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Elizabeth E.M. Bates · Wolf H. Fridman
Chris G.F. Mueller

The ADAMDEC1 (decysin) gene structure: evolution by duplication in a metalloprotease gene cluster on Chromosome 8p12

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Abstract Members of the ADAM superfamily of metalloprotease genes are involved in a number of biological processes, including fertilization, neurogenesis, muscle development, and the immune response. These proteins have been classified into several groups. The prototypic ADAM family is comprised of a pro-domain, a metalloprotease domain, a disintegrin domain, a cysteine-rich region, a transmembrane domain, and a variable cytoplasmic tail. We recently identified a novel member of this superfamily, ADAMDEC1 (decysin). Due to the partial lack of a disintegrin domain and the total lack of a cysteine-rich domain, this protein has been placed in a novel subclass of the ADAM gene family. We have investigated the gene structure of the human and mouse *ADAMDEC1* and have revealed a metalloprotease gene cluster on human Chromosome 8p12 comprising *ADAMDEC1*, *ADAM7*, and *ADAM28*. Our results suggest that *ADAMDEC1* has arisen by partial gene duplication from an ancestral gene at this locus and has acquired a novel function. *ADAMDEC1* is expressed in the immune system, by dendritic cells and macrophages. The relatedness of ADAMDEC1, ADAM7, and ADAM28 suggests that these proteases share a similar function.

Keywords Metalloprotease · ADAM · Promoter · Gene structure · Evolution

E.E.M. Bates
Schering-Plough, Laboratory for Immunological Research,
27 chemin des peupliers, BP11, 69571 Dardilly cedex, France

W.H. Fridman · C.G.F. Mueller (✉)
Laboratoire d'Immunologie Clinique et Cellulaire. INSERM U255.
Centre des Recherches Biomedicales des Cordeliers,
15, Rue de l'Ecole de Medécine, 75006 Paris, France
e-mail: chmuller@infobiogen.fr
Tel.: +33-1-53100405, Fax: +33-1-40510420

Introduction

The ADAM (a disintegrin and metalloprotease) family of zinc-binding proteins is a rapidly growing gene family composed of proteins that can function as adhesion proteins and/or endopeptidases. They are involved in a number of biological processes, including fertilization, neurogenesis, muscle development, and the immune response (Primakoff and Myles 2000; Wolfsberg et al. 1995; Yamamoto et al. 1999). (For recent updates see the following websites: http://www.uta.fi/%7Eloiika/ADAMs/HADAMs.htm; http://www.people.Virginia.EDU/%7Ejag6n/Table_of_the_ADAMs.html).

ADAMs are evolutionarily conserved from *Caenorhabditis elegans* to humans, and a typical ADAM comprises conserved structural elements: an N-terminal signal sequence followed by a pro-domain, a metalloprotease domain, a disintegrin domain, a cysteine-rich region, a transmembrane domain, and a variable cytoplasmic tail. The pro-domain shields the catalytic zinc-binding site and keeps the protease inactive (Grams et al. 1993; Loechel et al. 1999). The disintegrin domain and probably the cysteine-rich region (Jia et al. 2000) act as protein-binding domains, and the cytosolic tail has been shown to interact with SH3 domains involved in intracellular signaling (Kang et al. 2000; Nelson et al. 1999). As an example, ADAM17 (TACE, TNFα-converting enzyme) comprises all these structural features and has been shown to be catalytically active, cleaving pro-tumor necrosis factor-α (TNFα). This activity is the principal mechanism of release of TNFα from the cell membrane (Black et al. 1997; Moss et al. 1997). However, within the ADAM family there has been room for considerable variation. A great number of ADAMs, including ADAM2 and ADAM7, have a mutated catalytic consensus sequence (HEXG->HQXG) that would still permit zinc binding but renders the protein catalytically inactive. Their function is restricted to protein binding required, for instance, for cell adhesion in sperm-egg fusion (Primakoff et al. 1987; Wolfsberg and White 1996). Furthermore, ADAM11, ADAM12, and ADAM28 exist in

differently spliced isoforms that lack the transmembrane domain and would be secreted (Gilpin et al. 1998; Katagiri et al. 1995; Roberts et al. 1999).

Snake venom disintegrin metalloproteases (SVMP) show significant amino acid homology to the mammalian ADAMs and contain a pro-domain and a metalloprotease domain. The high molecular mass SVMPs contain, in addition, the disintegrin and cysteine-rich domains, but the low molecular mass SVMPs lack these domains (Matsui et al. 2000). Low and high molecular weight SVMPs commonly show potent fibrin(ogen)olytic and extracellular matrix-degrading (hemorrhagic) activity, although the high molecular weight SVMPs are more potent due to their ability to block integrin binding.

