Published in final edited form as:
Mol Aspects Med. 2008 October ; 29(5): 309–328. doi:10.1016/j.mam.2008.08.002.

Meprins, membrane-bound and secreted astacin metalloproteinases

Erwin E. Sterchi,
Institute of Biochemistry and Molecular Biology, University of Berne, CH-3012 Berne Switzerland. e-mail: erwin.sterchi@mci.unibe.ch

Walter Stöcker, and
Department of Cell and Matrix Biology, Institute of Zoology, Johannes Gutenberg University, D-55099 Mainz, Germany. e-mail: stoecker@uni-mainz.de

Judith S. Bond
Department of Biochemistry and Molecular Biology, The Pennsylvania State University, Hershey PA, USA 17033-0850. e-mail: jbond@psu.edu

Abstract

The astacins are a subfamily of the metzincin superfamily of metalloproteinases. The first to be characterized was the crayfish enzyme astacin. To date more than 200 members of this family have been identified in species ranging from bacteria to humans. Astacins are involved in developmental morphogenesis, matrix assembly, tissue differentiation and digestion. Family members include the procollagen C-proteinase (BMP1, bone morphogenetic protein 1), tolloid and mammalian tolloid-like, HMP (*Hydra vulgaris* metalloproteinase), sea urchin BP10 (blastula protein) and SPAN (*Strongylocentrotus purpuratus astacin*), the 'hatching' subfamily comprising alveolin, ovastacin, LCE, HCE ('low' and 'high' choriolytic enzymes), nephrosin (from carp head kidney), UVS.2 from frog, and the meprins. In the human and mouse genomes, there are six astacin family genes (two meprins, three BMP1/tolloid-like, one ovastacin), but in *Caenorhabditis elegans* there are 40.

Meprins are the only astacin proteinases that function on the membrane and extracellularly by virtue of the fact that they can be membrane-bound or secreted. They are unique in their domain structure and covalent subunit dimerization, oligomerization propensities, and expression patterns. They are normally highly regulated at the transcriptional and post-translational levels, localize to specific membranes or extracellular spaces, and can hydrolyse biologically active peptides, cytokines, extracellular matrix (ECM) proteins and cell-surface proteins. The *in vivo* substrates of meprins are unknown, but the abundant expression of these proteinases in the epithelial cells of the intestine, kidney and skin provide clues to their functions.

Keywords

metalloproteinase; metcincins; astacin; meprin; kidney; intestine; skin; epithelial cells

Address for correspondence: Erwin E. Sterchi, Institute of Biochemistry and Molecular Medicine, University of Bern, Buehlstrasse 28, CH-3012 Bern, Switzerland, Phone: +41 31 631 4199, Fax: +41 31 631 3737, e-mail: erwin.sterchi@mci.unibe.ch.

1. Introduction

Proteinases form the largest enzyme gene family in vertebrates (Fig. 1) (Lopez-Otin and Bond 2008). According to the Merops database (http://merops.sanger.ac.uk) they comprise 641 genes in the human and 677 in the mouse. Over 200 genes encode metalloproteinases, the majority of which are active in tissue differentiation and remodelling both during embryogenesis and in the adult as well as in various diseases. Most metalloproteinases are members of the metzincin superfamily (Stöcker, Grams et al. 1995). These enzymes share the conserved HExxHxxG/NxxH/D zinc binding sequence in their active site, a conserved methionine-containing turn (Met-turn) backing the zinc site and, despite low amino acid sequence identity, strikingly similar three dimensional structures of their catalytic domains (Fig. 1).

Typical metzincins are the ADAMs (a disintegrin and metalloproteinase), the MMPs (matrix metalloproteinases), the pappalysins (pregnancy associated plasma proteins), the serralysins (bacterial enzymes), the leishmanolysins (protozoan and metazoan proteinases), and the astacins (Gomis-Rüth 2003). In the last two decades research on metalloproteinases has been mostly focussed on MMP and ADAM proteinases due to their importance for pericellular proteolysis and their involvement in diseases such as cancer and rheumatoid arthritis. Recently, however, other groups of metzincins have also gained interest. These include the pappalysins (e.g. PAPP A pregnancy associated plasma protein A; (Glerup, Boldt et al. 2005)), the leishmanolysins (e.g. *Drosophila* invadolysin (McHugh, Krause et al. 2004)) and the astacins (Ge and Greenspan 2006), due to their roles in cell proliferation, cell migration, developmental patterning and tissue assembly.

1.1. Astacin Metalloproteinases

Todate, several hundred astacins have been identified in animals and bacteria, none as yet in plants and fungi. The number is increasing rapidly due to ongoing genome sequencing projects (for reviews cf. (Bond and Beynon 1995) (Stöcker, Grams et al. 1995; Gomis-Rüth 2003) (Bertenshaw and Bond 2004; Stöcker and Yiallouros 2004); the MEROPS proteinase database provides a comprehensive overview: http://merops.sanger.ac.uk; (Barrett, Rawlings et al. 2001) (Fig. 1). They are named after the digestive proteinase astacin from the crayfish *Astacus astacus* (Dumermuth, Sterchi et al. 1991) (Stöcker and Zwilling 1995). Most members of this family are secreted enzymes, which are found throughout the animal kingdom and also in some bacteria.

There are astacins, which are present in almost all animal phyla such as the tolloid family, but there are also others that seem to be restricted to distinct taxa of animals (Möhrlen, Hutter et al. 2003; Möhrlen, Maniura et al. 2006). The number of astacin genes in different species varies considerably. For example, in the human and mouse genomes, there are 6 astacin family genes including two meprins, three BMP1/tolloid-like and one ovastacin (Fig. 1), but in *Drosophila melanogaster* there are 16 and in *Caenorhabditis elegans* 40 astacins of which only a few have clearly assigned functions. Nematode parasites such as trichina and hookworms secrete a variety of unique astacins which are thought to pave the way through their host's extracellular matrix and which have not been observed so far outside the taxon nemathelmintes (Lun, Mak et al. 2003; De Maere, Vercauteren et al. 2005; Borchert, Becker-Pauly et al. 2007). Other proteinases are specifically observed in cnidarian species such as *Hydra vulgaris* (Yan, Fei et al. 2000; Yan, Leontovich et al. 2000), *Hydractinia echinata* (Möhrlen, Maniura et al. 2006) and *Podecoryne carnea* (Pan, Groger et al. 1998). As an example, *Hydra* metalloproteinase 1 (HMP-1) is involved in head regeneration and trans-differentiation of tentacle battery cells (Yan, Leontovich et al. 2000).

The vast majority of astacins have not been analyzed on the functional level but their localization and their domain structure can often be used to get clues about their physiological roles.

Crayfish astacin consists of a prodomain, which is cleaved off for activation (Yiallouros, Kappelhoff et al. 2002) and a 200 amino acid residue catalytic domain (Bode, Gomis-Rüth et al. 1992). There are functionally diverse astacin proteinases that merely have this minimal domain structure. However, most eumetazoan astacins are multidomain proteins that carry complex carboxyterminal elongations in addition to the astacin domain. These enzymes can be classified into three major groups, based on these elongations, which more or less also reflect different physiological functions (tolloids/BMP1 proteinases, hatching enzymes and meprin proteinases).

The **tolloid/BMP1** proteinases are named after the dorsoventral patterning protein from *Drosophila* (Shimell, Ferguson et al. 1991) and its mammalian counterpart BMP-1 (mTld; i.e. bone morphogenetic protein 1; mammalian tolloid; (Wozney, Rosen et al. 1988; Takahara, Lee et al. 1995). BMP-1 of vertebrates is identical with the procollagen C-proteinase (Kessler, Takahara et al. 1996; Li, Sieron et al. 1996), which activates pro-collagens, pro-proteoglycans and other precursors of matrix proteins and thereby triggers proper assembly of the extracellular matrix (Ge and Greenspan 2006; Hopkins, Keles et al. 2007). In addition, tolloids also cleave growth factors and their antagonists, which is crucial for dorso-ventral patterning in the embryo (Holley, Neul et al. 1996). Typically, tolloids are characterized by C-terminal CUB and EGF-like domains, which are thought to be important for substrate recognition and binding (Sieron, Tretiakova et al. 2000; Garrigue-Antar, Francois et al. 2004; Hintze, Höwel et al. 2006; Wermter, Höwel et al. 2007). By virtue of their similar but not identical domain composition, sea urchin astacins like SPAN and BP10 (blastula protein) are related to the tolloids (Lepage, Ghiglione et al. 1992; Reynolds, Angerer et al. 1992).

Another large and diverse group of astacins are the so called **hatching enzymes**, which degrade embryonic envelopes during hatching of crustaceans, fishes, frogs, birds and mammals. The proteinases of this heterogeneous group often contain C-terminal cysteine-rich and CUB domains, yet some consist of a single catalytic domain. Examples are crayfish embryonic astacin (Geier and Zwilling 1998), fish alveolin (Shibata, Iwamatsu et al. 2000), LCE and HCE ('low' and 'high' choriolytic enzymes from medaka), (Yasumasu, Shimada et al. 1996), nephrosin (from carp head kidney) (Hung, Huang et al. 1997), UVS.2 from frog (Fan and Katagiri 2001), CAM1 from birds (Elaroussi and DeLuca 1994) and mammalian ovastacin (Quesada, Sanchez et al. 2004).

The third defined group of astacin proteinases, the **meprins**, are distinguished due to their unique domain structure that includes a transmembrane domain. In contrast to other astacins, meprins have been discovered exclusively in vertebrates (human, mouse, rat, zebra fish) (Beynon, Shannon et al. 1981; Sterchi, Green et al. 1982; Kenny and Ingram 1987; Schötte, Lottaz et al. 2007).

This review will focus on the structure, function, regulation and expression of meprins. The abundance and location of meprin metalloproteinases in the kidney, intestine, skin and in leukocytes and certain cancer cells indicates functions at the interface of the host and external environment and in cell migration.

1.2. A short history of meprin metalloproteinases

The meprins were discovered in the 1980s as membrane-bound metalloproteinases in mouse and rat kidneys (Beynon, Shannon et al. 1981; Barnes, Ingram et al. 1989) They were distinct from other proteinases that had previously been described in the kidney in many ways

(molecular size, ability to cleave large proteins such as azocasein at neutral and basic pH values, localization to brush border membranes of kidney). In 1983, it was found that while kidneys of random bred mice and most inbred strains of mice had high meprin activity, certain inbred strains of mice had 'low activity' of meprin in the kidney (Beynon and Bond 1983). This led to the identification of two meprin subunits (α and β), and to the realization that meprin β was primarily latent in the mouse kidney (Butler and Bond 1988). It is now known that all mouse strains express meprin β, but only a subset express meprin α in the kidney. Meanwhile, an enzyme was discovered in rat and human intestine that hydrolyzed N-benzoyl-L-tyrosyl-p-aminobenzoic acid (PABA-peptide), a chymotrypsin substrate used in clinical medicine to assess exocrine pancreas function (Sterchi, Green et al. 1982; Sterchi, Green et al. 1983). Cloning and sequencing of the proteinase domains of mouse meprin and human PPH led to the realization that these proteins were 90% identical in amino acid sequence and were therefore orthologues. In addition`, homology to the catalytic domain of BMP-1 led to the identification of the 'astacin family of metalloproteinases (Dumermuth, Sterchi et al. 1991; Jiang, Gorbea et al. 1992; Dumermuth, Eldering et al. 1993; Bond and Beynon 1995).

The multidomain, evolutionarily related α and β subunits of meprins form disulphide-linked homo- or heterodimers (Bond and Beynon 1995). The heterodimers tend to form tetramers ($\alpha_2\beta_2$, $\alpha_3\beta_1$), whereas the meprin α homodimers form high molecular mass multimers of up to 6 million Daltons, among the largest extracellular proteolytic complexes known (Marchand, Tang et al. 1994; Becker, Kruse et al. 2003; Bertenshaw, Norcum et al. 2003). By contrast, only dimers of homooligomeric meprin β are formed. So, although the meprin α and β subunits have similar domain structures and are 42% identical at the amino acid level, they differ markedly in their ability to self-associate: the meprin α homodimer has a strong homophilic propensity, whereas the meprin β homodimer tends to form heterophilic interactions (Villa, Bertenshaw et al. 2003). There are several other important differences between the meprin α and β subunits with respect to proteolytic processing, substrate specificity, tissue expression patterns, and disease implications, which will be discussed later.

There is one gene each for the α and β subunit, and the two meprin genes give rise to different proteinases composed either of homodimers or a heterodimers with different properties, depending on whether or not they are coexpressed. The meprin subunits are capable of cleaving a wide range of substrates, from bioactive peptides to extracellular matrix proteins, using novel ways to concentrate their proteolytic activity at the cell surface and in extracellular compartments. As is implicit from the circumstances in which they were discovered, the proteinases are abundantly expressed in epithelial cells of the kidney tubules and intestine, and normally function in the lumen of these organs. Only one endogenous inhibitor, mannan-binding lectin, of these proteinases has been identified (Hirano, Ma et al. 2005). However, regulation of meprin is primarily achieved at the level of protein translation in specific cell types, targeting to specific extracellular compartments, proteolytic activation of the proenzymes, and concentration of the proteinases at specific locales.

2. Meprins

2.1. Structure of Meprins

2.1.1. Domain composition of meprin subunits—Meprins are translated as a unique combination of domains, including a signal sequence, a prodomain, a astacin-like proteinase domain, a MAM domain (meprin, A5 protein, receptor protein tyrosine phosphatase μ), a TRAF domain (tumor necrosis factor (TNF) receptor-associated factor), an EGF (epidermal growth factor)-like damain, a transmembrane and a cytosolic domain (Fig. 2). The signal sequence directs the protein into the rough endoplasmic reticulum (ER), the prodomain confers latency to these proteinases and has to be cleaved off for activation.

The catalytic (proteinase) domain of meprins comprises about 200 amino acid residues and shares 34% sequence identity with the crayfish proteinase astacin, the three-dimensional structure of which is known (Bode, Gomis-Rüth et al. 1992). An astacin-based homology model of the catalytic domains of meprins is shown in figure 3 (Stöcker, Gomis-Rüth et al. 1993). The model displays a five-stranded β-sheet, three α-helices and a coil structure in the lower subdomain as well as a typical Met-turn characteristic to all the metzincin metalloproteinases. The catalytic zinc is penta-coordinated by three histidines, the catalytic water and a tyrosine positioned by the Met-turn. The catalytic domain is stabilized by two intra-domain disulfide-bridges and several conserved salt bridges.

The MAM domain is present in a number of functionally diverse proteins such as neuropilins (Nakamura and Goshima 2002) and zonadhesins (Herlyn and Zischler 2005) and is thought to be involved in cell adhesion, protein–protein interactions and signal transduction (Beckmann and Bork 1993; Zondag, Koningstein et al. 1995; Aricescu, Hon et al. 2006). In neuropilins, the MAM domain participates in homo-oligomerization (Chen, He et al. 1998) and this is also the case in meprins. There are other astacins, like squid ‘myosinases’ (Tajima, Yokozawa et al. 1999; Yokozawa, Tamai et al. 2002), HMP2 from *Hydra* (Yan, Fei et al. 2000) and an enzyme from the sea anemone from *Nematostella vectensis* (Putnam, Srivastava et al. 2007) that share with meprins the presence of a MAM domain. However, their overall structure is different from that of meprins, which implies also different functions. They are not membrane anchored and they do not form dimers via their MAM domains as meprins do by an additional free cysteine.

The TRAF domain is found in several intracellular proteins and it has been suggested that it is involved in protein associations and signal transduction (Cheng, Cleary et al. 1995; Uren and Vaux 1996; Zapata, Pawlowski et al. 2001). Meprins are the only membrane proteins known so far that have a TRAF domain in their ectodomain. On the basis of structural similarities with other TRAFs, the domain is thought to be involved in the oligomeric association of meprins (Sunnerhagen, Pursglove et al. 2002; Zapata, Martinez-Garcia et al. 2007)

The C-terminal transmembrane domain anchors the meprins to the luminal side of the ER and, in the case of meprinβ, to the plasma membrane. The α subunit differs from the β subunit by the presence of an inserted (I) domain immediately N-terminal to the EGF-like domain. This domain is essential for the posttranslational proteolytic processing, which results in the loss of the cytosolic, the transmembrane and the EGF-like domains in meprin α (Grünberg, Luginbuhl et al. 1992; Marchand, Tang et al. 1995; Hahn, Lottaz et al. 1997). Therefore, meprin α is secreted if not coexpressed with β (Lottaz, Hahn et al. 1999).

