

# A Disintegrin-like and Metalloprotease (Reprolysin-type) with Thrombospondin Type 1 Motif (ADAMTS) Superfamily: Functions and Mechanisms\*

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Together with seven ADAMTS-like proteins, the 19 mammalian ADAMTS proteases constitute a superfamily. ADAMTS proteases are secreted zinc metalloproteases whose hallmark is an ancillary domain containing one or more thrombospondin type 1 repeats. ADAMTS-like proteins resemble ADAMTS ancillary domains and lack proteolytic activity. Vertebrate expansion of the superfamily reflects emergence of new substrates, duplication of proteolytic activities in new contexts, and cooperative functions of the duplicated genes. ADAMTS proteases are involved in maturation of procollagen and von Willebrand factor, as well as in extracellular matrix proteolysis relating to morphogenesis, angiogenesis, ovulation, cancer, and arthritis. New insights into ADAMTS mechanisms indicate significant regulatory roles for ADAMTS ancillary domains, propeptide processing, and glycosylation. ADAMTS-like proteins appear to have regulatory roles in the extracellular matrix.

## Evolutionary Perspective

Comparison of the human ADAMTS repertoire with invertebrates (1, 2) suggests that at least five human ADAMTS clades arose early in deuterostome evolution, whereas the largest human clade, which contains proteoglycanases, arose later in chordates. The six *Ciona intestinalis* ADAMTS proteases represent core evolutionary branches in chordates, and the mammalian ADAMTS clades they root are distinctive, implying neofunctionalization (*i.e.* distinct substrate specificities) of each clade (1). ADAMTS proteases in each mammalian clade have, however, a similar primary structure (Fig. 1), implying subfunctionalization, *i.e.* maintenance of substrate specificity, but specialization in different biological contexts (1). As an

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example, ADAMTS2, ADAMTS3, and ADAMTS14 each process the major fibrillar procollagen types I–III *in vitro*, but their tissue-specific expression manifests each activity in discrete tissues (3). ADAMTS2 and ADAMTS3 are coexpressed with procollagen III (a vascular collagen) and procollagen II (a cartilage collagen), respectively, and may have dominant roles in their maturation, whereas procollagen I processing may be undertaken by both enzymes in a tissue-specific or overlapping manner (3).

## Functions of ADAMTS Proteases

This minireview primarily discusses functions for which unequivocal genetic evidence exists and substrates whose deficient cleavage was implicated in a phenotype. *ADAMTS13* mutations cause inherited TTP<sup>2</sup> (4, 5), whereas acquired TTP is caused by ADAMTS13 autoantibodies. ADAMTS13 processes vWF at a single peptide bond (Tyr<sup>1605</sup>–Met<sup>1606</sup>) and has no other known substrates (4, 5). TTP results from failure to process ultra-large vWF to an optimal size for normal coagulation. ADAMTS13 deficiency leads to occlusion of the circulation by platelet thrombi, with serious consequences for vital organs.

*ADAMTS2* mutations cause Ehlers-Danlos syndrome (dermatosparactic type) (6), a connective tissue disorder first identified in cattle (7) and a consequence of failure to excise the amino propeptide of procollagen I in the dermis of skin. The retained propeptide hinders collagen fibril assembly, and the resulting thin branched fibrils are weak, leading to severe skin fragility (7). Other collagen-rich tissues (*e.g.* bone) are not fragile probably because of the compensating activity of ADAMTS3 (8) and/or ADAMTS14 (9). Procollagen processing is a specialized task, but unlike vWF processing, it is shared among three similar enzymes.

*ADAMTS10* mutations cause a rare connective tissue disorder, recessive WMS (10). Several clinical features of WMS are the opposite of Marfan syndrome, caused by mutations of *FBNI*, which encodes the matrix glycoprotein fibrillin-1. *FBNI* mutations lead to TGF $\beta$  dysregulation (11). Fibrillin-1 mutations can cause dominant WMS (12), suggesting that ADAMTS10 is functionally coupled to fibrillin-1. Indeed, we have found that ADAMTS10 specifically bound to fibrillin-1.<sup>3</sup> This places ADAMTS10 squarely within a TGF $\beta$ -regulating extracellular network centered in fibrillin microfibrils (13).

