ADAMTS-1 cleaves a cartilage proteoglycan, aggrecan

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Abstract A disintegrin-like and metalloproteinase with thrombospondin type I motifs-1 (ADAMTS-1) is an extracellular matrix-anchored metalloproteinase. In this study we have demonstrated that ADAMTS-1 is able to cleave a major cartilage proteoglycan, aggrecan. N-terminal sequencing analysis of the cleavage product revealed that ADAMTS-1 cleaves the Glu^{1871}–Leu^{1872} bond within the chondroitin sulfate attachment domain of aggrecan. In addition, deletional analysis demonstrated that the C-terminal spacer region of ADAMTS-1 is necessary to degrade aggrecan. These results suggest that ADAMTS-1 may be involved in the turnover of aggrecan in vivo.

Key words: Aggrecan; Disintegrin and metalloproteinase thrombospondin; Extracellular matrix; Cartilage degradation

1. Introduction

Disintegrin and metalloproteinases (ADAMs) represent a new family of gene products, which show a significant sequence similarity to snake venom metalloproteinase and disintegrin [1]. ADAMs have been shown to play a role in cell–cell interactions as well as in the ectodomain shedding of a variety of cell-surface proteins [1–3].

ADAMTS-1 is an ADAM family protein with both a reprolysin type metalloproteinase domain and three thrombospondin (TSP) type I motifs, which are conserved in TSP 1 and 2 [4]. The ADAMTS-1 gene was originally identified as a gene expressed in the colon 26 cachexigenic tumor in vivo and is mapped to mouse chromosome 16, region C3–C5 [4,5]. ADAMTS-1 consists of a proprotein, a metalloproteinase and a disintegrin-like domain, a central TSP type I motif, the spacing region, and the C-terminal TSP submotifs. While the type I motifs of ADAMTS-1 are functional in terms of binding to heparin, ADAMTS-1 itself is a secretory protein that is incorporated into the extracellular matrix (ECM) [6]. Analyses of deletion mutants have revealed that the spacing region of ADAMTS-1 as well as three TSP type I motifs are responsible for its anchoring to the ECM [6]. Furthermore, its capacity to form a covalent complex with α2-macroglobulin indicates the metalloproteinase domain of ADAMTS-1 being catalytically active [7]. These findings considered together, strongly suggest that ADAMTS-1 is an active metalloproteinase that is associated with the ECM. On the other hand, recent study showed that ADAMTS-1(METH-1) has anti-angiogenic activity [8].

Aggrecan is a major proteoglycan found in the ECM of articular cartilage. The core protein of aggrecan is substantially modified with chondroitin sulfate (CS) and keratan sulfate (KS) glycosaminoglycans, which serve to hydrate the cartilage tissue, providing properties of compression and elasticity [9,10]. Aggrecan comprises several segments: the N-terminal globular domains (G1 and G2), a long central glycosaminoglycan attachment region, and the C-terminal globular domain (G3) [11]. Aggrecan monomers interact with hyaluronic acid through the G1 domain to form aggregates with a large molecular weight, which keeps aggrecan within the cartilage matrix [12,13].

In cartilage destruction associated with arthritic diseases, measurable amounts of aggrecan are lost from cartilage tissue. This loss of aggrecan is attributed to the accelerated degradation within the interglobular domain (IGD) of aggrecan. Two major proteolytic cleavage sites, the Asn^{341}–Phe^{342} and Glu^{373}–Ala^{374} bonds, have been identified within the IGD of aggrecan. It is known that matrix metalloproteinases (MMPs) can cleave the Asn^{341}–Phe^{342} bond, whereas the enzymes responsible for the cleavage of the Glu^{373}–Ala^{374} bond, have been termed 'aggrecanases' [14–18]. The degradation products cleaved at the Glu^{373}–Ala^{374} bond have been detected in cartilage explants and chondrocyte cultures [14–16,19,20], and in the synovial fluids of patients with various joint diseases [21–23]. Therefore, it has been hypothesized that aggrecanases play an important role in the cartilage degradation of arthritis. Recently, Arner and her group have shown that aggrecanase-1 and -2 molecules (ADAMTS-4 and -5 (-11)) are structurally related to ADAMTS-1 [24,25]. However, the proteinases involved in the turnover of aggrecan in vivo, remain to be fully clarified.