The cytosolic regions of meprin α and β differ in several respects. While the cytosolic sequence of human meprin α consists of just 6 amino acids (SQRPRK) that of human meprin β is 28 amino acids (SVYCTRK KYRERMSSNRPNLTPQNQHAF) long. The human meprin β cytosolic region has a phosphorylation site on Ser687 within a PKC consensus sequence (Hahn, Pischitzis et al. 2003) and other potential interaction sequences for intracellular proteins.

2.1.2. Quaternary structure—Meprins are organized in extremely complex quaternary structures that vary considerably depending on whether or not the α and β subunits are co-expressed. If they are expressed alone, meprin β subunits form membrane-bound homodimers, which are crosslinked by two disulphide bonds between their MAM domains and an additional disulphide bridge between the TRAF domains (Fig. 4) (Ishmael, Shier et al. 2005). This arrangement of disulphide bonds is so delicately tuned that the exchange of any of the six cysteine residues involved for an alanine residue will preclude the formation of any intersubunit disulphide bridges. Meprin α subunits also form disulphide-linked dimers. However, mouse meprin α only has two intersubunit disulphide bonds because the third cysteine, which is

conserved in the meprin β TRAF domain, is missing. Human meprin α has an additional, unpaired TRAF cysteine that could also be involved in dimerization. In contrast to meprin β, α homodimers can interact noncovalently to form higher order structures, which represent the largest proteinases observed so far (Fig. 5). The largest meprin α homooligomers observed thus far are in the range of 1 to 6 mDa (Becker, Kruse et al. 2003;Bertenshaw, Norcum et al. 2003). When both subunits are coexpressed, meprin α and β subunits form predominantly disulphide-bonded heterodimers (see also figure 3), which can associate with other heterodimers or with α-subunit homodimers ($\alpha_2\beta_2$ and $\alpha_3\beta_1$) through homophilic interaction of their α subunits (Marchand, Tang et al. 1994;Bertenshaw, Norcum et al. 2003).

Meprins are expressed as monomers when any one of the cysteine residues that are involved in intersubunit crosslinking is mutated (Chevallier, Ahn et al. 1996; Marchand, Volkmann et al. 1996). For some of these mutants noncovalent, proteolytically active homooligomers form, but these are much less stable than their wild-type counterparts (Ishmael, Norcum et al. 2001; Ishmael, Shier et al. 2005). The disulphide-linked dimers are therefore essential for stability and the formation of higher-ordered structures. This could be important in the harsh environment of the intestinal and urinary tract lumens, and at sites of inflammation, infection and tumours. Both meprin subunits are heavily glycosylated, and can have a carbohydrate content of up to 25% of the total mass (Kounnas, Wolz et al. 1991; Dumermuth, Eldering et al. 1993; Grünberg, Dumermuth et al. 1993; Kadowaki, Tsukuba et al. 2000). Most of the oligosaccharides are N-linked but in the human β-subunit there are also O-linked glycans (Leuenberger, Hahn et al. 2003). A mutational analysis of the 10 potentially glycosylated asparagine residues in mouse meprin α and inhibition of O-glycosylation in human meprin β showed that the sugars are necessary for secretion and proteolytic activity (Kadowaki, Tsukuba et al. 2000; Leuenberger, Hahn et al. 2003). Mutational analyses has demonstrated that no single glycan is essential for oligomerization of homomeric meprin α; however, the absence of two glycans in the proteinase domain prevented intersubunit disulfide bond formation, noncovalent subunit associations and abolished enzymatic activity indicating these glycans are essential for correct folding (Ishmael, Ishmael et al. 2006).

2.1.3. Proenzyme activation—In contrast to other metzincins such as the matrix metalloproteinases (MMPs) and the ADAMs, meprins do not contain a ‘cysteine switch’ mechanism. Meprins are not capable of self-activation and require trypsin-like proteinases for proteolytic removal of the N-terminal prodomain (Grünberg, Dumermuth et al. 1993; Johnson and Bond 1997). Upon activation, the new N-terminus is salt-bridged with a glutamic acid residue that follows immediately after the third zinc-binding histidine (Bode, Gomis-Rüth et al. 1992). This conformation cannot be adopted by the inactive proprotein, which is elongated N-terminally by about 40 residues. The salt bridge is not a prerequisite for activity, however, but does seem to be extremely important for stability because mutant proteins in which the salt bridge was disrupted by site directed mutagenesis were considerably more heat labile than the wildtype protein (Johnson and Bond 1997; Yiallouros, Kappelhoff et al. 2002). Plasmin has also been identified as an activator of human promeprin β at cancer sites outside the gut, but it does not activate human promeprin β (Becker, Kruse et al. 2003). Most likely, this is because plasmin is about twice the size of trypsin and therefore cannot gain access to the activation site of the β subunit. This is consistent with a model of the β-subunit homodimer derived from studies using crosslinking and mutagenesis (Fig. 4). Differential activation of meprin subunits might occur through the action of kallikreins in tissues like skin. For example, human kallikrein-4 is a trypsin like enzyme, but it has a much higher preference for human promeprin β than for α (Becker-Pauly, Höwel et al. 2007).

2.2. Genomic structure of meprin α and β

Both meprin subunits are encoded by single genes in the mouse and the human genomes (Bond, Beynon et al. 1984; Bond and Beynon 1985;Gorbea, Marchand et al. 1993; Jiang, Sadler et al. 1993; Bond and Beynon 1995; Hahn, Illisson et al. 2000). The mouse structural gene for meprin α *Mep-1α*, is localized on chromosome 17 and the human gene, *MEP-1A*, on chromosome 6, near the major histocompatibility complex. The genes for the mouse and human meprin β subunits are both localized to chromosome 18. It is likely that the genes for the two subunits derived from a common ancestral gene that duplicated and diverged. The human gene for the α subunit is organized in 14 exons and 13 introns and spans about 40 kb (Hahn, Illisson et al. 2000; Jiang, Kumar et al. 2000) (Fig. 6). The gene for the meprin β subunit is approximately 27 kb in size and contains 15 exons. A comparison of both genes shows strong structural similarities — the exons are almost identical in size with the exception of exon 13 in meprin α, which codes for the additional I (inserted)-domain that is not present in β. The main difference is in the length of the introns. The genomic organization of the proteinase domains of human and mouse meprin α and β match in the positions and phases of the introns, and some of the introns are also conserved in other members of the astacin family of metalloproteinases (Finelli, Bossie et al. 1994; Takahara, Lee et al. 1995; Yasumasu, Shimada et al. 1996; Geier, Jacob et al. 1997).

The genes for the meprin α and β subunit can be transcribed independently or coordinately. For example, in the adult human colon only meprin α is expressed; by contrast, in the adult kidney of certain strains of inbred mice (e.g., C3H/He), only meprin α is expressed. In addition, in meprin β knockout mice, meprin α mRNA expression is unaffected in the kidney (Norman, Jiang et al. 2003). However, there are examples of coordinate expression of the meprin α and β genes that result in heterooligomeric meprin complexes (e.g., in the human small intestine, and in kidney of random bred and most inbred mouse strains).

The 5’ regions upstream of the transcription initiation sites for both meprin α and β genes contain putative binding sites for transcription factors that are expressed in different cells and tissues, and at different developmental stages. For example, the human meprin α promoter contains binding sites for GATA transcription factors that are expressed in developing and adult intestine and that have been implicated in the activation of a number of intestinal genes (Gao, Sedgwick et al. 1998; Dusing and Wiginton 2005). GATA-6 is also expressed in Caco-2 cells, a human colon carcinoma cell line that endogenously expresses meprin α, and only the meprin α promoter is capable of directing luciferase expression in Caco-2 cells. This indicates that GATA factors are involved in the tissue-specific expression of meprin α (Hahn, Illisson et al. 2000). There are also intestine-specific elements in the promoter region of the meprin β gene (Matters and Bond 1999). Interestingly, no kidney-specific elements have been identified in the 5’ upstream region of the meprin genes.

Some cancer cell lines (such as F9, Nulli SSC1, HT29-18-C_1) contain a larger mRNA species for meprin β, termed meprin β’ (Finelli, Bossie et al. 1994). In the mouse, the 5’ end of meprin β’ mRNA is encoded by an additional exon and the meprin β’ isoform is the result of alternative splicing of the 5’ exons. So independent promoter usage leads to meprin β expression in normal mouse tissues and meprin β’ expression in cancer cells (Matters and Bond 1999). In humans, a single exon contains the sequences of both the β and β’ mRNAs. Because of this, the transcription start used by the β mRNA is encoded by the same DNA sequence that also partially encodes the human β’ mRNA 5’ UTR. Mouse and human cells therefore employ a different strategy for generating the meprin β and meprin β’ mRNAs (Jiang and Flannery 1997; Matters and Bond 1999).

The promoter region of human meprin β contains potential transcription factor binding sites for activator protein-1 (AP-1), polyoma enhancer activator 3 (PEA3), CCAAT enhancer-

binding protein β and the oestrogen-receptor, as well as intestine-specific cdx-2 transcription factor (Matters and Bond 1999). Meprin β and β' promoter regions that contain PEA3 and AP-1 binding sites showed the highest activity in a luciferase reporter assay, indicating that the two promoter elements might have important functions in the regulation of meprin β expression (Gutman and Wasylyk 1990).

Both meprin α and β promoter regions also contain putative binding sites for steroid receptors. Meprin α shows high levels of mRNA expression during the suckling period of rats which then decline at weaning. In contrast, meprin β mRNA expression is low during the suckling period and is increased after weaning. For brush-border hydrolases that show increased expression at the time of weaning there is a large body of evidence showing that their expression can be activated precociously by glucocorticoids (Henning 1985),and this is also the case for meprin β mRNA (Henning, Oesterreicher et al. 1999). Steroid receptor binding sites may also be of importance in the context of a possible role of meprin in intestinal inflammatory disorders and their treatment with steroids (Lottaz, Hahn et al. 1999; Crisman, Zhang et al. 2004).

2.3. Substrate catalysis and inhibitor binding

2.3.1. Substrates—In the active site of astacin proteinases, substrates bind in an elongated manner, and this binding involves at least four amino acid residues on either side of the scissile peptide bond (Stöcker, Ng et al. 1990; Grams, Dive et al. 1996; Yiallouros, Vassiliou et al. 1998). Meprin α and β have strikingly different cleavage and substrate specificities (Fig. 7); they differ especially in the binding pocket, which harbours the residue directly C terminal to the cleaved bond in peptide substrates. In this pocket, meprin β prefers in negatively charged side chains of aspartic acid and glutamic acid, whereas meprin α binds neutral aliphatic and aromatic side chains (Bertenshaw, Villa et al. 2002; Villa, Bertenshaw et al. 2003; Kruse, Becker et al. 2004). In addition, meprin α exhibits a strong preference for proline two amino acids away from the scissile peptide bond, which is similar to the specificity of the prototype crayfish astacin (Stöcker, Ng et al. 1990; Grams, Dive et al. 1996; Yiallouros, Vassiliou et al. 1998).

Meprin subunits cleave a variety of biologically active peptides (Butler, McKay et al. 1987; Kenny and Ingram 1987; Sterchi, Naim et al. 1988; Wolz, Harris et al. 1991; Yamaguchi, Kido et al. 1991; Kaushal, Walker et al. 1994; Walker, Kaushal et al. 1998; Köhler, Kruse et al. 2000; Bertenshaw, Turk et al. 2001; Kruse, Becker et al. 2004). Gastrointestinal peptides like gastrin and cholecystokinin are among the best substrates for the meprin β subunit. Both peptides have a high content of negatively charged amino acid residues, which makes them excellent β substrates. On the other hand, substance P, a modulator of nociception, and cytokines are good substrates for meprin α (Norman, Matters et al. 2003). Meprins also cleave many cytokines and chemokines, some of which are grossly degraded while others are processed in a limited way that may lead to an alteration in biological function/activity. One such example is the processing of the interleukin-1β precursor by meprin β which results in the generation of an active form of this pro-inflammatory cytokine as was confirmed by measuring the proliferative response of helper T-cells (Herzog, Kaushal et al. 2005).

Of particular interest is the fact that meprins are capable of cleaving proteins of the extracellular matrix (ECM), in particular the basal lamina, as well as proteins that are involved in interactions between cells (Walker, Kaushal et al. 1998; Köhler, Kruse et al. 2000; Kruse, Becker et al. 2004; Ambort, Stalder et al. 2008) or between neighboring cells (Huguenin, Muller et al. 2008). Basal lamina proteins cleaved by meprins include collagen type IV, laminin-1, nidogen-1 and fibronectin (Köhler, Kruse et al. 2000; Kruse, Becker et al. 2004). While collagen IV is degraded completely by both meprin α and meprin β, human nidogen-1 and fibronectin are proteolytically cut by both meprin subunits at several sites. Nidogen-1, which connects laminins with the collagen IV network (Liddington 2001), is cleaved in the globular

G1 domain and in the linker between the G1 and G2 domains, and fibronectin is cut within the proteolytically vulnerable linker regions between fibronectin type III repeats 1 and 2, 3 and 4, and 5 and 6 (Pankov and Yamada 2002).

One example for the processing of proteins involved in cell-cell interaction is the processing of E-cadherin in epithelial cells that express meprin β. The extracellular cleavage of E-cadherin in meprin β-expressing MDCK cells leads to a weakening of adhesion between cells (Huguenin, Muller et al. 2008). A newer study indicates that other proteins involved in the maintenance and/or generation of of the extracellular matrix, such as lysyl oxidase, are also cleaved by meprins (Ambort, Stalder et al. 2008).

Comparisons between the mouse and human homomeric meprin α have shown that there are some species differences in substrate specificity and inhibitor potency (Bylander, Bertenshaw et al. 2007). For example, the human enzyme had lower activity against gelatin, bradykinin, α-melanocytes-timulating hormone and neurotensin, and a higher activity against secretin and orcokinin compared to the mouse enzyme. The human enzyme also shows a preference for acidic residues in the P1' position of the substrate unlike the mouse enzyme. Thus, while the mouse and human meprin α subunits are approximately 90% identical in amino acid sequenece, they display some specific differences in their catalytic sites.

2.3.2. Inhibitors—The most potent exogenous meprin inhibitor identified to date is a natural compound from *Streptomyces* termed actinonin, which binds to human meprin α with a K_i value of 20 nM (Kruse, Becker et al. 2004). The hydroxamate actinonin is about 36-fold more inhibitory for the human enzyme, compared to the mouse enzyme, whereas an analog of actinonin, CONA-65, is >600-fold more inhibitory for the mouse compared to the human enzyme (Bylander, Bertenshaw et al. 2007). Moreover, actinonin is a much more potent inhibitor of the meprin α activity than meprin β; the latter has a Ki of approximately 300 nM. Actinonin might therefore be useful as a lead compound for the development of selective, subunit-specific inhibitors. Meprins are also inhibited by a variety of peptide analogue inhibitors that were originally developed for the inhibition of MMPs (such as collagenases and gelatinases) or ADAMs (such as tumour necrosis factor-α (TNFα)-converting enzyme (TACE) (Kruse, Becker et al. 2004) (Fig. 7). Inhibitor binding reflects the different cleavage specificities of the two subunits. For example, the binding constant of the inhibitor Ro-327315 for meprin α and β differs by three orders of magnitude (Kruse, Becker et al. 2004).

Because meprins are inhibited by certain MMP and TACE inhibitors that have been used in human trials, the physiological functions of meprins might be impaired unwantedly in therapeutic approaches for the treatment of cancer or rheumatoid arthritis. On the other hand, if more selective inhibitors of MMPs and ADAMs were used, which would not affect meprins, this might retain the capability of certain epithelial tumour cells to invade through basal membranes into stromal tissues.

