

This chapter discusses the zinc-containing metalloendoproteases. These enzymes remodel the stroma during development and around tissues that have been injured or stressed. Section 1 describes the three major classes of metalloproteases, their metal ion cofactors, their functions in biology, and the metzincin catalytic mechanism in procollagen and stromal protein processing. Section 2 describes the astacin and adamalysin metzincin subclasses and how they process procollagen to tropocollagen. Section 3 describes the matrilysin metzincin subclass and their roles in collagen and stromal tissue degradation and in enamel synthesis.

## 8.1.1. The Zincin Enzyme Family

The zincin proteases remodel the stroma during development and around tissues that have been injured or stressed (Sects. 13.2.4). All proteases possess one of the three types of activities: (a) (Sects. 13.2.5) that cut within a polypeptide; (b) (Sects. 13.3.1) that cut at the C- or N-terminus; and (c) peptidases that cut small polypeptides. The five structural classes of proteases are listed in Table 8.1 and the enzymatic activities of the various metalloprotease classes and subclasses are listed in Table 8.2.

Most metalloproteases contain a catalytic zinc ion bound to two histidines within a conserved motif, usually HEXXH in the one letter amino acid code where X stands for any amino acid (Fig. 8.1). These enzymes are known as *zincins*, and they comprise by far the largest clan of metalloendoprotease families. The zincin clan assignments depend on the nature of a third zinc-binding residue: glutamate (E) in *gluzincins*, aspartate (D) in *aspzincins*, and histidine or aspartate (H/D) in *metzincins*. Aspzincins are absent from the human genome (Fig. 8.1) and gluzincins encode proteases other than endoproteases (Table 8.2).

All human metalloendoproteases are metzincins, named for a downstream methionine residue involved in regulating catalysis by mediating a critical turn that brings an adjacent tyrosine or proline residue close to the catalytic zinc ion. *Matrilysins* (also called matrix metalloendoproteases, MMPs) are the major class of metzincin endopeptidases involved in collagen and stromal degradation. The other two classes, *adamalysins* and

**Table 8.1** All the protease families

Catalytic site <sup>a</sup>	Genes <sup>b</sup>	Representative <sup>c</sup>	Catalytic mechanism
Serine	175	Trypsin/chymotrypsin/ caspases <sup>d</sup>	Residue forms <sup>-</sup> OH from bound H <sub>2</sub> O
Threonine	28	Proteasome enzymes <sup>e</sup>	Residue forms <sup>-</sup> OH from bound H <sub>2</sub> O
Aspartic acid	21	Pepsin/HIV retropepsin	Aspartyl residue
Cysteine	150	Cathepsins	Cysteiny residue
Metal ion (Zn <sup>2+</sup> )	187	Collagenase	Zn <sup>2+</sup> ion-bound water molecule <sup>f</sup>

<sup>a</sup>Protease structure is classified by its catalytic action. A subclass of the serine proteases requires, in addition, one or two histidine residues for catalytic activity

<sup>b</sup>Number of protease genes in the human genome

<sup>c</sup>Representative enzymes

<sup>d</sup>Subgroup requiring calcium ions for activity are called calpains

<sup>e</sup>Mostly exopeptidases

<sup>f</sup>Rarely, the catalytic ion is Ni<sup>2+</sup> or Co<sup>2+</sup> (not in humans)

(Adapted from Table 1 in Puente et al. "Human and mouse proteases: a comparative genomic approach." *Nat. Rev. Genet.* 4(7):544–558, 2003 and updated according to Puente et al., "A genomic view of the complexity of mammalian proteolytic systems." *Biochem Soc Trans.* 33 (Part 2):331–334, 2005)

**Table 8.2** Types of zincin metalloproteases in humans

Type of enzyme	Action	Typical enzyme	Structural class
Exoproteinase	Removes N-terminal amino acid	Alanyl Aminopeptidase <sup>a</sup>	Metzincin
Exoproteinase	Removes C-terminal amino acid	Carboxypeptidase A <sup>b</sup>	Gluzincin
Peptidase	Removes a C-terminal dipeptide	Angiotensin-converting enzyme <sup>c</sup>	Gluzincin
Peptidase	Cuts a peptide (<13 aa) internally	Neurolysin <sup>d</sup>	Gluzincin
Endoproteinase	Cuts a large polypeptide internally	Matrilysins, adamalysins and astacins <sup>e</sup>	Metzincin

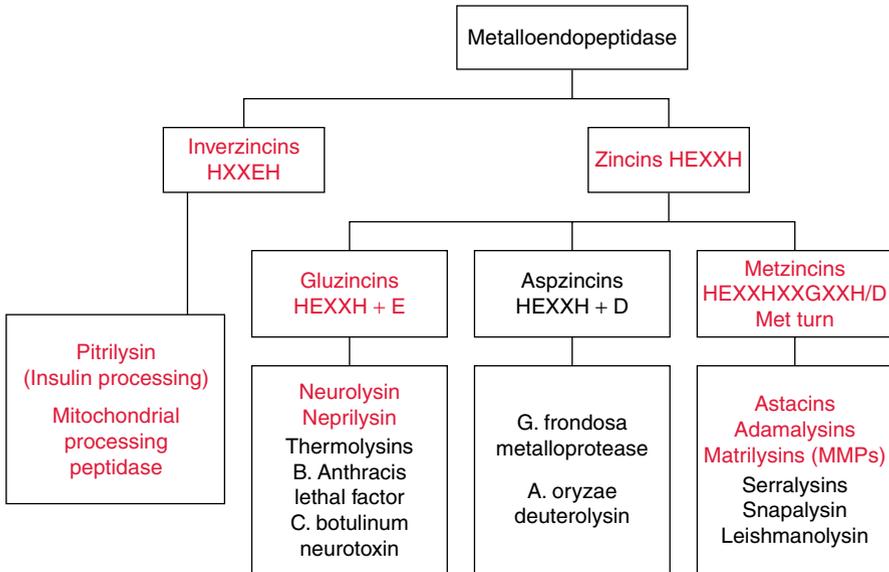
<sup>a</sup>Aminopeptidase N (APN or CD13) is expressed in many cells, tissues, and species. It cleaves the N-terminal amino acids from bioactive peptides, leading to their inactivation or degradation. It has putative involvement in antigen processing and presentation, cell adhesion, tumor invasion and metastasis, neurotransmitter degradation, and as a coronavirus receptor

