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The Bone Morphogenetic Protein 1/Tolloid-like Metalloproteinases

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Abstract

A decade ago, bone morphogenetic protein 1 (BMP1) was shown to provide the activity necessary for proteolytic removal of the C-propeptides of procollagens I–III: precursors of the major fibrillar collagens. Subsequent studies have shown BMP1 to be the prototype of a small group of extracellular metalloproteinases that play manifold roles in regulating formation of the extracellular matrix (ECM). Soon after initial cloning of BMP1, genetic studies showed the related *Drosophila* proteinase Tolloid (TLD) to be necessary for formation of the dorsal-ventral axis in early embryogenesis. It is now clear that the BMP1/TLD-like proteinases, conserved in species ranging from *Drosophila* to humans, act in dorsal-ventral patterning via activation of transforming growth factor β (TGF β)-like proteins BMP2, BMP4 (vertebrates) and decapentaplegic (arthropods). More recently, it has become apparent that the BMP1/TLD-like proteinases are key activators of a broader subset of the TGF β superfamily of proteins, with implications that these proteinases may be key in orchestrating formation of ECM with growth factor activation and BMP signaling in morphogenetic processes.

Keywords

BMP1; Procollagen; Extracellular matrix; Tolloid; Metalloproteinases; TGF β

1. Introduction

Collagens I–III, the major fibrous components of vertebrate extracellular matrix (ECM), are synthesized as procollagen precursors with amino- (N-) and carboxyl- (C-) terminal propeptides (Fig. 1). Cleavage of both N- and C-propeptides occurs via highly specific calcium-dependent metalloproteinases with neutral pH optima (Hojima et al., 1985; Hojima et al., 1994; Kessler et al., 1986), and is necessary for releasing the mature triple helical portion of the molecule for proper self-assembly into fibrils (Myllyharju and Kivirikko, 2001; Canty and Kadler, 2005). The smaller N-propeptides are processed by proteinases of the ADAMTS (A

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Disintegrin And Metalloproteinase with Thrombospondin repeats) family (Greenspan and Wang, 2005); specifically via the structurally similar proteinases ADAMTS2, 3 and 14, which have overlapping but differing distributions in tissues (Colige et al., 1997; Le Goff et al., 2006; Wang et al., 2003). Deficiencies in N-propeptide processing leads to the arthrochalasia and dermatosparaxis forms of Ehlers-Danlos Syndrome (EDS) (Byers, 1995). Interestingly, in these genetic connective tissue disorders, triple helical trimers are still incorporated into collagen fibrils, although the morphology of those fibrils can be aberrant (Byers, 1995). This is consistent with the observation that collagen monomers retaining N-propeptides are efficiently incorporated into elongating fibrils *in vitro* (Prockop and Hulmes, 1994). In contrast, retention of the bulkier C-propeptides precludes *in vitro* incorporation of monomers into growing fibrils (Prockop and Hulmes, 1994). The latter observation and the absence of identified genetic disorders in which procollagen C-propeptides remain uncleaved suggest that C-propeptide cleavage is a rate-limiting step necessary to both fibrillogenesis and development itself. Thus, enzymes responsible for removal of the procollagen I–III C-propeptides may be important control points in morphogenesis.

In seminal work, Urist and colleagues demonstrated that protein factor(s) from demineralized bone extracts could induce ectopic bone formation (Urist, 1965; Urist et al., 1973) via a process recapitulating the endochondral bone formation of normal development (Reddi and Huggins, 1972). These factor(s) were designated bone morphogenetic protein (BMP) (Urist et al., 1973), and extensive purification of bone inductive activity from bone extracts led to initial isolation of BMPs 1–7 (Celeste et al., 1990; Wang et al., 1988; Wozney et al., 1988). BMPs 2–7 are members of the TGF β superfamily of proteins, and over 30 TGF β -like BMPs have now been identified, primarily via sequence homology, in a broad range of species (Hogan, 1996; Ducky and Karsenty, 2000). BMP1 differs from all other BMPs, in that it is not a TGF β -like protein. Rather, it contains a metalloproteinase domain and initially was thought to act in bone morphogenesis via activation of the TGF β -like BMPs with which it co-purified (Wozney, et al., 1988). In 1996 BMP1 was independently shown by two groups to provide the procollagen C-proteinase (pCP) activity responsible for cleaving the C-propeptides from procollagens I–III (Kessler et al., 1996; Li et al., 1996). Subsequently, BMP1 has become the prototype of a small group of proteinases with conserved domain structure, shown to process a variety of precursors into mature functional proteins with roles in ECM formation. It has also become apparent that these proteinases activate a subset of the TGF β superfamily of proteins and that they play fundamental roles in development, perhaps via the orchestration of ECM formation and growth factor activity. This review details features of this group of proteinases and their functions, with emphasis on recent findings. The reader is directed to earlier reviews for more exhaustive coverage of biosynthetic processing of collagen molecules by various proteinases (Greenspan, 2005) and the developmental aspects of BMP1/TLD-proteinase activities (Ge and Greenspan, 2006).

2. BMP1/TLD family members

Subsequent to initial cloning of BMP1, the *Drosophila* protein Tolloid (TLD), the product of one of at least seven zygotically active genes necessary for dorsal-ventral patterning in gastrulation, was found to have a protein domain structure strikingly similar to that of BMP1 (Shimell et al., 1991). This finding demonstrated a previously unsuspected link between morphogenetic patterning and proteases involved in ECM formation. BMP1 has since become the prototype of a small group of metalloproteinases conserved in species ranging from *Drosophila* to humans. The distinct protein domain structure of BMP1 comprises an amino-terminal prodomain, followed by a conserved protease domain, characteristic of the astacin M12A family of metzincin metalloproteinases (Bond and Beynon, 1995; Stocker et al., 1995), and more carboxyl-terminal CUB (complement-uegf-BMP1) and EGF (epidermal growth factor)-like domains (Fig. 2). *Drosophila* TLD differs from BMP1 in having additional

carboxyl-terminal EGF and CUB motifs. The prodomains are not required for secretion of at least some BMP1/TLD-like proteinases (Marques et al., 1997; Sieron et al., 2000), but are required for maintaining latency (Marques et al., 1997; Sieron et al., 2000; Leighton and Kadler, 2003). The prodomains appear to be proteolytically removed via subtilisin-like proprotein convertases (SPCs), within the trans-Golgi compartments of some cells (Leighton and Kadler, 2003), although some cell types secrete BMP1/TLD-like proteinase as unprocessed forms (Lee et al., 1997; Jasuja et al., 2007). CUB domains are thought to mediate protein-protein interactions (Bork and Beckmann, 1993). EGF-like domains bind Ca^{2+} in some proteins (Handford et al., 1990), but the Ca^{2+} -dependent proteolytic activities of BMP1/TLD-like proteinases do not appear to rely on Ca^{2+} binding to EGF motifs (Garrigue-Antar et al., 2004; Ge et al., 2006). Rather, EGF domains bound to Ca^{2+} may confer a rigid configuration to portions of BMP1/TLD-like proteinase, thereby affecting their structural and functional properties. BMP1 contains a number of sites for Asn-linked glycosylation, some of which are conserved in all family members (Fig. 2). Blocking glycosylation at all sites or at all three CUB domain sites blocks secretion of BMP1, whereas blockage at some of the individual sites, while not blocking secretion, can affect thermal stability and levels of pCP activity (Garrigue-Antar et al., 2002).

