The mRNAs for the three chains of human collagen type XI are widely distributed but not necessarily co-expressed: implications for homotrimeric, heterotrimeric and heterotypic collagen molecules

Vincent C. H. LUI,* Richard Y. C. KONG,*† John NICHOLLS,† Annie N. Y. CHEUNG† and Kathryn S. E. CHEAH*§

Departments of *Biochemistry and †Pathology, The University of Hong Kong, Sassoon Rd., Hong Kong

In cartilage collagen type XI exists as heterotrimeric molecules composed of $\alpha 1(XI)$, $\alpha 2(XI)$ and $\alpha 3(XI)$ subunits. Messenger RNAs for some of the α chains of collagen type XI have also been found in non-chondrogenic tissues but the chain composition of the molecule in these sites is not known. Some non-chondrogenic tissues also contain heterotrimers containing collagen $\alpha 2(V)$ and $\alpha 1(XI)$ chains. We have explored the possibility that collagen type XI could exist in differing trimeric forms in non-chondrogenic tissues and aimed to predict the subunit composition of this collagen in those tissues. The distribution and relative levels of expression of collagen $\alpha 1(XI)$, $\alpha 2(XI)$ and $\alpha 3(XI)/\alpha 1(II)$ mRNAs in different human fetal tissues were studied. Expression of mRNAs for all three genes of collagen

type XI is not restricted to cartilage but is widespread. However, in some non-chondrogenic tissues, the mRNAs for all three α chains of collagen type XI were not co-expressed, but collagen $\alpha 1(XI)$ and $\alpha 2(XI)$ mRNAs were found either singly or without collagen $\alpha 3(XI)$ transcripts. Collagen type XI may therefore exist as homotrimers and/or heterotrimers composed of two collagen $\alpha(XI)$ chains in some tissues. The distribution of mRNAs for collagen $\alpha 2(V)$ and $\alpha 1(I)$ were also studied. Co-expression of collagen type XI, $\alpha 2(V)$ and $\alpha 1(I)$ mRNAs was found for many tissues. These findings have implications for the possibility of additional chain associations for collagen types XI and V in cross-type heterotrimers within heterotypic fibrils.

INTRODUCTION

The structural integrity and functional properties of the extracellular matrix (ECM) of different tissues is influenced by the presence of characteristic combinations and amounts of various collagen types and other matrix components which are expressed in a developmental and tissue-specific manner [1]. Collagens form supramolecular structures in the ECM such as fibrils, filaments or networks [2] and the 19 genetically distinct types can be sub-classified into various families, the largest of which are the fibril-forming collagens, i.e. types I, II, III, V and XI [3].

The fibrils in the ECM of bones, skin, tendons and ligaments are predominantly heterotypic, containing mainly collagen type I with collagen types III and V as quantitatively minor components [2]. Heterotypic fibrils in cartilage contain collagen type II as the quantitatively major component, with collagen type XI within the centre and collagen type IX decorating the outer surface [2]. It has been proposed that collagen types V and XI play a role in regulating fibrillogenesis and modulating fibril diameter [2,4]. This hypothesis is supported by recent findings that absence of collagen $\alpha 1(XI)$ chains results in abnormally thickened cartilage fibrils in the *cho* mouse mutant [5,6] and a consequence of targeted mutation of the collagen $\alpha 2(V)$ gene in mice is widened, disorganized fibrils [7].

In cartilage, collagen type XI is a heterotrimer composed of $\alpha 1(XI)$, $\alpha 2(XI)$ and $\alpha 3(XI)$ subunits [8]. The collagen $\alpha 1(XI)$ and $\alpha 2(XI)$ chains are genetically distinct products of separate genes

[9,10] while the collagen $\alpha 3(XI)$ chain is an over-glycosylated variant of collagen $\alpha 1(II)$ [11]. Although cartilage is the main site of expression of collagen type XI, recent findings show a more widespread tissue distribution. Collagen $\alpha 1(XI)$ mRNAs have been reported in a number of human cell-lines and non-chondrogenic chicken tissues [9,12–14]. Collagen $\alpha 2(XI)$ transcripts have been detected in a variety of human non-chondrogenic embryonic tissues [15]. The presence of mRNA implies, but does not prove, that the protein is also synthesized. Using immunological and protein extraction methods, collagen $\alpha 1(XI)$ chains have been found in heterotrimers with collagen $\alpha 2(V)$ in bovine bone, vitreous humour and human A204 rhabdomyosarcoma cells [16–18], suggesting that the mRNAs previously detected in these cells are translated. These findings suggest α chains of collagen types V and XI may not be strictly partitioned into trimeric molecules according to type and have implications for the possibility of other chain associations for collagen types XI and V.

