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Proteolytic Degradation of Amyloid β -Protein

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The amyloid β -protein ($A\beta$) is subject to proteolytic degradation by a diverse array of peptidases and proteinases, known collectively as $A\beta$ -degrading proteases ($A\beta$ DPs). A growing number of $A\beta$ DPs have been identified, which, under physiological and/or pathophysiological conditions, contribute significantly to the determination of endogenous cerebral $A\beta$ levels. Despite more than a decade of investigation, the complete set of $A\beta$ DPs remains to be established, and our understanding of even well-established $A\beta$ DPs is incomplete. Nevertheless, the study of known $A\beta$ DPs has contributed importantly to our understanding of the molecular pathogenesis of Alzheimer disease (AD) and has inspired the development of several novel therapeutic approaches to the regulation of cerebral $A\beta$ levels. In this article, we discuss the general features of $A\beta$ degradation and introduce the best-characterized $A\beta$ DPs, focusing on their diverse properties and the numerous conceptual insights that have emerged from the study of each.

Amyloid β -protein ($A\beta$) is a normal product of cellular metabolism (Haass et al. 1993) derived from the amyloid precursor protein (APP) by the successive action of the β - and γ -secretases (see Haass et al. 2011). As is true for any other peptide, the production of $A\beta$ is normally counterbalanced by its elimination via any of several processes operating in parallel, including proteolytic degradation, cell-mediated clearance, passive and active transport, and the aggregation and deposition of $A\beta$ into insoluble aggregates. Although the relative importance of these different pathways remains to be established, a growing body of evidence suggests that proteolytic degradation is a particularly significant determinant of cerebral $A\beta$ levels and, by extension, Alzheimer disease (AD) pathogenesis.

It has long been hypothesized that sporadic forms of AD may be attributable to defective clearance of $A\beta$ (Selkoe 2001; Tanzi et al. 2004). Nevertheless, despite the obvious appeal of this simple idea, it had remained little more than a theoretic possibility. Recently, using newly developed techniques for quantifying the rates of $A\beta$ production and clearance within the cerebrospinal fluid (CSF) in humans (Bateman et al. 2006), it was confirmed that sporadic AD patients do indeed exhibit significant defects in the clearance of CSF $A\beta$ (Mawuenyega et al. 2010). Although these experiments cannot distinguish precisely which clearance mechanisms are impaired in these patients, these findings—together with the evidence reviewed in this article—lend strong support to the

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idea that defective A β degradation may be operative in AD.

Widespread interest in A β degradation did not take hold until the turn of the 21st century. A key turning point in the field came with the first study that was explicitly designed to examine A β degradation in the living animal (Iwata et al. 2000). In addition to identifying neprilysin (NEP) as one of the principal A β -degrading proteases (A β DPs), this study highlighted the pathophysiological significance of A β degradation to AD pathogenesis generally, thereby igniting interest in this previously underappreciated aspect of A β metabolism. A growing list of A β DPs have been identified which, by virtue of their diverse features, contribute in unique ways to the overall economy of brain A β . In this article, we provide an

overview of the general features of A β degradation followed by a brief description of some of the best characterized A β DPs and their diverse properties. We conclude with a discussion of the feasibility of developing therapies targeting A β proteolysis.

GENERAL FEATURES

A β Levels Are Potently Regulated by Proteolytic Degradation

A β is degraded by a large set of proteases with diverse characteristics (Table 1). Abundant evidence shows that A β DPs, both collectively and in many cases individually, contribute substantially to the determination of cerebral A β levels (Eckman and Eckman 2005; Leissring 2008;

Table 1. Proteases implicated in the degradation of A β

Type	Protease	Max. relative brain A β levels in KO ^a		A β substrates ^b		Subcellular localization ^c
		A β 40	A β 42	Oligos	Fibrils	
Metallo	NEP	2.0	2.0	Synth	No	Ex, ER, G
	NEP2	1.3	1.6			Ex, ER, G
	hMMEL					Ex, ER, G
	ECE1	1.3 ^d	1.3 ^d			Ex, ER, G, Endo
	ECE2	1.3	1.3			Ex, ER, G, Endo
	ACE	N.S.	N.S.			Ex, ER, G
	MMP2	1.2	1.3		Yes	Ex, ER, G
	MMP9	N.S.	1.3		Yes	Ex, ER, G
	MMP14/MT1-MMP				Yes	Ex, ER, G
	CD147/EMMPRIN					Ex, ER, G, Endo
Serine	IDE	1.6	1.4	No	No	Ex, ER, Endo, Lyso, Mito
	Plasmin	N.S.	N.S.	Natural	Yes	Ex, ER, G
	Acylpeptide hydrolase			Natural		Ex, Cyto
	Myelin basic protein				Yes	Ex, ER, G
Aspartyl	Cathepsin D	N.S.	3.0		Yes	Endo, Lyso
	BACE1	0.0	0.0			Endo, Lyso
	BACE2	N.S.	N.S.		No	Endo, Lyso
Cysteine	Cathepsin B	N.S.	N.S.		Yes	Ex, Endo, Lyso
Threonine	Proteasome					Cyto
Other	Catalytic antibodies					—

^aData reflect the maximum published values for endogenous cerebral A β levels in mice lacking both copies of individual A β DPs, expressed relative to wild-type controls. KO, knockout; N.S., no significant difference.

^bAggregated forms of A β known to be degraded by individual A β DPs. Synth, synthetic A β oligomers; Natural, naturally secreted A β oligomers.

^cEx, extracellular space; ER, endoplasmic reticulum; Endo, endosomes; Lyso, lysosomes; Mito, mitochondria; Cyto, cytosol.

^dEffect induced by deletion of one copy of ECE1.

