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Procollagen II Amino Propeptide Processing by ADAMTS-3

INSIGHTS ON DERMATOSPARAXIS*

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The amino and carboxyl propeptides of procollagens I and II are removed by specific enzymes as a prerequisite for fibril assembly. Null mutations in procollagen I N-propeptidase (ADAMTS-2) cause dermatosparaxis in cattle and the Ehlers-Danlos syndrome (dermatosparactic type) in humans by preventing proteolytic excision of the N-propeptide of procollagen I. We have found that procollagen II is processed normally in dermatosparactic nasal cartilage, suggesting the existence of another N-propeptidase(s). We investigated such a role for ADAMTS-3 in Swarm rat chondrosarcoma RCS-LTC cells, which fail to process the procollagen II N-propeptide. Stable transfection of RCS-LTC cells with bovine ADAMTS-2 or human ADAMTS-3 partially rescued the processing defect, suggesting that ADAMTS-3 has procollagen II N-propeptidase activity. Human skin and skin fibroblasts showed 30-fold higher mRNA levels of ADAMTS-2 than ADAMTS-3, whereas ADAMTS-3 mRNA was 5-fold higher than ADAMTS-2 mRNA in human cartilage. We propose that both ADAMTS-2 and ADAMTS-3 process procollagen II, but ADAMTS-3 is physiologically more relevant, given its preferred expression in cartilage. The findings provide an explanation for the sparing of cartilage in dermatosparaxis and, perhaps, for the relative sparing of some procollagen I-containing tissues.

Collagens consist of the major structural proteins of the extracellular matrix (ECM)[1] and exist in both fibril-forming (*e.g.* collagens I–III, V, and XI) and nonfibrillar forms (1, 2). Molecules belonging to both categories are homotrimeric (*e.g.* collagen II) or heterotrimeric (*e.g.* collagen I) assemblies of specific α chains, each the product of a single gene (1, 2). The molecular types of collagen, as well as the specific supramolecular aggregates they form, are often tissue-specific and provide a specialized function. For example, collagen I, the principal collagen of skin, is arranged in randomly oriented bundles in the dermis but in parallel bundles in tendons. Collagen II, a specific component of cartilage ECM, is arranged in an open meshwork that traps proteoglycans and facilitates resistance to compression.

The synthesis, secretion, and assembly of collagens into specific supramolecular aggregates is a complex, multistep process (3, 4). Fibrillar collagens I–III are synthesized as a soluble procollagen monomer comprising a long triple helical "collagenous" region with smaller polypeptide extensions (propeptides) at the amino and carboxyl ends (4). Removal of the propeptides by specific enzymes, the N- and C-propeptidases (proteinases), is a prerequisite for the correct assembly of collagens I and II into growing fibrils (3, 4). The procollagen C-propeptidase is identical to bone morphogenetic protein-1 and processes all three of these fibrillar collagens (5). Biochemically distinct N-propeptidases with specificity for procollagens I and II or procollagen III are known (6). The bovine and human procollagen I N-propeptidases have been cloned (7, 8). This enzyme (designated ADAMTS-2, EC 3.4.24.14), is a zinc-containing, calcium-dependent metalloendopeptidase belonging to the recently described ADAMTS family (9, 10).

ADAMTS2[2] mutations cause the recessively inherited connective tissue disorder dermatosparaxis in animals and the Ehlers-Danlos syndrome (EDS)-VIIC or dermatosparactic type (Mendelian Inheritance in Man (OMIM) number 225410) in humans (8, 11–18). These disorders present clinically with severe fragility of skin. Their molecular hallmark is the presence of irregular, thin, branched collagen fibrils in the dermis that appear "hieroglyphic" in cross-section and contain collagen I with an intact N-propeptide, termed pN-collagen I (19). Sim-

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF247668.

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[1] The abbreviations used are: EDS, Ehlers-Danlos syndrome; ADAMTS, a disintegrin-like and metalloprotease domain (reprolysin type) with thrombospondin type I motifs; PCR, polymerase chain reaction; RT-PCR, reverse transcriptase-PCR; kb, kilobase pairs; ECM, extracellular matrix; TS, thrombospondin; PCNP, procollagen N-propeptidase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; RACE, rapid amplification of cDNA ends; X-gal, 5-bromo-4-chloro-3-indolyl-β-D-galactoside; PAGE, polyacrylamide gel electrophoresis.

[2] The approved nomenclature is used for human and mouse genes. *ADAMTS2* and *ADAMTS3* are human genes. *adamts2* and *adamts3* are the corresponding mouse orthologs. The protein products of the respective genes are designated ADAMTS-2 and ADAMTS-3. Suggested trivial names for the protein products of *ADAMTS2* and *ADAMTS3* are procollagen N-propeptidase 1 (PCNP1) and procollagen N-propeptidase 2 (PCNP2).

ilar findings have been described very recently in *adamts2* transgenic knockout mice (20).

Among the various members of the ADAMTS family (currently 19 gene products), the overall domain organization and amino acid sequence of ADAMTS-2 is most similar to that of ADAMTS-3 (10). *ADAMTS3* cDNA was originally cloned from human brain and named the *KIAA0366* gene[3] (21). The KIAA0366 cDNA was incomplete at the 5′ end, and the translation start codon had not been identified (21). Through molecular cloning described herein, we now provide the complete primary structure of ADAMTS-3.

Substrates for ADAMTS-3 have not been previously identified. In light of the new data presented here, showing that procollagen II in dermatosparactic cartilage is completely processed, we investigated a role for ADAMTS-3 in procollagen II processing. The model system we used to test this hypothesis was the Swarm rat chondrosarcoma-derived cell line, RCS-LTC. In monolayer culture, these cells deposit an ECM containing collagens II, IX, and XI. However, the collagen is organized into thin filaments instead of fibrils (22). RCS-LTC cells fail to process procollagen II beyond the stage of pN-collagen II, although the amino acid sequence of the N-propeptidase cleavage site in RCS-LTC procollagen II is normal (23). RCS-LTC pN-collagen II is, however, processed *in vitro* by addition of conditioned medium from cultures of chick chondrocytes (23). This suggested that RCS-LTC chondrocytes either fail to express procollagen II N-propeptidase or lack a soluble, essential cofactor.

In this article, we demonstrate that transfection of RCS-LTC cells with *ADAMTS3* or *ADAMTS2* results in conversion of some of the pN-collagen II to a fully processed form. The results establish that N-propeptidase deficiency is responsible, at least in part, for defective collagen processing in RCS-LTC cells. We further demonstrate that steady-state mRNA levels of *ADAMTS2* and *ADAMTS3* are different in normal human skin and in skin fibroblasts than in cartilage, with ADAMTS-3 being expressed at higher levels than ADAMTS-2 in cartilage. Together, these data suggest that ADAMTS-3, not ADAMTS-2, is the major procollagen II N-propeptidase.

EXPERIMENTAL PROCEDURES

Cloning of ADAMTS3—The previously reported 5774-base pair KIAA0366/*ADAMTS3* cDNA (21) was extended further in the 5′ direction by RACE using the Marathon™ system, and Marathon™ human fetal brain cDNA (reagents from CLONTECH, Palo Alto) as template, essentially as described previously (10). PCR was done with nested *ADAMTS3*-specific antisense oligonucleotide primers 5′-TCAAGGCCTTCCAGGTCCGACTCTC-3′ and 5′-GGGAGCCTGTTCTACAGCTGATCTC-3′ and with nested adapter primers at the 5′ end of the template. The RACE products were cloned and sequenced as described previously (10).

