Cloning and Developmental Expression of a Membrane-type Matrix Metalloproteinase from Chicken*

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We have cloned a novel matrix metalloproteinase (MMP) from cultured chicken embryo fibroblasts. The cDNA-derived protein sequence contains 608 amino acids including a C-terminal hydrophobic transmembrane domain of 24 amino acids and a cytoplasmic domain of 20 amino acids. This chicken MMP is 72% similar to a recently described membrane-type MMP (MT-MMP) from human placenta (Sato, H., Takino, T., Okada, Y., Cao, J., Shinagawa, A., Yamamoto, E., and Seiki, M. (1994) Nature 370, 61–65). Accordingly, we name this novel MMP chicken MT-MMP. As shown by Northern blotting, two MT-MMP mRNAs of 6 and 10 kilobases are constitutively expressed but only modestly regulated by growth factors and cytokines in cultured chicken embryo fibroblasts. Both mRNAs are abundant in the head and body of 8- and 9-day-old chicken embryos. As shown by in situ mRNA hybridization, MT-MMP is expressed in embryonic neural tube, spinal ganglia, and respiratory epithelium, as well as in developing cartilage and muscle. Using reverse transcription-polymerase chain reaction, we have found MT-MMP mRNA in 2-day-old chicken embryos and extraembryonic membranes. In addition, a strong correlation was observed between the mRNA expression of MT-MMP and 72-kDa type IV collagenase. Collectively, the early MT-MMP mRNA expression and its co-localization in several tissues with 72-kDa type IV collagenase mRNA suggest that the MT-MMP plays an important role in early development.

Matrix metalloproteinases (MMPs) constitute a large family of extracellular enzymes that degrade basement membranes and connective tissues (1–3). In cells in culture, MMPs are produced by many cell types, and their gene expression is regulated by several cytokines, growth factors, and transforming signals such as onogenes and the tumor-promoting 12-O-tetradecanoylphorbol-13-acetate (TPA) that activates protein kinase C enzymes (4–13). Since all MMPs are synthesized and secreted as inactive precursors, mechanisms that activate MMPs are particularly important in the regulation of MMP expression (14–19). MMPs are activated by proteolytic removal of their amino-terminal domain. Inactivation of MMPs is accomplished by TIMP (tissue inhibitor of metalloproteinases) proteins. In humans, three different TIMPs have been cloned and characterized that bind to active and inactive forms of MMPs in a 1:1 ratio with a \( K_d \) of \( 10^{-10} \). Several lines of evidence suggest that MMPs and TIMPs are involved in tissue remodeling during animal growth and development as well as playing a critical role in tumor cell invasion and metastasis (20–26). Overexpression of MMP activity is also observed in the pathogenesis of arthritis and other degenerative diseases (27–29).

In humans, 11 genetically distinct MMPs have been characterized and cloned thus far. These can be grouped into three categories: collagenases, gelatinases, and stromelysins (1–3). Collagenases are three different MMPs that cleave only fibril-forming collagen such as collagen types I, II, III, and X. 72- and 92-kDa type IV collagenases, also known as gelatinases A and B, digest denatured collagen (gelatin) and other extracellular matrix components including basement membrane collagen type IV and laminin. Stromelysins, also three different MMPs, are wider spectrum enzymes that can degrade almost any connective tissue matrix component including cartilage proteoglycans (49). Importantly, stromelysin-1 (MMP3) is also a potent activator of other MMPs such as interstitial collagenase (MMP1), pump-1 (matrilysin, MMP7), and 92-kDa type IV collagenase (MMP9), suggesting that stromelysin-1 plays a special role in connective tissue degradation and remodeling (18, 40, 54).

To study the role of MMPs in animal growth and development, we have cloned several MMPs from chicken embryos. We designed “universal” primers from the two sequence domains that are most conserved in the MMP family and used reverse transcription-coupled PCR to isolate cDNA clones of MMPs from cultured chicken embryo fibroblasts and other embryonic tissues. Here we describe the cloning and developmental expression of a novel member of the MMP family. While this work was in progress, Sato et al. (47) and Okada et al. (50) described a novel MMP, so-called membrane-type MMP (MT-MMP) from human placenta. The amino acid sequence of the chicken MMP reported here is 72% similar to the human MT-MMP sequence, and accordingly, we have named the new gene chicken MT-MMP. In this study, we demonstrate that the chicken MT-MMP gene is active in early development, in day 2 embryos (stages 12, 13, and 14), suggesting that the MT-MMP plays an important role in early embryogenesis and tissue remodeling.

