

CHANGES IN THE DISTRIBUTION OF A MAJOR FIBROBLAST PROTEIN, FIBRONECTIN, DURING MITOSIS AND INTERPHASE

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ABSTRACT

The distribution of a major fibroblast protein, fibronectin, was studied by immunofluorescence and immunoscanning electron microscopy in cultures of human and chicken fibroblasts during different phases of the cell cycle. The main findings were: (a) In interphase cells, the intensity of surface-associated fibronectin fluorescence correlated with that of intracellular fibronectin fluorescence. (b) The intensity of the fluorescence of both surface-associated and intracellular fibronectins was not changed in cells that were synthesizing DNA. (c) Mitotic cells had reduced amounts of surface-associated but not of intracellular fibronectin. The surface fibronectin that remained on meta-, ana-, or telophase cells had a distinct punctate distribution and was also localized to strands attaching the cells to the substratum. Fibronectin strands first reappeared on the surface of flattening cytoplasmic parts of telophase cells. (d) Fibronectin was also detected in extracellular fibrillar material on the growth substratum, particularly around dividing cells.

Thus, surface-associated fibrillar fibronectin was present during G₁, S, and G₂ but in cells undergoing mitosis the distribution was altered and the amount appeared to be reduced. The observations on the distribution of surface-associated fibronectin suggest that rather than being involved in growth control this fibronectin plays a structural role in interactions of cells with the environment.

Fibronectin is a glycoprotein composed of high molecular weight subunits (mol wt, 200–220 × 10³) and occurs in several immunologically cross-reactive forms in the plasma and connective tissue of vertebrates (11, 31). It is a major cell surface-associated protein in human (20) and chicken (22) fibroblasts and in human astroglial cells (32) cultivated *in vitro*. It has been demonstrated by immunofluorescence (28) and scanning electron microscopy to have an organized fibrillar distribution on the cell surface (33). Cells cultivated *in vitro*

shed or secrete fibronectin into the medium (22, 29, 32). The designation fibronectin emphasizes the interactions of the protein (fibra [lat.] fiber; nectere [lat.] connect, link) with fibrillar structures such as fibrin (16, 21), and possibly collagen (17), and its characteristic strandlike distribution on the surface of cultured fibroblasts (33). An analogous and, by several criteria, identical (10) surface-associated protein termed LETS protein or CSP has been detected by radiochemical surface labeling of fibroblasts of various species (2, 4–6, 10).

Fibronectin of human plasma (cold-insoluble globulin) can be extensively purified (16) and used to raise specific antisera. Such sera react with various forms of fibronectin (in plasma, connective tissues, and cultured cells) within a given species and cross-react with fibronectins of other vertebrates. Immunological cross-reactivity occurs even between avian and human fibronectins (11), suggesting that the protein is evolutionarily highly conserved.

The cellular and plasma (serum) fibronectins share several properties: they have similar mobility in immunoelectrophoresis (20, 22), they are dimers of disulfide bonded 200–220 × 10³ mol wt polypeptides (8, 15, 16), and both of them are substrates of the thrombin-activated cross-linking enzyme plasma transglutaminase (blood coagulation factor XIII) (9, 16).

Cell-surface associated fibronectin has been assigned a role in growth control. In mouse and hamster fibroblasts the amount of fibronectin, detected by radiochemical surface-labeling techniques, has been reported to vary during the cell cycle (3, 5, 7). Maximal labeling was detected in cells in the G1 phase and least labeling in mitotic cells (7). These observations suggested that fibronectin-like proteins might be involved in the regulation of cell proliferation and especially in the initiation of DNA synthesis in G1 cells (5). The abundant occurrence of fibronectin in the extracellular matrix of connective tissues (13, 34) and its interaction with fibrin (16, 21) and possibly with collagen (17), however, suggest that fibronectin is a structural element in cell interactions and in tissue organization.

The present experiments were designed to study fibronectin distribution during the cell cycle and cell division in human and chicken fibroblasts *in vitro*. To this end, immunofluorescence and immunoscanning electron microscope techniques were used.

MATERIALS AND METHODS

Cell Cultures

Human embryonic fibroblasts were used in their 10–15th passage *in vitro* when the cultures were homogeneous, containing only fibroblastic cells as judged by microscopy (stained cultures and phase contrast). The culture medium was RPMI 1640 supplemented with 10% fetal calf serum, 100 U/ml penicillin and 100 µg/ml streptomycin. Chicken embryo fibroblasts were prepared and used as secondary cultures in medium 199 supplemented with 10% tryptose phosphate broth and 5% calf serum

as described earlier (30). These cultures contained not less than 90% fibroblastic cells, judged as mentioned above. The stock cultures were free of mycoplasma, as tested by cytochemical DNA staining (23). Cells were cultivated on glass cover slips for immunofluorescence staining and on carbon-coated glass cover slips for scanning electron microscopy.

Identification of S-Phase Cells

Exponentially growing cultures were labeled with [³H]thymidine (3.0 µCi/ml, 6.7 Ci/mmol, 15 min) immediately before fixation for immunofluorescence staining. After photography the preparations were subjected to autoradiography (Kodak AR 10 stripping film, exposure time 10 days), and the fibronectin-specific fluorescence pattern of the cells that had [³H]thymidine label was identified from the immunofluorescence photographs.

Immunofluorescence Staining and Microscopy

Indirect immunofluorescence staining was made with rabbit antiserum against human plasma fibronectin purified in our laboratory by Dr. Deane Mosher, as described previously (16) with the modification that fibrinogen was removed by heat precipitation (56°C, 3 min) rather than by clotting. For the blocking experiments and immunodiffusion analysis, human plasma fibronectin was purified by the same procedure. Commercially available fluorescein isothiocyanate- (FITC-) conjugated sheep anti-rabbit γ-globulin was used to visualize rabbit anti-human fibronectin.

For simultaneous immunofluorescence visualization of surface-associated and intracellular fibronectin, the cultures were fixed with 3.5% formaldehyde in phosphate-buffered saline (PBS), pH 7.3, for 20 min at room temperature followed by acetone for 30 min at –20°C.

To study cell surface-associated fibronectin, cultures were fixed and stained in either of the following ways: (a) unfixed cells were indirectly stained at –0°C (30 min) by treating with rabbit anti-fibronectin serum, and then fixed in formaldehyde and stained with FITC-conjugated anti-rabbit IgG; and (b) formaldehyde-fixed cells were stained indirectly.

A Zeiss fluorescence microscope with phase-contrast optics and epi-illuminator III RS equipped with filters and beam splitter for specific FITC-fluorescence was used. Fluorescence micrographs (exposure time, 30 s) and phase-contrast micrographs were made on Kodak Tri-X pan film, developed in Kodak Microdol X to give normal contrast. The location of fibronectin fluorescence relative to the growth substratum and cell surfaces was determined by sequential use of phase-contrast and immunofluorescence optics without changing the objective or level of focus.