Because our previous study suggested that the ECM association of ADAMTS-1 occurs through binding to sulfated glycosaminoglycans [6], it has been hypothesized that proteoglycans could be the target molecules of the ADAMTS-1 metalloproteinase. In the present study, we examined whether

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Abbreviations: ADAM, disintegrin and metalloproteinase; CS, chondroitin sulfate; DMEM, Dulbecco's modified Eagle's medium; ECM, extracellular matrix; IGD, interglobular domain; KS, keratan sulfate; MMP, matrix metalloproteinase; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; TSP, thrombospondin

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ADAMTS-1 has the ability to cleave a major cartilage proteoglycan, aggrecan.

2. Materials and methods

2.1. Cell culture and materials

HEK293 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Nissui, Tokyo, Japan) supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 units/ml penicillin G, and 100 μg/ml streptomycin. Protease inhibitors, phenylmethylsulfonyl fluoride, pepstatin A, and *N*-ethylmaleimide were obtained from Sigma (St. Louis, MO, USA). Protease-free chondroitinase ABC (from *Proteus vulgaris*) and keratanase (from *Pseudomonas sp.*) were obtained from Seikagaku Co Ltd. (Tokyo, Japan).

2.2. Construction of expression vectors and expression of recombinant proteins

The expression vectors for the mouse ADAMTS-1 protein or its deletion mutants, X4 and X5, were constructed using pcDNA3 (Invitrogen, San Diego, CA, USA) as previously described [6]. In the present study, a $(His)_6$-tagged sequence was added prior to the FLAG-epitope tag at the C-terminus of each protein to facilitate purification.

Recombinant ADAMTS-1 proteins were prepared by means of transient expression using HEK293 cells. Briefly, the cells grown in 10-cm dishes were transfected with the ADAMTS-1 expression vector by means of a lipofectamine reagent (Life Technologies Inc., Gaithersburg, MD, USA) according to the manufacturer's instructions. 18 h after transfection, the cells were washed and further cultured for 2 days in serum-free DMEM medium containing soluble heparin (5 μg/ml).

$(His)_6$-tagged ADAMTS-1 proteins in the culture supernatants were purified by a HiTrap chelating column (Amersham Pharmacia Biotech, Uppsala, Sweden). Briefly, after culture supernatants supplemented with 20 mM HEPES (pH 7.5) and 0.5 M NaCl were applied to the HiTrap chelating column, the column was washed with HSB buffer (40 mM HEPES, pH 7.5, 1 M NaCl, 0.05% Brij 35) containing 10 mM imidazole. Recombinant ADAMTS-1 proteins were eluted with HSB buffer containing 500 mM imidazole.

2.3. Aggrecan degradation assay

Aggrecan was extracted from bovine nasal cartilage with 4 M guanidine hydrochloride and purified on a cesium chloride gradient centrifugation according to the methods reported by Hardingham [26].

Aggrecan was incubated with recombinant ADAMTS-1 proteins for 12 h at 37°C in the reaction buffer (20 mM Tris–HCl pH 7.6, 150 mM NaCl, 5 mM $CaCl_2$, 0.01% NaN_3). After digestion with the ADAMTS-1 proteinase, the samples were deglycosylated with chondroitinase ABC (0.05 unit/10 μg proteoglycan) and keratanase (0.05 unit/10 μg proteoglycan) for 3 h at 37°C in the buffer containing 50 mM Tris–HCl, pH 8.0, 30 mM sodium acetate, 10 mM EDTA, 5 mM phenylmethylsulfonyl fluoride, 0.36 mM pepstatin A, and 10 mM *N*-ethylmaleimide. Deglycosylated samples were heated at 100°C for 5 min in Laemmli sample buffer (60 mM Tris–HCl, pH 6.8, 2% sodium dodecyl sulfate (SDS), 5% mercaptoethanol, 10% glycerol), and were subjected to SDS-polyacrylamide gel electrophoresis (PAGE), followed by immunological detection.

To immunologically detect the core protein fragments, gels were transferred onto nitrocellulose membranes and blocked overnight in Block ACE (Dainippon Pharmaceutical Co. Ltd., Osaka, Japan). The membranes were then incubated with antibody 2-B-6 (anti-chondroitin-4-sulfate stubs generated by chondroitinase treatment) [27] (Seikagaku), followed by incubation with horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG polyclonal antibodies (Amersham Pharmacia Biotech). After washing, bound HRP-conjugated antibodies were detected with a chemiluminescence (ECL) system (Amersham Pharmacia Biotech).