There are four mammalian BMP1/TLD-like proteinases (Fig. 2), including mammalian TLD (mTLD), which is encoded by alternatively spliced mRNA produced by the *Bmp1* gene (Takahara et al., 1994), and mammalian TLD-like 1 and 2 (mTLL1 and mTLL2), which are genetically distinct (Scott et al., 1999; Takahara et al., 1996). The domain structures of all of these are essentially identical to that of *Drosophila* TLD. Genes that encode alternatively spliced RNAs for a shorter BMP1 form and a longer mTLD-like form appear to be conserved in vertebrates, as such an arrangement has also been reported for *Xenopus* (Lin et al., 1997) and chick (Reynolds et al., 2000).

Apparently due to partial genome duplication in teleosts, two genes exist for BMP1 in zebrafish (*bmp1a* and *bmp1b*) (Muraoka et al., 2006; Jasuja et al., 2006), although only sequences for longer mTLD-like proteinases have been described for these genes, and it has yet to be determined whether they also encode alternatively spliced mRNAs for shorter BMP1 forms. In contrast, cDNA encoding the short form of BMP1 (suBMP), has been described in the sea urchin *S. purpuratus* (Hwang et al., 1994), with a longer alternatively spliced form yet to be described. Interestingly, suBMP appears to play a role in spiculogenesis (Hwang et al., 1994), or formation of the sea urchin skeleton, thus paralleling at least one of the roles of vertebrate BMP1. Another sea urchin protein with a somewhat different configuration of astacin protease, CUB and EGF domains SpAN/BP10 (Fig. 2), is transiently expressed in the sea urchin blastula (Lepage et al., 1992; Reynolds et al., 1992), and may be involved in modulating BMP signaling (Wardle et al., 1999). Apparent orthologues for mTLL1 have been reported in zebrafish, *Xenopus*, and chick. In zebrafish this orthologue, TLL1, is the product of a gene originally designated *mini fin* (*mfn*) (Connors et al., 1999), but now also known as *tll1* (Zebrafish Information Network); whereas in *Xenopus* and chick, mTLL1 orthologues have been designated Xolloid-related (XLR) (Dale et al., 2002) and Colloid-like 1 (Liaubet et al., 2000), respectively. An apparent mTLL2 orthologue designated Xolloid (XLD) has been reported for *Xenopus* (Goodman et al., 1998), but an mTLL2 orthologue has yet to be reported for zebrafish.

In addition to the above, a second BMP1/Tolloid-like proteinase, tolloid-related 1 (TLR1, also known as *tolkin*) (Finelli et al., 1995; Nguyen et al., 1994) has been characterized in *Drosophila*, and differs from other members of the group in having a relatively elongated prodomain (Fig. 2). Sequence comparisons do not show either *Drosophila* TLD or TLR1 to be more related to one of the vertebrate BMP1/TLD-like proteinases than to another. Thus there is no clear orthologous relationship between either of the *Drosophila* proteinases and any one

of the vertebrate members of this proteinase subgroup. A single BMP1/TLD-like proteinase, *Aplysia* Tolloid/BMP1-like 1 (apTBL1) has been reported for the mollusk *Aplysia* (Liu et al., 1997) and has a domain structure identical to that of vertebrate and *Drosophila* members of this proteinase subgroup. However, apTBL1, which has an implied role in the synaptic plasticity associated with learning, is not a clear-cut orthologue of any one of the vertebrate proteinases, although some homology in the prodomain (Liu et al., 1997), and the placement of potential Asn-linked glycosylation sites in the protease domain (Fig. 2) suggest that it is more closely related to *Drosophila* TLR1 than to *Drosophila* TLD.

3. Direct roles of BMP1/TLD-like proteinases in ECM formation

Since the finding that BMP1/TLD-like proteinases provide pCP activity, it has become apparent that these proteinases process various precursors into functional structural proteins and into active enzymes involved in ECM formation in general and collagen fibrillogenesis in particular. A role has also been described for BMP1/TLD-like proteinases in the initiation of mineralization in hard connective tissues.

3.1 Fibrillar collagens

Subsequent to the finding that BMP1 provides pCP activity (Kessler et al., 1996; Li et al., 1996), it was shown that each of the mammalian BMP1/TLD-like proteinases has some level of pCP activity; with BMP1 and mTLL2 showing highest and lowest *in vitro* pCP levels, respectively (Pappano et al., 2003; Scott et al., 1999). In fact, mTLL2 pCP levels are not observable *in vitro*, unless procollagen substrates and mTLL2 are co-incubated in the presence of a third protein known to enhance the pCP activity of BMP1/TLD-like proteinases (Pappano et al., 2003; and see below). Nonetheless, small amounts of residual pCP activity in mouse embryos doubly null for the genes which encode BMP1, mTLD and mTLL1, suggest that mTLL2 may be an *in vivo* pCP (Pappano et al., 2003). BMP1/TLD-like proteinases are secreted molecules, pCP activity has a pH optimum of 8.0 – 8.5 (Hojima et al., 1985; Kessler et al., 1986), and various studies have demonstrated both pCP activity and unprocessed procollagen in the extracellular space in cell and organ cultures. However, it has recently been reported that processing of procollagen C-propeptides of the major fibrillar collagens occurs intracellularly, under presumably acidic conditions, in developing tendon (Canty et al., 2004). Thus, procollagen I-III C-propeptide processing may occur intracellularly or extracellularly, in a cell type-specific/tissue-specific manner. The major fibrillar procollagens appear to form intracellular aggregates prior to secretion (Birk and Trelstad, 1984; Bonfanti et al., 1998; Canty et al., 2004), and these aggregates may constitute the normal *in vivo* substrates for pCP activity. The latter possibility is supported by the finding that aggregated type I procollagen is processed more rapidly by pCP activity than is monomeric procollagen (Hojima et al., 1994).

