

FIBULINS: A VERSATILE FAMILY OF EXTRACELLULAR MATRIX PROTEINS

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Fibulins are a newly recognized family of extracellular matrix proteins. The five known members of the family share an elongated structure and many calcium-binding sites, owing to the presence of tandem arrays of epidermal growth factor-like domains. They have overlapping binding sites for several basement-membrane proteins, tropoelastin, fibrillin, fibronectin and proteoglycans, and they participate in diverse supramolecular structures. New insights into their biological roles are now emerging from studies of transgenic mice and of some inherited human diseases.

The production of various forms of extracellular matrix (ECM) was an important event in early metazoan evolution — it provided the basis for the organization of multicellular tissues with a high stability that was based on cell–cell and cell–matrix interactions. The ECM is very diverse in vertebrates. It includes structures such as the large collagen fibrils that endow tendons, bones and skin with considerable mechanical strength, the thin basement membranes that maintain the differentiation of sheets of epithelial and endothelial cells, and microfibrils and elastic fibrils that provide elasticity and resilience to specialized tissues such as arteries and lungs. Many proteins have been shown to be involved in these supramolecular structures and they have been classified into several families on the basis of related domain structures and/or functions. Several of the larger families include at least 27 types of collagen, 15 isoforms of the basement-membrane protein laminin and a similar number of large and small proteoglycans. They form complex structures by homotypic and heterotypic interactions, which can by far exceed the size of individual cells. Some of these proteins are also ligands for adhesive and/or signalling cellular receptors. Several smaller families of ECM proteins have been identified and they might, in part, have modulating cellular functions. A recent addition to the ECM proteins includes the fibulins, and five isoforms have been identified so far (TABLE 1). They share tandem arrays of calcium-binding consensus sequences and have a diverse repertoire of interaction

potentials, which makes them widespread components of the ECM.

The first member of the fibulin family — now referred to as **fibulin-1** — was identified in affinity chromatography experiments that used the short cytoplasmic tail of $\beta 1$ integrin receptors¹. The main component that was isolated from human placenta had a molecular mass of 90–100 kDa (from electrophoresis measurements), was shown to bind calcium and was considered to be a connecting element between $\beta 1$ integrins and some cytoskeletal structures. It was therefore named after the latin word *fibula*, which means clasp or buckle. This view of fibulin-1 was soon changed because complementary DNA cloning showed the presence of a signal peptide and a modular structure that is characteristic of ECM proteins. This was consistent with other observations, which found that fibulin-1 in fibroblast cultures was deposited as extracellular fibrils and was also secreted into the blood in substantial amounts². The same protein could be also isolated from a mouse tumour basement membrane, and was shown to be a ubiquitous basement-membrane protein by immunostaining³. Its incorporation into basement membranes is mediated by strong binding interactions with laminins and nidogens⁴. A second, larger isoform — **fibulin-2** — was subsequently identified by cDNA sequencing^{5,6}, and a disulphide-linked dimer of 200-kDa monomers was obtained by recombinant production in mammalian cells^{7,8}. Three further closely related proteins — **fibulin-3**, **fibulin 4** and **fibulin-5** — of ~50–60 kDa

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Table 1 | Size and sequence analysis of fibulin isoforms

Isoform*	Species	No. of amino-acid residues [‡]	Accession number [§]	References
Fibulin-1A	Human	537 (29)	NM_006487	2
Fibulin-1B	Human	572 (29)	NM_006485	2
Fibulin-1C	Human	654 (29)	NM_001996	2
	Mouse	656 (29)	X 70853	4
Fibulin-1D	Human	675 (29)	NM_006486	9
	Mouse	675 (29)	NM_010180	4
Fibulin-2	Human	1157(27)	NM_001998	6
		1204 (27)	AH011811	
	Mouse	1196 (26)	NM_007992	5
Fibulin-3 (S1-5, EFEMP1)	Human	466 (27)	NM_004105/ NM_018894	14
	Mouse	466 (27)	NM_146015	
Fibulin-4 (EFEMP2, PH1, MBP1)	Human	416 (27)	NM_016938	10
	Mouse	416 (27)	NM_021474	33
Fibulin-5 (DANCE, EVEC)	Human	367 (23)	NM_006329	69
	Mouse	367 (23)	NM_011812	69

*Alternative names are given in parentheses. †The number of amino acids in the predicted mature protein and in the signal peptide (in parentheses) is shown. §The accession number refers to information in the GenBank database (see Online links). DANCE, developmental arteries and neural crest epidermal growth factor (EGF)-like; EFEMP, EGF-containing fibulin-like extracellular matrix protein; EVEC, embryonic vascular EGF-like repeat-containing protein; MBP1, mutant p53-binding protein 1.

were identified more fortuitously by a systematic search for new secreted proteins, and were therefore initially named using various acronyms (TABLE 1) before they were classified as fibulins^{9,10}. The fibulin nomenclature has now been generally accepted and is used throughout this review.

This review covers the structure of the fibulin genes and their corresponding proteins, as well as their expression during embryonic development and in adult tissues. Studies of fibulin expression have shown the versatile incorporation of fibulins in different ECM compartments and have led to the extensive characterization of potential ligands, in particular for fibulin-1 and fibulin-2, which are discussed in detail. Finally, the potential interactions of fibulins with cellular receptors, as well as what we have learned about their biological roles from transgenic mouse models and inherited human disorders, are discussed.

The fibulin protein family

Complete sequences have been determined for the five fibulin isoforms of humans and mice (TABLE 1) and they have revealed that there is about 90% identity between

the fibulins of these species. The predicted mature proteins vary in size (367–1196 residues) and, as discussed below, can be further modified by alternative splicing. Sequences are also available for the fibulin-1C and fibulin-1D variants of zebrafish¹¹, *Caenorhabditis elegans*¹², chicken¹² and *Drosophila melanogaster* — these variants are somewhat larger than their mammalian counterparts and have a lower sequence similarity. This indicates that fibulin-1 is ancient in origin and that there has been a distinct modulation of its function during evolution.

Modular protein structure. Fibulin-1 was the first family member to be identified and it shows a distinct arrangement of typical ECM modules^{2,4} that are grouped together as domains I, II and III (FIG. 1). The amino-terminal domain I consists of three anaphylatoxin-like (AT) modules, each ~40 residues long and containing four or six cysteines. The structure of an AT module was determined previously for the complement-derived anaphylatoxin C3a (REF. 13), and it was found to be a compact α -helical fold that is stabilized by three disulphide bridges in the pattern Cys1–4, Cys2–5 and Cys3–6 (where Cys is cysteine). The central domain II is composed

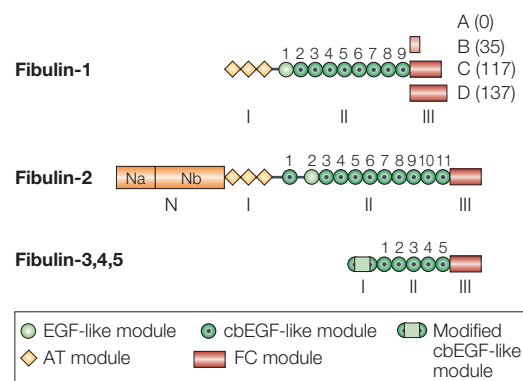


Figure 1 | The modular domain structure of fibulins as predicted from their sequence. The fibulins have a distinct arrangement of typical extracellular matrix modules that are grouped together as domains I, II and III. The four variants of fibulin-1 are shown, and variants C and D contain the fibulin-type carboxy-terminal (FC) module. Domain N, which can be subdivided into a cysteine (Cys)-rich segment (Na) and a Cys-free segment (Nb), is unique to fibulin-2. Fibulin-3, fibulin-4 and fibulin-5 have a modified calcium-binding (cb) epidermal growth factor (EGF)-like module at their amino terminus, which is referred to as domain I. As well as an extra Cys5–Cys6 loop at the beginning of the module, this modified cbEGF-like module has a link region of unusual length (28–88 residues) between the usual Cys2–Cys4 and Cys5–Cys6 loops¹⁰. AT, anaphylatoxin-like.

of nine epidermal growth factor (EGF)-like modules, which are also ~40 residues long with three disulphide bridges (in the pattern Cys1–3, Cys2–4 and Cys5–6). Most of these **EGF-like modules** have a consensus sequence for calcium ligation, and they are known as calcium-binding EGF (**cbEGF**)-like modules. The carboxy-terminal domain III, which is ~120–140 residues long with only two extra cysteine residues, resembles a new protein module (FC; fibulin-type carboxyl terminus) that is shared by fibulins and fibrillins at the carboxyl terminus¹⁰. A similar modular arrangement, with some modifications⁵, is also characteristic of the carboxy-terminal segment of fibulin-2 (FIG. 1), but, depending on the domain, it shows only 26–55% sequence identity with fibulin-1. Unique to fibulin-2 is a 400-residue amino-terminal domain — domain N — which can be subdivided into a cysteine-rich segment that is known as Na (12 cysteines in 150 residues) and a cysteine-free segment that is known as Nb. The shortest isoforms identified so far in the family are fibulin-3, fibulin-4 and fibulin-5, and they share a central segment of five cbEGF-like modules and a carboxy-terminal domain III of variable sequence identity with the other fibulins^{10,14} (FIG. 1). They have a unique amino-terminal domain I, which seems to be a modified cbEGF-like module that maintains its calcium-binding property (FIG. 1).

