Collagen XI Nucleates Self-assembly and Limits Lateral Growth of Cartilage Fibrils*

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Fibrils of embryonic cartilage are heterotypic alloys formed by collagens II, IX, and XI and have a uniform diameter of ~20 nm. The molecular basis of this lateral growth control is poorly understood. Collagen II subjected to fibril formation in vitro produced short and tapered tactoids with strong D-periodic banding. The maximal width of these tactoids varied over a broad range. By contrast, authentic mixtures of collagens II, IX, and XI yielded long and weakly banded fibrils, which, strikingly, had a uniform width of about 20 nm. The same was true for mixtures of collagens II and XI lacking collagen IX as long as the molar excess of collagen II was less than 8-fold. At higher ratios, the proteins assembled into tactoids coexisting with cartilage-like fibrils. Therefore, diameter control is an inherent property of appropriate mixtures of collagens II and XI. Collagen IX is not essential for this feature but strongly increases the efficiency of fibril formation. Therefore, this protein may be an important stabilizing factor of cartilage fibrils.

In vertebrates, hyaline cartilage occurs in specialized regions of the skeleton and comprises the structural tissue of other organs (e.g. the cartilaginous rings of the trachea or the avian scela). In the skeleton, cartilaginous regions either are permanent (e.g. in articular joints) or form a transient tissue template during bone development. The main functions of hyaline cartilage are biomechanical in nature and, essentially, are performed by its extracellular matrix, which occupies the major fraction of the tissue volume. Two matrix suprastructures can easily be distinguished in cartilage by electron microscopy. First are the collagen-containing fibrils with a periodic banding pattern of D = 67 nm. The other component is the electronlucent extrabrillar matrix, which is rich in immobilized anionic charges. Despite a high degree of molecular organization of its major components, aggrecan and hyaluronan, the extrabrillar cartilage matrix has no conspicuous morphological features. However, the anionic charges cause extensive binding of water, which results in the generation of substantial osmotic pressure. The fibrils serve as the essential tensile elements of the tissue and contain the swelling pressure exerted by the extrabrillar matrix.

Cartilage fibrils vary in their molecular organization, their width, and their orientation in the tissue in order to resist forces generated by external load. In adult articular cartilage, for example, thin fibrils near the joint cavity preferentially run parallel to the surface, since lateral forces predominate in this region. In the interterritorial regions of the deep zones, in contrast, wider fibrils are arranged perpendicularly to the surface to strengthen the tissue in the direction along the axis of the bones. A prominent feature of the fibrils in developing and in immature cartilage is their strictly uniform diameter of about 20 nm and their more random orientation.

Cartilage fibrils are often referred to as collagen II fibrils, since this protein primarily occurs in cartilage, where it is an abundant structural component. The biochemical composition of cartilage fibrils, however, is not only far more complex but also is nonuniform. Molecular heterogeneity correlates well with morphology and functional diversity. Thinner fibrils, such as those of fetal hyaline cartilage, contain not only collagen II but also collagen XI (1, 2) as well as collagen IX (3–5), which can occur in proteoglycan form carrying a single dermatan sulfate chain. In adult articular cartilage, wider fibrils arise in which collagen IX-proteoglycan is less abundant or even lacking. Instead, this type of cartilage fibril is coated with decorin, another proteoglycan, which is substituted by a single glycosaminoglycan chain in mammals but in chickens can carry two dermatan sulfate chains (6). Thus, cartilage fibrils have a highly anionic surface, albeit more stably so if they contain covalently cross-linked collagen IX (7, 8). Decorin, in contrast, is easily removed from the fibril surface by treatment with chaotropic agents at moderate concentrations (9), suggesting that decorin may be subject to greater turnover in the tissue than collagen IX.