Generation of ADAMTS3 and ADAMTS2 Expression Constructs—To generate a cDNA construct for expression of full-length ADAMTS-3, we first deleted the 5′ end of the KIAA0366 cDNA (in pBluescript II SK+ (Stratagene, La Jolla, CA), provided by Dr. Takahiro Nagase of the Kazusa DNA Institute). The deleted segment extended from the 5′ *Sal*I cloning (*i.e.* vector) site up to a unique internal *Acc*I site at nucleotide position 598 (KIAA0366 sequence enumeration). We replaced this fragment with a PCR-derived fragment of *ADAMTS3* cDNA extending from the 5′-untranslated sequence to just downstream of the *Acc*I site. Briefly, PCR was performed with Advantage PCR reagents (CLONTECH, Palo Alto, CA), using the RACE cDNA clone as template, the forward primer 5′-AACTCGAGGAAAGTGAACTCGACTCGTG-3′ (*Xho*I site underlined) and reverse primer 5′-AGCCTGTTCTACAGCTGATC-3′. The resulting amplicon was digested with *Xho*I and *Acc*I (at the internal *Acc*I site) and cloned into the *Sal*I-*Acc*I-restricted KIAA-0366 cDNA. This introduced the authentic *ADAMTS3* ribosome-binding sequence, translation start codon, and complete signal peptide into the KIAA0366 cDNA. This insert encoding full-length ADAMTS-3 was excised from pBluescript with *Xho*I and *Not*I and ligated into the corresponding sites in pcDNA 3.1 (+)-myc-His A (Invitrogen, San Diego, CA). In this mammalian expression construct, ADAMTS-3 is *not* in frame with the C-terminal *myc* and polyhistidine tags. For expression of ADAMTS-2, three overlapping bovine cDNA clones that have been reported previously (7) were appropriately digested (with *Not*I, *Bcl*I, *Eag*I, and *Kpn*I) and assembled to generate a construct encoding full-length bovine ADAMTS-2. The *ADAMTS2* cDNA was inserted into the *Not*I/*Xba*I sites of the pcDNA3 expression vector (Invitrogen, San Diego, CA) using an *Xba*I adapter.

Isolation of RNA—Skin samples obtained from the forearm of healthy volunteers were used to isolate dermal fibroblasts or were stored in liquid nitrogen until use (Laboratoire de Biologie des Tissus Conjonctifs, Sart-Tilman, Belgium, Ethics Committee Approval F94/14/1871). Dermal fibroblasts grown from skin explants were cultivated in Dulbecco's minimum essential medium supplemented with 10% fetal bovine serum. For Northern analysis, total RNA was purified from skin samples pulverized in liquid nitrogen or from dermal fibroblasts in culture, after solubilization of homogenates and cells in 0.1 M Tris-HCl, pH 7.5, 5 M guanidinium thiocyanate, 1% β-mercaptoethanol. These extracts were centrifuged at 100,000 × *g* for 18 h on a cesium chloride ($CsCl_2$) cushion (0.01 M EDTA, pH 7.5, 5.7 M $CsCl_2$). mRNA was subsequently purified using Poly(A)Tract mRNA Isolation System (Promega Benelux B.V.), according to the manufacturer's instructions. For quantitative RT-PCR, RNA was harvested from human fetal cartilage or from dermal fibroblasts using Trizol (Life Technologies, Inc.) and the manufacturer's recommended protocols. DNA was eliminated by treatment with DNase I (DNA-Free™, Ambion, Austin, TX).

Quantitative Reverse Transcriptase-PCR (RT-PCR) Analysis—Total RNA (2.5 μg) was reverse-transcribed with the SuperScript First-Strand Synthesis System for RT-PCR (Life Technologies, Inc.) using oligo(dT) as a primer. Real time PCR was performed in an ABI Prism 7700 Sequence Detector using SYBR Green PCR Core Reagents (Applied Biosystems, Foster City, CA). In this system, continuous, automated quantitation of the PCR product is performed by measuring the fluorescence generated by the binding of SYBR Green to double-stranded DNA. PCR amplifications were performed in triplicate for skin fibroblast RNA and in quadruplicate for cartilage RNA, along with parallel measurements of GAPDH cDNA (an internal control). Data were analyzed according to the comparative C_t method (Applied Biosystems protocols) and represented after normalization to GAPDH levels. To confirm these data, PCR products were also separated on a 2.0% agarose gel, visualized with UV light through a SYBR Green filter, and photographed. The following primers were used for amplification at a concentration of 300 nM: *ADAMTS2*, 5′-TGGGAAGCACAACGACATTG3-′ (forward) and 5′-CTCGGTCGTCGAGGGATTAG-3′ (reverse); *ADAMTS3*, 5′-TCAGTGGGAGGTCCAAATGCA-3′ (forward) and 5′-GCAAAGAAGGAAGCAGCAGCC-3′ (reverse); GAPDH, 5′-CCACTGCCAACGTGTCAGTGG-3′ (forward) and 5′-AAGGTGGAGGAGTGGGTGTCG-3′ (reverse). As an additional control, RT-PCR was also performed in the absence of template.

Northern Analysis of ADAMTS2 and ADAMTS3 Expression—A commercially available adult human multiple tissue Northern blot and a mouse embryo Northern blot (CLONTECH Inc. Palo Alto, CA) were hybridized as per manufacturer's instructions using ExpressHyb™ hybridization fluid (CLONTECH, Palo Alto, CA). The following cDNA probes were used after random-primed labeling with [α-^{32}P]dCTP: a fragment containing nucleotides 946–1379 of human ADAMTS-2 cloned in pCR4-TOPO (for human multiple tissue Northern blot); a 1.1-kb *Hin*dIII fragment from the KIAA0366/*ADAMTS3* cDNA (for human multiple tissue Northern blot); the insert of IMAGE clone 1246561, available with GenBank™ accession number AA832579 (mouse ADAMTS-2 probe for mouse embryo Northern blot); the insert of IMAGE clone 727026, available with GenBank™ accession number AA402760 (mouse ADAMTS-3 probe for mouse embryo Northern blot). Exposure of the blots to x-ray film was for 3–7 days.

Poly(A)$^+$ RNA (0.8 μg) from human skin or human skin fibroblasts was electrophoresed on a formaldehyde-agarose gel and blotted to Hybond N$^+$ nylon membrane (Amersham Pharmacia Biotech). cRNA probes for human *ADAMTS2* and *ADAMTS3* were generated by transcription from the respective clones using the Strip-EZ RNA kit (Ambion), T3 RNA polymerase, and [α-^{32}P]UTP as per manufacturer's instructions. Prehybridization (1 h) and hybridization (18 h) were performed at 65 °C in 0.2 M Na_2HPO_4 (pH 7.2), 1 mM EDTA, 1% bovine serum albumin, 7% SDS, and 20% formamide. Stringency washes were carried out at 65 °C in 40 mM Na_2HPO_4, 1 mM EDTA, and 1% SDS.

