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CELL-TO-SUBSTRATUM CONTACTS IN LIVING CELLS: A DIRECT CORRELATION BETWEEN INTERFERENCE-REFLEXION AND INDIRECT-IMMUNOFLUORESCENCE MICROSCOPY USING ANTIBODIES AGAINST ACTIN AND α-ACTININ

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SUMMARY

Rat mammary cells growing on glass coverslips were photographed first using interference-reflexion microscopy and then after processing for indirect-immunofluorescence microscopy with antibodies to actin or to α-actinin. A comparison of the images of the same cell given by the 2 microscopical procedures indicates that the focal contacts between the cell and the substratum correspond to distal ends of microfilament bundles, and that these bundles are only in limited areas close to the substratum. The focal contacts are rich in α-actinin which has been proposed as a membrane-anchorage protein for microfilament bundles. Use of stereo immunofluorescence microscopy allows a direct comparison between the interference-reflexion image, and the underside of the cell after staining with antibodies to actin or α-actinin.

INTRODUCTION

The interference-reflexion microscopy introduced by Curtis in 1964 (Curtis, 1964) and discussed in detail by Izzard & Lochner (1976) demonstrates directly on the light-microscopical level the patterns of spatial separation between the plasma membrane of the cell and the substratum on which it spreads. This technique has been used by several investigators to study cell-substratum contacts, which are clearly of importance in studies on cell movement (Izzard & Lochner, 1976; Abercrombie & Dunn, 1975; Rees, Lloyd & Thom, 1977; Lloyd, Smith, Woods & Rees, 1977). The results indicate either indirectly (Rees *et al.* 1977; Lloyd *et al.* 1977) or directly (Abercrombie & Dunn, 1975; Izzard & Lochner, 1976; Heath & Dunn, 1978) a relationship between focal contacts and microfilament bundles.

Microfilament bundles have been extensively studied by both electron microscopy and by immunofluorescence microscopy. These bundles are lateral aggregates of microfilaments (individual fibre diameter approximately 6 nm) since they can be specifically decorated by heavy meromyosin (Ishikawa, Bischoff & Holtzer, 1969) or by antibodies specific for actin (Lazarides & Weber, 1974). They correspond to phase-dense bundles visible in light microscopy (Lewis & Lewis, 1924; Goldman, Schloss & Starger, 1976) and are preferentially expressed underneath the plasmalemma of adhesive cells which grow and locomote on a solid substratum (see for instance Abercrombie, Heaysman & Pegrum, 1971; Buckley & Porter, 1967; Goldman, Lazarides,

Pollack & Weber, 1975). Electron-microscopical studies have indicated that some of these bundles may terminate at the lower plasma membrane, indicating regions where the cell attaches to the underlying substratum (see for instance Abercrombie *et al.* 1971; Heaysman & Pegrum, 1973; Goldman *et al.* 1976; Schollmeyer *et al.* 1976). In immunofluorescence microscopy the use of antibodies to actin has provided an overview of the arrangement of these bundles in a variety of cell types (see for example Lazarides & Weber, 1974; Weber, 1976; Osborn, Born, Koitzsch & Weber, 1978) and has demonstrated that this arrangement is different from that observed for microtubules and intermediate filaments (above references; Weber, Pollack & Bibring, 1975; Brinkley, Fuller & Highfield, 1975; Osborn, Franke & Weber, 1977). In addition such studies have allowed the construction of a protein chemical anatomy of the microfilament bundle and have shown that these microfilament bundles are not just F-actin in a storage form but in addition contain microfilament-associated proteins, of which currently myosin (Weber & Groeschel-Stewart, 1974), tropomyosin (Lazarides, 1975), filamin (Wang, Ash & Singer, 1975) and α-actinin (Lazarides & Burridge, 1975; Lazarides, 1976) have been identified. Thus actin cables or stressfibres (Goldman *at al.* 1975) can indeed be considered as potential 'cytomuscular structures' and in agreement with this hypothesis actin cables isolated from non-muscle cells are capable of ATP-induced *in vitro* contraction (Isenberg *et al.* 1976). In this context it is important to note that α-actinin has been widely proposed as a candidate for microfilament-membrane anchorage (Lazarides & Burridge, 1975; Lazarides, 1976; Schollmeyer *et al.* 1974, 1976).

Here we combine interference-reflexion microscopy with immunofluorescence microscopy on the same cell using antibodies against actin and α-actinin. We directly confirm the proposal of Heath & Dunn (1978) that focal contacts correspond to distal ends of microfilament bundles and that these bundles are only in limited areas connected with focal contacts. We show also that focal contacts relate to microfilament bundles especially rich in α-actinin. Furthermore we relate interference-reflexion microscopy images with stereo-immunofluorescence microscopy using antibodies against actin and α-actinin.

MATERIALS AND METHODS

Cells

A cloned permanent cell line, originally derived from a rat mammary adenocarcinoma (Rathke, Schmid & Franke, 1975), were grown in Dulbecco's modified Eagle's medium containing 10% foetal calf serum. Rat kangaroo PtK2 cells were maintained in Eagle's minimal essential medium supplemented with non-essential amino acids, glutamine, sodium pyruvate, and 10% foetal calf serum (Osborn *et al.* 1977). Cells were grown on marked coverslips (24 × 32 mm) subdivided into squares by scoring with a diamond pencil.

Interference-reflexion microscopy

The interference-reflexion microscope (LEITZ Diavert) is essentially a standard reflected-light microscope utilizing linearly polarized light for illumination (50-W mercury light source) and a crossed analyser in the viewing optics for reducing internal reflexions in the microscope's optics. The key accessory is a $\lambda/4$ plate between the objective and the object (combined in

the new LEITZ P1 Apo 40/1.00 oil-immersion contrast objective); it circularly polarizes the light reflected from the object (the information) to pass the analyser in the viewing optics. A further increase in contrast is achieved by inserting a central beam stop in the illumination path (Stach diaphragm); (for further details see Patzelt, 1977).

Living cells were examined and photographed in a temperature-regulated (37 °C) cell chamber in growth medium. Fixations were carried out at room temperature either with 3·7 % formaldehyde in phosphate-buffered saline (PBS) for 10 min, or with 2·5 % glutaraldehyde buffered with 0·1 M sodium cacodylate buffer pH 7·2 for 30 min. Fixed cells were photographed in these solutions as indicated. Interference-reflexion photography was performed using Kodak high copy contrast film at 50 ASA with a LEITZ Orthomat.

Immunofluorescence microscopy

Immediately after photographing the cells in the interference-reflexion microscope, the coverslips were processed for indirect immunofluorescence microscopy. When glutaraldehyde fixation was used excess aldehyde groups were reduced with sodium borohydride (Procedure II; Weber Rathke & Osborn, 1978). Formaldehyde-fixed cells were treated directly with methanol for 6 min at 10 °C (Weber *et al.* 1975; Weber, Wehland & Herzog, 1976). The rabbit antibody against actin (Weber *et al.* 1976), the rabbit antibody against α-actinin (Webster, Osborn & Weber, 1978) and the fluorescein-labelled goat anti-rabbit antibody (Miles-Yeda, Israel), as well as other details of the technique (Weber *et al.* 1975, 1976) have been described in detail. All immune IgGs were used after purification on their corresponding antigen covalently coupled to Sepharose 4B. The immune IgG concentration was approximately 0·05 mg/ml in phosphate-buffered saline.

