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Regional and temporal changes in the synthesis of matrix metalloproteinases and TIMP-1 during development of the rabbit mandibular condyle

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ABSTRACT

Connective tissues synthesise and secrete a family of matrix metalloproteinases (MMPs; collagenases, gelatinases and stromelysins) capable of degrading all the components of connective tissue matrices at physiological pH. We document the patterns of synthesis and distribution of MMPs and the tissue inhibitor of metalloproteinases-1 (TIMP-1) within the developing rabbit mandibular condyle using immunofluorescence microscopy. MMPs and TIMP-1 were detected both as bright intracellular accumulations within Golgi vesicles and also as diffuse matrix-bound extracellular deposits. Cells in the articular zone, proliferative zone, condylar cartilage and bone of the mandibular ramus were shown to produce all 3 classes of MMPs and TIMP-1 with the exception of stromelysin, which was not synthesised by cells of the bone of spongiosum. Temporal synthesis of MMPs and TIMP-1 within these regions varied during the period 18 d postcoitum to 14 d postnatum. Our results document unique patterns of MMP and TIMP-1 synthesis during embryonic and early postnatal development of condylar cartilage and support the concept that cells synthesise and secrete MMPs and TIMP-1 before undergoing proliferation and hypertrophy. A comparison of these results with data in the rabbit growth plate show many similarities, but some differences exist that probably reflect differences in the modes of growth of the 2 cartilages.

INTRODUCTION

Although the mandible is a membrane bone its development is complicated by the formation of 3 secondary cartilages, one of which, the condylar cartilage, persists throughout life. In the rat the condylar blastema appears at 17 d post coitum (pc) in the posterior region of the mandibular blastema (Bhaskar, 1953). Duterloo & Jansen (1969) observed that at 18 d pc the perichondrium covering the condylar blastema was continuous with the periosteum covering the bony sheath of the mandible. Since the chondrocytes of condylar cartilage do not divide (Blackwood, 1966), the only potential source of cells able to maintain growth of the mandible in this area is the cellular layer (proliferative zone) of the covering perichondrium. When placed in a mechanically nonfunctioning environment (intracerebral transplantation) cells of the proliferative zone differentiated into osteoblasts and not chondroblasts (Meikle, 1973), suggesting that the perichondrium of the condylar cartilage is a periosteum modified by functional articulation. Condylar cartilage, unlike the epiphyseal growth plate of a long bone, is therefore the product of periosteal chondrogenesis, the growth of which is appositional in character.

Connective tissue cells synthesise and secrete a family of matrix metalloproteinases (MMPs) capable of degrading all the components of connective tissue matrices at physiological pH. These enzymes have

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been implicated in many pathophysiological processes involving matrix degradation (Matrisian, 1990; Reynolds & Hembry, 1992; Murphy & Reynolds, 1993). Three groups of MMPs have been identified on immunological and biochemical criteria and sequence data. The specific collagenases (Goldberg et al. 1986; Whitham et al. 1986) cleave interstitial collagens types I, II and III into three-fourths and one-fourth fragments; 2 distinct gene products have been identified, both of which degrade fibrillar collagens. The 2nd group, known as gelatinases (type IV collagenases) degrade denatured interstitial collagens and collagen types IV, V and VII, as well as elastin. Two members have been described, a relative molecular mass (M_r) 72×10^3 species (gelatinase A) found in connective tissue cells and some tumour cells and a glycosylated M_r 92×10^3 species (gelatinase B) associated with monocytes, macrophages, polymorphonuclear leucocytes (Murphy et al. 1989*a*, *b*), tumour cells (Yamagata et al. 1988, 1989) and connective tissue cells in some circumstances. The stromelysins (proteoglycanases) comprise the 3rd group (Galloway et al. 1983; Murphy & Docherty, 1988; Murphy et al. 1988). They demonstrate wide substrate specificity and degrade proteoglycans, laminin, fibronectin and the nonhelical regions of collagens; 2 members, stromelysin-1 and stromelysin-2, showing considerable sequence homology have been described (Murphy & Reynolds, 1993).

MMPs are secreted as latent proenzyme forms and their extracellular activity is regulated in a complex fashion. In the case of collagenase and stromelysin (the mechanism of activation of gelatinases is uncertain), activation occurs via a plasminogen–plasmin–MMP cascade (Mignatti et al. 1986; Chapman et al. 1988; Thomson et al. 1989); their action is regulated by a family of specific inhibitors called TIMPs (tissue inhibitor of metalloproteinases; Sellers & Reynolds, 1977; Murphy et al. 1981; Docherty et al. 1985; Stetler-Stevenson et al. 1989). TIMP-1 is an M_r 28×10^3 glycoprotein synthesised by most connective tissue cells as well as macrophages (see Cawston, 1986, for a review), and TIMP-2 was originally isolated from human melanoma cells as an M_r 21×10^3 unglycosylated protein bound to progelatinase A (Stetler-Stevenson et al. 1989). Both TIMP-1 and TIMP-2 form inactive complexes with activated MMPs (Cawston et al. 1983). Previous biochemical and immunocytochemical studies of the epiphyseal growth plate have demonstrated an important role for MMP-mediated matrix degradation in both chondrogenesis and endochondral ossification. Collagenase activity has been identified in growth plate cartilage from embryonic chick bone (Yasui et al. 1981), collagenase and TIMP in normal and rachitic rat cartilage (Dean et al. 1985, 1990) and neutral proteoglycanase (stromelysin) activity in the bovine growth plate (Ehrlich et al. 1985). Collagenase, gelatinase, stromelysin and TIMP have also been immunolocalised in the distal femoral growth plate of the rabbit (Brown et al. 1989). Unique patterns in the synthesis and extracellular distribution of MMPs and TIMP were observed (Brown et al. 1989) at specific stages of chondrocyte differentiation, suggesting that extracellular matrix degradation within the growth plate is a carefully regulated event.