The major period of collagen synthesis is during fetal development. Direct protein analyses to test for molecules containing different combinations of collagen $\alpha(XI)$ and $\alpha(V)$ chains in fetuses is hampered by the small amount of tissue available and the lack of antibodies specific for each individual α chain. However, insight into the possible additional chain associations for collagen types V and XI may be obtained by studying the expression pattern of the mRNAs for their α chains. Previous studies of collagen type XI gene expression in non-chondrogenic

Abbreviations used: COL2A1, human collagen $\alpha 1(II)$ gene; COL1A1, human collagen $\alpha 1(I)$ gene; COL5A2, human collagen $\alpha 2(V)$ gene; COL11A1, human collagen $\alpha 1(XI)$ gene; COL11A2, human collagen $\alpha 2(XI)$ gene; Col11a-1, mouse collagen $\alpha 1(XI)$ gene; DMEM, Dulbecco's modified Eagle's medium; ECM, extracellular matrix; FCS, fetal calf serum.

† Present address: Department of Biology and Chemistry, City University of Hong Kong, Hong Kong.

§ To whom correspondence should be addressed.

tissues have focused on one or two of the collagen α (XI) transcripts. The expression pattern of mRNAs for all three collagen type XI chains and for collagen α 2(V) within each different non-chondrogenic tissue has not been reported. In this study, we aimed to test for different combinations of association of the individual α chains of collagen type XI by examining the distribution and levels of expression of mRNAs for collagen α 1(XI), α 2(XI) and α 3(XI)/ α 1(II) in different human fetal tissues. We show that expression of all three collagen type XI genes is not restricted to cartilage but is widespread. However, in some non-chondrogenic tissues, mRNAs for the three α chains of collagen type XI are not co-expressed. To investigate if intertype associations between collagen α 2(V) and other collagen α (XI) chains were possible, the distribution of collagen α 2(V) mRNAs was also studied. In general, the mRNAs for collagen type XI and collagen α 2(V) are co-expressed in non-chondrogenic tissues.

EXPERIMENTAL

Human fetal material

Human fetal tissues were obtained from therapeutic terminations of pregnancy with the approval of the Ethics Committee of the Medical Faculty of Hong Kong University. The gestation age of the fetuses was determined from the crown-rump length [19] and the estimated last menstrual period.

Plasmid probes

Recombinant plasmids used to produce antisense riboprobes for RNase protection assays were as described below.

(1) Collagen α 1(XI): pVL23, a 287 bp *HindIII/EcoRI* subclone of the *COL11A1* cDNA clone OK4 (gift of F. Ramirez, Mount Sinai School of Medicine, New York, U.S.A.), which contained the 3'-untranslated region of *COL11A1* in pBluescript/KS⁻ (Stratagene). RNase protection assays yielded a pair of 287 and 285 nt fragments.

(2) Collagen α 2(XI): pVL25, a 633 bp *PvuII/PstI* subclone containing 113 bp of exon 50 of the *COL11A2* gene subcloned into pKS⁻. RNase protection assays yielded 113–109 nt fragments.

Nucleotide fragments of shorter than expected size obtained for collagen α 1(XI) and α 2(XI) mRNAs respectively, were products of RNase overdigestion, which either disappeared or were significantly reduced in intensity when less enzyme was used.

(3) Collagen α 1(II)/ α 3(XI): probes from *COL2A1* were used since collagen α 3(XI) and α 1(II) chains are the products of the same gene. Plasmid pS5-PH1.2, containing the first exon of *COL2A1*, was used to detect both collagen α 3(XI) and α 1(II) mRNAs. An alternative riboprobe also used, pE7P2R, contained a 390 bp *RsaI* fragment encoding the 3'-untranslated region (exon 52) of the *COL2A1* gene subcloned in pGEM2 (Promega Biotec, U.S.A.).