Another related family is the ADAMTS protein family (Tang 2001). Its members contain numerous repeats of thrombospondin-1-like motifs, but do not have a transmembrane or a cytoplasmic domain. They are secreted and function in inflammation, angiogensis, and development. Recently, mutations in ADMAMTS13 have been found responsible for thrombotic thrombocytopenic purpura, characterized by abnormal platelet aggregation and blood clots (Levy et al. 2001).

In man and mouse, ADAM genes are localized to various chromosomes, and pseudogenes have been identified in humans for *ADAM1*, *ADAM4*, *ADAM6*, and *ADAM21*. It is likely that the ADAM-related gene families have arisen from an ancestral gene, created by exon shuffling, gene duplication, and genetic drift (Mourada-Silva et al. 1996). *ADAM1*, for which two genes have been detected on the same locus, may represent an example of an ADAM gene family member that has undergone recent duplication (Cho et al. 1997).

Human ADAMDEC1 mRNA has been cloned from dendritic cells purified ex vivo from human tonsils (Mueller et al. 1997) and its mouse orthologue has been cloned from mouse spleen using a sequence derived from an expressed sequencing tag (EST) (Mueller et al. 2001). In tissues, ADAMDEC1 is expressed in intestine, colon, and appendix, as well as in lymph nodes and spleen. In the mouse spleen, ADAMDEC1 is expressed in response to immunization, by dendritic cells, marginal metallophils, follicular dendritic cells, and tingible body macrophages (Mueller et al. 2001). By northern blot analysis, a single mRNA was detected, indicating that ADAMDEC1 does not produce alternative spliced isoforms (Mueller et al. 1997). ADAMDEC1 is distinct from the many other mammalian disintegrin metalloproteases by two features:

1. It possesses only about half of the disintegrin domain and lacks the cysteine-rich domain, the transmembrane domain, and the intracellular tail of other ADAM family members.
2. A conserved histidine residue in the zinc-binding consensus sequence is replaced by aspartate.

Apart from these particularities, ADAMDEC1 shares important homology with members of the ADAM gene family. ADAMDEC1 has therefore been assigned as the first representative of a new subclass of the ADAM gene family.

In an effort to investigate how ADAMDEC1 has acquired its atypical features, we characterized the genomic sequence of human and mouse *ADAMDEC1*. We studied the promoter sequence of human *ADAMDEC1* and predicted transcription factor binding sites. Human *ADAMDEC1* maps to Chromosome (Chr) 8 and, strikingly, *ADAM7* lies on the same genomic clone and *ADAM28* maps to the same locus as *ADAMDEC1*. These three genes show important homology at the amino acid and nucleotide level. However, *ADAMDEC1* lacks C-terminal exons that are conserved in *ADAM28* and *ADAM7*, and the final conserved exon (exon 13) shows only partial homology. We propose that recent gene duplication has given rise to an ADAM gene cluster comprising *ADAMDEC1*, *ADAM28*, and *ADAM7*. *ADAMDEC1* has been partially duplicated up to exon 13 and exon 14 added later. Furthermore, we present data suggesting that exon 13 of *ADAMDEC1* has been mutated, resulting in a frameshift within this exon, and subsequently an alternative stop codon has been acquired. *ADAMDEC1* may have evolved a new function that does not require a complete disintegrin domain.

Materials and methods

Sequence identification and analysis

ADAMDEC1 human and mouse cDNA sequences were compared by BLAST (Altschul et al. 1997) with the GenBank nucleotide sequences. ESTs with homology to the ADAMDEC1 sequences were identified. Sequences corresponding to genomic clones were noted. Intron/exon structure of the *ADAMDEC1*, *ADAM28*, and *ADAM7* genes was manually analyzed using Sequencher (Genecodes, Calif.) to ensure that the correct acceptor and donor boundaries were assigned. Genomic clones were also analyzed using Genscan (Burge et al. 1997) and BLAST to detect the presence of nearby coding regions. Only those coding regions that contained sequences similar to metalloprotease genes were examined. Alignments of small zones of nucleotide sequence and amino acid sequence analysis were performed with Lasergene software (DNASTAR, London, UK).

Promoter region analysis

The region directly upstream of the human *ADAMDEC1* gene was analyzed for potential transcription factor binding sites using MatInspector (Quandt et al. 1995), which compares a DNA sequence with the TRANSFAC database (Wingender et al. 2000). Significant matches were mapped onto a 1-kb region of DNA directly upstream of the transcriptional start site.