The aim of the present investigation was to determine whether the different mode of growth of condylar cartilage might be reflected in alterations in the distribution of MMPs and TIMP, and what temporal changes if any in their expression may be observed during development.

MATERIALS AND METHODS

Preparation of rabbit craniomandibular joint explants

Staged craniomandibular joint explants were prepared from embryonic tissues 18 to 28 d pc at 2 d intervals and from neonatal tissues at 1, 3, 7 and 14 d post natum (pn) from either New Zealand White or Old English rabbits under aseptic conditions. Noon following a morning mating was taken as T_0. Tissue (approximately 5 mm square for postnatal explants) was removed to include the condyle, disc and the zygomatic root of the squamosal bone. Postnatal joints were divided in an inclined coronal plane bisecting the centre of the disc (Fig. 1*A*) or disarticulated into condyle and disc portions. Prenatal specimens were disarticulated and condylar cartilages used either whole or divided. Explants were either cultured with monensin (monensin treated), frozen immediately (nonmonensin treated) or processed for routine histology as described below.

Histological examination

Frozen tissue sections were prepared for routine histological examination with connective tissue stains (von Kossa, van Gieson, toluidine blue and Alcian blue) and examined by bright field microscopy. Several 14 d pn specimens were decalcified and processed for routine paraffin wax embedding and stained with haematoxylin and eosin. All sections used for immunolocalisation were subsequently stained either with haematoxylin and eosin or tol-

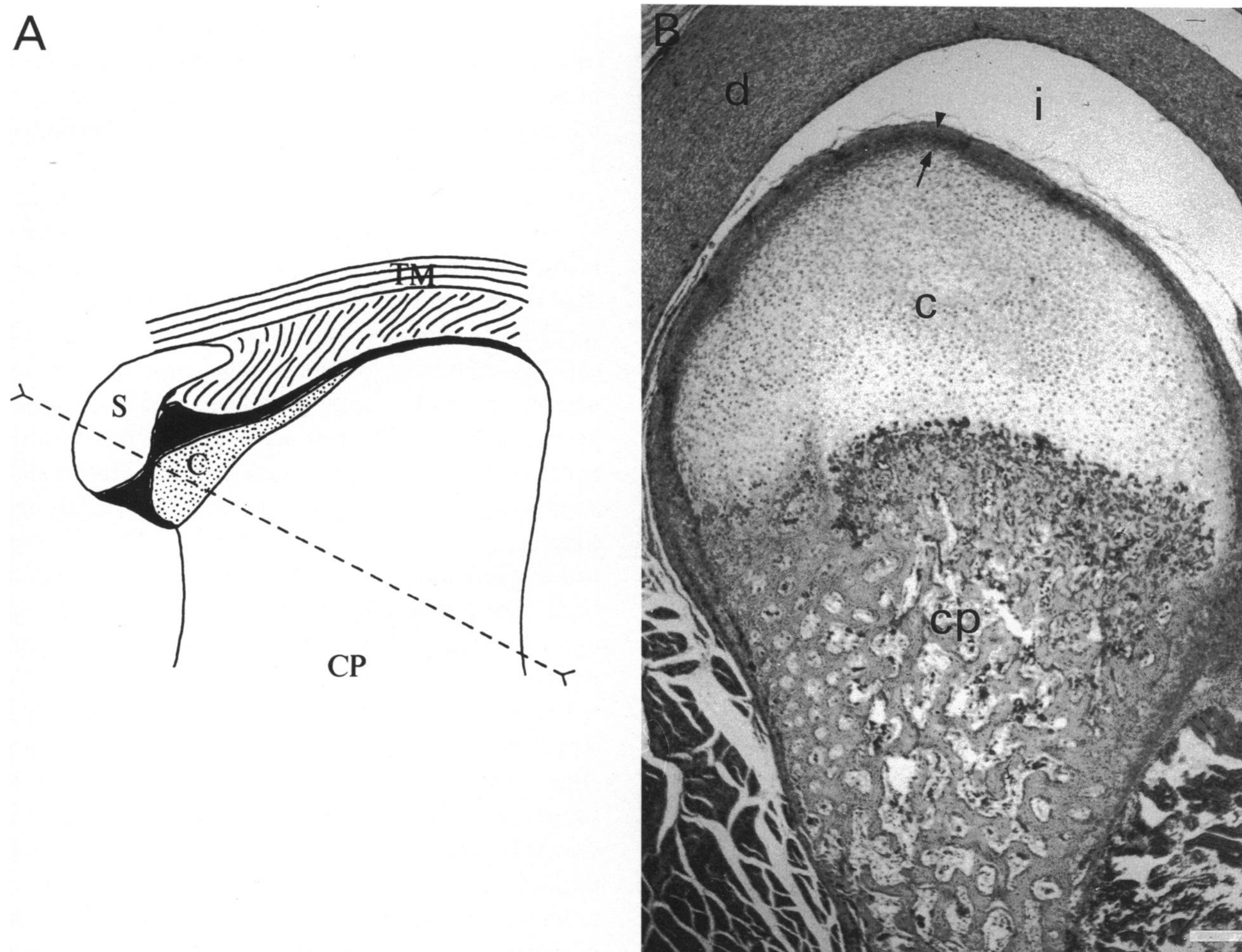

Fig. 1. (*A*) Diagram of 1-wk-old rabbit craniomandibular joint showing the inclined coronal plane used to section explants, condyloid process of the mandible (CP) the stippled part of which is the condylar cartilage (C), squamosal root of the zygomatic arch (sectioned, S) and temporalis muscle (TM). After Mills et al. (1988). (*B*) Light micrograph of paraffin-embedded section of an inclined coronal section from a 7-d rabbit mandibular condyle, stained with haematoxylin and eosin, showing condylar cartilage (c), articular zone (arrowhead), proliferative zone (arrow), inferior joint space (i), disc (d) and the condyloid process (cp). Bar, 200 μm.

uidine blue. Photographs were taken on a Zeiss standard WL microscope using Ektachrome 400X film.

