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ASSEMBLY AND ALIGNMENT OF FIBRONECTIN-COATED GOLD BEADS
INTO FIBRILS BY HUMAN SKIN FIBROBLASTS

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Abstract

The assembly of fibronectin into fibrils was monitored by high voltage electron microscopy using 18 nm colloidal gold beads bound to fibronectin (Au₁₈-fibronectin) or an amino terminal 70 kd fragment of fibronectin (Au₁₈-70 kd) that blocks the incorporation of fibronectin into disulfide bonded fibrils. Subconfluent cultures of human skin fibroblasts were incubated with the colloidal gold complexes for 0.25, 0.5, 1.5 and 5 h. In fibroblast cultures incubated with Au₁₈-fibronectin and Au₁₈-70 kd fragments for 0.25 and 0.5 h, the complexes of Au₁₈-fibronectin and Au₁₈-70 kd fragment were observed bound to the cell surface in clusters near bundles of intracellular microfilaments, usually along the lateral edges of the fibroblasts. Fibroblast cultures incubated with Au₁₈-fibronectin and Au₁₈-70 kd fragment for 1.5 and 5 h showed a more linear arrangement of the Au₁₈-fibronectin and Au₁₈-70 kd fragment along the lateral and retracting edges and on filopodia. This alignment of Au₁₈-fibronectin into linear arrangements on the fibroblast cell surface suggests that the assembly of fibronectin fibrils occurs on specific regions of the fibroblast.

KEY WORDS: Fibronectin, fibroblast, fibrils, colloidal gold, extracellular matrix, high voltage electron microscopy, critical point dried, filopodia, intracellular microfilaments

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Introduction

Fibronectin is a 500,000 dalton glycoprotein which is found as a soluble protein in plasma, cerebrospinal fluid, and amniotic fluid, and as an insoluble protein in the extracellular matrix of epithelial cells, connective tissue and a variety of cultured cells (for recent reviews see Mosher, 1984; Hynes, 1985). As part of the extracellular matrix, fibronectin plays an important role in cellular proliferation, differentiation, and organization. Fibronectin found in the extracellular matrix can come from either cellular fibronectin or from plasma fibronectin (Hayman and Ruoslahti, 1979; Oh et al., 1981). Both types of fibronectin are similar except that cellular fibronectin contains an additional Type III homology sequence (Kornblihtt et al., 1984).

It is of considerable interest to know how fibronectin is assembled into fibrils and incorporated into extracellular matrix. One possibility is that fibronectin associates with itself and other matrix constituents to form fibrils. Insoluble rod-like complexes of fibronectin can be formed in the absence of cells by precipitating the fibronectin with heparin (Jilek and Hörmann, 1979) or polyamines (Vuento et al., 1980). A second possibility is that fibronectin is incorporated into the extracellular matrix by the direct apposition of membrane associated fibronectin (Hedman et al., 1978). In this case the incorporation of fibronectin into fibrils can be mediated by the extracellular matrix which acts as a template for further matrix assembly or by a cell surface receptor which directs the assembly of fibronectin into fibrils. Recently, evidence for such a cell surface receptor has been obtained (McKeown-Longo and Mosher, 1983, 1985). The presumptive cell surface receptor appears to interact with disulfide-bonded Type I sequences especially those located in the 70 kd gelatin and heparin binding region of fibronectin (McKeown-Longo and Mosher, 1985) rather than the more extensively studied cell adhesion site of fibronectin.

Immunofluorescence studies of young cultures of NIL8 cells suggest that fibronectin fibrillogenesis starts on the cell surface (Hynes and Destree, 1978). In these cultures, short striae of fibronectin that co-aligned with actin bundles

were observed in subconfluent cultures by double immunofluorescence. As a more extensive fibrillar fibronectin network formed, the co-alignment between actin and fibronectin became less obvious. Similar short striae were observed in confluent cultures of human skin fibroblasts incubated with fluorescein-fibronectin for 20 minutes (McKeown-Longo and Mosher, 1983). These short striae of fluorescein-fibronectin were thought to represent soluble fibronectin bound to the cell layer.