In addition to their roles in processing the major fibrillar collagen types I–III, vertebrate BMP1/TLD-like proteinases play roles in biosynthetic processing of the minor fibrillar collagen types V and XI, which combine with collagens types I and II, respectively, and regulate the size and shapes of the resulting heterotypic fibrils (Birk et al., 1990; Li et al., 1995; Mendler et al., 1989; Toriello et al., 1996). The most abundant form of type V collagen monomer is a heterotrimer of two $\alpha 1(V)$ chains and one $\alpha 2(V)$ chain, although $\alpha 1(V)_3$ homotrimers and $\alpha 1(V)\alpha 2(V)\alpha 3(V)$ heterotrimers, with more restricted distributions of expression, are also found (Fessler and Fessler, 1987). Type XI collagen consists of $\alpha 1(XI)\alpha 2(XI)\alpha 3(XI)$ heterotrimers (Morris and Bachinger, 1987). In addition, heterotrimers containing α chains of both type V and type XI collagens have been reported (Kleman et al., 1992; Mayne et al., 1993), suggesting that V and XI may represent a single collagen type with various α chains expressed differentially in a tissue-specific manner. BMP1/TLD-like proteinases cleave the C-propeptides of pro $\alpha 2(V)$ chains (Unsold et al., 2002), which are more closely related in protein domain structure to the chains of the major fibrillar procollagens than are the pro $\alpha 1(V)$, pro $\alpha 1$

(XI), and pro α 2(XI) chains. Surprisingly, the BMP1/TLD-like proteinases appear to cleave the latter three chains solely within their relatively large amino-terminal globular sequences, leaving smaller, trimmed amino-terminal globular sequences (Imamura et al., 1998; Pappano et al., 2003; Unsold et al., 2002). These proteinases can also cleave within pro α 3(V) amino-terminal globular sequences (Gopalakrishnan et al., 2004).

Processing of minor and major fibrillar collagen propeptides by the same proteinases may coordinate deposition of the two types of fibrillar collagens into the same heterotypic fibrils. It is the proteolytically trimmed amino-terminal globular sequences of the minor fibrillar collagen chains that are thought to regulate the shapes and diameters of heterotypic fibrils (Linsenmayer et al., 1993). Thus, proteolytic trimming of the amino-terminal globular sequences of minor fibrillar procollagen chains appears to be another way in which the BMP1/TLD-like proteinases regulate collagen fibrillogenesis. Surprisingly, the cleavage sites at which BMP1/TLD-like proteinases process amino-terminal globular sequences of types V and XI procollagen chains contain glutamine residues at the P1' positions, rather than the aspartates observed at the P1' sites of all other known substrates (Ge and Greenspan, 2006; Imamura et al., 1998; Pappano et al., 2003) (Fig. 3). Thus, although there is a clear preference for certain residues at various positions adjoining the cleavage sites of BMP1/TLD-like proteinase substrates (Fig. 4), there is no absolute requirement for a particular residue at any site, and substrate tertiary configuration may play an important role in determining cleavage sites.

In addition to processing fibrillar procollagens, the BMP1/TLD-like proteinases affect fibrillogenesis by activating the zymogen of lysyl oxidase (Panchenko et al., 1996; Uzel et al., 2001), an enzyme necessary to forming the covalent crosslinks that provide collagen and elastic fibers with most of their tensile strength. Similarly, BMP1 has been shown to activate the related cross-linking enzyme lysyl oxidase-like (Borel et al., 2001). Another way in which BMP1/TLD-like proteinases affect fibrillar collagen-containing ECM is via cleavage of the non-collagenous **S**mall **I**ntegrin-**B**inding **L**igand **N**-linked **G**lycoprotein (SIBLING) protein, Dentin Matrix Protein-1 (DMP-1) (Steiglit et al., 2004), thereby releasing a highly acidic Ca²⁺ binding domain believed to help initiate mineralization of the type I collagen fibrils of bone and teeth (Butler, 1998; Veis, 1993).

3.2 Non-fibrillar collagens

Anchoring fibrils, consisting of type VII collagen, are important structural components that mediate attachment of epidermis to underlying stroma (Sakai et al., 1986). Synthesized and secreted by epidermal keratinocytes, type VII procollagen monomers self-assemble into anti-parallel dimers, which then associate laterally to form anchoring fibrils (Keene et al., 1987; Morris et al., 1986). Initial dimer formation and stabilization through disulfide bonding seems to require retention of the type VII procollagen C-propeptide, also known as the NC2 domain (Chen et al., 2001; Lunstrum et al., 1987). However, proteolytic removal of NC2 seems necessary to anchoring fibril formation, as a mutation that eliminates the cleavage site results in the severe blistering disease dystrophic epidermolysis bullosa (Bruckner-Tuderman et al., 1995). This mutation removes 29 amino acids containing a potential cleavage site with features found in known substrates of BMP1/TLD-like proteinases (Figs. 3 and 4). *In vitro* cleavage assays have confirmed that BMP1, mTLL1 and mTLL2 are capable of cleaving the NC2 domain (Rattenholl et al., 2002), however type VII collagen appears to be fully processed in the skin of mouse embryos null for the *Bmp1* gene, which encodes both BMP1 and mTLD (Rattenholl et al., 2002). Thus, mTLL1 and mTLL2 may fully compensate for loss of *Bmp1* in these mice, for removal of the procollagen VII NC2 domain. Direct testing of this possibility is not feasible at this time, as mice lacking the *Tll1* gene, which encodes mTLL1, die at ~13.5 days post conception (Clark et al., 1999), at which stage the dermal-epidermal basement membrane zone remains poorly developed; and *Tll2*^{-/-} mice have not yet been reported. Thus,

detailed analysis of *in vivo* roles for BMP1/TLD-like proteinases in processing type VII procollagen requires development of mice conditionally null for mTLL1 and null for mTLL2.

Gliomedin, a recently identified transmembrane collagen containing an olfactomedin domain, is essential to formation of the nodes of Ranvier (Eshed et al., 2005). Expressed on the surface of Schwann cells, gliomedin serves as a ligand for the neuronal Immunoglobulin Cell Adhesion Molecules (IgCAMs) Nr-CAM and neurofascin (Eshed, et al., 2005). Interaction of gliomedin with these IgCAMs induces Na⁺ channel clustering at nodes that enables saltatory conduction in peripheral myelinated nerve fibers. Although gliomedin is a transmembrane glycoprotein, it is also shed from the cell surface by furin-like proprotein convertases at a juxtamembrane site (Maertens et al., 2007). Thus, in various respects, gliomedin resembles other integral transmembrane proteins containing collagen domains, which are also shed from the cell surface. These include ectodysplasin A and collagens XIII, XVII and XXV (Franzke et al., 2005). It has recently been demonstrated that gliomedin ectodomains shed from the cell surface are further processed by BMP1/TLD-like proteinases, which cleave between the collagen-like and olfactomedin domains (Maertens et al., 2007). Although the physiological relevance of the latter cleavage is not yet clear, such cleavage appears to favor oligomerization of freed olfactomedin domains, which may in turn contribute to maintenance of the nodes of Ranvier (Maertens et al., 2007).

