

# Fibrillin, A New 350-kD Glycoprotein, Is a Component of Extracellular Microfibrils

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**Abstract.** A new connective tissue protein, which we call fibrillin, has been isolated from the medium of human fibroblast cell cultures. Electrophoresis of the disulfide bond-reduced protein gave a single band with an estimated molecular mass of 350,000 D. This 350-kD protein appeared to possess intrachain disulfide bonds. It could be stained with periodic acid-Schiff reagent, and after metabolic labeling, it contained [<sup>3</sup>H]glucosamine. It could not be labeled with [<sup>35</sup>S]sulfate. It was resistant to digestion by bacterial collagenase. Using mAbs specific for fibrillin, we demonstrated its widespread distribution in the connective tissue matrices of skin, lung, kidney, vasculature, cartilage, tendon, muscle, cornea, and ciliary zonule. Electron microscopic immunolocalization with colloidal gold conjugates specified its location to a class of extracellular structural elements described as

microfibrils. These microfibrils possessed a characteristic appearance and averaged 10 nm in diameter. Microfibrils around the amorphous cores of the elastic fiber system as well as bundles of microfibrils without elastin cores were labeled equally well with antibody. Immunolocalization suggested that fibrillin is arrayed periodically along the individual microfibril and that individual microfibrils may be aligned within bundles. The periodicity of the epitope appeared to match the interstitial collagen band periodicity. In contrast, type VI collagen, which has been proposed as a possible microfibrillar component, was immunolocalized with a specific mAb to small diameter microfilaments that interweave among the large, banded collagen fibers; it was not associated with the system of microfibrils identified by the presence of fibrillin.

THE extracellular matrix appears to contain a variety of fibrillar structural elements. In addition to the large banded collagen fibers, there are various nonstriated fibrils with small cross-sectional diameters. These fibrils can be classed on the basis of their average diameter as 8–10-nm microfibrils (24, 25) or as 3–5-nm microfibrils (16).

The larger 8–10-nm microfibril usually appears in cross-section as an outer electron-dense shell surrounding an inner hollow or lucid core, and in longitudinal section as a beaded chain. These microfibrils are most abundantly associated with basement membranes and with the elastic fiber, and are found in most tissues. They are the subject of a recent review (5). The smaller 3–5-nm microfibril or microfilament is often connected to proteoglycan granules (16, 10).

Many attempts have been made to specifically extract microfibrillar proteins. Ross and Bornstein, guided by electron microscopic analysis of the residue from each extraction step, have described a protocol for the isolation of microfibrillar proteins (30). After digestion of tissue with collagenase, the residue is extracted with guanidine. Microfibrillar proteins are thought to be finally solubilized with disulfide bond reducing agent in guanidine. Biochemical analysis of this microfibrillar extract from tissue (12) and immunological and biochemical studies of cell culture products

(34, 35) using an antiserum prepared with this microfibrillar extract as the immunogen (20) have led to the identification of a major periodic acid-Schiff-positive electrophoretic band with an apparent molecular mass of 140,000 D. This 140-kD glycoprotein has been shown to be a component of the undegraded tissue form of type VI collagen (18, 21, 14).

Electron microscopic studies of type VI collagen molecules suggest a model for type VI collagen filament formation involving an end-to-end rather than a lateral aggregation of a basic tetrameric unit (11, 8). This electron microscopical model has been useful in clarifying the relationship between type VI collagen and microfibrillar components. In fibroblast cultures of chick tendon and cornea and of human skin, 3-nm beaded filaments, which appear to be different from the chains of globules formed in 10-nm microfibrils, compare favorably with the filamentous model for type VI collagen (2, 3). Using a previously characterized mAb specific for type VI collagen (17), we demonstrate here immunolocalization of type VI collagen to small diameter microfilaments associated primarily with large banded interstitial collagen fibers and not with 10-nm microfibrils.

In this paper, we are presenting new information on a glycoprotein that we have isolated from the medium of human fibroblast cell cultures. Using a mAb specific for this glyco-

protein, we show that this protein is a component of extracellular structural elements described as 10-nm microfibrils, but not the 3-nm microfilaments, and we call this new protein "fibrillin."

## Materials and Methods

### mAbs

mAbs specific for fibrillin were prepared according to previously described methods (31, 32). A/J mice (Jackson Laboratories, Bar Harbor, ME) were immunized with a crude extract of human amnion that had been solubilized by pepsin digestion and salt precipitated and that had type VII collagen (I) as its principal species. Clones were screened by ELISA (7) and selected by immunofluorescence. Antibody was used either in the form of spent culture medium or as ascites produced in A/J × BALB/c hybrid mice. One of the hybridomas, clone 201, was used to produce the results shown here.

The mAb specific for type VI collagen was prepared as described (17).

### Indirect Immunofluorescence

Tissues were frozen in hexane in liquid nitrogen, and 8- $\mu$ m sections were cut using a histostat microtome (American Optics, Buffalo, NY) at  $-28^{\circ}\text{C}$ . Sections were air-dried for 30 min and fixed in cold acetone for 10 min. Primary antibodies, usually hybridoma supernatants, were incubated on the sections for 3 h at room temperature. After washing in PBS, fluorescein-conjugated anti-mouse IgG (Sigma Chemical Co., St. Louis, MO) was incubated on the sections for 30 min, and the unbound second antibody was removed by washing in PBS. The sections were covered with 90% glycerol in PBS and were viewed using a Zeiss Photomicroscope III.

With some tissue sections, immunofluorescent tests were conducted after enzymatic digestion with hyaluronidase (Worthington Biochemicals, Malvern, PA), employed at a concentration of 8,000 U/ml of 0.1 M phosphate buffer, pH 5.3, for 20 min at room temperature; 0.01% elastase (Worthington Biochemicals) in 0.067 M Tris buffer, pH 8.8, for 20 min at room temperature; and bacterial collagenase (a gift from Dr. Nicolas P. Morris, Shriners Hospital for Crippled Children, Portland, OR) in 0.05 M Tris buffer, pH 7.5, including 0.01 M  $\text{CaCl}_2$ , 50  $\mu\text{M}$  *N*-ethylmaleimide, and 50  $\mu\text{M}$  phenylmethylsulfonyl fluoride, for 30 min at room temperature.

For immunofluorescence of cell cultures, cells were plated onto glass chamber slides and were fixed either in cold acetone for 10 min or in 2% paraformaldehyde in PBS for 20 min. The assay then proceeded as above.