(4) Collagen α 2(V): pVL31, a 468 bp *EcoRI/PstI* subclone from the human collagen α 2(V) cDNA clone, Hf511 (gift of F. Ramirez), encoding the 3'-untranslated region of *COL5A2* in pKS⁻.

(5) Collagen α 1(I): pVL29 contained a 312 bp *EcoRI-PvuII* cDNA fragment encoding part of the C-propeptide of the *COL1A1* gene in pGEM2 (Promega Biotec).

RNA isolation

Total RNA was prepared from human fetal tissues and primary chondrocyte cultures by the LiCl/urea differential precipitation

method [20]. Primary chondrocyte cultures were prepared from 17-week fetal cartilage by digesting minced tissue with collagenase (1.5 mg/ml) for 16 h in Dulbecco's modified Eagle's medium (DMEM), 10% (v/v) fetal calf serum (FCS) containing antibiotics [1% (w/v) each of penicillin, streptomycin and nystatin]. Dispersed chondrocytes were grown in suspension culture in bacteriological dishes in DMEM with 10% (v/v) FCS, ascorbic acid (100 μ g/ml), β -aminopropionitrile fumarate (2.5 mM) and antibiotics. Total RNA was extracted from chondrocytes after 2 to 3 days in culture.

RNA was also extracted from the following tissues from five human fetuses at 15-, 16-, 17-, 19- and 24-week gestation: (a) 15-week, cartilage, kidney, lung, liver, skeletal muscle, calvaria, frontal lobe of brain, and parietal occipital lobe of brain; (b) 16-week, skin and bone; (c) 17-week, chondrocytes, heart, whole brain, kidney, gut, liver, tendon, skeletal muscle, lung, and spleen; (d) 19-week, cartilage; and (e) 24-week, brain dissected into parietal lobe, frontal lobe, parietal occipital lobe, temporal lobe, meninges, thalamus and caudate nucleus.

RNase protection assays

Syntheses of antisense riboprobes from plasmids and RNase protection assays were carried out as previously described [21]. T7 or T3 RNA polymerase (New England Biolabs) and [α -³²P]UTP (800 Ci/mmol, Amersham International) was used to generate riboprobes for RNase protection assays. Total RNA (20 or 40 μ g) were used in RNase protection assays, the RNase-protected fragments separated on 6% (w/v) polyacrylamide/8 M urea sequencing gels and the protected fragments were detected by autoradiography.

To determine the relative levels of the three collagen type XI mRNAs within a sample, equal amounts (5×10^5 c.p.m. each) of [α -³²P]UTP-labelled specific riboprobes for either collagen α 1(XI) plus α 3(XI) or α 1(XI) plus α 2(XI) were included in the same protection assay. For each set of determinations, autoradiography was for identical periods. An LKB 2202 Ultrascan Laser Densitometer was used for densitometry on autoradiograms and the areas under the absorbance peaks corresponding to the protected fragments were integrated. Since amounts of riboprobe were present in excess, the intensities of the protected RNA fragments, as determined by densitometry, were assumed to be directly proportional to their relative levels after making a correction for the number of protected labelled U's for each probe. Relative amounts of collagen α 1(XI), α 2(XI) and α 3(XI) mRNAs were calculated as follows: amount of collagen α (XI) mRNA = I/N ; where I = intensity of the protected RNA fragment and N = number of U's in the protected fragment.

Since collagen α 1(XI) riboprobe was common in each double riboprobe assay, levels of collagen α 2(XI) and α 3(XI) mRNAs were calculated relative to collagen α 1(XI) mRNA and fractional amounts were multiplied by a factor for clarity of representation. For example, the calculated transcript ratios for collagen α 1(XI): α 2(XI) and collagen α 1(XI): α 3(XI) in kidney were 1.5:1 and 1.5:10 respectively, which is equivalent to mRNA proportions for collagen α 1(XI): α 2(XI): α 3(XI) of 3:2:20.