Cell Culture and Stable Transfection of RCS-LTC Chondrocytes—Monolayer cultures of RCS-LTC cells were maintained in Dulbecco's

[3] The partial sequence of *ADAMTS3* (the *KIAA0366* gene) was previously reported with GenBank™ accession number AB002364.

minimum essential medium containing 4.5 g/liter glucose (Life Technologies, Inc.) and 10% fetal bovine serum (HyClone Labs, Logan, UT) at 37 °C in 5% CO_2. Culture medium was changed every other day, and confluent cultures were sub-cultured every 2 weeks as described previously (24, 25). LipofectAMINE Plus (Life Technologies, Inc.) was used for RCS-LTC transfections. Cells were plated at a density of 3×10^5 cells/well in 6-well plates (Falcon, Franklin Lakes, NJ). After 24 h, the wells were rinsed with Opti-MEM (Life Technologies, Inc.) and transfected with the human *ADAMTS3* or bovine *ADAMTS2* cDNA constructs (1 μg of DNA/well) as per the manufacturer's instructions. As a control for efficiency of transfection and to provide a negative control for procollagen II processing, cells were separately transfected with pcDNA3.1/Myc-His (+)-*lacZ* encoding the *Escherichia coli lacZ* gene. Mock transfections were performed without cDNA as a control for efficacy of antibiotic selection. After 4 days in culture, transfected cells were selected in media supplemented with 1 mg/ml G418 sulfate (geneticin, Life Technologies, Inc.). After 2 weeks in culture, the chondrocytes from the mock transfections did not survive selection. Geneticin-resistant *ADAMTS3*, *ADAMTS2*, and *lacZ* transfected chondrocytes were expanded and maintained as pools in serial monolayer culture as described previously for the RCS-LTC cell line but in the continued presence of 1 mg/ml geneticin.

β-Galactosidase expression in the *lacZ* stable transfectants was detected histochemically by staining the cells with 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal, Life Technologies, Inc.). Briefly, the cultures were rinsed with phosphate-buffered saline (150 mM NaCl, 15 mM sodium phosphate, pH 7.3), fixed with 0.2% glutaraldehyde, and incubated with 1 mg/ml X-gal, 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide, and 2 mM $MgCl_2$ in phosphate-buffered saline for 24 h at 37 °C.

Procollagen Analysis in RCS-LTC Cells—ADAMTS3, *ADAMTS2,* and *lacZ* stable transfectants as well as untransfected RCS-LTC chondrocytes were grown in 6-well plates (Falcon, Franklin Lakes, NJ) following sub-culture as described. For the last day of culture, the culture medium was supplemented with 10 mg/ml L-ascorbate and 100 mg/ml β-aminopropionitrile. Cells and ECM were rinsed with phosphate-buffered saline and extracted at 100 °C with Laemmli sample buffer (24) containing 100 mM dithiothreitol or with 0.4 M NaCl, 50 mM Tris-HCl, pH 7.5, containing 5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, and 0.1% Triton X-100. To identify procollagen II-processing intermediates, untransfected RCS-LTC chondrocytes were grown for 4 days in the absence of ascorbate and β-aminopropionitrile. The cell layers were extracted as above. Extracts were repeatedly passed through a 27.5-gauge needle to reduce viscosity and were centrifuged at 15,000 rpm for 30 min at 4 °C.

Collagen II in extracts of cartilage and RCS-LTC transfectants was analyzed by gel electrophoresis and immunoblotting. Extracts were heated to 100 °C for 3 min in Laemmli sample buffer (25) containing 100 mM dithiothreitol. Collagen II chains were resolved by 6% SDS-PAGE and detected after transfer to polyvinylidene difluoride membrane (Bio-Rad) using a monoclonal antibody to collagen II (1C10; 0.5 μg/ml) and the Renaissance Western blot detection reagents (PerkinElmer Life Sciences). Monoclonal antibody 1C10[4] recognizes an epitope in denatured α1(II) CB9,7. It detects procollagen II as well as processed collagen II on Western blots.

As controls, fully processed collagen II from a 4 M guanidine HCl extract of human fetal cartilage and pepsin-extracted collagen II from the RCS-LTC cell line were used (23, 24).

Procollagen Cleavage Analysis in Dermatosparactic Cartilage—Nasal cartilage from a dermatosparactic cow and normal fetal bovine epiphyseal cartilage were extracted in 4 M guanidine HCl, 50 mM Tris-HCl, pH 7.4, with 2 mM EDTA, 5 mM benzamidine, 2 mM phenylmethylsulfonyl fluoride and 10 mM 1,10-phenanthroline at 4 °C for 24 h. Collagen in this extract was separated by reducing SDS-PAGE and visualized by Coomassie Blue staining and by Western blot analysis using 1C10 as described above.

RESULTS

Molecular Cloning of Full-length ADAMTS-3 and Comparison with ADAMTS-2—By using 5′-RACE, we obtained a novel 720-base pair cDNA clone encoding the 5′-untranslated region, the translation start codon, signal peptide, and pro-domain of ADAMTS-3. The novel 5′ sequence we obtained has not been previously described and is deposited in GenBank™ with accession number AF247668. When incorporated into the KIAA0366 sequence, we were therefore able to identify the complete open reading frame and conceptual translation product of *ADAMTS3*. The predicted start codon is the 5′-most methionine codon (ATG) in the open reading frame (Fig. 1*B*) and is preceded by an in-frame stop codon, 16 nucleotides upstream. It is located within the context of a suitable Kozak consensus sequence for translation (contains purine (G) at position −3 with respect to the A of the ATG codon, and G at position +4) (25). The predicted ADAMTS-3 protein (1205 amino acids) is comparable in length to human and bovine ADAMTS-2 (1211 and 1205 amino acids, respectively) (7, 8). The predicted mature (furin-processed) forms of these proteases are also of comparable length, 957 residues (ADAMTS-3) and 953 residues (bovine and human ADAMTS-2) long. *ADAMTS3* predicts a full-length protein of molecular mass 135.6 kDa and a furin-processed form of 107.5 kDa, although it is likely that post-translational modification of ADAMTS-3 will increase its mass (see below).

ADAMTS-3 and ADAMTS-2 have a similar domain structure (Fig. 1*A*) and an overall sequence identity of 61% (Fig. 1*B*). From N to C terminus, each of these enzymes consists of the following domains (with percent sequence identity in parentheses): signal peptide, pro-domain (37%), catalytic domain (85%), disintegrin-like domain (77%), central thrombospondin type I (TS) repeat (63%), cysteine-rich domain (67%), spacer domain (56%), and three additional TS repeats (64%), followed by an essentially unique C-terminal extension (Fig. 1*A*).

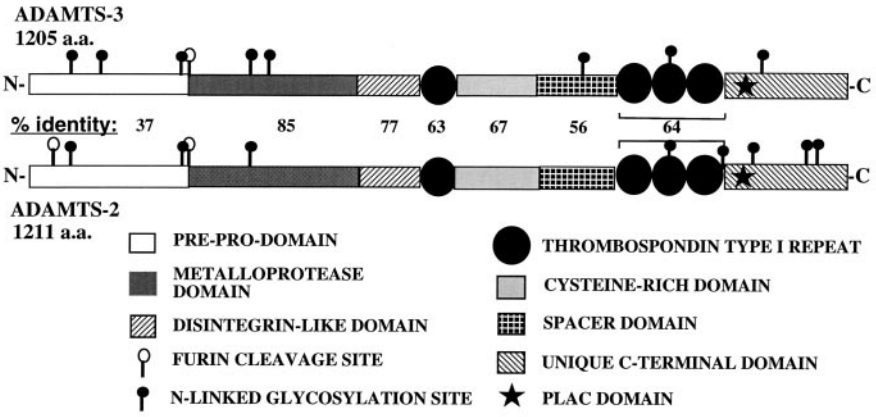
The putative ADAMTS-3 furin-processing site is at a location identical to the downstream of two furin consensus sites found in ADAMTS-2 (Fig. 1*B*). ADAMTS-3 and ADAMTS-2 catalytic domains have complete sequence identity (but for one amino acid) over 69 amino acids which includes the zinc-binding active site (Fig. 1*B*). ADAMTS-3 and ADAMTS-2 each have eight consensus sequences (Asn-*X*-(Ser/Thr)) for potential *N*-linked glycosylation (Fig. 1, *A* and *B*). Of these, four are conserved absolutely between ADAMTS-2 and ADAMTS-3, two conserved sites being in the pro-domain, and one site each in the catalytic domain and the second TS domain. One site in both ADAMTS-2 and ADAMTS-3 has the sequence Asn-Pro-Ser. It has been shown that the presence of proline between Asn and Ser/Thr inhibits *N*-glycosylation (26), and it is possible that this particular site may not be modified. In contrast to ADAMTS-2, which contains the potential cell-binding sequence CVRGDC, ADAMTS-3 has the sequence CVRGEC at the corresponding location (Fig. 1*B*).

