Elastic fibres

Cay M. Kielty[1,*], Michael J. Sherratt[2] and C. Adrian Shuttleworth[2]

Schools of [1]Medicine and [2]Biological Sciences, University of Manchester, Manchester M13 9PT, UK

*Author for correspondence (e-mail: cay.kielty@man.ac.uk)

Journal of Cell Science 115, 2817-2828 (2002) © The Company of Biologists Ltd

Summary

Elastic fibres are essential extracellular matrix macromolecules comprising an elastin core surrounded by a mantle of fibrillin-rich microfibrils. They endow connective tissues such as blood vessels, lungs and skin with the critical properties of elasticity and resilience. The biology of elastic fibres is complex because they have multiple components, a tightly regulated developmental deposition, a multi-step hierarchical assembly and unique biomechanical functions. However, their molecular complexity is at last being unravelled by progress in identifying interactions between component molecules, ultrastructural analyses and studies of informative mouse models.

Key words: Elastic fibres, Microfibrils, Elastin, Fibrillin, MAGP, Fibulin

Introduction

Tissue flexibility and extensibility have been essential requirements in the evolution of multicellular organisms. Elastic fibres are major insoluble extracellular matrix (ECM) assemblies that endow connective tissues with resilience, permitting long-range deformability and passive recoil without energy input. These properties are critical to the function of arteries, which undergo repeated cycles of extension and recoil, and to lungs, skin and all other dynamic connective tissues. This elastic function complements collagen fibrils, which impart tensile strength.

Genesis of elastic fibres in early development involves deposition of tropoelastin (the soluble precursor of mature elastin) on a preformed template of fibrillin-rich microfibrils (Figs 1, 2) (Mecham and Davis, 1994). Mature elastic fibres are thus a composite biomaterial comprising an outer microfibrillar mantle and an inner core of amorphous crosslinked elastin. Fibrillins and fibrillin-rich microfibrils are conserved in medusa jellyfish (Reber-Muller et al., 1995), invertebrates (Thurmond and Trotter, 1996) and vertebrates (Pereira et al., 1993; Zhang et al., 1994; Nagase et al., 2001). Tropoelastin evolved more recently to reinforce the high pressure closed circulatory systems of higher vertebrates (Faury, 2001). The distribution of microfibrils in dynamic elastic tissues such as blood vessels, lung, ligaments and skin implies a central biomechanical role. Microfibrils are also abundant in some flexible tissues that do not express elastin [e.g. the ciliary zonules that hold the lens in dynamic suspension (Ashworth et al., 2000)], which emphasises their independent evolutionary function.

Elastic fibres are designed to maintain elastic function for a lifetime. However, various enzymes (matrix metalloproteinases and serine proteases) are able to cleave elastic fibre molecules (Kielty et al., 1994; Ashworth et al., 1999c). Indeed, loss of elasticity due to degradative changes is a major contributing factor in ageing of connective tissues, in the development of aortic aneurysms and lung emphysema, and in degenerative changes in sun-damaged skin (Watson et al., 1999). The importance of elastic fibres is further highlighted by the severe heritable connective tissue diseases caused by mutations in components of elastic fibres (for reviews, see Robinson and Godfrey, 2000; Milewicz et al., 2000). Fibrillin-1 mutations cause Marfan syndrome, which is associated with cardiovascular, ocular (ectopia lentis) and skeletal defects; fibrillin-2 mutations cause congenital contractural arachnodactyly (CCA) with overlapping skeletal and ocular symptoms; and elastin mutations cause Williams syndrome, supravalvular stenosis (SVAS) and cutis laxa (Tassabehji et al., 1998). Recently, pseudoxanthoma elasticum (PXE), a heritable disease associated with elastic fibre calcification was linked to an ion channel protein (Le Saux et al., 2001; Ringpfeil et al., 2001).

The biology of elastic fibres is complex because of their multiple components, tightly regulated developmental pattern of deposition, multi-step hierarchical assembly, unique elastomeric properties and influence on cell phenotype. Below we discuss how the molecular complexity of the elastic fibre system is being unravelled by progress in identifying interactions between microfibrillar molecules and tropoelastin, detailed ultrastructural analyses and studies of mouse models.

Elastic fibre organisation

The microfibrillar component

Ultrastructural studies have provided insights into the complex organisation of fibrillin-rich microfibrils. Negative staining, rotary shadowing and atomic force microscopy (AFM) showed that isolated untensioned microfibrils have a 56 nm beaded periodicity (Fig. 3) (Keene et al., 1991; Kielty et al., 1991; Baldock et al., 2001). Calcium plays a key role in this microfibril organisation, since chelation of calcium induces a diffuse appearance and reduced beaded periodicity (Kielty and Shuttleworth, 1993; Cardy and Handford, 1998; Wess et al., 1998a). STEM analysis has defined microfibril mass and its axial distribution, predicted up to eight fibrillin molecules in cross-section, and shown that treatment of microfibrils with enzymes (chondroitinase ABC lyase, matrix metalloproteinase) can reduce mass and influence organisation (Sherratt et al., 1997; Sherratt et al., 2001). Automated electron

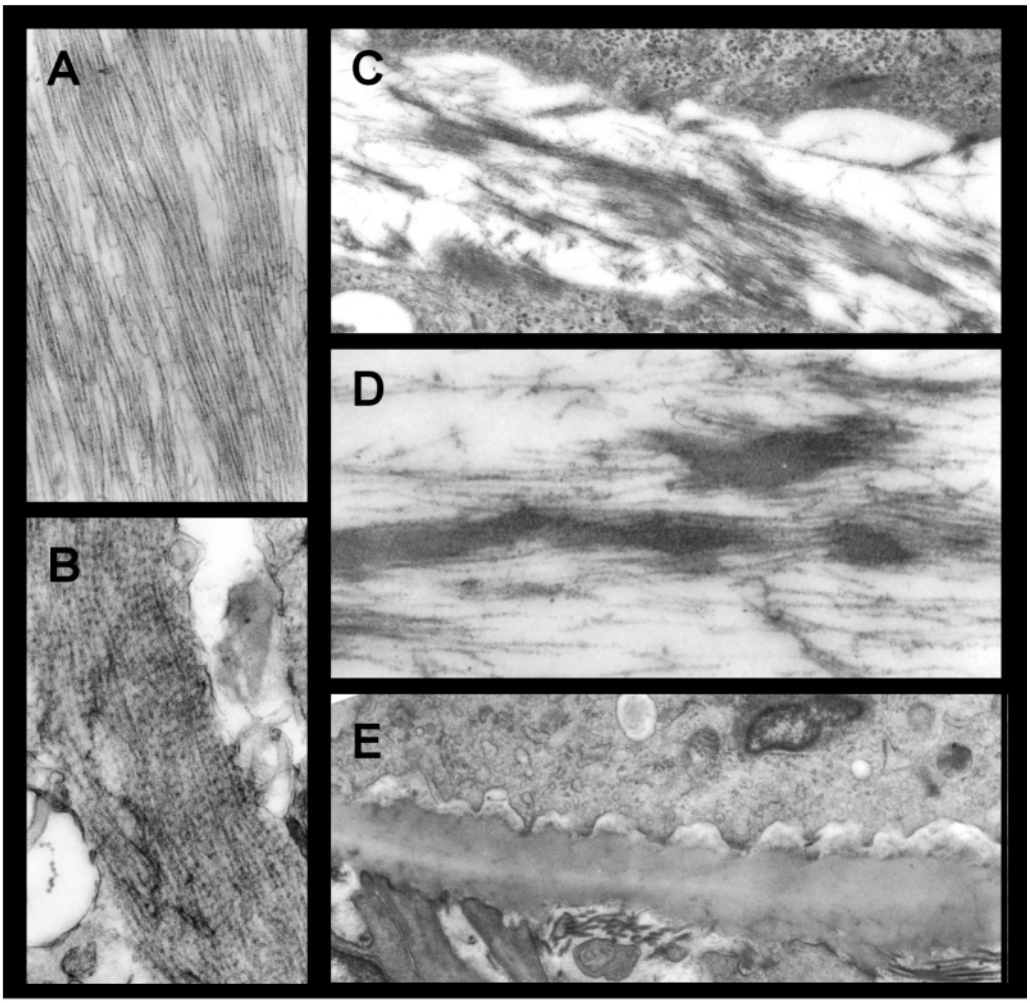

Fig. 1. Transmission electron microscopy of fibrillin-rich microfibrils and elastic fibres. (A) Human ciliary zonular microfibrils in loose, roughly parallel arrays. (B) Lobster aorta microfibrils, showing periodic striations. (C) Deposition of microfibrils and elastin by cultured bovine nuchal ligament fibroblasts. (D) Close-up of elastic fibres forming in bovine nuchal ligament fibroblast cultures. (E) Internal elastic lamina (IEL) of murine aorta, with associated endothelial cell (EC) and smooth muscle cell (SMC).

tomography (AET)-generated 3D reconstructions at 18.6Å resolution show that twisting occurs within untensioned microfibrils (Baldock et al., 2001). Localisation of antibody and gold-binding epitopes, and mapping of bead and interbead mass changes in untensioned and extended microfibrils have provided compelling evidence that fibrillin molecules, which form head-to-tail arrays in extended microfibrils (Reinhardt et al., 1996a), undergo intramolecular folding in untensioned microfibrils (Baldock et al., 2001).

These findings allowed us to develop a model of fibrillin alignment in extensible microfibrils in which intramolecular folding would act as a molecular ‘engine’ driving extension and recoil (Baldock et al., 2001; Kielty et al., 2002). The model predicts that maturation from initial parallel head-to-tail alignment (~160 nm) to an approximately one-third stagger (~100 nm) occurs by folding at the termini and the proline-rich region, which would align known fibrillin-1 transglutaminase crosslink sequences. Microfibril elasticity (in the range 56-100 nm) would require further intramolecular folding at flexible sites, which could be links between 8-cysteine motifs (also called ‘TB’ modules because of homology to TGFβ-binding modules in latent TGFβ-binding proteins) and calcium-binding epidermal-growth-factor-like (cbEGF) domains.

In tissues, microfibrils form loosely packed parallel bundles. X-ray fibre diffraction of hydrated zonular microfibril bundles has identified one-third-staggered ‘junctions’ that could modulate force transmission, and quick-freeze deep-etch analysis of zonules has detected links between microfibrils (R. P. Mecham, personal communication). X-ray studies and mechanical testing of microfibril bundles showed that bound calcium influences load deformation but is not necessary for high extensibility and elasticity (Wess et al., 1997; Wess et al., 1998a; Wess et al., 1998b; Eriksen et al., 2001). Thus, microfibril elasticity is modified by, but not dependent on calcium-induced beaded periodic changes, which is consistent with the molecular folding model.

