High Expression of 92-kD Type IV Collagenase (Gelatinase B) in the Osteoclast Lineage during Mouse Development

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Abstract. cDNA clones for murine 92 kD type IV collagenase (gelatinase B) were generated for the determination of its primary structure and for analysis of temporal and spatial expression in vivo. The mouse enzyme has 72% sequence identity with the human counterpart, the major difference being the presence of a 16-residue segment absent from the human enzyme. In situ hybridization analyses of embryonic and postnatal mouse tissues revealed intense signals in cells of the osteoclast cell lineage. Clear expression above background was not observed in macrophages, polymorphonuclear leukocytes, monocytes, or epithelial cells which have been shown to express the gene in vitro in cell cultures. Expression of the gene was first observed at early stage of cartilage and tooth development at E13, where signals were seen transiently in surrounding mesenchymal cells. At later developmental stages and postnatally strong expression was seen in large cells at the surface of bones. These cells were presumably osteoclasts as their location correlated with that of TRAP positive cells. Signals above background were not observed in a number of other tissues studied. The results represent the first demonstration of a highly osteoclast specific extracellular proteinase. The results suggest that during normal development of embryonic organs the 92-kD type IV collagenase does not have a major role in basement membrane degradation, but is rather mainly used for the turnover of bone matrix, possibly as a gelatinase required for the removal of denatured collagen fragments (gelatin) generated by interstitial collagenase.

TYPE IV collagenases (gelatinases) belong to a family of mammalian extracellular metalloproteinases which are the products of related genes (5, 46). The metalloproteinases share several structural and functional properties, and all of them exhibit the capacity to degrade one or more of the molecules that constitute the extracellular matrix. The enzymes are secreted as zymogens, they have a Zn^{2+}-binding site, and they are inhibited by specific tissue inhibitors of metalloproteinases (TIMPs)[1] (5, 46). The metalloproteinase family contains at least nine genetically distinct members: two interstitial collagenases which degrade fibrillar collagens of types I, II, and III (13–15), three stromelysins (3, 6, 24, 43) with activity against collagens with interrupted triple helices and some noncollagenous proteins, matrilysin (pump-1) a small proteinase with a wide range of substrates including fibronectin, laminin, casein, gelatin and proteoglycans (5, 30, 47), macrophage metalloelastase with activity against elastin (38), and the 72-kD (9, 16, 21, 33) and 92-kD (26, 44) type IV collagenases (gelatinases A and B, respectively) which degrade type IV collagen and denatured collagen (gelatin). The metalloproteinases presumably play an important role in tissue remodeling during embryonic development and in other conditions where matrix degradation occurs, such as in inflammation and tumor invasion.

The two type IV collagenases cleave native type IV collagen, a specific component of basement membranes, in the triple-helical region into 1/4- and 3/4-size fragments and they possess high activity against gelatin (11, 26). Additionally, both enzymes have been shown to be able to degrade native type V and VII collagens. The 72-kD type IV collagenase has also been shown to cleave type X collagen which is present in cartilage (46). However, neither enzyme degrades type I collagen, proteoglycan, or laminin. The high activity against gelatin has led to the hypothesis that the type IV collagenases play a role in the removal of denatured collagen fragments after cleavage of native collagen by the other metalloproteinases.

The question as to why two distinct type IV collagenases with seemingly identical substrate specifically are required is now beginning to become unraveled. It has previously been shown that the secretion of the two enzymes is not coordinately regulated in cultured cells (44, 48), and also that

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1. *Abbreviations used in this paper*: TIMP, tissue inhibitor of metalloproteinases; TRAP, tartrate resistant acid phosphatase.

their genes differ substantially with respect to regulation of gene expression (17, 18). In vivo both enzymes are likely to be important for tissue remodeling during embryonic morphogenesis. Increased proteinase activity has earlier been implicated in embryonic development (7). Using in situ hybridization, we have recently shown that the 72-kD enzyme is expressed primarily in mesenchymal cells during mouse development (31). In contrast, ectodermal and endodermal cells, with the exception of salivary gland, generally showed no expression. The high expression of the 72-kD type IV collagenase in mesenchymal cells indicates that this enzyme has a general role in the remodeling of extracellular matrix, not only in the degradation of the basement membrane type IV collagen, but probably also in the removal of denatured proteolytic fragments of fibrillar collagen molecules (gelatin). Knowledge about the physiological role of the 92-kD type IV collagenase is still limited, but it is known to be secreted by cultured lung alveolar macrophages, polymorphonuclear leukocytes, trophoblasts, and keratinocytes (4, 15, 22, 26, 34). Therefore, this enzyme might be used by macrophages and leukocytes for their penetration through the extracellular matrix and by trophoblasts during embryonic implantation. The expression of the 92-kD type IV collagenase in keratinocytes in vitro also suggests that it is used for the turnover of subepithelial basement membranes.

Both type IV collagenases have gained particular attention for their association with invasive tumor processes indicating that the tumor cells require the enzymes for the degradation of basement membranes during dissemination and formation of metastasis. A number of cultured tumor cells have been reported to secrete type IV collagenases, and recently numerous immunostaining and in situ hybridization studies have demonstrated activation of their genes in tumor tissues. Expression of the 92-kD type IV collagenase has been shown in macrophages located at the invading tumor front of human squamous cell (28) and colon carcinoma (29) by in situ hybridization and by immunostaining in breast carcinoma (42). In squamous cell carcinoma expression of the 92-kD enzyme has also been detected in the tumor cells themselves. The expression pattern of the 72-kD type IV collagenase is different in that expression, as judged by in situ hybridization, occurs in fibroblasts adjacent to the tumor front (27–29, 42), while the antigen is mainly localized by immunostaining to the tumor cells at the front of the invading tumor mass (2, 25).

To explore the physiological role of the 92-kD type IV collagenase, we have cloned the murine enzyme, determined its primary structure and studied its spatial and temporal expression during mouse development by in situ hybridization. Intense expression was observed in osteoclasts at sites of bone formation. Despite transient expression at embryonic days 12–13 in some mesenchymal cells, expression above background was not observed in macrophages or any other cells in numerous tissues studied.