Southern blotting of the *ADAMDEC1* genes

Genomic blots purchased from Clontech were hybridized for 16 h with ^{32}P-labeled cDNA probe in a solution consisting of 5× SSC, 1× Denhardt's, 100 µg/ml salmon sperm DNA, and 0.1% sodium dodecyl sulfate at 65°C. Membranes were washed four times in 2× SSC for 30 min at 65°C and exposed. The probe was generated by the random prime method and corresponds to an internal fragment of the human *ADAMDEC1* cDNA generated by PCR using primes U137 (5′-GTGGTGTGAAGAGCACTGACGGG) and L677 (5′-ACAACTGCCAGAGGGACACTTGG).

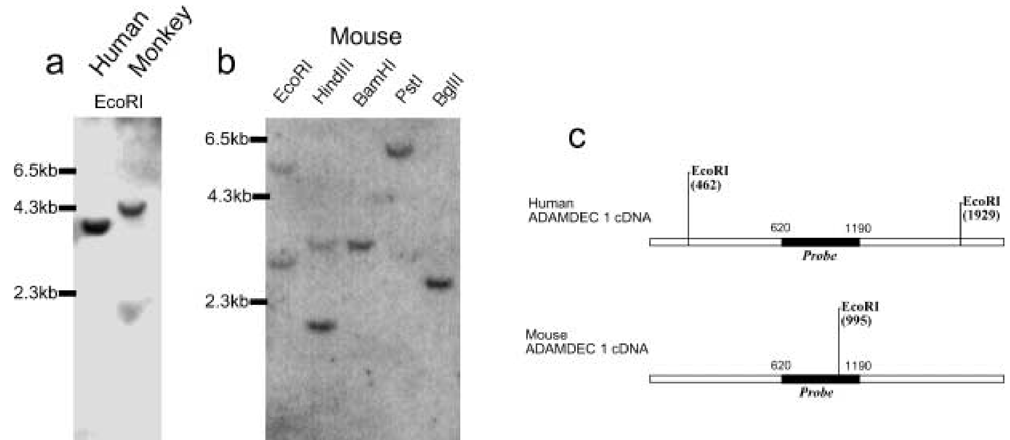

Fig. 1 Genomic Southern blot of *ADAMDEC1*. Nylon blots containing human, Rhesus monkey (**a**) and mouse (BALB/c) (**b**) genomic DNA were hybridized with a probe derived from human ADAMDEC1 cDNA. The position of the probe relative to the human and mouse ADAMDEC1 cDNAs is indicated in the schematic drawing (**c**). The region covered by the probe in mouse *Adamdec1* contains a *Eco*RI site. The *Hin*dIII site is present in the intron sequence

Fig. 2 **a** Amino acid alignment of the full-length human (*h*) and mouse (*m*) ADAMDEC1 sequences as well as partial porcine (*p*) and bovine (*b*) translated expressed sequence tag (EST) sequences. *Shaded* amino acids show sequence identity. *Boxed sequences* represent the conserved cysteine switch of the prodomain, the dibasic furin cleavage signal, and the zinc-binding motif. The *circle* indicates the altered aspartate residue. An *arrow* shows the start of the disintegrin domain. **b** Alignment of the consensus zinc-binding motifs of the different zinc-binding protease family members of the MEROPS classification (Rawlings and Barrett 2000) with the ADAMDEC1 sequences. Zinc-binding residues are *shaded*

Results

ADAMDEC1 is a single copy gene on Chr 8p12

A single gene for *ADAMDEC1* has been identified by the human genome project and mapped to Chr 8p12. A Southern genomic analysis of *ADAMDEC1* on human and monkey genomic DNA (Fig. 1a) and mouse genomic DNA (Fig. 1b), cut with multiple restriction enzymes, confirmed that *ADAMDEC1* is encoded by a single gene in these species.

ADAMDEC1 displays unique features

An amino acid alignment of human and mouse ADAMDEC1, including the deduced amino acid sequences of ESTs from porcine small intestine and bovine placenta, is shown in Fig. 2a. Unlike all other members of the ADAM metalloprotease family, ADAMDEC1 comprises two so-far unique features: (1) the third histidine residue in the zinc-binding site is replaced by an aspartate (circled amino acid), (2) the disintegrin domain is truncated due to a premature stop codon. ADAMDEC1 is otherwise highly homologous to the ADAM gene family, designated M12B in the MEROPS database (Rawlings and Barrett 2000). Comparison of the zinc-binding sequences of ADAMDEC1 and other metalloproteases of the MEROPS database showed that members of the M6 and M7 gene family also contain an aspartate instead of a histidine residue in their respective zinc-binding sites (Fig. 2b). The crystal structure of the metalloprotease ScNP of *Streptomyces caespitosus* (family M7) has shown that aspartate can functionally replace the third histidine residue as the fourth zinc-chelating residue (Kurisu et al. 2000). Members of the M6 and M7 gene family are active endopeptidases, hydrolyzing milk protein (Lampel et al. 1992), collagen (Yu et al. 2000), and insect antibacterial proteins (Dalhammar and Steiner 1984). These reports suggest that ADAMDEC1 should also be capable of binding zinc and be an active endopeptidase.

