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Review Article

Recent updates on the molecular network of elastic fiber formation

Seung Jae Shin[1,2] **and Hiromi Yanagisawa**[1,3]

[1]Life Science Center for Survival Dynamics, Tsukuba Advanced Research Alliance (TARA), University of Tsukuba, Tsukuba 305-8577, Japan; [2]Graduate School of Life and Environmental Sciences, University of Tsukuba, Ibaraki 305-8577, Japan; [3]Division of Biomedical Science, Faculty of Medicine, University of Tsukuba, Ibaraki 305-8575, Japan

Correspondence: Hiromi Yanagisawa (hkyanagisawa@tara.tsukuba.ac.jp)

Elastic fibers confer elasticity and recoiling to tissues and organs and play an essential role in induction of biochemical responses in a cell against mechanical forces derived from the microenvironment. The core component of elastic fibers is elastin (ELN), which is secreted as the monomer tropoelastin from elastogenic cells, and undergoes self-aggregation, cross-linking and deposition on to microfibrils, and assemble into insoluble ELN polymers. For elastic fibers to form, a microfibril scaffold (primarily formed by fibrillin-1 (FBN1)) is required. Numerous elastic fiber-associated proteins are involved in each step of elastogenesis and they instruct and/or facilitate the elastogenesis processes. In this review, we designated five proteins as key molecules in elastic fiber formation, including ELN, FBN1, fibulin-4 (FBLN4), fibulin-5 (FBLN5), and latent TGFβ-binding protein-4 (LTBP4). ELN and FBN1 serve as building blocks for elastic fibers. FBLN5, FBLN4 and LTBP4 have been demonstrated to play crucial roles in elastogenesis through knockout studies in mice. Using these molecules as a platform and expanding the elastic fiber network through the generation of an interactome map, we provide a concise review of elastogenesis with a recent update as well as discuss various biological functions of elastic fiber-associated proteins beyond elastogenesis *in vivo*.

Introduction

Elastic fibers are amorphous structures comprising insoluble polymerized elastin (ELN) core and a peripheral mantle of microfibrils (Figure 1). The primary function of elastic fibers is to provide elasticity and recoil to tissues and organs in response to mechanical stretch. Disruption of elastic fibers due to inflammatory insult or age-related wearing significantly reduces the function of elastic fibers. ELN is synthesized from mid-embryogenesis throughout the early postnatal period, and the expression is sharply down-regulated [1]. ELN has a long half-life (which is reported to be 40–80 years in humans) [2,3] and elastic fibers do not regenerate spontaneously. Abnormal or disrupted fragments of elastic fibers accumulate, resulting in deterioration of tissue function. Therefore, to develop strategies for the regeneration of elastic fibers, research in the elastic fiber field has been centered on elucidation of the molecular mechanism of elastogenesis. In this review, we will first provide an overview of core molecules in elastogenesis and revisit expanded protein–protein interactions involved in each step of this elegant cellular process. Then, we will explore the dynamic functions of elastic fibers beyond their role in structural support and maintenance of tissue integrity.

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Core elastic fiber network

Biochemical characterization of elastic fibers has been difficult because of its insoluble nature; however, use of chemical inhibitors of cross-linking and improvement of extraction methods by proteolytic degradation [4], together with the advancement of molecular biology facilitated identification of key proteins in elastic fibers. ELN and fibrillin-1 (FBN1) represent building blocks of elastic fibers that compose ELN

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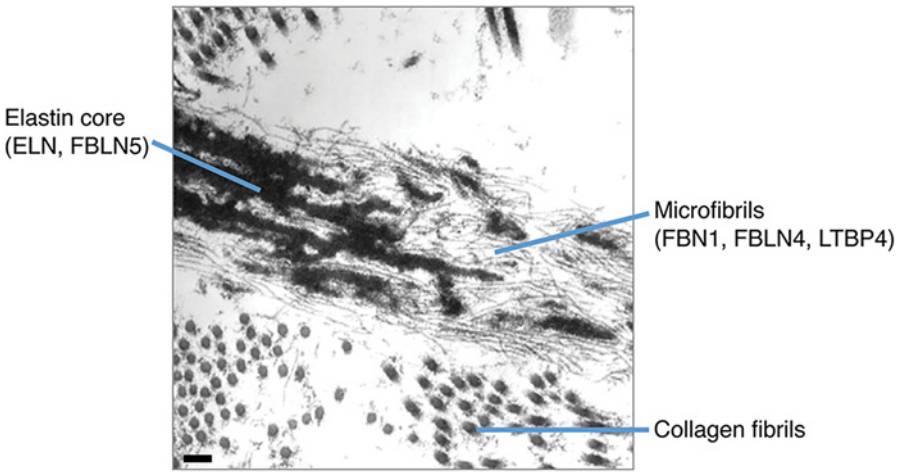