Materials and Methods

Isolation of RNA

For the preparation of cDNA, polyadenylated RNA was isolated directly from cultured mouse keratinocytes and 7-d old mouse skulls and leg bones. The mRNA was isolated directly from cultured cells with a slight modification of the Fast Track RNA isolation kit method (Invitrogen). Total-RNA from the 7-d old mouse tissues was first isolated by the acid guanidinium thiocyanate-phenol-chloroform extraction (8) and the mRNA was further isolated from total RNA by the Poly A Track mRNA Isolation System (Promega, Madison, WI).

Construction of cDNA Clones

The cDNA clones were generated from polyadenylated RNA using the PCR method. The sequence for the PCR oligonucleotide primers were based on the sequence of the mouse 92-kD type IV collagenase gene (Munaut, C., P. Huhtala, P. Reponen, and K. Tryggvason, unpublished results) and the RACE (rapid amplification of cDNA ends) technique (12). The first cDNA strand was synthesized with the Moloney murine leukemia virus (M-MLV) reverse transcriptase enzyme (BRL) using either specific primers or a "hybrid" primer consisting of oligo(dT) (17 bases) linked to a unique 18-base oligonucleotide ("adaptor") primer (5'-GACTCGAGTCGACATCGATTT-TTTTTTTTTTTTTT-3') (12). PCR amplification followed directly using the mRNA-cDNA hybrid as a template and the adaptor primer which binds to the 3' end of the cDNA and the specific primer from the 5' end of the template. Four overlapping cDNA clones generated with this method were compared by electrophoresis on an agarose gel and subcloned into the T-vector, derived from the Bluescript plasmid (23). The cDNA clones were sequenced from both strands by the dideoxynucleotide chain termination method (37) with Sequenase (USB). Either universal primers or specific primers were used, and the sequence was compared to the sequence from genomic clones.

Preparation of Tissue Sections

The embryonic age of the hybrid mice (CBA × C57BL) was set according to the day of the vaginal plug, which was designated day 0. Different organs or whole embryos from 12-d embryos to 11-d postnatal mouse pups were dissected under a stereo microscope in PBS, fixed in 4% paraformaldehyde in PBS (pH 7.2) at +4°C overnight, dehydrated and embedded in paraffin wax. Sections of 7 μm thickness were placed on silanized glass slides, dried overnight at +37°C, and stored in tight boxes at +4°C until use.

In Situ Hybridization

For the preparation of RNA probes the M92KD-2 cDNA clone was cut with *SmaI* and *EcoRI* restriction enzymes (SmaI site between nucleotides 1917-1918, see Fig. 1) and the 323 bp fragment was cloned into pSP64 and pSP65 plasmid vectors (Promega). The pSP64 (sense) and pSP65 (antisense) plasmid vectors were linearized with *EcoRI* and *BamHI* restriction enzymes, respectively, and the [^{35}S]-uridine S'-triphosphate (+1,000 Ci/nmol, Amersham) labeled RNA-probes were transcribed using a transcription kit (Promega). The labeled probes were precipitated with ethanol, dissolved in hybridization buffer, and used at 50,000–60,000 cpm/μl. The in situ hybridization was carried out according to Wilkinson and Green (45). In brief, the deparaffinized sections were pretreated with proteinase K (Sigma Chem. Co., St. Louis, MO), hybridized with the labeled probes in a humid chamber overnight at 50°C, and washed under high-stringency conditions. The dried slides were dipped in autoradiographic emulsion (Kodak NTB2) and exposed for 10 d at 10°C. After developing the film, the sections were stained with hematoxylin and mounted.

Tartrate Resistant Acid Phosphatase Staining

Tartrate resistant acid phosphatase (TRAP) staining was performed according to Thompson (41). Briefly, the deparaffinized slides were incubated in 50 mM tartrate in acetic buffer at 37°C for 2 h. Then, the sections were incubated in an acid phosphatase substrate buffer (25% Michaelis acetate buffer, 0.16% pararosaniline, 0.16% $NaNO_2$, 0.05% naphtol AS-BI phosphate), pH 5.0 with 20 mM tartrate at 37°C for 60 min, washed in distilled water, and mounted.

Results

Nucleotide Sequence and Comparison of the Mouse and Human Amino Acid Sequence

The 3152 bp sequence of the four overlapping cDNA clones generated in this study contained 19 bp encoding a 5' end untranslated region, a 2,190 bp open reading frame and 943 bp of a 3' end untranslated sequence (Fig. 1). The sequence was

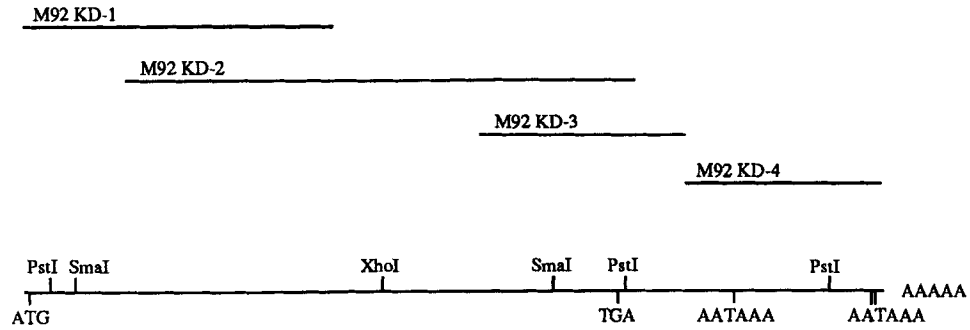


Figure 1. Partial map of the mouse 92-kD type IV collagenase cDNA clones, nucleotide sequence, and predicted amino acid sequence. On top, overlapping cDNA clones and partial restriction map. Below, nucleotide and amino acid sequences. The first line shows the nucleotide sequence starting from the transcription initiation site. The second line shows the predicted amino acid sequence. The third line shows the amino acid sequence of the human preproenzyme (44) with identical residues depicted by (−) and differing residues by one-letter code. Missing amino acids or nucleotides are illustrated by shaded boxes. The numbering of amino acids is shown starting from the first residue of the prepropeptide. The open box depicts the region of the cDNA used for the in situ hybridization probe and the polyadenylation signals (*AATAAA*) are underlined. The nucleotide sequence reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number Z27231.

verified by comparison with the sequence of the mouse gene (Munaut, C., P. Huhtala, P. Reponen, and K. Tryggvason, unpublished) and the mouse cDNA sequence reported while this work was in progress (40). The DNA sequence elucidated in the present study differs slightly from the reported sequence in that there are two differences in the predicted amino acid sequence. In the translated sequence the codon for residue 514 is GCC for alanine, not CCC for proline, and the codon for residue 711 is CCT for proline, not CAT for histidine. Furthermore, the present sequence contains an ACA codon for threonine 278, instead of ACG, the ACT codon for threonine 459 instead of ACA and the GAT codon for aspartate 654 instead of GAC. The two differences observed in the untranslated sequences were a lack of a C at position −5 in the 5′ end UTR and base 2607 was T not A. The presently elucidated cDNA sequence was identical to the exon sequences in the gene (Munaut, C., P. Huhtala, P. Reponen, and K. Tryggvason, unpublished). However, the differences from the reported sequence (40) could be due to variations within species.