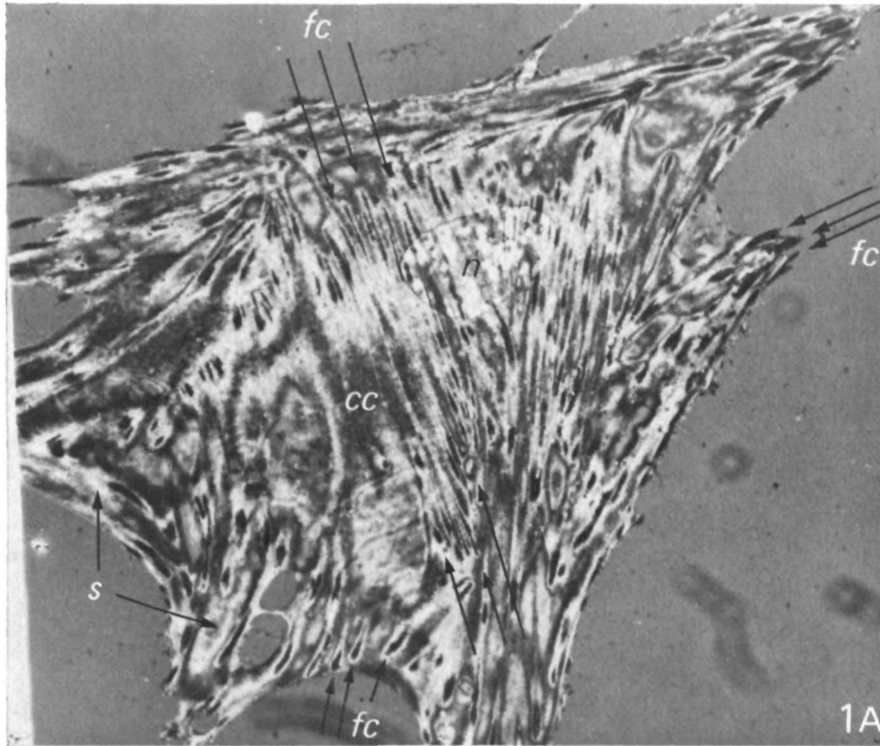
Stereo-immunofluorescence microscopy was performed as described (Osborn *et al.* 1978). The corresponding stereo pairs should be viewed with the help of a 2 × stereo viewer (Polaron, England).

RESULTS

To correlate the images in the interference-reflexion microscope with the images seen in the fluorescence microscope using selected antibodies we chose to study 2 cell lines: rat mammary cells, and rat kangaroo PtK2 cells. Rat mammary cells have extensively developed stress fibres; PtK2 cells have a typical epithelioid morphology, and display bundles of microfilaments as well as well developed arrays of tonofilament-like intermediate-sized filaments. Both cell lines have been extensively studied in this laboratory using antibodies against a variety of different structural proteins and the overall pattern of microfilament bundles, microtubules and intermediate filaments has been documented (Weber, 1976; Osborn *et al.* 1977, 1978). In addition these cell lines have been studied by electron-microscopical analysis (see for instance Franke, Grund, Osborn & Weber, 1978*a*; Franke, Schmid, Osborn & Weber, 1978*b*; Rathke *et al.* 1975).

Interference-reflexion microscopy

Typical interference-reflexion images of rat mammary cells growing on a glass substratum are shown in Figs. 1–3. These images show the three types of cell substratum approaches distinguished by Izzard & Lochner (1976) i.e. focal contacts (black streaks), close contacts (grey areas) and regions of greater separation (white areas; see Fig. 1). Focal contacts are thought to indicate a separation between the substratum and membrane of less than 15 nm (Izzard & Lochner, 1976). In addition larger light-grey areas can be seen (which are surrounded by concentric fringes of varying intensities; see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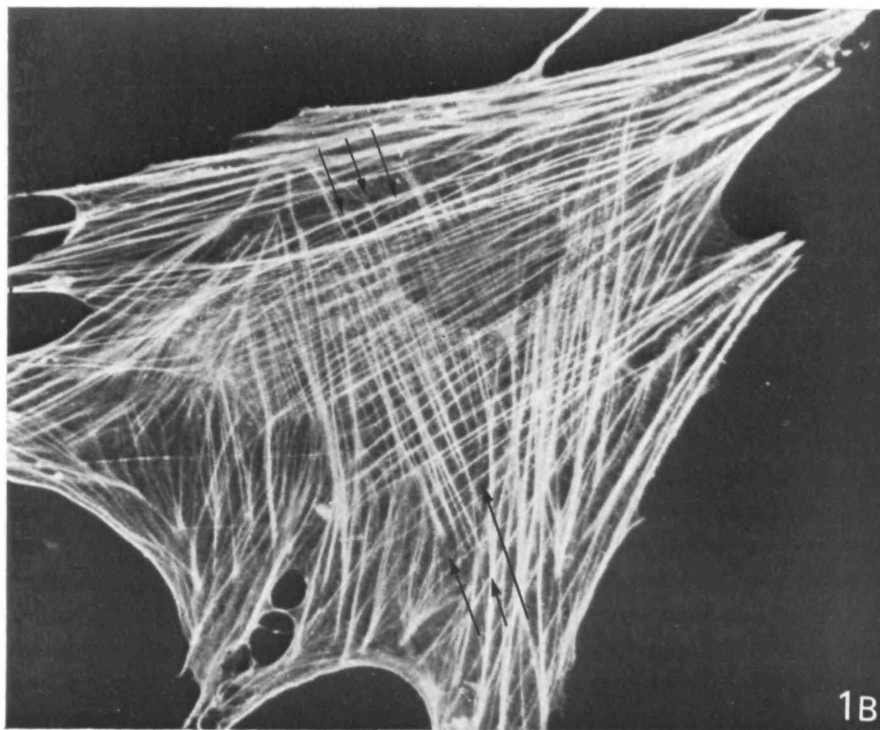

1B

areas marked in Figs. 2A, 3A). In the microscope using appropriate filters such images are partially coloured and range from blackish-blue to iron-grey. In the interference-reflexion images nuclei are readily identified when they are sufficiently close to the substratum (Figs. 1A, 3A, but not 2A).

A closer examination of the focal contacts in living cells (Figs. 1A–3A) shows that they are extensively distributed over the lower side of the cells. They are usually oblong in shape, and are of variable size. In Fig. 2A for example they range in length from 4 to 20 μm. In some cases (arrows in Fig. 1A, B) it is apparent that the long axes of the focal contacts can be arranged parallel to each other, although examination of the figures in detail shows that this orientation is often more random.

Since immunofluorescence microscopy is performed on fixed cells it was next necessary to assess whether changes in the interference-reflexion images of the living cell occurred after fixation. Fig. 2A, B show images of the same cell before and after fixation with paraformaldehyde, a fixation routinely used in fluorescence microscopy. The pattern of the focal contacts (black streaks) is preserved after the fixation step, and > 95% of the focal contacts correspond in Fig. 2A, B. However, the separation of some parts of the lower membrane from the glass surface does seem to be perturbed since some changes are observed in areas of close contacts (grey areas, Fig. 2A, B) and in the concentric fringes. Fig. 3A, B show the same cell before and after fixation with glutaraldehyde. The comparison indicates that glutaraldehyde also gives good preservation of the focal contacts and in addition allows a better preservation of the close contacts than does formaldehyde. In both Figs. 2 and 3 occasional focal contacts which do not correspond exactly before and after fixation can be detected (e.g. arrow in Fig. 2A, B). These small differences might result from the fixation, but we believe it more likely, that the changes occur in the living cell during the time interval between photography in the reflexion-contrast microscope and the time of fixation (1–10 min).

Immunofluorescence microscopy

Rat mammary cells documented by interference-reflexion microscopy in the living state (Figs. 1A–3A) were processed through immunofluorescence microscopy using specific antibodies against actin. Figs. 1B, 2C and 3C show the display of strongly developed microfilament bundles by immunofluorescence microscopy. The bundles are often orientated parallel to the long axis of the cell, although a number of them do not follow this direction (Lazarides & Weber, 1974; Goldman *et al.* 1975). The general pattern is that documented previously for this cell line (Osborn *et al.* 1978).