Leissring and Saido 2007; Turner and Nalivaeva 2007). In an illustrative study, the half-life of A β in brain interstitial fluid (ISF) was quantified in APP transgenic mice lacking or expressing NEP (Fig. 1A; Farris et al. 2007). This was accomplished by using in vivo microdialysis to quantify interstitial A β levels as a function of time before and after pharmacologic blockade of A β production (Farris et al. 2007). Genetic deletion of NEP resulted in a doubling of steady-state A β levels and, notably, a significant increase in the half-life of ISF A β (Fig. 1B). Conversely, transgenic overexpression of NEP in neurons by eightfold in an APP mouse model lowered A β levels by around 90% and, notably, prevented the development of any amyloid

plaques or downstream cytopathology when examined up to 14 months of age (Fig. 1C; Leissring et al. 2003). These and many other findings strongly suggest that A β DPs occupy an “upstream” position within the amyloid cascade that may be surpassed only by the proteases involved in A β production itself.

Net A β Levels Reflect the Balance between Rates of Production and Clearance

A β is generated and eliminated continuously, and the absolute concentration of A β , within a given compartment and at a given instant, is determined jointly by these opposing forces. An instructive analogy is that of a balance

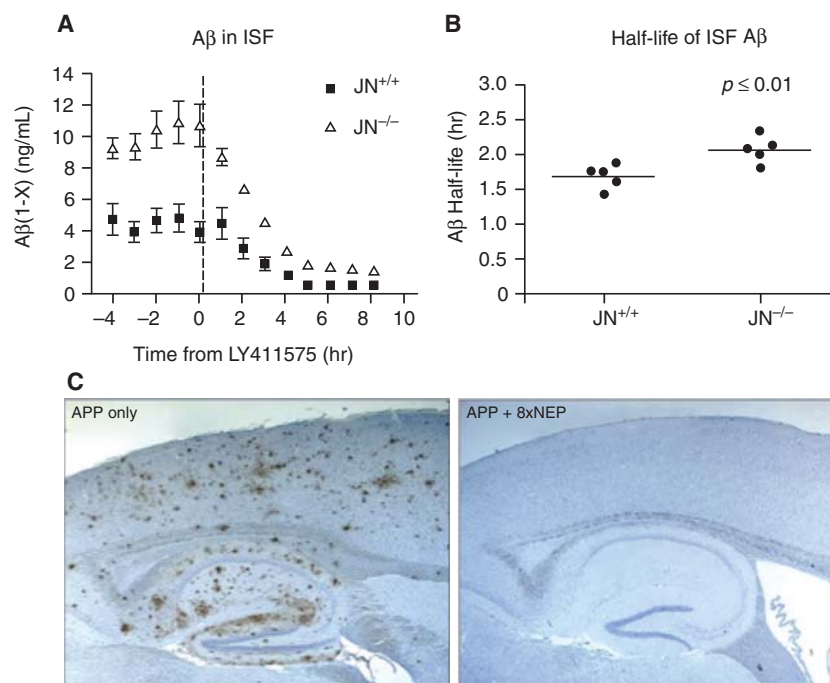


Figure 1. A β degradation is a potent determinant of brain A β levels and amyloid pathology. (A) Effects of genetic deletion of NEP on A β levels in the interstitial fluid (ISF) of the J9 line of APP transgenic mice monitored by in vivo microdialysis before and after blockade of A β production with the γ -secretase inhibitor, LY411575. Note that steady-state levels of A β are approximately doubled in J9 mice lacking NEP (JN^{-/-}). (Panel A is adapted from Farris et al. 2007; reprinted, with permission, from the authors.) (B) The half-life of ISF A β determined from the data in (A). Note that deletion of NEP results in a statistically significant ($P < 0.01$) 23% increase in the half-life of ISF A β , from 1.7 to 2.1 hours. (C) Transgenic overexpression of NEP by eightfold results in the complete prevention of amyloid plaque formation in the J20 line of APP transgenic mice up to 14 months of age. (Panel B is adapted from Leissring et al. 2003; reprinted, with permission, from the author.)

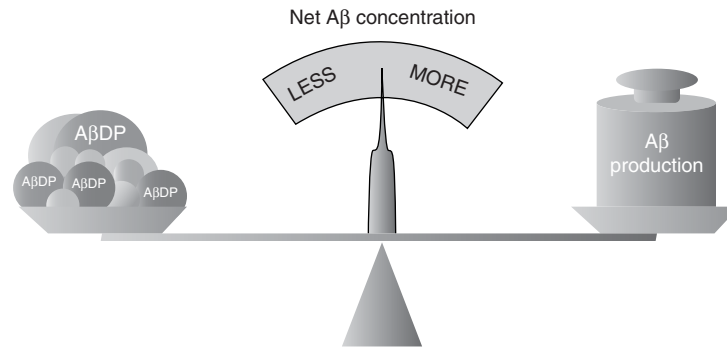


Figure 2. The balance analogy illustrates the relationship between A β DPs and net A β levels. By analogy with a balance, net A β concentrations (represented by the position of the pointer on the scale) are determined by the rate of A β production (represented by a single weight on one arm) relative to the overall rate of A β clearance (represented by a collection of counterweights on the other). A β clearance is performed by a collection of A β DPs (dark gray counterweights) working jointly with each other and with other eliminative processes (light gray counterweights). See text for additional details.

(Fig. 2), wherein the absolute rate of A β production, represented by a weight on one arm, is *counterbalanced* by the overall rate of A β clearance, represented by a large collection of diverse counterweights on the other arm.

The balance analogy serves to illustrate several general features of A β degradation:

1. *Net A β levels are determined by the relative, rather than absolute, rates of A β production and elimination.*

Net A β concentrations can be elevated either by an increase in A β production or by a decrease in the overall rate of its elimination, and the converse is also true. However, no change in net A β levels will occur if these opposing forces vary in indirect proportion to one another—only if one changes with respect to the other.