The mouse amino acid sequence contains several differences from the human enzyme. The most striking difference is the presence of a 16-residue segment (residues 481–496, Fig. 1) absent from man. This segment is located in the middle of 48-residue sequence which distinguishes the primary structure of the human 92-kD type IV collagenase. The sequence is contained in exon 9. Additionally, the human enzyme lacks amino acid residues corresponding to Ser-38, and residues 459–460 and 708–714.

Localization of 92 kD Type IV Collagenase Expression during Mouse Development by In Situ Hybridization

The in situ hybridization analyses revealed a remarkably cell and stage specific expression pattern in both embryonic and postnatal mouse tissues. No expression was observed in any tissues before E12 (data not shown). All control sections hybridized with the sense probes were also negative (data not shown).

In E12 embryos intense expression was detected in the limb buds in the mesenchymal cells around the developing cartilages of radius and ulna (Fig. 2, *a* and *b*). Similar staining pattern was detected around the incisor tooth germs in the E13 mandible (Fig. 3 *a*). This expression was quite intense and uniform in several layers of condensed mesenchymal cells which were TRAP negative (data not shown). However, the expression was transient and it appeared to be restricted to early stages of development. No expression was observed in any other tissues at this stage.

In E14 embryos intense expression of 92 kD type IV collagenase was detected in single cells in the center of the maxillary and mandibular processes (Fig. 3, *b–e*). During advancing embryonic development and postnatally the number of positive cells increased and they were localized throughout the mandibular and maxillary bones which form by intramembranous ossification (Fig. 4 *a*). Large numbers of positive cells were localized at the site of endochondral ossification beneath the condylar cartilage (Fig. 4 *b*). Numerous positive cells were localized at the bone surface around the growing tooth germs where bone resorption takes place (Fig. 4, *c–e*).

In the developing forelimbs single positive cells were localized in the perichondrium of the digits at E15 (Fig. 2, *c* and *d*). Similarly, in E16 forelimbs positive cells were present at many sites in the perichondrium/periosteum and in addition large aggregates of single positive cells were apparent in the growth plates at sites of endochondral ossification (Fig. 2, *e* and *f*). All other tissues in the limbs such as skin and muscle were devoid of signals above sense probe background.

Adjacent sections of the jaws and bones were analyzed with enzyme histochemical staining for TRAP which is a marker of osteoclasts. The distribution of TRAP positive cells adjacent to the bone closely resembled that of the single cells expressing 92 kD type IV collagenase (Fig. 5).

Expression of 92 kD type IV collagenase was examined in a variety of other tissues at different stages during development and postnatally. In the embryo expression was detected around the clavicle of an E14 embryo (Fig. 6, *c* and *d*) while signals above background level were not detected in skin, mammary gland, or kidney (Fig. 6). Also, salivary gland which expresses the 72-kD type IV collagenase (31) and brain were negative (not shown). After birth signals were seen in the bone marrow of adult mice, presumably osteoclasts (Fig. 7, *e* and *f*). However, signals above background were not observed postnatally e.g., in lung, heart, or intestine.

Discussion

The results of this in situ hybridization study showing expression of 92 kD type IV collagenase in mesenchymal cells around early development of cartilage and in single cells lining embryonic and postnatal bone, sharply contrast with current views on its tissue distribution and biological role. In previous studies 92 kD type IV collagenase activity has been shown to be secreted by cultured peripheral blood polymorphonuclear leukocytes and monocytes, as well as by cytotrophoblasts and alveolar macrophages in vitro (4, 5, 15, 20, 22, 26, 44). These cells, which are capable of migrating through connective tissue barriers, have been speculated to use the enzyme for the degradation of basement membrane and stromal collagens. The demonstration of 92 kD type IV collagenase expression in cultured keratinocytes (34) also suggested that the enzyme is normally needed for basement membrane turnover.

The present in situ hybridization results indicate different physiological roles for the 92-kD type IV collagenase than previously anticipated. Thus, during embryonic development and until 9 d postnatally, high expression was observed in osteoclasts, while it could not be detected above background levels in any epithelial cells of skin, lung, intestine or kidney, and not either in subepithelial mesenchymal cells. Therefore, this enzyme is not likely to play a major role in the normal turnover of basement membranes. Furthermore, clear signals were not observed in tissues usually rich in monocytes/macrophages such as liver, lung or intestine, even at 9 d postnatally. Only in bone marrow scattered cells, possibly representing the monocyte/macrophage/osteoclast lineage, were positive. Our interpretation of the present results is that the biological function of 92 kD type IV collagenase during embryogenesis is primarily to degrade the extracellular matrix around the growing cartilage and in the developing bone. These tissues are rich in type I, II, and III collagens and they also contain collagen types V, VI, VIII,