Culture of explants

The cut surface was placed downwards on a stainless steel grid in a 5 cm diameter Petri dish containing Dulbecco's modification of Eagle's medium (DMEM, 2.5 ml) supplemented with 10% fetal calf serum (Gibco, Grand Island, New York), antibiotics and 5 μM monensin (Sigma). Connective tissue cells normally synthesise and secrete low levels of MMPs and TIMP; the ionophore monensin, which inhibits the translocation and secretion of newly synthesised proteins while allowing synthesis to continue, was therefore used to accumulate intracellular MMPs and TIMP during explant culture (Nagase et al. 1983; Hembry et al. 1985, 1986). Explants were cultured at 37 °C in a humidified atmosphere of 5% CO_2/95% air for either 6 or 24 h. At the end of the culture period the explants were embedded in Tissue-Tek (OCT, Miles Laboratories Inc., Naperville, Illinois) and snap frozen in liquid nitrogen for 90 s. Samples were stored at −70 °C until sectioned.

Antisera

Specific polyclonal antibodies to rabbit collagenase (S-anti-CL), gelatinase (S-anti-GL; which recognises both gelatinase A and B), stromelysin (S-anti-SL) and TIMP-1 (S-anti-TIMP-1) were raised in sheep. The characterisation of these antisera, including species specificity, Western blots, inhibition curves and immunoabsorption experiments with purified antigen, have been reported in detail elsewhere (Hembry et al. 1986; Murphy et al. 1986, 1989*b*; Gavrilovic et al. 1987). Pooled normal sheep serum (NSS) was used as

a control. The 2nd antibody for indirect immunolocalisation was either a fluorescein isothiocyanate labelled monovalent Fab′ preparation from an antiserum raised in a pig (pig-FITC) as previously described (Hembry et al. 1985), or fluorescein isothiocyanate conjugated antisheep IgG (whole molecule) raised in a donkey (Sigma; donkey-FITC), and diluted 1:200 in 5% normal donkey serum (Sigma) in phosphate buffered saline (PBS, pH 7.4).

Immunolocalisation

Serial frozen tissue sections (4–6 μm) were cut from at least 3 samples from each time point. Sections were air dried for 5 min, fixed for 30 min in freshly prepared 4% paraformaldehyde in PBS, washed in PBS and permeabilised (0.1% Triton X-100, 5 min) to facilitate IgG penetration of cell membranes. They were then incubated with either NSS, S-anti-CL, S-anti-GL, S-anti-SL or S-anti-TIMP (IgG, 50 μg/ml in PBS, 30 min) in a humidified atmosphere. After repeated washing in PBS, sections were incubated with 2nd antibody (pig-FITC or donkey-FITC, 30 min), washed in PBS and counterstained with a nuclear stain (methyl green, 1 mg/ml, 2 min) which fluoresces red when visualised with a wide band FITC or rhodamine filter. Sections were mounted with Citifluor (Glycerol/PBS, City University, London, England), coverslipped and sealed with glyceel (Gurr, BDH). All sections were then examined within 24 h by fluorescence microscopy on either a Zeiss photomicroscope III with epifluorescence and standard wide band FITC filters or a Zeiss standard WL microscope equipped with rhodamine and standard narrow band FITC filter sets. Photographs were taken on Agfachrome RS 1000 film uprated to 2000 ASA or Ektachrome 400X. To semiquantify the intensity of staining observed the following scale was used: no staining detected (−), occasional faint intracellular staining (±), faint intracellular staining of most cells (+), faint intracellular staining of most cells with occasional bright intracellular staining (+ +), bright intracellular staining of most cells (+ + +), bright intracellular staining of most cells with positive matrix staining (+ + + +).

RESULTS

Histology

The soft tissue layers of the rabbit mandibular condyle were classified by morphology and are illustrated in Figures 1*B* and 2*A–C*. The surface layer, or perichondrium, was divided into an outer articular zone composed of fibrous connective tissue and an inner proliferative zone of undifferentiated mesenchyme cells. Chondrocytes were arbitrarily divided into upper hypertrophic and lower hypertrophic according to relative cell volume. Any bone included was identified as either cortical or spongiosum.

No recognisable condylar structures were identified in explants taken from the region of the craniomandibular joint in 18 d pc embryos. We assumed that these explants predate the appearance of the cartilage. In condyles taken from 20 d pc embryos, the cartilage consisting of hypertrophic chondrocytes was narrow in coronal section and covered by a broad perichondrium with both articular and proliferative zones (Fig. 2*A*). The transition between proliferative zone cells and hypertrophic chondrocytes was abrupt, suggesting little appositional growth. The cartilage was sleeved along its inferior third by a thin layer of bone. The appearance at 22 d pc was similar, except that there was erosion at the base of the cartilage with replacement by bone (Fig. 2*B*).

From 22–28 d pc there was a progressive increase in size during which the condyle broadened and rounded (Fig. 2*C*) with thickening of the cortical bone by subperiosteal osteogenesis and further cartilage erosion. The articular and proliferative zones, although thinner, showed increased cellularity. A spectrum of cellular development from undifferentiated cells of the proliferative zone through chondrocytes to hypertrophic chondrocytes had been established suggesting appositional growth.

In the postnatal joint the cartilage appeared as a broad, hemispherical cap of hyaline cartilage on top of the mandibular ramus (Fig. 1*B*). The perichondrium, now much reduced in thickness, had an articular zone of condensed fibrous tissue and a thin proliferative zone. The spectrum of chondrocyte phenotype was maintained.