The structures of fibulins or their individual domains have not, so far, been determined at atomic resolution. X-ray or NMR structures are, however, available for anaphylatoxin C3a (FIG. 2a) and for cbEGF-like domain pairs from **fibrillin-1** (FIG. 2b), and these can be used as models for the related structures in fibulins.

Alternative splicing. The structure of most of the fibulins can be modified further by alternative messenger RNA splicing. This was initially shown for domain III of human fibulin-1 (REFS 2,9), which can be completely eliminated in variant A or replaced by a short variant B (35 residues) in contrast to the longer variants C (117 residues) and D (137 residues) (FIG. 1). Mice⁴ and presumably most other species express only variants C and D, which have been shown to be produced in comparable amounts in most tissues and cell lines studied^{4,9}. Variants A and B have so far only been detected at low levels in the human placenta.

Alternative splicing of fibulin-2 causes the elimination of a single cbEGF-like module in domain II (cbEGF3; FIG. 1) and no variants have been detected for domain III (REF. 5). Five different splice variants have been described for human fibulin-3, which show a partial or complete deletion of the amino-terminal domain I (REF. 14). Only the largest and smallest variant are expressed in substantial amounts at the protein level, and two intermediate variants are predicted to lack signal peptides and therefore might not be secreted. A similar amino-terminal truncation with the loss of the signal peptide also results in a weak level of expression of human fibulin-4 (REF. 15).

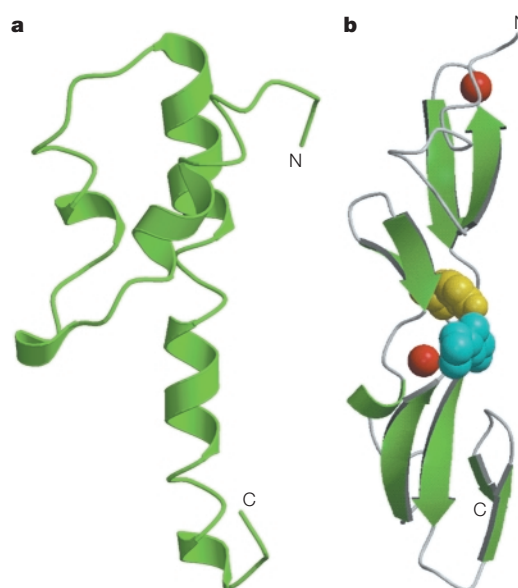


Figure 2 | Potential structural models for anaphylatoxin-like modules and calcium-binding epidermal growth factor-like domain pairs in fibulins. a | The structure of complement-derived anaphylatoxin C3a as determined by X-ray crystallography. The analogous part of fibulin anaphylatoxin-like (AT) modules comprises only the upper amino-terminal half of anaphylatoxin C3a, which contains the three disulphide bonds. This figure was kindly provided by R. Huber (Max Planck Institute for Biochemistry, Martinsried, Germany) and the original structure was published in REF. 13. **b** | The NMR structure of a pair of calcium-binding epidermal growth factor (cbEGF)-like modules from fibrillin-1. The red balls indicate calcium bound to the amino-terminal consensus sequence in each module. A hydrophobic interaction at the interface between the modules is highlighted in yellow and cyan. This figure was kindly provided by A. K. Downing (Oxford University, UK) and the original structure was published in REF. 17.

The fibulin genes. The genes that encode the fibulins (*FBLN1*, *FBLN2*, *FBLN3*, *FBLN4* and *FBLN5*) have been mapped to different autosomal human chromosomes and to some autologous regions in the mouse (TABLE 2). Their exon–intron structures have been determined (FIG. 3) and they explain the occurrence of alternative splice variants.

Calcium-binding properties. Many ECM proteins have been shown to bind calcium with moderate to high affinity and various types of protein modules can be involved in this ligation. This binding can be crucial for structural stability and is often required for efficient binding to other ECM ligands and cellular receptors. Furthermore, as the extracellular calcium concentration is maintained at ~1 mM, it is probable that most of these calcium-binding proteins are permanently saturated with calcium¹⁶.

A special class of EGF-like modules, which bind calcium and are referred to as cbEGF-like domains, are abundant in ECM proteins, including domain II of the fibulins (FIG. 1). They are characterized by the consensus sequence D-X-D/N-E (where D is aspartate, X is any

Table 2 | Chromosomal localization and structural organization of the fibulin genes

Fibulin gene*	Chromosome		Exon size and number		References
	Human	Mouse	Human	Mouse	
<i>FBLN1</i>	22q13.2–13.3	15E–F	96 kb, 20 exons	75 kb, 18 exons	84,85
<i>FBLN2</i>	3p24–p25	6D–E	89 kb, 18 exons	55 kb, 18 exons	6,86
<i>FBLN3</i>	2p16	11A3.3	58 kb, 13 exons	74 kb, 11 exons	87
<i>FBLN4</i>	11q13	19A	6.5 kb, 11 exons	ND	15,88
<i>FBLN5</i>	14q31	12F	79 kb, 11 exons	73 kb, 11 exons	69,89

*For more information on gene structure, please refer to the human and mouse genome resources at the National Center for Biotechnology Information web site (see Online links). ND, not determined.

amino acid, N is asparagine and E is glutamate) before the first cysteine residue¹⁷. Calcium binding has only been shown for fibulin-1 so far^{1,3}, but it is probable that all fibulins bind calcium, as indicated by the loss of several binding activities in the presence of EDTA (see below). Furthermore, domain II of fibulin-1 is resistant to matrix metalloproteinases and other tissue proteases in the presence of calcium, and only a few bonds are cleaved in domain II of fibulin-2 in the same conditions¹⁸. The addition of EDTA, however, increases protease sensitivity in both cases¹⁸.

The structure of a cbEGF-like domain pair from fibrillin-1 has been determined and it showed that calcium is ligated by several residues both of the consensus sequence and of the loop between Cys2 and Cys4, as well as by a strong, hydrophobic intermodular contact¹⁷ (FIG. 2b). These intermodular interactions are thought to endow such tandem arrays with a high rigidity, which stabilizes the long, rod-like structure of fibrillins and fibulins.

Electron-microscopy studies. The shapes of the various fibulin isoforms have been determined using electron microscopy^{8,10,19} (FIG. 4). This showed a 30-nm dumbbell-like structure for fibulin-1, in which domains I and III contribute small globular domains at opposite ends of the 20-nm-long rod of domain II. A minor fraction of fibulin-1 forms small non-covalent aggregates through numerous associations of one of its globular domains. Fibulin-3, fibulin-4 and, most likely, fibulin-5, on the other hand, consist of a rod (10–20 nm) that is connected to a single globular domain at the carboxyl terminus.

Fibulin-2 has a more complex structure, as it forms disulphide-linked dimers through an extra cysteine residue in the central AT module of domain I. Mutation of this cysteine, however, does not prevent non-covalent dimerization through interactions between the rod-like domain II and the N domain ($K_d = 0.2–0.7 \mu\text{M}$). The present model for the arrangement of fibulin-2 dimers⁸ proposes the anti-parallel association of two monomers through interactions between the central globular domains I and between the aligned domains II and the N domains, which gives a total dimer length of 70–80 nm (FIG. 4). This shape shows some plasticity and might change to a three- or four-arm structure by dissociation of one or both of the domain-II–N-domain associations. All of

the fibulins are glycoproteins that have several N-linked acceptor sites. Domain N of fibulin-2 can also contain ~20 O-linked oligosaccharides, which indicates that it has a mucine-like nature and, in part, explains the rod-like shape of this domain.

Expression and tissue deposition

The identification of a new family of ECM proteins usually generates questions about their specific functions and their associations with different tissue compartments as an initial approach to understanding their biological role. Information regarding the tissue deposition of all of the fibulin isoforms has been obtained using northern blots, *in situ* hybridization and immunohistology. The resulting data are contained in about 30 % of all of the fibulin publications that are available so far, and therefore only a selection of this information is discussed in detail here.

The most comprehensive analysis of expression and tissue deposition was reported for fibulin-1 in human adult tissues and cultured cell lines, and provided the first evidence for an association between fibulin-1 and elastic fibres²⁰. This association seems to occur through the amorphous elastin component of the elastic fibres and not through the microfibrillar structures (BOX 1). Fibulin-1 is also widely expressed in the ECM of various organs, in which it either associates with matrix fibres or with basement membranes³. Fibulin-2 shows a more restricted tissue distribution, which only partially overlaps with that of fibulin-1 (REFS 5,6). Fibulin-3 and fibulin-4 show a similar incomplete overlap in distribution in mouse tissues, and they were mainly detected in the walls of capillaries and larger blood vessels, in some basement membranes and, in part, in PERINEURAL TISSUE¹⁰. Some of these data were confirmed using northern blots^{14,15}, which also detected a particularly strong expression of fibulin-3 in the eye²¹. Fibulin-5 seems to be strongly expressed in large arteries and was localized to the elastic lamina close to endothelial cells^{22,23}. A broader study has not yet been carried out for fibulin-5.