3.3 Non-collagenous matrix proteins

Laminin-5, a trimer comprising α 3, β 3, and γ 2 chains, is a key component of anchoring filaments, which help keratinocytes adhere to the basement membrane (Rousselle, et al., 1991), is. Laminin-5 is secreted as a precursor that undergoes processing of its α 3 and γ 2 chains (Marinkovich et al., 1992; Rousselle et al., 1991). Matrix metalloproteinase 2 (MMP2) is capable of cleaving rat laminin-5 γ 2 chains *in vitro* (Giannelli et al., 1997), although this cleavage site is not conserved in human laminin-5. It has also been reported that Membrane type 1 matrix metalloproteinase (MT1-MMP) may be the primary enzyme responsible for cleaving the rat γ 2 chain (Koshikawa et al., 2000). However, BMP1/TLD-like proteinases are the only enzymes thus far demonstrated to cleave both rat and human γ 2 chains at the sites employed by keratinocytes (Amano et al., 2000). Moreover, analysis of laminin-5 in the skin of *Bmp1*^{-/-} mice has revealed a deficiency in γ 2 chain processing (Veitch et al., 2003). MMP2, MT1-MMP, plasmin, and BMP1/TLD-like proteinases are all capable of processing the α 3 chain to a size consistent with that observed in keratinocyte cultures (Amano et al., 2000; Goldfinger et al., 1998; Veitch et al., 2003). However, a small molecule inhibitor specific to BMP1/TLD-like proteinases is able to inhibit processing of both the γ 2 and α 3 chains in keratinocyte cultures, although inhibition of α 3 cleavage required higher inhibitor concentrations and was incomplete (Veitch et al., 2003). Thus, BMP1/TLD-like proteinases may be involved in processing both γ 2 and α 3 chains. Such processing is of importance, as processed and unprocessed forms of laminin 5 appear to have different properties in regard to cell adhesion and motility, and the invasiveness of squamous cell carcinomas (Giannelli et al., 1997; Tsubota et al., 2005).

Perlecan is a large proteoglycan that is a major structural component of basement membranes (Murdoch et al., 1994). The perlecan carboxyl-terminal domain, domain V, is responsible for most of the molecule's ability to bind cells and, when cleaved, corresponds to a fragment designated endorepellin, with angiostatic activity (Mongiat et al., 2003). Endorepellin comprises EGF-like repeats and laminin-like globular (LG) motifs. The most C-terminal LG3 motif appears to harbor most of the angiostatic activity (Bix et al., 2004) and is proteolytically removed from endorepellin and from full-length perlecan by BMP1/TLD-like proteinases (Gonzalez et al., 2005). The BMP1/TLD-like proteinase cleavage site for LG3 is identical to the site at which perlecan is cleaved *in vivo* to produce LG3 fragments found in the urine of patients with end-stage renal disease (Oda et al., 1996) and in the amniotic fluid of pregnant

women with ruptured fetal membranes (Vuadens et al., 2003), suggesting that cleavage by these proteinases is physiologically relevant. Recently, the LG3 domain was also shown to promote apoptosis-resistance in fibroblasts via interactions with $\alpha 2\beta 1$ integrins (Laplane et al., 2006). As accumulation of apoptosis resistant fibroblasts may be a hallmark of fibrosis (Arora and McCulloch, 1999; Desmouliere et al., 1995), proteolytic processing of perlecan by BMP1/TLD-like proteinases may have functional implications in both angiogenesis and fibrosis.

Small leucine-rich proteoglycans (SLRPs) are a small family of non-aggregating proteoglycans currently containing 11 members in mammals (Iozzo, 1998; Svensson et al., 2001). SLRPs can be subdivided into four classes. Members of class I (decorin and biglycan) and IV (epiphican, osteoglycin and opticin) SLRPs are synthesized as precursors that are processed *in vivo* to produce the mature proteins (Funderburgh et al., 1997; Johnson et al., 1997; Roughley et al., 1996). Biglycan has been shown to be processed by BMP1/TLD-like proteinases *in vitro* and *in vivo* (Scott et al., 2000). Since biglycan is thought to play a positive role in bone formation (Bianco et al., 1990) and to be involved in regulating TGF β signaling (Chen et al., 2002), its processing may represent novel mechanisms by which BMP1/TLD-like proteinases affect osteogenesis and TGF β signaling (also see below), respectively. It should be cautioned however that it is not yet known how processing affects biglycan function. Enzymes responsible for cleavage of decorin have yet to be determined.

Class IV SLRPs osteoglycin and epiphican N-propeptides are also proteolytically cleaved *in vivo* (Funderburgh et al., 1997; Johnson et al., 1997). All four BMP1/TLD-like proteinases are capable of cleaving pro-osteoglycin *in vitro* (Ge et al., 2004), and *Bmp1/Tll1* doubly homozygous null mice produce pro-osteoglycin exclusively in the unprocessed form (Ge et al., 2004). Osteoglycin is believed to function as a regulator of collagen fibril diameter (Ge et al., 2004; Tasheva et al., 2002), and *in vitro* fibrillogenesis assays revealed that mature osteoglycin is more efficient in regulating type I collagen fibrillogenesis than is the pro-form (Ge et al., 2004). This suggests another way in which BMP1/TLD-like proteinases may affect collagen fibrillogenesis. Epiphican is also processed at a site similar to those observed in substrates of BMP1/TLD-like proteinases (Johnson et al., 1997). However, further studies are required to ascertain whether BMP1/TLD-like proteinases are responsible for epiphican processing.

4. BMP1/TLD-like proteinase activation of a subset of TGF β -like proteins

One of the earliest functions ascribed to BMP1/TLD-like proteinases was the potentiation of signaling by TGF β -like BMPs in dorsal-ventral patterning. It has more recently become apparent that these proteinases are involved in activating a growing list of members of the TGF β superfamily.

4.1 BMPs 2 and 4

TGF β -like BMPs play a broad range of roles in processes that include dorsal/ventral patterning, organogenesis, neural development (Hogan, 1996; Furuta et al., 1997; De Robertis and Kuroda, 2004), and osteogenesis (Urist et al., 1973; Wozney et al., 1988). In particular, signaling gradients of BMP2 and BMP4 in vertebrates, and of the *Drosophila* orthologue decapentaplegic (DPP), are the major determinants for forming the dorsal-ventral axis in gastrulation (Hogan, 1996). It is now known that *Drosophila* TLD activates DPP by cleaving the extracellular antagonist Short Gastrulation (SOG) (Marques et al., 1997). Similarly, the vertebrate SOG orthologue Chordin is cleaved *in vitro*, at two sites (Fig. 5A), and counteracted in overexpression assays in *Xenopus* and zebrafish embryos, by various BMP1/TLD-like proteinases (Blader et al., 1997; Dale et al., 2002; Piccolo et al., 1996; Scott et al., 1999; Wardle et al., 1999). In mammals, BMP1 and mTLL1, but not mTLD or mTLL2, are capable of

cleaving Chordin *in vitro* (Scott et al., 1999); and products of the *Bmp1* and *Tll1* genes are responsible for cleaving Chordin *in vivo* (Pappano et al., 2003). It has been postulated that the two most carboxyl-terminal mTLD CUB domains are held by EGF-like motifs in a conformation that blocks Chordinase activity, as removal of one or both EGF motifs provides the truncated mTLD with readily detectable Chordinase activity (Garrigue-Antar et al., 2004).