2. *A β DPs work cooperatively with each other and with other catabolic processes to eliminate A β .*

The catabolism of A β is mediated not only by multiple A β DPs but also by a diverse array of eliminative processes, including diffusion, passive and active transport, protein–protein interactions, aggregation, and deposition. These processes all operate simultaneously, in complex combinations that vary regionally and by subcellular compartment.

3. *Net A β levels are determined by the sum total of all catabolic processes.*

Despite the complexity of A β catabolism, assuming production to be constant, the parameter most relevant to the determination of A β levels is the *overall rate* of A β catabolism, determined by the totality of all contributing processes. As a consequence, A β DPs and other A β -eliminating processes are *functionally interchangeable*, at least with respect to their influence determining net concentrations of A β .

4. *Proteolytic degradation of A β normally operates at or near its functional capacity.*

In mice, genetic deletion of any one of several, markedly different A β DPs can result in significant elevations in endogenous cerebral A β (Table 1). These increases in net A β levels occur in a gene dosage-dependent manner, and simultaneous deletion of two different A β DPs has also been shown to produce roughly additive effects. Taken together, these findings show that multiple A β DPs exist, each of which is *rate limiting* in the determination of cerebral A β concentrations. More significantly, these findings suggest that there is *little or no reserve capacity* in the overall catabolism of cerebral A β .

5. *The mechanistic relationship between A β and A β DPs is bidirectional.*

Not only do A β DPs regulate A β via proteolytic degradation, but A β itself can also disrupt the function of A β DPs, either directly, via *competitive inhibition*, or indirectly, via a wide range of secondary processes triggered by A β accumulation, such as oxidative damage. Conversely, aggregated A β can also stimulate the production or activation of certain A β DPs. In these and other ways, A β DPs and A β interact *bidirectionally*.

A β Production and Degradation Are Asymmetric

The balance analogy, although illustrative of the mutual interdependence of A β production and degradation, fails to completely capture several fundamental asymmetries between the two processes. Collectively, these asymmetries offer important insights into the contribution of A β DPs to the normal regulation of cerebral A β levels and, by extension, the pathogenesis and potential treatment of AD.

Few Sources versus Many Diverse Sinks

Perhaps the most fundamental asymmetry is the difference in sheer complexity between A β production and degradation. Full-length A β

peptides are produced by just two proteases, which act within a comparatively limited subset of subcellular compartments, primarily within neuronal cells. A β degradation, in contrast, is mediated by a considerably larger number of proteases, each with unique A β avidities, pH optima and, perhaps most critically, different regional, cellular, and subcellular localizations.

A β DPs Define Different Pools of A β

Proteolytic degradation of A β is the terminal event that defines the lifespan of a substantial portion of all A β peptides produced. By determining the temporal lifetime of individual A β molecules, proteolytic degradation also indirectly determines the spatial extent to which each molecule can be transported away from its site of production. As illustrated in Figure 3, A β DPs thus help to define specific *pools* of A β , the temporal and spatial extent of which is defined jointly by production and degradation. In light of the substantial variety in the regional and subcellular localization of many A β DPs (Table 1), it is evident that many different pools of A β exist, each contributing differently to overall A β levels and, potentially, to AD pathogenesis (Fig. 3). As such, functionally or spatially distinct A β DPs represent experimental probes for establishing the relative

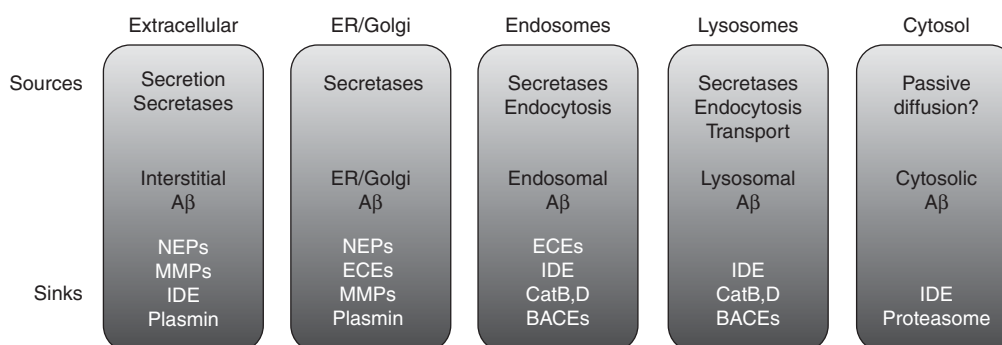


Figure 3. A β DPs regulate and help define distinct pools of A β . A β can be conceptualized as existing in distinct *pools* localized to different subcellular compartments (rounded rectangles). Each pool is characterized by different “sources” of A β (e.g., secretases, secretion) and different combinations of A β DPs. Because A β DPs vary considerably in terms of their subcellular localization, pH optima and other properties, A β within different subcellular compartments is regulated by diverse combinations of A β DPs.

importance of individual pools of A β , which might then be more selectively targeted for therapeutic benefit.

A β Degradation Is Catalytic and Irreversible

Proteolytic degradation is *catalytic* and *irreversible*, meaning that a single A β DP molecule can effect the permanent elimination of a large number of A β molecules, while itself remaining unchanged. Although it is true that A β production is also mediated by catalytic processes that can be rate limiting, in practice, A β production appears to be *substrate limited*. This can be seen from the fact that increases in APP expression—for instance, in Down's syndrome or in APP transgenic mice—result in roughly proportional increases in net A β production, both in the brain and in the periphery. Because small changes in the activities of multiple A β DPs can result in large changes in net A β levels, the catalytic nature of A β DPs suggests they are important both for the etiology and the potential treatment of AD.

