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Matrix metalloproteinases and the regulation of tissue remodelling

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Abstract

Matrix metalloproteinases (MMPs) were discovered because of their role in amphibian metamorphosis, yet they have attracted more attention because of their roles in disease. Despite intensive scrutiny *in vitro*, in cell culture and in animal models, the normal physiological roles of these extracellular proteases have been elusive. Recent studies in mice and flies point to essential roles of MMPs as mediators of change and physical adaptation in tissues, whether developmentally regulated, environmentally induced or disease associated.

The founding member of the matrix metalloproteinase (MMP) family, collagenase, was identified in 1962 by Gross and Lapiere, who found that tadpole tails during metamorphosis contained an enzyme that could degrade fibrillar collagen^{1,2}. Subsequently, an interstitial collagenase, collagenase-1 or MMP1, was found in diseased skin and synovium³. *In vitro*, MMP1 initiates degradation of native fibrillar collagens, crucial components of vertebrate extracellular matrix (ECM), by cleaving the peptide bond between Gly775–Ile776 or Gly775–Lys776 in native type I, II or III collagen molecules^{3,4}. Further research led to the discovery of a family of structurally related proteinases (23 in human, 24 in mice), now referred to as the MMP family.

Interest in MMPs increased in the late 1960s and early 1970s following observations that MMPs are upregulated in diverse human diseases including rheumatoid arthritis and cancer. Importantly, high levels of MMPs often correlated with poor prognosis in human patients (reviewed in REF. ⁵). However, recent clinical data indicate that the relationship between

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DATABASES

The following terms in this article are linked online to:

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DmMmp1 | DmMmp2

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MMP1 | MMP2 | MMP3 | MMP9 | MMP14 | MMP15 | MMP16 | MMP17 | MMP23 | MMP24 | MMP25

FURTHER INFORMATION

Andrea Page-McCaw's homepage: www.rpi.edu/research/biotech/researchers/page-mccaw.html

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International Proteolysis Society: <http://www.protease.org>

MEROPS: <http://merops.sanger.ac.uk>

MMPs and disease is not simple; for example, increased MMP activity can enhance tumour progression or can inhibit it (reviewed in REF. ⁶). This complex relationship between MMP expression and cancer has increased the basic and clinical interest in understanding MMP function *in vivo*, but it has also focused attention on MMPs and pathology, and relatively less attention has been focused on the normal roles of these enzymes. Surely we do not have 23 MMPs in our bodies just to promote tumours. A main question remains unanswered: what is the normal function of the MMP gene family in development? This Review focuses on what we have learned about the normal *in vivo* functions of MMPs from genetic analysis.

MMPs *in vivo*: analysis of MMP mutants

Analysis of genetic knockouts of MMPs offers opportunities to identify essential functions of an MMP and to validate candidate substrates by looking for cleavage products in the control animal and uncleaved proteins in the mutant animal. At least 14 mouse MMP mutants have been generated. The initial characterizations have described surprisingly subtle phenotypes, with all MMP-knockout lines surviving to birth (TABLE 1). Possible explanations for the seeming dispensability of MMPs during embryonic development include enzymatic redundancy, enzymatic compensation and adaptive development, although it is possible that MMPs are not important until after embryonic development. MMPs have many overlapping substrates *in vitro*⁵, which indicates possible genetic redundancy *in vivo*. Indeed, redundancy has now been shown with the recent generation of MMP double mutants^{7,8}. Compensation has also been shown in the MMP family^{9,10}. In the next sections, we will discuss mutant mouse phenotypes that rely on genetic approaches. Researchers can also now take advantage of simpler genetic model systems, such as *Drosophila melanogaster*, in which it is possible to mutate all MMP genes.

MMP proteolysis

MMPs are members of the metzincin group of proteases, which are named after the zinc ion and the conserved Met residue at the active site^{11,12}. Recent work has generated a unified peptidase nomenclature¹³ in which MMPs include the M10A subfamily, the M10 family and the MA clan of metallopeptidases. Mammalian MMPs share a conserved domain structure (FIG. 1) that consists of a catalytic domain and an autoinhibitory pro-domain. The pro-domain contains a conserved Cys residue that coordinates the active-site zinc to inhibit catalysis. When the pro-domain is destabilized or removed, the active site becomes available to cleave substrates. Most MMP-family members also contain a hemopexin domain, attached at their C termini by a flexible hinge. The hemopexin domain encodes a four-bladed β -propeller structure that mediates protein–protein interactions. This domain also contributes to proper substrate recognition, activation of the enzyme, protease localization, internalization and degradation¹⁴. The structures of the catalytic and hemopexin domains of several MMPs, including MMP1, MMP2, MMP3 and MMP14 (also known as membrane type 1 MMP (MT1-MMP)), have been solved (reviewed in REFS ^{13, 15}). MMP2 and MMP9 also have fibronectin type II repeats, which mediate binding to collagens, inserted into the catalytic domain. Most MMPs are secreted proteins; however there are six MT-MMPs: MMP14, MMP15, MMP16 and MMP24 have transmembrane domains and short cytoplasmic tails; MMP17 and MMP25 have glycosylphosphatidylinositol (GPI) linkages. Also, MMP23 is a type II transmembrane protein. The activity of MMPs is controlled at many levels and the regulation of MMP activity remains a topic of intense research (BOX 1).

Functions of MMP proteolysis

Historically, MMPs were thought to function mainly as enzymes that degrade structural components of the ECM. However, MMP proteolysis can create space for cells to migrate, can produce specific substrate-cleavage fragments with independent biological activity, can

regulate tissue architecture through effects on the ECM and intercellular junctions, and can activate, deactivate or modify the activity of signalling molecules, both directly and indirectly¹⁶ (FIG. 2). Because cells have receptors (for example, integrins) for structural ECM components, MMPs can also affect cellular functions by regulating the ECM proteins with which the cells interact¹⁷. In many cases, MMP cleavage of ECM substrates generates fragments that have different biological activities from their precursors. For example, the cleavage of laminin-5 or collagen IV results in the exposure of cryptic sites that promote migration^{18,19}. Type I collagen degradation that is mediated by MMP1 is necessary for epithelial cell migration and wound healing in culture models²⁰. Cleavage of ECM proteins by MMPs can also release ECM-bound growth factors, including insulin growth factors and fibroblast growth factors^{21,22}. Alternative mechanisms of action have also been observed, including functional intermolecular MMP complexes: MMP14 binds to tissue inhibitor of metalloproteinase-2 (TIMP2), which binds to pro-MMP2, thereby positioning it for activation by a second molecule of MMP14 (REF. 23). Furthermore, human MMP11 has an alternative splice isoform that functions as an intracellular proteinase and enters the nucleus²⁴.

MMP substrates include peptide growth factors, tyrosine kinase receptors, cell-adhesion molecules, cytokines and chemokines, as well as other MMPs and unrelated proteases (BOX 2). MMPs and the related families of proteinases, the ADAMs (a disintegrin and metalloproteinases) and ADAM-TSs (ADAMs with thrombospondin repeats), are important in shedding plasma-membrane-bound proteins. ADAMs and ADAM-TSs participate in shedding growth factors that are synthesized as cell-membrane-bound precursor forms, including heparin-binding epidermal growth factor (HB-EGF), neuregulin, amphiregulin and transforming growth factor- α (TGF α)^{25–28}. Cleavage of other membrane proteins, such as E-cadherin and CD44, results in the release of specific, biologically active fragments of their extra cellular domains, and in increased invasive behaviour^{29,30}. Cell-surface-adhesion molecules, such as syndecan-1, are also shed by soluble and membrane MMPs^{31,32}. MMP9 and MMP12 contribute to proteolytic shedding of the lipopolysaccharide (LPS) receptor CD14, and therefore influence innate host defense³³. TGF β is another important MMP substrate, the activation of which frequently alters cell migration; for example, MMP9 restrains corneal epithelial migration through the activation of TGF β ³⁴. Both MMP2 and MMP9 can release TGF β from an inactive extracellular complex that consists of TGF β , TGF β latency-associated protein (which is the pro-domain of TGF β), and latent TGF β -binding protein³⁵.

An important ongoing challenge is to link our understanding of MMP substrates that have been identified *in vitro* or in cell culture with the substrates that are cleaved by MMPs *in vivo*. A technical limitation of recent approaches is that MMPs are often expressed as isolated catalytic domains without their substrate-recognizing hemopexin domains. These catalytic domains have been proven to be capable of cleaving hundreds of substrates *in vitro*, including nearly all of the components of the ECM.