Figure 1. Electron micrograph showing bovine elastic fibers in the skin

Amorphous ELN core is seen within the bundles of microfibrils. Five key elastogenic molecules are indicated. The bar is 200 nm.

core and microfibrils, respectively, and are essential for development and maintenance of ELN-rich tissues such as blood vessels and lungs (reviewed in [5,6]). There are also a growing number of proteins associated with elastic fibers that are involved in different steps of elastogenesis, proteolysis of extracellular matrix (ECM) proteins, and regulation of intercellular signaling (reviewed in [7]).

Fibulin-5 (FBLN5), one of the elastic fiber-associated proteins and a member of the short fibulin family, was initially identified as a vascular remodeling factor (formally called EVEC/DANCE) [8,9]. FBLN5 contains a repeating array of calcium-binding EGF like motifs and a C-terminal fibulin module characteristic of the fibulin family [10]. FBLN5 was serendipitously found to be involved in elastic fiber organization *in vivo* through two independent knockout studies in mice [11,12]. Because of the dramatic phenotype of *Fbln5*-null mice, which exhibits emphysematous lungs, tortuous aorta at birth, progressive loose skin, and adult-onset pelvic organ prolapse together with disrupted elastic fiber morphology, FBLN5 was recognized as a crucial molecule in elastic fiber assembly *in vivo* [13].

Fibulin-4 (FBLN4 encoded by *EFEMP2*) is another member of the short fibulins. It shares high homology with FBLN5 and is localized on microfibrils [14,15]. Knockout mice revealed that FBLN4 plays a crucial role in elastic fiber formation as well as collagen fiber maturation, and the formation of ultrastructural connections, called ELN extensions, between elastic laminae and smooth muscle cells in the aorta [16–20]. *Fbln4*-null mice die soon after birth due to rupture of the aortic wall of an aneurysm, which begins to develop in mid-gestation and rapidly progresses, and is accompanied with degeneration of the aortic media, disruption of elastic fibers, and an increase in apoptotic cells [21]. Mutations in *FBLN4* and *FBLN5* in humans are found to be responsible for autosomal recessive cutis laxa type 1B and 1A, respectively [22,23].

Latent TGFβ binding protein-4 (LTBP4) is a member of the LTBP family, shares structural homology with FBN1, and weakly binds to TGFβ [24–26]. LTBP4 binds FBLN5 and promotes deposition of FBLN5–ELN microaggregates on to microfibrils in a TGFβ-independent manner, thereby aiding in elastic fiber assembly [27]. *Ltbp4* hypomorphic mice ($Ltbp4S^{-/-}$) exhibit age-dependent progression of emphysema and fragmentation of elastic fibers, particularly in the lung and colon [28]. Concordantly, humans with deletion of *LTBP4* develop a wide range of abnormalities including pulmonary, gastrointestinal, and craniofacial defects (Urban–Rifkin–Davis syndrome) [29]. In the skin of Urban–Rifkin–Davis syndrome patients, aggregation of ELN was observed near the microfibrils, which was recapitulated in $Ltbp4S^{-/-}$ mice.

Using these core molecules (ELN, FBN1, FBLN5, FBLN4, and LTBP4), a diagram illustrating the core molecular interactions in elastic fiber assembly is provided in Figure 2. We will next expand the network, build a comprehensive interactome of elastic fiber-associated molecules (Figure 3, and Tables 1 and 2), and provide a link to biological function and disease.

