Developmental regulation of mRNA species for types II, IX and XI collagens during mouse embryogenesis

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INTRODUCTION

Hyaline cartilage contains a network of collagen fibrils that provide structural strength to the tissue and entrap the proteoglycan molecules responsible for furnishing cartilage with resilience [1,2]. The fibrils form a multicomponent network where type II collagen, a homotrimer of $\alpha 1$(II), is the major component, and types IX and XI are minor components. Type XI collagen is a heterotrimeric fibrillar collagen that in cartilage contains $\alpha 1$(XI), $\alpha 2$(XI) and $\alpha 3$(XI) chains in a 1:1:1 ratio [2]. The $\alpha 1$(XI) and $\alpha 2$(XI) chains are products of COL11A1 and COL11A2 genes, whereas the $\alpha 3$(XI) chain is a product of the COL2A1 gene coding also for the type II collagen chains [2]. Type IX collagen, a heterotrimer of $\alpha 1$(IX), $\alpha 2$(IX) and $\alpha 3$(IX) chains, is a fibril-associated collagen attached laterally on the fibril surface [3] through covalent cross-links to type II and type XI collagens [4]. Small proteoglycans, decorin and fibromodulin, as well as cartilage matrix protein, have also been shown to interact with collagen fibrils in cartilage [2]. Interestingly the collagen fibrils in the vitreous and cornea of the eye share with cartilage a similar composition of collagen types II, IX and XI, except that the $\alpha 2$(V) chain replaces the $\alpha 2$(XI) chain and only one of the two alternative forms of the $\alpha 1$(IX) collagen chains is present [2,5,6].

The function of type IX collagen remains unknown. Its structure and location on the fibril surface have led to suggestions that type IX collagen has an important role in regulating the diameter of cartilage collagen fibrils and/or in mediating the interactions between the collagen network and proteoglycans. However, transgenic mice carrying a homozygous knock-out mutation of the $\alpha 1$(IX) gene have not been reported to exhibit any abnormalities in skeletal morphogenesis and growth, suggesting that type IX collagen is not essential for the formation of skeletal elements [7]. Development of early osteoarthritic lesions in these mice would instead suggest a structural function for type IX collagen in maintaining mechanical stability in articular cartilage. Osteoarthritic lesions and abnormally thin, randomly oriented collagen fibrils were observed in mice harbouring cDNA-based Col9a1 transgenes with a large deletion mutation [8]. Recent transgenic mice lacking type IX collagen have been implicated in the regulation of collagen fibril diameter in cartilage [9]. Taken together, the results available suggest that a correct balance of types II, IX and XI collagens, which in embryonic chick cartilage is 8:1:1 [3], is important for the maintenance of the correct fibril diameter.

Although much is known about the composition of cartilage collagen fibrils, the regulatory mechanisms controlling their synthesis are poorly understood. Maintenance of a correct balance of types II, IX and XI collagen molecules for fibrillogenesis presents interesting questions concerning the regulation of the corresponding genes (COL2A1, COL9A1, COL9A2, COL9A3, COL11A1 and COL11A2), which reside on four different chromosomes [10]. Molecular biological characterization of the type IX collagen has focused on the chick system because cDNA clones have been available for all three chains. Lack of cDNA or genomic clones for mammalian $\alpha 3$(IX) collagen has prevented systematic analyses of the regulation of type IX collagen gene expression in mammalian species. We have previously used reverse transcriptase-PCR (RT-PCR) technology to construct short cDNA clones for mouse and human $\alpha 1$(IX) and $\alpha 2$(IX) collagen mRNA species [11-13]. Here we report the sequences of the cDNA clones for the mouse and human $\alpha 3$(IX) collagens and mouse pro$\alpha$(XI) collagen. These and other clones were used for the determination of cartilage-specific collagen mRNA species and their ratios during mouse development.
**EXPERIMENTAL**