Elastic fibres

Ultrastructural analysis has shown that the elastic fibre core is not really amorphous but instead laterally packed, thin ordered filaments (Pasquali-Ronchetti and Baccarani-Contri, 1997). The architecture of mature elastic fibres is intricate and highly tissue specific, reflecting specific functions in different tissues. In the medial layer of the aorta and elastic arteries, elastic fibres form concentric fenestrated lamellae separated by smooth muscle cell (SMC) layers; this arrangement imparts elasticity and resilience to blood vessel walls. In lung, elastic fibres are present in blood vessel walls and as thin highly branched elastic fibres throughout the respiratory tree that support

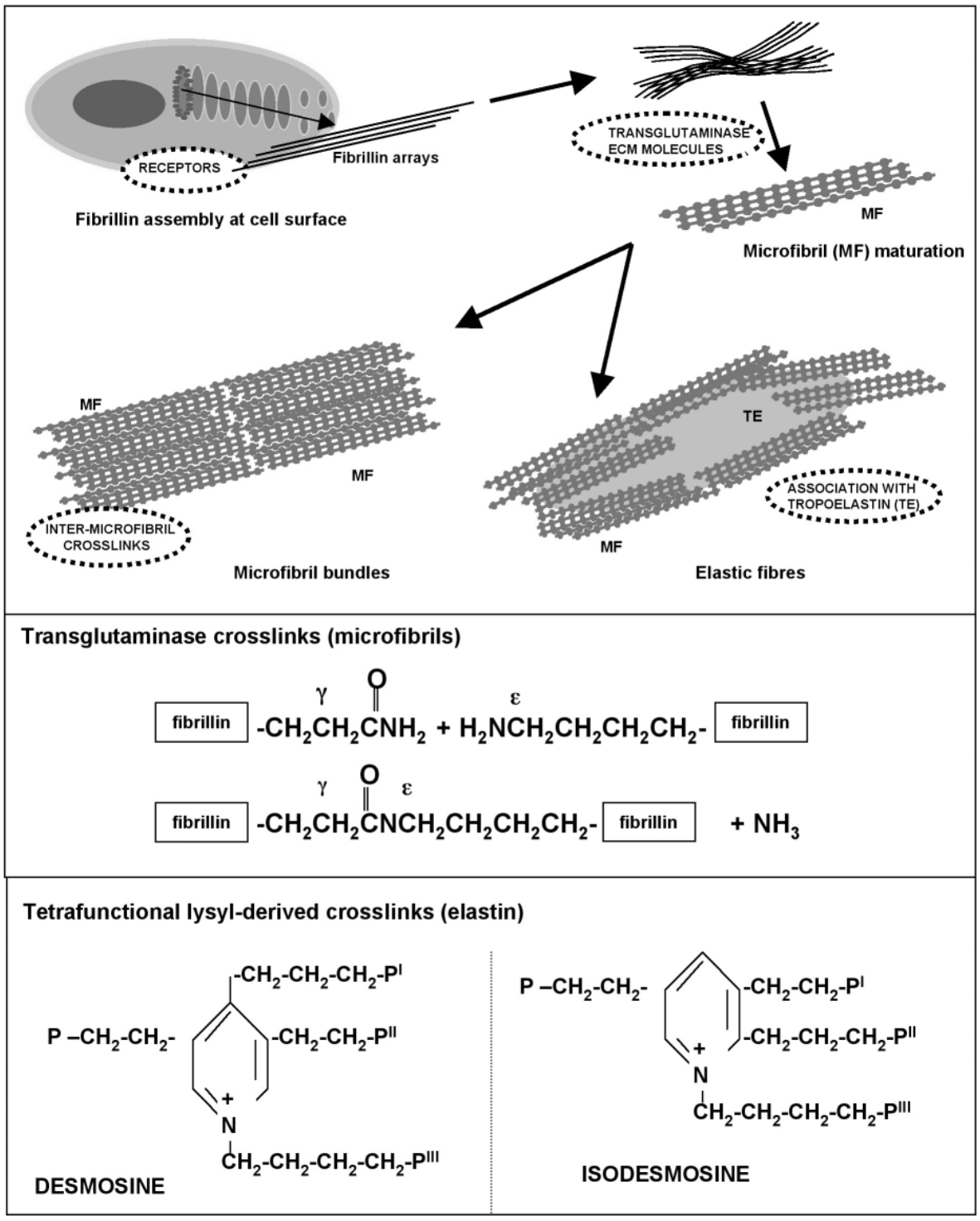

Fig. 2. Microfibril and elastic fibre formation. Fibrillin is assembled pericellularly into microfibrillar arrays that appear to undergo time-dependent maturation into beaded transglutaminase-crosslinked microfibrils. Mature microfibrils form parallel bundles that may be stabilised at inter-microfibrillar crosslinked regions. In elastic tissues, tropoelastin is deposited on microfibril bundles, and lysyl oxidase-derived crosslinks then stabilise the elastin core. Crosslinks catalysed by the actions of transglutaminase and lysyl oxidase are shown. Transglutaminase forms γ-glutamyl-e-lysine isopeptide bonds within or between peptide chains. Lysyl oxidase catalyses the oxidative deamination of certain lysine residues in elastin and subsequent, probably spontaneous, reactions lead to the formation of bifunctional crosslinks (dehydrolysinonorleucine and allysine aldol), a trifunctional crosslink (dehydromerodesmosine), and two tetrafunctional crosslinks (desmosine and isodesmosine, shown here).

alveolar expansion and recoil during breathing. The reticular dermis of skin contains thick, horizontally arranged elastic fibres, whereas the papillary dermis contains thinner perpendicular elastic fibres (elaunin fibres) that merge with the microfibrillar cascade (oxytalan fibres) that intercalates into the dermal-epidermal junction. This continuous elastic network

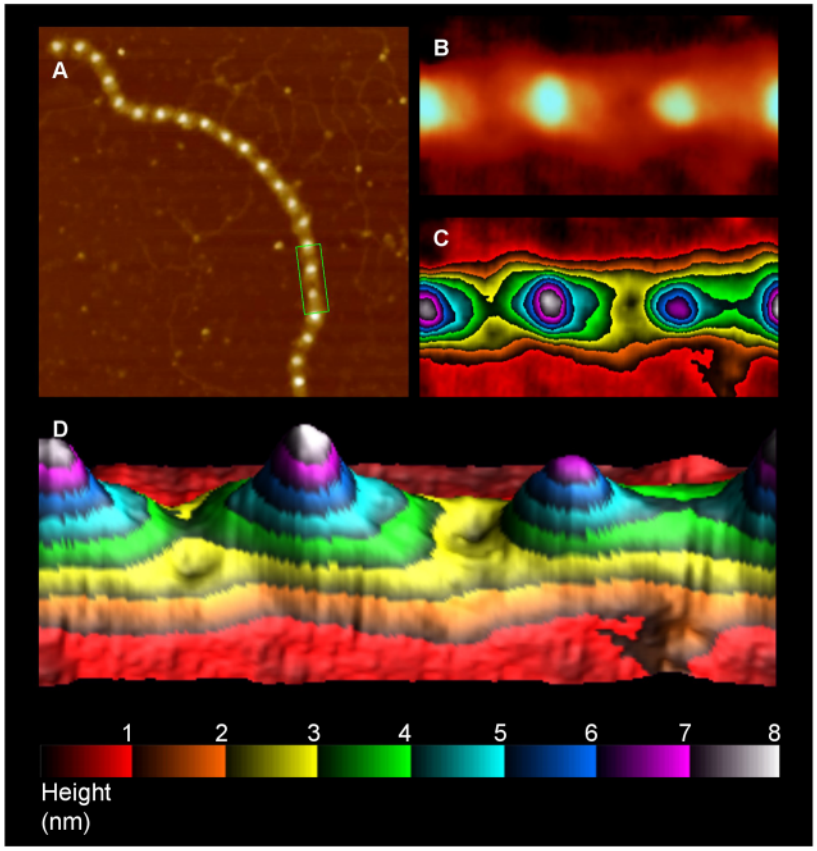

Fig. 3. Tapping mode atomic force microscopy (TMAFM) of isolated fibrillin-rich microfibrils on mica. (A) 1 μm×1 μm scan of beaded microfibrils isolated from fetal bovine nuchal ligament. (B,C) 180 nm×80 nm extracted region of three beaded repeats (C shows height contour map). (D) 3D height contour profile of the three beaded repeat units.

imparts elasticity throughout skin from the reticular and papillary dermis to the epidermis. In auricular cartilage, a thin network of elastic fibres interspersed with collagen fibrils in the interterritorial zone contributes to tissue deformability. Elastic fibres are abundant in flexible ligaments, but sparse in tendons. Tissue-specific arrangements are dictated by the mesenchymal cells that deposit and orientate the microfibril template, and by functional demands.

The biomechanical limitations in microfibrils that led to the evolution of elastin and the appearance of elastic fibres should be revealed once microfibril elastic properties are better understood. Thus, the molecular folding elastic 'motor' model needs rigorous assessment, and both flexible sites within fibrillin-1 and crosslinks that modulate force transmission within and between microfibrils must be identified and characterised. Although it has been recognised for many years that microfibrils form a template for elastin, precisely why microfibrils have this role is still a central question in elastic fibre biology. It is also not clear whether elastin-associated microfibrils differ from those that do not associate with elastin. New insights into the cellular and extracellular basis of tissue-specific elastic fibre architecture are also needed.

Molecular complexity

The inventory of known elastic-fibre-associated molecules has expanded dramatically in recent years following gene mapping of heritable elastic fibre defects, the development of mouse models and detailed immunohistochemical and biochemical studies of elastic tissues. However, the biological roles of many of these molecular players are far from clear. They can be broadly categorised as molecules that co-localise or co-purify with microfibrils, molecules that occur at the elastin-microfibril interface or the elastic-fibre-cell interface, and molecules that are involved in the process of elastic fibre formation. Table 1 lists all the molecules so far identified in these categories, and below we give details of the major players and their potential roles.

Microfibrillar molecules

Fibrillins are the principal structural components of elastic-fibre-associated microfibrils. Fibrillin-1 and fibrillin-2 are encoded by genes on chromosomes 15 and 5, respectively (Pereira et al., 1993; Zhang et al., 1994), and a third, closely related, fibrillin-3 gene has been identified on chromosome 19 (Nagase et al., 2001). The molecules are large glycoproteins (~350 kDa) whose primary structures are dominated by cbEGF domains that, in the presence of Ca^{2+}, adopt a rod-like structure (Downing et al., 1996). Fibrillin-1 and fibrillin-2 have distinct but overlapping patterns of expression (Zhang et al., 1995). Fibrillin-2 is generally expressed earlier in development than fibrillin-1 and may be particularly important in elastic fibre formation (Pereira et al., 1997). Fibrillin-3 was isolated from brain, and whether it is involved in elastic fibres is unknown (Nagase et al., 2001).