A β Degradation Is Prone to a Range of Environmental and Age-Associated Insults

The pathogenesis of AD is known to be influenced by a range of environmental insults, whereas aging itself is known to be characterized by the accrual of oxidative damage (Zhu et al. 2007), as well as a general decrease in the expression of many proteins (Lu et al. 2004). A β DPs, in turn, are known to be vulnerable to a range of potentially damaging exogenous influences, including pharmacological inhibition, environmental insults, and age-related oxidative damage (Wang et al. 2003; Caccamo et al. 2005; Shinall et al. 2005; Neant-Fery et al. 2008). Given that age is the principle risk factor for AD, these considerations suggest that defective clearance of A β is likely to be operative not only in sporadic forms of AD, as was recently confirmed experimentally (Mawuenyega et al. 2010), but even in those cases attributable to increased production of A β due to genetic disturbances.

A β Degradation Can Take Place Distal to Sites of Production

The study of A β DPs has confirmed other evidence suggesting that A β exists in a dynamic equilibrium between various compartments, such as the secretory pathway, the endolysosomal system, the interstitial space, CSF, and even compartments outside the brain such as the circulatory system. Because these compartments are interconnected, either through physical contiguity or through active and passive A β transport, the degradation of A β in one compartment can result in the lowering of A β in the others. As a consequence, A β DPs can regulate net A β levels at sites distal to its production. This principle has an important therapeutic corollary. Whereas therapies aimed at blocking A β production must necessarily act locally, within A β -producing cells, therapies aimed at increasing A β catabolism are capable of exerting their effect in multiple compartments, including compartments outside the blood–brain barrier. In a striking demonstration of this principle, overexpression of NEP exclusively in the periphery (in skeletal muscle) was recently shown to lower steady-state A β levels and amyloid plaque deposition in brain (Liu et al. 2009).

SPECIFIC A β -DEGRADING PROTEASES

A large number of A β DPs have been identified to date (Table 1), but the state of our knowledge about each varies considerably. A β DPs can be classified by enzymological type (e.g., metalloproteases, cysteine proteases, etc.), by the assembly state of the A β substrates they hydrolyze (e.g., peptidases, oligopeptidases, or fibril-lases), and by their subcellular localization (Table 1). There is a further, functional distinction between *endogenous regulators*, which regulate brain A β levels under physiological conditions, and *pathogenic regulators*, which are operative under pathological conditions, and these categories need not be mutually exclusive. In principle, a third functional category of A β DPs might be termed *therapeutic regulators*, which, it is important to emphasize, do

not necessarily need to belong to either of the former categories to be effective.

In the following subsections, we briefly introduce the best characterized A β DPs, together with catalytic antibodies and endogenous protease inhibitors, focusing on the distinguishing features of each and the principles that have been learned from their study. Experimental evidence strongly suggests that additional A β DPs remain to be identified. For instance, simultaneous inhibition of multiple zinc-metalloproteases by i.c.v. infusion of the broad-spectrum metalloprotease inhibitor, phosphoramidon, resulted in a remarkable >fivefold increase in endogenous cerebral A β levels (Eckman et al. 2006). The magnitude of this increase is far greater than that seen by genetic deletion of any single A β DP (Table 1) or even from simultaneous deletion of multiple A β DPs (Eckman et al. 2006). In a similar finding, i.c.v. infusion of thiorphan in mice lacking both NEP and a related protease NEP2, nevertheless resulted in large increases in cerebral A β (Hafez et al. 2011). These and other findings strongly suggest that additional A β DPs remain to be identified that normally participate in A β catabolism and/or that might be used therapeutically.

Zinc-Metalloproteases

Neprilysin

The most extensively investigated and best characterized A β DP is NEP, a member of the M13 clan of zinc-metalloproteases (Howell et al. 1995; Hersh and Rodgers 2008). NEP was once termed “enkephalinase” because enkephalin is one of its best substrates in vitro (Turner 1998). However, enkephalin levels in the cerebral cortex were unchanged in NEP knockout (KO) mice (Saria et al. 1997; Iwata and Saido, unpubl. data), suggesting that NEP alone does not determine the steady-state levels of enkephalin in vivo. This is probably because there exist redundant catabolic mechanism(s) that involve exopeptidase(s), other endopeptidase(s), or both. In contrast, levels of both A β 40 and A β 42 are twofold higher in NEP KO mice than the levels in wild-type controls (Table 1; Iwata

et al. 2001), suggesting that NEP is an important endogenous regulator of A β .

NEP was first identified as an important A β DP in an experimental paradigm in which the degradation of radiolabeled A β 42 injected into rat hippocampus was monitored in the presence or absence of different protease inhibitors (Iwata et al. 2000; Saido and Iwata 2006). NEP is a type II membrane-associated peptidase, the active site of which faces the luminal or extracellular side of membranes (Roques et al. 1993; Turner 2004; Turner et al. 2001), a topology that is ideally suited for the degradation of largely extracytoplasmic peptides such as A β . NEP is almost exclusively expressed in neurons, not in glia, and the peptidase, after synthesis in the soma, is axonally transported to presynaptic terminals (Fukami et al. 2002), presumably in a manner similar to that in which APP is transported. Therefore, presynaptic terminals and nearby intracellular (luminal) locations are likely to be the sites of A β degradation by NEP (Iwata et al. 2004). Importantly, the levels of A β inversely correlate with the gene dosage of NEP and thus with its enzymatic activity. These observations suggest that even partial loss of NEP expression/activity can cause the elevation of A β levels and could therefore induce amyloidosis on a long-term basis, in a similar manner to familial AD-causing gene mutations. The results also suggest that the rate constant for the intraparenchymal degradation of A β by NEP could account for as much as 50% of the total clearance activity (Saito et al., 2003).

Several insights have emerged from the study of NEP in APP transgenic mice. As discussed above, genetic deletion leads to an approximate doubling of steady-state levels of cerebral A β while accelerating amyloid deposition (see Fig. 1A). Qualitative pathological differences have emerged, as well. For example, deletion of NEP in the J9 line of transgenic mice led to the emergence of cerebral amyloid angiopathy that was not present in mice expressing two functional copies of NEP (Farris et al. 2007).