**RNA extraction, cDNA synthesis and amplification by PCR**

Total RNA species were extracted from several newborn mouse tissues including rib and epiphyseal cartilages, from mouse embryos at 9.5, 10.5, 12.5, 14.5, 16.5 and 18.5 days of development, and from human fetal cartilage, with the guanidinium isothiocyanate method [14]. Random hexamers and oligo(dT) were used to prime reverse transcription of 1 µg of total RNA by Moloney Murine Leukaemia Virus reverse transcriptase under conditions recommended by the supplier (Gibco BRL). Aliquots of cDNA were used for amplification by PCR (AmpliTaq®; Perkin Elmer) with oligonucleotide primers MP-23 [5'-GA(G/A)CAACA(C/T)AT(C/T)AGAGA(G/A)CT-3'] and MP-24 [5'-GCA(A/G)GCTGATGTGTCACA-3']. The reactions were cycled through denaturation at 94 °C for 1 min, annealing at 50 °C for 2 min, and extension at 72 °C for 2 min. After 30 amplification cycles, aliquots of the reactions were fractionated on 1.5 % (w/v) agarose gels and the specific fragments were purified and cloned by blunt-end ligation into the EcoRI site of the Bluescript KS® (Stratagene) vector. The cloned segments were sequenced with the Sanger dye-deoxy method (Sequenase* reagent kit; U.S. Biochemical).

**Northern hybridization**

For Northern hybridization 10 µg of total RNA from mouse embryos were denatured with formamide, subjected to electrophoresis on a 0.8 % (w/v) agarose gel and transferred to Hybond nylon membranes in 10 × SSC [1 × SSC is 0.15 M NaCl/0.015 M sodium citrate (pH 7.0)]. Membranes were hybridized with 32P-labelled cDNA probes in 5 × SSC/1 % Denhardt’s solution/50 % (w/v) formamide/0.1 % (w/v) SDS/250 µg/ml denatured calf thymus DNA at 42 °C overnight. Membranes were washed at high stringency and autoradiographed with intensifying screens at −70 °C.

**RT–PCR analysis**

Total RNA extracted from several tissues of newborn mice was used as templates for cDNA synthesis as described above. Reverse transcription reaction mixtures (5 µl) were used for PCR reactions for z1(IX), z2(IX) and z3(IX) with the primers complementary to the non-collagenous NC1 and NC2 domains of each chain under conditions also described above. Reaction products were subjected to electrophoresis on 1.5 % (w/v) agarose gels.

**RNase protection assay**

Linearized cDNA probes for z1(IX), z2(IX) and z3(IX) collagens were used as templates in transcription reactions with T3 or T7 RNA polymerase (Promega) and [α-32P]UTP (800 Ci/mmol; Amersham) as the labelled nucleotide. Full-length transcripts were purified on a denaturing polyacrylamide gel. Dried RNA samples (10 µg) were dissolved in 10 µl of hybridization buffer [80 % (v/v) formamide/40 mM Pipes (pH 6.4)/400 mM NaCl/1 mM EDTA] containing (2–5) × 106 c.p.m. of the cRNA probe. Hybridization was performed at 55 °C overnight, after which the samples were incubated for 1 h at 37 °C with 300 µl of digestion mixture (300 mM sodium acetate/5 mM EDTA/12 µg/ml RNase A/30 units/ml RNase T1). The digestion was terminated by adding 50 µg of protease K and 3.5 µl of 20 % (w/v) SDS followed by incubation for 15 min at 37 °C, phenol/chloroform (1:1, v/v) extractions and ethanol precipitation. The precipitates were dissolved, denatured and subjected to electrophoresis on an 8 M urea/4.5 % polyacrylamide gels. Dried gels were subjected to autoradiography at −70 °C for 1–8 days.