Apart from the fibrillins, microfibril-associated glycoprotein 1 (MAGP-1) (sometimes known as MFAP-2) is possibly the best candidate for an integral microfibril molecule important for structural integrity (Gibson et al., 1989; Trask et al., 2000a). It is associated with virtually all microfibrils and widely expressed in mesenchymal and connective tissue cells throughout development. Human MAGP-1 is a 183 residue molecule that has two distinctive domains: an acidic N-terminal half that is enriched in proline residues and has a clustering of glutamine residues, and a C-terminal portion that contains 13 cysteine residues and has a net positive charge. MAGP-1 localises to microfibril beads (sometimes two per bead) (Henderson et al., 1996; Kielty and Shuttleworth, 1997) and is probably disulphide bonded to microfibrils since reduction is required for its extraction.

MAGP-2, the other member of this small microfibrillar protein family, is a 170-173 residue protein structurally related to MAGP-1 mainly in a central region (Gibson et al., 1998; Segade et al., 2002). MAGP-2 is rich in serine and threonine residues and contains an RGD cell-recognition motif through which it binds to the αvβ3 integrin (Gibson et al., 1999). MAGP-2 localises to elastin-associated and elastin-free microfibrils in a number of tissues (Gibson et al., 1998). However, its restricted patterns of tissue localisation and developmental expression

Table 1. Reported microfibril and elastic fibre associated molecules

Molecule	Chromosome	Location	References
Fibrillin-1	15q21.1	Microfibrils	Pereira et al., 1993; Baldock et al., 2001
Fibrillin-2	5q23-q31	Microfibrils	Zhang et al., 1994
Fibrillin-3	19p	? Microfibrils	Nagase et al., 2001
MAGP-1	1pter – 1qter	Microfibrils	Brown-Augsberger et al., 1996
MAGP-2	12p13.1-p12.3	Some microfibrils	Gibson et al., 1998
LTBP-1	2p12-q22	Some microfibrils	Miyazono et al., 1988; Sinha et al., 1998
LTBP-2	14q22–q33	Microfibrils/elastic fibres	Gibson et al., 1995; Bashir et al., 1996
LTBP-3	11q12	?	Yin et al., 1995
LTBP-4	19q13.1	?	Giltay et al., 1997
Decorin	12q13.2	Microfibrils	Trask et al., 2000a
Biglycan	Xq28	Elastic fibre core	Reinboth et al., 2001
Versican	5q12-q14	Some microfibrils	Isogai et al., 2002
MFAP-1	15q15-q21	Some microfibrils	Liu et al., 1997
MFAP-3	5q32-q32.2	Some microfibrils	Abrams et al., 1995
MFAP-4 (MAGP-36)	17p11.2	Some microfibrils	Lausen et al., 1999; Hirano et al., 2002
Tropoelastin	7q11.2	Elastic fibre core	Mecham and Davis, 1994
Lysyl oxidase (LOX)	5q23.3-q31.2	Microfibrils/tropoelastin	Csiszar, 2001
LOXL	8p21.3-p21.2	?	Borel et al., 2001
LOXL2	15q22	?	Csiszar, 2001
LOXL3	2p13	?	Csiszar, 2001
BigH3 (keratoepithelin)	5q31	Elastic fibre/collagen interface	Gibson et al., 1997
Fibulin-1	22q13.3	Elastic fibre core	Kostka et al., 2001
Fibulin-2	3p24-25	Elastin-microfibril interface	Tsuda et al., 2001
Fibulin-5	14q32.1	Elastic fibre–cell interface	Nakamura et al., 2002; Yanagisawa et al., 2002
Emilin-1	2p23	Elastin-microfibril interface	Doliana et al., 1999
Emilin-2	18p11.3	Elastin-microfibril interface	Doliana et al., 2001
Elastin-binding protein	3p21.33	Newly secreted tropoelastin	Prody et al., 1998
Vitronectin	17q11	Some microfibrils	Dählback et al., 1990
Amyloid		Some microfibrils	Dählback et al., 1990
Collagen VIII [α1(VIII)]	3q12-q13.1	Some elastic fibres	Sadawa and Konomi, 1991
Collagen XVI [α1(XVI)]	1p34	Dermal microfibrils	Grässel et al., 1999
Endostatin [α1(XVIII)]	21q22.3	Vascular elastic fibres	Miosge et al., 1999
Collagen VI		Some microfibrils	Finnis and Gibson, 1997
[α1(VI) α2(VI)]	21q22.3		
[α3(VI)]	2q37		

suggest that MAGP-2 has a function related to cell signalling during microfibril assembly and elastinogenesis.

The latent TGFβ-binding proteins (LTBPs) are members of the fibrillin superfamily as a consequence of domain homology. LTBPs are smaller molecules than fibrillins but also comprise repeating cbEGF domains interspersed with TB modules, the latter being found only in the fibrillin superfamily (Sinha et al., 1998; Oklu and Hesketh, 2000). Specific TB modules in LTBP1, LTBP3 and LTBP4 can bind to TGFβ intracellularly, forming a large latent complex that is secreted and then crosslinked to the ECM by transglutaminase. Subsequent proteolytic release of the LTBP-TGFβ complex from ECM precedes TGFβ activation. Thus, LTBPs play an important role in tissue targeting of TGFβ. LTBP-1 colocalises with microfibrils in skin and cell layers of cultured osteoblasts and in embryonic long bone but not cartilage (Taipale et al., 1996; Raghunath et al., 1998; Dallas et al., 2000). Thus, LTBP-1 is unlikely to be an integral structural component, but its association implicates microfibrils in TGFβ targeting (see below). LTBP-2 colocalises with fibrillin-rich microfibrils in elastic-fibre-rich tissues especially in the response to arterial injury, and in trabecular bone (Gibson et al., 1995; Sinha et al., 2002; Kitahama et al., 2000). It is a good candidate for an integral microfibrillar molecule, although it does not bind to TGFβ. It will be of interest to establish whether LTBP-3 and LTBP-4 (Yin et al., 1995; Giltay et al., 1997; Saharinen et al., 1998), both of which can bind TGFβ, can interact with microfibrils and elastic fibres.

Several other microfibril-associated proteins have been identified immunohistochemically, but little is known about whether they are essential microfibrillar components and how they might influence microfibril function. Microfibril-associated protein (MFAP)-1 (also known as AMP), MFAP-3 and MFAP-4 (also known as MAGP-36) colocalise with elastic fibres in skin and other tissues (Horrigan et al., 1992; Abrams et al., 1995; Liu et al., 1997; Lausen et al., 1999; Toyashima et al., 1999; Hirano et al., 2002). In ageing and immune conditions, microfibrils can associate with amyloid deposits and accumulate a coating of adhesive glycoproteins such as vitronectin (Dählback et al., 1990).

Several proteoglycans (PGs) also engage in critically important interactions with microfibrils and contribute to their integration into the surrounding ECM. Early electron microscopy observations using polycationic dyes showed an association between PGs and elastic fibres (Baccarani-Contri et al., 1990). Two members of the small leucine-rich PG family, decorin and biglycan (PG I and PG II) were detected within elastic fibres in dermis; biglycan mapped to the elastin core and decorin mapped to microfibrils. More recent ultrastructural approaches have shown that chondroitin sulphate proteoglycans (CSPGs) are associated with microfibril beads, and a small CSPG co-immunoprecipitates with fibrillin from cultured smooth muscle cell medium (Kielty et al., 1996). Versican, a large CSPG of the lectican PG family, was immunolocalised to microfibrils in skin (Zimmermann et al., 1994). Small CSPGs may contribute to the beaded organisation

of microfibrils, and versican may influence microfibril integration into the surrounding ECM.

Elastic fibre interface molecules

Several molecules localise to the elastin-microfibril interface or to the cell-surface–elastic-fibre interface. These molecules could regulate tropoelastin deposition on microfibrils and link elastic fibres to cell surfaces. One such protein is emilin, a 136 kDa glycoprotein, that localises to the elastin microfibril interface (Bressan et al., 1993; Doliana et al., 1999). Four family members have now been identified: emilin-1, emilin-2, emilin-3 and multimerin (also known as emilin-4), all of which possess a long coiled-coil central region (Colombatti et al., 2000; Doliana et al., 2001). Emilin-1 and emilin-2 contain short triple-helical domains and trimerising C-terminal sequences similar to C1q and the NC1 domains of collagen VIII and collagen X. Apart from emilin-1, it remains to be determined which members of this family bind elastic fibres. Collagen VIII, a product of vascular smooth muscle cells and endothelial cells and a component of their pericellular basement membranes, localises to vascular elastic fibres and may link them to vascular cells (Sadawa and Konomi, 1991).

Members of the fibulin family of cbEGF-domain molecules are also present at elastic fibre interfaces. Three family members are strongly implicated in elastic fibre biology: fibulin-1, fibulin-2 and fibulin-5. Fibulin-1 is located within the amorphous core of elastic fibres but not in fibrillin-rich microfibrils (Kostka et al., 2001). Fibulin-5 localises to the elastin-microfibril interface (Nakamura et al., 2002; Yanagisawa et al., 2002). Fibulin-2 localises preferentially at the interface between microfibrils and the elastin core. It colocalises with fibrillin-1 in skin (except adjacent to the dermal-epithelial junction), perichondrium, elastic intima of blood vessels and the kidney glomerulus, although it does not appear to be present in ciliary zonules, tendon, and surrounding lung alveoli and kidney tubules (Reinhardt et al., 1996b; Utani et al., 1997; Raghunath et al., 1999b; Tsuda et al., 2001). Fibulin-2 is probably not needed for microfibril biomechanical integrity, since labelling is not linearly periodic, and it is absent from tissues subject to strong tensional forces (e.g. tendon, ciliary zonule). It is strongly expressed by smooth muscle cells during cardiovascular development and may be important in elastic fibre deposition and cell migration (Tsuda et al., 2001).

MP78/70 (also known as β-ig-h3 or keratoepithelin) is another molecule that occasionally appears at elastic fibre interfaces. Originally identified in bovine tissue extracts designed to solubilise microfibrils (Gibson et al., 1989), it localises to collagen fibres in ligament, aorta, lung and mature cornea, to reticular fibres in foetal spleen, and to capsule and tubule basement membranes in kidney (Gibson et al., 1997; Schorderet et al., 2000). No general localisation to elastic fibres was observed, but staining in most tissues closely resembles type VI collagen (see Table 1). In some elastic tissues, MP78/70 is present at the interface between collagen fibres and adjacent elastic fibre microfibrils, which suggests that it has a bridging function.