In the present study, we used 18 nm colloidal gold beads (Au₁₈) bound to fibronectin (Au₁₈-fibronectin) or the amino terminal 70 kd fragment of fibronectin (Au₁₈-70 kd) to identify cell surface binding sites and cellular processes associated with fibronectin fibrillogenesis. The Au₁₈-fibronectin or Au₁₈-70 kd fragment was incubated with subconfluent cultures of human skin fibroblasts and the binding sites of Au₁₈-fibronectin and Au₁₈-70 kd complexes over time were examined by high voltage electron microscopy (HVEM).

Materials and Methods

Cell cultures and Au₁₈-fibronectin labeling conditions

Embryonic human skin fibroblasts (S132; obtained from Dr. Michael Gould, University of Wisconsin) and neonatal human skin fibroblasts (A1-F; obtained from Dr. Lynn Allen-Hoffman, University of Wisconsin) were grown on sterile formvar-coated coverslips (Wolosewick and Porter, 1976) in Ham's F-12 media supplemented with 10% fetal calf serum and 15% fetal calf serum, respectively. After 3-4 days, cells were washed three times with Hank's balanced salt solution and placed in fresh media containing 0.2% bovine albumin and either 7 µg/ml of Au₁₈-fibronectin or 13 µg/ml Au₁₈-70 kd fragment for 0.25, 0.5, 1.5 or 5 h at 37°C. The labeled fibroblasts were washed three times with Hank's balanced salt solution and prepared for high voltage electron microscopy (HVEM).

Preparation of colloidal gold fibronectin and 70 kd complexes

Colloidal gold beads 18 nm in diameter (Au₁₈) were prepared by reducing 4% gold chloride with 1% tri-sodium citrate according to the procedure of Geoghegan and Ackerman (1977). The colloidal gold sol was filtered through a 0.45 µm millipore filter and stored at 4°C.

Human plasma fibronectin was purified using the procedure of Mosher and Johnson (1983). The 70 kd amino terminal fragment of fibronectin was prepared by digesting plasma fibronectin with cathepsin D (Balian et al., 1979) and placing the digest on a gelatin-sepharose column followed by gel filtration on a Sephadex G-100 column (Pesciotta Peters and Mosher, 1987).

Au₁₈-fibronectin was prepared by incubating 5-10 µg plasma fibronectin in 2mM Tris-HCL, pH 7.4, 15 mM sodium chloride, with 1 ml of 18 nm gold sol pH 7.0 for 5 minutes at 23°C (Geoghegan and Ackerman, 1977; Horisberger and Rosset, 1977). Unbound areas on the gold beads were blocked with 1% polyethyleneglycol (final concentration 0.05%) filtered through a 0.2 µm

filter. The Au₁₈-fibronectin was centrifuged at 28,000 g for 30 minutes at 4°C. The Au₁₈-fibronectin complex was resuspended into sterile 0.1 M Hepes, pH 7.0 (final Optical Density at 520nm ~ 3.6) and stored at 4°C. Au₁₈-70 kd complexes were prepared in a similar manner except that the pH of the gold sol was 7.2.

High voltage electron microscopy

Fibroblasts were fixed with 2.5% glutaraldehyde in 0.1M Hepes, pH 7.0, containing 0.05% saponin and 0.2% tannic acid (Maupin and Pollard, 1983), washed for 1 h (4 changes) with 0.1 M Hepes, pH 7.0 and 10 minutes (2 changes) with distilled water. Cells were stained for 10 minutes with 1% uranyl acetate in water, dehydrated in a graded series of ethanol (20%-100%), critical point dried with a Samdri PVT3 and examined with the AEI.EM7 high voltage electron microscope at Madison, WI (Ris, 1985).

Results

Localization of exogenous Au₁₈-fibronectin binding sites

To identify the binding sites of fibronectin involved in fibronectin fibrillogenesis, fibronectin or the 70 kd amino terminal fragment of fibronectin coupled to 18 nm colloidal gold beads were incubated with subconfluent cultures of fibroblasts which were in the process of assembling a fibronectin matrix. Subconfluent cultures of fibroblasts were used so that cell surface interactions could be better visualized and that possible low affinity interactions with preformed extracellular matrix could be reduced (Pesciotta Peters and Mosher, 1987).

