METALLOPROTEINASES: MEDIATORS OF PATHOLOGY AND REGENERATION IN THE CNS

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Abstract | The matrix metalloproteinases and related A disintegrin and metalloproteinase enzymes are implicated in various diseases of the nervous system. However, metalloproteinases are increasingly being recognized as having beneficial roles during nervous system development and following injury. This review discusses general principles that govern the expression of metalloproteinases in the nervous system and their detrimental outcomes. It then focuses on the roles of metalloproteinases and their mechanisms in regulating neurogenesis, myelin formation and axonal growth. It is clear that metalloproteinases are important determinants in enabling recovery from injury to the nervous system.

There has been an increase in research activity pertaining to metalloproteinases, which include the matrix metalloproteinases (MMPs) and A disintegrin and metalloproteinases (ADAMs), in the nervous system. Not only have these metalloproteinases now been convincingly implicated in a myriad of neurological conditions, they have also been associated with important neurophysiological functions, including synaptic remodelling and long-term potentiation (LTP). More recently, the demonstration that metalloproteinases impact on Nogo signalling, and regulate neural stem cell biology and remyelination suggests their importance in regeneration of the nervous system.

This review first introduces members of the metalloproteinase families and their biochemistry. This is followed by a description of the detrimental aspects of metalloproteinases in the CNS; this description is brief and encompasses only general principles given the many reviews that have already dealt with the subject. The article then focuses on the beneficial roles of metalloproteinases in the CNS during development and following injury, and discusses their functions and mechanisms in neurogenesis, axonal growth and myelin formation. Because this is a relatively new concept, the beneficial aspects of metalloproteinases are given extensive coverage. The determinants of metalloproteinases as either beneficial or detrimental are then explored. Finally, considerations of therapeutics that inhibit the excess metalloproteinase activity that occurs after CNS injury are discussed. Careful use of therapies that target metalloproteinases is advised in view of the increasing recognition of the beneficial functions of metalloproteinases in the CNS. It is hoped that this review will stimulate an expansion of research on the varied and novel roles of metalloproteinases in the nervous system.

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doi:10.1038/nrn1807
Published online
15 November 2005

Metalloproteinases and their properties

The metzincin metalloproteinases are those that bind a zinc ion in their active site using three histidine residues in the sequence motif HExxHxxGxxH (where x is any amino acid). In addition, there is a distinct β-turn at the active site, which is delineated by a methionine residue and is important for activity. There are four subgroups of metzincins: astacins, serralysins, MMPs and the adamalysins; the last comprise the snake venom metalloproteinases and ADAMs. There is ~20% homology between the metzincin subfamilies, although identity at the catalytic domain is much higher. This review addresses the MMPs and ADAM metalloproteinases.

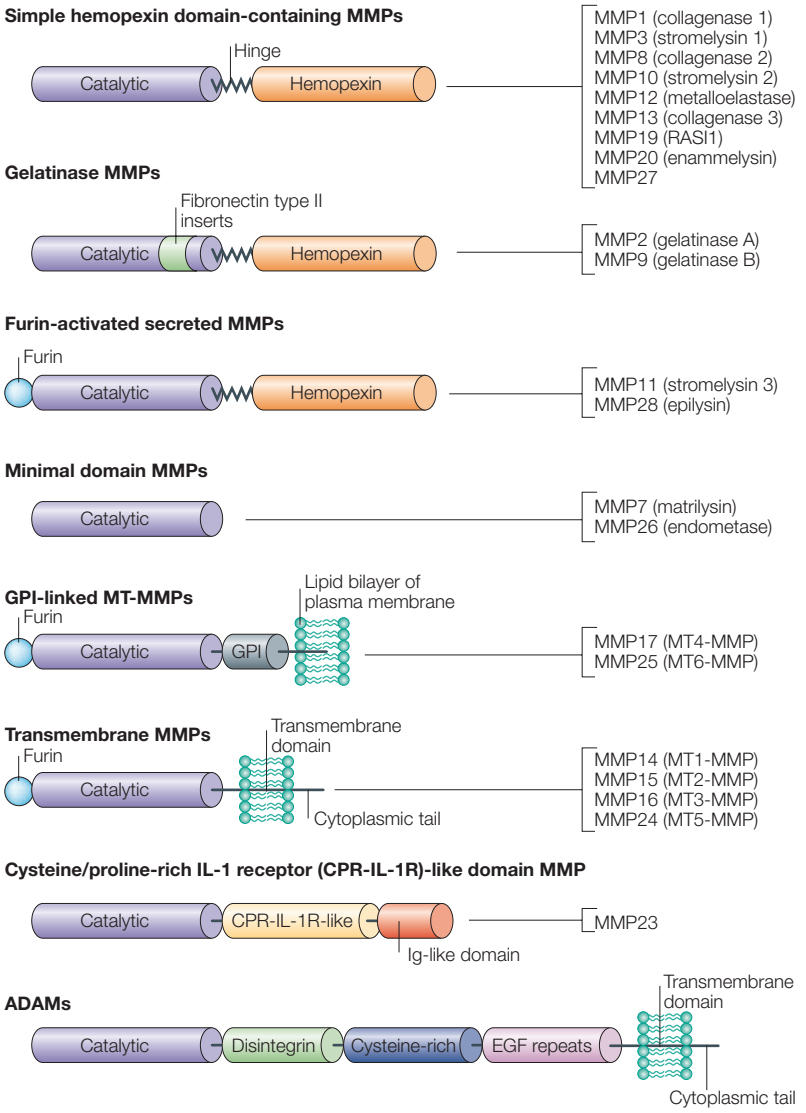

Figure 1 | **Structure of matrix metalloproteinases and A disintegrin and metalloproteinases.** The different domains of matrix metalloproteinases (MMPs) allow them to be subclassified into the categories depicted here (based on classifications listed in REF. 1). The figure lists both the MMP designation by numerals and the common names of the various MMPs (in parenthesis). The pre- and pro-domains that are found at the amino terminus adjacent to the catalytic region are not shown. The catalytic domain has also been referred as the metalloproteinase domain. There are various differences between members of each category, but these are not depicted. MMP21 (*Xenopus* MMP) is not included in this list. This has a vitronectin-like insert in the pro-domain, but is otherwise similar to the simple hemopexin domain MMPs, except that MMP21 lacks a hinge region. Molecular weights range from the smallest MMP (MMP7) of 28 kDa, to 92 kDa for human MMP9 in its pro-forms. A generic structure for A disintegrin and metalloproteinases (ADAMs) is shown, but there are several differences between members of this family[8,9,61]. EGF, epidermal growth factor; GPI, glycosyl phosphatidylinositol; Ig, immunoglobulin; IL-1, interleukin-1; MT, membrane type.

There are 24 mammalian MMP members (MMP1–28), each the product of a different gene. These can be subdivided on the basis of domain structure[1] (FIG. 1). The names MMP4–6 are no longer used, as the originally so-called members were subsequently found to be MMP2 or MMP3; MMP18 has only been cloned from *Xenopus* and there is no mammalian homologue. MMP22 is present in chickens, but not in mice or humans. In mice, there are two subtypes of MMP1, ColA and ColB. MMP26 is present in humans, but there is no mouse orthologue; there are also two versions of human MMP23. Therefore, there are 23 mouse MMPs and 24 human MMPs.

With the exception of the six membrane-type MMPs (MT-MMPs), MMP family members are generally secreted from cells. Nonetheless, these MMPs can be concentrated on the cell surface to improve their efficiency of proteolysis by virtue of binding to MT-MMPs, cell adhesion molecules, cell surface proteoglycans and INTEGRINS. Pro-MMP2 forms a complex consisting of MT1-MMP (MMP14) and tissue inhibitor of metalloproteinase 2 (TIMP2), which allows a neighbouring MMP14 to activate it on the cell surface. Pro-MMP2 can also be localized to cell surfaces by binding to $\alpha_2\beta_1$ integrin[2]. Similarly, active MMP9 binds to CD44, the hyaluronan receptor. Some MMPs, such as MMP7, can also be bound on the cell surface by association with membrane heparan sulphate proteoglycans[3].

The pericellular MMPs are believed to be the physiological mediators of matrix degradation or turnover and, collectively, the MMPs can cleave all protein components of the extracellular matrix (ECM)[4]. Indeed, many peptides liberated by the partial proteolysis of ECM macromolecules can regulate cell activities. Besides the ECM, other substrates of MMP processing include growth factors, receptors and adhesion molecules[5]. These activities confer on MMPs functions as diverse as cellular differentiation, migration, regulation of growth factor activity, survival or apoptosis, angiogenesis, inflammation and signalling[1,6].

MMPs have the potential for massive tissue destruction, and their expression and activity are tightly regulated[7]. MMP activity is controlled at three main levels: first, transcription (many MMPs are only expressed following cell activation or stimulation); second, proenzyme activation, during which MMPs — initially expressed as inactive ZYMOGENS — require processing to expose the active catalytic site; and third, inhibition by physiological inhibitors, including the four TIMPs (1–4) and α-2 macroglobulin in serum. There are additional mechanisms that control MMP activity, including internalization, post-translational modifications (for example, glycosylation of MMP9), storage of MMPs (for example, MMP9) in granules of neutrophils, and compartmentalization and availability of substrates.

The ADAMs are membrane-anchored enzymes with more domain structures than the MMPs (FIG. 1). The additional features include a disintegrin domain, a cysteine-rich region, an epidermal growth factor (EGF)-like domain and a cytoplasmic domain. The disintegrin domain can bind integrin and the cysteine-rich region can interact with proteoglycans; these structures therefore help target ADAMs to substrates[8,9]. The cytoplasmic domain of ADAMs often contains signalling motifs, thereby implicating ADAMs as mediators of signal transduction. Interestingly, some ADAM family members do not contain the HExxH catalytic site consensus sequence for metalloproteinases, and little is known about how these proteins function. There are 29 mammalian ADAM family members, 17 of which have

INTEGRINS
Receptors on cells that interact with ECM proteins or other cell surface molecules, and that regulate important functions such as growth and survival.

the HExxH consensus sequence. Not much is understood about the regulation of ADAM expression or their endogenous inhibitors; TIMP3, but not TIMP2, inhibits the activity of some ADAM metalloproteinases[10], and TIMP1 inhibits ADAM10 (REF. 11).

ADAMs can cleave and remodel components of the ECM[8]. However, their best characterized function is the proteolytic processing of membrane-anchored precursors and the subsequent release of mature proteins. This process is referred to as 'protein ECTODOMAIN SHEDDING', and substantially alters the activity of the substrate. Examples of proteins modulated in this manner include the cell-surface receptor Notch and its ligand Delta, which are important in nervous system development, amyloid precursor protein, which is implicated in Alzheimer's disease, tumour necrosis factor-α (TNFα), and the ligands of the EGF receptor[8,9]. The last category includes EGF, transforming growth factor-α, neuregulins and heparin-binding EGF-like growth factor. These ligands are synthesized as membrane-anchored precursor forms, many of which have some activity, and are shed by metalloproteinase-dependent cleavage to generate soluble ligands with increased activity. It should be noted that MMPs can also promote ectodomain shedding, although ADAMs might act as physiological mediators in this regard.

More recently, another group of ADAM-related proteases has been described. These proteins have the protease, disintegrin and cysteine-rich domains but differ from ADAMs in having thrombospondin (TS)-like repeats. These are referred to as ADAMTSs, of which 19 have been described[12]. Unlike the ADAMs, the ADAMTSs lack the transmembrane domain and are secreted molecules. As there is minimal literature on ADAMTSs in the nervous system, these metalloproteinases will not be discussed further. However, research in this area should be encouraged as many of the substrates for ADAMTSs, such as brevican and other proteoglycans, are upregulated in CNS injury sites where they are barriers to axonal regrowth (see below). At present, TIMP3 is the only known natural inhibitor of ADAMTSs.