The C-terminal extensions of these two molecules are of comparable length (184 and 191 amino acids in ADAMTS-2 and ADAMTS-3, respectively) but show little sequence similarity other than a highly conserved PLAC (protease and LACunin) domain (83% identity, Fig. 1*B*) (27). The PLAC domain was first described in an insect protein, lacunin (27), which contains all the ancillary domains of ADAMTS, as well as other domains and can therefore be considered a complex ADAMTS-like protein. The PLAC domains of human ADAMTS-3 and bovine ADAMTS-2 each contain six cysteine residues (Fig. 1*B*). In a human ADAMTS-2 sequence published previously (8) (GenBank™ accession number AJ003125), one of these cysteines (at position 1090, Fig. 1*B*) was substituted by serine. However, it is likely that this represents a sequence variation or error since there is a cysteine at this position in three independent *ADAMTS2* expressed sequence tag sequences (GenBank™ accession numbers AI417257, AI624388, and AI089232).

Differential Tissue-specific Expression of ADAMTS2 and ADAMTS3—adamts2 and *adamts3* were both expressed during mouse embryogenesis. *adamts2* expression was noted in mouse

[4] D. R. Eyre, unpublished data.

A



B

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hADAMTS-3 M--------------VLLSLWLIAAALVEVRTSADGQAGNEEMVQIDLPIKRYREYELVTPVSTNLEGRYLSHTLSASHK  66
hADAMTS-2 MDPPAGAARRLLCPALLLLLLLLPPPLLPPPPPPANARLAAAADPPGGPLGHGAERILAVPVRTDAQGRLVSHVVSAATS  80

hADAMTS-3 KRSARDVSSNP--------------EQLFFNITAFGKDFHLRLKPNTQLVAPGAVVEWHETSLVPGNITDPINNHQPGS 131
hADAMTS-2 RAGVRARRAAPVRTPSFPGGNEEEPGSHLFYNVTVFGRDLHLRLRPNARLVAPGATMEWQGEK---------------- 144

hADAMTS-3 ATYRIRKTEPLQTNCAYVGDIVDIP-GTSVAISNCDGLAGMIKSDNEEYFIEPLERGKQMEE-EKGRIHVVYKRSAVEQA 209
hADAMTS-2 GTTRV---EPLLGSCLYVGDVAGLAEASSVALSNCDGLAGLIRMEEEEFFIEPLEKGLAAQEAEQGRVHVVYRRPPTS-P 219

hADAMTS-3 PIDMSKDFHYRESDLEGLDDLGTVYGNIHQQLNETMRR-RRHAGENDYNIEVLLGVDDSVVRFHGKEHVQNYLLTLMNIV 288
hADAMTS-2 PLGGPQALD-TGASLDSLDSLSRALGVLEEHANSSRRRARRHAADDDYNIEVLLGVDDSVVQFHGKEHVQKYLLTLMNIV 298

hADAMTS-3 NEIYHDESLGVHINVVLVRMIMLGYAKSISLIERGNPSRSLENVCRWASQQQRSDLNHSEHHDHAIFLTRQDFGPAGMQG 368
hADAMTS-2 NEIYHDESLGAHINVVLVRIILLSYGKSMSLIEIGNPSQSLENVCRWAYLQQKPDTGHDEYHDHAIFLTRQDFGPSGMQG 378

hADAMTS-3 YAPVTGMCHPVRSCTLNHEDGFSSAFVVAHETGHVLGMEHDGQGNRCGDETAMGSVMAPLVQAAFHRYHWSRCSGQELKR 448
hADAMTS-2 YAPVTGMCHPVRSCTLNHEDGFSSAFVVAHETGHVLGMEHDGQGNRCGDEVRLGSIMAPLVQAAFHRFHWSRCSQQELSR 458
                                    Dis
hADAMTS-3 YIHSYDCLLDDPFDHDWPKLPELPGINYSMDEQCRFDFGVGYKMCTAFRTFDPCKQLWCSHPDNPYFCKTKKGPPLDGTE 528
hADAMTS-2 YLHSYDCLLDDPFAHDWPALPQLPGLHYSMNEQCRFDFGLGYMMCTAFRTFDPCKQLWCSHPDNPYFCKTKKGPPLDGTM 538

hADAMTS-3 CAAGKWCYKGHCMWKNANQQKQDGNWGSWTKFGSCSRTCGTGVRFRTRQCNNPMPINGGQDCPGVNFEYQLCNTEECQKH 608
hADAMTS-2 CAPGKHCFKGHCIWLTPDILKRDGSWGAWSPFGSCSRTCGTGVKFRTRQCDNPHPANGGRTCSGLAYDFQLCSRQDCPDS 618
                                                    1
hADAMTS-3 FEDFRAQQCQQRNSHFEYQNTKHHWLPYEHPDPKKRCHLYCQSKETGDVAYMKQLVHDGTHCSYKDPYSICVRGECVKVG 688
hADAMTS-2 LADFREEQCRQWDLYFEHGDAQHHWLPHEHRDAKERCHLYCESRETGEVVSMKRMVHDGTRCSYKDAFSLCVRGDCRKVG 698
                                  Spacer
hADAMTS-3 CDKEIGSNKVEDKCGVCGGDNSHCRTVKGTFTRTPRKLGYLKMFDIPPGARHVLIQEDEASPHILAIKNQATGHYILNGK 768
hADAMTS-2 CDGVIGSSKQEDKCGVCGGDNSHCKVVKGTFTRSPKKHGYIKMFEIPAGARHLLIQEVDATSHHLAVKNLETGKFILNEE 778

hADAMTS-3 GE-EAKSRTFIDLGVEWDYNIEDDIESLHTDGPLHDPVIVLIIPQENDTRSSLTYKYIIHEDSVPTINSNNVIQEELDTF 847
hADAMTS-2 NDVDASSKTFIAMGVEWEYRDEDGRETLQTMGPLHGTITVLVIPVG-DTRVSLTYKYMIHEDSL-NVDDNNVLEEDSVVY 856

hADAMTS-3 EWALKSWSQVSKPCGGGFQYTKYGCRRKSDNKMVHRSFCEANKKPKPIRRMCNIQECTHPLWVAEEWEHCTKTCGSSGYQ 927
hADAMTS-2 EWALKKWSPCSKPCGGGSQFTKYGCRRRLDHKMVHRGFCAALSKPKAIRRACNPQECSQPVWVTGEWEPCSQTCGRTGMQ 936
                    2
hADAMTS-3 LRTVRCLQPLLDGTNRSVHSKYCMGDRPESRRPCNRVPCPAQWKTGPWSECSVTCGEGTEVRQVLCRAGDH----CDGEK 1003
hADAMTS-2 VRSVRCIQPLHDNTTRSVHAKHCNDARPESRRACSRELCPGRWRAGPWSQCSVTCGNGTQERPVPCRTADDSFGICQEER 1016
                      3                                                    4
hADAMTS-3 PESVRACQLPPC-----------------------------NDEPCLGDKSIFCQMEVLARYCSIPGYNKLCCESCSKR 1053
hADAMTS-2 PETARTCRLGPCPRNISDPSKKSYVVQWLSRPDPDSPIRKISSKGHCQGDKSIFCRMEVLSRYCSIPGYNKLSCKSCN-- 1095