Meprins are not inhibited by the TIMPs (tissue inhibitors of metalloproteinases), and they are resistant to inhibition by the general scavenger α_2-macroglobulin (Kruse, Becker et al. 2004). The latter being an unspecific plasma inhibitor, which utilizes a unique trapping mechanism for removing a variety of endopeptidases from the circulation regardless their catalytic mechanism. However, there are indications for the presence of meprin inhibitors in serum, the identity of which is not known. The only endogenous inhibitor known for meprins so far is mannan-binding protein (MBP; also referred to as mannan-binding lectin, MBL) (Hirano, Ma et al. 2005). The interaction of MBP with meprins via high mannose- or complex- type N-glycans leads to the inhibition of the proteolytic activity and suggests that N-linked oligosaccharides are important for enzymatic activity. It has been suggested that MBP may be employed as a therapeutic agent metastatic disease, acute renal failure or inflammatory bowel

disease (Hirano, Ma et al. 2005). MBP is mainly found in the serum and at inflammation sites, and may play a role in regulating meprin activity at those sites. However, the primary mechanisms for regulation of meprins appear to be targeting to specific locations (e.g., the apical membrane of polarized cells), activation by proteolytic removal of the prosequence, and transcriptional regulation.

2.4. Biosynthesis, processing and secretion

The biosynthesis and post-translational processing of the meprin α and β subunits are complex processes that determine the expression of the mature, latent and active forms of the enzymes. The processes involve glycosylation, intra- and intersubunit disulphide bridging, interaction with chaperones, C-terminal proteolytic processing, oligomerization, phosphorylation, transport to the plasma membrane, activation by proteolysis, and shedding in specific situations (Sterchi, Naim et al. 1988; Grünberg, Dumermuth et al. 1993; Eldering, Grunberg et al. 1997; Hahn, Lottaz et al. 1997; Pischitzis, Hahn et al. 1999; Hahn, Pischitzis et al. 2003; Leuenberger, Hahn et al. 2003; Hengst and Bond 2004). There is an interdependence of the domains for correct folding, transport, secretion and activity (Tsukuba and Bond 1998). Expression of meprin transcripts in mammalian cells results in the synthesis of proteins with the domains depicted in figure 3. The N-terminal signal sequence is removed in the ER resulting in transmembrane-protein of type I with the C-terminal domain inside the cell, and the N-terminal prodomain containing the propeptide, the catalytic site, the MAM, TRAF and EGF-like domains outside the cell. This form of the enzyme is catalytically inactive. Activation requires the removal of the prodomain by a trypsin-like serine-proteinase (Grünberg, Dumermuth et al. 1993) (see also the section on proenzyme activation). Transfection of mammalian cells with a cDNA construct lacking the prodomain results in the biosynthesis of an immature and transport-incompatible protein which is degraded intracellularly and does not reach the plasma membrane. One interpretation of these results is that the prodomain functions as a chaperone that is essential for correct folding. Deletion of the MAM domain results in the degradation of the protein by the proteasome (Tsukuba and Bond 1998; Tsukuba, Kadowaki et al. 2002). Deletion of the TRAF domain, or parts of the TRAF domain, results in the expression of a protein that cannot be activated (Tsukuba and Bond 1998). So, unlike many modular proteins, such as the MMPs, in which the proteinase domain can be folded and expressed without the aid of noncatalytic domains, meprins require such domains for the expression of a stable, activatable proteinase.

Meprin α, when expressed alone in transfected cells, is synthesized as a 100 kDa protein that forms disulphide-linked dimers in the rough endoplasmic reticulum (ER). Before reaching the *cis*-Golgi each subunit is proteolytically processed to a 90 kDa soluble form that lacks the EGF-like domain, the transmembrane and the cytosolic domains. The transmembrane and cytosolic domains facilitate this processing by mediating a transitional association of meprin α with chaperones that are resident in the rough endoplasmic reticulum (Hahn, Lottaz et al. 1997; Tsukuba, Kadowaki et al. 2002). The meprin -specific I-domain is essential for the proteolytic processing of meprin α but the C terminus of the mature subunit is in the TRAF domain, which implies that further proteolytic trimming occurs (Hahn, Lottaz et al. 1997; Tang and Bond 1998). If the I-domain is deleted, the mutant protein is retained and accumulates in the rough endoplasmic reticulum and is probably degraded (Hengst and Bond 2004). In the Golgi complex, complex glycosylation occurs and meprin α is finally constitutively secreted as dimers and oligomers of a 95 kDa protein (see section on quaternary structure).

Meprin β is synthesized as a type I transmembrane protein and is inserted into the plasma membrane. Human meprin β is synthesized as a 95 kDa polypeptide, forms disulphide-linked dimers and is glycosylated to form the 105 kDa mature protein which is inserted into the plasma membrane as dimers. While mouse promeprin β remains membrane-bound, human promeprin

β is partially secreted into the culture medium of transfected cells and from organ-cultured human small intestinal mucosa by constitutive and regulated shedding processes (Eldering, Grunberg et al. 1997). This involves the proteolytic cleavage of membrane-bound meprin β N-terminal of the EGF-like domain by other metalloproteinases. The cleavage site lies within a heavily N- and O-glycosylated sequence and prevention of the formation of O-linked glycan chains increases the shedding of meprin β. The shedding of human meprin β is stimulated by phorbol esters and inhibited by protein kinase C (PKC) inhibitors, indicating a role of protein kinase C in the regulation of meprin β secretion. Although meprin β may be phosphorylated on a serine residue within the cytosolic region this is not implicated in the regulated secretion. ADAM17/TACE has been identified as being responsible for the regulated shedding of meprin β (Pischitzis, Hahn et al. 1999; Hahn, Pischitzis et al. 2003; Leuenberger, Hahn et al. 2003). If both meprin α and β are expressed in the same cell, covalent heterodimers are formed in the rough endoplasmic reticulum and inserted into the plasma membrane. In the case of mouse, the heterodimers remain membrane-bound, while human meprin α/β heterodimers may also be secreted analogous to meprin β alone (Sterchi, Naim et al. 1988). Regulated shedding of human meprin β/β and/or α/β therefore provides a unique means of either targeting the proteolytic activity to the cell surface or to the extracellular milieu (Lottaz, Maurer et al. 1999).

In polarized cells such as normal intestinal epithelial cells and transfected Madin-Darby-Kanine-Kidney cells (MDCK), meprin α and β are sorted to the apical brush-border membrane. The sorting signals for this apical transport are not known to date. In Caco-2 cells, a colon carcinoma cell line expressing meprin α only, the polarized sorting is disturbed resulting in secretion of meprin α to both the apical and the basolateral side of the monolayer and this may be of significance in the development/progression of colorectal cancer (see section on tissue expression).

2.5. Tissue expression

Meprins were first detected in the brush border membrane of kidney proximal tubular and small intestinal epithelial cells and these are the major sites of expression (Beynon, Shannon et al. 1981; Sterchi, Green et al. 1982; Barnes, Ingram et al. 1989).

2.5.1. Kidney—Tissue expression of the two subunits differs in mouse strains (Beynon and Bond 1983; Jiang, Sadler et al. 1993; Bankus and Bond 1996). There are random bred and inbred mouse strains (e.g., ICR or C57BL/6, respectively) that express both meprin α and β in adult kidney proximal tubular cells (also referred to as meprin A); these mice have a ‘high’ meprin activity in kidney. Other inbred mouse strains such as C3H/He or CBA have a ‘low’ kidney meprin activity which is due to the fact that they only express meprin β (also referred to as meprin B), mostly in a latent form (promeprin β). In both instances, cell surface localisation is via the transmembrane domain of meprin β to the brush border membrane of epithelial cells (Fig. 8A). Meprin α, after proteolytic removal of its transmembrane domain, is covalently tethered to meprin β by disulfide bonds in meprin α/β heterodimers. Alternatively, meprin α dimers may adhere noncovalently to α/β dimers. Recently, we also detected meprin α in glomeruli of rat kidney. Using quantitative RT-PCR of laser-capture microdissected glomeruli meprin β mRNA was detected, and with the aid of immuno-gold staining on electronmicroscopic sections, it was localized to the membrane of podocyte foot processes (Oneda, Lods et al. 2008).

There is still uncertainty about the expression of meprin in human kidney. Japanese investigators have reported the purification of meprin from human kidney and have assigned to it a major role in the degradation of parathyroid hormone (Yamaguchi, Fukase et al. 1994). Although meprin α mRNA has been detected in different human tissues including kidney in

an immunoblot (Jiang and Le 2000) the subunit was not detected in human kidneys of causasian origin in other studies (ES, unpublished data). It is not inconceivable that different human meprin expression phenotypes may exist among the world population.

2.5.2. Intestine—A differential expression pattern for the meprin subunits is also observed in the intestine (Sterchi, Naim et al. 1988; Bankus and Bond 1996). In mouse intestine low levels of meprin α mRNA and protein are detectable and are present in increasing concentrations from the duodenum to the ileum. In ICR mice that express both meprin α and β in kidney, less than 1% of meprin α mRNA (micrograms/g tissue) is expressed in duodenum relative to kidney; in the ileum it is approximately 20% of that in kidney. The large intestine contains approximately 10% of the meprin α message found in kidney. The C3H/He mouse strain that only express meprin β in kidney and intestine, display very low levels of meprin α mRNA (approximately 1% of that in ICR kidney) in both the kidney and the intestine. In contrast, intestinal expression of meprin β mRNA in ICR and C3H/He mice is at similar levels to that found in kidney, and for both strains there is a gradient in the meprin β message with an increase of two- to threefold from the proximal duodenum to the distal ileum. The expression pattern of the meprin α protein along the intestine is similar to that of meprin α mRNA, and the activity and response of intestinal meprin α to inhibitors is typical of the enzyme isolated from kidney (Bankus and Bond 1996). In human small intestine there is a similar gradient increase in meprin activity from the proximal duodenum to the distal ileum (Sterchi, Naim et al. 1988). In this tissue, meprin α and meprin β are are localized predominantly to the brush border membrane of villus epithelial cells but not in the crypts (Lottaz, Hahn et al. 1999) (Fig. 8B). In contrast to the small intestine, only meprin α is expressed significantly in human colon. Because the transmembrane and cytosolic domains of meprin α are removed by intracellular proteolytic processing it is secreted into the colon lumen and difficult to detect by immunohistochemial techniques (Lottaz, Hahn et al. 1999). Messenger RNA of meprin α can be detected in epithelial cells of human colon by *in situ* hybridisation (Fig. 8D).

2.5.3. Leukocytes—In mouse and human small intestine, meprins are also found in leukocytes of the lamina propria and this expression is elevated in inflammatory conditions of the intestine (Lottaz, Hahn et al. 1999; Crisman, Zhang et al. 2004; Lottaz, Buri et al. 2007). Figure 8C shows the presence of meprin α mRNA in small intestinal mucosa from a patient with active coeliac disease by *in situ* hybridization. In the intestine of coeliac disease patients, a marked shift of both meprin α and β mRNA expression from the epithelial cells to leukocytes in the lamina propria is observed. During this inflammatory disorder, meprin thereby gains access to a different substrate repertoire present beneath the epithelial cell layer (Lottaz, Buri et al. 2007).

2.5.4. Skin—In a recent finding, meprin expression was shown in human skin where the two subunits are localized in separate cell layers of the epidermis. While meprin α is expressed in the stratum basale, meprin β is found in cells of the stratum granulosum just beneath the stratum corneum (Becker-Pauly, Höwel et al. 2007) (Figs. 8E and 8F). In *psoriasis vulgaris*, a skin disease which is accompanied by an increased proliferation rate of skin cells the expression pattern of meprin α shifts from the basal to the uppermost layers of the epidermis. These expression patterns suggest that the two meprin subunits have distinct functions in the skin. This is supported by *in vitro* data on the effect of recombinant meprins on human keratinocytes which showed that meprin α leads to a change in cell morphology and reduced the number of cells while meprin β did not induce such changes. It is possible that meprin β is involved in the terminal differentiation, while meprin α plays a role in basal proliferation of keratinocytes (Becker-Pauly, Höwel et al. 2007).

2.5.5. Other tissues—In addition to kidney, intestine and skin, meprins are also expressed in other tissues. On the mRNA level, human meprin α has been detected in pancreas, testis and fetal liver Meprin β mRNA expression is more ubiquitous, and includes regions of the brain, the liver, as well as fetal liver and heart (Rösmann, S. and Sterchi, E.E., unpublished data). A mouse gene prediction database (http://mgpd.med.utoronto.ca) shows meprin β mRNA expression profile in the following order: small intestine, kidney, colon, large intestine, midbrain, embryonic stem cells, embryo 14.5 day, hindbrain, pancreas, bone marrow.

2.5.6. Cancer cells—Both the meprin α and β genes are expressed in various cancer cells (see genomic structure section). In colorectal tumour tissue meprin α mRNA, immunoreactive protein and enzymatic activity is detected. In contrast to normal colon, however, the meprin α subunit is secreted into the stroma of the tumour where it accumulates and may be detected by immuno-histochemical methods (Lottaz, Maurer et al. 1999). The mechanism of this abberant secretion was shown using a colon adenocarcioma cell line (Caco-2) expressing meprin α endogenously. When cultured on transwell filter supports meprin is equally secreted from the apical and the basolateral membrane domains. On the basolateral side of the epithelial cell layer, meprin α may be activated by plasmin, which is generated from plasminogen by an activation process catalyzed by uPA from intestinal fibroblasts (Rösmann, Hahn et al. 2002). Meprin expression may play a role in tumor cell invasion and migration and in doing so may be involved in tumor progression (Matters, Manni et al. 2005).

2.5.7. Meprin expression in zebra fish—The zebrafish *Danio rerio* is the most basal chordate known so far that contains meprins (Schötte, Lottaz et al. 2007). In contrast to higher vertebrates, there are three meprin subunits expressed in the zebra fish, two α subunits and one β subunit. The expression has been observed in kidneys, head kidney intestine, gills, brain, liver, heart and eidermis in adult fish. Therefore the zebra fish may provide a suitable model for developmental and functional studies of meprins.

2.6. Function of meprins

The potential physiological and/or pathological functions of these proteinases may be viewed from different viewpoints such as the pattern of tissue expression, the protein structure with the catalytic center and the various functional domains (such as the MAM, TRAF, EGF-like, the transmembrane and the cytosolic regions), as well as the potential substrates and binding partners. The generation of meprin null mice may shed more light on the function of meprins.

From the point of view of tissue expression, data are available from various tissues in different species and in health and disease (see above). The finding that meprin subunits are expressed in embryonic stem cells and in the embryo, coupled with the observation that meprin β null mice are underrepresented indicates that meprins function developmentally (Kumar and Bond 2001; Norman, Jiang et al. 2003). The critical embryonic stages and specific developmental functions of meprins are yet unexplored.

In situ hybridization studies of mouse embryos (both ICR and C3H/He strains) demonstrated expression of meprin α and β subunits by day 14 in the kidney and intestine (Kumar and Bond 2001). Postnatally, meprin subunits are expressed differentially. For example, in rats and mice intestinal meprin α mRNA levels were high throughout the suckling period and declined at weaning, a pattern similar to other brush border membrane hydrolases that are required for the assimilation of the milk diet (Henning, Oesterreicher et al. 1999; Kumar and Bond 2001). This indicates a unique function for meprin α during the suckling period, possibly by providing a specific digestive capacity for milk proteins or biologically active peptides in the milk. By contrast, intestinal meprin β mRNA increases at the time of weaning, implying a role for this proteinase when the supply of protein and peptide substrates is altered with solid food. The

meprin isoform changes may be associated with the diet, with the bacterial flora, or with glucocorticoid levels (Henning, Oesterreicher et al. 1999).

In adults (mouse and human), meprins are expressed most abundantly in epithelial cells that are part of barrier tissues such as kidney, intestine or the skin. In the kidney for example, meprin is predominantly found in the apical membrane of epithelial cells in proximal tubules (Craig, Reckelhoff et al. 1987; Barnes, Ingram et al. 1989) where it will cleave peptides and proteins either filtered from the plasma or secreted by tubular epithelial cells and render them suitable for reabsorption. Mepirn β is also present in the foot processes of podocytes in the glomeruli (Oneda, Lods et al. 2008). The podocytes maintain the integrity and the structure of the glomerular basement membrane (GBM) by a continuous replacment and recycling proess of its components (Mundel and Shankland 1999; Harvey, Jarad et al. 2007). Meprin with its activity towards ECM proteins may act as such a processing entity in breaking down GBM components, prior to being substituted by new matrix proteins.

