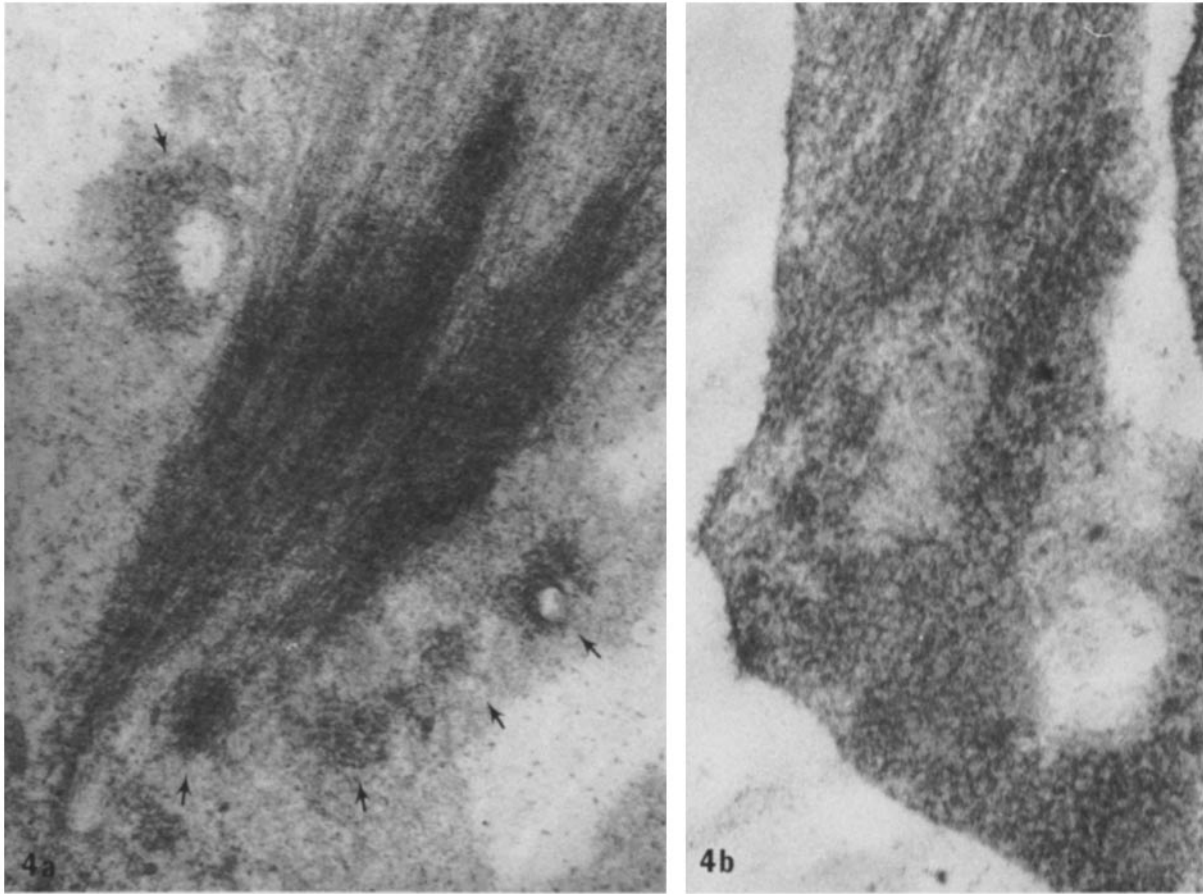
FIGURE 4   Two examples of microfilament-associated cell-substratum attachment regions observed in the leading edge of cells migrating into a wound area in a culture of HB cells. Sections are ~2,100–3,000 Å from the substratum. Arrows indicate lattice structures formed by densely packed hexagons or pentagons. (a) × 90,000. (b) × 220,000.

Comparison of the photographs taken by fluorescence microscopy and RIM demonstrates that the discrete actin-containing areas are located at sites of cell-substratum attachment. The gray regions seen by interference microscopy correspond in general to the brightly fluorescent spots or dots visualized after staining with fluoresceinated heavy meromyosin. In some instances, the images seen by fluorescence microscopy do not completely overlap those observed by reflection-interference optics. Review of many photographs shows that the correspondence between the regions revealed by interference vs. fluorescence microscopy is often not exact, which is to be expected as interference microscopy reveals the attachment sites of the cell membrane to the substratum, whereas staining with the antibodies reveals structures internal to the plasma membrane, photographed at a different focal plane.