### Electron Microscopic Immunolocalization

Freshly obtained tissue samples were cut into blocks and incubated with primary antibody for 7–15 h at  $4^{\circ}\text{C}$ . After washing away unbound antibody, the tissue blocks were incubated with colloidal gold conjugated to anti-mouse IgG (Janssen Life Sciences Products, Piscataway, NJ) in a 0.02-M Tris buffer, pH 8.0, containing 0.9% NaCl and 0.1% BSA, for 5–15 h at  $4^{\circ}\text{C}$ . The blocks were washed in PBS, rinsed in 0.1 M sodium cacodylate, pH 7.4, fixed in cacodylate-buffered Karnovsky's fix (19) for 60 min at  $4^{\circ}\text{C}$ , rinsed again, and finally fixed in 1%  $\text{OsO}_4$  in cacodylate for 60 min at  $4^{\circ}\text{C}$ . Samples were dehydrated at room temperature through 30, 50, 70, 90, 95, and 100% ethanol and were washed in 100% propylene oxide. Then they were infiltrated with Spurr's epoxy (hard formula) and polymerized at  $70^{\circ}\text{C}$  overnight. 60–90-nm sections were cut on an Ultramicrotome (Ultratrac E; Reichert Scientific Instruments, Buffalo, NY) and mounted on Formvar-coated grids. Grids were stained in 2% uranyl acetate in 50% ethanol for 15 min and Reynold's lead citrate (29) for 60 s. Specimens were examined at 60–80 kV, using a 30- $\mu\text{m}$  objective aperture with a Philips 410 LS transmission electron microscope.

The same protocol was used for preparing samples for immunolocalization by scanning electron microscopy, except that after dehydration through 100% ethanol, samples were critical point-dried through liquid  $\text{CO}_2$ , sputter-coated in an argon atmosphere with gold using an evaporator (MED 010; Balzers, Hudson, NH), and examined at 10 kV in the upper stage of a scanning electron microscope (DS-130; International Scientific Instruments, Inc., Milpitas, CA).

### Cell Cultures

Primary cultures of fibroblasts were established from normal human skin explants. They were then dissociated with trypsin (Sigma Chemical Co.) in

PBS and passaged. Primary cultures of human amniocytes were derived from amnion digested with 0.1% crude bacterial collagenase (Worthington Biochemicals) in PBS supplemented with 0.001 M  $\text{CaCl}_2$  and 0.0005 M  $\text{MgCl}_2$ . Amniocytes were mixed cultures of epithelial and fibroblastic cell types and were used only as primary cultures. Established cell lines, MG63, KB, FL, WISH, and HT1080 were obtained from the American Type Culture Collection, Rockville, Maryland. All cell cultures were maintained in Dulbecco's modified Eagles medium (Gibco, Grand Island, NY), supplemented with 10% FBS (Gibco), 50 U/ml of penicillin, and 50  $\mu\text{g}/\text{ml}$  of streptomycin (Gibco).

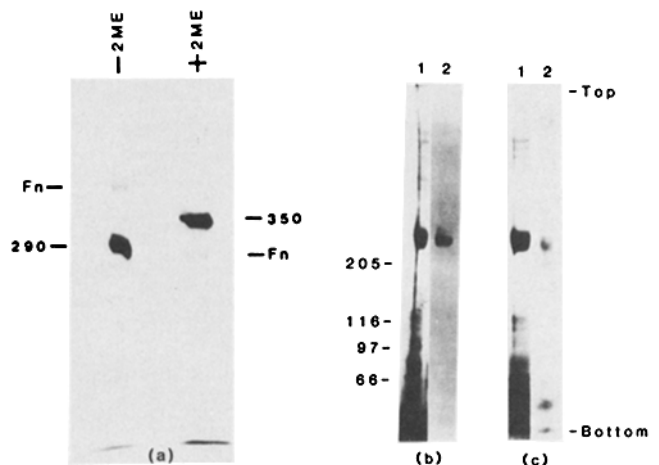
For metabolic labeling experiments, cell cultures were fed with serum-free medium containing 50  $\mu\text{Ci}/\text{ml}$  [ $^3\text{H}$ ]proline, [ $^3\text{H}$ ]glycine, [ $^3\text{H}$ ]glucosamine, [ $^{35}\text{S}$ ]sulfate, or [ $^{35}\text{S}$ ]cysteine (Amersham Corp., Arlington Heights, IL). In experiments measuring  $^{35}\text{S}$  incorporation, sulfate or cysteine were not included in the medium. Sometimes cultures were supplemented with 100  $\mu\text{g}/\text{ml}$  of ascorbic acid and 64  $\mu\text{g}/\text{ml}$  of  $\beta$ -aminopropionitrile. When cell culture medium was harvested, it was placed immediately on ice, and the following inhibitors were included: 5 mM EDTA, 50  $\mu\text{M}$  *N*-ethylmaleimide, and 50  $\mu\text{M}$  phenylmethylsulfonyl fluoride. Radiolabeled proteins were separated by polyacrylamide gel electrophoresis (23), and the bands were visualized by soaking the gel in Amplify (Amersham Corp.), drying it, and exposing it to Kodak XAR-5 x-ray film.

### Immunoisolation

Ascites antibody was partially purified using Affigel Blue (Bio-Rad Laboratories, Richmond, Ca) and was coupled to CNBr-activated Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ). Cell culture medium was incubated with antibody coupled to Sepharose for 3 h at room temperature. The gel was then washed extensively with 0.05 M Tris, pH 7.5, containing 0.5 M NaCl and 0.05% Tween 20. Bound material was eluted from the antibody beads with 1 M acetic acid. Contaminating fibronectin was removed by absorption with gelatin-sepharose.

### Immunoblotting

Proteins were separated by polyacrylamide gel electrophoresis, and the separated proteins were transferred from the gel to nitrocellulose paper with a transblot system (Bio-Rad Laboratories). After transfer, the paper was washed in PBS, blocked with 4% BSA in PBS, incubated with mAb for 3 h at room temperature, washed, and incubated with peroxidase-conjugated



**Figure 1.** (a) SDS PAGE using a 3–5% acrylamide gel of fibrillin isolated from [ $^{35}\text{S}$ ]cysteine-labeled fibroblast culture medium. Samples were run without (–2ME) or with (+2ME) 2-mercaptoethanol. Traces of contaminating fibronectin (Fn) in this preparation are used here for molecular mass estimation. (b and c) SDS-PAGE using a 3–5% acrylamide gel of 50% saturated  $(\text{NH}_4)_2\text{SO}_4$ -precipitated fibroblast culture medium proteins. Lane 1 in b and c is stained with Coomassie Blue. Lane 2 in b shows medium proteins transferred to nitrocellulose and immunoblotted with antifibrillin mAb. Lane 2 in c is stained with the periodic acid-Schiff reagent. Samples were prepared without reducing agent. Positions of molecular mass standards are marked.

anti-mouse IgG (Bio-Rad Laboratories) for 2 h. After the final washing, substrate (Bio-Rad Laboratories) was added, and the antibody-bound electrophoretic band was visualized.

### Collagenase Digestion

Immunoisolated or partially purified sample was incubated with collagenase (a gift from Dr. Nicholas P. Morris and Dr. Hans Peter Bachinger, Shriners Hospital for Crippled Children), 50  $\mu$ M *N*-ethylmaleimide, 50  $\mu$ M phenylmethylsulfonyl fluoride, and 0.01 M CaCl<sub>2</sub> in 0.05 M Tris, pH 7.5, for 30 min at 33°C. Proteins were separated by electrophoresis and visualized by fluorography or Coomassie Blue staining.

## Results

### Isolation of Fibrillin from Cell Cultures

Hybridomas were selected on the basis of their immunofluorescent staining pattern. Supernatants from two different clones yielded identical patterns. mAbs from the two clones bind to the same protein antigen, which we call fibrillin, in immunoblotting and immunoprecipitation experiments, but their epitopes are different. Both antibodies recognize epitopes in human and bovine tissues; one also binds to chick tissues. The results shown here were obtained using mAb 201, although similar data was produced using mAb F2.

