Review

Matrix metalloproteinases: old dogs with new tricks

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Abstract

The matrix metalloproteinase family in humans comprises 23 enzymes, which are involved in many biological processes and diseases. It was previously thought that these enzymes acted only to degrade components of the extracellular matrix, but this view has changed with the discovery that non-extracellular-matrix molecules are also substrates.

Four decades ago a collagenase was discovered that was responsible for involution of the tadpole tail in amphibian morphogenesis [1]. Today, the matrix metalloproteinase (MMP) family, to which this collagenase belongs, has expanded to include 23 gene products in humans (Table 1), which encode zinc-dependent and calcium-dependent proteases that cleave within a polypeptide (endopeptidases). There are also two other large families that have major roles in extracellular proteolysis, the ADAM family (A disintegrin and metalloprotease domain, with about 33 members in humans) and the ADAMTS family (A disintegrin-like and metalloprotease domain (reprolysin type) with thrombospondin type I repeats, with 19 members). Traditionally, the MMPs have always been thought to cleave components of the extracellular matrix (ECM). As the ECM was regarded for a long time as nothing more than a passive structure used for cell attachment, mechanical support and force transmission, extracellular proteases such as MMPs were thought to simply remodel the ECM for its homeostasis or to facilitate cell migration. This view has changed, however [2]: the ECM is now known to contain growth factors, their binding proteins, and other bioactive molecules, as well as binding sites for cell-surface molecules, some of which are revealed only after proteolysis. The proteases responsible for turnover of the ECM therefore contribute significantly to its dynamic interactions with cells. More recently, considerable evidence has emerged showing that the role of MMPs goes far beyond that of digesting ECM molecules alone; they are now known to process a number of cell-surface and soluble regulators of cell behavior (summarized in Table 1). Mouse genetic models and human diseases have revealed unexpected biological functions of MMPs (summarized in Table 2); many of the phenotypes of these animals are currently unexplained and suggest that there may be as yet unknown substrates for these enzymes that are not ECM components.

One consequence of the previously held view of MMPs as solely ECM-excavating enzymes was the belief that they were uniformly harmful in cancer and should therefore be targeted in cancer therapy. This was not entirely without basis, given that many MMPs are highly expressed in various cancers, both by tumor cells and in surrounding stromal cells such as macrophages. As recent reviews [3-6] have pointed out, however, the unexpectedly complex role of these enzymes in cancer is leading to their re-evaluation as drug targets, or at the very least, a pause while some of the evidence is analyzed in more detail. Although this is disappointing from the clinical perspective, the study of these enzymes has never been more exciting than it is today, because the human genome project has led to identification of all MMPs, and genetic alterations in individual MMP genes have provided new insights into their functions. In this article, we summarize some of the essential concepts in the MMP field at present and the new insights that are pouring in from biological studies.