<sup>b</sup>Carboxypeptidase is a digestive enzyme. It is synthesized in the pancreas, secreted into the small intestine, and hydrolyzes the C-terminal end of proteins and peptides

<sup>c</sup>Angiotensin-converting enzyme (ACE) cleaves dipeptides from the C-terminus of oligopeptides, notably converting angiotensin I (eight amino acids) to angiotensin II (six amino acids); angiotensin converting enzyme (ACE). The product of enzyme activity causes an increase in blood pressure

<sup>d</sup>Neurolysin acts only on substrates of less than about 19 amino acid residues (oligopeptides), with a particular preference for cleaving near the C-terminus. Neurolysin is known by many other names, including oligopeptidase M and soluble angiotensin II-binding protein

<sup>e</sup>Major metalloprotease families in humans (see text)

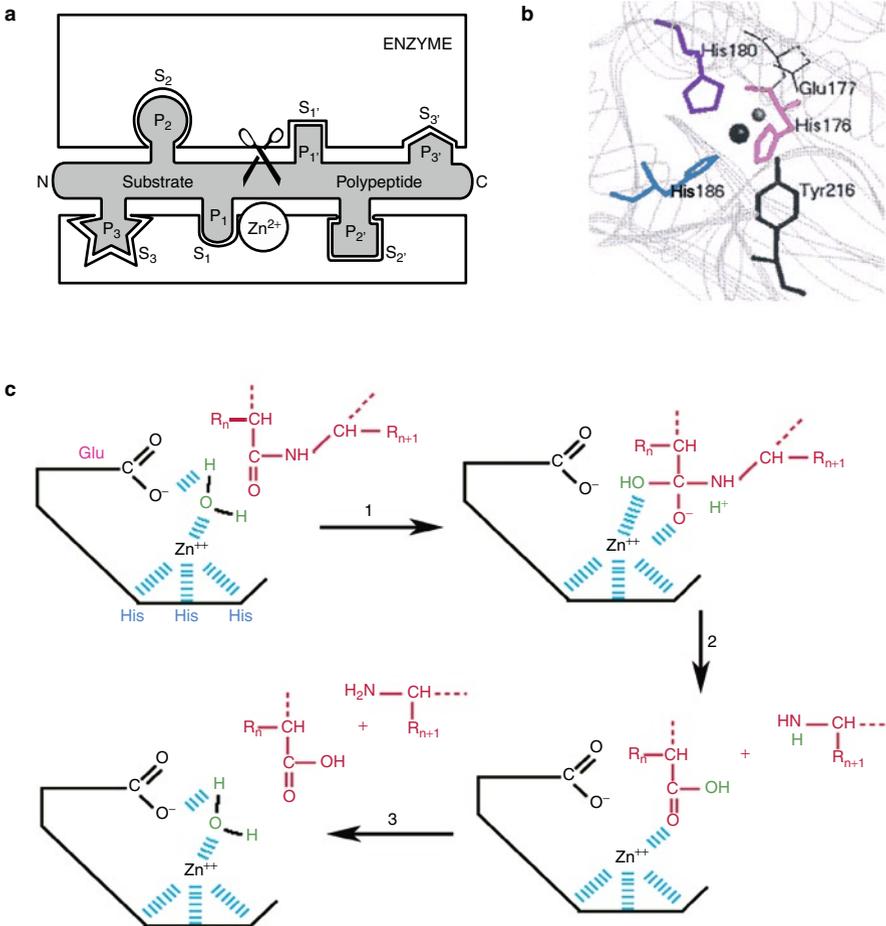


**Fig. 8.1** Classification of metallopeptidases by zinc-binding motifs. The major amino acid motif in zincins has two histidine residues that coordinate with the metal ion and a glutamate residue (E) for catalysis. A third residue that coordinates with the metal may be glutamate, aspartate (D), or histidine. In metzincins, the third coordinating residue is histidine or aspartate (H/D), but the name is taken from the presence of a downstream invariant methionine residue (see Fig. 8.2 and text). The *red type* indicates enzymes or enzyme subfamilies encoded in the human genome (Slightly modified from Fig. 1A of F.X. Gomis-Ruth, “Structural aspects of the metzincin clan of metalloendopeptidases.” *Mol. Biotechnol.* 24(2):157–202, 2003)

*astacins* (Fig. 8.1), are involved in procollagen processing. Metzincin classes not encoded in the human genome are mostly bacterial virulence factors such as anthrax lethal toxin, a serralysin. Additional metzincins have recently been identified in plants and bacteria.

## 8.1.2. Catalytic Action of the Metzincin Family

The metzincin catalytic domain consists of a flat surface within a small cleft within which peptide substrates bind and are hydrolyzed (Fig. 8.2a). In astacins, the catalytic domain is stable, but adamalysins require a calcium ion to stabilize the flat surface of the domain above the cleft. Matrilysins require *two* calcium ions and a second, *noncatalytic zinc* ion to stabilize this domain (Fig. 8.3). Table 8.3 reviews the roles of specific metal ions that participate in the various stages of collagen processing discussed in this chapter and Chap. 7.



**Fig. 8.2** Catalytic metzincin domain. **(a)** *Catalytic endopeptidase subunit showing the peptide binding cleft and surrounding domains.* The site binds six amino acids on either side of the bond to be hydrolyzed. Substrate is in an extended conformation and the bound amino acids are numbered (N- to C-terminus): ...S<sup>3</sup>-S<sup>2</sup>-S<sup>1</sup>-S<sup>1'</sup>-S<sup>2'</sup>-S<sup>3'</sup>... The products are two peptides: ...P<sup>3</sup>-P<sup>2</sup>-P<sup>1</sup>-COOH and NH<sub>2</sub>-P<sup>1'</sup>-P<sup>2'</sup>-P<sup>3'</sup>... (Slightly modified from Fig. 1B of F.X. Gomis-Ruth, "Structural aspects of the metzincin clan of metalloendopeptidases." *Mol. Biotechnol.* 24(2):157-202, 2003). **(b)** *Active site crevice of the metzincin catalytic domain from above.* The conserved zinc-binding motif has the structure: His<sup>176</sup>-Glu<sup>177</sup>-Xaa-Xaa-His<sup>180</sup>-Xaa-Xaa-Gly-Xaa-Xaa-His<sup>186</sup>. The numbering is for serralsin, an astacin family protein, but the relative positions of these amino acids within the motif are identical in all metzincins. The catalytic zinc ion (*large sphere*) has a coordinated water molecule (*small sphere*) whose hydrogen atoms are H-bonded to Glu<sup>177</sup> (bond shown in Fig. 8.2c). About 20 residues, downstream lies a tyrosine phenolic side chain that points back from beneath the active site as a result of a turn induced by the invariant methionine residue. The ligands involving tyrosine form a distorted trigonal bipyramid in which His<sup>176</sup>, His<sup>186</sup>, and the water molecule are equatorial. His<sup>180</sup> and Tyr<sup>216</sup>, respectively, form the upper and lower axial ends (peaks of each pyramid). The Zn<sup>2+</sup> ion is approximately 2.2 Å from the histidine ligands, 2 Å from the water molecule, and 2.8 Å from the phenolic O atom of Tyr<sup>216</sup>. Adamalysins and matrilysins (and some other