At first it seems difficult to compare the pattern of long microfilament bundles, revealed by immunofluorescence microscopy (Figs. 1B, 2C, 3C) with the corresponding

Fig. 1. A, interference-reflexion micrograph of a rat mammary cell spread on the substratum and photographed in growth medium prior to fixation. Note focal contacts (*fc*, dark black streaks) the areas of close contact (*cc*, light grey), and areas of greater separation (*s*, white). The arrows indicate areas where the focal contacts can be seen to be aligned parallel to one another. *n*, nucleus. B, the same cell after fixation with formaldehyde, and stained with antibody to actin to show the arrangement of microfilament bundles. Two layers of microfilament bundles can be seen. The ones running from top to bottom have focal contacts at both ends (arrows in A and B). × 660.

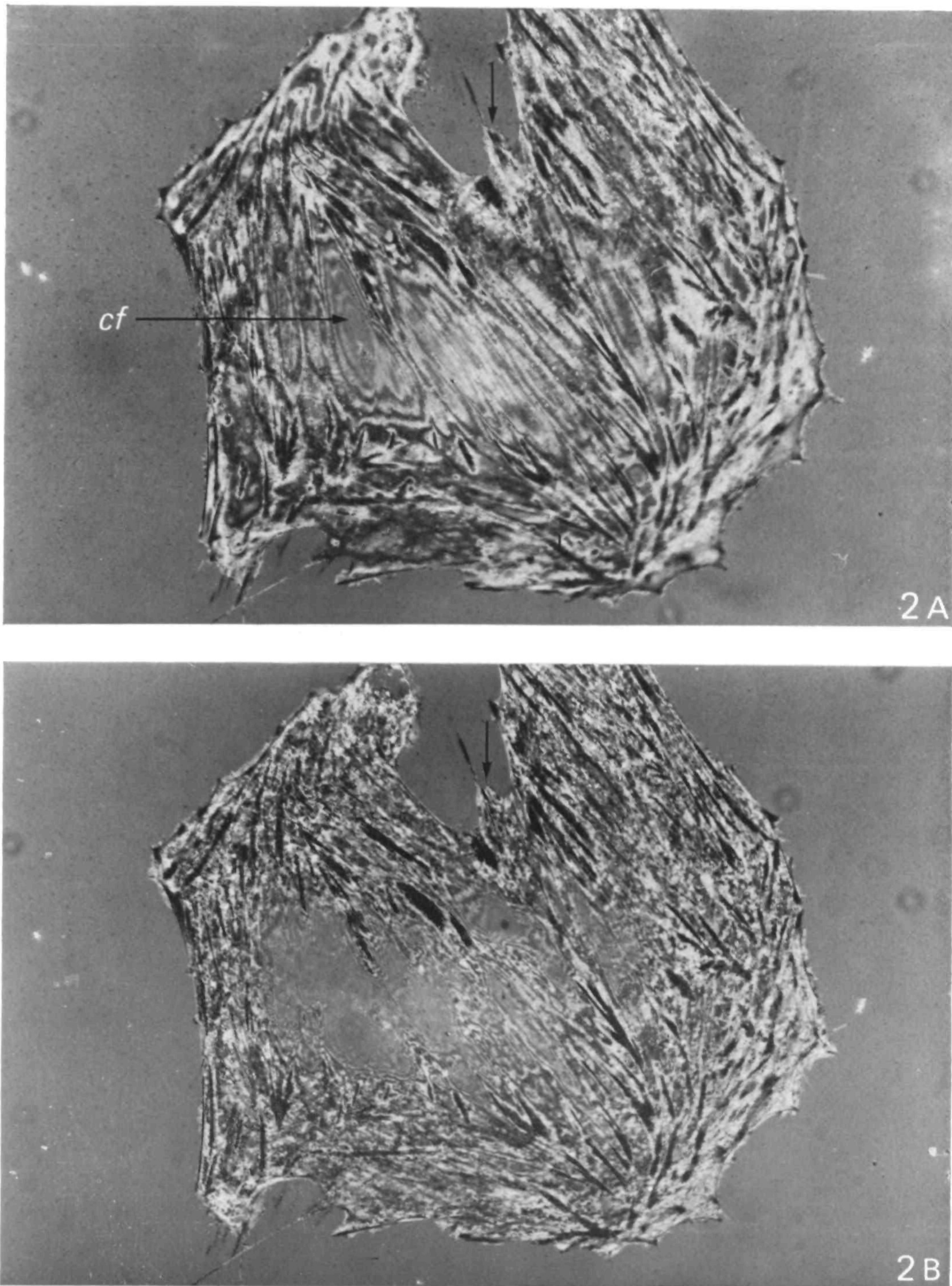
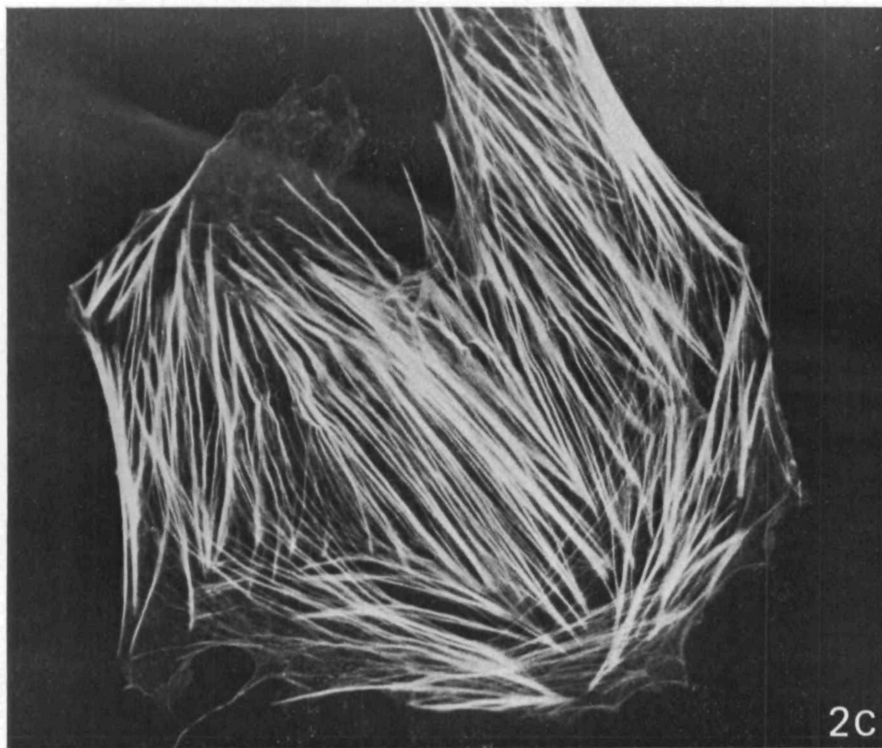


Fig. 2. Comparison of the interference-reflexion image and the fluorescent image of a rat mammary cell after formaldehyde fixation and staining with antibody to actin. A, living cell photographed in growth medium prior to fixation; B, same cell after fixation with formaldehyde; C, same cell after staining with antibody to actin. Almost all the focal contacts in A and B correspond although occasional differences can be seen (arrow Fig. 2A, B) and the concentric fringes (*cf*) are not well preserved. Superposition of the focal contacts in B and the stress fibres in C as shown for part of the cell in D shows that the majority of the focal contacts correspond to the proximal ends of microfilament bundles. × 500.