Although TLD-null *Drosophila* have severe dorsal-ventral patterning defects (Childs and O'Connor, 1994; Finelli et al., 1994), mice null for the *Bmp1* gene show what appears to be relatively mild dorsal-ventral patterning defects, reflected in failure to close the ventral body wall (Suzuki et al., 1996). The latter may also reflect deficient ECM production, as do abnormal collagen fibrillogenesis and defective membranous bone formation in these mice. *Tll1* knockout mice are embryonic lethal at 13.5 days post conception, with defects confined almost entirely to the heart (Clark et al., 1999). The cardiac defects in these mice do not appear to arise from defective collagen fibrillogenesis, which is not affected in an obvious way (Clark et al., 1999). Rather, *Tll1*-null cardiac abnormalities may arise due to failure to process Chordin in this tissue, resulting in aberrations in the BMP signaling normally involved in septation and looping of the vertebrate heart (Clark et al., 1999). Absence of severe dorsal-ventral patterning defects in *Bmp1* and *Tll1* null mice suggests functional redundancy. Such redundancy is confirmed by the observation that mouse embryos doubly null for both *Bmp1* and *Tll1* have less pCP activity than do embryos singly null for either gene; thereby showing products of both genes to be *in vivo* pCPs (Pappano et al., 2003). However, doubly null embryos die at the same point in development as do *Tll1*-null embryos, without obvious dorsal-ventral patterning defects (Clark et al., 1999), thus suggesting residual Chordinase activity from an unknown protease(s). mTLL2 may provide such activity *in vivo*, since it has Chordinase activity *in vitro* in the presence of the modulator protein twisted gastrulation (TSG) (Scott et al., 2001; also see below). In contrast to mammals, knockdown of the expression of the *bmp1a* and *tll1* genes in zebrafish is sufficient to severely dorsalize embryos (Jasuja et al., 2006; Muraoka et al., 2006), indicating less redundancy for Chordin cleavage in teleosts than in mammals. In addition to their roles in patterning, BMPs 2 and 4 are potently osteoinductive (Wozney et al., 1988). Thus, activation of BMPs 2 and 4 is another way in which BMP1/TLD-like proteinase affect skeletogenesis, in addition to their roles in maturing proteins necessary to normal bone formation (e.g. collagen type I, lysyl oxidase, biglycan, and DMP1).

4.2 Growth and differentiation factors 8 and 11 (GDFs 8 and 11)

All TGF β -like proteins are synthesized as proproteins, containing an amino-terminal prodomain that must be cleaved by SPCs for full activity of the mature protein (Ducy and Karsenty, 2000; Hogan, 1996). Although such cleavage is sufficient for activating most TGF β -like proteins (Hogan, 1996), prototypical family members TGF β 1–3 remain non-covalently bound to their cleaved prodomains in latent complexes (Massague, 1998). More recently it has been demonstrated that GDF8 and 11, which share ~90% sequence identity in their mature regions (Gamer et al., 1999; Ge et al., 2005; Nakashima et al., 1999), are also non-covalently bound to their respective prodomains in latent complexes (Ge et al., 2005; Hill et al., 2002; Lee and McPherron, 2001; Wolfman et al., 2003) (Fig. 5B). Secreted by myocytes, GDF8, also known as Myostatin, is an important negative regulator of skeletal muscle growth (Hill et al., 2002; Lee and McPherron, 2001; McPherron et al., 1997), whereas GDF11, secreted by mature neurons, serves a comparable function in inhibiting neurogenesis (Wu et al., 2003). GDF11 also appears to play roles in anterior-posterior patterning of the axial skeleton (McPherron et al., 1999). BMP1/TLD-like proteinases have been demonstrated to cleave within GDF8 and 11 prodomain sequences, thus liberating the mature proteins and enabling downstream signal transduction (Ge et al., 2005; Wolfman et al., 2003). Interestingly, recombinant GDF8 prodomain with a single amino acid substitution that renders it resistant to

cleavage by BMP1/TLD-like proteinases is capable of inducing increased muscle mass when injected into normal adult mice, whereas wild type GDF8 prodomain has no effect (Wolfman et al., 2003). This supports an *in vivo* role for activation of GDF8 by BMP1/TLD-like proteinases, and suggests mutated GDF8 and 11 prodomain sequences as possible therapeutic tools for reversing the pathologies of muscular dystrophies and degenerative diseases and injuries of neural tissues, respectively.

4.3 *Drosophila* TLD activates TGF β -like factor Daw and affects axon guidance

Two recent studies found mutations in the gene for the *Drosophila* BMP1/TLD-like proteinase TLR, also known as Tolkin, to cause strong defects in fasciculation and guidance of motor axons (Meyer and Aberle, 2006). Since TGF β -like molecules have been implicated in axon guidance in *C. elegans* and mouse, and because BMP1/TLD-like proteinases had previously been demonstrated to proteolytically activate GDF8 and 11 (see above), Serpe and O'Connor (Serpe and O'Connor, 2006) sought to determine whether the role of TLR in motor neuron axon guidance might involve activation of TGF β -like proteins. TLR was found to process the prodomains of three of the four *Drosophila* non-BMP TGF β -like proteins: Activin (Act), Dawdle (Daw), and Myostatin homolog Myoglianin (Myo), each of which contains at least one site resembling cleavage sites in known substrates of BMP1/TLD-like proteinases (Fig. 3). Both TLR and *Drosophila* TLD were capable of cleaving Act, Daw and Myo prodomains *in vitro*, to produce fragments consistent in size with cleavage having occurred at the predicted sites. Furthermore, alanine substitution for residues at the P3, P2, P1 and P1' positions at the predicted site of each prodomain inhibited processing by TLR and TLD. Surprisingly, only cleavage of the Daw prodomain was conclusively shown to result in increased signaling, as determined via Smad2 phosphorylation levels in a cell culture-based signaling assay (Serpe and O'Connor, 2006). The distribution of Daw expression in muscle and central nervous system is consistent with a role in fasciculation and axon guidance of motor neurons; whereas the finding that trans-heterozygous combinations of mutations in the *tlr* and *daw* genes had enhanced axon guidance defects compared with single heterozygous flies, was consistent with a role for TLR activation of Daw in axon guidance/fasciculation (Serpe and O'Connor, 2006). However, fasciculation defects are substantially milder and more transient in *daw* than in *tlr* mutants, suggesting additional roles in axon guidance and fasciculation for TLR, beyond activation of Daw. An additional interesting finding of the Serpe and O'Connor study (Serpe and O'Connor, 2006) was that endogenous TLR normally circulates in the hemolymph. It remains to be determined whether any vertebrate BMP1/TLD-like proteinases are found in the general circulation, although such a finding would have ramifications for our understanding of how these proteinases contribute to morphogenetic processes.