**Slot-blot analysis**

For RNA slot-blot analysis duplicate filters were prepared containing dilution series of cDNA inserts for z1(IX), z2(IX), z3(IX), z1(XI) and z1(II) collagens and total RNA species from newborn mouse limb cartilage and from 12.5-day and 16.5-day embryos. Yeast tRNA was also blotted as a negative control. Samples were blotted on Hybond N+ (Amersham) filters with a vacuum manifold (Schleicher & Schuell). The filters were hybridized with each of the probes under the same conditions as described above. Densitometric analyses of the hybridization signals of the dilution series were performed on an Ultrascan XL densitometer (LKB) to measure the molar amount of each mRNA in the RNA samples.

**Histology and hybridization in situ**

For histological examination and hybridization in situ the dissected limbs from mouse embryos were fixed in 4 % (v/v) formalin in PBS at room temperature overnight, or in 4 % (w/v) paraformaldehyde in PBS at 4 °C for 3–19 h, dehydrated in a graded ethanol series and embedded in paraffin for sectioning. Details of the in situ hybridization technique used have been published earlier [15]. The paraffin sections were pretreated with proteinase K (3.5 µg/ml for 30 min at 37 °C) and acetylated. The hybridizations were performed at 52 °C for 18 h with sense and anti-sense cRNA probes prepared as above except that [32P]Uridine 5'-[α-thio]triphosphate was used as the radiolabelled nucleotide. Hybridization was followed by washes and autoradiography for 2.5–14 days and by staining of the sections with haematoxylin.

**Hybridization probes**

The following mouse cDNA clones were used as hybridization probes: pMColl1a1-1 for proz1(I) collagen, pMColl2a1-1 for proz1(II) collagen, pMColl3a1-1 for proz1(III) collagen, pMColl9a1-1 for z1(IX) collagen [11], pMColl9a2-1 for z2(IX) collagen, pMColl10a1-1 for z1(X) collagen [12], pMColl9a3-1 for z3(IX) collagen and pMColl11a1-1 for z1(XI) collagen. For detection of the long form of type II collagen mRNA a genomic subclone containing exon 2 was used as the probe [16].

**RESULTS**

**cDNA cloning of mouse and human z3(IX) collagen mRNA species**

Two oligonucleotide primers (MP23 and MP24) were designed for the non-collagenous NC1 and NC2 domains of the z3 chain of the type IX collagen based on the available chick Col9a3 cDNA and bovine amino acid sequences [4,17–19]. The same degenerate oligonucleotides were used for the amplification of both mouse and human z3(IX) collagen cDNA covering sequences coding for the collagenous (COL) domain COL1 and parts of the non-collagenous domains NC1 and NC2. A sequence comparison of the 438 bp clones pMColl9a3 (mouse) and pHCOL9A3 (human) revealed nucleotide and amino acid sequence identities of 93 % and 82 % respectively between mouse and human (Figure 1). The length of the COL1 domain in mouse and human z3(IX) chains is 112 residues, as in the chick z3(IX)
Figure 1 Deduced amino acid sequences of clones pMCol9a3-1 and pHCOL9A3-1 for the mouse and human α3(IX) collagens respectively.

MO, deduced amino acid sequence of the mouse α3(IX) collagen clone and the corresponding human (HU) and chick (CH) sequences shown only at the positions where they differ from the mouse sequence. The oligonucleotides used for PCR amplification are underlined. The asterisk denotes Ala at residue 55 indicates Ala→Thr polymorphism detected in the human sequences analysed.

Nucleotide sequence of the mouse α1(XI) collagen cDNA

The 562 bp mouse α1(XI) collagen cDNA clone encoding a 3’ region of the triple helical domain was obtained accidentally in an RT–PCR reaction. The clone was discovered during the sequencing of putative RT–PCR clones for the 5’ end of the mouse α2(IX) collagen cDNA. The oligonucleotide MH-12 (5’-CTGGCCCTTGACTCCAGG-3’) for mouse α2(IX) collagen seemed to prime the amplification of the α1(XI) collagen cDNA from both the 3’ and the 5’ directions. Comparison of the mouse α1(XI) collagen sequence with the corresponding human sequence revealed an amino acid identity of 93% and nucleotide identity of 85%. Amino acid identity with the chick gene is 94% and nucleotide identity 79%. After clone pMCol11a1 had been sequenced and the data deposited in EMBL/GenBank, Yoshioka et al. [21] published the entire coding sequence of the mouse Col11a1 cDNA. In comparison with our cDNA fragment this sequence contains six nucleotide differences that result in two amino acid changes (results not shown).

