The spatial and temporal expression of cartilage matrix protein illustrates the molecular heterogeneity of cartilage

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Cartilages contain a common set of macromolecules, such as aggrecan, link protein and collagen II, but their relative proportions may vary to produce tissues with different biochemical and physical properties. Further, there exist matrix proteins that are present only in a subset of cartilages and which may be involved in the fine tuning of the properties of the matrix. Cartilage contains a number of proteins, that are also present in other tissues as well as a number of proteins with a more restricted tissue distribution which may even be specific for cartilage. Many proteins, such as link protein (Gardell et al. 1980), aggrecan (Wong et al. 1992) and cartilage oligomeric matrix protein (DiCesare et al. 1994) are found more widely distributed than initially assumed, albeit often at very low concentrations.

Cartilage matrix protein (CMP) was first described as cofractionating with cartilage proteoglycan (Paulsson and Heinegård 1979). It consists of three identical subunits of 50 kDa which are connected by disulfide bonds (Paulsson and Heinegård 1981). The primary structure of CMP, deduced from cDNA for chicken (Kiss et al. 1989) and human (Jenkins et al. 1990), shows that each subunit consists of two von Willebrand factor A-domains connected by one EGF-like domain and in addition containing a C-terminal extension peptide. The C-terminal extension was recently found to consist of heptad repeats, indicative of a coiled-coil α-helical structure, and shown to function in the assembly of trimeric CMP (Hauser and Paulsson 1994). Studies of the tissue distribution of CMP demonstrated that it occurs only in some, and not in all, cartilages (Paulsson and Heinegård 1982, Aszodi et al. 1994, Mundlos and Zabel 1994) and that the total amount of CMP in tracheal cartilage increases dramatically with maturation and aging (Paulsson et al. 1984).

Materials and methods

Six-week-old mouse tissues and 3 month gestational bovine tendon were frozen for immunhistochemistry in Tissue-Tek, cut in 5-μm sections, digested with 40 milliunits/ml chondroitinase ABC in TBS containing 0.01% BSA, incubated in methanol containing 1% (v/v) H_2O_2 followed by 1% BSA in TBS. Sections were probed with rabbit antiserum to bovine CMP, followed by peroxidase-coupled swine anti-rabbit IgG, developed with 3-amino-9-ethylcarbazole/H_2O_2 and counterstained with Mayers Hämalaun.

Adult and fetal tendon were sequentially extracted with 10 mM TrisHCl, pH 7.4 (twice); 10 mM EDTA, 10 mM TrisHCl, pH 7.4 (twice), 0.5 M NaCl, 10 mM EDTA, 10 mM TrisHCl, pH 7.4 (once) and with 1% Triton X100, 0.5 M NaCl, 10 mM EDTA, 10 mM TrisHCl, pH 7.4 (once), all containing protease inhibitors (1 mM PMSF and 1 mM NEM). Samples were run under nonreducing conditions on 4–15% SDS-polyacrylamide gels according to Laemmli (1970) and electrophoretically transferred to nitrocellulose. CMP was detected with polyclonal antibodies raised in rabbit to bovine CMP and ^{125}I-labelled protein A.

Results

The tissue distribution of CMP in mouse tissues as revealed by immunostaining (Figure 1) in most aspects confirmed the results obtained by both radioimmunoassay of bovine tissue extracts (Paulsson and Heinegård 1982) and the tissue specific expression of a chicken CMP transgene in mouse (Aszodi et al. 1994). Some features were particularly striking. In skeletal tissues CMP was strongly expressed throughout all epiphyseal growth plates as seen both

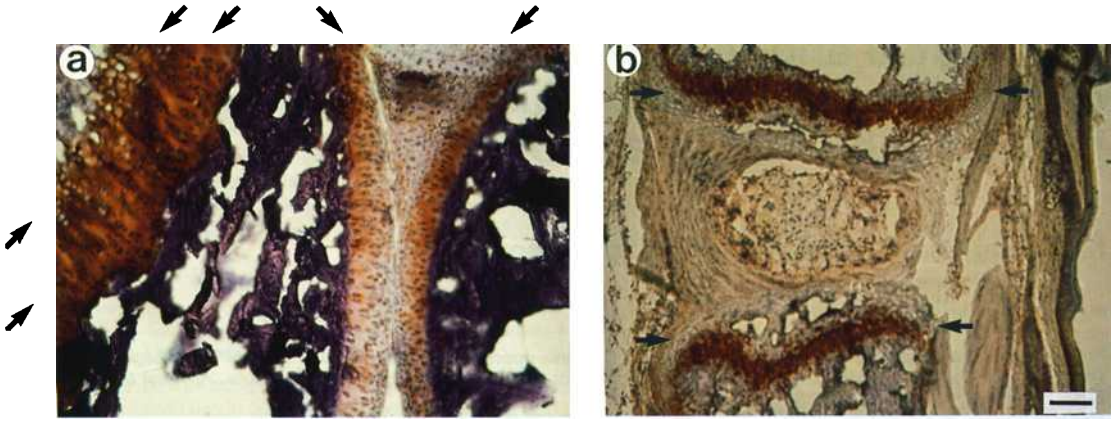

Figure 1. Indirect immunohistochemistry of the knee (a) and vertebral column (b) of a 6 week old mouse. Brown staining for CMP was seen in some but not all cartilagenous structures (see arrows). Original blue color was due to counter staining with Mayers Hämalaun. Bar, 100 µm.