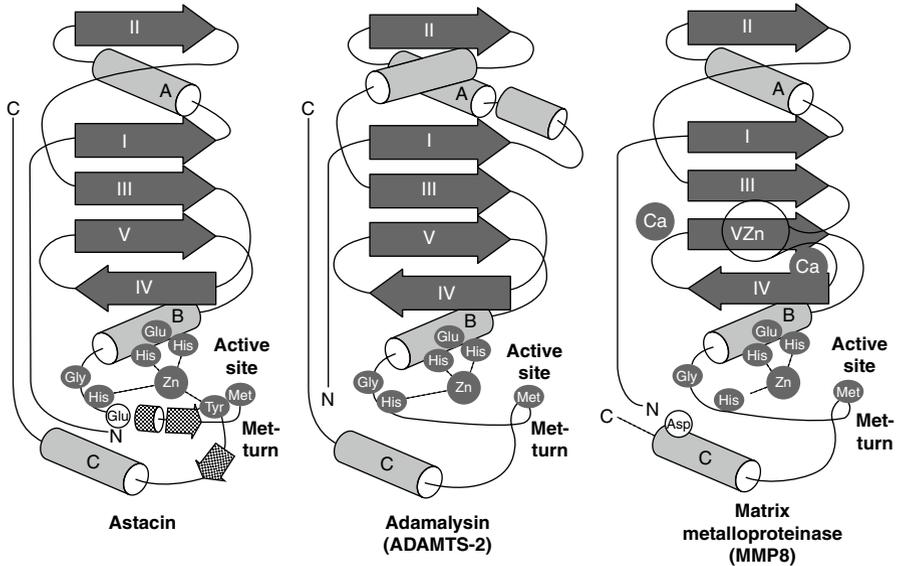
The glutamate (E) residue in the common zincin motif (HEXXH) participates in catalysis by attaching a water molecule that also coordinately bonds to the catalytic zinc ion (Fig. 8.2b). A likely catalytic mechanism involves the loss of a proton from the water molecule, followed by nucleophilic attack of  $\text{OH}^{-1}$  on the peptide bond (illustrated in Fig. 8.2c). During this process in astacins such as serralysin, a tyrosine residue flips back and forth during substrate anchoring, cleavage, and product release, in a motion referred to as a “*tyrosine switch*.” The phenol group of this tyrosine coordinates with the catalytic zinc ion and displaces the water molecule, thus inhibiting catalysis. Interactions of the enzyme away from the catalytic site are transmitted to the tyrosine residue and weaken or strengthen the coordination of its phenolic group to the catalytic zinc ion. In matrilysins and adamalysins, the “*tyrosine-switch*” is replaced by a “*cysteine-switch*.” The thiol group of cysteine coordinates to the catalytic zinc ion instead of tyrosine, similarly displacing the water molecule required for catalysis. Unlike the tyrosine switch, this “off” position is virtually permanent unless the N-terminal propeptide that contains this cysteine residue is removed, allowing the thiol group to be replaced on the zinc ion by the water molecule that participates in catalysis as in astacins (Fig. 8.2).

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### 8.1.3. Metzincin Activation

All human metzincins are secreted as proenzymes. Astacins and adamalysins are mostly activated by calcium-ion-dependent serine proteases (*pro-protein convertases*) that meet up with their substrates in trans-Golgi and secretory vacuoles. These proenzymes are known as furin-like convertases because of their homology to a serine protease called *furin* and a bacterial endoprotease called *subtilisin*. The furin-like enzymes require calcium ions to maintain structural stability whereas other serine proteases, represented by trypsin and chymotrypsin, do not. The *furin-like pro-protein convertases* autocleave their own N-terminal domain propeptide (self-activate) during secretion and then convert the N-terminal domains of co-secreted metzincins. Activation cascades also occur among the

metzincins) have proline instead of tyrosine at the turn. In these enzymes, a cysteine residue within the pro-domain blocks the cleft by binding to the catalytic zinc ion and excluding water molecule binding (see text) (Modified from Park, H.I. and Ming, L.J. “Mechanistic studies of the astacin-like *Serratia metalloendopeptidase serralysin*: highly active (>2,000%) Co(II) and Cu(II) derivatives for further corroboration of a “metallotriad” mechanism.” *J. Biol. Inorg. Chem.* 7(6):600–610. 2002). (c) *Mechanism of catalysis*. (1) The tyrosine residue moves away from the zinc ion and the water molecule (*green*) attacks the substrate polypeptide (*red*) under the influence of the deprotonated glutamate residue. (2) The C-terminal peptide amino group picks up the proton and is released. (3) The remaining hydroxide anion attacks the carboxyl group, which remains held by the zinc ion but it is quickly replaced by another water molecule (Modified from University of Tours, France Web site: <http://delphi.phys.univ-tours.fr/Prolysis/introprotease.html>)



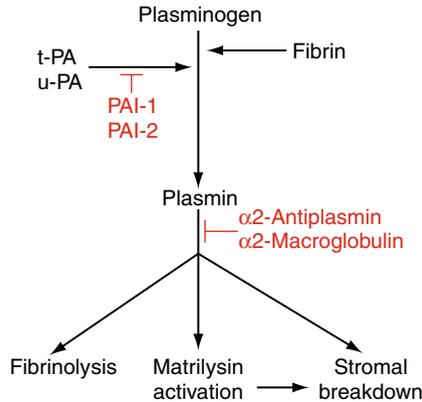
**Fig. 8.3** Architecture of astacins, adamalysin type-2, and matrix metalloproteinases. Topology scheme of astacins, adamalysin type-2, and matrix metalloproteinases. The  $\alpha$ -helices are shown as rods,  $\beta$ -sheet strands as *arrows*, and unstructured regions as *thin lines*. Key amino acids in the catalytic, zinc-binding motif are shown (*white in black ellipse*). Distinguishing features of each structure are shown as *lighter gray* for amino acids (*black letter*), and as unlabeled, secondary-structure elements. In addition to the catalytic zinc ion in all three structures, the calcium ion in adamalysin type-2, and the two calcium ions and second zinc ion in the matrix metalloproteinases (Abbreviated from Fig. 3 in F.X. Gomis-Ruth, “Structural aspects of the metzincin clan of metalloendopeptidases.” *Mol. Biotechnol.* 24(2):157–202, 2003)