The biogenesis of the molecular, suprastructural, and functional complexity in cartilage fibrils is incompletely understood. Collagen IX and the cartilage version of collagen XI, a heterotrimer composed of a1(XI)-, a2(XI)-, and a3(XI)-chains, are almost specific for cartilage. Other molecules, such as decorin, are far more ubiquitous. The role of each constituent has been tracked by several approaches. Skeletal development was severely compromised by overexpression of normal collagen II in mice. In young animals with this genotype, growth cartilage was highly disorganized and, notably, contained abnormally wide fibrils with a strong banding pattern. This pointed toward a crucial importance of the correct molar proportions of cartilage collagens in fibrillogenesis (10). A similar disorganization of cartilage matrix with uncharacteristically

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thick fibrils was seen in the cho/cho strain of mice (11, 12). These animals are deficient in the expression of α1(XI)-polypeptides and their cartilage contained abnormal fibrils with impaired diameter control. Comparable cartilage abnormalities were caused by point mutations in the human genes COL11A1 and COL11A2 in several families with Stickler syndrome, a severe form of chondrodysplasia (13). The mutations resulted in amino acid substitutions for glycine residues in the α1(XI)- or α2(XI)-chains, respectively, that, in turn, led to an absence or a functional incompetence of collagen XI. An exon-skipping mutation in the COL11A2 gene, presumably causing production of shortened α2(XI)-chains, had similar consequences (14, 15). The exclusive association of collagen XI with thin cartilage fibrils was observed by immunoelectron microscopy (2). Thus, several lines of circumstantial evidence suggest that collagen XI is essential for the regulation of the lateral fibril growth (12). Direct evidence for this concept came from studies of in vitro fibrillogenesis by mixtures of soluble cartilage collagens (16). It was found that collagen XI was essential in restricting lateral growth to a uniform width of about 20 nm typical for fibrils of embryonic or immature cartilage. However, it remained open whether collagen XI is sufficient for this limitation. Here, we have answered this question. We have purified collagens II and XI from chick embryo chondrocyte cultures in their native and fibril competent state and have demonstrated that uniform 20-nm fibrils can be reconstituted in vitro from solutions containing appropriate mixtures of the two collagens alone. In addition, we have gained further insight into how lateral growth beyond 20 nm may be controlled by two collagens alone. In addition, we have gained further insight into how lateral growth beyond 20 nm may be controlled by two collagens alone.

MATERIALS AND METHODS

Cell Culture—Sternal chondrocytes from 17-day-old chick embryos were isolated and cultured as described (17). Briefly, sterna from 17-day-old chick embryos were digested overnight with bacterial collagenase. Matrix-free chondrocytes were collected by centrifugation and embedded at a density of 2–3 × 10^6 cells/ml into gels containing 0.5% low melting agarose and Dulbecco’s modified Eagle’s medium. The medium that collagen XI is essential for the regulation of the lateral copy (2). Thus, several lines of circumstantial evidence suggest in vitro demonstrated that uniform 20-nm fibrils can be reconstituted into how lateral growth beyond 20 nm may be controlled by two collagens alone. In addition, we have gained further insight into how lateral growth beyond 20 nm may be controlled by two collagens alone.

Purification of Collagens—Aliquots containing 1–3 µg of mixed collagens were diluted with 50 µl of 0.1 M HCl and digested with 0.1 µg of pepsin at 4 °C for 24 h. Samples were neutralized with 1 M NaOH, and concentrated SDS-PAGE1 sample buffer was added to give a final concentration of 2% SDS. Collagens were separated by SDS-PAGE in 4.5–15% polyacrylamide gradient gels. After electrophoretic separation, the gels were stained with Coomassie Blue, and a densitometric quantification was performed on an Omni Media XRS scanner (MWG-Biotech, Ebersberg, Germany) and the Whole Band Analyzer image software (Bio Image™ version 3.2; MWG-Biotech). Collagen concentrations were calibrated on each gel by running in parallel standard samples of pepsin-digested, purified chicken collagen II with known concentrations as determined by circular dichroism at 221 nm (8,500 degrees·cm²/dmol (18)). The quantities of collagens II and IX in the gels were estimated by adding the relative intensities of the α1(II)- and the α1(XI)-band, respectively, assuming similar staining intensity for all collagen chains. Within the accuracy of the procedures used, relative mass and molar concentrations are equivalent for collagens II and XI.

