

## LTBPs, More Than Just an Escort Service

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### ABSTRACT

Latent transforming growth factor beta (TGF- $\beta$ ) binding proteins (LTBPs) are large extracellular glycoproteins structurally similar to fibrillins. They perform intricate and important roles in the extracellular matrix (ECM) and perturbations of their function manifest as a wide range of diseases. LTBPs are major regulators of TGF- $\beta$  bioavailability and action. In addition, LTBPs interact with other ECM proteins—from cytokines to large multi-factorial aggregates like microfibrils and elastic fibers, affecting their genesis, structure, and performance. In the present article, we review recent advancements in the field and relate the complex roles of LTBP in development and homeostasis. *J. Cell. Biochem.* 113: 410–418, 2012. © 2011 Wiley Periodicals, Inc.

**KEY WORDS:** TGF-BETA; LTBP; EXTRACELLULAR MATRIX

The extracellular matrix (ECM) represents a dynamic and multifarious environment that provides cells with support, anchorage, and instructive signals that guide essential cellular functions such as proliferation, migration, differentiation, and death. The scaffold of the vibrant extracellular space is made primarily of fibronectin, collagenous and elastic fibers, and microfibrils, which provide tissues with resilience and elasticity and also serve as docking platforms for soluble factors involved in inter-cellular communication. Latent transforming growth factor beta (TGF- $\beta$ ) binding proteins (LTBPs), components of microfibrils, represent a family of matrix proteins that exert important and complex functions in the extracellular space. Below, we discuss their characteristics, functions, as well as findings that might direct future research.

### LATENT TRANSFORMING GROWTH FACTOR BETA (TGF- $\beta$ ) BINDING PROTEINS (LTBPs)

The LTBPs are large secreted glycoproteins structurally related to fibrillins. The first described LTBP (LTBP1) was identified in platelet releasate as a component of a high molecular weight complex, which also included a dimer of TGF- $\beta$  and a dimer of its propeptide [Miyazono et al., 1988] (Fig. 2). TGF- $\beta$  is a pluripotent and omnipresent growth factor. Cells secrete this cytokine as a biologically inactive aggregate comprised of a disulfide linked

TGF- $\beta$  dimer non-covalently associated with its propeptide homodimer. The interaction of TGF- $\beta$  with its propeptide renders the cytokine inactive, hence the propeptide is dubbed the latency-associated peptide (LAP) and the complex between TGF- $\beta$  and LAP is named the small latent complex (SLC). LTBP1 associates with SLC, forming a tri-partite complex known as the large latent complex (LLC) (Fig. 2). Since the characterization of LTBP1, three other LTBPs were identified (LTBP2–4) (reviewed in [Rifkin, 2005]). LTBP3 and LTBP4 also interact with latent TGF- $\beta$ , whereas LTBP2 does not [Saharinen and Keski-Oja, 2000].

The four LTBP proteins have a modular and highly repetitive structure, primarily composed of two distinct cysteine-rich motifs: One, characterized by six cysteines, is similar to the epidermal growth factor (EGF) module, and the other defined by eight intramolecularly bound cysteines (8-cys or TB domain), represents the family's signature domain [Todorovic et al., 2005]. LTBPs can also possess an LTBP-exclusive 4-cys domain near the N-terminus. Each LTBP includes four 8-cys domains interspersed with numerous EGF-like motifs (15–20), many of which contain consensus Ca<sup>2+</sup>-binding sequences (Fig. 1). The first 8-cys domain is known as the hybrid domain, since it shares similarities both with 8-cys and EGF-like motifs. Between the second 8-cys domain and the stretch of repeating EGF-like motifs, there is an unstructured proline-rich region called the hinge domain, which shows the highest degree of sequence diversity amongst the four LTBPs [Annes et al., 2003]. Another hinge region is localized between the fourth 8-cys domain

