

Structure and Expression of Fibrillin-2, A Novel Microfibrillar Component Preferentially Located in Elastic Matrices

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Abstract. During the previous cloning of the fibrillin gene (FBN1), we isolated a partial cDNA coding for a fibrillin-like peptide and mapped the corresponding gene (FBN2) to human chromosome 5. (Lee, B., M. Godfrey, E. Vitale, H. Hori, M. G. Mattei, M. Sarfarazi, P. Tsipouras, F. Ramirez, and D. W. Hollister. 1991. *Nature [Lond.]* 352:330-334). The study left, however, unresolved whether or not the FBN2 gene product is an extracellular component structurally related to fibrillin. Work presented in this report clarifies this important point. Determination of the entire primary structure of the FBN2 gene product demonstrated that this polypeptide is highly homolo-

gous to fibrillin. Immunoelectron microscopy localized both fibrillin proteins to elastin-associated extracellular microfibrils. Finally, immunohistochemistry revealed that the fibrillins co-distribute in elastic and non-elastic connective tissues of the developing embryo, with preferential accumulation of the FBN2 gene product in elastic fiber-rich matrices. These results support the original hypothesis that the fibrillins may have distinct but related functions in the formation and maintenance of extracellular microfibrils. Accordingly, we propose to classify the FBN1 and FBN2 gene products as a new family of extracellular proteins and to name its members fibrillin-1 and fibrillin-2, respectively.

IT is now well established that fibrillin is the defective gene product responsible for the ophthalmic, cardiovascular, and skeletal manifestations of the Marfan syndrome (MFS)¹ (Dietz et al., 1991, 1992a,b, 1993a,b; Kainulainen et al., 1992; Hewett et al., 1993). Fibrillin was originally isolated from the medium of cultured fibroblasts using an antibody raised against pepsin-treated and salt-precipitated proteins from human amnion (Sakai et al., 1986). Biochemical analyses estimated fibrillin to be a 350-kD acidic glycoprotein with a large number of cysteine residues (Sakai et al., 1991). Additionally, fibrillin was shown to be an integral component of the non-collagenous microfibrils with an average diameter of 10 nm (Sakai et al., 1986, 1991). These extracellular aggregates are present in both elastic and non-elastic tissues (Low, 1962; Cleary and Gibson, 1983; Mecham and Heuser, 1991). Microfibrils of elastic tissues have been shown to assemble into a scaffold upon which elastin is subsequently deposited, whereas in

non-elastic tissues they are believed to serve an anchoring function (for reviews see Mecham and Heuser, 1991; Ramirez et al., 1993). Relevant to MFS pathology, microfibrils are a major structural component of the suspensory ligaments of the eye, the aortic wall, and the perichondrium and periosteum (Mecham and Heuser, 1991; Ramirez et al., 1993).

Cloning experiments have established that the primary structure of fibrillin consists mainly of repeats similar to the calcium-binding (CB) sub-class of the EGF motif (Maslen et al., 1991; Lee et al., 1991; Corson et al., 1993; Pereira et al., 1993). These experiments identified a partial cDNA potentially coding for a fibrillin-like peptide, thus suggesting that fibrillin may represent a novel gene family (Lee et al., 1991). The gene coding for this putative fibrillin-like transcript was mapped to human chromosome 5. Based on their respective chromosomal locations, the two fibrillin transcripts were provisionally termed Fib 15 and Fib 5 and the corresponding genes FBN1 and FBN2 (Lee et al., 1991). Interestingly, the latter was genetically linked to the MFS-like condition, congenital contractural arachnodactyly (CCA) (Lee et al., 1991; Tsipouras et al., 1992). Patients affected by this rare connective tissue disorder share some of the skeletal features of MFS, but display joint contractures in

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1. *Abbreviations used in this paper:* AMP, associated microfibril protein; CB, calcium-binding; CCA, congenital contractural arachnodactyly; MAGP, microfibril-associated glycoprotein; MSF, Marfan syndrome.

place of loose jointedness (Beals and Hecht, 1971). Aside from lacking the cardiovascular and ocular manifestations of MFS, these patients exhibit characteristically misshaped external ears (Beals and Hecht, 1971).

The association between phenotypically overlapping conditions and two potentially related macromolecules prompted us to suggest that Fib 5 and Fib 15 may have distinct but related extracellular functions (Lee et al., 1991). This postulate is supported by results presented in this study which demonstrates that Fib 5 is a microfibrillar constituent, whose structure and expression are related to those of Fib 15. Based on this evidence, we propose to classify the fibrillins as a new family of extracellular proteins and to name its members fibrillin-1 (Fib 15) and fibrillin-2 (Fib 5).