Molecules associated with forming elastic fibres

Tropoelastin is synthesised as a soluble precursor that has a molecular mass of ~70 kDa and alternating hydrophobic and crosslinking domains (Mecham and Davis, 1994; Brown-Augsberger et al., 1995). Interactions between hydrophobic domains are important in assembly and essential for elasticity (Bellingham et al., 2001; Toonkool et al., 2001). The formation of covalent lysyl-derived desmosine crosslinks by lysyl oxidase (Csiszar, 2001) stabilises the polymerised insoluble product (elastin). Five lysyl-oxidase-like proteins have now been characterised [LOX, LOXL, LOXL2 (or WS9-14), LOXL3 and LOXC]. All share homology in their catalytic C-terminal region, but the existence of distinct N-termini suggests different functions. Only LOX and LOXL have so far been shown, after processing from pro-forms by bone morphogenetic protein 1 (BMP-1), to crosslink insoluble elastin (Borel et al., 2001).

Significant progress has thus been made in identifying molecular components of the elastic fibre system, and the challenge now is to determine their biological roles. Of the long list of microfibril-associated molecules, it is highly unlikely that all are involved in assembly. Fibrillins form the backbone of microfibrils, but more sophisticated assembly assays are needed if we are to determine whether co-localising molecules are fundamental structural elements or associated components. In few cases (e.g. MAGP-1, versican) has a direct link been made between co-immunolocalisation with fibrillin and localisation on or within beaded microfibrils, and no molecules other than fibrillins have yet been shown to be necessary for microfibril assembly. New analytical approaches are needed to define microfibril composition in different tissues and to clarify the roles in elastin deposition of molecules at microfibril interfaces with elastin and cells. Much work will then be required to establish how these molecules modulate microfibril function and elastic fibre formation.

Molecular interactions

Recent in vitro binding studies using recombinant or purified molecules and isolated microfibrils have begun to shed light on how the elastic fibre ‘jigsaw’ fits together (Fig. 4). These ‘molecular maps’ are providing clues to structure-function relationships of molecules within elastic fibres.

Interactions between microfibrillar molecules

Expression of deletion constructs in a mammalian system has identified an MAGP-1 matrix-binding domain (MBD) that targets this molecule to the ECM (Segade et al., 2002). Although MAGP-2 contains a sequence similar to the MBD of MAGP-1, it does not associate with the ECM, because of an amino acid residue change. Refolded MAGP-1 produced in a bacterial system can bind to the fibrillin-1 N-terminus (within exons 1-10) in a calcium-dependent manner (Jensen et al., 2001). MAGP-1 and fibrillin-1 are both substrates for transglutaminase and, although only homotypic fibrillin-1 crosslinks have been identified to date, MAGP-1 might be crosslinked within microfibrils (Brown-Augsberger et al., 1996; Qian and Glanville, 1997). MAGP-1 also interacts with collagen VI (Finnis and Gibson, 1997) (Table 1).

A recent study showed that both MAGP-1 and fibrillin-1 interact with decorin, a sulphated CSPG (Trask et al., 2000a). The fibrillin-1-interacting sequence is within or adjacent to the proline-rich region, and the interaction is with the decorin core protein. Decorin can interact with both fibrillin-1 and MAGP-

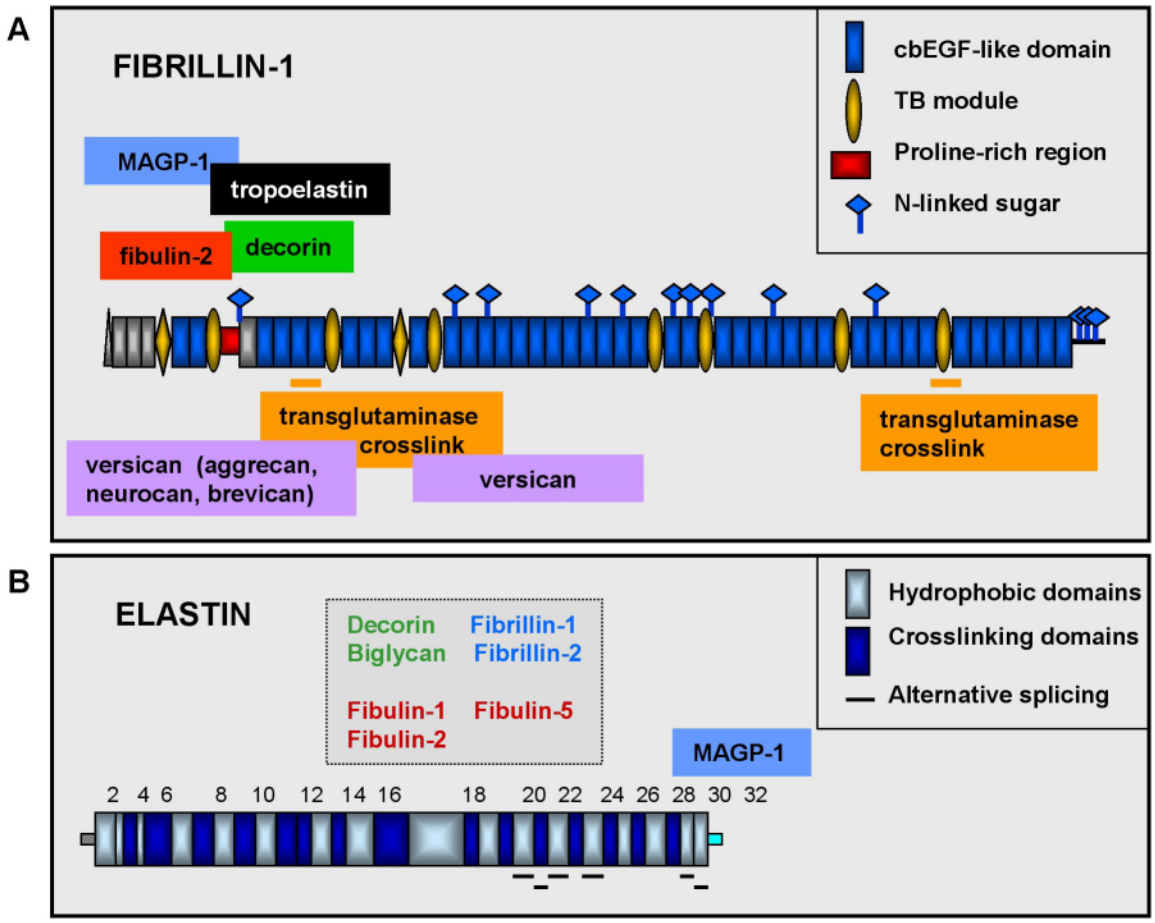

Fig. 4. Domain structures of fibrillin-1 and elastin, showing molecular interaction sites identified in vitro (see Molecular Interactions). (A) Fibrillin-1 has 47 cbEGF-like domains, interspersed with TB modules. A proline-rich region is towards the N-terminus. N-glycosylation sites are indicated. (B) Elastin contains alternating hydrophobic and crosslinking domains. The C-terminus has two cysteines and a negatively charged pocket.

1 individually, and together they form a ternary complex. Fibrillin-2 appears not to interact with MAGP-1 or decorin. Its inability to interact with MAGP-1 suggests either that fibrillin-2 does not support tropoelastin deposition or that MAGP-1 is not necessary for this process (see below). In a separate study, decorin and biglycan were shown not to bind to MAGP-1 and MAGP-2 in solid-phase assays, although MAGP-1 in solution interacted with biglycan but not with decorin (Reinboth et al., 2001). In these two studies, MAGP-1 was expressed in mammalian or bacterial systems, which could explain seemingly contradictory decorin-MAGP-1 interaction results.

The versican C-terminal lectin domain binds N-terminal fibrillin-1 sequences (Isogai et al., 2002). However, its non-periodic association with microfibrils indicates that versican is probably not an integral structural component. Instead, it may associate with microfibrils, and its negatively charged chondroitin sulphate chains may influence integration of microfibrils into the surrounding ECM.

Microfibrillar interactions with tropoelastin

MAGP-1 binds to tropoelastin as well as microfibrillar molecules and might be a critical elastic-fibre-linking molecule (Brown-Augsberger et al., 1996). The tropoelastin-binding site in MAGP-1 is a tyrosine-rich sequence within its positively charged N-terminal half, which may interact with a negatively charged pocket near the tropoelastin C-terminus. MAGP-1 may interact first with fibrillin-1 and decorin during microfibril assembly and then with tropoelastin during elastic fibre formation on the microfibrillar template. Sequences in fibrillin-1 and fibrillin-2 (within exons 10-16) interact with tropoelastin but only in solid-phase studies, which suggests that exposure of a cryptic site is needed (Trask et al., 2000b).

Both decorin and biglycan can bind to tropoelastin. Biglycan binds more avidly than decorin, and the biglycan core protein binds more strongly than the intact PG (Reinboth et al., 2001). The ability of biglycan to form a ternary complex with tropoelastin and MAGP-1 suggests that it has a role in the elastinogenesis phase of elastic fibre formation.

Fibulin-1 does not bind to fibrillin-1 but binds tropoelastin with low affinity (Sasaki et al., 1999). Fibulin-2 is not crosslinked within microfibrils but strongly binds a fibrillin-1 N-terminal sequence (within residues 45-450; exons 2-10) in a calcium-dependent interaction (Reinhardt et al., 1996b). This sequence is also reported to contain an MAGP-1 binding site (see above). Competition by two molecules for the same

fibrillin-1-binding site could represent an important mechanism for regulating microfibril function. Fibulin-2 has a particularly high affinity for tropoelastin and also binds to basement membrane molecules. Fibulin-1 and fibulin-2 interact with the versican C-terminal lectin domain, and fibulin-2 also binds to aggrecan and brevican (Olin et al., 2000). Fibulin-5 is a critical determinant of elastic fibre formation (see below). It binds strongly to tropoelastin in a calcium-dependent manner, but not to fibrillin-1, and colocalises with tropoelastin (Nakamura et al., 2002; Yanagisawa et al., 2002). It serves as a ligand for cell surface integrins αvβ3, αvβ5 and α9β1 through its N-terminal domain and might thus anchor elastic fibres to cells.

Current issues

A bewildering jigsaw of molecular interactions involving fibrillin, elastin and various microfibril-associated molecules has begun to emerge from in vitro binding studies, and it will be a major challenge to define the temporal hierarchy of interactions that drive microfibril and elastic fibre assembly in vivo. The extracellular appearance of fibrillin and assembled microfibrils precedes elastin deposition, and so the timeframe of secretion may provide clues to roles for associated molecules in microfibril assembly or elastin deposition. Comparisons between invertebrate microfibrils and vertebrate microfibril-elastin composites could help identify molecules required for elastin deposition. Cellular and in vitro assembly assays are needed to unravel the significance in assembly of homotypic fibrillin interactions and complexes of fibrillin-1, MAGP-1, decorin, biglycan and tropoelastin. Use of purified assembled microfibrils as ligands may be a useful approach to investigate interactions with associated molecules.