MATERIALS AND METHODS

Cell Culture—Fertile eggs from White Leghorn hens bred with a California Gray Leghorn rooster were obtained from Townline Poultry...
cDNA was then heat-denatured, and 1 mM molybdate as follows. The first cycle consisted of heating at 95°C for 1 min followed by a 15-s annealing with primers Y35 and Y36 and extension with Taq polymerase (Pharmacia) at 72°C for 1 min. The reverse transcribed cDNA was then amplified using the 3′-end anchor primer and an estero-specific primer (GAATTELGH) as shown: YAATHELGH. In the collagenase and stromelysin, MMP7, in which the second conserved domain is altered (underlined) as shown: VA(A/V)HE(F/I/L)GH. The two nucleotides shown in capitals were the same as the degenerate Y35 primer. A 23-mer 24-degenerate with five inosines, and its complementary sequence codes for VA(A/V)HE(F/I/L)GH. The two nucleotides shown in capitals were then amplified using the 3′-end primer and an estero-specific primer derived from the novel MMP sequence. These specific PCR products were then cloned into the mammalian expression vector (pET25b+; Novagen). b The vector was digested with NcoI and BamHI, so that the carboxyl-terminal Val residue of the chicken MT-MMP became fused in frame with the His tag sequence encoded by the pET25b+ vector. Two clones were selected and characterized by restriction mapping and sequencing. These chicken MT-MMP expression vectors were then transfected into the BL21(DE3)pLysS Escherichia coli bacterial host (Novagen). A single colony was inoculated into 2 ml of “terrific broth” medium (TB; 1.2% Bacto-tryptone, 2.4% Bacto-yeast extract, 0.1 M potassium phosphate, 0.5% glycerol) containing 25 μg/ml kanamycin and 34 μg/ml chloramphenicol and grown at 30°C until an A595 measurement of 0.5–0.8 was reached. 1 ml of culture was treated with 1 mM IPTG for 3 h at 30°C to induce the expression of MT-MMP. The remaining 1-ml culture served as a control. The bacteria were harvested by centrifugation and solubilized, and the total proteins were analyzed by SDS-polyacrylamide gel electrophoresis and Western blotting. Polyclonal rabbit antibodies against the synthetic chicken MT-MMP peptide MRSMDPGYP-KPITIWKGI (residues 468–485) were produced by Genetics Research, Inc. (Huntsville, AL). Potential antigenic sites of the chicken MT-MMP were kindly predicted by Dr. Steve Ledbetter (Upjohn Company, Kalamazoo, MI) on the basis of four end points, which included the length and content of a-helices, and Rosbon and Fansman prediction of a-helical probability. These programs were kindly provided by Dr. Roger Poorman (Upjohn Company).

In Situ mRNA Hybridization—Chicken embryos of Hamburger and Hamilton (43) stages 20 (E3), 30 (E7), and 31 (E7.5) were dissected, fixed, and processed as described (42). Single strand sense and antisense DNA probes of MT-MMP, 72-kDa type IV collagenase, and type I collagen were prepared and labeled with digoxigenin-DUTP using PCR. Sections were hybridized overnight at 45°C in a 50% formamide/0.1% SDS solution coupled to alkaline phosphatase (Boehringer Mannheim) at a dilution 1:2000. After washing in buffer I (Boehringer Mannheim), alkaline phosphatase activity was visualized with 5-bromo-4-chloro-3-indolyl phosphate coupled to alkaline phosphatase (Boehringer Mannheim) at a dilution 1:2000. After washing in buffer I (Boehringer Mannheim), alkaline phosphatase activity was visualized with 5-bromo-4-chloro-3-indolyl phosphate.
phosphate/nitro blue tetrazolium colorimetric reaction. In negative control experiments, sense probes gave minimal background staining. Positive controls using the collagen type I probe resulted in labeling of appropriate tissues known to express collagen type I mRNA (bones, ligaments, and tendons).