**Table 8.3** Metal ions required for collagen processing

Metal Ion/s	Enzyme/s
$\text{Fe}^{2+}$	Proline/lysine hydroxylase
$\text{Cu}^{+}$	Lysyl oxidase
$\text{Mg}^{2+}/\text{Mn}^{2+}$	Integrin attachment of collagen to cells
$\text{Zn}^{2+}/\text{Ca}^{2+}$	Metalloproteinases

zincins due to self- or hetero-catalysis. For example, an activated adamalysin can remove the propeptide from another adamalysin, a matrilysin or an astacin, all independently of the initiating furin-like activation.

A similar cascade is associated with inflammation following an injury, infection, or environmental stress (Fig. 8.4). It involves chymotrypsinogen-like serine proteases called



**Fig. 8.4** Matrilysin activation. Pro-matrilysins are activated by *plasmin*. This serine protease does not require  $\text{Ca}^{2+}$  for activation, unlike *furin*, which activates the procollagen peptidases (Fig. 8.5). Plasmin is obtained from a precursor, *plasminogen*, by plasminogen activators. Plasminogen is made in the liver and secreted into the blood plasma. *Plasminogen activators* are secreted in the tissues as urokinase related, u-PA, or as a tissue type, t-PA. tPA is made by fibroblasts and basal epithelial cells. It is present in small amounts in the stroma where it activates plasminogen released from injured capillaries and causes fibrinolysis (Sect. 11.4.2) (Copy of Fig. 1 from B. Kinbby, “The plasminogen activating system in periodontal health and disease”. *Biol. Chem.* 383:85–92, January 2002; copyright permission given by Walter de Gruyter, Berlin, New York and the author)

*tissue or urokinase plasminogen activators* (plasminogen pro-protein convertases). Plasminogen is made in the liver, secreted into the blood plasma, and activated its single polypeptide chain being cut into two disulfide linked chains, like the cutting of prothrombin by factor Xa (Sect. 11.3.4.). If plasminogen escapes from capillaries, it is converted by a plasminogen activator enzyme in the tissues (usually t-PA, Fig. 8.4) to plasmin. Plasmin degrades fibrin blood clots (Chap. 11). In the stroma, plasmin also cleaves fibronectin, thrombospondin (TS), laminin, and the matrilysins, especially procollagenase. These activities lyse a damaged stroma so that it can later be replaced with healthy tissue.

Excessive activation of matrilysin by plasmin is prevented by *tissue inhibitors of matrilysin proteases (TIMPs)* in a healthy stroma, or during the repair phase of inflammation (Sect. 13.2.5), or by thrombospondin-2. (Sect. 3.2.2), or during the repair phase of inflammation (Sect. 13.2.5). The N-terminal domains of one of four homologous TIMPs bind tightly and irreversibly to the catalytic site of activated matrilysins and adamalysins. Tissue damage must be maintained at sufficiently high levels for enough plasmin to have been activated before these inhibitors are significantly depleted along with the matrix. Uncontrolled matrilysin activation characterizes many chronic diseases including periodontal disease (Sect. 13.3.1).

Collagen processing and degradation are accomplished by zinc-containing metalloendoproteases (zincins) that cleave polypeptides into large fragments. The catalytic zinc ion is coordinated to two histidine residues in a motif (HEXXH) and subdivided by a third zinc-coordinated residue: glutamate (gluzincins), aspartate (aspzincins), or histidine or aspartate associated with a downstream methionine-mediated fold (metzincins). Human metzincin endoproteases are astacins, adamalysins and matrilysins. Catalysis involves a zinc-bound water molecule, the glutamate residue of the zincin motif, and an enzyme-specific recognition site. The coordinated water molecule may be displaced by a downstream phenol (tyrosine) in astacins, or thiol (cysteine) in adamalysins and matrilysins. The cysteine is part of the methionine-mediated fold, which is disconnected by activation when a large N-terminal peptide is removed by serine proteases: furin-like during development or plasminogen activators induced by stress. Stromal activation is resisted by tissue inhibitors of matrilysin proteases (TIMPs).

### 8.2.1. Fibrillar Procollagen Processing

Once the procollagen triple helix has assembled in the lumen of the endoplasmic reticulum (Chap. 6), it moves to the cis-Golgi cisternae in transport vesicles and then through the Golgi to the trans-Golgi, where it forms bundles before being released to secretory vacuoles (Fig. 7.1). The bundles develop as procollagen is cleaved to tropocollagen.