Metalloproteinases as mediators of disease

The adult mammalian CNS contains several metalloproteinases with detectable levels of transcripts or proteins[13–15]. Their upregulation, and increased expression of other metalloproteinases that are not detectable in the normal state (for example, MMP12), occur in all diseases of the CNS. As MMP upregulation in nervous system pathology and the detrimental effects of these proteins have been extensively reviewed[16–18], this article discusses only the principles of the elevation of metalloproteinases in CNS insults and their detrimental mechanisms.

General principles of the elevation of metalloproteinases. An increase in the expression of gelatinase B, or MMP9, has been well documented in insults to the CNS. Gelatinases can be easily detected by GELATIN ZYMOGRAPHY, an electrophoresis-based method in which the gel contains gelatin, resulting in the focus of their expression. However, it should be noted that the metalloproteinases constitute a large family and that the non-gelatinase members could serve more important roles. When techniques are used to detect the expression of multiple metalloproteinases — such as multi-probe RNAase protection assays — a typical result is the simultaneous elevation of several members. In an acute insult such as spinal cord compression injury, we have documented the increase in transcripts encoding MMP2, 3, 7, 10–13, 19 and 20 (see below for the comments on MMP9 and the release of preformed MMP9 by neutrophils; alteration of MMP9 transcript is not informative in spinal cord injury). This increase is time-dependent, with elevations in MMP3, 7, 10, 11, 19 and 20 observed within 24 h of injury, and the rest within 5 days[19]. During the period of peak clinical signs in EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE), an animal model of multiple sclerosis, the transcripts encoding the majority of MMPs are elevated, with the exception of MMP7, 20 and 28 (which are unchanged), and MMP15–17, 21 and 24 (which are reduced)[14]. In EAE, it is remarkable that 11 MMP family members are elevated in peak disease state; furthermore, although the significance is unclear, four of the five MMPs that are downregulated (MMP15–17 and 24) belong to the MT-MMP class. Expression of ADAM family members, especially ADAM12 (REF. 20), is also altered in EAE.

Comparisons across EAE and spinal cord injury show that not all metalloproteinases are altered in a similar manner. For example, the expression of MMP7 is increased in spinal cord injury but it is unaltered in EAE; the expression of MMP15–17 is unchanged in spinal cord injury but is reduced in EAE[14,19]. So, although many metalloproteinases are expected to be elevated at peak disease severity, those that are altered vary depending on the nature of the injury.

The increase of several metalloproteinase family members in nervous system pathology is the result of contributions from various cellular sources. In general, the different leukocyte subsets express a spectrum of metalloproteinases[21] and the CNS contains most of the ADAM family members and MMPs[14,15,19]. Therefore, in injuries/disorders such as stroke, multiple sclerosis and neurotrauma, in which there is an influx of leukocytes, the metalloproteinases that are detected might be the result of production and secretion by not only infiltrating cells, but also neural and endothelial cells. The prominent induction of MMP12 in cases of neurotrauma and intracerebral haemorrhage results from expression in CNS-intrinsic microglia, and peripheral macrophages that enter into the CNS[22,23].

CNS insults with neutrophil infiltration provide another example of metalloproteinase expression. In spinal cord injury and stroke, the influx of neutrophils is an early and significant event. Neutrophils have preformed stores of MMP9 (REF. 24), and the prompt elevation of MMP9 protein that is seen in CNS insults with neutrophil infiltration[25,26] might be the result of the release of preformed MMP9 into the injury site.

ZYMOGEN
An inactive pro-form of an enzyme. All metalloproteinases are initially expressed as zymogens that require processing to expose their active catalytic site.

ECTODOMAIN SHEDDING
Refers to the release of an active factor from the cell membrane, usually from an inactive form, by proteases. For instance, all EGF receptor ligands, which affect development and disease, are released in this manner.

GELATIN ZYMOGRAPHY
A gel-based method, using gels impregnated with gelatin, to measure the level of MMP2 and MMP9 through their ability to degrade gelatin.

EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS
(EAE). An animal model of multiple sclerosis that is initiated in animals by injecting myelin proteins or peptides to raise autoreactive T cells, or by the transfer of autoreactive T cells into naive recipients.

Table 1 | **Outcomes of adult MMP-null mice in CNS insults**

Genotype	Reported outcome	Reference
MMP2$^{-/-}$	Earlier onset and more severe EAE due to a compensatory increase in MMP9	27
	No difference from wild type after focal ischaemia	116
	Reduced glioma growth	117
MMP9$^{-/-}$	Better recovery from spinal cord injury	25
	Reduced apoptosis of retinal ganglion neurons after optic nerve ligation	49
	Less severe EAE disease course	118
	Improved histological and motor outcome in brain trauma	119
	Better histological outcome from ischaemic stroke	120
	Increased haemorrhage, neurological deficits and lethality after intracerebral haemorrhage	121
	Impaired remyelination after a spinal cord lesion	26
MMP12$^{-/-}$	Worse disease course in EAE	14
	Better recovery from spinal cord injury	23
	Better functional recovery from intracerebral haemorrhage	122

The varied outcomes highlight the influence of both the beneficial and detrimental properties of matrix metalloproteinases (MMPs) in the CNS. EAE, experimental autoimmune encephalomyelitis.

Therefore, when the MMP9 transcript is measured soon after these insults, its elevation is often not remarkable[19], which shows that the use of transcripts alone can under-represent the role of MMP9 at sites of neurotrauma. These observations highlight the importance of studying the expression of a metalloproteinase at several levels, including enzymic activity, and protein and transcript expression.

The increase of many metalloproteinases in CNS pathology raises the question of which of these enzymes are important for the pathophysiological process. The alleviation of disease pathology in response to metalloproteinase inhibitors[16] shows that the net effect of the expression of metalloproteinases in CNS injury is detrimental. As these metalloproteinase inhibitors (for example, BB94 and GM6001) are not specific to particular proteins, and often do not discriminate between the MMP and ADAM classes, their use might guide but cannot conclusively implicate specific members. Mice deficient in particular metalloproteinases are still the best tool with which to implicate that enzyme in CNS pathology. In this regard, MMP9-null mice have been found to be less afflicted than wild-type mice by EAE and stroke, whereas MMP9- and MMP12-null mice recover better from spinal cord injury (TABLE 1). MMP2-null mice have been found to be more susceptible to EAE, which has been attributed to a compensatory increase in MMP9 in these animals[27].

Different metalloproteinases might have distinct and opposite roles. We have noted in the mouse model of EAE that MMP9 promotes the development and progression of the disease (V. W. Y., unpublished observations), whereas MMP12 has a role in its resolution[14].

A convergence of data also implicates MMP9 in human diseases. For example, the upregulation of MMP9 in serum and cerebrospinal fluid in multiple sclerosis has been documented in several studies, especially when it is expressed as a ratio to TIMP1 (REF. 28). This corresponds to the development of gadolinium-enhancing lesions on brain MRI, which are reflective of inflammation and blood–brain barrier breakdown. The level of MMP9 in the serum of patients diagnosed with clinically isolated syndrome (which might represent the first recorded event of multiple sclerosis) is already raised from control levels, and is further increased in patients who register a second event to become conclusively diagnosed as having multiple sclerosis[29]. A polymorphism in the MMP9 promoter, which confers increased transcriptional activity, is correlated with an earlier onset of multiple sclerosis[30]. It should be noted that, in addition to MMPs, ADAM10 and 17 are also upregulated in brain tissues from patients with multiple sclerosis[31], whereas ADAMTS14 has been associated through genetic linkage with the disease[32].

Another feature of changes in metalloproteinase expression in neurological diseases is that they are accompanied by concordant changes in TIMP expression. For example, the expression of both TIMP1 and some metalloproteinases are elevated in EAE and after spinal cord injury[14,19,33], which raises the question of whether there is net proteolytic activity. Indeed, it has been revealed that there is with *in situ* gelatin zymography, in which the result of overlaying a tissue section with gelatin conjugated to fluorescein was that gelatin was degraded[25]. The improved outcome after injury noted above in mice that were deficient for particular metalloproteinases or that had been treated with inhibitors also points towards net proteolytic activity as being detrimental in several neurological diseases. Overall, although these findings show that the net effect of the acute upregulation of many metalloproteinases in nervous system pathology is detrimental, certain metalloproteinases that are discreetly expressed at specific sites, particularly during the repair or recovery phase, can have beneficial activity, which is noted above for MMP12 in EAE (see also discussion below).

How is the expression of metalloproteinases regulated after CNS injuries? A prominent stimulus is the inflammatory cytokines (FIG. 2) that are also upregulated in CNS injuries, expression of which typically precedes that of the metalloproteinases. In culture, interleukin-1 (IL-1) is a strong inducer of the expression of metalloproteinases[34,35], and the promoter region of many metalloproteinase genes contains binding elements for IL-1-regulated transcription factors. In brain trauma, the local application of IL-1-receptor antagonist reduces the expression of MMP9 (REF. 35). The promoter region of TIMP1 also contains binding elements for IL-1-regulated transcription factors, which might be responsible for the simultaneous elevation of metalloproteinases and TIMP1 during the acute or peak phases of a nervous system insult. Other inducers of metalloproteinase expression include alterations of ECM molecules, integrin signalling and CHEMOKINES.

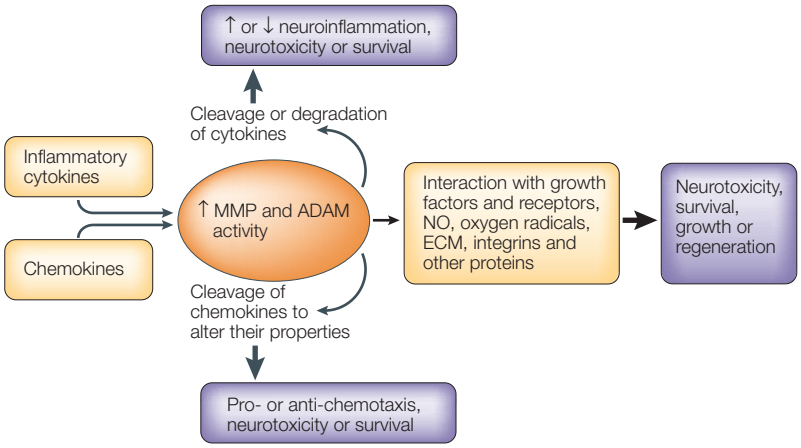

Figure 2 | **Interactions between metalloproteinases, cytokines, chemokines and other molecules present at a site of injury and their consequences.** The expression and activity of metalloproteinases can be upregulated by inflammatory cytokines and chemokines; the elevated protease activity can, in turn, process cytokines and chemokines to alter their properties. In addition, metalloproteinase activity can result in the processing of growth factors and receptors, free radicals, extracellular matrix (ECM), integrins and other proteins. The net result of these interactions includes the alteration of the extent of neuroinflammation, neurotoxicity or survival, and growth and regeneration. Therefore, it is not only important to determine whether there is a change in metalloproteinase activity, but also whether this affects the interactions of MMPs with other molecules in their microenvironment. ADAM, A disintegrin and metalloproteinase; MMP, matrix metalloproteinase; NO, nitric oxide.

As metalloproteinases can activate each other, the net effect of the simultaneous expression of several metalloproteinases is an overall increase in their activity. The interaction with non-metalloproteinase proteases is also significant; the serine protease plasmin activates MMP2, and this facilitation of MMP2 activity is one mechanism by which glioma cells exploit astrocytes to promote their invasiveness[36]. Reactive astrocytes and microglia also interact, which results in local MMP9 activation[37]. Generally, the interaction of proteases after injuries to the nervous system is an area that needs further investigation.

