

Chapter 19

Metalloproteases and Proteolytic Processing

Anthony J. Turner and Natalia N. Nalivaeva*

Abbreviations

A β	Amyloid β -peptide
ACE	Angiotensin converting enzyme
AICD	APP intracellular domain
APP	Amyloid precursor protein
ECE	Endothelin-converting enzyme
IDE	Insulysin (insulin-degrading enzyme)
MMP	Matrix metalloproteinase
PAI	Plasminogen-activator inhibitor
RAS	Renin-angiotensin system

19.1 Introduction

Proteolysis represents one of the key processes underlying biological events from fertilization through embryonic development, cell differentiation, ageing and ultimately death via the programmed route (apoptosis) or necrosis in the case of trauma, infection or disease. The term “peptidase” (or protease) is used to refer to the complement of enzymes that cleave peptide bonds and, as such, all known proteins and peptides represent their potential substrate repertoires. According to the location of the peptide bond cleaved by the peptidases, they are conventionally sub-divided into “endopeptidases” that cleave the bond within the polypeptide chain, or exopeptidases that release amino acids from the N- or C-terminus of the substrate. However, this is an over-simplification

A.J. Turner (✉)

Institute of Molecular and Cellular Biology, University of Leeds, LS2 9JT Leeds, UK

e-mail: a.j.turner@leeds.ac.uk

and

*Dr N.N. Nalivaeva’s

Institute of Evolutionary Physiology and Biochemistry, RAS, St.Petersburg, Russia

since some peptidases, e.g. ACE, can fulfill both endo- and exopeptidase functions (Skidgel and Erdös 1985). The term “limited proteolysis” was originally introduced by Linderstrom-Lang to distinguish the restricted specificity of certain peptidases, particularly those involved in post-translational modification of proprotein precursors, from the random proteolysis accompanying protein degradation (Neurath 1999).

The number of species in which peptidases have now been identified is approaching 5,000 (<http://merops.sanger.ac.uk/>) distributed among five classes (metallo-, serine, cysteine, aspartic and threonine) with cysteine-, serine- and metallopeptidases representing the major ones. Among these three main classes, the known or putative metallopeptidases are more consistently represented across species varying from 105, for example, in *Trypanosoma cruzi* to 164 in mouse (and 161 in the human) (Table 19.1). The other two classes exhibit a greater diversity ranging in the number of genes for cysteine proteases from 26 in *T. cruzi* to 162 in mouse (and 160 in the human). For the serine peptidases this distribution is even more pronounced: from 14 in *T. cruzi* to 245 in mouse (210 in human). There are a smaller number of aspartic peptidases although some are of key therapeutic importance (e.g. the β - and α -secretases implicated in Alzheimer pathology) and even less variety in the number of threonine peptidases. This greater consistency of representation of metallopeptidases across species probably reflects more generic cellular functions and their distinctive catalytic mechanisms which require a water molecule activated by a divalent metal ion, commonly zinc.

The complete repertoire of peptidases expressed by a tissue or organism is now referred to as the “protein degradome,” a concept originally introduced by Lopez-Otin and colleagues (Cal et al. 2003). Comparative analysis of mammalian degradomes has revealed that rodents generally have a more complex combination of peptidases compared with primates, mainly due to the expansion of peptidase families involved in reproduction and host defence (Puente et al. 2005). These authors also found several genes implicated in reproduction, nutrition and the immune system, which are functional in rat, mouse or chimpanzee, but have been inactivated by mutations in the human lineage. These findings suggested that conversion of specific peptidase genes into pseudogenes has contributed to the evolution of the human genome. A recent example of this “pseudogenisation” is found in the ACE3 gene in the human (Rella et al. 2007). Approx one-quarter of the 80 or so human inherited diseases caused by mutations in protease-coding genes (<http://www.uniovi.es/degradome/>) are linked to metallopeptidases (see Table 19.2). Abnormal functioning of these genes is implicated in such diverse pathologies as inflammatory and cardiovascular diseases, cancer, and neurodegeneration. Understanding the normal roles of these enzymes and their homologues is important for the design of novel and selective therapeutics. Animal venoms are a natural source of a wide range of metallopeptidases which have biological implications (Moura-da-Silva et al. 2007) and some bacterial toxins (e.g. botulinum, tetanus) exert their toxic actions as metalloproteases, in their case by blocking neurotransmitter release by proteolysis of synaptobrevin (Schiavo et al. 1992).

Table 19.1 Number of total peptidases and metallo-peptidases in genomes of some species^a

Species	Total number of known or putative peptidases	Total non-peptidase homologues	Unique peptidases	Total known or putative inhibitors	Total non-inhibitor homologues	Unique inhibitors	Total number of known or putative metallo-peptidases	Total number of non-metallo-peptidase homologues
<i>Homo sapiens</i> (human)	660	387	256	179	204	34	161	66
<i>Pan troglodytes</i> (chimpanzee)	438	120	388	107	83	89	108	38
<i>Rattus norvegicus</i> (rat)	519	148	114	218	162	25	144	57
<i>Mus musculus</i> (mouse)	679	189	660	225	215	225	164	59
<i>Drosophila melanogaster</i> (fruit fly)	494	245	389	62	86	62	126	71
<i>Caenorhabditis elegans</i> (roundworm)	350	126	350	49	138	11	137	50
<i>Trypanosoma cruzi</i> (parasitic flagellate)	153	66	133	1	2	1	105 (76 of M8 family)	35 (19 of M8 family)

^aData collected from the MEROPS database <http://merops.sanger.ac.uk/>

Table 19.2 Human hereditary diseases of metalloproteinases

Metalloproteinase	Class	Gene	Locus	Disease	Inheritance	Function	Animal model
Angiotensin converting enzyme	M02	ACE	17q23	Renal tubular dysgenesis	R	Loss	KO resembles disease
Collagenase 3	M10	MMP13	11q22	Spondyloepimetaphyseal dysplasia	D	(Gain)	KO resembles disease
Gelatinase A	M10	MMP2	16q13	Multicentric osteolysis with arthritis	R	Loss	KO does not resemble disease
Enamelysin	M10	MMP20	11q22	Amelogenesis imperfecta	R	Loss	KO resembles disease
ADAMTS-10	M12	ADAMTS10	19p13	Weill-Marchesani syndrome	R	Loss	–
ADAMTS-13	M12	ADAMTS13	9q34	Thrombotic thrombocytopenic purpura	R	Loss	–
Procollagen I N-endopeptidase	M12	ADAMTS2	5q23	Ehlers-Danlos syndrome type VIIC	R	Loss	KO resembles disease
Endothelin-converting enzyme 1	M13	ECE1	1p36	Hirschprung disease	D	Loss	KO partially resembles disease
Kell blood-group protein	M13	KEL	7q35	Kell blood group antigen	R	Loss	–
Neprilysin	M13	MME	3q26	Fetomaternal alloimmunisation	R	Loss	–
PHEX endopeptidase	M13	PHEX	Xp22	X-linked hypophosphatemia	D	Loss	Hyp mouse resembles disease
X-Pro dipeptidase	M24	PEPD	19q13	Prolidase deficiency	R	Loss	–
S2P protease	M50	MBTPS2	Xp22	Ichthyosis follicularis, atrichia, and photophobia syndrome (IFAP)	–	Loss	–

From a total of 80 human hereditary diseases caused by mutations in protease-coding genes, 13 are related to metalloproteinase classes discussed in this chapter. They are classified by loss or gain of function and by the type of inheritance: R (recessive) or D (dominant) (Adapted from: Quesada et al. (2009) and <http://www.uniovi.es/degradome/>)

19.2 Physiological Significance of Proteolysis

Organisms use proteolysis for a wide range of purposes. They include complete protein digestion of dietary proteins as a source of amino acids by the digestive proteases (trypsin, chymotrypsin, pepsin), degradation of structural components e.g. extracellular matrix by the matrix metalloproteases (MMPs), intracellular protein turnover important in post-mitotic cells to prevent accumulation of malfunctioning proteins (lysosome, ubiquitin-proteasome system) including partial digestion of external antigens in immune surveillance, degradation of cyclins at different stages of the cell cycle, conversion of precursor-proteins into their final biologically active structures (zymogens, pro-enzymes, prohormones, other cryptic peptides) and ectodomain shedding to initiate or modulate cell signalling. Highly specific proteolysis can regulate G protein receptor signalling (proteinase-activated receptors) (Bunnett 2006), and nuclear transcriptional events through regulated intramembrane proteolysis (Wolfe 2009). This chapter will predominantly focus on processing events that utilise metalloproteases, and their physiological and pathological consequences.

The process of protein biosynthesis itself involves proteolytic processing: the removal of initiator methionine, signal peptide, cell localization signal and propeptide, where present. A primary mechanism in post-translational modifications of proteins is hence the co-translational removal of the *N*-terminal methionine from a nascent protein, a process which ultimately determines the half-life of a protein by exposing a new *N*-terminal residue, as originally proposed by Varshavsky and colleagues (the *N*-end rule) (Bachmair et al. 1986). Proteins retaining the initiator methionine have shorter half-lives (Frottin et al. 2006). This has led to the definition of specific protein degradation signals or “degrons” that can allow rapid degradation through the ubiquitin-proteasome system in eukaryotes and the ClpAP protease in bacteria (Ravid and Hochstrasser 2008; Mogk et al. 2007). The initiating step, the methionine removal, is catalysed by methionyl aminopeptidases (MetAP) and, although the eukaryotic MetAP was originally thought to be cobalt-dependent, it is likely that the physiologically relevant cation for this enzyme is zinc (Bradshaw et al. 2004; Leopoldini et al. 2007). Methionine removal subsequently followed by modification of the *N*-terminal residue is often crucial for the function, or dysfunction, of a protein. In this context, the post-translational conversion of an *N*-terminal glutamate to a cyclized form (pyroglutamylation) is relatively common and is catalysed by the zinc metalloenzyme glutaminyl cyclase (QC) (Huang et al. 2005). This can modify, among other substrates, the Alzheimer’s amyloid- β (A β) peptide rendering it more hydrophobic with a greater propensity to aggregation (Cynis et al. 2008). Small molecule inhibitors of QC may provide novel therapeutics in the treatment of AD (Schilling et al. 2008) and see below (Sect. 19.7).