The following experiments indicated that the immunofluorescence methods specifically detected fibronectin,

and that the fibrillar fluorescence represented external surface-associated fibronectin, and that the patchy perinuclear fluorescence represented intracellular fibronectin: (a) the preparations of plasma fibronectin used in immunization to raise the antiserum, in blocking experiments, and in immunodiffusion analysis gave a single polypeptide band (apparent mol wt, 200,000 daltons) in polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS) and 2-mercaptoethanol (Fig. 1). (b) The anti-human fibronectin serum gave a single precipitation line against normal human plasma, extract of human fibroblasts, and the purified antigen in immunodiffusion analysis (Fig. 2), and in immunoelectrophoresis (not shown). (c) Immunofluorescence staining of unfixed cells in the cold, or of formaldehyde-fixed cells, as described above, resulted in fibrillary fluorescence only. The staining pattern was similar in cultures fixed with 1.5% glutaraldehyde, but this fixation procedure was not adopted because it resulted in strong background autofluorescence. (d) Staining of formaldehyde-fixed cells with anti-actin antibodies¹ under similar conditions resulted in no fluorescence, indicating that intracellular antigens were not appreciably exposed by these staining procedures. (e) Patchy perinuclear fluorescence was seen only in acetone-fixed cells, and was therefore judged to be intracellular, because under these conditions intracellular actin-containing microfilament bundles could be visualized by immunofluorescence. (f) Neither fibrillar nor perinuclear fluorescence was detected when rabbit anti-fibronectin serum was substituted with normal rabbit serum or rabbit antisera against a variety of human plasma proteins. (g) Immunofluorescence could be completely blocked by preincubation of the antiserum with purified fibronectin (Fig. 3). (h) The fibrillar pattern observed with anti-plasma fibronectin was identical to that observed with antisera against papain digest of live human fibroblasts (20), which contains mainly fibronectin determinants.

Immuno-Scanning Electron Microscopy

IgG-bound Latex particles were prepared essentially according to Linthicum et al. (14). Dow-Latex beads (0.9 μm in diameter, Serva Feinbiochemica, Heidelberg, W. Germany) were suspended in 0.2 M veronal buffer, pH 9.2, and incubated at room temperature for 30 min in the presence of different concentrations of sheep anti-chicken fibronectin IGG. The preparation and the specificity of the antiserum have been described earlier (22, 33). In immunodiffusion analysis similar to that shown in Fig. 2, that antiserum gave a single precipitation line against chicken plasma, extracts of chicken fibroblasts, and the immunizing antigen. The IgG fraction was isolated from serum by sodium sulfate precipitation and

¹ Kurkinen, M., Wartiovaara, J., and A. Vaheri. Manuscript in preparation.

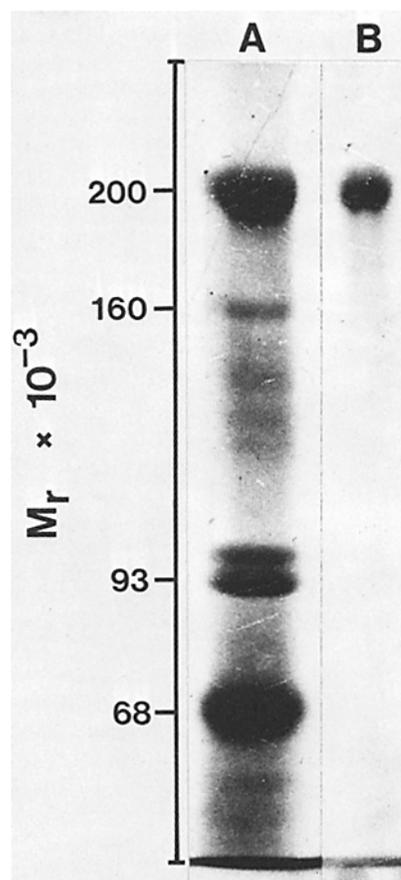


FIGURE 1 Gel electrophoresis of fibronectin purified from human plasma. (A) Molecular weight standards are human plasma fibronectin (2.0×10^5), α -2 macroglobulin (1.6×10^5), phosphorylase A (9.3×10^4) and human serum albumin (6.8×10^4). (B) Fibronectin. Before analysis the samples were treated with 4% SDS and 10% 2-mercaptoethanol for 3 min in boiling water bath. The polypeptides were separated on slab 3.0/5.0% discontinuous polyacrylamide gels, and the gels were stained for protein with Coomassie blue.

DEAE-cellulose chromatography. The optimal IgG:Latex ratio was determined experimentally. After centrifugation, the IgG-treated beads were passed through a 0.2- μm pore size Millipore filter (Millipore Corp., Bedford, Mass.) to remove aggregates. Cells, grown on carbon-coated cover slips, were prefixed for 10 min at 37°C in 0.5% glutaraldehyde in cacodylate buffer, pH 7.2. After washing with 0.2 M veronal buffer, pH 9.2, the cells were treated with different concentrations of the IgG/Latex preparation for 30 min at room temperature. After three washes with the above buffer, the cells were fixed with 1.5% glutaraldehyde in cacodylate buffer, pH 7.2, for 30 min at 4°C. The cells were

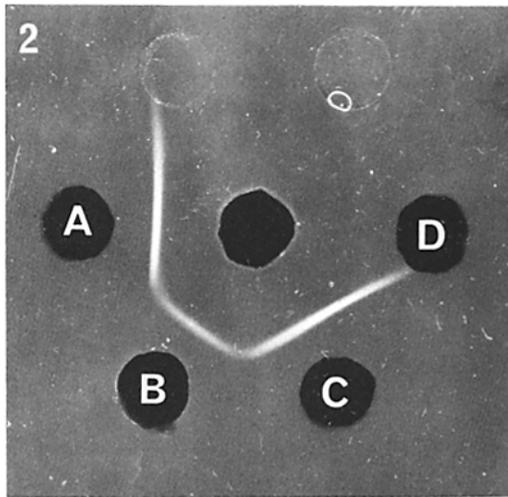


FIGURE 2 Immunodiffusion in agarose. (A) Normal human plasma. (B) Human fibroblasts extracted with 6 M urea and 1% Triton X-100. (C) Fibronectin purified from human plasma. (D) Fetal calf serum. Center well: anti-human fibronectin rabbit serum.

then washed in PBS, dehydrated in ethanol series, and critical point dried in CO_2 (1). After carbon-gold coating, the specimens were examined with a JSEM U-3 scanning electron microscope at 15 kV.

Preincubation of fixed cells with sheep anti-chicken fibronectin IgG followed by washing with veronal buffer before treatment with the sheep IgG-Latex preparation blocked the binding of Latex beads to the cell surface (Fig. 16), thus indicating that the IgG-Latex beads did not bind unspecifically.

RESULTS

Surface-Associated and Intracellular Staining of Fibronectin during Interphase and in DNA-Synthesizing Cells

Human interphase fibroblasts fixed with formaldehyde and acetone displayed both intracellular and surface-associated fibronectins in immunofluorescence staining (Fig. 4). The intracellular fluorescence was patchy, mainly perinuclear, and the cell surface-associated fluorescence was predominantly fibrillar. A variation in the pattern and intensity of fibronectin staining was seen among individual cells. The intensity of the two types of fibronectin-specific fluorescence in 305 interphase cells was classified as weak or strong (Table I). The distribution of fibronectin fluorescence in cells indicated that strong surface-associated fluorescence was associated with strong intracellular

fluorescence and weak surface-associated fluorescence with weak intracellular fibronectin staining ($P < 0.001$, χ^2 test).