Table 1

Human matrix metalloproteases and their substrates

Protein name*	Alternative names	Collagenous substrates	Non-collagenous ECM substrates	Non-structural ECM component substrates
MMP-1	Collagenase-1	Collagen types I, II, III, VII, VIII, X, and gelatin	Aggrecan, casein, nidogen, serpins, versican, perlecan, proteoglycan link protein, and tenascin-C	α_1-antichymotrypsin, α_1-antitrypsin/ α_1-proteinase inhibitor, IGFBP-3, IGFBP-5, IL-1β, L-selectin, ovostatin, recombinant TNF-α peptide, and SDF-1
MMP-2	Gelatinase-A	Collagen types I, IV, V, VII, X, XI, XIV, and gelatin	Aggrecan, elastin, fibronectin, laminin, nidogen, proteoglycan link protein, and versican	Active MMP-9, active MMP-13, FGF R1, IGF-BP3, IGF-BP5, IL-1β, recombinant TNF-α peptide, and TGF-β
MMP-3	Stromelysin-1	Collagen types II, IV, IX, X, and gelatin	Aggrecan, casein, decorin, elastin, fibronectin, laminin, nidogen, perlecan, proteoglycan, proteoglycan link protein, and versican	α_1-antichymotrypsin, α_1-proteinase inhibitor, antithrombin III, E-cadherin, fibrinogen, IGF-BP3, L-selectin, ovostatin, pro-HB-EGF, pro-IL-β, pro-MMP-1, pro-MMP8, pro-MMP-9, pro-TNFα, and SDF-1
MMP-7	Matrilysin-1, neutophil collagenase	Collagen types I, II, III, V, IV, and X	Aggrecan, casein, elastin, enactin, laminin, and proteoglycan link protein	β_4 integrin, decorin, defensin, E-cadherin, Fas-L, plasminogen, pro-MMP-2, pro-MMP-7, pro-TNFα, transferrin, and syndecan
MMP-8	Collagenase-2	Collagen types I, II, III, V, VII, VIII, X, and gelatin	Aggrecan, laminin, and nidogen	α_2-antiplasmin and pro-MMP-8
MMP-9	Gelatinase-B	Collagen types IV, V, VII, X, and XIV	Fibronectin, laminin, nidogen, proteoglycan link protein, and versican	CXCL5, IL-1β, IL2-R, plasminogen, pro-TNFα, SDF-1, and TGF-β
MMP-10	Stromelysin-2	Collagen types III, IV, V, and gelatin	Fibronectin, laminin, and nidogen	Pro-MMP-1, pro-MMP-8, and pro-MMP-10
MMP-11	Stromelysin-3		Laminin	α_1-antitrypsin, α_1-proteinase inhibitor, and IGFBP-1
MMP-12	Macrophage metalloelastase		Elastin	Plasminogen
MMP-13	Collagenase-3	Collagen types I, II, III, IV, V, IX, X, XI, and gelatin	Aggrecan, fibronectin, laminin, perlecan, and tenascin	Plasminogen activator 2, pro-MMP-9, pro-MMP-13, and SDF-1
MMP-14	MT1-MMP	Collagen types I, II, III, and gelatin	Aggrecan, dermatan sulphate proteoglycan, fibrin, fibronectin, laminin, nidogen, perlecan, tenascin, and vitronectin	$\alpha_v\beta_3$ integrin, CD44, gC1qR, pro-MMP2, pro-MMP-13, pro-TNFα, SDF-1, and tissue transglutaminase
MMP-15	MT2-MMP	Collagen types I, II, III, and gelatin	Aggrecan, fibronectin, laminin, nidogen, perlecan, tenascin, and vitronectin	Pro-MMP-2, pro-MMP-13, and tissue transglutaminase
MMP-16	MT3-MMP	Collagen types I, III, and gelatin	Aggrecan, casein, fibronectin, laminin, perlecan, and vitronectin	Pro-MMP-2 and pro-MMP-13
MMP-17	MT4-MMP	Gelatin	Fibrin and fibronectin	
MMP-19	RASI-1	Collagens types I, IV, and gelatin	Aggrecan, casein, fibronectin, laminin, nidogen, and tenascin	
MMP-20	Enamelysin		Aggrecan, amelogenin, and cartilage oligomeric protein	
MMP-21				α_1-antitrypsin
MMP-23	CA-MMP	Gelatin		
MMP-24	MT5-MMP	Gelatin	Chondroitin sulfate, dermatin sulfate, and fibronectin	Pro-MMP2 and pro-MMP-13
MMP-25	Leukolysin, MT6-MMP		Collagen type IV and gelatin	Fibrin and fibronectin Pro-MMP-2
MMP-26	Matrilysin-2, endometase	Collagen type IV and gelatin	Casein, fibrinogen, and fibronectin	β_1-proteinase inhibitor
MMP-28	Epilysin		Casein	

*Although there are 23 human MMPs, 29 numbers have been used in the literature. The symbols MMP-4, MMP-5, MMP-6 and MMP-29 are redundant in humans and are no longer in use; MMP-18 corresponds to a *Xenopus laevis* collagenase, for which no human ortholog is known, and a human protein published as MMP-18 is now called MMP-19. Two nearly identical human genes found in a segment of chromosome 1 that is duplicated were called *MMP21* and *MMP22* but are now referred to as *MMP23A* and *MMP23B*.