Expression of genes for cartilage-specific collagens in developing mouse embryos

Specific probes for types I, II, III, IX, X and XI collagen chains were first used to study how their expression is co-ordinated at the mRNA level. Northern analysis of total RNA isolated from 9.5–18.5-day mouse embryos and from newborn mice (Figures 2 and 3) revealed several different expression patterns. The mRNA levels for the three major fibrillar collagens (types I, II and III) increased rapidly from day 10.5 to day 14.5. Thereafter the levels of type I and II collagen mRNA remained relatively constant, but type II collagen mRNA continued to increase. The mRNA level of proα1(IX) collagen paralleled that of proα1(II) collagen, whereas those for the α1(IX), α2(IX) and α3(IX) chains reached their maximal level earlier than type II and type XI collagen mRNA species. Very little mRNA for the α1(X) collagen was detected until day 14.5, after which a rapid increase in the expression of this gene was observed, peaking at day 16.5. On extended exposure, the Northern analyses exhibited low levels of proα1(IX) mRNA for mouse α3(IX) collagen. The expression of the α1(IX) collagen paralleled that of proα1(IX), whereas those for the α3(IX) and α2(IX) collagens paralleled that of proα1(II) collagen. The α1(IX) and α2(IX) collagen mRNAs were first used to study how their expression is co-ordinated at the mRNA level. Northern analysis of total RNA isolated from 9.5–18.5-day mouse embryos and from newborn mice (Figures 2 and 3) revealed several different expression patterns. The mRNA levels for the three major fibrillar collagens (types I, II and III) increased rapidly from day 10.5 to day 14.5. Thereafter the levels of type I and II collagen mRNA remained relatively constant, but type II collagen mRNA continued to increase. The mRNA level of proα1(IX) collagen paralleled that of proα1(II) collagen, whereas those for the α1(IX), α2(IX) and α3(IX) chains reached their maximal level earlier than type II and type XI collagen mRNA species. Very little mRNA for the α1(X) collagen was detected until day 14.5, after which a rapid increase in the expression of this gene was observed, peaking at day 16.5. On extended exposure, the Northern analyses exhibited low levels of type II, IX and XI collagen mRNA species as early as day 9.5.

The two forms of the α1(IX) collagen mRNA arising from alternative promoters were developmentally regulated: the shorter approx. 3 kb α1(IX) mRNA appeared with type II collagen as early as day 9.5 and peaked at day 12.5, whereas the longer approx. 4 kb mRNA peaked at days 14.5–16.5. The size difference of the two forms of α1(IX) collagen mRNA allowed us to directly determine their ratio at the different time points (Figure 3F), whereas the two alternatively spliced proα1(II) collagen mRNA species could not be separated in Northern blots. The probe pMCol2a1-1 used for Figures 2(C) and 3(C) detected both the long and the short forms of proα1(II) collagen mRNA. The long form of proα1(II) collagen mRNA was detected...
Figure 3 Developmental expression of cartilage-specific collagen genes in mouse embryos

