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Biogenesis and function of fibrillin assemblies

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Abstract

Fibrillin-1 and fibrillin-2 are large cysteine-rich glycoproteins that serve two key physiological functions: as supporting structures that impart tissue integrity and as regulators of signaling events that instruct cell performance. The structural role of fibrillins is exerted through the temporal and hierarchical assembly of microfibrils and elastic fibers, whereas the instructive role reflects the ability of fibrillins to sequester transforming growth factor β (TGF β) and bone morphogenetic protein (BMP) complexes in the extracellular matrix. Characterization of fibrillin mutations in human patients and in genetically engineered mice has demonstrated that perturbation of either function manifests in disease. More generally, these studies have indicated that fibrillins are integral components of a broader biological network of extracellular, cell surface, and signaling molecules that orchestrate morphogenetic and homeostatic programs in multiple organ systems. They have also suggested that the relative composition of fibrillin-rich microfibrils imparts contextual specificity to TGF β and BMP signaling by concentrating the ligands locally so as to regulate cell differentiation within a spatial context during organ formation (positive regulation) and by restricting their bioavailability so as to modulate cell performance in a timely fashion during tissue remodeling/repair (negative regulation). Correlative evidence suggests functional coupling of the cell-directed assembly of micro-fibrils and targeting of TGF β and BMP complexes to fibrillins. Hence, the emerging view is that fibrillin-rich microfibrils are molecular integrators of structural and instructive signals, with TGF β and BMPs as the nodal points that convert extracellular inputs into discrete and context-dependent cellular responses.

Keywords

Elastic fibers; Extracellular matrix; Fibrillin; Marfan syndrome; TGF β

Introduction

Elastic fibers are key architectural elements of connective tissues that are normally subject to stretch and expansile forces, because they provide the mechanical basis of numerous body functions, such as phonation, respiration, and maintenance of vascular tone. Our understanding of the elastic fiber system has evolved throughout the past 50 years concomitantly with advances in protein biochemistry, imaging, gene cloning, and human and mouse genetics. During the original purification of elastin, Hall (1951) noted that an elastase-resistant mucoprotein-containing outercoat was tightly bound to the elastic fibers. At about the same

time, Karrer (1958) showed ultrastructural images of small fibrils (~10 nm in diameter), which lay around and within amorphous elastin, and which Ross and Bornstein (1969) later termed “elastic fiber microfibrils” to refine Low's broader definition of “20-nm-diameter fibers without characteristic collagen banding” (Low 1962). Parallel histochemical studies by Fullmer and Lillie (1958) and by Gawlik (1965) identified microfibrils without and with small amounts of elastin; these were named oxytalan (acid-resistant) and elaunin (stretchable) fibers, respectively. The early observations thus established the notion that the 10-nm-diameter microfibrils were distinct extracellular assemblies that could exist either as individual structures or in association with various amounts of elastin in elastic fibers. Furthermore, visual evidence that microfibrils were deposited into the matrix before tropoelastin was widely interpreted to indicate a role in guiding elastic fiber formation.

Several years later, Sakai et al. (1986) identified fibrillin-1 as the major structural component of the 10-nm micro-fibrils, which Hollister et al. (1990) reported to be significantly reduced in tissues and cultured cells from patients with Marfan syndrome (MFS; OMIM 154700). Dietz et al. (1991) corroborated this last observation by demonstrating that fibrillin-1 mutations cause MFS, whereas cloning work by Lee et al. (1991) led to the discovery of a second fibrillin protein (fibrillin-2) genetically linked with congenital contractural arachnodactyly (CCA; OMIM 121050). By analogy to collagenopathies, Aoyama et al. (1993) argued that heterozygous fibrillin-1 mutations in MFS might adversely affect the deposition, assembly, and/or function of wild-type molecules. Subsequent mouse studies by Pereira et al. (1997, 1999) refined the dominant-negative model of MFS pathogenesis with the observation that disease onset and progression depends on threshold levels of functional microfibrils that trigger secondary cellular events. Additional mouse work by Neptune et al. (2003) later demonstrated that perturbed transforming growth factor β (TGF β) activation is a critical determinant of MFS pathogenesis. A significant role of promiscuous TGF β signaling in driving MFS manifestations was consistent with early work by Dallas et al. (1995) who localized latent TGF β -binding protein-1 (LTBP-1) to non-collagenous fibrils in cultured cells and with concurrent studies by Isogai et al. (2003) who demonstrated that fibrillin-1 binds LTBP-1 and LTBP-4 in vitro. Based on gene expression patterns and the distinct phenotypes of MFS and CCA, Zhang et al. (1995) originally proposed that fibrillin-1 and fibrillin-2 might contribute to different steps in elastic tissue formation and homeostasis. In addition to these roles, mouse work by Arteaga-Solis et al. (2001) later documented the unique contribution of fibrillin-2 to bone morphogenetic protein-7 (BMP7)-driven digit formation, a finding more recently substantiated by Gregory et al. (2005) and by Sengle et al. (2008a) who reported in vivo co-localization of BMPs and microfibrils and in vitro interaction between BMP pro-domains and fibrillins. Thus, a new view has emerged whereby fibrillin-rich microfibrils perform both structural and instructive roles in organismal physiology, and that perturbation of these functions manifests in disease. The scope of the present review is to outline the current understanding of microfibril assembly and function, while pointing out old and new unresolved issues that are likely to constitute the focus of future investigations.