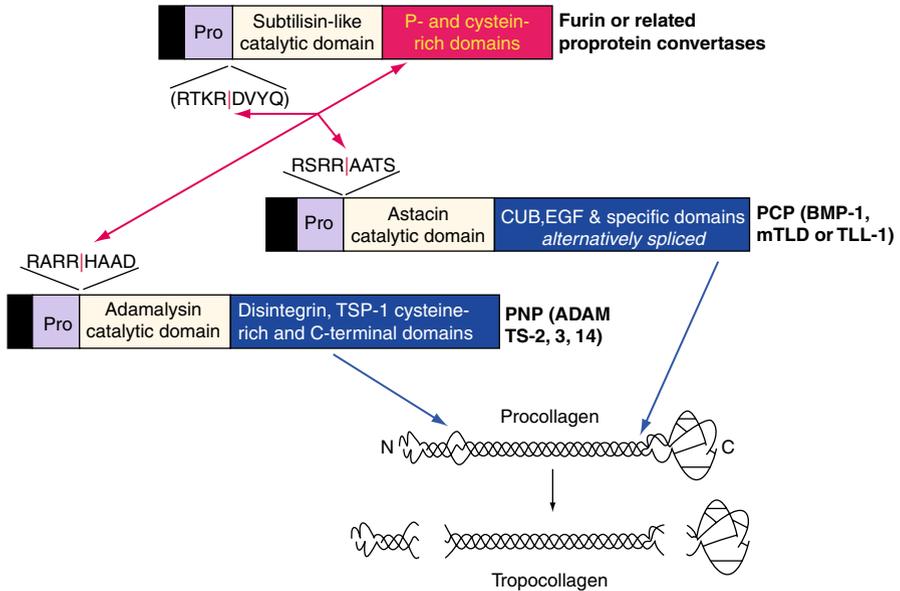
Collagen types I, II, III, and  $\alpha_2V$ , all use one of the three adamalysins as procollagen N-proteases (PNPs) and one of the three astacins as procollagen C-peptidases (PCPs). The adamalysin consensus sequence is pro-gln with the hydrolytic cleavage C-terminal to the proline residue as indicated by the down pointing arrow (P↓Q) in type I collagen, ala-gln (A↓Q) in types II and III collagens, and pro-ala (P↓A) in  $\alpha_2V$  collagen. The amino acid that follows the down-pointing arrow is the N terminal amino acid of the tropocollagen that is cleaved out by PNP. The  $\alpha_1V$ ,  $\alpha_1XI$ , and  $\alpha_2XI$  collagens use an astacin to remove the N-propeptide and a furin-like pro-protein convertase to remove the C-propeptide. Figure 8.5 indicates the polypeptide motifs of the nonadamalysin enzymes that cleave the various fibrillar collagens.

Furin-like pro-protein convertases activate all the procollagen processing enzymes (Fig. 8.6). Figure 8.7a shows the domain structure of the astacin family. The three common ones (grouped at the top) are bone morphogenetic protein 1 (BMP-1), mammalian tollid (mTld), and tollid like 1 protein (TLL-1). Protein mTld is the preferred PCP, but it cleaves slowly in secretory vesicles, preventing the bundles from growing too rapidly. Indeed, a separately secreted enhancer protein upregulates mTld activity after secretion at the cell surface, where a rapid self-assembly of collagen fibers occurs. All three astacins also process pro-lysyl oxidase (Sect. 4.2.2), the  $\gamma_2$  and  $\alpha_3$  chains of laminin-5, and pro-biglycan, a glycosaminoglycan similar to decorin (Sect. 6.5.1) but possessing two attached glycosaminoglycans residues instead of one.

<b>a</b> <b>BMP Procollagen C peptidase</b>							
Pro- $\alpha_1$ I	Tyr	Tyr	Arg	Ala	Asp	Asp	Ala
	Y	<b>Y</b>	R	A	<b>D</b>	<b>D</b>	<b>A</b>
Pro- $\alpha_2$ I	Phe	Tyr	Arg	Ala	Asp	Gln	Pro
	F	<b>Y</b>	R	A	<b>D</b>	<b>Q</b>	<b>P</b>
Pro- $\alpha$ II	Y	<b>M</b>	R	A	<b>D</b>	<b>Q</b>	<b>A</b>
Pro- $\alpha$ III	P	<b>Y</b>	Y	G	<b>D</b>	<b>E</b>	<b>P</b>
Pro- $\alpha_2$ V	E	<b>F</b>	T	E	<b>D</b>	<b>Q</b>	<b>A</b>
<b>b</b> <b>BMP Procollagen N peptidase</b>							
Pro- $\alpha_1$ V	T	P	<b>Q</b>	S	<b>Q</b>	D	<b>P</b>
Pro- $\alpha_1$ XI	A	A	<b>Q</b>	A	<b>Q</b>	E	<b>P</b>
Pro- $\alpha_2$ XI	R	P	<b>Q</b>	N	<b>Q</b>	<b>Q</b>	<b>P</b>
<b>c</b> <b>Furin Procollagen C peptidase</b>							
Pro- $\alpha_1$ V	R	<b>T</b>	<b>R</b>	<b>R</b>	N	I	D
Pro- $\alpha_1$ XI	K	<b>T</b>	<b>R</b>	<b>R</b>	H	T	E
Pro- $\alpha_2$ XI	K	<b>T</b>	<b>R</b>	<b>R</b>	S	V	D

**Fig. 8.5** Procollagen amino acid cleavage motifs other than adamalysins. See text for adamalysin cleavage sequences. All sequences are from human procollagens. The sequences around the cleavage site for Pro- $\alpha_1$ I and Pro- $\alpha_2$ I are given in both three-letter and (beneath) one-letter amino acid abbreviations. The sequences for the other procollagen chains are only the one-letter abbreviations. (a) *Astacin procollagen C peptidase* (PCP). Amino acids motifs cleaved in common fibrillar collagens by PCP, the astacin bone morphogenetic protein 1 (BMP1) and the homologous enzymes (Fig. 8.6). *Bold* indicates a motif, in this case:  $\phi$ XX↓DX(A/P) where  $\phi$  = hydrophobic residue; peptide bond cut = ↓ and X = any amino acid residue. (b) *Astacin procollagen N peptidase*. Amino acids motifs cleaved in type V and XI fibrillar collagens by procollagen N-protease (PNP) where PNP is BMP1 instead of adamalysin PNP (motif is QX↓QXP). (c) *Furin procollagen C peptidase*. Amino acids motifs cleaved in above fibrillar collagens where PCP is a furin-like enzyme instead of BMP1 (motif is BTRR↓XXX where B is a basic residue other than R). This motif is very similar to RXRR↓, one of two common furin consensus sequences (Sequences of all human procollagen polypeptides are public and can be downloaded from the Swiss-Protein Database. For example, Type I collagen alpha1 and alpha2 procollagen polypeptides, COL1A1 and COL1A2, are respectively at <http://www.uniprot.org/uniprot/P02452> and <http://www.uniprot.org/uniprot/P08123>). Data for type pro- $\alpha$ II, pro- $\alpha$ III, and pro- $\alpha_2$ V are from C. Unsold et al., J. Biol. Chem. 277(7):596–5602. Data for pro- $\alpha_1$ V, pro- $\alpha_1$ XI and pro- $\alpha_2$ XI are from Imamura et al., J. Biol. Chem. 273(42):27511–27517)