Graphs summarizing the densitometric analysis of several exposures of Northern blots (Figure 2). The panels show the levels of mRNA species for (A) pro\(\alpha\)1(I) collagen, (B) pro\(\alpha\)1(III) collagen, (C) pro\(\alpha\)1(II) collagen, (D) long form of pro\(\alpha\)1(II) collagen containing exon 2 sequences, (E) long form of \(\alpha\)1(IX) collagen containing the large NC4 domain (\(\wedge\)) and short form of \(\alpha\)1(IX) collagen without the large NC4 domain (\(\wedge\)), (F) \(\alpha\)2(IX) collagen, (G) \(\alpha\)3(IX) collagen, (H) \(\alpha\)1(X) collagen and (I) pro\(\alpha\)1(XI) collagen from embryonic day 9.5 to newborn. All values have been corrected for variations in RNA loading (levels of 28 S rRNA). (J) Short form of \(\alpha\)1(IX) collagen mRNA as a percentage of the total \(\alpha\)1(IX) collagen mRNA.

RT–PCR and RNase protection assays

We wished next to analyse the tissue distribution of the three type IX collagen mRNA species in different newborn mouse tissues by both RT–PCR and RNase protection assays (Figures 4 and 5). For RT–PCR, specific primers for the NC1 and NC2 domains of each chain were used. Approximately equal amounts of PCR products for \(\alpha\)1(IX), \(\alpha\)2(IX) and \(\alpha\)3(IX) collagens were obtained from total RNA prepared from cartilage and the eye. From brain RNA, PCR products were detected only for \(\alpha\)2 and \(\alpha\)3 chains, but not for \(\alpha\)1 chain. No expression of type IX collagen chains was detected in skin, kidney, muscle or liver with this technique (Figure 4).

To verify the tissue distribution of the three chains of type IX collagen, RNase protection analysis was performed (Figure 5). As well as in cartilage and the eye, low levels of type IX collagen
**Figure 5** Detection of $\alpha_1$(IX), $\alpha_2$(IX) and $\alpha_3$(IX) mRNA species in mouse tissues by RNase protection assay

Total RNA (10 $\mu$g) isolated from cartilage (lanes C), brain (lanes B), kidney (lanes K), eye (lanes E), skin (lanes S) and liver (lanes L) were hybridized with the cRNA probes for the $\alpha_1$(IX), $\alpha_2$(IX) and $\alpha_3$(IX) collagens. Protected fragments were subjected to electrophoresis on 8 M urea/4.5% (w/v) polyacrylamide gels with end-labelled MspI fragments of pBR322 as standards (lanes st). The 524 bp $\alpha_1$(IX) probe was protected as a 444 bp fragment, the 506 bp $\alpha_2$(IX) probe as a 444 bp and the 513 bp $\alpha_3$(IX) probe as a 437 bp fragment. Undigested probes (lanes p) were run on the gel, as were the probes hybridized with yeast tRNA (lanes t). The gel was exposed for 6 days (A) or 1 day (B).

**Figure 6** Quantification of mRNA levels of type II, type IX and type XI collagen mRNA species

Duplicate hybridization filters containing dilution series of cDNA inserts for $\alpha_1$(IX), $\alpha_2$(IX), $\alpha_3$(IX), $\alpha_1$(XI) and $\alpha_1$(II) collagens (from top to bottom: $5 \times 10^{-17}$, $10^{-17}$, $5 \times 10^{-18}$, $10^{-19}$, $5 \times 10^{-19}$, $10^{-18}$ and $5 \times 10^{-20}$ mol of each of the inserts) were prepared. Total RNA species from newborn mouse cartilage and from 12.5-day and 16.5-day embryos (from top to bottom: newborn, 12.5-day and 16.5-day) were blotted on the same filters. Similar amounts of yeast tRNA were also blotted as a control. The blotting arrangement is shown in (A). The figure shows one of the filters after consecutive hybridizations with the probes for the $\alpha_1$(IX) (B), $\alpha_2$(IX) (C), $\alpha_3$(IX) (D), $\alpha_1$(XI) (E) and $\alpha_1$(II) (F) collagen mRNA species.
Table 1 Quantification of the α1(IX), α2(IX), α3(IX), α1(XI) and α1(II) collagen mRNA species

The results are given in 10^{-19} mol/µg of total RNA as calculated from densitometric analyses of the slot-blot hybridizations of total RNA species extracted from mouse embryos at days 12.5 and 14.5 of development, and from limbs from newborn mice.