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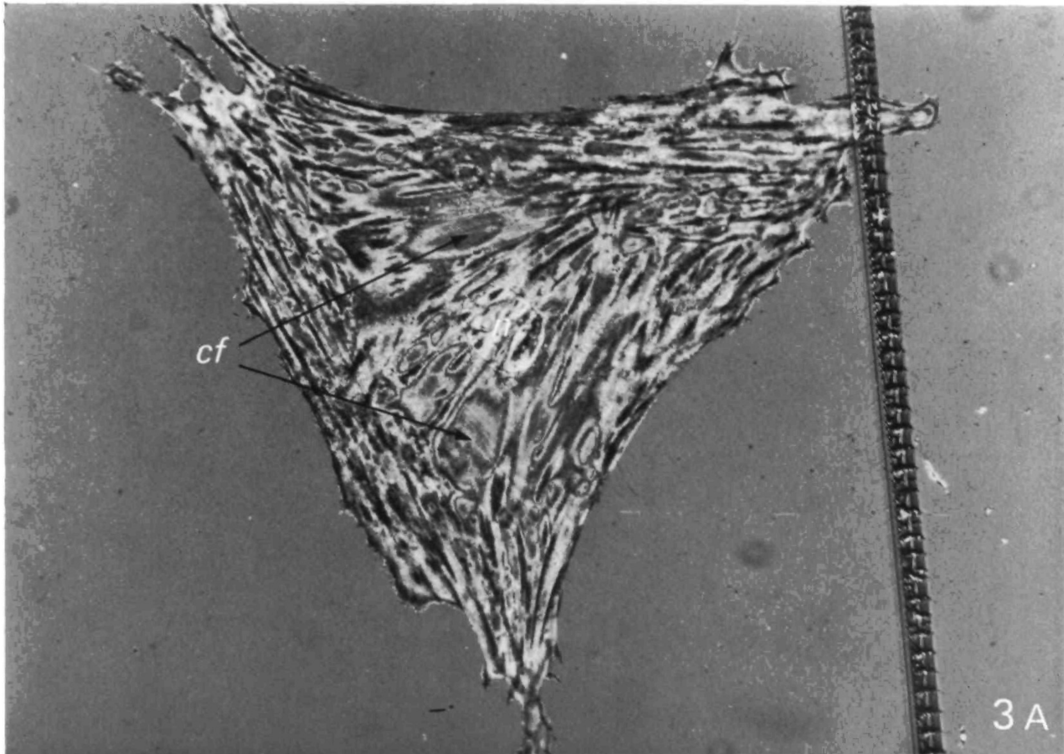
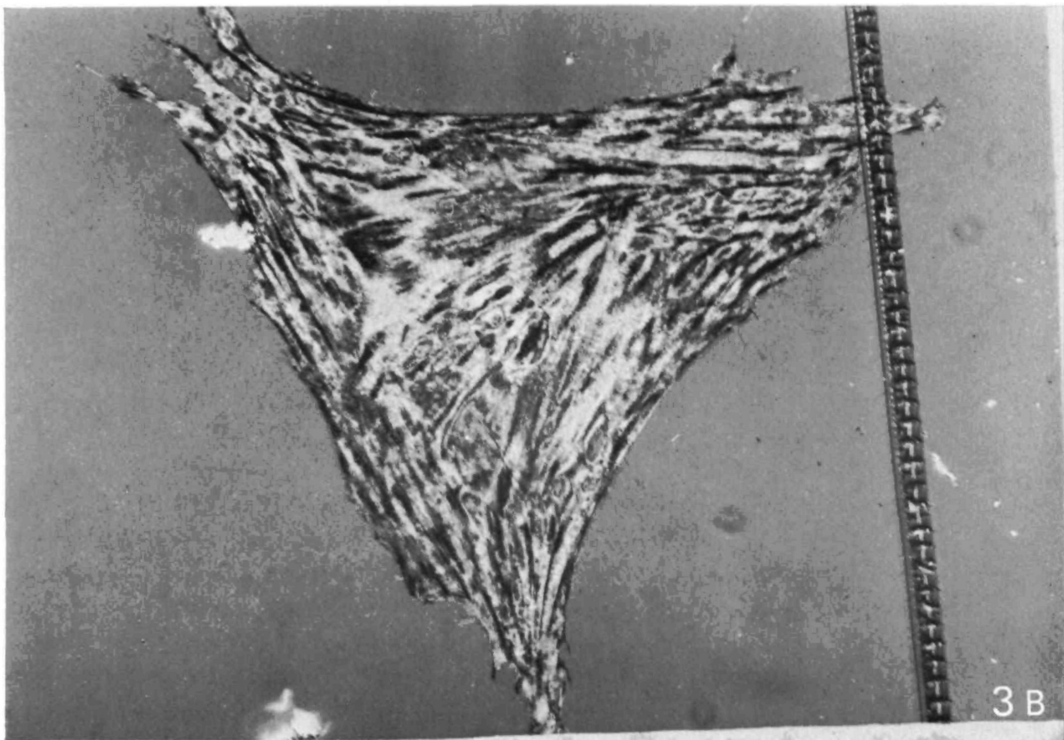




Figs. 3 A, 3 B. For legend see opposite page.

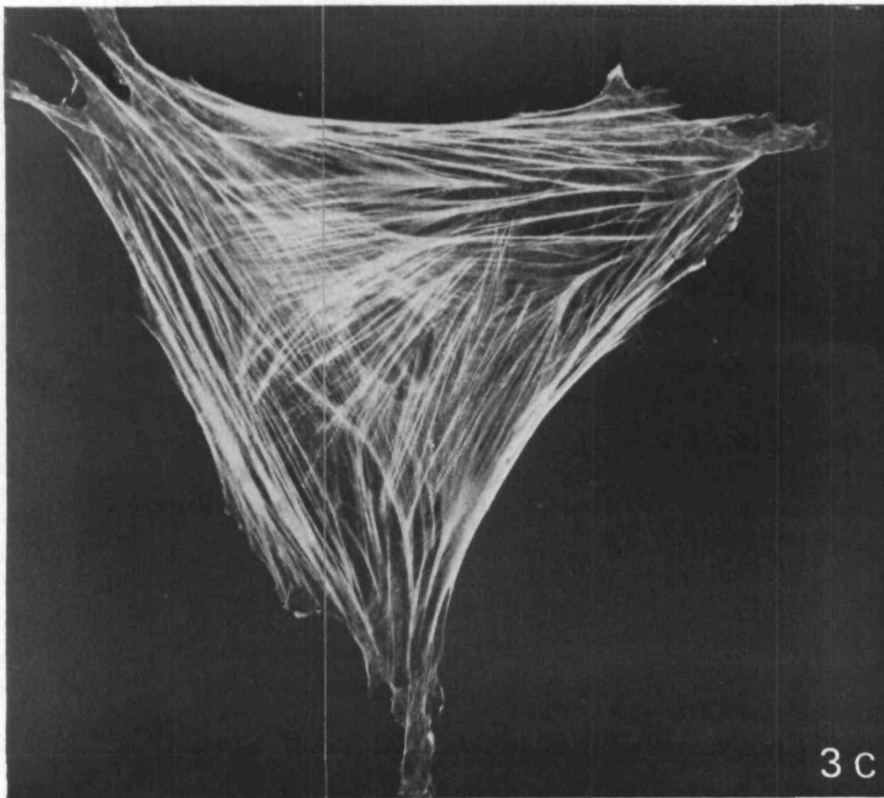
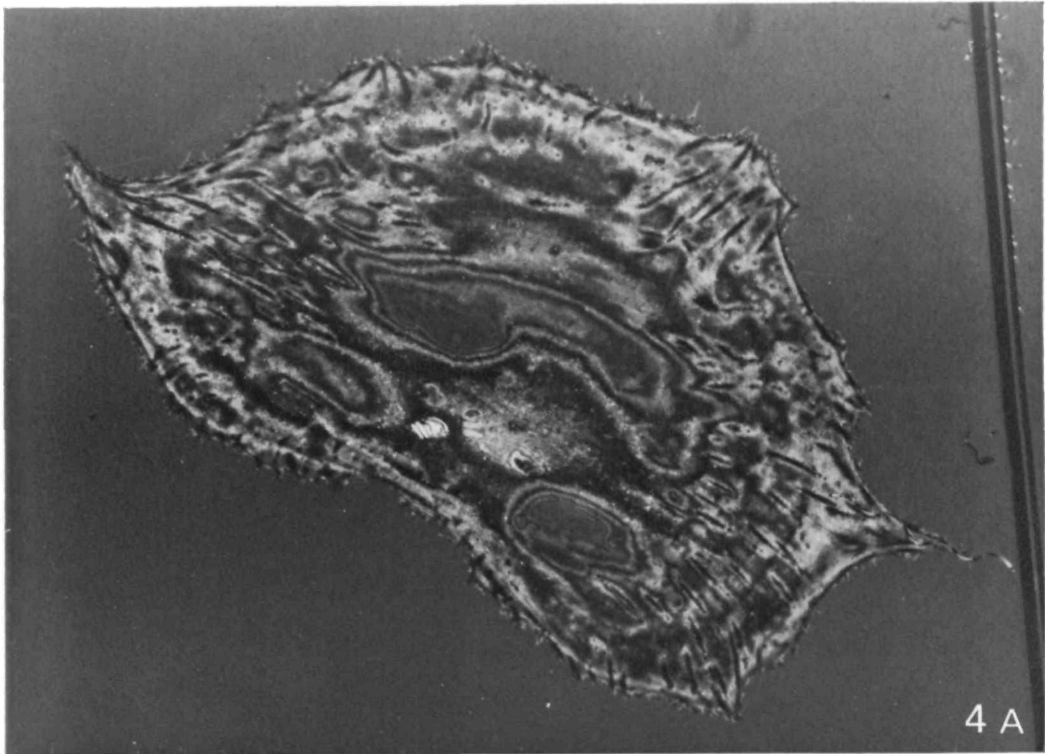


