Review

ADAMTS: A novel family of proteases with an ADAM protease domain and thrombospondin 1 repeats

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A disintegrin and metalloproteinase (ADAM) is a family of gene products with sequence homology to snake venom metalloproteinases and disintegrins [1,2]. Members of the family share several distinct protein modules, including a metalloprotease domain, a disintegrin domain, a cysteine-rich region and an EGF repeat [1,2]. These proteins, however, perform essential functions in cell adhesion and fusion in diverse systems [3]. The heterodimeric sperm protein fertilin participates in sperm-egg fusion [4], while meltrin-α is involved in myoblast fusion [5]. The *Drosophila* Kuzbanian (KUZ) plays a role in axonal extension and neural cell fate in fly neurogenesis [6,7]. The protease activity of some members is apparently important in ectodomain shedding of molecules such as the tumor necrosis factor α and the *Drosophila* signalling molecule Notch [3]. The tumor necrosis factor (TNF)-α converting enzyme (TACE, ADAM17), for example, has a role in processing multiple cell surface proteins as revealed by analysis of knockout mice [8]. The ADAM family members are generally cell surface molecules with a transmembrane domain spanning the plasma membrane.

We report here a bunch of proteins with a metalloprotease-disintegrin domain and an additional distinct feature not present in other ADAM proteins, a thrombospondin type 1 (TSP1) motif. The TSP1 motifs are repeats conserved in TSP 1 and 2, which are multifunctional extracellular matrix (ECM) proteins implicated in cell adhesion, motility and growth. Thrombospondin is a major constituent of platelet α-granules. It is also secreted by a wide variety of epithelial and mesenchymal cells and the levels of thrombospondin are correlated with developmental changes in the embryo and the response to injury in the adult [9]. TSP1 motifs can also be found in several proteins in the complement cascade, properdin [10] and f-spondin, a protein involved in neural cell adhesion and neurite outgrowth [11].

A schematic representation of the domain structures of four mammalian gene products with an ADAM type metalloprotease-disintegrin domain and TSP1 repeats are depicted in Fig. 1A. The respective metalloprotease-disintegrin domain and TSP1 repeats in these proteins were revealed by the pfscan program of the ISREC bioinformatics profilescan server (http://www.isrec.isb-sib.ch/software/PFSCAN_form.html). The first, ADAMTS-1, is a mouse gene cloned by differential display from a cachexigenic tumor cell line [12,13]. The authors coined the name ADAM with thrombospondin motifs (ADAMTS-1) for the gene product, an informative nomenclature which we shall adhere to. Two human cDNA deposited into the database by the Kazusa DNA research institute, KIAA0688 and KIAA0366, are designated ADAMTS-2 and ADAMTS-4 respectively. KIAA0688 shares a rather high homology (44%) with ADAMTS-1, whereas KIAA0366 shares a high homology (52%) with the bovine gene for procollagen I *N*-proteinase [14,15]. We propose to designate this bovine gene as ADAMTS-3. Fig. 1B is a phylogenetic tree illustrating the phylogenetic distances between representatives of the ADAM family with that of ADAMTS-1–4. The ADAMTS proteins clearly belong to a subfamily.

Fig. 2A shows the alignment of the metalloprotease domain of ADAMTS-1–4 with three ADAM proteins, fertilin-α (ADAM1), meltrin-α (ADAM12) and the monocyte surface antigen MS2 (ADAM8). Fig. 2B shows the alignment of the first TSP1 motif of ADAMTS-1–4 with the three TSP1 repeats found in thrombospondin 1. All the ADAMTS proteins with the exception of ADAMTS-2 have additional TSP1-like repeats (with lesser homology to that found in thrombospondin) at the carboxyl-terminal portion of the molecules.