Materials and Methods

Cloning Experiments and Northern Analysis

A cDNA library in the phage vector lambda-ZapII (Stratagene, La Jolla, CA) was generated using as a template poly-A⁺ RNA purified from cultured MG-63 cells. This human osteosarcoma cell line was previously shown to produce significant amounts of fibrillin (Sakai et al., 1986). Generation, screening, identification and purification of recombinant phages were accomplished by standard procedures (Sambrook et al., 1989). The 5' furthest cDNA was generated using the 5' RACE system for rapid amplification of cDNA ends, according to the manufacturer's recommendations (Life Technologies Inc., Gaithersburg, MD). Positive cDNA inserts were sequenced using the dideoxynucleotide chain termination procedure on double-stranded DNA (Zagursky et al., 1986). Sequences were obtained by multiple sequencing of both strands of cDNA inserts; they were analyzed using the computer program MacVector (International BioTechnologies, Inc., New Haven, CT). For Northern analysis, ~10 µg of total RNA was fractionated on a 0.8% agarose gel in formamide/formaldehyde, transferred onto a nylon membrane (Amersham Corp., Arlington Heights, IL), and hybridized at high stringency to fibrillin cDNA probes uniformly labeled by random priming (Sambrook et al., 1989). To insure specificity, we chose as probes two cDNA inserts of comparable size, ~1 kb; they are: clone F-35, which covers the 3' non-coding region of the FBNI mRNA (Pereira et al., 1993), and the internal EcoRI insert of clone A06-13 (see Fig. 1). The latter sequence exhibits 40% homology to the equivalent region of the FBNI gene, whereas there is only 6% homology in the untranslated segment of the two transcripts.

Antibody Generation

Fibrillin antibodies were raised against recombinant peptides generated in the *Escherichia coli*-based expression system pET-3xa (Novagen, Inc., Madison, WI). To this end, the sequences coding for region C of the two fibrillins (see Fig. 3) were adapted to *E. coli* codon usage by ligating several overlapping synthetic oligonucleotides prior to subcloning into the expression vector (Sambrook et al., 1989). Fusion products were expressed and isolated according to the manufacturer's protocol. Approximately 1 mg of gel-purified fusion protein was injected subcutaneously into rabbits. The injection was repeated at 2-wk intervals for a total of 12 wk and serum was collected by ear vein puncture. Pre-immune sera were also collected from the same rabbits to test for specificity.

Protein Analysis

For metabolic labeling experiments, MG-63 cells were cultured in cysteine-free and serum-free DME medium containing 60 µCi/ml [³⁵S]cysteine (Amersham Corp.) (Sakai et al., 1986). After an overnight incubation, the culture medium was harvested and placed immediately on ice with the addition of 5 mM EDTA, 50 µM *N*-ethylmaleimide, and 50 µM phenylmethylsulfonyl fluoride (Sakai et al., 1986). Labeled cell culture medium was incubated with each unpurified fibrillin antibody coupled to protein A-Sepharose for 3 h at 4°C. This was followed by extensive washing with PBS, and subsequently with RIPA buffer (150 mM NaCl, 1% NP-40, 0.5% DOC, 0.1% SDS, and 50 mM Tris, pH 8.0). Bound material was eluted with gel electrophoresis loading buffer (1% SDS, Tris 10 mM, pH 8, 5% glycerol,

and 2.5% β-mercaptoethanol) and analyzed by fractionation on a 4% polyacrylamide gel. For Western blot analysis, unlabeled immunoprecipitates were electrophoresed onto nitrocellulose paper after gel fractionation (Harlow and Lane, 1988). The filter was blocked with 3% BSA in 10 mM Tris, pH 8.0, 150 mM NaCl and 0.05% Tween-20, before incubation with each of the fibrillin antisera (Harlow and Lane, 1988). After 1-h incubation at room temperature the filter was washed, incubated for 30 min with alkaline phosphatase-conjugated anti-rabbit IgG (Promega Corp., Madison, WI), washed again, and incubated with the substrate to visualize the antibody-bound electrophoretic band (Harlow and Lane, 1988). Pre-immune serum was included as a negative control sample.

Immunohistochemistry

For indirect immunofluorescence studies, 20-wk gestation human tissues were immediately frozen, sectioned, fixed in 4% paraformaldehyde and processed as previously described (Sakai et al., 1986). Tissue specimens analyzed using the peroxidase anti-peroxidase method were initially fixed in paraformaldehyde, paraffin-embedded, and processed further using a commercial kit with chromogen AEC as a substrate (Zymed Laboratories, Inc., San Francisco, CA) following the manufacturer's recommendations. Tissue localization of elastic fibers was accomplished using standard Van Gieson elastic staining (Luna, 1968). All studies included preimmune serum as a negative control.

Immunoelectron Microscopy

Fetal bovine tissues were fixed in 4% paraformaldehyde in 0.1 M Sorensen's buffer (pH 7.4) for 4–6 h at 4°C and processed for reductive denaturation according to Gibson et al. (1989). Tissue specimens were dehydrated in a graded series of methanol to 90% at progressively lower temperatures from 4°C to –20°C. Samples were then infiltrated and embedded with Lowicryl K4M (SPI Supplies, West Chester, PA), which was subsequently polymerized by ultraviolet illumination for 24 h at –35°C and an additional 48 h at –10°C. Thin sections of tissue were cut with a diamond knife on a Reichert ultracut microtome and placed on formvar-coated nickel grids. After a 15-min blocking step on drops of 1% BSA in 50 mM Tris containing 100 mM NaCl (pH 7.4), the grids were incubated with primary antibody diluted in blocking solution overnight at 4°C. Following extensive washing and a second blocking step, the grids were transferred to 15-µl drops of secondary antibody for 1 h at room temperature. The secondary antibody, goat F(Ab) 2 anti-rabbit IgG conjugated to 10 nm colloidal gold (BioCell Research Lab., Cardiff, UK), was used at a 1:30 dilution in blocking solution. Immunolabeled sections were counterstained with methanolic uranyl acetate for 2 min followed by lead citrate for 30 s. Controls included the use of non-immunized rabbit serum and omission of primary antibody.