Several ADAMTS proteases collectively referred to as proteoglycanases (Fig. 1) participate in proteolysis of the large aggregating proteoglycans aggrecan, versican, and brevican and have been the subject of intense study because aggrecan loss is a major feature of osteoarthritis. Mice with deletion of the ADAMTS5 catalytic module (*Adamts5* $\Delta$ Cat) are resistant to induced arthritis (14, 15). Thus, ADAMTS5 is regarded as a

<sup>2</sup> The abbreviations used are: TTP, thrombotic thrombocytopenic purpura; vWF, von Willebrand factor; WMS, Weill-Marchesani syndrome; TGF $\beta$ , transforming growth factor- $\beta$ ; DTC, distal tip cell; TSR, thrombospondin type 1 repeat.

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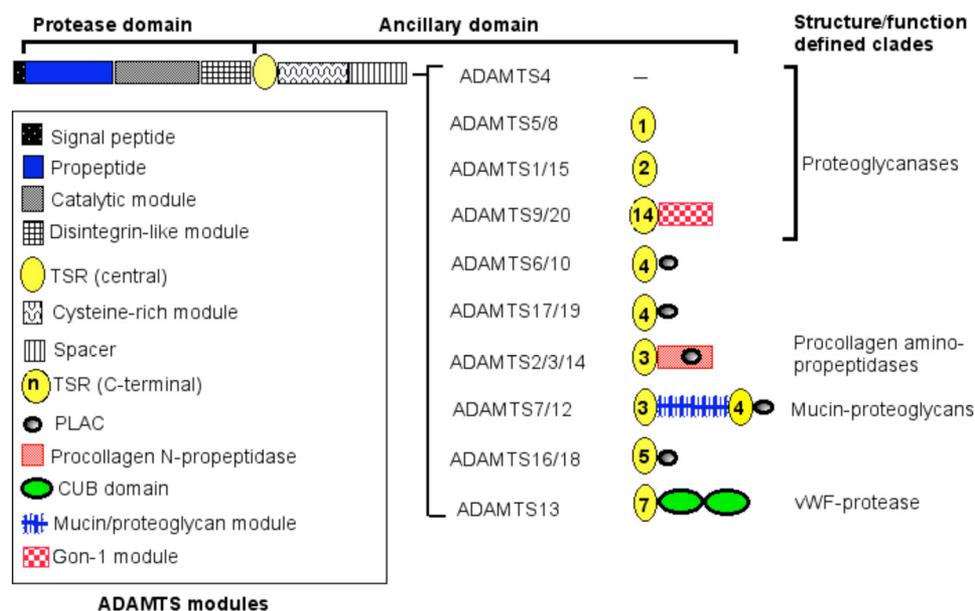


FIGURE 1. **Mammalian ADAMTS proteases.** The domain backbone shared by each ADAMTS protease is shown at the top. The unique structure of each ADAMTS protease C-terminal to the backbone is indicated on the right, and the key to these modules is located on the left. Some clades are named according to structural or functional characteristics that best define them; clades without a known function or a defining characteristic are not named. The proteoglycanases constitute a superclade comprising ADAMTS proteases with different domain structure. The figure is based on reference sequences obtained from GenBank<sup>TM</sup>. PLAC, protease and lacunin module.

major aggrecanase in mice and is a target for drug development in osteoarthritis.

Specific physiological roles of the proteoglycanases were sought using genetically engineered or naturally occurring mouse mutants. Although *Adamts4* $\Delta$ Cat and *Adamts5* $\Delta$ Cat mice and the doubly deleted mice are reportedly normal, 45% of *Adamts1* null mice die perinatally (16). Surviving mice are small, with low female fertility and urogenital anomalies (16–18). *Adamts1* is hormonally regulated and is essential for proteolysis of periovular versican during ovulation and for ovarian lymphangiogenesis (19, 20). ADAMTS1 is anti-angiogenic via several mechanisms. It sequesters VEGF<sub>165</sub> (21), binds fibroblast growth factor-2 via a heparin bridge (22), and releases anti-angiogenic fragments from thrombospondin-1 and thrombospondin-2 (23). In *Xenopus laevis*, *XADAMTS1* was found to regulate embryonic fibroblast growth factor signaling (24). ADAMTS1 is essential for versican turnover during mouse cardiac development and is regulated coordinately with myocardial trabecular growth and compaction (25).