What might the functions of the ADAMTS family members be? The ADAMTS-1 gene was isolated based on its selective expression in colon 26 adenocarcinoma cachexigenic sublines under in vivo tumor bearing states. ADAMTS-1 mRNA could be induced by an inflammatory cytokine interleukin-1 in vitro and by intravenous administration of lipopolysaccharide in vivo [12]. The expression of ADAMTS-1 is therefore associated with acute inflammation. Procollagen I *N*-proteinase cleaves the amino propeptides in the processing of type I and type II procollagens into collagens. The enzyme was first purified [14] from calf skin and the bovine gene was subsequently cloned [15]. It was noted by the authors that the mRNA transcript levels in some tissues are disproportionately high relative to the apparent rate of collagen biosynthesis and the proteins may have other roles in development that are independent of their role in procollagen processing [15].

The presence of a metalloprotease and a disintegrin domain and TSP1 repeats on the same protein suggests intriguing possibilities. It should be noted that ADAMTS-1–4 all possess a Zn-protease catalytic site consensus sequence (HEXXH), which suggests an intact catalytic activity [3]. Procollagen I *N*-proteinase was indeed isolated as an active enzyme [14]. The other ADAMTSs could therefore be responsible for proteolysis of yet unidentified substrates at the cell surface or the ECM, if they are expressed on the cell surface or are secreted. Both ADAMTS-1 and procollagen I *N*-proteinase precursors have putative N-terminal signal peptides, and both are secreted when expressed in cultured cells [13,15]. ADAMTS-2 has a hydrophobic N-terminal sequence and a potential signal cleavage site, as predicted by the PSORT II program

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(http://psort.nibb.ac.jp:8800/). The cDNA sequence of KIAA0366/ADAMTS-4 is probably not full length. However, it also has a potential hydrophobic N-terminus and a signal cleavage site. Therefore, although it remains to be determined experimentally, ADAMTS-2 and ADAMTS-4 are probably secreted as well. Also, a notable difference between ADAMTS-1, 3 and 4 with the ADAM family proteins is the lack of a distinct transmembrane domain.

The TSP1 motif of thrombospondins 1 and 2 has been thought to bind to ECM molecules. Kuno et al. first showed that the first TSP1 motif of ADAMTS-1 expressed as a glutathione-S transferase fusion protein can bind heparin in vitro [12]. The authors subsequently showed that ADAMTS-1 is indeed incorporated into the ECM and the three TSP1 motifs are important for a tight association with the ECM [13]. The bovine procollagen I *N*-proteinase functions to process procollagen into collagen, which is the major class of insoluble fibrous protein in the ECM. The TSP1 repeats on the other two ADAMTS proteins also suggests their location and function at the ECM.

Although sharing homology in key domains that characterized this new family of proteins, the four ADAMTSs are sufficiently divergent in sequences outside the metalloprotease and TSP1 motifs to suggest differences in their respective physiological roles. These are relatively large proteins, the smallest of which, ADAMTS-2, is predicted to have a molecular size of ~90 kDa. Their large size and the lack of a way for systematic identification based on function would have hindered their discovery. There may well be more members of the family that remain to be identified. It would be of great interest to examine the role of this novel family of proteins in the cell adhesion, fusion and modulation of the ECM.