<table>
<thead>
<tr>
<th>mRNA</th>
<th>Day 12.5</th>
<th>Day 16.5</th>
<th>Newborn</th>
</tr>
</thead>
<tbody>
<tr>
<td>α1(IX)</td>
<td>4.2</td>
<td>7.7</td>
<td>12</td>
</tr>
<tr>
<td>α2(IX)</td>
<td>3.3</td>
<td>2.8</td>
<td>11</td>
</tr>
<tr>
<td>α3(IX)</td>
<td>4.4</td>
<td>8.2</td>
<td>13</td>
</tr>
<tr>
<td>α1(XI)</td>
<td>2.4</td>
<td>3.1</td>
<td>11</td>
</tr>
<tr>
<td>α1(II)</td>
<td>68</td>
<td>120</td>
<td>380</td>
</tr>
</tbody>
</table>

species for cartilage collagens are assumed, the following ratios would be predicted for the type II/type IX/type XI collagens produced: 5.7:1:0.6 at day 12.5, 6.4:1:0.5 at day 16.5, and 10.6:1:0.9 in the newborn mouse limbs.

Localization of type IX collagen transcripts in mouse embryos by hybridization in situ

As a final approach to analysing the co-ordination of α1(IX), α2(IX) and α3(IX) collagen mRNA species, hybridizations of mouse limbs were performed in situ at 12.5, 14.5, 16.5 and 18.5 days of embryonic development. During initial chondrogenesis the expression patterns of the three mRNA species seemed identical in the cartilaginous anlage of bones (results not shown).

On subsequent endochondral ossification the distribution within the chondrocytes of epiphyseal growth plates remained similar; however, the mRNA species for the α1(IX) and α3(IX) collagens, but not that for the α2(IX) collagen mRNA, were also detected in peristeal cells (Figure 7).

DISCUSSION

In the present study we have examined the expression of genes coding for the constituent chains of types II, IX and XI collagens by determining their mRNA levels during mouse embryogenesis with several different techniques. The results indicate that the ratio of type II/type IX/type XI collagen mRNA species varies during embryonic development, whereas the three mRNA species coding for the α1, α2 and α3 chains of type IX collagen exhibit considerable co-ordination in cartilage but not in other tissues. In addition, the results reveal the extraskeletal expression of type IX collagen mRNA species during embryonic development analogous to the other two ‘cartilage-specific’ collagen types II and XI.

Non-co-ordinated expression of types II, IX and XI collagen mRNA species

We first used several hybridization techniques to determine the expression patterns and molar ratios of types II, IX and XI collagen mRNA species during mouse embryonic development (Figure 6 and Table 1). Assuming equal translational efficiency of the different mRNA species we extrapolated the ratio of types II, IX and XI collagens produced in newborn mouse limb cartilages to be 10.6:1:0.9, which is relatively close to that
Co-ordinated regulation of type IX collagen mRNA species in cartilage

Because each type IX collagen molecule is a heterotrimer of \(\alpha_1(IX), \alpha_2(IX)\) and \(\alpha_3(IX)\) chains, the synthesis of these molecules presents interesting questions about their co-ordinated regulation to yield a relatively constant mRNA ratio of 1:1:1. The gene for the human \(\alpha_1(IX)\) collagen (COL9A1) has been mapped to chromosome 6q12–q13 [31], whereas gene COL9A2 is localized in chromosome 1p32.3–p33 [32] and COL9A3 in chromosome 20q13.3 [20]. In mouse, the \(\alpha_1(IX)\) gene (Col9a1) has been mapped to chromosome 1 [31] and the gene for the \(\alpha_2(IX)\) to chromosome 4 [32]. The chromosomal localization of the mouse \(\alpha_3(IX)\) collagen chain remains unknown. Unfortunately the \(\text{Col9a2}\) gene is at present the only gene for which a mammalian promoter sequence is known [33]. Thus no comparisons or functional analyses between regulatory elements can be performed. Although the availability of new cDNA probes makes it possible to measure transcript levels of these genes in different tissues and developmental stages, functional analyses of the promoters are needed to understand how the type IX collagen genes are regulated.