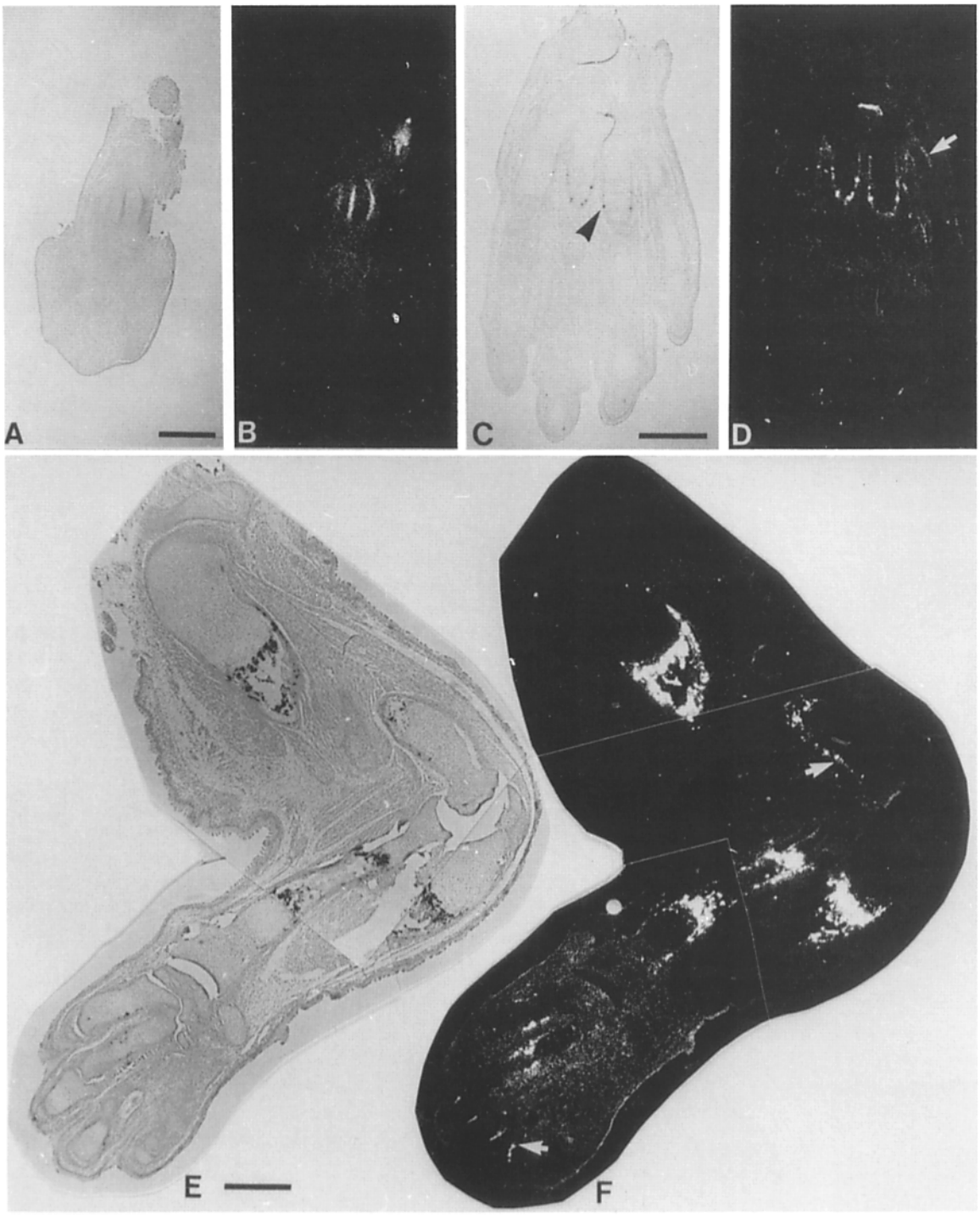
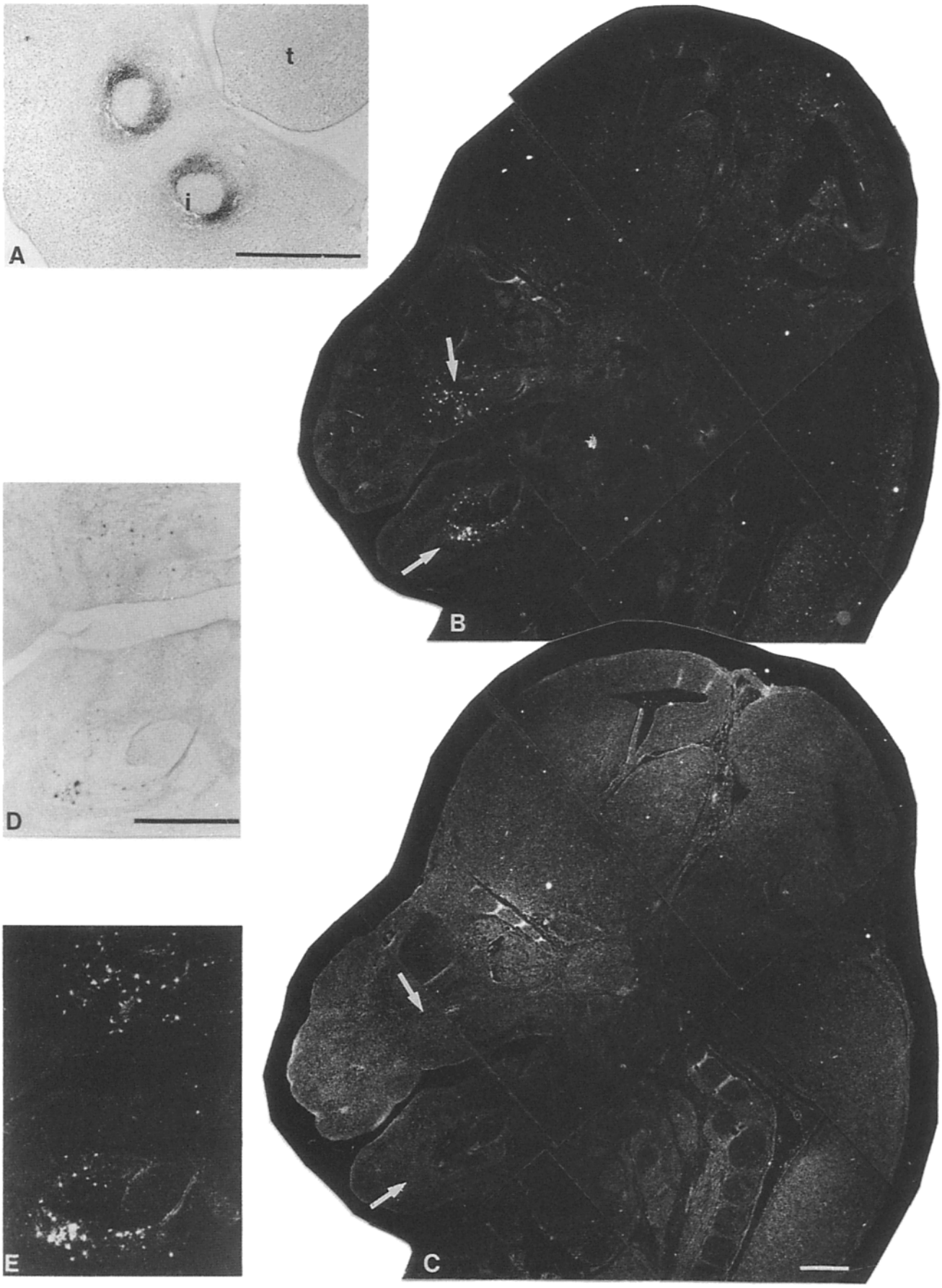