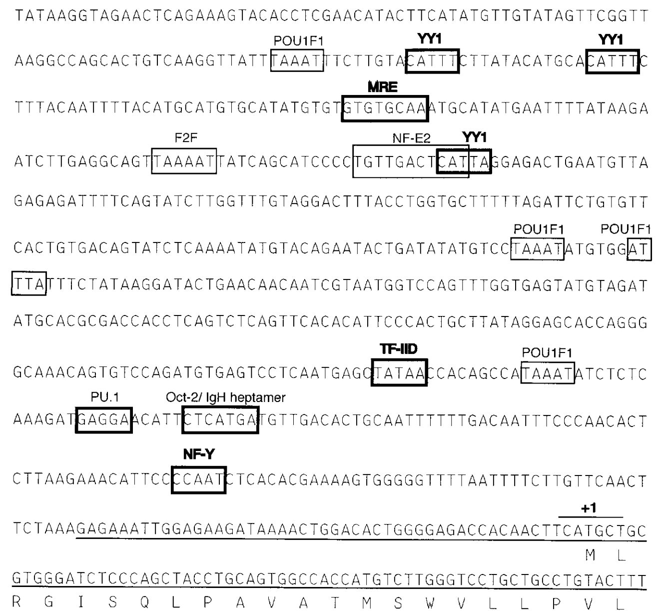

Fig. 3 Structure of the human *ADAMDEC1* promoter. The genomic DNA sequence around the human *ADAMDEC1* promoter is shown. Exon 1 is *underlined* and the predicted protein sequence is indicated. The consensus Kozak sequence of the first codon is *overlined* and marked +1. Consensus transcription factor binding sites are *boxed*; those of particular interest are boxed in *heavy print*. *POU1F1* Pou domain, class 1, transcription factor 1; *YY1* Yin Yang 1 transcription factor; *MRE* metal response element; *F2F* footprint II factor; *PU.1* ETS domain transcription factor, PU.1; *Oct-2* IgH heptamer; *NF-Y* CCAAT-box binding factor nuclear factor-Y

Characterization of the *ADAMDEC1* promoter sequence

The upstream sequence of *ADAMDEC1* was analyzed for transcription factor binding sites using the program MatInspector and the TRANSFAC database (Heinemeyer et al. 1998; Quandt et al. 1995) (Fig. 3). The promoter region is A plus T rich and contains multiple sequences similar to GATA family core binding sites. Two clusters of core binding sites were identified. The proximal cluster contains a NF-Y site, preceded by closely linked sites for PU.1 and the Oct-2/IgH heptamer, and a TATA box. Macrophage-specific genes are known to use the NF-Y transcription factor to initiate mRNA synthesis (Marziali et al. 1999), and this seems likely in this case. It is interesting to note that downstream of the Oct-2 site is the sequence CTGCAATT, which resembles the Oct-2 octamer site ATGCAAT (LeBowitz et al. 1989), although these sites may not have the correct spacing to be functional (Poellinger and Roeder 1989). Upstream lies a PU.1 consensus site. PU.1 is expressed by macrophages and B cells (Klemsz et al. 1990) and is required for macrophage and B-cell development (DeKoter and Singh 2000). PU.1 is known to be important for myeloid cell differentiation (Valledor et al. 1998). The binding of the PU.1 transcription factor to the *ADAMDEC1* promoter sequence may thus be responsible for the expression of *ADAMDEC1* in activated dendritic cells and macrophages. The second, more-distal cluster of binding sites involves YY1 elements (Ye et al. 1994) and a metal-responsive element (MRE) typical of metalloprotease promoters (Murata et al. 1999). YY1 is a transcription factor known to initiate or inhibit transcription, dependant on the promoter. This combination of transcription factors could thus conceivably drive tissue- and cell-specific gene expression from this promoter. Other core transcription factor binding sites are seen, such as POU1FI, F2F (Minami et al. 1998) and NF-E2 (Chan et al. 1993), and the significance of these sites in positive and negative regulation of this gene is not known.

