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The characterisation of six ADAMTS proteases in the basal chordate *Ciona intestinalis* provides new insights into the vertebrate ADAMTS family

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Abstract

ADAMTS, constituting a recently discovered family of secreted zinc-dependent metalloproteases, have been shown to have critical physiological roles through identification of a number of natural animal and human gene mutations. The identification of six ADAMTS genes in the basal chordate *Ciona intestinalis* provides new insight into how, when and in what order the vertebrate orthologues have evolved. The phylogenetic assignments, based on sequences conserved across all genes, are supported by conserved domain structures within defined sub-families. The phylogeny and the frequent localisation of ADAMTS genes in paralogous regions of the genome are consistent with the vertebrate lineages having arisen by large scale or genome duplication. The high level of conservation in the protease active site of vertebrate orthologues within some sub-families suggests subfunctionalisation, whereas the greater divergence in others would favour the evolution of novel substrate specificities and these observations are borne-out where substrate-specificity is known. The expansion and sub-specialization of the ADAMTS family is a component of the increased complexity of extracellular matrix that is associated with the evolution of vertebrates.

Keywords: Metalloprotease; *Ciona intestinalis*; Chordate; Vertebrate; Molecular evolution; Substrate specificity

Abbreviations: ADAMTS, a disintegrin-like and metalloprotease domain [reprolysin-type] with thrombospondin type-1 motif; ADAMTSL, ADAMTS-like; CUB, complement subcomponent C1r/C1s/embryonic sea urchin protein Uegf/Bone morphogenic protein 1; IGCAM, Immunoglobulin cell adhesion molecule; PLAC, protease and lacunin domain; PNP, procollagen n-proteinase; TSR, thrombospondin type 1 repeat

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1. Introduction

The ADAMTS genes (a disintegrin-like and metalloprotease domain [reprolysin-type] with thrombospondin type-1 motif) are a family of secreted proteases that contain both a catalytic metalloprotease domain and a large ancillary region characterised by a disintegrin-like module, cysteine rich module and one or more thrombospondin type 1 repeats (TSRs) (Kuno et al., 1997). Through the identification of naturally occurring human and animal mutations, essential functions have been discovered for several ADAMTS proteases, such that they are assuming considerable biological and medical significance (Porter, Clark, Kevorkian, & Edwards, 2005). *ADAMTS2* mutations cause dermatosparaxis in a number of animals and the Ehlers–Danlos syndrome type VIIc (or dermatosparactic type) in humans (Colige et al., 1999). Mutations in *ADAMTS13* (the von Willebrand factor protease) result in inherited thrombotic thrombocytopenic purpura (Furlan et al., 1997), while acquired forms of this disease may result from circulating autoantibodies to ADAMTS13 (Tsai & Lian, 1998). A recessive form of the Weill–Marchesani syndrome is a consequence of *ADAMTS10* mutations (Dagoneau et al., 2004). A white-spotting mutant in mice, named *Belted* (*Bt*), is caused by *Adamts20* mutations (Rao et al., 2003), and defective gonadal morphogenesis in *Caenorhabditis elegans* mutants reflects an essential role for GON-1, the *C. elegans* orthologue of mammalian ADAMTS9 and ADAMTS20 (Somerville et al., 2003). Furthermore, proteolysis of large aggregating, hyaluronan binding proteoglycans termed the hyalectans (aggrecan, brevican and versican) (Iozzo & Murdoch, 1996) by a subset of ADAMTS proteases (ADAMTS1, 4, 5, 8, 9, 15, 20) is felt to have pathophysiological roles in arthritis, brain tumours and atherosclerosis respectively (Nagase & Kashiwagi, 2003). Therefore, understanding how the vertebrate ADAMTS family evolved and acquired its broad spectrum of protease activities is of significance to both medical and cell biology.

Unlike matrix metalloproteases, the ADAMTS ancillary domain is thought to be a major factor determining substrate specificity since the protease domain alone, including the active site (HEXXHXXG/N/SXXHD) (Cal et al., 2002), appears to be unable to process native substrates. Relevant to this observation is the existence of a separate group of ADAMTS-like proteins whose composition mimics the ancillary domains of ADAMTS proteases, but lacks an obvious metalloprotease domain and disintegrin-like module (Hall, Klentoic, Anand-Apte, & Apte, 2003; Hirohata et al., 2002; Kramerova et al., 2000; Nardi et al., 1999). Previous phylogenetic analyses have clustered the 19 vertebrate ADAMTS and 4 ADAMTS-like genes into a number of sub-families that are loosely defined due to the absence of supporting statistical tests (Apte, 2004; Bolz, Ramirez, von Brederlow, & Kubisch, 2001; Collins-Racie et al., 2004; Somerville et al., 2003). Nevertheless, genes clustering together encode proteins with similar domain structures, and significantly, some appear functionally related, e.g., the procollagen N-proteases (ADAMTS2, 3 and 14), the aggrecanases (ADAMTS1, 4, 5, 8, 9, 15) and proteases involved in cell migration (GON-1-like - ADAMTS9 and 20) (Apte, 2004; Porter et al., 2005). Here we report that the genome of the urochordate *Ciona intestinalis*, one of the closest invertebrate relatives of vertebrates (Dehal et al., 2002), contains seven genes belonging to this supergene family. The six Ciona genes root and clearly define seven strongly supported vertebrate sub-families of ADAMTS genes and, in combination with other available data such as domain structure, provide new insight into the order and timing of vertebrate ADAMTS family evolution and function. The increased complexity of the ADAMTS family of matrix proteases is symptomatic of the co-ordinated increased complexity in extracellular matrix and other protein systems accompanying and enabling the evolution of vertebrates.

2. Materials and methods

The complete gene sequences of the human ADAMTS genes were used to probe the genome and TIGR gene index of *C. intestinalis* (http://www.genome.jgi-psf.org/ciona4/ciona4.home and http://www.tirg.org/tdb/tgi/cingi using TBLASTN and PSI-BLAST with cut-off expectancy values of $E = 1$) to identify homologous genes (Karlin & Altchul, 1990). Reciprocal BLAST searches of the Ciona, human, *C. elegans*, *D. melanogaster* and non-redundant databases were used to identify all the ADAMTS genes. Completion of Ciona gene sequences were performed by manually searching the JGI scaffolds and signal pep-