Cell cultures producing fibrillin were identified by indirect immunofluorescence. Using the medium from cell cultures of amniocytes, fibroblasts, or MG63, an osteosarcoma line, which had been incubated with radioactive amino acids, immunoisolation experiments yielded a protein, which, upon electrophoresis, had a slower mobility than fibronectin (250 kD) under disulfide bond-reducing conditions. The size of the polypeptide was estimated to be  $\sim$ 350,000 D (Fig. 1 *a*). When gels were run without reducing agent, the polypeptide

migrated faster, suggesting the presence of intrachain disulfide bonds. This electrophoretic identification of fibrillin was confirmed by immunoblotting (Fig. 1 *b*).

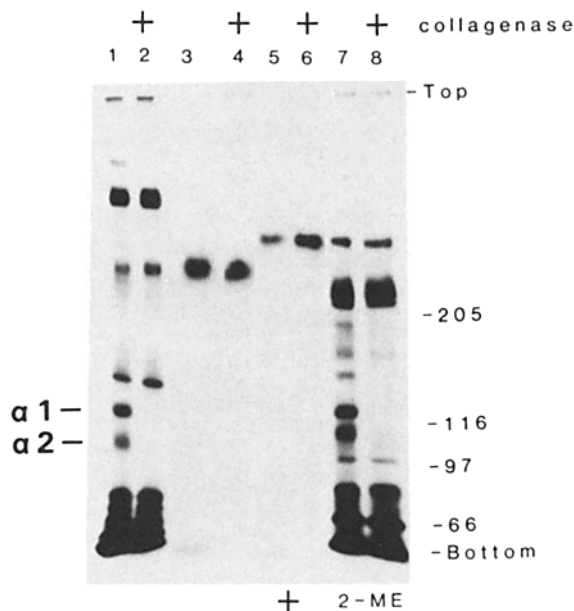
Reduction of disulfide bonds destroyed antibody binding in both immunoblotting and in ELISA as well as in immunofluorescence experiments. Therefore, the epitope recognized by this mAb (and also the different epitope recognized by mAb F2) appears to be stabilized by disulfide bonds.

When [<sup>3</sup>H]glucosamine was included in the medium, a radioactive protein with identical electrophoretic mobilities was immunoisolated. However, when the label was [<sup>35</sup>S]sulfate, no radioactive material was detected in the immunoisolation, although proteins in the medium did incorporate [<sup>35</sup>S]sulfate and the immunoisolated protein with its characteristic mobilities could be demonstrated after silver staining (data not shown). Presence of carbohydrate was also shown by the periodic acid-Schiff staining of partially purified culture medium separated by electrophoresis (Fig. 1 *c*).

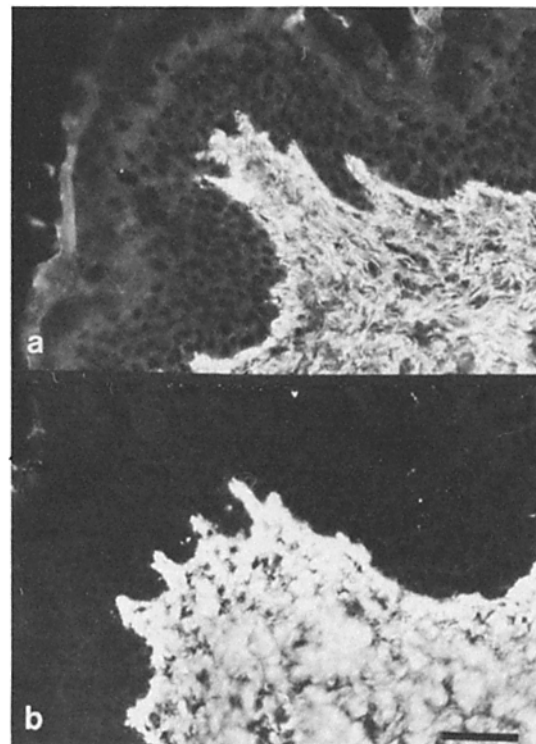
Incubation of immunoisolated fibrillin with bacterial collagenase did not result in degradation (Fig. 2). This data confirmed immunofluorescence experiments performed on tissue sections treated with collagenase.

### Distribution of Fibrillin in Tissues

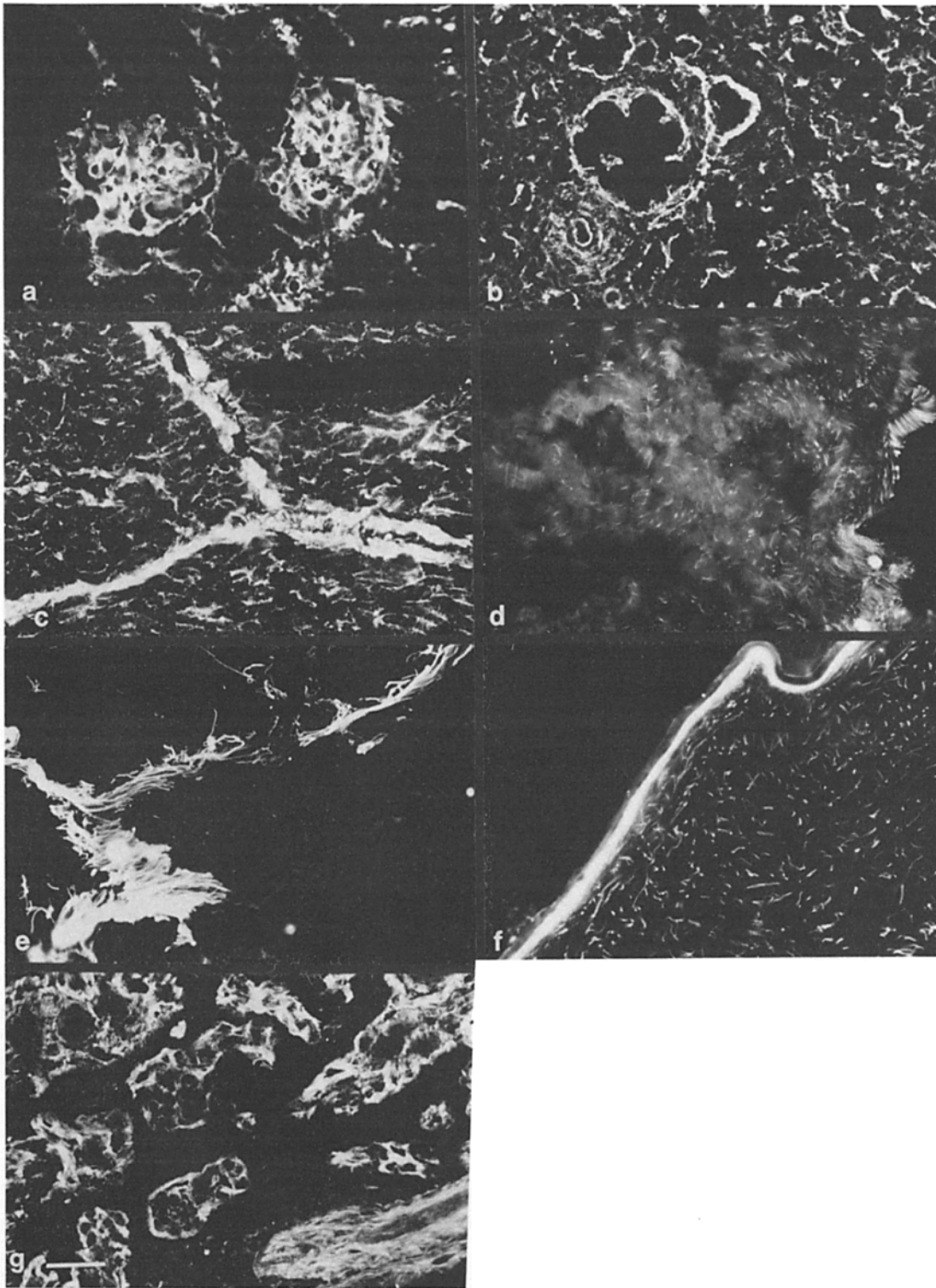
In human skin, the immunofluorescent staining pattern of fibrillin appeared to represent a network of discrete fibers extending from the region of the dermal-epidermal junction, where they often seemed to intersect perpendicularly, down into the deep dermis (Fig. 3 *a*). In contrast, type VI collagen immunofluorescence was more evenly distributed throughout the connective tissue stroma (Fig. 3 *b*).