Combined [^3H]thymidine labeling and immunofluorescence staining of the same cells was used to compare fibronectin patterns in G- and S-phase cells (Table II). Under the conditions used, about 50% of the cells were synthesizing DNA. No differences were seen either in surface-associated or in intracellular fibronectin patterns in S- vs. non-S-phase cells ($P > 0.3$).

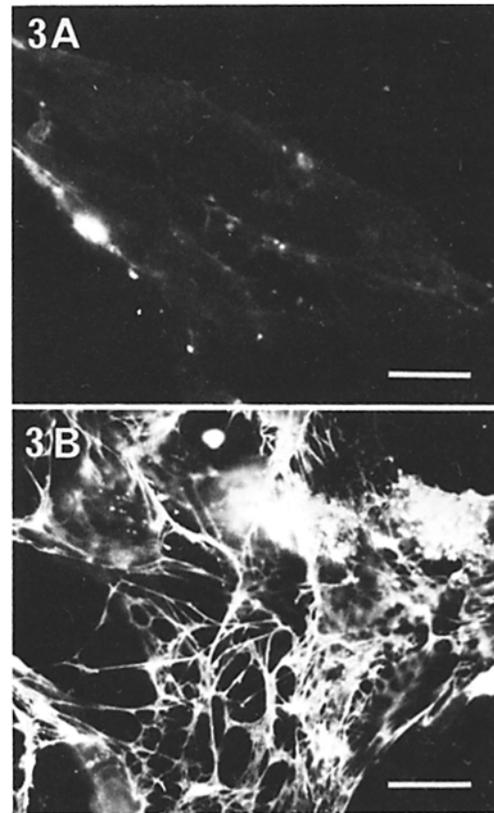


FIGURE 3 Blocking of fibronectin immunofluorescence staining by pretreatment of the antiserum with purified fibronectin. (A) Staining with fibronectin-treated antiserum. (B) Control-treated antiserum. 100 μg of purified human plasma fibronectin (A) or human serum albumin (B) were added to 1 milliliter of antifibronectin serum, in working dilution (1:20). The mixtures were kept first for 60 min at room temperature and overnight at 4°C. They were then clarified by centrifugation for 60 min at 10,000 g at 4°C and used in indirect immunofluorescence staining of formaldehyde-fixed cover slip cultures of human fibroblasts. $\times 540$. Bar, 20 μm .

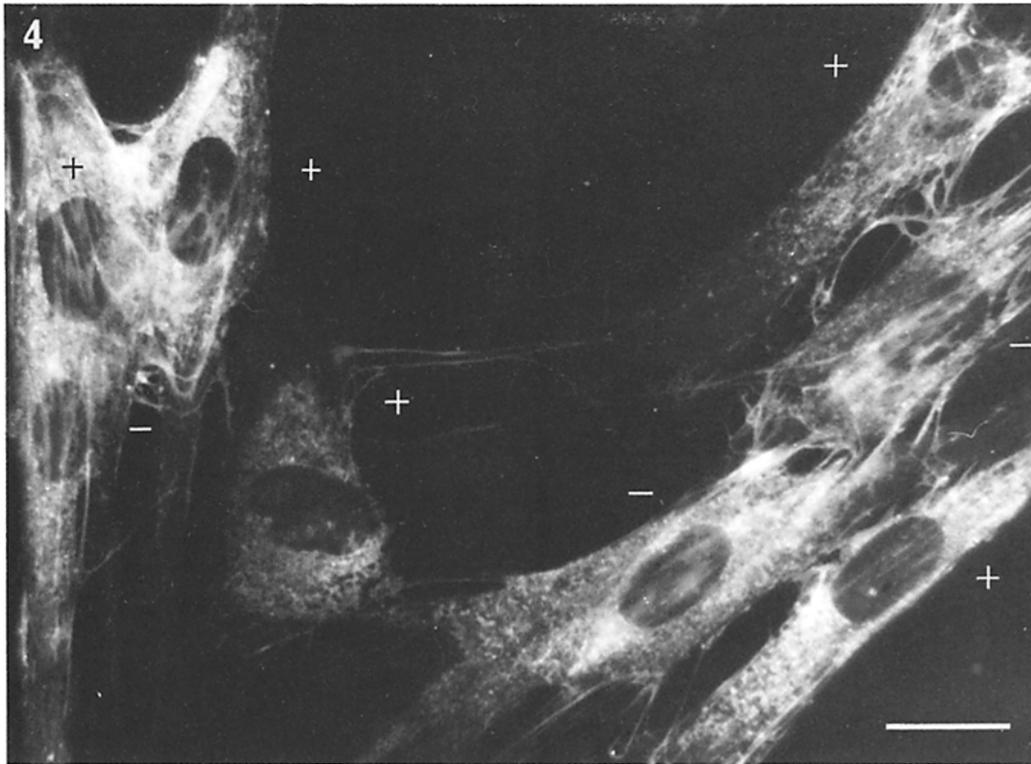


FIGURE 4 Immunofluorescence-stained fibronectin in exponentially growing human fibroblasts. Fixation in formaldehyde and acetone to visualize both fibrillar surface-associated and intracellular fibronectins. The intracellular fluorescence is patchy and predominantly perinuclear, giving a sharp contour to the unstained nucleus. Thymidine incorporation as detected by autoradiography indicated as + (positive, S-phase cells) or -. Various fluorescence patterns are seen in DNA-synthesizing cells. $\times 830$. Bar, 20 μm .

TABLE I
Fibronectin-Specific Fluorescence of Exponentially Growing Human Fibroblasts in Interphase

Intracellular fibronectin	Surface-associated fibronectin		Total no. of cells
	Weak	Strong	
Weak	55	18	73
Strong	85	147	232
Total	140	165	305

Rapidly growing cover slip cultures of human embryonic fibroblasts, 24 h after seeding, were fixed and stained for fibronectin by immunofluorescence. Interphase cells were selected for study under phase-contrast optics, and the intensity of intracellular and surface-associated fibronectins was evaluated either as weak (or negative) or strong under immunofluorescence optics.

Thus, S-phase cells had no particular fibronectin distribution as compared to cells not synthesizing DNA. This is exemplified in Fig. 4, in which S-phase cells display various fibronectin patterns.