Assembly

The dogma that tropoelastin is deposited on a preformed microfibrillar template (Fig. 2) has proved remarkably robust. However, it now needs updating in terms of the roles of newly identified component molecules and the control exerted by connective tissue cells, both intracellular and extracellular, over elastic fibre assembly and organisation.

Fibrillin-rich microfibrils

Microfibril assembly is, in part, a cell-regulated process that proceeds independently of tropoelastin. Fibrillin-1 may undergo limited initial assembly in the secretory pathway (Ashworth et al., 1999a; Trask et al., 1999), and in this respect is similar to other major ECM macromolecules such as collagens, laminins and proteoglycans. Using an in vitro transcription/translation system supplemented with semi-permeabilised cells as the source of secretory organelles, interactions were detected between recombinant fibrillin peptides and chaperones that play key roles in molecular folding and N-glycosylation (Ashworth et al., 1999b). Such associations may influence intracellular fibrillin assembly. Fibrillins have N- and C-terminal cleavage sites for furin convertase; extracellular deposition requires removal of the C-terminus (Raghunath et al., 1999a; Ritty et al., 1999), a process influenced by N-glycosylation and calreticulin (Ashworth et al., 1999b).

Microfibrils assemble close to the cell surface in a process that might require receptors, as shown for fibronectin, in which dimer interactions with α5β1 integrins induce a conformation change that leads to linear assembly (Sechler et al., 2001). Since RGD sequences in the fourth TB module of fibrillins interact with several integrins (Sakamoto et al., 1996), the latter might influence microfibril assembly in a similar manner. Tiedemann et al. have proposed that heparan sulphate proteoglycans (HSPGs), possibly in the form of cell surface HSPG receptors, have a role in assembly (Tiedemann et al., 2001). CSPGs may be needed for beaded microfibril formation (see above). Sulphation is needed for microfibril assembly, since chlorate treatment ablates microfibril and elastic fibre formation (Robb et al., 1999). This effect may reflect the absence of a PG or undersulphation of fibrillins or MAGP-1.

Different extracellular microfibril populations have been identified. The extracellular environment might thus play a major role in regulating microfibril fate. In human dermal fibroblast cultures, monoclonal antibody 11C1.3 (which binds to beaded microfibrils) does not detect microfibrils until 2 weeks in culture, but a polyclonal antibody (PF2) to a fibrillin-1 pepsin fragment can detect abundant microfibrils within 3 days (Baldock et al., 2001). The time-dependent appearance of 11C1.3-reactive microfibrils suggests a form of maturation that might be due to conformational changes, transglutaminase crosslinking (Fig. 2) or unmasking of a cryptic epitope. In developing vascular tissues, 11C1.3 detects microfibrils associated with medial elastic fibres, but another monoclonal antibody, 12A5.18, which also binds to beaded microfibrils, recognises microfibrils only in collagen-fibril-rich tissues (S. Kogake, S. M. Hall, C.M.K. and S. G. Haworth, unpublished). Different microfibril-associated molecules may influence epitope availability and commit microfibrils to distinct extracellular fates.

Elastin and fibrillin self-assembly

Under appropriate in vitro conditions of temperature and ionic strength, elastin undergoes a process of ordered self-aggregation called coascervation caused by multiple and specific interactions of individual hydrophobic domains, which are usually induced by an increase in temperature (Bellingham et al., 2001; Toonkool et al., 2001). The elastin aggregates formed through coascervation appear as ordered fibrillar structures resembling the elastic fibre core, indicating that the protein has an intrinsic ability to organise into polymeric structures. In vivo, tropoelastin probably binds microfibrils, and then coascervates and becomes crosslinked by lysyl oxidase (Fig. 2).

The molecular form of fibrillin secreted from cells is controversial. As in the case of most major ECM polymers, fibrillins can undergo limited intracellular assembly to form dimers or trimers that could be intermediates during extracellular assembly. However, monomers that have been excluded from assembly are detected in cell culture medium. Difficulties in expressing full-length fibrillin-1 have so far precluded detailed assessment of whether fibrillin can self assemble. However, microscopy studies indicate that assembly occurs in association with cell surfaces and predict a key role for receptors in this process. Subsequent time-dependent microfibril maturation in the ECM could reflect association with

other molecules or transglutaminase crosslink formation. Both fibrillin and elastin interact with chaperones in the secretory pathway, but more work is needed if we are to understand how cells coordinate the production of microfibrillar molecules and elastin during elastic fibre formation, and how they prevent uncontrolled or inappropriate intracellular interactions.

Mouse models

Mouse models are proving to be powerful tools for identifying elastic fibre components and unravelling their biological roles (Dietz and Mecham, 2000). Below, we describe models that show clear elastic fibre defects. Knockout mice lacking some elastic fibre components (decorin, biglycan, LTBP-2, LTBP-3) have no obvious elastic fibre defects and are not covered here (Danielson et al., 1997; Xu et al., 1998; Shipley et al., 2000; Dabovic et al., 2001).

Fibrillin-1

Pereira and co-workers have created a gene-targeted mouse model for Marfan syndrome to test a dominant-negative pathogenesis model in which they replaced central exons 19-24 of the fibrillin-1 gene with a neomycin-resistance (neo) expression cassette, reducing the expression of the *fbn*1 mutant allele (designated mgΔ) by more than tenfold. Heterozygous *fbn*1$^{wt/mut}$ (mgΔ/+) mice express very low levels of mutant protein and are morphologically and histologically indistinguishable from wild-type mice. Homozygous *fbn*1$^{mut/mut}$ (mgΔ/mgΔ) mice produce only small amounts of mutant fibrillin-1, appear normal at birth but die of vascular complications prior to weaning. Some mice show thinning of the proximal aortic wall, which suggests that they experience aneurysmal dilatation as in human Marfan syndrome. Substantially reduced extracellular fibrillin-1 staining but normal elastin staining suggests that organised elastic fibres could accumulate in the absence of normal fibrillin-1-rich microfibrils.

A second fibrillin-1 mutant mouse line (designated the mgR allele), accidentally created as a result of aberrant ES cell targeting, has an 80% reduction in expression of normal fibrillin-1 (Pereira et al., 1999). Heterozygous mice appear normal at birth and throughout adult life. Homozygous mice gradually develop severe kyphosis and die of Marfan-like vascular complications at about 4 months. Newborn homozygous mice have normal vascular anatomy and architecture, including aortic medial elastic lamellae. However, fibrillin hypomorphism appears to trigger a secondary sequence of cell-mediated events, which begin with focal calcifications in the aortic elastic lamellae, progress to intimal hyperplasia, monocytic infiltration of the media, fragmentation of elastic lamellae and loss of elastin content, and finally result in aneurysmal dilatation of the aortic wall.

Tight skin *(Tsk)* mice are a naturally occurring strain that harbours a large in-frame insertion in the fibrillin-1 gene (Kielty et al., 1998; Gayraud et al., 2000). The mutant protein, which is synthesised, secreted and incorporated into the ECM, is ~450 kDa rather than 350 kDa. Heterozygous mice provide a well documented model of scleroderma, and perturbations in the extracellular organisation of fibrillin-1 correlate with increased TGFβ availability.

Chaudry and co-workers recently described fibrillin-2 null and fibrillin-2 mutant mice (Chaudry et al., 2001). The classical shaker-with-syndactyly (*sy*) mice harbour a radiation-induced mutation that results in auditory/vestibular defects together with fusion of the digits (syndactyly) and early lethality. Positional cloning demonstrated that the syndactyly phenotype is caused by loss-of-function mutations in the fibrillin-2 gene. Mutations in the *sy* allelic series of mice include deletion of the fibrillin-2 gene, premature termination in which homozygotes have no detectable fibrillin-2 protein, and in-frame exon 38 deletion (part of the fourth TB module), which may cause a molecular kink; the resulting syndactyly ranges in severity from hard tissue to soft tissue fusion. The syndactyly could be due to aberrant fibrillin-2-rich microfibrils in the cartilaginous limb skeleton or altered availability of TGFβ family growth factors. A second fibrillin-2-knockout mouse has now been described (Arteaga-Solis et al., 2001), revealing that syndactyly is caused by defective mesenchyme differentiation rather than reduced apoptosis of interdigital tissues. Further analysis identified a functional interaction between fibrillin-2-rich microfibrils and bone morphogenetic protein 7 (BMP-7) signalling which, when disrupted, may lead to syndactyly.

Studies of elastin-null mice have confirmed that elastin is an essential determinant of arterial morphogenesis (Li et al., 1998a; Li et al., 1998b). The mice die of obstructive arterial disease, which results from subendothelial cell proliferation and reorganisation of smooth muscle, but not endothelial damage, thrombosis or inflammation. Hemizyous mice have an increased number of lamellar units in the ascending and descending aorta consistent with early developmental compensatory alterations in vessel wall structure. The hemizygous mice phenotype is similar to SVAS, which may be a disease of haploinsufficiency.

Fibulin-1-null mice exhibit vascular wall weakness, which could involve elastic fibre defects (Kostka et al., 2001). Homozygotes die 1-2 days after birth owing to rupture of blood vessels and massive haemorrhages, and also display kidney (glomerular malformation or podocyte disorganisation) and lung pathology (delayed alveolar development). Unexpectedly, fibulin-2-null mice have no obvious phenotype. Fibulin-5-null mice exhibit a severely disorganised elastic fibre system throughout the body (Nakamura et al., 2002; Yanagisawa et al., 2002). They survive to adulthood but have a tortuous aorta with loss of compliance, severe emphysema and loose skin. These tissues contain fragmented elastin but no increased elastase activity, which suggests they have defects in elastic fibre assembly rather than stability.

Fibrillin-1-null mouse models have provided new insights into the Marfan phenotype. Haploinsufficiency or expression of low levels of an allele product that has dominant-negative potential is associated with mild skeletal phenotypes, whereas abundant expression of a dominant-negative allele product leads to a more severe Marfan phenotype. The fibrillin-1 *Tsk* mice phenotype does not overlap with Marfan syndrome, reflecting instead a gain-of-function that has altered TGFβ regulation and excessive fibrosis. Fibrillin-2-null and -mutant mice exhibit recessive syndactyly, indicating a loss-of-function possibly associated with altered BMP-7 activity. Targeted deletion of elastin causes structural and cellular vessel wall abnormalities and altered haemodynamics, indicating a role for elastin in regulating smooth muscle cell proliferation and stabilising arterial structure. Defects in elastic fibre formation in fibulin-5-null mice suggest a key role for this pericellular matrix molecule

in cell-matrix interactions. Although these models have provided valuable insights into the physiological roles of several microfibril and elastic fibre molecules, the lack of elastic fibre phenotype in other knockout mice could either reflect compensatory mechanisms or the fact that these molecules are not critical to elastic fibre formation and function.