Genomic structure of the *ADAMDEC1* genes

We identified a number of genomic clones by a BLAST search (Altschul et al. 1997) that contained entire or partial sequences for the human and mouse *ADAMDEC1* genes. Two of the clones were selected (accession numbers AC091249 for the mouse *Adamdec1* gene and AC024958 for the human *ADAMDEC1* gene) as having the most-complete sequence data at this locus. These clones were analyzed and the intron/exon structures of the complete genes were obtained (Fig. 4). The mouse gene is 18,646 bp long and contains 14 exons. The human gene is shorter, although the size of the first intron cannot be determined as the sequence is unfinished in this zone, and contains 14 exons in a very similar structure (Fig. 4). There are, however, size differences be-

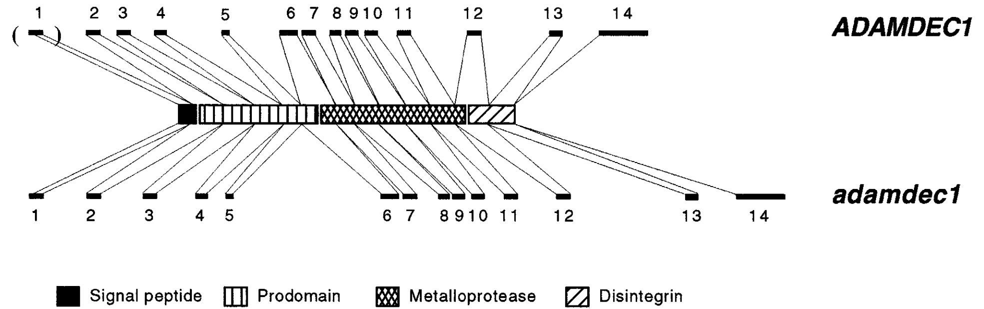

Fig. 4 Comparative intron/exon structure of the human and mouse *ADAMDEC1* genes. The gene structure is shown to scale. The positions of the exons on the protein sequence indicate the split domains. Exon 1 of human *ADAMDEC1* is in brackets as its position could not be precisely determined

Table 1 Intron/exon boundaries of the human and mouse *ADAMDEC1* genes. Comparison of the gene structures shows an identical arrangement of exons and exon boundaries. The mouse gene is slightly larger due to longer introns. The intron splice sites all follow the GT-AG type U2 rule (*ND* not determined)

Exon	Exon length	5′ Junction		3′ Junction		Junction type	Intron	Intron length
		Intron sequence	Exon sequence	Exon sequence	Intron sequence			
ADAMDEC1								
1	135		GAGAAA	CTCAAG	gtacgtaccatc	I	1	ND
2	119	tatttctctttctctag	CAATAG	AAAGAG	gtaagcaaggtg	0	2	880
3	77	taattaaatgttttcag	GAAAGG	AACCAA	gtaagttgtacc	II	3	730
4	79	ctctctgctctctacag	GCACCT	AACATG	gtagggtccgaa	0	4	1572
5	77	tcttttttccactccag	GAACAC	GTTGAG	gtaagaactacc	II	5	1473
6	187	gtctctatatctcccag	AGGATA	CCAGAG	gtgaatacaatt	II	6	226
7	63	ttttattctttgtaaag	AAAGAA	GCCTTT	gtgagtatgaaa	0	7	734
8	72	tgttttgtattttacag	TATAAG	AATGTG	gtaagacattag	0	8	322
9	167	aaatgctctttccacag	ATATAT	TCTCAG	gtgagcacatgc	II	9	352
10	82	tgtaacttttcatacag	CGGGAT	ATTGAG	gtttgtaaattt	0	10	695
11	131	atatctactttttccag	GCTAAA	TCTGAG	gtgagaccttgt	II	11	1614
12	178	ttttcttctgattacag	TTCAAA	CCTAAG	gtattatttatt	0	12	1910
13	86	gtgtgtgtgctttgaag	GAGTGT	TACCAC	gtaagacctttt	II	13	1203
14	721	atttttccttcaatcag	AGAGTG	TGAGGC				
adamdec1								
1	114		CTGGCC	TCCAAG	gtaagaataggc	I	1	1303
2	119	tttctgactttctctag	TGATAG	AAAGAG	gtaagttatgtt	0	2	1306
3	77	tcattaaatattttcag	GAAAAA	AACCAA	gtaaggacaatt	II	3	1224
4	79	ctttattctctgcacag	GCATCT	GATGTG	gtaagctctgaa	0	4	687
5	77	tgtattttctactctag	AAACCC	GTTGAG	gtgagaaatgaa	II	5	3821
6	187	aaactcatatcccacag	AGGGTA	CCAAAT	gcaagtacagct	II	6	193
7	60	tgttatttggaaaacag	GAAGAC	GCCTAT	gtgagtatgaaa	0	7	1001
8	72	tattttgtaattttcag	TATAAG	AATATG	gtaagacatttg	0	8	254
9	167	aaatgttctttccacag	ATTTAT	TCTCAG	gtgaatcccttc	II	9	358
10	82	ctctacctttcacacag	TGGAGC	ATTGAG	gtttgtatactt	0	10	690
11	131	tgttttttttttccag	GCTAAA	CCTGAG	gtaagatggtat	II	11	1185
12	175	tactttttctgttacag	TTCAAA	CCTGAG	gtatgaaaatac	0	12	3110
13	83	ttgtttgttctttgaag	GAATGT	CATCAC	gtgagcaccatc	II	13	1142
14	989	gtgttttgatttcctag	AGAATG	CTATCA				
	Consensus	ccttttcttttttttag	NNNNNN	NNNCAG	gtaagtgccggg			