Figures 1A and 1B are micrographs of whole mounts of human skin fibroblasts that had been incubated with Au₁₈-fibronectin for 15 minutes. Approximately 63% of the fibroblasts show a clustering arrangement of the Au₁₈-fibronectin on the cell surface similar to the one seen in these micrographs. The clusters of Au₁₈-fibronectin were found along the lateral edges of the cell (Figure 1A) on both the ventral surface (Figure 1B, arrowheads) facing the substratum and on the dorsal surface (Figure 1B, arrows). Some of the clusters of Au₁₈-fibronectin co-align with bundles of intracellular microfilaments (Figure 1B). Binding of Au₁₈-fibronectin on a cell surface could be competitively inhibited with an excess of unlabeled fibronectin (700 µg/ml) indicating that Au₁₈-fibronectin was specifically bound to the cell surface (Figure 2). As an additional control, fibroblasts were incubated with 18 nm gold beads bound to albumin for 15 minutes. In those experiments, only an occasional gold bead was observed on the cell surface and no gold beads were found along actin bundles (Pesciotta Peters and Mosher, 1987).

During longer incubations with Au₁₈-fibronectin (1.5 h), the exogenous Au₁₈-fibronectin bound to the cell surface took on a more linear fibril-like arrangement (Figure 3A,B). The linear arrangements of Au₁₈-fibronectin showed an extensive co-alignment with intracellular bundles of microfilaments along the lateral edges of the fibroblast (Figure 3A; large arrow). In addition, linear arrangements of Au₁₈-fibronectin

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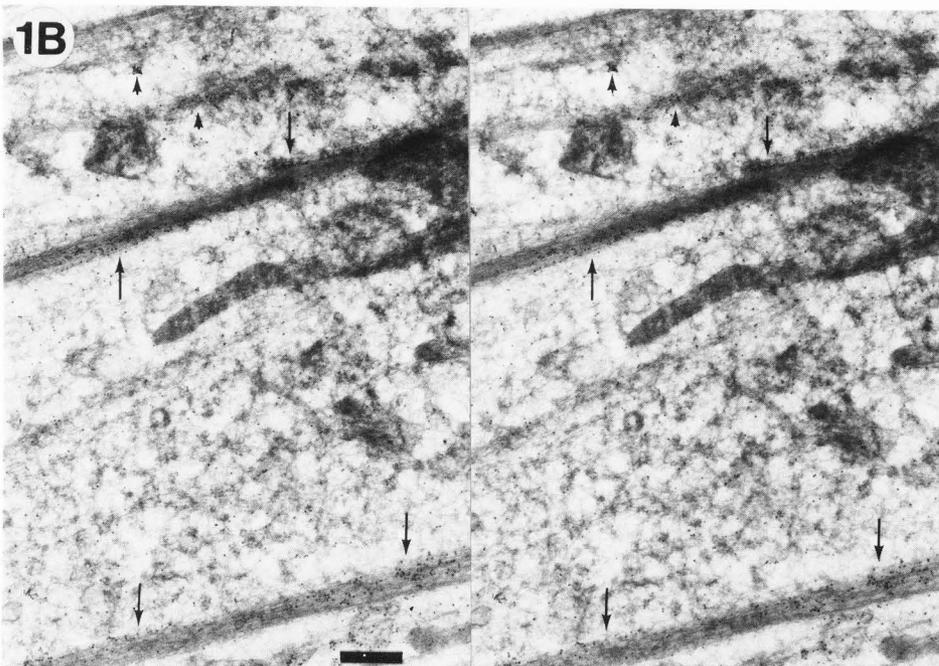
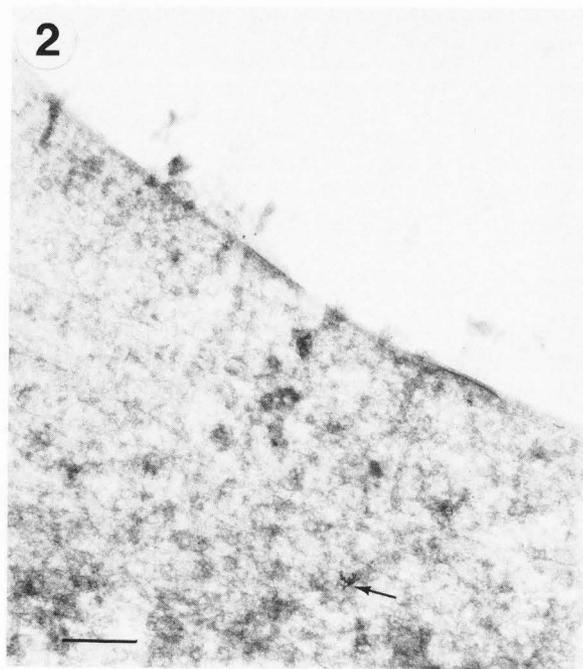
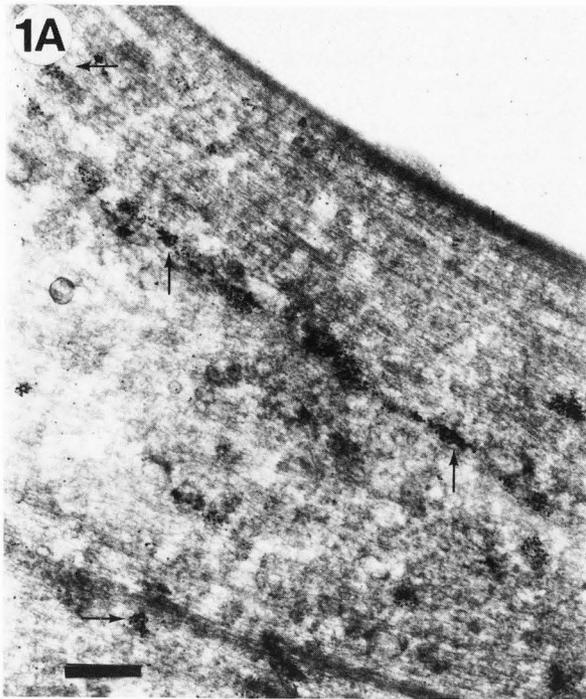