MMPs in bone modelling and remodelling

Bone is an important site of ongoing tissue remodelling during development, homeostasis and repair. Bone develops according to one of three distinct processes: intramembranous ossification, endochondral ossification (FIG. 3a) or pseudo-metamorphic ossification. The clavicle and some skull bones develop by intramembranous ossification, in which mesenchymal precursors differentiate directly into osteoblasts. The appendicular and axial skeleton, including the long bones, develops by endochondral ossification, whereby a cartilage template forms first and is then resorbed and replaced by mineralized bone. Cartilage cells, or chondrocytes, differentiate and are replaced at the growth plate, where they follow a stereotyped progression of proliferation, differentiation, hypertrophy, angiogenic invasion and apoptosis. In pseudo-metamorphic ossification, a cartilage template functions as a temporary

mould to shape the deposition of bone. Even after bones have formed, they are continually remodelled throughout life.

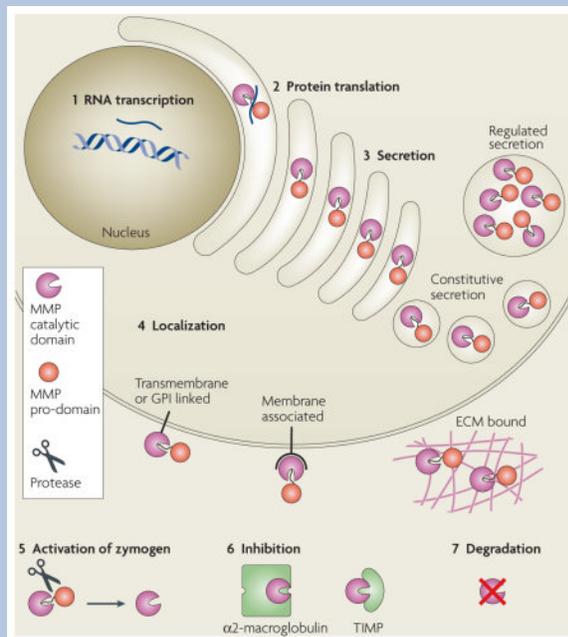
Loss of MMP9 is associated with growth-plate defects

The first developmental phenotype reported in an MMP-knockout mouse was a defect in endochondral ossification of long bones³⁶. Deletion of *Mmp9* results in expansion of the zone of hypertrophic chondrocytes in the growth plate³⁶ because of a failure of apoptosis (FIG. 3b). A relevant MMP9 substrate seems to be galectin-3, a lectin with anti-apoptotic activity that can be secreted and localized to the ECM. MMP9 cleaves and inactivates galectin-3. Indeed, galectin-3-mutant mice display growth-plate defects that are opposite of the defects of *Mmp9*-deficient mice, with premature apoptosis of the hypertrophic chondrocytes³⁷. Uncleaved galectin-3 is also enriched specifically at the growth plate in *Mmp9* mutants. Uncleaved galectin-3, added exogenously to wild-type bone explants, causes an expansion of the growth plates that is similar to the phenotype of the *Mmp9* mutants, whereas cleaved galectin-3 has no effect³⁸.

MMP9 is also highly expressed during bone healing after fracture, and bone fractures in *Mmp9*-mutant mice heal more slowly than in controls. Exogenous vascular endothelial growth factor (VEGF) rescues the repair defects, indicating that MMP9 processing of VEGF might be rate limiting in bone repair³⁹. Intriguingly, the *Mmp9*-null animals heal from stabilized fractures through endochondral ossification rather than through intramembranous ossification as in wild type³⁹, indicating that a different mechanism is used to heal the fracture in the absence of MMP9.

Box 1

Regulation of extracellular proteolysis



It is easier to assay for the presence or absence of matrix metalloproteinase (MMP) RNA transcripts than for protein abundance or enzyme-activity levels. However, MMP function can be regulated at many levels. In addition to (1) RNA transcription and (2) protein synthesis, MMP function can be regulated at the levels of (3) secretion, intracellular trafficking, (4) subcellular or extracellular localization, (5) activation of the zymogen form,

(6) expression of their endogenous protein inhibitors, such as tissue inhibitors of metalloproteinases (TIMPs) and α 2-macroglobulin¹¹⁸, and (7) protease degradation. Also, substrate availability and accessibility determine the degree to which MMP activity is used. This regulatory complexity has made it especially difficult to infer the spatial and temporal details of MMP activity from RNA-expression patterns. For details on the regulation of MMP activity, see REFS^{2,16,119} and references therein. For a specific example of the regulation of MMP13 activity, see REF.¹²⁰ ECM, extracellular matrix; GPI, glycosylphosphatidylinositol.

MMP13 functions in bone formation and remodelling

Mmp13 mutants show an expansion of the zone of hypertrophic chondrocytes and a delay in apoptosis, indicating that MMP13 is required for the transition from cartilage to bone at the growth plates of long bones^{7,40} (FIG. 3b). MMP13 cleaves type II collagen and aggrecan *in vivo*, and these cleavage products can be recognized in a tight zone that is just distal to the zone of ossification and angiogenesis. The primary defect in *Mmp13* mutants is a failure of chondrocytes to remodel the ECM that is rich in type II collagen and aggrecan. Collagen cleavage does not occur in the absence of MMP13, whereas both MMP13 and MMP9 cleave aggrecan. The cleavage product of aggrecan is absent in the *Mmp9 Mmp13* double mutant⁷. It is worth noting that there are no phenotypic consequences of replacing endogenous aggrecan with aggrecan that is resistant to MMP cleavage, indicating that other mechanisms exist for the removal of aggrecan at the cartilage–bone transition⁴¹.

The second function of MMP13 in long-bone development occurs during ossification. The cartilage ECM becomes the scaffold for mineralization, forming bone spicules or trabeculae. *Mmp13* mutants exhibit irregularly shaped spicules, demonstrating that MMP13 is required for their initial modelling⁷. MMP13 also functions in the continual remodelling of spicules; *Mmp13* mutants have an abnormal increase in trabecular bone mass that persists into adulthood. Conditional *Mmp13* mutants in bone or cartilage show that the regulation of hypertrophic chondrocyte apoptosis by MMP13 and the initial MMP13-mediated spicule-modelling function reside in chondrocytes. The MMP13-mediated remodelling function that regulates bone mass resides in osteoblasts. Therefore, MMP13 functions in bone formation, both at the growth plate and at the spicules, and in bone remodelling.

MMP14 deficiency results in lethality

MMP14 has various roles in skeletal development. *Mmp14* knockouts are the only lethal MMP-mutant mice; the mice are normal at birth but develop multiple abnormalities and die by 3–12 weeks^{42,43}. *Mmp14* mutants are grossly defective in the remodelling of the connective tissue. Loss of an ECM-degrading enzyme would be expected to result in increased bone deposition; paradoxically, *Mmp14* mutants instead show secondary effects of increased bone resorption and defective secondary ossification centres^{42,43}. Osteogenic cells from *Mmp14*-mutant mice cannot degrade collagen and do not form bone when transplanted subcutaneously into host immunodeficient mice⁴². Taken together, the phenotypes of *Mmp14*-mutant mice strongly support the physiological relevance of the *in vitro* data, which show that collagen types I, II and III are substrates for MMP14 (REF.⁴⁴).

Some initial bone modelling is also dependent on MMP14. The pseudo-metamorphic form of bone development was discovered only after aberrant residual cartilaginous tissues were found in *Mmp14* mutants⁴⁵. In wild-type mice, some skull bones and the diaphysis of long bones are formed by osteoblasts that lay down mineralized bone in close apposition to pre-existing cartilage that functions as a mould and later undergoes apoptosis. These bones develop by the

replacement of a juvenile tissue with an adult tissue, a process comparable to metamorphosis. In the absence of MMP14, these cartilaginous templates persist.

Mmp2- Mmp14-null mice die at birth⁸. These double mutants resemble much older *Mmp14* single mutants, indicating that MMP2 and MMP14 function redundantly. It is clear that MMP14 activates MMP2, because MMP2 activity is reduced, but not eliminated, in *Mmp14*-null mice^{43,46}. However, other activators also exist, probably including MMP15 and MMP16. Last, MMP14 has MMP2-independent roles in bone and connective-tissue remodelling and during angiogenesis^{8,43,46}.

Human MMP mutations lead to defects in bone development

Three human skeletal disease conditions are associated with loss-of-function MMP mutations. These diseases are a rare osteolytic syndrome, which is caused by *MMP2* mutations⁴⁷, the Missouri variant of spondyloepimetaphyseal dysplasia (SEMD), which is caused by point mutations in *MMP13* (REF. ⁴⁸), and the tooth enamel defect amelogenesis imperfecta, which is caused by splice-acceptor mutations in *MMP20* (REF. ⁴⁹).