Table 2

Phenotypes of knockout mice lacking MMP genes

Genotype	Phenotype
MMP2$^{-/-}$	Suppression of experimentally induced pancreatic carcinogenesis [85]; suppression of angiogenesis and tumor growth [86]; delayed mammary gland differentiation; mild growth retardation.
MMP3$^{-/-}$	Accelerated mammary gland adipogenesis [87]; delayed incisional wound healing [88]; resistance to contact dermatitis [89]; impaired *ex vivo* herniated disc resorption [90].
MMP7$^{-/-}$	Suppression of experimental adenoma growth [91]; defective innate intestinal immunity [70]; impaired tracheal wound re-epithelization [92]; defective prostate involution after castration [93]; impaired *ex vivo* herniated disc resorption [94]; impaired transepithelial migration of neutrophils [32]; reduced syndecan-1 shedding [32].
MMP9$^{-/-}$	Suppression of experimentally induced skin carcinogenesis [95]; suppression of experimentally induced pancreatic carcinogenesis [85]; decreased experimental metastasis [86]; delayed growth plate vascularization [96]; abnormal endochondral ossification [96]; defective in osteoclast recruitment [97]; resistance to bullous pemphigoid (skin blistering) [98]; resistance to experimentally induced aortic aneurysms [99]; prolonged contact dermatitis [89]; protection from ventricular enlargement after infarction [100]; protection from cardiac rupture after infarction [101]; diminished peripheral mononucleocyte infiltrate in glomerular nephritis [102]; impaired smooth muscle cell migration and geometrical arterial remodeling [103,104]; impaired cellular infiltration and bronchial hyper-responsiveness during allergen-induced airway inflammation [105]; impaired recruitment of stem and progenitor cells from the bone marrow following irradiation [106]; myocardial protection from ischemia and/or reperfusion [107].
MMP11$^{-/-}$	Suppression of experimentally induced mammary carcinogenesis [108]; decreased tumor cell survival and growth [109]; accelerated neointima formation after vessel injury [110].
MMP12$^{-/-}$	Resistance to cigarette-smoke-induced emphysema [111].
MMP14$^{-/-}$	Severe abnormalities in bone and connective tissue [76,77]; reduced collagen turnover [76]; impaired endochondral ossification [76,77]; defective angiogenesis [76,77].

Data in the table are modified from [5,77,83,112].

Classification and evolution

There are 23 human MMP genes (Table 1). Before this genetic diversity was known, it was customary to divide the MMPs into collagenases, gelatinases, stromelysins, elastase and others, on the basis of the known substrates, but as some MMPs have overlapping substrate specificities, the boundary between the previously used enzyme classes is now blurred. Nevertheless, the trivial names are often useful, particularly if they reflect a function or a distinct structural feature or location, and they have therefore been retained (and are indicated in Table 1). MMP genes are structurally similar to each other, indicating that they evolved by duplication of a common ancestral gene followed by divergent evolution. A number of MMP genes (*MMP1, MMP3, MMP7, MMP8, MMP10, MMP12, MMP13, MMP20*, and *MMP26*) are found in a cluster on human chromosome 11 (11q21-23) [7,8]. The human and mouse genomes appear not to contain an identical complement of MMPs, which may complicate the use of transgenic mice for insights into functions of the human genes. For example, human *MMP1* does not have a clear-cut ortholog in the mouse but instead is most similar to two genes, *mColA* and *mColB*, whose functions are at present unknown [9].

MMP genes have been identified in a number of non-vertebrate species, and the number will undoubtedly increase as more genome projects are completed. The nematode *Caenorhabditis elegans* has a number of MMPs, including C31, H19 and Y19 [10]; there is a *Drosophila* MMP [11]; sea urchins contain an MMP essential for hatching, known as envelysin [12]; and the hydra has an MMP that is required for foot-process development [13]. Interestingly, there is an MMP in green algae [14] as well as in plants such as the soybean [15] and *Arabidopsis thaliana* [16].

Domain organization and protein structure

The close evolutionary relationship between MMPs is further reflected in the conservation of their domain structure (Figure 1) and of their mechanisms of catalysis and regulation. Most MMPs are secreted, although some are anchored to the cell surface by a transmembrane segment (MMP-14, MMP-15, MMP-16 and MMP-24, referred to as membrane-type MMPs or MT-MMPs) [17] or via a glycosylphosphoinositol (GPI) anchor (MMP-17 and MMP-25, sometimes also included in the MT-MMPs) [18]. Most of the MT-MMPs are type I transmembrane proteins - oriented with the amino terminus outside the cell - although one, MMP-23, is unusual in apparently having the amino terminus positioned intracellularly (a type II orientation) [19]. The MT-MMPs have a single transmembrane domain followed by a short, highly conserved cytosolic segment of 20 amino acids. 'Soluble' MMPs may also be located at the cell surface via interaction with membrane-bound molecules or complexes such as integrin $\alpha_v\beta_3$ [20], the extracellular matrix metalloproteinase inducer EMMPRIN/CD147 [21], the hyaluronan receptor CD44 [22],