Both enhanced and low levels of meprins are associated with kidney disease. In experimental models of acute renal failure affecting mainly the tubular structures (e.g., mouse and rat models of ischemia-reperfusion), mislocation of meprins in epithelial cells of proximal tubules results in enhanced kidney tissue damage and treatment of animals with the inhibitor actinonin can prevent such damage (Trachtman, Valderrama et al. 1995; Carmago, Shah et al. 2002; Kieran, Doran et al. 2003). High levels of active meprins can therefore be cytotoxic to a kidney that has been subjected to acute injury/stress when the proteinases are found in inappropriate places such as the cytoplasm, the basolateral membrane, cell–cell adhesion complexes or basolateral in the extracellular space. This is supported by data with meprin β-null mice, which show that the absence of active meprin β in the kidney protects against renal ichemia-reperfusion injury (Bylander, Li et al. 2008)

In contrast to acute kidney failure, meprins are markedly downregulated in chronic conditions that lead to cell death (necrosis and apoptosis), and result in fibrosis. Conditions in which meprin mRNA and/or protein levels are dramatically decreased and which lead to fibrosis and cell death include: hydronephrosis (unilateral urethral ligation), adriamycin-induced nephropathy, experimental diabetes (genetic or chemically-induced), passive Hymann nephritis, anti-Thy 1.1 nephritis, after kidney transplantation, and in collagen IVA3 knockout mice that develop Alport's syndrome (Ricardo, Bond et al. 1996; Sampson, Ryan et al. 2001; Sadlier, Connolly et al. 2004; Mathew, Futterweit et al. 2005; Berthier, Lods et al. 2006; Oneda, Lods et al. 2008). Interestingly, in rats with anti-Thy 1.1 glomerulonephritis, a reversible glomerular disease, meprin β expression is decreased in the glomeruli and, at the same time, its localization in the proximal tubules is altered in a way simillar to that seen in acute renal failure (Oneda, Lods et al. 2008). In passive Heymann nephritis (PHN), a model of membranous glomerulonephritis, induced by the administration of antibodies generated against a membrane fraction of rat renal proximal tubular cells (sheep or rabbit anti-Fx1A antibodies), this is not the case. In PHN subepithelial immune complexes at the glomerular basement membrane (GBM) are deposited and this results in the disruption of the functional integrity of the GBM and the filtration barrier and, ultimately, in proteinuria (Kamata, Baird et al. 1985). The direct detection of meprin β by anti-Fx1A antibodies (Western blot analysis) and the subcellular localization of meprin β in the membrane of podocyte foot processes suggest that meprin β acts as an auto-antigen and contributes to the pathogenesis of PHN (Oneda, Lods et al. 2008).

Meprin β is a candidate gene for diabetic nephropathy. Sequencing of the *MEP1B* gene from Pima Indians revealed 19 single nucleotide polymorphisms (SNPs) (Red Eagle, Hanson et al. 2005). No overall associations were found for individual SNPs. However, within-family association tests found significant results for nine SNPs such that the more common allele was

more frequently observed in those with nephropathy than in their unaffected siblings. The associated polymorphisms are in the 5' untranslated region of the gene and in the C-terminal tail of the protein, and could affect transcription or trafficking of the protein, and ultimately result in enhanced fibrosis.

In the intestine, the expression pattern of meprins, both longitudinal and developmental, also suggests different functions of the subunits. As a barrier tissue, the intestinal epithelium is exposed to the luminal environment that contains enteric bacteria, both commensal and pathogenic, as well as peptides potentially harmful to the epithelial cells. The intestinal epithelium acts as a "mucosal defense" that modulates damage and/or subsequent repair via a complex system involving different inflammatory mediators such as growth factors, cytokines as well as components of cell-cell and cell-ECM interactions. It is in these mechanisms that meprin may play a role by degrading components of bacterial cell surfaces, ECM, or potentially toxic peptides such as gliadin fragments.

Damage of intestinal epithelial cells as a result of an inflammatory or mechanical challenge may lead to a redistribution of the proteolytic potential of meprins from the luminal cell surface to the balsolateral membrane domain or to secretion into the *lamina propria.* In inflammation, the infiltration of meprin-expressing leukocytes further adds to a shift in the proteolytic activity from the brush border membrane to the lamina propria (Lottaz, Buri et al. 2007). This eventually leads to the cleavage of proteins that are not normally accessible to meprin, including cell-adhesion and ECM proteins or cytokines/chemokines released by other cells of the *lamina propria*. Meprin β may also be directly involved in the dissemination of the leukocytes into the *lamina propria* (Crisman, Zhang et al. 2004). Deletion of the *Mep-1b* gene decreased the ability of leukocytes to migrate in matrigel compared with wild-type leukocytes (Crisman, Zhang et al. 2004).

Colon carcinoma cells endogenously express only meprin α and secrete this in a non-polarized fashion both from the apical and the baolateral membrane (Lottaz, Maurer et al. 1999). Meprin α secreted to the basolateral side of these epithlelial cells can be activated by plasmin (Rösmann, Hahn et al. 2002) and this may lead to the observed proteolytic activity in colorectal tumor stroma. Through the degradation of ECM components, meprin may therefore contribute to tumor progression by facilitating migration, intravasation, and metastasis of carcinoma cells (Lottaz, Hahn et al. 1999; Lottaz, Maurer et al. 1999).

In human skin, meprins are expressed in different cell layers (Becker-Pauly, Höwel et al. 2007). While meprin α is found in the *stratum basale*, meprin β is present in cells of the *stratum granulosum* immediately underneath the *stratum corneum*. In the skin of patients with psoriasis vulgaris, a disease acompanied by a hyperproliferative epidermis, a shift in the location of meprin α from the basal to the uppermost layers of the epidermis is observed. This too implies distinct functions for the two meprin subunits in skin, a notion that is supported by *in vitro* data on cultured human keratinocytes. When added to keratinocytes recombinant meprin β (but not meprin α) is toxic, inducing a dramatic change in the morphology and a decrease in the number of these cells. It has been suggested that meprin β may have a function in terminal differentiation, whereas meprin α may be involved in the proliferation of basal keratinocytes (Becker-Pauly, Höwel et al. 2007).

From a structural point of view, the different functional meprin domains need to be considered. As discussed in the section on substrate catalysis and inhibitor binding, both subunits are capable of cleaving the same protein substrates. However, they do this by cleaving different peptide bonds and this results in the generation of different degradation products, which in turn may or may not have their own biological functions. The activation and inhibition profiles also differ. In the intestinal lumen, trypsin is responsible for activation of both meprin subunits

(Grünberg, Dumermuth et al. 1993). In addition to trypsin, other proteinases exist that can activate meprin. One of these, plasmin, may be responsible for the activation of human meprin α in colorectal tumours (Rösmann, Hahn et al. 2002). Meprin β has been shown to be activated by kallikrein-4, another serine proteinase but the significance of this is unknown (Becker-Pauly, Höwel et al. 2007).

The extracellular MAM, TRAF domains have been implicated in protein-protein interactions. The non-covalent interactions seen with meprins in the generation of oligmers suggests that other cis- or trans-cellular binding partners may interact with meprins. Such interactions may trigger cellular processes that are not necessarily dependent on the proteolytic activity of meprins. In this way, meprins may also act as receptors. The role of the EGF-like domain is not known. It is interesting, however, that in human serum an EGF-like peptide homologous to mepirn α has been identified (Richter, Schulz-Knappe et al. 1999), indicating that the EGF-like domain remaining in the plasma membrane after cleavage of meprin α is shed. It is not known if this has any functional consequences.

Meprin β, in addition to the ecto-domains described above, also has potential binding regions in the cytosolic domain. The latter is essential for correct maturation of meprin β (Litovchick, Chestukhin et al. 1998) and has been shown to interact transiently with OS-9, a protein that is associated with the membrane of the endoplasmic reticulum (ER). It has been suggested that OS-9 may be involved in the ER-to-Golgi transport of meprin β (Litovchick, Friedmann et al. 2002). Another interesting aspect of the cytosolic domain of meprin β is the fact that it contains a serine residue that may be phosphorylated (Hahn, Pischitzis et al. 2003). Although the significance of these findings remain unclear it seems that meprin β, via the cytosolic domain, interacts with other intracellular proteins. This has been shown by the processing of E-cadherin in meprin β-expressing cells. Concomitant with the E-cadherin ectodomain cleavage, degradation of the E-cadherin cytosolic domain was observed and, interstingly, β- and γ-catenin that interact directly with the cytosolic domain of E-cadherin were also processed (Huguenin, Muller et al. 2008). From these data it is becoming clear that, there is probably more to meprin β than simple cell-surface proteolyis.

Many substrates that are cleaved by meprins *in vitro* have been identified (see section on substrate catalysis). The significance of protein cleavage depends on the co-localization of the proteinase with a particular substrate. Furthermore this may be the case only in a relatively short time window such as during development or tissue remodelling. For the maintenance and/or repair of epithelia or in carcinogenesis, proteins involved in cell-cell (e.g. E-cadherin) and cell-matrix interaction (e.g. laminin, nidogen, collagen IV, fibronecting, etc.) are of particular interest. Recently, a proteomic approach was used in a cell culture system using meprin β-expressing MDCK cells to identify functionally relevant substrates (Ambort, Stalder et al. 2008). A total of 22 proteins, including proteins such as lysyl oxidase, collagen V, annexin A1 and vinculin, were identified. It is noteworthy that in that study, intracellular proteins were identified that were processed only in cells that expressed meprin β. Ten of these proteins were classified as being involved in cell growth and maintenance, four in immune response, and two each in cell communication/signal transduction, transport, and energy or protein metabolism.

Meprinβ-null mice—The targeted disruption of the meprin β gene does not result in an overtly altered phenotype but does lead to changes in the renal gene expression profile. Microarray analysis of kidney RNA allowed the classification of altered genes into functional groups. These included immune response related, cell growth or cell cycle related, nervous system related, post-translational modification related and cytoskeleton related genes, which were, with a few exceptions upregulated in meprin β-null mice (Norman, Jiang et al. 2003).

3. Concluding remarks

Studies of the structure, biochemistry, genetics and cell biology of meprin metalloproteinases have provided a firm base to probe further the function of these enzymes in health and disease. The structural properties of the meprins have been particularly intriguing because of the insights they provide into how proteolytic activity can function and be concentrated in the environments of the intestine, kidney and at sites of inflammation and tumours. We are now poised to discover the role of meprins in embryogenesis, in the suckling and weaning phases of development, and in the adult kidney, skin, and intestine and other tissues using genetically altered mice. The involvement of meprins in cancer progression, inflammatory bowel disease as well as acute and/or chronic renal disease indicates that meprins might be targets for therapy through the development of potent and specific inhibitors.

Acknowledgments

The work of the authors was supported by the Swiss National Science Foundation (ES), the National Institutes of Health (DK 19691 to JSB), and the Deutsche Forschungsgemeinschaft (WS).

References

Ambort D, Stalder D, Lottaz D, Hguenin M, Oneda B, Heller M, Sterchi EE. A novel 2D-based approach to the discovery of candidate substrates for the metalloendopeptidase meprin. FEBS J. 2008

Aricescu AR, Hon WC, Siebold C, Lu W, van der Merwe PA, Jones EY. Molecular analysis of receptor protein tyrosine phosphatase mu-mediated cell adhesion. Embo J 2006;25(4):701–712. [PubMed: 16456543]

Bankus JM, Bond JS. Expression and distribution of meprin protease subunits in mouse intestine. Arch. Biochem. Biophys 1996;331(1):87–94. [PubMed: 8660687]

Barnes K, Ingram J, Kenny A. Proteins of the kidney microvillar membrane. Structural and immunochemical properties of rat endopeptidase-2 and its immunohistochemical localization in tissues of rat and mouse. Biochem. J 1989;264(2):335–346. [PubMed: 2690825]

Barrett AJ, Rawlings ND, O'Brien EA. The MEROPS database as a protease information system. J. Struct. Biol 2001;134(2–3):95–102. [PubMed: 11551172]

Becker C, Kruse MN, Slotty KA, Köhler D, Harris JR, Rösmann S, Sterchi EE, Stöcker W. Differences in the activation mechanism between the alpha and beta subunits of human meprin. Biol. Chem 2003;384(5):825–831. [PubMed: 12817480]

Becker-Pauly C, Höwel M, Walker T, Vlad A, Aufenvenne K, Oji V, Lottaz D, Sterchi EE, Debela M, Magdolen V, Traupe H, Stöcker W. The alpha and beta subunits of the metalloprotease meprin are expressed in separate layers of human epidermis, revealing different functions in keratinocyte proliferation and differentiation. J. Invest. Dermatol 2007;127(5):1115–1125. [PubMed: 17195012]

Beckmann G, Bork P. An adhesive domain detected in functionally diverse receptors. Trends Biochem. Sci 1993;18(2):40–41. [PubMed: 8387703]

Bertenshaw, GP.; Bond, JS. Meprins alpha and beta. Handbook of Proteolytic Enzymes. Barrett, AJ.; Rawlings, ND.; Woessner, FJ., jr, editors. San Diego: Academic Press; 2004.

Bertenshaw GP, Norcum MT, Bond JS. Structure of homo- and hetero-oligomeric meprin metalloproteases. Dimers, tetramers, and high molecular mass multimers. J. Biol. Chem 2003;278 (4):2522–2532. [PubMed: 12399461]

Bertenshaw GP, Turk BE, Hubbard SJ, Matters GL, Bylander JE, Crisman JM, Cantley LC, Bond JS. Marked differences between metalloproteases meprin A and B in substrate and peptide bond specificity. J. Biol. Chem 2001;276(16):13248–13255. [PubMed: 11278902]

Bertenshaw GP, Villa JP, Hengst JA, Bond JS. Probing the active sites and mechanisms of rat metalloproteases meprin A and B. Biol. Chem 2002;383(7–8):1175–1183. [PubMed: 12437103]

Berthier CC, Lods N, Joosten SA, van Kooten C, Leppert D, Lindberg RL, Kappeler A, Raulf F, Sterchi EE, Lottaz D, Marti HP. Differential regulation of metzincins in experimental chronic renal allograft

rejection: potential markers and novel therapeutic targets. Kidney Int 2006;69(2):358–368. [PubMed: 16408127]

Beynon RJ, Bond JS. Deficiency of a kidney metalloproteinase in inbred mouse strains. Science 1983;219 (4590):1351–1353. [PubMed: 6338590]

Beynon RJ, Shannon JD, Bond JS. Purification and characterization of a metallo-endoproteinase from mouse kidney. Biochem. J 1981;199:591–598. [PubMed: 7041888]

Bode W, Gomis-Rüth FX, Huber R, Zwilling R, Stöcker W. Structure of astacin and implications for activation of astacins and zinc-ligation of collagenases. Nature 1992;358(6382):164–167. [PubMed: 1319561]

Bond JS, Beynon RJ. Mammalian metalloendopeptidases. Int. J. Biochem 1985;17(5):565–574. [PubMed: 3896890]

Bond JS, Beynon RJ. The astacin family of metalloendopeptidases. Protein Sci 1995;4(7):1247–1261. [PubMed: 7670368]

Bond JS, Beynon RJ, Reckelhoff JF, David CS. Mep-1 gene controlling a kidney metalloendopeptidase is linked to the major histocompatibility complex in mice. Proc. Natl. Acad. Sci. U. S. A 1984;81 (17):5542–5545. [PubMed: 6382265]

Borchert N, Becker-Pauly C, Wagner A, Fischer P, Stöcker W, Brattig NW. Identification and characterization of onchoastacin, an astacin-like metalloproteinase from the filaria Onchocerca volvulus. Microbes Infect 2007;9(4):498–506. [PubMed: 17347015]

Butler P, Bond J. A latent proteinase in mouse kidney membranes. Characterization and relationship to meprin. J. Biol. Chem 1988;263(26):13419–13426. [PubMed: 3047123]