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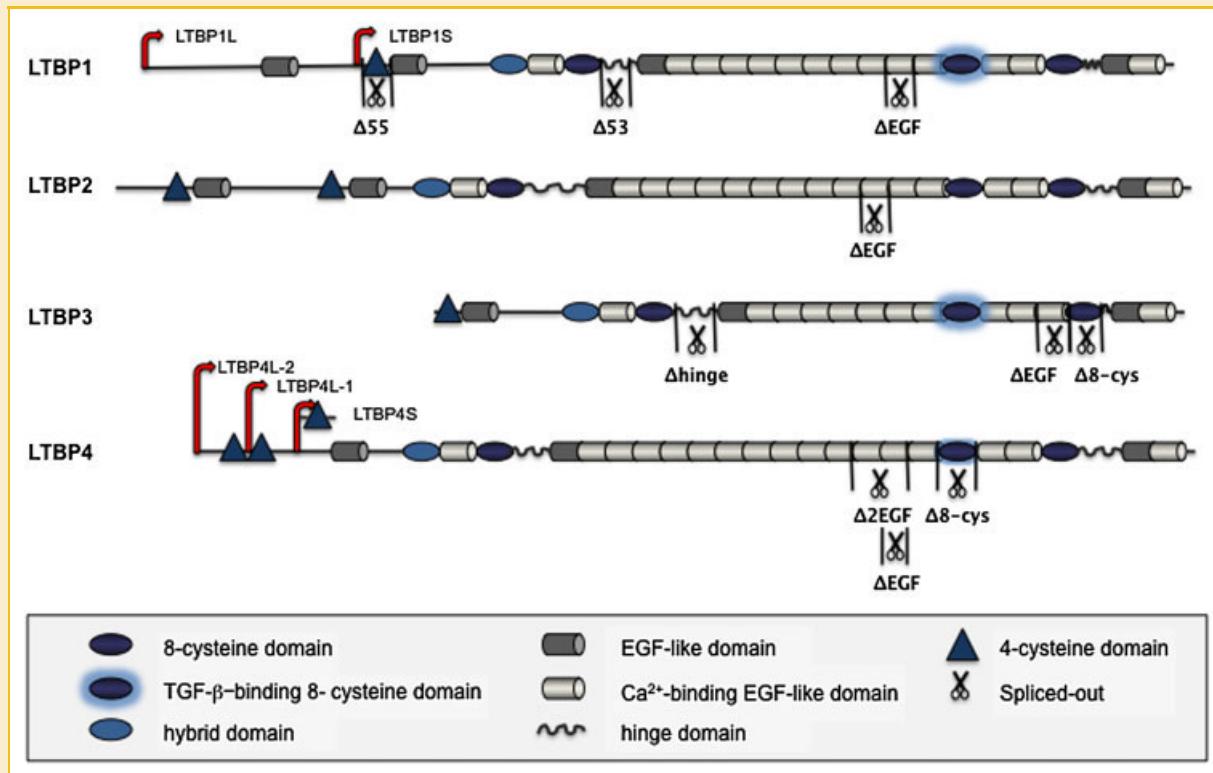


Fig. 1. Schematic representation of the LTBP family domain organization. Four TBPs are shown, with domain symbols depicted in the boxed area below the figure. Major isoforms and known splice variants of TBPs are also illustrated [Robertson et al., 2010].

and the downstream EGF-like motif of LTBP-1. The hinge sequences are sensitive to proteolytic cleavage [Taipale et al., 1995; Unsold et al., 2001; Dallas et al., 2002; Ge and Greenspan, 2006], which plays an important role in regulation of LTBP function.

In addition to proteolytic processing, alternative splicing and usage of different promoters contribute to the structural variability of TBPs (Fig. 1). LTBP1 and LTBP4 exist as two major isoforms—long and short, transcribed from independent promoters [Koski et al., 1999; Kantola et al., 2010]. Transcriptional regulation of *LTBP2* and *LTBP3* genes might be similar, considering that Northern blot analysis of *LTBP2* and *LTBP3* mRNA in different tissues shows the presence of two major transcripts [Moren et al., 1994; Shipley et al., 2000; Dabovic et al., 2002b]. Different variants of each LTBP show distinct expression patterns and diverse affinities toward their binding partners [Koli et al., 2001]. The interactions of TBPs with their intra- and extra-cellular ligands determine their localization, function, and action.

## LTBP INTERACTIONS

The best-studied LTBP interaction is the one that gave this protein family its name, the association with TGF- $\beta$  LAP. LTBP and TGF- $\beta$  proteins evolved in sea urchins and subsequently co-evolved, developing into families of four TBPs and three TGF- $\beta$  isoforms [Robertson et al., 2010]. Three out of four TBPs bind LAP,

indicating that this interaction might have been the driving force for their co-evolution. LTBP1 and 3 bind all three TGF- $\beta$  LAP isoforms with high affinity, whereas LTBP4 shows a weak binding capacity only for TGF- $\beta$ 1 LAP. LAP binds to the third 8-cys domain (8-cys-3) in LTBP [Gleizes et al., 1996; Saharinen et al., 1996]. Close inspection of all 8-cys domains in fibrillins and TBPs demonstrated that the 8-cys-3 motifs of LTBP1, 3, and 4 possess a two amino-acid insertion between the sixth and the seventh cysteine [Saharinen and Keski-Oja, 2000]. Deletion of this short insert in the 8-cys-3 motif of LTBP1 results in loss of LAP binding ability, while its inclusion in the non-TGF- $\beta$  binding 8-Cys-3 repeat of LTBP2 results in TGF- $\beta$  binding. This further emphasizes the importance of this short sequence in disulfide bridging with LAP. The solution structure of the LTBP1 8-cys-3 domain suggests that the dipeptide insertion just before cys7 results in exposure of the 2–6 disulfide bond, allowing covalent linkage to the pair of cys33 residues in the LAP dimer [Lack et al., 2003]. Furthermore, the 8-cys-3 solution structure suggested that a “ring” of five negatively charged amino acids surrounds the reactive disulfide bond and this electrostatic interaction facilitates covalent bonding of LAP and LTBP [Chen et al., 2005]. A recent mutational analysis showed that the crucial amino acids within LAP’s LTBP-binding epitope are four positively charged residues that surround cys33 [Walton et al., 2010]. The pentameric amino acid ring in LTBP4 8-cys-3 motif has only one negative charge, which might account for the weak and exclusive covalent bonding of LTBP4 and TGF- $\beta$ 1 LAP. The poor and restricted interaction between LTBP4 and TGF- $\beta$  LAP implies that LTBP4 might have a

