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ARTICULAR CARTILAGE COLLAGEN: AN IRREPLACEABLE FRAMEWORK?

David R. Eyre, Mary Ann Weis and Jiann-Jiu Wu
University of Washington, Seattle

Abstract

Adult articular cartilage by dry weight is two-thirds collagen. The collagen has a unique molecular phenotype. The nascent type II collagen fibril is a heteropolymer, with collagen IX molecules covalently linked to the surface and collagen XI forming the filamentous template of the fibril as a whole. The functions of collagens IX and XI in the heteropolymer are far from clear but, evidently, they are critically important since mutations in COLIX and COLXI genes can result in chondrodysplasia syndromes. Here we review what is known of the collagen assembly and present new evidence that collagen type III becomes covalently added to the polymeric fabric of adult human articular cartilage, perhaps as part of a matrix repair or remodelling process.

Keywords: Cartilage, collagen type III, collagen cross-linking, heterotypic fibrils, osteoarthritis

Address for Correspondence
David R. Eyre, Ph.D.
University of Washington
Dept. of Orthopaedics and Sports Medicine
Box 356500
Seattle, WA 98195-6500 USA

Tel 206-543-4700
Fax 206-685-4700
E-mail: deyre@u.washington.edu

Introduction

Osteoarthritis (OA) has multiple causes and risk factors, but once the articular cartilage is lost, the joint fails. The collagen framework of adult cartilage is turned over very slowly and any severe damage appears to be irreversible and a critical step in the process of joint failure. There is growing evidence that proteolytic damage to cartilage collagen can occur very soon after joint injury (Lohmander *et al*., 2003).

The material strength and biological properties of articular cartilage depend heavily on its uniquely and extensively cross-linked collagen network and characteristic fibrillar organization that varies with tissue depth and proximity to the cells. Once laid down during development, there appears to be little capacity for articular chondrocytes to recapitulate the overall collagen architecture if the mature tissue is mechanically injured or goes through advanced stages of degeneration. The ability of chondrocytes to remodel the collagen at ultrastructural and molecular levels is poorly understood. There may be greater capacity than previously thought. Potential mechanisms for proteolytic remodelling and molecular repair are of growing interest (Eyre, 2002). In this review, we present what is known of the molecular organization of cartilage collagen as a basis for understanding mechanisms of normal growth, remodelling and potential replacement of the cartilage fibrillar matrix and its proteolytic destruction in osteoarthritis.

Collagen ultrastructure

Collagen fibrils seen by transmission electron microscopy (TEM) in articular cartilage form a random, loose network compared with collagen in most other connective tissues. Patterns of preferred fibril orientation are evident, however (Chen and Broom, 1998). In the surface zone (0-2mm), the fibrils are thin and tend to run primarily parallel to the plane of the articular surface with some degree of preferred orientation in that plane. A greater range of fibril diameters is seen in the deeper zones, and the organization appears more random when viewed by TEM. In the radial zone of some joint regions, a preferred orientation of fibril bundles orthogonal to the surface can be seen by scanning electron microscopy, also visible by TEM in regions of pathologically softened cartilage (Chen and Broom, 1998). The arcade-like macro-architecture of collagen responsible for this zonal appearance described by Benninghoff (1925) appears, on scanning electron microscopy, to reflect a folding over of radial fibre bundles to lie in the plane of the surface in a series of layers or leaflets that makes up the tangential zone (Notzli and Clark, 1997). In mammalian articular cartilage, the primary polymer-forming components (collagens II, IX and XI) do not appear to alter dramatically in proportion between these macroscopic zones.