Fibrillin structure

Fibrillins are large (~350-kDa) glycoproteins that are grouped together with LTBPs and fibulins into a structurally related family of extracellular matrix (ECM) proteins (Kielty et al. 2005; Hubmacher et al. 2006; Wagensell and Mecham 2007; Ramirez et al. 2007). Three functional fibrillin genes have been identified in all metazoan genomes sequenced thus far; the sole exception is the rodent fibrillin-3 gene that is inactivated by a chromosomal rearrangement (Corson et al. 2004). The function of fibrillin-3 is yet to be determined, and therefore this protein will not be discussed in subsequent sections of this review. All three fibrillins display superimposable modular structures that consist of 46/47 epidermal growth factor (EGF)-like domains (42/43 of which are of the calcium-binding type; cb-EGF) interspersed with seven 8-

cysteine containing modules (TB/8-Cys; Fig. 1a). Whereas cbEGF-like domains are found in numerous other proteins, TB/8-Cys modules are unique to fibrillins and LTBPs. Additionally, fibrillins contain two hybrid domains composed of TB/8-Cys and cb-EGF sequences, and N- and C-terminal regions with sequence homologies with the respective segments of LTBPs and fibulins. The only major sequence differences between the fibrillins reside in an internal domain, which is enriched in either proline residues (fibrillin-1) or glycine residues (fibrillin-2) or both proline and glycine residues (fibrillin-3), and in the number of integrin-binding Arg-Gly-Asp (RGD) sequences and putative glycosylation sites (Kielty et al. 2005; Hubmacher et al. 2006; Ramirez et al. 2007).

Calcium binding has been shown to stabilize contiguous cb-EGF domains into a rigid linear structure that is thought to be necessary for the assembly of microfibrils and interaction with proteins and for protection from proteolysis (Downing et al. 1996; Reinhardt et al. 1997a, 1997b). The unique RGD sequence in the TB/8-Cys4 module of fibrillin-1 (and by extrapolation the corresponding RGD sequence of fibrillin-2) resides within a flexible loop that apparently favors binding to integrin receptors $\alpha_5\beta_1$, $\alpha_v\beta_3$, and $\alpha_v\beta_6$ (Sakamoto et al. 1996; Pfaff et al. 1996; Bax et al. 2003; Jovanovic et al. 2007). Cell culture experiments have suggested that the RGD sequence in the TB/8-Cys3 module of fibrillin-2 and the common RGD in the TB/8-Cys4 module of fibrillin-1 and -2 contribute differently to platelet-derived growth-factor-induced migration of lung fibroblasts (McGowan et al. 2008). Other in vitro investigations have implicated discrete sequences of fibrillin-1 in the interaction with molecules that modify structural properties of microfibril networks, such as elastin, proteoglycans (PGs), fibulins, and microfibril-associated proteins (MAPGs), and that confer instructive properties to micro-fibrils, such as the LTBPs and BMPs (Fig. 1b; Isogai et al. 2003; Gregory et al. 2005; Sengle et al. 2008a, 2008b; Kielty et al. 2005; Hubmacher et al. 2006; Wagensell and Mecham 2007; Ramirez et al. 2007). Binding with microfibril-associated glycoprotein (MAGPs), LTBPs, and BMPs has also been reported for fibrillin-2 (Isogai et al. 2003; Sengle et al. 2008a; Penner et al. 2002; Werneck et al. 2004; Hanssen et al. 2004). Whereas genetic studies have validated the physiological significance of fibrillin interactions with TGF β and BMP complexes, similar results have not been revealed for molecules that have been implicated in microfibril assembly. However, a recent study of mice in which *Magp-1* was inactivated indicated phenotypes that might be related to improper TGF β signaling (Weinbaum et al. 2008).

Fibrillin assemblies

Microfibrils and elastic fibers display a large variety of tissue-specific architectures that fulfill the mechanical requirements of individual organ systems (Kielty et al. 2005; Ramirez et al. 2007). Illustrative examples include: (1) the long continuous elastic fibers of the perichondrium/ periosteum, which participate in imparting physal constraint and bone elasticity, (2) the parallel bundles of microfibrils in the ocular suspensory ligament, which anchors the lens to the ciliary body, (3) the concentric rings of elastic fibers in the arterial wall, which normalize blood flow throughout the cardiac cycle, and (4) the loose meshwork of microfibrils and elastic fibers in the dermis, which contribute to skin pliability. Assembly of fibrillin monomers into microfibrils and microfibrils into elastic fibers are complex multistep processes that are yet to be fully delineated. As previously mentioned, ultrastructural evidence originally suggested that microfibrils provide the extracellular scaffold that guides tropoelastin alignment and cross-linking (Kielty et al. 2005; Hubmacher et al. 2006; Wagensell and Mecham 2007; Ramirez et al. 2007). In vitro analyses have implicated MAGP-1 and fibulin-5 in this process as bridging molecules between tropoelastin and fibrillins and between them and integrin receptors, respectively (Jensen et al. 2001; Yanagisawa et al. 2002; Nakamura et al. 2002; Rock et al. 2004; Freeman et al. 2005; Lomas et al. 2007; El-Hallous et al. 2007). However, data from mutant mice indicate that elastin cross-linking proceeds normally in the

absence of either fibrillin-1 or fibrillin-2 (Carta et al. 2006), and that elastic fiber morphology is apparently unaffected by the loss of MAGP-1 production (Weinbaum et al. 2008). These findings can be explained by invoking functional redundancy between the fibrillins in guiding elastin assembly and maturation (a point that has not been addressed by the study of doubly null *Fbn* mice) and between MAGPs and other microfibril-associated molecules (a point that may be resolved by the characterization of mice with graded deficiencies of elastic fiber components).