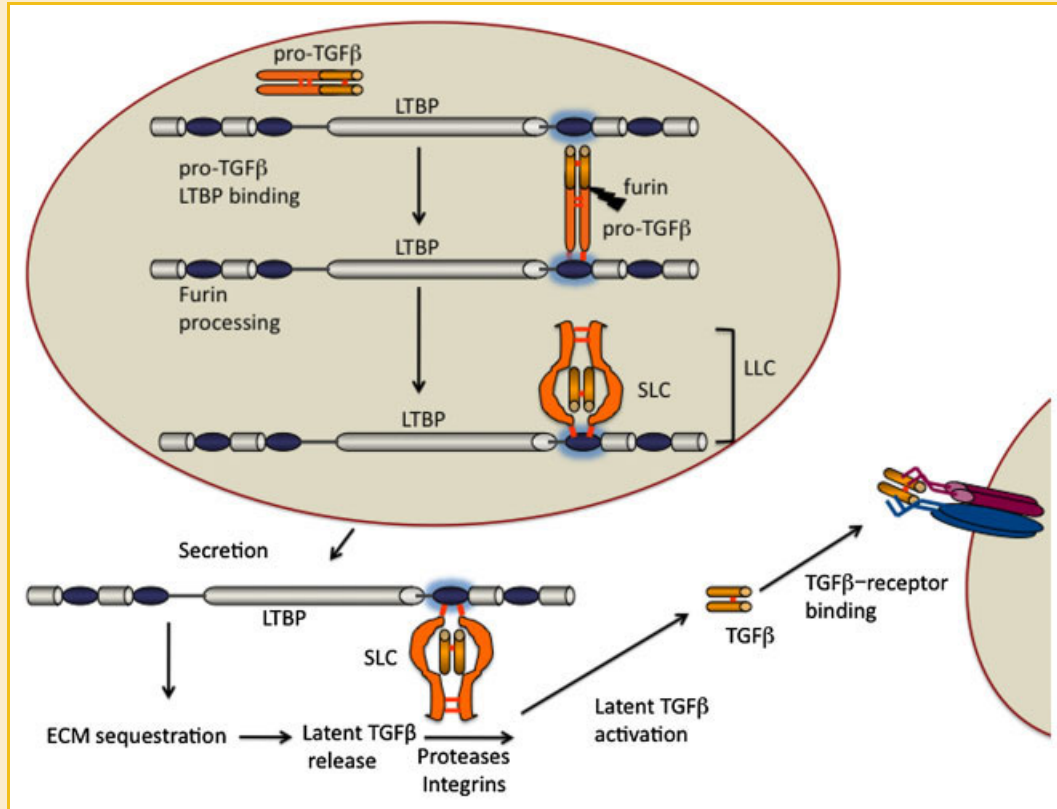


Fig. 2. TGF- $\beta$ -LTBP assembly, processing, and activation. After dimerization in the endoplasmic reticulum, pro-TGF- $\beta$  molecules form disulfide bonds to LTBP molecules early in the secretory pathway. In the trans-Golgi, furin-like enzymes process the pro-TGF- $\beta$  yielding mature TGF- $\beta$ , the TGF- $\beta$  propeptide (LAP), and an LTBP. This complex is called the large latent complex (LLC). When complexed with LAP, TGF- $\beta$  cannot bind to its receptor. The LLC is secreted, binds to matrix components, and the latent TGF- $\beta$  is activated by proteases/integrins that modify LAP or/and LTBP. The liberated TGF- $\beta$  then binds to its receptor.

role independent of TGF- $\beta$ . The fact that LTBP4 is the only LAP-binding LTBP that can be produced as a splice-variant without the LAP-binding domain (Fig. 1), supports this hypothesis [Koli et al., 2001]. A recent study showed that the long form of LTBP4 binds TGF- $\beta$ 1 LAP much more efficiently than the short isoform, although the reason for this difference is unclear [Kantola et al., 2010].

LTBPs function as chaperones by binding LAP within the endoplasmic reticulum (Fig. 2). In the absence of LTBP, the reactive cysteine (cys33) within LAP bonds with a free cysteine of the mature cytokine, causing misfolding and slow secretion of the SLC [Rifkin, 2005]. Interestingly, LTBP3 cannot be secreted from cells if not complexed to SLC [Chen et al., 2002; Penttinen et al., 2002]. Secreted LLC is either soluble or sequestered within the ECM through LTBP interactions with multiple extracellular proteins. Latent TGF- $\beta$  is then stored in the extracellular space until required, and when the need arises it is liberated from its propeptide, in a process commonly referred to as "latent TGF- $\beta$  activation" (Fig. 2). Several different mechanisms of TGF- $\beta$  activation have been described, involving diverse activators such as proteases, the integrins  $\alpha_v\beta_6$ ,  $\alpha_v\beta_3$ ,  $\alpha_v\beta_5$ , and  $\alpha_v\beta_8$ , thrombospondin-1, F-spondin, neuropilin-1, reactive oxygen species, and low or high pH [Chandramouli et al., 2011]. The molecular targets of these divergent latent TGF- $\beta$  activators can be LTBPs and/or LAPs. Binding of SLC to an LTBP is required for proper activation of latent TGF- $\beta$ 1 as mutation of cys33 to ser yields