hADAMTS-3 SSTLPPPYLLEAAETHDDVISNPSDLPRSLVMPTSLVPYHSETPAKKMSLSSISSVGGPNAYAAFRPNSKPDGANLRQRS 1133
hADAMTS-2 --------LYNNLTNVEGRIEPPPGKHNDIDV------FMPTLPVPTVAMEVRPSPSTPLE-VPLNASSTNATEDHPETN 1159

hADAMTS-3 AQQAGSKTVRLVTVPSSPPTKRVHLSSASQMAAASFFAASDSIGASSQARTSKKDGKIIDNRRPTRSSTLER          1205
hADAMTS-2 AVDEPYKIHGL--------EDEVQPPNLIPRRPSPY----------EKTRNQRIQELIDEMRKKEMLGKF          1211
```

FIG. 1. *A*, domain organization of ADAMTS-3 and ADAMTS-2. Key for the domains is shown at the *bottom*. Amino acid identity (in percent) between ADAMTS-3 and ADAMTS-2 is shown for each domain (except the C-terminal domain). *a.a.*, amino acids. *B*, alignment of the primary structures of ADAMTS-3 and ADAMTS-2 using the single-letter amino acid code. The likely furin-processing site for generation of mature ADAMTS-3 and ADAMTS-2 is indicated by the *flat arrowhead*. Another consensus furin-processing site in ADAMTS-2 is indicated by an *arrowhead*. The sequence encoded by the RACE clone is *overlined*. TS repeats are *underlined* and indicated by *numbers*. The zinc-binding histidine triad is enclosed in a *box*. The boundaries of a region of complete amino acid sequence identity in the catalytic domain are indicated by the *vertical arrows*. The N termini of the disintegrin-like (*Dis*) and spacer domains are indicated. The cysteine-rich domain extends from TS repeat 1 to the start of the spacer domain. Potential cell-binding RG(D/E) sequences are indicated by the *thick overline*. The PLAC domains are shown by the *dashed underline*. Potential sites for *N*-linked glycosylation are indicated by *stars*.

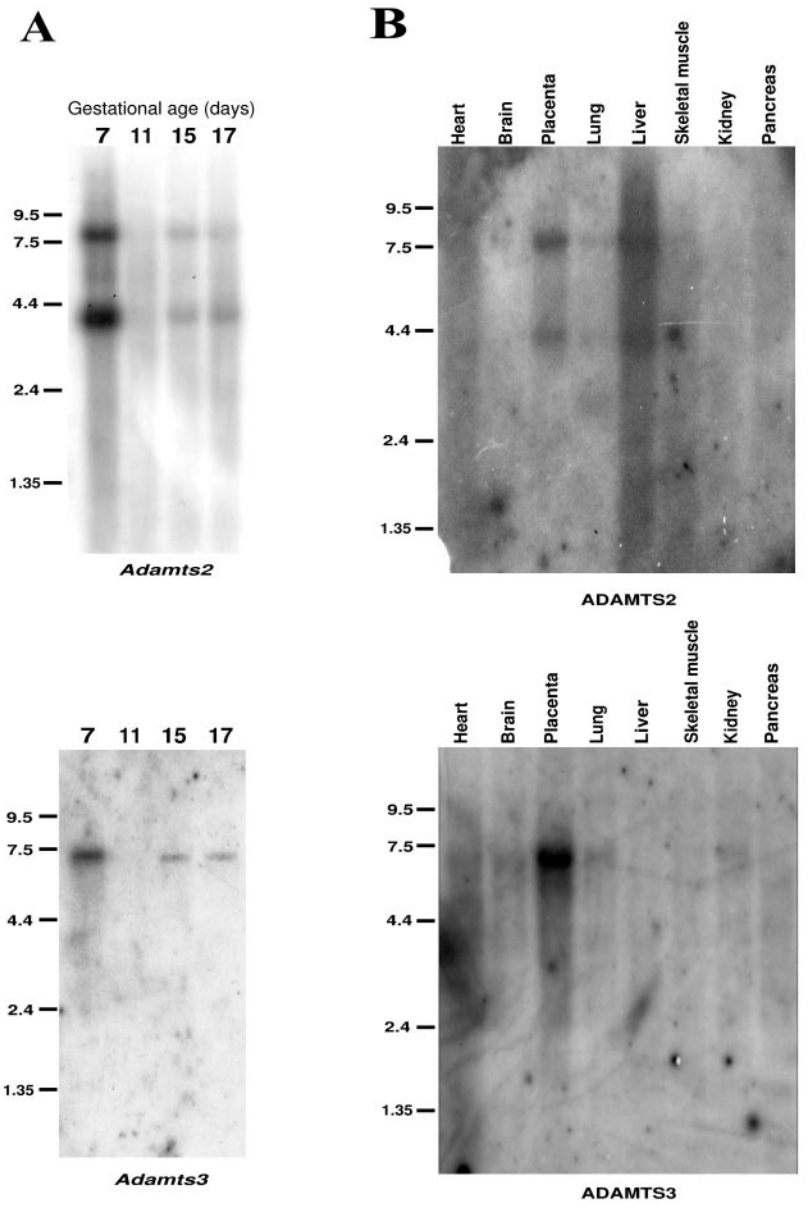

FIG. 2. **Northern blot analysis of ADAMTS-2 and ADAMTS-3 expression.** The tissue origin is indicated *above* the autoradiogram. RNA kilobase markers are shown at *left*. *A,* mouse embryo Northern blot; *B,* adult multiple tissue Northern blots. The probes used are indicated *below* each blot.

embryos at 7, 15, and 17 days but not at 11 days. Two mRNA species (7.8 and 4.0 kb) were detected (Fig. 2). A single *adamts3* mRNA species (~7.2 kb in size) was also detected in mouse embryos at 7, 15, and 17 days but not at 11 days of gestation (Fig. 2*A, bottom panel*). Expression of *ADAMTS2* and *ADAMTS3* was restricted among eight normal human adult tissues examined by Northern analysis (Fig. 2*B*). In human placenta, lung, and liver, two *ADAMTS2* transcripts were present, migrating at ~7.8 and 4.4 kb similar to those described previously (10). The previously described 2-kb transcript that encodes a truncated form of ADAMTS-2 was not detected on these blots (10). The highest expression of *ADAMTS3* was noted in placenta with lower level expression in lung, brain, and heart, with a single mRNA species migrating at ~7.0 kb.

In skin samples and in skin fibroblasts, Northern analysis with equivalent amounts of cRNA probes for *ADAMTS2* or *ADAMTS3* demonstrated a differential prevalence of steady-state mRNA levels. Fig. 3 shows autoradiograms generated by 1 (*ADAMTS2*) and 18 h of exposure (ADAMTS-3). Based on the different exposure times, a substantially stronger signal is evident with an *ADAMTS2* probe than with *ADAMTS3* in skin and skin fibroblasts. All three previously reported *ADAMTS2*

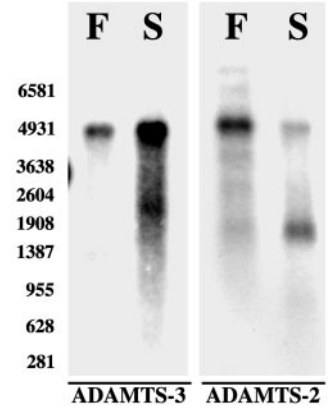

FIG. 3. **Northern analysis of *ADAMTS2* and *ADAMTS3* in RNA from skin (*S*) or skin fibroblasts (*F*).** RNA markers are shown at *left*.

transcripts (7.0, 4.5, and 2.0 kb) as well as several other minor transcripts were seen in skin fibroblasts, whereas only the 4.5- and 2.0-kb mRNAs were found in skin. With the *ADAMTS3* probe, we identified a 4.5-kb band plus 2.3-kb band in skin but

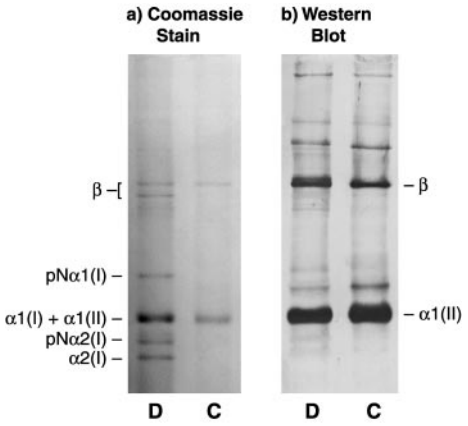

FIG. 4. **SDS-PAGE of guanidine-extracted proteins from nasal cartilage of a dermatosparactic cow (*D*) and control bovine articular cartilage (*C*).** *a,* Coomassie Blue-stained gel. *b,* Western blot of gel identical to that shown in *a* using a monoclonal antibody to collagen II (1C10). The position of the β chain, the cross-linked α1(II) dimer is also indicated.

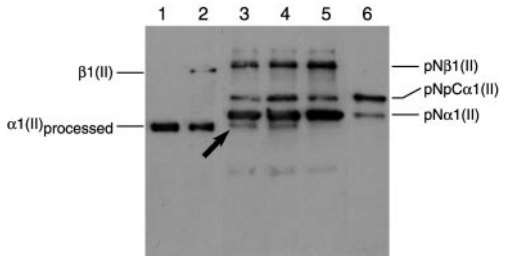

FIG. 5. **Procollagen II processing in RCS-LTC chondrocytes.** Immunoblot analysis of procollagen II processing demonstrated using monoclonal antibody 1C10. Equal protein loading was determined by Coomassie Blue staining (not shown). *Lanes 3–5* represent collagen II from *ADAMTS2*-, *ADAMTS3*-, and *lacZ*- transfected cells, respectively. Procollagen II from the RCS-LTC cell line is shown in *lane 6.* Pepsin-treated collagen II from the RCS-LTC cell line, and fully processed human fetal collagen II are shown in *lanes 1* and *2*, respectively. The *arrow* points to processed collagen II chains in *lanes 3* and *4*. Note that these co-migrate with pepsinized collagen II in *lane 1* and processed collagen II in *lane 2.* The different molecular forms indicated are as follows: $\alpha1(II)_{processed}$, the fully processed form; pN α1(II), pN-collagen II; pNpC α1(II), unprocessed procollagen II, and the β forms pNβ1(II) and β1(II). The identity of the bands migrating proximal to the pNβ1(II) form and distal to the processed forms in *lanes 3–5* is not known.

only a 4.5-kb band in skin fibroblasts. The discrepancy in number and size of these ADAMTS-3 bands with those evident in multiple tissue Northern blots (Fig. 2) is presently unexplained, but it is possible that, like *ADAMTS2*, *ADAMTS3* also generates multiple transcripts in a tissue-specific fashion. It was also noted that, whereas *ADAMTS3* signal is stronger in skin than in skin fibroblasts, the reverse was true for *ADAMTS2* (Fig. 3).