Results

Fibrillins Are Structurally Related Proteins

We previously reported the isolation of MF-23, a partial cDNA that codes for a peptide consisting mostly of EGF-CB repeats (Lee et al., 1991). The conceptual product of this 2.8-kb clone is remarkably similar to the fibrillin protein originally identified by Sakai et al. (1986), and subsequently characterized in full by molecular cloning experiments (Maslen et al., 1991; Lee et al., 1991; Corson et al., 1993; Pereira et al., 1993). To complete the characterization of Fib 5, appropriate sub-fragments of MF-23 were used to screen the MG-63 cDNA library. This and subsequent screenings led to the isolation of several overlapping cDNAs covering the entire coding sequence of Fib 5 and almost 1.5 kb of 3' untranslated sequence (Fig. 1). The experiments also revealed that the original MF-23 cDNA represents the reverse transcribed product of a partially spliced transcript with a long intronic sequence at its 3' end (Fig. 1). Overall, the Fib 5 and Fib 15 sequences are highly homologous (Fig. 1); in addition, Northern analysis showed that the two proteins are

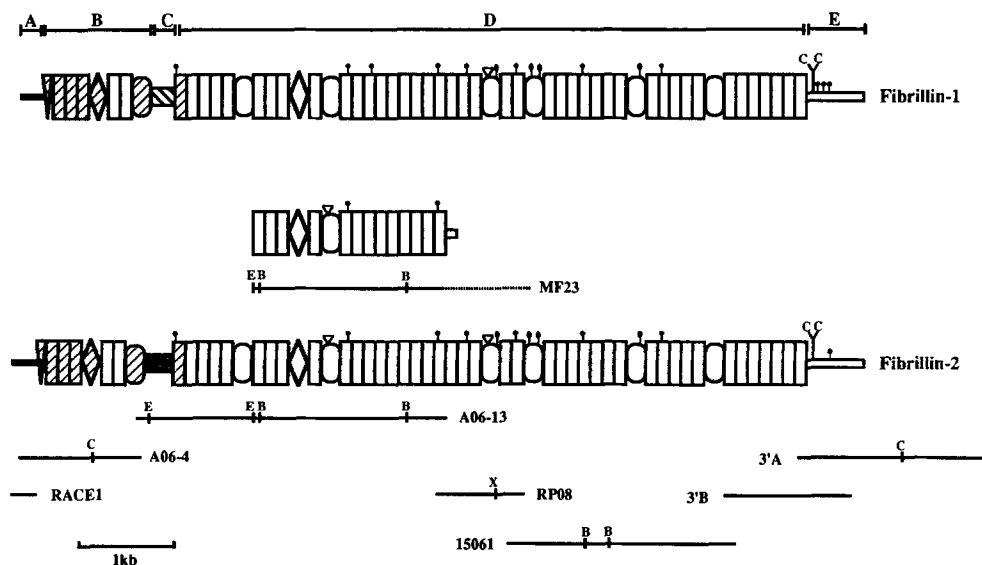


Figure 1. Restriction map of fibrillin-2 cDNAs with a schematic illustration of the fibrillin-1 and fibrillin-2 proteins. Letters indicate the five structurally distinct regions of the latter. Symbols designate the following structural elements: EGF-like repeat (cross-hatched rectangle); EGF-CB repeat (white rectangle); TGF- β repeat (white oval); Fib-motifs (other symbols). Note that region C is depicted differently in the two fibrillins in order to emphasize its compositional diversity. A similar representation of the translational product of the original clone MF-23 is also shown for comparison; the dotted line indicates the unspliced intron sequence

in MF-23. Letter on the cDNAs indicate the following restriction enzymes: Bam HI (B), Cla I (C), Eco RI (E) and Xho I (X). The open triangle and the pin-heads symbolize putative cell attachment and glycosylation sites, respectively (see also Fig. 3).

encoded by transcripts of similar size (Fig. 2). Based on this evidence, Fib 15 and Fib 5 were re-named fibrillin-1 and fibrillin-2, respectively.

Fibrillin-1 is a multidomain protein consisting of five structurally distinct regions termed A, B, C, D, and E (Fig. 1) (Corson et al., 1993; Pereira et al., 1993). Although the precise start site of transcription remains to be determined, a consensus has been reached on the putative start site of translation (Corson et al., 1993). The predicted amino acid sequence of fibrillin-2 shows that this 2,889 amino acid polypeptide can be divided into five regions analogous to domains A-E of fibrillin-1 (Fig. 1).

Pairwise alignment of the fibrillins further emphasizes the close kinship between these two gene products (Fig. 3). In region D, for example, fibrillin-1 and fibrillin-2 contain 41 EGF-CB repeats similarly interspersed among eight non-EGF-CB motifs (Fig. 1). Both fibrillins exhibit the same number of amino acids between the cysteinyl residues in nearly all of the corresponding 49 pairs of cysteine-rich repeats (Fig. 3). In addition, all but one of the putative *N*-gly-

cosylation sites and the potential cell attachment sequence previously noted in fibrillin-1 (Maslen et al., 1991) are conserved in fibrillin-2 (Fig. 3). Fibrillin-2 contains a second RGD sequence within another non-EGF-CB repeat of region D (Fig. 3). The overall level of amino acid homology of region D is $\sim 81\%$.