Electron microscopic examination of serial sections cut in the plane parallel to the substratum shows that in the region of cell attachment or directly above it (0–1,400 Å), there is present a paracrystalline lattice of fairly uniformly spaced unit structures that appear to be hexagonal or pentagonal. These structures appear to be associated with the plasma membrane (Fig. 3 a). Measurement of more than 20 of such paracrystalline lattices reveals that the individual hexagonal or pentagonal structures measure, on the average, 180 × 220 Å. At higher planes (1,400–6,000 Å), small microfilament bundles, often several in number, are seen radiating from the paracrystalline structures (Fig. 3, b and c). Similar structures with associated microfilament bundles are also observed in the

leading edge of cells that are migrating into a wound area in a culture (Fig. 4, a and b).

Association of Gelsolin with Areas Involved in Cell Attachment to the Substratum

In RSV-transformed cells, gelsolin displays a patchy distribution corresponding to cell-substratum attachment sites, as determined by immunofluorescence and reflection interference microscopy (Fig. 5, a and b). In stationary cells, the bulk of the protein appears to be localized in spots that form a circular pattern in the cytoplasm. The punctate fluorescence staining pattern was not seen when the cell samples were stained with serum that had been preabsorbed with purified gelsolin (Fig. 5c). The specificity of the antigelsolin antibody has been documented previously (46).

In cells in motion, gelsolin is frequently visualized in the form of spots dotting the periphery of fan-shaped membrane ruffles. Time-lapse cinematography has shown that these membrane ruffles represent the leading edge of a cell in motion and, thus, commonly indicate the direction of cell locomotion. Fig. 6, a and b, demonstrate the location of the fluorescent gelsolin-containing punctate regions in areas of cell to substratum contact revealed by RIM. There is an abundance of gelsolin-containing spots in the leading edge of a forming pseudopod.

