

Expression of mRNAs Coding for the $\alpha 1$ Chain of Type XIII Collagen in Human Fetal Tissues: Comparison with Expression of mRNAs for Collagen Types I, II, and III

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Abstract. This paper describes the topographic distribution of the multiple mRNAs coding for a novel human short-chain collagen, the $\alpha 1$ chain of type XIII collagen. To identify the tissues and cells expressing these mRNAs, human fetal tissues of 15–19 gestational wk were studied by Northern and in situ hybridizations. The distribution pattern of the type XIII collagen mRNAs was compared with that of fibrillar collagen types I, II, and III using specific human cDNA probes for each collagen type. Northern hybridization showed the bone, cartilage, intestine, skin, and striated muscle to contain mRNAs for type XIII collagen. An intense in situ hybridization signal was obtained with the type XIII collagen cDNAs in the epidermis, hair follicles, and nail root cells of the skin, whereas the fibrillar collagen mRNAs were detected in the dermis.

Cells in the intestinal mucosal layer also appeared to contain high levels of $\alpha 1$ (XIII) collagen mRNAs, but contained none of the fibrillar collagen mRNAs. In the bone and striated muscle, $\alpha 1$ (XIII) collagen mRNAs were detected in the mesenchymal cells forming the reticulin fibers of the bone marrow and endomycium. The hybridization signal obtained with the $\alpha 1$ (XIII) collagen cDNA probe in cartilaginous areas of the growth plates was similar, but less intense, to that obtained with the type II collagen probe. A clear hybridization signal was also detected at the (pre)articular surfaces and at the margins of the epiphyses, whereas it was weaker in the resting chondrocytes in the middle of the epiphyses. The brain, heart, kidney, liver, lung, placenta, spleen, testis, tendon, and thymus did not appear to contain $\alpha 1$ (XIII) collagen mRNAs.

THE collagen gene family of proteins now consists of at least thirteen different types with characteristic tissue distributions and functions (7, 11–13, 23). The major fibrillar collagens include types I, II, and III (11, 12, 23). Type I collagen is a heterotrimer of two $\alpha 1$ (I) chains and one $\alpha 2$ (I) chain, while types II and III collagens are homotrimers of $\alpha 1$ (II) and $\alpha 1$ (III) chains, respectively. These four chains share considerable homology at the amino acid, mRNA, and gene structure levels (11, 12, 23). Type I collagen is the major structural component in bone, skin, tendon, and other fibrous tissues and is the most abundant in the human body. Type II collagen is found only in cartilage, whereas type III collagen is also present in a wide variety of tissues, with the exception of bone, in association with type I collagen.

We have recently characterized cDNA clones coding for a unique collagen with alternatively spliced transcripts, the $\alpha 1$ chain of collagen type XIII (13; Pihlajaniemi, T., and M. Tamminen, unpublished data). The $\alpha 1$ (XIII) collagen polypeptide as depicted in the cDNA clones consists of three collagenous domains joined by noncollagenous segments, short NH₂- and COOH-terminal noncollagenous domains and a putative signal sequence. The collagenous domains COL 1,

COL 2, and COL 3 contain 95, 172, and 235 amino acid residues, respectively. Comparison of nucleotide sequences encoded by overlapping cDNA clones indicates that there are at least four different $\alpha 1$ (XIII) collagen mRNAs with different coding capacities in HT-1080 human fibrosarcoma cells, normal human skin fibroblasts, and endothelial cells from human umbilical cord veins. Nuclease S1 protection experiments and partial characterization of the corresponding gene suggest that the different mRNAs arise by alternative splicing of the precursor RNA at five locations within the coding region (20; Pihlajaniemi, T., and M. Tamminen, unpublished observations). This property makes type XIII collagen unique among all the collagen types studied so far. The polypeptide length varies between 654 and 566 amino acid residues, depending on which internal splicing has taken place. The sizes of the cDNA-deduced polypeptides are in good agreement with the 67,000- and 62,000-*M_r* polypeptides observed in Western blot transfer analysis with antibodies to a synthetic peptide derived from the cDNA sequences (13). Small amounts of mRNAs for the $\alpha 1$ (XIII) collagen chain have been found in cultured human skin fibroblasts and HT-1080 cells. Nothing is known about the general distribution of this collagen in tissues, however.

In the present work we used cDNA clones specific for the $\alpha 1$ chain of type XIII collagen (Pihlajaniemi, T., and M. Tamminen, unpublished data) to locate its mRNAs in developing human fetal tissues by Northern and in situ hybridizations and compared its topographic expression patterns with those of types I, II, and III collagen mRNAs.

Materials and Methods

RNA Extraction and Northern Blotting

Total RNAs were extracted as described previously (1, 14) from the calvarial bones, diaphysis of long bones, growth plates, epiphyseal cartilages, skeletal striated muscles, cardiac muscle, skin, brain, placenta, lungs, kidney, testis, liver, spleen, thymus, colon, and small intestine of 15–19-wk human fetuses obtained from therapeutic abortions. 15- μ g aliquots of total RNA were fractionated on 1% agarose gels containing 2 M formaldehyde, blotted onto GeneScreen Plus transfer membranes (New England Nuclear, Boston, MA) and hybridized with 32 P-labeled random primed cDNA inserts at 42°C for 20 h. The hybridization mixture contained 2 ng/ml 32 P-labeled probe, 1 M NaCl, 10% (wt/vol) dextran sulfate, 1% SDS, 0.1% (wt/vol) Ficoll, 0.1% (wt/vol) polyvinyl pyrrolidone, 0.1% (wt/vol) BSA, 100 μ g/ml sonicated calf thymus DNA, and 50% deionized formamide. The filters were washed sequentially in 2 \times SSC (twice at room temperature for 5 min), 2 \times SSC and 1% (wt/vol) SDS (twice at 60°C for 30 min), and 0.1 \times SSC (twice at room temperature for 30 min). The bound probe was detected by autoradiography at -70°C using x-ray films and intensifying screens.