Figure 2. Expression of 92 kD type IV collagenase during limb development. (*A* and *B*) E12 fore limb. The mesenchymal cells around the radius and ulna show expression of 92 kD type IV collagenase. (*C* and *D*) In the E15 fore limb expression is detectable around the cartilage (*white arrow*). Single cells in the perichondrium of the metacarpal cartilage as well as at the tips of the digits intensely express 92 kD type IV collagenase. In the E16 fore limb (*E* and *F*), the expression of 92 kD type IV collagenase is very intense in the ossifying regions of the long bones. Also the perichondrium and the periosteum show some expression (*arrows*). (*A*, *C*, and *E*) bright field; (*B*, *D*, and *F*) dark field. Bar, 400 μm.

IX, X, XI, and XII, but not the basement membrane type IV collagen (for review see 36). Although the actual substrate of the 92 kD type IV collagenase is not known, it seems plausible that the enzyme acts as a gelatinase involved in the removal of denatured collagen fragments (gelatin) formed by the action of interstitial collagenases that are considered the only enzymes capable of degrading collagen fibres which contain interstitial collagens. Once cleaved into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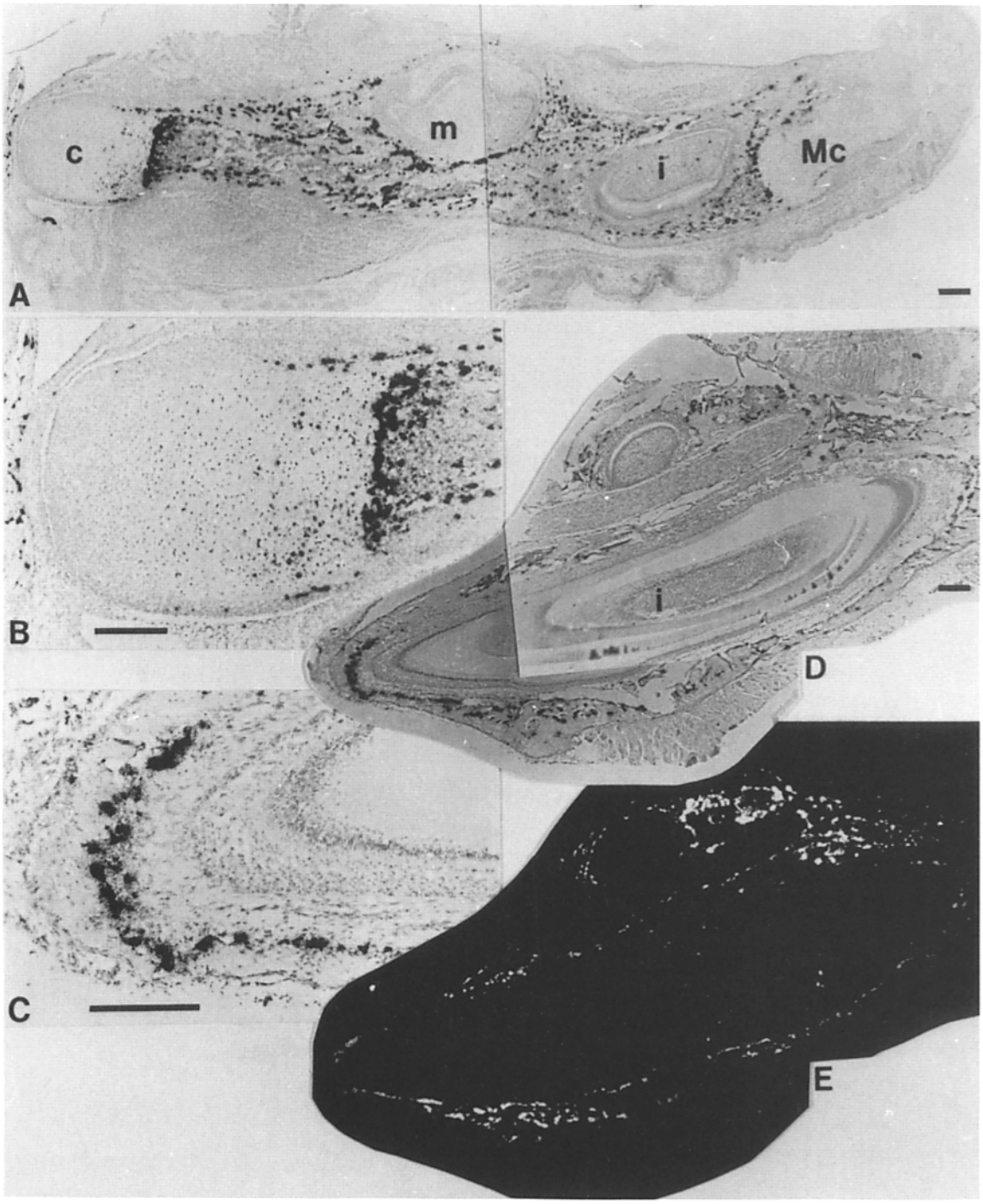

Figure 4. Expression of 92 kD type IV collagenase during fetal and postnatal development of the jaw. (*A*) In the E18 mandible, the 92-kD type IV collagenase gene expression is intense throughout the developing bone of the mandible. The developing teeth are completely negative. (*B*) Higher magnification of the condylar process. (*D* and *E*) 11 d postnatal mandible with incisor tooth. The expression in the bone is most intense at the resorptive surface around the growing tooth germ. (*C*) Magnification of the tip of the incisor. The area of the bone through which the incisor is about to erupt shows especially intense 92 kD type IV collagenase expression. *c*, condylar process; *i*, incisor; *m*, molar; *Mc*, Meckel's cartilage. (*A*, *B*, *C*, and *D*) bright field; (*E*) dark field of *D*. Bar, 200 μm.