The synthesis of fibulin-1 starts at early stages of embryonic development and it is a constituent of most basement membranes in the avian embryo²⁴. In zebrafish, it can be detected as early as the blastula stage and later during heart and mesoderm development¹¹. Although fibulin-2 synthesis starts somewhat later than fibulin-1 synthesis, both of these proteins are already at relatively high levels at the onset of organogenesis in the

EDTA
(ethylenediamine tetra-acetic acid). A strong chelator of bivalent cations.

K_d VALUE
The equilibrium dissociation constant of bimolecular reactions.

PERINEURAL TISSUE
The tissue around a nerve or group of nerves.

ELASTOTIC SKIN
Skin that shows degenerative changes of the elastic fibres. The condition can be associated with skin diseases, ageing or prolonged exposure to sunlight.

CONTACT DERMATITIS

Itching, redness or inflammation of the skin that is caused by direct exposure to irritating substances, such as chemicals, metals, clothing, cosmetics and plants.

mouse (embryonic day 10)²⁵ and in humans²⁶. Both fibulins are subsequently expressed at many sites of epithelial–mesenchymal interactions, in several basement membranes and vessels, and fibulin-2 is a specific marker of the early stages of cartilage development and bone calcification. High levels of fibulin-1 and fibulin-2

expression were observed during avian^{24,27,28} and murine^{29–31} heart development in the endocardial cushion tissue, which consists of a hyaluronan-rich matrix (cardiac jelly) and represents the precursor of aortic valves and heart septa. There is evidence that the fibulins bind to the proteoglycan **versican** that associates with the hyaluronan-rich matrix, a pattern that changes on further maturation³². Early expression (embryonic day 7–9.5) has also been observed for mouse fibulin-4 (REF 33) and fibulin-5 (REFS 22,34). Their synthesis decreases during adult stages, but is restimulated on arterial injury.

There are several interesting aspects of fibulin expression. Fibulin-1 can be detected in human and mouse serum²³ at relatively high concentrations (10–50 µg/ml), whereas the concentration of fibulin-2 is 1000-fold lower⁵, which is more typical of an ECM protein. This indicates that the circulating form of fibulin-1 has specific functions, which are so far not known, and the sites of its synthesis are also as yet unknown. Another interesting aspect is the deposition of fibulin-1 and fibulin-2 in various, but different, neuronal structures and the synthesis of fibulin-1 by several neuronal cell lines^{20,26–35}. As the brain shares only some of the ECM proteins that are found in the body, this indicates a specific neuronal function for the fibulins. Several immunohistological studies have shown a close colocalization of some fibulins and other prominent ECM proteins, which is indicative of molecular interactions. The results include the colocalization of fibulin-2 with fibrillin-1 in various tissues³⁶ and of fibulin-1 and fibulin-2 with **tropoelastin** in the aorta³⁷. A similar colocalization was also observed between **fibronectin** and fibulin-1 and fibulin-2 in fibroblast cultures² and was restricted, in the case of fibulin-2, to a particular class of amorphous microfibrils³⁸. There might also be this latter colocalization in certain basement membranes such as in those of the testis, but it might not necessarily involve microfibrils³⁹. Two types of microfibril that contain fibulin-2 and either fibrillin-1 or fibronectin are anchored to the epidermal basement membrane and their abundance changes during skin regeneration⁴⁰. There could be similar switches in associations under pathological conditions. An upregulation of fibulin-1 has been detected during cutaneous wound healing⁴¹, in sun-damaged **ELASTOTIC SKIN**⁴² and in **CONTACT DERMATITIS**⁴³. An increased synthesis of fibulin-1 has also been identified in ovarian carcinoma⁴⁴. There might be many more pathological changes that have not yet been identified.

Protein ligands and supramolecular structures

The broad tissue distribution of the fibulins indicates that they have complex binding repertoires for other important ECM proteins (BOX 2), and the binding repertoires of fibulin-1 and fibulin-2 have been extensively studied over the past 10 years (TABLE 3). These studies identified considerable overlap in their binding patterns, with only a couple of ligands being specific for fibulin-1 (**fibrinogen** and **laminin-1**) and for fibulin-2 (**fibrillin-1** and **perlecan**). The affinity of the

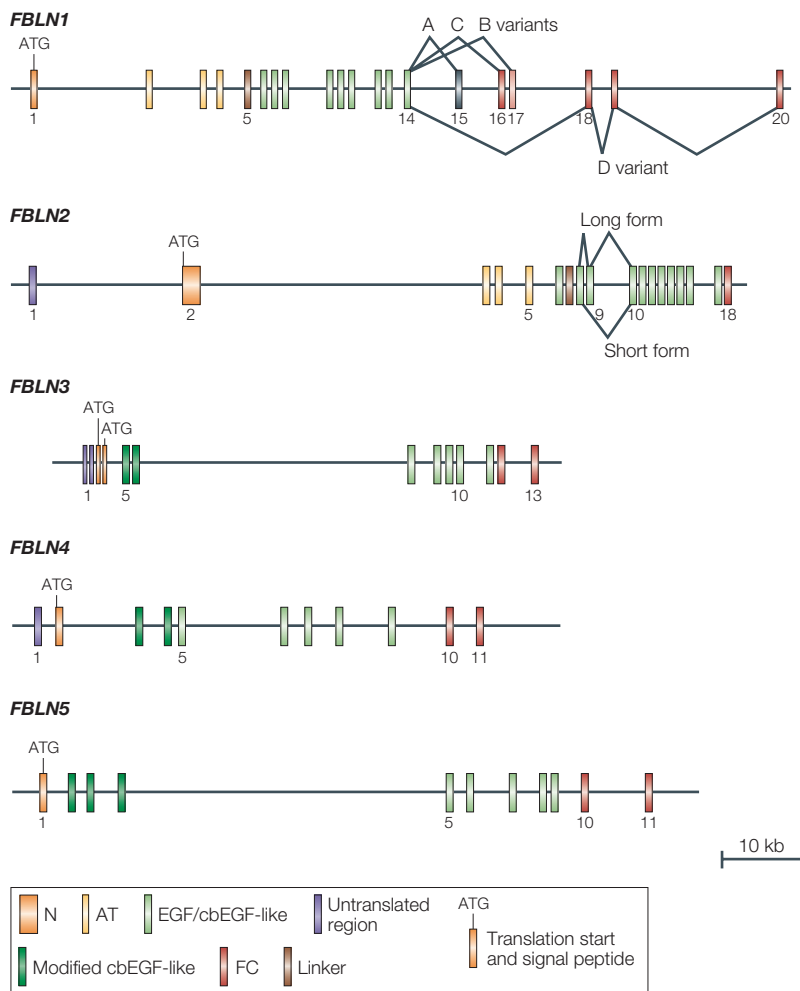


Figure 3 | The exon–intron structure of the human fibulin genes. The maps of each gene are drawn to the same scale except for the short fibulin-4 gene (*FBLN4*) that is enlarged tenfold (see TABLE 2 for gene sizes). The epidermal growth factor (EGF)-like modules and the calcium-binding EGF (cbEGF)-like modules are each encoded by a single exon, except for the last cbEGF-like module of all fibulins. In this case, the sixth cysteine residue of this module is encoded by the exon that encodes the carboxy-terminal domain III. The fibulin-type carboxy-terminal (FC) module in domain III is common to all fibulins (except for variants A and B of fibulin-1). It is encoded by a single exon in the genes for the fibulin-1C variant and fibulin-2 (*FBLN1* and *FBLN2*, respectively), but by two exons in the genes for fibulin-3, -4 and -5 (*FBLN3*, *FBLN4*, and *FBLN5*, respectively) and by three exons in the gene for the fibulin-1D variant (*FBLN1*). An unusually long exon 2 in *FBLN2* encodes the amino-terminal domain N, which is unique to fibulin-2. The modified cbEGF-like module is encoded by two exons in *FBLN3* and *FBLN4* and three exons in *FBLN5*. Most of the fibulin genes undergo alternative splicing that results in messenger RNA variants with altered coding and/or non-coding sequences. In human *FBLN1*, exons 15, 16, and 17 encode domain III of the A, C and B variants, respectively, whereas exons 18–20 together encode domain III of the D variant. Exon 15 starts with a stop codon (TGA) and therefore encodes no extra protein for variant A of fibulin-1. The B, C and D variants of fibulin-1 are colour-coded to match FIG. 1. Similar gene structures were determined for the fibulin-1 genes from mouse⁸⁴ and *Caenorhabditis elegans*¹². In *FBLN2*, exon 9 can be alternatively spliced, so that a short form (cbEGF3 shown in FIG. 1 is absent) or a long form (cbEGF3 shown in FIG. 1 is present) can be produced. In *FBLN3*, the four exons at the 5'-end are used alternatively¹⁴. AT, anaphylatoxin-like.