Although the *Mmp2*-knockout mouse has only a subtle skeletal defect⁵⁰, human *MMP2* mutants have an osteolytic (vanishing bone) syndrome, which involves progressive destruction and resorption of bone, as well as dwarfism and arthritis⁴⁷. The defects of this syndrome are similar to the phenotype of *Mmp14*-mutant mice. As with the *Mmp14* mice, the paradoxical observation is that a loss-of-function mutation in an ECM-degrading protease causes, rather than inhibits, bone degradation. This phenotype correlates with an excessive number of osteoclasts, which might compensate for the primary failure of another type of ECM degradation.

The similarity between the human disease caused by *MMP2* mutation and the *Mmp14*-knockout mice is particularly interesting in light of the biochemical interaction between these two proteases. Membrane-bound MMP14 activates secreted MMP2 by cleaving its pro-domain^{51,52}. Indeed, active MMP2 is significantly decreased in *Mmp14*-null mice, strongly indicating that MMP2 is an *in vivo* substrate of MMP14 (REF. ⁴³). Therefore, it is clear that MMPs have many important roles in bone development.

MMP2 and MMP3 in mammary development

The epithelial ductal network of the mammary gland expands greatly during puberty in response to both local and systemic signals⁵³ (FIG. 4a). This process requires degradation of the basement membrane and ECM, restructuring of the endogenous vascular network and large-scale epithelial morphogenesis — all these processes suggest MMP involvement.

Combined pharmacological and genetic analysis of MMP2 and MMP3 revealed a role for MMP activity during mammary development⁵⁴. Branching morphogenesis in the mammary gland occurs by two spatially and temporally distinct mechanisms: terminal end bud (TEB) elongation and secondary branching. The length of the mammary ductal network is established through the invasion of TEBs through the mammary fat pad. Secondary branches initiate laterally from the main ducts. *Mmp2*- and *Mmp3*-null mammary glands have reciprocal phenotypes in branching morphogenesis. *Mmp2*-null mammary glands are deficient in TEB invasion, but have an excess of secondary branches, whereas *Mmp3*-null mammary glands have normal invasion, but are characterized by deficient secondary branching⁵⁴ (FIG. 4b). Inhibition of MMP function with a chemical inhibitor (GM6001) results in deficient primary invasion and an excess of secondary branches. Overexpression of the endogenous MMP inhibitor TIMP1 limits TEB invasion, but has a minor effect on secondary branching⁵⁴. These

observations led to a model in which MMP2 promotes ductal invasion and inhibits secondary branching, whereas MMP3 promotes secondary-branch initiation.

Box 2

Biological consequences of MMP proteolysis

Converting structural matrix proteins to signalling molecules*

- Collagen II: fragment is a bone morphogenetic protein (BMP) antagonist^{125,126}
- Collagen IV $\alpha 1$ – $\alpha 6$ noncollagenous-1 (NC1) domains are anti-angiogenic after cleavage^{121,124,127}
- Collagen 18 has an NC1 domain (endostatin), which is anti-angiogenic^{121,128}

Structural changes to matrix proteins

- Cleavage of perlecan¹²⁹
- Cleavage of laminin-5 (332)¹⁸ and laminin-10 (511)¹³⁰
- Procollagen to collagen processing for assembly^{131,132}

Changes in tissue architecture

- Cleavage of E-cadherin^{29,84}
- Cleavage of desmogleins¹³³
- Epithelial–mesenchymal transition (EMT)^{134–138}
- Invasion and metastasis^{29,138,139}
- Vasoconstriction¹⁴⁰

Chemoattraction

- Increase in chemokine activity following cleavage: interleukin-8 (IL8, also known as CXC-motif ligand-8 (CXCL8); the mouse LIX)^{93,94}
- Decrease in chemokine activity following cleavage: monocyte chemoattractant protein-1 (MCP1, also known as CCL2 or JE)^{92,99–101}
- Change in chemotaxis: gradients form by shedding of syndecan^{32,141}

Proliferation

- Epidermal-growth-factor receptor (EGFR) ligands need to be processed to function^{28,142–144}

Cell survival

- Neuronal survival factor: stromal-cell derived factor-1 (SDF1, also known as CXCL12); HIV dementia¹⁴⁵
- E-cadherin and desmosomes^{84,133}

Activate latent signalling molecules

- Cleavage of insulin-growth-factor (IGF)-binding protein (IGFBP) to release active IGF²¹
- Latent transforming growth factor- β (TGF β) to active TGF β ³⁵
- Tumour necrosis factor- α (TNF α) shedding¹⁴⁶

- Shedding of Ninjurin A to signal release of cell adhesion⁷⁸

Change in the range of action of a signalling molecule

- Vascular endothelial growth factor (VEGF): change in the range of diffusion through the modulation of heparin binding⁶⁴
- Membrane versus soluble TNF α ¹⁴⁷

Differentiation

- *Mmp2 Mmp14* double-knockout animals are synthetic lethal and the defect is due to a failure in muscle differentiation⁸
- Adipocyte maturation requires the replacement of the surrounding interstitial matrix with a basement membrane^{148,149}

*Reviewed in REFS ^{121–124}.

MMPs in blood-vessel remodelling

Analysis of a broad range of MMP mutants shows that embryonic vascular development proceeds normally, yet defects are observed in both normal and pathological postnatal vascular remodelling and angiogenesis (TABLE 1). These data imply that MMPs might have a specific role in postnatal refinement, remodelling and neoangiogenesis, but not in the original construction of the embryonic vascular networks.

Mmp9-mutant mice show defects in angiogenesis at the growth plate of long bones³⁶. Similarly, *Mmp14*-mutant mice lack appropriate vascular invasion at secondary ossification centres, despite normal levels of VEGF and its receptor VEGFR2 (also known as FLK1) (REF. ⁴³). This lack of vascular growth is recapitulated in an experimentally induced corneal angiogenesis assay⁴³, which induces new blood-vessel growth in control mice. Blood-vessel growth is not observed in *Mmp14* mutants⁴³, whereas it is reduced but not eliminated in *Mmp2*-mutant animals⁵⁵. In a laser-induced injury model of retinal degeneration, neovascularization is reduced in single *Mmp2* and *Mmp9* mutants and is strongly reduced in the *Mmp2 Mmp9* double mutant, indicating that these MMPs function redundantly⁵⁶. *Mmp2 Mmp14* double mutants also have developmental vascular defects, including capillaries with extremely small lumens. These defective blood vessels are sufficient to support embryonic growth and development, but not postnatal survival.

How do MMPs contribute to vascular remodelling? Likely mechanisms include proteolysis of type I collagen, modification of platelet-derived growth factor (PDGF) signalling, regulation of perivascular cells and processing of VEGF. Invading blood vessels in postnatal tissues encounter ECM that is rich in type I collagen, a protein that is not highly expressed in embryos. When control aortic explants are embedded in three-dimensional collagen matrices⁵⁷, they sprout neovessels and the endothelial cells invade the collagen matrix in a growth-factor-dependent manner. Strikingly, *Mmp14*-null explants do not sprout neovessels or invade the collagen matrix, whereas explants from *Mmp2*- and *Mmp9*-null mice are indistinguishable from control explants. However, when *Mmp14*-null explants are embedded in a fibrin matrix (similar to the provisional ECM at a wound site) they form capillaries, demonstrating matrix specificity. So, MMP14 contributes to postnatal vascular development by cleaving type I collagen; the relative absence of type I collagen in the embryo might partially explain the lack of embryonic vascular defects.

MMP9 and MMP14 have another effect on the vasculature: the perivascular (or smooth muscle) cells that ensheath the endothelial cells of the blood vessels are missing or their density is

significantly decreased in normal vessels and during tumour neoangiogenesis^{57–59}. This defect is especially evident in the smaller arterioles of the brain, and many of the remaining perivascular cells have irregular morphology. This phenotype is similar to that of mice that carry weak PDGF-B alleles. Signalling that occurs downstream of PDGF is attenuated in *Mmp14*-null mice, and PDGF receptor- β (PDGFR β) co-immunoprecipitates with MMP14, demonstrating that they form a physical complex⁶⁰. These data indicate a novel and direct function for MMP14 in PDGF signalling.

MMPs and VEGF signaling

MMP processing of VEGF might have an important role in physiological and tumour angiogenesis. VEGF is stored extracellularly: after secretion, VEGF binds to the ECM, from where it must be released to initiate angiogenesis⁶¹. Small insulinomas form constitutively in the RIP1-Tag (rat insulin promoter 1-T-antigen) mouse pancreatic islet cancer model, but only 1–2% of these develop into angiogenic adenomas and carcinomas⁶². Despite continuous expression of VEGF and its receptor, VEGFR2, VEGF availability is limited and cannot bind to its receptor in pre-angiogenic tumours. MMP9 mobilizes VEGF and initiates angiogenesis. Importantly, when the *Mmp9* mutation is crossed into the RIP1-Tag background, fewer tumours become angiogenic, supporting the role of MMP9 in mobilizing VEGF⁶³.