RESULTS

Purification of Collagens—Collagen IX was purified from an authentic mixture of cartilage collagens by ion exchange chromatography on DEAE-cellulose (Fig. 1). The protein (fraction B) still contained small amounts of α1(II)-chains as judged by SDS-PAGE after staining with Coomassie Blue (Fig. 1B, lanes 2 and 3). Pure collagen II does not bind to DEAE-cellulose under the conditions applied. Therefore, the contaminant α1(II)-chains could be derived from triple helical collagen II-molecules in which at least one chain is covalently bound to collagen IX (7, 8, 19), thereby liberating free α1(II) chains after denaturation in the presence of SDS. β-Components, i.e. dimers of covalently cross-linked collagen polypeptides, were apparent in SDS-PAGE after reduction of the disulfide bonds of collagen IX (Fig. 1B, lane 4). Thus, covalent cross-linking was a likely explanation for our inability to eliminate the residual collagen II under conditions preserving the native and fibril-competent structure of the proteins. The chemistry of the cross-linking is not easily explained, since lysyl oxidase activity should have been effectively inhibited by 60 µg/ml of the lathyrogen β-aminopropionitrile in the culture medium. However, substantially

1 The abbreviation used is: PAGE, polyacrylamide gel electrophoresis.
more collagen could be extracted from cultures treated with the lathyrogen. Further, the quantity of β-components in crude collagen preparations was drastically reduced when β-aminopropionitrile was added to the culture medium. Increasing this supplement up to 100 μg/ml was tolerated by the chondrocytes but did not further reduce cross-linking. Thus the single experiment described below that involved collagen IX was performed with the partially purified preparation (fraction B).

The other cartilage collagens, i.e. types II and XI, were purified to essential homogeneity by subjecting crude collagen mixtures to a scheme of consecutive steps of ion exchange chromatography as outlined in Fig. 1A. This led to preparations of native collagens II and XI that were not only free of other collagen types (Fig. 1B, lanes 2 and 5) but also retained their competence to undergo fibrillogenesis in vitro (see below). Even at concentrations above 4 mg/ml, the proteins did not contain proteoglycans or glycosaminoglycans detectable by standard procedures using dimethylene blue. In this way, contamination by glycosaminoglycans at levels of 1 part per 1000 would have been detected (20).

Collagen XI appeared as several bands on SDS-PAGE (Fig. 1B, lane 5). However, this electrophoretic pattern was converted to a single heterotrimer of α1(XI)-, α2(XI)-, and α3(XI)-polypeptides upon treatment with pepsin (Fig. 1B, lane 7), which results in the removal of the entire amino-terminal propeptide domain. During and after incorporation into cartilage fibrils in situ, collagen XI undergoes several successive steps of proteolytic processing within the amino-terminal propeptide domain (21–24) and also occurs in several variants due to alternative splicing (25, 26), resulting in several matrix forms of the protein. Interestingly, the α3(XI)-polypeptide of our purified collagen XI preparation was distinctly less mobile in SDS-PAGE than the α1(II)-chain (Fig. 1B, compare lanes 2 and 5), although the two polypeptides are products of the same gene (27). This suggested that the amino-terminal propeptide domain was retained in α3(XI)-chains but not in α1(II)-chains. As expected, therefore, most of the difference in electrophoretic mobility between α1(II)- and α3(XI)-chains was eliminated by pepsin-treatment. The small remaining difference presumably is due to the more extensive post-translational modification of collagen XI. Therefore, the material was suitable for fibril reconstitution experiments in vitro, since it included several, if not all, states of processing as they occur in the newly deposited matrix in chondrocyte cultures in agarose.

In Vitro Fibrillogenesis—Native collagens IX or XI were soluble at concentrations adequate for fibrillogenesis experiments neither in dilute acids nor in neutral buffers with a quasiphysiological composition. However, the proteins were well solubilized in 100 mM Tris HCl, pH 7.4, containing 400 mM NaCl (storage buffer). Their final concentrations were 1–2 mg/ml as determined by densitometric analysis of SDS-PAGE patterns after pepsin digestion. Samples of pepsin-treated collagen II, at concentrations determined by circular dichroism spectroscopy (18), were used as standards. The solutions in storage buffer did not contain aggregated collagens observable by electron microscopy after negative staining (not shown). In vitro fibrillogenesis was initiated by diluting appropriate mixtures of collagen solutions in storage buffer with equal volumes of distilled water and by warming the reaction mixtures to 37 °C. Aggregation was followed by monitoring turbidity at 313 nm. At given intervals, the newly formed fibrillar aggregates were examined by electron microscopy after negative staining.