To obtain additional evidence of the preferential localization of gelsolin in the region of the ruffling and advancing
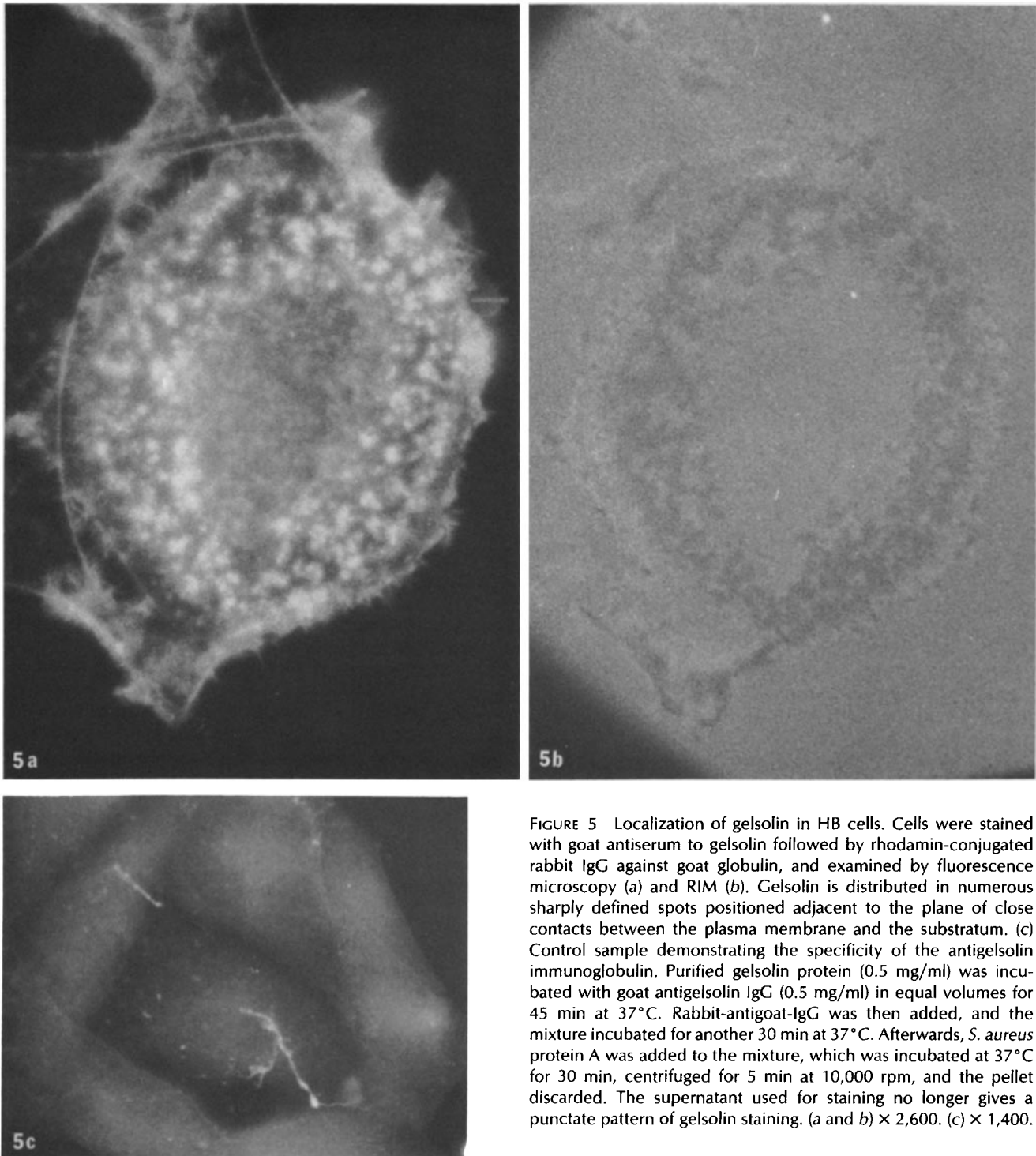
FIGURE 5 Localization of gelsolin in HB cells. Cells were stained with goat antiserum to gelsolin followed by rhodamin-conjugated rabbit IgG against goat globulin, and examined by fluorescence microscopy (a) and RIM (b). Gelsolin is distributed in numerous sharply defined spots positioned adjacent to the plane of close contacts between the plasma membrane and the substratum. (c) Control sample demonstrating the specificity of the antigelsolin immunoglobulin. Purified gelsolin protein (0.5 mg/ml) was incubated with goat antigelsolin IgG (0.5 mg/ml) in equal volumes for 45 min at 37°C. Rabbit-antigoat-IgG was then added, and the mixture incubated for another 30 min at 37°C. Afterwards, S. aureus protein A was added to the mixture, which was incubated at 37°C for 30 min, centrifuged for 5 min at 10,000 rpm, and the pellet discarded. The supernatant used for staining no longer gives a punctate pattern of gelsolin staining. (a and b) × 2,600. (c) × 1,400.

edge of the cell, we performed wounding experiments in cultures of HB cells as described in Materials and Methods. Fig. 7, a and b, illustrates the patchy distribution of gelsolin in the areas of active membrane ruffling in the advancing pseudopods of cells that are moving into the wound in the confluent culture.

Simultaneous staining with fluorescent heavy meromyosin and a selected monospecific antibodies to either gelsolin or α-actinin indicates that α-actinin is also present in cell-substratum attachment areas in addition to actin and gelsolin (Fig. 8, a–d).