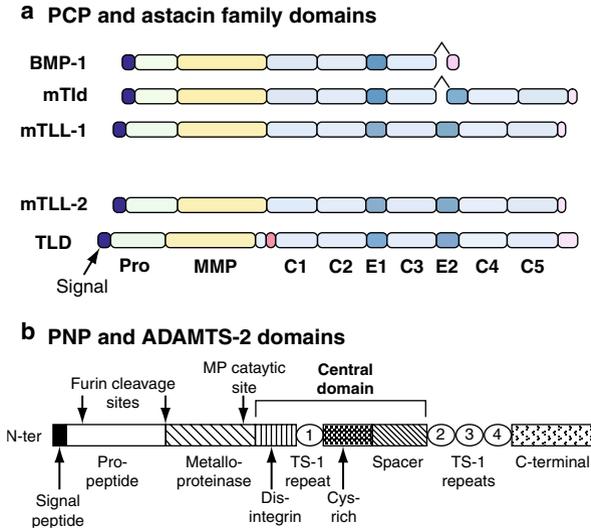
Note: Astacins were named from two unrelated sources. Bone morphogenetic protein 1 (BMP-1) was originally identified as a zinc metalloprotease in extracts of demineralized bovine bone together with TGF $\alpha$ -like growth factors (described in Chap. 3 and 8) termed BMP-2A and BMP-3. Amino acid sequencing and cDNA cloning demonstrated that mouse PCP-1 was identical to BMP-1 and that chicken PCP-2 was identical to a protein named mammalian tolloid (mTld) after a homologous *Drosophila* proteinase tolloid (TLD). BMP-1 and mTld are two of six splice variants of the *bmp1* gene. Two related genes encoding proteases similar to mTld have been identified in *bmp1* null mice: mammalian tolloid like-1 and -2 (mTLL-1 and mTLL-2). The *bmp1* null mice make abnormal collagen fibrils in the skin and fail to close the ventral body wall. Homozygous loss of *bmp1* is lethal in utero, but a skeleton develops because mTll-1 has PCP activity that partially compensates for BMP1.



**Fig. 8.6** Activation of procollagen N- and C-proteinases. The signal sequence for secretory targeting is represented as a *black rectangle* and is removed in the endoplasmic reticulum prior to secretion. Each of the C-terminal domains is labeled in the figure. The activator pro-protein convertases furin, procollagen C-peptidase (PCP) and procollagen N-protease (PNP) each contain a pro-domain (Pro; purple) that ensures proper folding. The pro-domain of furin is auto-catalytically removed, but the pro-domains of PNP and PCP are not. The first domain of furin after its signal- and pro-domains is the protease domain, related to the subtilase superfamily of  $\text{Ca}^{2+}$ -dependent proteases. Similarly, the first domain of PCP and PNP after their signal and pro-domains is a metalloendoprotease. Following the catalytic domain of each protein is a mixture of C-terminal domains. In furin and other pro-protein convertases, the so-called P-domain is essential for activity C-terminal extensions for correct localization. The C-terminal domains of the PCPs modulate activity, whereas those of PNP specify collagen-type substrate (Modified slightly from Fig. 4 in E.C. Canty and K.E. Kadler. "Procollagen Trafficking, Processing, and Fibrillogenesis." *J. Cell Sci.* Vol. 118(7):1341–1353, 2005; Reproduced with permission of the Company of Biologists)

Removal of the less bulky N-propeptide of procollagen proceeds similarly. The released collagen N-terminal propeptide is reabsorbed back into the cytosol where it inhibits collagen translation and prevents excessive fiber formation (feedback inhibition). The most common PNP is an *adamalysin of the thrombospondin type-2* class (ADAMTS-2), which is structurally large and complex, containing nine domains (Fig. 8.7b). The pro-domain is essential for correct folding during polypeptide synthesis. The thrombospondin (TS) domains through the C-terminus of the protein specify the proper orientation of enzyme binding for catalysis. Replacing the three C-terminal TS repeats with those in a closely related protein, ADAMTS-14 (PNP for type  $\alpha_2\text{V}$  collagen), prevents all enzymatic activity toward type I collagen, whereas removing only the C-terminal domain enhances the type I collagen activity.

Snake venoms cause a rapid disintegration of the stroma (*disintegrin*) due to short peptides each containing an RGD integrin-binding sequence. The RGD sequence displaces



**Fig. 8.7** Domain organization of the two procollagen peptidases. **(a)** *Domain organization of procollagen C-peptidase (PCP) and related astacins.* Signal peptides are dark purple, prodomains are green, proteinase domains are yellow, CUB domains are light blue, EGF-like domains are light purple, and domains unique to each protein are shown in pink. The CUB domains are labeled C1 through C5 and the EGF domains are labeled E1 and E2. CUB domains are present in functionally diverse, mostly developmentally regulated proteins and also in peptidases belonging to astacin and chymotrypsin families. It is an extracellular domain of approximately 110 amino acid residues containing four conserved cysteines that form adjacent disulfide bridges. The CUB domain is predicted to have a beta-barrel structure similar to that of immunoglobulins (antibody molecules). The EGF domain does not include the N-terminal  $\text{Ca}^{2+}$ -binding subdomain discussed in relation to fibrillin (Sect. 6.1.1) (Slightly modified from Fig. 1 in G. Ge. and D.S. Greenspan, *Developmental Roles of the BMP1/TLD Metalloproteinases*. Birth Defects Research (Part C), 78:47–68, 2006). **(b)** *Domain organization of procollagen N-peptidase, ADAMTS-2 adamalysin.* The four thrombospondin type 1 (TS-1) repeats (circles) are numbered as indicated Thrombospondins are discussed in Sect. 3.2.2). ADAMTS-3 and ADAMTS-14 proteins are homologous (Slightly modified from Fig. 1 in A. Colige et al. “Domains and maturation processes that regulate the activity of ADAMTS-2, a metalloproteinase cleaving the aminopropeptide of fibrillar procollagens types I-III and V.” *J. Biol. Chem.* 280(41):34397–34408, 2005)

adamalysins from integrins on the cell surface (Sect. 3.2.1). The released adamalysins float freely in the stroma and behave like activated nonspecific matrilysins. Integrin-bound metalloproteases are critical for ovum fertilization, and so the name of this group of proteins was cleverly transformed into a biochemical name: A Disintegrin And Metalloprotease Domain (ADAM), or ADAMalysin. The ADAMTS-2 proteins comprise a subfamily of adamalysins possessing thrombospondin domains. The ADAM family proper consists of over 40 proteins, one of which is described in Chap. 13 (Sect. 13.2.2). These latter proteins possess a canonical disintegrin domain that keeps them integrin-bound at the outer cell surface. A different disintegrin domain in the ADAMTS family enables their secretion instead of remaining cell surface bound.