MMPs can also cleave VEGF, separating the matrix-binding domain from the receptor-binding domain. Uncleaved VEGF is enriched in at least one MMP9-mutant postnatal tissue, the hypertrophic chondrocyte zone³⁸, which indicates that VEGF is an important downstream effector, and possibly a substrate, of MMP9. Truncated VEGF has different effects on tumour blood vessels than does uncleavable VEGF; truncated VEGF increases vessel diameter, whereas uncleavable VEGF increases vessel sprouting⁶⁴. *Mmp9*-null mice display defective post-embryonic neovasculature, suggesting that in wild-type mice, postnatal blood vessels respond differently to cleaved and uncleaved forms of VEGF. Perhaps the sprouting-initiation function of uncleaved VEGF is the only VEGF function required in embryos; however, this function of VEGF is unaffected in *Mmp9*-mutant embryos.

MMPs regulate tissue remodelling in flies

The results from mouse mutants indicate that during development individual MMPs are required specifically for tissue-remodelling events — bone remodelling, mammary development and vascular remodelling. Although the mouse double mutants that have been examined survive to birth^{7,8,56}, it is not feasible to knockout all the MMPs in a mouse to test the general role of the MMP family. MMP expression and inhibition have been examined in many other model systems, including *Xenopus laevis*^{65,66}, hydra⁶⁷, zebrafish^{68–70} and *Caenorhabditis elegans*^{71,72}; in *C. elegans*, genetic analysis indicates that a worm MMP contributes to a developmentally regulated cell-invasion event⁷¹. The fruitfly *D. melanogaster* has fewer MMPs than any other model system studied, with only two MMPs^{73,74}, and mutants have been generated that lack both genes⁷⁵. *D. melanogaster* MMP double mutants eliminate concerns about redundancy and compensation.

The *D. melanogaster* MMP genes, *DmMmp1* and *DmMmp2*, maintain the conserved MMP domain structure, and *DmMmp2* also encodes a predicted GPI membrane anchor^{73,74} (FIG. 1). Although neither fly gene has a single orthologue among the mammalian MMPs (despite the nomenclature), fly TIMP can inhibit mammalian MMPs⁷⁶ and mammalian TIMPs can inhibit fly MMPs⁷⁴, indicating conservation between the systems. Double mutants made from null alleles can complete embryonic development and can progress partway through the larval stages, a time of tremendous growth. Even when the maternal contribution of both MMP genes is removed from the double-mutant embryo, MMPs are not required for viability or morphogenesis until the middle of larval development⁷⁵. Therefore, fly experiments confirm

and extend the mammalian MMP-mutant results, which indicate that the MMP family specializes in remodelling, rather than in the regulation of the initial cell and tissue migrations that are required during embryogenesis. It is worth noting, however, that morpholino-inhibition experiments indicate that MMPs are essential for embryonic axis formation in zebrafish⁷⁰.

Loss of *DmMmp1* results in defects in tracheal remodeling

What are the fundamental roles of MMPs if an organism that lacks MMPs can complete embryo genesis? Although the single and double fly mutants can grow to the larval stages, their phenotypes indicate that even in insects, MMPs participate in tissue remodelling. *DmMmp1* mutants are unable to remodel the breathing tubes known as tracheae. The tracheae are a system of branched interconnected tubes that allow the diffusion of oxygen to all internal tissues; during normal larval growth, tracheal tubes grow 14-fold in length and 7–40-fold in diameter⁷⁷. In *DmMmp1* mutants, as the larva grows, the tracheal tubes cannot grow concomitantly, they stretch inappropriately, and within a few days, they break from the stretching tension (FIG. 5a). These tubes also cannot dilate properly, and instead display inappropriate constrictions. At a cellular level, it seems that the epithelial cells that comprise the tubes cannot release their attachments from the ECM that lines the tubes⁷⁵.

Recently, a new signalling protein that is likely to participate in the normal remodelling of the tracheal tubes was identified⁷⁸. This signalling protein, Ninjurin A, physically associates with DmMMP1 and colocalizes with it at tracheal cell surfaces. In cell culture, the Ninjurin A ectodomain is shed by DmMMP1 protein, and the liberated ectodomain signals cells to release adhesion. It is likely that in larvae, DmMMP1 releases the Ninjurin A ectodomain, which signals a release of cell adhesion from the tracheal cuticle⁷⁸. The Ninjurin-family proteins were first identified because of their upregulation in injured rat neurons. Therefore, it seems that this new MMP substrate, the ectodomain of which functions as a previously unsuspected cell–cell signal, is conserved between insects and mammals.

DmMmp2 is required for tissue histolysis

The requirement for *DmMmp2* in *D. melanogaster* is reminiscent of the original MMP identification in metamorphic tadpole-tail histolysis. Like frogs, insect metamorphosis requires extensive tissue histolysis, and this histolysis requires an MMP: in the *DmMmp2* mutants, tissues that are normally slated for histolysis persist inappropriately. *DmMmp2* is also required for the fusion of two epithelial sheets that meet at the midline of the fly during metamorphosis, as the mutants display cleft thoraxes⁷⁵ (FIG. 5b). Further work on identifying the cellular defect in these mutants might shed light on the roles of MMPs in other epithelial fusion events, such as palate formation, which goes awry in cleft palate birth defects.

That embryonic development can be successfully completed without MMP function does not preclude the possibility that MMPs normally participate in embryogenesis. Further analysis might elucidate specific MMP functions that are non-essential or that might be obscured by adaptive development. For example, DmMMP2 is expressed in the central nervous system during development, and during motor-neuron outgrowth, *DmMmp2*-mutant axons inappropriately lose adhesion to one another. These data indicate that MMPs cleave a substrate that is important for axon guidance (A.P.-M. and H. Broihier, unpublished results).

Whereas the study of mammalian MMPs began many decades ago, the study of fly MMPs is still new and the topics of research are still expanding. Recent studies in flies have focused on the role of fly MMPs in tumour metastasis, and *DmMmp1* was found to contribute significantly to the invasiveness of tumours^{79,80}. Additionally, the fruitfly offers opportunities for forward genetic screening as a method of identifying new substrates and pathways, methods not available in mammalian systems. Yet even in these early days, fly experiments have genetically

identified a limited role for the MMP family in embryogenesis, they have highlighted a crucial role for MMPs in tissue remodelling and they have uncovered a new conserved substrate, the Ninjurin signalling protein.

MMPs in inflammation and wounding

The role of MMPs is not limited to developmental processes, and mouse-mutant experiments are beginning to show that MMPs are also required to maintain homeostasis in response to environmental challenges, such as wounding and infection. This topic has been reviewed recently⁸¹, but here we will discuss a few of the most important findings.

MMP7 is involved in innate immunity and wound healing

MMP7 (or matrilysin) has no known role in development and is not expressed at high levels in embryonic development. In healthy adult tissue, MMP7 is expressed only in mucosal epithelia and it is upregulated and proteolytically activated in response to bacterial exposure in epithelia⁸². Consistent with this expression pattern, *Mmp7*-mutant mice are more easily infected with intestinal bacteria. This susceptibility is partly caused by the inability of *Mmp7* mutants to proteolytically activate an endogenous antibiotic peptide, pro-cryptdin. *In vivo*, the precursor, but not the mature forms, of cryptdin is found in samples of intestinal epithelia from *Mmp7*-mutant mice⁸³. These experiments firmly establish a role for MMP7 in innate immunity.

MMP7 is also required for wound healing. Epithelial cells at the edges of wounded tracheal explants migrate towards each other and fuse. MMP7 seems to mediate wound-induced epithelial migration by cleaving E-cadherin⁸⁴. MMP7 colocalizes with E-cadherin and cleaves its extracellular domain in wounded epithelia, which results in a loosening of cell-cell attachments. In wounded *Mmp7*-mutant mice, epithelial cells do not migrate and E-cadherin cleavage does not occur⁸⁵. MMP3 also functions in epidermal wound healing, as skin wounds of *Mmp3*-mutant mice heal more slowly than those of control mice, owing to a deficit in actin purse-string formation⁸⁶.

Pro-inflammatory and anti-inflammatory MMP functions

Both injury and infection induce inflammation, another physiological response to environmental challenge that requires MMPs. Lung epithelium controls the recruitment of inflammatory cells to alveoli, through the production of chemoattractants, following injury, infection or exposure to allergens or chemicals. Injury induces leukocytes to migrate across the epithelium into the alveolar air spaces in response to a chemoattractant gradient. The transmembrane heparan sulphate proteoglycan syndecan-1 sequesters many chemokines. The formation of the chemoattractant gradient requires MMP-mediated shedding of the syndecan-1 ectodomain. Subsequent chemokine release results in the migration of leukocytes into the air spaces and epithelial repair. In the absence of MMP7, neutrophils remain stuck outside the epithelia in injured lungs, and subsequent inflammation-mediated lung damage is attenuated³². Neutrophil migration is defective in *Mmp7* mutants owing to the absence of the neutrophil attractant CXC-motif ligand-1 (CXCL1; also known as keratinocyte-derived chemokine (KC); or GRO α in humans) in the fluid of the alveolar lumens. In summary, injury promotes MMP7 expression, MMP7 cleaves syndecan-1 and syndecan-1 shedding releases CXCL1, which recruits neutrophils.