4.4 BMP1/TLD-like proteinases activate TGF β 1 via cleavage of LTBP

TGF β s 1–3 play fundamental roles in regulating cell differentiation, apoptosis, immune responses, tissue repair and ECM formation (Massague, 1990; Border and Ruoslahti, 1992; Derynck et al., 2001; Letterio and Roberts, 1998), and are key players in the etiology of fibrosis (Border and Ruoslahti, 1992). TGF β signaling induces a net increase in ECM formation in development, tissue repair, and fibrosis via inhibiting expression of ECM-degrading proteases; and by increasing expression of structural components of the ECM, lysyl oxidase, and the proteinases that process ECM components and lysyl oxidase to their mature and functional forms (Lee et al., 1997; Massague, 1990; Wang et al., 2003). The latter include the BMP1/TLD-like proteinases (Lee et al., 1997). Thus, if BMP1/TLD-like proteinases were involved in activating TGF β signaling, it would complete a positive feed-back loop in ECM formation. Binding of TGF β 1–3 prodomains to their cognate mature proteins in non-covalent latent complexes, subsequent to cleavage by SPCs, has garnered these prodomains the designation Latency Associated Peptides (LAPs) (Annes et al., 2003). The complex formed by TGF β and LAP is known as the small latent complex (SLC) (Annes et al., 2003). However, most TGF β

is secreted as a Large Latent Complex (LLC), comprising SLC disulfide bonded, via LAP, to the genetically distinct protein Latent TGF β -binding Protein (LTBP) (Annes et al., 2003) (Fig. 5C). LLC is in turn bound to the ECM via LTBP, perhaps by covalent binding of LTBP to ECM components (Annes, et al., 2003). TGF β can be activated via cleavage by MMPs (D'Angelo et al., 2001; Maeda et al., 2001; Yu and Stamenkovic, 2000) or via interactions with thrombospondin (Crawford, et al., 1998) or integrins (Mu et al., 2002; Munger et al., 1999; Yang et al., 2007). However, a role has also recently been described for BMP1/TLD-like proteinases in activating TGF β LLCs (Ge and Greenspan, 2006). Specifically, although BMP1/TLD-like proteinases do not cleave LAP, they were found to cleave LTBP1 (one of four mammalian LTBPs) at two sites (Figs. 3 and 5C) *in vitro* (Ge and Greenspan, 2006). In addition, *Bmp1/Tld1* doubly homozygous null mouse embryo fibroblasts were found to produce only unprocessed LTBP1, in contrast to wild type (Ge and Greenspan, 2006). Although LTBP1 cleavage by BMP1/TLD-like proteinases does not directly activate TGF β 1, it does appear to liberate LLCs from the ECM, thereby rendering LAP susceptible to cleavage by MMP2, and possibly by MMPs 9 and 14, with consequent TGF β 1 activation (Ge and Greenspan, 2006).

TGF β 1 activation by BMP1/TLD-like proteinases, MMP2, and potentially MMP9, all of which are potently up-regulated by TGF β 1 (Brown et al., 1990; Lee et al., 1997; Marti et al., 1994; Overall et al., 1991; Sehgal and Thompson, 1999) suggests a fast-forward loop of potential importance to tissue remodeling (Fig. 6). Since mammals appear to possess various molecular mechanisms for latent TGF β activation, the mechanism involving activation by BMP1/TLD-like proteinases may be limited to some subset of cell types and/or physiological/pathological circumstances. One candidate set of circumstances is development, as LTBP1 is clearly cleaved by BMP1/TLD-like proteinases in mouse embryo fibroblast cultures (Ge and Greenspan, 2006). Another set of circumstances may involve neoplasia, given the key roles of MMP2, other MMPs and TGF β in the tissue remodeling associated with growth, angiogenesis and invasiveness of tumors (Derynck et al., 2001; Sternlicht and Werb, 2001; Yu and Stamenkovic, 2000), and the identification of BMP1 as among the most up-regulated genes in activated endothelia associated with tumor angiogenesis (St Croix et al., 2000).

5. Modulators of BMP1/TLD-like Proteinase Activity

It is apparent that the BMP1/TLD-like proteinases play manifold roles in morphogenetic processes. Clearly, modulators of the activities of these proteinases would therefore be important regulators of the same morphogenetic processes. Interestingly, separate positive regulators have now been reported for the pCP and Chordinase activities of these proteinases. More recently, endogenous inhibitors have been described as well.

5.1 Procollagen C-Proteinase Enhancers

While not a proteinase itself, the glycoprotein Procollagen C-Proteinase Enhancer 1 (PCOLCE 1) was found to increase the pCP activity of BMP1/TLD-like proteinases ~10-fold (Kessler and Adar, 1989). More recently, a second PCOLCE (PCOLCE 2) was identified, with an identical protein domain structure and similar levels of pCP-enhancing activity (Xu et al., 2000; Steiglitz et al., 2002). Both PCOLCEs contain two amino-terminal CUB domains followed by a carboxyl-terminal Netrin-like (NTR) domain (Steiglitz et al., 2002; Takahara et al., 1994). Full pCP enhancing activity appears to reside in the CUB domains (Takahara et al., 1994), through which PCOLCEs are thought to bind directly to procollagen C-propetides and -telopeptide sequences, thereby inducing conformational changes that render procollagens more readily cleaved by BMP1/TLD-like proteinases (Kessler and Adar, 1989; Moali et al., 2005; Takahara et al., 1994). The enhancing properties of the PCOLCEs appear to be restricted to pCP activity, as these proteins show no enhancement of cleavage of other known substrates of BMP1/TLD-like proteinases (Petropoulou et al., 2005). Recently, it was shown that PCOLCE1 is bound via the most carboxyl-terminal EGF and/or CUB domains of mTLL1 (Ge

et al., 2006) and that full-length, but not carboxyl-terminal truncated forms of BMP1 and mTLL1 are fully-enhanced by PCOLCE1 (Ge et al., 2006; Petropoulou et al., 2005). Thus, PCOLCEs may act via forming a ternary complex between the most carboxyl-terminal domains of BMP1/TLD-like proteinases and their procollagen substrates. The PCOLCE1 NTR domain possesses inhibitory activity against MMPs, and perhaps other proteinases (Mott et al., 2000), and thus may play some role as an inhibitor of collagen catabolism. PCOLCE1-null mice have profoundly abnormal collagen fibrils in both bone and soft tissues, suggesting that PCOLCE1 may play additional *in vivo* roles beyond enhancement of pCP activity (Steiglitz et al., 2006).