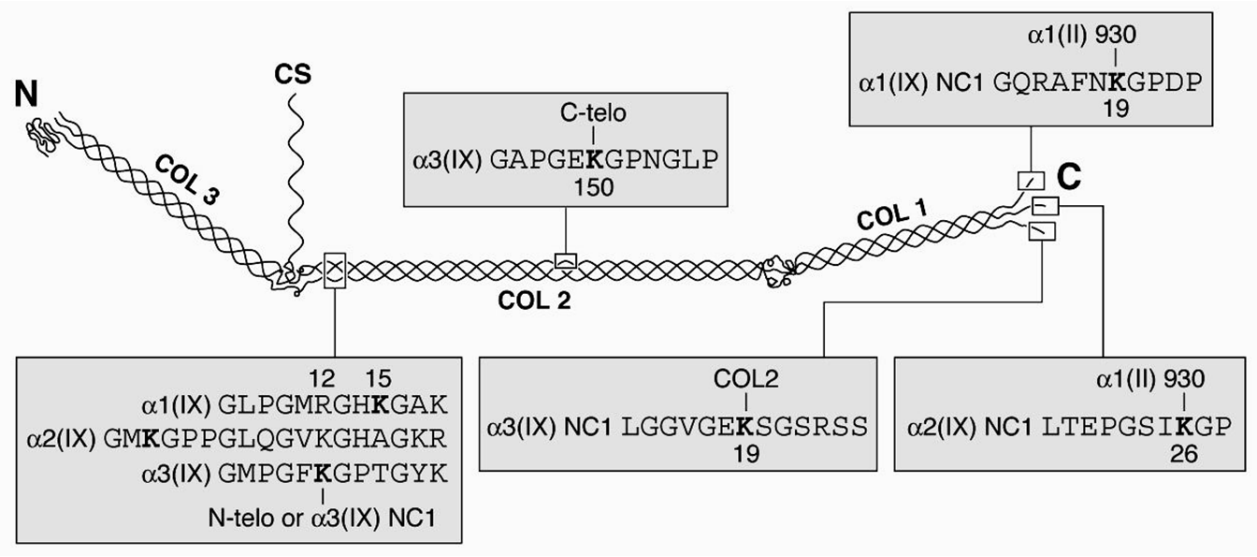

Fig. 1 Seven cross-linking sites (lysines) have been identified in the mammalian type IX collagen molecule, which consists of three triple-helical segments interrupted by and ending in four non-collagen-helical sequences (NC1 to NC4 going from right to left in this figure). The chondroitin sulphate (CS) chain shown attached to NC3 is found on type IX collagen from some sources but not from articular cartilage (Diab *et al.*, 1996).

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In finer detail, the fibrillar appearance of the mature tissue differs between the pericellular and the intercellular (interterritorial) matrix. Fibrils become coarser and more obviously banded, as seen by TEM, going farther from the chondrocytes (Lane and Weiss, 1975). The proportion of type IX (Poole *et al*., 1987) and type XI (Vaughan-Thomas *et al*., 2001) collagens is highest in the thinnest fibrils that form the pericellular basket, or the chondron described by Poole *et al*. (1987). Remodelling and maturation of thin, newly made fibrils presumably involves removal of collagens IX and XI, and/or their dilution by addition of new type II collagen. To what degree thin fibrils fuse laterally in the matrix versus growing by accretion of new monomers is unclear, although both processes are thought to occur (Hunziker *et al*., 1997; Holmes *et al*., 2001).

The collagen II/IX/XI heteropolymeric template

Many different types of collagen molecules are expressed in articular cartilage but the backbone polymeric template during development is a copolymer of collagens II, IX and XI (Table 1). Collagen IX molecules decorate the surface of type II collagen fibrils, particularly the thin fibrils forming the basket (chondron) around chondrocytes (Hagg *et al*., 1998). Seven cross-linking sites have been defined in the collagen IX molecule (Fig. 1). These interact with type II collagen and with other collagen IX molecules, presumably on the surface of fibrils as illustrated (Fig. 2) (Diab *et al*., 1996; Ichimura *et al*., 2000; Wu *et al*., 1992). The cross-links are either trivalent pyridinolines or borohydride-reducible divalent structures formed on the lysyl oxidase pathway typical of collagen. In an extension of one potential packing model (Miles *et al*., 1988), the NC1/COL2 domain of collagen IX docks in the hole region of the fibril and the molecule folds back through its NC2 domain (Fig. 2). This arrangement allows all seven cross-links (Fig. 1) to form between a single collagen IX molecule and the surface of a type II collagen polymer.