Procollagen N-terminal processing is performed mostly by adamalysins and C-terminal processing mostly by astacins. Processing begins with procollagen bundle formation. Some cleavage of all three polypeptides at mostly the N-terminus occurs within the secretory vacuole, but secretion activates rapid C-terminal cleavage and spontaneous fiber formation. The adamalysin responsible for procollagen N-terminal processing is held to integrins at the cell surface following secretion and this also promotes faster extracellular procollagen processing to tropocollagen.

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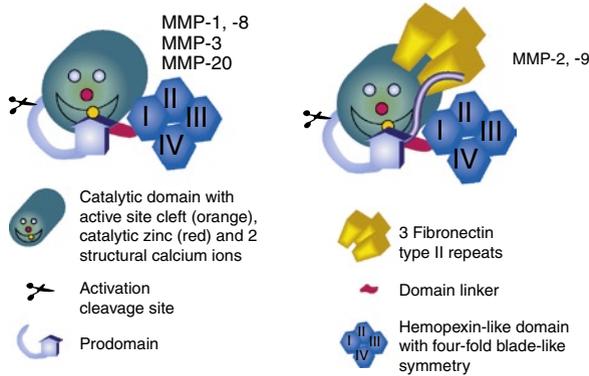
### 8.3.1.

#### **Matrilysins (MMPs) Hydrolyze Collagen and Stromal Proteins**

Matrilysins (MMPs) are required for stromal remodeling during development, pregnancy, and growth, and also following trauma or infection. Different classes degrade different extracellular matrix protein components: fibers, anchoring and basement membrane collagens, proteoglycans, laminin, fibronectin, and other stromal proteins. Many also participate in proteolytic events required to control diverse physiological processes: cell surface release of growth factors, activation of cytokines and receptors, and the inactivation of proteinase and angiogenesis inhibitors.

As noted in the previous section, matrilysin catalysis is held in check by endogenous tissue inhibitors of metalloproteinases (TIMPs), which irreversibly bind to the active site. Different TIMPs first bind to hemopexin-like domains on almost all matrilysins before they can bind to the active site, thus providing some TIMP specificity. Hemopexin is a plasma protein that binds to heme and transports it to the liver for conversion to bile. It is formed by the repetition of a variable length unit of 35 to 45 residues, the hemopexin-like domain. TIMPs are the ligands for a modified, homologous hemopexin-like domain on matrilysins. Figure 8.8 diagrams the structures of matrilysins most relevant to the topics in this book.

There are at least 28 matrilysins that participate in connective tissue degradation as collagenases, gelatinases, elastases, and stromelysins. All 28 matrilysin enzymes are listed in Table 8.4 along with their matrilysin (MMP) number and cell expression. The molecular weights of the most relevant pro- and activated enzymes, and their substrate specificities, are listed in Table 8.5. Collagen is primarily degraded by MMP-1 and -8 (fibroblast and neutrophilic granulocyte collagenase) and MMP-2 and -9 (fibroblast and neutrophilic granulocyte gelatinase). Fibroblast gelatinase (MMP-2) secretion is inhibited by thrombospondin-2, causing excessive collagen synthesis (fibrosis), which not only limits the spread of an infection, but also destroys tissue architecture and causes implants rejection (foreign body reaction; Sect. 3.2.2).



**Fig. 8.8** The domain arrangement of the matrilysins. *White dots* represent two calcium ions that contribute to the structural integrity of the zincin catalytic domain. The *red dot* represents the zinc ion, the *yellow dot* represents the active site cleft with substrate binding sites represented by the ‘smile’. The thick light-blue arrow represents the prodomain in the binding cleft and the scissors represent where the prodomain is removed by plasmin. Figure is composed of the right top two parts of Fig. 1 in W. Bode and K. Maskos, Structural basis of the matrix metalloproteinases and their physiological inhibitors, the tissue inhibitors of metalloproteinases. *Biol. Chem.* 384 (June):863–872, 2003; Copyright permission given by Walter de Gruyter, Berlin, New York and both authors

**Table 8.4** Cellular sources of matrix metalloproteases

Protease class	MMP number	Keratinocyte/leukocyte expression
Collagenases	MMP-1 <sup>a</sup> , 8 and 13	Keratinocyte <sup>b</sup> : MMP-1, 3, 9, 10, and 28
Gelatinases	MMP-2 <sup>a</sup> and 9	Granulocytes <sup>c</sup> : MMP-8, 9, and 25
Stromelysins	MMP-3 and 10	B Cells <sup>d</sup> : MMP-11, 26, and 27
Membrane-type MMPs	MT-MMPs 14 <sup>a</sup> – 17; 24, and 25	T cells <sup>e</sup> : MMP-15,16, 24, and 28
Others	MMP-7, 11, 12, 19, 20, 21, 23, 26, 27, and 28	Monocytes <sup>f</sup> : MMP-1, 2, 3, 9, 10, 14, 17, 19, and 25

<sup>a</sup>Major MMPs expressed by fibroblasts

<sup>b</sup>Expression enhanced in proliferative/migratory basal keratinocytes at wound edge. Around ulcerations of mucosal tissues, such as lung and intestine, MMP-28 (epilysin) is absent but MMP-7 (matrilysin - from which all this group of MMPs takes its name.) is present instead MMP-7 degrades most major non-collagen proteins in a stromal matrix

<sup>c</sup>Mostly in neutrophilic granulocytes attracted to a region of stromal injury

<sup>d</sup>Antibody-producing lymphocytes that become attracted to a site of infection

<sup>e</sup>Non-antibody producing lymphocytes also attracted to a site of infection

<sup>f</sup>Macrophage precursors that develop from the white blood cell infiltrates at sites of stromal injury or infection

**Table 8.5** Matrilysin connective tissue degrading enzyme specificities

MMP#	Enzyme name	Molecular mass, latent (kDa)	Molecular mass, active (kDa)	Substrates
MMP-1	Collagenase-1 (fibroblasts)	55	45	Fibrillar collagens, gelatin, proteoglycans
MMP-8	Collagenase-2 (neutrophils)	75	58	Fibrillar collagens
MMP-13	Collagenase-3 (many cells)	65	55	Collagen type II
MMP-2	Gelatinase A (fibroblasts)	72	66	Gelatin, collagen type IV, elastin, fibronectin
MMP-9	Gelatinase B (neutrophils)	92	86	Gelatin, collagen type IV, elastin
MMP-7	Matrilysin	28	19	Matrix components except fibrillar collagens
MMP-3	Stromelysin-1	57	45	All matrix components except elastin and fibrillar collagen
MMP-10	Stromelysin-2	57	44	Matrilysin without elastase or laminin activity
MMP-11	Stromelysin-3	59	44	Laminin
MMP-12	Metalloelastase	53	45/22	Elastin, fibronectin, collagen type IV
MMP-20	Enamelysin	54	43	Amelogenin