MMPs can be both pro-inflammatory and anti-inflammatory. MMPs facilitate inflammatory cell recruitment^{87,88} and clearance of inflammatory cells^{89–91} by cleaving inflammatory mediators, resulting in a tightly regulated inflammatory response⁹². Several chemokines, including C–C motif ligand-7 (CCL7) and CXCL12 (REF. ⁹²), are substrates for MMP2. MMP9 cleaves and activates CXCL6 and CXCL8 (also known as interleukin-8 (IL8)), whereas

it inactivates CXCL1 and CXCL4 (REFS^{93,94}). In asthma models, eosinophils from *Mmp2*- or *Mmp9*-deficient mice fail to migrate into the airways and accumulate in the interstitium, thereby predisposing the animals to asphyxiation^{95,96}. MMP2 and MMP9 participate in a regulatory loop that dampens allergic inflammation. The interstitial eosinophil accumulation might be explained by the disruption of transepithelial chemokine gradients, which affect CCL7 (also known as MCP3 or MARC), CCL11 (also known as eotaxin) and CCL17 (also known as TARC). MMP2 and MMP9 also process S100A8 and S100A9, two neutrophil- and macrophage-specific chemoattractant proteins that are found in the bronchioalveolar fluid of asthmatic mice⁹⁷. These MMPs establish the chemotactic gradient that is required for clearance of lung inflammatory cells and the prevention of lethal asphyxiation. In a mouse model of the autoimmune skin blistering disease, bullous pemphigoid, *Mmp9*-null mice have decreased recruitment of neutrophils and fail to develop epidermal blistering. This defect is caused by a lack of inactivation of $\alpha 1$ -proteinase inhibitor by MMP9, which does not allow neutrophil elastase to function⁹⁸.

Truncation of macrophage-derived CCL7 by MMP2 and MMP14 results in the formation of peptides that can bind to the CC chemokine receptor and function as antagonists^{99–101}. Limited N-terminal proteolytic processing of CXCL8 and the CXC chemokine LIX (the mouse equivalent of CXCL5 and CXCL6) by MMP9 and MMP8, respectively, results in the generation of chemoattractants that are more potent than the full-length molecules^{102,103}. Accordingly, *Mmp8*-null animals¹⁰⁴ are protected from tumour necrosis factor- α (TNF α)-induced lethal hepatitis because of impaired LIX release and impaired leukocyte influx into the liver¹⁰³. A tripeptide neutrophil chemo-attractant *N*-acetyl Pro-Gly-Pro, derived from the breakdown of ECM, shares sequence and structural homology with an important domain on α -chemokines and causes chemotaxis through CXCR2 (REF.¹⁰⁵).

MMPs in human inflammatory disease models

Most human pathologies have an inflammatory component, as do their mouse models. The same MMP can have different roles in different conditions. For example, MMP9 contributes to inflammation in mouse models of stroke, heart attack, Alzheimer's disease, some aspects of asthma and other lung inflammatory conditions, aortic aneurysms and autoimmune encephalomyelitis^{9,106–110}; however, it functions as an anti-inflammatory agent in models of inflammatory skin and kidney diseases^{89,111,112}. Current data indicate that MMP12 contributes to emphysema^{113,114}, whereas MMP3 and MMP9 contribute to skin inflammatory conditions^{89,115}. By contrast, MMP8 protects against skin inflammatory responses¹⁰⁴ and MMP2 protects against inflammation of the brain and spinal cord¹¹⁶. Collectively, these observations indicate that MMPs function non-redundantly to regulate inflammation, which is potentially of great medical significance. Indeed, tumour progression also triggers inflammation, and the clinical observations that MMPs are upregulated in cancer⁵ might reflect the roles of MMPs in triggering and controlling inflammation.

Conclusions and future directions

The MMP family of extracellular proteinases is present in most multicellular organisms, including plants and animals¹³. MMP function can be most simply analysed in *D. melanogaster*, which has only two MMP genes. The fly mutants demonstrate that MMPs are dispensable, both individually and together, for embryonic fly development, but are crucial for tissue growth and tissue-ECM remodelling in the larvae and during larval development. In mammals, the 24 MMP genes seem to share redundant functions. Analysis of single-MMP-mutant mice (TABLE 1) has identified developmental phenotypes in postnatal mammary, skeletal and circulatory development, three prominent sites of postnatal tissue and ECM remodelling. A particularly striking aspect of these phenotypes is that MMPs are not required

to build blood vessels or bones in the embryo, but are required for their postnatal development and tissue remodelling. Examination of mouse mutants also reveals an important function of MMPs in regulating tissue response to environmental challenges, such as wounding, infection and inflammation. The genetic analysis from humans and model systems argues for a crucial role for MMPs as active regulators of post-natal tissue development, tissue remodelling and tissue repair in response to injury, infection or disease.

Current efforts to delete the remaining mouse MMPs and continuing analyses of existing mutants will reveal how MMPs are deployed in development and disease. Further studies with compound knockouts might yield lethal phenotypes, thereby revealing essential roles in development. Complementing the analysis of compound mouse mutants will be experiments that use pharmacological manipulations of both individual and classes of MMPs, as well as analyses in other genetically tractable model systems. Biochemical challenges include the visualization of MMP activity in living cells and tissues, the development of non-cleavable substrates and the determination of the complete *in vitro* MMP degradome¹¹⁷. An important question is the extent to which MMPs have a structural role in remodelling tissues versus a role in regulating access to signalling molecules.

Another challenge is to determine the relative importance of extracellular proteolysis in general, and MMP-mediated proteolysis in particular, as a post-translational regulatory mechanism in development and disease. Which stages in normal development, which cellular processes and which signalling pathways are strongly influenced by MMPs? A key to answering these questions will be to increase our understanding of the relevant *in vivo* substrates for specific MMPs. The challenge is to link our *in vitro* understanding of potential functions of MMPs with an *in vivo* understanding of how MMPs function in a given cellular process. Joining together biochemical approaches and MMP genetics with mutant substrates should make it possible to make progress on this important problem.

Conceptually, one of the main advances in MMP biology has been the realization that extracellular proteolysis need not simply be a mechanism of destroying structure or information. Instead, diverse studies demonstrate that MMPs can release growth factors from the ECM and cell surface, activating latent proteins and generating new bioactive molecules through proteolysis (BOX 2). We view extracellular proteolysis as another form of post-translational modification. From this perspective, proteolysis can function to destroy a protein, but it can equally provide a mechanism to grant cells conditional access to a particular signalling molecule. Significantly, the products of MMP cleavage can function locally (for example, cleavage of E-cadherin) or at a long distance from the site of proteolysis (for example, chemokine activation). Given that many MMP phenotypes have been observed in the remodelling of tissues that do not require MMPs for their initial formation, it is tempting to speculate that MMPs might function specifically as regulators of post-embryonic cell motility and tissue architecture.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

Fibrillar collagen

Polymerized, supramolecular collagen that has been organized into fibrils

collagen types I

II and III form fibrils

Extracellular matrix

Complex, ordered mixture of structural and signalling molecules that surrounds cells

Enzymatic redundancy

Two enzymes that are expressed in the same time and place that can fully substitute for each other's essential functions

Enzymatic compensation

Upregulation of an enzyme, which is normally not expressed (or is expressed at a low level) to substitute for the absence of a mutated enzyme

Adaptive development

Alternative developmental trajectory whereby an organism compensates for the loss of a gene by doing some essential function in a reproducibly different manner

Catalytic domain

A domain that cleaves other proteins

Pro-domain

An autoinhibitory domain that prevents the catalytic domain from functioning

Hemopexin domain

A four-bladed β -propeller domain that mediates protein-protein interactions

Tissue inhibitor of metalloproteinases

(TIMPs). Endogenous protein inhibitors of matrix metalloproteinase function

Intramembranous ossification

Direct differentiation of mesenchymal precursors into osteoblasts, as in the clavicle and in some skull bones

Endochondral ossification

Bone development in which a cartilage template forms first and is then replaced by mineralized bone in the growth plate, as in the long bones

Pseudo-metamorphic ossification

Bone development in which a cartilage template functions as a temporary mould to shape the deposition of bone, as in the mandible

Osteoblast

A cell that secretes unmineralized type-I-collagen-rich extracellular matrix and builds bone

Growth plate

The region of developing appendicular and axial skeleton that is responsible for growth in length of bones