Fig. 3. Comparison of interference-reflexion images and fluorescent image of a rat mammary cell after fixation with glutaraldehyde and staining with antibody to actin. A, living cell photographed prior to fixation; B, same cell after fixation with glutaraldehyde; C, same cell after staining with antibody to actin. Again almost all the focal contacts in A and B correspond, and in less complicated areas of the cell good correspondence can be seen between the proximal ends of stress fibres and the focal contacts (*cf*, concentric fringes; *n*, nucleus). × 510.

images of focal contacts provided in the interference-reflexion microscope. The difficulty is, however, readily overcome upon closer inspection. In order to reveal the relationship more easily we found it helpful to trace the focal contacts on a transparent sheet of plastic and to superimpose this graph on to the fluorescent image. A part of Fig. 2B and C in which both the focal contacts and many of the stress fibres have been traced is shown in Fig. 2D. This and similar figures showed that almost all the focal contacts correspond to terminating tips of microfilament bundles. Although the cells have gone through multiple experimental steps during the immunofluorescence procedure the overall agreement is excellent. The central regions of the microfilament bundles in general do not have focal contacts (see Fig. 2D), although focal contacts can sometimes be detected along the length of microfilament bundles at the cellular margins.

The region close to the nucleus in Fig. 1B (arrows) shows a group of relatively short fluorescent fibres nearly all of which show focal contacts at both ends of the fibres in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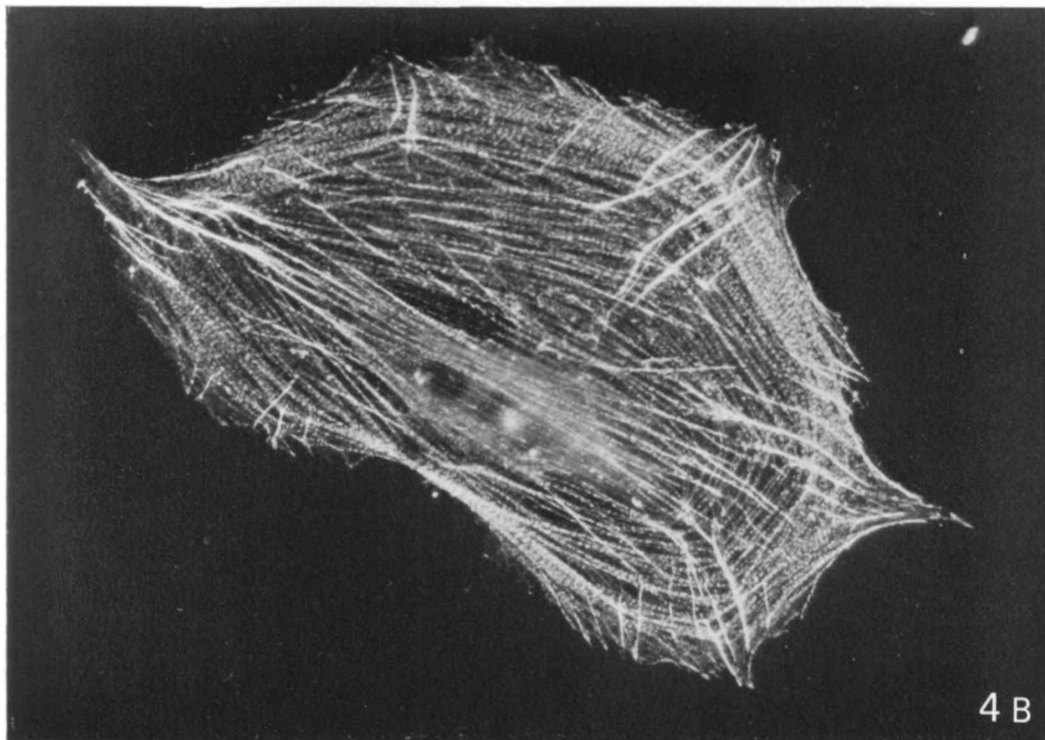
4 B

Fig. 1 A (arrows). In the immunofluorescence image a second layer of fibres is often observed crossing the first one. Some of these microfilament bundles run the length of the cell with focal contacts at both ends of the bundle. Other fibres often extend from the edge of the cell where focal contacts can be observed towards the cell centre where no focal contacts are visible in the interference-reflexion image and instead only close contact of the lower membrane is observed. Thus in this cell region the fibres of the second layer might be anchored in the upper membrane with no resulting contacts in the interference-reflexion image. This interpretation is in agreement with results obtained by stereo immunofluorescence microscopy (Osborn *et al.* 1978).

When the fluorescent images of cells stained with antibodies to other structural proteins were compared to the interference-reflexion image it became clear that the focal points are strongly stained by antibodies against α-actinin. Fig. 4 shows a rat mammary cell during respreading which was photographed first in interference-reflexion optics and was then stained with antibody to α-actinin. The interference-reflexion image of this cell shows several areas surrounded by concentric fringes. Three types of staining patterns by the antibody against α-actinin can be observed (cf. Lazarides, 1976). First a striated staining of stress fibre material throughout the cell, second a continuous staining of short and often radially arranged fibres often close to the cell periphery, and third a spot-like staining of prominent areas on the lower plasma membrane (not shown in Fig.4). Comparison of the corresponding interference-reflexion and the α-actinin images (Fig. 4A and B) shows a correlation between the focal contacts and the second and third types of α-actinin staining. Superimposition of the focal contacts and the continuously stained stress fibres suggests that in general the focal contacts correspond to the ends of the continuously stained stress fibres closest to the cell margin. Since these stress fibres stop part way across the cell it seems probable that the ends closest to the cell nucleus terminate close to the upper cell membrane (see below).