Lack of Phosphorylation of Gelsolin in Normal and Transformed Cells

Phosphorylation of cytoskeletal proteins has been proposed as a mechanism for regulation of assembly and polymerization of actin-containing microfilaments (40). To investigate the possibility that gelsolin may be phosphorylated, we cultured both normal rat fibroblasts (rat 1) and the transformed HB cells in ^{32}P-containing medium under conditions similar to those used successfully to demonstrate phosphorylation of known phosphoproteins. We found no ^{32}P-label associated
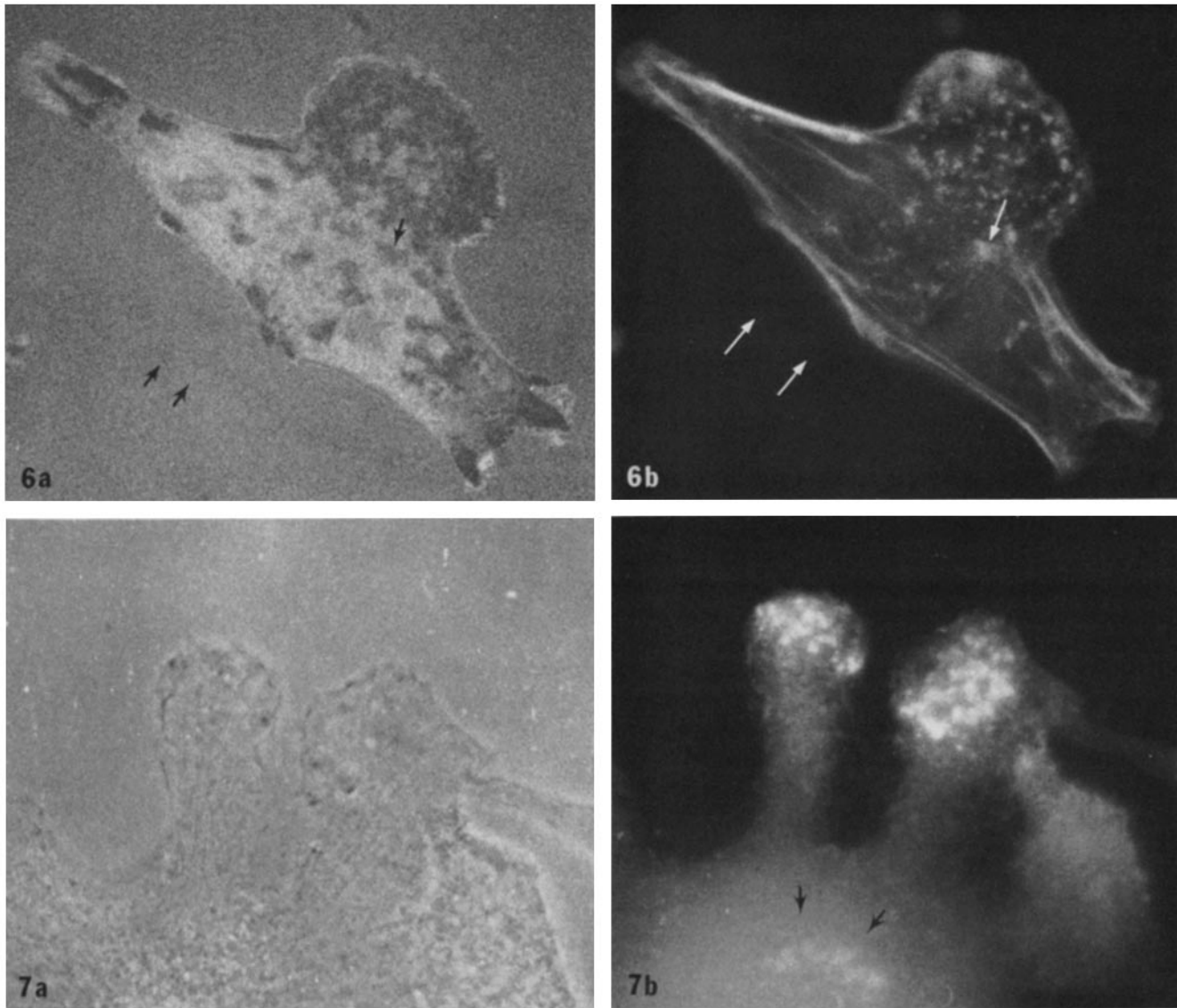
FIGURE 6 Gelsolin localization in HB cells that are moving across the substratum. (a) RIM; (b) immunofluorescence with gelsolin antibody. Single arrows indicate a corresponding region in a and b. Double arrows indicate the direction of cell movement. The photographs were taken as described in Materials and Methods and in the legend for Fig. 2. × 1,400.

FIGURE 7 Gelsolin localization in HB cells migrating into a "wound" area. (a) Phase contrast; (b) immunofluorescence with gelsolin antibody. In addition to the patchy distribution of gelsolin in the main part of the cell body (arrows), there are also numerous prominent spots of this protein in the frontal regions of the advancing pseudopods, which are exhibiting active membrane ruffling. × 1,800.

with gelsolin immunoprecipitated by antigelsolin antibody (Fig. 9, lanes 5 and 7). That the immunoprecipitation protocol used here can separate gelsolin from the cell lysate is demonstrated by the precipitation of [^{3}H]gelsolin from cells grown in [^{3}H]leucine (Fig. 9, lanes 1 and 3).

DISCUSSION

On the basis of RIM, Izzard and Lochner (23) described two kinds of cell-substratum contacts between cultured chick heart fibroblasts and glass substrates: (a) Focal contacts appear as black images and are small, elongated adhesions, separated by 10–15 nm from the substrate; cytoplasmic fibers commonly associate with the focal contacts, and can be resolved by electron microscopy as microfilament bundles attached to cytoplasmic plaques. (b) Close contacts appear as gray images

and consist of broad areas of cell-to-substrate separation of ~30 nm.