Immunolocalisation of collagenase, gelatinase, stromelysin and TIMP-1

Sections from 24 h monensin-treated explants incubated with antisera to collagenase, gelatinase, stromelysin and TIMP-1 followed by FITC-labelled second antibody showed red counterstained nuclei and bright green fluorescence indicating antigen synthesis. MMPs and TIMP-1 were detected both as bright intracellular accumulations within Golgi vesicles and also as diffuse matrix-bound extracellular deposits (e.g. Fig. 3*A*). The patterns of distribution of each antigen are described below and summarised in Tables 1–4. Sections from nonmonensin treated

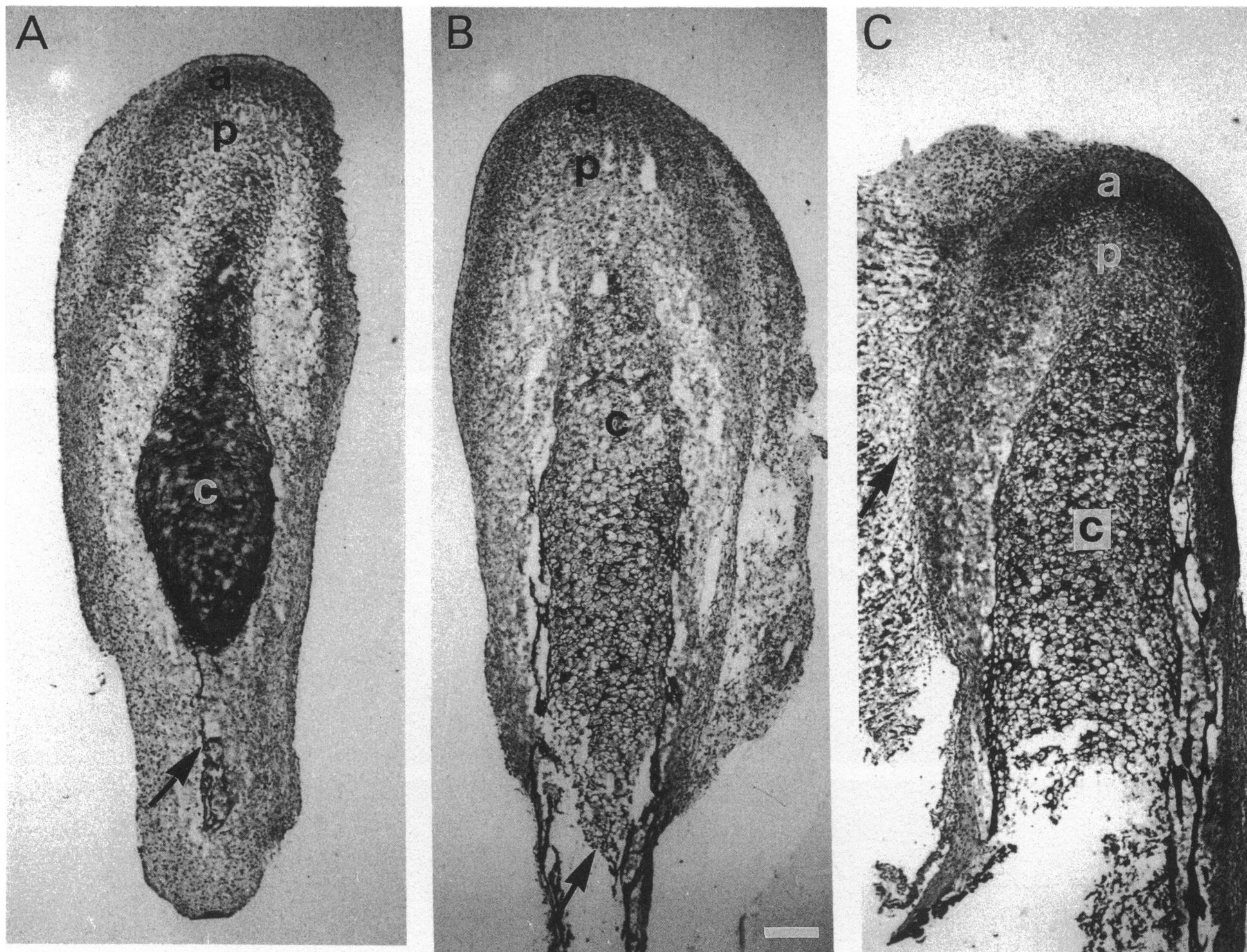

Fig. 2. Light micrographs of frozen sections of monensin treated explants of mandibular condyles taken from rabbit embryos at 20 d pc (*A*), 22 d pc. (*B*) and 26 d pc (*C*), stained with toluidine blue. Bar, 200 µm. (*A*) Inclined coronal section of 20 d pc mandibular condyle showing condylar cartilage (c), articular zone (a), proliferative zone (p) and bone of the condyloid process (arrow). (*B*) Inclined coronal section of 22 d pc mandibular condyle showing condylar cartilage (c), articular zone (a), proliferative zone (p) and early cartilage erosion with bone replacement (arrow). (*C*) Inclined coronal section of 26 d pc mandibular condyle showing condylar cartilage (c), articular zone (a), proliferative zone (p) and insertion of lateral pterygoid muscle (arrow).

explants showed only occasional very faint intracellular and matrix staining (data not shown), indicating that the bright green staining seen in sections from monensin-treated explants represents antigen synthesised and accumulated during culture. Control sections from monensin-treated and nonmonensin-treated explants incubated with NSS IgG followed by FITC-labelled second antibody showed only red counterstained nuclei.

Collagenase

Collagenase was detected at 20 d pc as general faint intracellular fluorescence in upper and lower hypertrophic chondrocytes with 1 or 2 lower hypertrophic chondrocytes per section showing bright staining. From 22 d pc to 14 d pn most lower hypertrophic chondrocytes continued to show bright intracellular staining (Fig. 3*B*). Upper hypertrophic chondrocytes showed only general faint intracellular staining with occasional bright cells during the remainder of embryonic development (see Table 1); however, most stained brightly during the postnatal period (Fig. 3*C*). In the articular and proliferative zones collagenase was first detected at 22 d pc as faint intracellular staining with occasional brightly fluorescent cells and small areas of faint, diffuse extracellular matrix staining. Most cells in the perichondrium stained brightly during final embryonic development, 26 to 28 days pc, but during early postnatal development there was strong immunofluorescence in many cells and adjacent matrix (Fig. 3*D*). Cells lining the trabeculae of the spongiosum stained weakly at all times whilst within the narrow spaces occasional foci of brightly staining cells (Fig. 3*E*) were visible from 22 d pc onwards.