2.4. N-terminal sequence analysis of an aggrecan fragment

For the determination of the N-terminal sequence of the 100-kDa aggrecan fragment, the deletion mutant X3 of ADAMTS-1 was used for the cleavage of aggrecan. After elution from a HiTrap chelating column as indicated above, the ADAMTS-1 X3 protein was applied to a HiTrap heparin column (Amersham Pharmacia Biotech) equilibrated with 25 mM Tris–HCl, pH 7.6, 0.2 M NaCl, and 0.05% Brij 35 and was eluted with the same buffer containing 1 M NaCl. Aggrecan fragments produced by digestion with the ADAMTS-1-X3 proteinase were deglycosylated with both chondroitinase ABC and keratanase and subjected to 6% SDS–PAGE, followed by electroblotting onto PVDF membrane. After staining with Coomassie Blue, the region corresponding to the 100-kDa aggrecan fragment was cut out and

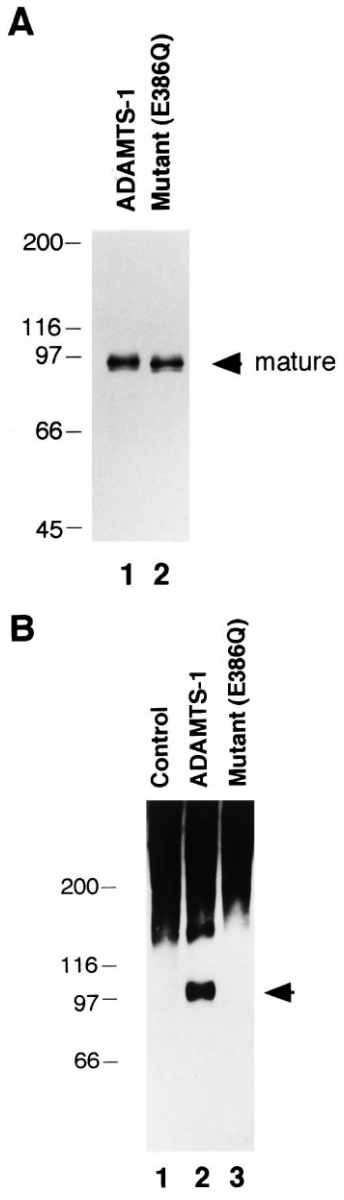

Fig. 1. Aggrecan cleavage by the recombinant ADAMTS-1 proteinase A: The recombinant ADAMTS-1 proteins were expressed as the mature forms in the HEK293 cell expression system. Recombinant ADAMTS-1 (lane 1) or the mutant (E386Q) (lane 2) purified with a Ni-chelating column (see Section 2) was analyzed by Western blotting with anti-FLAG M2 antibody. B: Aggrecan-cleaving assay for ADAMTS-1. Bovine aggrecan monomer (10 μg) was incubated with the recombinant ADAMTS-1 protein (0.5 μg) (lane 2) or the mutant (E386Q) (0.5 μg)(lane 3) purified with a Ni-chelating column at 37°C for 12 h. The Ni-chelating column-bound fraction prepared from the culture media of the control vector-transfected cells was used for the control reaction (lane 1). After treatment with chondroitinase ABC and keratanase, the samples were subjected to 6% SDS–PAGE, and the cleavage products of aggrecan were analyzed by Western blotting using the 2-B-6 antibody. The arrow indicates the 100-kDa fragment of the aggrecan core protein generated by the ADAMTS-1 proteinase.

subjected to N-terminal amino acid sequencing using Protein Sequencing System G1005A (Hewlett-Packard Co., CA, USA).

3. Results

3.1. Expression of recombinant ADAMTS-1 protein

To investigate the proteolytic activity of ADAMTS-1 against proteoglycan, the recombinant ADAMTS-1 protein was prepared by means of the transient expression system using HEK293 cells. Both the FLAG epitope tag and the histidine tag were added at its C-terminus for detection and rapid purification of the recombinant protein. Because ADAMTS-1 is an ECM-anchored protease [6], heparin was added to the culture to allow ADAMTS-1 to be released from the ECM into the culture supernatant. Under the expression condition employed in our study, the majority of the recombinant ADAMTS-1 was produced as the processed active form without a proprotein domain (Fig. 1A, lane 1). Therefore, the recombinant ADAMTS-1 protein purified by the Ni-chelating column could be directly used in the proteinase assay without separation of the precursor form.