19.3 Metalloproteases: Their Properties and Roles in Disease

All known metalloproteases can be divided into 12 clans according to structural and catalytic similarities, for example in the type and number of metal ions required for their activity (Rawlings and Barrett 2004). They are also subdivided into distinct classes and in the human genome practically all classes are present. In this chapter we shall focus on zinc metalloprotease families belonging to one of these clans, namely MA, since these are principally involved in the cell-surface or extracellular post-translational modifications of proteins and peptides. Representatives of this clan require zinc for their activity and are often referred to as “zincins.” They contain histidine residues as zinc ligands in the typical zinc binding motif, His-Glu-Xaa-Xaa-His (HEXXH). They are further sub-divided according to the nature of the third zinc ligand: gluzincins use a glutamyl residue which lies 18–72 amino acids C-terminal to the HEXXH motif; metzincins use a His or Asp within an extended zinc motif HEXXHXXGXXH/D. They are termed metzincins because of a strictly conserved methionine-containing turn (the Met-turn) in their structure which is thought to provide a hydrophobic base at the zinc-binding site (Stöcker et al. 1995).

The metalloprotease families that will be considered in particular detail are (i) the M13 family typified by neprilysin [or neutral endopeptidase (NEP)], (ii) the M2 family represented by angiotensin-converting enzyme [or peptidyl dipeptidase (ACE)], (iii) the matrix metalloproteases (MMPs) which include collagenases and gelatinases. Finally, we shall consider the related ADAMs (A disintegrin and metalloproteinase) family involved in the shedding of surface proteins and including the ADAMs with thrombospondin motifs (ADAMTS family), which play a particular role in extracellular matrix turnover and arthritis (Jones and Riley 2005). Members of each of these families have served as potential drug targets, particularly in cardiovascular disease and cancer, but more recently have provided insight into mechanisms involved in neurodegeneration and neuroinflammation, especially from the point of view of processing of the amyloid precursor protein (APP) and its products in Alzheimer’s disease.

While the metalloproteases above utilise a single zinc ion for activity, there are examples where two zinc ions are required in a co-catalytic (binuclear) process. The first example of these to be discovered was the cytosolic enzyme, leucyl aminopeptidase (Burley et al. 1990). A key function for this enzyme is in “trimming” proteasome-produced peptides for class I antigen presentation and it is up-regulated by cytokines, e.g. γ -interferon (Beninga et al. 1998). Another example of key pathophysiological significance is the type II membrane glycoprotein, glutamate carboxypeptidase II (GCPII; NAALADase), which has two distinct roles: in the brain it inactivates the neuropeptide *N*-acetyl-aspartyl-glutamate by cleaving off the α -linked glutamate whereas in the intestine it sequentially releases γ -linked glutamates from folyl-poly- γ -glutamates (folate hydrolase activity). It is also identical with the prostate cancer marker PSMA (prostate-specific membrane antigen), which is highly expressed in aggressive prostate tumours and which might provide

a target for immunological or other therapeutic approaches to the disease (Elsässer-Beile et al. 2009). The recent structural solution of a catalytically inactive mutant (E424A) of GCPII in complex with its substrate (*N*-Ac-Asp-Glu) has, for the first time, revealed the detailed reaction mechanism for this dual zinc-containing metallopeptidase (Klusák et al. 2009).

19.4 Classes of Metallopeptidases Present in the Human Genome

A brief and selected overview of the characteristics of some of the metallopeptidase families discussed herein follows before a more detailed description of individual peptidases and their roles in peptide and protein modifications in health and disease. For a full description of all classes and categories of metallopeptidases, the reader should consult (Rawlings and Barrett 2004).

19.4.1 Class M2

Family M2 contains angiotensin-converting enzyme (ACE) best known for its key role in the processing cascade of the renin-angiotensin system (RAS) and, until recently, this was the only known member of the family in humans. Arguably, ACE is better termed peptidyl-dipeptidase-A because, as a carboxydipeptidase, it has a number of important substrates and functions in addition to the processing of angiotensin, but it is generally referred to as “ACE.” Relatively recently in the timescale of vertebrate evolution the ACE gene underwent an internal duplication producing the so-called somatic form of the enzyme which has two catalytic sites (the N- and C-domains) which, while similar, show some differences both in substrate specificity and inhibitor sensitivity. X-ray crystal structures for each of these domains in complex with selective inhibitors have revealed the molecular basis for this selectivity in the two peptide processing domains (Watermeyer et al. 2008). Use of an alternative initiation site produces a form of ACE with only a single catalytic site expressed exclusively in mammalian testis which is critical to male reproduction (Hagaman et al. 1998).

Vertebrates are now known to express the homologous peptidase ACE2 (Tipnis et al. 2000) whose actions seem to counterbalance those of ACE. ACE is, of course, of great importance in the regulation of blood pressure through its conversion of the decapeptide angiotensin I into the vasopressor octapeptide angiotensin II by removal of the C-terminal dipeptide. Potent inhibitors of ACE, such as captopril or lisinopril, are hence highly effective anti-hypertensive agents (Menard and Patchett 2001) and do not inhibit ACE2 (Tipnis et al. 2000). Figure 19.1 illustrates the similarities between ACE and ACE2 and their counter-regulatory roles in angiotensin metabolism and hence cardiovascular biology. Most notably, ACE2 comprises only a single catalytic metalloprotease extracellular domain and its transmembrane and C-terminal

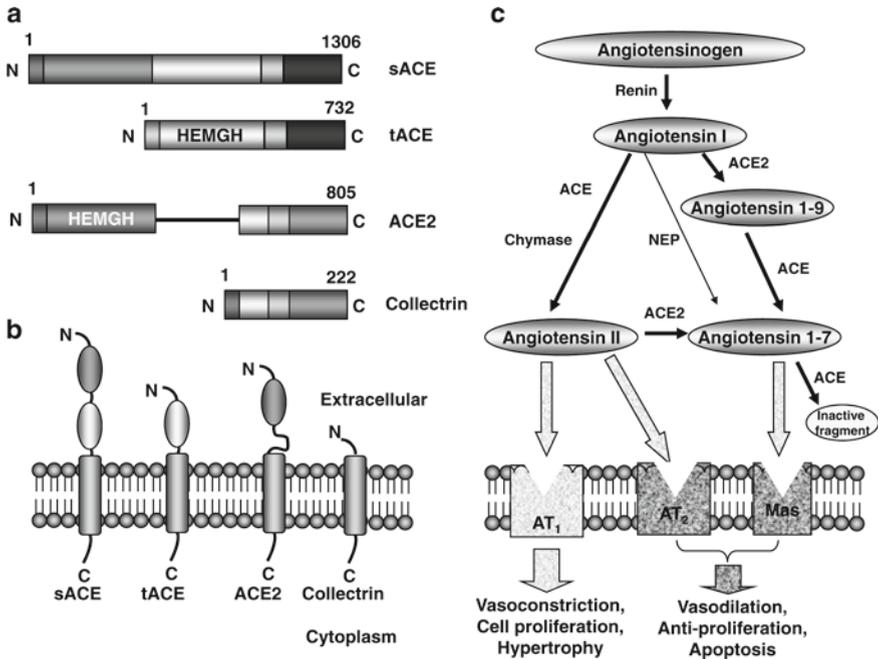


Fig. 19.1 The domain organisation (a) and cellular topology (b) of ACE isoforms and collectrin, and a schematic representation (c) of proteolytic processing events involved in the functioning of the renin-angiotensin system (RAS). (a and b) ACE isoforms (somatic – sACE, testicular – tACE, and ACE2) and collectrin, are type I integral membrane proteins with a signal peptide at the N-terminus and a short, C-terminal cytoplasmic domain. The numbers above the proteins represent the amino acid numbering of the sequences. tACE and ACE2 possess a single HEXXH Zn-binding motif (HEMGH in both), which forms the active site, whereas sACE possesses two of these. Collectrin lacks a catalytic site but has 47.8% sequence identity with the non-catalytic extracellular, transmembrane and cytosolic domains of ACE2 and no similarity to ACE. (c) shows the processing pathway in the RAS from the protein precursor angiotensinogen (452 amino acids in the human), to the biologically active angiotensin peptides (the octapeptide angiotensin II and the heptapeptide angiotensin-(1-7). ACE – angiotensin-converting enzyme, NEP – neprilysin, AT₁ – type 1 angiotensin II receptor), AT₂ – type 2 angiotensin II receptor and Mas – angiotensin (1-7) receptor. The conversion of angiotensin I to angiotensin (1-9) by ACE2 is kinetically 400 times less favourable than the conversion of angiotensin II to angiotensin (1-7) (Rice et al. 2004)

cytoplasmic domain share no similarity with ACE but with a small membrane protein, collectrin, involved in amino acid transport in the kidney (Danilczyk et al. 2006). ACE2 appears to be a dual function protein since it also is involved in amino acid transport through interactions with its collectrin-like domains but functions as a protease (a strict carboxypeptidase) through its extracellular ACE-like domain (Kowalczyk et al. 2008). ACE2 has also been subverted into acting as the cellular receptor for the severe acute respiratory syndrome (SARS) coronavirus and the associated down-regulation of surface ACE2 following infection predisposes to the

acute, and potentially fatal, lung injury in the disease. Hence in a number of distinct disorders: SARS infection, cardiovascular disease, liver fibrosis and diabetes, effects all mediated through impaired angiotensin peptide metabolism, upregulation of ACE2 expression and activity can be beneficial (Turner 2008).

19.4.2 *Class M10*

The peptidases of family M10 represent a large family of metzincins usually occurring as secreted metalloendopeptidases, although there are some membrane-bound forms in mammals. These enzymes are principally involved in degradation of the extracellular matrix, important in tissue remodeling and repair, and hence have trivial names such as collagenases, gelatinases, etc. but are generically referred to as matrix metalloproteases, matrixins or MMPs. Excessive activation of MMPS can result in significant pathological tissue damage as seen, for example, in arthritic conditions. The matrixins are generally synthesized as inactive precursors although differences exist in the activation mechanism. The matrixin proenzymes are held in a catalytically inactive state due to the presence of a “cysteine switch” (Van Wart and Birkedal-Hansen 1990), which involves interaction between a conserved cysteine in the propeptide and the catalytic zinc ion hence preventing the binding and activation of a crucial water molecule. Because of the importance of cysteine in the latency of the proenzymes, they can commonly be activated by thiol-blocking agents such as aminophenylmercuric acetate (APMA) (see e.g. Allinson et al. 2004).