To obtain a numerical comparison of relative mRNA levels, we performed quantitative RT-PCR analysis of *ADAMTS2* and *ADAMTS3* mRNA in cultured skin fibroblasts and human cartilage. The data demonstrate considerably higher steady-state *ADAMTS2* mRNA levels in human skin fibroblasts relative to *ADAMTS3* levels (mean, 30.6-fold; range, 20.41–45.84-fold *ADAMTS2* over *ADAMTS3*). In contrast, quantitative RT-PCR analysis of RNA from human fetal cartilage demonstrates an approximately 5-fold higher steady-state level of ADAMTS-3 mRNA compared with *ADAMTS2* (mean, 4.86-fold; range, 2.89–7.88-fold *ADAMTS3* over *ADAMTS2*).

Procollagen II Is Completely Processed in Dermatosparactic Cartilage—Dermatosparactic animals have no functioning ADAMTS-2 (10). Despite this, Coomassie Blue staining of collagen extracted from dermatosparactic nasal cartilage demonstrated α chains migrating to the expected position of α1(II) chains from control cartilage (Fig. 4*a*). This was confirmed by Western blot analysis of these extracts using a monoclonal antibody that recognizes an epitope in the triple helical domain of α 1(II) (Fig. 4*b*), showing that essentially all of the immunoreactive collagen was fully processed. Some pN-collagen I was visible in the extract from dermatosparactic cartilage (Fig. 4*a*).

ADAMTS3 and ADAMTS2 Process Procollagen II in Transfected RCS-LTC Cells—The efficiency of RCS-LTC transfection was monitored by β-galactosidase staining. *lacZ* -transfected cells also served as a negative control for analysis of procollagen processing. Cells stably transfected with *lacZ* showed dark blue staining (representing about 10% of the population), whereas no staining was seen in the *ADAMTS3* and *ADAMTS2* stably transfected populations as expected. A similar efficiency of transfection and/or expression was assumed for the *ADAMTS3*- and *ADAMTS2*-transfected cells as for the *lacZ*-transfected cells.

To determine if ADAMTS-3 was capable of enzymatically removing the N-propeptide of procollagen II, lysates of *ADAMTS3*-, *ADAMTS2*-, and *lacZ*-transfected RCS-LTC chondrocytes were blotted, and procollagen II and collagen II were identified using monoclonal antibody 1C10. The results show some processing of pN-collagen II to mature collagen II in the *ADAMTS2*- and *ADAMTS3*-transfected cells (Fig. 5, *lanes 3* and *4*) but none in *lacZ*-transfected cells (Fig. 5, *lane 5*) or in untransfected cells (Fig. 5, *lane 6*). Following N-propeptide excision, the α1(II) chains migrate faster than the pN-collagen II chains (*e.g.* Fig. 5, *lane 3*) and at a position similar to the naturally processed α1(II) chain (Fig. 5, *lane 2*) or pepsinized collagen II (Fig. 5, *lane 1*).

DISCUSSION

Unsolved Enigmas in Dermatosparaxis and EDS-VIIC—Procollagen I processing in dermatosparaxis is most deficient in skin, although mature skin has some processed collagen (28). Many collagen I-containing dermatosparactic tissues such as tendon, ligament, sclera, and aorta show the presence of significant amounts of fully processed collagen I (12, 28). None of these tissues, nor bone, which relies on collagen I for its mechanical strength, have been noted to be fragile (13). Very recently, *adamts2* knockout mice have been reported to have significant amounts of processed collagen in skin (20).

These anomalies were attributable to the presence of residual pN-collagen processing activity, due to either the incompleteness of the genetic defect or to compensation by another enzyme (13, 28). The demonstration that the causative mutations were functionally null favored the existence of one or more additional procollagen N-propeptidases (8). The presence of processed procollagen I in many dermatosparactic tissues, including skin, suggested that this putative alternative propeptidase(s) might be regulated differently from ADAMTS-2 in skin and other tissues or that it may not be as efficient in procollagen I processing as ADAMTS-2 (28).

Procollagen II Processing Is Normal in Dermatosparaxis—ADAMTS-2 can process procollagen II (29, 30). However our finding of processed procollagen II in dermatosparaxis supported the existence of an enzyme other than ADAMTS-2 that actually removes the N-propeptide of collagen II *in vivo*. Although EDS-VIIC patients are of short stature, they do not have chondrodysplasia or premature arthritis (11, 13, 15–19). Failure of collagen II processing comparable to that of collagen I in dermatosparactic skin might be expected to cause a severe chondrodysplasia, given the critical role of collagen II in the structural stability of cartilage matrix (31, 32). Our studies thus provide an explanation for the absence of cartilage fragil-

ity and/or chondrodysplasia in dermatosparaxis or EDS-VIIC.

The presence of procollagen I in dermatosparactic nasal cartilage (Fig. 4) but not in normal cartilage may be explained by differences in composition of these cartilages, the inclusion of perichondrium in the extract, or by up-regulation of collagen I gene expression, which has been previously noted in dermatosparaxis (33). In contrast to our data, the recently described *adamts2* knockout mice retain some unprocessed collagen II in their cartilage (20).

ADAMTS-2 and ADAMTS-3 Comprise of a Structurally and Functionally Distinct Subfamily of ADAMTS Proteases but Are Regulated Differently—ADAMTS-2 and ADAMTS-3 are the only two members of the ADAMTS family to have three C-terminal TS domains and the only published members of the ADAMTS family to have a substantial C-terminal extension downstream of these TS domains. The location of the PLAC domain within this C-terminal extension is also unique, because in other ADAMTS family members where it is present, such as in ADAMTS-7B and ADAMTS-10,[5] it is usually at the C-terminal end of the protein.