Stereo-fluorescence microscopy

The interpretation of corresponding interference-reflexion micrographs and fluorescent micrographs revealing the distribution of actin or α-actinin antibodies is often complicated, because although interference-reflexion microscopy reflects the lower membrane-substratum distance, the fluorescent images show an overview of the fibre systems in the whole cytoplasm. To overcome this difficulty we have used stereo-immunofluorescence microscopy which, as we have shown elsewhere (Osborn *et al.* 1978), can also be used to selectively view the arrangement of fibrous structures from the adhesive side of the cell. Fig. 5 shows a comparison between the interference-reflexion image after fixation with formaldehyde (Fig. 5C) and a stereo pair of a cell stained with actin antibody (Fig. 5A) and viewed from underneath. Examination of

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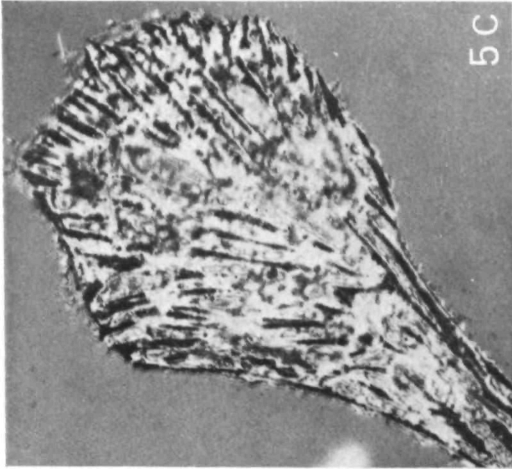
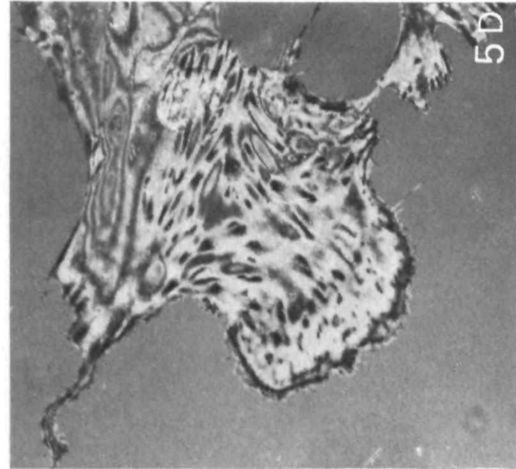
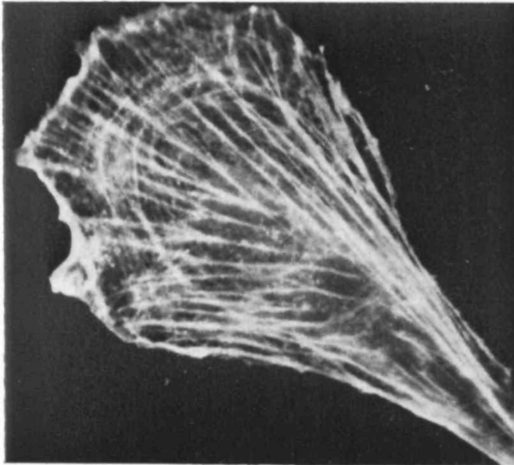
Fig. 4A, interference-reflexion micrograph of a living respreading rat mammary cell, 4 h after replating; B, the immunofluorescence micrograph of the same cell shows the different types of staining by the α-actinin antibody. Note the correlation between the focal contacts in A and the distal ends of the short and often radially arranged fibres in B that are stained in an uninterrupted fashion by the antibody to α-actinin. ×710.







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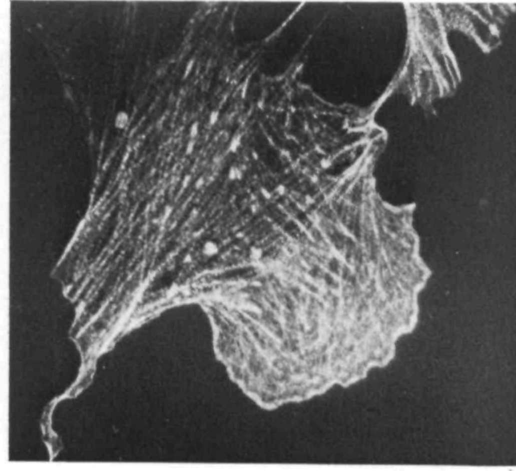

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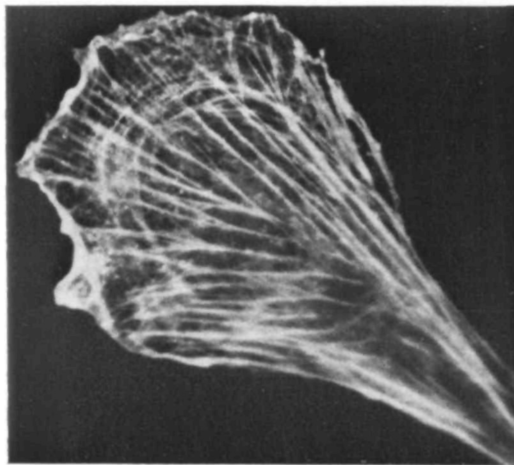

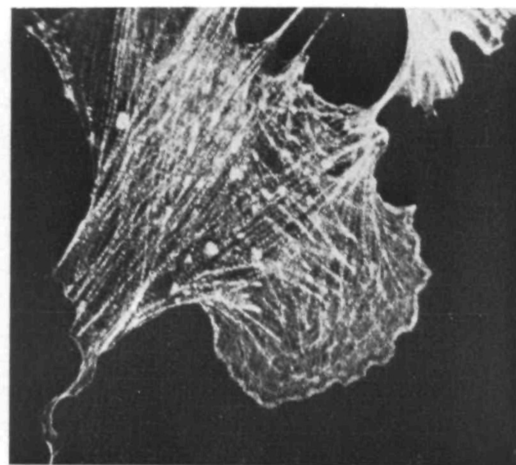

these figures shows again the correlation between focal contacts and the ends of microfilament bundles on the lower surface of the cell. In addition the figure indicates that some fibres do indeed terminate in a region close to the upper membrane of the cell (see above).

The same figure shows a comparison between the interference-reflexion image (Fig. 5D) and a stereo pair of the same cell stained with antibody to α-actinin (Fig. 5B). The continuously stained structures correlate well with focal contacts in the interference-reflexion microscope. Stereo views (Fig. 5B) show 2 layers of bundles in the thicker part of the cell. One layer of fibres appears to be parallel to the substratum throughout the whole cell and all the continuous fluorescent parts of the bundles seem to be in this layer and correspond to focal contacts in the interference-reflexion microscope. The second (upper) layer of fibres seems to have a concave form as if they are associated with the upper cell membrane. These filament bundles can occasionally also terminate in focal contacts at both ends of the cell.

Other cell types

In contrast to the fibroblastic rat mammary cells, PtK2 cells which are epithelioid in nature give a quite different pattern in the organization of microfilament bundles (Fig. 6B). There is only one prominent layer of fibres, and these fibres do not show such a directed organization as their counterparts in fibroblastic cells. Most of the bundles run over short distances. When the interference-reflexion image of PtK2 cells (Fig. 6A) is compared with the images typical of rat mammary cells (Figs. 1–3) the following differences are revealed: the areas of focal contacts are limited to the edges of the cell (Fig. 6A, arrows), even though the microfilament bundles do not run the full length of the cell (Fig. 6B). The resolution of the focal contacts in the interference-reflexion image is rather poor. White streaks in the interference-reflexion image often correspond to actin-containing fibres in the immunofluorescence image. Lochner & Izzard (1973) consider these streaks as indication that the membrane is adherent to the bundle and therefore is lifted away from the substratum. In other more-rounded cell types such as HeLa and SV40-transformed 3T3 cells (clone SV101) the resolution of the adhesion pattern in the interference-reflexion microscope is much poorer than that shown for rat mammary cells and for PtK2 cells.

Fig. 5. Stereo views of rat mammary cells treated in A, with antibody to actin or in B, with antibody to α-actinin. These stereo pairs should be viewed with a stereo viewer placed symmetrically over the stereo pair in order to obtain a 3-dimensional impression, and are mounted so the cells are viewed from the underneath. Thus the immunofluorescent profiles can be compared directly to the interference-reflexion images of the same cells (C, D). Fig. 5C was photographed after formaldehyde fixation, Fig. 5D in the living state. Note the correlation between the distal ends of the stress fibres present on the lower surface in A and the focal contacts in C and also that areas on the lower surface strongly stained with antibody to α-actinin in B correspond to focal contacts visible in D. A, C, $\times 760$; B, D, $\times 450$.