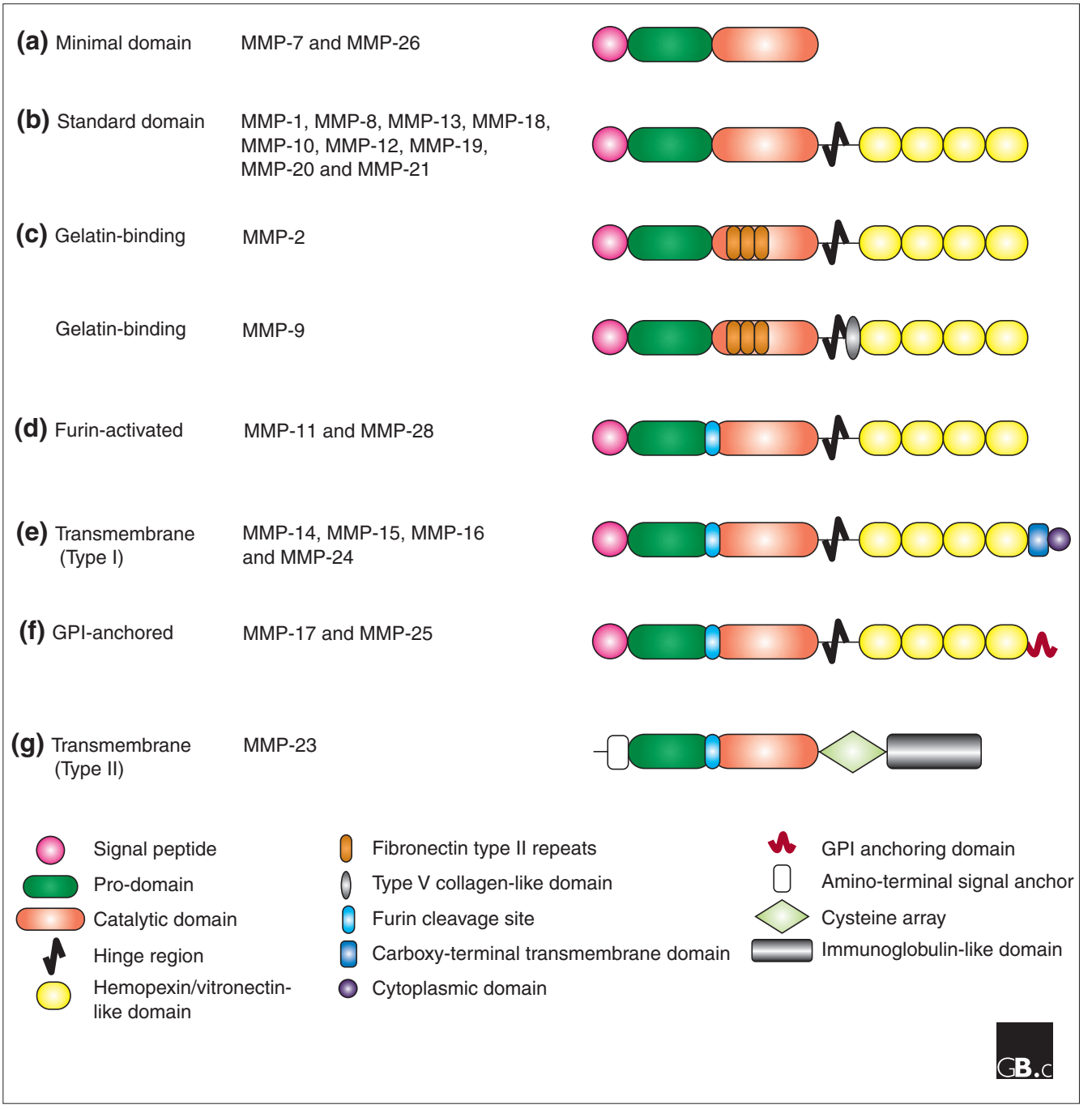

Figure 1
The domain composition and important structural features of the various subtypes of MMPs.

cell-surface heparan-sulfate proteoglycans such as syndecans [23], or transmembrane MMPs [17].

All MMPs except MMP-7 (matrilysin-1) and MMP-26 (endometase or matrilysin-2) consist of two domains connected by a flexible proline-rich hinge peptide. The domains comprise a protease domain and an ancillary (helper) domain - most commonly a domain similar to sequences in the heme-binding protein hemopexin and the soluble fibronectin-related protein vitronectin [24,25] (see Figure 1). The protease domain contains a signal peptide, the pro-domain, and the catalytic module, in that order. MMP-2 and MMP-9, which are the main gelatinases, differ from other MMPs in that they have three tandem fibronectin type II

repeats within the amino terminus of the catalytic module that mediate gelatin binding (Figure 1c) [26-28]. MMP-9 has an additional insert within its hinge region similar to collagen V; its function is unknown. MMP-7 and MMP-26 [29] lack the hemopexin/vitronectin-like domain (Figure 1a), whereas MMP-23 has a unique cysteine- and proline-rich sequence and a domain similar to the immunoglobulin-like domains of the interleukin-1 receptor instead of the hemopexin/vitronectin-like domain (Figure 1g) [30].