Fibronectin in Mitotic Cells

Exponentially growing human fibroblasts were fixed in different ways (see Materials and Methods) to study by immunofluorescence the distribution of surface-associated and intracellular fibronectins of dividing cells as compared to interphase cells. In formaldehyde-fixed interphase cells only, the characteristic surface-associated strands were visualized (Fig. 5) indicating that no intracellular fibronectin was stained (cf. Fig. 4). Of cells in meta-, ana-, or telophase, identified in phase-contrast microscopy, the majority (42 out of 49 cells examined) showed some fibronectin surface staining. Seven of the 49 cells were immunofluorescence-negative. The fluorescence of singly lying cells was distributed as small "commas" on the surface (Figs. 6 and 7) and it appeared much weaker than that on interphase cells. Cells dividing in the vicinity or on top of interphase cells were often interwoven by fibronectin-containing strands

TABLE II
Phase of Cell Cycle as Judged by [³H]thymidine labeling and Distribution of Fibronectin-Specific Fluorescence in Exponentially Growing Human Fibroblasts in Interphase

Phase of cell cycle	No. of cells	Staining of fibronectin			
		Surface-associated		Intracellular	
		Strong	Weak	Strong	Weak
S-phase	160	93	67	121	39
Non-S phase	145	72	73	111	34
Total	305	165	140	232	73

The cultures, prepared as in Table I, were pulse-labeled with [³H]thymidine, fixed, immunofluorescence-stained for fibronectin, photographed and then subjected to autoradiography to localize DNA-synthesizing cells. Evaluation of fibronectin fluorescence as in Table I.

and cell extensions connecting mitotic cells with the surrounding interphase cells (Figs. 6-8) or with the substratum (Fig. 9). In these cells, the fluorescence intensity was higher than that in singly lying dividing cells which had only comma-shaped fluorescence (compare the two daughter cells in Fig. 6). In flattening, early G1 cells, weak surface-associated strands were seen (Fig. 10).

Both surface-associated and intracellular fibronectins were visualized in fibroblasts fixed with formaldehyde and acetone (Figs. 11-14). In all meta-, ana-, and telophase cells studied, a fluorescence-negative area corresponding to the condensed chromosomes was seen (Figs. 11-13). In addition, fluorescent strands connecting the dividing cells to the glass and neighboring cells were observed (Figs. 11 and 12). In flattening G1 cells, an intracellular perinuclear fluorescence was conspicuous (Fig. 14) but frequently less intensive than in interphase cells in general (cf. Figs. 13 and 4). In the periphery of spreading cells, weak fibronectin strands were also seen (Figs. 13 and 14).

In all preparations, some mitotic cells were surrounded by a zone of fibrillar fluorescence on the level of the glass surface (Fig. 11). In general, these extracellular areas did not contain material visible in phase-contrast microscopy.

In addition to the above experiments on human fibroblasts, studies were carried out on cultures of chick fibroblasts. The data (not shown) confirmed the findings made on human cells; the presence of intracellular fibronectin throughout the cell cycle of surface-associated fibronectin in all interphase cells and its reduction during mitosis.

Visualization of Fibronectin in Scanning Electron Microscopy

Chicken fibroblast cultures fixed briefly with glutaraldehyde (0.5%, 10 min) were incubated with anti-fibronectin IgG-Latex beads. In scanning electron microscopy, Latex beads were seen attached to interphase cells (Figs. 15, 17, and 19). Beads were found located both on the cell body and on cytoplasmic extensions. The beads were distributed partly in a random fashion or aligned on cell surface ridges or strands (Figs. 15 and 17). The cells also had surface microspikes with blebs that differed from the beads by their uneven shape and larger size (Fig. 17). Pretreatment of the cultures with free antifibronectin IgG blocked the binding of the beads (Fig. 16).

Cells in different phases of division (metaphase, Fig. 15; early and late telophase, Figs. 18 and 19) had only few beads on the surface of the cell body. In metaphase cells, the surface was covered with numerous microvilli which may have obscured possibly attached beads. Beads were often located on thin and thick cytoplasmic extensions of the dividing cells (Figs. 18 and 19). In late telophase cells, beads were again seen in larger numbers on the surface of the flattening cell body (Fig. 19).

Latex beads were detected also in association with extracellular fibrillar material particularly surrounding dividing cells (Fig. 19). This localization corresponds with the extracellular immunofluorescence detected at the level of the growth substratum (Fig. 11).

DISCUSSION

These experiments demonstrate that in fibroblast cultures fibronectin is present in three localizations in agreement with previous findings (32, 33): (a) in all cells intracellularly, (b) in association with the cell surface, and (c) in extracellular fibrillar structures on the growth substratum. A fourth in vitro localization of fibronectin, not studied here, is the extracellular fibronectin released from cultured fibroblast in large quantities into the medium (22, 29, 32).

The present studies were designed to evaluate the expression of intracellular and surface-associated fibronectins in different phases of the cell cycle including mitosis. In interphase cells, a clear correlation between the amounts of intracellular and surface-located fibronectins was seen (Table I). Furthermore, in individual cells the expression