**Figure 2.** SDS PAGE using a 3–5% acrylamide gel of fibroblast culture medium proteins labeled with [<sup>3</sup>H]proline and [<sup>3</sup>H] glycine (lanes 1, 2, 7, and 8) and of immunoisolated fibrillin (lanes 3, 4, 5, and 6). Lanes 2, 4, 6, and 8 show samples that were incubated with bacterial collagenase. Lanes 1, 2, 3, and 4 were run without reducing agent, and lanes 5, 6, 7, and 8 were run with reducing agent.



**Figure 3.** Indirect immunofluorescent staining of human skin using antifibrillin mAb (*a*) and monoclonal anti-type VI collagen (*b*). Bar, 50  $\mu$ m.



**Figure 4.** Fibrillin immunofluorescence of sections of (a) bovine kidney, (b) bovine lung, (c) human muscle, (d) human tendon, (e) bovine ciliary zonule, (f) bovine cornea, and (g) human placenta. Bar, 50  $\mu$ m.

Immunofluorescent studies of various tissues revealed a wide distribution of fibrillin (Fig. 4). Antifibrillin staining demonstrated the presence of discrete fibers in the bovine corneal stroma and emphasized a broad band of immunofluorescence in Descemet's membrane (Fig. 4 f). Bovine kidney glomeruli (Fig. 4 a) were brightly stained. Bovine

bronchioles, adjoining blood vessel walls, and alveolar connective tissue (Fig. 4 b), human muscle perimyseum and endomyseum (Fig. 4 c), and human placental vessels and trophoblastic connective tissue (Fig. 4 g) displayed immunofluorescence. Discrete fibers were stained in the human tendon (Fig. 4 d). In bovine ciliary zonule, the suspensory liga-

ment of the lens, extraordinarily fibrillar structures were identified (Fig. 4 *e*).

Fibrillin immunofluorescence in aorta was typical of elastic fiber staining (Fig. 5, *a* and *c*). However, when tissue sections of bovine aorta were incubated with elastase using conditions that abolished (Fig. 5 *b*) immunofluorescence generated by anti-elastin antiserum (a gift from Dr. Judith Foster, Syracuse University, Syracuse, NY), staining with antifibrillin mAb was unaltered (Fig. 5 *c*). Fibrillin was also present in elastic cartilage of human ear (Fig. 6, *a* and *b*). However, hyaluronidase digestion was necessary to unmask antigenic sites; staining in the perichondrium and dermal connective tissue were not affected by treatment with hyaluronidase (Fig. 6 *b*). The immunofluorescence pattern was undisturbed by prior digestion of the tissue sections with bacterial collagenase, using conditions where the immunofluorescence obtained with mAbs specific for type IV collagen (31) and for type III collagen were completely abolished (data not shown).

Cell matrices were examined by immunofluorescence (Fig. 7). Fibrillin was identified in the matrices of cultures of human amniocytes (Fig. 7 *a*), fibroblasts (Fig. 7 *b*), and MG63, an osteosarcoma cell line (Fig. 7 *c*). In the primary cultures of amniocytes, some fibroblastic cells were present amidst the epithelial cells; immunofluorescence appeared only around the fibroblastic cells. In addition, the F1- and WISH-transformed epithelial cell lines from human amnion displayed no immunofluorescence. KB, an epidermal oral carcinoma, and HT1080, a fibrosarcoma that synthesizes basement membrane constituents, were also unreactive with the mAb.

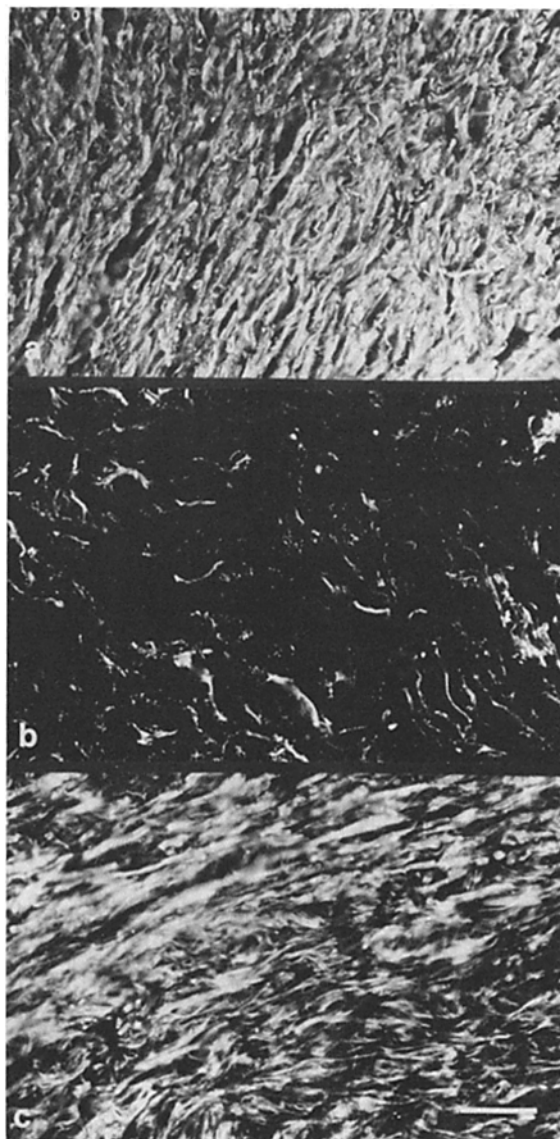
### ***Electron Microscopic Immunolocalization***

Electron microscopic immunolocalization studies demonstrated the presence of fibrillin in fibrous structural elements described as microfibrils (Figs. 8 and 9). In human skin, antifibrillin mAb directed the deposition of gold specifically to microfibrils whose average diameter was 10 nm. Gold-decorated microfibrils were seen around amorphous cores of the elastic fiber system (Fig. 8). In addition, bundles of microfibrils without amorphous cores were equally well labeled. At the dermal-epidermal junction, these bundles of microfibrils appeared to intersect the basal lamina (Fig. 9). There was no labeling of any other connective tissue components.