5.2 Twisted gastrulation (TSG)

Drosophila TSG is the product of a gene necessary to dorsal-ventral patterning (Mason et al., 1994). TSG orthologues have now been identified in various species, and in which they bind BMP4/DPP and Chordin/SOG in a ternary complex, and appear capable of either promoting or antagonizing BMP signaling in different *in vivo* contexts (Oelgeschlager et al., 2000; Chang et al., 2001; Ross et al., 2001; Scott et al., 2001). Although TSG does not appear to bind BMP1/TLD-like proteinases, it does enhance the Chordinase activity of BMP1 and mTLL1 (Scott et al., 2001). In fact mTLD, which does not have detectable *in vitro* Chordinase activity in the absence of TSG (Scott et al., 1999), has such activity when TSG is present (Scott et al., 2001). Perhaps TSG induces a conformational change in Chordin that makes it more readily cleavable by BMP1/TLD-like proteinases. It has also been reported that TSG may increase cleavage of Chordin at an alternative site not used in the absence of TSG (Scott et al., 2001), although this may not be the case in all systems (Xie and Fisher, 2005).

5.3 Inhibitors of BMP1/TLD-like Proteinases

α_2 -Macroglobulin (α_2 M) is a homotetrameric member of the α -macroglobulin family (Sottrup-Jensen, 1989). Synthesized primarily in the liver, α_2 M circulates at high levels (2–4 mg/ml) in blood and is also produced by astrocytes, monocytes/macrophages, lung fibroblasts and some tumor cells (Baker et al., 2002; Borth, 1992). An irreversible suicide inhibitor, each α_2 M subunit has a “bait region” containing cleavage sites for a wide variety of proteases (Borth, 1992; Feinman, 1994). Cleavage within these bait regions causes a conformational change, such that proteases are entrapped in the α_2 M interior, thereby inhibiting protease activity via steric hindrance (Baker et al., 2002; Borth, 1992). The conformational change also exposes previously buried reactive thioester groups, which can covalently bind proteases (Baker et al., 2002; Borth, 1992). In addition, the conformational change transforms α_2 M to an “activated” form capable of binding various cytokines, and of binding to the cell surface receptor, low-density lipoprotein receptor-related protein (LRP) (Borth, 1992). The result of LRP binding is rapid clearance of α_2 M-protease complexes from the extracellular space and degradation, although activated α_2 M may also be able to bind cytokines in a reversible manner, such that it serves as a carrier for targeting such proteins to appropriate cell types (Borth, 1992). α_2 M has been shown to inhibit a wide variety of proteases, including MMP and ADAM family metalloproteinases, but it does not inhibit the astacin-like metalloproteinases α - and β -meprin (Kruse et al., 2004). Thus, α_2 M was not believed to inhibit any astacin family members (Baker et al., 2002; Kruse et al., 2004), including the BMP1/TLD-like proteinases. However, the α_2 M bait region contains sequences that resemble previously characterized BMP1/TLD-like proteinase cleavage sites (Fig. 3); and it was recently demonstrated that this site is indeed cleaved by BMP1/TLD-like proteinases, with consequent inhibition of these proteinases by plasma α_2 M (Fig. 6), with a K_i of 25 nM (Zhang et al., 2006). Interestingly, replacing the bait region with the cleavage site and flanking sequences of probiglycan, which is very efficiently cleaved by BMP1/TLD-like proteinases, produced a recombinant version of α_2 M that is 16-fold more effective at BMP1/TLD-like proteinase inhibition than is native plasma α_2 M (and 24-fold more effective than wild type recombinant α_2 M prepared via the same system as the

mutant α_2M) (Zhang et al., 2006). Future modified versions of α_2M may be further optimized for cleavage by BMP1-like proteinases by substituting in preferred residues (Fig. 4) at positions flanking bait cleavage sites.

Sizzled, a secreted frizzled-related protein (sFRP) originally described as a Wnt signaling antagonist, has surprisingly been identified as the product of the *ogon/mercedes* locus, that acts as an inhibitor of BMP signaling in zebrafish dorsal-ventral patterning (Martyn and Schulte-Merker, 2003; Yabe et al., 2003). It has recently been shown that *Xenopus* and zebrafish Sizzled can act by binding BMP1/TLD-like proteinases and inhibiting their chordinase activity (Lee et al., 2006) (Fig. 6). Since Sizzled was effective at inhibiting cleavage of a small fluorogenic peptide by BMP1 and XLR, it has been inferred that it acts exclusively by binding the active sites of these proteinases (Lee et al., 2006). It has also been reported that the related protein mouse sFRP2 is capable of inhibiting the chordinase activity of XLR proteinases (Lee et al., 2006). However, further studies are needed to determine whether sFRPs are physiologically relevant inhibitors of BMP1/TLD-like proteinases in mammals.

6. Perspectives

Biosynthetic processing by the same small group of proteinases of a wide range of proteins involved in ECM formation in general and collagen fibrillogenesis in particular, doubtless provides an element of coordination to these processes. Similarly, the activation of a subset of TGF β -like proteins by the BMP1/TLD-like proteinases may serve to coordinate signaling by these factors, and to orchestrate such signaling with ECM formation in morphogenetic events (Fig. 6). In addition to previously known roles of the BMP1/TLD-like proteinases in ECM formation and patterning, new roles are beginning to emerge. Among these are the roles that these proteinases appear to play in formation of neural tissues (Liu et al., 1997; Ge et al., 2005; Meyer and Aberle, 2006; Maertens et al., 2007). To further understand the varied roles of the BMP1/TLD-like proteinases will require a further cataloguing of their substrates, which may require high-throughput approaches, such as mass-spectrometric analyses of protein cleavage products produced by wild type cells, but not by cells null for individual, or combinations of BMP1/TLD-like proteinases.

Of great potential interest is the possibility of inhibiting BMP1/TLD-proteinase actions in ways that will constitute therapeutic interventions in human disease. In this regard, inhibition of GDF8 activation by BMP1/TLD-like proteinases has recently been shown to result in increased muscle mass, and represents a possible approach to treating muscular dystrophies (Wolfman et al., 2003). Importantly, the multiple roles of BMP1/TLD-like proteinases in collagen fibrillogenesis make them attractive targets for anti-fibrotic interventions. Approaches towards therapeutic inhibition of BMP1/TLD-like proteinases may take advantage of the manipulation of recently reported endogenous inhibitors (Lee et al., 2006; Muraoka et al., 2006; Zhang et al., 2006) and/or development of small molecule inhibitors (Veitch et al., 2003).

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Fig. 1. Processing of the N- and C-propeptides of type I procollagen by ADAMTS-2 and BMP-1, respectively. The single, shorter pro- $\alpha 2$ chain and two pro- $\alpha 1$ chains of type I procollagen are shown in grey and black, respectively.

Fig. 2.