Fig. 1: A whole mount of an embryonic human skin fibroblast (S132) incubated with Au_{18} -fibronectin for 15 min at 37 C. Fig. 1A: Clusters of Au_{18} -fibronectin are observed along the lateral edges of the fibroblast (arrows). Fig. 1B: A stereo micrograph. The Au_{18} -fibronectin on the dorsal (arrows) and ventral surface of the fibroblast (arrowheads) co-aligns with bundles of intracellular microfilaments. Bar = 0.5 μ m.

Fig. 2: A whole mount of an A1-F skin fibroblast incubated with Au_{18} -fibronectin and a 100-fold excess of plasma fibronectin for 30 minutes at 37 C. Arrow shows cluster of Au_{18} -fibronectin on the dorsal surface. Bar = 0.5 μ m.

were observed at the retracting end of the fibroblast (figure not shown). In some instances, the Au_{18} -fibronectin was seen incorporated into fibrils (Figure 3B; small arrow) on the cell surface and in the extracellular space (Figure 3B; large arrow). Both the linear arrangements and the fibrils of Au_{18} -fibronectin occurred predominantly on the dorsal surface of the fibroblast. Morphometric analysis showed that the

area of the cell covered by these linear arrangements of Au_{18} -fibronectin increased with incubation time whereas the cluster arrangement remained constant (Pesciotta Peters and Mosher, 1987)

Extensive linear arrangements of Au_{18} -fibronectin were also observed on short, thin cytoplasmic extensions that appeared to be filopodia. These linear arrangements were found primarily in

fibroblast cultures that had been incubated with Au₁₈-fibronectin for 1.5 h (Figure 3A, small arrow). Some of these filopodia were observed overlapping a neighboring fibroblast (Figure 3A) while others were observed to be intermeshed with fibrils in the extracellular space (Figure 3B; large arrow). The localization of Au₁₈-fibronectin to filopodia was even more evident in cultures that had been incubated with Au₁₈-fibronectin for 15 minutes, washed and then incubated for an additional 1 h at 37°C in the absence of Au₁₈-fibronectin. In these instances, the majority of the Au₁₈-fibronectin was observed bound to filopodia (figures not shown).

Localization of binding sites for Au₁₈-70 kd fragment

The amino terminal 70 kd fragment of fibronectin coupled to 18 nm colloidal gold beads was added to subconfluent cultures of A1-F fibroblasts for 30 minutes and 5 h in order to distinguish between sites on the cell surface involved in matrix formation (McKeown-Longo and Mosher, 1983; 1985) and sites involved in cell adhesion (Pytela et al., 1985; Akiyama et al., 1986).