Scanning electron microscopic immunolocalization displayed a three-dimensional image of antifibrillin-labeled fibers (Fig. 10). In a region of human dermis that has been shown by transmission electron microscopy to contain mature elastic fibers, gold identified the presence of fibrillin on fibers of varying diameters that branch and that appear to form a discrete fiber system.

In contrast to the localization of fibrillin, type VI collagen was immunolocalized in human skin to the fine filamentous structures that lie among the large banded interstitial collagen fibers (Fig. 11). Type VI collagen-directed gold deposition did not occur on bundles of microfibrils or on microfibrils around elastin cores.

The epitope on fibrillin that is recognized by mAb 201 seemed to occur periodically along the microfibrils (Fig. 12). Interestingly, the fibrillin periodicity, as detected by gold deposition, matched the interstitial collagen band periodicity

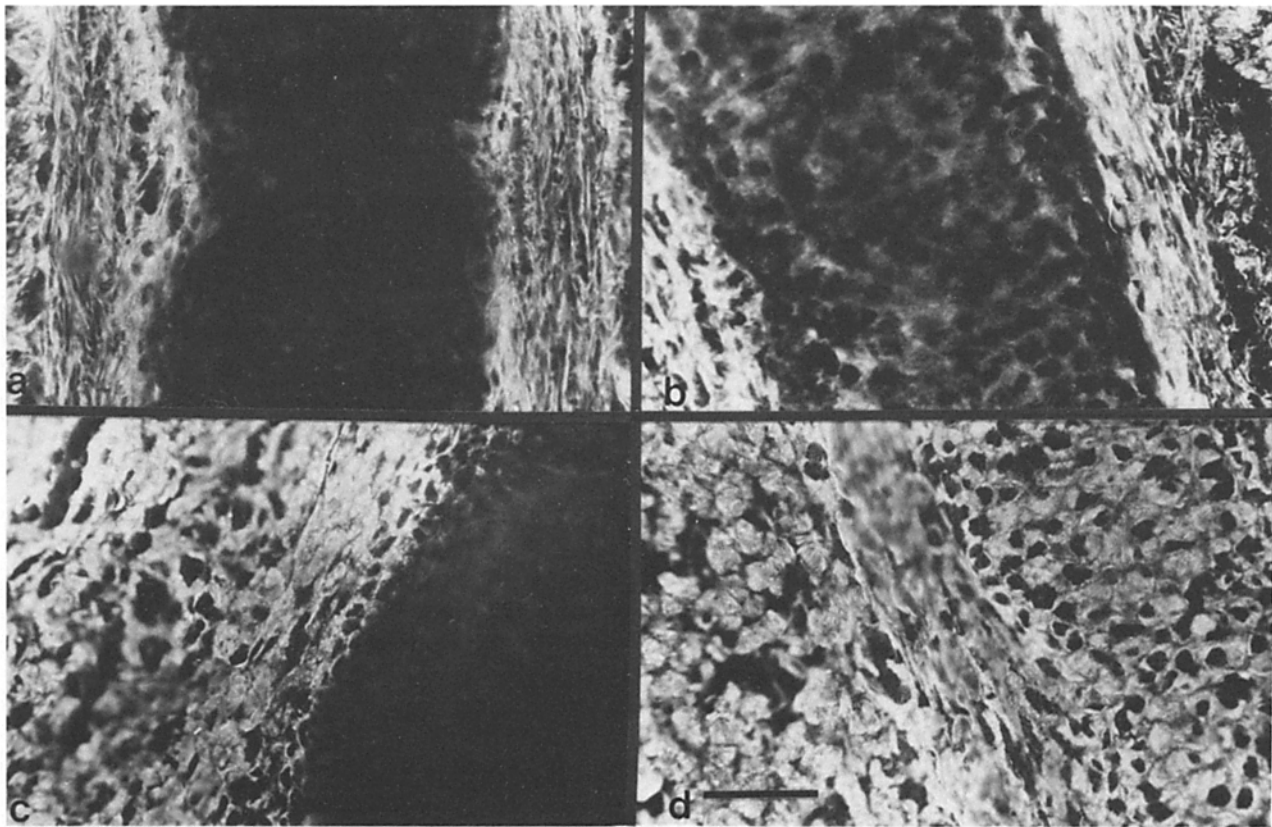


**Figure 5.** Resistance of fibrillin immunofluorescence to elastase digestion. Bovine aorta tissue sections were stained with anti-elastin antiserum before (*a*) and after (*b*) elastase treatment of the sections. *c* shows antifibrillin staining of an elastase-digested tissue section. Bar, 50  $\mu$ m.

of 67 nm. This periodicity appeared along the length of individual microfibrils as well as across bundles of individual microfibrils, suggesting an ordered aggregation of this component into microfibrils and microfibrillar bundles. However, it is also possible that the seemingly ordered aggregation of individual microfibrils into bundles may be due in part to aggregation and alignment by the antibodies.

### ***Discussion***

We have demonstrated that a new 350-kD glycoprotein, which we call fibrillin, is a major structural component of a special class of microfibrils. Fibrillin is present in both elastin-associated microfibrils and in bundles of microfibrils without a visible amorphous element. Because of their visual similarities, it has been thought that elastin-associated mi-



**Figure 6.** Hyaluronidase digestion of human elastic (ear) cartilage. *a* and *b* show immunofluorescence using monoclonal antifibrillin, and *c* and *d* show staining using monoclonal anti-type VI collagen. Immunofluorescence of hyaluronidase-digested tissue sections is shown in *b* and *d*. Bar, 50  $\mu$ m.

crofibrils might be related to the oxytalan fibers of skin (6, 22, 38), to microfibrils described in the basement membrane region of the alveolar wall (24), to the bundles of discrete tubular fibrils in the subendothelial spaces of kidney glomeruli (9), and to the microfibrils of the ciliary zonule (28, 36). Our data supports these ideas. Using a mAb and electron microscopic immunolocalization, we have shown that fibrillin is common to microfibrils at the basement membrane of the dermal-epidermal junction and to microfibrils at the periphery of true elastic fibers in skin, to microfibrils in elastic tissues such as aorta and ear cartilage (data not shown), and to microfibrils in elastin-poor tissues such as cornea, tendon, and ciliary zonule (data not shown). In addition, immunofluorescence localized this glycoprotein to alveolar connective tissue, kidney glomeruli, and muscle sheaths. Thus, our immunological data has specified the presence of fibrillin in all morphologically similar 10-nm microfibrils that we have so far examined and suggests that a unique system of microfibrils exists in the connective tissue space.

In addition, our data, using a mAb and immunogold electron microscopy, localized type VI collagen specifically to small diameter microfilaments. Immunofluorescence microscopy has shown type VI collagen to be distributed in tissues in a pattern similar to the interstitial collagens (17, 13). Electron microscopic immunolocalization using monospecific antisera directed against the pepsinized form of type VI collagen has shown ferritin labeling very close to 5–10-nm

microfilaments present in the amorphous ground substance of aortic media and placental villi (39). In that study, some ferritin can be seen along elastic fibers, but the labeling is not prominent. In our study, the microfibrillar component of the elastic fiber, as well as the bundles of 10-nm microfibrils, were never labeled by monoclonal anti-type VI collagen and immunogold.