Like other metallopeptidases, the peptidases in family M10 are inhibited by chelating agents, and many potent inhibitors such as batimastat have been synthesized as possible drugs. These seemed to offer great prospects as anti-metastatic drugs in cancer chemotherapy although they have failed to live up to expectations as yet (Tu et al. 2008). The TIMP (tissue inhibitor of metalloproteases) proteins are endogenous inhibitors of these peptidases.

19.4.3 *Class M12*

The study of a crayfish digestive enzyme (astacin) and its mammalian homologue (meprin) (Bond and Beynon 1995) led to the subsequent discovery of one of the largest families of metallopeptidases, containing the adamalysin (ADAM) peptidases, the snake venom metallopeptidases (reprolysins) and many other related enzymes (Dumermuth et al. 1991; Seals and Courtneidge 2003). Because of their potential involvement in inflammatory and other conditions, a number of synthetic inhibitors have been described, although there is a lack of highly specific inhibitors and those available also tend to inhibit MMPs (Zhang et al. 2004; Delaet et al. 2003; Skotnicki et al. 2003). Some members of the family are inhibited by TIMP-3 (Lee et al. 2002).

The peptidases are generally membrane or secreted proteins. Two key mammalian examples are ADAM17, or tumour necrosis factor- α converting enzyme (TACE), which is important for post-translational processing of certain membrane proteins and ADAMTS13, also known as von Willebrand factor protease, the activity of which normally prevents von Willebrand disease.

19.4.4 Class M13

The prototype of this family is neprilysin, an endopeptidase (neutral endopeptidase; endopeptidase-24.11) originally discovered in renal brush border membranes by Kenny and colleagues (e.g. Kerr and Kenny 1974) and subsequently rediscovered in the brain as a key neuropeptide-degrading enzyme (“enkephalinase”) (Roques et al. 1980; Matsas et al. 1983). NEP is a type II integral membrane protein composed of a short, cytoplasmic tail, a transmembrane region and a large extracellular region that folds into two domains that enclose a large, central cavity that contains the catalytic site (Oefner et al. 2000). Ten cysteine residues in the extracellular domain are conserved between all family members and are involved in intrachain disulphide bonds required for maintenance of structure and activity (Turner et al. 2001). NEP has important roles in modulating pain (through its actions on enkephalin peptides), blood pressure (through inactivation of atrial natriuretic peptide), prostate cancer (through metabolism of the mitogenic peptide, endothelin-1) and the immune system, being also identical with the common acute lymphoblastic leukaemia antigen, CALLA or CD10 (LeTarte et al. 1988). It was for some long time the only known peptidase in family M13, but additional homologues are now known, including the endothelin converting enzymes (ECE-1 and ECE-2) responsible for the post-translational processing of the protein precursors of the endothelin family of hypertensive peptides (Fig. 19.2). This processing pathway, like many protein processing events involving hormones, neuropeptides and growth factors, involves the initial removal of the N-terminal signal prepeptide to generate a physiologically inactive proprotein subsequently processed in the secretory pathway at dibasic cleavage sites to its active form by one of the seven subtilisin-like proprotein convertases, which include furin (Rholam and Fahy 2009). As intracellular, serine proteases they are not considered further in this chapter. In the endothelin pathway, it is likely that either furin or PC7 catalyses this processing event (Blais et al. 2002). However, the endothelin processing pathway is unusual in protein processing events since the product of prohormone convertase action is another physiologically inert intermediate, termed “big endothelin,” which is in turn converted to the vasoactive endothelin by the metallopeptidase, ECE-1. When first identified, as the most potent, endogenous human vasoconstrictor known at the time, the endothelin system held much hope for development of novel cardiovascular therapeutics such as ECE-1 inhibitors, a promise that has yet to be fulfilled (Kirkby et al. 2008).

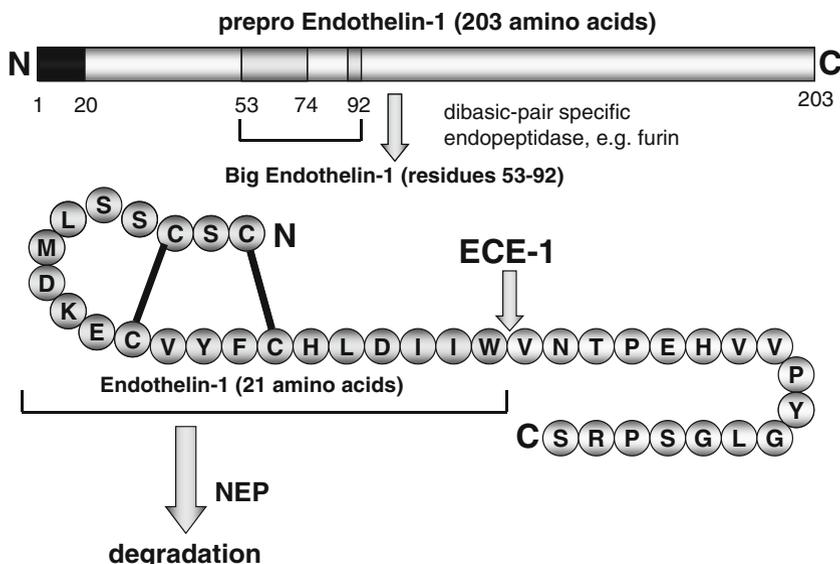


Fig. 19.2 Proteolytic processing pathway of endothelin-1. Pre-proendothelin-1 (203 amino acids) is cleaved by a dibasic endopeptidase such as furin to generate biologically inactive big endothelin-1 (39 amino acids) (Yanagisawa et al. 1988). Big ET-1 is then cleaved at a Trp-Val bond by the metalloproteinase ECE-1 to release endothelin-1 (21 amino acids). Active ET-1 contains 2 intramolecular disulphide bonds (shown as *straight lines*)

A detailed bioinformatic analysis of the phylogeny of the M13 family has recently been described (Bland et al. 2008). The human genome is now known to contain at least seven NEP-related enzymes, including ECE-1 and ECE-2, as well as a close homologue of NEP, termed NEP2 (Whyteside and Turner 2008). Several of the NEP-like enzymes are, as yet, orphan peptidases with no recognized peptide substrates. Invertebrate species contain considerably more NEP-like proteases (more than 20 genes in *Drosophila*), which are involved in events such reproduction, embryogenesis, renal function, as well as other behavioural traits (Thomas et al. 2005; Bland et al. 2009). The 3D structures of both NEP and ECE-1, each complexed with the inhibitor phosphoramidon, have been solved providing detailed mechanistic insight into the similarities and differences between these two metalloproteinases (Oefner et al. 2000; Schulz et al. 2009).

There has been much work towards the development of selective inhibitors of NEP and ECE as possible cardiovascular therapeutics and the synthetic compound thiorphan is currently the most specific and potent inhibitor of NEP (Roques et al. 1980). Endogenous physiological inhibitors of NEP have been described – sialorphan and opiorphan – which have analgesic activity of comparable potency to morphine, which is consistent with their inhibition of enkephalin inactivation by NEP (Rougeot et al. 2003; Wisner et al. 2006). In recent years, much attention has focused on a newly discovered physiological activity of both NEP and ECE,

namely their ability to degrade the neurotoxic Alzheimer's amyloid peptide, A β (Iwata et al. 2000). Up-regulation of NEP (or ECE) may therefore provide an option for the treatment of Alzheimer's disease by stimulating clearance of A β – a therapeutic example of enhancing protein processing (Belyaev et al. 2009). While the homologous NEP and ECE serve complementary roles in A β metabolism, they play opposing roles in the progression of androgen-insensitive prostate cancer through their respective effects on endothelin maturation (ECE-1) and inactivation (NEP) (Fig. 19.3). Hence, upregulation of prostatic NEP or downregulation of ECE-1 could provide therapeutic options for this untreatable and aggressive stage of prostate cancer (Papandreou et al. 1998; Dawson et al. 2004).

19.4.5 Class M16

Peptidase family M16 (the pitrilysin family) contains metalloendopeptidases with varied specificity and physiological roles. These range from the mitochondrial processing peptidase (MPP) to the insulin-degrading enzyme (insulysin; IDE). MPP removes an N-terminal targeting signal from mitochondrial precursor proteins hence targeting nuclear-encoded proteins to their correct location within mitochondria. IDE plays a role in insulin metabolism but also may be another physiologically significant amyloid (A β)-degrading enzyme (Edland 2004). This family are characterized by an inverted zinc-binding domain (HX \bar{X} EH rather than HEXXH) although the reaction mechanism is similar, namely nucleophilic attack of the carbonyl carbon of the peptide bond by a water molecule activated by the zinc and polarized by an active site glutamate residue. The lack of potent or specific inhibitors for peptidases of this family has significantly hindered studies of their physiological functions, particularly in the case of IDE. However, mice with targeted deletion of the IDE gene show hyperinsulinaemia and glucose intolerance as well as increased accumulation of A β in the brain supporting the proposed protein processing roles for the enzyme (Farris et al. 2003).

19.4.6 Class M24

Peptidase family M24 contains exopeptidases that were thought to require co-catalytic ions of cobalt or manganese. They are either aminopeptidases or dipeptidyl-peptidases. The methionyl aminopeptidases (see above) fall into this class, although it is likely that they use zinc physiologically as their cations as does the structurally related X-Pro aminopeptidase (aminopeptidase P) (Cottrell et al. 2000). Fumagillin-related angiogenesis inhibitors of type II methionyl aminopeptidase are of interest to the pharmaceutical industry for the treatment of solid tumours. Related processing enzymes include the eukaryotic X-Pro dipeptidase which plays a role in collagen recycling. Deficiency results in an increase of these proline dipeptides to toxic levels (Myara et al. 1994) (Table 19.2).