Fibrillins polymerize into microfibrils in which individual molecules are organized in a head-to-tail arrangement and also associate laterally (Sakai et al. 1991; Reinhardt et al. 1996). Rotary-shadowing electron microscopy has visualized fibrillin polymers as multiple strings with regularly spaced beads (Fig. 2a), the periodicity of which can extend from ~50 to ~150 nm depending on the tissue source and extraction procedure (Keene et al. 1991; Ren et al. 1991). Whereas intermolecular disulfide bonding is widely accepted as an important initial step in microfibril assembly (Reinhardt et al. 2000), the precise sequence of events leading to the formation of the beads-on-a-string structure remains controversial. Some investigators have argued that N-terminally mediated formation of homo-dimers potentially drive fibrillin polymerization, even though they disagree on whether this step occurs intracellularly or extracellularly (Trask et al. 1999; Ashworth et al. 1999). Other investigators have more recently demonstrated that C-terminally promoted interactions are required first for fibrillin-1 oligomerization into bead-like structures and subsequently for their incorporation of N-terminal sequences (Hubmacher et al. 2008). Similarly, early investigations have suggested that C-terminal processing of fibrillin-1 by furin/PACE enzymes is a precondition for microfibril formation, without however resolving whether cleavage occurs in the secretory pathway or pericellularly after precursor molecules somehow escape processing in the trans-Golgi network (Ritty et al. 1999; Raghunath et al. 1999; Wallis et al. 2003). Furthermore, proteomic analyses have recently detected unprocessed fibrillin-1 peptides in native microfibrils (Cain et al. 2006).

Several cell culture experiments have implicated heparin/heparan sulfate proteoglycans (HSPGs) and fibronectin in the driving of the initial pericellular steps of microfibril assembly *in vitro*. Some of the studies have shown that a heparin-binding site of fibrillin-1 (conceivably recognized by cell surface HSPGs) synergizes with the proximate RGD sequence to support $\alpha_5\beta_1$ -mediated focal adhesion of dermal fibroblasts, and that inhibition of heparan sulfate attachment to core proteins or glycosaminoglycan sulfation disrupts microfibril assembly (Tiedemann et al. 2001; Ritty et al. 2003; Cain et al. 2005; Bax et al. 2007; Cain et al. 2008). Similar *in vitro* analyses have demonstrated that fibronectin fibrils are absolutely required to drive microfibril formation (Kinsey et al. 2008; Sabatier et al. 2009). Interestingly, one of the studies has reported that fibronectin fibrils interact with the same C-terminally assembled fibrillin oligomers that have been shown to drive the initial formation of microfibrils (Sabatier et al. 2009). This and the additional finding that HSPGs and fibronectin fibrils also support LTBP assembly raise the intriguing possibility that these molecules coordinate the pericellular targeting of latent TGF β complexes to the nascent microfibril scaffold (Dallas et al. 2005; Chen et al. 2007; Kantola et al. 2008). Indeed, dynamic imaging analyses have recently revealed the coupling of cell-mediated assembly and the reorganization of fibronectin and LTBP fibrils during the formation of the provisional matrix (Sivakumar et al. 2006).

Gene expression data indicate that fibrillin-2 is generally produced during organ development and tissue remodeling, but consistently in lower amounts than fibrillin-1, which continues to be produced throughout postnatal growth and in the adult organism (Zhang et al. 1995; Quondamatteo et al. 2002; Kelleher et al. 2004). Studies in chicken, frog, and zebrafish, in particular, have traced the onset of fibrillin gene expression to the beginning of gastrulation (and thus earlier than elastin production), in addition to showing restricted fibrillin production

in mesenchymal tissues (such as notochord, heart, blood vessels, somites, lungs, and limbs) later in development (Gallagher et al. 1993; Skoglund et al. 2006; Miao et al. 2007; Gansner et al. 2008). Comparable tissue-specific patterns have been observed during fetal development of higher vertebrates (Zhang et al. 1995; Quondamatteo et al. 2002; Kelleher et al. 2004). Additional work with recombinant polypeptides has documented that the N- and C-termini of human fibrillin-1 and -2 can interact with each other to form both homotypic and heterotypic macro-aggregates (Lin et al. 2002; Charbonneau et al. 2003). However, in spite of sharing similar expression patterns and identical macromolecular assemblies, the discrete phenotypes of MFS and CCA patients and of *Fbn1*-null and *Fbn2*-null mice indicate that the two microfibril subunits play both unique and overlapping roles in organ formation and function (Arteaga-Solis et al. 2001; Ramirez et al. 2007; Carta et al. 2006). Although the significance of these phenotypic differences is yet to be fully understood, preliminary evidence suggests that they might reflect the relative location of fibrillin-1 and fibrillin-2 within a given macro-aggregate and/or fibrillin-specific interactions with cells and signaling molecules.

The organization of fibrillin molecules within microfibrils and microfibril elasticity remains controversial. On one hand, there is general agreement from epitope-mapping studies that fibrillins are organized in a head-to-tail arrangement in which the beads correspond to the positions at which the N- and C-termini of contiguous parallel monomers reside (Maddox et al. 1989; Reinhardt et al. 1996). On the other hand, three different models have been proposed to explain the way that such an arrangement could afford microfibril extensibility while preserving the multiple intermolecular interactions of fibrillins (Fig. 2; Kielty et al. 2005; Hubmacher et al. 2006; Ramirez et al. 2007). The intramolecular pleating model envisions that un-staggered fibrillin monomers progressively fold into the ~50-nm microfibril period and unfold into longer periods when microfibrils are extended (Fig. 2b; Baldock et al. 2001; Wang et al. 2009). The 1/3-staggered model proposes that each fibrillin molecule is in a 1/3-staggered configuration that accommodates putative trans-glutaminase cross-links (Fig. 2c; Qian and Glanville 1997; Lee et al. 2004). The half-staggered model postulates that fibrillins are half-staggered with the N-terminal halves forming an outer surface on the microfibrils that mediates binding to multiple proteins (Fig. 2d; Kuo et al. 2007). Whereas the intramolecular pleating model seeks to explain the highly extended 150-nm microfibril period (Baldock et al. 2001; Wang et al. 2009), the half-staggered model is based on non-enzymatically extracted microfibrils whose periods are only rarely extended to 80 nm (Kuo et al. 2007). Even in populations of collagenase-digested microfibrils, most periods are between 46 and 66 nm, and only 10% of the population exceeds 70 nm (Wang et al. 2009). The question of microfibril elasticity and the contribution of microfibrils to the elasticity of elastic fibers have been directly addressed by Koenders et al. (2009), but these studies have also been hampered by the difficulties in separating elastin fibers from microfibrils.