The interaction of metalloproteinases with other molecules at injury sites is also important. Nitric oxide production is increased at lesion sites, where it might contribute to the evolution of an injury. Significantly, nitric oxide activates MMP9 through S-nitrosylation and the resultant product is neurotoxic[38]. Similarly, chemokines are also present at injury sites and there is a complex but fascinating interaction between these and metalloproteinases (FIG. 2). For example, MMP2 (as well as MMP13 and 14 but not MMP7–9) removes an amino (N)-terminal tetrapeptide from macrophage chemoattractant protein 3 (MCP3, also known as chemokine (C–C motif) ligand 7, CCL7) and the cleaved MCP3 becomes an antagonist at chemokine receptors; this supports a role for the MMP-processed chemokine to terminate inflammation[39]. By contrast, MMP9 removes 6 amino acids from the N terminus of interleukin-8 (IL-8) and the truncated form becomes a more potent stimulus for the recruitment of neutrophils[40]. Similarly, it has been observed that in damaged lungs the shedding of the complex of syndecan 1 and the chemokine (C–X–C motif) ligand 1 (KC) by MMP7 establishes a gradient for the infiltration of neutrophils into the lungs[41], and similar concepts might apply to the nervous system. The processing of stromal cell-derived factor 1 (SDF1) by MMP2 results in a shortened SDF1 that acquires neurotoxic properties[42].

The general features of expression and activity of metalloproteinases in CNS injury might be summarized as follows. First, several metalloproteinases are upregulated simultaneously, particularly shortly after an acute insult (for example, spinal cord injury) or at peak pathological state of a chronic injury (for example, EAE). Second, different metalloproteinases might be more prominently represented at different phases of the pathology. Third, particular metalloproteinases are elevated in specific diseases. Fourth, there are many cellular sources of metalloproteinases. Fifth, proteases work in concert with or activate one another. Finally, the metalloproteinases interact with other molecules (FIG. 2) in the vicinity of an insult to confer various outcomes. Overall, metalloproteinases are key molecules that help to determine the outcome of an insult.

Mechanisms of metalloproteinases in pathology. The abundant expression of some metalloproteinase members in nervous system injuries can lead to several undesirable outcomes. The breakdown of the blood–brain barrier has been alluded to above. Other outcomes include the development of demyelination and axonal injury[16], as the deposition of purified MMPs in the brain parenchyma results in the loss of myelin[43] and axonal injury[44]. However, it is not clear whether these outcomes are primary or secondary to other processes that develop as the result of large amounts of metalloproteinases injected into the brain parenchyma.

The modulation of neuroinflammation is another significant outcome of changes in metalloproteinase expression and activity after injury. Several MMPs can generate encephalogenic fragments from CNS proteins, examples of which include the cleavage of myelin basic protein into fragments that produce neuroinflammation in animals[45]. Many inflammatory molecules, such as TNFα, are synthesized as precursors that require metalloproteinase processing to generate the active form. The interaction of metalloproteinases with chemokines to produce pro- or anti-inflammatory molecules has been alluded to above. Finally, metalloproteinases can also degrade inflammatory molecules, such as IL-1β (REF. 46), to terminate their actions.

Perhaps the most significant consequence of the overexpression of metalloproteinases in the CNS is the regulation of cell survival and death. Various mechanisms contribute to metalloproteinase-mediated cell death (FIG. 2). The adherence of cells to the ECM provides survival signals through mechanisms that include the activation of integrin receptors that have engaged particular ECM proteins. When such anchored cells are detached from the substratum, the loss of integrin signalling can result in apoptosis, a phenomenon that has been referred to as anoikis ('homelessness'). An advantage of anoikis is that cells that are detached from their usual locations do not become the seed of a tumour

CHEMOKINES
A subfamily of inflammatory molecules that were initially described in regulating the chemotaxis of inflammatory cells, but that also have important roles in other processes, such as cell growth and differentiation.

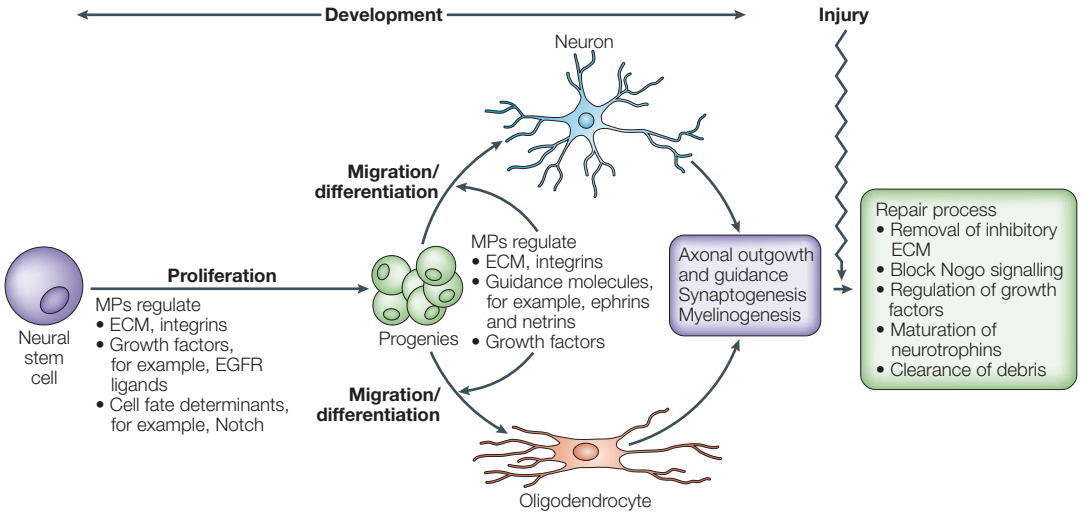

Figure 3 | **The metalloproteinases regulate developmental and regenerative events in the CNS.** The figure depicts the genesis of progenies from a neural stem cell, the migration and differentiation of the progenies into neurons and oligodendrocytes, and the subsequent formation of axons and myelin. The mechanisms by which metalloproteinases (MPs) regulate these events are listed. Similarly, following an injury, metalloproteinases are involved in the repair process through specific mechanisms. ECM, extracellular matrix; EGFR, epidermal growth factor receptor.

elsewhere. Therefore, in the CNS, the degradation of ECM proteins and loss of integrin signalling by abnormally expressed MMPs can affect cell survival[38,47]. Notably, the disintegrin domain of ADAMs can interact with integrins[48] and MMPs can engage integrin signalling[2]. In optic nerve injury in mice, the upregulation of MMP9 and subsequent degradation of laminin from the underlying inner membrane are thought to be the mechanisms that accounts for the apoptosis of retinal ganglion cells. This is consistent with the fact that MMP9-deficient mice are protected from laminin degradation and apoptosis of retinal ganglion neurons after optic nerve injury[49].

Kainic acid is a neurotoxin that is used extensively to produce damage to neurons. The kainate-induced excitotoxicity in the rat hippocampus is accompanied by a net increase in MMP activity in hippocampal neurons, and broad spectrum metalloproteinase inhibitors protect these neurons[50]. Intravitreal injection of kainate increases MMP9 activity in astrocytes, which correlates with a decrease in laminin immunoreactivity in the ganglion cell layer and apoptosis of retinal ganglion neurons; this toxicity is protected by GM6001 (REF. 51).

The results of tissue culture studies have also shown that direct application of purified MMPs results in neuronal death. Human fetal neurons are destroyed by MMP1, and Conant *et al.* speculate that MMP1 disrupts ECM and affects membrane receptors that modulate cell survival[52]. In more recent work, the same group[53] showed that pro-MMP1 interacts with $\alpha_2\beta_1$ integrin on the neuronal surface, which results in a rapid reduction in the phosphorylation of Akt, a kinase that influences caspase activity and cell survival.

The interaction of metalloproteinases with other molecules present in the injured CNS is also crucial for determining whether toxicity occurs. As discussed above, nitric oxide activates MMP9; the stable S-nitrosylated MMP9 product, which is toxic to neurons[38], has been detected *in vitro* and *in vivo* in a model of stroke injury. Reactive oxygen species produced after CNS injuries might contribute to the induction and/or activation of MMPs, thereby exacerbating injury, although inactivation of metalloproteinases by oxidants, through modification of amino acids crucial for catalysis, can also occur[6]. As noted above, active MMP2 cleaves SDF1 to form a neurotoxic product[42].

Metalloproteinases in growth and regeneration

Although many metalloproteinases have detrimental effects when they are overexpressed in CNS injuries, they also have various roles in conferring beneficial activities. This section describes the roles of metalloproteinases in neurogenesis, axonal growth and myelinogenesis (FIG. 3). These beneficial functions occur not only during development but also in repair of the CNS after insult in adult animals.

Metalloproteinases and neurogenesis. *In vitro*, neural stem cells express MMP2 and all four TIMPs[54,55]. *In situ*, TIMP3 mRNA is expressed in areas of extensive neurogenesis, including the embryonic ventricular zone, the postnatal subventricular zone and the rostral migratory stream to the olfactory bulb[56]. Although the expression of TIMP3 suggests roles for metalloproteinases during development, TIMPs might exert their effects through functions unrelated to protease regulation. For example, TIMP2, through an MMP-independent mechanism, promotes neuronal differentiation *in vitro* and *in vivo*[57]. However, reports of metalloproteinase expression in areas of neurogenesis, which relate expression to function, have since come to light. In *Xenopus*, neural crest cells express ADAM13, and cranial neural crest cell grafts that express protease-defective ADAM13 fail to migrate along the hyoid and branchial pathways[58].

In postnatal development of the cerebellum, the expression of MMP9 is spatiotemporally related to granule cell migration, which is delayed in MMP9-deficient mice[59]. Both MMP2 and 9 are expressed and involved in postnatal morphogenesis of the cerebellum[60].

The mechanisms by which metalloproteinases regulate neurogenesis have not yet been defined. One possibility is the modulation of the ECM and/or other guidance molecules. In the rostral migratory stream to the olfactory bulb, the pattern of expression of TIMP3 is similar to the ECM proteoglycan brevican[56]. Furthermore, many of the molecules that effect cell fate decisions in neurogenesis are substrates for metalloproteinases, an example of which is the Notch pathway, which is mediated by ADAM10 and 17 signalling[61]. Another mechanism of metalloproteinases in neurogenesis might involve interaction with integrins, growth factors and other molecules to confer activities such as survival, differentiation and signalling[62]. Finally, the EGF receptor ligands, which include EGF and neuregulins, are potent mediators of proliferation and lineage specification of neural stem cells[63]; ADAMs regulate the activity of these ligands through ectodomain shedding[9], as can MMPs.

We await reports of the roles of metalloproteinases in neural development, and whether neural stem cells and their differentiation into committed progenies is altered in the CNS of metalloproteinase-deficient mice. Such reports would help us to define which metalloproteinase family members are crucial for neural development. Similarly, experiments involving the use of metalloproteinase inhibitors during neural stem cell proliferation and differentiation could help further establish a role for metalloproteinases in neurogenesis.

Metalloproteinases and axonal growth. There is substantial evidence that metalloproteinases regulate axonal growth. The metalloproteinase activity is localized to the growth cones of neurons, and inhibition of metalloproteinase activity reduces growth cone motility[64]. In PC12 cells treated with nerve growth factor (NGF), neurite extension correlates with the localization of MMP3 by immunohistochemistry to the growth cones[65]; PC12 lines that stably express MMP3 antisense RNA have reduced capacity to penetrate matrigel, a reconstituted basement membrane[66]. Active proteolytic activity demonstrated by *in situ* zymography with gelatin-FITC (fluorescein isothiocyanate) as a substrate has also been shown in cerebellar neurons and at the edge of growth cones from dorsal root ganglion neurons[67]. MT5-MMP expression is closely associated with the formation of dendritic trees of Purkinje neurons[68]. Growth factors and ECM molecules that promote neurite outgrowth, such as NGF and laminin, increase the expression of MMP2 in neurons[69].

Mutational analyses have also shown that metalloproteinases have roles in axonal elongation. In *Drosophila melanogaster*, defects in kuzbanian, which is homologous to mammalian ADAM10, lead to stalled axons during development[70]. In a phenotype-based gene-trap screen, ADAM23 was identified as a regulator that controls axonal wiring in the murine CNS[71], and ADAM23 mutant mice show tremor and ataxia[72]. Indeed, ADAM23 expression is enriched in neurons throughout the rat brain and at all stages of postnatal development [73].