Collagen XI molecules are primarily cross-linked to each other in a head-to-tail manner, and are believed to form a template that constrains the lateral growth of the type II collagen hetero-fibril (Blaschke *et al*., 2000). Retained N-propeptide domains on collagen XI are thought to inhibit fibril lateral growth (Gregory *et al*., 2000). The N-telopeptide cross-linking lysines responsible for XI-to-XI cross-linking are located external to candidate metalloproteinase cleavage sites. Such cleavages could depolymerise collagen XI (Wu and Eyre, 1995), perhaps

TABLE I. Collagen Types in Cartilage Matrix (% of total collagen)

- Type II (75% foetal, >90% adult)
- Type III (>10% in adult human articular)
- Type IX (covalently fibril-associated collagen, 10% foetal, 1% adult)
- Type X (hypertrophic cartilage only)
- Type XI (fibril template, 10% foetal, 3% adult)
- Type VI (chondron basket, microfilaments <1%)
- Type XII/XIV (non-covalently fibril-associated collagens)
- Type XIII (transmembrane)

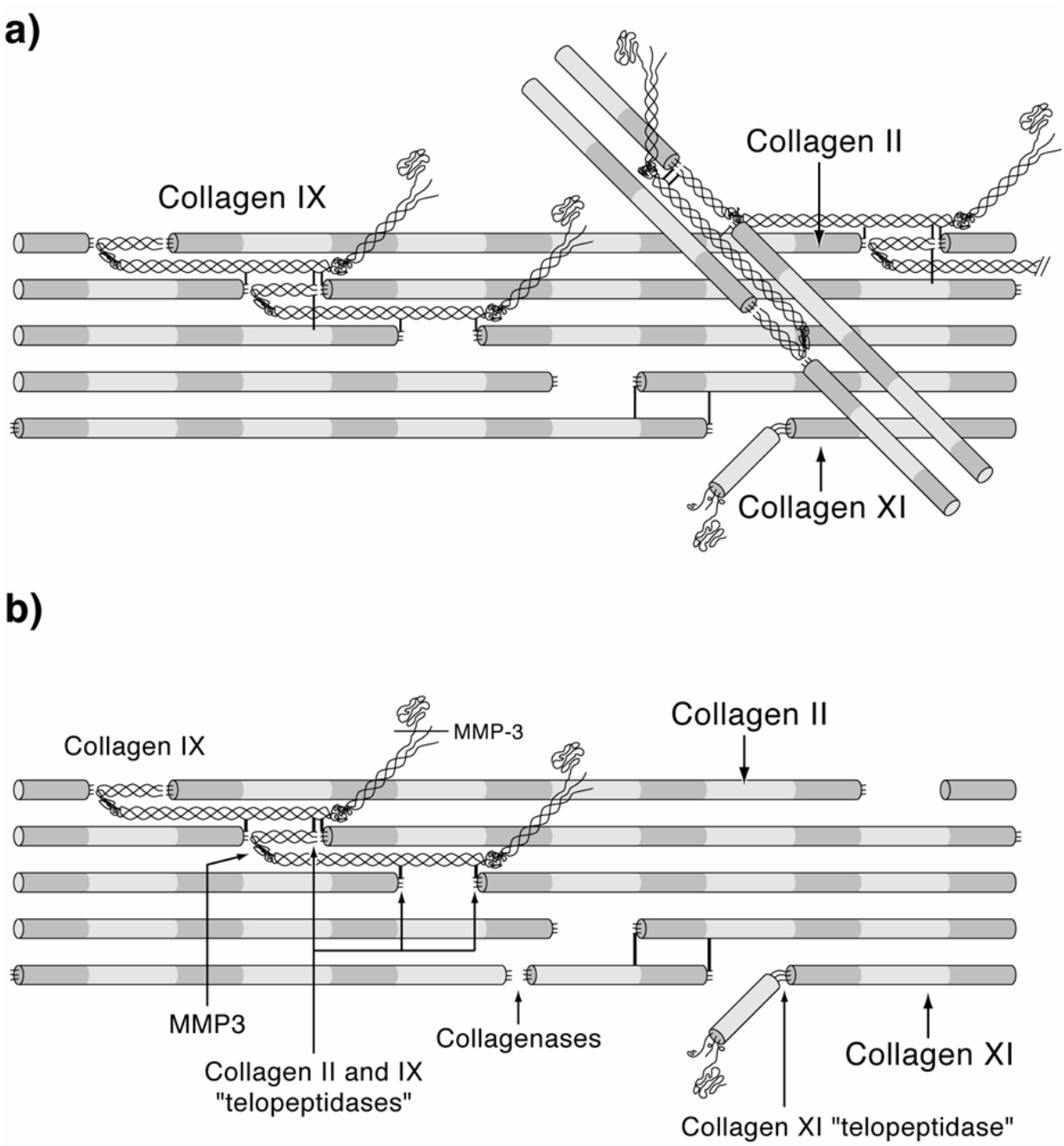

Fig. 2 The collagen II/IX/XI heterofibril. (a) An interaction model between surface collagen IX molecules and the collagen II polymer that can accommodate all known IX-to-IX and IX-to-II cross-links and potential interfibrillar cross-links. (b) Known and speculated sites of peptide bond cleavage in the heteropolymer required for degradation and/or lateral growth of fibrils

TABLE II. Molecular Isoforms of Type V/XI Collagen in Different Tissues.

Hyaline Cartilage	α1(XI)α2(XI)α3(XI), α1(XI)α1(V)α3(XI)	(Eyre and Wu, 1987)
Bone, Tendon	$[\alpha1(V)]_2\alpha2(V)$, α1(V)α1(XI)α2(V)	(Niyibizi and Eyre, 1989)
Vitreous humor	$[\alpha1(XI)]_2\alpha2(V)$	(Mayne *et al*., 1993)
Rhabdomyosarcoma		(Kleman *et al*., 1992)