Butler PE, McKay MJ, Bond JS. Characterization of meprin, a membrane-bound metalloendopeptidase from mouse kidney. Biochem. J 1987;241(1):229–235. [PubMed: 3105525]

Bylander J, Li Q, Ramesh G, Zhang B, Reeves WB, Bond JS. Targeted disruption of the meprin metalloproteinase beta gene protects against renal ischemia-reperfusion injury in mice. Am. J. Physiol. Renal Physiol 2008;294(3):F480–F490. [PubMed: 18172000]

Bylander JE, Bertenshaw GP, Matters GL, Hubbard SJ, Bond JS. Human and mouse homo-oligomeric meprin A metalloendopeptidase: substrate and inhibitor specificities. Biol. Chem 2007;388(11): 1163–1172. [PubMed: 17976009]

Carmago S, Shah SV, Walker PD. Meprin, a brush-border enzyme, plays an important role in hypoxic/ ischemic acute renal tubular injury in rats. Kidney Int 2002;61(3):959–966. [PubMed: 11849450]

Chen H, He Z, Bagri A, Tessier-Lavigne M. Semaphorin-neuropilin interactions underlying sympathetic axon responses to class III semaphorins. Neuron 1998;21(6):1283–1290. [PubMed: 9883722]

Cheng G, Cleary AM, Ye ZS, Hong DI, Lederman S, Baltimore D. Involvement of CRAF1, a relative of TRAF, in CD40 signaling. Science 1995;267(5203):1494–1498. [PubMed: 7533327]

Chevallier S, Ahn J, Boileau G, Crine P. Identification of the cysteine residues implicated in the formation of alpha 2 and alpha/beta dimers of rat meprin. Biochem. J 1996;317(Pt 3):731–738. [PubMed: 8760356]

Craig SS, Reckelhoff JF, Bond JS. Distribution of meprin in kidneys from mice with high- and low-meprin activity. Am. J. Physiol 1987;253(4 Pt 1):C535–C540. [PubMed: 3310653]

Crisman JM, Zhang B, Norman LP, Bond JS. Deletion of the mouse meprin beta metalloprotease gene diminishes the ability of leukocytes to disseminate through extracellular matrix. J. Immunol 2004;172 (7):4510–4519. [PubMed: 15034068]

De Maere V, Vercauteren I, Geldhof P, Gevaert K, Vercruysse J, Claerebout E. Molecular analysis of astacin-like metalloproteases of Ostertagia ostertagi. Parasitology 2005;130(Pt 1):89–98. [PubMed: 15700760]

Dumermuth E, Eldering J, Grunberg J, Jiang W, Sterchi E. Cloning of the PABA peptide hydrolase alpha subunit (PPH alpha) from human small intestine and its expression in COS-1 cells. FEBS Lett 1993;335(3):367–375. [PubMed: 8262185]

Dumermuth E, Sterchi EE, Jiang WP, Wolz RL, Bond JS, Flannery AV, Beynon RJ. The astacin family of metalloendopeptidases. J. Biol. Chem 1991;266(32):21381–21385. [PubMed: 1939172]

Dusing MR, Wiginton DA. Epithelial lineages of the small intestine have unique patterns of GATA expression. J. Mol. Histol 2005;361(1–2):15–24. [PubMed: 15703995]

Elaroussi MA, DeLuca HF. A new member to the astacin family of metalloendopeptidases: a novel 1,25-dihydroxyvitamin D-3-stimulated mRNA from chorioallantoic membrane of quail. Biochim. Biophys. Acta 1994;1217(1):1–8. [PubMed: 8286408]

Eldering JA, Grunberg J, Hahn D, Croes HJ, Fransen JA, Sterchi EE. Polarised expression of human intestinal N-benzoyl-L-tyrosyl-p-aminobenzoic acid hydrolase (human meprin) alpha and beta subunits in Madin-Darby canine kidney cells. Eur. J. Biochem 1997;247(3):920–932. [PubMed: 9288916]

Fan TJ, Katagiri C. Properties of the hatching enzyme from Xenopus laevis. Eur. J. Biochem 2001;268 (18):4892–4898. [PubMed: 11559358]

Finelli A, Bossie C, Xie T, Padgett R. Mutational analysis of the Drosophila tolloid gene, a human BMP-1 homolog. Development 1994;120(4):861–870. [PubMed: 7600963]

Gao X, Sedgwick T, Shi YB, Evans T. Distinct functions are implicated for the GATA-4, -5, and -6 transcription factors in the regulation of intestine epithelial cell differentiation. Mol Cell Biol 1998;18 (5):2901–2911. [PubMed: 9566909]

Garrigue-Antar L, Francois V, Kadler KE. Deletion of epidermal growth factor-like domains converts mammalian tolloid into a chordinase and effective procollagen C-proteinase. J. Biol. Chem 2004;279 (48):49835–49841. [PubMed: 15381708]

Ge G, Greenspan DS. Developmental roles of the BMP1/TLD metalloproteinases. Birth Defects Re.s C. Embryo Today 2006;78(1):47–68.

Geier G, Jacob E, Stöcker W, Zwilling R. Genomic organization of the zinc-endopeptidase astacin. Arch. Biochem. Biophys 1997;337(2):300–307. [PubMed: 9016826]

Geier G, Zwilling R. Cloning and characterization of a cDNA coding for Astacus embryonic astacin, a member of the astacin family of metalloproteases from the crayfish Astacus astacus. Eur. J. Biochem 1998;253(3):796–803. [PubMed: 9654081]

Glerup S, Boldt HB, Overgaard MT, Sottrup-Jensen L, Giudice LC, Oxvig C. Proteinase inhibition by proform of eosinophil major basic protein (pro-MBP) is a multistep process of intra- and intermolecular disulfide rearrangements. J. Biol. Chem 2005;280(11):9823–9832. [PubMed: 15647258]

Gomis-Rüth FX. Structural aspects of the metzincin clan of metalloendopeptidases. Mol Biotechnol 2003;24(2):157–202. [PubMed: 12746556]

Gorbea CM, Marchand P, Jiang W, Copeland NG, Gilbert DJ, Jenkins NA, Bond JS. Cloning, expression, and chromosomal localization of the mouse meprin beta subunit. J. Biol. Chem 1993;268(28):21035–21043. [PubMed: 8407940]

Grams F, Dive V, Yiotakis A, Yiallouros I, Vassiliou S, Zwilling R, Bode W, Stöcker W. Structure of astacin with a transition-state analogue inhibitor. Nat. Struct. Biol 1996;3(8):671–675. [PubMed: 8756323]

Grünberg J, Dumermuth E, Eldering JA, Sterchi EE. Expression of the alpha subunit of PABA peptide hydrolase (EC 3.4.24.18) in MDCK cells. Synthesis and secretion of an enzymatically inactive homodimer. FEBS Lett 1993;335(3):376–379. [PubMed: 8262186]

Grünberg J, Luginbuhl U, Sterchi EE. Proteolytic processing of human intestinal lactase-phlorizin hydrolase precursor is not a prerequisite for correct sorting in Madin Darby canine kidney (MDCK) cells. FEBS Lett 1992;314(3):224–228. [PubMed: 1468552]

Gutman A, Wasylyk B. The collagenase gene promoter contains a TPA and oncogene-responsive unit encompassing the PEA3 and AP-1 binding sites. Embo J 1990;9(7):2241–2246. [PubMed: 2162765]

Hahn D, Illisson R, Metspalu A, Sterchi EE. Human N-benzoyl-L-tyrosyl-p-aminobenzoic acid hydrolase (human meprin): genomic structure of the alpha and beta subunits. Biochem. J 2000;346(Pt 1):83–91. [PubMed: 10657243]

Hahn D, Lottaz D, Sterchi EE. C-cytosolic and transmembrane domains of the N-benzoyl-L-tyrosyl-p-aminobenzoic acid hydrolase alpha subunit (human meprin alpha) are essential for its retention in the endoplasmic reticulum and C-terminal processing. Eur. J. Biochem 1997;247(3):933–941. [PubMed: 9288917]

Hahn D, Pischitzis A, Roesmann S, Hansen MK, Leuenberger B, Luginbuehl U, Sterchi EE. Phorbol 12-myristate 13-acetate-induced ectodomain shedding and phosphorylation of the human meprinbeta metalloprotease. J. Biol. Chem 2003;278(44):42829–42839. [PubMed: 12941954]

Harvey SJ, Jarad G, Cunningham J, Rops AL, van der Vlag J, Berden JH, Moeller MJ, Holzman LB, Burgess RW, Miner JH. Disruption of glomerular basement membrane charge through podocyte-specific mutation of agrin does not alter glomerular permselectivity. Am. J. Pathol 2007;171(1):139–152. [PubMed: 17591961]

Hengst JA, Bond JS. Transport of meprin subunits through the secretory pathway: role of the transmembrane and cytoplasmic domains and oligomerization. J. Biol. Chem 2004;279(33):34856–34864. [PubMed: 15187079]

Henning SJ. Ontogeny of enzymes in the small intestine. Annu. Rev. Physiol 1985;47:231–245. [PubMed: 3888075]

Henning SJ, Oesterreicher TJ, Osterholm DE, Lottaz D, Hahn D, Sterchi EE. Meprin mRNA in rat intestine during normal and glucocorticoid-induced maturation: divergent patterns of expression of alpha and beta subunits. FEBS Lett 1999;462(3):368–372. [PubMed: 10622727]

Herlyn H, Zischler H. Identification of a positively evolving putative binding region with increased variability in posttranslational motifs in zonadhesin MAM domain 2. Mol Phylogenet Evol 2005;37 (1):62–72. [PubMed: 15927490]

Herzog C, Kaushal GP, Haun RS. Generation of biologically active interleukin-1beta by meprin B. Cytokine 2005;31(5):394–403. [PubMed: 16095909]

Hintze V, Höwel M, Wermter C, Grosse Berkhoff E, Becker-Pauly C, Beermann B, Yiallouros I, Stöcker W. The interaction of recombinant subdomains of the procollagen C-proteinase with procollagen I provides a quantitative explanation for functional differences between the two splice variants, mammalian tolloid and bone morphogenetic protein 1. Biochemistry 2006;45(21):6741–6748. [PubMed: 16716085]

Hirano M, Ma BY, Kawasaki N, Okimura K, Baba M, Nakagawa T, Miwa K, Kawasaki N, Oka S, Kawasaki T. Mannan-binding protein blocks the activation of metalloproteases meprin alpha and beta. J. Immunol 2005;175(5):3177–3185. [PubMed: 16116208]

Holley SA, Neul JL, Attisano L, Wrana JL, Sasai Y, O'Connor MB, De Robertis EM, Ferguson EL. The Xenopus dorsalizing factor noggin ventralizes Drosophila embryos by preventing DPP from activating its receptor. Cell 1996;86(4):607–617. [PubMed: 8752215]

Hopkins DR, Keles S, Greenspan DS. The bone morphogenetic protein 1/Tolloid-like metalloproteinases. Matrix Biol 2007;26(7):508–523. [PubMed: 17560775]

Huguenin M, Muller EJ, Trachsel-Rosmann S, Oneda B, Ambort D, Sterchi EE, Lottaz D. The metalloprotease meprinbeta processes E-cadherin and weakens intercellular adhesion. PLoS ONE 2008;3(5):e2153. [PubMed: 18478055]

Hung CH, Huang HR, Huang CJ, Huang FL, Chang GD. Purification and cloning of carp nephrosin, a secreted zinc endopeptidase of the astacin family. J. Biol. Chem 1997;272(21):13772–13778. [PubMed: 9153232]

Ishmael FT, Norcum MT, Benkovic SJ, Bond JS. Multimeric structure of the secreted meprin A metalloproteinase and characterization of the functional protomer. J. Biol. Chem 2001;276(25): 23207–23211. [PubMed: 11301339]

Ishmael FT, Shier VK, Ishmael SS, Bond JS. Intersubunit and domain interactions of the meprin B metalloproteinase. Disulfide bonds and protein-protein interactions in the MAM and TRAF domains. J. Biol. Chem 2005;280(14):13895–13901. [PubMed: 15695509]

Ishmael SS, Ishmael FT, Jones AD, Bond JS. Protease domain glycans affect oligomerization, disulfide bond formation, and stability of the meprin A metalloprotease homo-oligomer. J. Biol. Chem 2006;281(49):37404–37415. [PubMed: 17040911]

Jiang W, Flannery A. Correlation of the exon/intron organization to the secondary structures of the protease domain of mouse meprin alpha subunit. Gene 1997;189(1):65–71. [PubMed: 9161413]

Jiang W, Gorbea CM, Flannery AV, Beynon RJ, Grant GA, Bond JS. The alpha subunit of meprin A. Molecular cloning and sequencing, differential expression in inbred mouse strains, and evidence for divergent evolution of the alpha and beta subunits. J. Biol. Chem 1992;267(13):9185–9193. [PubMed: 1374387]

Jiang W, Kumar JM, Matters GL, Bond JS. Structure of the mouse metalloprotease meprin beta gene (Mep1b): alternative splicing in cancer cells. Gene 2000;248(1–2):77–87. [PubMed: 10806353]

Jiang W, Le B. Structure and expression of the human MEP1A gene encoding the alpha subunit of metalloendopeptidase meprin A. Arch Biochem Biophys 2000;379(2):183–187. [PubMed: 10898933]

Jiang W, Sadler P, Jenkins N, Gilbert D, Copeland N, Bond J. Tissue-specific expression and chromosomal localization of the alpha subunit of mouse meprin A. J. Biol. Chem 1993;268(14): 10380–10385. [PubMed: 7683677]

Johnson GD, Bond JS. Activation mechanism of meprins, members of the astacin metalloendopeptidase family. J. Biol. Chem 1997;272(44):28126–28132. [PubMed: 9346968]

Kadowaki T, Tsukuba T, Bertenshaw GP, Bond JS. N-Linked oligosaccharides on the meprin A metalloprotease are important for secretion and enzymatic activity, but not for apical targeting. J. Biol. Chem 2000;275(33):25577–25584. [PubMed: 10837482]

Kamata K, Baird LG, Erikson ME, Collins AB, McCluskey RT. Characterization of antigens and antibody specificities involved in Heymann nephritis. J. Immunol 1985;135(4):2400–2408. [PubMed: 2411795]

Kaushal GP, Walker PD, Shah SV. An old enzyme with a new function: purification and characterization of a distinct matrix-degrading metalloproteinase in rat kidney cortex and its identification as meprin. J. Cell Biol 1994;126(5):1319–1327. [PubMed: 8063866]

Kenny AJ, Ingram J. Proteins of the kidney microvillar membrane. Purification and properties of the phosphoramidon-insensitive endopeptidase ('endopeptidase-2') from rat kidney. Biochem. J 1987;245(2):515–524. [PubMed: 3117045]

Kessler E, Takahara K, Biniaminov L, Brusel M, Greenspan DS. Bone morphogenetic protein-1: the type I procollagen C-proteinase. Science 1996;271(5247):360–362. [PubMed: 8553073]

Kieran NE, Doran PP, Connolly SB, Greenan MC, Higgins DF, Leonard M, Godson C, Taylor CT, Henger A, Kretzler M, Burne MJ, Rabb H, Brady HR. Modification of the transcriptomic response to renal ischemia/reperfusion injury by lipoxin analog. Kidney Int 2003;64(2):480–492. [PubMed: 12846743]

Köhler D, Kruse M, Stöcker W, Sterchi EE. Heterologously overexpressed, affinity-purified human meprin alpha is functionally active and cleaves components of the basement membrane in vitro. FEBS Lett 2000;465(1):2–7. [PubMed: 10620696]

Kounnas MZ, Wolz RL, Gorbea CM, Bond JS. Meprin-A and -B. Cell surface endopeptidases of the mouse kidney. J. Biol. Chem 1991;266(26):17350–17357. [PubMed: 1894622]

Kruse MN, Becker C, Lottaz D, Köhler D, Yiallouros I, Krell HW, Sterchi EE, Stöcker W. Human meprin alpha and beta homo-oligomers: cleavage of basement membrane proteins and sensitivity to metalloprotease inhibitors. Biochem. J 2004;378(Pt 2):383–389. [PubMed: 14594449]