In Situ Hybridization

In situ hybridizations were carried out as described previously (15). In brief, tissue samples were fixed with formalin and embedded in paraffin for sectioning. The sections for in situ hybridization were pretreated with proteinase K and HCl and acetylated. The hybridizations were carried out at 42°C for 50 h using 35 S-deoxy(thio)ATP-labeled probes followed by washing, autoradiography at 4°C for 2–10 d, and staining of the sections with hematoxylin as described previously in detail (15).

Hybridization Probes

Selected 300–400-bp fragments of cloned human cDNAs exhibiting the lowest degree of homology were used as Northern and in situ hybridization probes to detect mRNAs corresponding to collagen types I–III, as described earlier (15, 17). Specifically, a 372-bp Xho I–Pvu II fragment of cDNA clone pHCAL1 corresponding to part of the C-propeptide coding region of the $\alpha 1$ (I) collagen mRNA (10, 24), a 400-bp Dra I–Eco RI fragment of clone pHCAR3 corresponding to the untranslated region of the $\alpha 1$ (II) mRNA (2), and a 300-bp Pst I–Pst I fragment of clone pHFS3 corresponding to part of the C-propeptide region of the $\alpha 1$ (III) mRNA (18) were used. Two cDNA clones coding for human $\alpha 1$ (XIII) collagen mRNAs were used: clone HT-125, containing a 550-bp Pst I–Pst I insert (13); and clone E-3, containing a 1800-bp Eco RI–Eco RI insert (Pihlajaniemi, T., and M. Tamminen, unpublished data). The HT-125 insert codes almost entirely for -Gly-X-Y- sequences and E-3 covers most of the coded sequences of the respective mRNA in addition to 3' untranslated sequences. The entire inserts of cDNA clones HT-125 and E-3 were used as probes in both the Northern and in situ hybridizations. The probes were labeled with [32 P]dCTP by the random priming method for the Northern hybridizations and with 35 S-deoxy(thio)ATP for the in situ hybridizations (4). Bgl I-generated 100–790-bp fragments of bacteriophage λ DNA were labeled similarly and used as a negative control probe.

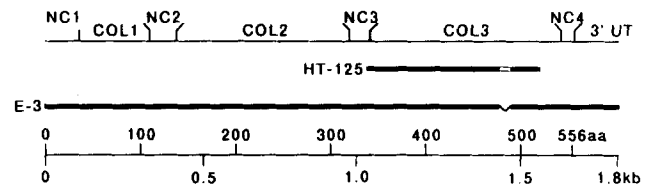


Figure 1. The cDNA clones encoding the $\alpha 1$ chain of human type XIII collagen used in this study. The various domains of the polypeptide encoded by the clones are shown at the top: the three collagenous (COL1–COL3) and the four noncollagenous domains (NC1–NC4). 3' UT, the 3' untranslated region encoded by cDNA clone E-3. (□) A stretch of 36 nucleotides found in cDNA clone HT-125 but absent (V) from the corresponding region of E-3. The nucleotide (kb) and amino acid (aa) scales are shown at the bottom.