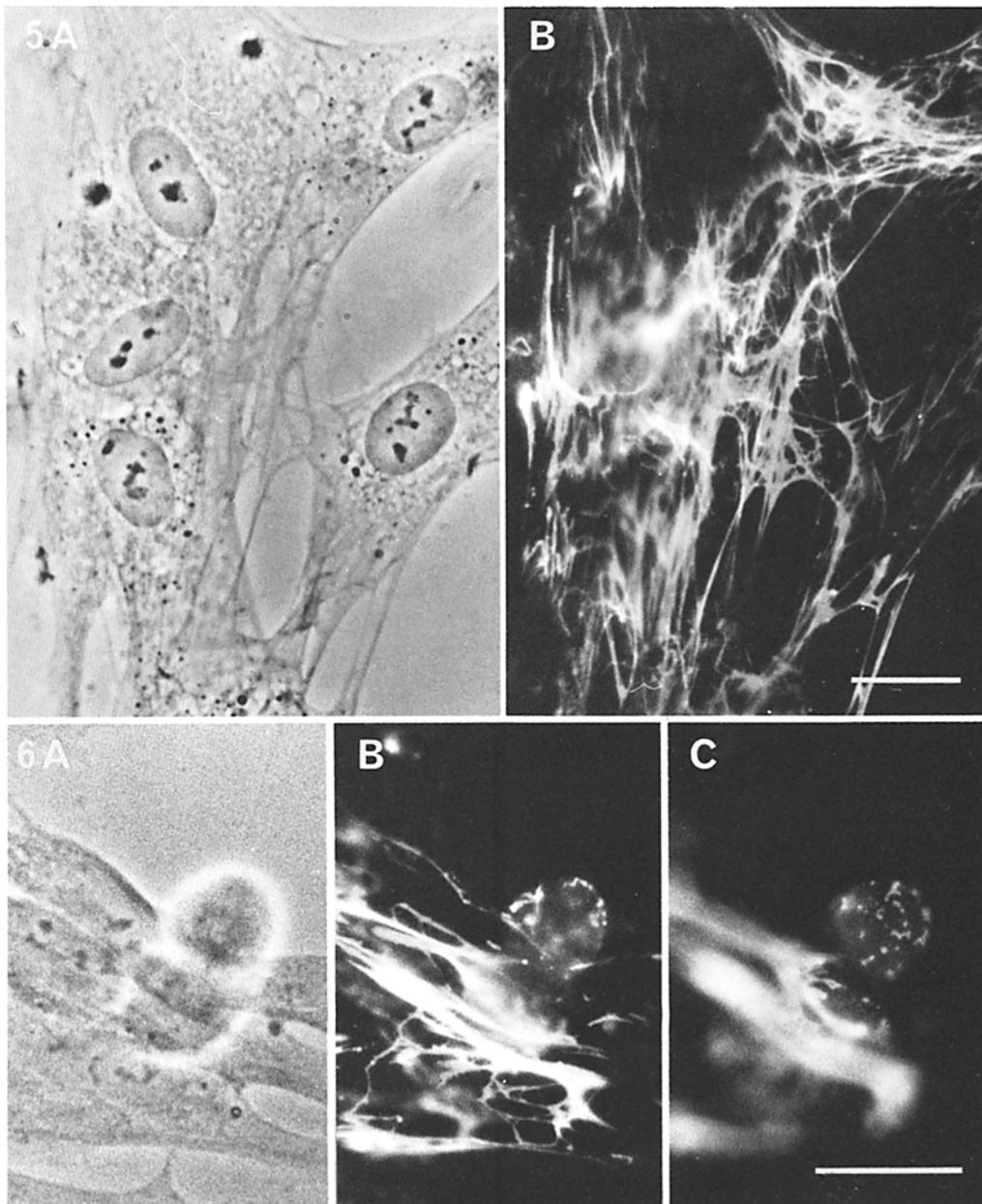
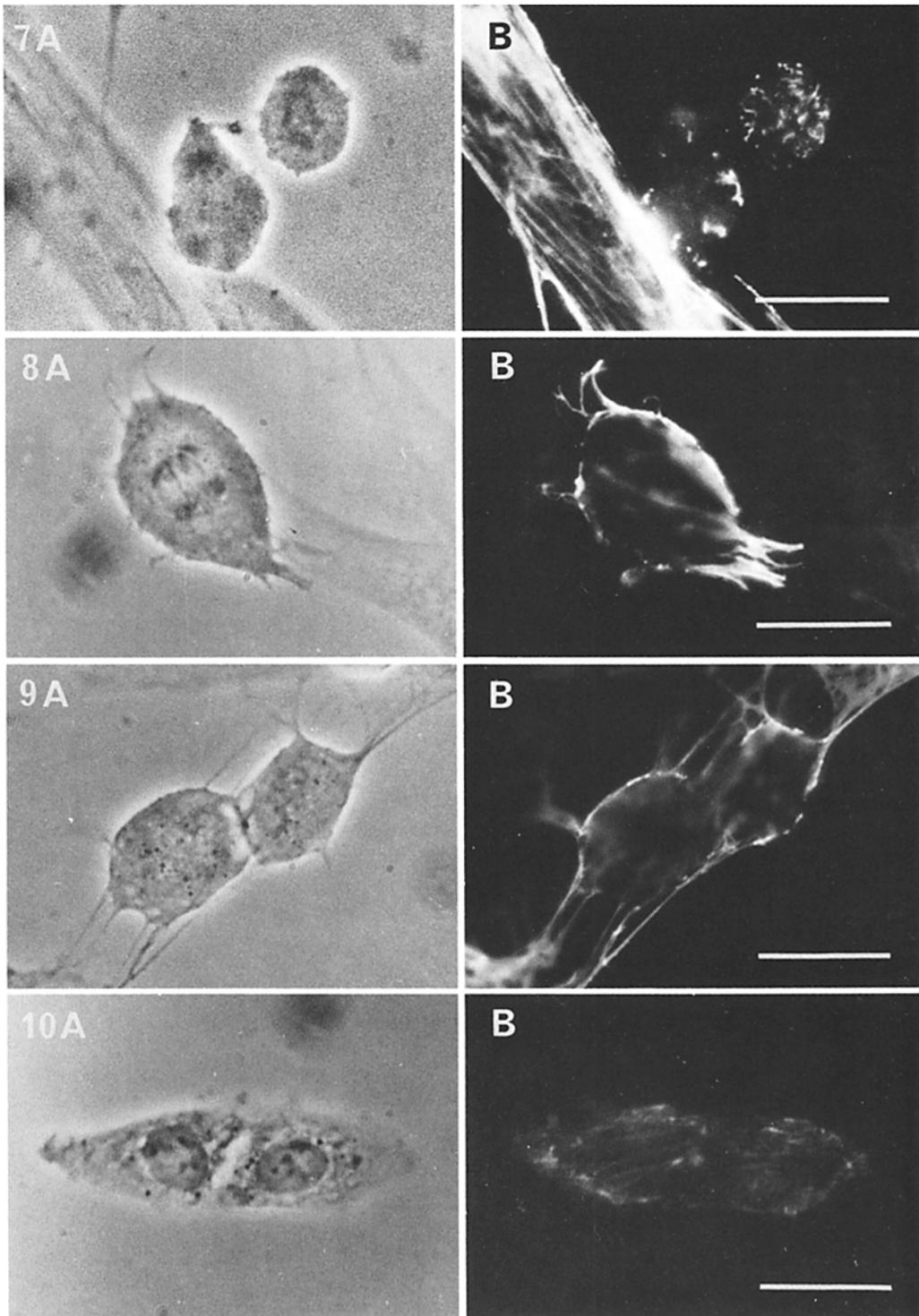


FIGURE 5 Phase-contrast (A) and immunofluorescence (B) photographs of human fibroblasts in monolayer. Staining of human fibronectin in cells fixed in formaldehyde only, to visualize surface-associated fibronectin which has a fibrillar distribution. Nuclear contours are not visible, indicating that no intracellular fibronectin is stained (cf. Fig. 1). $\times 800$. Bar, $20 \mu\text{m}$.

FIGURE 6 Phase-contrast (A) and immunofluorescence (B and C) photographs of a human fibroblast in telophase and neighboring interphase cells. The culture was fixed with formaldehyde, and only surface-associated fibronectin is visualized. One of the daughter cells lies on top of an interphase cell and is connected to the network of surface-associated fibronectin of this cell. The other daughter cell has only the comma-like surface fluorescence, which is typical for singly lying mitotic cells. Figs. 6 A and B are focused to the same level close to the growth substratum. Fig. 6 C is focused at a level about $4 \mu\text{m}$ higher. The figures show the distribution of the commas on the cell periphery, and the location of the strong fibrillar fluorescence close to the surface of the growth substratum. $\times 480$. Bar $20 \mu\text{m}$.



of fibronectin, either intracellular or surface-associated, was independent of DNA synthesis (Table II). This is in contrast to the results obtained with radioactive labeling of surface-exposed fibronectin-like proteins in synchronized populations. Hynes and Bye (7) and Hunt et al. (5) reported a decrease of surface-labeling as cells move from G1 to S phase. Surface fibronectin-like proteins were therefore suggested to play a direct role in the regulation of cell growth (5). We could not make such an association. Our results are in agreement with the observation that protease- or insulin-evoked stimulation of cell proliferation is not necessarily coupled to extensive removal of fibronectin-like proteins from the cell surface during the first hours after release from density-dependent inhibition of growth (10, 27).

There are several possible explanations for the discrepancy between our observations and the previous studies in which radiolabeling was used. Synchronization procedures, such as double thymidine block (5) or hydroxyurea (7), are known to lead to unbalanced protein synthesis unlinked to DNA metabolism (12, 26). This is in line with our unpublished observations on the use of double thymidine block to synchronize human fibroblasts which led to aberrant increase in cell size and fibronectin content in all phases of the cell cycle. Hunt et al. (5) used suspension cultures, and in the other study (7) the cells were grown for several days before analysis. After long-term cultivation a large proportion of external fibronectin is not directly associated with cells, but is part of an extracellular matrix network visualized by immunofluorescence staining (13, 33). In the present experiments in which 24-h-old cell cultures were used,

the influence of extracellular fibronectin was minimized. It should be pointed out that fibronectin detected by surface radiolabeling and surface immunofluorescence may not be identically distributed.