mice with a phenotype similar to that of Tgf- $\beta$ 1 null mice [Yoshinaga et al., 2008]. Moreover, activation of TGF- $\beta$ 1 by the integrins  $\alpha_v\beta_6$  and  $\alpha_v\beta_5$  requires an LLC [Annes et al., 2004; Wipff et al., 2007]. The recent x-ray structure of TGF- $\beta$ 1 LAP is consistent with the integrin binding to one end of the complex and thereby transmitting a distortional force through the SLC to the LTBP, which is also bound to the matrix [Shi et al., 2011]. The requirement for LLC may indicate a mechanism for force-induced latent TGF- $\beta$  activation in organs exposed to recurrent stretching or movement.

In summation, LTBPs orchestrate TGF- $\beta$  action at multiple levels: (1) They ensure correct folding and efficient secretion, (2) they direct temporal and spatial deposition in the extracellular space through their own secretion dynamics and interaction with ECM proteins, and (3) they regulate activation.

Incorporation of LTBPs into the ECM is crucial for regulation of latent TGF- $\beta$  storage and activation. However, significant amounts of LTBP1 and LTBP4 are secreted free of SLC, much like LTBP2, which cannot interact with SLC, suggesting that LTBPs perform roles unrelated to TGF- $\beta$ . LTBPs interact with many extracellular proteins (Fig. 3), but interactions with fibronectin and fibrillins seem to be crucial for proper localization and function. The initial studies of LTBP1 deposition into the ECM identified three potential ECM-interacting domains, two localized at the N-terminus and one localized at the C-terminus [Unsold et al., 2001]. N-terminal

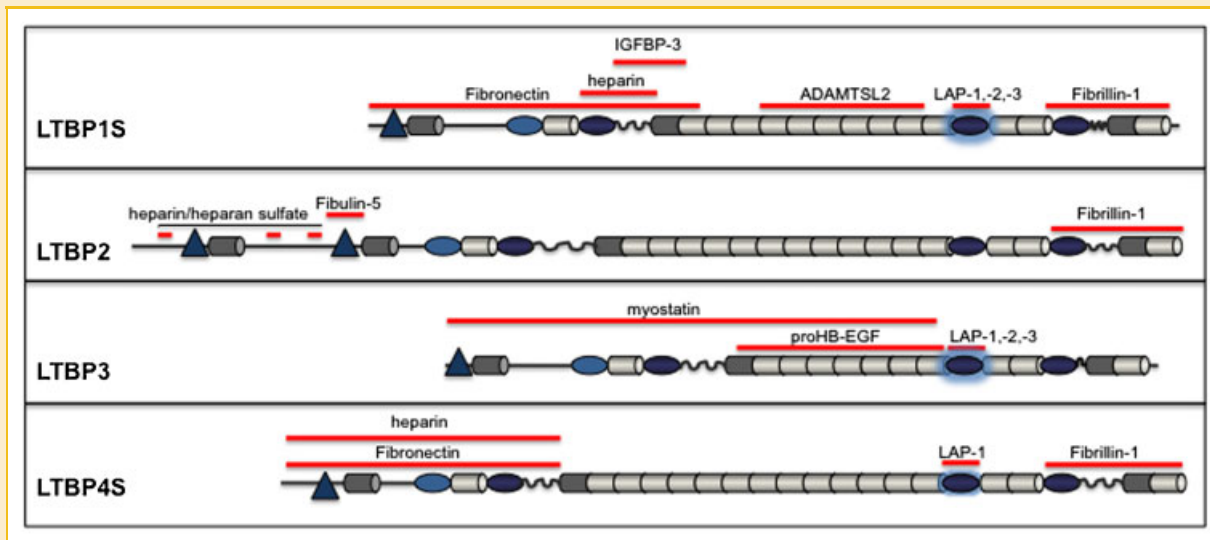


Fig. 3. Protein interactions of LTBP and ECM proteins. LTBP sequences that interact with given ECM proteins are depicted as red bars. Some LTBP interactions with certain ECM components are not depicted because the fine mapping of the interaction site has not been done.

fragments were readily incorporated into the matrices and association of the binding domains with the ECM was enhanced by soluble, cell-derived factors. Similar results were obtained with binding peptides from LTBP2, LTBP3, and LTBP4. The N-terminal ECM-binding sequences of LTBP1, 2, and 4 associate with fibronectin [Taipale et al., 1996; Hyytiainen and Keski-Oja, 2003; Fontana et al., 2005; Chen et al., 2007; Kantola et al., 2008] (Fig. 3) and this interaction is promoted by transglutaminase [Rifkin, 2005]. The purified N-terminal fragment of LTBP3 co-localized with fibronectin in pre-formed lung fibroblast ECM, and so did the endogenous LTBP3 at the time of its initial incorporation into mature matrices [Koli et al., 2005], suggesting an association of LTBP3 and fibronectin. However, no biochemical studies were performed thus far to investigate this possibility.