Control of TGF β and BMP bioavailability by fibrillin

Multiple mechanisms that operate within and outside the cell and at the cell surface regulate TGF β and BMP signaling in a context-specific temporal (developmental) and spatial (tissue-specific) manner (Derynck and Miyazono 2008). Fibrillin-rich microfibrils contribute to the extracellular regulation of endogenous TGF β activity by providing a structural platform that controls the diffusion, storage, presentation, and release (i.e., bioavailability) of the ligands. TGF β -1, -2, and -3 (hereafter collectively referred to as TGF β unless otherwise indicated) are each secreted either as a small latent complex (SLC) that consists of the bioactive homodimer non-covalently associated with its pro-peptide (latency-associated protein; LAP) or as a large latent complex (LLC) in which the SLC is covalently bound to LTBP-1, -3, or -4 (Fig. 3; Rifkin 2005). Whereas LTBP-1 and -3 can associate with all TGF β isotypes, LTBP-4 binds only to TGF β -1 and at a lower affinity than the other LTBPs. With the notable exception of osteoblastic cells (Dallas et al. 1994), the SLC is usually secreted as part of the larger LTBP-

bound complex (Rifkin 2005). Recent mouse work has validated the earlier hypothesis that LTBP s might also have a structural role in the ECM in agreement with the finding that only a fraction of them are bound to the SLC (Dallas et al. 1995; Dabovic et al. 2009). Association of TGF β with LAP blocks interaction with its receptors, whereas association with LTBP s promotes the targeting of latent TGF β to fibronectin or fibrillin assemblies (Rifkin 2005). With regard to the latter structures, in vitro data indicate that fibrillin-1 and -2 bind LTBP-1 and -4 (Isogai et al. 2003). Several extracellular molecules (including some that are associated with fibrillin-rich microfibrils, such as small leucine-rich PGs, fibulin-4, MAGP-1, emilin-1, and lysyl oxidase) have been implicated in modulating TGF β bioavailability and signaling (Weinbaum et al. 2008; Schaefer and Iozzo 2008; Zacchigna et al. 2006; Hanada et al. 2007; Atsawasuwan et al. 2008). Additionally, integrins α v β ₆ and α v β ₈ have been shown to bind the RGD sequence of LAPs for TGF β -1 and -3 and promote LLC activation through non-proteolytic and proteolytic mechanisms, respectively (Wipff and Hinz 2008).

BMPs are also stored in the ECM and synthesized as complexes containing pro-domains associated with bioactive dimers (Koenders et al. 2009). Albeit less well understood, the extracellular control of BMP bioavailability differs from the mechanisms that modulate endogenous TGF β signaling because of two main features. First, BMPs can be targeted directly to microfibrils through non-covalent interactions between their pro-domains and the N-terminal regions of fibrillin-1 and -2 (Fig. 3; Sengle et al. 2008a). Pro-BMP tethering to microfibrils is probably uncoupled from the process of fibronectin-directed fibrillin assembly or fibronectin-mediated sequestration of latent TGF β complexes. Second, BMP signaling can be activated simply through the competitive displacement of the pro-domain by type II receptors (Sengle et al. 2008b). Hence, soluble antagonists and their modulators might be the only extracellular molecules required to control the activity of free (ECM-unbound) BMP complexes. We can however reasonably predict that microfibril-bound BMPs require proteolytic or non-proteolytic release from fibrillins in order to signal.

In general, several variables appear to influence fibrillin-directed control of endogenous TGF β and BMP signaling. These include the heterogeneous and differential binding affinities of LTBP s for TGF β and for fibrillins (including a role for LTBP-2, which cannot escort TGF β but nevertheless may compete with other LTBP s for binding to fibrillin-1; Hirani et al. 2007), the spatiotemporal expression patterns of activators and inhibitors of matrix-bound and free ligands, and the molecular mechanisms that target TGF β and BMP complexes to selected fibrillin molecules.

Fibrillins in organ development

The transient appearance of an elastic scaffold that demarcates prospective chondrogenic sites in the developing chick limb was originally interpreted to indicate a role of elastic macro-aggregates in tissue compartmentalization (Hurle et al. 1994). In support of such a notion, the disruption and reorganization of the elastic scaffold was noted to precede the experimentally induced formation of ectopic digits in the chick embryo (Hurle and Colombatti 1996). Subsequent analyses of fibrillin mutant mice have corroborated and extended the concept that elastic macro-aggregates define physically discrete tissue compartments and/or guide functionally distinct developmental programs (Ramirez et al. 2007). More recent genetic perturbations in frog and zebrafish embryos have demonstrated the phylogenetic importance of microfibrils in vertebrate development, in addition to reiterating the organ-specific roles of fibrillin proteins (Skoglund and Keller 2007; Gansner et al. 2008).