Pairwise comparison of regions B, A, E, and C revealed several more similarities between fibrillin-1 and fibrillin-2, and at least one important difference. A high level of sequence homology (87%) was noted in region B of the two molecules. Region A, on the other hand, is substantially less homologous (19%) and it contains a novel proline-rich sequence, immediately after the postulated signal peptide cleavage site (Fig. 3). The significance of this finding, if any, is unclear at present. Despite the compositional difference, region A of fibrillin-2 is as basic as region A of fibrillin-1 (est. pIs, 12 versus 11.1). Region E of fibrillin-2 displays also relatively low homology (50%) to the corresponding domain of fibrillin-1. In addition to two polylysine stretches at the carboxy terminus of region E, two cysteine residues found in this domain of fibrillin-1 are maintained in the same position in fibrillin-2 and in the context of a nearly identical sequence (Fig. 3). Finally, region C shows the most striking and important sequence divergence in that this short sequence is glycine rich in fibrillin-2 but proline rich in fibrillin-1.

Fibrillin-2 Is a Microfibrillar Component

The structural diversity of region C provided an experimental means to characterize further the fibrillin proteins. Accordingly, two polyclonal antibodies were raised against recombinant peptides of region C from fibrillin-1 and fibrillin-2 and used to immunoprecipitate radioactively labeled proteins from the medium of cultured MG-63.

Consistent with the cloning and Northern blot data, electrophoretic analysis of the immunisolates revealed that the antibodies interact with proteins of similar size, about 350

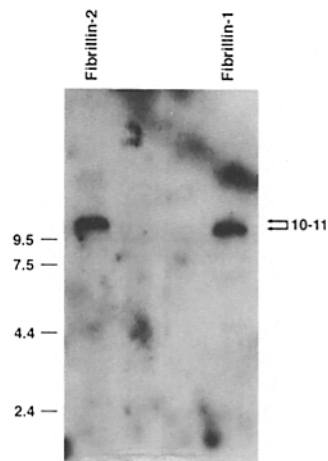


Figure 2. Northern blot hybridization of MG-63 RNA to fibrillin-1 and fibrillin-2 probes. RNA size markers (in kb) are indicated on the left of the autoradiogram, while the estimated range of the two transcripts is shown on the right.

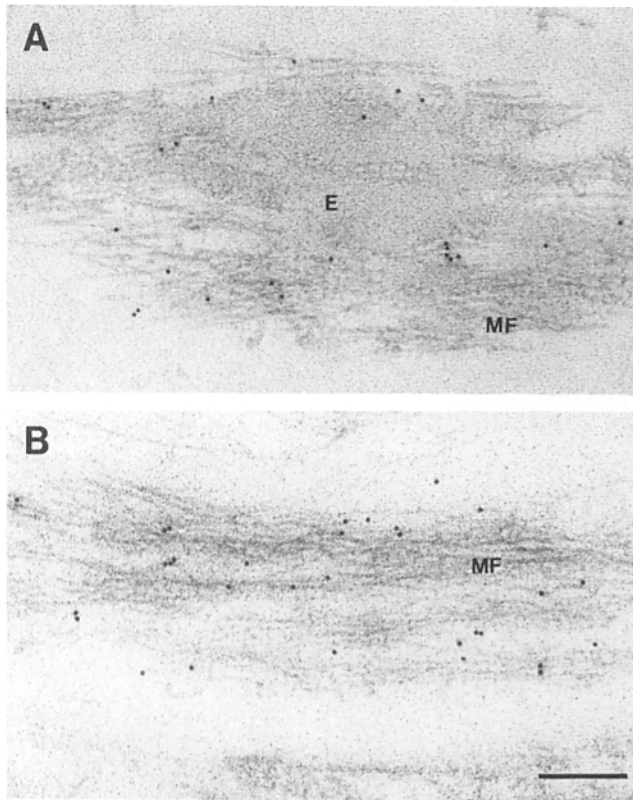


Figure 5. Immunolocalization of fibrillin-2 (*A*) and fibrillin-1 (*B*) on a developing elastic fiber in 170-d gestation bovine ligamentum nuchae. Gold particles label the peripheral mantle of microfibrils (*MF*) of the elastic fiber demonstrating the specific association of fibrillins with the microfibrils. Note that the central core of amorphous elastin (*E*) is devoid of label. Bar, 0.2 μm .

connective tissue across all three layers of the fetal aorta (Fig. 7 *A*), staining with the anti-fibrillin-2 antibody was more intense in the media where elastic fibers are most abundant (Fig. 7 *B*). In elastic cartilage, fibrillin-1 staining was strong to moderate in the connective tissue adjacent to the central elastic cartilaginous core, but the core itself remained unreactive (Fig. 8 *A*). In contrast, this same core region stained strongly and evenly with the anti-fibrillin-2 antibody (Fig. 8 *B*). The same antibody also stained intensely the network of fibrils in the perichondrium directly adjoining the cartilage core (Fig. 8 *B*). In hyaline cartilage, fibrillin-1 epitopes were distributed throughout the cartilage and surrounding matrices (Fig. 8 *C*), while fibrillin-2 epitopes were mostly identified in the peripheral areas of the cartilage and in the perichondrium (Fig. 8 *D*). Interestingly, staining with the anti-fibrillin-1 antibody was somewhat augmented in the hypertrophic zone of the osteogenic cartilage where fibrillin-2 was nearly undetectable (Fig. 8, *E* and *F*).