An A1-F fibroblast labeled with Au₁₈-70 kd for 30 minutes is shown in Figure 4. In contrast to Au₁₈-fibronectin, complexes of Au₁₈-70 kd were usually found clustered on the dorsal surface along the lateral edges of the fibroblast. However, as in the case with Au₁₈-fibroblast longer incubations with the Au₁₈-70 kd complexes resulted in the formation of extensive linear arrangements along the lateral edges of the fibroblast (Pesciotta Peters and Mosher, 1987) and near the retracting end of the fibroblast (Figure 5).

Discussion

In this study, we used 18 nm colloidal gold beads coupled to fibronectin, or the amino terminal 70 kd fragment of fibronectin to study the cell surface events involved in the assembly of fibronectin fibrils. We found that exogenously added Au₁₈-fibronectin and the Au₁₈-70 kd fragment of fibronectin bound to the cell surface in small clusters along specific regions of the fibroblast surface. More specifically, we observed that Au₁₈-fibronectin bound to both the ventral and dorsal surfaces of the cell along the lateral edges of the fibroblast. In contrast to this, Au₁₈-70 kd fragments were found predominantly clustered on only the dorsal surface of the lateral edges of the fibroblast. These clusters of Au₁₈-fibronectin and Au₁₈-70 kd are reminiscent of the short striae of fibronectin observed by immuno-electron microscopy (Hedman et al., 1978), immunofluorescence microscopy (Hynes and Destree, 1978) and fluorescence microscopy studies (McKeown-Longo and Mosher, 1983). In the earlier two studies, and in this study, a close association with actin bundles was observed.

Longer incubations with Au₁₈-fibronectin or Au₁₈-70 kd fragments show that Au₁₈-fibronectin and Au₁₈-70 kd fragments assume a more linear arrangement with time. The increase in the amount of Au₁₈-fibronectin and Au₁₈-70 kd fragments seen in this arrangement suggests that

Au₁₈-fibronectin and Au₁₈-70 kd fragments initially binds to the cell surface in clusters, and with time, are arranged into linear fibril-like arrangements. These linear arrangements were observed on only the dorsal surface of the cell along the lateral and retracting edges of the fibroblast and on the filopodia. This suggests that fibronectin fibril formation occurs predominately along these regions of the fibroblast. A similar observation has been made by Birk and Trelstad (1984) where specific regions of the fibroblast surface have been shown to be involved in collagen fibril formation.

The binding of only Au₁₈-fibronectin to the ventral surface of the cell, suggests that Au₁₈-fibronectin may be involved in cell surface interactions other than fibril formation. One such interaction may be with the 140 kd surface glycoprotein shown to be the receptor cell adhesion (Pytela et al., 1985; Akiyama et al., 1986). Further studies will be necessary before any conclusions can be drawn.

The linear fibril-like arrangements of Au₁₈-fibronectin and Au₁₈-70 kd fragment on the cell surface may represent intermediate stages in fibronectin fibrillogenesis where fibronectin molecules bound to the cell surface are being rearranged prior to being assembled into a fibril. The arrangement of fibronectin into fibrils on the cell surface may be mediated by the matrix assembly receptor (McKeown-Longo and Mosher, 1983; 1985) which together with actin filaments align the complexes of Au₁₈-fibronectin and Au₁₈-70 kd fragments along these specific regions of the cell surface. Once assembled into a linear fibril-like arrangement, filopodia or the retracting edges of the fibroblast may translocate these arrangements from the cell surface to the extracellular space where fibronectin can be crosslinked to itself or other matrix constituents to form the extracellular matrix.

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References

- Akiyama SK, Yamada SS, Yamada, KM. (1986). Characterization of a 140-kd avian cell surface antigen as a fibronectin-binding molecule. *J. Cell Biol.* 102, 442-448.
- Balian GE, Click EM, Crouch E, Davidson JM, Bornstein P. (1979). Isolation of a collagen-binding fragment from fibronectin and cold-insoluble globulin. *J. Biol. Chem.* 254, 1429-1432.
- Birk DE, Trelstad RL. (1984) Extracellular Compartments in Matrix Morphogenesis: Collagen Fibril, Bundle, and Lamellar Formation by Corneal Fibroblasts. *J. Cell Biol.* 99, 2024-2033.
- Geoghegan WD, Ackerman A. (1977). Absorption of horseradish peroxidase, ovomucoid, and anti-immunoglobulin to colloidal gold for the indirect detection of Concanavalin A, wheat germ agglutinin