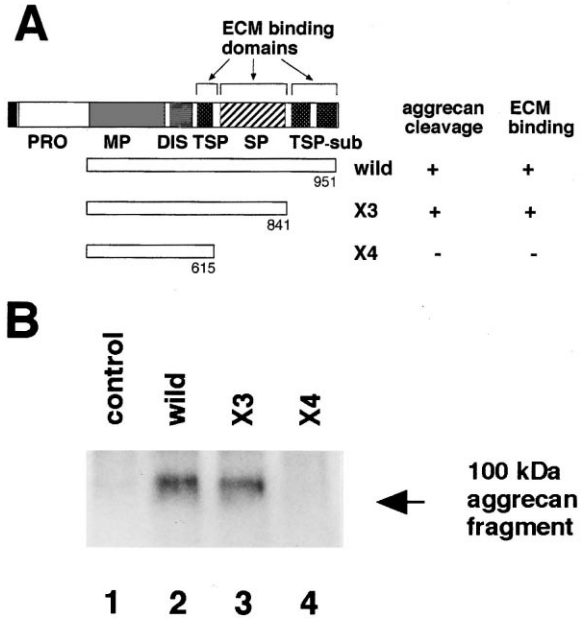

Fig. 2. Requirement of the C-terminal spacer region of ADAMTS-1 for aggrecan-degrading activity. A: The domain organization of ADAMTS-1 and construction of the C-terminal deletion mutants. (PRO, proprotein domain; MP, metalloproteinase domain; DIS, disintegrin-like domain; TSP, TSP type I motif; SP, the spacer region; TSP-sub, TSP submotifs). Open bars: represent the portion present in the processed forms of wild-type and deletion mutants with the amino acid number at the end. The aggrecan-degrading activities of deletion mutants are summarized on the right side of the figure, together with their binding capacity to ECM, which was determined in our previous study [6]. B: Aggrecan-cleaving assay for the C-terminal deletion mutants of ADAMTS-1. Bovine aggrecan (10 μg) was incubated with recombinant ADAMTS-1 (0.5 μg) (lane 2), mutant ×3 (0.4 μg) (lane 3), or mutant ×4 (0.3 μg) (lane 4) at 37°C for 12 h. After treatment with chondroitinase ABC and keratanase, generation of the 100-kDa aggrecan fragment in the reaction mixtures was monitored by Western blotting analysis using the 2-B-6 antibody. The control reaction (lane 1) was carried out as described in Fig. 1B.

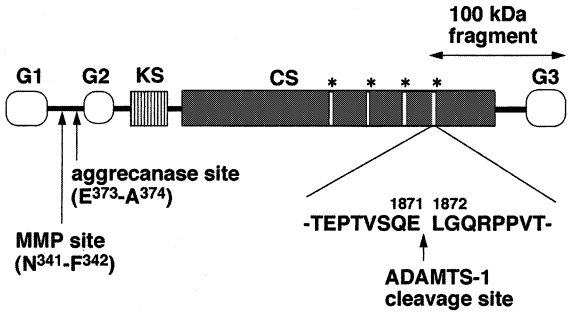

Fig. 3. Schematic representation of the cleavage site in the bovine aggrecan by ADAMTS-1. The gap regions in the CS attachment domain are indicated by asterisks. CS, chondroitin sulfate attachment domain; KS, keratan sulfate attachment domain.

3.2. Aggrecan cleavage by ADAMTS-1

To examine whether ADAMTS-1 has the ability to cleave aggrecan, the recombinant ADAMTS-1 proteinase was incubated with bovine aggrecan monomer. After the deglycosylation of aggrecan with both chondroitinase ABC and keratanase, the degradation products of the aggrecan core protein were analyzed by means of Western blotting with the antibody 2-B-6, which recognizes chondroitin 4-sulfate stubs of the core protein. As shown in Fig. 1B, upon incubation of the full-length ADAMTS-1 with the aggrecan substrate, an immunoreactive band migrating at approximately 100-kDa was generated (lane 2). In contrast, the control sample prepared from the culture supernatant of the control vector-transfected cells lacked the activity to generate the 100-kDa core fragment (Fig. 1B, lane 1). In addition, the inclusion of 10 mM EDTA into the reaction mixture inhibited the generation of the 100-kDa core fragment, whereas aprotinin and leupeptin had no inhibitory effects (data not shown).