Kumar JM, Bond JS. Developmental expression of meprin metalloprotease subunits in ICR and C3H/He mouse kidney and intestine in the embryo, postnatally and after weaning. Biochim. Biophys. Acta 2001;1518(1–2):106–114. [PubMed: 11267665]

Lepage T, Ghiglione C, Gache C. Spatial and temporal expression pattern during sea urchin embryogenesis of a gene coding for a protease homologous to the human protein BMP-1 and to the product of the Drosophila dorsal-ventral patterning gene tolloid. Development 1992;114(1):147–163. [PubMed: 1339338]

Leuenberger B, Hahn D, Pischitzis A, Hansen MK, Sterchi EE. Human meprin beta: O-linked glycans in the intervening region of the type I membrane protein protect the C-terminal region from proteolytic cleavage and diminish its secretion. Biochem. J 2003;369(Pt 3):659–665. [PubMed: 12387727]

Li SW, Sieron AL, Fertala A, Hojima Y, Arnold WV, Prockop DJ. The C-proteinase that processes procollagens to fibrillar collagens is identical to the protein previously identified as bone morphogenic protein-1. Proc. Natl. Acad. Sci. U. S. A 1996;93(10):5127–5130. [PubMed: 8643539]

Liddington RC. Mapping out the basement membrane. Nat. Struct. Biol 2001;8(7):573–574. [PubMed: 11427880]

Litovchick L, Chestukhin A, Shaltiel S. The carboxyl-terminal tail of kinase splitting membranal proteinase/meprin beta is involved in its intracellular trafficking. J. Biol. Chem 1998;273(44):29043–29051. [PubMed: 9786910]

Litovchick L, Friedmann E, Shaltiel S. A selective interaction between OS-9 and the carboxyl-terminal tail of meprin beta. J. Biol. Chem 2002;277(37):34413–34423. [PubMed: 12093806]

Lopez-Otin C, Bond JS. Proteases: Multifunctional enzymes in life and disease. J. Biol. Chem. Jul 2008;23[Epub ahead of print]

Lottaz D, Buri C, Monteleone G, Rosmann S, Macdonald TT, Sanderson IR, Sterchi EE. Compartmentalised expression of meprin in small intestinal mucosa: enhanced expression in lamina propria in coeliac disease. Biol. Chem 2007;388(3):337–341. [PubMed: 17338642]

Lottaz D, Hahn D, Muller S, Muller C, Sterchi EE. Secretion of human meprin from intestinal epithelial cells depends on differential expression of the alpha and beta subunits. Eur. J. Biochem 1999;259(1–2):496–504. [PubMed: 9914532]

Lottaz D, Maurer CA, Hahn D, Buchler MW, Sterchi EE. Nonpolarized secretion of human meprin alpha in colorectal cancer generates an increased proteolytic potential in the stroma. Cancer Res 1999;59(5):1127–1133. [PubMed: 10070973]

Lun HM, Mak CH, Ko RC. Characterization and cloning of metallo-proteinase in the excretory/secretory products of the infective-stage larva of Trichinella spiralis. Parasitol. Res 2003;90(1):27–37. [PubMed: 12743801]

Marchand P, Tang J, Bond JS. Membrane association and oligomeric organization of the alpha and beta subunits of mouse meprin A. J. Biol. Chem 1994;269(21):15388–15393. [PubMed: 8195177]

Marchand P, Tang J, Johnson GD, Bond JS. COOH-terminal proteolytic processing of secreted and membrane forms of the alpha subunit of the metalloprotease meprin A. Requirement of the I domain for processing in the endoplasmic reticulum. J. Biol. Chem 1995;270(10):5449–5456. [PubMed: 7890660]

Marchand P, Volkmann M, Bond JS. Cysteine mutations in the MAM domain result in monomeric meprin and alter stability and activity of the proteinase. J. Biol. Chem 1996;271(39):24236–24241. [PubMed: 8798668]

Mathew R, Futterweit S, Valderrama E, Tarectecan AA, Bylander JE, Bond JS, Trachtman H. Meprin-alpha in chronic diabetic nephropathy: interaction with the renin-angiotensin axis. Am. J Physiol. Renal Physiol 2005;289(4):F911–F921. [PubMed: 15942051]

Matters GL, Bond JS. Expression and regulation of the meprin beta gene in human cancer cells [In Process Citation]. Mol. Carcinog 1999;25(3):169–178. [PubMed: 10411143]

Matters GL, Bond JS. Meprin B: transcriptional and posttranscriptional regulation of the meprin beta metalloproteinase subunit in human and mouse cancer cells. Apmis 1999;107(1):19–27. [PubMed: 10190276]

Matters GL, Manni A, Bond JS. Inhibitors of polyamine biosynthesis decrease the expression of the metalloproteases meprin alpha and MMP-7 in hormone-independent human breast cancer cells. Clin. Exp. Metastasis 2005;22(4):331–339. [PubMed: 16170669]

McHugh B, Krause SA, Yu B, Deans AM, Heasman S, McLaughlin P, Heck MM. Invadolysin: a novel, conserved metalloprotease links mitotic structural rearrangements with cell migration. J. Cell Biol 2004;167(4):673–686. [PubMed: 15557119]

Möhrlen F, Hutter H, Zwilling R. The astacin protein family in Caenorhabditis elegans. Eur. J. Biochem 2003;270(24):4909–4920. [PubMed: 14653817]

Möhrlen F, Maniura M, Plickert G, Frohme M, Frank U. Evolution of astacin-like metalloproteases in animals and their function in development. Evol. Dev 2006;8(2):223–231. [PubMed: 16509900]

Mundel P, Shankland SJ. Glomerular podocytes and adhesive interaction with glomerular basement membrane. Exp. Nephrol 199;7(2):160–166. [PubMed: 10213869]

Nakamura F, Goshima Y. Structural and functional relation of neuropilins. Adv. Exp. Med. Biol 2002;515:55–69. [PubMed: 12613543]

Norman LP, Jiang W, Han X, Saunders TL, Bond JS. Targeted disruption of the meprin beta gene in mice leads to underrepresentation of knockout mice and changes in renal gene expression profiles. Mol Cell Biol 2003;23(4):1221–1230. [PubMed: 12556482]

Norman LP, Matters GL, Crisman JM, Bond JS. Expression of meprins in health and disease. Curr Top Dev Biol 2003;54:145–166. [PubMed: 12696749]

Oneda B, Lods N, Lottaz D, Becker-Pauly C, Stöcker W, Pippin J, Huguenin M, Ambort D, Marti HP, Sterchi EE. Metalloprotease meprin beta in rat kidney: glomerular localization and differential expression in glomerulonephritis. PLoS ONE 2008;3(5):e2278. [PubMed: 18509531]

Pan T, Groger H, Schmid V, Spring J. A toxin homology domain in an astacin-like metalloproteinase of the jellyfish Podocoryne carnea with a dual role in digestion and development. Dev. Genes Evol 1998;208(5):259–266. [PubMed: 9683741]

Pankov R, Yamada KM. Fibronectin at a glance. J. Cell Sci 2002;115(Pt 20):3861–3863. [PubMed: 12244123]

Pischitzis A, Hahn D, Leuenberger B, Sterchi EE. N-Benzoyl-L-tyrosyl-p-aminobenzoic acid hydrolase beta (human meprinbeta). A 13-amino-acid sequence is required for proteolyticprocessing and subsequent secretion. Eur. J. Biochem 1999;261(2):421–429. [PubMed: 10215852]

Putnam NH, Srivastava M, Hellsten U, Dirks B, Chapman J, Salamov A, Terry A, Shapiro H, Lindquist E, Kapitonov VV, Jurka J, Genikhovich G, Grigoriev IV, Lucas SM, Steele RE, Finnerty JR, Technau U, Martindale MQ, Rokhsar DS. Sea anemone genome reveals ancestral eumetazoan gene repertoire and genomic organization. Science 2007;317(5834):86–94. [PubMed: 17615350]

Quesada V, Sanchez LM, Alvarez J, Lopez-Otin C. Identification and characterization of human and mouse ovastacin: a novel metalloproteinase similar to hatching enzymes from arthropods, birds, amphibians, and fish. J. Biol. Chem 2004;279(25):26627–26634. [PubMed: 15087446]

Red Eagle AR, Hanson RL, Jiang W, Han X, Matters GL, Imperatore G, Knowler WC, Bond JS. Meprin beta metalloprotease gene polymorphisms associated with diabetic nephropathy in the Pima Indians. Hum. Genet 2005;118(1):12–22. [PubMed: 16133184]

Reynolds SD, Angerer LM, Palis J, Nasir A, Angerer RC. Early mRNAs, spatially restricted along the animal-vegetal axis of sea urchin embryos, include one encoding a protein related to tolloid and BMP-1. Development 1992;114(3):769–786. [PubMed: 1618141]

Ricardo SD, Bond JS, Johnson GD, Kaspar J, Diamond JR. Expression of subunits of the metalloendopeptidase meprin in renal cortex in experimental hydronephrosis. Am. J. Physiol 1996;270(4 Pt 2):F669–F676. [PubMed: 8967345]

Richter R, Schulz-Knappe P, Schrader M, Standker L, Jurgens M, Tammen H, Forssmann WG. Composition of the peptide fraction in human blood plasma: database of circulating human peptides. J. Chromatogr. B. Biomed. Sci. Appl 1999;726(1–2):25–35. [PubMed: 10348167]

Rösmann S, Hahn D, Lottaz D, Kruse MN, Stöcker W, Sterchi EE. Activation of human meprin-alpha in a cell culture model of colorectal cancer is triggered by the plasminogen-activating system. J. Biol. Chem 2002;277(43):40650–40658. [PubMed: 12189145]

Sadlier DM, Connolly SB, Kieran NE, Roxburgh S, Brazil DP, Kairaitis L, Wang Y, Harris DC, Doran P, Brady HR. Sequential extracellular matrix-focused and baited-global cluster analysis of serial transcriptomic profiles identifies candidate modulators of renal tubulointerstitial fibrosis in murine adriamycin-induced nephropathy. J. Biol. Chem 2004;279(28):29670–29680. [PubMed: 15033991]

Sampson NS, Ryan ST, Enke DA, Cosgrove D, Koteliansky V, Gotwals P. Global gene expression analysis reveals a role for the alpha 1 integrin in renal pathogenesis. J. Biol. Chem 2001;276(36): 34182–34188. [PubMed: 11447218]

Schechter I, Berger A. On the size of the active site in proteases. I. Papain. Biochem. Biophys. Res. Commun 1967;27(2):157–162.

Schütte A, Lottaz D, Sterchi EE, Stöcker W, Becker-Pauly C. Two alpha subunits and one beta subunit of meprin zinc-endopeptidases are differentially expressed in the zebrafish Danio rerio. Biol. Chem 2007;388(5):523–531. [PubMed: 17516848]

Shibata Y, Iwamatsu T, Oba Y, Kobayashi D, Tanaka M, Nagahama Y, Suzuki N, Yoshikuni M. Identification and cDNA cloning of alveolin, an extracellular metalloproteinase, which induces chorion hardening of medaka (Oryzias latipes) eggs upon fertilization. J. Biol. Chem 2000;275(12): 8349–8354. [PubMed: 10722665]

Shimell MJ, Ferguson EL, Childs SR, O'Connor MB. The Drosophila dorsal-ventral patterning gene tolloid is related to human bone morphogenetic protein 1. Cell 1991;67(3):469–481. [PubMed: 1840509]

Sieron AL, Tretiakova A, Jameson BA, Segall ML, Lund-Katz S, Khan MT, Li S, Stöcker W. Structure and function of procollagen C-proteinase (mTolloid) domains determined by protease digestion,

circular dichroism, binding to procollagen type I, and computer modeling. Biochemistry 2000;39 (12):3231–3239. [PubMed: 10727214]

Sterchi E, Naim H, Lentze M, Hauri H, Fransen J. N-benzoyl-L-tyrosyl-p-aminobenzoic acid hydrolase: a metalloendopeptidase of the human intestinal microvillus membrane which degrades biologically active peptides. Arch. Biochem. Biophys 1988;265(1):105–118. [PubMed: 3261961]

Sterchi EE, Green JR, Lentze MJ. Non-pancreatic hydrolysis of N-benzoyl-l-tyrosyl-p-aminobenzoic acid (PABA-peptide) in the human small intestine. Clin. Sci 1982;62(5):557–560. [PubMed: 7042181]

Sterchi EE, Green JR, Lentze MJ. Nonpancreatic hydrolysis of N-benzoyl-L-tyrosyl-p-aminobenzoic acid (PABA peptide) in the rat small intestine. J. Pediatr. Gastroenterol. Nutr 1983;2(3):539–547. [PubMed: 6352892]

Sterchi EE, Naim HY, Lentze MJ. Biosynthesis of N-benzoyl- L-tyrosyl-p-aminobenzoic acid hydrolase: disulfide-linked dimers are formed at the site of synthesis in the rough endoplasmic reticulum. Arch. Biochem. Biophys 1988;265(1):119–127. [PubMed: 3261962]

Stöcker W, Gomis-Rüth FX, Bode W, Zwilling R. "mplications of the three-dimensional structure of astacin for the structure and function of the astacin family of zinc-endopeptidases. Eur. J. Biochem 1993;214(1):215–231. [PubMed: 8508794]

Stöcker W, Grams F, Baumann U, Reinemer P, Gomis-Rüth FX, McKay DB, Bode W. The metzincins--topological and sequential relations between the astacins, adamalysins, serralysins, and matrixins (collagenases) define a superfamily of zinc-peptidases. Protein Sci 1995;4(5):823–840. [PubMed: 7663339]

Stöcker W, Ng M, Auld DS. Fluorescent oligopeptide substrates for kinetic characterization of the specificity of Astacus protease. Biochemistry 1990;29(45):10418–10425. [PubMed: 2261483]

Stöcker, W.; Yiallouros, Y. Astacin. Handbook of Proteolytic Enzymes. Barrett, AJ.; Rawlings, ND.; Woessner, F., jr, editors. New York: Academic Press; 2004. p. 595-598.

Stöcker W, Zwilling R. Astacin. Meth. Enzymol 1995;248:305–325. [PubMed: 7674929]

Sunnerhagen M, Pursglove S, Fladvad M. The new MATH: homology suggests shared binding surfaces in meprin tetramers and TRAF trimers. FEBS Lett 2002;530(1–3):1–3. [PubMed: 12387856]

Tajima T, Yokozawa Y, Kanzawa N, Tsuchiya T. "Cloning and sequence of a novel astacin family metalloproteinase, myosinase. Mol. Biol. Cell 1999;10:72A–72A.