RESULTS

Cloning of a New Member of the MMP Family—All MMPs characterized thus far, from a variety of species, contain two sequence domains that are highly conserved across species and between the different members of the MMP family. The first domain, PRCG(V/N)PD, the so-called Cys switch region, is at the end of the amino-terminal propeptide that is proteolytically removed from the active MMPs. The second domain, VA(A/V)HE(F/L)L/GH, is in the catalytic zinc and has two of the three His residues that coordinate the Zn$^{2+}$ atom in MMPs (51). For example, in a sea urchin hatching enzyme, which is a collagenase-like MMP, these domains are PRGVPD and VAAHFGH (52). The only exception to the rule appears to be pump-1 (matrilysin, MMP7), which contains an altered second domain YAATHELGH (the underlined T is an insertion; Ref. 53).

To generate cDNA clones for MMPs from chicken, we first synthesized two “universal” inosine-containing degenerate oligonucleotide primers that cover all codons and permutations of amino acids found in the two conserved MMP domains. Using total RNA of cultured primary chicken embryo fibroblasts treated with TPA for 16 h, we synthesized cDNA using reverse transcriptase and random hexamer primers and followed this with PCR amplification using the “universal” MMP primers. As shown in Fig. 1, we obtained at least seven distinct PCR products corresponding to DNA sizes between 350 and 900 bp. We also used total RNA from TPA-treated Drosophila embryos for reverse transcription and PCR, but cloning and sequencing of the small size Drosophila PCR products (Fig. 1, lane 2) showed that they were not related to MMPs. Attempts to identify and clone MMPs from Drosophila continue.

The chicken PCR products were cloned into the pCR-Script SK(+) vector, and 24 clones were randomly selected and characterized by nucleotide sequencing. Three clones containing a 900-bp cDNA insert were found to be homologous to mouse 72-kDa type IV collagenase (MMP2, Ref. 38). Subsequently, Aimes et al. (41) reported the cloning of chicken 72-kDa type IV collagenase, which is almost identical with the clone we have isolated.\(^2\) Another two clones with a 400-bp cDNA insert were the chicken homologue of collagenase-1/stromelysin-1.\(^2\) In addition, among the products of the chicken RT-PCR we observed one major band at about 450 bp (Fig. 1, lane 3). When this PCR product was cloned and sequenced, the deduced amino acid sequence confirmed its MMP-origin but also revealed significant differences from other known MMP sequences. In particular, it contained a novel eight-residue insertion not found in any other MMPs characterized thus far (boxed in Fig. 2). As described in this paper, further characterization of this chicken MMP revealed that it represented a new member of the MMP family.

Using the 5‘- and 3‘-end RT-PCR strategy of cDNA cloning (see “Materials and Methods”) allowed us to determine 5736 bp and the complete sequence of 608 amino acids for this novel chicken MMP (Fig. 2). The sequence contains nine Cys residues and four potential sites for N-linked sugars (Asn\(^{63}\), Asn\(^{138}\), Asn\(^{151}\), and Asn\(^{171}\)). At the carboxyl-terminal end (residues 565–588) there is a stretch of 24 hydrophobic amino acids, a potential transmembrane domain, followed by a 20-residue long cytoplasmic domain (Fig. 2). After the conserved PRGVPD domain, the sequence contains an 11-residue insertion that has an RRKR motif for the intracellular proteinase furin. A similar 10-residue insertion containing an RQKR motif is also found in stromelysin-3 (MMP11). Pei and Weiss (36) have shown that this motif is necessary for intracellular activation of stromelysin-3 by a furin-dependent mechanism.

The chicken MMP sequence reported here contains similarities to the sequence for a novel MMP from human placenta (47, 50) that was reported while this work was in progress. A sequence alignment reveals 72% similar and 54% identical amino acids (Fig. 2). Since the novel human MMP contained a potential hydrophobic transmembrane domain and was expressed on the cell surface, it was named MT-MMP (47). Accordingly, we name this chicken MMP sequence chicken MT-MMP. The human MT-MMP sequence also contains an 11-residue insertion with an RRKR motif. In addition, both sequences contain a unique eight-residue insertion (boxed in Fig. 2) that further characterizes this new class of MMPs. However, one distinct feature of the chicken MT-MMP is a 19-amino acid-long domain (residues 315–333) rich in proline, lysine, and arginine that is absent from the human placenta MT-MMP. Also, only four of the nine Cys residues of the chicken MT-MMP are present in the human MT-MMP (Fig. 2).