Besides the elongation of axons, metalloproteinases are also implicated in their guidance. *In vivo* studies of development of the retinotectal projection of *Xenopus* embryos found that the addition of metalloproteinase inhibitors at low concentrations disrupted guidance cues, whereas higher concentrations reduced axonal outgrowth[74]. Ephrins are guidance molecules that bind to Eph family receptors and Hattori *et al.*[75] found that the ephrin–Eph interaction was disrupted by ADAM10. The engagement of another guidance molecule, netrin 1, with its receptor, DCC (deleted in colorectal carcinoma), was altered by metalloproteinase activity through the shedding of DCC; it was proposed that proteolytic activity might regulate axonal growth by controlling the number of functional extracellular axon guidance receptors[76]. Kuzbanian may act partly through the proteolytic activation of the Slit and Robo receptor complex that regulates axonal guidance[77]. In *Caenorhabditis elegans*, mutants with loss-of-function of UNC-71 (which is probably a homologue of mammalian ADAM14) have defects in the guidance of motor axons; double mutant analysis of UNC-71 with other axon guidance signalling molecules implicates a mechanism involving integrins and netrins that provide distinct axon guidance cues[78]. Different metalloproteinases are now thought to regulate axon behaviour at distinct 'choice points' during the development of retinal ganglion neurons: one is thought to provide guidance at the optic chiasm and another is proposed to guide axons in the mid-diencephalon *en route* to the tectum[79].

In conclusion, the formation of neurites and the activity of growth cones during development are associated with the expression of several metalloproteinases. This relationship is functionally coupled, as the reduction of metalloproteinase activity decreases neurite outgrowth and affects guidance decisions.

Metalloproteinases and axonal regeneration. If metalloproteinases are expressed during development to guide axonal growth, does the same occur following an injury? Can some of the metalloproteinases that are expressed abundantly after nervous system injury be used for repair? Evidence that metalloproteinases facilitate regeneration of axons *in vivo* is lacking, but various lines of evidence point towards this potential. First, expression of metalloproteinases has been shown to correspond with periods of recovery. In regenerating sciatic nerve fibres, MMP9 expression is co-localized with phosphorylated neurofilament M, a marker for regenerative elongation; the phosphorylated neurofilament M is also induced by MMP9 treatment and inhibited by an anti-MMP9 antibody treatment[80]. Regenerating axons show immunoreactivity for MMP2 and 3 (REF. 81). Ahmed *et al.*[82] report that MMP1, 2 and

9 levels are higher in a regenerating (non-scarring) versus a non-regenerating (scarring) model of optic nerve injury in rats.

A second line of evidence that implicates metalloproteinases in axonal regeneration comes from studies of their application to non-permissive substrates. When primary dorsal root ganglion neurons are grown on cryostat sections of normal adult nerves, neurite outgrowth is poor as the adult nerves constitute an unfavourable substrate. However, when the nerve sections are treated with active MMP2, which degrades inhibitory CHONDROITIN SULPHATE PROTEOGLYCANS (CSPGs) to expose permissive laminin, neurite outgrowth from dorsal root ganglion neurons is promoted[83]. The inhibitory effect of CSPGs on neurite extension from dorsal root ganglion neurons is also eliminated by the addition of MT5-MMP[67]. In a chronically denervated distal tibial nerve segment, successful regeneration of axons is facilitated by supernatant from a neural stem cell line containing large quantities of secreted MMP2, which degrades the CSPGs in the chronically denervated nerve sections[55].

There is also data that indicates a role for metalloproteinases in synaptic plasticity. Both the MMP9 transcript and protein are upregulated in the cell bodies and dendrites of hippocampal neurons within hours of intraperitoneal kainate administration, and the protease appears to have a role in the dendritic remodelling[84]. In traumatic brain injury involving the hippocampus, the resultant reactive synaptic plasticity corresponds to increased MMP3 expression[85]. In adult rats with unilateral lesions of the entorhinal cortex, those receiving vehicle showed normal sprouting and synaptogenesis, with the emergence of the capacity for LTP within the sprouting pathway; by contrast, lesioned rats receiving a metalloproteinase inhibitor fail to develop the capacity for LTP[86].

Wallerian degeneration occurs after injury and involves the breakdown of myelin and axons, and removal of degenerating nerve components. This clearance is necessary for eventual repair. MMP levels increase during Wallerian degeneration, and this correlation is functionally significant as Wallerian degeneration of a transected nerve is delayed by hydroxamate metalloproteinase inhibitors[87].

In summary, the data for metalloproteinases in axonal regeneration and dendritic remodelling is scant but suggestive. More definitive evidence, perhaps in the form of application of metalloproteinases to an area of injury *in vivo* to improve axonal regrowth, is required. In this regard, the complementary work of a glycosidase, chondroitinase-ABC, is of interest. Chondroitinase-ABC, a bacterial enzyme that removes the glycosylaminoglycan side chain but not the core protein of CSPGs, facilitates axonal regeneration when applied to the lesion site in rats with spinal cord injury[88]. As the core proteins of CSPGs also contain inhibitory domains[89], it is possible that improved regeneration can occur when both chondroitinase-ABC and metalloproteinases are used in tandem, as the latter can clear the core proteins of CSPGs[26] left behind by the chondroitinase-ABC treatment. The *in vivo* application of proteases must be conducted carefully, as it is necessary to prevent the toxic properties of metalloproteinases from becoming manifest.

CHONDROITIN SULPHATE PROTEOGLYCANS (CSPGs). Important components of the ECM. The deposition of these proteins at sites of CNS injury is an important impediment to axonal regrowth.

Mechanisms of metalloproteinases in axonal growth. There are several mechanisms by which metalloproteinases might facilitate the growth of axons (FIG. 3). The interaction with ECM proteins and guidance molecules has been mentioned earlier. Another possibility is through the activity of metalloproteinases on the Nogo and Nogo receptor (NgR, also termed NgR1) complex (FIG. 4). Besides the axon inhibitory properties of CSPGs described above, members of the other major group of molecules involved in inhibiting axonal regeneration are components found in CNS myelin, including oligodendrocyte myelin glycoprotein, myelin associated glycoprotein (MAG) and NogoA (reticulon 4)[90]. These myelin components inhibit neurite outgrowth by binding NgR on axons, which then transduces inhibitory signals intracellularly through an interaction with a transmembrane co-receptor, the p75 neurotrophin receptor ($p75^{NTR}$); a third component, LINGO, might also be necessary.

$p75^{NTR}$ has been the subject of extensive research focused on processing by metalloproteinases. $p75^{NTR}$ undergoes proteolysis mediated by ADAM17, which results in the release of an N-terminal fragment containing the binding domain for NGF[91]; this reduction of $p75^{NTR}$ signalling by increasing metalloproteinase activity might reduce the inhibitory signals for axonal regeneration. The situation is complicated, however, by a recent report that MAG initiates the cleavage of $p75^{NTR}$ through sequential activation of an α-secretase (which is probably an ADAM) and a γ-secretase, and that the combined proteolysis leads to the MAG-inhibition of neurite outgrowth[92]. In this context, increasing metalloproteinase activity could enhance inhibitory signalling for axonal regeneration.

For NgR, metalloproteinase activity generates an N-terminal fragment that binds the Nogo66 domain of NogoA, thereby blocking its binding to intact NgR[93]; therefore the result of enhancing metalloproteinase activity could be improved axonal regrowth. This metalloproteinase activity probably resides in the MT-MMPs[94] based on profiles of antagonism by various inhibitors. Interestingly, the N-terminal fragment of NgR generated by metalloproteinase activity *in vitro* was detected in human brain cortex and cerebrospinal fluid, which indicates that NgR proteolysis by metalloproteinases is a physiological or pathological event in humans[93].

It is not clear whether metalloproteinases can remove oligodendrocyte myelin glycoprotein and MAG, thereby clearing these myelin inhibitors from sites of CNS injury. Previously, it was found that MT1-MMP could digest NI-220 (REF. 95), an inhibitory protein on myelin that was subsequently found to be a fragment of NogoA. So, besides inhibiting signalling by NgR and $p75^{NTR}$, metalloproteinases might also remove the ligands that trigger inhibitory signalling.

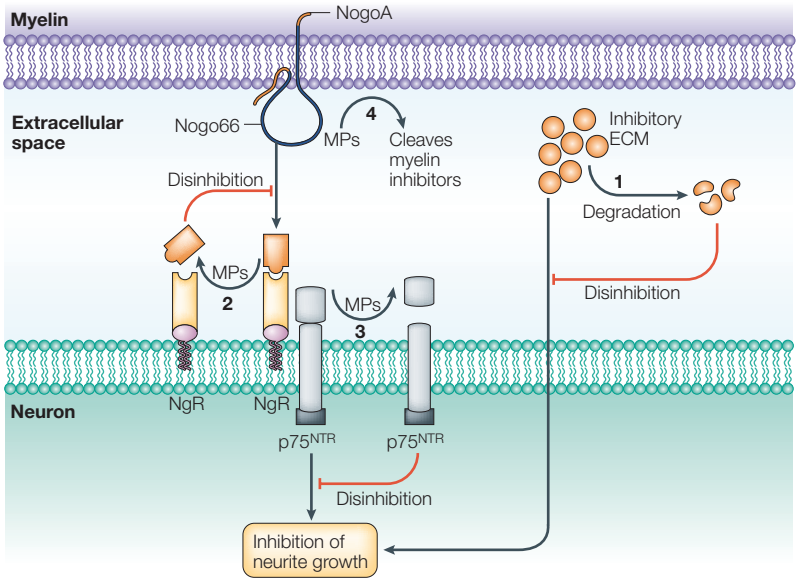

Figure 4 | **Mechanisms by which metalloproteinases might regulate axonal regeneration of neurons.** Among extracellular matrix (ECM) molecules, various proteoglycans are responsible for the activity that inhibits axonal regrowth. By removing these inhibitory ECM components (1), it might be possible to achieve disinhibition of neurite outgrowth. The Nogo66 domain of NogoA interacts with the Nogo receptor (NgR), which then associates with the p75 neurotrophin receptor ($p75^{NTR}$) to mediate inhibitory signalling for axonal regrowth. Metalloproteinases (MPs) can remove the amino (N)-terminal fragment (2) that binds Nogo66, thereby blocking its interaction with intact NgR. Metalloproteinases can mediate the release of an N-terminal fragment of $p75^{NTR}$ (3), which contains the binding domain for nerve growth factor (NGF), thereby reducing inhibitory signalling through $p75^{NTR}$. Finally, metalloproteinases can cleave and remove molecules such as NogoA (4) and possibly other inhibitory myelin proteins such as myelin associated glycoprotein (MAG). Overall, these activities of metalloproteinases could enhance axonal regeneration.

There are other activities of metalloproteinases that are relevant to CNS regeneration. First, metalloproteinases impact on many aspects of cell survival and apoptosis[96]. In neurons, MMP3 has been found to confer neuronal survival properties by virtue of removing FasL from the neuronal surface, thereby protecting against Fas-induced apoptosis[97]. ADAM8 also has a role in suppressing neuronal death. The CHL1 (close homologue of L1) protein, a neural cell adhesion molecule, is processed by ADAM8, which results *in vitro* in the stimulation of neurite outgrowth from cerebellar granule neurons and the suppression of neuronal apoptosis; this activity of ADAM8 was not mimicked by ADAM10 and 17 (REF. 98). Second, metalloproteinases interact significantly with integrins as the disintegrin domain of ADAMs can interact with integrins and several MMPs bind integrins[2]. As noted earlier, integrins are vital to the development of the nervous system[62]. Third, metalloproteinases can potentially remove growth factors that are anchored to ECM or plasma membranes[99], making these bioavailable for regenerating structures. Fourth, metalloproteinases have remarkable interactions with neurotrophins, which are initially generated as a pro-form requiring proteolytic conversion to the active molecule. Both forms can have different properties: the pro-neurotrophins and active growth factors differ in their affinity for $p75^{NTR}$ and Trk receptors, and although pro-NGF has been found to kill neurons, NGF promotes neuronal survival and neurite extension[100]. Of particular interest is that the conversion of pro-NGF to NGF, and of pro-brain-derived neurotrophic factor (BDNF) to BDNF, is facilitated by metalloproteinases[100,101]. These observations provide an interesting environment of neurotrophins and metalloproteinases at sites of injury whereby the concurrent absence or presence of metalloproteinases may result in different outcomes.