The release of surface-associated fibronectin has been detected in transformed, in protease-treated (2, 4, 6, 10, 28, 29, 32, 33) and in cytochalasin B-treated cells,² and now a decrease in the amount of surface-associated fibronectin has been noted on mitotic cells. All these conditions are characterized by a rounding up of cells and by a disorganization of plasma membrane-associated microfilament bundles (18, 19, 24, 25). The findings suggest that there are complex interactions between surface proteases, microfilaments, and external fibronectin.

The observation that mitotic cells in general have less surface fibronectin than interphase cells is in agreement with the data of Hynes and Bye (7), who used radiolabeling techniques. The present results show, however, that mitotic cells contain fibronectin both intracellularly and on the surface particularly on cytoplasmic extensions (Figs. 7-14 and 18). One has to consider also the possibility that the fluorescence seen as comma-like structures can result from optical superimposition of membrane areas of even but low fluorescence intensity. This cannot be completely ruled out, but, by using oil immersion objectives with high numerical apertures (1.3 or 1.4) to give a

² Kurkinen, M., J. Wartiovaara, and A. Vaheri. 1976. Cytochalasin B releases major surface-associated glycoprotein, fibronectin, from cultured fibroblasts. Manuscript submitted for publication.

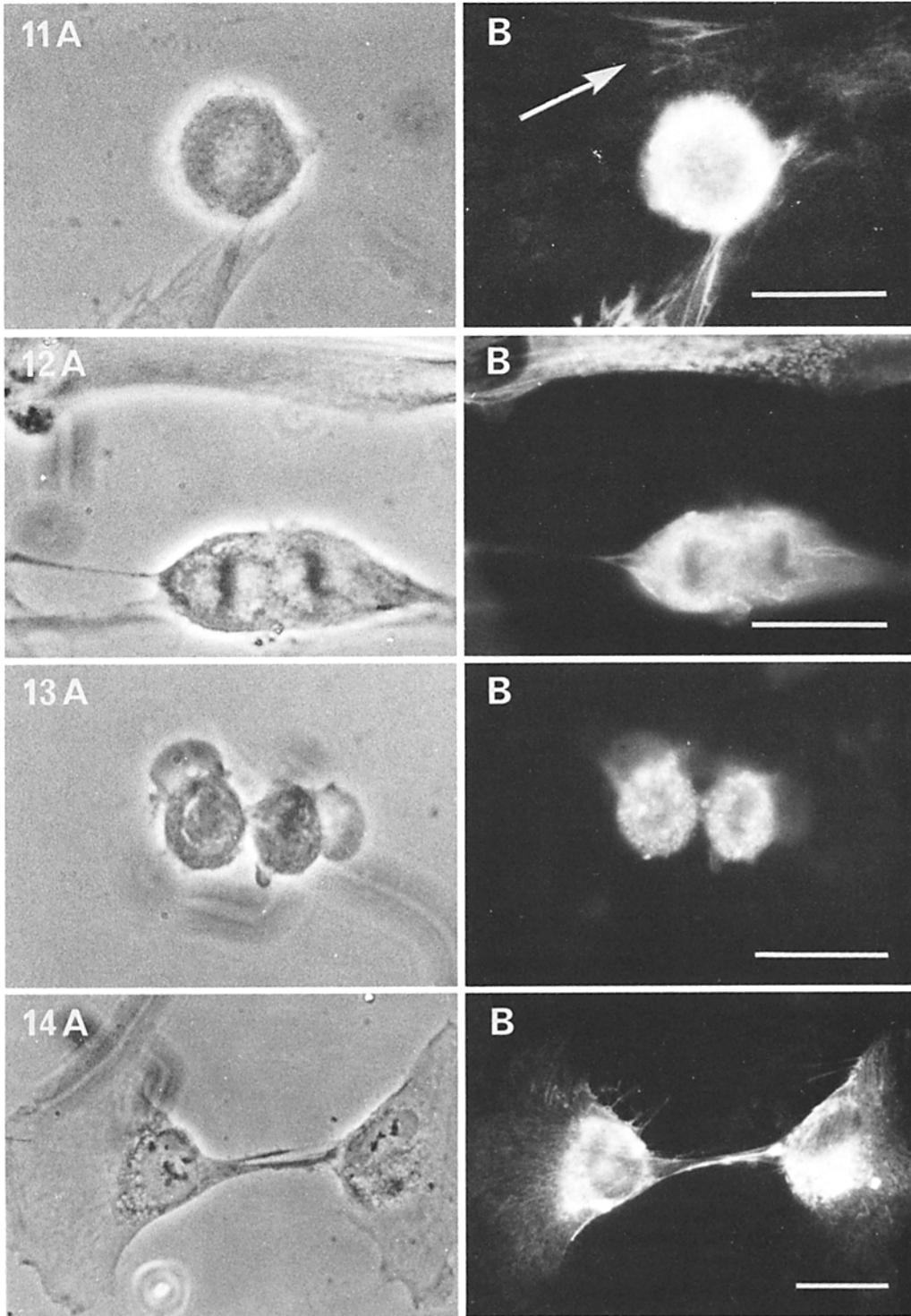
FIGURES 7-10 Phase-contrast (A) and immunofluorescence (B) photographs of dividing human fibroblasts. Staining of human fibronectin in cells fixed in formaldehyde only, to visualize surface-associated but not intracellular fibronectin. Surface fluorescence is seen throughout mitosis, mainly around cell border and in cytoplasmic strands. $\times 990$. Bar, 20 μm .

FIGURE 7 Comma-like fluorescence on two metaphase cells and bright fibrillar fluorescence on neighboring interphase cells.

FIGURE 8 Fluorescence on strands connecting anaphase cell to the neighboring interphase cells on the right and to the growth substratum.

FIGURE 9 Fluorescence on strands connecting two telophase cells to each other and to neighboring interphase cells.

FIGURE 10 Faint fibrillar surface-associated fluorescence on pair of early G1 cells, spreading out after cell division.