Collagen II—Native rat or chicken collagens II at concentrations above 300–400 μg/ml (16, 28, 29) or more than 50–100 μg/ml of human recombinant collagen II (30, 31) have been shown to aggregate at 30–38 °C, accompanied by an increase in turbidity of the reconstitution mixtures. In our hands (Fig. 2), collagen II solutions gave rise to turbidity development at concentrations above 100 μg/ml, but the final values of turbidity were not easily determined, since the aggregates tended to precipitate from the solutions rather than to yield uniform gels. Characteristically, however, a prominent lag phase of about 2 h occurred in which neither light-scattering gels nor aggregate structures recognizable in the electron microscope were formed. The morphology of the aggregates emerging after the lag period was profoundly different from that of chick embryo cartilage fibrils. Instead of the unbanded, cylindrical objects formed with the partially purified preparation (fraction B), flowchart representation of the protocol for purification of cartilage collagens from chondrocyte cultures in agarose. The sample run in lane 1 represents the crude mixture of collagens extracted with neutral buffer containing 1 mM NaCl. The samples in the other lanes correspond to the purified fractions specified in panel A. The proteins in lanes 6 and 7 were subjected to limited digestion with pepsin prior to electrophoresis.
(Fig. 3A) and maximal diameters of up to 2 μm. No diameter control was apparent. The tactoids also displayed a strong D-periodic banding with a pattern characteristic for fibrils of collagen (32). The fine structure in the banding pattern persisted throughout the tactoids, indicating that the polarity of the collagen II molecules within the tactoids was uniform.

Native Mixtures of Collagens II, IX, and XI—Heterotypic fibrils from chick embryo sternal cartilage contain collagens II, IX, and XI in molar proportions of 8:1:1 (1), and fibrils in the newly formed extracellular matrix of our chondrocyte cultures are closely similar in appearance and collagen composition (33). Therefore, the unfractionated mixture of cartilage collagens II, IX, and XI extracted from the cultures was subjected to in vitro fibrillogenesis. In such mixtures, turbidity developed without a lag period and reached half-maximal values after $t_{1/2} = 5 \times 10^{-14}$ min, regardless of total collagen concentrations (Fig. 4A). As reported earlier (16), thin and faintly banded fibrils were apparent in the electron microscope that strikingly resembled authentic cartilage fibrils (Fig. 3B). Lateral growth appeared to be tightly regulated, which resulted in uniform fibrils of 21.3 ± 0.6 nm ($n = 225$) width. This value agrees with our previous results (19.7 ± 4.7 nm (16)) and is only slightly larger than the value seen in authentic cartilage fibrils (16.7 ± 1.1 nm (5)). Thus, either collagen IX or collagen XI, or both, seemed to be essential for the stringent diameter control.

Collagen IX—Solutions of collagen IX alone generated neither turbidity increases nor aggregate structures discernible in the electron microscope (not shown).