Collectively, there are many mechanisms through which metalloproteinases can potentially alter the capacity of neurons and axons to regenerate after nervous system injury (FIGS. 3,4). This is a field that must be investigated further to bring about successful nerve regeneration in the CNS.

Metalloproteinases and myelin formation. In the CNS, myelin is formed by oligodendrocytes. Myelin facilitates efficient saltatory conduction of nerve impulses along axons and also provides axons with nutritional and survival factors. Successful remyelination following injuries to the nervous system is therefore a prerequisite for successful recovery.

The mechanisms leading to successful remyelination have been well reviewed[102]. Because an early step in remyelination is the production of extensive processes by an oligodendrocyte to contact several axons, a requirement for metalloproteinase activity in remodelling the ECM seems likely. In this regard, we first described that oligodendrocytes express MMP9 coincident with their extension of processes along an astrocyte ECM *in vitro*[103]. For tissue culture studies, oligodendrocytes cultured from adult human or mouse brains are stripped of their processes during the cell isolation process, and the regrowth of processes provides a model of potential remyelinating capacity. We found that process re-extension by adult murine oligodendrocytes is impaired in cells derived from MMP9-null mice compared with wild-type controls and, furthermore, that inhibitors of MMP9 reduce process extension[104]. These *in vitro* experiments were extended to models of remyelination in adult mice, in which a demyelinating insult was produced in the spinal cord by a lipid disrupting agent, lysolecithin. We showed that after demyelination in adult mice the subsequent remyelination in MMP9-null mice was retarded compared with that in wild-type animals[26]. The mechanism for the requirement of MMP9 in remyelination *in vivo* was attributed in part to the requirement of MMP9 to clear NG2, a CSPG that was deposited into the ECM following injury. In the absence of MMP9, the NG2 in the matrix persisted and provided an inhibitory barrier that prevented the maturation of oligodendrocytes into myelin forming cells[26].

More recently, the expression of several metalloproteinases was investigated in the optic nerve and corpus callosum of mice during the period of postnatal developmental myelinogenesis. MMP9 and 12 were found to be elevated during this period (P. H. Larsen

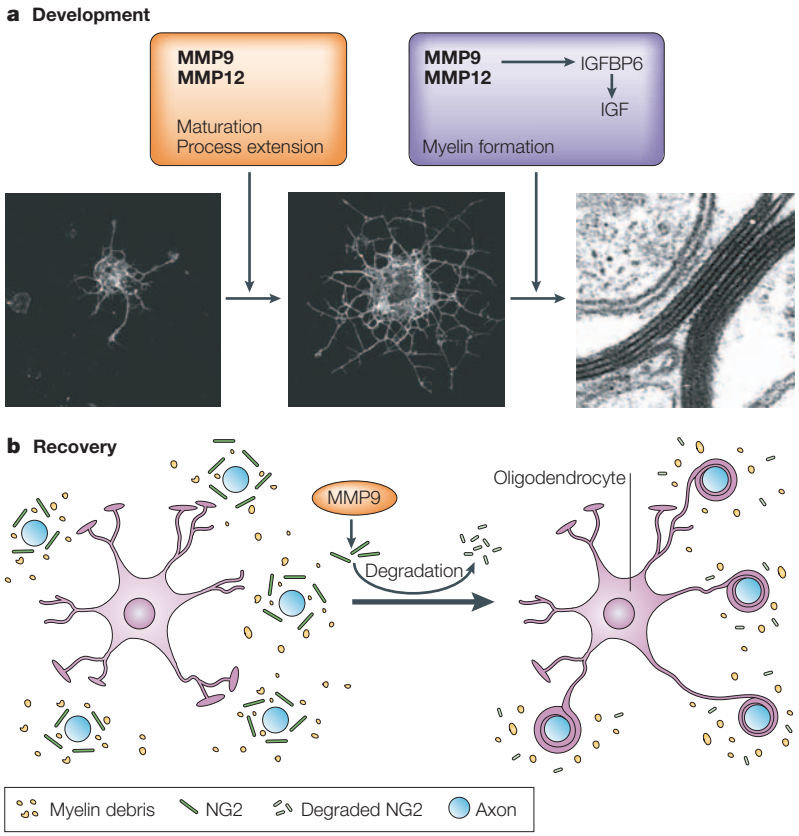

Figure 5 | **Metalloproteinases regulate myelinogenesis. a** | Evidence is emerging that implicates matrix metalloproteinases 9 and 12 (MMP9/12) in the maturation of oligodendrocyte precursor cells to oligodendrocytes and the extension of their processes. MMP9 and 12 are known to influence myelin formation during development, as there is a deficiency of myelination in the development of mice that are genetically deficient for MMP9 and 12; this is probably achieved through the effect of these MMPs on the bioavailability of insulin-like growth factor 1 (IGF1). IGFBP6, IGF binding protein 6. **b** | Following demyelination in the adult mouse spinal cord, MMP9 regulates remyelination. One mechanism through which it does this is the clearance of the NG2 proteoglycan, which constitutes an inhibitory barrier for maturation of oligodendrocytes and their subsequent reformation of myelin.

and V. W. Y., unpublished observations). The absence of these metalloproteinases in MMP9- and 12-null mice led to a delay in myelin formation, as the lack of these MMPs prevented the degradation of a substrate, insulin-like growth factor (IGF) binding protein 6 (IGFBP6), which normally sequesters IGF1. In the absence of MMP9 and 12, the persistence of IGFBP6 reduces the bioavailability of IGF1, an important maturation factor for cells of the oligodendrocyte lineage (P. H. Larsen and V. W. Y., unpublished observations). A role for MMP12 in myelin formation has also been suggested previously from *in vitro* experiments in which cells from adult MMP12-null mice were less able to reform morphologically mature processes[105].

Overall, these studies emphasize that when MMPs are expressed in a spatially and temporally restricted manner during development or after an insult, they can have important roles. Indeed, the elevation of these MMPs might be so restricted that the use of tissue homogenates from an injury site could fail to detect their increase. We were able to show a localized upregulation of MMP9 at an area of remyelination in the adult mouse spinal cord only with immunohistochemistry, and not through gelatin zymography of tissue homogenate[26].

It is likely that other metalloproteinases also have important roles in myelin formation. ADAM22 null mice were recently found to have hypomyelinated peripheral but not central nerves, and altered differentiation of Schwann cells might underlie the defect[106]. Another metalloproteinase with possible links to myelination is ADAM12, which has been found in oligodendrocytes; in adult rat and human brains, the density of ADAM12-positive oligodendrocytes is higher in grey than in white matter, which suggests that there are different subgroups of oligodendrocytes with specific metalloproteinase functions[107].

There may be other mechanisms accounting for the effect of metalloproteinases in remyelination. Metalloproteinases regulate Notch signalling, and the Notch–jagged 1 pathway has been implicated in limiting remyelination in multiple sclerosis[108]. Integrins have a well established role in oligodendrocyte biology[62], and integrin functions are impacted by metalloproteinase activity, as noted above. Furthermore, there is an interaction between metalloproteinases and the chemokine GRO-α (CXCL1), which deserves attention. GRO-α has been shown to regulate the proliferation and positioning of oligodendrocyte precursors[109] and it is of interest that GRO-α can be degraded by MMP9 (REF. 40).

Overall, evidence is mounting in support of a role for metalloproteinases in regulating myelin formation. Several mechanisms may be involved (FIG. 5), and the unravelling of these mechanisms could lead to new targets for enhancing remyelination in demyelinating conditions such as multiple sclerosis.

Determinants of beneficial or detrimental roles

It is clear that metalloproteinases perform both beneficial and detrimental activities in the CNS (BOX 1). For example, MMP9 is detrimental in multiple sclerosis and in acute insults to the nervous system, such as stroke and spinal cord injury. However, MMP9 has a beneficial effect on repair processes such as axonal regeneration and remyelination. MMP12 is detrimental in acute injuries, as noted for spinal cord injury and intracerebral haemorrhage, but it is beneficial to recovery from EAE (TABLE 1). How might we resolve the duality of metalloproteinase function?

At least three factors might determine whether metalloproteinases are beneficial or detrimental after nervous system injuries: the stage of CNS injury, the type of injury inflicted, and the pathophysiology of the disorder involved. With respect to the stage of CNS injury, it is likely that in the early phase after an insult, when many metalloproteinases are induced or released into a milieu that normally has low levels of proteases, the metalloproteinases are detrimental. Indeed, in rodents, the early and short-term treatment (for 3 days) of spinal cord injury or intracerebral haemorrhage with metalloproteinase inhibitors attenuates the extent of

Box 1 | **Effects of metalloproteinases in the CNS**

Metalloproteinases can have beneficial and detrimental effects on the developing and adult brain and spinal cord.

Detrimental effects

Blood–brain barrier dysfunction | Demyelination | Neuroinflammation | Neurotoxicity | Roles in CNS injuries and diseases, such as spinal cord injury, multiple sclerosis and stroke

Beneficial effects

Development of nervous system | Cell fate determination and neurogenesis | Proliferation and migration of precursors | Axonal growth and regeneration | Myelinogenesis and remyelination | Angiogenesis | Survival | Terminate neuroinflammation

neurotoxicity and subsequent loss of function[25,110]. More latterly after injury, when metalloproteinases are expressed discreetly and locally but studies of homogenates might not reveal any increase in metalloproteinase expression, metalloproteinases might be important in the remodelling and repair process. This is highlighted by the observation that, 7 days after lysolecithin-induced demyelination of the spinal cord, the increase in MMP9 expression is detectable only by immunohistochemistry and not by zymography analyses of homogenates; MMP9 promoted remyelination in this study[26].

The second factor that might determine whether metalloproteinases are beneficial or detrimental relates to the type of injury, as this affects the profile of inflammatory cells present at the injury site and the available metalloproteinase substrates expressed in that microenvironment. For example, chronic diseases such as multiple sclerosis and EAE entail significant representation by T lymphocytes, which promote inflammation and neurotoxicity, whereas in acute insults such as spinal cord injury the injury site is infiltrated with neutrophils and macrophages rather than T cells. In different cellular contexts, and with the differences in gene expression in these cell types, the substrates for, and functions of, metalloproteinases therefore differ.

The third factor that might determine the outcomes of metalloproteinase activity lies in the pathophysiology of the disorder in question. For example, in EAE, recovery from the disease depends on a switch of $CD4^+$ T lymphocytes from a proinflammatory T-helper 1 (Th1) subset to a T-helper 2 (Th2) environment in order for inflammation to subside[14]. MMP12 might be required for this switch, and its absence results in animals with a more inflamed CNS and a greater EAE severity[14]. By contrast, in spinal cord injury, which represents an insult less dependent on T cells, MMP12 would not be required to alter the Th1:Th2 ratio. Rather, MMP12 expression increases the activation state of microglia/macrophages, which exacerbate damage to an injured spinal cord[19].

To date, a systematic analysis of the reasons why metalloproteinase activities lead to different outcomes in different diseases, or at particular phases of a specific disorder, is lacking. Studies on this subject should be encouraged, as the capacity to reveal biological substrates in a given microenvironment is improving at an increasing rate. Such studies not only have the capacity to unravel the biology and mechanisms of metalloproteinases, but could also lead to therapies to target substrates for increased selectivity of action.