Hybridization analyses in situ revealed that the mRNA species for \(\alpha_1(IX), \alpha_2(IX), \alpha_3(IX)\) collagen mRNA species were very similarly distributed in the cartilaginous anlage of long bones as well as in the epiphyses and growth plates during endochondral ossification. The presence of \(\alpha_1(IX)\) and \(\alpha_3(IX)\) collagen mRNA species in the perichondrium and periesteam of embryonic long bones was surprising, although the expression of \(\alpha_1(IX)\) collagen mRNA has been shown to be an early phenotypic feature of osteogenic cells in rat calvarial bones [34]. As type IX collagen is believed to exist only as a heterotrimer of three different chains, no protein product would be expected to be secreted by these cells. This suggests that the presence of the \(\alpha_2(IX)\) chains might be a limiting factor for type IX collagen production in some tissues. Interestingly, differential regulation of the three genes coding for type XI collagen has also been observed during mouse embryonal development [35].

Non-chondrogenic expression of type IX collagen mRNA species

In Northern analyses faint hybridization signals of types II, IX and XI collagen mRNA species were seen as early as day 9.5 (Figures 2 and 3). The expression of type II collagen gene has previously been reported in prechondrogenic mesenchyme and in extraskeletal locations during embryonic development [26,36]. In the present study we also detected low levels of type IX collagen transcripts in brain, skin and kidney of newborn mice by RT–PCR and RNase protection assays (Figures 4 and 5). As the ratios of the \(\alpha_1(IX), \alpha_2(IX)\) and \(\alpha_3(IX)\) collagen mRNA species seemed to vary considerably in these tissues it is possible that no type IX collagen protein is actually synthesized in these tissues. Interestingly, transient low-level expression of type II collagen has also been detected in brain, skin and kidney, as well as in several other tissues of mouse embryos [36,37]. Extraskeletal expression of \(\alpha_1(IX)\) collagen mRNA has also been reported in mouse heart [38]. It remains to be determined whether this expression of type IX and other cartilage collagen represents illegitimate transcription or serves some unknown biological function. The expression of the mRNA species for types II, IX and XI collagen in the eye will be reported elsewhere (M. Savontaus, T. Ihnammäki, M. Perala, M. Metsäranta, M. Sandberg-Lalli and E. Vuorio, unpublished work).

Implications for the function of type IX collagen

Molecular cloning of type IX collagen cDNA species and genes forms the basis for studies on their molecular genetic analyses. The genes for type IX collagen are clearly candidate genes in various hereditary diseases affecting cartilage (chondrodysplasias) and degenerative diseases of joints and spine. Recently
the first mutation affecting type IX collagen causing multiple epiphyseal dysplasia (EDM2) was characterized in the α2(IX) collagen gene [39].

Transgenic mice harbouring specific mutations of type IX collagen genes have also been used for defining the role of this collagen type in cartilage and other tissues. A dominant negative mutant of the α1 chain of type IX collagen [8] and α1(IX) gene inactivation in mice [7] are characterized by degenerative joint disease and mild chondrodysplasia. An osteoarthritic phenotype has been observed in transgenic mice harbouring Col9a2 transgenes with a deletion mutation in the COL2 domain (M. Perälä, unpublished work). The availability of molecular probes for all three constituent chains of mouse type IX collagen and the slot-blot technique employed here allow more reliable quantitative analyses of mRNA levels (including statistical analyses) and should be of use for detailed analyses of existing and future transgenic mice and thus direct us to a better understanding of the roles of the individual constituent molecules of cartilage.

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