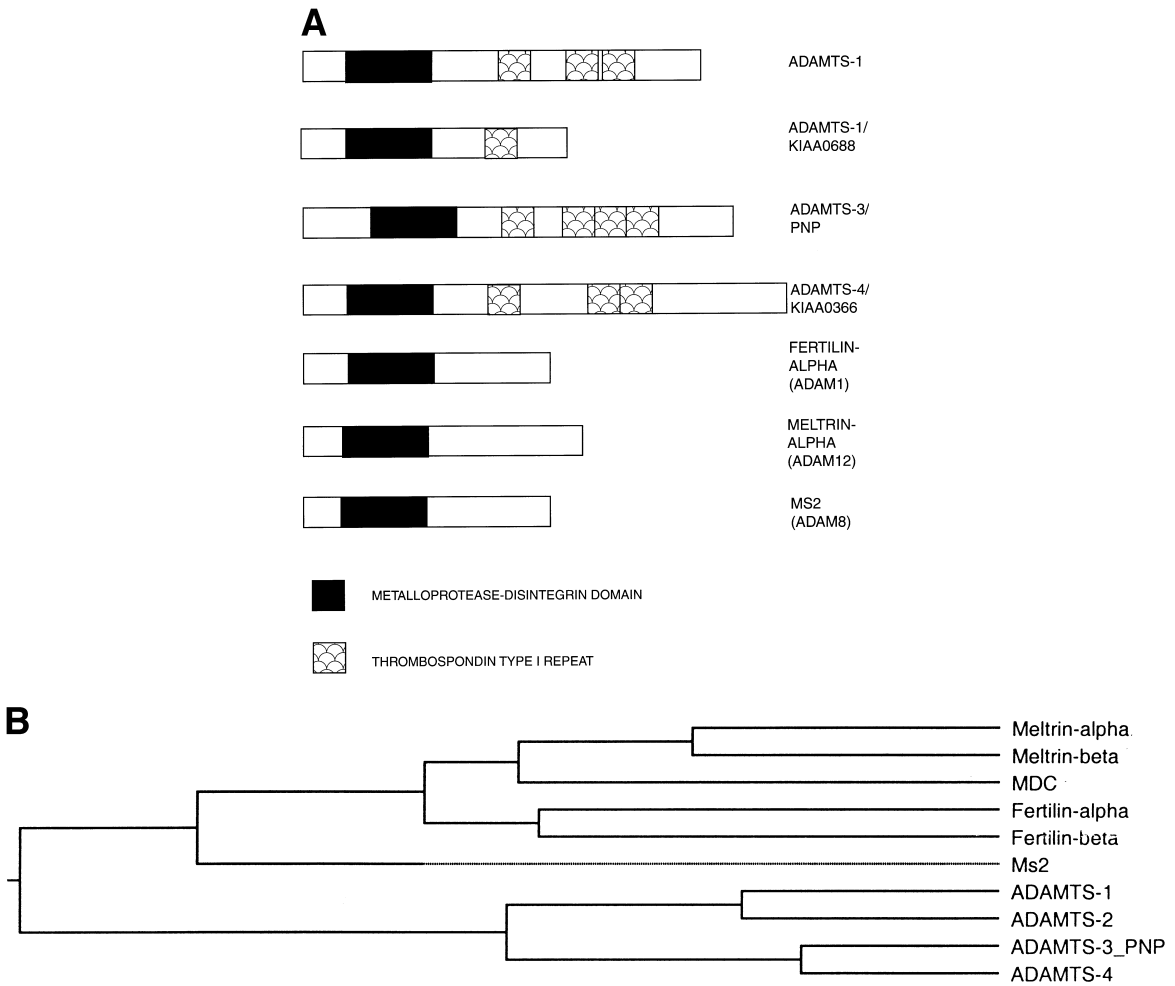

Fig. 1. A: Schematic representation of the domain structure of the four members of the ADAMTS family and three members of the ADAM family, fertilin-α (ADAM1, EMBL Y08616), meltrin-α (ADAM12, PIR S60257) and the monocyte surface antigen MS2 (ADAM8, PIR A60385). ADAMTS-1 (AB001735) is the product of a mouse gene (cloned from a cachexigenic tumor line) [12]. Two human cDNA clones from the Kazusa DNA research institute, KIAA0688 (AB014588) and KIAA0366 (AB002364), also belong to this family. KIAA0688 is designated ADAMTS-2 because of its relative high homology to ADAMTS-1. KIAA0366, on the other hand, is more homologous to the bovine gene for procollagen I *N*-proteinase [14]. We propose to designate procollagen I *N*-proteinase as ADAMTS-3 and KIAA0366 as ADAMTS-4, respectively. The translated amino acid sequences of all the respective cDNAs (obtained by searching the GeneBank database) were subjected to analysis by the pfscan program of the ISREC bioinformatics profilescan server (http://www.isrec.isb-sib.ch/software/PFSCAN_form.html). The green boxes represent the