Therapeutic implications

If metalloproteinases have diverse and widespread functions, what are the prospects for metalloproteinase inhibitors in the treatment of neurological diseases? This is a complex issue that requires careful consideration. It seems that metalloproteinase inhibitors would be useful in treating acute neurological insults, in which the expression of several metalloproteinases is elevated and their overall activity is detrimental. Such insults would include stroke and spinal cord injury, in which a brief period of treatment with metalloproteinase inhibitors could be sufficient to reduce subsequent loss of neurological functions. It is instructive that the use of the metalloproteinase inhibitor GM6001 for only the first 3 days after spinal cord injury in rodents was sufficient to improve neurological functions[25]. Similarly, in mice, treatment with GM6001 for the first 2 days after haemorrhagic stroke was adequate to reduce neutrophil infiltration, oxidative stress, brain oedema and neurotoxicity[110].

Chronic usage of selective metalloproteinase inhibitors, in diseases such as multiple sclerosis, will be more problematic as the long-term application of these inhibitors might affect reparative processes such as remyelination. Interferon-β, an immunomodulator that has beneficial effects in the treatment of multiple sclerosis, inhibits the production of MMP9 (REFS 111,112). However, this is a medication with significant activity in the periphery and at the level of the blood–brain barrier, and it does not appear to gain access to the CNS parenchyma to affect metalloproteinase-dependent repair processes within[113]. Another medication that is currently in clinical testing for long-term usage in multiple sclerosis is minocycline, an antibiotic that affects several aspects of CNS neurobiology, including the inhibition of MMP activity[114]. Minocycline does have access to the CNS and its long-term impact and potential for inhibiting repair need to be carefully reviewed.

As different metalloproteinases might carry out beneficial or detrimental activities in chronic diseases, and as a given metalloproteinase might have disparate functions at different phases of the disease state, the outcome of targeting metalloproteinases using a nonspecific inhibitor might be complex. To achieve selectivity of function, the first step should be a thorough understanding of the pathophysiology of metalloproteinases in a given disorder, so that inhibitors with selectivity to particular metalloproteinases, used at specific times, can be considered. Another approach is the understanding of the stimulus that has led to the increased activity of a given metalloproteinase, so that the removal or targeting of that stimulus might offer a means of controlling metalloproteinase activity (FIG. 6). The inhibition of particular metalloproteinases,

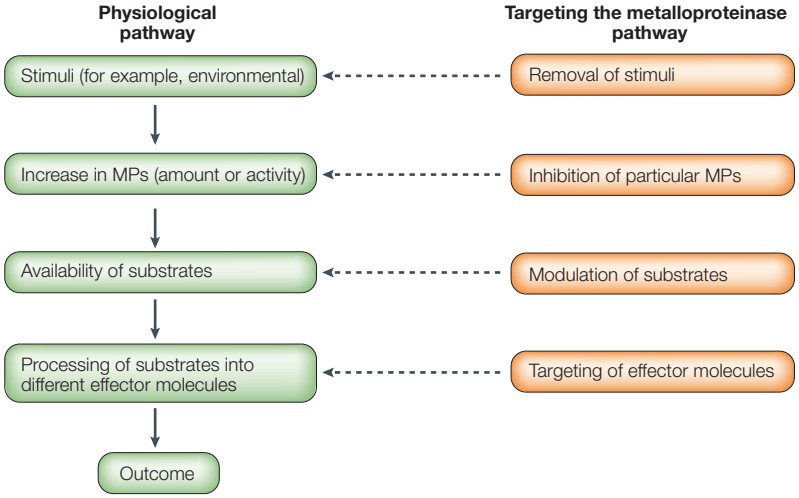

Figure 6 | **Multiple ways of targeting the metalloproteinases.** If the stimuli, including environmental cues, for upregulating the expression of metalloproteinases (MPs) can be identified, these could be removed to normalize the expression of metalloproteinases. Various pharmacological inhibitors are available that could be used to target the increase in the content or activity of metalloproteinases. At present, many inhibitors are relatively nonspecific and fail to discriminate between matrix metalloproteinases (MMPs) and A disintegrin and metalloproteinases (ADAMs), let alone between members of a particular subclass of MMP or ADAM. However, it is anticipated that pharmacological inhibitors will become more selective, as this is a drug discovery goal in the pharmaceutical industry. A perhaps more selective approach to targeting the metalloproteinases is to identify the substrates that modulate the action of a particular metalloproteinase in a given context, and to either modulate the availability of these substrates or to target the effector pathway that results from them. The ability to inhibit the detrimental aspects of metalloproteinases in the CNS while maintaining or even enhancing the beneficial ones will require improved selectivity for targeting particular metalloproteinases and their substrates.

using inhibitors selective to individual metalloproteinases, offers another level. Although the early generation metalloproteinase inhibitors might lack selectivity or the ability to discriminate between MMPs and ADAMs, let alone between members of the same class, newer inhibitors are being chosen for improved selectivity. Finally, the identification of the substrates that mediate the activity of a given metalloproteinase in a particular disease might be the most crucial endeavour, as these substrates are probably unique to a given disease context as noted above. The modulation of the availability of substrates, as well as the specific targeting of these effector substrates, could conceivably lend selectivity even if one inhibitor could not target the upstream metalloproteinase because of its varied functions. The methods with which to identify metalloproteinase substrates are becoming more sophisticated and manageable (for an example, see REF. 115), making it possible to derive means of affecting the substrate of interest so as to block a specific action of a metalloproteinase rather than targeting the enzyme and all of its associated activities.

In summary, metalloproteinases are implicated in diseases of the nervous system, and it is important to target their activity. Although the long-term impact of metalloproteinase inhibitors in neurological conditions is not clear because of the complexity of metalloproteinase function, the evidence suggests that the short-term use of inhibitors after acute insults such as stroke and spinal cord injury will have beneficial outcomes. Clinical trials for testing the effects of metalloproteinase inhibitors in these conditions should be encouraged.

Conclusions

Metalloproteinases have both beneficial and detrimental functions in the CNS. The challenges for a better understanding of metalloproteinase biology are considerable: which member or members of the MMP, ADAM or ADAMTS classes are important in a particular condition or pathological state, how do they achieve their effects, and what roles does each metalloproteinase have in the overall scheme? Therapeutically, a non-selective metalloproteinase inhibitor might alleviate neuroinflammation and neuropathology, but it might also inhibit the neuroprotective or reparative functions of metalloproteinases. The nature of the metalloproteinase–substrate interaction, which is dictated by the availability of particular substrates, might control the role of a particular metalloproteinase; it is possible that an important level of selectivity in targeting diseases attributed to metalloproteinase overactivity is the identification and targeting of relevant substrates. At present, the evidence suggests that acute rather than chronic diseases or injuries are more amenable to metalloproteinase inhibitor therapy. The study of metalloproteinase functions in the nervous system should open up new vistas in nervous system physiology and pathology, and its findings will shed light on metalloproteinase function throughout the human body, both in health and in pathological conditions.

1. Sternlicht, M. D. & Werb, Z. How matrix metalloproteinases regulate cell behavior. *Annu. Rev. Cell Dev. Biol.* **17**, 463–516 (2001).
A very thorough review on the many roles of metalloproteinases.
2. Dumin, J. A. *et al.* Pro-collagenase-1 (matrix metalloproteinase-1) binds the $\alpha_2\beta_1$ integrin upon release from keratinocytes migrating on type I collagen. *J. Biol. Chem.* **276**, 29368–29374 (2001).
3. Yu, W. H., Woessner, J. F. Jr, McNeish, J. D. & Stamenkovic, I. CD44 anchors the assembly of matrilysin/MMP-7 with heparin-binding epidermal growth factor precursor and ErbB4 and regulates female reproductive organ remodeling. *Genes Dev.* **16**, 307–323 (2002).
4. Mott, J. D. & Werb, Z. Regulation of matrix biology by matrix metalloproteinases. *Curr. Opin. Cell Biol.* **16**, 558–564 (2004).
5. McCawley, L. J. & Matrisian, L. M. Matrix metalloproteinases: they're not just for matrix anymore! *Curr. Opin. Cell Biol.* **13**, 534–540 (2001).
6. Parks, W. C., Wilson, C. L. & Lopez-Boado, Y. S. Matrix metalloproteinases as modulators of inflammation and innate immunity. *Nature Rev. Immunol.* **4**, 617–629 (2004).
This review introduces many aspects of MMPs and links the fields of inflammation and metalloproteinase biology in a cohesive manner.
7. Overall, C. M. & Lopez-Otin, C. Strategies for MMP inhibition in cancer: innovations for the post-trial era. *Nature Rev. Cancer* **2**, 657–672 (2002).
8. White, J. M. ADAMs: modulators of cell–cell and cell–matrix interactions. *Curr. Opin. Cell Biol.* **15**, 598–606 (2003).
9. Blobel, C. P. ADAMs: key components in EGFR signalling and development. *Nature Rev. Mol. Cell Biol.* **6**, 32–43 (2005).
This review considers several aspects of protein ectodomain shedding and discusses several mechanistic aspects of metalloproteinase biology.
10. Murphy, G. *et al.* Role of TIMPs (tissue inhibitors of metalloproteinases) in pericellular proteolysis: the specificity is in the detail. *Biochem. Soc. Symp.* **70**, 65–80 (2003).
11. Baker, A. H., Edwards, D. R. & Murphy, G. Metalloproteinase inhibitors: biological actions and therapeutic opportunities. *J. Cell Sci.* **115**, 3719–3727 (2002).
12. Porter, S., Clark, I. M., Kevorkian, L. & Edwards, D. R. The ADAMTS metalloproteinases. *Biochem. J.* **386**, 15–27 (2005).
13. Brundula, V., Rewcastle, N. B., Metz, L. M., Bernard, C. C. & Yong, V. W. Targeting leukocyte MMPs and transmigration: minocycline as a potential therapy for multiple sclerosis. *Brain* **125**, 1297–1308 (2002).