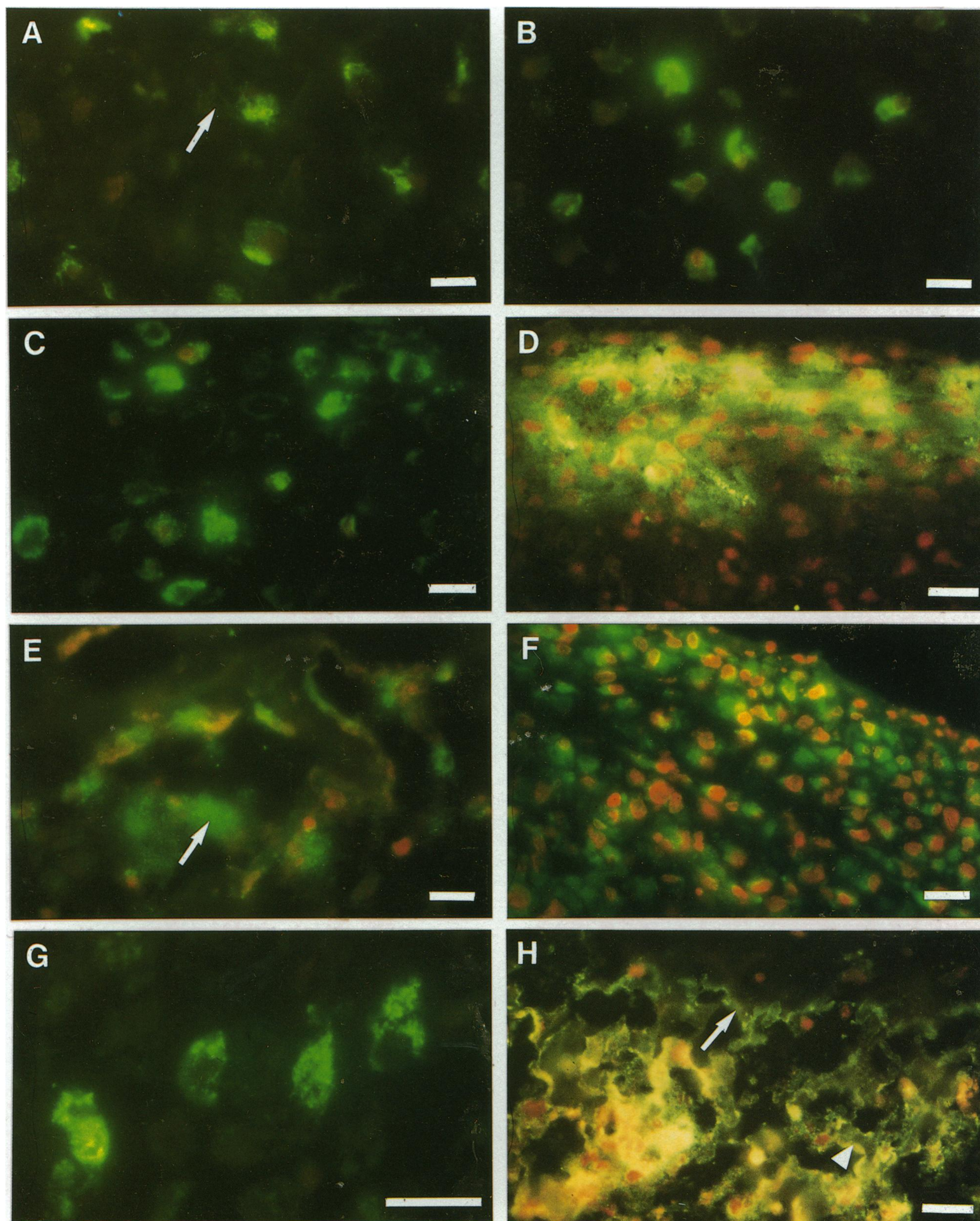

Fig. 3. Immunofluorescent detection of collagenase (*B–E*) and gelatinase (*A*, *F–H*) in frozen sections of monensin treated explants of mandibular condyle taken from 20 d pc to 14 d pn rabbits. Bar, 20 µm. (*A*) Gelatinase in lower hypertrophic chondrocytes at 26 d pc. Cells show bright intracellular fluorescent and diffuse, weak extracellular staining (see arrow). (*B*) Collagenase in lower hypertrophic chondrocytes at 14 d pn. Cells show bright intracellular staining. (*C*) Collagenase in upper hypertrophic chondrocytes at 1 d pn. Cells show bright intracellular staining. (*D*) Collagenase in the articular and proliferative zones at 1 d pn. Many cells stain brightly and there is extensive, bright extracellular matrix staining. (*E*) Collagenase in bone cells. There is weak fluorescence of cells lining the trabeculae of the spongiosum and bright staining of cells within the marrow spaces (arrow) at 3 d pn. (*F*) Gelatinase in the articular and proliferative zones at 28 d pc. Cells show bright intracellular staining and there are areas of diffuse extracellular matrix staining. (*G*) Gelatinase in upper hypertrophic chondrocytes at 7 d pn. Cells show bright intracellular staining. (*H*) Gelatinase in calcified cartilage and bone underlying the condylar cartilage at 1 d pn. There is matrix staining along the margin of the inferior surface of the condylar cartilage (arrow) and adjacent trabecular bone (arrowhead) and bright intracellular staining of cells within the marrow spaces.