RESULTS AND DISCUSSION

Tissue distribution of the mRNAs for collagen type XI

As shown in Figures 1 and 2, collagen α 1(XI), α 2(XI) and α 3(XI)/ α 1(II) mRNAs were clearly present in 15-week fetal cartilage and in 17-week isolated chondrocytes. Messenger RNAs encoding the three collagen α (XI) chains were also found in a variety of non-chondrogenic tissues as shown in Figures 1–3 and

Figure 1 Distribution of collagen α (XI), α 2(V) and α 1(I) mRNAs in chondrogenic and non-chondrogenic tissues

RNase protection assays on RNAs from 15 to 19 gestation week fetal tissues. For (a) to (e), 20 μ g of total RNA from 16- and 17-week fetuses were assayed in each track except for chondrocytes (2 μ g). For (f) and (g), 40 μ g of total RNA from a 15-week fetus were assayed in each track except for 19-week fetal cartilage (2 μ g). Yeast tRNA (20 μ g) was included as the no RNA control (N). The protected fragments (arrows) for collagen α 1(XI), α 2(XI), α 3(XI)/ α 1(II), α 2(V) and α 1(I) mRNAs were 285 and 287, 109–113, 140–154, 468 and 312 nt, respectively. Numbers refer to nt. Abbreviations: Ch, chondrocytes; H, heart; Br, brain; Ki, kidney; G, gut; Li, liver; Te, tendon; Mu, skeletal muscle; Lu, lung; Sp, spleen; Sk, skin; Bo, bone; C, cartilage (from 19-week fetus); Ca, calvaria; F, frontal lobe of brain; PO, parietal occipital lobe of brain. Markers (M) were 32 P-labelled *Hpa*II-digested pGEM2. Stage of fetal tissues (weeks gestation) are indicated above the appropriate panels. The low amount of collagen α 1(I) mRNAs detected in the chondrocyte sample may reflect incomplete removal of contaminating perichondrial fibroblasts. Alternatively, partial dedifferentiation could account for the collagen α 1(I) mRNAs in chondrocyte RNA since the differentiated phenotype of cultured chondrocytes is sensitive to culture conditions which can cause expression of collagen type I and a decrease in collagen type-II synthesis [29,30].

summarized in Table 1. Strong hybridization signals were obtained using 2 μ g of total RNA compared with 20–40 μ g of RNA for the other tissues, confirming cartilage was the major site of expression of collagen type-XI mRNAs. Messenger RNAs for the three collagen α (XI) chains were found in combinations which varied between tissues and with developmental stage. Three combinations of collagen α (XI) mRNA expression were found.

First, co-expression of all three collagen α (XI) mRNAs was

Figure 2 Double riboprobe RNase protection assays for estimating relative levels of mRNAs for α chains of collagen type XI in cartilage and non-chondrogenic tissues

Forty micrograms of total RNA was assayed for each track except for cartilage (1 μ g). RNA was hybridized with two antisense riboprobes within each assay (see the Experimental section) as follows: (a) collagen α 1(XI) and α 2(XI); and (b) collagen α 1(XI) and α 3(XI)/ α 1(II). An assay using yeast tRNA (20 μ g) was included as the no RNA control (N). Specific protected fragments are indicated with arrows. Abbreviations are as for Figure 1.

found in many tissues such as 15-week calvaria, kidney, skeletal muscle and brain; 16-week long bone; 17-week tendon, lung and brain; and 24-week brain (Figures 1–3 and Table 1) pointing to the presence of collagen type XI heterotrimers composed of the three α chains in these tissues. Secondly, there was co-expression of only two of the three collagen α (XI) mRNA species in different combinations such as: (a) collagen α 1(XI) with α 3(XI) mRNAs in 15-week lung; (b) collagen α 1(XI) with α 2(XI) mRNAs in parts of 24-week brain; and (c) collagen α 2(XI) with α 3(XI) mRNAs in 17-week kidney (Table 1). These observations point to the presence of heterotrimers containing only two of the collagen α (XI) chains.