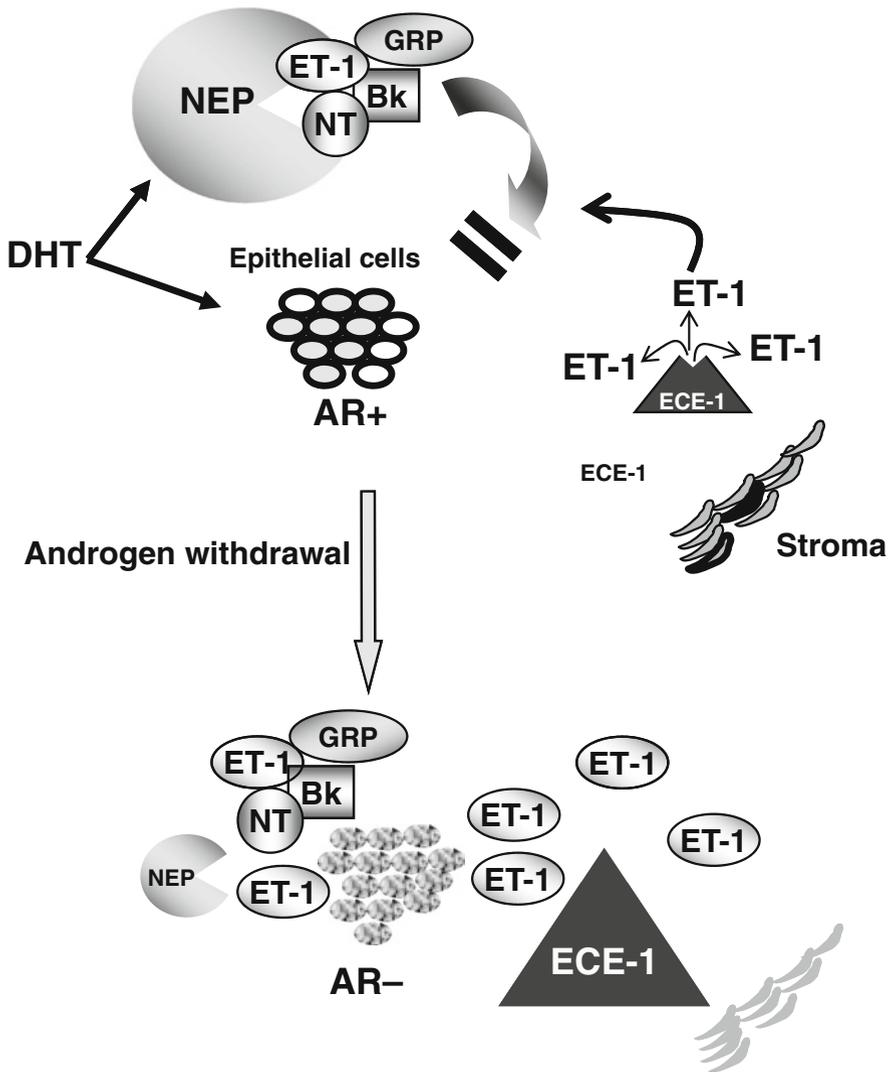


Fig. 19.3 Reciprocal roles of the metallopeptidases neprilysin (NEP) and endothelin-converting enzyme (ECE-1) in the development of androgen-independent prostate cancer. In androgen-dependent prostate cells, epithelial cells express high levels of NEP. NEP levels are up-regulated by androgens which also stimulate epithelial growth. Although epithelial cells produce the mitogenic peptide ET-1 it is inactivated by NEP and does not stimulate cell growth. The stromal cells produce low levels of ECE-1 converting big ET-1 into ET-1 and this action is again opposed by the action of NEP. When androgen levels are low as in typical therapeutic surgical or drug regimes, NEP levels decrease allowing mitogenic peptides produced by epithelial cells to exhibit their growth-promoting effect. Also, under these conditions, the stromal cells express higher levels of ECE-1 producing even more ET-1 and encouraging cell invasion. ECE-1 levels then increase in epithelial cells which become metastatic and are no longer influenced by stroma. Hence, inhibition of ECE-1 activity in the stroma (or up-regulating the activity of NEP in epithelial cells) could provide a novel therapeutic strategy in prostate cancer by manipulating metallopeptidase levels. DHT, dihydrotestosterone; ET-1, NT, Bk, GRP, the mitogenic peptides endothelin-1, neurotensin, bradykinin and gastrin-releasing peptide, respectively. AR+/-, in the presence/absence of androgen receptor

19.4.7 Class M48

These enzymes are mainly metalloendopeptidases. Eukaryotic peptidases from family M48 have a requirement for substrates that are prenylated at a C-terminal motif known as a CAAX box, in which A is an aliphatic residue, and the lipid is attached to the cysteine residue. Protein prenylation enhances membrane association of proteins. Human farnesylated-protein converting enzyme 1 may be important for processing prelamin A, a farnesylated protein that is cleaved twice. In cells with a defective peptidase gene, incompletely processed prelamin A accumulates in the nuclear envelope. Farnesylated-protein converting enzyme 1 performs the second processing step for prelamin A, releasing a 15-residue peptide containing the prenylated C-terminal Cys (Pendas et al. 2002). The Oma1 endopeptidase is believed to degrade misfolded membrane proteins, thereby helping to maintain the integrity of the mitochondrial inner membrane (Kaser et al. 2003). Deficiencies in lamin A, a component of the nuclear lamina, lead to abnormalities in the nuclear architecture, and knockouts of farnesylated-protein converting enzyme 1 in mice cause growth retardation, muscular dystrophy and premature death (Bergo et al. 2002).

19.4.8 Class M50

This family is also known as the site 2 protease (S2P) family and is represented by S2P peptidase (human), sporulation factor SpoIVFB and RseP peptidase (*Escherichia coli*). They are all integral membrane proteins, and their extensive hydrophobicity, even within the sequences containing the active site residues, indicates that they cleave their substrates either within the membrane or very close to it. These peptidases are involved in the regulation of gene expression by the two-step proteolysis of transcriptional regulators. For example, following an initial cleavage in the lumen of the Golgi by the serine protease, (site-1 protease, S1P) (Bartz et al. 2008), the zinc metallopeptidase S2P releases the N-terminal transcription factor domain, sterol regulatory element binding protein (SREBP), from its membrane-bound precursor (Fig. 19.4). The released domain then enters the nucleus and activates genes that control cellular levels of cholesterol and phospholipids (Zelenski et al. 1999).

Bacterial RseP peptidase also cleaves transmembrane sequences and it now seems very likely that intramembrane proteolysis is a general activity of peptidases in family M50 related to cell regulation and signalling. The structure of a bacterial S2P-like protease has revealed elements of this unusual catalytic mechanism in which zinc and water-mediated peptide bond hydrolysis occurs in the hydrophobic environment of the lipid bilayer (Feng et al. 2007). Intramembrane proteolysis is involved in such diverse physiological and pathological events as bacterial sporulation, hepatitis C viral infection and Alzheimer's disease (Weihofen and Martoglio 2003). In the latter case, it is an aspartic protease, γ -secretase, that catalyses the intramembrane proteolytic processing event that is the final step in production of the neurotoxic amyloid A β peptide (see below).

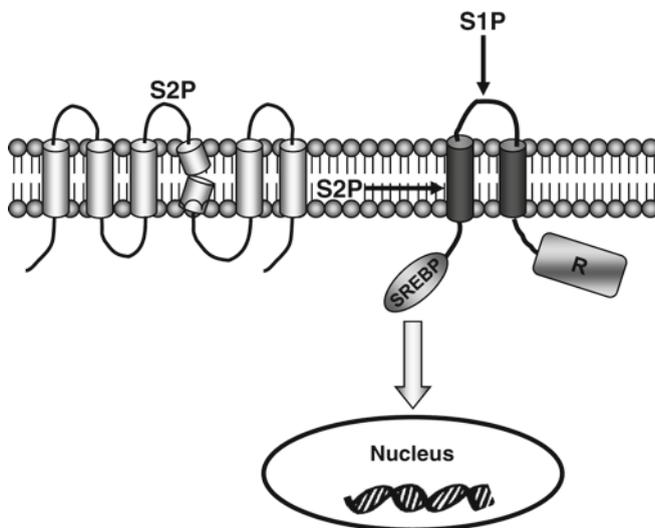


Fig. 19.4 Schematic presentation of two-step proteolytic processing of sterol regulatory element binding protein (SREBP) precursor protein – an example of intramembrane proteolysis. First, the SREBP precursor protein is cleaved within the luminal loop between two transmembrane regions by a membrane-anchored serine protease termed S1P (the site-1 protease). Then, the site-2 protease (S2P) cleaves the processed protein within the transmembrane domain but close to the cytoplasmic membrane surface, releasing the SREBP transcription factor. S2P contains the conserved HEXXH motif common for metalloproteases. The regulatory cytoplasmic domain (R) of the SREBP precursor protein interacts with a cholesterol-sensor protein. The processing events are activated only if the cholesterol level is low (adapted from Wolfe 2009)

19.5 Membrane Protein Shedding: A Primary Role for ADAMs Proteins

The limited proteolysis of precursor proteins, such as prohormones and zymogens, has been understood for decades. However, the selective proteolysis of cell-surface proteins by proteases, commonly referred to as secretases or sheddases, is a more recent discovery, which is providing insight into a number of pathological conditions (see e.g. van Goor et al. 2009 for recent review). We first observed that the metalloproteinase ACE, which itself is an ectoenzyme at the endothelial cell-surface, could be released or shed from the membrane through the action of another distinct integral membrane proteinase, inhibitable by EDTA, and hence presumably a metalloenzyme (Hooper et al. 1987). Hence, the soluble form of ACE found in plasma and used diagnostically to assess the effectiveness of ACE inhibitor therapy is derived as a result of a highly specific post-translational processing of the membrane-bound form of ACE. The physiological function of soluble ACE, if other than a down-regulation mechanism, is unknown although the soluble ectodomain could conceivably act as a ligand for another protein. ACE secretase, as we subsequently named the proteolytic activity, has turned out to be the prototype for a

family of such membrane protein secretases or sheddases, which cause the release of specific membrane proteins, normally from the cell-surface into the extracellular medium (Hooper et al. 1997; Huovila et al. 2005).

A wide variety of cell-surface proteins are cleaved by membrane protein secretases, like ACE secretase, and these function in processes as diverse as cell fusion, adhesion and cell signalling. The substrates include some receptors, receptor ligands, cell adhesion molecules and ectoenzymes. The cleavage normally occurs at a single, specific site in the extracellular domain close to the membrane surface, in the case of ACE at Arg⁶³⁸-Ser⁶³⁹ (Alfalah et al. 2001). In many cases, these shedding processes are inhibited by certain hydroxamate compounds originally developed as MMP inhibitors (e.g. batimastat). Principally through studies on one of these secretases, the tumour necrosis factor- α converting enzyme (TACE, ADAM17), which causes the post-translational release of active TNF- α into the blood from its membrane-bound precursor, pro-TNF, these secretases have mainly been identified as members of the ADAM gene family: multi-domain, membrane proteins that generally (but not always) have both metalloproteinase and adhesion activities (Seals and Courtneidge 2003).