Results

Tissue Distribution of Human $\alpha 1$ (XIII) Collagen mRNAs

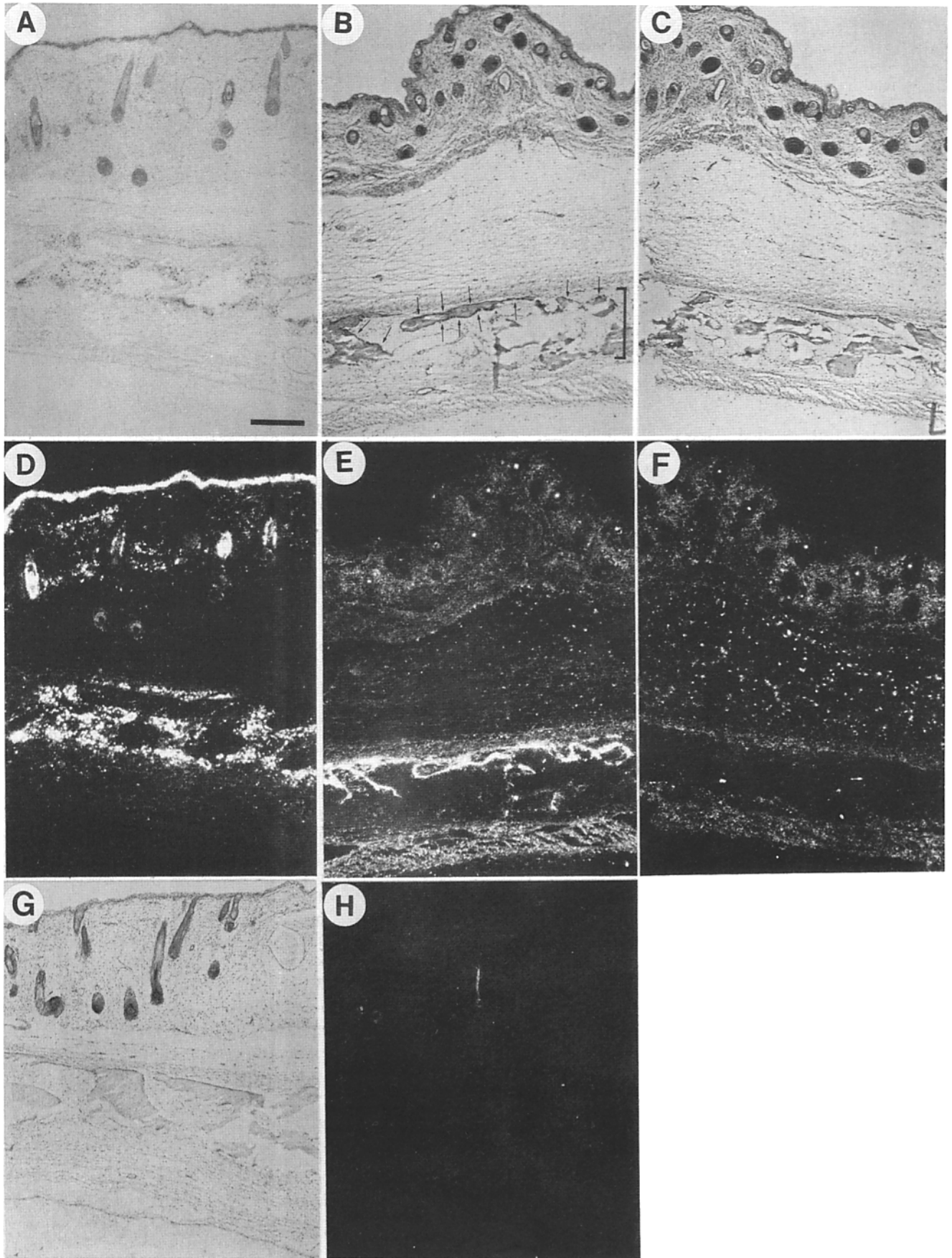
Two cDNA clones were used to detect human $\alpha 1$ (XIII) collagen mRNAs (Fig. 1). The cDNA clone HT-125 (14) covers part of the NC3 domain and most of the COL3 domain, which consists of repeating -Gly-X-Y- sequences. The cDNA clone E-3 (Pihlajaniemi, T., and M. Tamminen, unpublished data) covers most of the coded sequences of the respective mRNA (it is lacking the 5' untranslated region and the beginning of the coded sequences) and 150 nucleotides of 3' untranslated sequences. HT-125 is entirely covered by sequences encoded by E-3, except for one difference due to alternative splicing of a 36-bp segment of the gene coding for the $\alpha 1$ (XIII) collagen mRNAs (20; Pihlajaniemi, T., and M. Tamminen, unpublished data). Identical results were obtained with these two probes except that the hybridization signals were weaker with HT-125 than with E-3 and therefore E-3 was used in the experiments shown here.

Northern analysis of total RNAs extracted from various human fetal tissues was first used to detect tissues expressing the $\alpha 1$ (XIII) collagen gene. The 2.6–3-kb bands characteristic of the $\alpha 1$ (XIII) collagen mRNAs were detected in total RNAs extracted from the calvarial bones, diaphysis of long bones, growth plates, epiphyseal cartilages, skeletal muscle, skin, colon, and small intestine (not shown); however, no hybridization signals were obtained from RNAs extracted from the brain, cardiac muscle, kidney, liver, lung, placenta, spleen, testis, or thymus (not shown).

Distribution of the $\alpha 1$ (XIII) Collagen mRNAs In Vivo in Calvaria, Skin, and Long Bones

To identify further the location of the cell types containing

Figure 2. Location of types I, III, and XIII collagen mRNAs in fetal calvarial bone, periosteum, dura mater, and scalp by in situ hybridization. The probes used were inserts from clones E-3 for $\alpha 1$ (XIII) collagen mRNAs (A and D), pHCAL1U for $\alpha 1$ (I) collagen mRNA (B and E), and pHFS3 for $\alpha 1$ (III) collagen mRNA (C and F), with restriction fragments of bacteriophage λ DNA (G and H) as a negative control. For better visualization of the autoradiographic grains, dark field images of the phase-contrast fields are shown (D–F and H). The cranial side on each section is towards the top of the panel. Loose connective tissue and blood vessels are present between the bone spicules. The fibrous connective tissue (periosteum) borders the calvaria on both surfaces, merging with the dura mater on the inside and with the deep layers of the dermis on the outer surface. Structures marked in B: arrows show osteoblasts along bone spicules and the bracket indicates the calvarial bone. Bar, 300 μ m.