providing a mechanism for fibril maturation and remodelling. These findings can be interpreted if collagen XI forms a head-to-tail self-cross-linked filamentous template that initiates the growth of collagen II fibrils (Wu and Eyre, 1995).

Table II shows three examples of differential chain usage from the collagen V/XI family of gene products. The molecular variants appear to be associated with tissue-specific forms of fibril organization.

Deposition of type III collagen, cross-linked to type II collagen, in adult human articular cartilage

Collagen type III is consistently detected by immunofluorescence in samples of normal and osteoarthritic human articular cartilage (Aigner *et al*., 1993; Wotton and Duance, 1994; Fig. 3). By transmission electron microscopy, type III collagen was found co localized with type II collagen in the same banded fibrils and retaining its N-propeptide domain (Young *et al*., 2000). In another study of osteoarthritic cartilage, collagen III tended to be concentrated in the superficial and upper middle zones and to be synthesized by the chondrocytes in the absence of collagen I expression (Aigner *et al*., 1993).

Using an antibody that specifically recognizes the C-telopeptide domain of type II collagen and Western blot analysis (Fig. 4), we could show that the pool of type III collagen extractable by pepsin from adult human and bovine articular cartilage included molecules that had been covalently linked to type II collagen in the tissue. The pool from 5-yr bovine articular cartilage had to be enriched by molecular sieve chromatography in order to be seen on SDS-PAGE by Coomassie Blue staining (Fig. 4, left panel). By isolating cross-linked peptides and determining their structure by sequence analysis and mass spectrometry, we established that type III collagen is copolymerized and linked to collagen II in adult human articular cartilage in significant amounts (Fig. 5) (Wu *et al*., 1994). It is tempting