ADAM17 is one of 23 genes encoding ADAMS proteins in humans. Ironically, given it was the first protein shedding event to be described at a molecular level, the precise identity of the ACE secretase is still unknown. The ADAMS are complex proteins featuring a pro-domain with cysteine switch like MMPs, a zinc metallo-protease domain which is inactive in some family members, a disintegrin domain involved in adhesion processes, a cysteine-rich region, an EGF-like sequence, a transmembrane region and a cytoplasmic tail. The structures of the catalytic domains of ADAM17 and ADAM33 have been solved and show many similarities, typical aspects being a central five-stranded β -sheet surrounded by five α -helices including the Met-turn, a feature of all metzincin proteases (Maskos et al. 1998; Orth et al. 2004).

A sub-group of the ADAMs comprising 19 members is the ADAMTS family in which the transmembrane domain of ADAMs proteins is replaced by one or more thrombospondin (TS) repeats and hence they act as soluble, secreted proteases. They also lack the EGF sequence of ADAMs. They were originally identified as the long sought after proteases involved in processing of pro-collagen (procollagen N-endopeptidase, ADAMTS2) and of aggrecan (ADAMTS4). Hence, members of this proteinase family are potential therapeutic targets in inflammatory and arthritic conditions (Fosang and Little 2008). Genetic deficiency of, or autoimmunity against, one member of this family (ADAMTS13) leads to the severe haematological disorder, thrombotic thrombocytopenic purpura, through its failure to process the platelet protein, von Willebrand factor (Raife and Montgomery 2000) (Table 19.2).

In addition to the shedding of the pro-inflammatory cytokine TNF α from its membrane precursor by ADAM17, many of the ligands for the EGF receptor family involved in cellular proliferation and survival are derived from membrane-bound precursors through ADAM-mediated shedding. Examples include heparin-binding EGF (HB-EGF), TGF α and neuregulin. ADAM17 seems to be important (but not

exclusive) to the shedding of these ligands and itself can be activated through activation of G protein-linked receptors by, for example, thrombin, endothelin-1 and angiotensin II. This process of indirect, receptor-mediated activation of the EGF receptor through ADAM-mediated protein processing has been termed transactivation and has particular implications in vascular remodelling and in cancer. In some cases, MMPs rather than ADAMs may contribute to shedding events. For example, MMP-9 may mediate the shedding of the $\beta 2$ integrin subunit (CD18) from macrophages, N-cadherin and also the soluble CD40 ligand involved in platelet activation (Vaisar et al. 2009; Dwivedi et al. 2009; Menchén et al. 2009).

Protein shedding plays a part in the processing of two key neuronal proteins involved in neurodegeneration: the prion protein and the Alzheimer's amyloid precursor protein (APP) and the latter system serves as a finale to this discussion of protein processing events combining elements of both ADAM-mediated shedding, intramembrane proteolysis and dysregulation of cell signalling. The cellular prion protein, which is essential for the development of a prion disease occurs both as a glycolipid-anchored membrane form and as a soluble, secreted form (Parkin et al. 2004). Both ADAM9 and ADAM10, but not ADAM17, contribute to prion shedding and, intriguingly, the ADAM9-mediated shedding is an indirect process acting via ADAM10, which cleaves the prion protein only three residues away from the site of addition of the lipid anchor (Gly²²⁸-Arg²²⁹) (Taylor et al. 2009). However, this shedding process does not appear to regulate the conversion of the prion protein to its transmissible neurotoxic form. Not surprisingly, in the light of its close involvement in the development of dementia, the complex processing of APP has been studied far more intensively and has led to a number of possible therapeutic avenues involving manipulation of protease activity or expression for treatment of the disease, some of which are currently in clinical trials.

19.6 APP Processing and Alzheimer's Disease

The sequential proteolysis of the large, transmembrane, amyloid precursor protein (APP) by the two aspartic proteases, β - and γ -secretase, releases a soluble APP β (sAPP β) ectodomain and generates from the C-terminal membrane fragment the 39-42 amino acid amyloid- β peptide (A β) together with the transcriptional regulator, APP intracellular domain (AICD) (Fig. 19.5) (Wolfe 2007). It was the A β peptide which was originally identified as the main constituent of the extracellular neuritic plaques which characterize AD (Glennner and Wong 1984; Masters et al. 1985) and led to the formulation of the "amyloid cascade" hypothesis of Alzheimer's disease (Hardy and Higgins 1992). Accumulation of A β is a characteristic feature of AD and its prevention is a primary target in therapeutic strategies. Late-onset forms of AD may primarily be due to deficiencies in A β clearance rather than its formation (Hama and Saido 2005) and hence understanding clearance mechanisms and their regulation could be of fundamental importance and provide novel approaches to AD treatment. The exact role of the C-terminal proteolytic product

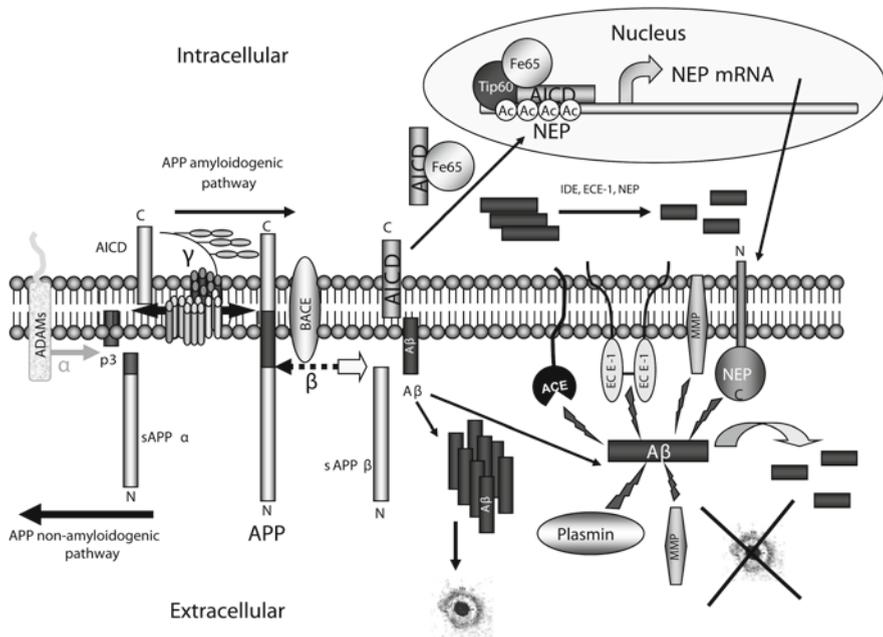


Fig. 19.5 Schematic representation of the proteolytic processing of the Alzheimer's amyloid precursor protein (APP), regulation by AICD of *NEP* gene expression and degradation of A β peptide by a number intracellular, membrane-bound and extracellular metalloproteinases. APP is a constitutive, transmembrane protein which undergoes proteolytic cleavage by β - and γ -secretases producing a soluble ectodomain referred to as sAPP β , the neurotoxic amyloid β peptide (A β) and the APP intracellular domain (AICD). A β has the property to form neurotoxic oligomers and then to aggregate further, ultimately forming the so-called senile plaques, which are one of the pathological hallmarks of Alzheimer's disease. This APP processing pathway is considered amyloidogenic in contrast with the cleavage of APP by the metalloproteinase α -secretase (ADAM protein) within the A β domain, which prevents formation of A β . This non-amyloidogenic processing leads to formation of the soluble ectodomain sAPP α , a short peptide p3 and AICD. AICD binds to the neprilysin (*NEP*) promoter and activates neprilysin expression (*NEP*) (Belyaev et al. 2009). This binding requires AICD stabilisation by Fe65 and also involves the histone-acetyl transferase, Tip60. AICD also controls expression of some other genes. If the *NEP* gene is silenced through histone deacetylation by HDACs, expression of *NEP* is inhibited. However, incubation of cells with VA, and hence inhibition of HDACs, allows AICD to bind to the *NEP* promoter and re-activate *NEP* expression (Belyaev et al. 2009). *NEP*, a cell-surface ectopeptidase, in turn, degrades A β and reverses amyloid plaque accumulation. A β can also be cleaved by ECE-1, ACE, matrix metalloproteinases (MMPs) and by the serine peptidase, plasmin. Cleavage of A β can occur within the cells, at the cell surface (both along the neurites and cell bodies) and in the extracellular space. *NEP* and plasmin between them can cleave monomeric and oligomeric forms of A β (Nalivaeva et al. 2008)

AICD remains unclear and controversial but it probably acts as a transcriptional regulator together with the APP-tail binding protein Fe65 and the histone acetyltransferase (HAT), Tip60 (Cao and Südhof 2001; Belyaev et al. 2009) (Fig. 19.5).

It is well established that APP is cleaved by selective proteases (α -, β -, and γ -secretase) into several fragments some of them possessing physiological properties

(Fig. 19.5). Thus, the action of α - or β -secretases produces the largest products of APP – the soluble ectodomain proteins sAPP $_{\alpha}$ and sAPP $_{\beta}$, which play an important role in proliferation of neuronal cells and development of the nervous system (Caille et al. 2004; Conti and Cattaneo 2005; Taylor et al. 2008). Metabolism of APP can undergo either an “amyloidogenic” pathway through the action of β - and γ -secretases and production of A β , or a non-amyloidogenic pathway, when APP is cleaved by α -secretase (Hooper and Turner 2002). α -Secretase cleaves APP inside of the A β sequence between lysine and leucine in positions 16 and 17, and prevents formation of the A β peptide. Hence, inhibition of the amyloidogenic processing of APP and activation of the activity of α -secretase are possible approaches for prevention of AD (Lichtenthaler and Haass 2004).

The main candidates as α -secretases are ADAM10 and ADAM17; ADAM9 may also participate but in an indirect manner via ADAM10 (Allinson et al. 2004; Hooper and Turner 2002). The constitutive process of secretion of sAPP $_{\alpha}$ under the action of α -secretase occurs at the cell surface (Parvathy et al. 1999). However, the regulated α -secretase activity, stimulated by cholinergic agonists such as muscarine and carbachol (Canet-Aviles et al. 2002) or phorbol esters (Zhu et al. 2001), is reported to be localised mainly intracellularly (Jolly-Tornetta and Wolf 2000). In transgenic mice expressing human APP moderate neuronal overexpression of ADAM10 was shown to reduce the formation of A β and prevent its deposits in the plaques. In contrast, expression of a mutant inactive ADAM10 led to an increase in the number and size of amyloid plaques in the brain of the same transgenic mice (Postina et al. 2004). Transgenic mice lacking ADAM10 or ADAM17 genes are lethal, suggesting that α -secretase plays an important role in the viability of the organism (Killar et al. 1999), although these enzymes do have other important developmental and regulatory roles.