A number of sequence hallmarks are unique to this PCNP subfamily of ADAMTS enzymes as follows. (*a*) The pro-domain contains only two cysteines, in contrast to other ADAMTS enzymes, which usually contain three. (*b*) The catalytic domain contains six cysteines as opposed to eight for the other ADAMTS, where the usual arrangement is five cysteines upstream of the zinc-binding site and three downstream. In the PCNP subfamily, only three cysteines occur upstream of the zinc-binding sequence; the three downstream cysteines, however, are at absolutely conserved positions with regard to other ADAMTS enzymes. This arrangement of cysteines suggests that the catalytic domain of this subfamily may be structurally different from that of the other ADAMTS enzymes. (*c*) The sequence of the zinc-binding triad (HETGHLGMEHD) in this subfamily is unique in having threonine in the 3rd position (underlined), whereas all other ADAMTS enzymes have a hydrophobic residue with a long side chain (leucine or isoleucine) at this position. (*d*) The spacer domains of the ADAMTS family vary in length and sequence. Within the PCNP subfamily, they are significantly similar to each other (56% amino acid identity).

On the basis of domain and amino acid sequence homology, this subfamily appears to contain no more than three members as determined by a search of the complete human genome sequence (Celera Genomics, Rockville, MD, and GenBank™ at NCBI). A third, equally closely related member of this family is located on human chromosome 10 (encoded by genomic sequences with GenBank™ accession numbers AC069538, AC016043, and AC007484). We previously mapped *ADAMTS3* to human chromosome 4, distinct from the *ADAMTS2* locus on human chromosome 5 (10). Thus, although these are the most closely related enzymes within the ADAMTS family, they are not clustered within the human genome.

Whereas the similarities in the catalytic domains of this subfamily suggest similar catalytic mechanisms, differences in their ancillary domains (*i.e.* the TS, disintegrin-like, cysteine-rich, and spacer domains) may affect substrate preferences, intermolecular interactions, or compartmentalization in ECM. For example, ADAMTS-1 ancillary domains are responsible for ECM binding (34), and the TS domains of aggrecanase-1 (ADAMTS-4) are required for binding to native aggrecan (35). A splice variant of *ADAMTS2* that generates a short form of ADAMTS-2 lacking the ancillary domains is functionally inactive in procollagen I processing (8).

[5] S. Apte, unpublished data.

Transfection with ADAMTS2 and ADAMTS3 Leads to Procollagen II Processing in RCS-LTC Cells—We have taken a genetic approach to identify a function for ADAMTS-3 and to determine the nature of the underlying processing defect in RCS-LTC cells. Transfection of these cells with *ADAMTS2* and *ADAMTS3*, but not with *lacZ* (a negative control), results in processing of some pN-α1(II) to α1(II). N-propeptide removal was detected by the co-migration of the processed form and pepsin-treated RCS-LTC collagen II (pepsin removes the propeptides) compared with collagen II extracted from human cartilage. Our results suggest that in quantitative terms the activity of ADAMTS-3 is roughly equivalent to that of ADAMTS-2. One caveat in our studies is that we have not performed experiments with purified procollagen II and recombinant ADAMTS-3. However, given the fact that ADAMTS-2 is an established procollagen I/II-processing enzyme (29, 36) and that there is a high degree of similarity of ADAMTS-3 to ADAMTS-2, it is very likely that ADAMTS-3 directly processes procollagen II.

There are several possible explanations for the persistence of pN-collagen II in *ADAMTS2*- or *ADAMTS3*- transfected cells. The transfected cells were maintained as a pooled population rather than as clonally selected lines, so that there may be transfected cells that do not express the construct at all or do so at low levels. In support of this possibility, only a small proportion of *lacZ*-transfected cells expressed β-galactosidase activity. We believe it unlikely that cells not containing the cDNA constructs survived because control, untransfected cells subjected to geneticin selection pressure were killed after 2 weeks. Other possibilities include an inadequate access of enzymes to pN-collagen II, an excess of procollagen II over the amount of enzyme required for processing, or that the culture conditions were not optimized for maximal processing.

Previous studies have shown that procollagen I processing in dermatosparactic fibroblasts is enhanced by including dextran sulfate (37, 38) or polyethylene glycol (38) in the culture medium. However, despite the increased efficiency, processing was still incomplete (37, 38). In previous studies in which culture medium conditioned by chick chondrocytes was added to pN-collagen II synthesized by RCS-LTC cells, we were unable to get complete processing to collagen II even after 18 h of incubation *in vitro* (23). A final possibility, which we consider to be unlikely, is that there may be some species specificity in the enzyme-substrate interactions that may affect the efficiency of processing. Our studies used bovine ADAMTS-2 and human ADAMTS-3 against rat collagen II. The procollagen II aminopropeptidase site is identical in a number of species, including rat and human (39). Furthermore, ADAMTS-2 is shown to have essentially similar activity against procollagen I from unrelated species. We therefore believe that effects resulting from species specificity are likely to be negligible.

Failure of RCS-LTC cells to produce a functional processing enzyme could result from a structural mutation in ADAMTS-2 or ADAMTS-3 or because of transcriptional repression of these genes in RCS-LTC cells. We have been unable to amplify *ADAMTS2* or *ADAMTS3* mRNA in RCS-LTC cells by RT-PCR suggesting the latter mechanism (data not shown; PCR was done using mouse primers, since rat sequences are currently not available for these two genes. However, rat and mouse show considerable genetic identity, about 96–98%, and these data may thus be valid.).

Is ADAMTS-3 Responsible for Procollagen II Processing in Dermatosparaxis?—We propose that, on the basis of either substrate preference for procollagen II *in vivo* or of a higher expression than ADAMTS-2 in cartilage, ADAMTS-3 is the principal collagen II N-propeptidase *in vivo*. Data from our