Figure 3. Localization of 92 kD type IV collagenase expression by in situ hybridization in the head of E13 (*A*) and E14 (*B*, *C*, *D*, and *E*) mouse embryos. (*A*) Transverse section through the anterior part of the lower jaw. Intense expression is seen in the mesenchyme around the incisor tooth germ. (*B* and *C*) Sagittal section of E14 anterior embryo hybridized with antisense (*B*) and control sense (*C*) probes. All tissues are negative for 92 kD type IV collagenase, except in the central areas in the upper and lower jaw where spots of strong expression can be seen. The spots are localized to single cells in the developing jaw bones (*D* and *E*) in the area where the first ossification can be seen at 15 d p.c. (*A* and *D*) bright field; (*B*, *C*, and *E*) dark field. *i*, incisor; *t*, tongue. Bar, 400 μm.

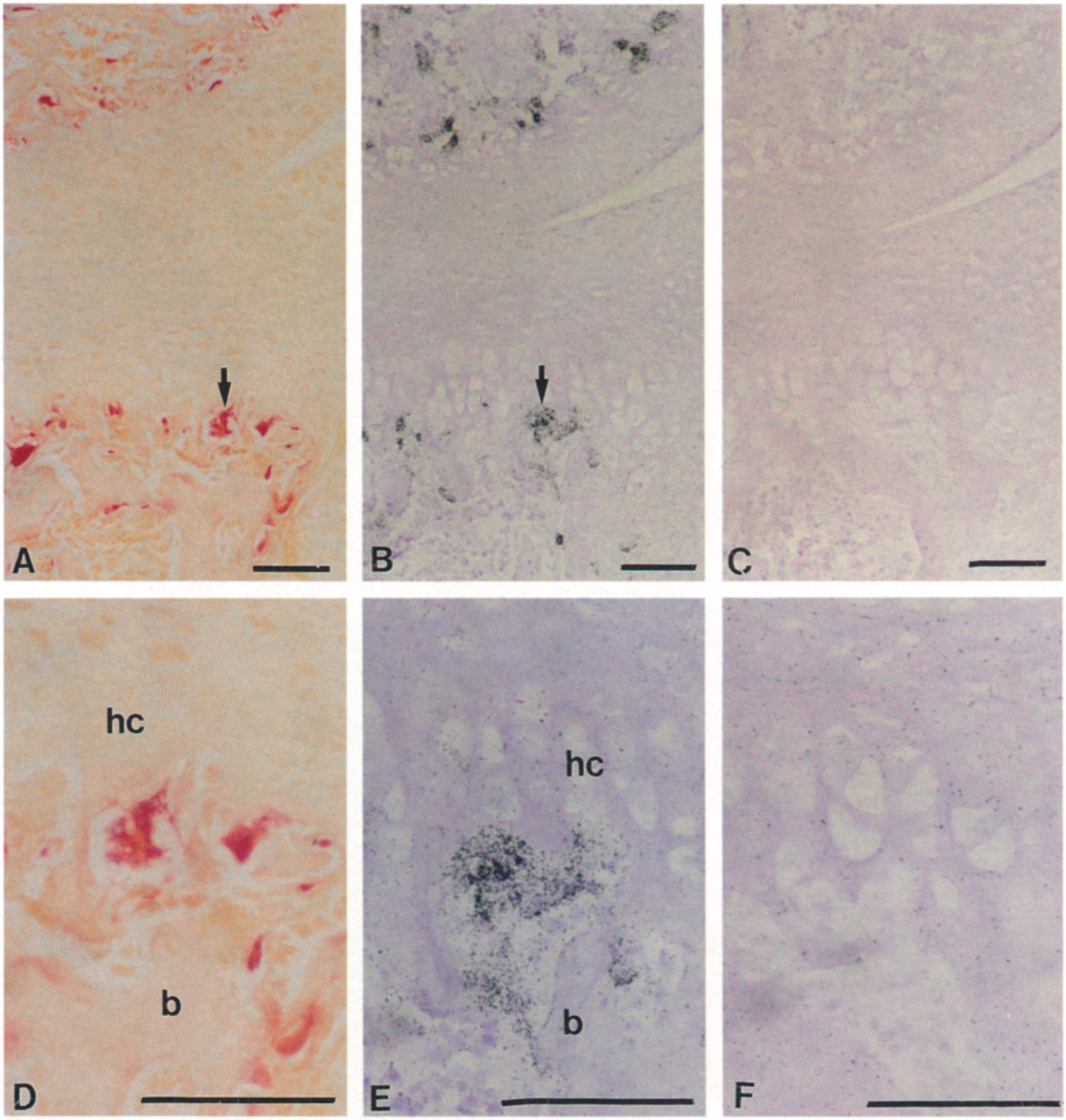

Figure 5. Identification of osteoclasts in the fore limb by TRAP staining and colocalization with 92 kD type IV collagenase expression. (*A* and *D*) TRAP-stained osteoclasts (*red*) are evident in the epiphyseal plate in the bone of 2-wk-old mouse forelimb. (*B*) In situ hybridization of an adjacent section to *A* shows intense expression of 92 kD type IV collagenase in cells with similar distribution as osteoclasts (*arrows*). (*C* and *F*) Negative in situ hybridization control of an adjacent section to *B* with sense probe. *D*, *E*, and *F* are magnifications of *A*, *B*, and *C*, respectively. *b*, bone; *hc*, hypertrophic chondrocytes. Bar, 100 μm.

two 1/4 and 3/4 size triple-helical fragments, the collagen fragments denature at body temperature and can subsequently be degraded further by the gelatinases.