Future perspectives

Elastic fibres are large and complex, but still surprisingly poorly understood, ECM macromolecules. They are important because they endow critical mechanical properties on elastic tissues and regulate cell fate in developing tissues such as blood vessels. The major challenges ahead are to establish how cells regulate microfibril and elastic fibre assembly, to define the temporal hierarchy and repertoire of molecular interactions in assembly and to resolve their molecular composition. The biomechanical properties of tissue microfibrils and microfibril-elastin composites, and their molecular basis, must be better understood. At the whole organism level, virtually nothing is yet known about how elastic fibres influence cell behaviour, and so identification of cellular receptors, signalling responses and growth factor relationships is a priority. Together, these approaches will provide a new level of understanding of elastic fibre biology that, in turn, should lead to new strategies for elastic fibre repair and regeneration in ageing and disease.

Studies from our laboratory that are described here were supported by the Medical Research Council, UK.

References

Abrams, W. R., Ma, R. I., Kucich, U., Bashir, M. M., Decker, S., Tsipouras, P., McPherson, J. D., Wasmuth, J. J. and Rosenbloom, J. (1995). Molecular cloning of the microfibrillar protein MFAP3 and assignment of the gene to human-chromosome 5q32-q33.2. *Genomics* **26**, 47-54.

Arteaga-Solis, E., Gayraud, B., Lee, S. Y., Shum, L., Sakai, L. Y. and Ramirez, F. (2001). Regulation of limb patterning by extracellular microfibrils. *J. Cell Biol.* **154**, 275-281.

Ashworth, J. L., Kelly, V., Wilson, R., Shuttleworth, C. A. and Kielty, C. M. (1999a). Fibrillin assembly: dimer formation mediated by amino-terminal sequences. *J. Cell Sci*. **112**, 3549-3558.

Ashworth, J. L., Kelly, V., Rock, M. J., Shuttleworth, C. A. and Kielty, C. M. (1999b). Regulation of fibrillin carboxy-terminal furin processing by N-glycosylation, and association of amino- and carboxy-terminal sequences. *J. Cell Sci*. **112**, 4163-4171.

Ashworth, J. L., Sherratt, M. J., Rock, M. J., Murphy, G., Shapiro, S. D., Shuttleworth, C. A. and Kielty, C. M. (1999c). Fibrillin turnover by metalloproteinases: implications for connective tissue remodelling. *Biochem. J.* **340**, 171-181.

Ashworth, J. L., Kielty, C. M. and McLeod, D. M. (2000). Fibrillin and the eye. *Br. J. Ophthalmol.* **84**, 1312-1317.

Baccarani-Contri, M., Vincenzi, D., Cicchetti, F., Mori, G. and Pasquali-Ronchetti, I. (1990). Immunocytochemical localization of proteoglycans within normal elastic fibres. *Eur. J. Cell Biol.* **53**, 305-312.

Baldock, C., Koster, A. J., Ziese, U., Sherratt, M. J., Kadler, K. E., Shuttleworth, C. A. and Kielty, C. M. (2001). The supramolecular organisation of fibrillin-rich microfibrils. *J. Cell Biol.* **152**, 1045-1056.

Bashir, M. M., Han, M. D., Abrams, W. R., Tucker, T., Ma, R. I., Gibson, M., Ritty, T., Mecham, R. and Rosenbloom, J. (1996). Analysis of the human gene encoding latent transforming growth factor-β-binding protein-2. *Int. J. Biochem. Cell Biol.* **28**, 531-542.

Bellingham, C. M., Woodhouse, K. A., Robson, P., Rothstein, S. J. and Keeley, F. W. (2001). Self-aggregation of recombinantly expressed human elastin polypeptides. *Biochim. Biophys. Acta* **1550**, 6-19.

Borel, A., Eichenberger, D., Farjanel, J., Kessler, E., Gleyzal, C., Hulmes, D. J. S., Sommer, P. and Font, B. (2001). Lysyl oxidase-like protein from bovine aorta. *J. Biol. Chem.* **276**, 48944-48949.

Bressan, G. M., Daga, G., Colombatti, A., Castellani, I., Marigo, V. and Volpin, D. (1993). Emilin, a component of elastic fibers preferentially located at the elastin-microfibril interface. *J. Cell Biol.* **121**, 201-213.

Brown-Augsburger, P., Tisdale, C., Broekelmann, T., Sloan, C. and Mecham, R. P. (1995). Identification of an elastin crosslinking domain that joins three peptide chains. *J. Biol. Chem.* **270**, 17778-17783.

Brown-Augsberger, P., Broekelmann, T., Mecham, L., Mercer, R., Gibson, M. A., Cleary, E. G., Abrams, W. R., Rosenbloom, J. and Mecham, R. P. (1996). Microfibril-associated glycoprotein binds to the carboxyl-terminal domain of tropoelastin and is a substrate for transglutaminase. *J. Biol. Chem.* **269**, 28443-28449.

Cardy, C. M. and Handford, P. A. (1998). Metal ion dependency of microfibrils supports a rod-like conformation for fibrillin-1 calcium-binding epidermal growth factor- like domains. *J. Mol. Biol.* **276**, 855-860.

Chaudry, S. S., Gazzard, J., Baldock, C., Dixon, J., Rock, M. J., Skinner, G. C., Steel, K. P., Kielty, C. M. and Dixon, M. J. (2001). Mutation of the gene encoding fibrillin-2 results in syndactyly in mice. *Hum. Mol. Genet.* **10**, 835-843.

Colombatti, A., Doliana, R., Bot, S., Canton, A., Mongiat, M., Mungiguerra, G., Paron-Cilli, S. and Spessotto, P. (2000). The EMILIN protein family. *Matrix Biol*. **19**, 289-301.

Csiszar, K. (2001). Lysyl oxidases: a novel multifunctional amine oxidase family. *Prog. Nucleic Acid Res. Mol. Biol.* **70**, 1-32.

Dallas, S. L., Keene, D. R., Saharinen, J., Sakai, L. Y., Mundy, G. R. and Bonewald, L. F. (2000). Role of the latent transforming growth factor beta binding protein family in fibrillin-containing microfibrils in bone cells in vitro and in vivo. *J. Bone Mineral Res.* **15**, 68-81.

Dabovic, B., Chen, Y., Colarossi, C., Obata, H., Zambuto, L., Perle, M. A. and Rifkin, D. B. (2001). Bone abnormalities in latent TGF-β binding protein (Ltbp)-3-null mice indicate a role for Ltbp-3 modulating TGF-β availability. *J. Cell Biol.* **156**, 227-232.

Dahlbäck, K., Ljungquist, A., Lofberg, H., Dahlbäck, B., Engvall, E. and Sakai, L. Y. (1990). Fibrillin immunoreactive fibers constitute a unique network in the human dermis: immunohistochemical comparison of the distributions of fibrillin, vitronectin, amyloid P component, and orcein stainable structures in normal skin and elastosis. *J. Invest. Dermatol.* **94**, 284-291.

Danielson, K. G., Baribault, H., Holmes, D. F., Graham, H., Kadler, K. E. and Iozzo, R. V. (1997). Targeted disruption of decorin leads to abnormal collagen fibril morphology and skin fragility. *J. Cell Biol.* **136**, 729-743.

Dietz, H. C. and Mecham, R. P. (2000). Mouse models of genetic diseases resulting from mutations in elastic fiber proteins. *Matrix Biol.* **19**, 481-488.

Doliana, R., Bucciotti, F., Giacomello, E., Deutzmann, R., Volpin, D., Bressan, G. M. and Colombatti, A. (1999). EMILIN, a component of the elastic fiber and a new member of the C1q/Tumor necrosis factor superfamily of proteins. *J. Biol. Chem.* **274**, 16773-16781.

Doliana, R., Bot, S., Mungiguerra, G., Canton, A., Cilli, S. P. and Colombatti, A. (2001). Isolation and characterization of EMILIN-2, a new component of the growing EMILINs family and a member of the EMI domain-containing superfamily. *J. Biol. Chem.* **276**, 12003-12011.

Downing, A. K., Knott, V., Werner, J. M., Cardy, C. M., Campbell, I. D. and Handford, P. A. (1996). 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.

Eriksen, T. A., Wright, D. M., Purslow, P. P. and Duance, V. C. (2001). Role of Ca^{2+} for the mechanical properties of fibrillin. *Proteins: Struct. Funct. Genet.* **45**, 90-95.

Faury, G. (2001). Function-structure relationship of elastic arteries in evolution: from microfibrils to elastin and elastic fibres. *Pathol. Biol. (Paris)* **49**, 310-325.

Finnis, M. L. and Gibson, M. A. (1997). Microfibril-associated glycoprotein-1 (MAGP-1) binds to the pepsin-resistant domain of the alpha 3 (VI) chain of type VI collagen. *J. Biol. Chem.* **272**, 22817-22823.

Gayraud, B., Keene, D. R., Sakai, L. Y. and Ramirez, F. (2000). New insights into the assembly of extracellular microfibrils from the analysis of the fibrillin 1 mutation in the tight skin mouse. *J. Cell Biol.* **150**, 667-680.

Gibson, M. A., Kumaratilake, J. S. and Cleary, E. G. (1989). The protein components of the 12-nanometer microfibrils of elastic and non-elastic tissues. *J. Biol. Chem.* **264**, 4590-4598.

Gibson, M. A., Hatzinikolas, G., Davis, E. C., Baker, E., Sutherland, G. R. and Mecham, R. P. (1995). Bovine latent transforming growth factor β1-binding protein-2: molecular cloning, identification of tissue isoforms, and immunolocalization to elastin-associated microfibrils. *Mol. Cell. Biol.* **15**, 6932-6942.

Gibson, M. A., Kumaratilake, J. S. and Cleary, E. G. (1997).

Immunohistochemical and ultrastructural localization of MP 78/70 (beta ig-H3) in extracellular matrix of developing and mature bovine tissues. *J. Histochem. Cytochem.* **45**, 1683-1696.

Gibson, M. A., Finnis, M. L., Kumaratilake, J. L. and Cleary, E. G. (1998). Microfibril-associated glycoprotein-2 (MAGP-2) is specifically associated with fibrillin-containing microfibrils and exhibits more restricted patterns of tissue localisation and developmental expression than its structural relative MAGP-1. *J. Histochem. Cytochem.* **46**, 871-885.

Gibson, M. A., Leavesley, D. I. and Ashman, L. K. (1999). Microfibril-associated glycoprotein-2 specifically interacts with a range of bovine and human cell types via alphaVbeta3 integrin. *J. Biol. Chem.* **274**, 13060-13065.

Giltay, R., Kostka, G. and Timpl, R. (1997). Sequence and expression of a novel member (LTBP-4) of the family of latent transforming growth factor-β binding proteins. *FEBS Lett.* **411**, 164-168.