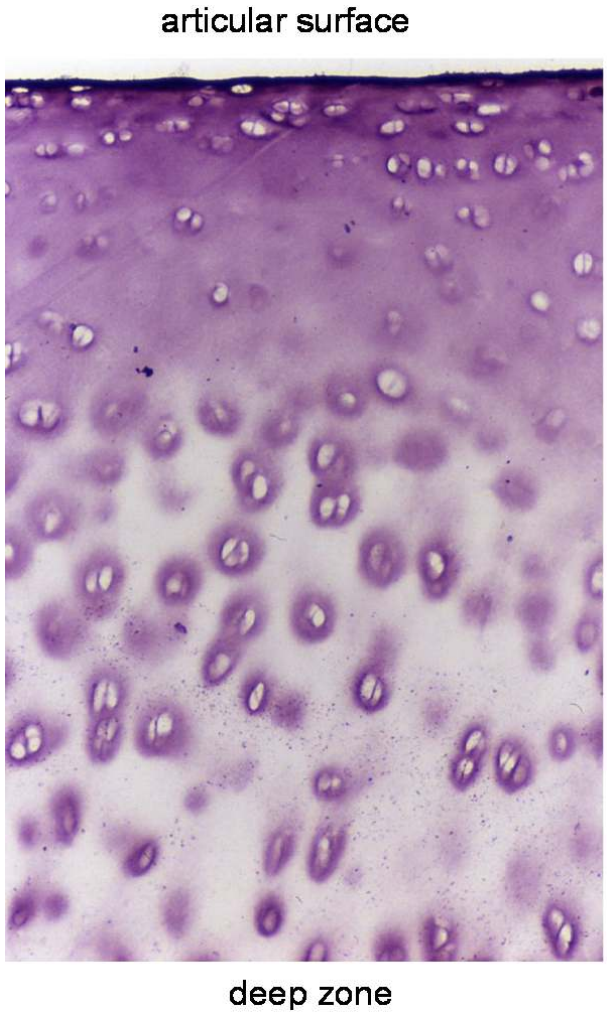

Fig. 3 Immunochemical localization of type II collagen in an intact articular cartilage region from the knee joint of a 59 yr old female with familial OA. mAb 4G9 specifically sees the N-propeptide of collagen type III, which is concentrated in the surface zone and around chondrocytes at depth

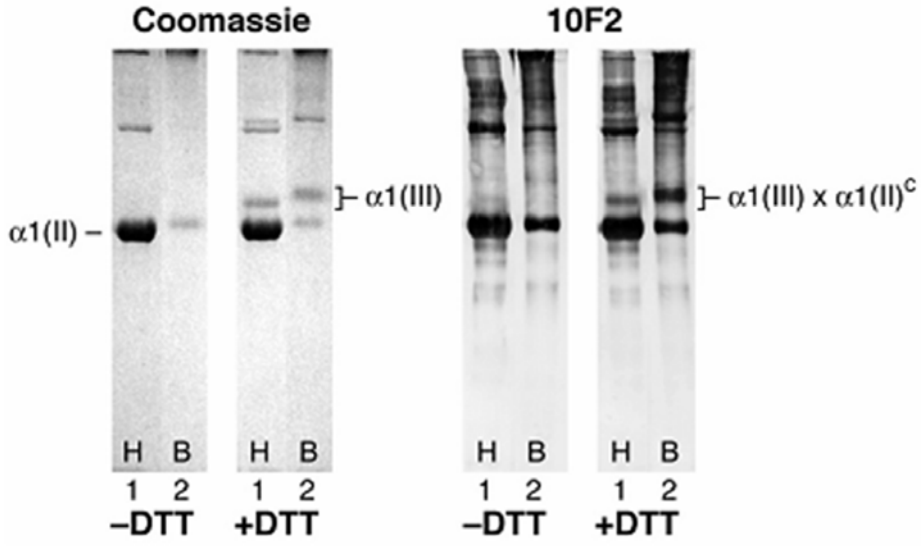

Fig. 4 SDS-PAGE/Western blot analysis of type III collagen from mature human and bovine (5-yr cow) articular cartilage using a type II collagen C-telopeptide-specific antibody (10F2). mAb 10F2 recognizes a pepsin-generated neoepitope in the cleaved C-telopeptide from type II collagen linked to an α1(III) chain.