In the cornea, fibrillin-2 expression was found to be more restricted than that of fibrillin-1, notably to the anterior third of Descemet's membrane (Fig. 9, *A* and *B*). This morphologically distinct portion of Descemet's membrane is organized in a characteristic succession of banded nodes interconnected by strands and is known to be rich in type II collagen fibrils (von der Mark et al., 1977; Hendrix et al., 1982). Fibrillin-1 was predominantly found in the outer layer and in-

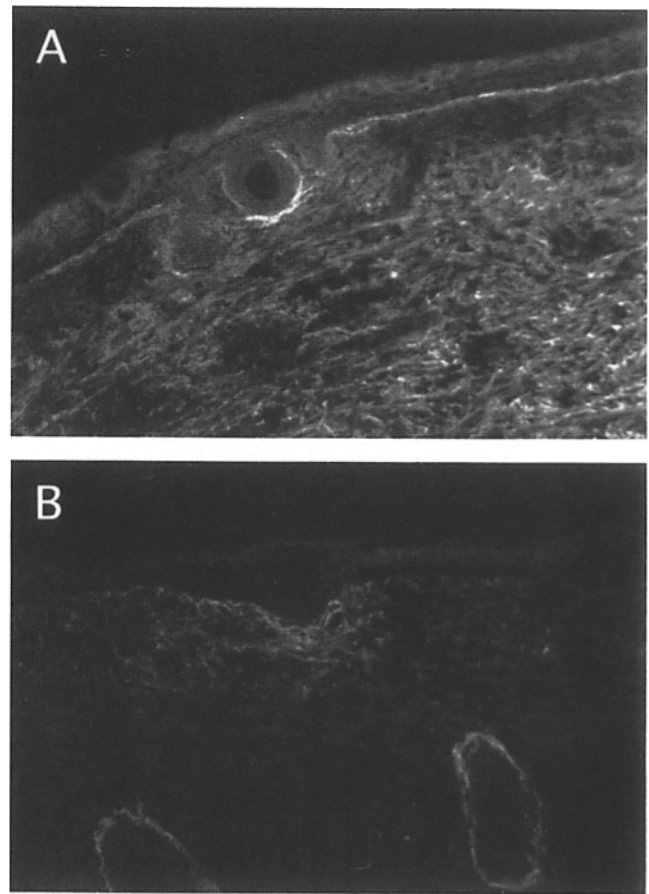
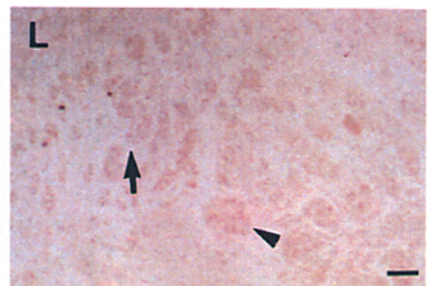
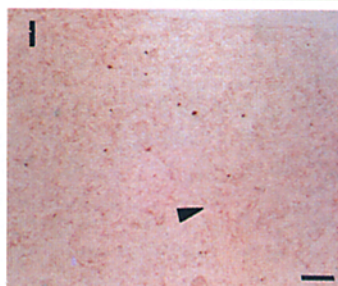
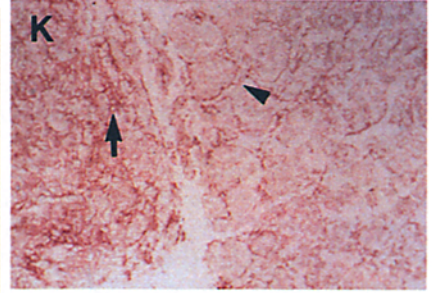
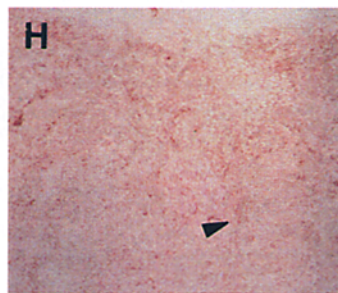
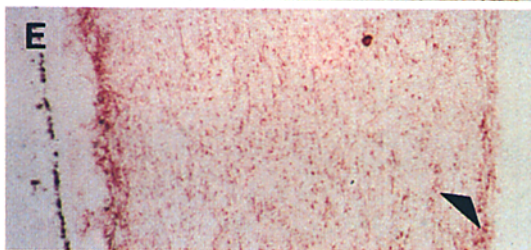
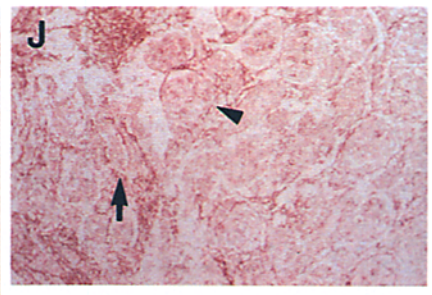
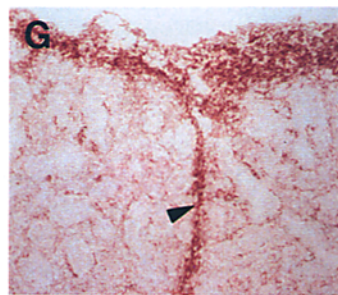
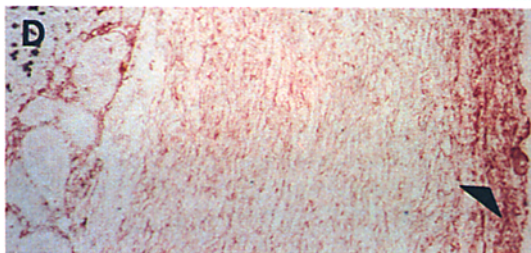
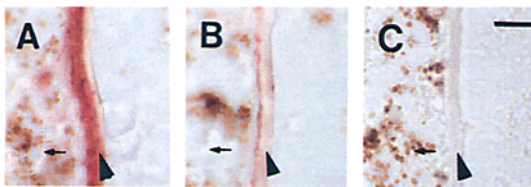