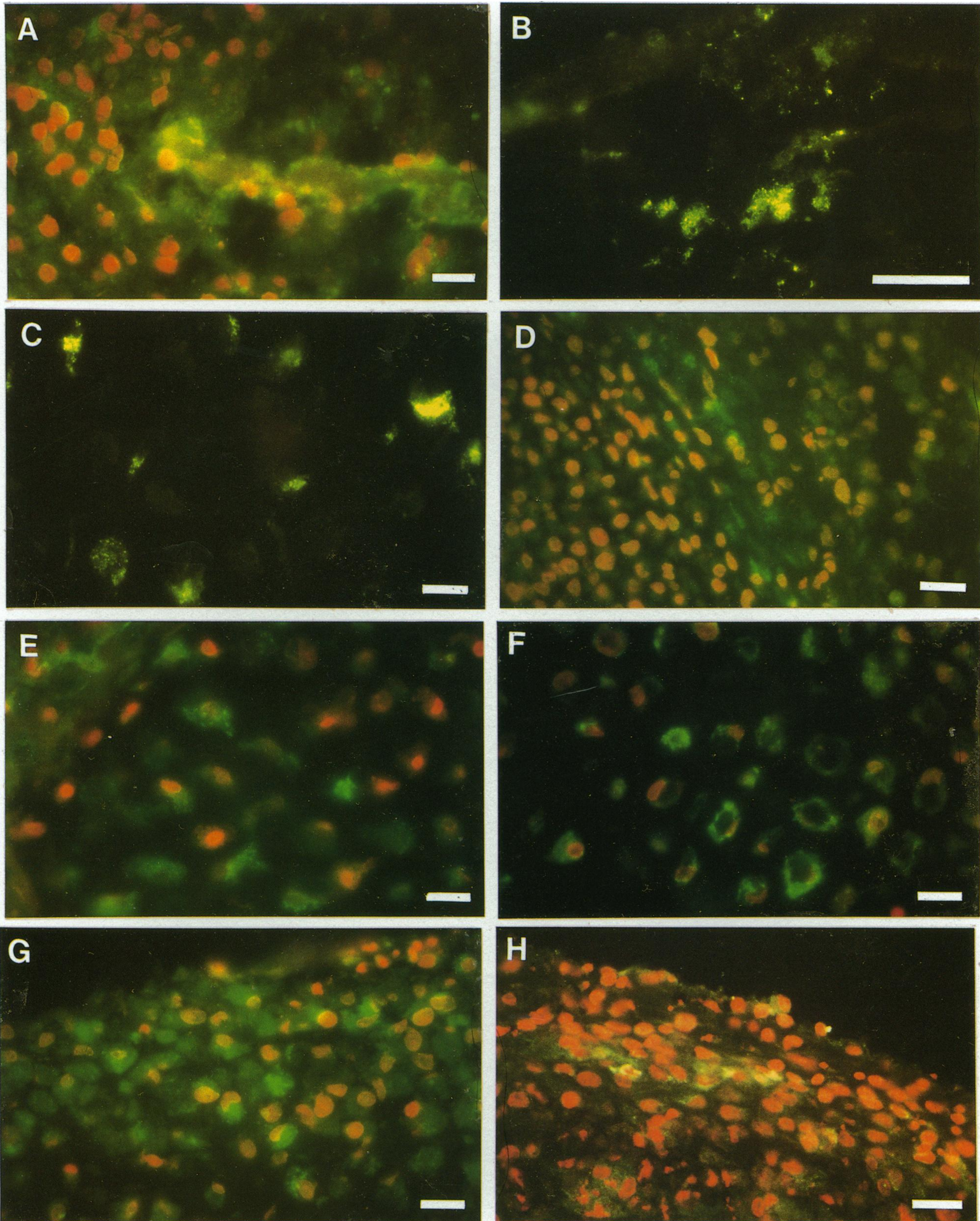

Fig. 4. Immunofluorescence detection of gelatinase (*A*, *B*), stromelysin (*C*, *D*) and TIMP-1 (*E–H*) in monensin treated explants of mandibular condyle taken from 20 d pc to 14 d pn rabbit. Bar, 20 µm. (*A*) Gelatinase in cells along the periosteal surface of bone at 20 d pc. (*B*) Gelatinase in cells lining the trabeculae of the spongiosum and marrow spaces at 22 d pc. (*C*) Stromelysin in lower hypertrophic chondrocytes at 22 d pc. Cells show earliest detection as faint staining with occasional bright cells. (*D*) Stromelysin in the articular and proliferative zones at 28 d pc. Cells show earliest detection by faint intracellular staining. (*E*) TIMP-1 in lower hypertrophic chondrocytes at 20 d pc. Cells show faint intracellular staining with occasional brighter cells. (*F*) TIMP-1 fluorescence in lower hypertrophic chondrocytes at 3 d pn. Cells have bright intracellular staining. (*G*) TIMP-1 in articular and proliferative zones at 28 d pc. Cells show bright intracellular staining. (*H*) TIMP-1 in articular and proliferative zones at 1 d pn showing intracellular staining in the flattened cell layer.

Table 1. *Summary of relative distribution of collagenase in the zones of the condylar cartilage during development from 20 d postcoitum (pc) to 14 d postnatum (pn)**

	20 d pc	22 d pc	24 d pc	26 d pc	28 d pc	1 d pn	3 d pn	7 d pn	14 d pn
Articular/proliferative zones	—	+ +	+ +	+ + +	+ + +	+ + + +	+ + + +	+ + + +	+ + + +
Upper hypertrophic chondrocytes	+	+ +	+ +	+ +	+ +	+ + +	+ +	+ + +	+ +
Lower hypertrophic chondrocytes	+ +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
Calcified cartilage/bone	—	+ +	+ +	+ + +	+ +	+ + +	+ + +	+ +	+ +

* At least 3 sections from each of 3 samples were examined and the average (modal) score recorded.
± occasional faint cell staining, + general faint cell staining, + + general faint with occasional bright cells, + + + bright cells, + + + + many bright cells with positive matrix staining.