Bacterial Expression of the Chicken MT-MMP—A full-length 1.6-kb cDNA of the chicken MT-MMP was cloned into the pET25b bacterial expression vector, and MT-MMP synthesis was induced in E. coli using 1 mM IPTG. The cells were lysed, and total proteins were analyzed by SDS-gel electrophoresis and Western blotting. Polyclonal rabbit antibodies raised against the synthetic chicken MT-MMP peptide MRSMDP-GYPKPTITWKG (residues 468–485) reacted with one major polypeptide of 57 kDa. This is significantly smaller than the calculated molecular weight of 69,407. However, this may be due to an increased mobility of MT-MMP because of its high proline content (8.7%). As expected, synthesis of the MT-MMP polypeptide was induced by IPTG (Fig. 3). Detection of the MT-MMP polypeptide was completely blocked by the synthetic peptide (1 μg/ml) or when the primary antibody was omitted in the staining reaction (data not shown).

Expression of the Chicken MT-MMP mRNA in Cultured Cells—In Northern blotting (Fig. 4), MT-MMP cDNA probe hybridized to two bands of 6 and 10 kb that were constitutively expressed in primary chicken embryo fibroblast cultures. The levels of these MT-MMP mRNAs were similarly but only modestly increased in cells treated with TPA, basic FGF, or TNF. Surprisingly, their level was not affected by retinoic acid, which is generally known to repress the expression of MMP genes (6,\(^2\))\(^{-}\).
Similarly, we saw little if any effect of human IL-1 on the level of these two mRNAs. In contrast to the human MT-MMP mRNA, which is detected in Northern blot as a single 4.5-kb band (30, 47), the chicken MT-MMP mRNA is detected in cultured cells and embryonic tissues as two bands of 6 and 10 kb (Figs. 4 and 5). These two mRNAs are expressed together, and their level is similarly regulated in embryonic tissues and cultured cells. This suggests that the 6- and 10-kb mRNAs are derived from the same chicken MT-MMP gene rather than being transcribed from two separate genes that hybridize with the same probe.

FIG. 2. Comparison of the chicken and human MT-MMP amino acid sequences. The aligned sequences reveal 54% identical and 72% chemically similar amino acids between the chicken (first) and human (second) MT-MMP sequences (from Ref. 50). The conserved amino acids are shown on the third line. The top triangle indicates a putative signal peptide cleavage site, and the bottom triangle shows the propeptide cleavage site. The nine Cys residues are highlighted. The two double underlines refer to conserved MMP sequences and were used in primer design to clone chicken MMPs. The boxed sequence shows an eight-residue insertion in the MT-MMPs. The underline indicates an 11-residue insertion in MT-MMPs.

FIG. 3. Expression of chicken MT-MMP in E. coli. A full-length chicken MT-MMP cDNA was cloned into the pET25b vector (Novagen), and expressed in E. coli bacterial host. Total proteins were studied by SDS-gel electrophoresis and Western blotting. A, Coomassie Blue-stained proteins from two separate clones (cMT1 and cMT2) that were grown with (+) or without (−) 1 mM IPTG. B, Western blotting of the same gel with antibodies that were raised against a synthetic chicken MT-MMP peptide. In both clones, synthesis of one major 57-kDa polypeptide was induced by IPTG. Lane M shows prestained protein marker (New England Biolabs). From top to bottom, 175, 83, 62, 47.5, and 32.5 kDa.

FIG. 4. Expression of chicken MT-MMP mRNA in cell cultures. Confluent primary chicken embryo fibroblasts were cultured for 16 h with TNF, TPA, basic FGF, IL-1, or retinoic acid (RA), and total RNA (10 μg) was analyzed for MT-MMP mRNA expression by Northern blotting. Two mRNA species of 6 and 10 kb are constitutively expressed. In all lanes, a “ghost band” of 3 kb is an artifact that moves in front of the 28 S ribosomal RNA. It was not seen in Northern blotting using poly(A)1 RNA. Numbers on the left show migration of the RNA size standards (Life Technologies, Inc.). As a control for RNA loading and transfer, the bottom panel shows the 18 S ribosomal RNA on the same Northern filter visualized by EtBr staining and UV light.
days 8 and 9. Expression in the head is equally robust during both of these periods. Using RT-PCR with unique primers that were designed from the chicken MT-MMP sequence, we have detected MT-MMP mRNA expression in 2-day-old embryos and extraembryonic membranes, at stages 12, 13, and 14 (Fig. 6). In stage 12 extraembryonic membranes, however, MT-MMP mRNA (and 72-kDa type IV collagenase mRNA) is barely detectable. In control experiments, we found comparable expression of glyceraldehyde-3-phosphate dehydrogenase mRNA in 2-day-old embryos and extraembryonic membranes (Fig. 6).