tween human and mouse in the completed introns (Table 1). The gene structure is typical of ADAMs, with a split signal peptide and all introns obeying the GT-AG splice rule typical of U2-type introns (Burge et al. 1998). However, unlike the more-typical members of the ADAM gene family *ADAM2* and *ADAM8* that comprise over 19 exons (Cho et al. 1997) and 28 exons (Kataoka et al. 1997) respectively, *ADAMDEC1* contains only 14 exons, of which the last contains a single translated codon followed by the stop codon. This suggests that *ADAMDEC1* is truncated at the disintegrin domain by the loss of exons.

ADAMDEC1, ADAM28, and *ADAM7* form a novel gene cluster

We searched for other ADAMs mapped to Chr 8p12 in the literature and using the following databases: UNIGENE (Ermolaeva et al. 1998), OMIM (Hamosh et al. 2000) (http://www.ncbi.nlm.nih.gov/omim/). We found that human *ADAM28* maps to the same locus. Homology analysis showed that ADAM28 and ADAMDEC1 share 47% identity at the amino acid level. The study of a partial *ADAM28* genomic sequence shows that this gene also shares with ADAMDEC1 an almost

Fig. 5 Comparison of the ADAM28, ADAM7, and ADAMDEC1 amino acid sequences showing the intron/exon boundaries by a *vertical line* and indicating the splice acceptor type (O, I, II). Identical residues are *shaded*. In almost all cases, the boundaries are conserved in type and position (small displacements of the sequence are due to the difficulty in aligning poorly conserved regions). The 10 residues in ADAMDEC1 that show no homology with ADAM7 and ADAM28 are *boxed*. Exons following exon 13 for ADAM7 and ADAM28 are not shown

identical intron/exon organization (Fig. 5). Among other ADAM gene family members, ADAM7 displays strong homology with ADAMDEC1 (36%) and ADAM28 (42%). We therefore wondered if *ADAM7* also mapped to this locus. We used the GENSCAN algorithm (Burge and Karlin 1997) to predict genes close to the human and mouse *ADAMDEC1* genes. Of the predicted coding sequences from the human BAC clones, one corresponded clearly to exons derived from human *ADAM7*. Certain exons of the mouse *Adam7* gene could also be mapped to the mouse genomic clone containing *Adamdec1*. An intron/exon structure could be predicted for human *ADAM7* and showed 22 exons, of which the first 13 correspond very closely to the first 13 exons of *ADAMDEC1* and *ADAM28* with respect to their size, relative position on the protein sequence, and splice junction type (Fig. 5).

The amino acid sequence comparison of ADAMDEC1, ADAM28, and ADAM7 also showed that the last nine residues of exon 13 of ADAMDEC1 show no identity with ADAM28 or ADAM7 (boxed residues). In all other members of the ADAM gene family this region is highly conserved. We wondered how these C-terminal residues could have been altered, and analyzed the nucleotide sequences of exon 13 in *ADAM7, ADAM28*, and *ADAMDEC1* (Fig. 6a). The nucleotide sequences of exon 12 and 13 are highly conserved, except, as expected, the nucleotides encoding the last nine residues of exon 13. However, when gaps are introduced in the *ADAMDEC1* genes just upstream of this region, the homology score is