LTBPs and fibrillins are highly homologous proteins, but are easily distinguished since fibrillins are much larger (~350 kDa) with almost double the number of EGF-like and 8-cys repeats compared to LTBPs. Fibrillins are major structural components of connective tissue microfibrils and elastic fibers [Olivieri et al., 2010]. Microfibril extracts from tissues do not contain LTBPs, indicating that LTBPs are not disulfide bonded to microfibrils [Isogai et al., 2003]. However, all LTBPs, with the exception of LTBP3, bind to fibrillin-1 (Fig. 3), the major component of microfibrils through their C-terminus [Isogai et al., 2003; Hirani et al., 2007]. LTBP3 is unique because it cannot be secreted if not bound to latent TGF- $\beta$ . Because the binding capacity of LTBP3 to fibrillin-1 was assessed only with a C-terminal peptide homologous to the fibrillin-1-binding LTBP1 and LTBP4 domains, which lack the TGF- $\beta$  LAP-binding sequence (8-cys-3), this C-terminal peptide may have assumed a conformation different from the one of LTBP3-LLC. It would be advisable to use the full length LTBP3-LLC for testing its binding abilities toward fibrillins. LTBP1 and LTBP4 can also bind fibrillin-2, whereas LTBP2 cannot [Isogai et al., 2003]. Competitive binding studies showed that the binding site on fibrillin-1 for LTBP2 is the same or in close proximity

to that for LTBP1 [Hirani et al., 2007], suggesting that LTBP2 might be a negative regulator of LTBP1 interaction with microfibrils. LTBP4 may also compete with its family members for binding to fibrillin-1, since its binding site lies within the same fibrillin-1 peptide as that of LTBP1 and LTBP2 (Fig. 3). Competitive binding of LTBPs to microfibrils may represent an additional level of control of microfibril composition and function.

Some LTBPs (LTBP1, 2, and 4) associate with heparin and heparan sulfate, in a manner that might affect cell adhesion, migration, and micro- and elastic fiber assembly [Chen et al., 2007; Kantola et al., 2008; Parsi et al., 2010]. Elastogenesis and structural organization of elastic fibers also depend on fibulin-4 and -5. LTBP2 interacts with fibulin-5 and plays an important role in the targeting of fibulin-5 to microfibrils to form elastic fibers [Hirai et al., 2007]. LTBP1 binds to fibulin-4 [Massam-Wu et al., 2010], whose role in elastogenesis is to tether lysyl-oxidase (LOX) to tropoelastin to facilitate cross-linking [Horiguchi et al., 2009], whereas LTBP4 interacts with fibulin-5 (T. Nakamura, personal communication). Fibulin-2, -4, and -5 can bind to fibrillin-1 and their binding sites are within the domain responsible for interaction with LTBPs [Ono et al., 2009]. Therefore, LTBPs and fibulins may compete for interaction with fibrillin 1. However, Kielty's group showed that fibulin-4 can simultaneously bind fibrillin-1 and LTBP1, suggesting that fibulin-4 might function as a mediator of LTBP1 association with microfibrils [Massam-Wu et al., 2010].

Recently, a few diverse factors have been shown to bind to LTBPs. For example, LTBP1 interacts with ADAMTSL2, a secreted glycoprotein of unknown function [Le Goff et al., 2008]. ADAMTSL2 mutations in humans cause geleophysic dysplasia, an autosomal recessive disorder characterized by thick skin, short stature, fingers and toes, and cardiac valve malfunction that can lead to premature death. The functional significance of LTBP1 interaction with ADAMTSL2 is unclear, but skin fibroblasts from affected individuals secrete enhanced levels of active TGF- $\beta$ ,

suggesting that ADAMTSL2 might regulate TGF- $\beta$  activity, possibly in an LTBP1-dependent fashion.

At least three LBTPs: LBTP1, 2, and 3, interact non-covalently with growth factors from the TGF- $\beta$  super-family other than TGF- $\beta$ s. In *Xenopus* embryos LBTP1 interacts with activin and modulates its action [Altmann et al., 2002], whereas LBTP2 and LBTP3 associate with a pro-form of myostatin, a TGF- $\beta$ -like hormone that regulates the size of skeletal muscle [Anderson et al., 2008]. Pro-myostatin is secreted and retained in the ECM in an LBTP3-dependent manner and upon cleavage by furin, the mature growth factor is released from the complex with LBTP3. LBTP2 and LBTP3 bind pro-GDF11 and proprotein convertase 5/6A (PC5/6A), which cleaves the GDF11 propeptide from the mature cytokine. LBTP2 and LBTP3 may act as chaperons for PC5/6A zymogen in the ECM and prevent its maturation, therefore regulating the processing of GDF11 [Sun et al., 2011]. Yeast two-hybrid system experiments revealed a potential association between LBTP1 and insulin-like growth factor binding protein-3 (IGFBP-3) (Fig. 3) and LBTP3 and heparin-binding EGF-like growth factor (HB-EGF) precursor, but these interactions have not been confirmed biochemically [Brooke et al., 2002; Gui and Murphy, 2003].