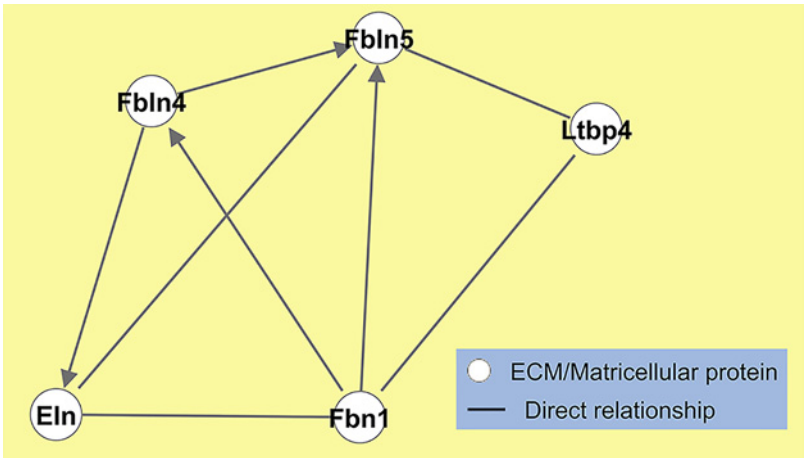

Figure 2. Interactions among core elastogenic genes/gene products

Network generation algorithm in Ingenuity Pathway Analysis (IPA®, Qiagen) allows the transformation of a list of genes into a network [105] using the ingenuity knowledge database (IPKB). IPKB is generated from scientific literature between 1954 and 2019, and each connection is supported by previous publication. IPA is performed to explore the interactome among fibrillin-1 (*Fbn1*), Elastin (*Eln*), fibulin-4 (*Fbln4*, encoded by *Efemp2*), fibulin-5 (*Fbln5*) and LTBP4 (*Ltbp4*), all of which are involved in elastogenesis *in vivo*. Path explorer, which calculates the shortest path between two molecules, is used to explore the direct and indirect connections among five molecules. A solid line indicates direct interaction, and an arrow indicates the direction of interaction and upstream regulator.