Among the proteoglycanases, ADAMTS9 and ADAMTS20 constitute a highly conserved subset given their strong resemblance to the GON-1 protease in *Caenorhabditis elegans*. *Adamts20* mutations occur in the white spotting mutant, *Belted (bt)* (26). Expression of *Adamts20* mRNA in dermal mesenchyme coordinately with melanoblast migration during embryogenesis initially suggested that ADAMTS20 facilitated melanoblast migration (26). However, new evidence suggests that *Adamts20* promotes survival of melanoblasts via versican proteolysis and Kit signaling (27). *Adamts9* is widely expressed in mesoderm derivatives (28). *Adamts9* null mice die by 7.5 days of gestation (27), currently precluding full analysis of its developmental

role. *ADAMTS9* was shown to be a tumor suppressor in esophageal and nasopharyngeal cancer using a functional complementation approach (29), and another proteoglycanase, *ADAMTS15*, was identified as a tumor suppressor gene in colorectal cancer (30). In addition to its tumor suppressor role, ADAMTS9 is a product of microvascular endothelial cells that acts cell-autonomously to suppress angiogenesis.<sup>4</sup>

Mutations of the *C. elegans* ADAMTS proteases GON-1 and MIG-17 have offered several novel insights. *gon-1* mutants have gonad defects due to failed migration of pathfinding DTCs along the ventral body wall (31, 32), whereas *mig-17* mutants have defects in gonadal arm migration along the dorsal body wall (33). *gon-1* defects may result from lack of basement membrane degradation or release of migratory cues and can be rescued by transgenic overexpression of

ADAMTS9 or ADAMTS4. RNA interference and engineered deletions of fibulin, but not of several other extracellular matrix proteins, suppressed the *gon-1* phenotype (34). The *mig-17* phenotype was suppressed only by dominant fibulin mutations, but not by fibulin inactivation (35). Fibulin mutations in the worm lead to expansion of the gonad and loss of integrity of the basement membrane separating it from the gut lumen (35); the protease mutations suppressed, in turn, the fibulin phenotype (34). Thus, fibulin and the two *C. elegans* proteases appear to interact in the gonadal basement membrane (34, 35), but neither GON-1 nor MIG-17 cleaves fibulin (34, 35). Intriguingly, MIG-17 controls cell migration by recruitment of nidogen to the basement membrane (36).

### Cooperative Functions of ADAMTS Proteases

Evolutionary subfunctionalization suggests that closely related ADAMTS proteases may cooperatively maintain proteolysis above a functionally critical threshold in specific pathways. In support of a cooperative proteolysis model, hemizygosity of *Adamts9* increased the depigmented area in *bt* mice (27). Furthermore, 100% of *bt/bt:Adamts9*<sup>+/-</sup> mice develop cleft palate, whereas *bt* and *Adamts9*<sup>+/-</sup> mice have delayed palatal closure but a very low incidence of cleft palate.<sup>5</sup> ADAMTS5, ADAMTS9, and ADAMTS20 work cooperatively in resorption of interdigital webs in mice.<sup>6</sup> In interdigital webs, the cardiac outflow tract, embryonic myocardium, and ovarian

<sup>4</sup> B.-H. Koo and S. S. Apte, manuscript in preparation.

<sup>5</sup> H. Enomoto, C. Nelson, and S. S. Apte, unpublished data.

<sup>6</sup> McCulloch, D. R., Nelson, C. M., Dixon, L. J., Silver, D. L., Wylie, J. D., Lindner, V., Sasaki, T., Cooley, M. A., Argraves, W. S., and Apte, S. S. (2009) *Dev. Cell*, in press.

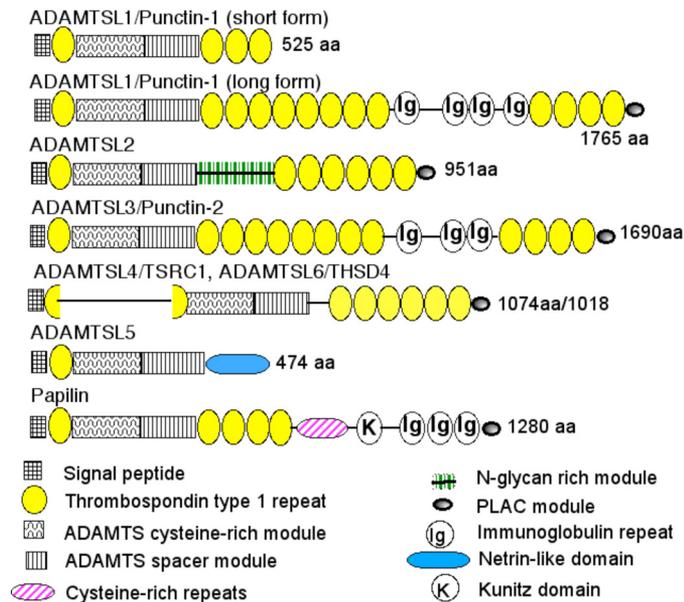


FIGURE 2. **Mammalian ADAMTSL proteins.** The domain structure of each ADAMTSL is shown according to the key at the bottom. The two forms of ADAMTSL1 shown are splice variants, and the long form composes a clade with ADAMTSL3. ADAMTSL4 and ADAMTSL6 compose a distinct clade in which TSR1 is split by an insertion. The figure is based on reference sequences obtained from GenBank™. aa, amino acids. PLAC, protease and lacunin module.