X-ray crystal structures are now available for a number of MMPs, including full-length activated enzymes and zymogen forms that include the pro-domain [31]. Crystallography has revealed how a cleft in the active site contributes to enzyme specificity [32]. The catalytic domain of all MMPs has a unique signature of amino-acid residues (His-Glu-X-Gly-His-X-X-Gly-X-X-His-Ser, where X is any amino acid) within the active site [25] that coordinates the catalytic zinc atom. The hemopexin/vitronectin-like domain consists of four parts arranged symmetrically around a central axis, forming a four-bladed propeller-like structure that helps to confer substrate-binding specificity on the enzyme.

Regulation of MMPs

In keeping with their potential for tissue destruction, MMPs are stringently regulated at multiple levels, including transcription, activation of the zymogen forms, extracellular inhibitors, location inside or outside the cell and internalization by endocytosis. The pro-domain keeps the enzyme latent using the thiol group of a highly conserved, unpaired cysteine at its carboxyl terminus. This conserved cysteine acts as a fourth inactivating ligand for the catalytic zinc atom in the active site, resulting in the exclusion of water and rendering the enzyme inactive (Figure 2). For the enzyme to be activated, this cysteine-zinc pairing needs to be disrupted by a conformational change or by proteolysis (such as by the protease plasmin or by other MMPs). Once the thiol group is replaced by water, the enzyme is able to hydrolyze the propeptide to complete the activation process and can then cleave the peptide bonds of its substrates. This system of regulation is referred to as the 'cysteine-switch' mechanism [33]. Most MMPs are not activated until they are outside the cell, but the MT-MMPs and MMP-11, MMP-23 and MMP-28 are activated by a proprotein convertase (such as furin) within the secretory pathway [34-37].

Once activated, there are multiple mechanisms that can inactivate the MMPs [38]. Four classes of metalloproteinase inhibitors are found in extracellular spaces and body fluids that have broad inhibitory activity against many MMPs. One class is the tissue inhibitors of metalloproteinases (TIMPs), which are disulfide-bonded proteins of 20-30 kDa that directly interact with the MMP active site through a small number of their amino acids. An unrelated small inhibitor derived by proteolysis of the procollagen C-proteinase enhancer has structural similarity to TIMPs and may inhibit MMPs through a similar mechanism [39]. Recently, a membrane-anchored molecule, reversion-inducing cysteine-rich protein with Kazal motifs (RECK), has been discovered that appears to regulate MMP-2, MMP-9 and MMP-14 post-transcriptionally by affecting secretion and activation as well as by inhibition of the active site [40]. In the circulation, the protease inhibitor α2-macroglobulin inactivates active MMPs by a 'bait and trap' mechanism [38,41]: when protease-sensitive sites within the inhibitor are cleaved, it springs closed around the proteinase and isolates it from potential substrates.

MMP zymogens can also be activated by MMPs themselves; for example, MMP-2 is activated by MT-MMPs such as MMP-14 [17]. Paradoxically, the inhibitor TIMP-2 is absolutely required for this process, which requires it to bind to the active site of MMP-14 and also, through its carboxy-terminal domain, to the hemopexin/vitronectin-like domain of MMP-2. The essential role of TIMP-2 is emphasized by the lack of pro-MMP-2 activation in *Timp2*-null mice [42] as well as by the exquisite co-regulation of the *Mmp14* and *Timp2* genes (but not other TIMP genes) during mouse development [43]. (MMP-14 also activates MMP-13 at the cell surface, but this does not seem to require TIMP-2 [44].)