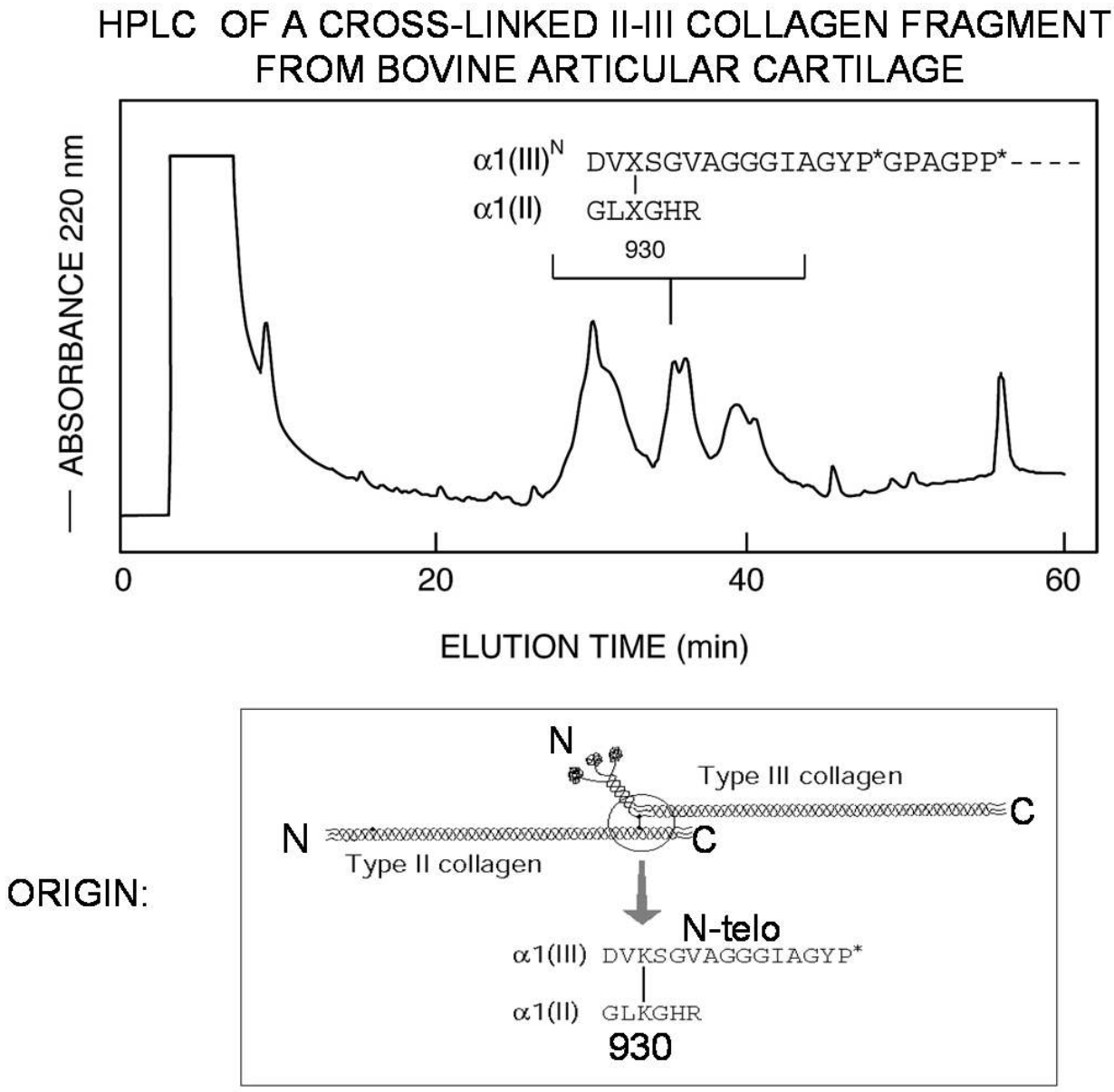

Fig. 5 Isolation and preliminary identification of a cross-linked peptide from a site of interaction between type III collagen and type II collagen from bovine articular cartilage. The collagen III N-telopeptide is linked to the residue 930 site in type II collagen by a divalent lysyl oxidase pathway cross-link.

to speculate that collagen III is made by chondrocytes in addition to collagen II in response to matrix damage akin to the wound-healing role of collagen III in type I collagen-based tissues.