Thirdly, expression of only one of the collagen α (XI) mRNAs was found: collagen α 1(XI) mRNAs in 17-week skeletal muscle; collagen α 2(XI) mRNAs in 17-week heart and in parts of 24-week brain; collagen α 3(XI)/ α 1(II) mRNAs in 15-week liver, and in 17-week gut and liver. In these tissues homotrimers of collagen α 1(XI) and α 2(XI) may exist. Since both collagen α 1(II) mRNA and/or protein have been found in non-chondrogenic tissues in avians, *Xenopus*, mouse and man (reviewed in [22] and [23]), sole expression of collagen α 1(II)/ α 3(XI) mRNAs probably reflects the synthesis of collagen type II.

Collagen type XI mRNAs could not be detected in 16-week fetal skin and 17-week gut and spleen (Figure 1). In contrast, co-expression of collagen α 2(XI) and α 3(XI)/ α 1(II) transcripts in the epidermis of developing fetal skin and intestine detected by *in situ* hybridization and RNase protection assays has been reported [15]. Absence of expression in the fetal skin and gut samples studied here cannot be accounted for by RNA degradation since collagen α 2(V) and α 1(I) transcripts were clearly present in the same mRNA preparation (Figure 1). Transcripts for collagen α 3(XI)/ α 1(II) in the frontal and parietal occipital lobes of the brain were present at 15 weeks gestation but absent in these regions at week 24. These findings also differ from the

Figure 3 Distribution of collagen α (XI), α 2(V) and α 1(I) mRNAs in 24-week fetal brain

RNase protection assays on RNA from 24-week brain. Total RNA (40 μ g) was assayed for each track. Yeast tRNA (20 μ g) was included as the no RNA control (N). Sizes of specific protected fragments in nt (arrows) for collagen α 1(XI) and α 2(XI) were as for Figure 1. For collagen α 3(XI)/ α 1(II), a riboprobe for the 3'-untranslated region of *COL2A1* mRNA was used instead, yielding a protected fragment of 390 nt. Abbreviations: P, parietal lobe; F, frontal lobe; PO, parietal occipital lobe; T, temporal lobe; Me, meninges; Th, thalamus; CN, caudate nucleus; M, 32 P-labelled *Hpa*II-digested pGEM2 markers.

recent report where transcripts were detected only in meninges [15].

Transient expression of collagen α 3(XI)/ α 1(II) mRNAs in the developing skin of chick [24] and mouse [22] and in the brain of human and mouse has been reported [22,23,25]. Although the fetal stages of the individual tissues studied by Sandberg et al. [15] were not specified, our different observations could reflect transient expression of collagen type XI during development. Other examples of possible developmentally transient expression of collagen α (XI) mRNAs was found for kidney, lung and skeletal muscle. Transcripts for collagen α 2(XI) were absent in 15-week but present in 17-week lung; collagen α 1(XI) transcripts were absent in 17-week but present in 15-week kidney (Figures 1 and 2; Table 1). In contrast, collagen α 2(XI) and α 3(XI) mRNAs were present in skeletal muscle at week 15 but absent at week 17 (Table 1).

Co-expression of collagen type XI, α 2(V) and α 1(I) mRNAs

Collagen α 2(V) mRNA was found in all non-chondrogenic tissues studied but expression was transient in parts of the brain, being absent in the parietal occipital lobe at 24-week but present at 15-week gestation (Figures 1 and 3). Collagen α 2(V) mRNAs were found with one or more of the collagen type XI mRNAs with the exception of 17-week spleen and 16-week skin in which collagen α 2(V) mRNA was expressed in the absence of collagen α (XI) mRNAs (Figures 1 and 3; Table 1). Collagen α 2(V), α 1(I), and the three α (XI) mRNAs were co-expressed in many non-chondrogenic tissues such as 15-week calvaria, kidney, muscle, and parts of the brain, 16-week bone, 17-week tendon and lung, 24-week meninges and caudate nucleus (Figures 1 and 2 and Table 1). Co-expression of all these mRNAs was transient in some tissues such as lung, kidney, muscle and brain (Table 1).