The therapeutic value of overexpressing NEP has also been investigated in APP transgenic

mouse models. For example, as mentioned above, a cross between the J20 line of APP transgenic mice and a transgenic mouse that expresses eightfold higher levels of NEP (8xNEP) resulted in up to a 90% reduction in steady-state A β levels and the complete prevention of amyloid plaque formation and associated cytopathology when examined at up to 14 months of age (Fig. 1C; Leissring et al. 2003). NEP has been reported to degrade A β oligomers that impair neuronal plasticity and cognitive function in APP-Tg mice (Huang et al. 2006), although a different study saw no decrease in oligomers (Meilandt et al. 2009) (discussed below). As another potential therapeutic benefit, neuropeptide Y fragments generated by NEP-catalyzed proteolysis have been shown to be neuroprotective (Rose et al. 2009). Although these and other findings illustrate the potential benefits of therapeutic overexpression of NEP, there may also be risks. For example, the 8xNEP transgenic line has been shown to alternatively prevent or promote premature lethality in a strain-dependent manner (Leissring et al. 2003; Meilandt et al. 2009).

Like all known A β DPs, NEP degrades monomeric A β . Interestingly, some of the pathogenic APP mutations that reside within the A β sequence render A β monomers more resistant to NEP-catalyzed proteolysis (Tsubuki et al. 2003; Betts et al. 2008). It is less clear whether NEP can directly degrade oligomeric A β species. In vitro, NEP was reported to degrade oligomeric forms of synthetic A β (Kanemitsu et al. 2003), but it was incapable of degrading naturally secreted A β oligomers isolated from cultured cells (Leissring et al. 2003), suggesting that differences in the A β oligomer preparation might matter. Two findings in APP transgenic mice raise additional questions. On the one hand, deletion of NEP in 2 different mouse models was found to increase the concentration of A β oligomers (Huang et al. 2006; Farris et al. 2007). On the other hand, a cross between the 8xNEP line and the J20 line of APP transgenic mice resulted in dramatic decreases in monomeric A β levels and prevented all plaque formation (as reported previously by Leissring et al.

2003), yet oligomeric A β levels were unchanged (Meilandt et al. 2009). Moreover, in the latter study, NEP overexpression failed to reverse the learning and memory deficits present in the J20 line (Meilandt et al. 2009). Because different promoters were used, the extent to which the NEP and APP transgenes were coexpressed in the same population of neurons is not clear. Nevertheless, whether coexpressed appreciably or not, this result implies that NEP might not be capable of clearing at least some naturally produced A β oligomers.

NEP-Like Peptidases

Several close homologs of NEP are also implicated as candidate A β DPs (Table 1; Shirotani et al. 2001). For example, genetic ablation of NEP2 produces net increases in cerebral A β levels that are additive with those produced by deletion of NEP (Hafez et al. 2011). Another phosphoramidon-sensitive NEP homolog, human membrane metalloendopeptidase-like protein (hMMEL), was recently found to degrade A β in cultured cells (Huang et al. 2008). Although the exact contribution of each is still under investigation, it seems likely that the *collective* action of these and other NEP-like peptidases contribute significantly to the determination of cerebral A β levels.

Endothelin-Converting Enzymes

Two additional members of the M13 family of zinc metalloproteases, endothelin-converting enzymes 1 and 2 (ECE1, ECE2), are also known to be endogenous regulators of A β (Table 1; Eckman et al. 2001, 2003). In contrast to NEP and NEP-like peptidases, which are most active at neutral pH, ECEs have an acidic pH optimum and are therefore active primarily within acidic subcellular compartments (Table 1). As a consequence, ECEs primarily degrade A β at intracellular sites (Eckman et al. 2003). This point is important, because, together with other evidence (Leissring 2008), it serves to show that the vast majority of A β degradation likely occurs *before* the secretion of the monomer into the extracellular space.

Angiotensin-Converting Enzyme

Another important vasopeptidase implicated in the degradation of A β is angiotensin-converting enzyme (ACE) (Carvalho et al. 1997; Hu et al. 2001). Because pharmaceutical ACE inhibitors are widely used to treat hypertension, the question of whether ACE is an endogenous regulator of A β is a critical one. At present, the balance of the evidence suggests that it is not. Oral administration of the widely used ACE inhibitor, captopril, to APP transgenic mice resulted in no significant elevation in cerebral A β levels (Hemming et al. 2007b). Moreover, genetic deletion of ACE failed to produce any significant elevation in steady-state levels of endogenous A β (Table 1; Eckman et al. 2006). Nevertheless, because there is also genetic evidence that variants in the *Ace* gene are associated with the risk for late-onset AD (Bertram et al. 2007), it will be important to gain further clarity about the exact role of ACE in the degradation of A β under physiological and pathophysiological conditions.

Matrix-Metalloproteinases

Matrix-metalloproteinases (MMPs) represent another important group of A β DPs that can be distinguished, in part, by their ability to degrade both monomeric and fibrillar forms of A β (Table 1; Yan et al. 2006a). Multiple MMPs have been implicated in the degradation of A β , including MMP2 (Roher et al. 1994), MMP9 (Yan et al. 2006a) and MMP14 (a.k.a. MT1-MMP) (Liao and Van Nostrand 2010) but only a subset have been investigated in vivo. Relative to other A β DPs, MMPs are comparatively weak endogenous regulators of A β . For example, deletion of MMP2 or MMP9 in mice resulted in modest but statically significant increases in endogenous cortical and hippocampal A β (Yin et al. 2006) (Table 1). However, some special properties of MMPs suggest they are likely to be of considerably greater importance in a pathological context. First, MMPs normally exist as latent pro-enzymes that can be proteolytically processed to become fully active (Van Wart and Birkedal-Hansen 1990).