Collagen turnover

After skeletal growth has ceased, the rate of type II collagen synthesis by articular chondrocytes drops dramatically as assessed by proline labelling *in vivo*. In the adult tissue, however, some synthesis continues, and this can be accelerated up to 10-fold within 2 weeks after joint injury, for example after anterior cruciate ligament section in the mature dog (Eyre *et al*., 1980). Little is known of synthetic rates of the other collagen types in adult articular cartilage. Observations based on the synthetic rate of hydroxyproline indicate very little turnover of the collagenous component of the matrix as a whole, with an estimated turnover time of 400 years for human femoral head cartilage (Maroudas, 1979). This still leaves the possibility that a sub fraction of the collagenous matrix (e.g., fibril surface molecules and the pericellular domain) is remodelled more rapidly by chondrocytes in response to mechanical and molecular signals. If the bulk of the collagen mass, which is embodied in the thicker, mature fibrils of the interterritorial matrix, persists in maturity without turnover, then the average turnover rate of the collagen as a whole would still be very slow. Indeed, the mean diameter of banded collagen fibrils in mature human articular cartilage increases with age (Lane and Weiss, 1975), consistent with this remodelling concept.

Mechanisms of collagen degradation

Tissue sites of proteolysis and denaturation of matrix type II collagen can be observed in normal and osteoarthritic joint surfaces (Poole *et al*., 1995) using specific antibodies. The classical concept of collagen fibril degradation is