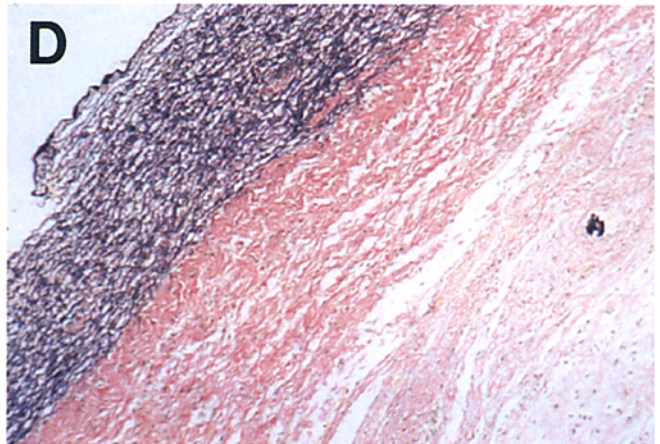
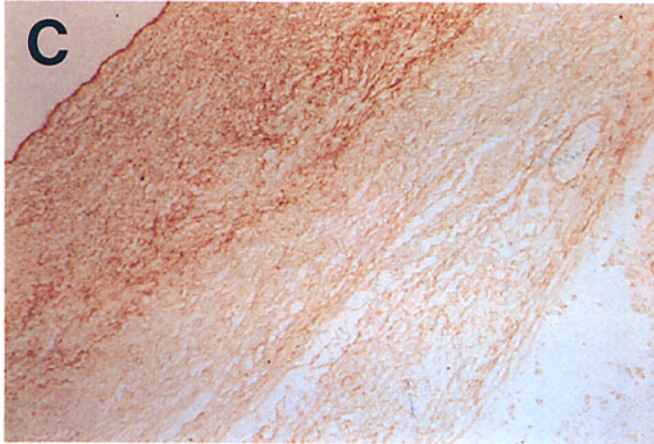
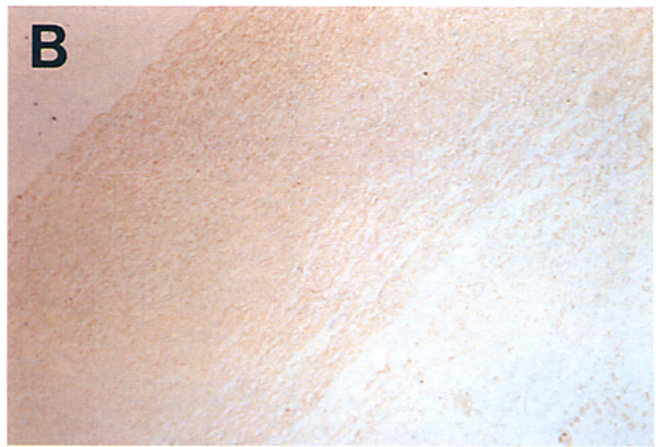
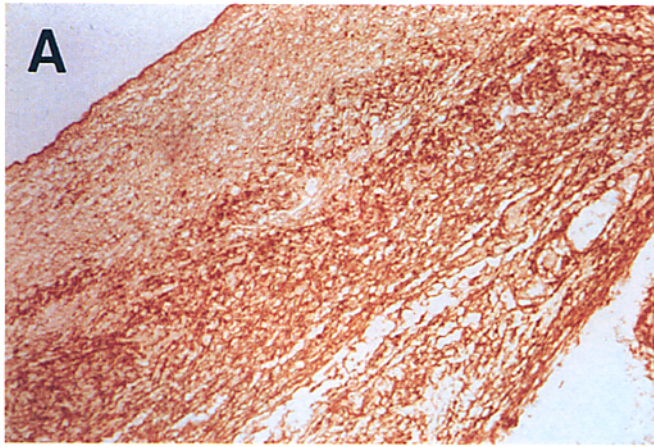


Figure 6. Indirect immunofluorescence localization of fibrillins epitopes in human fetal skin using anti-fibrillin-1 (*A*) and anti-fibrillin-2 (*B*) antibodies. Bar, 100 μm .

ner vascular choroid layer of the sclera, while fibrillin-2 was evenly distributed throughout (Fig. 9, *D* and *E*). A similar pattern of even, albeit low, fibrillin-2 distribution was observed in the lung where fibrillin-1 staining was significantly higher, particularly in the interlobular septa (Fig. 9, *G* and *H*). In contrast, the anti-fibrillin antibodies exhibited similar staining patterns in the basement membrane of the glomeruli and in the developing collecting ducts of the fetal kidney (Fig. 9, *J* and *K*).