Grässel, S., Unsold, C., Schacke, H., Brucner-Tuderman, L. and Bruckner, P. (1999). Collagen XVI is expressed by human dermal fibroblasts and keratinocytes and is associated with the microfibrillar apparatus in the upper papillary dermis. *Matrix Biol.* **18**, 309-317.

Henderson, M., Polewski, R., Fanning, J. C. and Gibson, M. A. (1996). Microfibril-associated glycoprotein-1 (MAGP-1) is specifically located on the beads of the beaded-filament structure for fibrillin-containing microfibrils as visualized by the rotary shadowing technique. *J. Histochem. Cytochem.* **44**, 1389-1397.

Hirano, E., Fujimoto, N., Tajima, S., Akiyama, M., Ishibashi, R. and Okamoto, K. (2002). Expression of 36-kDa microfibril-associated glycoprotein (MAGP-36) in human keratinocytes and its localization in skin. *J. Dermatol. Sci.* **28**, 60-67.

Horrigan, S. K., Rich, C. B., Streeten, B. W., Li, Z. Y. and Foster, J. A. (1992). Characterization of an associated microfibril protein through recombinant DNA techniques. *J. Biol. Chem.* **267**, 10087-10095.

Isogai, Z., Aspberg, A., Keene, D. R., Ono, R. N., Reinhardt, D. P. and Sakai, L. Y. (2002). Versican interacts with fibrillin-1 and links extracellular microfibrils to other connective tissue networks. *J. Biol. Chem.* **277**, 4565-4572.

Jensen, S. A., Reinhardt, D. P., Gibson, M. A. and Weiss, A. S. (2001). Protein interaction studies of MAGP-1 with tropoelastin and fibrillin-1. *J. Biol. Chem.* **276**, 39661-39666.

Keene, D. R., Maddox, B. K., Kuo, H.-J., Sakai, L. Y. and Glanville, R. W. (1991). Extraction of extensible beaded structure and their identification as extracellular matrix fibrillin-containing microfibrils. *J. Histochem. Cytochem.* **39**, 441-449.

Kielty, C. M. and Shuttleworth, C. A. (1993). The role of calcium in the organisation of fibrillin microfibrils. *FEBS Lett.* **336**, 323-326.

Kielty, C. M. and Shuttleworth, C. A. (1997). Microfibrillar elements of the dermal matrix. *Microsc. Res. Tech.* **38**, 407-427.

Kielty, C. M., Cummings, C., Whittaker, S. P., Shuttleworth, C. A. and Grant, M. E. (1991). Isolation and ultrastructural analysis of intact high-M_r assemblies of type VI collagen and fibrillin. *J. Cell Sci.* **99**, 797-807.

Kielty, C. M., Woolley, D. E., Whittaker, S. P. and Shuttleworth, C. A. (1994). Catabolism of intact fibrillin microfibrils by neutrophil elastase, chymotrypsin and trypsin. *FEBS Lett.* **351**, 85-89.

Kielty, C. M., Whittaker, S. P. and Shuttleworth, C. A. (1996). Fibrillin: evidence that chondroitin sulphate proteoglycans are components of microfibrils and associate with newly synthesised monomers. *FEBS Lett.* **386**, 169-173.

Kielty, C. M., Raghunath, M., Siracusa, L. D., Sherratt, M. J., Shuttleworth, C. A. and Jimenez, S. (1998). The *Tight skin* mouse: demonstration of mutant fibrillin-1 production and assembly into microfibrillar abnormalities. *J. Cell Biol.* **140**, 1159-1166.

Kielty, C. M., Baldock, C., Lee, D., Rock, M. J., Sherratt, M. J., Ashworth, J. L. and Shuttleworth, C. A. (2002). Fibrillin: from microfibril assembly to biomechanical function. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **357**, 207-217.

Kitahama, S., Gibson, M. A., Hatzinikolas, G., Hay, S., Kuliwaba, J. L., Evdokiou, A., Atkins, G. J. and Findlay, D. M. (2000). Expression of fibrillins and other microfibril-associated proteins in human bone and osteoblast-like cells. *Bone* **27**, 61-67.

Kostka, G., Giltay, R., Bloch, W., Addicks, K., Timpl, R., Fassler, R. and Chu, M.-L. (2001). Perinatal lethality and endothelial cell abnormalities in several vessel compartments of fibulin-1-deficient mice. *Mol. Cell. Biol.* **21**, 7025-7034.

Lausen, M., Lynch, N., Schlosser, A., Tornoe, I., Sackmose, S. G., Teisner, B., Willis, A. C., Crouch, E. Schwaeble, W. and Holmskov, U. (1999). Microfibril-associated protein 4 is present in lung washings and binds to the collagen region of lung surfactant protein D. *J. Biol. Chem.* **274**, 32234-32240.

Le Saux, O., Beck, K., Sachsinger, C., Silvestri, C., Treiber, C., Goring, H. H., Johnson, E. W., de Paepe, A., Pope, F. M., Pasquali-Ronchetti, I. et al. (2001). A spectrum of ABCC6 mutations is responsible for pseudoxanthoma elasticum. *Am. J. Hum. Genet.* **69**, 749-764.

Li, D. Y., Brooke, B., Davis, E. C., Mecham, R. P., Sorenson, L. K., Boak, B. B., Eichwald, E. and Keating, M. T. (1998a). Elastin is an essential determinant of arterial morphogenesis. *Nature* **393**, 276-280.

Li, D. Y., Faury, G., Taylor, D. G., Davis, E. C., Boyle, W. A., Mecham, R. P., Stenzel, P., Boak, B. and Keating, M. T. (1998b). Novel arterial pathology in mice and humans hemizygous for elastin. *J. Clin. Invest.* **102**, 1783-1787.

Liu, W. G., Faraco, J., Qian, C. P. and Francke, U. (1997). The gene for microfibril-associated protein-1 (MFAP1) is located several megabases centromeric to FBN1 and is not mutated in Marfan syndrome. *Hum. Genet.* **99**, 578-584.

Mecham, R. P. and Davis, E. C. (1994). Elastic fiber structure and assembly. In *Extracellular Matrix Assembly and Structure* (ed. P. D. Yurchenco, D. E. Birk and R. P. Mecham), pp. 281-314. New York: Academic Press.

Miyazono, K., Hellman, U., Wernstedt, C. and Heldin, C. H. (1988). Latent high molecular-weight complex of transforming growth factor-β1–purification from human-platelets and structural characterization. *J. Biol. Chem.* **263**, 6407-6415.

Milewicz, D. M., Urban, Z. and Boyd, C. (2000). Genetic disorders of the elastic fiber system. *Matrix Biol.* **19**, 471-480.

Miosge, N., Sasaki, T. and Timpl, R. (1999). Angiogenesis inhibitor endostatin is a distinct component of elastic fibers in vessel walls. *FASEB J.* **13**, 1743-1750.

Nagase, T., Nakayama, M., Nakajima, D., Kikuno, R. and Ohara, O. (2001). Prediction of the coding sequences of unidentified human genes. XX. The complete sequences of 100 new cDNA clones from brain with code for large proteins in vitro. *DNA Res.* **8**, 85-95.

Nakamura, T., Lozano, P. R., Ikeda, Y., Iwanaga, Y., Hinek, A., Minamisawa, S., Cheng, C.-F., Kobuke, K., Dalton, N., Takada, Y. et al. (2002). Fibulin-5/DANCE is essential for elastogenesis in vivo. *Nature* **415**, 171-175.

Oklu, R. and Hesketh, R. (2000). The latent transforming growth factor beta binding protein (LTBP) family. *Biochem. J.* **352**, 601-610.

Olin, A. I., Morgelin, M., Sasaki, T., Timpl, R., Heinegärd, D. and Aspberg, A. (2000). The proteoglycans aggrecan and versican form networks with fibulin-2 through their lectin domain binding. *J. Biol. Chem.* **276**, 1253-1261.

Pasquali-Ronchetti, I. and Baccarani-Contri, M. (1997). Elastic fiber during development and aging. *Microsc. Res. Tech.* **38**, 428-435.

Pereira, L., D'Alessio, M., Ramirez, F., Lynch, J. R., Sykes, B., Pangilinan, T. and Bonadio, J. (1993). Genomic organization of the sequence coding for fibrillin, the defective gene product in Marfan syndrome. *Hum. Mol. Genet.* **2**, 961-968.

Pereira, L., Andrikopoulos, K., Tian, J., Lee, S. Y., Keene, D. R., Ono, R., Reinhardt, D. P., Sakai, L. Y., Biery, N. J., Bunton, T. et al. (1997). Targetting of the gene encoding fibrillin-1 recapitulates the vascular aspect of Marfan syndrome. *Nat. Genet.* **17**, 218-222.

Pereira, L., Lee, S. Y., Gayraud, B., Andrikopoulos, K., Shapiro, S. D., Bunton, T., Biery, N. J., Dietz, H. C., Sakai, L. Y. and Ramirez, F. (1999). Pathogenetic sequence for aneurysm revealed in mice underexpressing fibrillin-1. *Proc. Natl. Acad. Sci. USA* **96**, 3819-3823.

Prody, P. S., Callahan, J. W. and Hinek, A. (1998). The 67-kDa enzymatically inactive alternatively spliced variant of beta-galactosidase is identical to the elastin/ laminin-binding protein. *J. Biol. Chem.* **273**, 6319-6326.

Qian, R. Q. and Glanville, R. W. (1997). Alignment of fibrillin molecules in elastic microfibrils is defined by transglutaminase-derived crosslinks. *Biochemistry* **36**, 15841-15847.

Raghunath, M., Unsold, C., Kubitscheck, U., Bruckner-Tuderman, L., Peters, R. and Meuli, M. (1998). The cutaneous microfibrillar apparatus contains latent transforming growth factor-beta binding protein-1 (LTBP-1) and is a repository for latent TGF-beta 1. *J. Invest. Dermatol.* **111**, 559-564.

Raghunath, M., Putnam, E. A., Ritty, T., Hamstra, D., Park, E. S., Tschodrich-Rotter, M., Peters, R., Rehemtulla, A. and Milewicz, D. M. (1999a). Carboxy-terminal conversion of profibrillin to fibrillin at a basic site by PACE/furin-like activity required for incorporation in the matrix. *J. Cell Sci.* **112**, 1093-1100.

Raghunath, M., Tschodrich-Rotter, M., Sasaki, T., Meuli, M., Chu, M.-L. and Timpl, R. (1999b). 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.

Reber-Muller, S., Spissinger, T., Schubert, P., Spring, J. and Schmid, V. (1995). An extracellular matrix protein of jellyfish homologous to mammalian fibrillins forms different fibrils depending on the life stage of the animal. *Dev. Biol.* **169**, 662-672.