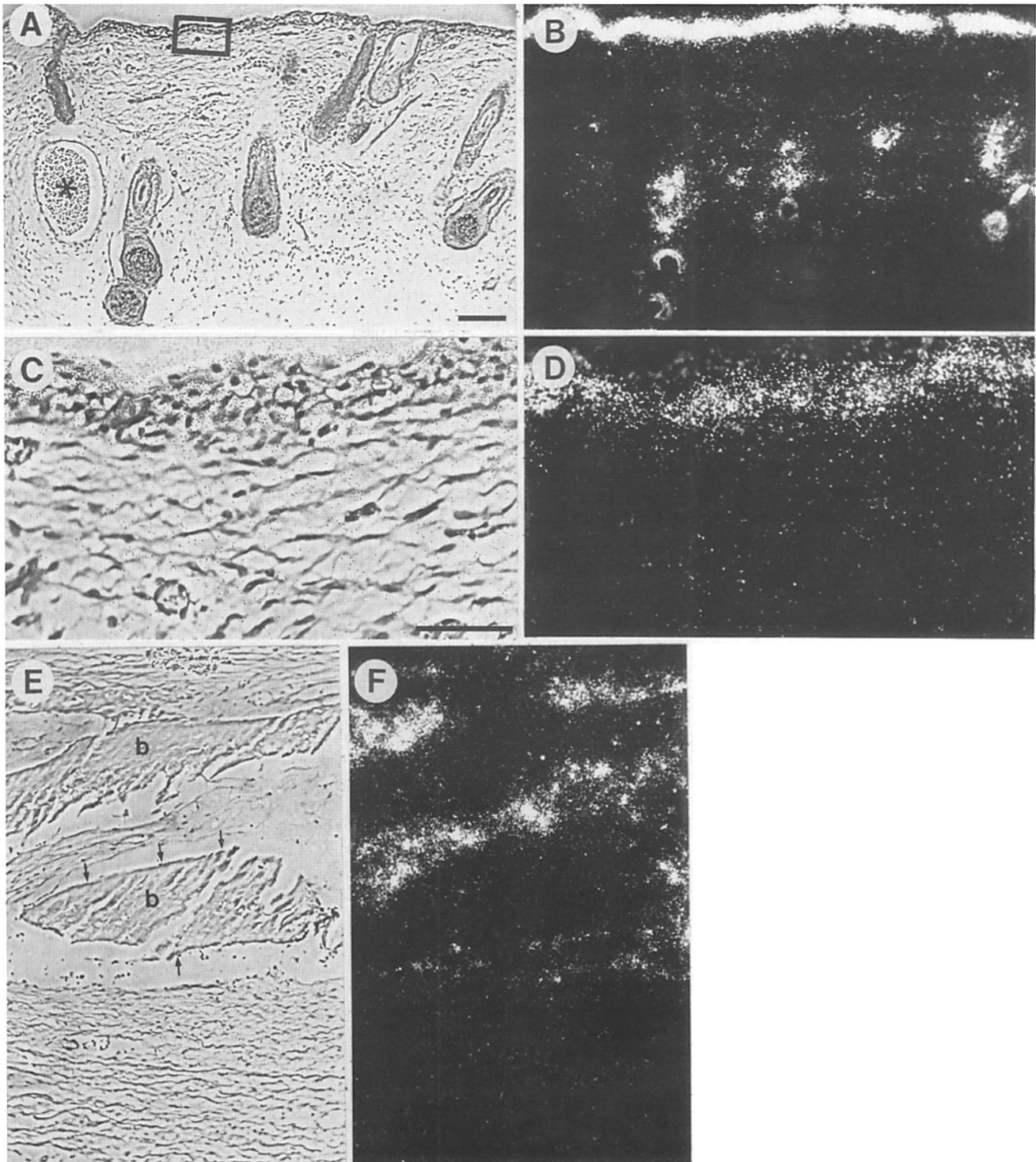
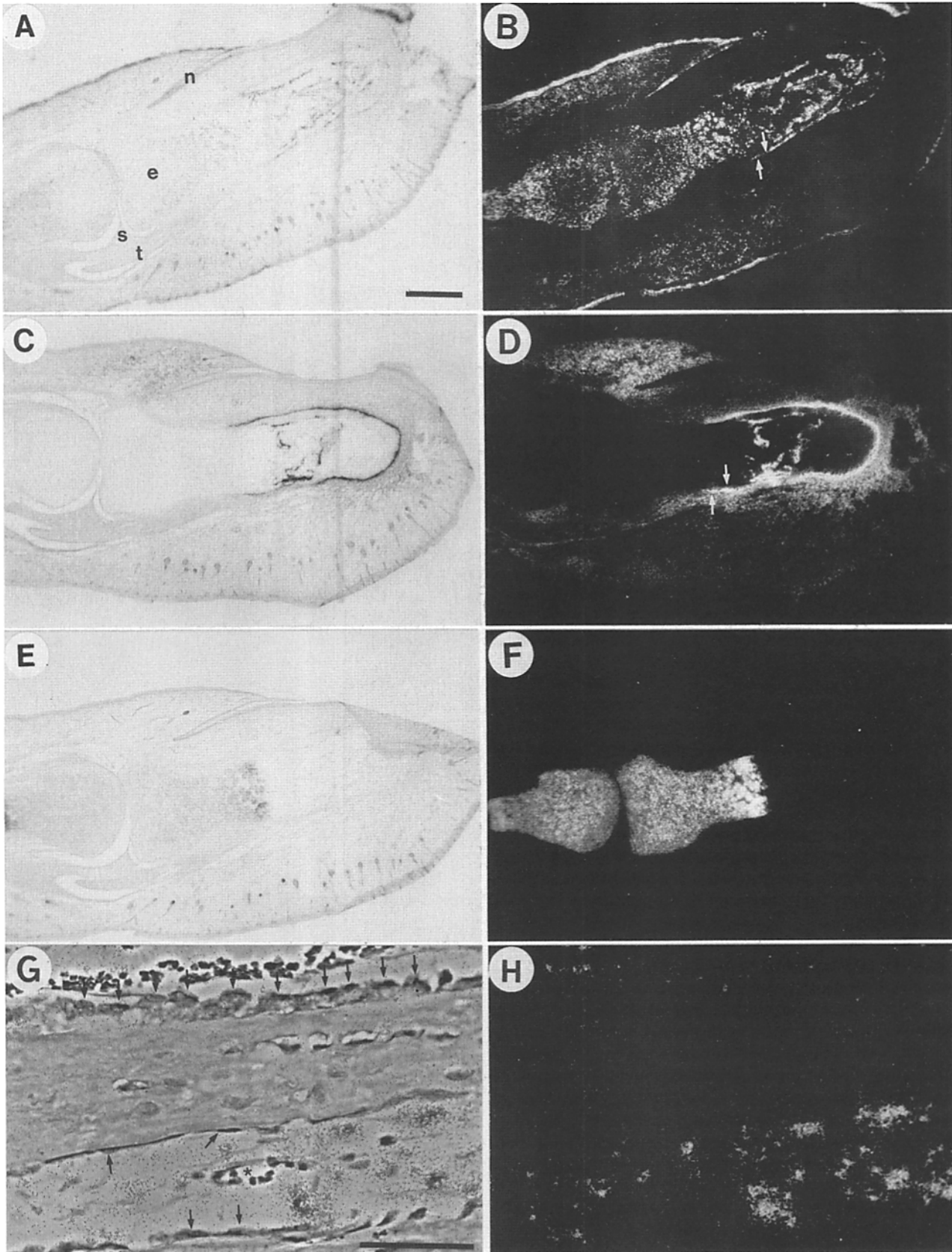