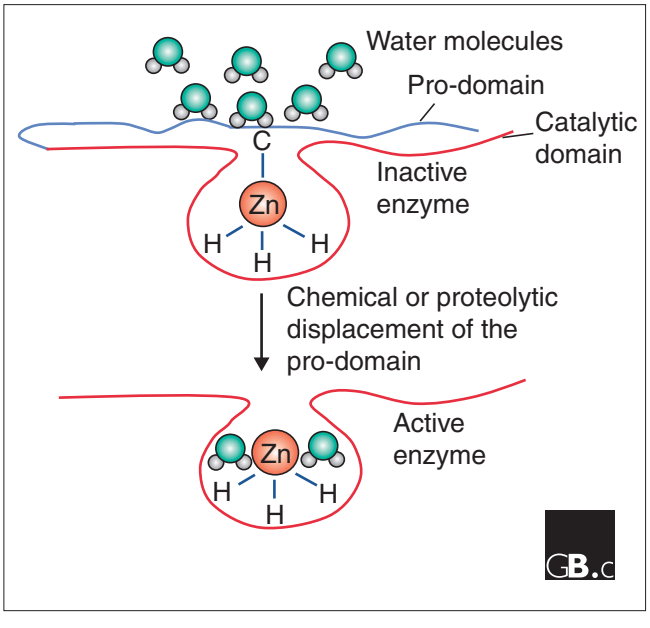

Figure 2
The 'cysteine-switch' mechanism regulating the MMP zymogen. The thiol group of a conserved cysteine (C) at the carboxyl terminus of the pro-domain acts as a fourth inactivating ligand for the catalytic zinc atom in the active site; this results in the exclusion of water and keeps the enzyme latent. Displacement of the pro-domain by conformational change or proteolysis disrupts this cysteine-zinc pairing and the thiol group is replaced by water. The enzyme can then cleave the peptide bonds of its substrates.

The further activation of MMP-2 bound to TIMP2 probably involves complex but highly regulated repositioning of membrane-bound MMPs through their cytoplasmic tails or transmembrane domains or through interaction with cellular receptors. Lehti *et al.* [45,46] have suggested that oligomerization may occur between the cytoplasmic domains of the two MMP-14 molecules, an observation that ties in with a report that a conserved cysteine in the MMP-14 cytoplasmic tail is essential for dimerization [47].

In addition to a possible role in oligomerization, the cytoplasmic domain of MT-MMPs is essential for regulating the activity of these enzymes; it acts by altering their spatial distribution in the cell in response to intracellular signaling events. The cytoplasmic sequence targets the MT-MMPs to specific domains within the cell membrane, including invadopodia (surface protrusions of invasive cells) [48] and caveolae (specialized membrane invaginations) [49]. Recently, a role for the cytoplasmic domain has been identified in the cycling of MT-MMPs between an intracellular pool and the cell surface via clathrin-coated pits and vesicles [50,51]; this may be one of several cellular mechanisms by which the amount of enzyme at the cell surface is regulated. Studies of *thrombospondin-2* null mice have indicated that thrombospondin-2 has a role in targeting active MMP-2 to the scavenger-receptor pathway [52], and it has been shown that MMP-13 binds to a specific cell-surface receptor before it is internalized via the low-density lipoprotein receptor [53].

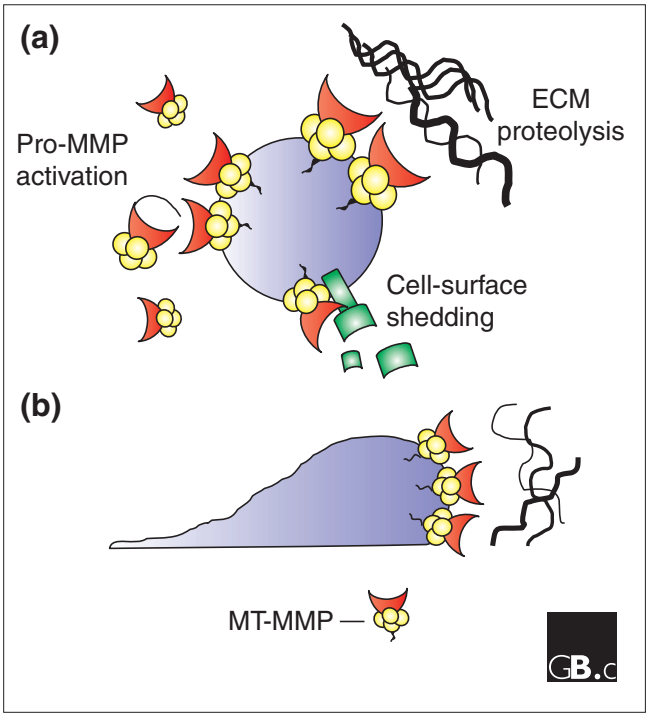

Figure 3
The locations of MT-MMPs. **(a)** The location of MT-MMPs lends them critical biological roles at the cell surface: they cleave components of the ECM, other MMPs and receptors for growth factors (which leads to shedding of the receptors from the cells). **(b)** Mobilization of MT-MMPs to the leading edge of cancer cells, where they remodel the ECM, facilitates cell migration and tumor invasion.

Functions of MMPs

The substrates of MMPs are given in Table 1, and the functions of MT-MMPs in particular are illustrated in Figure 3.