Formation of amyloidogenic A β requires the activity of the transmembrane aspartic proteases, β - and γ -secretases (Fig. 19.5). β -secretase is also known as BACE-1 and its biochemistry and cell biology are described in detail (Hunt and Turner 2009). Therapeutically, BACE1 provides an attractive drug target for AD since BACE knockout mice do not form A β and appear to be healthy (Luo et al. 2001). Numerous approaches have been undertaken to find a clinically effective inhibitor of human β -secretase activity, mostly in the field of peptidomimetic, noncleavable substrate analogues (Hunt and Turner 2009). However, new endogenous protein substrates are continually being discovered for BACE-1, e.g. it cleaves membrane proteins APLP-1 and -2, P-selectin glycoprotein ligand-1 (PSGL-1), membrane-bound sialyltransferase and LDL-receptor-related protein (LRP), and so caution is needed since BACE may have unexpected physiological roles. Most recently, BACE-1 is known to be required for proper myelination to occur through its processing of neuregulin (Willem et al. 2006). The cytosolic fragment of APP formed by BACE (C99) is then a substrate for γ -secretase (Fig. 19.5). The latter represents a complex of several proteins involving presenilin proteins as the catalytic core. They are intramembrane proteases like the S2P protein but representing a different catalytic class of proteinase (aspartic rather than metallo). The signal peptide peptidases also represent aspartic intramembrane proteases. There are also examples of serine-type intra-membrane proteases, e.g. rhomboid proteases (Wolfe 2009).

19.7 Amyloid-Degrading Metallopeptidases

Until recently, accumulation of A β in the brain and other tissues was thought to be an irreversible process, leading to development of AD. However, in the brain there are biochemical pathways for elimination of A β (Nalivaeva et al. 2008). In the last few years, several proteases, such as the metalloenzymes NEP, IDE, ECE-1 and ECE-2, as well as the serine proteinase plasmin have been found to be capable of degrading A β in vitro and in vivo (Leissring et al. 2003; Turner et al. 2004). Most of these act extracellularly either as membrane-bound (ECE-1, NEP) or as soluble extracellular proteins (IDE, plasmin). IDE is anomalous in terms of its subcellular distribution since it is largely cytosolic (Akiyama et al. 1988) but also occurs within some organelles (mitochondria, peroxisomes) as well as in a soluble, secreted form yet it lacks any secretory signal in its sequence (Zhao et al. 2009). The mechanism of its secretion remains an enigma and it has been termed an “unconventionally secreted protein” (Zhao et al. 2009). Figure 19.5 shows schematically A β peptide aggregation and its proteolytic degradation by these various enzymes. Pathological down-regulation of these enzymes could predispose to accumulation of A β and the development of AD. Thus, a possible therapeutic approach for treatment of AD might be a chronic up-regulation of one or more of these proteases, either pharmacologically or through a gene therapy approach. These proteases are localised in a variety of subcellular compartments and cell types in the brain and remove A β prior to its accumulation.

While intra-membrane proteolysis is now a well established mechanism of gene regulation through the action of “intracellular domains” in for example the notch signalling pathway, such a role for the ICD released from the APP tail (AICD) through the action of the presenilin/ γ -secretase complex has proved controversial. This has particularly been the case in relation to the potential feedback regulation of the A β -degrading enzyme, NEP (Pardossi-Piquard et al. 2005; Chen and Selkoe 2007). However, chromatin immunoprecipitation studies have established that NEP expression is directly regulated through acetylation of core histones on the NEP promoter mediated via AICD and the inhibition of histone deacetylation with histone deacetylase inhibitors such as the anti-convulsant drug, valproate, or trichostatin A can potentiate NEP expression and activity (Belyaev et al. 2009). Valproate treatment in vivo in mice can reduce amyloid plaque formation (Qing et al. 2008) (Fig. 19.5). These observations add to the growing body of literature (Saha and Pahan 2006; Fischer et al. 2007) emphasizing the potential beneficial effects of HDAC inhibitors in the treatment of AD and other neurodegenerative disorders, in this case through manipulation of metalloprotease expression.

19.8 Concluding Remarks

This chapter has reviewed some of the diverse roles of metalloproteases in the cell biology and physiology of protein processing and has described aspects of their mechanisms and regulation. It is clear that metalloproteases represent

important therapeutic targets not only in cardiovascular disease (ACE inhibitors being the prime example) but also with potential in inflammatory diseases, neurodegeneration and cancer. While potential targets are being explored, there are numerous “orphan” metallopeptidases whose substrates and functions are currently unknown that may themselves provide novel therapeutic opportunities. Understanding their intricate interrelationships is still a major goal of modern molecular and cell biology.

Acknowledgements We thank the Medical Research Council of Great Britain and Russian Academy of Sciences Programme “Fundamental Sciences to Medicine” for financial support.

References

- Akiyama, H., Shii, K., Yokono, K., et al. (1988). Cellular localization of insulin-degrading enzyme in rat liver using monoclonal antibodies specific for this enzyme. *Biochem. Biophys. Res. Commun.* 155:914–922.
- Alfalah, M., Parkin, E.T., Jacob, R., et al. (2001). A point mutation in the juxtamembrane stalk of human angiotensin I-converting enzyme invokes the action of a distinct secretase. *J. Biol. Chem.* 276: 21105–21109.
- Allinson, T.M., Parkin, E.T., Condon, T.P., et al. (2004). The role of ADAM10 and ADAM17 in the ectodomain shedding of angiotensin converting enzyme and the amyloid precursor protein. *Eur. J. Biochem.* 271:2539–2547.
- Bachmair, A., Finley, D., Varshavsky, A. (1986). In vivo half-life of a protein is a function of its amino-terminal residue. *Science* 234:179–186.
- Bartz, R., Sun, L.P., Bisel, B., et al. (2008). Spatial separation of Golgi and ER during mitosis protects SREBP from unregulated activation. *EMBO J.* 27:948–955.
- Belyaev, N.D., Nalivaeva, N.N., Makova, N.Z., et al. (2009). Nephrylysin gene expression requires binding of the amyloid precursor protein intracellular domain to its promoter: implications for Alzheimer disease. *EMBO Rep.* 10:94–100.
- Beninga, J., Rock, K.L., Goldberg, A.L. (1998). Interferon-gamma can stimulate post-proteasomal trimming of the N terminus of an antigenic peptide by inducing leucine aminopeptidase. *J. Biol. Chem.* 273:18734–18742.
- Bergo, M.O., Gavino, B., Ross, J., et al. (2002). Zmpste24 deficiency in mice causes spontaneous bone fractures, muscle weakness, and a prelamin A processing defect. *Proc. Natl. Acad. Sci. USA.* 99:13049–13054.
- Blais, V., Fugère, M., Denault, J.B., et al. (2002). Processing of proendothelin-1 by members of the subtilisin-like pro-protein convertase family. *FEBS Lett.* 524:43–48.
- Bland, N.D., Pinney, J.W., Thomas, J.E., et al. (2008). Bioinformatic analysis of the neprilysin (M13) family of peptidases reveals complex evolutionary and functional relationships. *BMC Evol. Biol.* 8:16.
- Bland, N.D., Robinson, P., Thomas, J.E., et al. (2009). Locomotor and geotactic behavior of *Drosophila melanogaster* over-expressing neprilysin 2. *Peptides* 30:571–574.
- Bond, J.S., and Beynon, R.J. (1995). The astacin family of metalloendopeptidases. *Protein Sci.* 4:1247–1261.
- Bradshaw, R.A., Yi, L., Walker, K.W. (2004). Methionyl aminopeptidase type 1. In: Barrett, A.J., Rawlings, N.D., Woessner, J.F. (eds) *Handbook of Proteolytic Enzymes*, 2 edn. Elsevier, London, pp. 911–917.
- Bunnett, N.W. (2006). Protease-activated receptors: how proteases signal to cells to cause inflammation and pain. *Semin. Thromb. Hemost.* 32(Suppl 1):39–48.