Mice lacking fibrillin-2 gene (*Fbn2*) expression display a limb-patterning defect (syndactyly) that is not observed in *Fbn1*-null mice, even though both proteins are abundantly deposited in the ECM of the forming autopods (Arteaga-Solis et al. 2001; Penner et al. 2002). Digit

formation is the combined result of chondrogenic outgrowth and interdigital cell death, which are under the control of several signaling molecules, including BMPs (Dahn and Fallon 2000). Syndactyly in *Fbn2*-null mice is accounted for by the impaired commitment of mesenchymal cells in the prospective interdigital tissue to undergo apoptosis, because of failed activation of BMP7-induced cell death determinants (Arteaga-Solis et al. 2001). Consistent with the latter point, syndactyly and polydactyly is also observed in *Bmp7*-null mice (Luo et al. 1995) but not in mice haploinsufficient for either *Fbn2* or *Bmp7*; however, combined *Fbn2* and *Bmp7* haploinsufficiency yields syndactyly and polydactyly in the absence of additional manifestations in other organ systems (Arteaga-Solis et al. 2001). These findings demonstrate that, in the developing autopod, the predominant effect of fibrillin-2 on BMP-7 signaling is positive regulation, and that, in spite of robust expression, fibrillin-1 cannot compensate for the loss of fibrillin-2 in the interdigital space of the forming autopod. Ongoing studies of muscle development and bone formation in microfibril-deficient mice support the notion that fibrillin-1 and -2 differentially regulate signaling by TGF β and BMPs depending on temporal and spatial contexts.

The thoracic aorta is another example of a tissue in which fibrillins play organ-specific roles. The development of the aorta involves the change of vascular smooth muscle cells (VSMCs) from a biosynthetic to a contractile phenotype concomitantly with ECM assembly and maturation (Kelleher et al. 2004). VSMCs can however revert to a biosynthetic phenotype in response to tissue injury. At about mid-gestation, VSMCs begin to deposit and organize fibrillins and tropoelastin into elastic fibers. Whereas fibrillin-2 is largely synthesized during the fetal period, the production of fibrillin-1 increases steadily until neonatal life parallel to the gradual growth of elastic fibers into mature elastic lamellae that separate parallel layers of quiescent VSMCs (Kelleher et al. 2004). The resulting organization of the tunica media into a multilayered structure of alternating VSMCs and elastic lamellae (a.k. a.: the lamellar unit) is the functional determinant of aortic compliance. Impaired maturation of the aortic matrix accounts for dissecting aneurysm and neonatal death of *Fbn1*-null mice (Arteaga-Solis et al. 2001); by contrast, the loss of fibrillin-2 has no impact on vessel maturation and, consequently, on post-natal survival and fitness (Carta et al. 2006). However, mice lacking both fibrillins die at mid-gestation, significantly earlier than either of the parental strains, and exhibit a poorly developed aortic media, implying functional cooperation between fibrillins in promoting elastic matrix assembly (Carta et al. 2006). Furthermore, in contrast to *Fbn1*^{-/-};*Fbn2*^{+/-} mice, which survive through neonatal and early postnatal life, half of the *Fbn1*^{+/-};*Fbn2*^{-/-} embryos die *in utero* suggesting either that a threshold amount of microfibrils is required to drive aortic matrix formation, and that fibrillins contribute differentially to the total amount of fetal microfibrils, or that the fibrillins play functionally distinct roles in vessel morphogenesis (Carta et al. 2006). Collectively, the above studies indicate that the structural and instructive roles of microfibrils are stage-specific, tissue-specific, and fibrillin-specific.

Fibrillins in disease processes

Heterozygous mutations that affect the structure or decrease the expression of fibrillin-1 are responsible for the pleiotropic manifestations of MFS; these principally involve the ocular, skeletal, and cardiovascular systems (Ramirez et al. 2007). A number of MFS patients also display chronic obstructive lung disease and predisposition for pneumothorax, manifestations that were originally equated with destructive emphysema caused by impaired tissue integrity. The finding that newborn *Fbn1*-deficient mice exhibit developmental emphysema without signs of inflammation or tissue destruction later questioned this interpretation of the lung phenotype (Neptune et al. 2003). Indeed, the mouse studies have revealed that impaired lung development is associated with constitutive Smad2/3 signaling and heightened apoptosis, and that systemic administration of TGF β -neutralizing antibodies can rescue this phenotype (Neptune et al. 2003). The causal association between fibrillin-1 mutations and dysregulated

TGF β signaling was subsequently extended to the progression of mitral valve prolapse, muscle hypoplasia, and aortic aneurysm (Ng et al. 2004; Habashi et al. 2006; Cohn et al. 2007). A new model of MFS pathogenesis has therefore emerged whereby *FBN1* mutations are believed to preclude or decrease the matrix sequestration of latent TGF β complexes, thus rendering them more prone to or accessible for activation, and this in turn leads to unproductive tissue remodeling, as evidenced by improper cell behavior and the loss of matrix integrity. An important corollary to this model is that the amount of substrate (LLC) available to activators is the limiting factor in MFS pathogenesis, or stated differently, that physiological levels of LLC activators are sufficient to drive disease progression.

Additional events have been identified that exacerbate TGF β -driven aneurysm progression in MFS. Some investigators have reported that a C-terminal third fragment of fibrillin-1 can apparently displace LTBPs from microfibrils, thus contributing to LLC release from the ECM (Chaudhry et al. 2007). Others have shown that synthetic fibrillin-1 peptides or protein extracts from *Fbn1* mutant aortas can stimulate metalloproteinase production and macrophage chemotaxis, thus increasing TGF β activation (Booms et al. 2005; Guo et al. 2006). In accordance with these last findings, systemic administration of doxycycline to *Fbn1* mutant mice has been reported to improve aortic wall architecture and to delay aneurysm rupture (Chung et al. 2008; Xiong et al. 2008). Analysis of *Fbn1*-null mice (which only exhibit molecular markers of tissue remodeling because of the catastrophic collapse of the microfibril-deficient aortic matrix) has implicated p38 MAPK (mitogen-activated protein kinase) as an early determinant of promiscuous Smad2/3 signaling (Carta et al. 2009). This study has however left unresolved whether stimulation of p38 MAPK in the *Fbn1*-null state is secondary to improper TGF β activation or occurs in response to a structurally abnormal matrix, and whether this and/or other MAPKs may be dysregulated in progressively severe mouse models of MFS.