Figure 3. Location of type XIII collagen mRNAs in the epidermis (*A–D*) and hair follicles of the scalp (*A* and *B*) and in the mesenchymal tissue between bone spicules (*E* and *F*). The micrographs are from the same sample as shown in Fig. 2, *A* and *D*, but at greater magnification to allow identification of the epidermis, hair follicles, mesenchymal fibroblasts, and blood capillaries (marked with an asterisk in *A*). The dark field images corresponding to the phase-contrast micrographs are shown on the right. *C* and *D* show the framed area in *A* at a greater magnification. The arrows in *E* show osteoblasts along bone spicules (*b*). Bars: (*A*, *B*, *E*, and *F*) 100 μm ; (*C* and *D*) 50 μm .

Figure 4. Location of types I, II, and XIII collagen mRNAs in developing human fingers at 19 gestational wk. The sections were hybridized with the same probes for $\alpha 1(\text{XIII})$ (*A*, *B*, *G*, and *H*), $\alpha 1(\text{I})$ (*C* and *D*), and $\alpha 1(\text{II})$ collagen mRNAs (*E* and *F*) as in Fig. 2. The dark field views on the right correspond to the phase-contrast fields on the left. Structures marked in *A*: nail root (*n*), epiphysis (*e*), synovial (*s*) tissue, and structures developing into tendons and ligaments (*t*). The narrow periosteal layers synthesizing mRNAs for type XIII and type I collagens are marked by white arrows in *B* and *D*. *G* and *H* are higher magnifications of the area of growing bone in *A* and *B*, showing



osteoblasts lining the bone spicules (*black arrows*), osteocytes within the bone spicules, blood capillaries (*asterisks*), and mesenchymal fibroblastic cells between the bone spicules which contain type XIII collagen mRNAs, as visualized better in the dark field in *H*. Bars: (*A-F*) 500 μm ; (*G* and *H*) 100 μm .

the $\alpha 1(\text{XIII})$ collagen transcripts, the tissues which had proved positive in Northern hybridization were subjected to in situ hybridization, with serial sections hybridized with fragments of bacteriophage λ DNA as negative controls. Only an evenly distributed background of autoradiographic grains was observed with this λ DNA probe. The brain and kidney tissues, which were negative for $\alpha 1(\text{XIII})$ mRNAs in Northern hybridization, similarly showed only background hybridization in the in situ hybridization experiments with the $\alpha 1(\text{XIII})$ cDNA probe (data not shown). Hybridization patterns with cDNA probes coding for the fibrillar collagen types I–III served as additional internal controls since the patterns were distinctly different with each probe, as illustrated by the low power micrographs and dark field images of the autoradiograms (Figs. 2 and 4).

An intense hybridization signal was observed with the $\alpha 1(\text{XIII})$ cDNA probe in the epidermis and hair follicles of the scalp (Fig. 2, *A* and *D*, and Fig. 3, *A–D*); scattered cells in the dermis were also positive. The same pattern of hybridization was observed in the skin of the developing fingers, and the nail root cells were also shown to contain $\alpha 1(\text{XIII})$ collagen mRNAs (Fig. 4, *A* and *B*). mRNAs for type I and III collagens were detected in the dermis and hypodermis of the skin, but not in the epidermis, hair follicles, or nail roots (Figs. 2 and 4). In calvarial bones autoradiographic grains from the $\alpha 1(\text{XIII})$ collagen probe were observed in the reticulin framework, mesenchymal tissue between bone spicules (Fig. 2, *A* and *D*, and Fig. 3, *E* and *F*). The type XIII signal was present in only a few scattered cells of the calvarial periosteum, while none of the osteoblasts lining the spicules of the calvarial bone contained the mRNAs (Fig. 3, *E* and *F*). In contrast, the autoradiographic signal with the $\alpha 1(\text{I})$ collagen probe was abundant in these cells and over the periosteal fibroblasts (Fig. 2, *B* and *E*). Type III collagen mRNA was present in the periosteum and, to a lesser extent, in some mesenchymal cells between the bone spicules (Fig. 2, *C* and *F*).

