The matrilins: a growing family of A-domain-containing proteins
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Structure of matrilins
The matrilins might be the protein family whose properties are the most dominated by A-domains. They contain one or two of these domains and, in addition, a varying number of EGF-like modules and a C-terminal set of heptad repeats, allowing the formation of a coiled-coil α-helix (Figure 1). In addition, matrilins-2 and -3 carry a positively charged stretch of amino acids at their N-terminal ends, possibly designed to allow interactions with negatively charged polymers. Matrilin-4 occurs in alternatively spliced forms; in the mouse, the N-terminal A1-domain may be spliced out while all four EGF repeats are retained, and in humans the number of EGF repeats may vary between one and three.

The prototype member, matrilin-1, was originally called cartilage matrix protein [1] or 148 kDa cartilage protein [2]. The purified protein was shown to be a trimer of subunits of 52 kDa each [3], and determination of the complete amino acid sequence of the protein from several species revealed a domain structure as shown in Figure 1 [4–6]. The functional importance of the C-terminal coiled-coil α-helical domain for trimer formation was demonstrated by electron microscopy [7] and mutational analysis [8], and confirmed by assembly studies using a synthetic peptide corresponding to the C-terminal 36 residues of human matrilin-1 [9]. NMR spectroscopy studies confirmed that the coiled-coil is stabilized by a ring of interchain disulphide bonds formed by two closely spaced cysteine residues close to the most N-terminal heptad repeat [10,11].

Systematic searches for other proteins of related structure led to the discovery and cloning of matrilin-2 [12], matrilin-3 [13,14] and matrilin-4 [15,16]. They all have a domain structure related to that of matrilin-1 (Figure 1) and assemble via their C-terminal coiled-coil regions in a similar manner, although matrilin-2 can form tetramers [17] and matrilin-3 can form heterotetramers together with matrilin-1 [18]. The presence of four known family members, together with the alternative splicing of matrilin-4, and the propensity of matrilin-1 to form both homotrimers and heterotetramers with matrilin-3, provides extensive structural variability within the matrilin family. The different matrilins have presumably evolved from a common ancestor gene through duplication of EGF modules and deletion or insertion of other modules [19].

Expression of matrilins
Matrilin-1 was first described as a protein specific for cartilage extracellular matrix [2] and it was therefore surprising that matrilin-2 is found in many other tissues but not cartilage, except for weak expression in the growth plate [12,17]. Matrilin-2 is expressed by connective tissue cells, but also by myocytes and epithelial cells, which secrete the protein into the basement membrane [17]. Information on the tissue distribution of matrilins-3 and -4 is at present more limited, but it appears that matrilin-3 has an expression pattern similar to that of matrilin-1 [13,14], while matrilin-4 is often found in the same tissues as matrilin-2 [15,16]. An example of the complementary expression of matrilins-1 and -2 is shown in Figure 2.

Gene regulation has only been studied for chicken matrilin-1. This gene contains a chondrocyte-specific enhancer in the first intron, and two negative and two positive control regions upstream of a promiscuous minimal promoter [20–22]. The concerted action of these elements allows full promoter activity in stage Ib proliferative chondrocytes [22]. This results in a more restricted expression of matrilin-1 than that of most other cartilage proteins, as reflected in its absence from articular cartilage [2] and its zonal expression in the growth plate [23]. We expect that similar studies of the genes for the other matrilins will reveal a related regulation for matrilin-3, but very different mechanisms for matrilins-2 and -4.

Functions of matrilins
Matrilin-1 was first isolated as a protein tightly associated with aggrecan molecules [1]. Later work has shown that the interaction is with the aggrecan core protein in the E2 domain, which is highly
substituted with chondroitin sulphate chains [24]. By use of specific antibodies to matrilin-1 in rotary shadowing electron microscopy of aggrecan-matrilin-1 complexes, several distinct binding sites could be mapped in this domain of the core protein. A portion of the matrilin-1 molecules are covalently bound to the core protein by a non-reducible bond, while some may be dissociated from the core by high concentrations of guanidinium chloride [24]. It could be that a tight non-covalent bond is first formed which is later stabilized by covalent cross-linking. The amount of matrilin-1 present in cartilage increases with the maturation and aging of the animal [25], and the number of molecules bound to each aggrecan increases in a similar fashion [24].

In addition, matrilin-1 has been detected in filamentous structures in the pericellular matrix of cultured chondrocytes [26, 27]. Culturing cells in the presence and absence of ascorbate shows that one set of matrilin-1-containing filaments depends on collagen secretion for their formation [27]; in addition, antibodies to matrilin-1 have been shown in immunoelectron microscopy to decorate collagen fibrils [26]. A second network of thinner filaments is not altered upon ascorbate deprivation, and may represent a pure matrilin-1 polymer or a co-polymer of matrilin-1 and another non-collagenous protein [27]. Recombinant matrilin-1 lacking the N-terminal A1-domain does not assemble into filaments, indicating a crucial role for this A-domain in fibril formation [27].

Little is known about the functions and
interactions of other matrilins. The matrilin-2 expressed in cultured smooth muscle cells is integrated into a pericellular filamentous network in a manner analogous to that of matrilin-1 in chondrocytes, but at present it is not known if the network surrounding the smooth muscle cells also contains collagens, or how matrilin-2 interacts with itself and/or other molecules to form the filaments [17]. Based on the fact that matrilin-1 may bind to both collagen fibrils and aggrecan molecules, it is tempting to speculate that it may mediate interactions between these two major structural components of cartilage. In analogy, it may well be that the other matrilins mediate similar interactions in the other tissues where they are expressed. At present we are testing this hypothesis by systematically expressing full-length and truncated recombinant forms of the various matrilins and studying their interactions both in defined in vitro systems and after transfection into matrix-forming cells.

We gratefully acknowledge the skilful technical assistance of Birgit Kobbe and Christian Frie, and the close collaboration with Dr. Ferenc Deák and Dr. Ibolya Kiss. Our work on matrilins is supported by grants from the Deutsche Forschungsgemeinschaft, the Volkswagen Foundation and the Köln Fortune program of the Medical Faculty, University of Cologne.


Received 21 June 1999

Integrin I domains and their function
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Background
The integrins are a family of cell adhesion receptors, which recognize multiple protein ligands and mediate cell–cell and cell–extracellular-matrix interactions [1]. The family members are hetero-dimeric cell surface glycoproteins composed of non-covalently associated α and β subunits. The ectodomains of integrins consist of several types of domains (Figure 1). While the N-termini of all α subunits contain seven homologous repeats of