LBTPs form multiple intra- and extra-cellular associations with divergent proteins. These interactions affect the bioavailability of TGF- $\beta$ s as well as other TGF- $\beta$  super-family members like activin, myostatin, and GDF11. In addition, LBTPs play an important structural role in microfibril and elastic fibril assembly and organization.

## DYNAMIC INTEGRATION OF LBTPs INTO THE ECM

The backbone of the ECM is composed of fibrillar proteins, such as collagen, fibronectin, and elastin to which many adaptor proteins and proteoglycans associate, together forming a complex and dynamic 3D structure. The building blocks of elastic matrices are fibrillins and tropoelastin, but elastogenesis and maintenance of elastic fibers would be impossible without a number of other factors, including specific LBTPs and fibulins.

LBTPs initially incorporate into the ECM via interaction with fibronectin. Fibronectin is one of the first molecules to be deposited in the extracellular space, and regulates the initial and continued assembly and stability of several other ECM components (reviewed in Dallas et al. [2006]). Without a pre-laid fibronectin matrix, fibrillins, LBTPs, and tropoelastin are not integrated into the ECM.

Cultured human lung fibroblasts deposit LBTP1 immediately after formation of fibronectin matrices; LBTP1 is clearly detectable after 2 days of culture and it co-localizes with the fibronectin meshwork [Koli et al., 2005]. LBTP2 forms extracellular fibers on day 4 [Vehvilainen et al., 2009], whereas an LBTP4 network is not visible until day 7, and both proteins initially co-localized with fibronectin. LBTP3 is the last LBTP to be deposited into fibroblast matrices at about 10–14 days of culture [Koli et al., 2005]. After an extended period of culture, the localization of LBTPs starts to diverge from that of fibronectin, with the LBTPs being organized into fibrillar arrays in a layer above the fibronectin fibers. This phenomenon was previously described for fibrillin deposition in the ECM and, indeed, in mature matrices LBTPs co-localized with

fibrillin-1. Experiments following the dynamics of ECM formation and reorganization explained this change in localization of fibronectin and LBTPs/fibrillins upon long culture periods, since LBTP matrices are much more stable and less prone to cell-mediated reorganization than the fibronectin meshwork, which is constantly assembled and turned over [Dallas et al., 2006].

Fibrillin-1 and -2 self-associate both longitudinally and through lateral binding, giving rise to microfibrils with an average diameter of 10 nm (reviewed in Olivieri et al. [2010]). Lack of fibrillin-1 microfibrils affects ECM deposition of all LBTPs ([Ono et al., 2009; Vehvilainen et al., 2009; Massam-Wu et al., 2010]; L. Zilberberg, personal communication). Abnormal LBTP-fibrillin interactions in mice and humans may yield promiscuous TGF- $\beta$  signaling and changes in p38 MAPK, ERK1/2, and JNK1 signaling pathways [Carta et al., 2009; Holm et al., 2011].

LBTP2 and LBTP4 have been implicated in the formation of elastic fibers. Elastogenesis requires coacervation of tropoelastin and is modulated by fibulin-4 and -5, cell-surface glycosaminoglycans, and cross-linking enzymes LOX and LOX-like (LOXL) (Fig. 4). Fibulin-5 and elastin deposition in cell culture is dependent on fibrillin-1 [Hirai et al., 2007]. However, in the absence of LBTP2, fibulin-5 targets tropoelastin mainly to fibrillin-2. Therefore, LBTP2 might function as a molecular switch that regulates the differential usage of microfibrils during assembly of elastic matrices.

*Ltbp4S<sup>-/-</sup>* mice show impaired elastogenesis because elastin coacervates cannot integrate into microfibril bundles. LBTP4 colocalizes with both fibrillins and fibulin-5 (B. Dabovic, personal communication) and might operate as a tethering factor between the microfibrils and tropoelastin aggregates. In the absence of LBTP4, elastin coacervates and microfibril bundles do not interact to form functional elastic fibers. Studies of *Ltbp4S* null cells and mice showed that the absence of LBTP4 causes both impaired elastin and fibulin-5 deposition, further emphasizing the importance of LBTP4-fibulin-5 interaction in elastogenesis (B. Dabovic; T. Nakamura, personal communications).

## LBTP-RELATED PATHOLOGIES IN MICE AND HUMANS

Genetic disorders arising as a result of mutations in *LBTP* genes in mice and humans underline the importance of the LBTP family. Deficiency of the long form of *Ltbp1* (*Ltbp1L*) in mice causes serious disruption of great vessels and cardiac valve development, resulting in perinatal death [Todorovic et al., 2007; Todorovic et al., 2011]. These defects stem from decreased TGF- $\beta$  activity that occurs at specific times during heart and great vessel formation and within defined cellular populations such as cardiac neural crest cells and valvular mesenchymal cells. However, mice with deletion of the first exon of the *Ltbp1* gene common for both the long and the short form display only mild cranio-facial abnormalities and live a normal lifespan [Drews et al., 2008]. The sequence deleted in these mice codes for a 4-cys domain that can be spliced out giving rise to an *Ltbp1L* splice variant called *Ltbp1L $\Delta$ 55* (Fig. 1). It is possible that mice with deletion of the first exon shared by *Ltbp1L* and *Ltbp1S* make *Ltbp1L $\Delta$ 55*, which might explain the discrepancy between phenotypes of these mutants