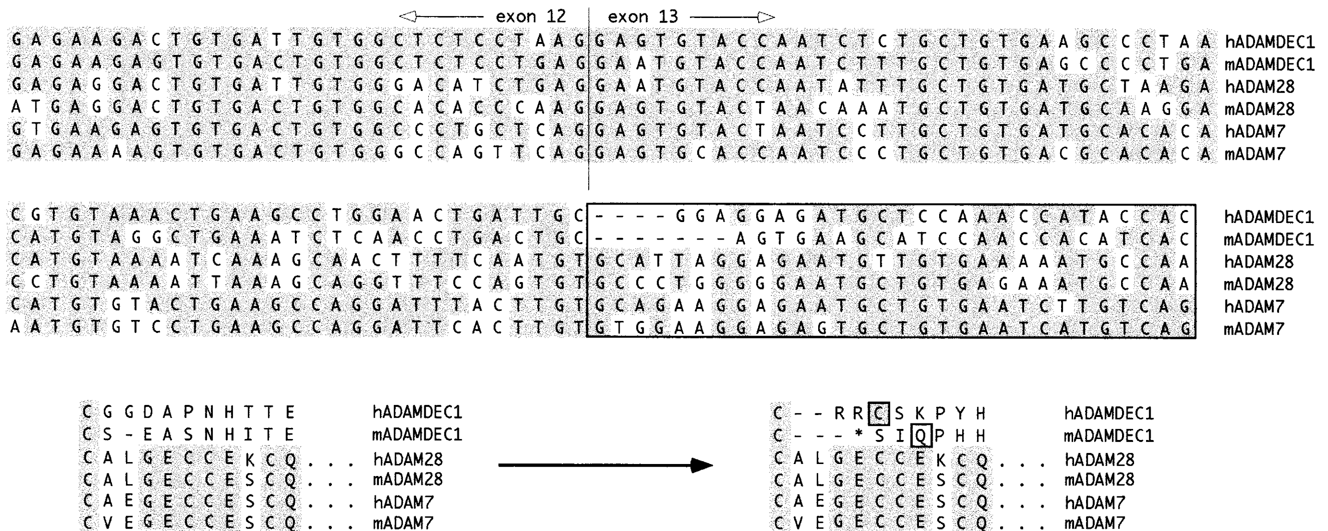

Fig. 6 **a** Nucleotide sequence of exon 13 and part of exon 12 of human and mouse ADAMDEC1, ADAM28, and ADAM7. A deletion of 4 and 7 nucleotides, respectively, in the human and mouse ADAMDEC1 exon 13 is introduced, leading to a greatly improved homology score of the downstream nucleotide sequence. **b** Amino acid comparison of the translated nucleotides *boxed* in **a** before (*left*) and after (*right*) introduction of the gaps. The cysteine residue in human ADAMDEC1 and the glutamine residue in mouse ADAMDEC1 are *highlighted*. The stop codon is indicated by an *asterisk*

greatly improved. [From 32% (human) and 36% (mouse) nucleotide identity to 64% (human) and 50% (mouse) identity after introduction of gaps.] Translation of the nucleotide sequence containing gaps shows that a highly conserved cysteine residue could be recovered in human ADAMDEC1 and a glutamate residue is positioned in place of a glutamine in murine ADAMDEC1. This suggests that, after gene duplication, exon 13 of *ADAMDEC1* has suffered deletion(s) in its C-terminal region, which introduce a frameshift in the remaining coding sequence.

Discussion

ADAMDEC1 (previously called decysin) is expressed by activated human and mouse dendritic cells (Mueller et al. 1997). In addition, in response to immunization in the mouse, it has been found to be expressed by marginal macrophils, follicular dendritic cells, and tingible body macrophages (Mueller et al. 2001) ADAMDEC1 mRNA has also been detected in human follicular dendritic cells (C. Mueller, unpublished observation). ADAMDEC1 is therefore likely to play an important role in the control of the immune response, like ADAM28 (Roberts et al. 1999), ADAM8 (CD156) (Yoshida et al. 1990), ADAM17 (Black et al. 1997; Moss et al. 1997), and MADAM (Fritsche et al. 2000), which are also expressed in the immune system.

ADAMDEC1 presents two features that are unique among the ADAM gene family: (1) its reading frame prematurely terminates in the disintegrin domain, thus deleting about half of the disintegrin domain, the cysteine-rich domain, the transmembrane domain, and the intracellular tail, typically found in other ADAM family members; (2) within its zinc-binding site, the third histidine residue is replaced by an aspartate.

Studies employing antibodies specific for the disintegrin domain and peptides that mimic the disintegrin and the cysteine-rich domain have shown that these domains interact with other membrane proteins (presumably integrins). For instance ADAM1, ADAM2, and cryostatin, expressed by spermatocytes, bind cell surface proteins present on oocytes (Blobel et al. 1990). Furthermore, snake disintegrin peptides bind to integrins (Matsui et al. 2000). Because ADAMDEC1 is soluble and lacks a complete disintegrin domain and the cysteine-rich domain, it is unlikely to function as a cell adhesion factor. The disintegrin domain appears, however, not to be required for protease function as low-molecular-weight SVMP, such as Ht-d, degrade basement membrane proteins or the cartilage aggrecan core protein (Matsui et al. 2000; Tortorella et al. 1998). It is likely that, in the absence of a complete disintegrin domain and a cysteine-rich domain, ADAMDEC1 is still capable of functioning as a protease.

The replacement of the histidine residue by an aspartate as the forth ligand for zinc should not negatively affect its capacity to chelate the zinc ion. Members of the M6 and M7 metalloprotease gene family contain an aspartate in place of histidine, and the crystal structure resolved for the M7 protease from *Streptomyces caespitosus* has shown that the aspartate acts as the forth zinc chelator amino acid (Kurisu et al. 2000). Furthermore, many of the M6 and M7 metalloproteases have proteolytic activity (Dalhammar and Steiner 1994; Lampel et al. 1992; Yu et al. 2000). It is not clear why the histidine of the zinc-binding site is replaced by an aspartate. It is likely that there are very few substitutions at this position that can be tolerated without loss of function. This, as well as the low level of overall homology to the M6

and M7 protein family, should be considered as evidence for convergent evolution rather than acquisition of a gene from another species. In the light of these features that distinguish *ADAMDEC1* from other members of the ADAM family, *ADAMDEC1* has been assigned as the first member of a novel ADAM gene family subclass.