follicles, several proteoglycanases are coexpressed in versican-rich contexts and may work cooperatively to degrade this proteoglycan (25, 37–39). Cooperation of ADAMTS2 with ADAMTS3 and/or ADAMTS14 is suggested by the presence of varying amounts of completely processed procollagen I and III in dermatosparactic tissues (3). The contribution of GON-1 and MIG-17 to gonadogenesis is also a cooperative role, albeit at discrete stages of DTC migration.

### Structure, Post-translational Modification, and Proteolytic Mechanisms

ADAMTS proteases consist of a protease domain and an ancillary domain with a characteristic modular structure containing at least one TSR (Fig. 1). In general, ADAMTS ancillary domains provide substrate-binding specificity, and the protease domain provides cleavage site specificity. ADAMTS catalytic modules have a reprolysin active-site motif (HEXXH + HD), but their cysteine signatures differ from ADAM proteins (40). Inhibition of ADAMTS4 and ADAMTS5 by TIMP3, which also inhibits ADAM proteins (41), suggests structural similarities between the active sites of ADAMTS proteases and ADAM proteins despite several specific differences in catalytic cleft topography (42, 43). The crystal structure of ADAMTS1 (42) suggests that the disintegrin-like module is functionally a part of the protease domain. This is supported by absence of the disintegrin-like domain in all ADAMTS-like proteins (Fig. 2) and its presence in MIG-17, which lacks an ancillary domain. Indeed, residues in the ADAMTS13 disintegrin-like domain comprise an exosite that interacts with vWF (44).

All ADAMTS proteases (except ADAMTS4) and ADAMTSL are modified by addition of *N*-linked carbohydrate. *N*-Glycosylation of the MIG-17 propeptide is critical for its spa-

tial targeting during gonadal morphogenesis (45), and *N*-glycosylation of the ADAMTS9 propeptide is essential for its secretion (46). WXXW and CXX(S/T)CG motifs in TSRs are consensus sites for *C*-mannosylation (of Trp) and *O*-fucosylation (of Ser and Thr), respectively (47). Prevention of *O*-fucosylation of ADAMTSL1 and ADAMTS13 severely restricts their secretion (48, 49). Because *O*-fucosylation requires prior protein folding, it functions as a quality control mechanism in biosynthesis. *C*-Mannosylation was demonstrated in ADAMTSL1 and ADAMTS5, and it also appears to be a potential quality control mechanism (50). ADAMTS7 and ADAMTS12 are unusual in being mucins as well as chondroitin sulfate proteoglycans, with both modifications being present in a distinct module (Fig. 1) (51).

Excision of metalloprotease propeptides, a process termed “activation,” is a key post-translational step. ADAMTS propeptide excision is undertaken by proprotein convertases, e.g. furin. ADAMTS1 and ADAMTS4 are conventionally processed by furin because their processing occurs in the *trans*-Golgi and is required for proteolytic activity (52, 53). However, there are several idiosyncrasies in ADAMTS propeptide processing. For example, removal of the short ADAMTS13 propeptide is not essential for vWF cleavage (54). Furin processing of several ADAMTS proteases occurs extracellularly (51, 55, 56), and pro-ADAMTS9 specifically undergoes cell-surface furin processing (56). In contrast to most metalloproteases, cell-surface processing of the ADAMTS9 propeptide by furin reduces its proteolytic activity (46). MIG-17 undergoes a stage-specific extracellular activation during DTC migration (57). The furin-processing site in ADAMTS10 lacks a P4 or P6 Arg residue and is inefficiently processed by furin (55).

ADAMTS proteases require their ancillary domains for substrate recognition and correct tissue compartmentalization (58–63). The ancillary domain of several ADAMTS proteases is modified by *C*-terminal proteolysis (52, 55, 64), which potentially alters substrate recognition and enzyme localization. *C*-terminal fragments thus released may have independent biological activities, as exemplified by oxidative platelet fragmentation caused by a fragment of ADAMTS18 (65).