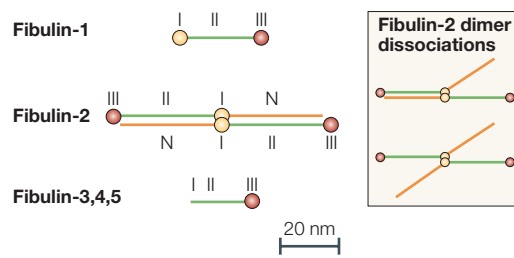


Figure 4 | The shapes of fibulins. The figure shows a schematic representation of the shapes of fibulins as determined by electron microscopy after rotary shadowing^{8,10,19}. The shape of the anti-parallel fibulin-2 dimer is depicted with fully aligned N/II domains. The individual dissociation of these domains can give rise to the three-arm and four-arm structures that are shown, which are still covalently connected through the central domain I (REF. 8).

interactions has been shown to vary over a broad range ($K_d = 0.1$ nM to 3 μ M) and to require, in most cases, the ligation of calcium. This was, in part, supported by the mapping of the binding epitopes to fibulins and/or to their corresponding ligands.

Fibronectin microfibrils. The ECM and serum protein fibronectin (BOX 2) was one of the first fibulin ligands to be identified by showing that it colocalizes with fibulin-1 (REF. 2) and fibulin-2 (REF. 38) in microfibrils that are deposited in fibroblast cultures and by using various direct binding assays^{7,45,46}. The microfibrils were shown to consist of equal amounts of fibronectin and fibulin-2 and to have a lower content of fibulin-1. About 60–70% of fibulin-2 can be extracted from these microfibrils using EDTA, whereas denaturing conditions are required to solubilize fibronectin³⁸. This indicates that the microfibril core structure is formed by fibronectin, to which the fibulins attach. This idea is supported by the lack of fibulin-1 deposition on inhibition of fibronectin assembly in a fibroblast culture^{47,48}. The fibulin-1 binding site has been mapped to the 12–14 type III modules of fibronectin, which have an immunoglobulin (Ig)-like fold and a heparin-binding site⁴⁵. The complementary binding site has been localized to cbEGF5 and cbEGF6 of fibulin-1 (FIG. 1), which are also involved in the limited oligomerization of fibulin-1 (how this oligomerization relates to the non-covalent aggregates mentioned above is still unclear)⁴⁶. This oligomerization, but not the fibronectin binding, is dependent on calcium. No epitopes have yet been mapped for the fibronectin–fibulin-2 interaction.

Elastic fibres. Elastic fibres have a unique structure and special functions in tissues (BOX 1), and they have been shown to contain fibulin-1 (REF. 20) and fibulin-2 (REFS 36,37). Immunogold staining of vessels showed that fibulin-1 and fibulin-2 colocalize with either the amorphous core or the microfibrillar component of these elastic fibres, and that this colocalization was stronger for fibulin-2 than for fibulin-1. This might reflect, in part, distinct differences in their affinity for tropoelastin, which is high for fibulin-2 ($K_d = 0.6$ nM) and 30-fold

lower for fibulin-1 (REF. 37). Studies with recombinant fragments of fibrillin-1 showed distinct binding to fibulin-2, but not to the fibulin-1 C or D variants. Binding of fibulin-2 could be mapped to a restricted amino-terminal region of fibrillin-1, which contains several cbEGF-like modules, and was dependent on calcium³⁶. In the elastic fibres of the skin, fibulin-2 was localized to these microfibrils. However, microfibrils that consist of fibrillins and fibulin-2 are not restricted to elastic fibres, and they are found at many other places such as at basement-membrane zones and in some cartilage structures^{36,40}. Fibulin-5, which binds to tropoelastin but not fibrillin, seems to be another important component of elastic fibres, as its absence in transgenic mice leads to severe ELASTINOPATHIES^{22,23}. The fact that fibulin-5 binding to elastic fibres can be blocked by EDTA indicates that the interaction occurs through domain I and/or II of fibulin-5. The binding activities of fibulin-3 and fibulin-4 have not yet been examined.

Basement-membrane proteins. Various basement membranes in many organs have been shown to contain fibulin-1 and/or fibulin-2 by immunohistology^{4,25,26}, and several important basement-membrane proteins — such as **nidogen-1**, some laminin isoforms, the heparan sulphate proteoglycan perlecan and **collagen IV** (BOX 2) — have been identified as potential ligands for these associations^{4,7,19}. Nidogen-1 is the only ligand identified so far that binds to fibulin-1 splice variants with different affinities, with its affinity being around 30-fold higher for the C, compared to the D, variant¹⁹. The fibulin-1–nidogen-1 association was confirmed in subsequent epitope-mapping studies, which showed that domain III and cbEGF6–9 of domain II of fibulin-1 participate in this interaction⁴⁹. Fibulin-2 was also shown to bind to nidogen-1 with a comparable affinity, but its binding sites have not yet been mapped. The fact that dimeric fibulin-2 has two binding sites is consistent with its participation in the formation of ternary complexes that include nidogen-1, perlecan, laminin and/or fibulin-1 (REF. 7). The binding sites for both fibulins have been mapped to the central globular domain (G2) of nidogen-1, which has a high affinity binding site for perlecan, and to its carboxy-terminal G3 domain, which has a high affinity for the laminin γ 1 chain⁵⁰. These data indicate that fibulin-1 and fibulin-2 have a versatile repertoire of binding properties that they can use to integrate themselves into basement membranes, depending on the composition of these membranes and on the relative affinities of the fibulins for the membrane components.

Perlecan (BOX 2) is another important constituent of basement membranes, as well as of cartilage and other MESENCHYMAL TISSUES. Perlecan was shown to bind fibulin-2, but not fibulin-1, and the binding site was mapped to two EGF-like modules in the carboxy-terminal domain V of the perlecan core protein (480 kDa)⁵¹ and to several Ig-like modules of domain IV, including Ig2 and Ig10–12 (REF. 52). Ig2 is located next to two more Ig-like modules (Ig3 and Ig4), which have high affinity binding sites for nidogens and fibronectin. This cluster of

ELASTINOPATHY

Pathological changes of the elastic fibres that lead to various degenerative diseases.

MESENCHYMAL TISSUE

The tissue that originates from mesenchymal cells, which are unspecified cells that are derived mainly from the primitive mesoderm during embryogenesis. The connective tissues of the body develop from the mesenchymal cells.

Box 1 | The structure and biology of elastic fibres

Elastic fibres are special forms of extracellular matrix assembly, which provide resilience to dynamic connective tissues and are crucial for the proper functioning of the lungs, arteries and skin. They can be readily identified at the ultrastructural level by their amorphous appearance, as they are frequently associated with numerous microfibrils. The amorphous core consists mainly of elastin — a highly hydrophobic protein that is generated from the soluble precursor tropoelastin (70 kDa) and is extensively crosslinked after the enzymatic oxidation of lysyl residues. Important components of the microfibrils are several fibrillin isoforms (~350 kDa, length ~150 nm), which have around 40 calcium-binding sites and some elastic properties. These microfibrils are believed to provide a scaffold for the deposition of elastin. More than 30 proteins, including some fibulins and collagens, have so far been identified as other components of some or all elastic fibres⁸¹. Elastic fibres are known to lose their regenerative potential during ageing and in certain obstructive diseases such as lung emphysema. Mutations in elastin and fibrillins are known to lead to various human disorders with skeletal, cutaneous, vascular and ocular malformations, and similar elastinopathies can be generated in transgenic mice^{82,83}.

modules therefore seems well suited for making numerous associations that can produce the large complexes that are formed by perlecan, fibulin-2 and nidogens or fibronectin.

Further candidates for forming basement-membrane interactions with fibulins are several laminin isoforms (BOX 2). Such an interaction was first shown for laminin-1 that binds fibulin-1 through its E3 fragment, a distinct fragment that is formed by cleavage with elastase. The E3 fragment consists of the α 1-chain laminin G-type (LG)4–5 modules and is derived from the α 1-chain carboxyl terminus^{4,53}. The same site of the laminin α 2 chain and the adjacent α 2LG1–3 modules were also shown to bind fibulin-1 and fibulin-2 with moderate affinities⁵⁴. The fibulin-binding properties of other laminin α -chain isoforms (α 3– α 5) have not yet been examined. Laminin LG modules provide important cell-adhesion sites for integrin, dystroglycan and heparan sulphate proteoglycan receptors⁵³, and their potential modulation by fibulins remains an interesting possibility to be studied.

The γ 2 chain, which is unique to laminin-5, was also shown to bind fibulin-2 in affinity chromatography experiments, and the binding was attributed to a short peptide sequence in the central region of the γ 2 short-arm structure⁵⁵. Subsequent studies showed that this central domain also binds fibulin-1 and that the interacting epitopes have a more complex structure, which includes a second binding site for fibulin-2 on a laminin-type EGF-like (LE) module⁵⁶. These two binding sites can be separated on proteolytic processing of laminin-5, which is essential for its matrix deposition.

Other ligands that bind fibulin-1 and fibulin-2 are endostatins, which are released from the carboxy-terminal end of collagens XV and XVIII and deposited into basement membranes and elastic tissues^{57–59}. Endostatins are potent inhibitors of ANGIOGENESIS and their binding to fibulins might be required for their localization to the ECM, particularly in vessel walls.