Collagen XI—Collagen XI alone, like native collagen mix-
Mixtures of Collagens II and XI—In different sets of experiments, the concentration of one collagen type was kept constant while the other collagen concentration was varied. The amount of collagen II in the reconstitution mixtures was 10–14 min; see above). The final plateau levels of turbidity were increased by the addition of collagen II when the quantity of collagen XI was kept constant and as long as $\theta_{\text{II}}/\text{XI}$ increases above a value of 8. For the molar ratios $\theta_{\text{II}}/\text{XI} = 8, 18,$ and 64, the concentrations of collagen XI were 60, 20, and 7.5 $\mu$g/ml, respectively. Note that biphasic characteristics comprising a hyperbolic and a sigmoidal phase gradually appear as $\theta_{\text{II}}/\text{XI}$ increases above a value of 8. The inset shows hyperbolic turbidity development at early time points for $\theta_{\text{II}}/\text{XI} = 64$. B and C, plateau values of turbidity at the end of the hyperbolic phase ($t = 40$ min). D and E, final plateau values of turbidity at the end of the experiment. Note that final turbidity values in E decrease as the total collagen concentration increases from 490 to 540 $\mu$g/ml due to the higher intensity of light scattering elicited by tactoids.
in a linear fashion (Fig. 6D). In striking analogy to the two phases of turbidity development, the aggregates eventually formed had two types of morphology. Spindle-shaped, strongly banded tactoids resembling those formed by collagen II alone coexisted with cartilage-like fibrils having a shape similar to those produced by reaction mixtures with higher collagen XI contents (Fig. 7A). This could be explained as an independent aggregation of collagens II and XI, with the two proteins giving rise to tactoids and fibrils, respectively. Due to their large mass-to-length ratio, tactoids formed by collagen II alone would contribute proportionally more to the turbidity development in the second phase of the aggregation. This notion was unlikely, however, for the following reasons. It was frequently observed that the two shapes were contiguous within the same aggregate (Fig. 7B). Thus, if the two collagens indeed gave rise to type-specific suprastructures, these could be joined directly by inconspicuous transition regions that probably comprised both proteins. Furthermore, the addition of increasing amounts of collagen XI to a constant amount of collagen II resulted in a decrease in final turbidity values, although the overall concentration of collagens increased (Fig. 6E). This can best be explained by the concept that the increasing amounts of collagen XI recruit proportionally more of the collagen II into weakly scattering heterotypic fibrils. For \( f_{II/IX} < 12 \), a smooth transition occurred between the two types of turbidity curves.

Fibrillogenesis of a mixture with \( f_{II/IX} = 18 \) was analyzed in detail, since, under these conditions, the two phases of turbidity evolution were well resolved in time (Fig. 6A). Examination by electron microscopy of the shapes of aggregates formed during the early hyperbolic phase (when solutions of pure collagen II showed no evidence of aggregation) clearly revealed a coexistence of tactoids and cartilage-like fibrils (Fig. 7). The maximal width of the tactoids was 60–70 nm after 25 min, i.e. well before the initiation of the second phase of turbidity increase (Fig. 6A). The width then gradually increased, became more heterogeneous, and often exceeded 1 \( \mu m \) at \( t = 300 \) min when turbidity had reached its final plateau level. These observations are entirely inconsistent with the notion of independent suprastructures formed by the two collagen types and, instead, demonstrate that both tactoids and cartilage-like fibrils developing from mixtures of collagens II and XI were heterotypic. To determine whether the transition range of \( f_{II/IX} < 12 \) depended on the absolute quantities of collagen XI, experiments were conducted with mixtures containing constant amounts of collagen XI and variable quantities of collagen II. At all concentrations of collagen XI, the results were closely comparable with those described above (not shown).

From these observations, we concluded that fibrillogenesis of mixtures of the two collagen types was determined by collagen XI in a saturable manner. At 8-fold or lower excesses of collagen II over collagen XI, the lateral growth of the fibrils was tightly controlled in that the formation of tactoids did not occur.

Collagen solutions were used rapidly for fibril reconstitution experiments, since their competence to form fibrils was lost after storage at 4 °C for more than 1 week or after freezing and thawing. This was not due to noticeable degradation by contaminant proteinases, since the fresh and the stored proteins produced indistinguishable electrophoretic patterns, although loss of telopeptides from collagen II would not readily be detected by SDS-PAGE. Similarly, no conspicuous differences were detected when the collagen preparations were examined by electron microscopy after rotary shadowing (not shown). This phenomenon remains unexplained but is consistent with the observation by Lee (34) that purified collagen I lost its capacity to form fibrils upon storage even in the frozen state at \(-20 \) or \(-80 \) °C. Interestingly, however, this “aging” of collagens II and XI could be reversed by the addition of collagen IX in a dose-dependent manner. As judged by turbidimetry (Fig. 8), the competence to form fibrils was fully restored when the fraction of collagen IX reached 10\%, i.e. the amount present in the original collagen mixtures.