Aggrecan

The main chondroitin sulphate proteoglycan in cartilage

Trabeculae

A fine network of bony spicules in trabecular bone

Osteoclast

A cell that resorbs the mineralized matrix of the bone

Terminal end bud

A highly proliferative epithelial structure at the end of invading mammary ductal epithelium during puberty

Secondary branching

Also known as side branching. A process during mammary development whereby secondary ducts initiate laterally off of main ducts during puberty

Corneal angiogenesis assay

Stimulated neovascularization assay in which test substances are implanted in the cornea of a rabbit or mouse and the cornea is monitored for the development of new blood vessels

Perivascular cells

Also known as pericytes, mural cells or smooth muscle cells. These cells are tightly associated with endothelial cells and aid in the function and regulation of vascular networks

Endothelial cells

Cells that constitute the lining of the blood vessels

MMP degradome

The complete list of matrix metalloproteinase proteolytic substrates

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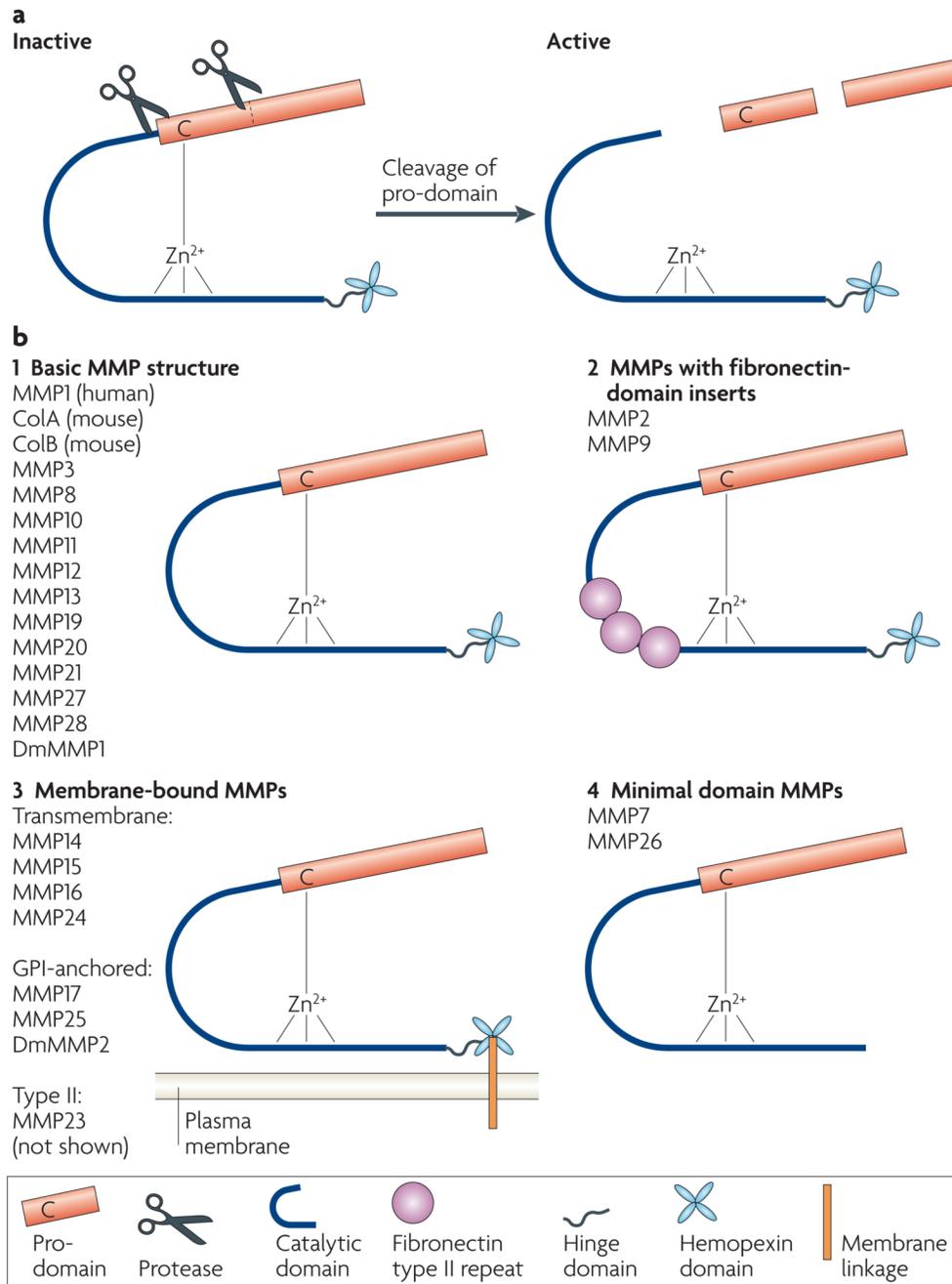


Figure 1. Schematic structure of MMPs

a | Matrix metalloproteinases (MMPs) are expressed as pro-proteins. A conserved Cys residue in the pro-domain coordinates the zinc ion, which would otherwise be used for catalysis. The pro-domain is removed by a combination of a cleavage in the domain and a cleavage between the pro-domain and the catalytic domain. **b** | Most MMPs share a conserved domain structure of pro-domain, catalytic domain, hinge region and hemopexin domain (**1**). All MMPs are synthesized with a signal peptide, which is cleaved during transport through the secretory pathway. MMP2 and MMP9 have three fibronectin type II repeats in their catalytic domains (**2**). Membrane type MMPs (MT-MMPs) are linked to the plasma membrane either by a transmembrane domain or by a glycosylphosphatidylinositol (GPI) linkage, attached to the

hemopexin domain (3). Minimal MMPs lack the hinge and hemopexin domains (4). MMP21 has a truncated hinge domain. *Drosophila melanogaster* DmMMP2 has an insertion of 214 amino acids into its hinge domain. MMP23 (not shown) has a non-conserved N-terminal domain that consists of an immunoglobulin IgC2 domain and a ShKT domain; it is unclear if MMP23 contains a Cys residue switch.

