Review:

The role of endoproteolytic processing in neurodegeneration

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List of Abbreviations:

aa = amino acids

Aβ = Amyloid-β protein

AD = Alzheimer's disease

ADAM = A Disintegrin And Metalloprotease

AEP = asparagine endopeptidase

AICD = APP intracellular domain

Aph-1 = anterior pharynx-defective 1

APP = amyloid precursor protein

BDNF = brain-derived neurotrophic factor

CALM = clathrin assembly lymphoid myeloid leukaemia

CNS = central nervous system

GPI = glycosyl-phosphatidylinositol

GSIs = γ-secretase inhibitors

HD = Huntington's disease

Htt = huntingtin gene

IMP = intramembrane proteases

LGMN = legumain

MAPT = Microtubule-Associated Protein Tau

MBD = Microtubule-binding domain

MMP-10 = matrix metalloproteinase 10

NES = nuclear export signal

ND = neurodegenerative diseases

PICALM = phosphatidylinositol binding clathrin assembly protein

Pen-2 = presenilin enhancer-2

PrP^C = cellular prion protein

PS = presenilin

SPP = signal peptide peptidase

SPPL = SPP-like proteases

TMD = transmembrane domain

Abstract

Endoproteolysis is a normal post-translational process in the eukaryotic cell that had played a role early on in protein evolution allowing protein catabolism and the generation of amino acids. Endoproteolytic cleavage regulates many crucial cellular processes including the activity of many proteins, their protein-protein interactions and the amplification of cell signals. Not surprisingly, disruption or alternation of endoproteolytic cleavage maybe the root cause of many human diseases such as Alzheimer's disease, Huntington's disease and prion diseases. Most neurodegenerative diseases (ND) are caused by the build-up of misfolded proteins and the promotion of aggregation events. A common event that occurs in these ND is the alteration of endoproteolytic cleavage due to genetic mutations of the associated-proteases or in the target substrate. Endoproteolytic cleavage resulting in protein truncation has significant effects on the structure and function of a protein representing a common feature of ND. In this review, we will discuss the

endoproteolytic cleavage events that lead to ND, namely Alzheimer's disease, Huntington's disease and prion diseases.

Overview of endoproteolytic cleavage

Endoproteolytic cleavage is the break down of proteins into smaller polypeptides or amino acids. This process is accelerated mainly through the action of proteases. Under normal circumstance, proteolysis is the physiological function that provides nutrients to the organism it may serve as a means to control the activity of a protein after protein synthesis. This process is important for cellular homeostasis thus allowing the regulation of cellular processes as well as removing the build up of unwanted protein products that could be toxic to the cell. Not surprisingly, deregulation of endoproteolysis can result in a number of human diseases such as pancreatitis, diabetes mellitus, Alzheimer's disease (AD), prion diseases, emphysema, muscular dystrophy, to name a few. In this review, we shall focus on the deregulation of proteolysis in neurodegenerative diseases, namely Alzheimer's disease, Huntington's disease (HD), and Prion diseases.

Alzheimer's disease

Alzheimer disease (AD) is neuropathologically characterized by neuronal loss and the emergence of two forms of protein aggregates: senile plaques and neurofibrillary tangles [1]. AD is a sequential and progressive disease of the brain resulting in irreversible loss of neurons. The effect has a profound consequence on the affected individual with gradual loss of intellectual abilities especially the memory and the

ability to reason, thus hindering the person's normal life activities and cognitive function.

APP processing

Amyloid-β protein (Aβ) is mainly found in senile plaques. Aβ is generated from its precursor called amyloid precursor protein (APP) via sequential cleavage by the β- and γ-secretases [2] (Figure 1). Notably, genetic mutations observed in patients of mainly the inherited form of familial AD resulted in increased production or aggregation of Aβ, indicating that Aβ is a seed of AD pathogenesis. Specifically, the C-terminal region of Aβ shows diversity and the longer Aβ peptide is principally deposited in AD patient brains [3]. Therefore, the γ-secretase has become a major molecular target in drug discovery of AD. The putative APP γ-cleavage sites related to amino acid residues are located at the transmembrane domain (TMD). In fact, it has taken more than a decade of research to advance our current understanding of the γ-secretase, which performs endoproteolysis within the TMD [4]. Firstly, the genes to be identified by genetic analyses of the familial form of AD were those of the multi-spanning membrane protein encoding called presenilin (PS) 1 and 2 [5]. Remarkably, the missense mutations causing disease are likely to change γ-secretase cleavage of APP resulting in elevated production of aggregation-prone Aβ-form (Aβ42) as compared with the other main Aβ species, Aβ40 [6, 7]. Secondly, the ablation of presenilin eliminated cleavage by the γ-secretase cleavage of APP [8, 9] and the intra-membrane aspartates found in PS TMD-6 and TMD-7 are necessary for Aβ production [10]. A number of cross-linking approaches showed that γ-secretase

inhibitors (GSIs) directly implicated and targeted the PS proteins [11, 12]. Lastly, the perturbed function of the clathrin assembly lymphoid myeloid leukaemia protein gene (CALM) that is encoded by the phosphatidylinositol binding clathrin assembly protein (PICALM) was shown to cause alteration of the lysosomal localisation of the γ-secretase and in turn the production of Aβ42 [13, 14]. These studies demonstrate that PS possesses the catalytic activity resulting in γ-secretase intramembrane cleavage of APP, thus affecting AD pathogenesis.