Interestingly, extracellular matrix metalloproteinase inducer (EMMPRIN; CD147), one of the proteases responsible for activating MMPs by this mechanism, was found to lower A β levels in cultured cells by inducing multiple MMPs (Vetrivel et al. 2008). Second, basal expression of MMPs is low but can be stimulated by pathological insults, including A β itself (Deb and Gottschall 1996). Consistent with these features, in APP transgenic mice, MMPs were found to be up-regulated in astrocytes adjacent to amyloid deposits (Yin et al. 2006). Moreover, in the same mice, i.c.v. infusion of the broad-spectrum MMP inhibitor, GM6001, resulted in significant increases (~50%) in both the steady-state levels and the half-life of ISF A β (Yin et al. 2006).

Insulin-Degrading Enzyme

Insulin-degrading enzyme (IDE) is another well-established A β DP that has been extensively investigated for its role in A β degradation using a wide array of experimental approaches, ranging from enzymological analyses to human molecular genetics (Hersh 2006). Although IDE is a zinc-metalloprotease, it belongs to a separate superfamily with distinct evolutionary origins, referred to as “inverzincins” because they feature a zinc-binding motif (HxxEH) that is inverted with respect to the canonical one (HExxH) present in most known zinc-metalloproteases (Becker and Roth 1992). The crystal structure of IDE is unusual, resembling a clam shell, with a large internal chamber formed from two bowl-shaped halves connected by a flexible linker (Shen et al. 2006). Because oligomeric and fibrillar forms of A β are too large to fit completely into its internal chamber, IDE is strictly a peptidase, i.e., it exclusively degrades monomeric A β .

Although functionally similar to vasopeptidases (e.g., ACE) in showing a preference for monomeric A β , IDE differs substantially in terms of its subcellular localization. It is well established that IDE is most abundant in the cytosol (Falkevall et al. 2006) and also present within mitochondria (Leissring et al. 2004; Faris et al. 2005), but there is less certainty about

its presence in other subcellular compartments (Leissring et al. 2004), with various studies reporting its presence in peroxisomes (Kuo et al. 1994), endosomes (Hamel et al. 1991), the endoplasmic reticulum (Carpenter et al. 2010), and lysosomes (MA Leissring, unpubl.). Like most other A β DPs, IDE is also present in the extracellular space (Table 1), both in secreted (Qiu et al. 1998) and cell-associated (Vekrellis et al. 2000) forms. IDE lacks a canonical signal peptide sequence (Leissring et al. 2004) and it is exported independent of the classical secretory pathway (Zhao et al. 2009). The precise nature of the underlying secretion mechanism remains obscure, but accruing evidence suggests that it is mediated at least partly by exosomes (Bullock et al. 2010; Tamboli et al. 2010).

Abundant evidence suggests that IDE is the major A β DP secreted into the medium of cultured cells (Qiu et al. 1998). For example, in cultured primary neurons, genetic deletion of IDE resulted in >90% decrease in the initial degradation rate of physiological levels of exogenous A β monomers (Farris et al. 2003), and similar results are seen with a wide variety of different cultured cells (MA Leissring, unpubl.). However, in vivo, genetic deletion of IDE resulted in elevations in cerebral A β levels which, although comparable to those induced by many A β DPs, are smaller than might be expected from results in cultured cells (Table 1; Farris et al. 2003). Two factors may contribute to this interesting disparity. First, although IDE is present in CSF (Qiu et al. 1998), it is likely that IDE accumulates in the medium of cultured cells to a greater extent than it does in extracellular fluids in vivo. Second, IDE KO mice suffer from chronic hyperinsulinemia (Farris et al. 2003; Abdul-Hay et al. 2011), which triggers age-dependent compensatory adaptations, including severe insulin and glucose intolerance (Abdul-Hay et al. 2011). The secondary consequences of IDE ablation thus obscure the impact of this important A β DP on brain A β levels. New pharmacologic inhibitors of IDE (Leissring et al. 2010) should make it possible to circumvent these compensatory changes and determine the direct contribution of IDE to cerebral brain A β levels.

Serine Proteases

Plasmin

Three functionally related serine proteases have been linked directly and indirectly to A β degradation: plasmin and urokinase-type and tissue-type plasminogen activators (uPA and tPA, respectively). Of these, only plasmin has been shown to directly degrade A β ; like MMPs, it can degrade both monomeric and fibrillar forms (Table 1; Van Nostrand and Porter 1999; Tucker et al. 2000). tPA and uPA, however, are responsible for converting the inactive zymogen of plasmin (plasminogen) into its active form. The latter process is normally inhibited by the endogenous inhibitor, plasminogen activator inhibitor1 (PAI1; Myohanen and Vaheri 2004), and it is of great interest that pharmaceuticals which disrupt PAI1 have been developed that effectively lower brain A β in APP transgenic mice (Jacobsen et al. 2008). tPA is an excellent example of a pathologic regulator of A β , because it is stimulated by fibrillar proteins including A β (Van Nostrand and Porter 1999). uPA is of interest because of evidence linking variability around the gene for uPA (PLAU) to late-onset AD (Serretti et al. 2007).

Acylpeptide Hydrolase

A second serine protease implicated in the degradation of A β is acylpeptide hydrolase (APH), a predominantly cytosolic enzyme that catalyzes the hydrolysis of amino-terminally acetylated amino acids from small peptides (Table 1; Yamin et al. 2007). Intriguingly, APH has been reported to show a preference for degrading naturally secreted A β dimers and trimers (Yamin et al. 2009).

Myelin Basic Protein

In rather remarkable discovery, myelin basic protein (MBP), which is known to possess endogenous serine protease activity, was recently identified as a *bona fide* A β DP (Liao et al. 2009). As is true for plasmin and APH, MBP can degrade both monomeric and fibrillar forms of A β (Table 1; Liao et al. 2009).