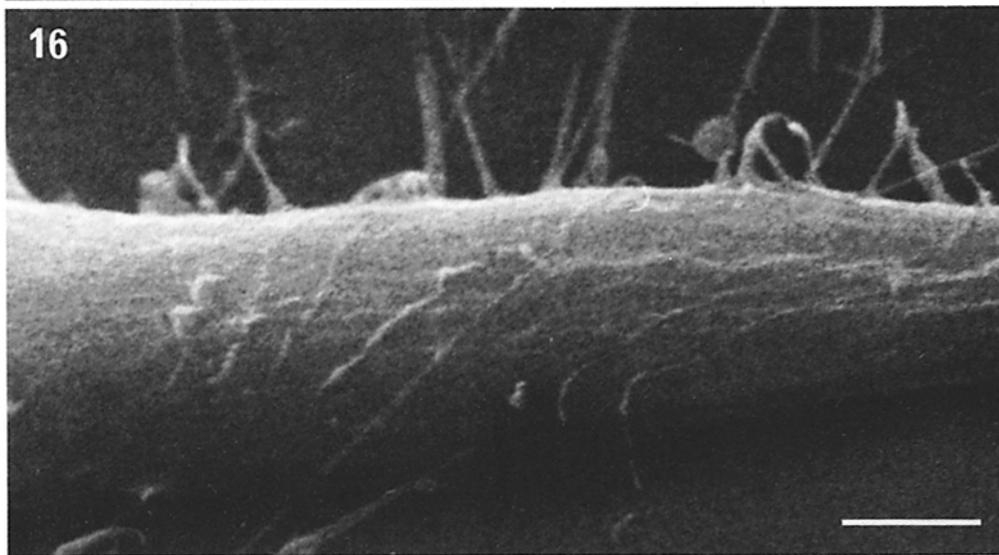
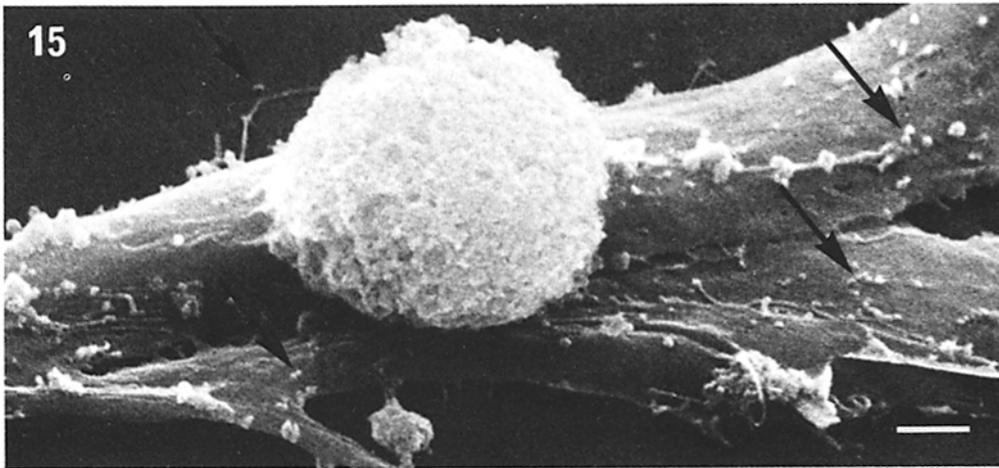


FIGURE 15 Scanning electron micrograph of a rounded chicken fibroblast in metaphase on top of a flat interphase cell. Antichicken fibronectin IgG-coated Latex beads (arrows) are discernible on the surface of the cell body and on cytoplasmic extensions of the interphase cell. Some of the beads are found on cell surface ridges. The surface of the mitotic cell is covered with numerous microvilli. $\times 4,800$. Bar, $2 \mu\text{m}$.

FIGURE 16 Control of IgG-Latex labeling. Typical interface cell in chicken fibroblast culture pretreated, after fixation, with free antifibronectin before incubation with IgG-Latex beads. The cell has surface ridges and fibrillary strands but lacks attached IgG-Latex beads. $\times 9,000$. Bar, $2 \mu\text{m}$.

FIGURES 11-14 Phase-contrast (A) and immunofluorescence (B) photographs of dividing human fibroblasts. Staining of human fibronectin in cells fixed in formaldehyde and acetone to visualize both intracellular and surface-associated fibronectins. In addition to surface-associated fibronectin intracellular fibronectin also is visible in all phases of mitosis. Figs. 11-13, $\times 990$; Fig. 14, $\times 670$. Bar, $20 \mu\text{m}$.

FIGURE 11 Strong intracellular fluorescence of metaphase cell and strands connecting it to the neighboring interphase cell. Faint extracellular fluorescence on the growth substratum (arrow) in material partly invisible in phase contrast.

FIGURE 12 Fluorescence of an anaphase cell and a neighboring interphase cell. The fluorescence is weaker in the chromosome region.

FIGURE 13 Telophase cells starting to spread out on the substratum. The flattening areas show weak fluorescence.

FIGURE 14 Pair of early G1 cells spreading out after cell division. Perinuclear intracellular fluorescence and weak fibrillary fluorescence of peripheral cytoplasmic regions.

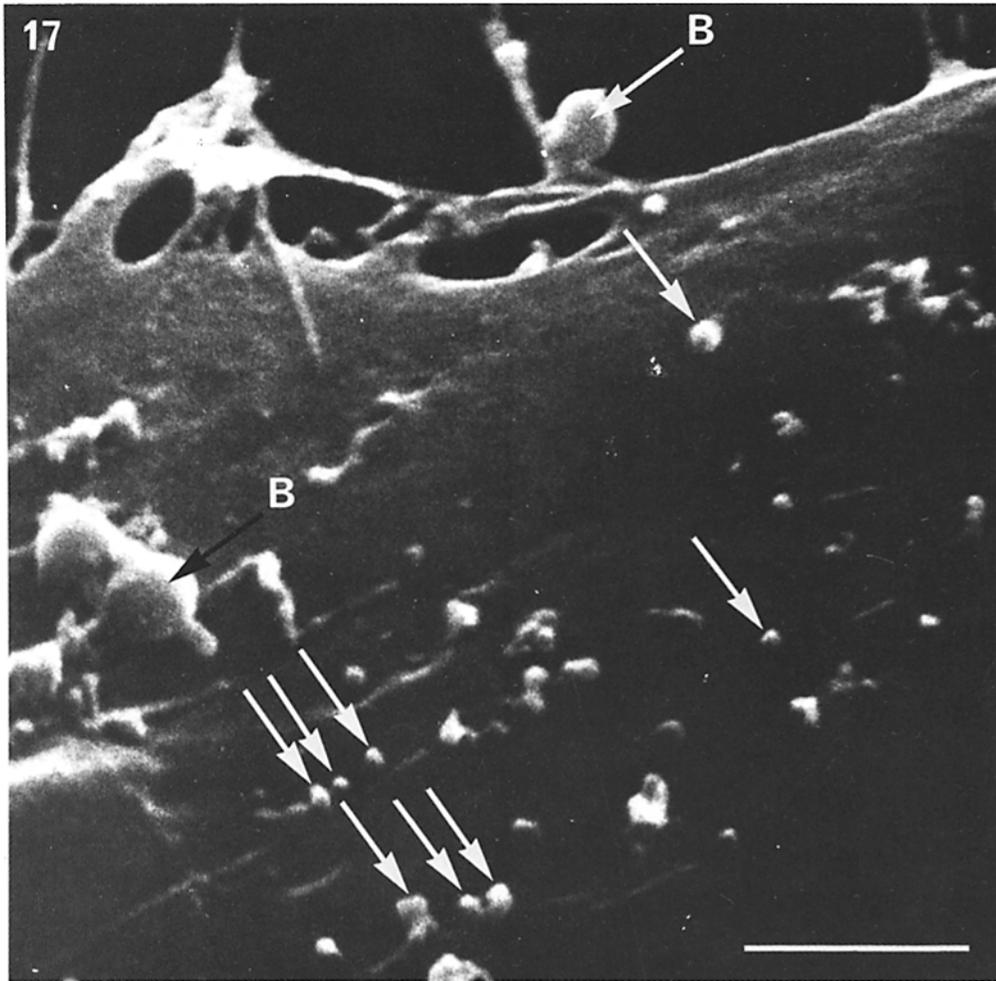


FIGURE 17 High magnification of chicken fibroblast in interphase. Numerous IgG-Latex beads (arrows) distributed either randomly or in rows on cell surface ridges and strands. Larger-sized blebs (*B*) are seen on a number of surface microspikes. $\times 15,000$. Bar, $2 \mu\text{m}$.

shallow depth of field, we could not detect any decrease in the intensity of these fluorescing images relative to the surrounding nonfluorescing cell surface. This makes it likely that the intense comma-like fluorescence in fact corresponds to locally increased amounts of fibronectin.