Lectican proteoglycans. Another group of highly potent fibulin ligands is represented by the lectican family of large chondroitin sulphate proteoglycans, which includes the cartilage-specific **aggrecan**, the ubiquitously deposited versican and the brain-specific **neurocan** and **brevican**⁶⁰. The lectican family core protein structure includes an amino-terminal globular domain, which has a high binding affinity for hyaluronan, a central elongated region that is used for chondroitin sulphate attachment, and a carboxy-terminal globular domain that has a complex modular structure, which includes a **C-type lectin domain**. This lectin domain in aggrecan and versican has been shown to bind fibulin-1 with moderate affinity. This binding is calcium-dependent, but independent of the N-glycosylation of fibulin-1 (REF. 61). The same two lectin domains also bind fibulin-2 with high affinity. These interactions are specific, as no, or only low, binding activity was found for the lectin domains of neurocan and brevican⁶². The binding epitopes were mapped to a short stretch of cbEGF-like modules in domain II of both fibulins. Furthermore, fibulin-2 was shown to form networks with aggrecan and versican, but only the four-arm structure of fibulin-2 seemed to be active⁶² (FIG. 4). An important conclusion from these data is that there is a prominent role for fibulins (in addition to that of hyaluronan) in the formation of huge proteoglycan networks. Some *in vivo* evidence for such networks was discussed above for the endocardial cushion tissue³². Another function for these interactions is indicated by the binding of fibulin-1 to aggrecanase (a disintegrin and metalloproteinase with thrombospondin motifs 1; **ADAMTS-1**), which might target this specific protease to proteoglycans for their controlled degradation (S. Agraves and M. Iruela-Arispe, unpublished observations).

Specific fibulin-1 ligands. A few more interactions have been described for fibulin-1, which do not seem to be shared by fibulin-2. A weak affinity ($K_d = 3 \mu\text{M}$) was described for the fibrinogen β B chain, and this interaction causes fibulin-1 to associate with, and to inhibit the formation of, fibrin clots^{63,64}. However, fibulin-1-deficient mice that lack the circulating form of this protein show no obvious defect in coagulation⁶⁵, which indicates that this function is not crucial. Two more ligands were initially identified by yeast two-hybrid screening and were subsequently confirmed using recombinant fibulin-1 in other binding assays. The first ligand was connective-tissue growth factor (CTGF), as well as related members of this protein family, which correlates well with the co-expression of fibulin-1 and these growth factors⁶⁶. The other ligand was β -amyloid precursor protein (APP), which binds to cbEGF3–7 of fibulin-1 in a calcium-dependent fashion³⁵. This interaction inhibits APP stimulation of neurite outgrowth and neuronal stem-cell proliferation. A similar role *in vivo* is indicated by the presence of fibulin-1 in some neurons²⁰, but it is not yet known whether fibulin-1 is also incorporated into amyloid deposits in the brain.

ANGIOGENESIS

A process of blood-vessel branching, in which blood vessels sprout from small capillaries.

Box 2 | The properties of important protein ligands for fibulins

Fibronectin is an important microfibrillar protein. It is an elongated (61 nm) disulphide-linked homodimer of 450 kDa, which consists of unique fibronectin type I (F1), F2 and F3 modules that have various binding properties.

Laminins are heterotrimers ($\alpha\beta\gamma$) of $\alpha 1$ – $\alpha 5$, $\beta 1$ – $\beta 3$ and $\gamma 1$ – $\gamma 3$ chains in various combinations and they have molecular masses of 400–750 kDa. Principal domains include the laminin G-type (LG)1–LG5 modules at the carboxyl terminus of the α -chains and numerous laminin-type epidermal growth factor (EGF)-like (LE) modules that are related to EGF-like domains. Laminins have a cross-like shape (36–77 nm along their short and long arms) and they form important network structures in basement membranes.

Perlecan is an important proteoglycan of basement membranes and of other extracellular matrix structures, and it consists of an elongated (80 nm) core protein of 480 kDa, which is modified by 3–4 heparan sulphate chains. It has a multidomain structure and is composed of LG, LE, EGF-like and immunoglobulin-like modules.

Nidogen is a ubiquitous basement-membrane protein of 150 kDa. It consists of three globular domains (G1–G3) that have high binding affinities for laminins, perlecan and collagen IV.

Effects on cellular activities

Interactions with integrins. Because of their broad occurrence in the ECM, several of the fibulins were also studied for their ability to promote integrin-mediated cell adhesion and migration. Mouse fibulin-2 was shown to bind to purified platelet α IIb β 3 and α v β 3, which are both RGD-DEPENDENT INTEGRINS, and this interaction was confirmed in adhesion assays with activated platelets and other cells⁶⁷. The RGD sequence in the N domain of mouse fibulin-2 was shown to be involved in this interaction, but this RGD sequence is not conserved in human fibulin-2, which still binds the α IIb β 3 integrin. Together, the data indicate that there is a role for fibulin-2 in haemostatic control. Fibulin-1 lacks RGD sequences and had no activity in cell-adhesion assays^{67,68}. However, fibulin-1 inhibited cell adhesion to, and migration on, fibronectin. The binding of fibulin-1 does not, however, block the integrin-binding sites of fibronectin, which led to the proposal that the formation of this complex generates a new anti-adhesive site, which repulses cellular interactions rather than promotes them⁶⁸. Finally, a single RGD site in the amino-terminal domain I of fibulin-5 has also been shown to be cell adhesive, and it interacts, in particular, with α v β 3, α v β 5 and α 9 β 1 integrins^{22,69}.

Effect on cell proliferation. There is an increasing amount of data that indicate that most fibulins have the ability to interfere with several cellular activities, and these data were interpreted to reflect their control of cellular proliferation and malignant transformation. Most of these data are, however, preliminary and, in part, controversial, and no general concept has emerged for their interpretation. This is perhaps best illustrated by a recent study on fibulin-5, which came to the conclusion that regulation occurs in a context-specific manner and depends on the cell type and assay conditions used⁷⁰.

The first evidence for a cellular proliferation activity was described for fibulin-3, which is upregulated

in senescent and WERNER SYNDROME fibroblasts, as well as in quiescent young fibroblasts¹⁴. This indicated that there was an effect on DNA synthesis, but the data have so far remained controversial. A similar change in quiescent fibroblasts has also been reported for fibulin-4 (REF. 10), although fibulin-4 has also been shown to stimulate growth⁷¹. A few further interesting activities have been reported for fibulin-1, which, as already discussed, include modulation of neurite outgrowth through binding to APP³⁵, the binding of growth factors such as CTGF⁶⁶, and the regulation of signal transduction by inhibiting the phosphorylation of the extracellular-signal-regulated kinase (ERK)⁶⁸. The latter activity is also shared by fibulin-5 (REF. 70). The cellular receptors that are involved remain to be identified.

Effect on malignant transformation. A variable set of data has indicated that there is a correlation between fibulin expression and certain types of malignant cells, and these data were interpreted to indicate that there is a role for fibulins in tumour suppression^{70,72}. These correlations include an upregulation of fibulin-4 in colon carcinomas¹⁵ and of fibulin-1 in ovarian carcinomas^{44,73}. This upregulation might inhibit the mobility of cancer cells, which would suppress their invasiveness⁷⁴. The most interesting observation, however, is that high concentrations of the fibulin-1D variant selectively delay tumour transformation and invasive potential⁷². The mechanism is still unclear, but this indicates that there is a specific binding function for fibulin-1D that is not shared by fibulin-1C. This specific-binding function for fibulin-1D is also indicated by genetic evidence (see below), but remains to be defined.

Genetic evidence for biological function

Insights into the *in vivo* functions of several fibulins have emerged from the identification of human diseases that involve fibulin loci and from the analysis of gene-targeted mutant mouse strains. Targeted inactivation of the fibulin-1 gene in mice (*Fbln1*) leads to severe haemorrhages in skin, muscle and perineural tissues, which start from midgestation and result in the death of almost all homozygous embryos at birth⁶⁵. The defect is caused by ruptures in the endothelial lining of small, but not large, blood vessels. Capillary endothelial cells in the *Fbln1*-null mice have very irregular cytoplasmic processes, but the underlying basement membrane is intact. Further defects were found in the kidneys and lungs, which might also contribute to the lethal phenotype. The mouse model therefore indicates that fibulin-1 is important in the stabilization of blood vessel walls and that it is also involved in diverse biological processes during embryogenesis.

Fibulin-1 has also been implicated in limb malformations. Three patients in a family affected with a complex type of SYNPOLYDACTYLY have been found to carry a balanced chromosomal translocation that involves an alternatively spliced exon of *FBLN1* and a gene of unknown function that is located on the short arm of chromosome 12 (REF. 75). This results in reduced

RGD-DEPENDENT INTEGRINS

A specific group of cellular integrin receptors that bind to the arginine-glycine-aspartate (RGD) sequences of their ligands.

WERNER SYNDROME

A premature ageing disorder that is inherited in an autosomal recessive mode. The clinical symptoms can include short stature, wrinkled skin, baldness, cataracts and muscular atrophy.