We have identified and studied the genomic sequences for human and mouse *ADAMDEC1*. The human promoter sequence was characterized and transcription factor binding sites were predicted. In addition to a metal response element, typical of metalloproteases, and sites suggestive of hematopoieitic transcription, binding sites for the transcription factors PU.1 and Oct-2 were predicted. These factors are specifically involved in promoting gene transcription in macrophages and B cells, and may be responsible for the observed gene transcription of *ADAMDEC1* in activated marginal macrophils, tingible body macrophages, and dendritic cells. We have not been able to detect *ADAMDEC1* gene transcription in normal mouse or human B cells, however, we have observed *ADAMDEC1* transcription in the human B-cell myeloma ARH77 and U266 (C. Mueller, unpublished observations). It is unclear which transcription factor may be involved in *ADAMDEC1* transcription in follicular dendritic cells, which are thought to be of a mesenchymal cell lineage. The study of *ADAMDEC1* expression in PU.1-deficient mice may help to address this question.

Using gene prediction software, we found that human and mouse *ADAMDEC1*, *ADAM7,* and probably *ADAM28,* are located on the same chromosomal locus. These genes share important homology on a nucleotide and amino acid basis and display an almost-identical intron/exon organization. The intron/exon organization also closely resembles that reported for *ADAM2* (Cho et al. 1997) and *ADAM8* (Kataoka et al. 1997). However, in contrast to *ADAM2, 7, 8* and *28*, which comprise 20, 22, 28, and 22 exons respectively, the *ADAMDEC1* genes have only 14 exons, with the last exon encoding a single amino acid. Because alternatively spliced forms of ADAMDEC1 have not been observed (Mueller et al. 1997), it is likely that the *ADAMDEC1* gene has arisen from a partial gene duplication of an ancestral gene that has also given rise to *ADAM7* and *ADAM28*. The final exon 14 may then have been acquired from its novel chromosomal environment. The simplest hypothesis to explain the continued existence of this gene is that the newly duplicated partial sequence was immediately suited to a new function and may have in fact taken over certain functions of the multifunctional ancestor gene. It is also possible that the partial duplication of *ADAMDEC1* altered its original function and a completely novel function was acquired.

The last nine residues of exon 13 display no homology with *ADAM7, ADAM28*, or any other members of the ADAM gene family. We investigated the cause of these mutations and found that by introducing a gap in exon 13, upstream of these nine residues, the homology score at the nucleotide level can be significantly improved. When these frame-shifted nucleotides are translated, a highly conserved cysteine residue could be recovered in human ADAMDEC1 and a glutamate residue is positioned in place of a glutamine in murine ADAMDEC1. This suggests that exon 13 has suffered nucleotide deletions, giving rise to a frame shift. The fact that the bovine ADAMDEC1 EST has deleted four of the nine residues supports our hypothesis. However, other point mutations have also been acquired in this region, indicated by the fact that, after introducing the gaps, not all conserved amino acids could be recovered and the mouse open reading frame contained a stop codon. Because ADAMDEC1 probably does not require an intact disintegrin domain for its function, mutations in this domain could be tolerated.

In conclusion, we defined a novel ADAM gene cluster on the short arm of human Chr 8 comprising *ADAMDEC1*, *ADAM7*, and probably *ADAM28*. Analysis of the genomic structure of *ADAMDEC1* and homology comparison of the three genes has allowed us to propose a model to explain truncation and mutation of the disintegrin domain of ADAMDEC1. The role of ADAMDEC1 is as yet not clear. It may function in a similar manner to ADAM17, as a protease-cleaving TNF/TNFR family member. It is worth noting that there are many known TNF/TNFR family members expressed by dendritic cells or in germinal centers, and ADAMDEC1 is a good candidate for cleavage of these molecules. In the light of the findings that ADAMDEC1, ADAM28, and ADAM7 share important amino acid identity and may have evolved from a common ancestral gene, it is plausible that their substrates may also be related and belong to a same gene family. In contrast to ADAM7 and ADAM28, ADAMDEC1 is exclusively secreted (Mueller et al. 2001), which would allow ADAMDEC1 to proteolyse substrates that are also soluble and/or produced at a distant site. Further studies will help to verify this idea and elucidate the functions of ADAMDEC1, ADAM28, and ADAM7.

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