The realization that improper TGF β signaling is central to MFS pathogenesis has prompted the idea to test, in *Fbn1* mutant mice, the therapeutic efficacy of losartan, an angiotensin II type I receptor antagonist that also blunts TGF β signaling. The results document the ability of the drug to mitigate histological signs of aortic aneurysm and to improve alveolar septation and muscle hypoplasia (Habashi et al. 2006). Although the precise mechanism of action remains to be elucidated, therapy with losartan has subsequently been shown significantly to reduce the rate of aortic growth in a small cohort of children affected by a particularly severe and rapidly progressive form of MFS (Brooke et al. 2008). Whereas these findings are extremely encouraging, both theoretical and practical limitations are apparent that justify pursuing the identification of additional biological targets that may prove amenable to combinatorial therapies in MFS patients who may be refractive or resistant to losartan treatment. Characterization of the precise mechanisms responsible for improper latent TGF β activation in MFS tissues may yield these biological targets.

Conclusions and perspectives

Since the initial purification of fibrillin-1, extraordinary progress has been made in our understanding of the biogenesis and function of extracellular microfibrils. Fibrillins are now widely accepted to participate in the assembly of specialized matrices that confer structural properties to connective tissues, and they provide contextual specificity for TGF β and BMP signals, which regulate matrix formation and remodeling. A new paradigm has thus emerged whereby the fibrillins are integral components of a broader biological network of extracellular, cell surface, and signaling molecules that orchestrate morphogenetic and homeostatic programs in multiple organ systems (Figs. 1b, 3). In this view, fibrillin-rich microfibrils are molecular integrators of structural and instructive signals, with TGF β and BMPs as the nodal points that convert extracellular inputs into discrete and context-dependent cellular responses. It follows

that a temporally regulated hierarchical assembly of fibrillin-rich macro-aggregates might form tissue-specific gradients of effector molecules that are released and activated to different extents during physiological (productive) and pathological (unproductive) tissue remodeling.

We have previously discussed some of the unresolved issues that are likely to direct future research effort. Additional new questions that have emerged include the molecular and cellular mechanisms responsible for targeting TGF β and BMP complexes to selected fibrillin molecules, the physiological significance of these diverse interactions for tissue morphogenesis and homeostasis, and the role of other fibrillin-interacting proteins in specifying structural and instructive properties of microfibrils. Relevant to the second point, preliminary evidence strongly suggests that fibrillin-1 and fibrillin-2 regulate TGF β and BMP signaling differently depending on the tissue and developmental stage, and by either concentrating the ligands at sites of intended function (positive regulation) or inhibiting their bioavailability (negative regulation). The finding that mutations in *FBNI* or *ADAMTS10* and *FBNI* or *ADAMTSL4* can cause Weill-Marchesani syndrome or isolated ectopia lentis, respectively, raises the possibility of potentially important interactions between fibrillin-1 and the two related families of extracellular proteins (Faivre et al. 2003; Dagoneau et al. 2004; Kainulainen et al. 1994; Ahram et al. 2009). Similarly, the heightened TGF β signaling in geleophysic dysplasia resulting from *ADAMTSL2* mutations (Le Goff et al. 2008) supports the conclusion that these families of related proteins perform significant roles in modulating or specifying the functional properties of fibrillin-rich microfibrils.

In summary, three questions relevant to the physiology and pathophysiology of the elastic fiber system remain unanswered. (1) How do tissue- and stage-specific interactions between fibrillins, other ECM molecules, and cell surface receptors translate into morphologically distinct macro-aggregates and matrices with discrete mechanical properties? (2) How does the temporal hierarchical assembly of microfibril macro-aggregates influence the proper sequestration and release of TGF β and BMP ligands during organ formation and tissue remodeling/repair? (3) How do fibrillin mutations lead to dysregulated TGF β and BMP signaling and do these mutations also promote additional cellular responses that sustain and/or exacerbate disease onset and progression in different tissues? Answering these questions will significantly advance our knowledge of the roles of microfibrils (and, more generally, of the ECM) in organ formation and function, in addition to improving the clinical management of congenital and acquired disorders of the connective tissue.