Sections of several long bones from the 16–19-wk human fetuses (including the humerus, radius, ulna, tibia, fibula, and fingers) were hybridized in situ with cDNA probes for types I, II, and XIII collagens. The signal distributions over the finger sections from a 19-wk fetus are representative of these hybridizations (Fig. 4). Expression of the $\alpha 1(\text{XIII})$ collagen mRNAs in the developing human long bones was associated with cartilaginous and osseal areas, the signal being accentuated in the hypertrophic and proliferating chondrocytes of the growth plates in the cartilage and in the chondrocytes near the perichondrium or articular surfaces, as compared with the zone of resting chondrocytes in the middle of the epiphyses (Fig. 4, *A* and *B*). In the osseal areas, the $\alpha 1(\text{XIII})$ collagen mRNAs were located in the mesenchymal cells that form the reticulin framework between the bone spicules—i.e., analogously to their distribution in the calvar-

ial bone sections. No mRNA was detected in the osteoblasts lining the bone spicules, the osteocytes within them (Fig. 4, *G* and *H*), or the osteoclasts (not shown). A narrow layer of cells actively synthesizing the $\alpha 1(\text{XIII})$ collagen mRNAs was observed in the periosteum (Fig. 4, *A* and *B*). This expression pattern is clearly distinct from that of the fibrillar collagen types. The $\alpha 1(\text{I})$ collagen mRNA was identified to the osteoblasts lining the bone spicules and those of the inner cambial layer of the periosteum (Fig. 4, *C* and *D*). The type II collagen signal was observed only over the cartilaginous areas of the developing bones, with accentuation over the zones of lower proliferating and upper hypertrophic chondrocytes. The type II collagen cDNA probe did not hybridize with any of the soft connective tissues (Fig. 4, *E* and *F*), whereas the type III collagen mRNA was confined to these structures in the finger sections studied (not shown).

Distribution of the $\alpha 1(\text{XIII})$ Collagen mRNAs In Vivo in Other Tissues

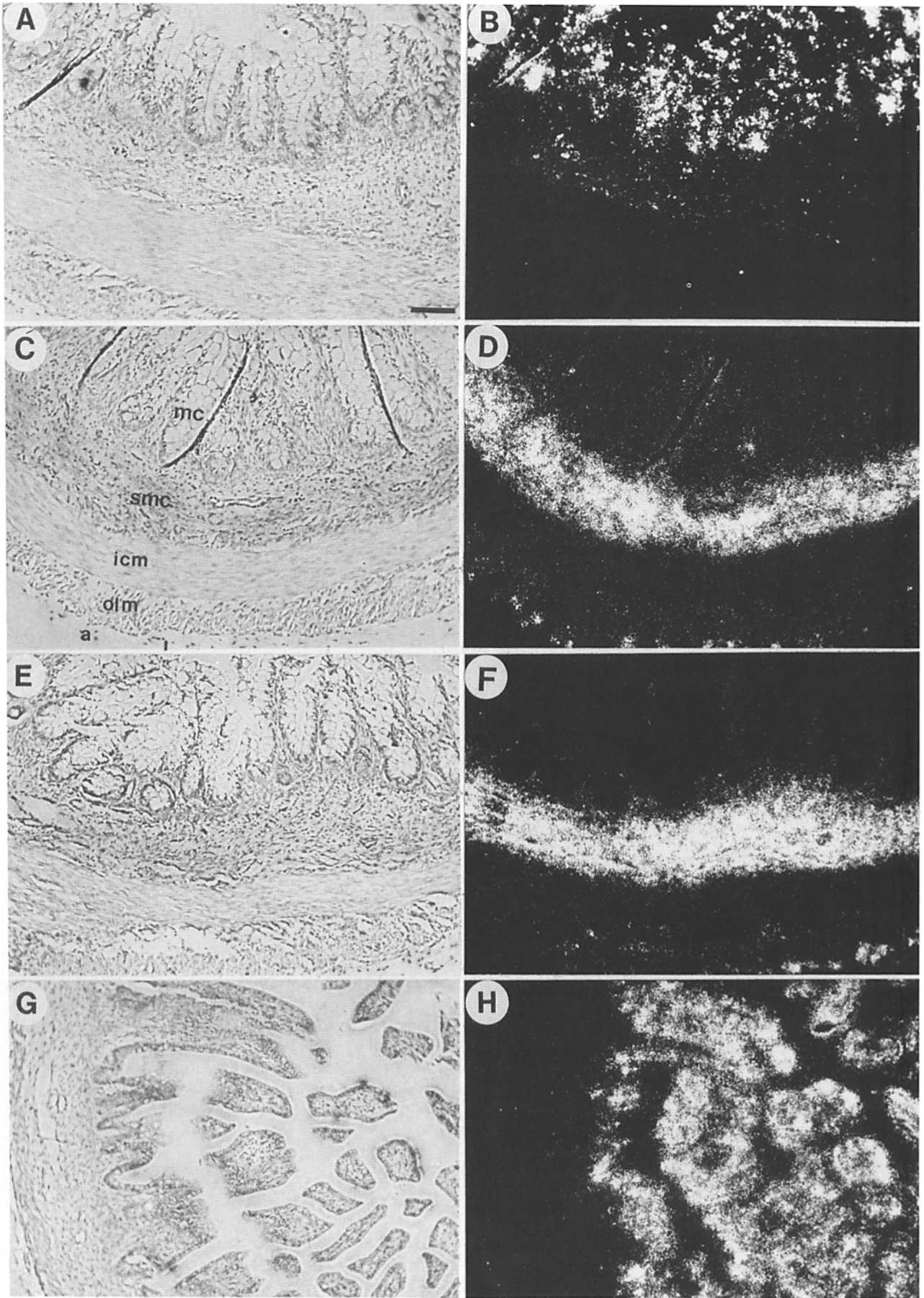
Cells expressing high amounts of $\alpha 1(\text{XIII})$ collagen mRNAs were also identified in the mucosal layer of the colon and the small intestine (Fig. 5, *A*, *B*, *G*, and *H*), and some scattered cells of the submucosa of the colon were likewise positive. High levels of type I and III collagen mRNAs were observed in the submucosa and the adventitia of the colon and the small intestine, but not in the mucosal layer (Fig. 5, *C–F*).