Takahara K, Lee S, Wood S, Greenspan D. Structural organization and genetic localization of the human bone morphogenetic protein 1/mammalian tolloid gene. Genomics 1995;29(1):9–15. [PubMed: 8530106]

Tang J, Bond JS. Maturation of secreted meprin alpha during biosynthesis: role of the furin site and identification of the COOH-terminal amino acids of the mouse kidney metalloprotease subunit. Arch. Biochem. Biophys 1998;349(1):192–200. [PubMed: 9439598]

Trachtman H, Valderrama E, Dietrich JM, Bond JS. The role of meprin A in the pathogenesis of acute renal failure. Biochem. Biophys. Res. Commun 1995;208(2):498–505. [PubMed: 7695599]

Tsukuba T, Bond JS. Role of the COOH-terminal domains of meprin A in folding, secretion, and activity of the metalloendopeptidase. J. Biol. Chem 1998;273(52):35260–35267. [PubMed: 9857066]

Tsukuba T, Kadowaki T, Hengst JA, Bond JS. Chaperone interactions of the metalloproteinase meprin A in the secretory or proteasomal-degradative pathway. Arch. Biochem. Biophys 2002;397(2):191–198. [PubMed: 11795871]

Uren AG, Vaux DL. TRAF proteins and meprins share a conserved domain. Trends Biochem. Sci 1996;21 (7):244–245. [PubMed: 8755243]

Villa JP, Bertenshaw GP, Bond JS. Critical amino acids in the active site of meprin metalloproteinases for substrate and peptide bond specificity. J. Biol. Chem 2003;278(43):42545–42550. [PubMed: 12888571]

Walker PD, Kaushal GP, Shah SV. Meprin A, the major matrix degrading enzyme in renal tubules, produces a novel nidogen fragment in vitro and in vivo. Kidney Int 1998;53(6):1673–1680. [PubMed: 9607199]

Wermter C, Höwel M, Hintze V, Bombosch B, Aufenvenne K, Yiallouros I, Stöcker W. The protease domain of procollagen C-proteinase (BMP1) lacks substrate selectivity, which is conferred by non-proteolytic domains. Biol. Chem 2007;388(5):513–521. [PubMed: 17516847]

Wolz RL, Harris RB, Bond JS. Mapping the active site of meprin-A with peptide substrates and inhibitors. Biochemistry 1991;30(34):8488–8493. [PubMed: 1883833]

Wozney JM, Rosen V, Celeste AJ, Mitsock LM, Whitters MJ, Kriz RW, Hewick RM, Wang EA. Novel regulators of bone formation: molecular clones and activities. Science 1988;242(4885):1528–1534. [PubMed: 3201241]

Yamaguchi T, Fukase M, Kido H, Sugimoto T, Katunuma N, Chihara K. Meprin is predominantly involved in parathyroid hormone degradation by the microvillar membranes of rat kidney. Life Sci 1994;54(5):381–386. [PubMed: 8289600]

Yamaguchi T, Kido H, Fukase M, Fujita T, Katunuma N. A membrane-bound metallo-endopeptidase from rat kidney hydrolyzing parathyroid hormone. Purification and characterization. Eur J Biochem 1991;200(2):563–571. [PubMed: 1889419]

Yan L, Fei K, Zhang J, Dexter S, Sarras MP Jr. Identification and characterization of hydra metalloproteinase 2(HMP2): a meprin-like astacin metalloproteinase that functions in foot morphogenesi. Development 2000;127(1):129–141. [PubMed: 10654607]

Yan L, Leontovich A, Fei K, Sarras MP Jr. Hydra metalloproteinase 1: a secreted astacin metalloproteinase whose apical axis expression is differentially regulated during head regeneration. Dev. Biol 2000;219(1):115–128. [PubMed: 10677259]

Yasumasu S, Shimada H, Inohaya K, Yamazaki K, Iuchi I, Yasumasu I, Yamagami K. Different exon-intron organizations of the genes for two astacin-like proteases, high choriolytic enzyme (choriolysin H) and low choriolytic enzyme (choriolysin L), the constituents of the fish hatching enzyme. Eur. J. Biochem 1996;237(3):752–758. [PubMed: 8647122]

Yiallouros I, Kappelhoff R, Schilling O, Wegmann F, Helms MW, Auge A, Brachtendorf G, Berkhoff EG, Beermann B, Hinz HJ, König S, Peter-Katalinic J, Stöcker W. Activation mechanism of pro-astacin: role of the pro-peptide, tryptic and autoproteolytic cleavage and importance of precise amino-terminal processing. J. Mol. Biol 2002;324(2):237–246. [PubMed: 12441103]

Yiallouros I, Vassiliou S, Yiotakis A, Zwilling R, Stöcker W, Dive V. Phosphinic peptides, the first potent inhibitors of astacin, behave as extremely slow-binding inhibitors. Biochem. J 1998;331(Pt 2):375–379. [PubMed: 9531473]

Yokozawa Y, Tamai H, Tatewaki S, Tajima T, Tsuchiya T, Kanzawa N. Cloning and biochemical characterization of astacin-like squid metalloprotease. J. Biochem 2002;132(5):751–758. [PubMed: 12417025]

Zapata JM, Martinez-Garcia V, Lefebvre S. Phylogeny of the TRAF/MATH domain. Adv. Exp. Med. Biol 2007;597:1–24. [PubMed: 17633013]

Zapata JM, Pawlowski K, Haas E, Ware CF, Godzik A, Reed JC. A diverse family of proteins containing tumor necrosis factor receptor-associated factor domains. J. Biol. Chem 2001;276(26):24242–24252. [PubMed: 11279055]

Zondag GC, Koningstein GM, Jiang YP, Sap J, Moolenaar WH, Gebbink MF. Homophilic interactions mediated by receptor tyrosine phosphatases mu and kappa. A critical role for the novel extracellular MAM domain. J. Biol. Chem 1995;270(24):14247–14250. [PubMed: 7782276]

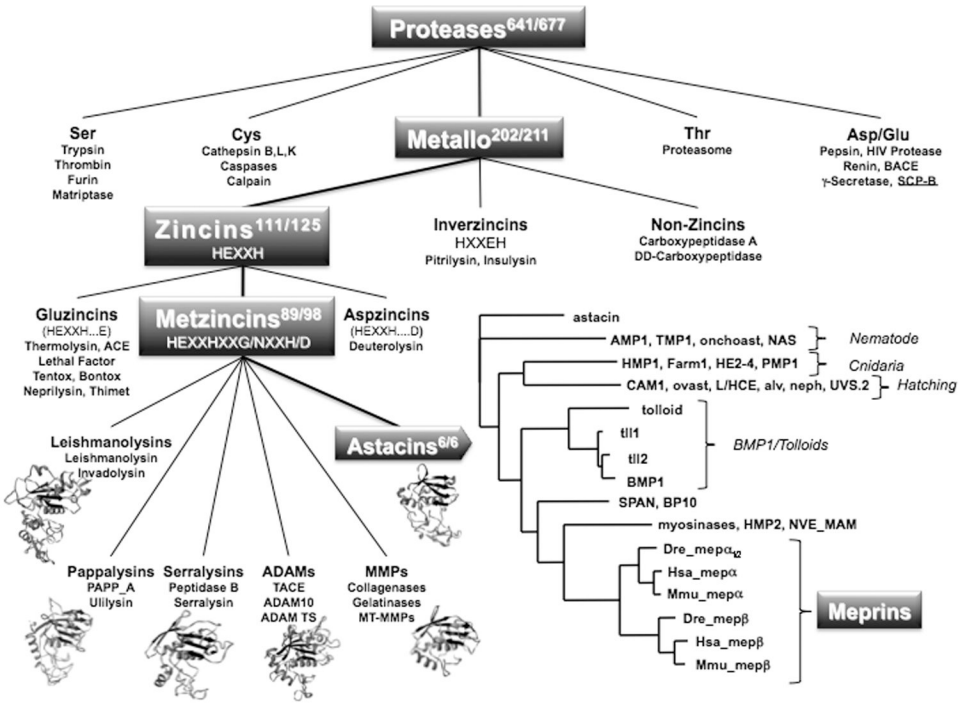


Figure 1. Proteases, metzincins, astacins, meprins

Schematic overview of proteolytic enzymes with special emphasis on metzincin metalloproteinases. The superscripts indicate the numbers of human and mouse protease genes (http://merops.sanger.ac.uk). Enzymes are indicated with UniProtKB/Swiss-Prot code.

Ser, serine proteases (trypsin P07478; thrombin P00734; furin P09958, matriptase Q9Y5Y6);

Cys, cysteine proteinases (cathepsins B P07711, L P07858, K P43235, caspase 8 Q14790, calpain P07384);

Thr, threonine proteinases (human proteasome β4 P28070);

Asp/Glu, aspartate and glutamate proteinases (pepsin A P00790, HIV proteinase P04586, renin P00797, BACE P56817, γ secretase P49810, SCP-B P15369, i.e. scytalidoglutamic peptidase);

Metallo = metalloproteases; **Non-Zincins**, metalloproteinases with alternate zinc motifs (carboxypeptidase A P00730, DD-carboxypeptidase P00733);

Inverzincins, metalloproteinases with the inverted zinc binding motif HXXEH (pitrilysin P05458, insulysin P14735);

Zincins, metalloproteinases with the zinc binding consensus sequence HEXXH (single letter code, X = any amino acid residue);

Gluzincins, metalloproteinases with the zinc binding motif HEXXH…E (thermolysin P00800; ACE P12821, i.e. angiotensin converting enzyme, *Anthrax* lethal factor P15917; Tentoxilysin P04958, Bontoxilysin P10844, neprilysin P08473, thimet oligopeptidase P24155);

Aspzincins, metalloproteinases withe the zinc binding motif HEXXH.…D (deuterolysin, P46073);

Metzincins, metalloproteinases with the zinc binding motif HEXXHXXG/NXXH/D);

Leishmanolysins (leishmanolysin, P08148; invadolysin, Q9VH19);

Pappalysins (PAPP_A, Q13219);

Serralysins (peptidase B P16316, serralysin P23694);

ADAMs (A Disintegrin And Metalloproteinase; TACE i.e. ADAM17 P78536, ADAM10 i.e. α secretase Q10741, ADAMTS2 P79331);

MMPs (matrix metalloproteinases, MMP1 collagenase P79331, MMP2 gelatinase P08253, MT1-MMP P50281);

Astacins (astacin P07584; hook worm AMP1 1 Q9GTJ6, trichina TMP1 Q8T5Z5, Onchocerca onchoastacin Q2YFS7, C. elegans NAS36 Q18206, Hydra HMP1 Q25174, Hydra FARM1; Hydractinia HE2-4 Q2MCX8 Q2MCX7 Q2MCX6, Podecoryne PMP1 O62558, quail CAM1 P42662, carp alveolin Q9IBE7, carp nephrosin O42326, medaka HCE P31580, medaka LCE P31579, frog UVS2 P42664, human ovastacin Q6HA08, fly tolloid P25723, human BMP1 P13497, human Tll1 O43897, human Tll2 Q9Y6L7, sea urchin SPAN P98068, sea urchin BP10 P42674, squid myosinases Q8IU47 Q8IU44 Q8IU46 Q8IU45, Hydra HMP2 Q9XZG0, sea anemone NVE_MAM A7SJ13, fish meprin a1 Q5RHM1, fish meprin a2 Q5RHM2, mouse meprin a P28825, human meprin a Q16819, fish meprin b Q08CC, human meprin b Q16820, mouse meprin b Q61847.

Below the different metzincin subfamilies, ribbon plots are depicted of representative catalytic domain structures: leishmanolysin (1lml.pdb), ulilysin (2cki.pdb), serralysin (1srp.pdb), TACE (1bck.pdb), MMP8 (1mnc.pdb), and astacin (1ast.pdb).

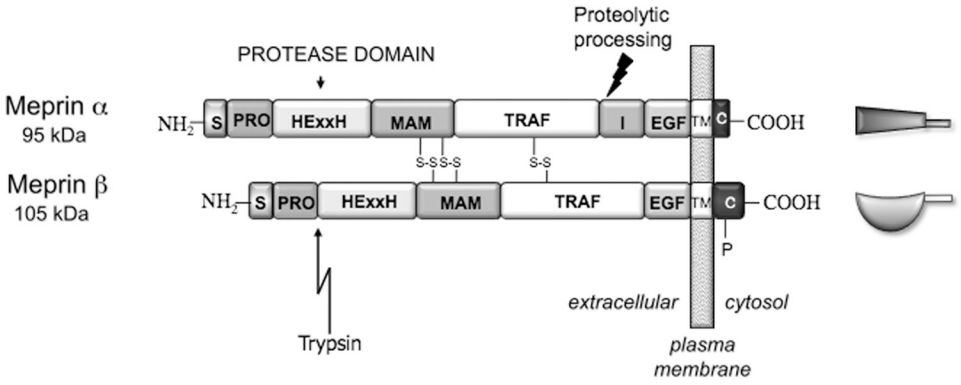


Figure 2. Domain structure of human meprins

Meprins consist of an N-terminal signal peptide (S), a prodomain (PRO), a proteinase domain containing the metzincin motif HxxLHxxGxxH, a MAM domain (meprin, A5 protein, receptor protein tyrosine phosphatase μ), a TRAF domain (tumor necrosis factor receptor-associated factor), an epidermal growth factor-like domain (EGF), a transmembrane domain (TM) and a cytosolic tail sequence (C), which is only 6 amino acid residues long in the α subunit and 28 amino acid residues in the β subunit. The α subunit contains an additional inserted sequence (I), which is essential for the constitutive proteolytic processing of this subunit. Three intersubunit S-S bridges are proposed for the human heterodimer; only two intersubunit bridges are possible for the mouse heterodimer because this subunit has no Cys residues in the TRAF domain. Cleavage by trypsin or trypsin-like enzymes results in removal of the prodomain and activation of the meprin subunits (Jiang, Gorbea et al. 1992; Dumermuth, Eldering et al. 1993; Eldering, Grunberg et al. 1997).

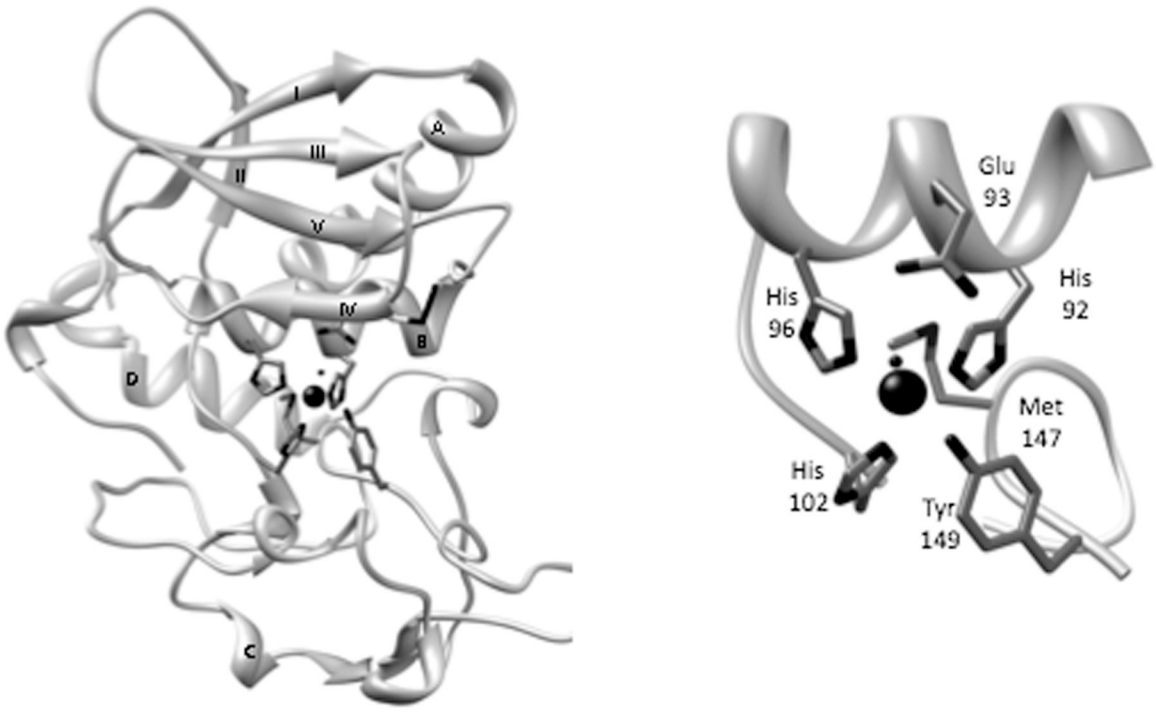


Figure 3. Model of the proteinase domain structure of meprins

Based on the structure of astacin (Bode, Gomis-Rüth et al. 1992) (1ast.pdb), the homologous proteinase domain of meprins consists of a five-stranded β-sheet, three α-helices and coil structure in the lower subdomain (Stöcker, Gomis-Rüth et al. 1993). The catalytic zinc (large black sphere) is penta-coordinated by three histidines, the catalytic water (small black sphere) and a tyrosine positioned by the Met-turn, containing the eponymous methionine typical for metzincin proteinases. The structure of the catalytic domain is stabilized by two intradomain disulfide-bridges and several conserved salt bridges.