SYNPOLYDACTYLY

A developmental defect that is characterized by the fusion (syndactyly) and splitting (polydactyly) of fingers or toes. It is usually an autosomal dominant disease and can result from mutations in the homeobox genes.

Table 3 | The binding properties of fibulin-1 and fibulin-2 for extracellular proteins

Interacting ligand	Affinity (K _d)*	Calcium dependence [†]	Epitope mapped	References
Fibulin-1				
Fibronectin	139 nM	+	Yes	19,45,46
Nidogen-1	2–60 nM	+	Yes	19,50
Laminin-1	80 nM	+	Yes	19
Fibrinogen	3 μM	ND	Yes	63
Aggrecan, versican	10–30 nM	+	Yes	61
Tropoelastin	18 nM	+	No	37
Endostatin	46 nM	–	No	57
Laminin γ2 chain	20 nM	ND	Yes	56
Laminin α2 chain	14 nM	ND	Yes	54
Connective-tissue growth factor	ND	ND	Yes	66
β-amyloid precursor protein	ND	+	Yes	35
Fibulin-2				
Fibronectin	1 μM	+	Yes	7
Nidogen-1	0.5 μM	–	No	7
Perlecan	22 nM	ND	Yes	52
Fibrillin-1	56 nM	+	Yes	36
Tropoelastin	0.6 nM	+	Yes	37
Endostatin	33 nM	–	No	57
Aggrecan, versican	0.1 nM	+	Yes	62
Laminin γ2 chain	20–40 nM	ND	Yes	56
Laminin α2 chain	13 nM	ND	Yes	54

*The affinities were determined using surface plasmon resonance. [†]The calcium dependence was examined by the addition of EDTA. ND, not determined.

expression of the D, but not the C, variant of fibulin-1 in patients' fibroblasts. As fibulin-1 is expressed in the digits of the developing limb, altering the expression of fibulin-1 splice variants is thought to be responsible for the digit abnormality⁷⁵. This possibility, however, remains to be tested.

As a result of positional cloning, a single mutation in the fibulin-3 gene (*FBLN3*), which results in an R345W substitution (where W is tryptophan), was found to be the cause of two inherited forms of MACULAR DEGENERATIVE DISEASE — so-called Malattia Leventinese and Doyme honeycomb retinal dystrophy²¹. Macular degenerative disease is characterized by extracellular deposits, which are known as drusen, beneath the retinal pigment epithelium. R345 is located in the last cbEGF-like domain of fibulin-3 and follows the second cysteine residue. Interestingly, this mutation in fibulin-3 was found in 161 patients from 37 families in the initial study, although recent evidence indicates that there is molecular heterogeneity within the inherited forms of macular degenerations⁷⁶. Mutations in fibulin-3 are not associated with the sporadic form of early onset drusen or with age-related macular degeneration, which is the most common cause of blindness and is also characterized by drusen deposition²¹. In both inherited and age-related macular degeneration diseases, fibulin-3 is not located at the site of drusen formation, but is aberrantly accumulated with and/or beneath the retinal

pigment epithelium⁷⁷. A R345W mutant fibulin-3 protein produced by a mammalian expression system is misfolded, secreted inefficiently and retained in cells⁷⁷. It has therefore been postulated that the misfolding and aberrant accumulation of fibulin-3 might have a role in the etiology of macular degeneration⁷⁷.

An essential role for fibulin-5 in elastic-fibre assembly *in vivo* was highlighted by the abnormal phenotypes of mice lacking fibulin-5 (REFS 22,23). The mutant mice showed markedly disrupted and disorganized elastic fibres, which resulted in loose skin, emphysematous lungs and tortuous aortas with loss of function. Fibulin-5, which is expressed in tissues that are enriched in elastic fibres and binds tropoelastin and cell-surface integrins *in vitro*, is thought to anchor elastic fibres to cells and to be required for elastic-fibre assembly during development. It has also been postulated that fibulin-5 might function as a chaperone that tethers tropoelastin to the cell surface during elastic-fibre assembly⁷⁸.

Although *Fbln5*-null mice have highly disorganized elastic fibres in their internal organs, they survive to adulthood. The phenotypes of the mutant mice resemble those of human patients with cutis laxa — a heterogeneous group of disorders that are characterized by loose skin and that have variable internal-organ involvement, such as pulmonary emphysema and cardiovascular abnormalities. This phenotype similarity led to the identification of a homozygous missense mutation

MACULAR DEGENERATIVE DISEASE

An incurable eye disease that is caused by the deterioration of the central portion of the retina, which is known as the macula. The disease is heterogeneous and includes the rare heritable forms, the sporadic early-onset form and the age-related form.

MARFAN SYNDROME

A heritable connective tissue disease that affects several organ systems, including the skeleton, eyes, lungs, heart and blood vessels. The disease is caused by dominant mutations in the fibrillin-1 gene.

(which results in an S227P substitution; where S is serine and P is proline) in *FBLN5* in a family that was affected by a severe form of cutis laxa⁷⁹. S227 is located between Cys3 and Cys4 in cbEGF3 of fibulin-5. This amino-acid substitution is probably the cause of cutis laxa in this family, as the corresponding position in most of the cbEGF-like modules in fibrillin-1 is occupied by a serine, and an analogous mutation in fibrillin-1 results in MARFAN SYNDROME. Although the heterozygous carriers of the missense mutation in this family are not affected, cutis laxa can result from a heterozygous mutation in fibulin-5. A patient with a milder phenotype of cutis laxa carries a heterozygous tandem gene duplication of 22 kb in *FBLN5*, which results in the duplication of exons 5–8 that encode four cbEGF-like domains⁸⁰. The mutant fibulin-5 protein with four extra cbEGF-like domains is synthesized and secreted by the patient's fibroblasts in amounts that are somewhat greater than for a normal counterpart, so the mutation probably exerts a dominant-negative effect.

Conclusions and perspectives

The fibulins represent a newly characterized family of calcium-binding ECM proteins and five isoforms have so far been characterized from mammalian species. The fibulins do not form large homotypic aggregates, in contrast to many other ECM proteins, but they have the ability to join other supramolecular structures as diverse as basement-membrane networks, elastic fibres, several types of microfibrils and proteoglycan aggregates. Their inclusion, in part, stabilizes these structures, but also extends their repertoire of biological functions. The latter point is highlighted by genetic studies of inherited diseases and of transgenic animals. Some fibulins also have the ability to interact with cells, in part, through interactions with RGD-dependent integrins. There is also increasing evidence that fibulins control the growth of normal and malignant cells. These studies are still in a preliminary stage and, in the future, a comprehensive characterization of the receptors and signal-transduction pathways involved will be required in order to evaluate their biological significance.