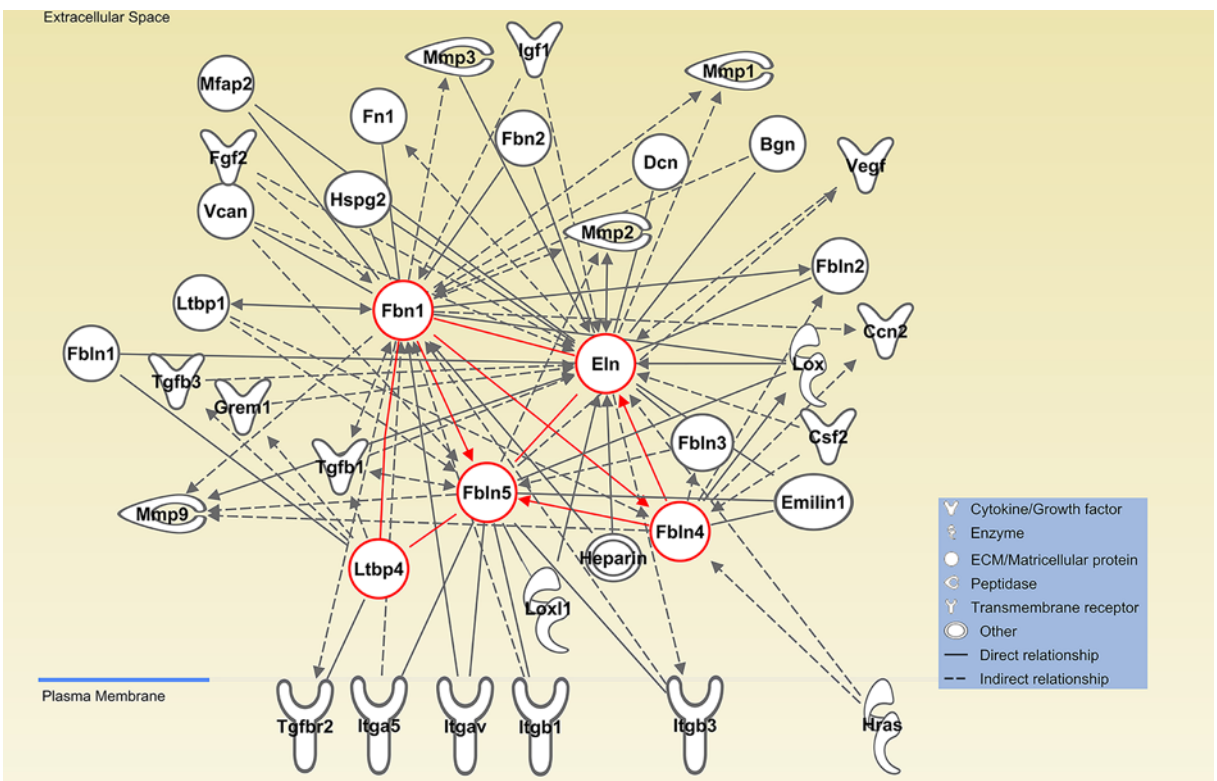

Figure 3. Interactions amongelastic fiber-associated genes/gene products

IPA is performed to explore the extended interactome among elastic fiber-associated molecules using IPKB as described in Figure 2. Path explorer (shortest paths +1), which calculates the shortest path with one additional molecule in between two molecules, is used for five core molecules: fibrillin-1 (*Fbn1*), *Eln*, fibulin-4 (*Fbln4*, encoded by *Efemp2*), fibulin-5 (*Fbln5*), and LTBP4 (*Ltbp4*), that are connected by red solid line. Genes/gene products expressed in extracellular space and plasma membrane are selected (Table 1). A solid line indicates direct interaction, and an arrow indicates the direction of interaction and upstream regulator. Direct interactions that are supported by previous experiments are listed in Table 2 with references.

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Table 1 List of genes identified in IPA

Symbol	Entrez gene name	Function
Extracellular space		
Bgn	Biglycan	ECM/matricellular-protein
Dcn	Decorin	
Efemp1 (*Fbln3*)	EGF containing fibulin ECM protein 1	
Efemp2 (*Fbln4*)	EGF containing fibulin ECM protein 2	
Eln	Elastin	
Emilin1	ELN microfibril interfacer 1	
Fbln1	Fibulin 1	
Fbln2	Fibulin 2	
Fbln5	Fibulin 5	
Fbn1	Fibrillin 1	
Fbn 2	Fibrillin 2	
Fn1	Fibronectin	
Grem1	Gremlin 1, DAN family BMP antagonist	
Ltbp1	Latent transforming growth factor β binding protein 1	
Ltbp4	Latent transforming growth factor β binding protein 4	
Mfap2	Microfibril-associated protein 2	
Vcan	Versican	
Hspg2	Heparan sulfate proteoglycan 2	
Ccn2	Connective tissue growth factor	Growth factor and cytokine
Fgf2	Fibroblast growth factor 2	
Igf1	Insulin-like growth factor 1	
Tgfb1	Transforming growth factor β 1	
Tgfb3	Transforming growth factor β 3	
Vegf	Vascular endothelial growth factor	
Csf2	Colony stimulating factor 2	
Lox	Lysyl oxidase	Enzyme
Loxl1	Lysyl oxidase like 1	
Mmp1	Matrix metallopeptidase 1	
Mmp2	Matrix metallopeptidase 2	
Mmp3	Matrix metallopeptidase 3	
Mmp9	Matrix metallopeptidase 9	
Plasma membrane		
Itga5	Integrin subunit α 5	Transmembrane receptor
ItgaV	Integrin subunit α V	
Itgb1	Integrin subunit β 1	
Itgb 3	Integrin subunit β 3	
Tgfbr2	Transforming growth factor β receptor 2	
Hras	HRas proto-oncogene, GTPase	Enzyme

ELN deposition and roles of elastic fiber-associated proteins

ELN is secreted from elastogenic cells as a 60–70 kDa monomer tropoelastin, which undergoes self-aggregation and cross-linking and is then deposited on to microfibrils. The self-aggregation property of tropoelastin is called coacervation. Coacervation includes phase separation from monomer to multimer and an irreversible coalescence of coacervated tropoelastin multimers; processes that occur spontaneously under physiological conditions [30]. FBLN5 binds tropoelastin with high affinity and facilitates coacervation, but limits coacervate maturation [31,32], thereby rendering the coacervates to subsequent cross-linking and assembly. FBLN4 also promotes coacervation and suppresses maturation, but less effectively than FBLN5, even though FBLN4 and FBLN5 exhibit similar half-maximal binding concentrations to tropoelastin (8 nM and 1–2 nM, respectively) [14].