Using in situ mRNA hybridization, we studied the cell and tissue type-specific expression of MT-MMP. In stage 30/31 embryos (E7/E7.5), MT-MMP was abundantly expressed in neural tube and spinal ganglia (Fig. 7, A and C). Sporadic expression is also found in areas of the developing lung, occurring in intriguing asymmetric patches in the respiratory epithelium of newly forming bronchi (Fig. 7D). In control experiments, hybridization with an MT-MMP sense probe produced very low levels of background staining (Fig. 7B). Interestingly, in stage 30/31 embryos, MT-MMP expression continues to be associated with developing limb. Expression is observed in the developing muscle fibers as well as in chondrocytes and perichondrium of limb buds (Fig. 8C). In stage 20 embryos (E3), MT-MMP transcripts are expressed prominently in the myotome of somites and, in this stage, to a lesser extent in the neural tube and neural crest derivatives (Fig. 8A).

Interestingly, we have detected early expression of the 72-kDa type IV collagenase gene in chicken embryos in patterns similar to that of MT-MMP. Using RT-PCR and unique primers designed from the chicken 72-kDa type IV collagenase gene (41), 72-kDa type IV collagenase mRNA was present in 2-day-old embryos, at stages 12, 13, and 14 (Fig. 6). We note that detection of the PCR products (visualized by EtBr staining) in these early stage embryos required only 25 cycles of amplification, suggesting a high level of 72-kDa type IV collagenase and MT-MMP mRNA expression. As visualized by in situ hybridization, 72-kDa type IV collagenase mRNA expression correlated closely with the expression of MT-MMP mRNA as illustrated by their co-localization in stage 20 somitic myotomes (Fig. 8, A and B).

DISCUSSION
We have cloned a novel MMP, which we named chicken MT-MMP and studied its expression during chicken embryo development. Here, we show that the MT-MMP mRNA is abundantly expressed in the head and body of 8- and 9-day-old chicken embryos. By in situ mRNA hybridization, MT-MMP was expressed in embryonic neural tube, spinal ganglia, and respiratory epithelium as well as in developing cartilage and muscle.

Although the chicken MT-MMP is 72% similar in sequence to a recently described MT-MMP from human placenta (47, 50) (Fig. 2), the two genes show some clear differences in their expression pattern. For example, Takino et al. (30) reported that while human MT-MMP mRNA is expressed in heart, placenta, lung, liver, skeletal muscle, kidney, and pancreas, very little MT-MMP mRNA was found in the human fetal brain. In contrast, the chicken MT-MMP we have cloned is abundantly expressed in the chicken brain also (Fig. 5). Thus, they are not the same gene in chicken and human, rather they may represent different genes of the MT-MMP gene group.

We have recently cloned and characterized a mouse MT-MMP from F9 embryonal carcinoma cells. This gene also is abundantly expressed in the mouse brain. This mouse MT-MMP has almost exactly the same basic and proline-rich 19-residue domain that is missing from the human placental MT-MMP (Fig. 2). We note that the mouse MT-MMP sequence we have cloned is different from the GenBank mouse MT-MMP sequence (X83536) deposited by Okada et al. (50), indicating that it is another member in the MT family of MMPs.

The chicken MT-MMP was cloned using RT-PCR and two...
“universal” degenerate oligodeoxynucleotide primers. These primers were designed to cover all codon usage and amino acid permutations in the two conserved MMP sequence domains. To reduce the primer sequence complexity, we used inosine in positions that otherwise would have required all four nucleotides for complete codon usage. For example, for the sequence VA(A/V)HE(F/I/L)GH, we designed a 23-mer 24-degenerate primer containing five inosines (see “Materials and Methods”). Without the use of inosines, the complexity of this primer (24,576-degenerate) would have increased 1024-fold. We expect that the “universal” MMP primers (Y35 and Y36) will facilitate the cloning of other novel MMPs as well as MMPs from many distantly related species.