Cysteine Proteases

Cathepsin B

Cysteine proteases were initially implicated in A β degradation by in vivo pharmacological studies (Frautschy et al. 1998). However, only one cysteine protease, cathepsin B (CatB), has so far been specifically implicated in the degradation of A β in vivo (Mueller-Steiner et al. 2006). Interestingly, CatB is predominantly present within the endolysosomal protein degradation pathway (Mort and Buttle 1997), which is known to degrade A β and which is compromised in AD (Glabe 2001). CatB is secreted by exocytosis in certain pathological conditions (Mort and Buttle 1997) and has also been found to be present within extracellular amyloid plaques in AD (Mueller-Steiner et al. 2006). However, it is unclear whether CatB is operative in these compartments, because it exhibits optimal activity at pH 5–6 (Koga et al. 1991). Unlike most other known A β DPs, CatB is an endoprotease (Mort and Buttle 1997), but it is unusual for also having dipeptidyl carboxypeptidase activity (Mueller-Steiner et al. 2006).

Aspartyl Proteases

Cathepsin D

A second lysosomal protease implicated in A β degradation is the aspartyl protease, cathepsin D (CatD) (Leissring and Saido 2007). This role for CatD was initially discovered from analysis of brain homogenates, where it was shown to be the principal protease responsible for A β degradation at acidic pH (Hamazaki 1996; McDermott and Gibson 1996). Confirming its physiological relevance, CatD KO mice were recently found to have significant elevations in steady-state endogenous brain A β (Leissring et al. 2009). Consistent with its high activity in brain homogenates, deletion of CatD resulted in cerebral brain A β 42 levels threefold higher than those in wild-type littermates, the largest increase observed in any A β DP KO mouse model (Table 1). Intriguingly, A β 40 levels were unaffected in these mice, resulting in increases in the A β 42/40 ratio that are comparable to those induced by presenilin mutations

(Leissring et al. 2009). Consistent with an effect on the critical A β 42/40 ratio, deletion of CatD, unlike that of any other known A β DP, accelerates the onset of plaque formation. In the TgCRND8 APP transgenic mice, which normally develop amyloid plaques beginning at 3 months of age, deletion of CatD elicits plaque formation by just 3 weeks of age (MA Leissring, unpubl.). The differential increase in A β 42 seen in the CatD KO mice has an intriguing mechanistic basis. Unlike CatB, CatD does not convert A β 42 to A β 40. Rather, CatD degrades A β 42 and A β 40 in a highly differential manner, with the affinity for A β 42 and A β 40 CatD being in the low nanomolar and low micromolar range, respectively, a factor that may drive preferential degradation of A β 42 at low concentrations. At the same time, the turnover rate of A β 42 is very slow, around 100-fold lower than that of A β 40. Quite interestingly, the strong affinity of A β 42 together with its slow turnover rate render A β 42 a potent competitive inhibitor of CatD, even at relatively low (midnanomolar) concentrations (Leissring et al. 2009). Together with accumulating human molecular genetic evidence linking CatD to late-onset AD (Bertram et al. 2007), these findings suggest that CatD is a physiological and pathological regulator of A β , and they further suggest that CatD might be a downstream target of A β 42 itself.

BACE1

Ironically, the major protease implicated in β -secretase activity, β -site APP cleaving enzyme 1 (BACE1; a.k.a. memapsin 2), is also capable of directly degrading A β (Fluhrer et al. 2003). Given BACE1's key role in A β production, the physiological relevance of this finding is difficult to assess but may explain the finding that transgenic overexpression of very high levels of BACE1 paradoxically resulted in reduced A β deposition in vivo (Lee et al. 2005).

BACE2

BACE2, a close homolog of BACE1, also avidly degrades A β in vitro, exhibiting a catalytic efficiency that is around 50-fold greater than

BACE1 (Abdul-Hay and Leissring 2011), higher in fact than the published values for any other known A β DP. Nevertheless, BACE2 KO mice show no net elevation in endogenous cerebral A β levels (Table 1; MA Leissring and SO Abdul-Hay, unpubl.). This is likely because BACE2 is expressed in astrocytes and other glia but not in neurons, which carry out the majority of A β production (Dominguez et al. 2005). Although these results suggest that BACE2 is not a physiologic regulator of A β , BACE2 might play some role in a pathological context because adult astrocytes are known to avidly degrade A β (Wyss-Coray et al. 2003).

The Proteasome

A β is also degraded by the proteasome (a.k.a., multicatalytic proteinase) by as-yet undetermined catalytic subunits (Lopez Salon et al. 2003). The proteasome is localized to the cytosol (Table 1) and, given that A β is produced in luminal compartments, might therefore be assumed to play no physiologic role in A β degradation. However, some experimental evidence suggests that A β 42 can diffuse passively from the lumen of the ER into the cytosol, where it is degraded jointly by the proteasome and IDE (Fig. 3; Schmitz et al. 2004). These and other findings—including evidence that A β accumulates within other intracellular organelles such as mitochondria (Yan et al. 2006b)—suggest that ill-defined pools of A β may exist that are degraded by certain A β DPs.

A β -Degrading Catalytic Antibodies

As is true for the secretases involved in A β production, the therapeutic targeting of A β DPs is complicated by the fact that each degrades multiple substrates besides A β . Catalytic antibodies have been suggested as an alternative that, by virtue of their higher specificity for particular antigenic targets, might improve the selectivity for A β . A surprisingly large number of A β -degrading immunoglobulins (Igs) and Ig-fragments have been discovered or engineered (Taguchi et al. 2008a). Although the catalytic efficiencies of most A β -degrading antibodies

is currently orders of magnitude slower than A β DPs, the technology exists to engineer existing antibodies or select new ones with improved properties (Taguchi et al. 2008b). Interestingly, A β -degrading antibodies are present in the sera of normal subjects and, notably, are increased in AD patients (Paul et al. 2010). Such antibodies can be harvested and may have therapeutic potential (Taguchi et al. 2008a). The exciting potential of catalytic A β -degrading antibodies makes this a topic worthy of continued investigation.