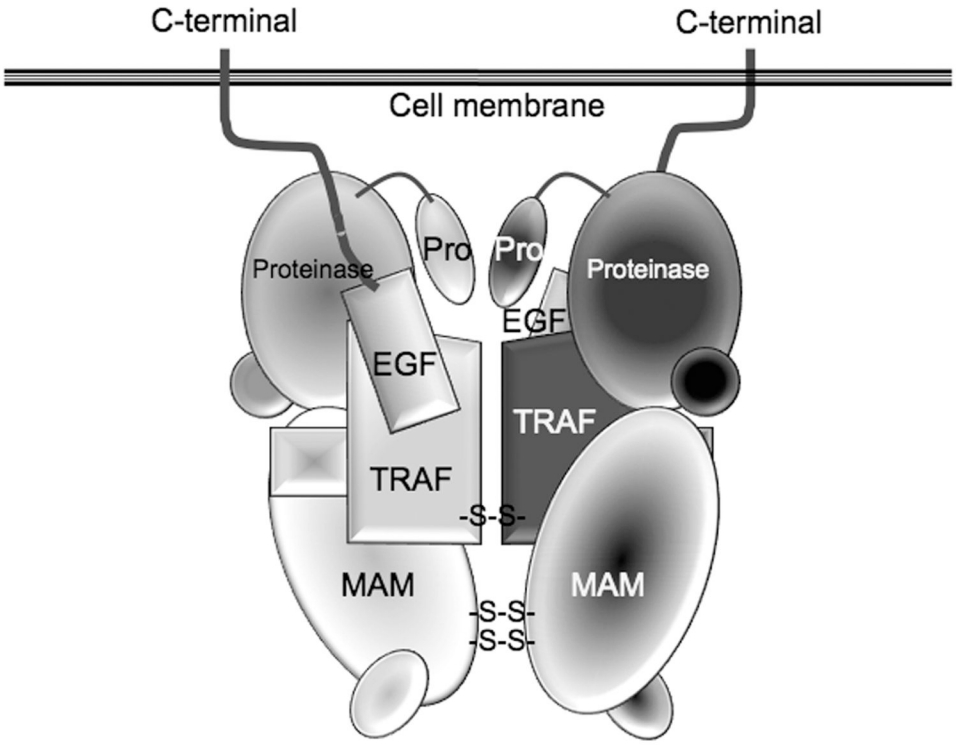


Figure 4. Model of the meprin β dimer

A model of the dimeric structure is proposed based upon disulfide and cross-linking mapping experiments. The dimmer exhibits D2 symmetry and is arranged such that the subunit interface is formed between the MAM domains, the TRAF domains, and the prosequences. The protease domain interacts with the TRAF domain as well as the EGF domain, placing it near the COOH-terminal region of the protein close to the cell membrane (Ishmael, Shier et al. 2005).

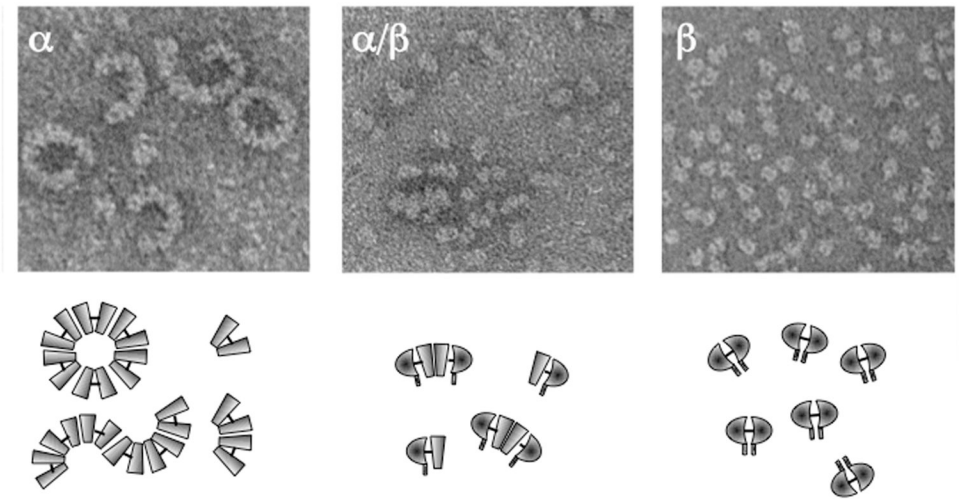


Figure 5. Oligomerization of meprin proteinases

α Electron micrographs of meprin α expressed in human embryonic kidney 293 show disulphide-bridged homodimers which can further associate noncovalently in long, curved chains to form rings and spirals of variable sizes that reach molecular masses in the mega Dalton range. By contrast, meprin β homooligomers form only disulphide-linked dimers that are incapable of further aggregation. α/β When coexpressed, meprin α and β subunits show disulphide-linked heterodimers which mostly associate non-covalently as heterotetramers. Below the electron micrographs the oligomeric arrangements of the individual subunits is schematically depicted (Becker, Kruse et al. 2003; Bertenshaw, Norcum et al. 2003).

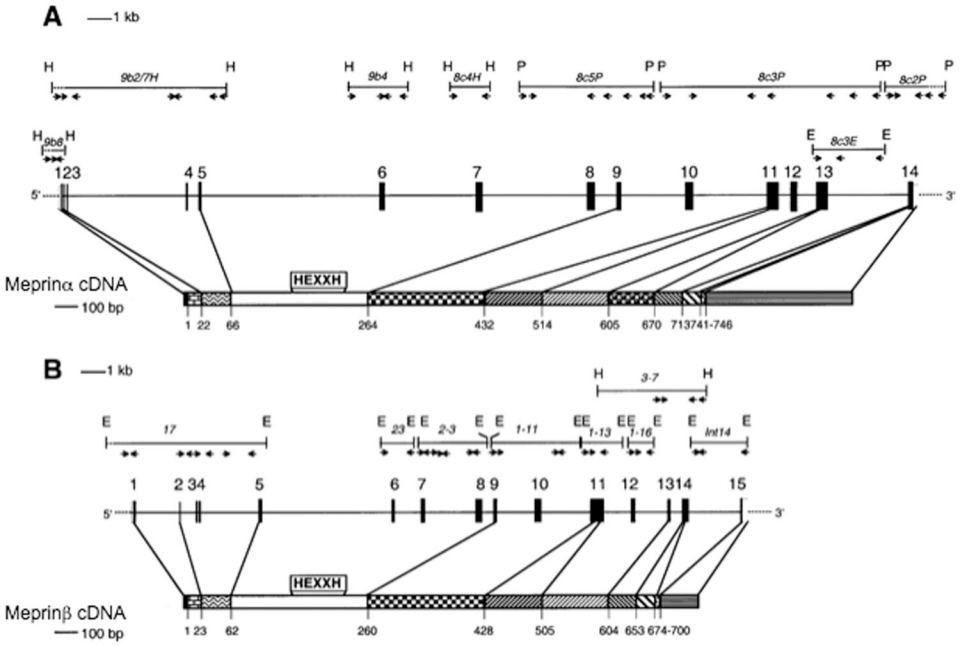
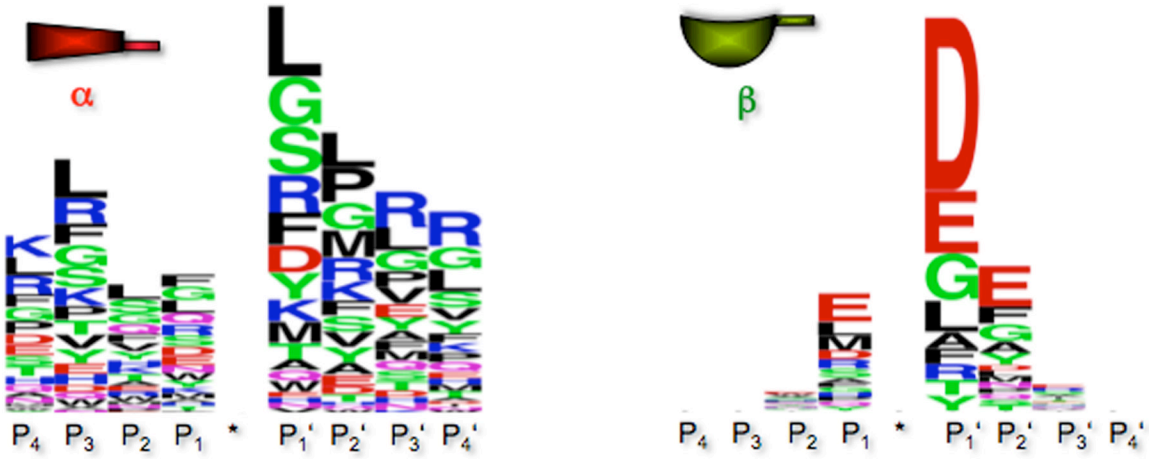


Figure 6. Gene organization of meprins

The top line in each panel shows a schematic diagram of the respective gene. Exons are numbered and are represented as follows: black boxes, coding regions; open boxes, non-coding regions. Introns are presented as dashes. pKS clones containing genomic sequences are shown above the line. Arrows indicate the position and direction of primers used during the sequencing project.

The bottom line in each panel demonstrates a schematic diagram of meprin α and meprin β protein derived from the exons. The amino acid numbers at the beginning of each domain are also presented.

Abbreviations : H, *Hin* dIII ; E, *Eco* RI ; P, *Pst* I. Regions of the protein are indicated as follows: 5' untranslated region; signal peptide; propeptide; proteinase; MAM (meprin subunit domain/A5 protein/receptor protein tyrosine phosphatase l domain); MATH (meprin- and tumour-necrosis-factor-receptor-associated factors homology domain); intervening domain; I-domain (inserted domain); EGF-like domain; transmembrane domain; cytosolic domain; 3' untranslated region (Hahn, Illisson et al. 2000).



Substrates of α only
bombesin, neurotensin, substance P, angiotensin I, luteinizing hormone releasing hormone, valosin, vasoactive intestinal peptide, bradykinin, α-melanocyte stimulating hormone

Substrates of β only
orcokinin, gastrin 17, peptide YY, kinetensin osteopontin, interleukin 1β

Substrates of both α and β
gastrin releasing peptide, cholecystokinin, secretin, glucagon, neuropeptide Y, cerulein, laminin 1 α1, laminin 5 α3, collagen IV, gelatin, nidogen, fibronectin

Substrates of neither α nor β
[Lys^{3}]-vasopressin, Somatostatin, Kassinin, Oxytocin, α-Neurokinin, Collagen I (mouse meprin β but not human meprin bcleaves collagen I at 37°C

Inhibitors of both α and β
Actinonin, Batimastat, Galardin, Pro-Leu-Gly-NHOH, NNGH (N-Isobutyl-N-(4-methoxy-phenylsulfonyl)glycyl hydroxamic acid), Ro-327315, TAPI-0, TAPI-2 (tumor necrosis factor α protease inhibitor), 1,10-phenanthroline EDTA, Captopril (weak), mannan binding protein (MBP)

Inhibitors of neither α nor β
Ro-282653, α_2-macroglobulin, TIMPs

Figure 7. Substrate and Inhibitor specificity of meprins

At the top the strikingly different subsite specificities of mouse meprin α and β are depicted as a WEBLOGO (http://weblogo.berkeley.edu/). The size of the letters reflects the relative abundance of the respective single letter coded amino acid residues in substrate proteins at the P4 through P4' position (Schechter and Berger 1967). The substrates analyzed are those listed in the paper by (Bertenshaw, Turk et al. 2001). Listed below are substrates and inhibitors of meprin subunits (Bertenshaw, Turk et al. 2001; Kruse, Becker et al. 2004; Herzog, Kaushal et al. 2005; Hirano, Ma et al. 2005).

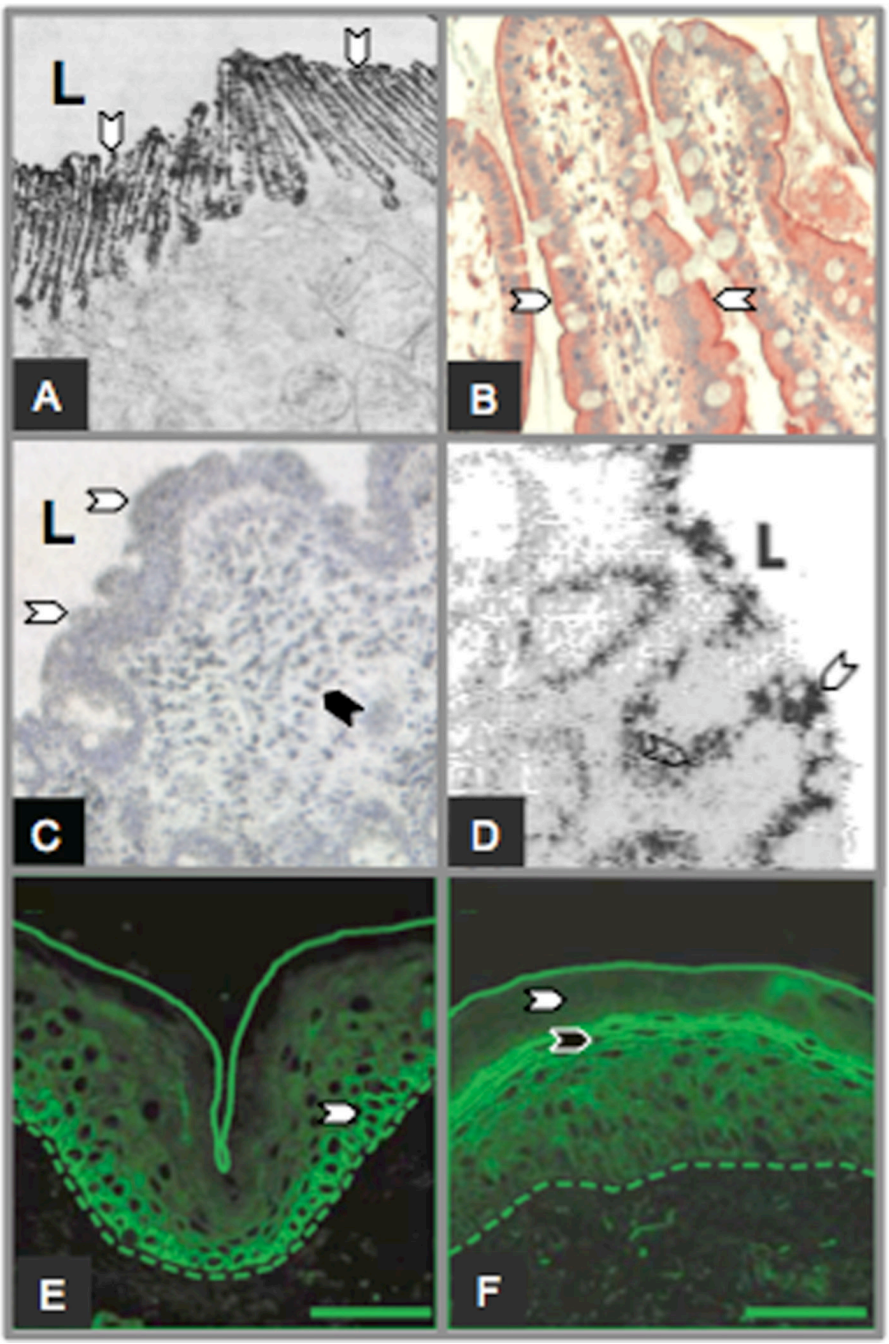


Figure 8. Tissue expression of meprins

A Immuno-electronmicrograph of proximal tubular cells from ICR mice showing brush border membrane localization of meprin α (Craig, Reckelhoff et al. 1987).
B Immunohistochemical staining for meprin β in the brush border membrane of small intestinal epithelial cells (Lottaz, Hahn et al. 1999). **C** *In situ* hybridization for meprin mRNA α in small intestinal mucosa from a patient with active coeliac disease. The presence of meprin α shifts from the epithelial cells to leukocytes in the lamina propria (Lottaz, Buri et al. 2007). **D** *In situ* hybridization for meprin α mRNA in normal human colon (Lottaz, Hahn et al. 1999). **E** and **F** show immunofluorescence staining of human skin for meprin α and β, respectively. The continuous line indicates the outer most border of the *stratum corneum*, the dashed line

indicates the basal membrane of the epidermis. Panel E: Perinuclear fluorescence signal (white arrow head) for meprin α in cells of the *stratum basale*. Panel F: Signal for meprin β (white arrow head) in membranes of cells of the *stratum granulosum* beneath the *stratum corneum* (black arrow head) (Becker-Pauly, Höwel et al. 2007).