The present findings on the distribution of external fibronectin are consistent with the proposal by Yamada et al. (35) that this protein may function in cell adhesion. Through interactions with other fibronectin molecules (8, 9, 16), with fibrin (16, 21) and possibly with collagen (17), fibronectin may in vivo have an important adhesive role as a component of the connective tissue matrix, nota-

bly of basement membranes in which it is characteristically found (13, 34).

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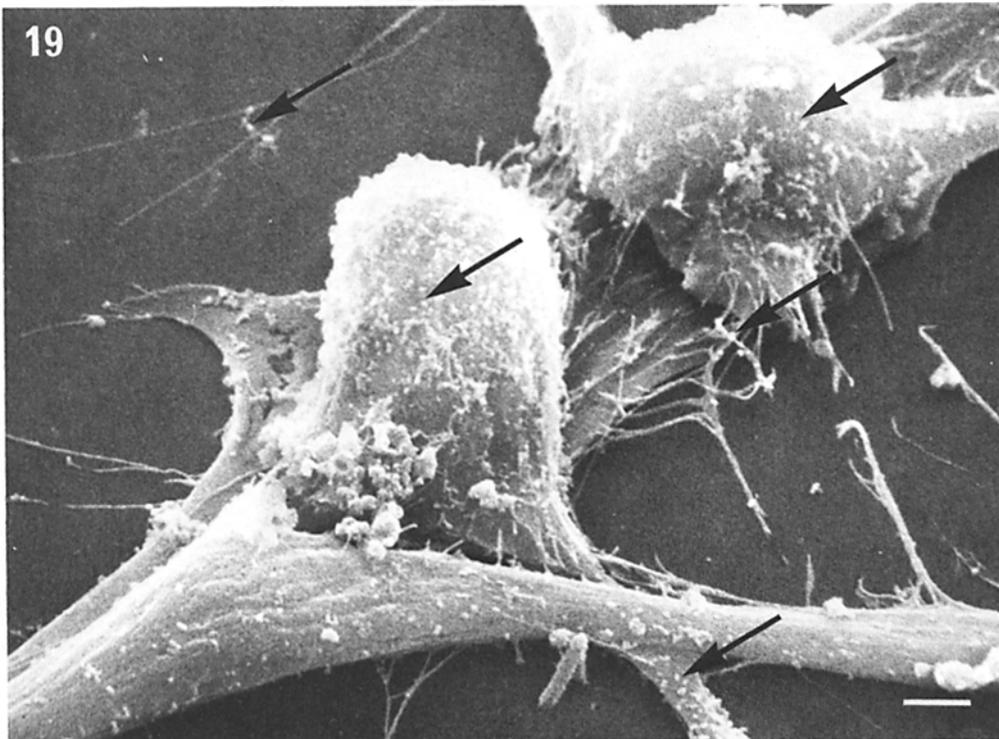
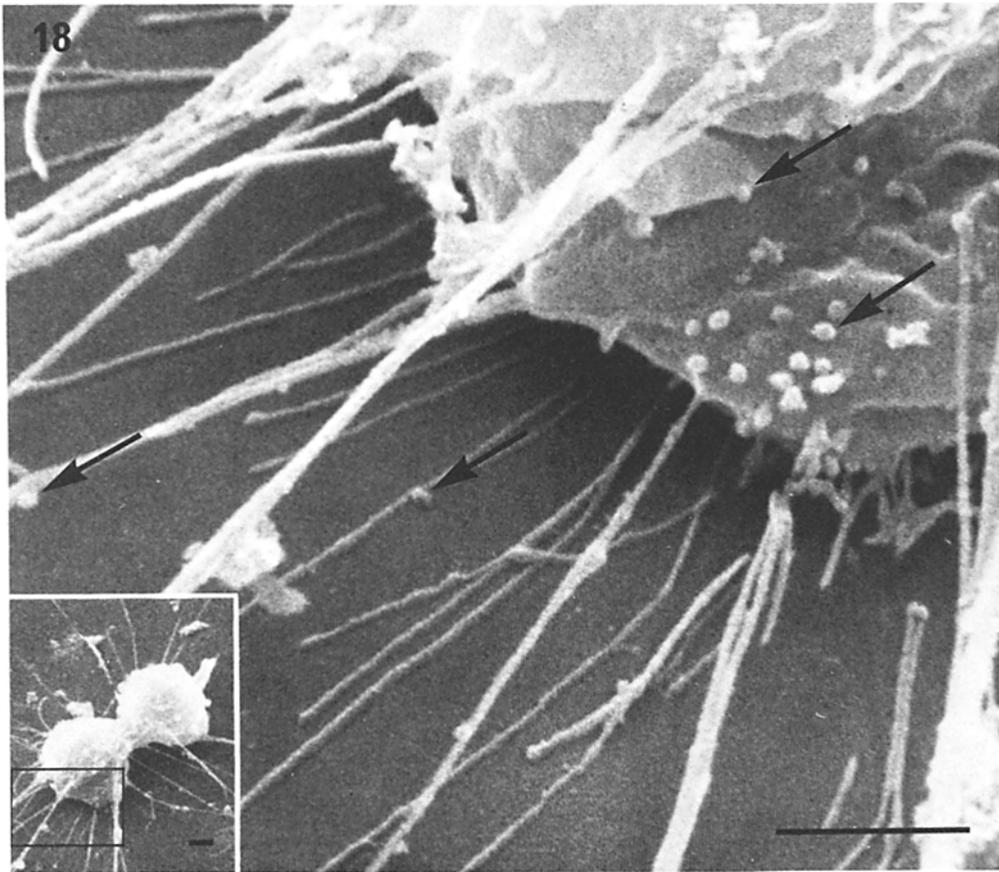


FIGURE 18 *Inset*: Chicken fibroblast in early telophase. $\times 1,500$. Bar, $2 \mu\text{m}$. *Blow up*: IgG-Latex beads (arrows) are seen on surface of cell body and on thin and thick cytoplasmic extensions $\times 13,000$. Bar, $2 \mu\text{m}$.

FIGURE 19 Two flattening chicken fibroblasts in late telophase—early G1 cells have numerous attached IgG-Latex beads (arrows) which can similarly be seen on flat interphase cells. Fibrillar extracellular material with attached beads probably represents broken-off cell extensions. $\times 4,500$. Bar, $2 \mu\text{m}$.

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