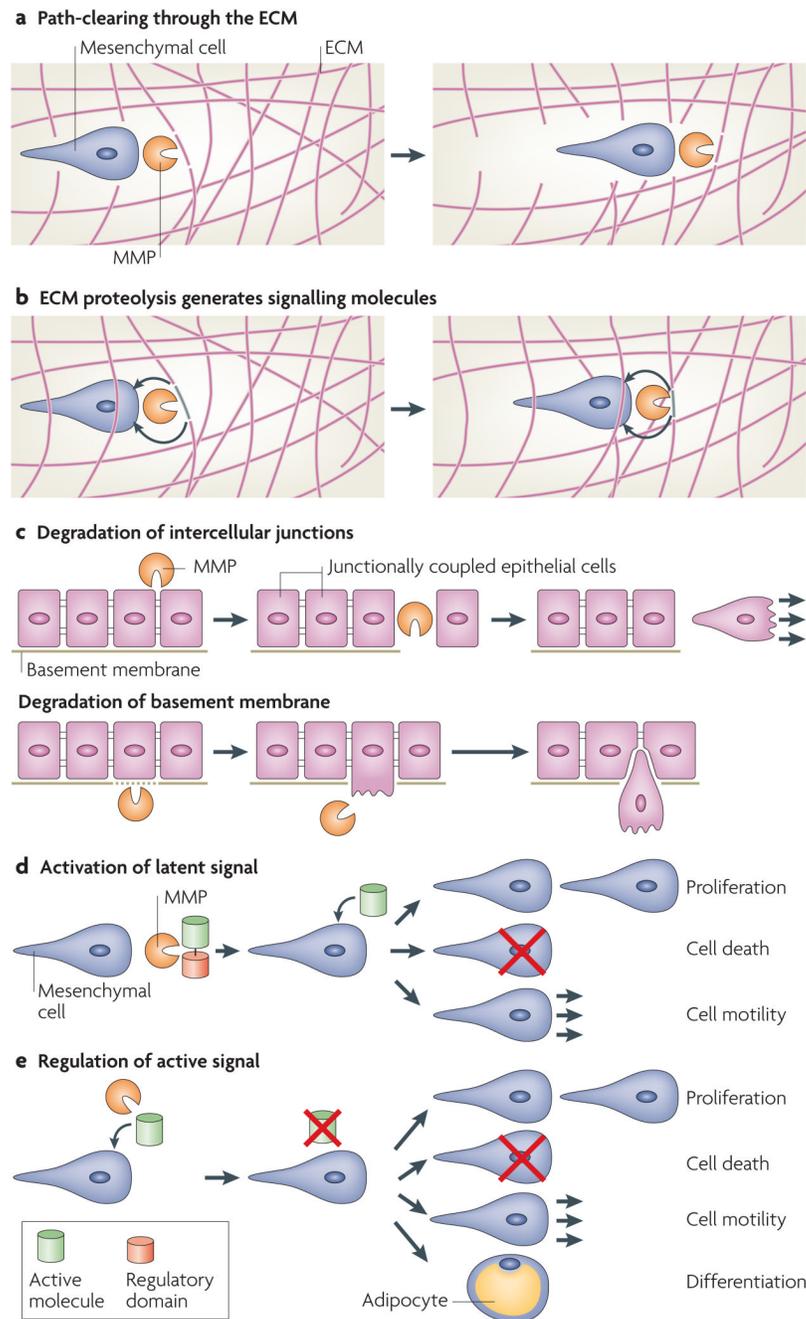


Figure 2. Possible modes of MMP action

Recent work has dramatically expanded our understanding of the possible substrates that can be subject to matrix metalloproteinase (MMP) cleavage, and has similarly expanded our awareness of the means by which MMPs can affect cell behaviour. **a** | MMPs can cleave components of the extracellular matrix (ECM), resulting in increased space for cell or tissue movement. **b** | Alternatively, MMP proteolysis can generate specific cleavage products that then signal in an autocrine or paracrine manner (for example, cleavage of collagen IV $\alpha 3$ chain by MMP9 yields tumstatin, an anti-angiogenic peptide that functions by binding to the $\alpha \nu \beta 3$ integrin¹²⁷). **c** | MMPs can also directly regulate epithelial tissue architecture through cleavage of intercellular junctions or the basement membrane. **d** | MMPs can activate or modify the

action of latent signalling molecules, resulting in diverse cellular consequences. For example, cleavage of vascular endothelial growth factor (VEGF) by MMPs changes angiogenic outcome by modifying the binding and diffusion properties of VEGF⁶⁴. e | MMPs can deactivate or modify the action of active signalling molecules, resulting in changes in proliferation, cell death, differentiation or cell motility. For example, MMP2 cleavage of stromal-cell derived factor-1 (SDF1, also known as CXC-motif ligand-12 (CXCL12)) results in its inactivation¹⁰⁰.

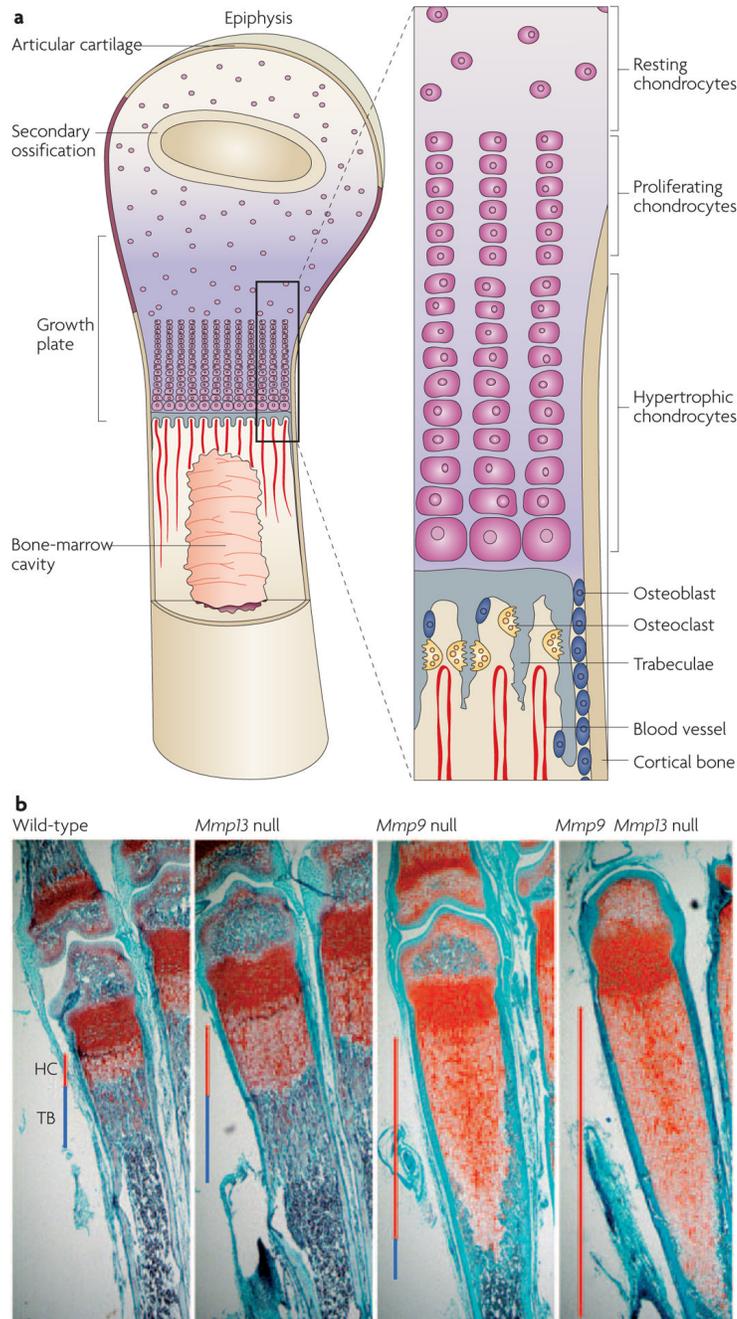


Figure 3. Skeletal phenotypes of MMP mutants

a | Long bones in mice and humans develop through the process of endochondral ossification, in which a cartilage template forms first and then is resorbed and replaced by mineralized bone. This process requires extensive matrix remodelling and invasion of new blood vessels. The schematic is adapted from REF. ¹⁵⁰ © (2000) Elsevier, and REF. ¹⁵¹ © (1999) Macmillan Magazines Ltd. **b** | Matrix metalloproteinase-9 (*Mmp9*)- and *Mmp13*-null femurs display greatly expanded hypertrophic cartilage zones (HC; red line) and altered trabecular bone (TB; blue line). Despite this expansion, *Mmp9*- and *Mmp13*-null phenotypes eventually resolve, resulting in good bone formation. The *Mmp9 Mmp13* double mutant has an even greater expansion of hypertrophic cartilage, and significantly and persistently shorter long bones.

Images courtesy of D. Stickens, D. Behonick and N. Ortega, University of California, San Francisco, USA.

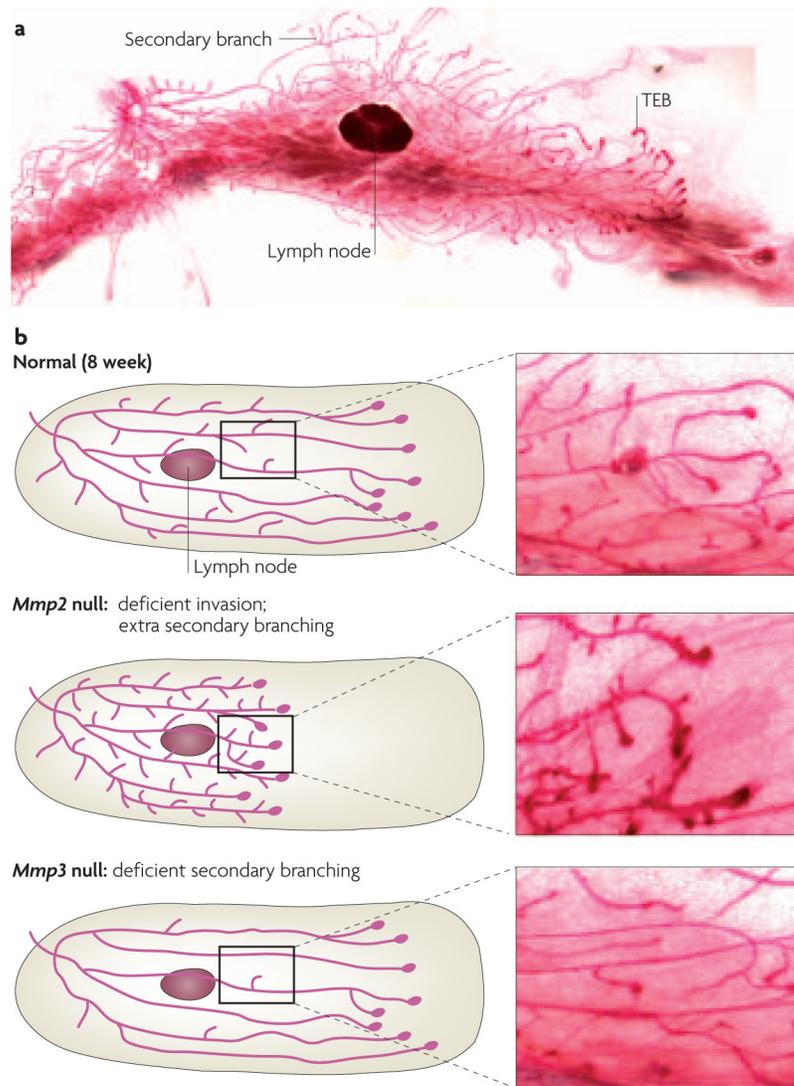


Figure 4. Mammary gland phenotypes of MMP mutants

a | The length of the ductal network of the mammary gland is formed through the invasion and bifurcation of terminal end buds (TEBs). New branches initiate off of these primary ducts, in a process known as secondary branching. **b** | *Mmp2*- and *Mmp3*-null animals have reciprocal phenotypes; *Mmp2*-null mammary glands have deficient primary invasion and excess secondary branching, whereas *Mmp3*-null mammary glands have normal invasion and deficient secondary branching. These findings show that different proteases are used to accomplish these tissue-invasion processes. Images courtesy of A.J.E. and J. Trumbull, University of California, San Francisco, USA.