We report, in the present study, that structures involved in cell-substratum attachment in a line of RSV-transformed rat cells are composed of a paracrystalline lattice of hexagons and pentagons and contain microfilament-associated proteins such as actin, gelsolin and α-actinin. The paracrystalline structures reported here have been seen in other cell types including adenovirus-transformed hamster embryo cells, baby hamster kidney (BHK-21) cells, and human ENSON cells (see Fig. 25 and 26 of reference 18). At present, it is not clear whether the paracrystalline structures shown in Figs. 3, a and b, and 4 represent clathrin basketwork in the form similar to that of flat patches on the adhesive surface of macrophages (2), or distinctive structures located at the interface between microfilament bundles and the plasmalemma. These struc-
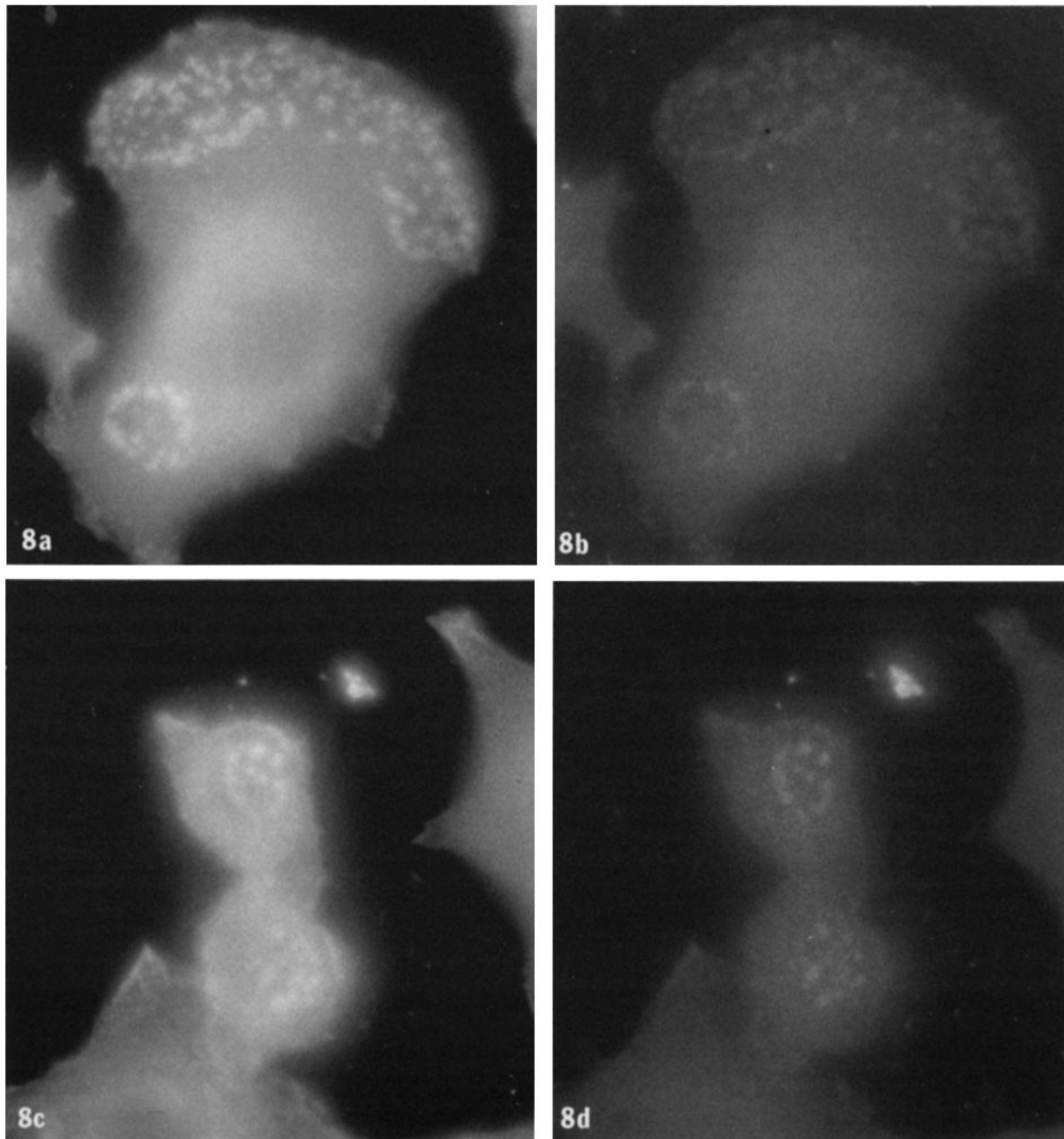
FIGURE 8 Colocalization of actin and gelsolin (a and b), and actin and α-actinin (c and d) in HB rat cells. Double fluorescence microscopy was performed by using fluorescence-conjugated heavy meromyosin for detection of actin, and a primary antiserum to gelsolin or α-actinin in combination with a rhodamin-labeled second antiserum. Microscopic visualization of fluorescein was obtained with light at the wave length of 485 nm, and of rhodamin at 546 nm. To insure the complete separation between fluorescein and rhodamin staining, we used a selective interference fluorescein barrier filter (520–540 nm) when the samples were viewed for double-label immunofluorescence microscopy. (a and b) × 3,000. (c and d) × 1,300.