metalloprotease-disintegrin domain and the red boxes TSP1-like repeats. Note: KIAA0366 is probably not full length. For convenience, the numbering of amino acid residues begins at the first available methionine of the open reading frame. B: A phylogenetic tree of representatives of the ADAM family with that of ADAMTS-1–4. Alignment of representatives of the ADAM family (fertilin-α (EMBL Y08616), fertilin-β (X99794), meltrin-α (PIR S60257), meltrin-β (GeneBank AF019887), MS2 (PIR A60385)) with ADAMTS-1–4 and phylogenetic analysis was performed by MegAlign of DNAstar (clustal method).

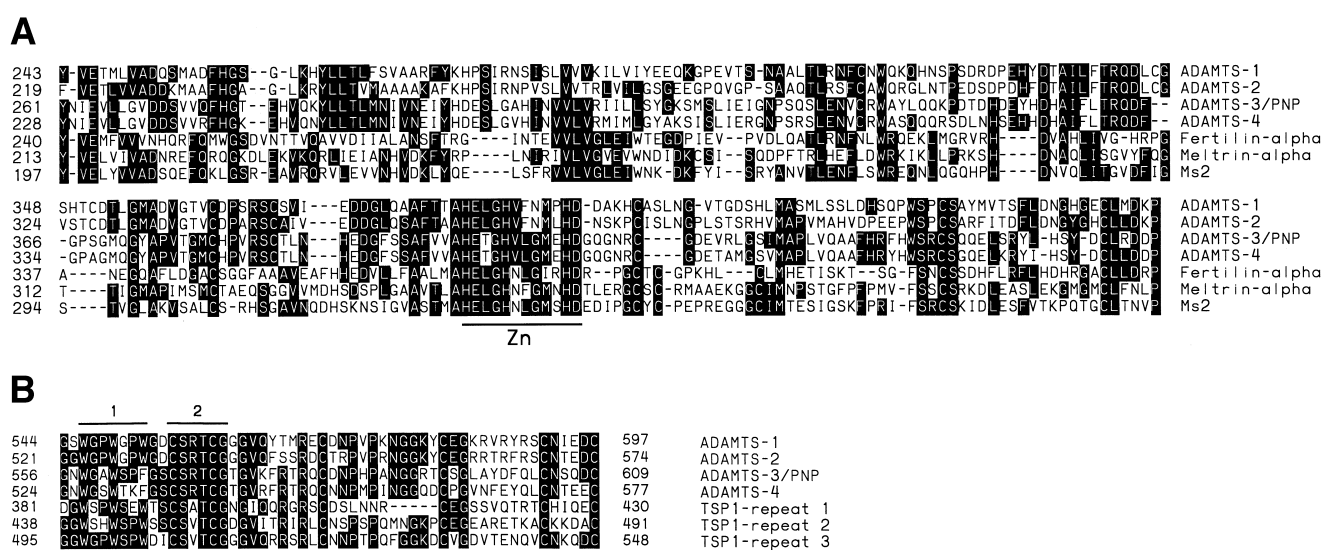

Fig. 2. A: Alignment of the metalloprotease motif of ADAMTS-1–4 with fertilin-α, meltrin-α and MS2. Alignment was performed by MegAlign of DNAstar (clustal method). A bar with the symbol Zn marks the Zn^{2+}-binding signature sequence. B: Alignment of the first TSP1 motifs of ADAMTS-1–4 with the three TSP1 repeats found in thrombospondin 1 [9] by the clustal method. The two conserved heparin-binding segments are marked by bars labeled 1 and 2.

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