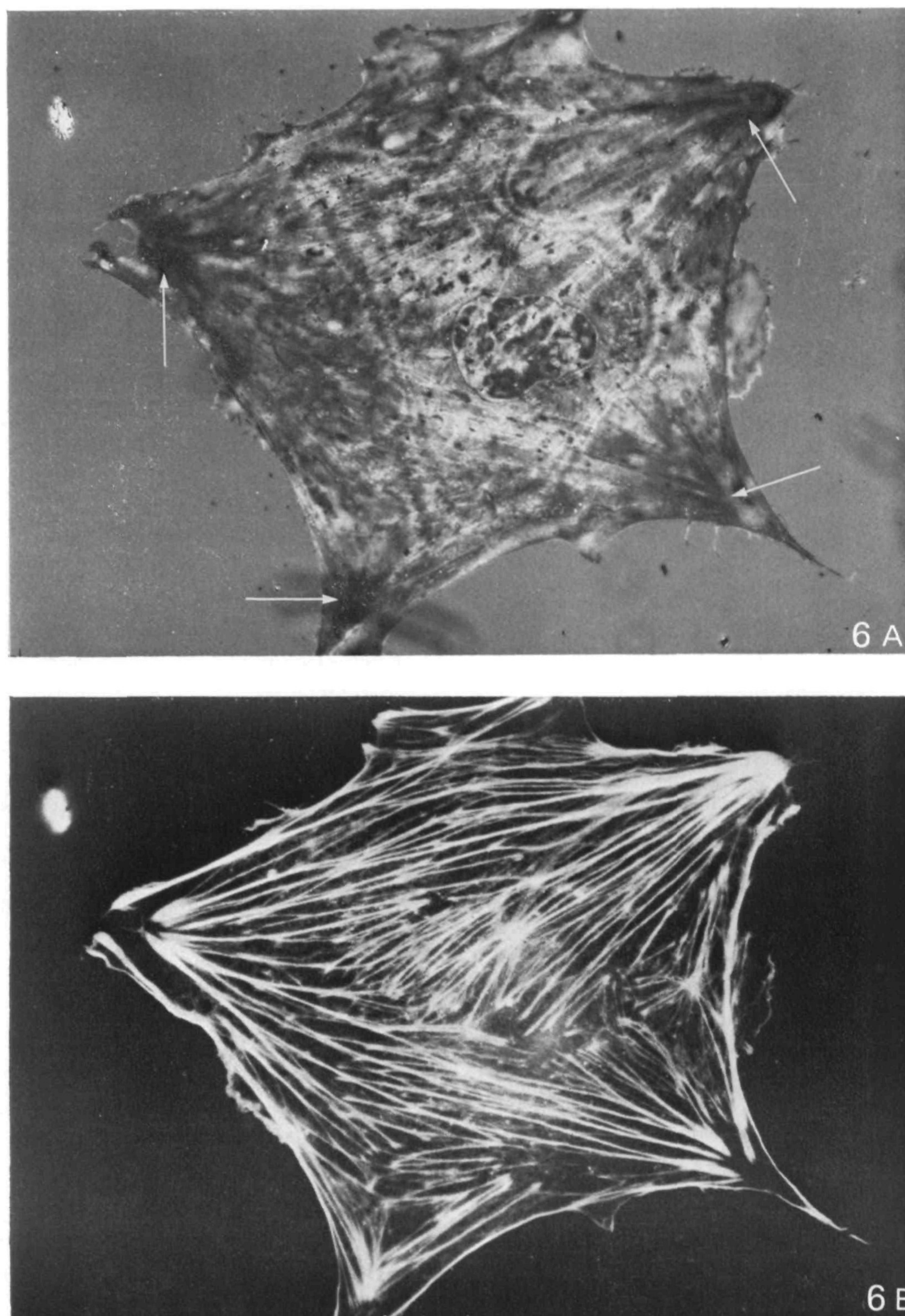

Fig. 6. A, interference-reflexion micrograph of a living rat kangaroo PtK2 cell; B, the immunofluorescence micrograph of the same cell, stained by the actin antibody. Note the poor definition of focal contacts in comparison to that shown in previous figures of rat mammary cells. Although the microfilament bundles do not run the full length of the cell, the focal contacts are limited to the edges of the cell (arrows in A). × 1050.

DISCUSSION

The results presented in this paper represent part of our continuing effort to determine the arrangement of specific fibrous elements in tissue culture cells using a variety of microscopic techniques. The interference-reflexion micrographs presented above have been taken using optics specially developed for this type of microscopy (Patzelt, 1977). More detail seems to be present in our micrographs than in those of some earlier studies (for references see Introduction). Our results show in the same cell a direct correlation between focal contacts in the interference-reflexion images and restricted portions of the microfilament bundles revealed by indirect-immunofluorescence microscopy using specific antibodies to actin and to α-actinin. The conclusion that stress fibres contact the substratum only at their ends is in agreement with the results of others who have compared interference-reflexion images with results of other techniques in studies on chick heart fibroblasts. Thus Izzard & Lochner (1976) showed a correlation between focal contacts and stress fibres visible with differential-interference optics, which was also noted by Abercrombie & Dunn (1975) using phase-contrast optics. In an elegant study, reported while our work was in progress, Heath & Dunn (1978) showed a correlation between focal contacts visible in interference-reflexion microscopy and bundles of microfilaments viewed by high-voltage electron microscopy.

The use of immunofluorescence microscopy has the advantage that one can construct a protein chemical anatomy for a biological structure such as the microfilament bundle (Weber, 1976). In an attempt to understand further the anatomy of focal contacts we stained cells with antibodies to α-actinin. Use of normal and stereo-immunofluorescence microscopy has allowed us to correlate focal contacts with parts of microfilament bundles especially rich in α-actinin suggesting that α-actinin may be required for a focal contact to be established. This result is in line with the proposal that α-actinin can act as membrane anchorage protein for microfilaments in non-muscle cells (Lazarides & Burridge, 1975; Lazarides, 1976; Schollmeyer *et al.* 1976). However currently it is not known whether such anchorage is mediated by direct insertion of α-actinin into or through the membrane or whether the linkage occurs through membrane protein(s) linked to α-actinin.

The results presented here further document for rat mammary cells the existence of at least 2 layers of microfilament bundles (see also Osborn *et al.* 1978). The first layer exists parallel to the lower plasma membrane, and contains those microfilament bundles most frequently observed when sections are made parallel to the substratum and are examined in the electron microscope (see for example Goldman *et al.* 1975). The second layer is often found above the nucleus, and appears to lie underneath the upper plasma membrane, as far as can be judged by light-microscopical procedures. Some of the microfilament bundles present in the upper layer can terminate on the upper membrane; others run over the nucleus and terminate at both ends in well defined focal contacts. Although microfilament bundles displayed above the nucleus have occasionally been reported in other cell lines the relative contribution of the upper and lower layers of microfilament bundles to processes such as cell movement remains to be determined. This problem, and the changes in focal contacts that occur

as the cell moves along the substratum will be discussed elsewhere. Our hope is that as the changes in these structural elements and their membrane interaction upon cell movement and spreading are further understood, it will be possible to construct a picture at the molecular level of the interactions which result in the locomotion of a cell along the substratum.

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REFERENCES

ABERCROMBIE, M. & DUNN, G. A. (1975). Adhesions of fibroblasts to substratum during contact inhibition observed by interference reflection microscopy. *Expl Cell Res.* **92**, 57–62.

ABERCROMBIE, M., HEAYSMAN, J. E. M. & PEGRUM, S. M. (1971). The locomotion of fibroblasts in culture. IV. Electron microscopy of the leading lamella. *Expl Cell Res.* **67**, 359–367.