Weak binding between tropoelastin and fibulin-3 (FBLN3, another short fibulin encoded by *EFEMP1*) has been demonstrated [14]. The contribution of FBLN3 to deposition of ELN, however, has not been reported and *in vivo* role of FBLN3 seems tissue-specific, affecting development of elastic fibers in the visceral fascia and the pelvic floor

Table 2 Interactome generated by IPA

Interaction	References
Eln–Fbn1	[15,51,64]
Eln–Fbn2	[64]
Eln–Fbln1	[14,93]
Eln–Fbln2	[14,93]
Eln–Fbln3	[14]
Eln–Fbln4	[14,16,51]
Eln–Fbln5	[12,14,51]
Eln–Lox	[49]
Eln–Loxl1	[49,50]
Eln–Mfap2	[66,94]
Eln–Emilin1	[45]
Eln–Bgn	[95]
Eln–Dcn	[95]
Eln–Mmp2/Mmp3/Mmp9	[96,97]
Eln–Hspg2	[93]
Eln–heparin	[98]
Fbn1–Fbn2	[99]
Fbn1–Fn1	[59,60,98]
Fbn1–Ltbp1	[67,100]
Fbn1–Ltbp4	[67,100]
Fbn1–heparin/heparan sulfate	[61,98]
Fbn1–Fbln2	[101]
Fbn1–Fbln4	[15,51]
Fbn1–Fbln5	[15,51]
Fbn1–Vcan	[68]
Fbn1–Hspg2	[61,102]
Fbn1–Mfap2	[66]
Fbn1–Lox	[51]
Fbn1–Itgav	[70]
Fbln4–Emilin1	[46]
Fbln4–Fbln5	[51]
Fbln4–Lox	[17,51]
Fbln5–Loxl1	[50]
Fbln5–Emilin1	[45]
Fbln5–Ltbp4	[27]
Fbln5–Itga5	[14]
Fbln5–Itgav	[8]
Fbln5–Itgb1	[103]
Fbln5–Itgb3	[8]
Ltbp4–Tgfbr2	[104]

Direct interactions shown in Figure 3 that are confirmed by experiments are listed.
Symbols are shown for mouse genes.

[33,34]. Fibulin-2 (FBLN2) belongs to the long fibulin family. Together with its prototype fibulin-1 (FBLN1), FBLN2 exhibits a high tropoelastin binding affinity, similar to that of FBLN5 [14]. Nevertheless, FBLN2 seems to play a limited role in elastogenesis as *Fbln2*-null mice do not exhibit any discernible elastic fiber phenotypes, most likely due to compensation by FBLN5 [35,36]. The isoform, FBLN1C exhibits an intermediate binding strength to tropoelastin (i.e., less than FBLN4 but greater than FBLN3) [14], but an elastic fiber phenotype has not been reported so far in the transgenic mouse models. In addition to fibulins, microfibril-associated protein 4 (MFAP4) has been shown to promote coacervation *in vitro*; however, *Mfap4*-null mice do not show a reduced ELN content, nor ultrastructural abnormalities in elastic fibers [37,38].

FBLN5- and FBLN4-mediated coacervates are deposited on to microfibrils. Whereas FBLN5 is known to use LTBP4 for deposition of the coacervates on to microfibrils, the role of LTBP4 in a coacervate deposition is complex. For example, splice variants LTBP4L (long form) interacts with FBLN4, whereas LTBP4S (short form) interacts with FBLN5.

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Interestingly, the absence of both types of LTBP4 results in severe elastic fiber defects *in vivo* [39,40]. LTBP2, a member of the LTBP family, was previously shown to bind FBLN5 [41]. A study analyzing *Ltbp2*-null mice revealed that LTBP2 is essential for stabilization of microfibrils in ciliary zonules [42]. Furthermore, LTBP2 and LTBP4 have overlapping functions in the formation of microfibrils in various tissues *in vivo* [43]. On the other hand, the role of LTBP3 appears to be distinct from that of LTBP2 or LTBP4, and it is critical in regulating latent TGFβ complexes and TGFβ levels in the tissues [44]. To add to the complexity of these interactions, ELN microfibril interface-located protein (EMILIN1), which was previously shown to co-localize with FBLN5 in the aorta [45], binds FBLN4 and is involved in deposition of FBLN4 *in vitro* [46]. *Emilin1*-null mice exhibit a mild alteration of elastic fibers, including irregular elastic lamellar surface and decreased anchorage of endothelial cells and smooth muscle cells to elastic laminae [45]. Taken together, the initial stages of elastogenesis involve a process in which FBLN4 and FBLN5 bind spontaneously coacervated ELN utilizing different LTBP4 variants to deposit ELN on to microfibrils.