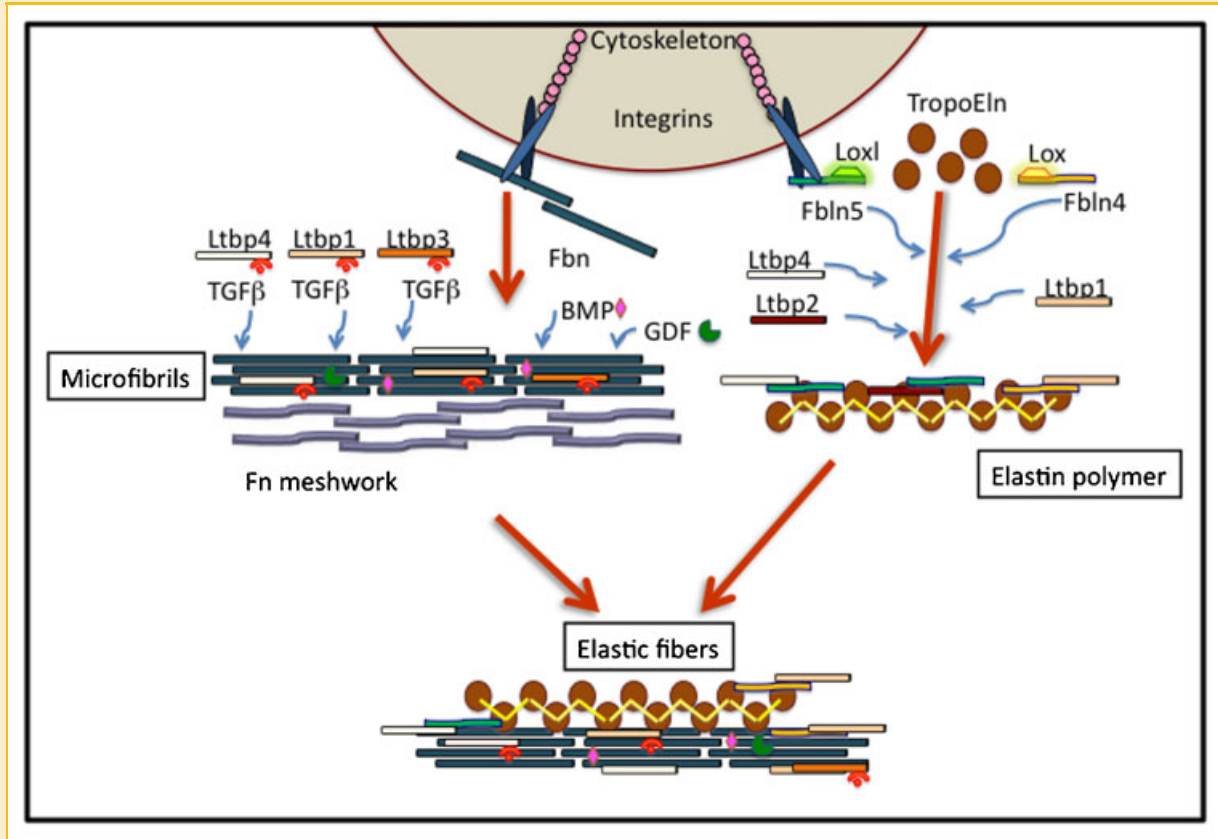


Fig. 4. Organization of microfibrils and elastic assemblies. The initial step in the progressive and hierarchical building of microfibrils and elastic fibers is the deposition of the fibronectin (Fn) meshwork. Fibrillin (Fbn) –1 and –2 self-associate on head-to-tail principle resulting in longitudinal polymerization, but also through lateral binding, altogether giving rise to microfibrils. BMPs, GDFs and TGF- $\beta$  are probably incorporated at this point because of their direct binding to Fbn and association with LTBP. As the initial microfibrils form, a second series of reactions in which tropoelastin (TropoEln) binds to complexes of fibulins (Fbln-4 and Fbln-5), each of which is associated with lysyl-oxidase (LOX) and LOX-like (Loxl) molecules. Fbln-4-LOX and Fbln-5-LOXL promote TropoELN conversion to elastin polymers. The microfibrils and elastin polymers combine to form elastic fibers in a manner that is Fbln- and LTBP4-dependent. LTBP, with their associated TGF- $\beta$ s, bind to the microfibrils independently of Fbln incorporation. However, LTBP might associate with Fbln-4 and -5 during elastin polymerization. LTBP are illustrated within the microfibrils as both with and without TGF- $\beta$  because a significant amount of LTBP is found without any bound TGF- $\beta$ .

and *Ltbp1L*<sup>-/-</sup> mice. The *Ltbp1L* isoform is expressed mostly during embryonic development, whereas *Ltbp1S* expression initiates late during organogenesis and becomes the major isoform in adults. The lack of the long form at the time when it represents the only *Ltbp1* isoform expressed might have grave consequences on development, whereas the lack of the short form later in life when a splice variant of *Ltbp1L* is still expressed might result only in mild developmental defects. Currently, no *LTBP1* mutation has been linked to any human pathology.