tures are consistently found in the cell regions where intense membrane activity occurs for cell spreading, the formation of protruding pseudopods, or locomotion across the substratum. By RIM, these cell substratum attachment-associated structures differ from "adhesion plaques," which are located primarily at the termini of large actin fibers (1, 5, 7, 9, 10–12, 15–17, 24).

David-Pfeuty and Singer (13) have reported that after transformation of fibroblasts by RSV, a majority of the cells have fewer focal adhesion plaques, and instead exhibit clusters of small gray areas in the RIM images, suggesting that these are

sites of close (but not focal) contact of the ventral cell surface with the substratum. In most of the transformed cells, these close contact regions form a rosette localized in the subnuclear area, which we have also observed in stationary HB cells. David-Pfeuty and Singer (13) reported that in transformed cells, vinculin and α-actinin are in large part associated with the rosettes of small round patches, although they are also associated with the relatively few focal adhesion plaques that are present. These findings are consistent with the well known disorganization of microfilament bundles and the decrease in cell-substratum adhesion in transformed cells (13, 38–41, 43).
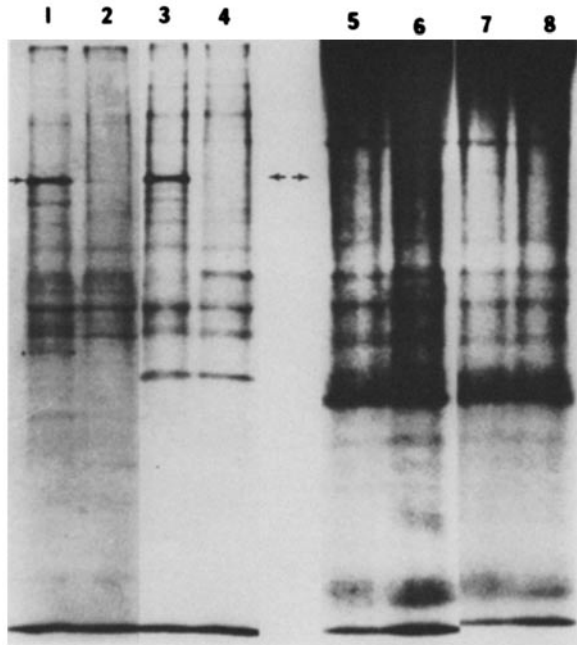
FIGURE 9 Immunoprecipitation of gelsolin from ^{3}H-leucine or ^{32}P-labeled transformed (HB) or normal (Rat 1) rat cells. Lanes 1–4: ^{3}H-leucine label; (1) HB cells, goat anti-gelsolin antibody (Ab); (2) HB cells, nonimmune serum; (3) Rat 1 cells, antigelsolin Ab; (4) Rat 1 cells, nonimmune serum. Lanes 5–8: ^{32}P-label; (5) HB cells, anti-gelsolin Ab; (6) HB cells, nonimmune serum; (7) Rat 1 cells, anti-gelsolin Ab; (8) Rat 1 cells, nonimmune serum. Arrows indicate the position of immunoprecipitated gelsolin protein in ^{3}H- or ^{32}P-labeled cells, as determined by the use of purified gelsolin.