- Burley, S.K., David, P.R., Taylor, A., et al. (1990). Molecular structure of leucine aminopeptidase at 2.7-Å resolution. *Proc. Natl. Acad. Sci. USA.* 87:6878–6882.
- Caille, I., Allinquant, B., Dupont, E., et al. (2004). Soluble form of amyloid precursor protein regulates proliferation of progenitors in the adult subventricular zone. *Development* 131:2173–2181.
- Cal, S., Quesada, V., Garabaya, C., et al. (2003). Polyserylase-I, a human polyprotease with the ability to generate independent serine protease domains from a single translation product. *Proc. Natl. Acad. Sci. USA.* 100:9185–9190.
- Canet-Aviles, R.M., Anderton, M., Hooper, N.M., et al. (2002). Muscarine enhances soluble amyloid precursor protein secretion in human neuroblastoma SH-SY5Y by a pathway dependent on protein kinase C(α), src-tyrosine kinase and extracellular signal-regulated kinase but not phospholipase C. *Brain Res. Mol. Brain Res.* 102:62–72.
- Cao, X., and Südhof, T.C. (2001). A transcriptionally active complex of APP with Fe65 and histone acetyltransferase Tip60. *Science* 293:115–120.
- Chen, A.C., and Selkoe, D.J. (2007). Response to: Pardossi-Piquard et al., “Presenilin-dependent transcriptional control of the A β -degrading enzyme neprilysin by intracellular domains of β APP and APLP.” *Neuron* 53:479–483.
- Conti, L., and Cattaneo, E. (2005). Controlling neural stem cell division within the adult subventricular zone: an APpealing job. *Trends Neurosci.* 28:57–59.
- Cottrell, G.S., Hyde, R.J., Lim, J., et al. (2000). Identification of critical residues in the active site of porcine membrane-bound aminopeptidase P. *Biochemistry* 39:15129–15135.
- Cynis, H., Scheel, E., Saido, T.C., et al. (2008). Amyloidogenic processing of amyloid precursor protein: evidence of a pivotal role of glutaminyl cyclase in generation of pyroglutamate-modified amyloid- β . *Biochemistry* 47:7405–7413.
- Danilczyk, U., Sarao, R., Remy, C., et al. (2006). Essential role for collectrin in renal amino acid transport. *Nature* 444:1088–1091.
- Delaet, N.G., Robinson, L.A., Wilson, D.M. (2003). Novel inhibitors of procollagen C-terminal proteinase. Part 1: Diamino acid hydroxamates. *Bioorg. Med. Chem. Lett.* 13:2101–2104.
- Dawson, L.A., Maitland, N.J., Turner, A.J., et al. (2004). Stromal-epithelial interactions influence prostate cancer cell invasion by altering the balance of metallopeptidase expression. *Br. J. Cancer* 90:1577–1582.
- Dumermuth, E., Sterchi, E.E., Jiang, W.P., et al. (1991). The astacin family of metalloendopeptidases. *J. Biol. Chem.* 266:21381–21385.
- Dwivedi, A., Slater, S.C., George, S.J. (2009). MMP-9 and -12 cause N-cadherin shedding and thereby beta-catenin signalling and vascular smooth muscle cell proliferation. *Cardiovasc. Res.* 81:178–186.
- Edland, S.D. (2004). Insulin-degrading enzyme, apolipoprotein ϵ , and Alzheimer’s disease. *J. Mol. Neurosci.* 23:213–217.
- Elsässer-Beile, U., Bühler, P., Wolf, P. (2009). Targeted therapies for prostate cancer against the prostate specific membrane antigen. *Curr. Drug Targets* 10:118–125.
- Farris, W., Mansourian, S., Chang, Y., et al. (2003). Insulin-degrading enzyme regulates the levels of insulin, amyloid β -protein, and the β -amyloid precursor protein intracellular domain in vivo. *Proc. Natl. Acad. Sci. USA.* 100:4162–4167.
- Feng, L., Yan, H., Wu, Z., et al. (2007). Structure of a site-2 protease family intramembrane metalloprotease. *Science* 318:1608–1612.
- Fischer, A., Sananbenesi, F., Wang, X., et al. (2007). Recovery of learning and memory is associated with chromatin remodelling. *Nature* 447:178–182.
- Fosang, A.J., and Little, C.B. (2008). Drug insight: aggrecanases as therapeutic targets for osteoarthritis. *Nat. Clin. Pract. Rheumatol.* 4:420–427.
- Frottin, F., Martinez, A., Peynot, P. (2006). The proteomics of N-terminal methionine cleavage. *Mol. Cell Proteomics* 5:2336–2349.
- Glenner, G.G., and Wong, C.W. (1984). Alzheimer’s Disease: Initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem. Biophys. Res. Commun.* 120:885–890.

- Hagaman, J.R., Moyer, J.S., Bachman, E.S., et al. (1998). Angiotensin-converting enzyme and male fertility. *Proc. Natl. Acad. Sci. USA.* 95:2552–2557.
- Hama, E., and Saïdo, T.C. (2005). Etiology of sporadic Alzheimer's disease: somatostatin, neprilysin, and amyloid beta peptide. *Med. Hypotheses* 65:498–500
- Hardy, J.A., and Higgins, G.A. (1992). Alzheimer's disease: the amyloid cascade hypothesis. *Science* 256: 184–185.
- Hooper, N.M., and Turner, A.J. (2002). The search for α -secretase and its potential as a therapeutic approach to Alzheimer's disease. *Curr. Med. Chem.* 9:1107–1119.
- Hooper, N.M., Karran, E.H., Turner, A.J. (1997). Membrane protein secretases. *Biochem J.* 321:265–279.
- Hooper, N.M., Keen, J., Pappin, D.J., et al. (1987). Pig kidney angiotensin converting enzyme. Purification and characterization of amphipathic and hydrophilic forms of the enzyme establishes C-terminal anchorage to the plasma membrane. *Biochem. J.* 247:85–93.
- Huang, K.F., Liu, Y.L., Wang, A.H. (2005). Cloning, expression, characterization, and crystallization of a glutaminyl cyclase from human bone marrow: a single zinc metalloenzyme. *Protein Expr. Purif.* 43:65–72.
- Hunt, C.E., and Turner, A.J. (2009). Cell biology, regulation and inhibition of β -secretase (BACE-1). *FEBS J.* 276:1845–1859.
- Huovila, A.P., Turner, A.J., Pelto-Huikko, M. (2005). Shedding light on ADAM metalloproteinases. *Trends Biochem. Sci.* 30:413–422.
- Iwata, N., Tsubuki, S., Takaki, Y., et al. (2000). Identification of the major A β 1–42-degrading catabolic pathway in brain parenchyma: suppression leads to biochemical and pathological deposition. *Nature Med.* 6:143–150.
- Jolly-Tornetta, C., and Wolf, B.A. (2000). Protein kinase C regulation of intracellular and cell surface amyloid precursor protein (APP) cleavage in CHO695 cells. *Biochemistry* 39:15282–15290.
- Jones, G.C., and Riley, G.P. (2005). ADAMTS proteinases: a multi-domain, multi-functional family with roles in extracellular matrix turnover and arthritis. *Arthritis Res. Ther.* 7:160–169.
- Kaser, M., Kambacheld, M., Kisters-Woike, B., et al. (2003). Oma1, a novel membrane-bound metallopeptidase in mitochondria with activities overlapping with the m-AAA protease. *J. Biol. Chem.* 278:46414–46423.
- Kerr, M., and Kenny, A.J. (1974). The purification and specificity of a neutral endopeptidase from rabbit kidney brush border. *Biochem. J.* 137:477–488.
- Killar, L., White, J., Black, R. (1999). Adamalysins. A family of metzincins including TNF-alpha converting enzyme (TACE). *Ann. N Y Acad. Sci.* 878:442–452.
- Kirkby, N.S., Hadoko, P.W., Bagnall, A.J., et al. (2008). The endothelin system as a therapeutic target in cardiovascular disease: great expectations or bleak house? *Br. J. Pharmacol.* 153:1105–1119.
- Klusák, V., Barinka, C., Plechanová, A., et al. (2009). Reaction mechanism of glutamate carboxypeptidase II revealed by mutagenesis, X-ray crystallography, and computational methods. *Biochemistry* 48:4126–4138.
- Kowalczyk, S., Bröer, A., Tietze, N. (2008). A protein complex in the brush-border membrane explains a Hartnup disorder allele. *FASEB J.* 22:2880–2887.
- Lee, M.H., Verma, V., Maskos, K., et al. (2002). The C-terminal domains of TACE weaken the inhibitory action of N-TIMP-3. *FEBS Lett.* 520:102–106.
- Leissring, M.A., Lu, A., Condrón, M.M., et al. (2003). Kinetics of amyloid β -protein degradation determined by novel fluorescence- and fluorescence polarization-based assays. *J. Biol. Chem.* 278:37314–37320.
- Leopoldini, M., Russo, N., Toscano, M., et al. (2007). Which one among Zn(II), Co(II), Mn(II), and Fe(II) is the most efficient ion for the methionine aminopeptidase catalyzed reaction? *J. Am. Chem. Soc.* 129:7776–7784.
- Letarte, M., Vera, S., Tran, R., et al. (1988). Common acute lymphocytic leukemia antigen is identical to neutral endopeptidase. *J. Exp. Med.* 168:1247–1253.
- Lichtenthaler, S.F., and Haass, C. (2004). Amyloid at the cutting edge: activation of α -secretase prevents amyloidogenesis in an Alzheimer disease mouse model. *J. Clin. Invest.* 113:1384–1387.

- Luo, Y., Bolon, B., Kahn, S., et al. (2001). Mice deficient in BACE1, the Alzheimer's β -secretase, have normal phenotype and abolished β -amyloid generation. *Nat. Neurosci.* 4:231–232.
- Maskos, K., Fernandez-Catalan, C., Huber, R., et al. (1998). Crystal structure of the catalytic domain of human tumor necrosis factor- α -converting enzyme. *Proc. Natl. Acad. Sci. USA.* 95:3408–3412.
- Masters, C.L., Simms, G., Weinman, N.A., et al. (1985). Amyloid plaque core protein in Alzheimer disease and Down Syndrome. *Proc. Natl. Acad. Sci. USA.* 82:4245–4249.
- Matsas, R., Fulcher, I.S., Kenny, A.J., et al. (1983). Substance P and [Leu]enkephalin are hydrolyzed by an enzyme in pig caudate synaptic membranes that is identical with the endopeptidase of kidney microvilli. *Proc. Natl. Acad. Sci. USA.* 80:3111–3115.
- Menard, J., and Patchett, A.A. (2001). Angiotensin-converting enzyme inhibitors. *Adv. Protein Chem.* 56:13–75.
- Menchén, L., Marín-Jiménez, I., Arias-Salgado, E.G., et al. (2009). Matrix metalloproteinase 9 is involved in Crohn's disease-associated platelet hyperactivation through the release of soluble CD40 ligand. *Gut* 58:920–928.
- Mogk, A., Schmidt, R., Bukau, B. (2007). The N-end rule pathway for regulated proteolysis: prokaryotic and eukaryotic strategies. *Trends Cell Biol.* 17:165–172.
- Moura-da-Silva, A.M., Butera, D., Tanjoni, I. (2007). Importance of snake venom metalloproteinases in cell biology: effects on platelets, inflammatory and endothelial cells. *Curr. Pharm. Des.* 13:2893–2905.
- Myara, I., Cosson, C., Moatti, N., et al. (1994). Human kidney prolidase -purification, preincubation properties and immunological reactivity. *Int. J. Biochem.* 26:207–214.
- Nalivaeva, N.N., Fisk, L.R., Belyaev, N.D., et al. (2008). Amyloid-degrading enzymes as therapeutic targets in Alzheimer's disease. *Curr. Alzheimer Res.* 5:212–224.
- Neurath, H. (1999). Proteolytic enzymes, past and future. *Proc. Natl. Acad. Sci. USA.* 96:10962–10963.
- Oefner, C., D'Arcy, A., Hennig, M., et al. (2000). Structure of human neutral endopeptidase (Nepilysin) complexed with phosphoramidon. *J. Mol. Biol.* 296:341–349.
- Orth, P., Reichert, P., Wang, W., et al. (2004). Crystal structure of the catalytic domain of human ADAM33. *J. Mol. Biol.* 335:129–137.
- Papandreou, C.N., Usmani, B., Geng, Y., et al. (1998). Neutral endopeptidase 24.11 loss in metastatic human prostate cancer contributes to androgen-independent progression. *Nature Med.* 4:50–57.
- Pardossi-Piquard, R., Petit, A., Kawarai, T., et al. (2005). Presenilin-dependent transcriptional control of the A β -degrading enzyme neprilysin by intracellular domains of β APP and APLP. *Neuron* 46:541–554.
- Parkin, E.T., Watt, N.T., Turner, A.J., et al. (2004). Dual mechanisms for shedding of the cellular prion protein. *J. Biol. Chem.* 279:11170–11178.
- Parvathy, S., Hussain, I., Karran, E.H. (1999). Cleavage of Alzheimer's amyloid precursor protein by α -secretase occurs at the surface of neuronal cells. *Biochemistry* 38:9728–9734.
- Pendas, A.M., Zhou, Z., Cadinanos, J., et al. (2002). Defective prelamin A processing and muscular and adipocyte alterations in Zmpste24 metalloproteinase-deficient mice. *Nat. Genet.* 31:94–99.
- Postina, R., Schroeder, A., Dewachter, I. (2004). A disintegrin-metalloproteinase prevents amyloid plaque formation and hippocampal defects in an Alzheimer disease mouse model. *J. Clin. Invest.* 113:1456–1464.
- Puente, X.S., Sánchez, L.M., Gutiérrez-Fernández, A., et al. (2005). A genomic view of the complexity of mammalian proteolytic systems. *Biochem. Soc. Trans.* 33:331–334.
- Qing, H., He, G., Ly, P.T., et al. (2008). Valproic acid inhibits A β production, neuritic plaque formation, and behavioral deficits in Alzheimer's disease mouse models. *J. Exp. Med.* 205:2781–2789.
- Quesada, V., Ordóñez, G.R., Sánchez, L.M., et al., (2009). The Degradome database: mammalian proteases and diseases of proteolysis. *Nucleic Acids Res.* 37 (Database issue):D239–D243.
- Raife, T.J., and Montgomery, R.R. (2000). von Willebrand factor and thrombotic thrombocytopenic purpura. *Curr. Opin. Hematol.* 7:278–283.