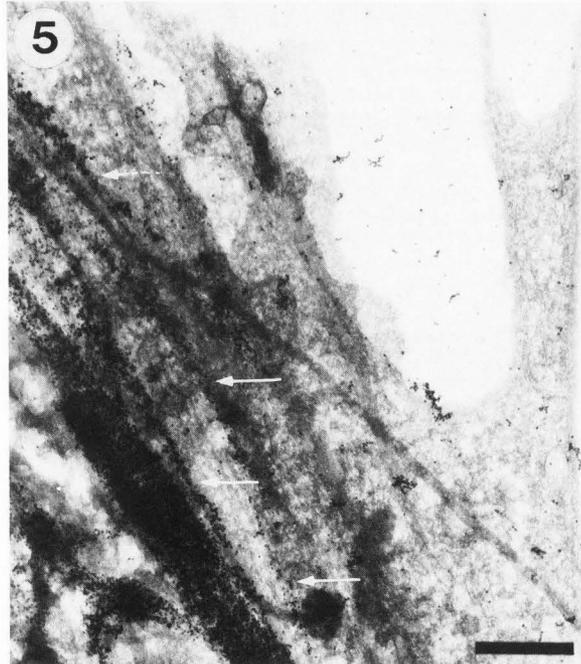
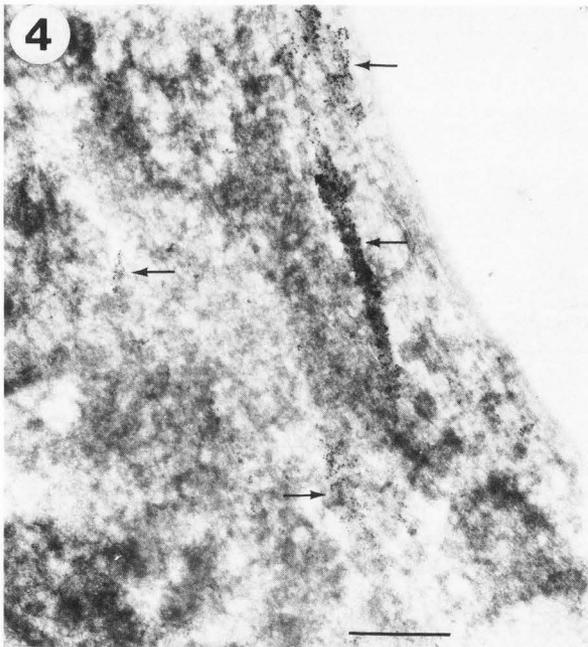
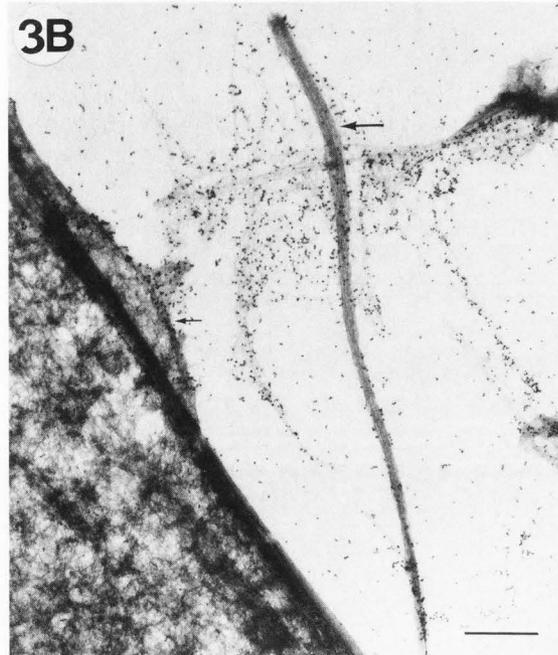
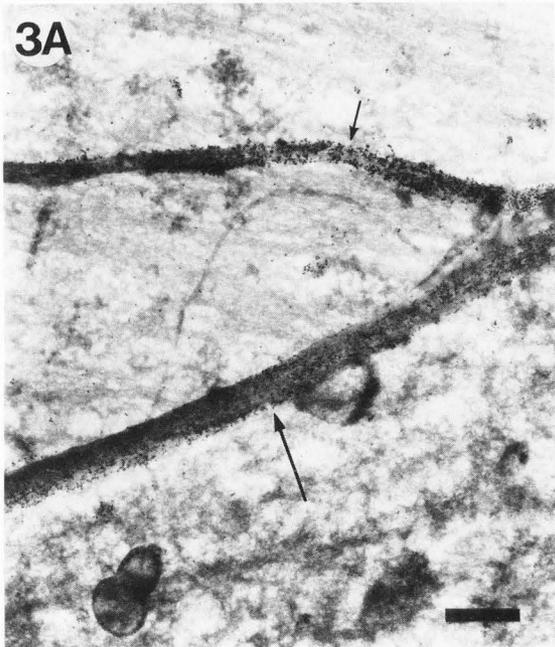