Furthermore, the metalloproteinase-inactive mutant of ADAMTS-1, E386Q, in which the essential Glu residue in the zinc-binding motif was converted to Gln (Fig. 1A, lane 2), lost its capacity to generate the 100-kDa fragment of aggrecan (Fig. 1B, lane 3), confirming that the metalloproteinase activity of ADAMTS-1 is necessary for the generation of the 100-kDa fragment. These findings demonstrate that ADAMTS-1 has the ability to cleave the aggrecan core protein.

3.3. Requirement of the spacer region for aggrecan cleaving activity

The C-terminal half region of ADAMTS-1 consists of the three type I motifs and the intervening spacer region, both of which are responsible for binding to the ECM [6]. To investigate the involvement of the C-terminal domains of ADAMTS-1 in the aggrecan cleavage, digestion was carried out with the C-terminal deletion mutants, X3 and X4 (Fig. 2A). As shown in Fig. 2B, mutant X3, which lacks the C-terminal TSP submotifs, retained the activity to generate the 100-kDa fragment of aggrecan (lane 3). In contrast, when aggrecan was incubated with mutant X4, deleting both the spacer region and the C-terminal TSP submotifs, the 2-B-6 reactive 100-kDa band was not generated (lane 4), indicating that deletion of the spacer region of ADAMTS-1 results in a loss of ability or a very low ability to cleave aggrecan. This finding demonstrates that the spacer region in the C-terminal

half is required for recognition of the aggrecan substrate by ADAMTS-1.

3.4. N-terminal sequencing of an aggrecan fragment generated by ADAMTS-1

In order to determine the site at which aggrecan is cleaved by the ADAMTS-1 proteinase, the 100-kDa aggrecan core fragment generated by ADAMTS-1 was subjected to N-terminal amino acid analysis. The 100-kDa fragment yielded the sequence L-QRP-VTYT, which corresponds to a site in the CS attachment region starting at Leu^{1872}. This result suggests that the ADAMTS-1 proteinase cleaves the Glu^{1871}–Leu^{1872} bond within the CS attachment region of aggrecan.

4. Discussion

ADAMTS-1 is a metalloproteinase associated with the ECM [6,7] and is characterized by the presence of TSP type I motifs and a potential sulfated glycosaminoglycan-binding region. The present study has demonstrated that the ADAMTS-1 proteinase is able to degrade a cartilage proteoglycan, aggrecan.

The turnover of aggrecan is an important process for maintenance of homeostasis of the articular cartilage matrix. Any increase in the degradation rate or a decrease in the biosynthesis of aggrecan may have pathological consequences. The IGD between the G1 and G2 domains is sensitive to proteolytic attack, and a proteolysis of the core protein within the IGD is up-regulated under pathological conditions such as rheumatoid arthritis and osteoarthritis. Several MMPs (MMP-1–3,7–9,13) have been shown to cleave the Asn^{341}–Phe^{342} bond within the IGD, whereas 'aggrecanases' are responsible for the cleavage at the Glu^{373}–Ala^{374} bond within the IGD [14–18]. An important finding in recent studies is that both aggrecanase-1 and -2 [ADAMTS-4, and -5 (-11)], purified from interleukin-1-stimulated bovine nasal cartilage culture, are members of the ADAMTS family [24,25]. Aggrecan fragments resulting from cleavage at the Glu^{373}–Ala^{374} bond are found in the synovial fluids of patients with osteoarthritis, inflammatory joint disease and joint injury [21–23]. These observations suggest that 'aggrecanases' are involved in the degradation of aggrecan in various joint diseases.