Cross-linking

After ELN coacervates are deposited on to microfibrils, cross-linking is mediated by lysyl oxidase (LOX) and LOX-like 1 (LOXL1), two enzymes that belong to the LOX family, comprising five members. LOX and LOXL1 mediate cross-linking of ELN via oxidative deamination of peptidyl lysine residues in a copper- and lysyl-tyrosyl-quinone (LTQ)-dependent manner thereby yielding covalent ELN-specific cross-links desmosine and isodesmosine [47]. Recently, LOXL2 has been reported to directly bind and cross-link tropoelastin *in vitro* [48]. LOX also mediates cross-linking of collagen whereas LOXL1 seems to be more specific for ELN compared with collagen. LOX is regulated not only at a transcriptional level, but also at a post-translational level through proteolytic activation of the pro- to mature-form, mediated by bone morphogenetic protein-1 (BMP1). LOX and LOXL1 are secreted as proenzymes and tethered to ECM. It was reported that pro-regions of LOX and LOXL1 were required for their localization on to elastic fibers [49]. Later, it has been shown that the N-terminal domain of FBLN4 binds the N-terminal propeptide domain of LOX, whereas the C-terminal domain of FBLN5 binds the propeptide domain of LOXL1 [17,50,51]. Based on biochemical data and observations in animals lacking FBLN4, direct interactions between FBLN4 and LOX promote binding of LOX on to tropoelastin [17] as well as regulate LOX enzymatic activity [20,52]. Hence, FBLN4 not only participates in the deposition of ELN coacervates on to microfibrils but also regulates ELN cross-linking.

Fbln4-null mice die at birth due to severe elastic fiber and collagen fiber defects, which result in aortic aneurysm rupture and herniation of the diaphragm, essentially phenocopying *Lox*-null animals [53,54]. Similarly, *Loxl1*-null mice show overlapping phenotypes with *Fbln5*-null mice, which includes loose skin, emphysematous lungs, and genital prolapse [50]. Since elastic fibers in *Fbln5*-null mice were reported to contain the uncleaved, inactive form of LOXL1, it was suggested that FBLN5 potentially promotes activation of LOXL1 [55]. In addition to FBLN4, LOX is regulated directly by matricellular protein thrombospondin-1 (THBS1) [56] and indirectly by thrombospondin-2 (THBS2) via microRNA, miR-29 [57]. The absence of THBS1 or THBS2, however, affects collagen fibers predominantly as these proteins have not been reported to co-localize with elastic fibers. It is conceivable that elastic fiber-associated proteins regulate the enzymatic activity of LOX or LOXL1, leading to effective spatial distribution and processing of enzymes in close proximity to their substrates and facilitates cross-linking and assembly of tropoelastin.

Microfibrils as scaffolds of elastic fibers

ELN always coexists with microfibrils *in vivo*. Microfibrils are believed to provide a scaffold function based on the molecular interactions between FBN1 and ELN directly, or via elastic fiber-associated proteins. FBN1 is the major component of microfibrils and a large protein with a molecular mass of approximately 320 kDa. Fibrillin-2 (FBN2) is also a component of microfibrils but is predominantly synthesized during embryogenesis [58]. Mutations in the fibrillin-1 gene (*FBN1*) are responsible for Marfan syndrome, an autosomal dominant connective tissue disorder involving cardiovascular, skeletal, and ocular tissues, manifesting aortic root aneurysms, arachnodactyly, and lens dislocation.

FBN1 undergoes homophilic interactions to form microfibrils with a 10–12 nm diameter and a 'beads-on-a-string' appearance (reviewed in [5]). Fibronectin is required for the formation of the FBN1 scaffold [59,60] and heparan sulfate regulates polymerization of FBN1 [61]. FBN1 oligomers extend and retract in response to mechanical stretch and support mechanical functions of microfibril-rich tissues as a component of elastic fibers (i.e., cardiovascular, lungs) or independent of elastic fibers, such as in ocular zonules. The biological function of microfibrils as a scaffold for elastic fiber assembly was confirmed by knockout studies of *Fbn1* and *Fbn2* in mice, both of which are major components of microfibrils. Deletion of *Fbn1* alone led to neonatal lethality due to aortic aneurysms and elastic fiber

defects, and deletion of both genes abolished elastic fiber formation in the aortic wall and led to embryonic lethality, indicating that FBN1 and FBN2 scaffolds are required for proper assembly of elastic fibers [62].