The $\alpha 1(\text{XIII})$ collagen mRNAs were widely distributed along the fibers of striated skeletal muscle (Fig. 6), whereas type I and III collagen mRNAs were mainly located in the loose connective tissue of the perimysium separating fasciculi (not shown). At greater magnification the $\alpha 1(\text{XIII})$ collagen mRNAs (Fig. 6, *C* and *D*) were seen to be located in the endomysium, a delicate connective tissue separating individual muscle fibers (25). Only a background signal was observed with types I, III, and XIII collagen cDNA probes over the visceral muscles of the small and large intestine, comprised of inner circular and outer longitudinal layers (Fig. 5). The endothelial cells of the capillaries were devoid of type XIII collagen mRNAs (Figs. 3, *A* and *B*, and 4, *G* and *H*), and the developing synovial tissue, tendons, and ligaments around the long bones likewise did not express the $\alpha 1(\text{XIII})$ collagen mRNAs (Fig. 4, *A* and *B*).

Discussion

Human fetal tissues were studied to identify tissues and cells expressing the type XIII collagen gene. Northern hybridizations showed type XIII collagen mRNAs to exist in the skin, colon, small intestine, and skeletal tissues (bones, cartilages, and striated muscle). In situ hybridization was then used to determine the topographic distribution of this unusual collagen at the cellular level in certain fetal tissue sections. The

Figure 5. Location of mRNAs for collagen types I, III, and XIII in the fetal colon (*A–F*) and small intestine (*G* and *H*). The hybridizations were for $\alpha 1(\text{XIII})$ (*A*, *B*, *G*, and *H*), $\alpha 1(\text{I})$ (*C* and *D*), and $\alpha 1(\text{III})$ collagen mRNAs (*E* and *F*) using the same probes as in Fig. 2. The dark field images on the right correspond to the phase-contrast micrographs on the left. The different intestinal layers are marked in *C*: mucosa (*mc*), submucosa (*smc*), inner circular layer of the smooth intestinal muscle (*icm*), outer longitudinal layer of the smooth intestinal muscle (*olm*), and adventitia (*a*). Bar, 100 μm .