Relative levels of collagen type XI mRNAs in cartilage

Relative levels for collagen α 1(XI), α 2(XI) and α 3(XI)/ α 1(II) mRNAs were determined in cartilage to test for a correlation with the known chain composition of collagen type XI. However, levels of collagen α 3(XI) and α 1(II) chains are encoded by the same gene, the proportion of *COL2A1* transcripts accounting for collagen types II or XI can only be estimated if the relative amounts and rates of assembly of collagen type II and collagen type XI are known. Both the relative abundance of collagen type XI in human fetal cartilage and the rates of trimer assembly for collagen types II and XI have not been determined. Since post-translational modification of collagen occurs prior to helix formation [26] and collagen α 3(XI) and α 1(II) chains are post-translationally modified to different degrees, rates of assembly of collagen types II and XI are likely to differ. Therefore only the amounts of collagen α 1(XI) to α 2(XI) mRNAs were compared in testing for a correlation between transcript levels and relative proportions of collagen α (XI) chains. However, levels of collagen α 3(XI)/ α 1(II) mRNAs could be used to indicate if collagen type II was also present, if those transcripts were significantly more abundant than collagen α 1(XI) and α 2(XI) mRNAs.

RNase protection analyses using two different riboprobes within a single assay were carried out using cartilage mRNAs (see the Experimental section) and the relative amounts of collagen α 1(XI), α 2(XI) and α 3(XI) mRNAs found to be 1:1:4, respectively (Table 2). The 1:1 ratio of collagen α 1(XI) to α 2(XI) mRNAs found, corresponds to that expected for a heterotrimeric collagen type XI molecule containing collagen α 1(XI), α 2(XI) and α 3(XI) chains [8]. The fourfold higher level for collagen α 3(XI)/ α 1(II) probably reflects the added presence of collagen type II.

Relative amounts of collagen α 1(XI), α 2(XI) and α 3(XI) mRNAs in non-chondrogenic tissues

The relative levels of mRNAs for the three pro- α chains of collagen type XI was determined in non-chondrogenic tissues. With the exception of skeletal muscle, the proportions of the different collagen α (XI) mRNAs relative to each other differed significantly from that found for cartilage (Table 2). In one category one of the collagen α (XI) mRNAs was greatly in excess of the other two transcripts. For example, collagen α 3(XI)/ α 1(II) mRNAs in kidney and lung were greatly in excess (Table 2). In these tissues collagen α 2(XI) mRNAs were either absent (lung) or were approximately equal in level to collagen α 1(XI) mRNA (kidney). In calvaria and brain, levels of collagen α 2(XI) and

Table 1 Summary of expression of collagen α (XI), α 2(V) and α 1(I) mRNAs in chondrogenic and non-chondrogenic tissues in fetuses at different developmental stagesAbbreviations: ND, not determined; ++, strong expression; +, expression; $\pm$, very low level expression; —, no expression.

Developmental stage (weeks)	Fetal tissue	Collagen gene				
		α 1(XI)	α 2(XI)	α 3(XI)/ α 1(II)	α 2(V)	α 1(I)
15	Cartilage	++	++	++	—*	+
	Calvaria	++	+	++	++	++
	Kidney	+	$\pm$	++	++	++
	Lung	++	—	++	++	++
	Liver	—	—	++	++	+
	Skeletal muscle	++	++	++	++	++
	Brain (frontal lobe)	+	$\pm$	$\pm$	+	$\pm$
	Brain (parietal occipital lobe)	+	$\pm$	$\pm$	+	$\pm$
16	Skin	—	—	—	+	+
	Long bone	+	+	++	++	++
17	Cultured chondrocytes	++	++	++	—	$\pm$
	Tendon	++	++	+	++	++
	Whole brain	+	$\pm$	$\pm$	$\pm$	—
	Lung	$\pm$	$\pm$	$\pm$	+	+
	Kidney	—	$\pm$	$\pm$	+	+
	Heart	—	+	—	+	—
	Skeletal muscle	$\pm$	—	—	$\pm$	+
	Spleen	—	—	—	+	—
	Gut	—	—	$\pm$	+	+
	Liver	—	—	+	+	—
	Parietal lobe	—	+	—	—	—
	Frontal lobe	$\pm$	+	—	$\pm$	—
24 (brain only)	Parietal occipital lobe	—	+	—	—	+
	Temporal lobe	$\pm$	+	—	—	$\pm$
	Meninges	++	$\pm$	+	++	++
	Thalamus	+	$\pm$	$\pm$	ND	ND
	Caudate nucleus	+	+	$\pm$	$\pm$	$\pm$

* 19-week fetus.