FBN1 interacts with various structural and elastic fiber-associated proteins through its multiple binding domains [63]. *In vitro* binding assays demonstrated that N-terminal domains of FBN1 bind tropoelastin [64]. High-affinity binding between tropoelastin and the central domain of FBN1 is also documented [65]. In addition to the binding between FBN1 and tropoelastin, FBN1 binds various proteins associated with microfibrils. For example, microfibril-associated glycoprotein-1 (MAGP1) encoded by the *MFAP2* gene has been shown to bind FBN1 and tropoelastin moderately, suggesting a possible interaction between MAGP1 and tropoelastin on the microfibrils [65,66]. MFAP4 has been recently shown to bind both FBN1 and tropoelastin, as well as desmosine, an ELN-specific cross-link [37]. LTBP1 interacts with the N-terminal region of FBN1 via its C-terminal regions, although LTBP1 is not an integral structural component of microfibrils. LTBP1 is tethered to the surrounding ECM by transglutaminase [67].

Versican, a large chondroitin sulfate proteoglycan, is covalently bound to FBN1 microfibrils through its C-terminus [68], where it inhibits tropoelastin synthesis, thereby regulating elastogenesis [69].

FBN1 supports adhesion of elastic fibers in the matrix to surrounding cells using arginine-glycine-aspartate (RGD) domain to bind cell surface integrins $\alpha_5\beta_1$ and $\alpha_v\beta_3$ [70–72]. Interestingly, *Fbn1*-deficient mice exhibit loss of cell attachments of elastic fibers in the aorta, leading to abnormal morphological changes in smooth muscle cells [73]. Further, increased secretion of matrix metalloproteinase (MMP) 2 (MMP2) and MMP9 by vascular smooth muscle cells was also reported in *Fbn1*-deficient mice, followed by elastolysis and collapse of the vessel wall [74]. Thus, it is suggested that integrin–FBN1 interactions are crucial for the suppression of the elastolytic enzymes MMP2 and MMP9 and may be important in the well-described anti-inflammatory properties of intact elastic fibers [75]. Mechanistically, it has been suggested that dense plaques (or focal adhesions) form connections with integrins, activate focal adhesion kinase (FAK) and maintain intracellular signaling [76]. The observation that compound heterozygous mutants for *Fbn1* and integrinβ1 gene (*Itgb1*) exhibit marked down-regulation of phosphorylated (p-) FAK and an increase in p-ERK signaling in the heart demonstrates the importance of FBN1-mediated cell attachment in transducing signals and maintenance of cellular phenotypes. It has also been shown that FBN1 controls focal adhesion through integrin-mediated microRNA regulation, which involves miR-612 and miR-3185 [77]. It is of note that heterozygous point mutation that disrupts RGD-mediated signaling in FBN1 causes increased deposition of collagens and fibrosis by compensatory activation of integrin-mediated signaling [78]. Collectively, connections between cells and ECM elastic fibers play pivotal roles in cellular phenotypes, proper mechanotransduction, and maintenance of cellular function. [77]

Interactions of elastic fiber-associated proteins outside of elastogenesis

Beyond structural roles, elastic fibers and elastic fiber-associated proteins participate in the regulation of intercellular signaling. FBN1 has been shown to play a central role in extracellular regulation of TGFβ (reviewed in [79]). The first evidence came from the observation that *Fbn1* hypomorphic mice showed increased TGFβ activity without an increase in the synthesis of pro-TGFβ, and administration of anti-TGFβ neutralizing antibody ameliorated the emphysema phenotype of these mice [80]. It was also shown that an anti-TGFβ neutralizing antibody improved disruption of elastic fibers in aortic aneurysm lesions with a marked decrease in p-Smad2/3 [81]. Mechanistically, it was suggested that tethering of large latency complex (LLC), which is formed by covalent binding of LTBP1, 3, and 4 with latency-associated peptide of TGFβ, to FBN1 microfibrils regulates the bioavailability of TGFβ [67,82]. These findings lead to the notion that excess TGFβ signaling due to the increased availability of extracellular LLC is responsible for Marfan-related pathology, and inhibition of angiotensin II, which is an upstream regulator of TGFβ, is effective in inhibiting TGFβ signaling and preventing aortic aneurysms in *Fbn1* hypomorphic mutant mice [81]. Recently, however, conflicting results have emerged, showing that deletion of TGFβ receptor type II gene in Marfan mutant mice deteriorates the aneurysm phenotype [83,84]. Furthermore, the effect of TGFβ is dimorphic. It may support homeostasis of the aortic wall or exert deleterious effects depending on the time of administration of TGFβ in the postnatal period or the cell type responsive to angiotensin II [85,86].