It has become increasingly clear that, in addition to the large banded collagen fibers, different collagen types can form different extracellular structures. In basement membranes, type IV collagen may aggregate in an end-to-end manner to form a network (37) and may associate laterally as well (40). Type VII collagen appears to be a major structural component of the anchoring fibril in the basement membranes of certain epithelia like skin and cornea (32). In accord with Bruns' analysis (2, 3) of cell culture microfilaments, immunolocalization of type VI collagen to extracellular microfilaments supports the suggestion that type VI collagen may be the major structural element of a special class of microfilaments. It is not known whether these type VI collagen microfilaments are identical to the small microfilaments observed in early chick development or what function they serve in the extracellular matrix, particularly with respect to the large banded collagen fibers among which they appear to interweave.

We have characterized fibrillin as a microfibrillar component and as a fibroblast cell culture product. Fibrillin is a collagenase-resistant nonsulfated glycoprotein with an esti-

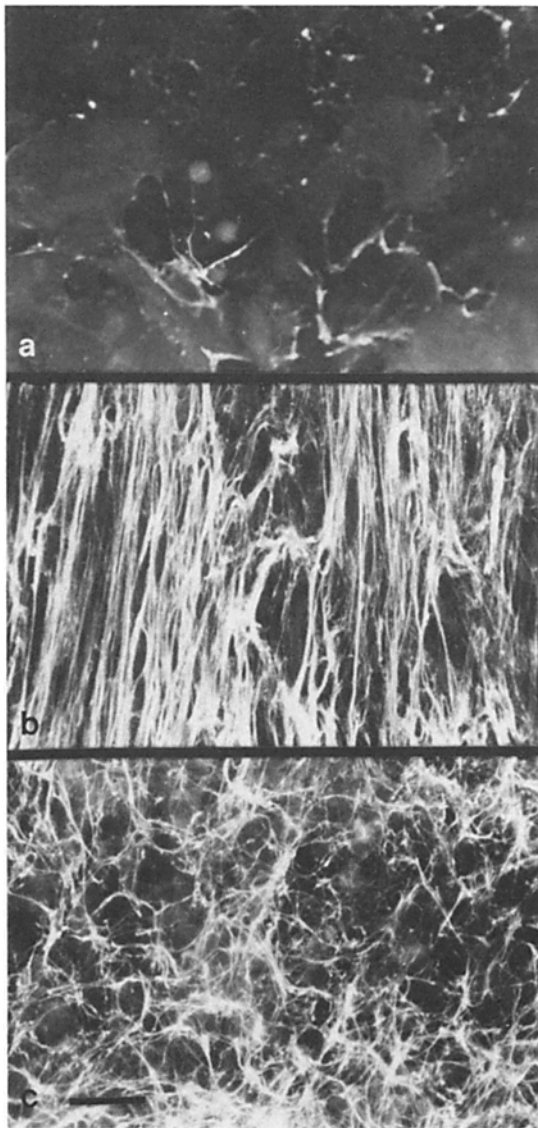


Figure 7. Immunofluorescence showing fibrillin in the matrices of cultured cells: human amniocytes (a), fibroblasts (b), and MG63 (c). Bar, 50  $\mu$ m.

mated molecular mass of  $\sim 350,000$  D. A comparison with Sear's MFPII (34, 35) cannot be adequately made, except to say that MFPII possessed a similar apparent molecular mass (300 kD) by electrophoresis under disulfide bond-reducing conditions and that it appeared to be resistant to collagenase digestion.

The tissue form of fibrillin is currently under investigation. Ross and Bornstein (30) have demonstrated the resistance of microfibrillar structural components to extraction by collagenase digestion and dissociating agents. Their data also suggests that disulfide bridges form the principal cross-links that stabilize the fibril structure. Our preliminary studies (not shown) indicate that, in chick aorta organ cultures, in cell culture matrices, and in extracts of aorta, immunodetection of fibrillin results in an electrophoretic band similar in size to the fibroblast product in the culture medium and that, in organ cultures, fibrillin forms intermolecular disulfide-bonded aggregates that become insoluble.

It has been speculated that microfibrils consist of a precursor elastin core surrounded by glycoprotein (4). Fibronectin may be associated with microfibrils (26, 33, 15). The association of types III and IV collagens and laminin with microfibrils in the cornea has also been reported (27). The 67-nm periodicity of fibrillin that we have described may be suggestive of a relationship between fibrillin and an interstitial collagen. The identification of fibrillin as a principal structural component of microfibrils will allow the exploration of potential interactions of microfibrils with other extracellular matrix constituents.