Table 2 Relative levels of mRNAs for α chains of collagen type XI in cartilage and non-chondrogenic tissuesIntensities of protected fragments shown in Figure 2 were quantified by densitometry and relative levels of the collagen α (XI) mRNAs calculated as described in the Experimental section.

Tissue	Relative amount of collagen mRNAs [α 1(XI): α 2(XI): α 3(XI)/ α 1(II)]
Cartilage	1:1:4
Skeletal muscle	2:1:1
Lung	3:0:10
Kidney	3:2:20
Calvaria	10:1:3
Brain (frontal lobe)	10:1:1
Brain (parietal occipital lobe)	10:2:1

α 3(XI)/ α 1(II) mRNAs were approximately equivalent, but collagen α 1(XI) mRNA was in great excess.

Distribution of collagen type XI and collagen α 2(V) mRNAs: Implications for homotrimeric, heterotrimeric and heterotypic collagen molecules

Our detailed study of the distribution and levels of expression of mRNAs of collagen type-XI and collagen α 2(V) mRNAs in

human fetuses show that these transcripts are widely but not necessarily co-expressed. The varied combinations of co-expression of collagen α (XI) and collagen α 2(V) mRNAs and the marked differences in relative levels of the different transcripts, raise questions whether homotrimeric molecules composed of either collagen α 1(XI) or α 2(XI); or heterotrimeric molecules composed of collagen α 1(XI) and α 2(XI) alone; or additional kinds of heterotypic collagen α 2(V)/ α (XI) molecules exist in the fibrils of non-chondrogenic tissues. It is not known if the translational efficiencies for all three collagen α (XI) mRNAs were equal in these tissues. The relative rates of assembly of homotrimers and heterotrimers are also unknown. But it would be reasonable to suggest that where one of the transcripts was vastly in excess of the other two, homotrimeric molecules as well as heterotrimers could also be present. Direct demonstration that these varied trimeric forms of collagen type XI exist in the ECM awaits development of appropriate tools for detecting and distinguishing between the individual collagen α (XI) chains. Once such tools are available, choice of target tissues for study can be made on the basis of the findings described here. Since fibrils can contain collagen types I, III and V [2,27], and co-expression of collagen α 1(I), α 2(V) and α (XI) mRNAs was found, it would also be important to determine if different trimeric forms of collagen type XI and heterotypic type XI-V collagen are present within fibrils containing collagen type I.

Questions also arise whether collagen α (XI) and α 2(V) chains may be functionally interchangeable in some non-chondrogenic

tissues, in which case loss of one collagen α (XI) chain may be compensated for by another, at least partially. Mutations in the human *COL11A2* gene have recently been shown to cause several autosomal dominant and recessive osteochondrodysplasias [28]. The chondrodysplasia in the *cho* mouse has also been shown to be caused by a mutation in *Coll1a-1* [6]. It is interesting that although collagen type XI is expressed in many non-chondrogenic tissues, apart from the skeletal abnormalities, other defects have not been described for these chondrodysplasias. However, compensation may be partial or possible only in particular tissues, since mutation of the collagen α 2(V) gene causes abnormal fibril assembly in skin and cornea [7]. In this context it is interesting that we could not detect collagen type-XI transcripts in skin. To address these questions it would be important to determine whether changes in the trimeric composition of collagen type XI and V molecules are associated with disorders caused by mutations in the genes for these collagens.

This work was supported by grants from the Arthritis and Rheumatism Council (U.K.), the Hong Kong University Committee for Conference and Research Grants and the Croucher Foundation (Hong Kong). V.C.H.L. is a Croucher Foundation Scholar. We also thank Dr. K. H. Fu for help with the early stages of this work.