Table 2. *Summary of relative distribution of gelatinase in the zones of the condylar cartilage during development from 20 d postcoitum (pc) to 14 d postnatum (pn)**

	20 d pc	22 d pc	24 d pc	26 d pc	28 d pc	1 d pn	3 d pn	7 d pn	14 d pn
Articular/proliferative zones	+ +	+ + +	+ + +	+ + +	+ + + +	+ + +	+ + +	+ + +	+ + +
Upper hypertrophic chondrocytes	—	+ +	+ +	+ + +	+ +	+ + +	+ +	+ + +	±
Lower hypertrophic chondrocytes	—	±	+ + +	+ + +	+ + +	+ + +	—	—	±
Calcified cartilage/bone	—	+ + +	+ + +	+ + +	+ + + +	+ + + +	+ + + +	+ + +	+ + + +

* At least 3 sections from each of 3 samples were examined and the average (modal) score recorded.

Table 3. *Summary of relative distribution of stromelysin in the zones of the condylar cartilage during development from 20 d postcoitum (pc) to 14 d postnatum (pn)**

	20 d pc	22 d pc	24 d pc	26 d pc	28 d pc	1 d pn	3 d pn	7 d pn	14 d pn
Articular/proliferative zones	—	±	—	—	+	+ + +	+ +	—	+ + +
Upper hypertrophic chondrocytes	—	±	—	—	—	+ +	+	—	+ +
Lower hypertrophic chondrocytes	—	+ +	+ +	+ +	+ +	+ +	+	+	+ +
Calcified cartilage/bone	—	—	—	—	—	—	—	—	—

* At least 3 sections from each of 3 samples were examined and the average (modal) score recorded.

Table 4. *Summary of relative distribution of TIMP-1 in the zones of the condylar cartilage during development from 20 d postcoitum (pc) to 14 d postnatum (pn)**

	20 d pc	22 d pc	24 d pc	26 d pc	28 d pc	1 d pn	3 d pn	7 d pn	14 d pn
Articular/proliferative zones	—	+	+	+ +	+ + +	+ +	±	+ +	+ +
Upper hypertrophic chondrocytes	—	+ +	+ +	+ + +	+ +	+ +	±	+ +	—
Lower hypertrophic chondrocytes	+ +	+ +	+ +	+ + +	+ +	+ + +	+ + +	+ + +	+ + +
Calcified cartilage/bone	—	—	—	—	—	—	—	+ +	+ + +

* At least 3 sections from each of 3 samples were examined and the average (modal) score recorded.

Gelatinase

Gelatinase synthesis was detected at 20 d pc as general faint intracellular fluorescence in the articular and proliferative zones of the perichondrium. From 22 d pc to 14 d pn cells in this region showed bright staining; in addition, areas of diffuse extracellular matrix staining were noted during the final stages of embryonic development at 28 d pc (Fig. 3*F*). Upper hypertrophic chondrocytes showed a variable pattern of synthesis from faint staining with occasional bright cells at 22 d pc to bright fluorescence at 7 d pn (Fig.

3 *G*); only occasional faint intracellular fluorescence was noted at 14 d pn. Gelatinase synthesis by lower hypertrophic chondrocytes exhibited an interesting pattern with faint staining at 22 d pc and bright positive intracellular fluorescence from 24 d pc to 1 d pn similar to that shown in Figure 3 *A* and *G*; little or no staining was detected in this region during later postnatal development (see Table 2). There was consistently bright matrix staining along the margin of the inferior surface of the condylar cartilage (Fig. 3 *H*) from 22 d pc onwards. In bone, cells along the periosteal surface (Fig. 4 *A*) and adjacent to trabecular bone/calcified cartilage (Fig. 4 *B*) stained brightly for gelatinase.

Stromelysin

The earliest time at which stromelysin could be detected was 22 d pc, when faint staining was observed in lower hypertrophic chondrocytes (see Table 3) with an occasional bright cell (Fig. 4 *C*). Upper hypertrophic chondrocytes showed an occasional bright cell staining at 1 d pn. The earliest detection of stromelysin synthesis in the articular and proliferative zones was at 28 d pc (Fig. 4 *D*) and synthesis continued through postnatal development with brightest staining at 1 d pn. At no time was stromelysin synthesis detected in bone or marrow cells.

TIMP-1

TIMP-1 was detected at 20 d pc in lower hypertrophic chondrocytes as generalised faint intracellular staining with very occasional bright cells (Fig. 4 *E*). This pattern continued during the remainder of the embryonic period (Table 4), but during postnatal development most cells had bright intracellular fluorescence (Fig. 4 *F*). The earliest detection in upper hypertrophic chondrocytes was at 22 d pc showing occasional bright cells. Synthesis continued through the embryonic period but showed a variable pattern during postnatal development with no detectable synthesis by 14 d pn. In the articular and proliferative zones very faint cell staining was seen at 22 d pc. TIMP-1 synthesis in this region was variable with strongest bright cell staining at 28 d pc (Fig. 4 *G*). During the postnatal period TIMP-1 fluorescence was strongest in the flattened cell layer described by Marchi et al. (1991) (see Fig. 4 *H*). TIMP-1 was not seen in either bone or marrow cells until 7 d pn when a number of cells within the marrow spaces showed bright intracellular fluorescence (data not shown). Occasional intracellular staining was noted adjacent to bone of periosteal origin at 20 d pc (data not shown).

DISCUSSION

This paper documents the patterns of synthesis and distribution of MMPs and TIMP-1 within the developing rabbit mandibular condyle. Our results demonstrate unique patterns of MMP and TIMP synthesis during embryonic and early postnatal development of condylar cartilage in the craniomandibular joint and support the concept that before undergoing proliferation and hypertrophy cells synthesise and secrete MMPs and TIMP-1.