Some ADAMTS proteases are operational cell-surface proteases via the affinity of their ancillary domain or propeptides for cell-surface molecules (55, 59, 66) and may participate in cell-surface ectodomain shedding; indeed, ADAMTS1 sheds syndecan-4 (67) and increases the shedding of two heparin-binding epidermal growth factors, amphiregulin and heparin-binding epidermal growth factor (68). Processing of the ADAMTS4 ancillary domain is initiated at the chondrocyte cell surface within a complex comprising a heparan sulfate proteoglycan (syndecan-1) and a membrane-anchored metalloprotease (66).

ADAMTS activity may be influenced by cofactors or substrate modification. ADAMTS1 associates with fibulin-1 and, like GON-1, does not cut it, but the interaction enhances its aggrecanase activity by 10-fold (69). ADAMTS1 and ADAMTS4 bind fibronectin, but whereas the aggrecanase activity of ADAMTS4 is inhibited, that of ADAMTS1 is enhanced 2-fold by fibronectin (69, 70). The heparin-binding property of some ADAMTS proteases such as ADAMTS1 provides a basis for cell binding and matrix association. Therefore, intermolecular interactions may define

niches for ADAMTS proteases, bring them into defined networks, and act as cofactors for their activity. As shown by MIG-17 recruitment of nidogen (36), ADAMTS proteases may also be instrumental in forming networks.

Post-translational modification of some ADAMTS substrates is essential for their cleavage. ADAMTS2 processes heat-denatured procollagen inefficiently, requiring the triple-helical conformation of procollagen or the hairpin conformation of the propeptide folded back onto the triple helix (71). ADAMTS4 requires the glycosaminoglycan side chains on aggrecan for efficient processing (62). ADAMTS13 processing is enhanced by hydrodynamic shear-mediated stretching of the vWF A1 and A3 domains in circulating blood, which exposes the scissile bond (72); thus, in test tube assays, vWF processing by ADAMTS13 is inefficient and requires mild denaturation for optimization.

### ADAMTS-like Proteins

ADAMTS-like proteins (Fig. 2) are products of distinct genes, not alternatively spliced products of ADAMTS genes, and they appear to be components of the extracellular matrix (73, 74). Their resemblance to ADAMTS proteases and matrix-binding properties suggest a potential role in ADAMTS regulation. Indeed, bovine ADAMTS2 is inhibited non-competitively by *Drosophila* papilin (74), and isoforms of MIG-6, a *C. elegans* papilin ortholog, influence DTC migration and act in the same pathway as MIG-17 (75). Alternatively, ADAMTSL may have architectural or regulatory functions in the matrix that are independent of ADAMTS proteases. Human *ADAMTSL2* mutations cause geleophysic dysplasia, a rare disorder that resembles WMS and that is characterized by high levels of TGF $\beta$  activity (76). ADAMTSL2 binds to latent TGF $\beta$ -binding protein-1 (76), a fibrillin-1 ligand, as well as to fibrillin-1,<sup>7</sup> suggesting that ADAMTSL2 and ADAMTS10 operate in the same pathway. A homozygous *ADAMTSL4* mutation was recently identified in isolated ectopia lentis (dislocation of the ocular lens) (77). The suspensory ligament of the lens, which centers the lens in the path of light, is composed primarily of fibrillin-1. Intriguingly, ectopia lentis is a major clinical feature of both WMS and Marfan syndrome, and dominant ectopia lentis is caused by *FBN1* mutations (11). Taken together, the role of ADAMTSL2 and ADAMTSL4 in TGF $\beta$  regulation and maintenance of the lens, respectively, suggests that these ADAMTSL proteins may regulate fibrillin supramolecular assembly and/or architecture.

### Conclusions

Beginning with the discovery of ADAMTS1 only a dozen years ago (78), the ADAMTS field has advanced considerably. We now appreciate that some of these molecules work in highly specialized contexts (vWF and procollagen processing), whereas broad subsets of the superfamily may operate within fibrillin networks and in proteoglycan cleavage. The concept of cooperative proteolysis is likely to apply to several biological contexts. The concepts and knowledge that have been developed will be invaluable for investigating several superfamily members (e.g. ADAMTS17, ADAMTS19, ADAMTSL5, and

ADAMTSL6) of whom little is currently known beyond their primary structure.

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