Previously, Shapiro et al. (48) used RT-PCR and degenerate inosine primers to clone mouse macrophage metalloelastase, which is a matrix metalloproteinase (MMP12) with elastolytic activity. However, in their approach, only partial sequences were used in primer design, which did not represent all amino acid permutations in the conserved MMP sequences. Also, their degenerate primers did not cover all codon usage. Of note is that, at the time of their report (1992), mouse macrophage metalloelastase was the only MMP where valine was found in the third position in the conserved sequence VA(A/V)HE(F/I/L)GH (underlined residues are for the mouse macrophage metalloelastase). Chicken MT-MMP and human MT-MMP have valine in this position (Fig. 2). It is also valine in MT-MMPs from mouse and rat (GenBank™ numbers X83536 and X83537; Ref. 50) and in the mouse MT-MMP we have cloned.3 In all other MMPs that have been cloned thus far, alanine is found in this position.

In an attempt to streamline MMP cloning even more, we also tried the “universal base” that was recently described by Nichols et al. (33). Here, a 3-nitropyrrrole-derived base is used in place of inosine and in other positions of codon degeneracy. We synthesized and tested several primers in RT-PCR, but, disappointingly, none of these primers gave us any specific PCR products from MMPs.2 Recently, Loakes et al. (34) also reported that primers containing the “universal base” may not be of that general value in PCR or dideoxy sequencing as originally suggested (33).

Human MT-MMP is an activator of the 72-kDa type IV collagenase (32, 47, 57). In tissue culture, COS-1 cells expressing MT-MMP activated pro-72-kDa type IV collagenase (B) mRNA in myotomes (arrows). MT-MMP also appears to be expressed in the walls of the dorsal aorta. MT-MMP mRNA in developing muscle and cartilage tissue is shown in C. The top arrows point to a distinct expression in perichondrium. The bottom arrows refer to newly forming myotubes that express MT-MMP mRNA. Sections of a 7-day-old embryo were studied as in Fig. 7. Scale bar is 100 μm.
During mouse development, 72-kDa type IV collagenase is widely expressed by mesenchymal cells but not at all by cells of ectodermal or endodermal origin including the epithelial component of different organs (38). These results are somewhat surprising, since it would be expected that epithelial cells that produce basement membrane components such as laminin and collagens type IV would also make the 72-kDa type IV collagenase for the degradation and remodeling of basement membranes (38). It is also striking that 72-kDa type IV collagenase has recently been found to be a true collagenase (35) (i.e. it cleaves fibrillar collagens with the same specificity and kinetics as the interstitial collagenase (MMP1)).

The cooperation of activity of MT-MMP and 72-kDa type IV collagenase enzymes may be regulated at the level of gene expression. Using RT-PCR, we have found strong correlation between the MT-MMP and 72-kDa type IV collagenase gene expression in very early stages of chicken embryo development (Fig. 6). Also, in preliminary in situ hybridization studies, 72-kDa type IV collagenase mRNA often co-localized with the MT-MMP mRNA in chicken embryo tissues (Fig. 8). Similarly, Okada et al. (50) have shown co-expression of the MT-MMP and 72-kDa type IV collagenase mRNAs in different tumor tissues, and by in situ mRNA hybridization they were found to be co-localized in the stromal component of tumor tissues. Of note is that no significant mRNA expression of either MT-MMP or 72-kDa type IV collagenase was found in the cancerous component of tumor tissues or in the surrounding normal tissue (50).

In conclusion, we have cloned and characterized a MT-MMP from chicken that is differentially expressed in specific embryonic tissues. Three sets of results suggest that this MT-MMP plays an important role in embryogenesis and tissue remodeling. We have found that MT-MMP is expressed at high levels during early stages of development when large scale cell migration occurs. Furthermore, the level of expression from one embryonic period to the other changes, indicating developmental regulation of MT-MMP. Finally, in situ mRNA hybridization has revealed MT-MMP expression in tissues such as myotomes that are known to undergo massive changes during embryonic development. Experiments to examine how MT-MMPs are involved in normal tissue remodeling during animal growth and development are currently in progress.

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Addendum—Three novel human MT-MMPs were recently cloned, MT2-MMP by Will and Hinmann (57), MT3-MMP by Takino et al. (59), and MT4-MMP by Puente et al. (56). The chicken MT-MMP described here is almost identical (89%) to the human MT3-MMP sequence. Accordingly, we name it chicken MT3-MMP (GenBank™ number U66463).

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