At 18 d pc no recognisable condylar structures could be identified histologically, and neither enzymes nor inhibitor could be detected. On d 20 pc the condyle consisted of hypertrophic chondrocytes covered by the articular and proliferative zones of the perichondrium: collagenase and TIMP-1 were immunolocalised within hypertrophic chondrocytes, and gelatinase was detected in both layers of the perichondrium. By d 22 pc all 3 MMPs and TIMP-1 were localised within hypertrophic chondrocytes, but only collagenase, gelatinase and TIMP-1 within cells of both layers of the perichondrium. From d 22 pc until birth the condyle increased in size, broadened and rounded. Collagenase, gelatinase and TIMP-1 were immunolocalised within cells of all zones, with foci of bright intracellular staining. However, stromelysin synthesis was confined to lower hypertrophic chondrocytes until d 28 pc, when cells of the articular and proliferative zones also demonstrated weak intracellular fluorescence. After birth, the broad hemispherical cap of hyaline cartilage was covered by a relatively thin perichondrium of articular and proliferative zones. Strong collagenase intracellular immunofluorescence was present in all zones, and adjacent matrix immunofluorescence was frequently observed in the articular and proliferative zones. Gelatinase was also present in all zones, but from 3 d pn there was little evidence for gelatinase synthesis by lower hypertrophic chondrocytes. Stromelysin and TIMP-1 synthesis was also observed in some cells of all zones with foci of stronger staining.

In the articular zone, the cells have a low rate of proliferation (Blackwood, 1966; Luder et al. 1988), display fibroblastic features and the extracelluar matrix contains collagen I (Marchi et al. 1991). The proliferative zone contains the majority of the mitoses (Luder et al. 1988), and collagen II secretion begins in the basal cells of this layer (Marchi et al. 1991).

Synthesis and secretion of collagenase and gelatinase by cells in these zones at all times probably represents local degradation of extracellular matrix to provide space for the increasing cell population. Interestingly, significant TIMP-1 synthesis was also observed within the basal flattened layer, suggesting that remodelling is tightly controlled. In the upper hypertrophic cell layer the cells have typical chondrocyte features, matrix immunostaining for collagen II reaches a peak (Marchi et al. 1991) as do cellular protein production (Luder et al. 1988) and proteoglycan synthesis (Marchi et al. 1991): the cells gradually enlarge. This enlarging cell volume also requires local matrix remodelling which correlated with the secretion of MMPs. In the lower hypertrophic zone mineralisation of the cartilage matrix takes place and the chondrocytes ultimately degenerate (Marchi et al. 1991). The observation that proteoglycan inhibits calcification (DeBernard et al. 1977; Blumenthal et al. 1979; Axelsson et al. 1983; Mercier et al. 1987; Brown et al. 1989) is supported by our data. Stromelysin as well as collagenase and gelatinase are present in lower hypertrophic chondrocytes, implying that degradation of proteoglycan prior to mineralisation of the matrix may be mediated by MMPs.

A comparison of these data with a study of the patterns of synthesis of MMPs and TIMP-1 in the rabbit growth plate (Brown et al. 1989) shows many similarities and some important differences. All 3 MMPs and TIMP-1 are synthesised at varying rates by chondrocytes of the resting and proliferative zones of the growth plate, and by cells of the articulating and proliferative zones of the condyle, although only the basal layer of the condyle proliferating zone are true chondrocytes synthesising collagen II (Marchi et al. 1991). Stromelysin synthesis in the growth plate was ubiquitous, with a relative increase in the proximal hypertrophic zone where changes in proteoglycan structure and the appearance and disappearance of type-X collagen have been reported (DeBernard et al. 1977; Buckwalter, 1983; Grant et al.1985; Schmid & Linsenmayer, 1985). Stromelysin synthesis by lower hypertrophic chondrocytes of the condyle probably serves a similar function. Lower hypertrophic chondrocytes in the condyle consistently secrete collagenase, as they do in the growth plate. Isolated cells in both tissues were observed with strong intracellular immunofluorescence of either MMP or TIMP-1, demonstrating that although many biosynthetic events occur in synchrony, each chondrocyte—or perichondrial cell—is capable of individual synthesis, possibly in response to local variations in matrix and growth factor composition. However, TIMP-1 synthesis was not observed in the lower hypertrophic zone of the growth plate but was observed at all times in the condyle. Hypertrophic chondrocytes of the growth plate did not synthesise gelatinase, whereas those of the condyle were positive for gelatinase, apart from lower hypertrophic cells from d 3 pn onwards which were negative. These differences are probably due to differences in modes of growth: condylar growth is appositional (Blackwood, 1966) whereas growth by the epiphyseal plate is interstitial and predominantly longitudinal. Synthesis of TIMP-1 by condylar lower hypertrophic chondrocytes may indicate that the chondrocyte maintains tight control over the extracellular activity of MMPs. The observation that the lower hypertrophic zone matrix contains abundant collagen fibrils (Marchi et al. 1991) supports this hypothesis.

In the condyle from d 20 pc the base of the cartilage was progressively replaced by bone. From d 22 pc onwards this area contained considerable gelatinase immunofluorescence, both in cells and on matrix, and cells lining the trabeculae of the spongiosum also stained weakly for collagenase. No stromelysin was detected in this area. In a recent study, M_r 72×10^3 gelatinase was localised by in situ hybridisation in a 4-d mouse, and found to be expressed strongly by osteoblasts depositing alveolar bone and in the bone and perichondrium of the angular process (Sahlberg et al. 1992). Osteoblasts from several species produce MMPs in culture, including gelatinase (Heath et al. 1984; Overall & Sodek, 1987; Meikle et al. 1992). Gelatinase was also observed in cells lining the trabeculae of the spongiosum of the epiphyseal plate (Brown et al. 1989). Taken together, these data suggest that gelatinase has an important role in endochondral ossification.

Our results also demonstrated the presence of TIMP-1 in condylar bone from d 7 pn. In separate studies using single strand antisense RNA probes temporal expression of TIMP mRNA has been documented during mouse embryogenesis. Earliest transcript detection was at 13.5 d pc in sites of bone formation (Nomura et al. 1989; Flenniken & Williams, 1990). As development progressed levels increased and transcripts were detected at additional sites of both endochondral and intramembranous osteogenesis but at no stage was TIMP mRNA detected in adjacent proliferating, resting or hypertrophic cartilage. Further work is required to establish whether these differences are due to species variation, or differences in sensitivity of the methods of detection of mRNA and protein, or whether there is an additional level of control at translation.

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