Reinboth, B., Hanssen, E., Cleary, E. G. and Gibson, M. A. (2001). Molecular interactions of biglycan and decorin with elastic fiber components. Biglycan forms a ternary complex with tropoelastin and MAGP-1. *J. Biol. Chem.* **277**, 3950-3957.

Reinhardt, D. P., Keene, D. R., Corson, G. M., Pöschl, E., Bächinger, H. P., Gambee, J. E. and Sakai, L. Y. (1996a). Fibrillin-1: organization in microfibrils and structural properties. *J. Mol. Biol.* **258**, 104-114.

Reinhardt, D. P., Sasaki, T., Dzamba, B. J., Keene, D. R., Chu, M.-L., Göhring, W., Timpl, R. and Sakai, L. Y. (1996b). Fibrillin-1 and fibulin-2 interact and are colocalized in some tissues. *J. Biol. Chem.* **271**, 19489-19496.

Ringpfeil, F., Pulkkinen, L. and Uitto, J. (2001) Molecular genetics of pseudoxanthoma elasticum. *Exp. Dermatol.* **10**, 221-228.

Ritty, T. M., Broekelmann, T., Tisdale, C., Milewicz, D. M. and Mecham, R. P. (1999). Processing of the fibrillin-1 carboxyl-terminal domain. *J. Biol. Chem.* **274**, 8933-8940.

Robb, B. W., Wachi, H., Schaub, T., Mecham, R. P. and Davis, E. C. (1999). Characterization of an in vitro model of elastic fiber formation. *Mol. Biol. Cell* **10**, 3595-3605.

Robinson, P. N. and Godfrey, M. (2000). The molecular genetics of Marfan syndrome and related microfibrillopathies. *J. Med. Genet.* **37**, 9-25.

Sadawa, H. and Konomi, H. (1991). The alpha 1 chain of type VIII collagen is associated with many but not all microfibrils of elastic fiber system. *Cell Struct. Funct.* **16**, 455-466.

Saharinen, J., Taipale, J., Monni, O. and Keski-Oja, J. (1998). Identification and characterization of a new latent transforming growth factor-β-binding protein, LTBP-4. *J. Biol. Chem.* **273**, 18459-18469.

Sakamoto, H., Broekelmann, T., Cheresh, D. A., Ramirez, F., Rosenbloom, J. and Mecham, R. P. (1996). Cell-type specific recognition of RGD- and non-RGD-containing cell binding domains in fibrillin-1. *J. Biol. Chem.* **271**, 4916-4922.

Sasaki, T., Göhring, W., Miosge, N., Abrams, W. R., Rosenbloom, J. and Timpl, R. (1999). Tropoelastin binding to fibulins, nidogen-2 and other extracellular matrix proteins. *FEBS Lett.* **460**, 280-284.

Schorderet, D. F., Menasche, M., Morand, S., Bonnel, S., Buchillier, V., Marchant, D., Auderset, K., Bonny, C., Abitbol, M. and Munier, F. L. (2000). Genomic characterisation and embryonic expression of the mouse BigH3 (Tgfbi) gene. *Biochim. Biophys. Acta* **274**, 267-274.

Sechler, J. L., Rao, H., Cumiskey, A. M., Vega-Colón, I., Smith, M. S., Murata, T. and Schwarzbauer, J. E. (2001). A novel fibronectin binding site required for fibronectin fibril growth during matrix assembly. *J. Cell Biol.* **154**, 1081-1088.

Segade, F., Trask, B. C., Broekelmann, T. J., Pierce, R. A. and Mecham, R. P. (2002). Identification of a matrix binding domain in the microfibril-associated glycoproteins 1 and 2 (MAGP1 and 2) and intracellular localization of alternative splice forms. *J. Biol. Chem.* **277**, 11050-11057.

Sherratt, M. J., Holmes, D. F., Shuttleworth, C. A. and Kielty, C. M. (1997). Scanning transmission electron microscopy mass analysis of fibrillin-containing microfibrils from foetal elastic tissues. *Int. J. Biochem. Cell Biol.* **29**, 1063-1070.

Sherratt, M. J., Wess, T. J., Baldock, C., Ashworth, J. L., Purslow, P. P., Shuttleworth, C. A. and Kielty, C. M. (2001). Fibrillin-rich microfibrils of the extracellular matrix: ultrastructure and assembly. *Micron* **32**, 185-200.

Shipley, J. M., Mecham, R. P., Maus, E., Bonadio, J., Rosenbloom, J., McCarthy, R. T., Baumann, M. L., Frankfater, C., Segade, F. and Shapiro, S. D. (2000). Developmental expression of latent transforming growth factor β binding protein 2 and its requirement early in mouse development. *Mol. Cell. Biol.* **20**, 4879-4887

Sinha, S., Nevett, C., Shuttleworth, C. A. and Kielty, C. M. (1998). Cellular and extracellular biology of the latent transforming growth factor-beta binding proteins. *Matrix Biol.* **17**, 529-545.

Sinha, S., Shuttleworth, C. A., Heagerty, A. M. and Kielty, C. M. (2002). Expression of latent TGFbeta binding proteins and association with TGFbeta-1 and fibrillin-1 in the response to arterial injury. *Cardiovasc. Res.* **53**, 971-983.

Taipale, J., Saharinen, J., Hedman, K. and Keski-Oja, J. (1996). Latent transforming growth-factor-β1 and its binding-protein are components of extracellular-matrix microfibrils. *J. Histochem. Cytochem.* **44**, 875-889.

Tassabehji, M., Metcalfe, K., Hurst, J., Ashcroft, G. S., Kielty, C. M., Wilmot, C., Donnai, D., Read, A. P. and Jones, C. J. P. (1998). An elastin gene mutation producing abnormal tropoelastin and abnormal elastic fibres in a patient with autosomal dominant cutis laxa. *Hum. Mol. Genet.* **7**, 1021-1028.

Thurmond, F. A. and Trotter, J. A. (1996). Morphology and biomechanics of the microfibrillar network of sea cucumber dermis. *J. Exp. Biol.* **199**, 1817-1828.

Tiedemann, K., Bätge, B., Müller, P. K. and Reinhardt, D. P. (2001). Interactions of fibrillin-1 with heparin/heparan sulphate, implications for microfibrillar assembly. *J. Biol. Chem.* **276**, 36035-36042.

Toonkool, P., Jensen, S. A., Maxwell, A. L. and Weiss, A. S. (2001). Hydrophobic domains of human tropoelastin interact in a context-dependent manner. *J. Biol. Chem.* **275**, 44575-44580.

Toyashima, T., Yamashita, K., Shishibori, T., Itano, T. and Kobayashi, R. (1999). Ultrastructural distribution of 36-kDa microfibril-associated glycoprotein (MAGP-36) in human and bovine tissues. *J. Histochem. Cytochem.* **47**, 1049-1056.

Trask, T. M., Ritty, T. M., Broekelmann, T., Tisdale, C. and Mecham, R. P. (1999). N-terminal domains of fibrillin 1 and fibrillin 2 direct the formation of homodimers: a possible first step in microfibril assembly. *Biochem. J.* **340**, 693-701.

Trask, B. C., Trask, T. M., Broekelmann, T. and Mecham, R. P. (2000a). The microfibrillar proteins MAGP-1 and fibrillin-1 form a ternary complex with the chondroitin sulfate proteoglycan decorin. *Mol. Biol. Cell* **11**, 1499-1507.

Trask, T. M., Trask, B. C., Ritty, T. M., Abrams, W. R., Rosenbloom, J. and Mecham, R. P. (2000b) Interaction of tropoelastin with the amino-terminal domains of fibrillin-1 and fibrillin-2 suggests a role for the fibrillins in elastic fiber formation. *J. Biol. Chem.* **275**, 24400-24406.

Tsuda, T., Wang, H., Timpl, R. and Chu, M.-L. (2001). Fibulin-2 expression marks transformed mesenchymal cells in developing cardiac valves, aortic arch vessels, and coronary vessels. *Dev. Dyn.* **222**, 89-100.

Utani, A., Nomizu, M. and Yamada, Y. (1997). Fibulin-2 binds to the short arms of laminin-5 and laminin-1 via conserved amino acid sequences. *J. Biol. Chem.* **272**, 2814-2820.

Watson, R. E. B., Griffiths, C. E. M., Craven, N. M., Shuttleworth, C. A. and Kielty, C. M. (1999). Fibrillin-rich microfibrils are reduced in photoaged skin. Distribution at the dermal-epidermal junction. *J. Invest. Dermatol.* **112**, 782-787.

Wess, T. J., Purslow, P. and Kielty, C. M. (1997). Fibrillin-rich microfibrils: an X-ray diffraction study and elastomeric properties. *FEBS Lett.* **413**, 424-428.

Wess, T. J., Purslow, P. P., Sherratt, M. J., Ashworth, J. L., Shuttleworth, C. A. and Kielty, C. M. (1998a). Calcium determines the supramolecular organization of fibrillin- rich microfibrils. *J. Cell Biol.* **141**, 829-837.

Wess, T. J., Purslow, P. P. and Kielty, C. M. (1998b). X-ray diffraction studies of fibrillin-rich microfibrils: effects of tissue extension on axial and lateral packing. *J. Struct. Biol.* **122**, 123-127.

Xu, T., Bianco, P., Fisher, L. W., Longenecker, G., Smith, E., Goldstein, S., Bonadio, J., Boskey, A., Heegaard, A. M., Sommer, B. et al. (1998). Targeted disruption of the biglycan gene leads to an osteoporosis-like phenotype in mice. *Nat. Genet.* **20**, 78-82.

Yanagisawa, H., Davis, E. C., Starcher, B. C., Ouchi, T., Yanagisawa, M., Richardson, J. A. and Olsen, E. N. (2002). Fibulin-5 is an elastin-binding protein essential for elastic fibre development in vivo. *Nature* **415**, 168-171.

Yin, W., Smiley, E., Germiller, J., Mecham, R. P., Florer, J. B., Wenstrup, R. J. and Bonadio, J. (1995). Isolation of a novel latent transforming growth factor β-binding protein gene (LTBP-3). *J. Biol. Chem.* **270**, 10147-10160.

Zhang, H., Apfelroth, S. D., Hu, W., Davis, E. C., Sanguineti, C., Bonadio, J., Mecham, R. P. and Ramirez, F. (1994). Structure and expression of fibrillin-2, a novel microfibrillar component preferentially located in elastic matrices. *J. Cell Biol.* **124**, 855-863.

Zhang, H., Hu, W. and Ramirez, F. (1995). Developmental expression of fibrillin genes suggests heterogeneity of extracellular microfibrils. *J. Cell Biol.* **129**, 1165-1176.

Zimmermann, D. R., Dours-Zimmermann, M., Schubert, M. and Bruckner-Tuderman, L. (1994). Versican is expressed in the proliferating zone in the epidermis and in association with the elastic network of the dermis. *J. Cell Biol.* **124**, 817-825.