The highly restricted spatial and temporal expression indicates two major functions of the 92-kD type IV collagenase during embryonic development. First, at E12 and E13, the earliest stages of expression, signals were only observed in TRAP negative mesenchymal cells around the developing cartilages and tooth germs. This expression was restricted only to early stages of development suggesting that the enzyme functions in the removal of gelatin at the growing end of the cartilage and tooth allowing their rapid growth. Second, the localization of expression to cells on the surface of the developing bones from E14 onwards implies a specific

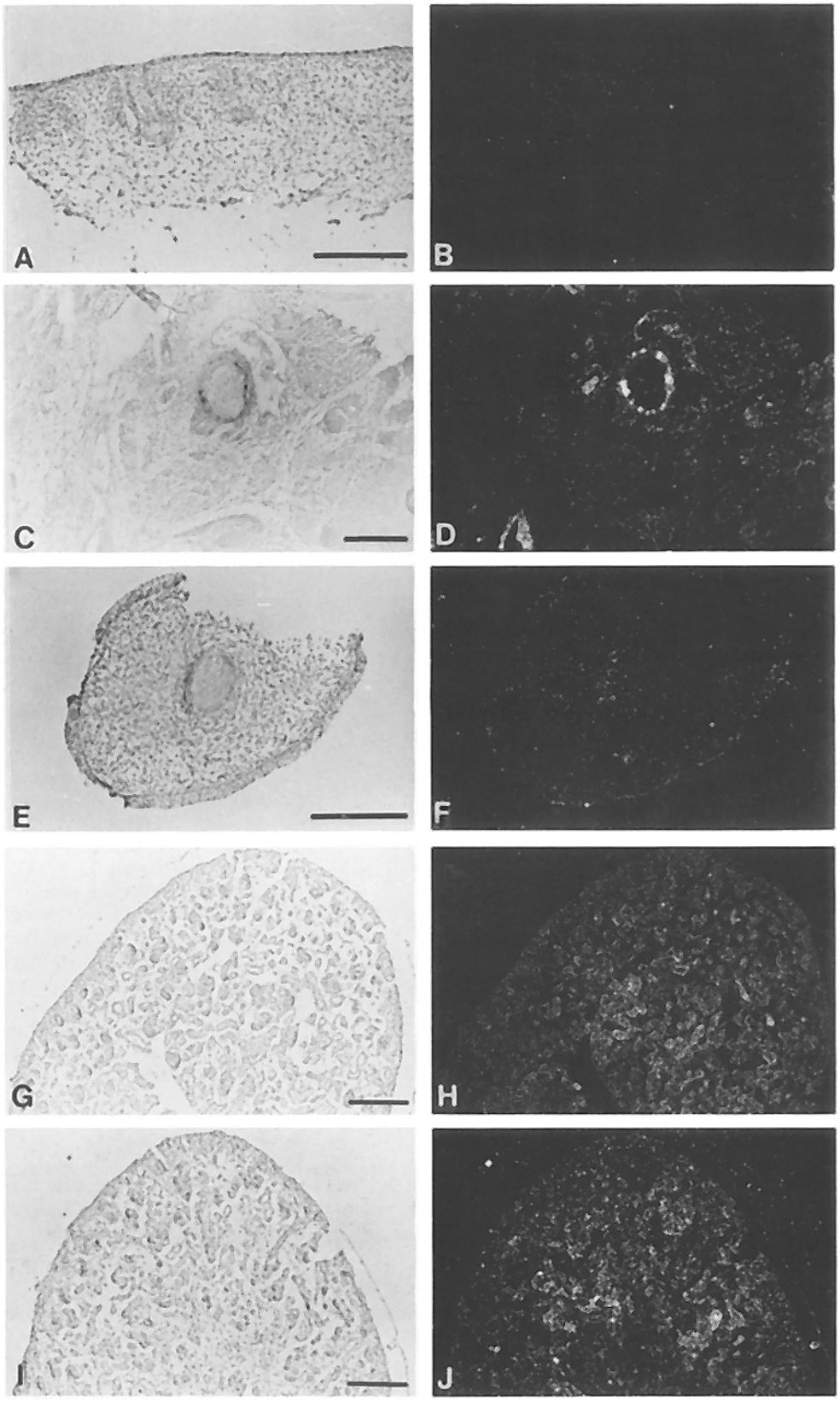

Figure 6. Expression of 92 kD type IV collagenase in various tissues during mouse development. (*A* and *B*) E14 skin with hair follicle, (*C* and *D*) E14 clavicula, (*E* and *F*) E16 mammary gland, (*G* and *H*) E18 kidney hybridized with antisense probe, and (*I* and *J*) E 18 kidney hybridized with control sense probe. Skin with hair follicle, kidney, and mammary gland do not show expression of the 92-kD type IV collagenase gene above sense control background. In the clavicula, one of the earliest ossification centers, spots of expression are seen in the periosteum which presumably represent osteoclasts. (*A*, *C*, *E*, *G*, and *I*) bright field; (*B*, *D*, *F*, *H*, and *J*) dark field. Bar, 200 μm.

role of the enzyme in the resorption of bone, presumably in the removal of gelatinous polypeptides. The localization of these positive cells showed close correlation with osteoclasts which were localized in adjacent sections by staining for TRAP which is generally considered a marker of osteoclasts (41). Our preliminary observations (unpublished) in older bones indicate that TRAP positivity in the osteoclasts does not always correlate with 92 kD type IV collagenase expres-