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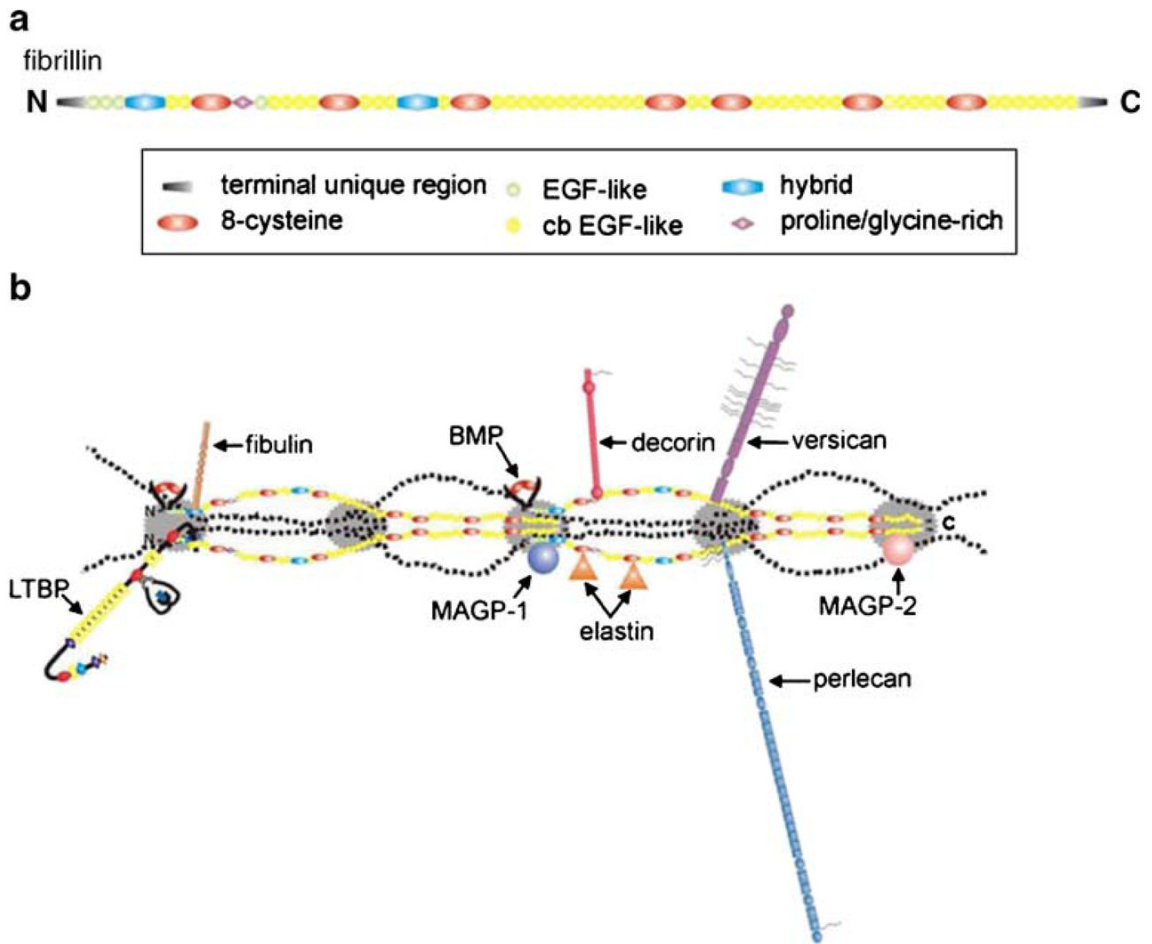


Fig. 1.
a Representation of fibrillin domain structure. All three fibrillins are composed of the same types of domains arranged in the same sequential order. A region of unique sequence is proline-rich in fibrillin-1, glycine-rich in fibrillin-2, and proline- and glycine-rich in fibrillin-3. This region lies between the first 8-cysteine module and the fourth epidermal growth factor (*EGF*)-like domain. **b** Representation of fibrillin ligands as they might appear when bound to microfibrils (*LTBR* latent TGF β -binding protein-1, *BMP* bone morphogenetic protein, *MAGP* microfibril-associated glycoprotein). Ligands and their approximate binding sites are positioned according to published data, using the model for microfibrils proposed by Kuo et al. (2007)