14. Weaver, A. *et al.* An elevated matrix metalloproteinase in experimental autoimmune encephalomyelitis is protective by affecting Th1/Th2 polarization. *FASEB J.* **19**, 1668–1670 (2005).
15. Karkkainen, I., Rybnikova, E., Pelto-Huikko, M. & Huovila, A. P. Metalloprotease-disintegrin (ADAM) genes are widely and differentially expressed in the adult CNS. *Mol. Cell. Neurosci.* **15**, 547–560 (2000).
The first article to emphasize that several metalloproteinases, particularly those of the ADAM family, are found in the CNS.
16. Yong, V. W., Power, C., Forsyth, P. & Edwards, D. R. Metalloproteinases in biology and pathology of the nervous system. *Nature Rev. Neurosci.* **2**, 502–511 (2001).
Contains a comprehensive list of the detrimental roles of metalloproteinases in the nervous system, and summarizes the roles of metalloproteinases in several neurological diseases.
17. Lo, E. H., Wang, X. & Cuzner, M. L. Extracellular proteolysis in brain injury and inflammation: role for plasminogen activators and matrix metalloproteinases. *J. Neurosci. Res.* **69**, 1–9 (2002).
18. Cunningham, L. A., Wetzel, M. & Rosenberg, G. A. Multiple roles for MMPs and TIMPs in cerebral ischemia. *Glia* **50**, 329–339 (2005).
19. Wells, J. E., Hurlbert, R. J., Fehlings, M. G. & Yong, V. W. Neuroprotection by minocycline facilitates significant recovery from spinal cord injury in mice. *Brain* **126**, 1628–1637 (2003).
20. Toft-Hansen, H., Nuttall, R. K., Edwards, D. R. & Owens, T. Key metalloproteinases are expressed by specific cell types in experimental autoimmune encephalomyelitis. *J. Immunol.* **173**, 5209–5218 (2004).
21. Bar-Or, A. *et al.* Analyses of all matrix metalloproteinase members in leukocytes emphasize monocytes as major inflammatory mediators in multiple sclerosis. *Brain* **126**, 2738–2749 (2003).
22. Power, C. *et al.* Intracerebral hemorrhage induces macrophage activation and matrix metalloproteinases. *Ann. Neurol.* **53**, 731–742 (2003).
23. Wells, J. E. *et al.* An adverse role for matrix metalloproteinase 12 after spinal cord injury in mice. *J. Neurosci.* **23**, 10107–10115 (2003).
24. Opdenakker, G. *et al.* Gelatinase B functions as regulator and effector in leukocyte biology. *J. Leukoc. Biol.* **69**, 851–859 (2001).
25. Noble, L. J., Donovan, F., Igarashi, T., Goussev, S. & Werb, Z. Matrix metalloproteinases limit functional recovery after spinal cord injury by modulation of early vascular events. *J. Neurosci.* **22**, 7526–7535 (2002).
26. Larsen, P. H., Wells, J. E., Stallcup, W. B., Opdenakker, G. & Yong, V. W. Matrix metalloproteinase-9 facilitates remyelination in part by processing the inhibitory NG2 proteoglycan. *J. Neurosci.* **23**, 11127–11135 (2003).
The first report to emphasize that some MMPs, which are upregulated after injuries to the adult CNS, can have beneficial functions.
27. Esparza, J., Kruse, M., Lee, J., Michaud, M. & Madri, J. A. MMP-2 null mice exhibit an early onset and severe experimental autoimmune encephalomyelitis due to an increase in MMP-9 expression and activity. *FASEB J.* **18**, 1682–1691 (2004).
28. Waubant, E. *et al.* Serum MMP-9 and TIMP-1 levels are related to MRI activity in relapsing multiple sclerosis. *Neurology* **53**, 1397–1401 (1999).
29. Correale, J. & de los Milagros Bassani Molinas, M. Temporal variations of adhesion molecules and matrix metalloproteinases in the course of MS. *J. Neuroimmunol.* **140**, 198–209 (2003).
30. Fiotti, N. *et al.* MMP-9 microsatellite polymorphism and multiple sclerosis. *J. Neuroimmunol.* **152**, 147–153 (2004).
31. Kieseier, B. C., Pischel, H., Neuen-Jacob, E., Tourtellotte, W. W. & Hartung, H. P. ADAM-10 and ADAM-17 in the inflamed human CNS. *Glia* **42**, 398–405 (2003).
32. Goertsches, R., Comabella, M., Navarro, A., Perkal, H. & Montalban, X. Genetic association between polymorphisms in the ADAMTS14 gene and multiple sclerosis. *J. Neuroimmunol.* **164**, 140–147 (2005).
33. Pagenstecher, A., Stalder, A. K., Kincaid, C. L., Shapiro, S. D. & Campbell, I. L. Differential expression of matrix metalloproteinase and tissue inhibitor of matrix metalloproteinase genes in the mouse central nervous system in normal and inflammatory states. *Am. J. Pathol.* **152**, 729–741 (1998).
34. Gottschall, P. E. & Deb, S. Regulation of matrix metalloproteinase expressions in astrocytes, microglia and neurons. *Neuroimmunomodulation* **3**, 69–75 (1996).
35. Vecil, G. G. *et al.* Interleukin-1 is a key regulator of matrix metalloproteinase-9 expression in human neurons in culture and following mouse brain trauma *in vivo*. *J. Neurosci. Res.* **61**, 212–224 (2000).
36. Le, D. M. *et al.* Exploitation of astrocytes by glioma cells to facilitate invasiveness: a mechanism involving matrix metalloproteinase-2 and the urokinase-type plasminogen activator-plasmin cascade. *J. Neurosci.* **23**, 4034–4043 (2003).
37. Rosenberg, G. A. *et al.* Immunohistochemistry of matrix metalloproteinases in reperfusion injury to rat brain: activation of MMP-9 linked to stromelysin-1 and microglia in cell cultures. *Brain Res.* **893**, 104–112 (2001).
38. Gu, Z. *et al.* S-nitrosylation of matrix metalloproteinases: signaling pathway to neuronal cell death. *Science* **297**, 1186–1190 (2002).
Reveals a mechanism by which metalloproteinases regulate cell death.
39. McQuibban, G. A. *et al.* Inflammation dampened by gelatinase A cleavage of monocyte chemoattractant protein-3. *Science* **289**, 1202–1206 (2000).
40. Van den Steen, P. E., Proost, P., Wuyts, A., Van Damme, J. & Opdenakker, G. Neutrophil gelatinase B potentiates interleukin-8 tenfold by aminoterminal processing, whereas it degrades CTAP-III, PF-4, and GRO-α and leaves RANTES and MCP-2 intact. *Blood* **96**, 2673–2681 (2000).
41. Li, Q., Park, P. W., Wilson, C. L. & Parks, W. C. Matrilysin shedding of syndecan-1 regulates chemokine mobilization and transepithelial efflux of neutrophils in acute lung injury. *Cell* **111**, 635–646 (2002).
42. Zhang, K. *et al.* HIV-induced metalloproteinase processing of the chemokine stromal cell derived factor-1 causes neurodegeneration. *Nature Neurosci.* **6**, 1064–1071 (2003).
43. Anthony, D. C. *et al.* Matrix metalloproteinase expression in an experimentally-induced DTH model of multiple sclerosis in the rat CNS. *J. Neuroimmunol.* **87**, 62–72 (1998).
44. Newman, T. A. *et al.* T-cell- and macrophage-mediated axon damage in the absence of a CNS-specific immune response: involvement of metalloproteinases. *Brain* **124**, 2203–2214 (2001).
45. Proost, P., Van Damme, J. & Opdenakker, G. Leukocyte gelatinase B cleavage releases encephalitogens from human myelin basic protein. *Biochem. Biophys. Res. Commun.* **192**, 1175–1181 (1993).
46. Ito, A. *et al.* Degradation of interleukin 1β by matrix metalloproteinases. *J. Biol. Chem.* **271**, 14657–14660 (1996).
47. Zalewska, T., Ziemka-Nalecz, M., Sarnowska, A. & Domanska-Janik, K. Involvement of MMPs in delayed neuronal death after global ischemia. *Acta Neurobiol. Exp. (Wars.)* **62**, 53–61 (2002).
48. Bridges, L. C. & Bowditch, R. D. ADAM–integrin interactions: potential integrin regulated ectodomain shedding activity. *Curr. Pharm. Des.* **11**, 837–847 (2005).
49. Chintala, S. K., Zhang, X., Austin, J. S. & Fini, M. E. Deficiency in matrix metalloproteinase gelatinase B (MMP-9) protects against retinal ganglion cell death after optic nerve ligation. *J. Biol. Chem.* **277**, 47461–47468 (2002).
50. Jourquin, J. *et al.* Neuronal activity-dependent increase of net matrix metalloproteinase activity is associated with MMP-9 neurotoxicity after kainate. *Eur. J. Neurosci.* **18**, 1507–1517 (2003).
51. Zhang, X., Cheng, M. & Chintala, S. K. Kainic acid-mediated upregulation of matrix metalloproteinase-9 promotes retinal degeneration. *Invest. Ophthalmol. Vis. Sci.* **45**, 2374–2383 (2004).
52. Vos, C. M. *et al.* Cytotoxicity by matrix metalloprotease-1 in organotypic spinal cord and dissociated neuronal cultures. *Exp. Neurol.* **163**, 324–330 (2000).
53. Conant, K. *et al.* Matrix metalloproteinase 1 interacts with neuronal integrins and stimulates dephosphorylation of Akt. *J. Biol. Chem.* **279**, 8056–8062 (2004).
54. Frolichsthal-Schoeller, P. *et al.* Expression and modulation of matrix metalloproteinase-2 and tissue inhibitors of metalloproteinases in human embryonic CNS stem cells. *Neuroreport* **10**, 345–351 (1999).
55. Heine, W., Conant, K., Griffin, J. W. & Hoke, A. Transplanted neural stem cells promote axonal regeneration through chronically denervated peripheral nerves. *Exp. Neurol.* **189**, 231–240 (2004).
56. Jaworski, D. M. & Fager, N. Regulation of tissue inhibitor of metalloproteinase-3 (Timp-3) mRNA expression during rat CNS development. *J. Neurosci. Res.* **61**, 396–408 (2000).
57. Perez-Martinez, L. & Jaworski, D. M. Tissue inhibitor of metalloproteinase-2 promotes neuronal differentiation by acting as an anti-mitogenic signal. *J. Neurosci.* **25**, 4917–4929 (2005).
58. Alfandari, D. *et al. Xenopus* ADAM 13 is a metalloprotease required for cranial neural crest-cell migration. *Curr. Biol.* **11**, 918–930 (2001).
59. Vaillant, C. *et al.* MMP-9 deficiency affects axonal outgrowth, migration, and apoptosis in the developing cerebellum. *Mol. Cell. Neurosci.* **24**, 395–408 (2003).
60. Ayoub, A. E., Cai, T. Q., Kaplan, R. A. & Luo, J. Developmental expression of matrix metalloproteinases 2 and 9 and their potential role in the histogenesis of the cerebellar cortex. *J. Comp. Neurol.* **481**, 403–415 (2005).
61. Schlondorff, J. & Blobel, C. P. Metalloprotease-disintegrins: modular proteins capable of promoting cell–cell interactions and triggering signals by protein-ectodomain shedding. *J. Cell Sci.* **112**, 3603–3617 (1999).
62. Baron, W., Colognato, H. & ffrench-Constant, C. Integrin–growth factor interactions as regulators of oligodendroglial development and function. *Glia* **49**, 467–479 (2005).
63. Xian, C. J. & Zhou, X. F. EGF family of growth factors: essential roles and functional redundancy in the nerve system. *Front. Biosci.* **9**, 85–92 (2004).
64. Sheffield, J. B., Krasnopolsky, V. & Dehlinger, E. Inhibition of retinal growth cone activity by specific metalloproteinase inhibitors *in vitro*. *Dev. Dyn.* **200**, 79–88 (1994).
65. Machida, C. M., Rodland, K. D., Matrisian, L., Magun, B. E. & Ciment, G. NGF induction of the gene encoding the protease transin accompanies neuronal differentiation in PC12 cells. *Neuron* **2**, 1587–1596 (1989).
66. Nordstrom, L. A., Lochner, J., Yeung, W. & Ciment, G. The metalloproteinase stromelysin-1 (transin) mediates PC12 cell growth cone invasiveness through basal laminae. *Mol. Cell. Neurosci.* **6**, 56–68 (1995).
67. Hayashita-Kinoh, H. *et al.* Membrane-type 5 matrix metalloproteinase is expressed in differentiated neurons and regulates axonal growth. *Cell Growth Differ.* **12**, 573–580 (2001).
68. Sekine-Aizawa, Y. *et al.* Matrix metalloproteinase (MMP) system in brain: identification and characterization of brain-specific MMP highly expressed in cerebellum. *Eur. J. Neurosci.* **13**, 935–948 (2001).
69. Weeks, B. S., Nomizu, M., Ramchandran, R. S., Yamada, Y. & Kleinman, H. K. Laminin-1 and the RKRLQVQLSIRT laminin-1 α1 globular domain peptide stimulate matrix metalloproteinase secretion by PC12 cells. *Exp. Cell Res.* **243**, 375–382 (1998).
70. Fambrough, D., Pan, D., Rubin, G. M. & Goodman, C. S. The cell surface metalloprotease/disintegrin Kuzbanian is required for axonal extension in *Drosophila*. *Proc. Natl Acad. Sci. USA* **93**, 13233–13238 (1996).
71. Leighton, P. A. *et al.* Defining brain wiring patterns and mechanisms through gene trapping in mice. *Nature* **410**, 174–179 (2001).
72. Mitchell, K. J. *et al.* Functional analysis of secreted and transmembrane proteins critical to mouse development. *Nature Genet.* **28**, 241–249 (2001).
73. Goldsmith, A. P., Gossage, S. J. & ffrench-Constant, C. ADAM23 is a cell-surface glycoprotein expressed by central nervous system neurons. *J. Neurosci. Res.* **78**, 647–658 (2004).
74. Webber, C. A., Hocking, J. C., Yong, V. W., Stange, C. L. & McFarlane, S. Metalloproteases and guidance of retinal axons in the developing visual system. *J. Neurosci.* **22**, 8091–8100 (2002).
The first report to demonstrate that the inhibition of metalloproteinases *in vivo* affects axonal guidance and growth.
75. Hattori, M., Osterfield, M. & Flanagan, J. G. Regulated cleavage of a contact-mediated axon repellent. *Science* **289**, 1360–1365 (2000).
Using *in vitro* systems, the authors showed a role for metalloproteinases in axonal guidance.
76. Galko, M. J. & Tessier-Lavigne, M. Function of an axonal chemoattractant modulated by metalloprotease activity. *Science* **289**, 1365–1367 (2000).
77. Schimmelpfeng, K., Gogel, S. & Klambt, C. The function of leak and kuzbanian during growth cone and cell migration. *Mech. Dev.* **106**, 25–36 (2001).
78. Huang, X., Huang, P., Robinson, M. K., Stern, M. J. & Jin, Y. UNC-71, a disintegrin and metalloprotease (ADAM) protein, regulates motor axon guidance and sex myoblast migration in *C. elegans*. *Development* **130**, 3147–3161 (2003).
79. Hehr, C. L., Hocking, J. C. & McFarlane, S. Matrix metalloproteinases are required for retinal ganglion cell axon guidance at select decision points. *Development* **132**, 3371–3379 (2005).
80. Shubayev, V. I. & Myers, R. R. Matrix metalloproteinase-9 promotes nerve growth factor-induced neurite elongation but not new sprout formation *in vitro*. *J. Neurosci. Res.* **77**, 229–239 (2004).
81. Demestre, M. *et al.* Characterisation of matrix metalloproteinases and the effects of a broad-spectrum inhibitor (BB-1101) in peripheral nerve regeneration. *Neuroscience* **124**, 767–779 (2004).
82. Ahmed, Z. *et al.* Matrix metalloproteases: degradation of the inhibitory environment of the transected optic nerve and the scar by regenerating axons. *Mol. Cell. Neurosci.* **28**, 64–78 (2005).