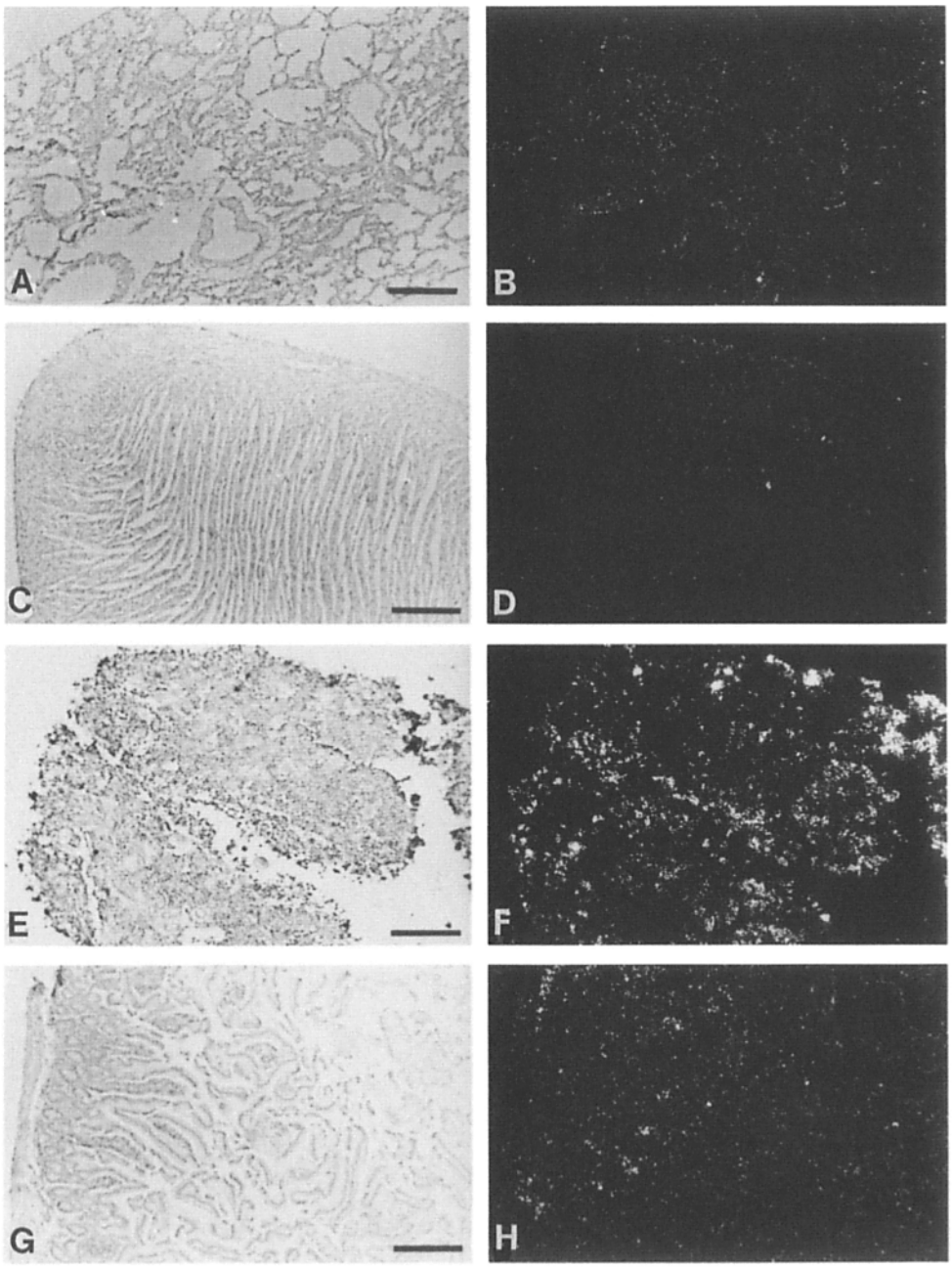

Figure 7. Expression of 92 kD type IV collagenase in various postnatal and adult tissues. (*A* and *B*) 9D postnatal lung, (*C* and *D*) 9D postnatal heart, (*E* and *F*) adult bone marrow, (*G* and *H*) adult intestine. The lung, heart, and intestine do not show signals for 92 kD type IV collagenase gene expression above sense control background (not shown). The bone marrow (*E* and *F*) from the adult femur shows some single cells expressing the 92-kD type IV collagenase gene. (*A*, *C*, *E*, and *G*) bright field; (*B*, *D*, *F*, and *H*) dark field. Bar, 200 μm.

sion. Hence, the 92-kD enzyme expressing cells may represent only a subpopulation of cells in the osteoclast cell lineage. Metalloproteinase activity has been suggested to occur in osteoclasts (10) but our study is the first to demonstrate the expression of a metalloproteinase highly specific for osteoclasts. Although it has not yet been demonstrated, it is likely that osteoclasts secrete interstitial collagenases which are presumably essential for dissolution of the collagen fibrils. Lysosomal cathepsins have also been proposed to participate in the extracellular degradation of bone matrix (10).

Surprisingly, with the exception of bone marrow, clear expression of the 92-kD type IV collagenase above background was not observed in this study in embryonic or postnatal tissues normally containing macrophages. The question therefore rises, whether findings showing that macrophages, monocytes, and polymorphonuclear leukocytes secrete the enzyme in vitro have any relevance to the situation in vivo. It is possible that although transcripts are absent from macrophages of normal tissues, they possess the potential to express the 92-kD type IV collagenase e.g., upon stimulation by cytokines. In fact, we have shown by in situ hybridization that macrophages located adjacent to invading cells of human tumors of skin (28), colon (29), and ovary (1) express the gene. The enzyme antigen has also been localized specifically to macrophages in human breast cancer by monoclonal antibodies (42). Furthermore, there is evidence that the 92-kD type IV collagenase is involved in other pathological processes such as periodontal inflammation (19, 39) and wound healing (35) where the activity may be present in macrophages. Hence, the absence of signals in macro-

phages of the normal mouse tissues presently studied may simply reflect the quiescent state of the cells. Since osteoclasts, like macrophages, have their origin in hematopoietic cells, it can be speculated that cells of the hematopoietic cell lineage can in certain situations be induced to express the 92-kD type IV collagenase gene. The localization of 92-kD type IV collagenase mRNA by in situ hybridization in tumor cells of human squamous cell carcinoma (28), but not of colon cancer (29), also indicates that the gene may be induced in malignant transformation of some tissues.

Several studies have indicated that the 92-kD type IV collagenase is critical for the invasion of cytotrophoblasts during embryonic implantation. Presence of the enzyme has been shown in these cells by zymography and their invasion into extracellular matrix could be inhibited by specific antibodies (4, 20). We have also shown by in situ hybridization analysis that the 92-kD type IV collagenase gene is expressed solely in the trophoblast population (Reponen, P., C. Sahlberg, I. Thesleff, and K. Tryggvason, unpublished data). These observations suggest that the 92-kD type IV collagenase also has a key role in the degradation of the maternal extracellular matrix by the extraembryonic trophoblast cells.

In conclusion, the results of the present study suggest that during normal morphogenesis, the 92-kD type IV collagenase is mainly secreted by osteoclasts where it is used for bone remodeling. However, the enzyme can also be involved in physiological tissue remodeling by extraembryonic trophoblasts during embryonic implantation and in pathological states such as tumor invasion and inflammation where it may be secreted by macrophages. The expression pattern of the 92-kD type IV collagenase differs completely from that of the 72-kD enzyme the expression of which has been shown to be widely distributed mainly in mesenchymal tissues during development of all organs (31). The 72-kD enzyme is intensely expressed in osteoblasts (32), but we do not know whether it is expressed by osteoclasts also. This question can be studied in isolated osteoclasts. Both enzymes have similar substrate specificities, but the restricted distribution of the 92-kD enzymes implies the requirement for the availability of a gene transcribing a type IV collagenase (gelatinase) for a more specific purpose.

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