- Ravid, T., Hochstrasser, M. (2008). Diversity of degradation signals in the ubiquitin-proteasome system. *Nature Rev. Mol. Cell Biol.* 9:679–690.
- Rella, M., Elliot, J.L., Revett, T.J., et al. (2007). Identification and characterisation of the angiotensin converting enzyme-3 (ACE3). gene: a novel mammalian homologue of ACE. *BMC Genomics* 8:194.
- Rawlings, N.D. and Barrett, A.J. (2004). Introduction: metalloproteases and their clans. In: Barrett, A.J., Rawlings, N.D., Woessner, J.F. (eds) *Handbook of Proteolytic Enzymes*, 2 edn. Elsevier, London, pp. 231–268.
- Rice, G.L., Thomas, D.A., Grant, P.J. et al. (2004). Evaluation of angiotensin-converting enzyme (ACE), its homologue ACE2 and neprilysin in angiotensin peptide metabolism. *Biochem. J.* 383:45–51.
- Rholam, M., and Fahy, C. (2009). Processing of peptide and hormone precursors at the dibasic cleavage sites. *Cell Mol. Life Sci.* 66:2075–2091
- Roques, B.P., Fournié-Zaluski, M.C., Soroca, E., et al. (1980). The enkephalinase inhibitor thiorphan shows antinociceptive activity in mice. *Nature* 288:286–288.
- Rougeot, C., Messaoudi, M., Hermitte, V., et al. (2003). Sialorphin, a natural inhibitor of rat membrane-bound neutral endopeptidase that displays analgesic activity. *Proc. Natl. Acad. Sci. USA.* 100:8549–8554.
- Saha, R.N., and Pahan, K. (2006). HATs and HDACs in neurodegeneration: a tale of disconcerted acetylation homeostasis. *Cell Death Differ.* 13:539–550.
- Schiavo, G., Benfenati, F., Poulain, B., et al. (1992). Tetanus and botulinum-B neurotoxins block neurotransmitter release by proteolytic cleavage of synaptobrevin. *Nature* 359:832–835.
- Schilling, S., Zeitschel, U., Hoffmann, T., et al. (2008). Glutaminyl cyclase inhibition attenuates pyroglutamate A β and Alzheimer's disease-like pathology. *Nature Med.* 14:1106–1111.
- Schulz, H., Dale, G.E., Karimi-Nejad, Y., et al. (2009). Structure of human endothelin-converting enzyme I complexed with phosphoramidon. *J. Mol. Biol.* 385:178–187.
- Seals, D.F., and Courtneidge, S.A. (2003). The ADAMs family of metalloproteases: multidomain proteins with multiple functions. *Genes Dev.* 17:7–30.
- Skidgel, R.A., and Erdös, E.G. (1985). Novel activity of human angiotensin I converting enzyme: release of the NH₂- and COOH-terminal tripeptides from the luteinizing hormone-releasing hormone. *Proc. Natl. Acad. Sci. USA.* 82:1025–1029.
- Skotnicki, J.S., DiGrandi, M.J., Levin, J.I. (2003). Design strategies for the identification of MMP-13 and TACE inhibitors. *Curr. Opin. Drug Discov. Devel.* 6:742–759.
- Stöcker, W., Grams, F., Baumann, U., et al. (1995). The metzincins - topological and sequential relations between the astacins, adamalysins, serralysins, and matrixins (collagenases) define a superfamily of zinc-peptidases. *Protein Sci.* 4:823–840.
- Taylor, D.R., Parkin, E.T., Cocklin, S.L., et al. (2009) Role of ADAMs in the ectodomain shedding and conformational conversion of the prion protein. *J. Biol. Chem.* 284(34):22590–22600.
- Taylor, C.J., Ireland, D.R., Ballagh, I., et al. (2008). Endogenous secreted amyloid precursor protein- α regulates hippocampal NMDA receptor function, long-term potentiation and spatial memory. *Neurobiol. Dis.* 31:250–260.
- Thomas, J.E., Rylett, C.M., Carhan, A., et al. (2005). Drosophila melanogaster NEP2 is a new soluble member of the neprilysin family of endopeptidases with implications for reproduction and renal function. *Biochem. J.* 386:357–366.
- Tipnis, S.R., Hooper, N.M., Hyde, R., et al. (2000). A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. *J. Biol. Chem.* 275:33238–33243.
- Tu, G., Xu, W., Huang, H., et al. (2008). Progress in the development of matrix metalloproteinase inhibitors. *Curr. Med. Chem.* 15:1388–1395.
- Turner, A.J. (2008). Angiotensin-converting enzyme 2: cardioprotective player in the renin-angiotensin system? *Hypertension* 52:816–817.
- Turner, A.J., Isaac, R.E., Coates, D. (2001). The neprilysin (NEP) family of zinc metalloendopeptidases: genomics and function. *Bioessays* 23:261–269.
- Turner, A.J., Fisk, L., Nalivaeva, N.N. (2004). Targeting amyloid-degrading enzymes as therapeutic strategies in neurodegeneration. *Ann. N Y Acad. Sci.* 1035:1–20.

- Vaisar, T., Kassim, S.Y., Gomez, I.G., et al. (2009). MMP-9 sheds the $\beta 2$ integrin subunit (CD18) from macrophages. *Mol. Cell Proteomics* 8:1044–1060.
- van Goor, H., Melenhorst, WB, Turner, A.J., et al. (2009). Adamalysins in biology and disease. *J. Pathol.* 219(3):277–286.
- Van Wart, H.E., and Birkedal-Hansen, H. (1990). The cysteine switch: a principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family. *Proc. Natl. Acad. Sci. USA.* 87:5578–5582.
- Watermeyer, J.M., Kröger, W.L., O’Neill, H.G., et al. (2008). Probing the basis of domain-dependent inhibition using novel ketone inhibitors of angiotensin-converting enzyme. *Biochemistry* 47:5942–5950.
- Weihofen, A., and Martoglio, B. (2003). Intramembrane-cleaving proteases: controlled liberation of proteins and bioactive peptides. *Trends Cell Biol.* 13:71–78.
- Wisner, A., Dufour, E., Messaoudi, M., et al. (2006). Human Opiorphin, a natural antinociceptive modulator of opioid-dependent pathways. *Proc. Natl. Acad. Sci. USA.* 103:17979–17984.
- Whyteside, A.R., and Turner, A.J. (2008). Human neprilysin-2 (NEP2) and NEP display distinct subcellular localisations and substrate preferences. *FEBS Lett.* 582:2382–2386.
- Willem, M., Garratt, A.N., Novak, B., et al. (2006). Control of peripheral nerve myelination by the beta-secretase BACE1. *Science* 314:664–666.
- Wolfe, M.S. (2007). When loss is gain: reduced presenilin proteolytic function leads to increased A β 42/A β 40. Talking point on the role of presenilin mutations in Alzheimer disease. *EMBO Rep.* 8:136–140.
- Wolfe, M.S. (2009). Intramembrane proteolysis. *Chem. Rev.* 109:1599–1612.
- Yanagisawa, M., Kurihara, H., Kimura, S., et al. (1988). A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 332:411–415.
- Zelenski, N.G., Rawson, R.B., Brown, M.S., et al. (1999). Membrane topology of S2P, a protein required for intramembranous cleavage of sterol regulatory element-binding proteins. *J. Biol. Chem.* 274:21973–21980.
- Zhang, Y., Xu, J., Levin, J. (2004). Identification and characterization of 4-[[4-(2-butynyloxy)phenyl]sulfonyl]-N-hydroxy-2,2-dimethyl-(3S)thiomorpholinecarboxamide (TMI-1), a novel dual tumor necrosis factor- α -converting enzyme/matrix metalloproteinase inhibitor for the treatment of rheumatoid arthritis. *J. Pharmacol. Exp. Ther.* 309:348–355.
- Zhao, J., Li, L., Leissring, M.A. (2009). Insulin-degrading enzyme is exported via an unconventional protein secretion pathway. *Mol. Neurodegener.* 4:4.
- Zhu, G., Wang, D., Lin, Y.H., et al. (2001). Protein kinase C ϵ suppresses A β production and promotes activation of γ -secretase. *Biochem. Biophys. Res. Commun.* 285:997–1006.