**DISCUSSION**

As a salient feature of many tissues, shape control in collagen containing fibrils is essential for tissue and organ functions. In the corneal stroma, for example, fibrils have a very small and strictly uniform width as a pivotal prerequisite for the unhindered passage of visible light. Fibril diameters are similarly restricted in embryonic cartilage, but upon alteration of the biomechanical requirements during postnatal life, the thickness of fibrils may become more heterogeneous (e.g. in the interterritorial matrix regions of mammalian joint or growth plate cartilage). The structure of heterotypic collagen assemblies is well studied in fibrils from these two tissues (35–37), but the mechanisms underlying lateral growth control still are poorly understood for collagen containing fibrils in general. An attractive hypothesis has been that processing of procollagens
by two metalloproteinases, the procollagen N- and/or C-proteinases, is a crucial element of the determination of fibril shapes (38–42). Apposition of collagen molecules to nascent fibrils is prevented by propeptides of partially processed or unprocessed procollagen molecules incorporated into the fibril surface. Therefore, an opportunity arises for the procollagen N- and/or C-proteinases to release lateral growth restriction by proteolytic removal of the propeptides. However, in many tissues, including cornea and cartilage, the quantitatively major collagens I and II, respectively, occur as fully processed molecules. By contrast, the minor collagens V and XI, respectively, retain their amino-terminal extensions as functional components throughout the life span of the fibrils. Thus, while procollagen proteinases may well be a transient element of growth control, it remains unclear how shapes are permanently determined by the enzymes. In this study, we have shown that self-assembly in vitro of the tissue forms of collagens II and XI is a tightly regulated process. Thus, procollagen proteinases are essential for fibrillogenesis in cartilage as a part of the cellular machinery supplying molecular fibril subunits at adequate rates. They are, however, not directly involved in lateral growth control, which is a property of the self-assembly by the processed collagens II and XI themselves. Corneal fibrils are similar in shape to those of embryonic cartilage but contain the homologous collagens I and V. However, mixtures of these collagen types do not yield fibrils of strictly defined thickness, although lateral growth appears to be reduced in heterotypic fibrils formed by collagens I and V (43).

As described previously (28–31, 44), collagen II could be reconstituted in vitro into aggregates sharing important characteristics with collagen-containing fibrils. The longitudinal packing of collagen molecules appeared to resemble that of authentic cartilage fibrils and resulted in a strong D-periodic banding pattern. Unlike cartilage fibrils, however, collagen II-aggregates were relatively short and had an elongated rhomboid shape. Lateral growth control was completely absent, since the maximal width of the aggregates was highly variable. Because of their patent suprastructural dissimilarities, we have designated the collagen II aggregates as tactoids rather than as fibrils.

Earlier studies (28–30) also have reported the formation of thin, nonstriated fibrils at early stages of the assembly process. Here, thin fibrils were only observed in the presence of collagen XI. The reasons for this discrepancy are not obvious, but it could be due to the presence of trace amounts of collagen XI in collagen II preparations (28, 29) and/or the use of different buffer conditions, which also applies to the studies of Fertala et al. (30, 31) on fibril reconstitution by recombinant collagen II. The relative insolubility of collagen XI, either in dilute acids or at neutral pH and physiological ionic strength, precluded direct comparison with other buffer conditions.

The introduction of more than 10% of collagen XI into the reconstitution mixtures caused profound suprastructural changes in the products formed. They became conspicuously similar to fibrils of embryonic cartilage in that they displayed a uniform diameter of about 20 nm and a weak banding pattern. They also became very long to the extent that their ends were rarely observable in the electron microscope. Thus, the presence of collagen XI appeared to be sufficient for a stringent diameter control in fibrillogenesis of cartilage collagens.

When the weight proportion of collagen II exceeded 90%, the lateral growth control was gradually lost. Scarcity of collagen XI did not result, however, in a generalized increase in width of the reconstitution products but, instead, led to a striking suprastructural heterogeneity. An increasing number of tactoids coexisted with fibrils, and in many cases, the two types of suprastructures were contiguous. Thus, tactoids and fibrils reconstituted from collagen mixtures represent different types of suprastructures (i.e. biological alloys in which the manner of lateral packing depends on the molecular composition). The decision on the type of suprastructure to be formed by any given mixture of collagens II and XI takes place early in fibrillogenesis during the lag phase of the turbidity development. By contrast, reconstitution of collagen II alone resulted in a concomitant evolution of tactoids and turbidity, corroborating the differences in the suprastructures of tactoids formed by collagen II alone and by the mixtures of collagens II and XI, respectively.