We found that a 100-kDa fragment is generated by aggrecan cleavage by the ADAMTS-1 proteinase. Detection of this 100-kDa fragment by the 2-B-6 antibody suggests that it is derived from the CS attachment domain of aggrecan. Furthermore, N-terminal sequencing of the fragment revealed that the ADAMTS-1 proteinase cleaves the Glu^{1871}–Leu^{1872} bond within the CS attachment domain (Fig. 3). To date, it has been shown that, in addition to two major proteolytic cleavage sites within the IGD, proteolysis of aggrecan in vivo also occurs within the CS attachment domain [15,19,22]. The proteolysis within the CS attachment domain is reported in cartilage explant cultures treated with interleukin-1 or retinoic acid [15,19] and in the synovial fluids from arthritis patients [22]. Sequencing analyses revealed that these cleavage sites are located in gap regions, which are relatively devoid of glycosaminoglycan chains, of the CS attachment domain. Since these cleavage sites in the gap regions show a sequence highly similar to that around the Glu^{373}–Ala^{374} aggrecanase site, 'aggrecanase'-type proteinases have been postulated to be involved in the cleavage within the CS attachment domain [28]. The ADAMTS-1 cleavage site (E^{1871}–L^{1872}) is located in one of these gap regions (Fig. 3). In view of these considerations, it is reasonable that ADAMTS-1 is involved in the degradation of the CS attachment domain of aggrecan because ADAMTS-1 shows a high sequence homology to aggrecanase-1 and -2 (ADAMTS-4 and ADAMTS-5) among the ADAMTS family, eight members of which have been identified [29–32].

The aggrecan degrading ability of ADAMTS-1 deletion mutants is consistent with their capacity to bind to ECM, as summarized in Fig. 2A. The deletional analyses revealed that the spacer region in the C-terminal half of ADAMTS-1 is required both for the tight interaction with the ECM and for the aggrecan-cleaving activity. Our previous finding that the full-length ADAMTS-1 protein is released from the ECM into culture supernatant in the presence of soluble heparin suggests that the spacer region of ADAMTS-1 as well as the TSP type I motifs may interact with sulfated glycosaminoglycans in the ECM [6]. It is therefore presumed that the spacer region of ADAMTS-1 plays a role in the recognition of sulfated glycosaminoglycans, CS and/or KS of aggrecan and that such an interaction between the spacer region of ADAMTS-1 and glycosaminoglycan chains is necessary for the cleavage of aggrecan. The recent finding that the deglycosylation of aggrecan reduces the ability of aggrecanase-1 (ADAMTS-4) to cleave the Glu^{373}–Ala^{374} bond [24] supports this notion.

Further investigation is required to establish the physiological importance of aggrecan cleavage by ADAMTS-1. In this connection, there have been recent reports that ADAMTS-1 mRNA is detectable by RT-PCR analysis in human articular cartilage and arthritic joint tissues [25,32]. Therefore, our finding that ADAMTS-1 has aggrecan-degrading activity strongly suggests the possibility that ADAMTS-1 plays a role in the turnover of aggrecan in normal physiological processes and/or the degradation of aggrecan under pathological conditions.

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References

[1] Wolfsberg, T.G., Primakoff, P., Myles, D.G. and White, J.M. (1995) J. Cell Biol. 131, 275–278.
[2] Blobel, C.P. (1997) Cell 90, 589–592.
[3] Yamamoto, S., Higuchi, Y., Yoshiyama, K., Shimizu, E., Kataoka, M., Hijiya, N. and Matsuura, K. (1999) Immunol. Today 278, 278–284.
[4] Kuno, K., Kanada, N., Fujiki, F., Ichimura, F., Nakashima, E. and Matsushima, K. (1997) J. Biol. Chem. 272, 556–562.
[5] Kuno, K., Iizasa, H., Ohno, S. and Matsushima, K. (1997) Genomics 46, 466–471.
[6] Kuno, K. and Matsushima, K. (1998) J. Biol. Chem. 273, 13912–13917.
[7] Kuno, K., Terashima, Y. and Matsushima, K. (1999) J. Biol. Chem. 274, 18821–18826.
[8] Vazquez, F., Hastings, G., Ortega, M., Lane, T.F., Oikemus, S., Lombardo, M. and Iruela-Arispe, M.L. (1999) J. Biol. Chem. 274, 23349–23357.
[9] Hardingham, T.E. and Fosang, A.J. (1992) FASEB J. 6, 861–870.
[10] Vertel, B.M. (1995) Trends Cell Biol. 5, 458–465.
[11] Doege, K., Sasaki, M., Horigan, E., Hassell, J.R. and Yamada, Y. (1987) J. Biol. Chem. 262, 17757–17767.
[12] Heiegard, D. and Hascall, V.C. (1974) J. Biol. Chem. 249, 4250–4256.
[13] Hardingham, T.E. (1979) Biochem J. 177, 237–247.