- Argraves, W. S., Dickerson, K., Burgess, W. H. & Ruoslahti, E. Fibulin, a novel protein that interacts with the fibronectin receptor β subunit cytoplasmic domain. *Cell* **58**, 623–629 (1989).
- Argraves, W. S., Tran, H., Burgess, W. H. & Dickerson, K. Fibulin is an extracellular matrix and plasma glycoprotein with repeated domain structure. *J. Cell Biol.* **111**, 3155–3164 (1990).
Fibulin-1 is identified and is found to be a new class of ECM protein.
- Kluge, M., Mann, K., Dziadek, M. & Timpl, R. Characterization of a novel calcium-binding 90-kDa glycoprotein (BM-90) shared by basement membranes and serum. *Eur. J. Biochem.* **193**, 651–659 (1990).
- Pan, T.-C. *et al.* Sequence of extracellular mouse protein BM-90/fibulin and its calcium-dependent binding to other basement membrane ligands. *Eur. J. Biochem.* **215**, 733–740 (1993).
- Pan, T.-C. *et al.* Structure and expression of fibulin-2, a novel extracellular matrix protein with multiple EGF-like repeats and consensus motifs for calcium-binding. *J. Cell Biol.* **123**, 1269–1277 (1993).
The first evidence that there is a fibulin protein family that contains different fibulin isoforms.
- Zhang, R.-Z. *et al.* Fibulin-2 (*FBLN2*): human cDNA sequence, mRNA expression and mapping of the gene on human and mouse chromosomes. *Genomics* **22**, 425–430 (1994).
- Sasaki, T., Göhring, W., Pan, T.-C., Chu, M.-L. & Timpl, R. Binding of mouse and human fibulin-2 to extracellular matrix ligands. *J. Mol. Biol.* **254**, 892–899 (1995).
- Sasaki, T. *et al.* Dimer model for the microfibrillar protein fibulin-2 and identification of the connecting disulfide bridge. *EMBO J.* **16**, 3035–3043 (1997).
The first extensive model of the fibulin-2 structure.
- Tran, H., Mattei, M., Godyna, S. & Argraves, W. S. Human Fibulin-1D: molecular cloning, expression and similarity with S1-5 protein, a new member of the fibulin-1 gene family. *Matrix Biol.* **15**, 479–493 (1997).
- Giltay, R., Timpl, R. & Kostka, G. Sequence, recombinant expression and tissue localization of two novel extracellular matrix proteins, fibulin-3 and fibulin-4. *Matrix Biol.* **18**, 469–480 (1999).
- Zhang, H.-Y., Lardelli, M. & Ekblom, P. Sequence of zebrafish fibulin-1 and its expression in developing heart and other embryonic organs. *Dev. Genes Evol.* **207**, 340–351 (1997).
- Barth, J. L., Argraves, K. M., Roark, E. F., Little, C. D. & Argraves, W. S. Identification of chicken and *C. elegans* fibulin-1 homologs and characterization of the *C. elegans* fibulin-1 gene. *Matrix Biol.* **17**, 635–646 (1998).
- Huber, R., Scholze, H., Paques, E. P. & Deisenhofer, J. Crystal structure analysis and molecular model of human C3a anaphylatoxin. *Hoppe-Seyler's Z. Physiol. Chem.* **361**, 1389–1399 (1989).
- Lecka-Czernik, B., Lumpkin, C. K. & Goldstein, S. An overexpressed gene transcript in senescent and quiescent human fibroblasts encoding a novel protein in the epidermal growth factor-like repeat family stimulates DNA synthesis. *Mol. Cell Biol.* **15**, 120–129 (1995).
- Gallagher, W. M. *et al.* Human fibulin-4: analysis of its biosynthetic processing and mRNA expression in normal and tumour tissues. *FEBS Lett.* **489**, 59–66 (2001).
- Maurer, P. & Hohenester, E. Structural and functional aspects of calcium-binding in extracellular matrix proteins. *Matrix Biol.* **15**, 569–580 (1997).
- Downing, A. K. *et al.* Solution structure of a pair of calcium-binding epidermal growth factor-like domains: implications for the Marfan syndrome and other genetic disorders. *Cell* **85**, 597–605 (1996).
This paper explains how the structure of cbEGF-like modules is stabilized by calcium ligation.
- Sasaki, T., Mann, K., Murphy, G., Chu, M.-L. & Timpl, R. Different susceptibilities of fibulin-1 and fibulin-2 to cleavage by matrix metalloproteinases and other tissue proteases. *Eur. J. Biochem.* **240**, 427–434 (1996).
- Sasaki, T. *et al.* Structural characterization of two variants of fibulin-1 that differ in nidogen affinity. *J. Mol. Biol.* **245**, 241–250 (1995).
- Roark, E. F. *et al.* The association of human fibulin-1 with elastic fibers: an immunohistological, ultrastructural, and RNA study. *J. Histochem. Cytochem.* **43**, 401–411 (1995).
- Stone, E. M. *et al.* A single *FEFMP1* mutation associated with both Malattia Leventinese and Doyme honeycomb retinal dystrophy. *Nature Genet.* **22**, 199–202 (1999).
- Nakamura, T. *et al.* Fibulin-5/DANCE is essential for elastogenesis *in vivo*. *Nature* **415**, 171–175 (2002).
- Yanagisawa, H. *et al.* Fibulin-5 is an elastin-binding protein essential for elastic fibre development *in vivo*. *Nature* **415**, 168–171 (2002).
This work describes, together with reference 22, the role of fibulin-5 in stabilizing elastic fibres.
- Spence, S. G., Argraves, W. S., Walters, L., Hungerford, J. E. & Little, C. Fibulin is localized at sites of epithelial-mesenchymal transitions in the early avian embryo. *Dev. Biol.* **151**, 473–484 (1992).
- Zhang, H.-Y., Timpl, R., Sasaki, T., Chu, M.-L. & Ekblom, P. Fibulin-1 and fibulin-2 expression during organogenesis in the developing mouse embryo. *Dev. Dyn.* **205**, 348–364 (1996).
- Miosge, N. *et al.* The extracellular matrix proteins fibulin-1 and fibulin-2 in the early human embryo. *Histochem. J.* **28**, 109–116 (1996).
- Hungerford, J. E., Owens, G. K., Argraves, W. S. & Little, C. D. Development of aortic vessel wall as defined by vascular smooth muscle and extracellular matrix markers. *Dev. Biol.* **178**, 375–392 (1996).
- Bouchev, D., Argraves, W. S. & Little, C. D. Fibulin-1, vitronectin expression during avian cardiac valve and septa development. *Anat. Rec.* **244**, 540–551 (1996).
- Zhang, H.-Y., Kluge, M., Timpl, R., Chu, M.-L. & Ekblom, P. The extracellular matrix glycoproteins BM-90 and tenascin are expressed in the mesenchyme at sites of endothelial-mesenchymal conversion in the embryonic mouse heart. *Differentiation* **52**, 211–220 (1993).
- Zhang, H.-Y. *et al.* Extracellular matrix protein fibulin-2 is expressed in the embryonic endocardial cushion tissue and is a prominent component of valves in adult heart. *Dev. Biol.* **167**, 18–26 (1995).
- Tsuda, T., Wang, H., Timpl, R. & Chu, M.-L. Fibulin-2 expression marks transformed mesenchymal cells in developing cardiac valves, aortic arch vessels and coronary vessels. *Dev. Dyn.* **222**, 89–100 (2001).
- Miosge, N., Sasaki, T., Chu, M.-L., Herken, R. & Timpl, R. Ultrastructural localization of microfibrillar fibulin-1 and fibulin-2 during heart development indicates a switch in molecular associations. *Cell. Mol. Life Sci.* **54**, 606–613 (1997).
- Gallagher, W. M. *et al.* MBP1: a novel mutant p53-specific protein partner with oncogenic properties. *Oncogene* **18**, 3608–3616 (1999).
- Kowal, R. C., Richardson, J. A., Miano, J. M. & Olsen, E. N. EVEC. A novel epidermal growth factor-like repeat containing protein upregulated in embryonic and injured adult vasculature. *Circ. Res.* **84**, 1166–1176 (1999).
- Ohsawa, I., Takamura, C. & Kohsaka, S. Fibulin-1 binds the amino-terminal head of β -amyloid precursor protein. *J. Neurochem.* **76**, 1411–1420 (2001).
- Reinhardt, D. P. *et al.* Fibrillin-1 and fibulin-2 interact and are colocalized in some tissues. *J. Biol. Chem.* **271**, 19489–19496 (1996).
- Sasaki, T. *et al.* Tropoelastin binding to fibulins, nidogen-2 and other extracellular matrix proteins. *FEBS Lett.* **460**, 280–284 (1999).
- Sasaki, T., Wiedemann, H., Matzner, M., Chu, M.-L. & Timpl, R. Expression of fibulin-2 by fibroblasts and deposition with fibronectin into a fibrillar matrix. *J. Cell Sci.* **109**, 2895–2904 (1996).
- Loveland, K. *et al.* Developmental changes in the basement membrane of the normal and hypothyroid postnatal rat testis: segmental localization of fibulin-2 and fibronectin. *Biol. Reprod.* **58**, 1123–1130 (1998).
- Raghunath, M. *et al.* Confocal laser scanning analysis of the association of fibulin-2 with fibrillin-1 and fibronectin define different stages of skin regeneration. *J. Invest. Dermatol.* **112**, 97–101 (1999).
- Fässler, R., Sasaki, T., Timpl, R., Chu, M.-L. & Werner, S. Differential regulation of fibulin, tenascin and nidogen expression during wound healing of normal and glucocorticoid-treated mice. *Exp. Cell Res.* **222**, 111–116 (1996).