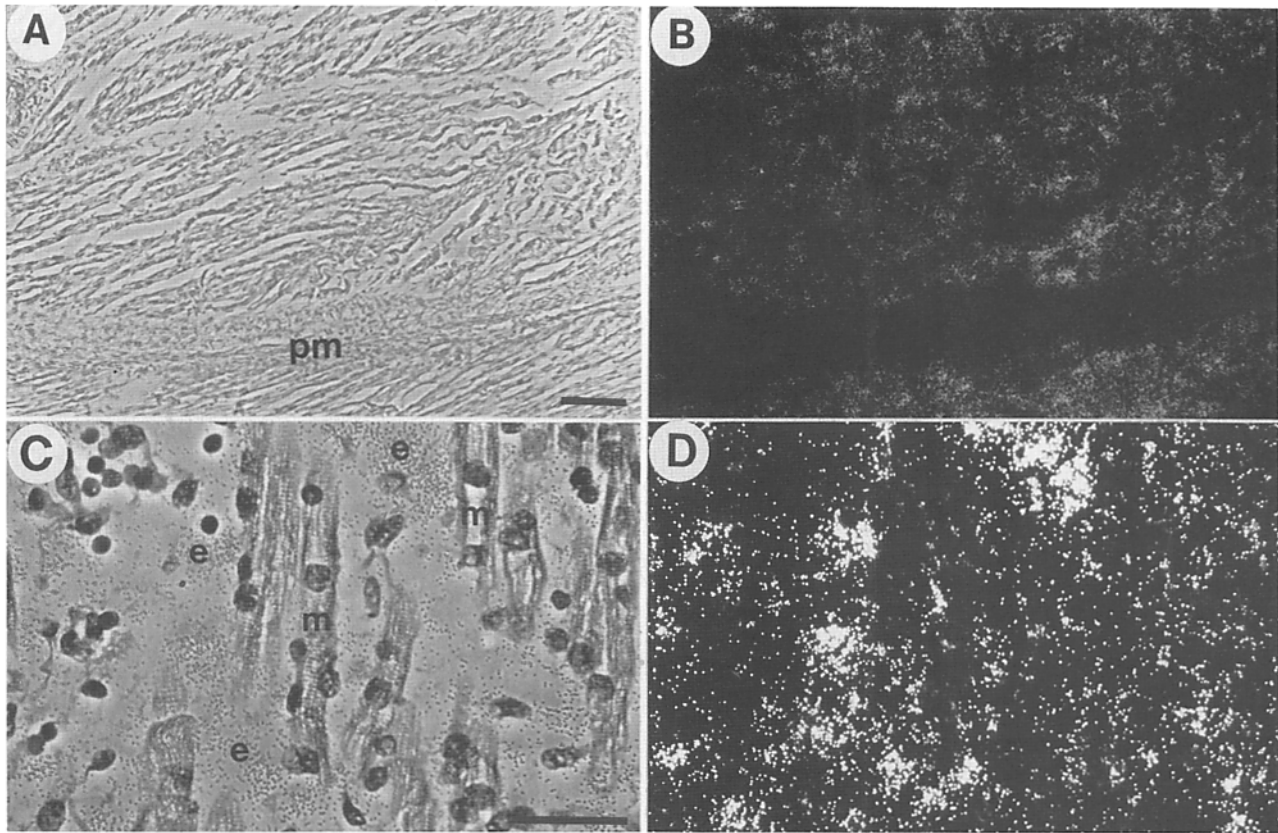


Figure 6. Location of type XIII collagen mRNAs in developing striated skeletal muscle. The sections were hybridized with cDNA clone E-3 coding for the $\alpha 1(\text{XIII})$ collagen mRNAs. The dark field images on the right correspond to the phase-contrast micrographs on the left. The perimysium (*pm*) is seen at the low magnification (*A* and *B*). A greater magnification is shown for better visualization of the hybridization signal in the endomysium (*e*) separating muscle fibers (*m*) (*C* and *D*). Bars: (*A* and *B*) 100 μm ; (*C* and *D*) 50 μm .

expression pattern of the $\alpha 1(\text{XIII})$ collagen mRNAs was compared with that of collagen types I, II, and III.

An intense hybridization signal was observed in the skin and gut with the $\alpha 1(\text{XIII})$ collagen cDNA probe. The expression of $\alpha 1(\text{XIII})$ collagen mRNAs in the developing human epidermis, hair follicles, and nail roots may be explained by their embryological origin. Like the sweat glands and sebaceous glands, they originate from down-growths of the epidermal epithelium into the dermis and hypodermis (25). At least some of the scattered cells in the dermis expressing type XIII collagen mRNAs may be due to the down-growth of the epidermal epithelium from which the epidermal appendages originate. In the case of the colon and small intestine, $\alpha 1(\text{XIII})$ collagen mRNAs were found in the mucosa, which developmentally originates from the endoderm (6). The expression of the type XIII collagen gene in both intramembranously formed calvaria and enchondrally formed long bones was confined to the fibrous mesenchymal tissue, the so-called reticulin framework, situated between the bone spicules. Type III collagen and the corresponding mRNA is similarly located in this tissue (16, 25). The present observation of $\alpha 1(\text{XIII})$ mRNAs in cells within reticulin fibers is further supported by the finding that mRNAs for type XIII collagen are also detected in the endomysium of skeletal muscle, another location where reticulin fibers have been suggested to be present (25). Type III and XIII collagen mRNAs do not, however, coexist in certain other tissues: synovial tissue, thick fibrous periosteum, and perichondrium contain type III

(18) but not type XIII collagen mRNAs, whereas growth plate cartilage, epidermis, and intestinal mucosa contain type XIII but not type III collagen mRNAs.

The major collagen in cartilage is type II, and other apparently cartilage-specific collagens, namely types IX, X, and XI, are present in smaller amounts (12). Collagen types II and IX are distributed throughout the cartilage (5, 11, 12, 22), while type X is a product of hypertrophic chondrocytes (19). Surprisingly, mRNAs for the $\alpha 1$ chain of type XIII collagen were also found in both proliferating and hypertrophic chondrocytes. Resting chondrocytes in the middle of epiphyses expressed type XIII collagen mRNAs to a somewhat lesser degree. We have previously observed mRNA for type III collagen in some sections of developing (pre)articular surfaces (18), and both this region and the margins of the epiphyses also contain mRNAs for type XIII collagen.

The locations in which type I and XIII collagen mRNAs may coexist in the same cell are the dermis and the periosteum of long bones where a narrow layer of cells express both mRNAs. The cells expressing type XIII collagen mRNAs in the dermis, however, may be down-growths of the epidermal epithelium, as discussed above, whereas cells expressing type I collagen mRNA are fibroblasts. The periosteum is generally regarded as consisting of two layers: an inner cambial or osteogenic layer and an outer fibrous layer (8). Some workers even describe a third layer: a zone of transition between these two which provides progenitor cells for both (3, 9). Thus collagen types I and XIII are not necessar-

ily produced by the same cells even in the periosteum. We have previously detected type II collagen mRNA in a narrow layer of the periosteum of some developing human long bones, whereas type III collagen mRNA was identified only in the thick fibrous periosteum and perichondrium (15), which were devoid of any signal when the type XIII collagen probe was used.

Type IV collagen is found only in basement membranes and is synthesized by epithelial and endothelial cells (21). Antibodies to type IV collagen stain basement membranes from the epidermis and cutaneous appendages (16), for example; epithelial cells from the gut are thought to synthesize type IV collagen (21). We found type XIII collagen mRNAs in the same locations in both the skin and gut. Type IV collagen is not present in cartilage (21) or in the fibrous mesenchymal tissue of bone (16), which we have found to contain $\alpha 1(\text{XIII})$ collagen mRNAs. Neither do the two collagen types coexist in the endothelial cells of blood capillaries. Previous in situ hybridization experiments indicated that endothelial cells of the capillaries of human fetal calvarial bone are stained by a $\alpha 1(\text{IV})$ collagen cDNA probe (16), while analogous in situ hybridization experiments here did not show any staining with the $\alpha 1(\text{XIII})$ collagen cDNA probe.

In conclusion, our Northern and in situ hybridization data show distinct expression patterns for types I, II, III, and XIII collagens in developing human fetal tissues. Altogether five alternative splicing sites have been described for the $\alpha 1$ chain of type XIII collagen (13, 20; Pihlajaniemi, T., and M. Tamminen, unpublished data). Whether these differentially spliced mRNAs bear a correlation to their tissue distribution or different developmental stages, is not known at the present.

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