ENDOGENOUS INHIBITORS OF A β DEGRADATION

Several endogenous protease inhibitors have also been implicated in the regulation of A β degradation. Certainly the most interesting example is the nonneuronal isoform of APP itself (APP₇₅₁), which was in fact identified initially as a serine protease inhibitor (protease nexin II; Van Nostrand and Cunningham 1987; Van Nostrand et al. 1989) due to the presence of a Kunitz-type serine protease inhibitor (KPI) domain present in the longer APP isoforms (Ponte et al. 1988). The KPI domain inhibits A β degradation in cell culture by as-yet undetermined serine proteases (Naidu et al. 1995) and, intriguingly, transgenic mice overexpressing KPI-containing APP isoforms were found to have more severe amyloid pathology than mice expressing equivalent levels of APP lacking this domain (Higgins et al. 1993). It was later discovered that a second inhibitor domain exists within all isoforms of APP (Miyazaki et al. 1993), which has been mapped (to residues 579–601 of APP₇₇₀; Higashi and Miyazaki 2003) and shown to potently and selectively inhibit MMP2 (Higashi and Miyazaki 2008). Another serine protease inhibitor, alpha-1 antichymotrypsin, which was identified as a constituent of amyloid plaques (Abraham et al. 1988), has also been shown to inhibit the degradation of A β in vitro and in vivo (Abraham et al. 2000). The cysteine protease inhibitor cystatin C, which has been genetically linked to late-onset AD (Bertram et al. 2007), also regulates A β degradation by inhibiting CatB (Sun

et al. 2008), although other mechanisms may also contribute to cystatin C's overall effect on amyloid plaque formation (Gauthier et al. 2011). Finally, as noted already, pharmacologic inhibitors of PAI1, which normally blocks that conversion of plasminogen to plasmin by tPA and uPA, have been shown to attenuate amyloid deposition in APP transgenic mice (Jacobsen et al. 2008).

THERAPEUTIC APPROACHES BASED ON A β DEGRADATION

One strategy for the treatment of chronically elevated A β levels in AD would be gene therapy using an A β DP. The introduction of NEP into the brains of APP transgenic mice using viral vectors has been shown to attenuate A β pathology, leading to improved cognitive function (Marr et al. 2003; Iwata et al. 2004; El-Amouri et al. 2008; Spencer et al. 2008). Although gene therapy for the treatment of Parkinson's disease in humans has already gained substantial momentum (Feng and Maguire-Zeiss 2010), its application to AD has not been as prominent, presumably due to the difference in the size and extent of the affected brain regions. However, in the very early stage of disease development, introduction of the NEP gene into the entorhinal cortex, which leads to expression of NEP in the hippocampus (Iwata et al. 2004), might generate a useful therapeutic effect. On the other hand, a significant reduction in cerebral A β levels and plaque formation has been achieved by expression of NEP in transplanted astrocytes (Hemming et al. 2007a), suggesting that neurons do not necessarily need to be directly infected with A β DPs to be effective. Substantial advances in gene therapy technology are anticipated in the coming years. IDE, ECEs, MMPs, BACE2, or other A β DPs could also be used in a similar manner to NEP, whereas the plasmin system should be more cautiously considered due to potential adverse side effects caused by hemorrhages (Murray et al. 2011).

A β DPs can be targeted by pharmacological therapies as well. Compared to the approach of inhibiting A β production, the notion that drugs

could be developed which chronically stimulate A β degradation would seem to be impractical. However, because many A β DPs are regulated at least in part by endogenous inhibitors, it is feasible to enhance A β degradation via drugs that disrupt protease–inhibitor interactions. Indeed, this approach has been pursued pre-clinically, as illustrated by the development of PAI1 inhibitors that effectively promote A β degradation by plasmin (Jacobsen et al. 2008). Pharmacologic enhancement of the expression of A β DPs is another potential strategy. For example, neuronal NEP activity has been shown to be controlled by a neuropeptide, somatostatin (Saito et al. 2005), likely involving the phosphorylation status of the cytoplasmic domain of NEP (Kakiya R, Saito T, and Saido T, unpubl.). It should be feasible to develop synthetic small-molecule agonists that stimulate NEP by activating somatostatin receptors, such as the type four receptor, which is present exclusively in brain. Finally, for certain A β DPs, it may be feasible to develop compounds that directly activate proteolytic degradation. Consistent with this, compound screening has identified drug-like molecules that increase A β degradation by IDE several-fold (Cabrol et al. 2009).

CONCLUSIONS

Perhaps the most fundamental question yet to be answered is why A β is deposited in sporadic AD, which accounts for >99% of AD cases. It should be noted that the number of sporadic AD patients will grow as the average life expectancy increases, whereas the number of early-onset familial AD patients should remain proportional to the total population. The hypothesis that A β accumulation results at least in part from an age-dependent decline of A β degradation provides a plausible mechanism that may account for a substantial portion of AD cases. Virtually all humans accumulate A β in the brain as they age (Funato et al. 1998; Morishima-Kawashima et al. 2000), suggesting that A β deposition may be an unavoidable consequence of aging which may in turn place fundamental limits on the health of the brain. Because the conversion of “normal aging” to

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AD via mild cognitive impairment appears to be a continuous process caused primarily by the gradual acceleration of A β accumulation, we may ultimately be able to implement pre-symptomatic interventions which include A β -reducing strategies utilizing degradation and clearance mechanisms to maintain lower A β levels during later life (Saito et al. 2003).

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