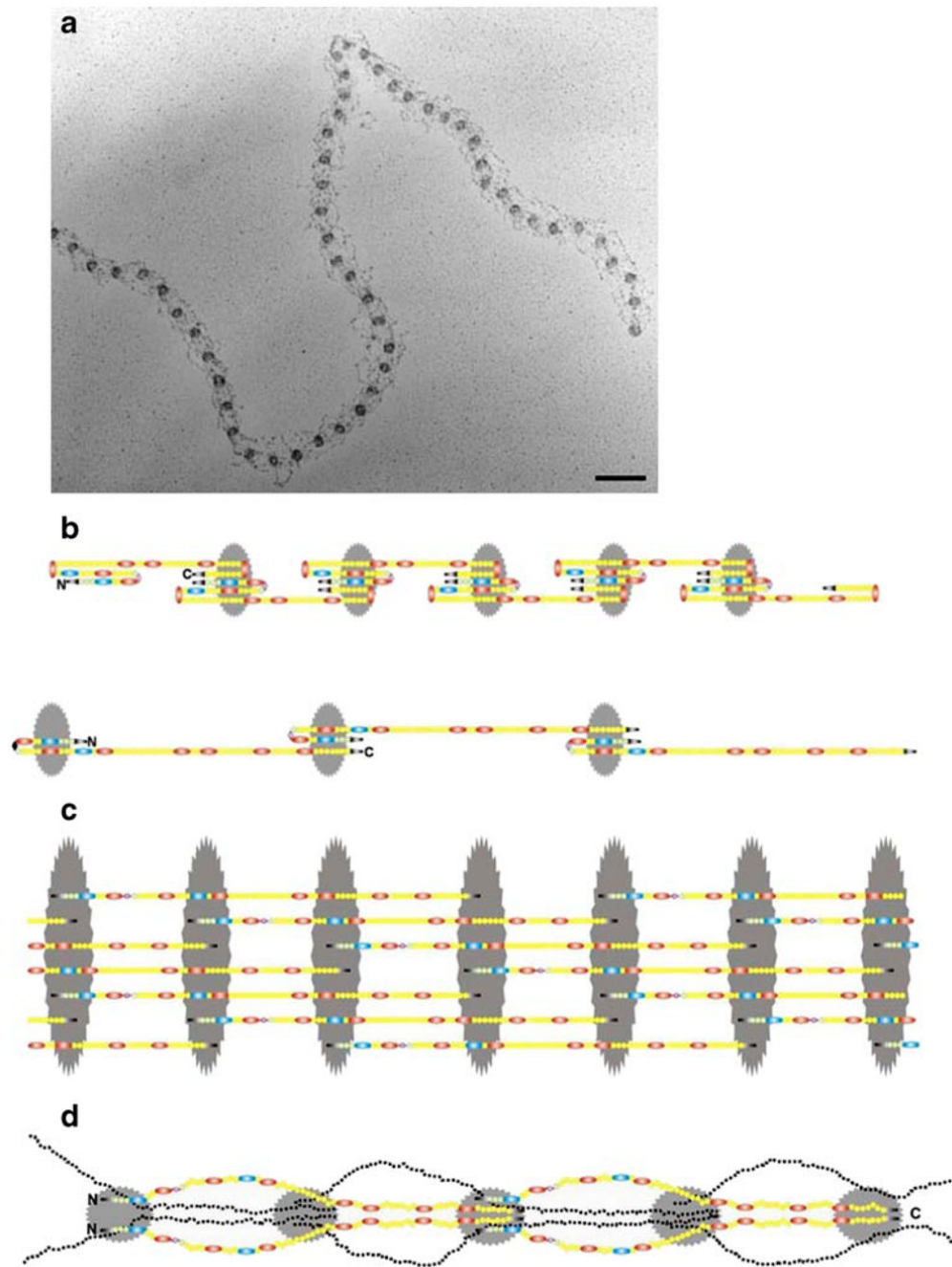


Fig. 2.
a Rotary-shadowed image of a fibrillin microfibril extracted from connective tissue. The bead-to-bead periodicity is approximately 55 nm. *Bar* 100 nm. **b** The intramolecular pleating model. This model allows for the extreme extensibility of extracted microfibrils to periodicities of 150 nm (*bottom*), while also accommodating a 150-nm fibrillin monomer within a 55-nm period (*top*). **c** The 1/3-staggered model. This model is based on molecular studies indicating limited flexibility of interdomain regions in fibrillin. It accommodates crosslinks that suggested a 1/3 stagger. **d** The half-staggered model with N-terminal halves on the surface of the microfibril. This model is based on antibody epitope mapping by using extracted microfibrils and on the

identification of crude collagenase cleavage sites in fibrillin-1. It accommodates the findings of ligand-binding sites to N-terminal halves of fibrillin

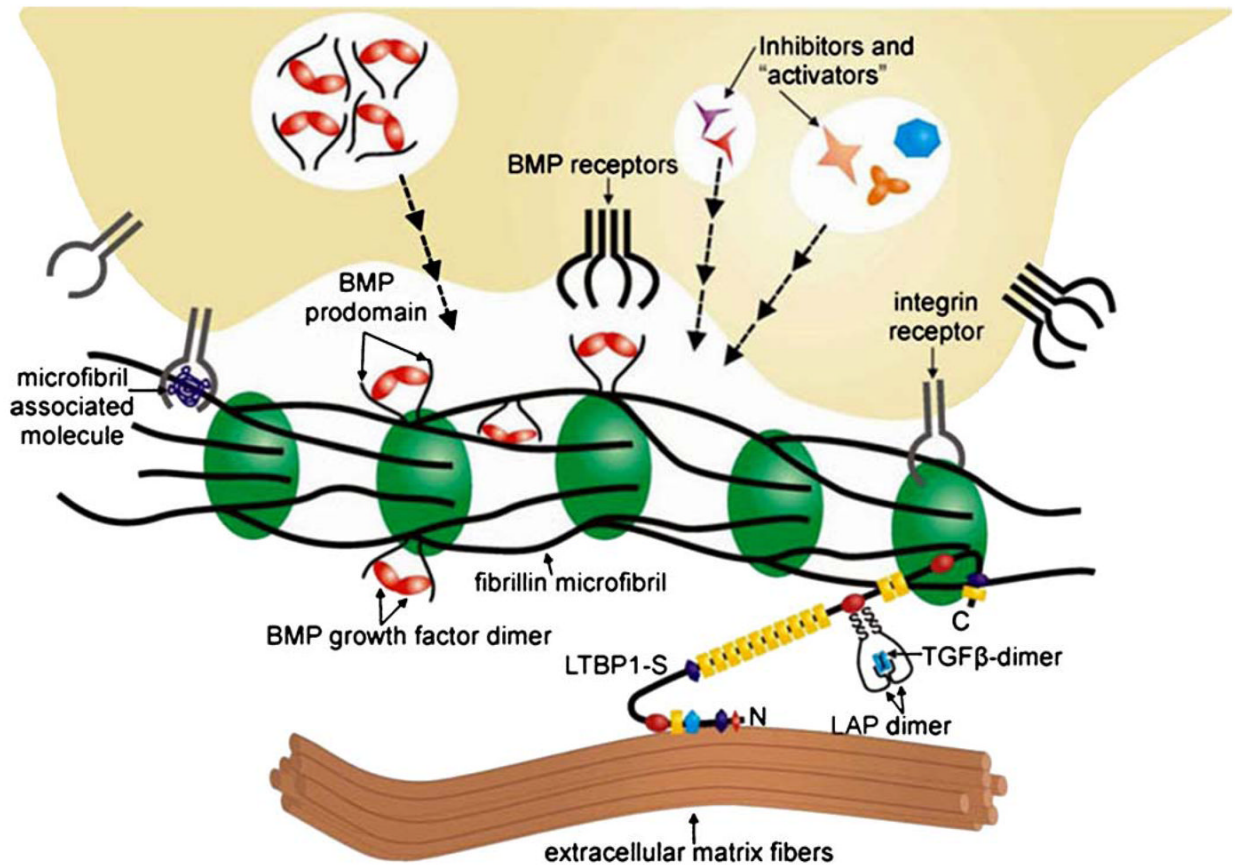


Fig. 3.

Representation of cellular and molecular interactions with fibrillin microfibrils that work in concert to control growth factor signaling. The large latent TGF β complex is associated with fibrillin microfibrils through interactions between LTBPs and fibrillin. BMP complexes are targeted to fibrillin microfibrils through direct interactions with fibrillin. Cells receive positional information through integrin interactions with fibrillin or with microfibril-associated proteins and can activate ECM-sequestered growth factors or inhibit activated growth factors as required by the tissue or developmental context. Defects in fibrillin microfibril structure, perceived by the cell, can result in the secretion of activators of growth factor signaling, leading to MFS and related disorders of the connective tissue