studies that provide a rationale for this speculation are as follows. (*a*) Collagen II is processed in dermatosparactic cartilage despite a null mutation in *ADAMTS2*. (*b*) pN-collagen II is equally processed in RCS-LTC cells transfected with ADAMTS-2 or ADAMTS-3. (*c*) The ratio of *ADAMTS3* to *ADAMTS2* mRNA in human cartilage is about 5:1.

More definitive proof is required: for example, the identification of ADAMTS-3 mutations in a human chondrodysplasia or from the targeted inactivation of *adamts3* in mice and analysis of the phenotype in cartilage. Such studies may uncover other functions for ADAMTS-3, such as the recently described role of ADAMTS-2 in male fertility (20). It is also possible that other enzymes of the ADAMTS family or other families contribute substantially to collagen N-propeptide processing in tissues other than skin. Finally, it is possible that the existence of procollagen N-proteinase(s) such as ADAMTS-3 can be exploited in the treatment of dermatosparactic patients. Before this can be done, it will be necessary to demonstrate procollagen I processing activity by ADAMTS-3 and to identify factors that up-regulate it in skin fibroblasts.

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REFERENCES

1. Fukai, N., Apte, S. S., and Olsen, B. R. (1994) in *Extracellular Matrix Components* (Erkki, R., and Engvall, E., eds) Vol. 245, pp. 3–28, Academic Press, New York
2. van der Rest, M., and Garrone, R. (1991) *FASEB J.* **5,** 2814–2823
3. Birk, D. E., Silver, F. H., and Trelstad, R. L. (1991) in *Cell Biology of Extracellular Matrix* (Hay, E. D., ed) 2nd Ed., pp. 221–254, Plenum Publishing Corp., New York
4. Olsen, B. R. (1991) in *Cell Biology of Extracellular Matrix* (Hay, E. D., ed) 2nd Ed., pp. 177–220, Plenum Publishing Corp., New York
5. Li, S. W., Sieron, A. L., Fertala, A., Hojima, Y., Arnold, W. V., and Prockop, D. J. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 5127–5130
6. Lapiere, C. M., Lenaers, A., and Kohn, L. D. (1971) *Proc. Natl. Acad. Sci. U. S. A.* **68,** 3054–3058
7. Colige, A., Li, S. W., Sieron, A. L., Nusgens, B. V., Prockop, D. J., and Lapiere, C. M. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 2374–2379
8. Colige, A., Sieron, A. L., Li, S. W., Schwarze, U., Petty, E., Wertelecki, W., Wilcox, W., Krakow, D., Cohn, D. H., Reardon, W., Byers, P. H., Lapiere, C. M., Prockop, D. J., and Nusgens, B. V. (1999) *Am. J. Hum. Genet.* **65,** 308–317
9. Kuno, K., Kanada, N., Nakashima, E., Fujiki, F., Ichimura, F., and Matsushima, K. (1997) *J. Biol. Chem.* **272,** 556–562
10. Hurskainen, T. L., Hirohata, S., Seldin, M. F., and Apte, S. S. (1999) *J. Biol. Chem.* **274,** 25555–25563
11. Fujimoto, A., Wilcox, W. R., and Cohn, D. H. (1997) *Am. J. Med. Genet.* **68,** 25–28
12. Hanset, R., and Lapiere, C. M. (1974) *J. Hered.* **65,** 356–358
13. Lapiere, C. M., and Nusgens, B. V. (1993) *Arch. Dermatol.* **129,** 1316–1319
14. O'Hara, P. J., Read, W. K., Romane, W. M., and Bridges, C. H. (1970) *Lab. Invest.* **23,** 307–314
15. Petty, E. M., Seashore, M. R., Braverman, I. M., Spiesel, S. Z., Smith, L. T., and Milstone, L. M. (1993) *Arch. Dermatol.* **129,** 1310–1315
16. Reardon, W., Winter, R. M., Smith, L. T., Lake, B. D., Rossiter, M., and Baraitser, M. (1995) *Clin. Dysmorphol.* **4,** 1–11
17. Smith, L. T., Wertelecki, W., Milstone, L. M., Petty, E. M., Seashore, M. R., Braverman, I. M., Jenkins, T. G., and Byers, P. H. (1992) *Am. J. Hum. Genet.* **51,** 235–244
18. Wertelecki, W., Smith, L. T., and Byers, P. (1992) *J. Pediatr.* **121,** 558–564
19. Nusgens, B. V., Verellen-Dumoulin, C., Hermanns-Le, T., De Paepe, A., Nuytinck, L., Pierard, G. E., and Lapiere, C. M. (1992) *Nat. Genet.* **1,** 214–217
20. Li, S. W., Arita, M., Fertala, A., Bao, Y., Kopen, G. C., Langsjo, T. K., Hyttinen, M. M., Helminen, H. J., and Prockop, D. J. (2001) *Biochem. J.* **355,** 271–278
21. Nagase, T., Ishikawa, K., Nakajima, D., Ohira, M., Seki, N., Miyajima, N., Tanaka, A., Kotani, H., Nomura, N., and Ohara, O. (1997) *DNA Res.* **4,** 141–150
22. Fernandes, R. J. (1993) An Analysis of Collagen Synthesized by a Swarm Rat Chondrosarcoma Cell Line in Monolayer Culture, Ph.D. thesis, Dept. of Biochemistry, Rush University, Chicago, IL
23. Fernandes, R. J., Schmid, T. M., Harkey, M. A., and Eyre, D. R. (1997) *Eur. J. Biochem.* **247,** 620–624
24. Laemmli, U. K. (1970) *Nature* **227,** 680–685
25. Kozak, M. (1989) *J. Cell Biol.* **108,** 229–241
26. Gavel, Y., and von Heijne, G. (1990) *Protein Eng.* **3,** 433–442
27. Nardi, J. B., Martos, R., Walden, K. K., Lampe, D. J., and Robertson, H. M. (1999) *Insect Biochem. Mol. Biol.* **29,** 883–897
28. Lenaers, A., Ansay, M., Nusgens, B. V., and Lapiere, C. M. (1971) *Eur. J. Biochem.* **23,** 533–543
29. Dombrowski, K. E., and Prockop, D. J. (1988) *J. Biol. Chem.* **263,** 16545–16552
30. Fertala, A., Sieron, A. L., Hojima, Y., Ganguly, A., and Prockop, D. J. (1994) *J. Biol. Chem.* **269,** 11584–11589
31. Spranger, J., Winterpacht, A., and Zabel, B. (1994) *Eur. J. Pediatr.* **153,** 56–65
32. Metsaranta, M., Garofalo, S., Decker, G., Rintala, M., de Crombrugghe, B., and Vuorio, E. (1992) *J. Cell Biol.* **118,** 203–212
33. Wiestner, M., Rohde, H., Helle, O., Krieg, T., Timpl, R., and Muller, P. K. (1982) *EMBO J.* **1,** 513–516
34. Kuno, K., and Matsushima, K. (1998) *J. Biol. Chem.* **273,** 13912–13917
35. Tortorella, M. D., Pratta, M., Liu, R. Q., Abbaszade, I., Ross, H., Burn, T., and Arner, E. (2000) *J. Biol. Chem.* **275,** 25791–25797
36. Fertala, A., Holmes, D. F., Kadler, K. E., Sieron, A. L., and Prockop, D. J. (1996) *J. Biol. Chem.* **271,** 14864–14869
37. Bateman, J. F., and Golub, S. B. (1990) *Biochem. J.* **267,** 573–577
38. Hojima, Y., Behta, B., Romanic, A. M., and Prockop, D. J. (1994) *Anal. Biochem.* **223,** 173–180
39. Baldwin, C. T., Reginato, A. M., Smith, C., Jimenez, S. A., and Prockop, D. J. (1989) *Biochem. J.* **262,** 521–528