BRINKLEY, B. R., FULLER, G. M. & HIGHFIELD, D. P. (1975). Cytoplasmic microtubules in normal and transformed cells in culture: Analysis by tubulin antibody immunofluorescence. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4981–4983.

BUCKLEY, I. K. & PORTER, K. R. (1967). Cytoplasmic fibrils in living cultured cells. A light and electron microscope study. *Protoplasma* **64**, 349–380.

CURTIS, A. S. G. (1964). The mechanism of adhesion of cells to glass. A study by interference reflection microscopy. *J. Cell Biol.* **20**, 199–215.

FRANKE, W. W., GRUND, C., OSBORN, M. & WEBER, K. (1978*a*). The intermediate-sized filaments in rat kangaroo PtK2 cells. I. Morphology *in situ*. *Cytobiologie* **17**, 365-391.

FRANKE, W. W., SCHMID, E., OSBORN, M. & WEBER, K. (1978*b*). The intermediate-sized filaments in rat kangaroo PtK2 cells. II. Structure and composition of isolated filaments. *Cytobiologie* **17**, 392–411.

GOLDMAN, R. D., LAZARIDES, E., POLLACK, R. & WEBER, K. (1975). The distribution of actin in non-muscle cells. *Expl Cell Res.* **90**, 333–344.

GOLDMAN, R. D., SCHLOSS, J. A. & STARGER, J. M. (1976). Organizational changes of actinlike microfilaments during animal cell movement. In *Cell Motility, vol. A* (ed. R. D. Goldman, T. Pollard & J. Rosenbaum), pp. 217–246. Cold Spring Harbor Laboratory.

HEATH, J. P. & DUNN, G. A. (1978). Cell to substratum contacts of chick fibroblasts and their relation to the microfilament system. A correlated interference-reflexion and high-voltage electron-microscope study. *J. Cell Sci.* **29**, 197–212.

HEAYSMAN, J. E. M. & PEGRUM, S. M. (1973). Early contacts between fibroblasts – an ultrastructural study. *Expl Cell Res.* **78**, 71–78.

ISENBERG, G., RATHKE, P. C., HÜLSMANN, N., FRANKE, W. W. & WOHLFARTH-BOTTERMANN, K. E. (1976). Cytoplasmic actomyosin fibrils in tissue culture cells. Direct proof of contractility by visualization of ATP-induced contraction in fibrils isolated by laser microbeam dissection. *Cell Tiss. Res.* **166**, 427–433.

ISHIKAWA, H., BISCHOFF, R. & HOLTZER, H. (1969). Formation of arrowhead complexes with heavy meromyosin in a variety of cell types. *J. Cell Biol.* **43**, 312–328.

IZZARD, C. S. & LOCHNER, L. R. (1976). Cell-to-substrate contacts in living fibroblasts: an interference-reflexion study with an evaluation of the technique. *J. Cell Sci.* **21**, 129–159.

LAZARIDES, E. (1975). Tropomyosin antibody: the specific localisation of tropomyosin in non-muscle cells. *J. Cell Biol.* **65**, 549–561.

LAZARIDES, E. (1976). Actin, α-actinin, and tropomyosin interaction in the structural organisation of actin-filaments in non-muscle cells. *J. Cell Biol.* **68**, 202–219.

LAZARIDES, E. & BURRIDGE, E. (1975). α-Actinin: immunofluorescent localisation of actin filaments in non-muscle cells. *Cell* **6**, 289–298.

LAZARIDES, E. & WEBER, K. (1974). Actin antibody: the specific visualization of actin filaments in non-muscle cells. *Proc. natn. Acad. Sci. U.S.A.* **71**, 2268–2272.

LEWIS, W. H. & LEWIS, M. R. (1924). Behaviour of cells in tissue cultures. In *General Cytology* (ed. E. V. Cowdry), pp. 385–447. Chicago, Illinois: The University of Chicago Press.

LLOYD, C. W., SMITH, C. G., WOODS, A. & REES, D. A. (1977). Mechanisms of cellular adhesion. II. The interplay between adhesion, the cytoskeleton and morphology in substrate-attached cells. *Expl Cell Res.* **110**, 427–437.

LOCHNER, L. & IZZARD, C. S. (1973). Dynamic aspects of cell-substrate contact in fibroblast motility. *J. Cell Biol.* **59**, 199*a*.

OSBORN, M., BORN, T., KOITZSCH, H. J. & WEBER, K. (1978). Stereo immunofluorescence microscopy: I. Three dimensional arrangement of microfilaments, microtubules and tonofilaments. *Cell* **14**, 477–488.

OSBORN, M., FRANKE, W. W. & WEBER, K. (1977). Visualization of a system of filaments 7–10 nm thick in cultured cells of an epithelioid line (PtK2) by immunofluorescence microscopy. *Proc. natn. Acad. Sci. U.S.A.* **74**, 2490–2494.

PATZELT, W. J. (1977). Reflexionskontrast: Eine neue lichtmikroskopische Technik. *Mikrokosmos* **3**, 78–81.

RATHKE, P. C., SCHMID, E. & FRANKE, W. W. (1975). The action of cytochalasins at the subcellular level. I. Effects and binding of cytochalasin B in cells of a line derived from a rat mammary adenocarcinoma and in rat erythrocytes. *Cytobiologie* **10**, 366–396.

REES, D. A., LLOYD, C. W. & THOM, D. (1977). Control of grip and stick in cell adhesion through lateral relationships of membrane glycoproteins. *Nature, Lond.* **267**, 124–128.

SCHOLLMEYER, J. V., GOLL, D. E., TILNEY, L., MOOSEKER, M., ROBSON, R. & STROMER, M. (1974). Localization of α-actinin in nonmuscle material. *J. Cell Biol.* **63**, 304*a*.

SCHOLLMEYER, J. E., FURCHT, L. T., GOLL, D. E., ROBSON, R. M. & STOMER, M. H. (1976). Localization of contractile proteins in smooth muscle cells and in normal and transformed fibroblasts. In *Cell Motility*, Vol. A (ed. R. D. Goldman, T. Pollard & J. Rosenbaum), pp. 361–386. Cold Spring Harbour Laboratory.

WANG, K., ASH, F. & SINGER, S. (1975). Filamin: A new high-molecular-weight protein found in smooth muscle and nonmuscle cells. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4483–4486.

WEBER, K. (1976). Biochemical anatomy of microfilaments in tissue culture cells using immunofluorescence microscopy. In *Contractile Systems in Non-Muscle Tissues* (ed. S. V. Perry, A. Margreth & R. S. Adelstein), pp. 51–66. Amsterdam: North-Holland Publishing.

WEBER, K. & GROESCHEL-STEWART, U. (1974). Antibody to myosin: the specific visualization of myosin-containing filaments in non-muscle cells. *Proc. natn. Acad. Sci. U.S.A.* **71**, 4561–4564.

WEBER, K., POLLACK, R. & BIBRING, T. (1975). Antibody against tubulin: The specific visualization of cytoplasmic microtubules in tissue culture cells. *Proc. natn. Acad. Sci. U.S.A.* **72**, 459–463.

WEBER, K., RATHKE, P. C. & OSBORN, M. (1978). Cytoplasmic microtubular images in glutaraldehyde-fixed tissue culture cells by electron microscopy and by immunofluorescence microscopy. *Proc. natn. Acad. Sci. U.S.A.* **75**, 1820–1824.

WEBER, K., WEHLAND, J. & HERZOG, W. (1976). Griseofulvin interacts with microtubules both *in vivo* and *in vitro*. *J. molec Biol.* **102**, 817–829.

WEBSTER, R. E., OSBORN, M. & WEBER, K. (1978). Visualization of the same PtK2 cytoskeletons by both immunofluorescence and low power electron microscopy. *Expl Cell Res.* **117**, 47–61.

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