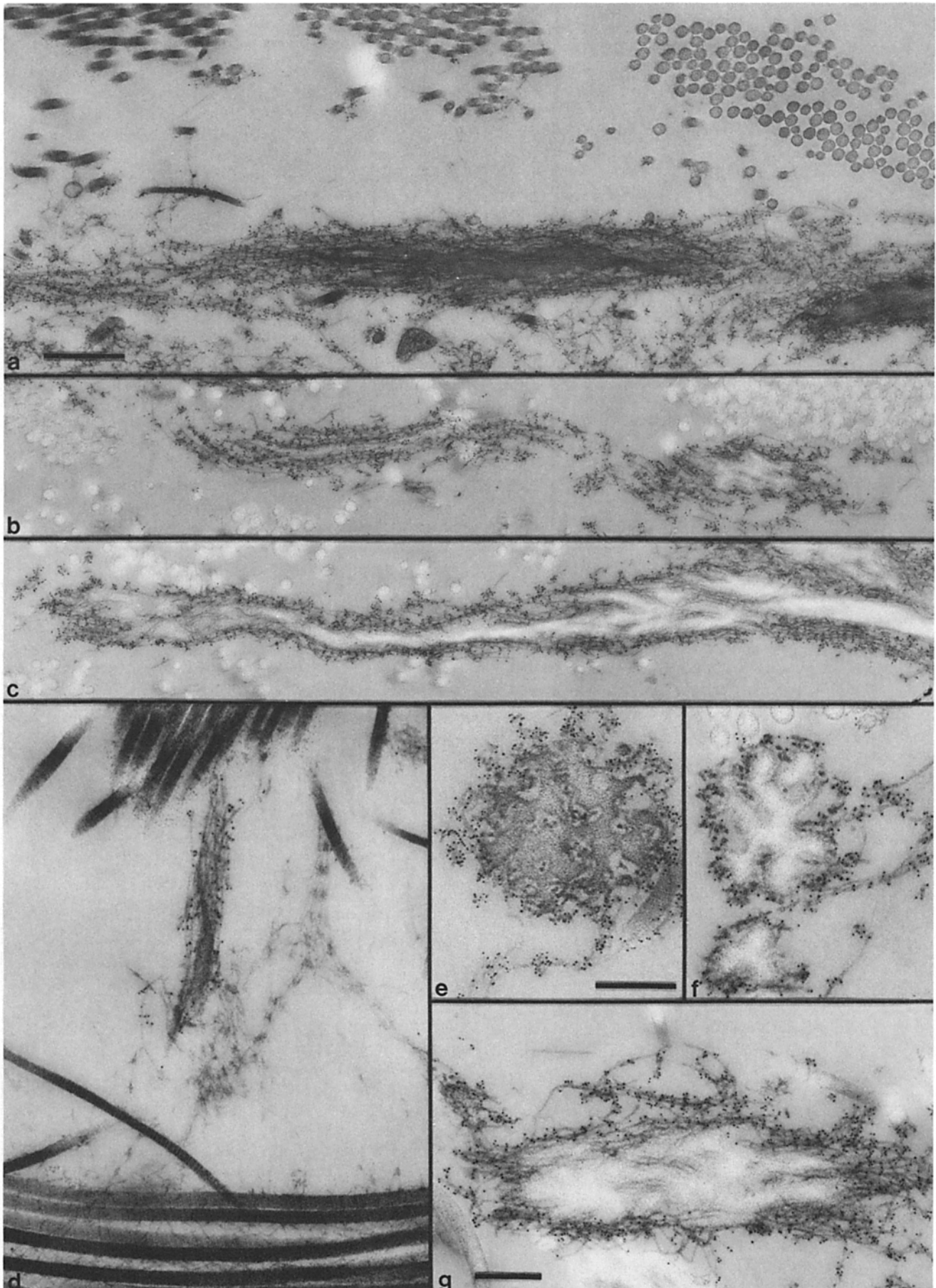
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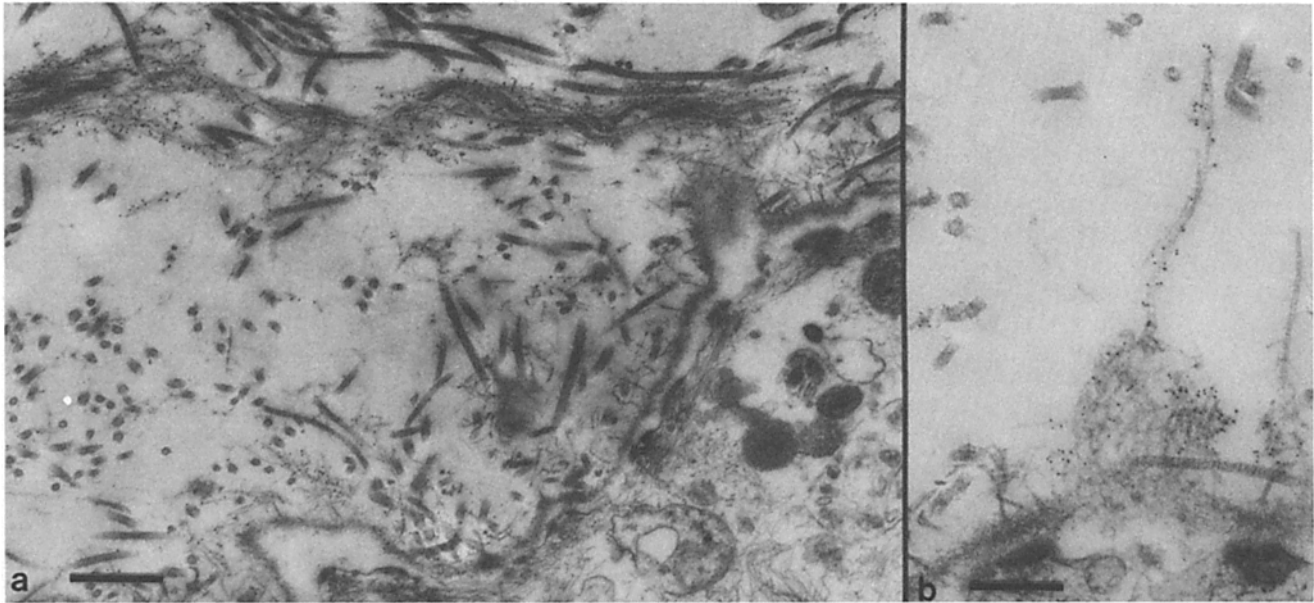
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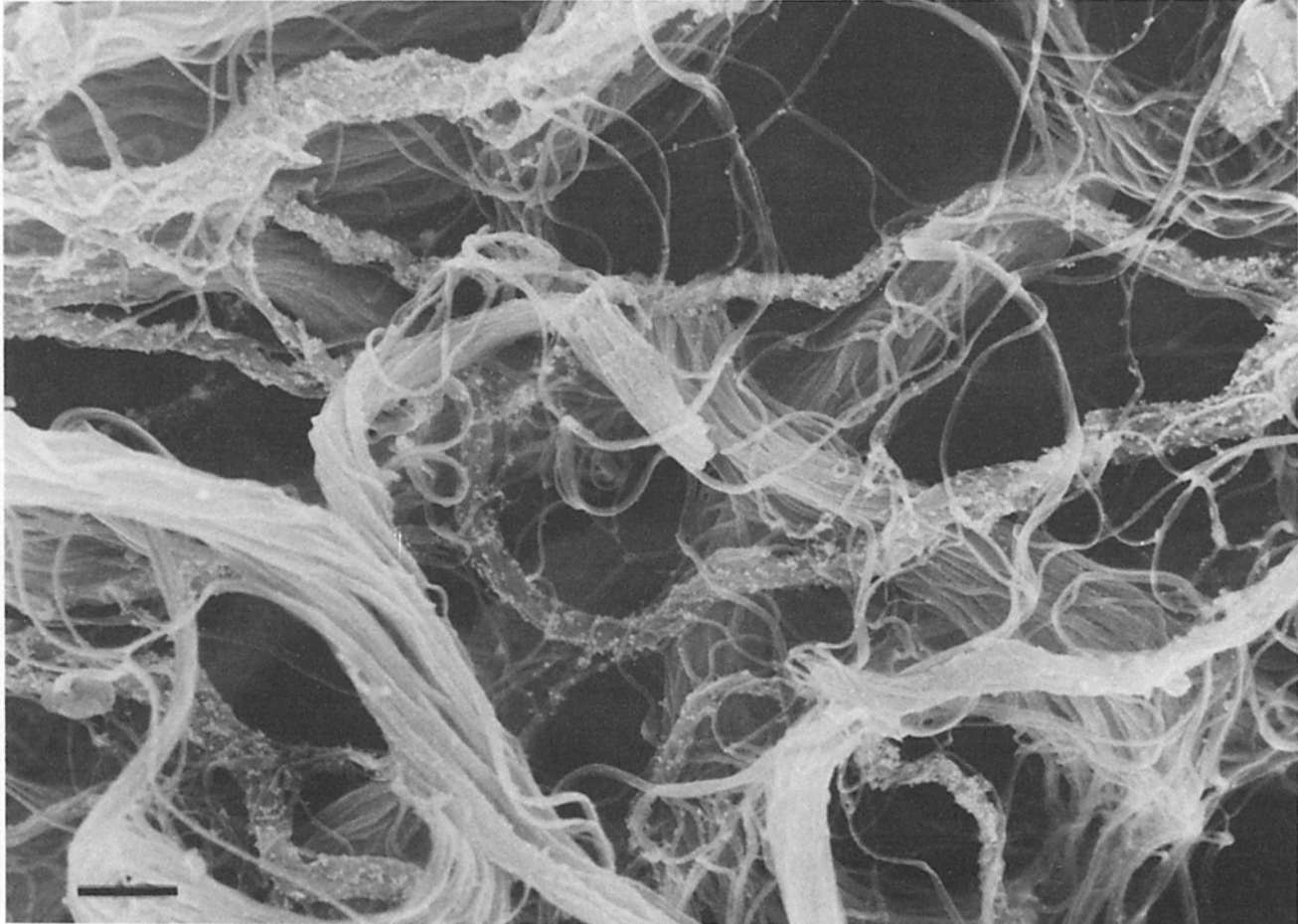