83. Zuo, J., Neubauer, D., Dyess, K., Ferguson, T. A. & Muir, D. Degradation of chondroitin sulfate proteoglycan enhances the neurite-promoting potential of spinal cord tissue. *Exp. Neurol.* **154**, 654–662 (1998)
Introduces the concept that a MMP can be used to remove inhibitory ECM molecules to expose the permissive ones.
84. Szklarczyk, A., Lapinska, J., Rylski, M., McKay, R. D. & Kaczmarek, L. Matrix metalloproteinase-9 undergoes expression and activation during dendritic remodeling in adult hippocampus. *J. Neurosci.* **22**, 920–930 (2002).
85. Kim, H. J., Fillmore, H. L., Reeves, T. M. & Phillips, L. L. Elevation of hippocampal MMP-3 expression and activity during trauma-induced synaptogenesis. *Exp. Neurol.* **192**, 60–72 (2005).
86. Reeves, T. M., Prins, M. L., Zhu, J., Povlishock, J. T. & Phillips, L. L. Matrix metalloproteinase inhibition alters functional and structural correlates of deafferentation-induced sprouting in the dentate gyrus. *J. Neurosci.* **23**, 10182–10189 (2003).
87. Siebert, H., Dippel, N., Mader, M., Weber, F. & Bruck, W. Matrix metalloproteinase expression and inhibition after sciatic nerve axotomy. *J. Neuropathol. Exp. Neurol.* **60**, 85–93 (2001).
88. Bradbury, E. J. *et al.* Chondroitinase ABC promotes functional recovery after spinal cord injury. *Nature* **416**, 636–640 (2002).
89. Ughrin, Y. M., Chen, Z. J. & Levine, J. M. Multiple regions of the NG2 proteoglycan inhibit neurite growth and induce growth cone collapse. *J. Neurosci.* **23**, 175–186 (2003).
90. Lee, D. H., Strittmatter, S. M. & Sah, D. W. Targeting the Nogo receptor to treat central nervous system injuries. *Nature Rev. Drug Discov.* **2**, 872–878 (2003).
91. Weskamp, G. *et al.* Evidence for a critical role of the tumor necrosis factor α convertase (TACE) in ectodomain shedding of the p75 neurotrophin receptor ($p75^{NTR}$). *J. Biol. Chem.* **279**, 4241–4249 (2004).
92. Domeniconi, M. *et al.* MAG induces regulated intramembrane proteolysis of the p75 neurotrophin receptor to inhibit neurite outgrowth. *Neuron* **46**, 849–855 (2005).
93. Walmsley, A. R. *et al.* Zinc metalloproteinase-mediated cleavage of the human Nogo-66 receptor. *J. Cell Sci.* **117**, 4591–4602 (2004).
Lays the foundation for considering the involvement of metalloproteinases in overcoming inhibition of axonal regrowth by Nogos.
94. Walmsley, A. R., Mir, A. K. & Frentzel, S. Ectodomain shedding of human Nogo-66 receptor homologue-1 by zinc metalloproteinases. *Biochem. Biophys. Res. Commun.* **327**, 112–116 (2005).
95. Belien, A. T., Paganetti, P. A. & Schwab, M. E. Membrane-type 1 matrix metalloprotease (MT1-MMP) enables invasive migration of glioma cells in central nervous system white matter. *J. Cell Biol.* **144**, 373–384 (1999).
96. Mannello, F., Luchetti, F., Falcieri, E. & Papa, S. Multiple roles of matrix metalloproteinases during apoptosis. *Apoptosis* **10**, 19–24 (2005)
A thorough review of metalloproteinases in life and death decisions of a cell.
97. Wetzel, M., Rosenberg, G. A. & Cunningham, L. A. Tissue inhibitor of metalloproteinases-3 and matrix metalloproteinase-3 regulate neuronal sensitivity to doxorubicin-induced apoptosis. *Eur. J. Neurosci.* **18**, 1050–1060 (2003).
98. Naus, S. *et al.* Ectodomain shedding of the neural recognition molecule CHL1 by the metalloprotease-disintegrin ADAM8 promotes neurite outgrowth and suppresses neuronal cell death. *J. Biol. Chem.* **279**, 16083–16090 (2004).
99. Fowlkes, J. L. & Winkler, M. K. Exploring the interface between metallo-proteinase activity and growth factor and cytokine bioavailability. *Cytokine Growth Factor Rev.* **13**, 277–287 (2002).
100. Lee, R., Kermani, P., Teng, K. K. & Hempstead, B. L. Regulation of cell survival by secreted proneurotrophins. *Science* **294**, 1945–1948 (2001).
Implicates the metalloproteinases in the biology of the neurotrophins.
101. Hwang, J. J., Park, M. H., Choi, S. Y. & Koh, J. Y. Activation of the Trk signaling pathway by extracellular zinc. Role of metalloproteinases. *J. Biol. Chem.* **280**, 11995–12001 (2005).
102. Franklin, R. J. Why does remyelination fail in multiple sclerosis? *Nature Rev. Neurosci.* **3**, 705–714 (2002).
103. Uhm, J. H., Dooley, N. P., Oh, L. Y. & Yong, V. W. Oligodendrocytes utilize a matrix metalloproteinase, MMP-9, to extend processes along an astrocyte extracellular matrix. *Glia* **22**, 53–63 (1998).
104. Oh, L. Y. *et al.* Matrix metalloproteinase-9/gelatinase B is required for process outgrowth by oligodendrocytes. *J. Neurosci.* **19**, 8464–8475 (1999).
One of the first papers to suggest beneficial roles of metalloproteinases in the nervous system.
105. Larsen, P. H. & Yong, V. W. The expression of matrix metalloproteinase-12 by oligodendrocytes regulates their maturation and morphological differentiation. *J. Neurosci.* **24**, 7597–7603 (2004).
106. Sagane, K. *et al.* Ataxia and peripheral nerve hypomyelination in ADAM22-deficient mice. *BMC Neurosci.* **6**, 33 (2005).
107. Bernstein, H. G. *et al.* ADAM (a disintegrin and metalloprotease) 12 is expressed in rat and human brain and localized to oligodendrocytes. *J. Neurosci. Res.* **75**, 353–360 (2004).
108. John, G. R. *et al.* Multiple sclerosis: re-expression of a developmental pathway that restricts oligodendrocyte maturation. *Nature Med.* **8**, 1115–1121 (2002).
109. Tsai, H. H. *et al.* The chemokine receptor CXCR2 controls positioning of oligodendrocyte precursors in developing spinal cord by arresting their migration. *Cell* **110**, 373–383 (2002).
110. Wang, J. & Tsirka, S. E. Neuroprotection by inhibition of matrix metalloproteinases in a mouse model of intracerebral haemorrhage. *Brain* **128**, 1622–1633 (2005).
111. Stuve, O. *et al.* Interferon β-1b decreases the migration of T lymphocytes *in vitro*: effects on matrix metalloproteinase-9. *Ann. Neurol.* **40**, 853–863 (1996).
112. Leppert, D., Waubant, E., Burk, M. R., Oksenberg, J. R. & Hauser, S. L. Interferon β-1b inhibits gelatinase secretion and *in vitro* migration of human T cells: a possible mechanism for treatment efficacy in multiple sclerosis. *Ann. Neurol.* **40**, 846–852 (1996).
113. Yong, V. W. Differential mechanisms of action of interferon-β and glatiramer aetate in MS. *Neurology* **59**, 802–808 (2002).
114. Yong, V. W. *et al.* The promise of minocycline in neurology. *Lancet Neurol.* **3**, 744–751 (2004).
115. Overall, C. M. *et al.* Protease degradomics: mass spectrometry discovery of protease substrates and the CLIP-CHIP, a dedicated DNA microarray of all human proteases and inhibitors. *Biol. Chem.* **385**, 493–504 (2004).
Lays the foundation for a key area of future metalloproteinase research, that of identifying substrates of metalloproteinases in particular contexts.
116. Asahi, M., Sumii, T., Fini, M. E., Itohara, S. & Lo, E. H. Matrix metalloproteinase 2 gene knockout has no effect on acute brain injury after focal ischemia. *Neuroreport* **12**, 3003–3007 (2001).
117. Takahashi, M. *et al. In vivo* glioma growth requires host-derived matrix metalloproteinase 2 for maintenance of angioarchitecture. *Pharmacol. Res.* **46**, 155–163 (2002).
118. Dubois, B. *et al.* Resistance of young gelatinase B-deficient mice to experimental autoimmune encephalomyelitis and necrotizing tail lesions. *J. Clin. Invest.* **104**, 1507–1515 (1999).
119. Wang, X. *et al.* Effects of matrix metalloproteinase-9 gene knock-out on morphological and motor outcomes after traumatic brain injury. *J. Neurosci.* **20**, 7037–7042 (2000).
120. Asahi, M. *et al.* Effects of matrix metalloproteinase-9 gene knock-out on the proteolysis of blood–brain barrier and white matter components after cerebral ischemia. *J. Neurosci.* **21**, 7724–7732 (2001).
121. Tang, J. *et al.* Mmp-9 deficiency enhances collagenase-induced intracerebral hemorrhage and brain injury in mutant mice. *J. Cereb. Blood Flow Metab.* **24**, 1133–1134 (2004).
122. Wells, J. E. A., Biernaskie, J., Szymanska, A., Corbett, D. R. & Yong, V. W. Matrix metalloproteinase (MMP)-12 expression has a negative impact on sensorimotor function following intracerebral haemorrhage in mice. *Eur. J. Neurosci.* **21**, 187–196 (2005).

Acknowledgements

The author wishes to thank the numerous trainees who worked on metalloproteinase biology while in the Yong laboratory. A grateful acknowledgement is also due to funding agencies, particularly the Canadian Institutes of Health Research and the Multiple Sclerosis Society of Canada. The author thanks R. Nuttall, A. Fournier and D. Edward for reading through parts of this manuscript, and F. Yong for help with the figures.

Competing interests statement

The author declares no competing financial interests.

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