Figure 3A: A micrograph of a S132 human skin fibroblast incubated with Au₁₈-fibronectin for 1.5 h at 37°C. A linear arrangement of Au₁₈-fibronectin is observed above intracellular bundles of micro-filaments along the lateral edge of the cell (large arrow). Au₁₈-fibronectin is also observed connected to a filopodia (small arrow). Bar = 0.5 μm.

Figure 3B: A S132 skin fibroblast incubated with Au₁₈-fibronectin for 1.5 h at 37°C. Au₁₈-fibronectin is arranged in a fibrillar network on the dorsal surface of the cell (small arrow) and in the extracellular space where the network is

connected to a filopodia (large arrow). Bar = 0.5 μm.

Figure 4: An A1-F human skin fibroblast incubated with Au₁₈-70 kd fragments for 30 minutes at 37°C. Clusters of Au₁₈-70 kd fragments are observed on the dorsal surface along the lateral edges of the fibroblast (arrows). Bar = 0.25 μm.

Figure 5: An A1-F skin fibroblast incubated with Au₁₈-70 kd fragments for 5 h. Linear arrangements of Au₁₈-70 kd fragments are observed along the retracting edge of the cell (arrows). Bar = 0.5 μm

and goat anti-human immunoglobulin G on cell surfaces at the electron microscopic level: a new method, theory and application. *J. Histochem. and Cytochem.* 25, 1187-1200.

Hayman EG, Ruoslahti E. (1979). Distribution of fetal bovine serum fibronectin and endogenous rat cell fibronectin in extracellular matrix. *J. Cell Biol.* 83, 255-259.

Hedman K, Vaheri A, Wartiovaara J. (1978). External fibronectin of cultured human fibroblasts is predominantly a matrix protein. *J. Cell Biol.* 76, 748-760.

Horisberger M, Rosset J. (1977). Colloidal gold, a useful marker for transmission and scanning electron microscopy. *J. Histochem. and Cytochem.* 25, 295-305.

Hynes RO, (1985). Molecular biology of fibronectin. *Ann. Rev. Cell Biol.* 1, 67-90.

Hynes RO, Destree AT. (1978). Relationship between fibronectin (LETS protein) and actin. *Cell* 15, 875-886.

Jilek F, Hörmann H. (1979). Fibronectin (cold insoluble globulin) VI. Influence of heparin and hyaluronic acid on the binding of native collagen. *Hoppe-Seyler's Z. Physiol. Chem.* 360, 597-603.

Kornblihtt AR, Vibe-Pederson K, Baralle FE. (1984). Human fibronectin: molecular cloning evidence for two mRNAs species differing by an internal segment coding for a structural domain. *EMBO J.* 3, 221-226.

Maupin P, Pollard TD. (1983). Improved preservation and staining of HeLa cell actin filaments, clathrin-coated membranes, and other cytoplasmic structures by tannic acid-glutaraldehyde-saponin fixation. *J. Cell Biol.* 96, 51-62.

McKeown-Longo PJ, Mosher DF. (1983). Binding of plasma fibronectin to cell layers of human skin fibroblasts. *J. Cell Biol.* 97, 466-472.

McKeown-Longo PJ, Mosher DF. (1985). Interactions of the 70,000-mol-wt amino-terminal fragment of fibronectin with the matrix-assembly receptor of fibroblasts. *J. Cell Biol.* 100, 364-374.

Mosher DF, (1984). Physiology of fibronectin. *Ann. Rev. Med.* 35, 561-575.

Mosher DF, Johnson RB (1983). In vitro formation of disulfide-bonded fibronectin multimers. *J. Biol. Chem.* 258, 6595-6601.