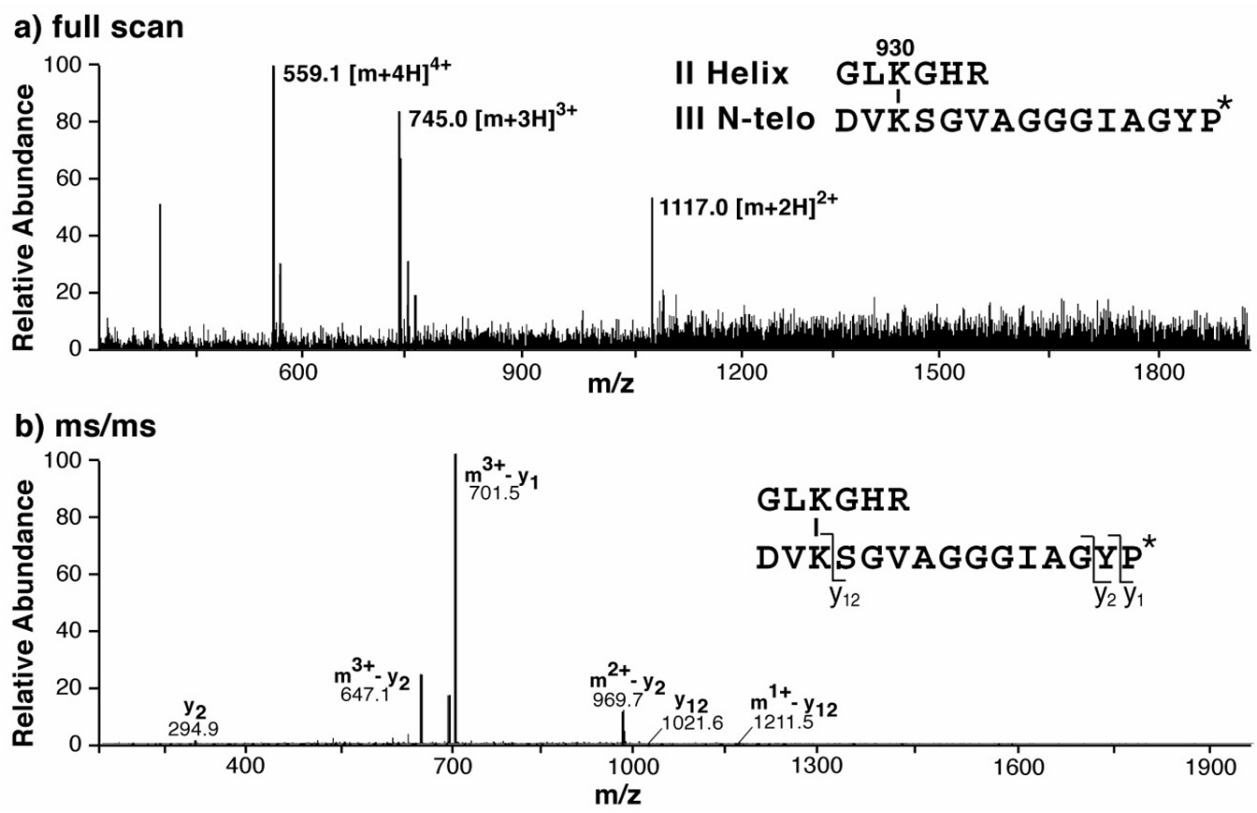

Fig. 6 Mass spectral confirmation of the structure of the collagen II-III cross-linked peptide after further proteolysis by bacterial collagenase.

through an initial cleavage of the collagen molecule (type I, II or III) by collagenase into three-quarter and one-quarter length fragments. Articular chondrocytes can express collagenases, including collagenase-3 (MMP13) (which is the most active in cleaving type II collagen), as demonstrated in culture under interleukin-1 stimulation or directly in tissue removed from arthritic joints (Murphy *et al*., 1999). This enzyme therefore is implicated in the breakdown of cartilage collagen in osteoarthritis. Of the growing number of matrix metalloproteinases that may contribute to matrix protein metabolism (Krane *et al*., 1996), the collagenases are perhaps the best understood in terms of the natural substrate. However, an essential role for collagenases in all forms of collagen breakdown and turnover is becoming less certain. For instance, in mice genetically engineered to express type I collagen lacking a functional cleavage sequence at the three-quarter site, no phenotype was evident at birth. Only later did mild skin thickening and uterine fibroses develop, implying that alternative degradation mechanisms not requiring the three-quarter cleavage can provide for essentially normal development, growth and remodelling of most collagen type I-based tissues (Eyre and Wu, 1987).

Clinical significance

Knowledge of the molecular structure of the collagenous framework of articular cartilage and how it grows, remodels and matures through its various pericellular, territorial, and interterritorial domains is essential for understanding mechanisms of its breakdown in disease. The extent to which chondrocytes can remodel this cross-linked polymer in the adult joint and whether pericellular/territorial collagen is the primary target of such remodelling or the remote interterritorial matrix as well, are open questions. Accelerated synthesis (and breakdown) of collagen is evident in OA and begins within hours after joint injury, but needs to be understood better in terms of where new deposition occurs in the microarchitecture of the matrix and in molecular detail. If increased collagen synthesis is directed at the deposition of new fibrils having the high proportion of collagen types IX and XI seen in developing cartilage, then control mechanisms of gene expression and protein assembly in the adult may be more susceptible to the effects of genetic variations (polymorphisms) than during skeletogenesis. Osteoarthritic disease progression might be particularly rapid in individuals with a genotype that compromises the ability of mature chondrocytes to repair and remodel their matrix after joint trauma.

The covalent addition of type III collagen to the polymeric matrix of articular cartilage of adult human joints suggests an active remodelling process. It occurs in both normal and osteoarthritic joints, but whether it signals pathological events or a healthy repair mechanism is not known. In considering tissue engineering strategies and the feasibility of promoting articular cartilage repair or formation *de novo* in an adult joint, the complex collagen architecture and unique molecular phenotype are clearly a challenge to recreate and a candidate benchmark of success.

Acknowledgments

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