Null mutations in *LTBP2* have been associated with ocular malformations, such as development of a small and dislocated lens and glaucoma (reviewed in Robertson et al. [2010]. An earlier report indicating that *Ltbp2* null mice died at the time of implantation has not been reproduced. However, ectopia lentis but not glaucoma has been found in recently designed *Ltbp2* null mice, emphasizing the importance of *LTBP2/Ltbp2* in development of microfibrillar bundles of the ciliary zonules in the eye (T. Nakamura, personal communication).

Homozygous mutation within the seventh EGF-like domain in *LTBP3* caused a dramatic decrease in *LTBP3* expression and was

manifested as oligodontia (agenesis of  $\geq$ six teeth), short stature and scoliosis [Noor et al., 2009]. *Ltbp3* null mice are also shorter than their littermates and have multiple skeletal and craniofacial abnormalities, including kyphosis, extension of the mandible beyond the maxilla, and cranial doming. As the animals age, their long bones and vertebrae become osteoarthritic and osteopetrotic and they develop pulmonary emphysema [Dabovic et al., 2002a; Colarossi et al., 2005]. Similar to *Ltbp1L*<sup>-/-</sup> mice, developmental defects and pathologies that arise in the adulthood of *Ltbp3*<sup>-/-</sup> mice are consistent with decreased TGF- $\beta$  levels.

Autosomal recessive mutations in *LTBP4* cause Urban-Rifkin-Davis syndrome (URDS), which is characterized by multi-organ developmental defects, such as aberrant lung, gastrointestinal, genitourinary, musculoskeletal, and dermal organogenesis [Urban et al., 2009]. Human patients with a mutation in *LTBP4* were identified based upon comparative electron microscopy studies of patients' tissues and tissue samples from mice lacking the short form of *Ltbp4* (*Ltbp4S*). Tissues from both sources showed strikingly similar elastic fiber pathology—elastin appeared as large globules positioned next to the microfibrils with only a few elastin polymers

integrated within the microfibril bundle, instead of having the microfibrils enmeshed into elastin [Dabovic et al., 2009]. Lack of *Ltbp4S* in mice results in defective differentiation of terminal air sacs, cardiomyopathy, and colon carcinomas [Sternier-Kock et al., 2002]. The lung abnormalities in *Ltbp4S*<sup>-/-</sup> mice are rescued when *Tgfb2* expression is genetically abolished, implicating excess TGF- $\beta$  signaling in the pathogenesis of the phenotype. Similarly, skin fibroblasts from URDS patients produce more active TGF- $\beta$  than controls. However, genetic ablation of *Tgfb2* expression in *Ltbp4S*<sup>-/-</sup> mice did not improve the organization of elastic matrices, indicating a role for LTBP4 in elastogenesis independent of its role in modulating TGF- $\beta$  activity. Collectively, the human and mouse findings imply that LTBP4 may perform two roles: (1) A structural role in promoting elastogenesis and (2) a functional role in regulating TGF- $\beta$  activity. However, it is possible that LTBP4 plays exclusively a structural role and that increased TGF- $\beta$  activity is actually a consequence of abnormal elastic fibers and perturbed extracellular milieu, which, in turn, leads to a promiscuous activation of latent TGF- $\beta$ , similarly to what is seen in human and mouse mutants for fibrillin-1 (reviewed in [Olivieri et al., 2010]). The fact that attenuation of TGF- $\beta$ 2 activity, a TGF- $\beta$  isoform that does not interact directly with LTBP4, partially rescues abnormal lung development in *Ltbp4S*<sup>-/-</sup> mice, favors the latter hypothesis.

Taken together, the human and mouse pathologies arising from mutations in *LTBP* genes underscore the importance of TGF- $\beta$  interactions in the biology of these ECM proteins. However, LTBPs (especially LTBP4) are also important structural elements of the ECM.

## CONCLUSIONS AND PERSPECTIVES

Amongst ECM proteins, LTBPs occupy a special place warranted by their interaction with the omnipresent latent TGF- $\beta$ . LTBPs participate and regulate every step in TGF- $\beta$ 's biology—from its folding and secretion, through its localization, to, finally, its activation. To rephrase an old proverb: Behind every successful TGF- $\beta$  there is an LTBP. Yet, the LTBPs are more than just TGF- $\beta$  chaperones: they interact with other proteins—from potent growth factors to structural proteins of the ECM and in doing so, they play a vital role in organ formation and tissue homeostasis. The biological importance of LTBPs is emphasized by the pathology of mouse and human LTBP mutants. Though much work has been done to understand the mechanism(s) of LTBP action, these efforts have been primarily related to studies of TGF- $\beta$  activity. However, not all pathologies that arise from *LTBP* mutations and, consequently, lack or change of this protein's function, can be explained by changes in TGF- $\beta$  activity. LTBPs also play important roles in elastogenesis and in maintenance of ECM structure. Further investigations are required to determine the sequence and molecular players that yield LTBP-related pathologies in order not only to better understand LTBP function, but also to find suitable tools to prevent or meliorate the devastating consequences caused by LTBP malfunction.

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