Oh E, Pierschbacher M, Ruoslahti E. (1981). Deposition of plasma fibronectin in tissues. *Proc. Natl. Acad. Sci. USA.* 78, 3218-3221.

Pesciotta Peters D, Mosher DF, (1987). Localization of Cell Surface Sites Involved in Fibronectin Fibrillogenesis. *J. Cell Biol.* 104, 121-130.

Pytela R, Pierschbacher MD, Ruoslahti E. (1985). Identification and isolation of a 140 kd cell surface glycoprotein with properties expected of a fibronectin receptor. *Cell* 40, 191-198.

Ris H, (1985). The cytoplasmic filament system in critical point-dried whole mounts and plastic-embedded sections. *J. Cell Biol.* 100, 1474-1487.

Vuotto M, Vartio T, Saraste MT, von Bonsdorff CH, Vaheri A. (1980). Spontaneous and polyamine-induced formation of filamentous polymers from soluble fibronectin. *Eur. J. Biochem.* 105, 33-42.

Wolosewick JJ, Porter KR. (1976). Stereo high voltage electron microscopy of whole cells of the human diploid line, WI-38. *Am J. Anat.* 147, 303-324.

Discussion with Reviewers

J. Wartiovaara: Can one be sure what is a retracting edge of a fibroblast?

Authors: The retracting edge of a fibroblast is identified the same way a retracting edge on any cell in culture is identified. It is the trailing edge of the cell and has a large retracting fiber (although smaller retracting fibers are observed). In addition, it, unlike the leading edge of the cell, does not have a ruffling edge. These features can easily be identified in a whole mount since the cells are not sectioned.

J. Wartiovaara: Did the authors find that removing cell membranes by saponin treatment for visualization of intracellular actin filaments also cause dislocation of Au₁₈-complexes from the cell surface into the interior of the cell?

Authors: From stereo tilt analysis of whole mounts, the only Au₁₈-complexes in the interior of the cell were those that were inside vesicles. We assumed that those Au₁₈-complexes had been endocytosized and did not feel that the saponin treatment (which only permeabilizes the membrane and does not completely remove it) cause any dislocation of the Au₁₈-complexes into the interior of the cell.

P. B. Bell: Please discuss the rationale behind the fixative solution used. How does it compare with standard glutaraldehyde protocols? What is the function of saponin in this mixture?

Authors: We added tannic acid and saponin to our fixative to improve staining of actin filaments and protect actin filaments against surface tension distortion when cells were critical point dried. Saponin was added in order to make the cells permeable to the tannic acid.

P. B. Bell: How do the fibronectin-gold clusters form? Do they form in solution and attach to the cell as performed clusters? Do the fibronectin-gold particles attach to the cells as single beads and secondarily form clusters as the result of a capping-like process or are the binding sites on the cell pre-clustered?

Authors: We do not believe that the Au₁₈-fibronectin complexes form in solution because a spread of Au₁₈-fibronectin shows that the Au₁₈-fibronectin exists mostly as single beads. Therefore, the clusters of Au₁₈-fibronectin must form when they come into contact with the cells. We do not know whether the clusters of Au₁₈-fibronectin on the cell surface are formed as a result of a capping-like process or the binding sites are pre-clustered. Studies to determine this are currently being done.

P. B. Bell: What proportion of the gold particles are actually in clusters? What proportion of the clusters and gold particles are associated with filaments?

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Authors: At the present moment we do not know what proportion of the gold particles are in clusters. From preliminary studies, it appears that at least 15% of the particles are found as single beads on the cell's surface. We are in the process of doing morphometric studies to compare the percentage of clusters and single beads on the dorsal surface of the fibroblast that co-align with actin filaments.

G. Albrecht-Buehler: How do the authors distinguish between incorporation of the labelled fibronectin into newly forming fibronectin fibrils and their binding to newly forming collagen fibrils?

Authors: To determine if Au₁₈-fibronectin was binding to cell surface collagen, we digested fibroblasts with bacterial collagenase to remove the collagen and then incubated the cells for 15 minutes with Au₁₈-fibronectin. We observed that Au₁₈-fibronectin still bound the cell surface. At the present moment, we are using fibroblasts that are not producing collagen fibrils to further determine if the Au₁₈-fibronectin is being incorporated into newly forming collagen fibrils.