Authentic cartilage fibrils with a uniform diameter of 20 nm contain not only collagens II and XI but also a third collagenous component (i.e. collagen IX). However, based on the data presented here, participation of collagen IX or other macromolecular fibril components, such as decorin (9), is not required to achieve the morphology of prototypic 20-nm cartilage fibrils. This makes collagen IX and decorin seemingly optional components of cartilage fibrils that are dispensable for fibrillogenesis. At first glance, this conclusion appears to be supported by the phenotypic consequences of several genetic mutations in transgenic animals or in patients with genetic skeletal disorders. Collagen XI genes appear to be important for skeletal morphogenesis (12). By contrast, decorin seems to be dispensable, since decorin-null mice exhibit no conspicuous abnormalities in their skeleton (45).

Our observations lead us to propose a model of heterotypic collagen II/XI fibrils as biological alloys that may help to visualize the structural requirements of lateral growth control. The model (Fig. 9) is based on current knowledge of collagen I fibril structure (49), which indicates that molecules are concentrically arranged. As molecules within a fibril are staggered by integral multiples of $D$, it is convenient to consider each molecule as made up of 4.5 D-length segments (Fig. 9A). In this way, the relative axial positions of molecules can be identified in the fibril cross-section (Fig. 9B). Following the model of Chapman (50), when the number of molecules in a fibril cross-section is 270, one-fifth of these (i.e. 54) can be surface-located. In a fibril composed entirely of collagen XI, such an arrangement would allow all of the bulky N-terminal regions to be exposed on the fibril surface, thereby maximizing close packing and stereochemically blocking further accretion. We show here that heterotypic fibrils are limited to diameters of approximately 20 nm when formed from molar ratios of collagen II/XI of up to 8:1.
This suggests that the bulky N-terminal domain of collagen XI is large enough to cover eight adjacent collagen II molecules on the fibril surface, thereby preventing further growth in diameter. The conformation of the N-terminal domain of mature collagen XI is unknown, although the presence of the NC2 triple-helical region ensures a minimum length of 25 nm, which is more than sufficient to straddle eight molecular widths. In the model shown in Fig. 9, when the collagen II/XI molar ratio exceeds 8:1, not all accretion sites would be blocked, thus permitting further diameter growth.

In contrast to fibrils from immature cartilage, fibrils of adult joint cartilage are homogeneous in diameter and composition (9). The latter tissues also contain reduced quantities of minor collagens, consistent with our finding reported here that at least 10% of collagen XI was required to restrict the diameters of reconstituted fibrils to 20 nm. We postulate that the control of lateral fibril growth is an intrinsic property of appropriate collagen mixtures not only during fibrillogenesis in vitro but also in cartilage tissue in vivo. The question then arises as to how the prototypic fibrils mature into the wider fibrils of the territorial zone of cartilage matrix. Conceivably, large banded fibrils could arise by accretion of collagen molecules onto 20 nm fibrils or by fusion of such fibrils. Evidence for fusion in situ may be seen in electron micrographs showing small fibrils merging into larger banded structures (51). Interestingly, the fibrils formed by collagen II/XI mixtures (e.g. those shown in Fig. 3D) displayed a conspicuous propensity to coalesce in register into larger banded fibrils. This action could potentially be regulated by the presence of collagen IX on the surface of fibrils, which is consistent with the reduced content of collagen IX in mature fibrils.

Larger fibrils are distinctly different from tactoids formed by mixtures with a large collagen II content. This necessitates that macromolecules other than collagens II and XI are required to allow formation of authentic fibrillar alloys in cartilage. This is supported by our observations that well banded, large cartilage fibrils contain decorin (9). Altered expression levels of genes for these and other matrix macromolecules are likely to result in defects in the fibrillar organization in cartilage underlining cartilage disorders, including osteoarthritic degeneration.

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