It is of note that defects in FBN1 can also exhibit clinical phenotypes opposite of Marfan syndrome, which includes short stature (brachydactyly) and ectopic lenses, and is called Weill–Marchesani syndrome 2 (WMS2) [87]. A similar phenotype was reported in patients carrying an autosomal recessive mutation in a disintegrin and metalloproteinases with thrombospondin motif 10 (ADAMTS10) [88]. Although our interactome analysis did not identify the ADAMTS family of proteases, a high degree of binding between FBN1 and ADAMTS10 has been reported, and the activated

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ADAMTS10 is shown to accelerate microfibril biogenesis [89]. ADAMTS-related protein ADAMTSL4, which lacks enzymatic activity, also binds FBN1 and accelerates FBN1 deposition in ECM [90]. The molecular mechanism of WMS is not entirely understood, but it has been suggested that an abnormal microfibril scaffold caused by WMS mutations disrupted the ternary complex formation among FBN1, ADAMTS10 and ADAMTSL proteins and alters the microenvironment, including collagen fibers [91]. In addition, WMS mutant mice generated by introducing the S236X human mutation using CRISPR/Cas9 system showed up-regulation of FBN2 and down-regulation of FBN1, as well as down-regulation of growth differentiation factor 8 (GDF8) and BMP2, 4, 5, 7, recapitulating the WMS phenotype [92]. These findings indicate that FBN1 interacts with numerous binding partners, some of which are involved in elastic fiber formation, whereas others are involved in extra-elastogenic functions through multiple binding sites in FBN1.

Future directions

Researchers in the field have begun to elucidate protein–protein interactions of key molecules involved in elastic fiber formation. While validation of the binding and identification of binding domains are necessary, expanding the binding protein network will be helpful to grasp the complete picture of elastic fiber assembly and organization with high resolution. The network will be expanded to categorize binding partners according to their biological functions. Missing pieces of information include the 'timing' of interactions and how the order of protein–protein interaction is determined in a tissue-specific manner. A search for new players in elastic fiber formation will also be important to fully understand this process. An unbiased genetic screen using CRISPR/Cas9 to find molecules for enhanced or disrupted elastogenesis using *in vitro* elastogenesis assays may be designed. The development of new tools to indicate and evaluate the quantity and quality of elastic fibers *in vitro* will also be needed for further advancement of elastic fiber biology.

Summary

- ELN core and microfibrils are building blocks for elastic fibers.
- Key molecules involved in elastic fiber formations are ELN, FBN1, FBLN4, FBLN5, LTBP4, and cross-linking enzymes, LOX and LOXL1.
- FBN1 has elastogenic and non-elastogenic functions *in vivo* and the latter includes regulation of intercellular signaling in ECM.

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Author Contribution

S.J.S. performed Ingenuity pathway analyses and helped to draft the manuscript, and H.Y. contributed to the conception and wrote the manuscript.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations

ADAMTS10, a disintegrin-like and metalloproteinase with thrombospondin type 1 motif 10; ECM, extracellular matrix; EGF, epidermal growth factor; ELN, elastin; FAK, focal adhesion kinase; FBN1, fibrillin-1; FBN2, fibrillin-2; FBLN2, fibulin-2; FBLN4, fibulin-4; FBLN5, fibulin-5; LLC, large latency complex; LOX, lysyl oxidase; LOXL1, LOX-like 1; LTBP4, latent TGFβ-binding

protein-4; MAGP1, microfibril-associated glycoprotein-1; MFAP4, microfibril-associated protein-4; MMP, matrix metalloproteinase; RGD, arginine-glycine-aspartate; THBS1, thrombospondin-1; THBS2, thrombospondin-2.

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