Domain structures of BMP1/TLD-like proteinases. Light blue, red, green, dark blue, yellow and pink represent signal peptides, prodomains, metalloprotease, CUB and EGF domains and domains unique to each protein, respectively. Chevrons denote alternative splicing that produces BMP1 and mTLD from the same gene. Diamond shapes atop schematics represent potential Asn-linked glycosylation sites conserved in orthologues across all species represented in the figure. In addition, zebrafish BMP1 has two, XLR has one, and XLD has 4 additional prodomain sites; and mTLL2 has one additional site in the fourth CUB domain, not found in corresponding orthologues of other species, and not displayed in the figure.

BMP1/TLD-like Substrates		
Procollagen C-Propeptides		
$\alpha 1$ (I)	(h)	DGGR Y YRA-DDANVVRD
$\alpha 2$ (I)	(h)	YDGD F YRA-DQPR S APS
$\alpha 1$ (II)	(h)	DPLQ Y MRA-DQAAGGLR
$\alpha 1$ (III)	(h)	GGFAP Y YG-DEPMDFKI
$\alpha 2$ (V)	(h)	DPLPE F TE-DQAAPDDK
$\alpha 1$ (VII)	(h)	RPLPS Y AA-DTAGSQLH
Procollagen N-Propeptides		
$\alpha 1$ (V)	(h)	AVPDT P QS-QDPNPDEY
$\alpha 3$ (V)	(h)	GPPVS F QQ-AQAQAVLQ
$\alpha 1$ (XI)	(h)	SAPKAA Q A-QEPQID E Y
$\alpha 2$ (XI)	(h)	GQRER P QN-QQPHRAQR
Cell Surface Collagens		
Gliomedin	(m)	GETCVIPN-DDTLVGRA
Basement Membrane Proteins		
Laminin 5 $\gamma 2$	(h)	DTGDC Y SG-DENPDIEC
Perlecan	(h)	HLEGSGGN-DAPGQYGA
SLRPs		
Probiglycan	(h)	DDG P FMN-DEEASGAD
Osteoglycin	(m)	EKSLQLQK-DEVIPSLP
Mineralization		
Dentin Matrix Protein1	(m)	F DDEGMQS-DDPESTRS
Enzymes		
Prolysyl oxidase (b)		SHVDRMV G -DDPYN P YK
Prolysyl oxidase-like(b)		NWREVA V G-DSTGMARA
		P YLPVRSS-DAPPPGSE
Growth Factor Related		
Chordin	N-Terminus (m)	DPEHRS Y S-DRGEPGVG
	C-Terminus (m)	KLGD P MQA-DGPRGCRF
LTBP-1	N-Terminus (h)	P EKEIPSL-DQEKTKLE
	C-Terminus (h)	EAD P YFIQ-DRFLNSFE
Myostatin	(m)	IDQ Y DVQR-DDSSDGSL
GDF11	(m)	LDLHDFQ G -DALQPEDF
Myoglianin	(d)	SSSSQ M QG-DDANTVNE
Dawdle	(d)	VEL P IMYG-DAPEESSL
Activin	(d)	HND Y EYFN-DYSVQTHD
Inhibitors		
$\alpha 2$ -Macroglobulin	(h)	LRVG F YES-DVMGRGHA

Fig. 3.

Cleavage sites of known substrates of BMP1/TLD-like proteinases. Residues in boldface type have previously been reported to be conserved either at the cleavage sites of the N-propeptides of minor fibrillar procollagens, or at the cleavage sites of the majority of other substrates.

Fig. 4.

Amino acid conservation adjoining cleavage sites in known substrates of BMP1/TLD-like proteinases. Amino acid sequence specificity was examined for a 36 amino acid region flanking each cleavage site of the 29 substrates of Fig. 3. The sequence logo of these sites was plotted by the WebLogo software of Crooks et al. (2004). At each position of the logo, in which the cleavage site is between positions 18 and 19, amino acids are arranged in the order of their occurrence frequency from top to bottom, with the highest-frequency amino acid being on top of the stack. The height of the entire stack is proportional to the information measured in bits at that position, which helps in easily identifying the most important positions, whereas the height of each letter is proportional to the occurrence frequency of the corresponding amino acid at that position. For each position, the null hypothesis was tested that the amino acid composition of the site follows a random distribution, which assigns an equal occurrence probability to each amino acid. Nominal p-values from the chi-square goodness-of-fit test of each position showed positions 10 and 16–21 to exhibit strong evidence against the null hypothesis. In addition, although positions 7 and 23 did not pass the Bonferroni adjusted p-value cut-off, which accounts for multiple testing (Westfall et al., 1993), they had notably small p-values (0.0016 and 0.002, respectively).

Fig. 5.

Activation of TGF β -like growth factors by BMP1/TLD-like growth factors. Scissors represent cleavage by BMP1/TLD-like proteinases within Chordin (A), the prodomains of GDF8 and 11 (B), and LTBP1 (C). Chordin comprises four cysteine-rich (CR) domains connected by linker regions, and binds dimeric BMP4 or BMP2 (A). Cleaved GDF8/11 prodomains (blue) form non-covalent latent complexes with their cognate mature regions (disulfide-linked dimers, shown in purple) (B). LTBP1 has three 8-cysteine domains (C1-3). The LAP prodomain (red) of TGF β is disulfide bound to C2, and the mature TGF β dimer (black) is noncovalently bound to LAP. Other LTBP1 domains are Ca²⁺-binding (green) and non-binding (purple) EGF-like motifs, and a “hybrid” domain (H).

Fig. 6.

An overview of roles of BMP1/TLD-like proteinases in morphogenetic events. BMP1/TLD-like proteinases process ECM precursors (e.g. procollagen) to mature functional ECM components. They also activate TGF β by processing LTBP1, thereby releasing truncated LLC from the ECM, leading to consequent activation via cleavage of LAP cleavage by non-BMP1/TLD-like metalloproteinases (e.g. MMP 2). Activated TGF β binds cell surface receptors, which activate R-Smads 2 and 3, which combine with Smad4 for translocation to the nucleus and up-regulation (arrows) of BMP1, ECM precursors (e.g. procollagen), MMP 2, and TGF β itself. TGF β also down-regulates some MMPs that degrade ECM (e.g. MMP1). The BMP1/TLD-like proteinases thus appear to be involved in a positive feedback/feed-forward loop for tissue remodeling. BMP1-like proteinases also activate BMPs 2/4 by cleaving the antagonist Chordin, thus inducing activation of R-Smads 1, 5, and 8. These may compete with R-Smads 2 and 3 for limiting amounts of Smad4. Chordin might also compete with ECM

precursors and LTBP1 for BMP1/TLD-like proteinases. Smad4 and BMP1-like proteinases may thereby represent two levels at which cross-talk between TGF β and BMP signaling pathways orchestrates tissue remodeling with patterning.