[14] Sandy, J.D., Neame, P.J., Boynton, R. and Flannery, C.R. (1991) J. Biol. Chem. 266, 8683–8685.
[15] Loulakis, P., Shrikhande, A., Davis, G. and Maniglia, C.A. (1992) Biochem. J. 284, 589–593.
[16] Ilic, M.Z., Handley, C.J., Robinson, H.C. and Mok, M.T. (1992) Arch. Biochem. Biophys. 322, 22–30.
[17] Arner, E.C., Pratta, M.A., Trzaskos, J.M., Decicco, C.P. and Tortorella, M.D. (1999) J. Biol. Chem. 274, 23443–23450.
[18] Sugimoto, K., Takahashi, M., Yamamoto, Y., Shimada, K. and Tanzawa, K. (1999) J. Biochem. 126, 449–455.
[19] Ilic, M.Z., Mok, M.T., Williamson, O.D., Campbell, M.A., Hugh, C.E. and Handley, C.J. (1995) Arch. Biochem. Biophys. 322, 22–30.
[20] Lark, M.W., Gordy, J.T., Weidner, J.R., Ayala, J., Kimura, J.H., Williams, H.R., Mumford, R.A., Flannery, C.R., Carlson, S.S., Iwata, M. and Sandy, J.D. (1995) J. Biol. Chem. 270, 2550–2556.
[21] Sandy, J.D., Flannery, C.R., Neame, P.J. and Lohmander, L.S. (1992) J. Clin. Invest. 89, 1512–1516.
[22] Lohmander, L.S., Neame, P.J. and Sandy, J.D. (1993) Arthritis Rheum. 9, 1214–1222.
[23] Lark, M.W., Bayne, E.K., Flanagan, J., Harper, C.F., Hoerrner, L.A., Huchinson, N.I., Singer, I.I., Donatelli, S.A., Weidner, J.R., Williams, H.R., Mumford, R.A. and Lohmander, L.S. (1997) J. Clin. Invest. 100, 93–106.
[24] Tortorella, M.D., Burn, T.C., Pratta, M.A., Abbaszade, I., Hollis, G.F., Liu, R-Q., Rosenfeld, S.A., Copeland, R.A., Decicco, C.P., Wynn, R., Rockwell, A., Yang, F., Duke, J.L., Solomon, K., George, H.J., Bruckner, R., Nagase, H., Itoh, Y., Ellis, D.M., Ross, H., Wiswall, B.H., Murphy, K., Hillman, M.C., Hollis, J.M., Newton, R.C., Magolda, R.L., Trzaskos, J.M. and Arner, E.C. (1999) Science 284, 1664–1666.
[25] Abbaszade, I., Liu, R.-Q., Yang, F., Rosenfeld, S.A., Ross, H., Link, J.R., Ellis, D.M., Tortorella, M.D., Pratta, M.A., Hollis, J.M., Wynn, R., Duke, J.L., George, H.J., Hillman, M.C., Murphy, K., Wiswall, B.H., Copeland, R.A., Decicco, C.P., Bruckner, R., Nagase, H., Itoh, Y., Newton, R.C., Magolda, R.L., Trzaskos, J.M., Hollis, G.F. and Burn, T.C. (1999) J. Biol. Chem. 274, 23443–23450.
[26] Hardingham, T.E. (1979) Biochem. J. 177, 237–247.
[27] Caterson, B., Christner, J.E. and Baker, J.R. (1983) J. Biol. Chem. 258, 8848–8854.
[28] Sandy, J.D., Plaas, A.H.K. and Koob, T.J. (1995) Acta Orthop. Scand. 266 (Suppl.), 26–32.
[29] Colige, A., Beschin, A. and Samyn, B. et al. (1997) Proc. Natl. Acad. Sci. USA 94, 2374–2379.
[30] Hurskainen, T.L., Hirohata, S., Seldin, M.F. and Apte, S.S. (1999) J. Biol. Chem. 274, 25555–25563.
[31] Tang, B.L. and Hong, W. (1999) FEBS Lett. 445, 223–225.
[32] Flannery, C.R., Little, C.B., Hugh, C.E. and Caterson, B. (1999) Biochem. Biophys. Res. Commun. 260, 318–322.