### 8.3.2. Stromelysins

Stromelysins-1, -2, and -3 (MMP-3, MMP10, and MMP-11) degrade stromal components other than collagen. Skin fibroblasts constitutively express progelatinase (MMP-2), and activate it by co-secreting a membrane-adherent matrilysin on their cell surface, especially MMP-14. In contrast, following exogenous stresses or exposure to cytokines and ultraviolet irradiation, fibroblasts secrete procollagenase (MMP-1) and neutrophils secrete progelatinase (MMP-9). These enzymes are activated by plasmin from stress-activated *plasminogen pro-protein convertases* (see Sect. 8.1.3).

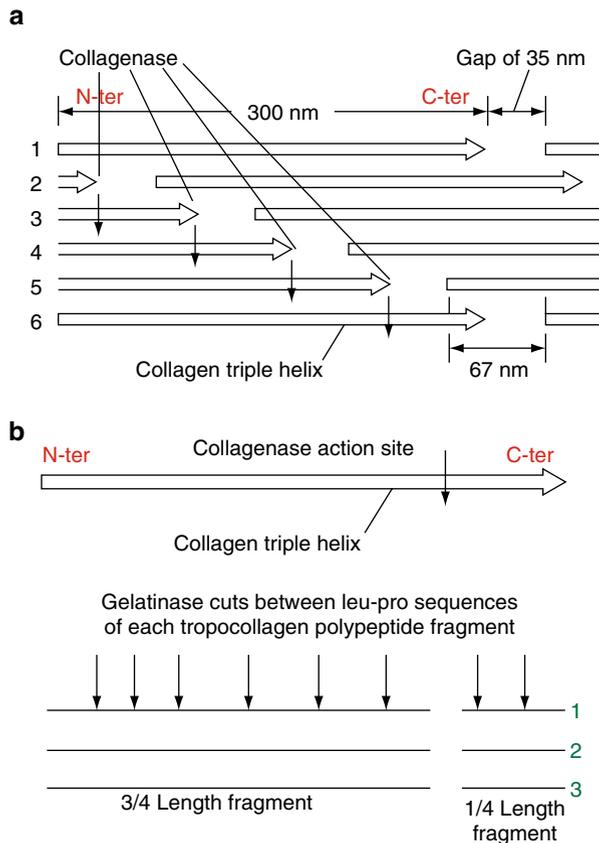
### 8.3.3. Enamelysin

Enamelysin (MMP-20) has major domains and an overall structure identical to fibroblast collagenase, gelatinase, and some stromelysins, but it lacks conserved residues that

determine collagenase or stromelysin specificity. A shorter amino acid sequence of the catalytic domain's C-terminal region further distinguishes it from collagenase and gelatinase. Enamelysin cleaves amelogenin, a major protein that determines enamel crystallization (Chap. 9). Except for collagenases (Sect. 8.3.4), the roles of the remaining matrilysins are not yet known.

### 8.3.4. Collagenases and Gelatinases

Collagenases act on collagen fibers at neutral pH. They recognize a three-dimensional structure that recurs at the gaps in the quarter-staggered array of tropocollagen molecules and cleave all three polypeptides at that point. This cut (Fig. 8.9a) causes the tropocollagen triple helix to spontaneously unwind, exposing individual one-quarter and three-quarter



**Fig. 8.9** Modes of action of neutral collagenase and gelatinase on collagen fiber. **(a)** *Initial step*: Collagen degradation begins with neutral collagenase cutting a triple helix into N-ter  $\frac{3}{4}$  and C-ter  $\frac{1}{4}$  fragments. **(b)** *Final step*: The  $\frac{3}{4}$  and  $\frac{1}{4}$ -length tropocollagen  $\alpha$ -chain fragments unwind, exposing its leu-pro bonds to gelatinase and it is degraded to small peptides

length polypeptides to gelatinase. The gelatinase cleaves exposed leucine–proline bonds, which are common in the tropocollagen sequence (Fig. 8.9b). The resultant small peptides are taken up by local cells and degraded to free amino acids in their lysosomal vesicles. Excessive collagen fiber cross-linking slows the unwinding of the three chains and their rate of degradation is slowed considerably. Collagen fibers are therefore difficult to turn over in old age because of extensive cross-linking. (Sect. 4.2.2), resulting in tissue malfunctions associated with senescence.

Collagenase and gelatinase are produced by fibroblasts and neutrophils. Although catalytically identical, the respective cells utilize different genes with homologous but non-identical amino acid sequences (Table 8.5). The fibroblast enzymes are larger and produced in a different environment from the neutrophil enzyme. Fibroblast gelatinase cleaves monocyte chemoattractant protein-3 (MCP-3), which prevents leukocyte infiltration of developing or remodeling tissues. The MCP-3 cleavage products bind to and inhibit a monocyte receptor that intact MCP-3 activates on monocytes and neutrophils. MCP3 is cleaved because it binds to the hemopexin domain of fibroblast gelatinase (MMP-2), but not to the corresponding domain of neutrophil gelatinase (MMP-9). Neutrophilic granulocytes are absent during development, but present in large numbers following tissue damage or infection when intact MCP3 actively recruits granulocytes to the affected region (Sect. 13.3.1).

There are 28 matrilysins that degrade various stromal proteins, most importantly fibrous collagen: MMP-1 and -8 (collagenases) and MMP-2 and -9 (gelatinases). Collagenases cleave all three tropocollagen polypeptides into large N-terminal and small C-terminal fragments that spontaneously unwind, exposing leu-pro bonds. Gelatinase cleaves these bonds to short peptides that are endocytosed and digested to amino acids in lysosomal vesicles. The collagenases and gelatinases are expressed by fibroblasts and neutrophils, respectively. They are separately encoded: fibroblast gelatinase will hydrolyze monocyte chemoattractant protein-3 (MCP-3), preventing inflammation during development when neutrophils are absent. Neutrophil gelatinase cannot cleave MCP-3.