Enzyme-substrate co-localization and interactions

The collagenases MMP-1, MMP-8, MMP-13, and MMP-14 are the only MMPs that can efficiently degrade the fibrillar collagens (types I, II and III) in their triple-helical domains [54]. Cleavage by these enzymes renders the collagen molecules thermally unstable, so that they unwind to form gelatin, after which they can be degraded by other members of the MMP family such as the major gelatinases MMP-2 and MMP-9. The collagenase active site is unable to accommodate the entire cross-section of the collagen triple helix, an observation that has generated much interest in how this substrate is actually cleaved.

The two major gelatinases, MMP-2 and MMP-9, have several distinctive features. They can be distinguished by the fact that MMP-2 binds preferentially to TIMP-2, which is required for its activation, whereas MMP-9 is preferentially inhibited by TIMP-1 [38]. MMP-2 becomes located at the cell surface by binding of its carboxyl terminus to the integrin $\alpha_v\beta_3$ [29] or the MMP-14-TIMP-2 complex; when bound, the catalytic site of MMP-2 is exposed and can be cleaved and activated. The α2 chains of collagen IV bind MMP-9 with a high affinity even when MMP-9 is inactive [55]; this juxtaposition of enzyme and substrate means that a pool of the enzyme is rapidly available upon activation for any remodeling events.

Non-matrix substrates and consequences of MMP activity

The extracellular matrix contains sites that can bind growth factors, either directly and via growth-factor-binding proteins. When bound, growth factors - such as transforming growth factor β (TGF-β), fibroblast growth factor 1 (FGF-1) and insulin-like growth factor 1 (IGF-1), tumor necrosis factor α, and heparin-binding epidermal growth factor-like growth factor (HB-EGF) [56] - are unable to bind their receptors and signal to the nucleus [57-61]. Several MMPs are able to release growth factors by cleaving either the growth-factor-binding protein or the matrix molecule to which these proteins attach. In addition, MMP-3 and MMP-7 can cleave the adherens-junction protein E-cadherin, thus promoting cell invasion by disrupting cell aggregation [62,63]. MMP-3 can release a soluble form of the adhesion molecule L-selectin [64] from leukocytes. It also sheds membrane-bound HB-EGF, so that it can exert signaling functions [56]. MMP-7 releases soluble Fas ligand; this occurs during involution of

the prostate after castration and induces apoptosis [65]. In contrast to the activation of growth factors by proteolytic release, many growth factors are proteolytically inactivated by MMPs, including the chemokine connective tissue activating peptide III (CTAP-III), monocyte chemoattractant protein and stromal cell-derived factor 1 (SDF-1) [66,67]. A second mechanism by which growth factors and cytokines are negatively regulated is when MPPs cause the shedding of their receptors from the cell membranes, as in the case of surface FGF receptor 1 [68].

The immune system is also influenced by MMPs. Firstly, the defensins are a family of polar antimicrobial peptides that make up part of the innate immune system of some animals. Defensins are synthesized in an inactive form and activated by the proteolytic removal of the pro-domain by MMP-7, which allows them to insert into the bacterial membrane and disrupt its integrity [69,70]. Secondly, MMP-3 (stromelysin-1) and MMP-7 (matrilysin-1) can cleave all immunoglobulin G proteins; this cleavage is important as it prevents the initiation of the complement cascade and is potentially beneficial in the removal of the immunoglobulin G from damaged or inflamed tissue [71]. And finally, the receptor of the complement component C1q, gC1qR, exists in both a membrane-bound form and a soluble form that may inhibit the hemolytic activity of C1q. By releasing gC1qR, MT1-MMP may help tumor cells to avoid targeted destruction by the complement system and may thus facilitate tumor-cell survival [72-74].