**Figure 8.** Electron microscopic immunolocalization of fibrillin to microfibrils around amorphous cores of elastic fibers in human skin. Longitudinal sections (*a-d* and *g*) and cross-sections (*e* and *f*) of 5-nm gold-decorated microfibrils are shown. Undecorated collagen fibers (*a-d*) as well as undecorated filamentous structures (*d*) can be seen. Bars: (*a-c*) 0.5  $\mu\text{m}$ ; (*d-g*) 0.25  $\mu\text{m}$ .

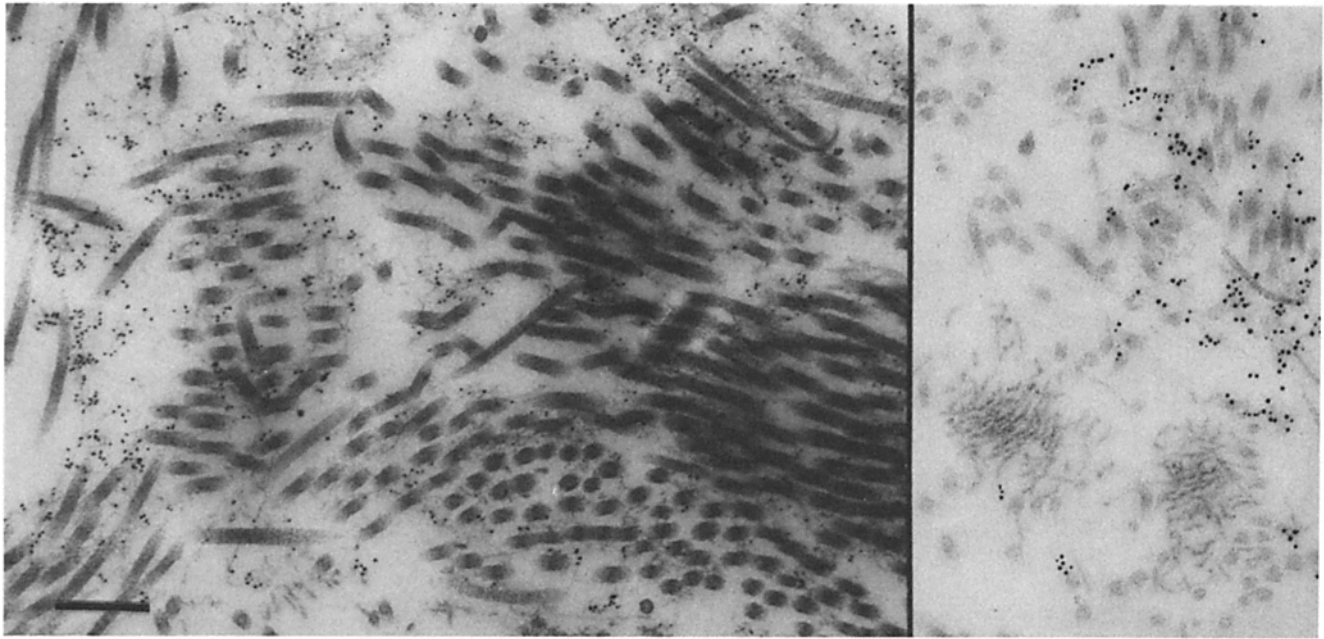




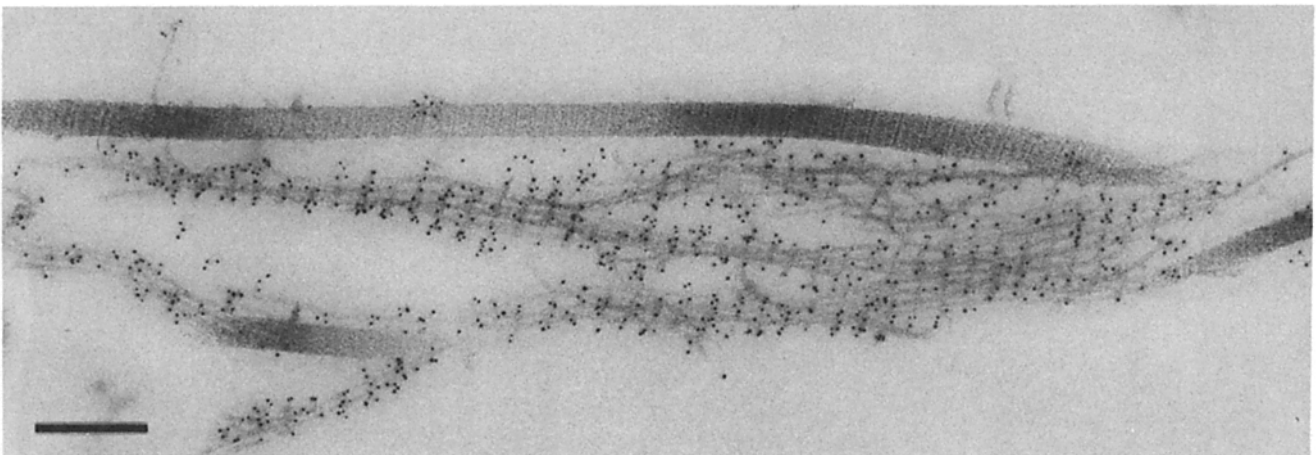
**Figure 9.** Antifibrillin-labeled microfibrils in the region of the dermal-epidermal junction of human skin. *a* shows a microfibrillar bundle, amidst unlabeled collagen fibers, in its approach toward the basal lamina; anchoring fibrils, the lamina densa, and basal cell structures are also unlabeled. *b* depicts 5-nm gold-decorated microfibrils appearing to insert into the lamina densa. Bars: (*a*) 0.5  $\mu\text{m}$ ; (*b*) 0.25  $\mu\text{m}$ .



**Figure 10.** Immunolocalization of fibrillin by scanning electron microscopy of 40-nm gold-decorated fibers, among unlabeled collagen fibers, in human dermis. Bar, 1  $\mu\text{m}$ .



**Figure 11.** Electron microscopic localization of type VI collagen to fine filaments associated with banded collagen fibers in human skin. Unlabeled microfibrils can be seen. Bar, 0.25  $\mu\text{m}$ .



**Figure 12.** Longitudinal periodicity of fibrillin demonstrated by 5-nm gold deposition. Similarity to the interstitial collagen banding period can be seen by comparison with the adjoining collagen fiber. Bar, 0.2  $\mu\text{m}$ .

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