Discussion

The term "microfibrils" was originally used to identify morphologically similar matrix aggregates lacking the characteristic banding periodicity of interstitial collagen fibers (Low, 1962). This broad definition was subsequently narrowed to include only microfibrils with a diameter of 10 nm that may or may not be associated with elastic fibers (Cleary and Gibson, 1983). The complete inventory of the macromolecular constituents of the microfibrils is presently unknown, chiefly because of the highly insoluble nature of these structures. Biochemical and immunochemical techniques have nevertheless identified a number of distinct microfibrillar-associated macromolecules (Gibson et al., 1989). Structural information about some of these microfibrillar components has



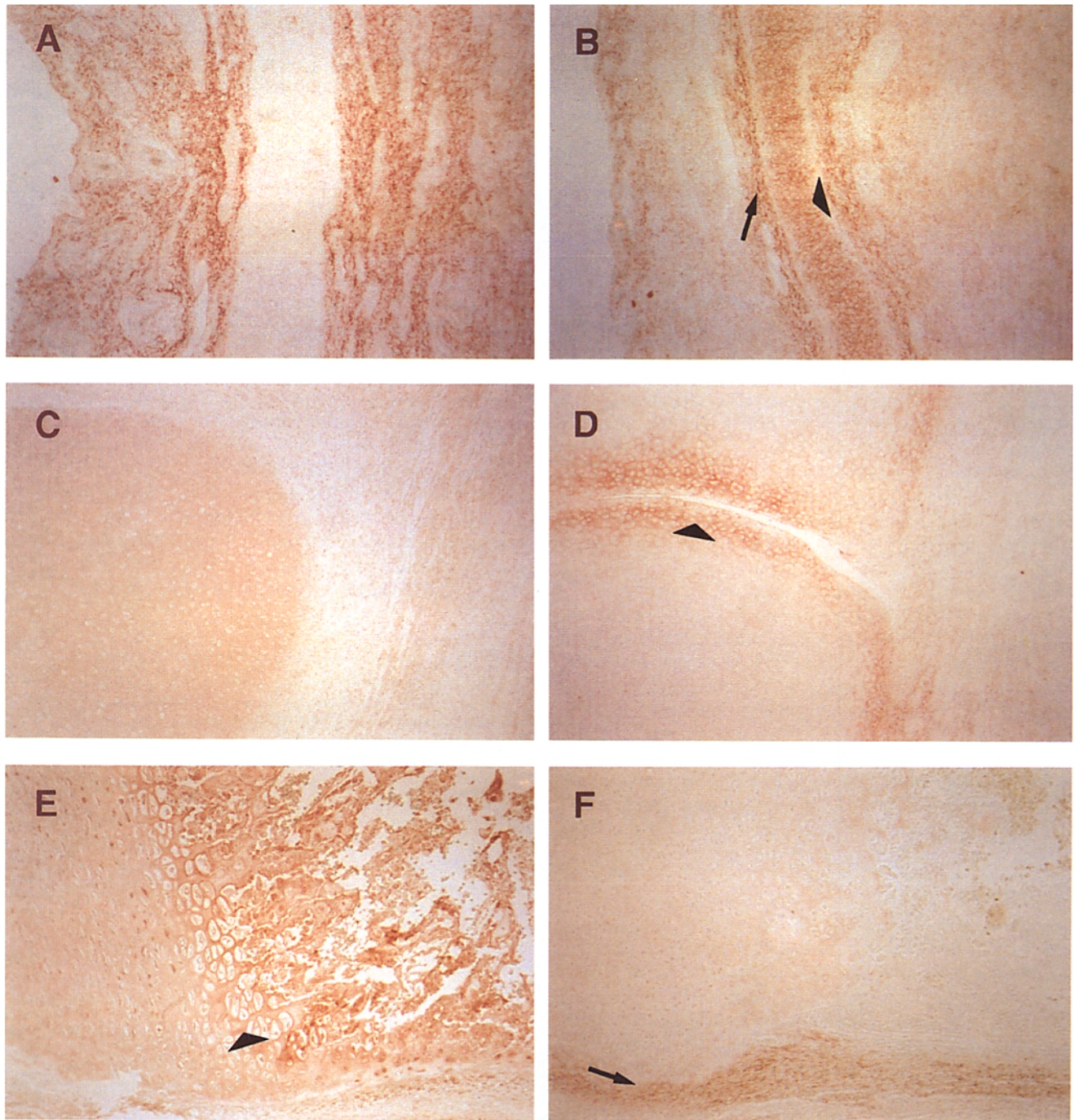


Figure 8. Immunohistochemical localization of fibrillins in human fetal ear (A and B) and toe (C–F) using anti-fibrillin-1 (A, C, and E) and anti-fibrillin-2 (B, D, and F) antibodies. In all panels the arrow highlights the perichondrium. On the other hand, the arrowhead points to the cartilaginous core in B, the peripheral areas of cartilage in D, and the hypertrophic zone in E. Staining with pre-immune serum was negative and localization of elastic fiber-containing areas was as for the analyses shown in Fig. 5 (data not shown). Bar, 100 μm .