42. Hunzelmann, N., Nischt, R., Brenneisen, P., Eickert, A. & Krieg, T. Increased deposition of fibulin-2 in solar elastosis and its colocalization with elastic fibers. *Brit. J. Dermatol.* **145**, 217–222 (2001).
43. Kusubata, M. *et al.* Spatiotemporal changes of fibronectin, tenascin-C, fibulin-1, and fibulin-2 in the skin during the development of chronic contact dermatitis. *J. Invest. Dermatol.* **113**, 906–912 (1999).
44. Roger, P., Pujol, P., Lucas, A., Baldet, P. & Rochefort, H. Increased immunostaining of fibulin-1, an estrogen-regulated protein in the stroma of human ovarian epithelial tumors. *Am. J. Pathol.* **153**, 1579–1588 (1998).
45. Balbona, K. *et al.* Fibulin binds to itself and to the carboxyl-terminal heparin-binding region of fibronectin. *J. Biol. Chem.* **267**, 20120–20125 (1992).
46. Tran, H., Van Dusen, W. J. & Argraves, W. S. The self-association and fibronectin-binding sites of fibulin-1 map to calcium-binding epidermal growth factor-like domains. *J. Biol. Chem.* **272**, 22600–22606 (1997).
47. Roman, J. & McDonald, J. A. Fibulin's organization into the extracellular matrix of fetal lung fibroblasts is dependent on fibronectin matrix assembly. *Am. J. Respir. Cell Mol. Biol.* **8**, 538–545 (1993).
48. Godyna, S., Mann, D. M. & Argraves, W. S. A quantitative analysis of the incorporation of fibulin-1 into the extracellular matrix indicates that fibronectin assembly is required. *Matrix Biol.* **14**, 467–477 (1994).
49. Adam, S. *et al.* Binding of fibulin-1 to nidogen depends on its C-terminal globular domain and a specific array of calcium-binding epidermal growth factor-like (EG) modules. *J. Mol. Biol.* **272**, 226–236 (1997).
50. Ries, A., Göhring, W., Fox, J. W., Timpl, R. & Sasaki, T. Recombinant domains of mouse nidogen-1 and their binding to basement membrane proteins and monoclonal antibodies. *Eur. J. Biochem.* **268**, 5119–5128 (2001).
51. Friedrich, M. V. K. *et al.* Structural basis of glycosaminoglycan modification and of heterotypic interactions of perlecan domain V. *J. Mol. Biol.* **294**, 259–270 (1999).
52. Hopf, M., Göhring, W., Mann, K. & Timpl, R. Mapping of binding sites for nidogens, fibulin-2, fibronectin and heparin to different IG modules of perlecan. *J. Mol. Biol.* **311**, 529–541 (2001).
53. Timpl, R. *et al.* Structure and function of laminin LG modules. *Matrix Biol.* **19**, 309–317 (2000).
54. Talts, J. F., Andac, Z., Göhring, W., Brancaccio, A. & Timpl, R. Binding of the G domains of laminin $\alpha 1$ and $\alpha 2$ chains and perlecan to heparin, sulfatides, α -dystroglycan and several extracellular matrix proteins. *EMBO J.* **18**, 863–870 (1999).
55. Utani, A., Nomizu, M. & Yamada, Y. Fibulin-2 binds to the short arms of laminin-5 and laminin-1 via conserved amino acid sequences. *J. Biol. Chem.* **272**, 2814–2820 (1997).
56. Sasaki, T. *et al.* Short arm region of laminin-5 γ -2 chain: structure, mechanism of processing and binding to heparin and proteins. *J. Mol. Biol.* **314**, 751–763 (2001).
57. Sasaki, T. *et al.* Structure, function and tissue forms of the C-terminal globular domain of collagen XVIII containing the angiogenesis inhibitor endostatin. *EMBO J.* **17**, 4249–4256 (1998).
58. Sasaki, T. *et al.* Endostatins derived from collagens XV and XVIII differ in structural and binding properties, tissue distribution and anti-angiogenic activity. *J. Mol. Biol.* **301**, 1179–1190 (2000).
59. Sasaki, T., Hohenester, E. & Timpl, R. Structure and function of collagen-derived endostatin inhibitors of angiogenesis. *IUBMB Life* **53**, 77–84 (2002).
60. Ruoslahti, E. Brain extracellular matrix. *Glycobiology* **6**, 489–492 (1996).
61. Aspberg, A., Adam, S., Kostka, G., Timpl, R. & Heinegard, D. Fibulin-1 is a ligand for the C-type lectin domains of aggrecan and versican. *J. Biol. Chem.* **274**, 20444–20449 (1999).
62. Olin, A. J. *et al.* The proteoglycans aggrecan and versican form networks with fibulin-2 through their lectin domain binding. *J. Biol. Chem.* **276**, 1253–1261 (2001).
63. Tran, H. *et al.* The interaction of fibulin-1 with fibrinogen. A potential role in hemostasis and thrombosis. *J. Biol. Chem.* **270**, 19458–19464 (1995).
64. Godyna, S., Dias-Ricart, M. & Argraves, W. S. Fibulin-1 mediates platelet adhesion via a bridge of fibrinogen. *Blood* **88**, 2569–2577 (1996).
65. Kostka, G. *et al.* Perinatal lethality and endothelial cell abnormalities in several vessel compartments of fibulin-1 deficient mice. *Mol. Cell. Biol.* **21**, 7025–7034 (2001).
- This paper shows that a fibulin-1 deficiency causes a haemorrhagic phenotype.**
66. Perbal, B. *et al.* The C-terminal domain of the regulatory protein NOVH is sufficient to promote interaction with fibulin-1C: a clue for a role of NOVH in cell-adhesion signalling. *Proc. Natl Acad. Sci. USA* **96**, 869–874 (1999).
67. Pfaff, M., Sasaki, T., Tangemann, K., Chu, M.-L. & Timpl, R. Integrin-binding and cell-adhesion studies of fibulins reveal a particular affinity for $\alpha 5\beta 3$. *Exp. Cell Res.* **219**, 87–92 (1995).
68. Twal, W. O. *et al.* Fibulin-1 suppression of fibronectin-regulated cell adhesion and motility. *J. Cell Sci.* **114**, 4587–4598 (2001).
69. Nakamura, T. *et al.* DANCE, a novel secreted RGD protein expressed in developing, atherosclerotic, and balloon-injured arteries. *J. Biol. Chem.* **274**, 22476–22483 (1999).
70. Schiemann, W. P., Blobbe, G. C., Kalume, D. E., Pandey, A. & Lodish, H. F. Context-specific effects of fibulin-5 (DANCE/EVEC) on cell proliferation, motility, and invasion. Fibulin-5 is induced by transforming growth factor- β and affects protein kinase cascades. *J. Biol. Chem.* **277**, 27367–27377 (2002).
71. Heine, H., Delude, R. L., Monks, B. G., Esperik, T. & Golenbock, D. T. Bacterial lipopolysaccharide induces expression of the stress response genes *hop* and *H411*. *J. Biol. Chem.* **274**, 21049–21055 (1999).
72. Qing, J. *et al.* Suppression of anchorage-independent growth and matrigel invasion and delayed tumor formation by elevated expression of fibulin-1D in human fibrosarcoma-derived cell lines. *Oncogene* **15**, 2159–2168 (1997).
73. Clinton, G. M. *et al.* Estrogens increase the expression of fibulin-1, an extracellular matrix protein secreted by human ovarian cancer cells. *Proc. Natl Acad. Sci. USA* **93**, 316–320 (1996).
74. Hayashido, Y. *et al.* Estradiol and fibulin-1 inhibit motility of human ovarian- and breast-cancer cells induced by fibronectin. *Int. J. Cancer* **75**, 654–658 (1998).
75. Debeer, P. *et al.* The fibulin-1 gene (*FLBN1*) is disrupted in a t(12;22) associated with a complex type of synpolydactyly. *J. Med. Genet.* **39**, 98–104 (2002).
76. Tartellin, E. E. *et al.* Molecular genetic heterogeneity in autosomal dominant drusen. *J. Med. Genet.* **38**, 381–384 (2001).
77. Marmorstein, L. Y. *et al.* Aberrant accumulation of EFEMP1 underlies drusen formation in Malattia Leventinese and age-related macular degeneration. *Proc. Natl Acad. Sci. USA* **99**, 13067–13072 (2002).
78. Midwood, K. S. & Schwarzbauer, J. E. Elastic fibres: building bridges between cells and their matrix. *Curr. Biol.* **12**, R279–R281 (2002).
79. Loeys, B. *et al.* Homozygosity for a missense mutation in fibulin-5 (*FBLN5*) results in a severe form of cutis laxa. *Hum. Mol. Genet.* **11**, 2113–2118 (2002).
80. Markova, D. *et al.* Genetic heterogeneity of cutis laxa: a heterozygous tandem duplication within the fibulin-5 (*FBLN5*) gene. *Am. J. Hum. Genet.* **72**, 998–1004 (2003).
81. Kielty, C. M., Sherratt, M. J. & Shuttleworth, C. A. Elastic fibers. *J. Cell Sci.* **115**, 2817–2828 (2002).
82. Dietz, H. C. & Mecham, R. P. Mouse models of genetic diseases resulting from mutations in elastic fiber proteins. *Matrix Biol.* **19**, 481–488 (2000).
83. Milewicz, D. M., Urban, Z. & Boyd, C. Genetic disorders of the elastic fiber system. *Matrix Biol.* **19**, 471–480 (2000).
84. Pan, T.-C., Kostka, G., Zhang, R.-Z., Timpl, R. & Chu, M.-L. Complete exon-intron organization of the mouse fibulin-1 gene and its comparison with the human fibulin-1 gene. *FEBS Lett.* **444**, 38–42 (1999).
85. Mattei, M. G., Pan, T.-C., Zhang, R.-Z., Timpl, R. & Chu, M.-L. The fibulin-1 gene (*FBLN1*) is located on human chromosome 22 and mouse chromosome 15. *Genomics* **22**, 437–438 (1994).
86. Grässel, S., Sicot, F.-X., Gotta, S. & Chu, M.-L. Mouse fibulin-2 gene. Complete exon-intron organization and promoter characterization. *Eur. J. Biochem.* **263**, 471–477 (1999).
87. Ikegawa, S., Toda, T., Okui, K. & Nakamura, Y. Structure and chromosomal assignment of the human S1-5 gene (*FBLN*) that is highly homologous to fibrillin. *Genomics* **35**, 590–592 (1996).
88. Katsanis, N., Venable, S., Smith, J. R. & Lupski, J. R. Isolation of a paralog of the Doyme honeycomb retinal dystrophy gene from the multiple retinopathy critical region on 11q13. *Hum. Genet.* **106**, 66–72 (2000).
89. Kowal, R. C., Jolsin, J. M., Olson, E. N. & Schultz, R. A. Assignment of fibulin-5 (*FBLN5*) to human chromosome 14q31 by *in situ* hybridization and radiation hybrid mapping. *Cytogenet. Cell Genet.* **87**, 2–3 (1999).

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