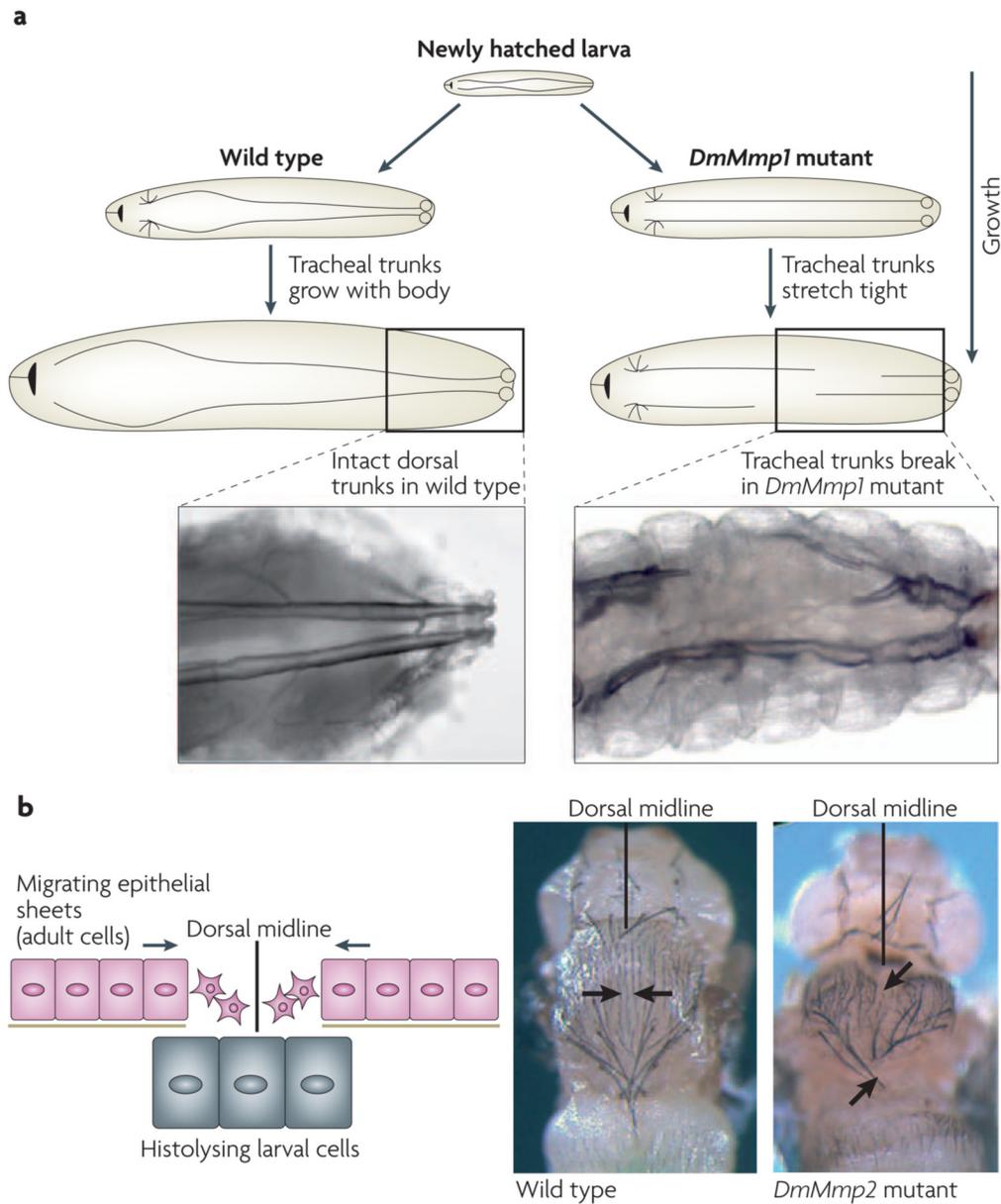


Figure 5. Phenotypes of *Drosophila melanogaster* *DmMmp1* and *DmMmp2* mutant flies
a | *Drosophila melanogaster* tracheal development requires the dorsal tracheal trunks to extend with the growth of the larva. In *DmMmp1*-mutant flies, the tracheal trunks stretch tight and finally break, resulting in larval lethality. **b** | During *D. melanogaster* metamorphosis, larval tissue is histolysed and adult dorsal epithelium migrates to the midline and fuses. In *DmMmp2* mutants, the midline closure of the epithelium fails. Images adapted from REF. ⁷⁵ © (2003) Elsevier.

Table 1

Selected MMP- and TIMP-null mutant phenotypes

MMP gene	Mutant phenotypes (mouse, unless noted)
<i>Mmp2</i>	Reduced body size ¹⁵² ; reduced neovascularization ⁵⁵ ; decreased primary ductal invasion in the mammary gland ⁵⁴ ; reduced lung saccular development ¹⁵³
<i>Mmp3</i>	Altered structure of neuromuscular junctions ¹⁵⁴ ; reduced purse stringing during wound healing ⁸⁶ ; altered secondary branching morphogenesis in the mammary gland ⁵⁴
<i>Mmp7</i>	Innate immunity defects ⁸³ ; decreased re-epithelialization after lung injury ⁸⁵
<i>Mmp8</i>	Increased skin tumours ¹⁰⁴ ; resistance to tumour necrosis factor (TNF)-induced lethal hepatitis ¹⁰³
<i>Mmp9</i>	Bone-development defects ³⁶ ; defective neuronal remyelination after nerve injury ¹⁵⁵ ; delayed healing of bone fractures ³⁹ ; impaired vascular remodelling ¹⁵⁶ ; impaired angiogenesis ³⁶
<i>Mmp10</i>	Increased inflammation and increased mortality in response to infection or wounding (W. C. Parks, personal communication)
<i>Mmp11</i>	Delayed mammary tumorigenesis ¹⁵⁷
<i>Mmp12</i>	Diminished recovery from spinal cord crush ¹⁵⁸ ; increased angiogenesis due to decreased angiostatin ¹²⁸
<i>Mmp13</i>	Bone remodelling defects ^{7, 40} ; reduced hepatic fibrosis ¹⁵⁹ ; increased collagen accumulation in atherosclerotic plaques ¹⁶⁰
<i>Mmp14</i>	Skeletal remodelling defects ^{42, 43, 45, 161} ; angiogenesis defects ⁴⁶ ; inhibition of tooth eruption and root elongation ¹⁶² ; defects in lung and submandibular gland ^{46, 163}
<i>Mmp19</i>	Obesity ¹⁶⁴
<i>Mmp20</i>	Defects in tooth enamel ¹⁶⁵
<i>Mmp23</i>	No phenotype reported
<i>Mmp24</i>	Abnormal response to sciatic nerve injury ¹⁶⁶
<i>Mmp28</i>	Increased inflammatory response (W. C. Parks, personal communication)
<i>DmMmp1</i>	Defective tracheal tube growth (see text) ⁷⁵ ; failure of head eversion at metamorphosis ⁷⁵
<i>DmMmp2</i>	Failure of tissue histolysis at metamorphosis ⁷⁵
<i>Timp1</i>	Accelerated endometrial gland formation ¹⁶⁷ ; impaired learning and memory ¹⁶⁸ ; accelerated hepatocyte cell-cycle progression ¹⁶⁹ ; increased resistance to <i>Pseudomonas</i> infection ¹⁰⁷
<i>Timp2</i>	Motor defects ¹⁷⁰
<i>Timp3</i>	Accelerated apoptosis in mammary glands ¹⁷¹ ; impaired bronchiole branching ¹⁷² ; enhanced metastatic dissemination ¹⁷³
<i>Timp4</i>	No phenotype reported ¹⁷⁴
<i>DmTimp</i>	Inflated wings ¹⁷⁵ ; autodigesting gut ¹⁷⁵

Dm, *Drosophila melanogaster*; *Mmp*, matrix metalloproteinase; *TIMP*, tissue inhibitor of metalloproteinase.