Genetic alterations in human and mouse MMPs

An extensive body of literature indicates an association of MMPs with cancer, arthritis, numerous other inflammatory or autoimmune disorders, cardiovascular and cerebrovascular diseases, and fibrotic diseases. Despite this, there are relatively few instances in which MMPs have proved to be the primary cause of disease. One of these is a rare inherited disorder of bone (nodulosis arthropathy osteolysis syndrome), one of a set of 'vanishing bone syndromes', in which there is severe resorption and destruction of bones, primarily those of the hands and feet [75]. The affected individuals also have chronic arthritis and subcutaneous nodules in the hands and feet. The disease is inherited in an autosomally recessive manner, and loss-of-heterozygosity analysis mapped the responsible gene to 16q12-q22, an interval containing the *MMP2* gene. Analysis of the serum and fibroblasts of affected individuals showed a complete absence of *MMP2*. The family was found to have two homoallelic *MMP2* mutations, resulting in the ablation of *MMP2* activity. The presence of collagenous nodules fits with the role of MMPs in removing excess ECM; the paradox in these patients, however, is that excessive destruction of bones and arthritis are caused by an absence of a destructive enzyme, not an excess as might have been expected. There are a number of mechanisms by which this may occur: there may be compensatory overproduction of another MMP or protease, MMP-2 may be essential for processing of an inductive factor required for the activity of bone-forming cells (osteoblasts), or it may be required to regulate the activity of bone-degrading cells (osteoclasts) in tissues. Interestingly, the *Mmp2* knockout mouse is not known to recapitulate the human disease, which resembles more closely the phenotype of *Mmp14* knockout mice [76,77]. Given that MMP-14 and TIMP-2 are required for MMP-2 activation, it is possible that mutations in the genes encoding these enzymes may result in a similar human disease.

The chromosome 1p36.3 region that contains the two closely related *MMP23* genes (see Table 1) is altered or frequently deleted in neuroblastomas in which the oncogene *MYCN* has been amplified, in a subset of malignant melanomas and in a 1p35 deletion disorder [78], but the role of the two MMP genes in these disorders has not been investigated.

A polymorphism in the *MMP1* promoter can result in one allele having a sequence of either one or two guanines in a particular position. The presence of two guanines results in the *de novo* appearance of a binding site for the ETS-1 transcription factor that cooperates with an adjacent site for the AP-1 transcription factor to enhance the expression of *MMP1* [79-81]. It is interesting to note that cancer patients, on average, have a higher incidence of the two-guanine allele than have people in the unaffected population. A regulatory polymorphism is also present in the *MMP3* promoter, leading to the presence of either five or six adenosines [82]. The allele with six (6A) has a reduced transcriptional activity compared to the allele with five. A study on patients with coronary artery atherosclerosis revealed that patients homozygous for the 6A allele showed a more rapid progression of both global and focal atherosclerotic lesions.

The creation of transgenic mice that are null for specific genes has been useful in attributing functions to a number of MMP family members (Table 2). In general, most MMP gene knockouts are viable, with subtle phenotypic differences from their wild-type counterparts [83]. This could reflect either a lack of function in vital developmental processes or a significant amount of redundancy amongst these enzymes for substrates, with the consequence that other family members can be upregulated to compensate for the loss of an individual enzyme. Although the lack of a dramatic phenotype in many of the knockout mice may initially have seemed a disappointing outcome, it has in fact been very valuable for dissecting the role of MMPs in cancer and inflammation. The exception to the rule is the *Mmp14*-knockout mouse [76,77], which has severe postnatal growth-retardation and skeletal anomalies and dies before the onset of sexual maturity (Figure 4). The severity of the *Mmp14* knockout, in contrast to the other MMP knockout animals, may reflect the position of this enzyme as an initiator of a number of proteolytic cascades, as well as its obligate location at the cell surface. (Note that none of the other MT-MMPs has yet been inactivated in mice.) The defects seen in these animals are probably attributable to both deficiencies in the turnover of direct MMP-14 substrates

Figure 4
The phenotype of *Mmp14*-null mice, which is the most dramatic developmental phenotype of all MMP null transgenes. A rare example of an *Mmp14*-null mouse that has survived to 10 weeks of age is shown (front, alongside a normal littermate); note the severe dwarfism and craniofacial anomalies.

and abnormalities in the indirect substrates that are mediated by the activation of intermediary enzymes such as MMP-2 and MMP-13.

The importance of MMPs in physiological processes and human disease is now undisputed. What is not known, however, is the full extent to which they are involved in every process in mammals. The failure of broad-spectrum and even relatively specific MMP inhibitors in cancer therapy is not surprising, because the complete effects for a given inhibitor depend on the functions of its targets, and these are mostly unidentified and unknown. The fact that there are two other large families of related proteases with important physiological functions, the ADAM and ADAMTS families, suggests that additional caution should be taken when using MMP inhibitors to treat disease states. More subtle approaches are going to be required to modulate the functions of these enzymes in a more selective fashion [84]. By understanding the precise mechanisms by which MMPs are regulated and their interactions with various binding partners, it may be possible to block the deleterious functions of these enzymes without the concomitant loss of beneficial functions. This underscores the need for a greater investment than ever before in MMP biology and biochemistry. The next five years are expected to bring many exciting insights into the role of MMPs in development and human disease and may lead to even broader interest in what was once thought to be a highly specialized and restricted field.

Acknowledgements

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