REFERENCES

- Adamson, E. D. (1982) in *Collagen in Health and Disease* (Weiss, J. B. and Jayson, M. W., eds.), pp. 218–243. Churchill Livingstone, Edinburgh.
- Van der Rest, M. and Bruckner, P. (1993) *Curr. Opin. Struct. Biol.* **3**, 430–436.
- Kivirikko, K. I. (1993) *Ann. Med.* **25**, 113–126.
- Birk, D. E., Fitch, J. M., Barbiarz, J. P., Doane, K. J. and Linsenmayer, T. F. (1990) *J. Cell Sci.* **95**, 649–657.
- Seegmiller, R. E., Fraser, F. C. and Sheldon, H. (1971) *J. Cell Biol.* **48**, 580–593.
- Li, Y., Lacerda, M. L., Warman, M. L. et al. (1995) *Cell* **80**, 423–430.
- Andrikopoulos, K., Liu, X., Keene, D. R., Jaenisch, R. and Ramirez, F. (1995) *Nature Genet.* **9**, 31–36.
- Burgeson, R. E. and Hollister, D. W. (1979) *Biochem. Biophys. Res. Commun.* **87**, 1124–1131.
- Bernard, M., Yoshioka, H., Rodriguez, E. et al. (1988) *J. Biol. Chem.* **263**, 17159–17166.
- Kimura, T., Cheah, K. S. E., Chan, S. D. H. et al. (1989) *J. Biol. Chem.* **264**, 13910–13916.
- Eyre, D. and Wu, J. J. (1987) in *Structure and Function of Collagen Types* (Mayne, R. and Burgeson, R. E., eds.), pp. 261–281. Academic Press, Orlando.
- Yoshioka, H. and Ramirez, F. (1990) *J. Biol. Chem.* **265**, 6423–6426.
- Brown, K. E., Lawrence, R. and Sonenshein, G. E. (1991) *J. Biol. Chem.* **266**, 23268–23273.
- Nah, H.-D., Barenbaum, M. and Upholt, W. B. (1992) *J. Biol. Chem.* **267**, 22581–22586.
- Sandberg, M. M., Hirvonen, H. E., Elima, K. J. M. and Vuorio, E. I. (1993) *Biochem. J.* **294**, 595–602.
- Niyibizi, C. and Eyre, D. R. (1989) *FEBS Lett.* **242**, 314–318.
- Kleman, J.-P., Hartmann, D. J., Ramirez, F. and Van der Rest, M. (1992) *Eur. J. Biochem.* **210**, 329–335.
- Mayne, R., Brewton, R. G., Mayne, P. M. and Baker, J. R. (1993) *J. Biol. Chem.* **268**, 9381–9386.
- O'Rahilly, R. and Muller, F. (1987) *Developmental Stages in Human Embryos*, Carnegie Institution of Washington, Washington.
- Lovell-Badge, R. H., Bygrave, A. E., Bradley, A., Robertson, E. J., Tilley, R. and Cheah, K. S. E. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 2803–2807.
- Lau, E. T., Kong, R. Y. C. and Cheah, K. S. E. (1993) *Anal. Biochem.* **209**, 360–366.
- Cheah, K. S. E., Lau, E. T., Au, P. K. C. and Tam, P. P. L. (1991) *Development* **111**, 945–953.
- Lui, V. C. H., Ng, L. J., Nicholls, J., Tam, P. P. L. and Cheah, K. S. E. (1995) *Dev. Dynamics* **203**, 198–211.
- Kosher, R. A. and Solursh, M. (1989) *Dev. Biol.* **131**, 558–566.
- Ng, L. J., Tam, P. P. L. and Cheah, K. S. E. (1993) *Dev. Biol.* **159**, 403–417.
- Torre-Blanco, A., Adachi, E., Hojima, Y., Wootton, J. A. M., Minor, R. R. and Prockop, D. J. (1992) *J. Biol. Chem.* **267**, 2650–2655.
- Birk, D. E., Silver, F. H. and Trelstad, R. L. (1991) in *Cell Biology of Extracellular Matrix* (Hay, E. D., ed.), pp. 221–254. Plenum Press, New York.
- Vikkula, M., Mariman, E. C. M., Lui, V. C. H. et al. (1995) *Cell* **80**, 431–438.
- Benya, P. D. and Brown, P. D. (1986) in *Articular Cartilage Biochemistry* (Kuettner, K. E., Schleyerbach, R. and Hascall, V. C., eds.), pp. 219–230. Raven Press, New York.
- Askew, G. R., Wang, S. and Lukens, L. N. (1991) *J. Biol. Chem.* **266**, 16834–16841.