It is of interest to note that the clusters of cell-substratum contact-associated structures observed by David-Pfeuty and Singer (13) and us have also been observed in fibroblasts transformed by a variety of avian sarcoma viruses (26). The actin-containing clusters of contact regions are abundant in all of these transformed cell systems. The HB subclone of the RR1022 line and other permanent RSV-transformed cell lines provide suitable model systems for the investigation of questions concerned with the structural rearrangement of cell-substratum attachment sites as the cell performs its motile functions. The precise physiological role of the subnuclear clusters of contact regions in stationary cells remains to be defined.

Regions of close contact between the cell and the substratum have been observed not only in transformed, but also in untransformed cell systems (5, 9, 11, 19). It has been reported that, during cell locomotion, the structures involved in cell-substratum contacts are the areas where loosely organized microfilament bundles terminate (5, 9, 11, 19, 31). The formation of fixed points between the cell and the substratum may serve the function of nucleation sites for the formation of bundles from microfilaments present in the vicinity. Structures involved in transient cell contact or attachment are probably most abundant in cells that are highly motile, either in terms of cell spreading or translocation across solid substratum. The motile activity involved in membrane movement and in the rearrangement of cellular compartments entails breaking of old cell-substratum contacts and formation of new sites for anchoring the membrane onto the newly

gained substratum territory, and therefore probably requires less permanent structures than the focal adhesion plaques.

Immunofluorescence studies show that several proteins that interact with actin are located within focal adhesion plaques with which large actin fibers are associated and that may therefore play a role in determining actin organization. These include vinculin (3, 16) and α-actinin (7, 13, 17, 30). Tropomyosin, another protein which binds actin, is excluded from these structures (29). Of the proteins present, vinculin has received the most attention so far as the possible mediator of microfilament-membrane interaction and actin bundle formation within the plaques (8, 24). The mechanism for regulation of vinculin activity, however, is not known. In transformed cells, vinculin may be phosphorylated by pp60^{src}, a src-gene product with tyrosine-specific phosphotransferase activity (21, 40), which is found in the cell-substratum contact regions (38, 39), among a number of other sites (25, 26). However, no evidence has so far been reported that fluctuations in vinculin phosphorylation directly affect the assembly of actin bundles.

The present identification of gelsolin in the cell-substratum contact regions suggests a mechanism for the dissolution of actin filaments that is independent of protein phosphorylation but regulated by changes in Ca^{2+} concentration within the physiological range. Unlike vinculin and α-actinin, gelsolin has no actin-bundling activity. Instead, it shortens actin filaments in the presence of Ca^{2+} concentrations above 10^{-7} M (46). The interaction of gelsolin with actin, and its regulation by Ca^{2+} have been examined in detail in vitro. Gelsolin binds to Ca^{2+} with high affinity ($K_a = 1.09 \times 10^{-6}$ M) and the gelsolin-Ca^{2+} complex binds both to actin filaments and actin monomers (44, 45, 47).

Binding of gelsolin to actin filaments leads to shortening of the filaments. The shortened filaments are capped by gelsolin at the barbed end, which is the preferred for filament elongation (47). Binding of gelsolin to actin monomers leads to enhanced actin nucleation with formation of short actin filaments under appropriate salt conditions. These short filaments are also capped at the barbed end. Shortening of actin filaments has profound implications for the organization of actin-containing structures, as demonstrated by the effectiveness of gelsolin in solating actin gel networks formed by cross-linking with a high molecular weight actin-binding protein from macrophages (45).

Based on the in vitro studies of the interaction of gelsolin with actin, we propose that gelsolin can affect the organization of actin in the cell-substratum contact regions in two ways, as follows: (a) by shortening actin filaments that makes for inefficient linking of the filaments by the bundling proteins; (b) by capping the shortened filaments that prevents the elongation of the filaments at the barbed end and at the same time, their attachment to the membrane (either directly, or indirectly through a putative linkage protein). Since gelsolin interacts with both polymeric and monomeric actin, this scheme encompasses both the dismantling of preexisting contact areas as well as the assembly of new areas de novo. The fact that gelsolin is reversibly activated by Ca^{2+} within a concentration range believed to occur in vivo makes this hypothesis of regulation of the transient cell-substratum attachments by localized changes in cytoplasmic Ca^{2+} concentration particularly attractive. Furthermore, since gelsolin has been identified in many different types of cells (46), this mechanism should be applicable to other cells as well.

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