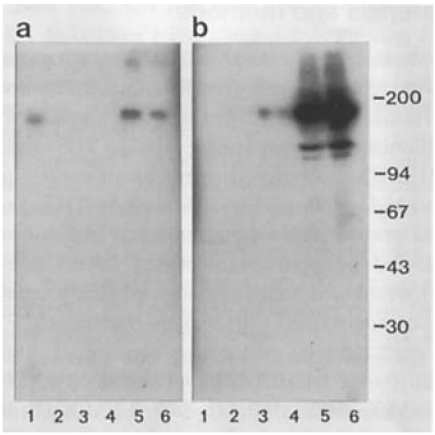

Figure 2. Immunoblotting for CMP in extracts of adult (a) and fetal (b) bovine tendon. SDS-PAGE was run under non-reducing conditions where CMP is detected as a band running slightly below the 200 kDa marker. Extraction was done with 10 mM TrisHCl (lanes 1 and 2), 10 mM EDTA, 10 mM TrisHCl (lanes 3 and 4), 0.5 M NaCl, 10 mM EDTA, 10 mM TrisHCl (lane 5) and 1% Triton X100, 0.5 M NaCl, 10 mM EDTA, 10 mM TrisHCl (lane 6) all at pH 7.4.

for the distal end of the femur and for the vertebral bodies. In articular cartilage, staining was only seen in deep and peripheral areas and not in weight-bearing areas. In the intervertebral disc, CMP could neither be detected in anulus fibrosus nor in nucleus pulposus.

The presence and tissue distribution of CMP in tendon was investigated in steer. By immunoblotting under non-reducing conditions CMP could be detected as a band migrating with an apparent molar mass slightly lower than 200 kDa (Figure 2). In both fetal and adult tissue, CMP was extracted mainly by the combination of EDTA and high salt, indicating a rather stable, divalent-cation-dependent anchorage in the tissue (see also Hauser and Paulsson 1994). Much more CMP was extracted from fetal as compared to adult tendon (Figure 2). This was somewhat surprising as the CMP concentration in bovine tracheal cartilage increases dramatically with aging. The result could, however, be confirmed by immunohistochemistry which showed little to no staining in adult tendon and strong specific staining throughout the extracellular matrix of fetal tendon (Figure 3).

Discussion

Our results show that CMP is a protein expressed in some, but not all, cartilages as well as in tendon. While tissue concentration of CMP in cartilage increases during aging (Paulsson and Heinegård 1982), in tendon the highest CMP concentration is found in the fetus. Similar results have been obtained for a number of proteins that were at one time considered "cartilage specific." Indeed, the spatial and temporal expression patterns for extracellular matrix proteins are often highly complex.

As a result, the use of such proteins as serum markers for cartilage disease is difficult. Saxne and Heinegård (1989) could show that CMP concentrations were higher in serum than in synovial fluid and that CMP release in rheumatoid arthritis reflects involvement of tissues other than articular cartilage. Cartilage antigens have a considerable potential as disease markers, but only, after their tissue distribution and site of release into the circulation are properly characterized.

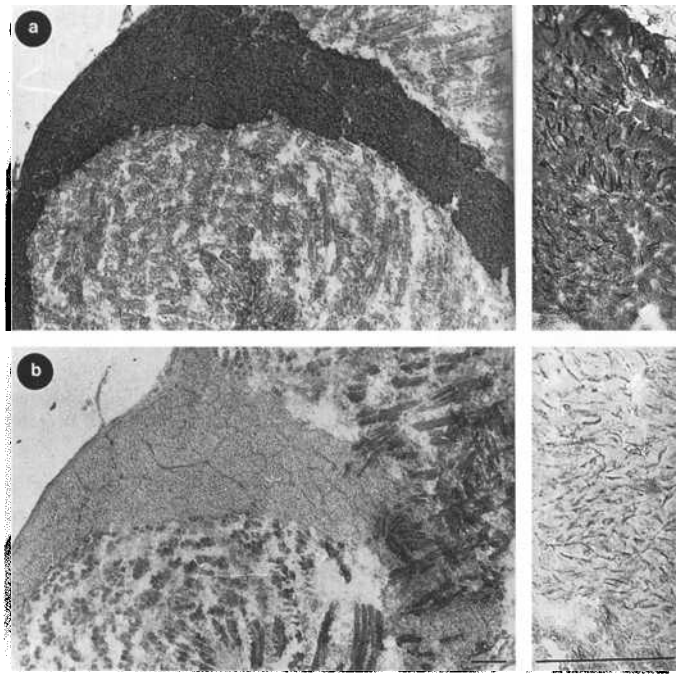

Figure 3. Indirect immunohistochemistry of fetal bovine tendon with antibodies to bovine CMP (a) and with a control antiserum to an irrelevant antigen (b). Bar, 100 µm.

Acknowledgements

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