Figure 7. Immunohistochemical localization of fibrillins in human fetal aorta using anti-fibrillin-1 (A) and anti-fibrillin-2 (C) antibodies. B and D show samples treated with preimmune serum and stained for elastic fibers, respectively. Note that the lumen of the aorta is always at the top left corner. Bar, 200 μm .

Figure 9. Immunolocalization of fibrillins in human fetal ocular tissues (A–F), lung (G–I) and kidney (J–L) using anti-fibrillin-1 (A, D, G, and J) and anti-fibrillin-2 (B, E, H, and K) antibodies. C, F, I, and L show samples treated with pre-immune serum. The arrowhead highlights the Descemet's membrane (A–C), the outer layer of the sclera (D–F), the interlobular septa of the lung (G–I) and the glomeruli of the kidney (J–L). In A–C, the arrow points to the anterior portion of the cornea, in which stained necrotic debris are noticeable, and to the developing collecting ducts in J–L. Bars: (A–C) 10 μm ; (D–L) 50 μm .

been recently gathered from the cloning of the genes coding for the 31-kD microfibril-associated glycoprotein (MAGP), the 58-kD associated microfibril protein (AMP) and the 350-kD fibrillin (Gibson et al., 1991; Chen et al., 1993; Horrigan et al., 1992; Maslen et al., 1991; Lee et al., 1991; Corson et al., 1993; Pereira et al., 1993). Contrary to previous evidence, cloning of a partial transcript suggested that fibrillin may represent a small family of extracellular proteins (Lee et al., 1991). Results presented in this paper confirm and extend this observation; in addition, they provide the first comparison of the tissue distribution of the fibrillin family members.

The sequencing data indicate that the structure of fibrillin-2 replicates very closely that recently established for fibrillin-1 (Maslen et al., 1991; Lee et al., 1991; Corson et al., 1993; Pereira et al., 1993). As a result, the structural/functional criteria defining a fibrillin molecule are beginning to emerge. Accordingly, such a macromolecule is expected to be made of five structurally distinct regions preceded by a short signal peptide. Two of these regions are composed of cysteine-rich sequences that can be segregated into three distinct groups. The first and largest, comprises numerous EGF-CB repeats and fewer EGF-like repeats (Davis, 1990). The second group features an 8-cysteine motif which was first described interspersed among the EGF-like repeats of the TGF- β 1-binding protein, and is termed the TGF- β p motif (Kanzaki et al., 1990). The third class of fibrillin repeats is heterogeneous in composition and apparently unique to this protein (Corson et al., 1993; Pereira et al., 1993). We have referred to these repeats as the Fib motifs and recently postulated their evolution from various rearrangements of ancestral exons coding for EGF-like and TGF- β p repeats (Pereira et al., 1993). We based our evolutionary considerations on structural homologies and the organization of the coding sequence of the FBN1 gene, which preliminary evidence suggests to be similar in the FBN2 gene.

In our previous work on fibrillin-1, we suggested that region C may be of some potential relevance to microfibril assembly (Pereira et al., 1993). Based on its relative position and composition, we argued that region C may bend the fibrillin molecule and thus facilitate the postulated protein interactions of the cysteine-rich regions (Pereira et al., 1993). Such a scenario needs not to be changed for fibrillin-2, since the sequence of region C could theoretically form a poly-glycine hinge and thus provide the protein with a high degree of flexibility at that position (Claassen and Grossmann, 1991). Region E is also of potential relevance to microfibril assembly, for it contains the same two cysteines already noted in the corresponding segment of fibrillin-1 (Maslin et al., 1981). The maintenance of these residues in two different chains, together with the extensive identity of the sequence around them, may conceivably indicate that they participate in the polymerization of individual fibrillin monomers.

In general, the tissue distribution of fibrillin-1 resembles that of fibrillin-2. There are however some notable differences which are likely to imply distinct functions of the two glycoproteins in the assembly and maintenance of the microfibrillar network. The most obvious of these differences is the preferential localization of fibrillin-2 to extracellular matrices that are rich in elastic fibers. This feature is best exemplified in the elastic cartilage of the external ear.

This preferential distribution of fibrillin-2 is also consistent with the abnormal presentation of the CCA auricle which is characterized by flattening of the helix and crumpled appearance of the antihelix with resulting partial obliteration of the concha (Beals and Hecht, 1971). Hyaline cartilage is another tissue that exhibits the intriguing feature of a differential distribution of the two fibrillins, plausibly a reflection of distinct contributions of these proteins to the process of skeletogenesis. Along this line, it is of some interest to note the restricted localization of fibrillin-2 to the type II collagen-rich layer of Descemet's membrane.

It is likely that the contribution of fibrillin-2 to tissue integrity will be eventually clarified with the analysis of naturally occurring mutations in humans and artificially generated ones in transgenic mice. Along these lines, the complete characterization of the FBN2 gene and the detailed examination of its early developmental pattern of expression are both necessary preconditions to undertaking these investigations. From this and related work, it is becoming apparent that the function of the 10-nm microfibrils is likely to depend on the integrity and proper spatio-temporal expression of several distinct gene products. More generally, the microfibrillar aggregates with or without elastin cores are increasingly becoming appreciated as critical players in providing biomechanical properties to a wide variety of tissues and organ systems.

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