PS is part of a family of intramembrane proteases (IMP) that are distinguished by the topology of their transmembrane domain and also by the nature of the membrane active site amino acid residues. The aspartate IMPs are located near two catalytic aspartate amino acid residues found in PSs, signal peptide peptidase (SPP), and SPP-like (SPPL) proteases [15, 16]. The catalytic aspartate of IMPs is located within the Gly-X-Gly-Asp motif and, hence, these proteases are known as the GXGD-type proteases [17]. The GXGD motif is conserved in type 4 prepilin peptidase proteins, and is prerequisite to many bacterial protease cleavages. Interestingly, SPP was originally identified by a chemical biology approach and contains the same GXGD motif but differs according to its primary sequence and topology [18]. Nevertheless, the expression of PS alone is not sufficient to reconstitute the γ-secretase activity. A number of genetic and biochemical studies have demonstrated that PS required three further partners, nicastrin [19], anterior pharynx-defective 1 (Aph-1), and presenilin enhancer-2 (Pen-2) [20]. These subunits of the γ-secretase associate in a 1:1:1:1 stoichiometry [21] and co-expression of these binding partners along with PS was sufficient to reconstitute the γ-secretase activity [22, 23]. The activity of the γ-secretase complex is regulated by an auto-endoproteolysis cleavage

event of PS between the TMD-6 and TMD-7 generating N- and C-terminal fragments [24] that only associate with γ-secretase inhibitors or demonstrate strong proteolytic activity [12, 25]. The activity through this auto-endoproteolysis is regulated by an unknown mechanism. Our studies have defined that subunits of the oligosaccharyltransferase DC2 and KCP2) complex normally involved in N-glycosylation of proteins at the ER can regulate PS endoproteolysis to promote γ-secretase activity [26].

Tau protein and neurofibrillary tangles

The other contributing factor to AD is the emergence of tau protein snarls found in neurofibrillary tangles that accumulate in AD and other dementias. Increasing evidence suggests that the soluble form of tau causes the most damage in neurodegeneration [27]. On one hand, a study has shown that tau 'stickiness' is because of its ability to aggregate that contributes to its toxicity [28].

Tau truncation

Tau protein is naturally an intrinsically disordered protein singly encoded by Microtubule-Associated Protein Tau (MAPT) gene located on chromosome 17q21 in humans [29] (Figure 2). Tau protein is mainly expressed in the axons of neurons of the central nervous system (CNS) and also found in lower amounts in other cell types in the CNS [30]. In the adult human brain, tau mRNA is spliced with or without three regions encoded by exon 2, exon 3 and exon 10, resulting in 6 different isoforms of tau [31, 32]. There is also alterative splicing of exon 10 yielding to tau isoforms

containing 3 (3R) or 4 (4R) imperfect MT-binding repeats in the C-terminal domain, both of which are found in the tangles of patient brains with tauopathy [33, 34]. The 3R and 4R isoforms are found in equivalent amounts in the healthy adult brain [35]. The longest form of human tau is 441 amino acids long (Figure 2). Tau can be subdivided into two domains, the N-terminal projection domain and the C-terminal microtubule-binding domain (MBD). The N-terminal domain of tau plays an important role in signal transduction pathways through its interaction with proteins such as Phosphoinositide phospholipase C-γ (PLC-γ) and Sarcoma (Src)-kinases. The C-terminal domain, also known as the microtubule-binding domain (MBD), controls the rate of polymerization of microtubules and that is directly involved in the association of functional proteins such as protein phosphatase 2A (PP2A) or PS1 [36]. Mutations found at the gene level in MAPT linked to inherited tauopathies are mainly grouped into two categories: mutations that affect the protein levels or mutations affecting mRNA splicing, leading to elevated 4R tau production. In addition, many mutations found in exon 10 can effect both the protein and the RNA levels of tau [37].

In the eukaryotic cell, the MT cytoskeleton is an important network that plays a pivotal role in many cellular processes such as motility, morphogenesis, mitosis, meiosis, and the trafficking of organelles and macromolecules. The major constituent of MTs is the heterodimer of α- and β-tubulin proteins. The nature of MTs is highly dynamic showing a non-equilibrium behaviour often termed dynamic instability [38]. The MTs adopt rapid stochastic transition through growth and shortening as an effect of the interaction of the heterodimer of tubulin dimers at its ends. This provides a mechanism for Tau protein to promote tubulin polymerisation and thus stabilize MTs [39-41]. The functional role of tau was initially shown to promote the assembly and stability of MTs [39] and to associate with the cytoskeleton. Tau interacts strongly

with MTs [42], and after associating with the MT via the repeat regions, stabilizes the MTs at the plus end, thus providing stability to the MTs during growth phases, whilst tau N-terminal domain may act as a 'spacer' region between tau and the MTs ensuring the correct distance between them [43-46]. Also, there are a number of studies which implicate and corroborate that tau enhances the stabilization and assembly MTs on one hand, and tau mutations alter the association of tau with MTs on the other hand. For example, tau mutations such as G272V, DK280, P301L, P301S, L315R, G335V, V337M, and R406W alter tau's ability to interact with the MTs affecting MT assembly with a direct affect upon the cytoskeleton and all the activities associated with this network [44, 47-51]. This could be due to an alteration in the phosphorylation status of tau that could be essential to regulate tau-MT interactions [52, 53], and, at the same time, to changes to the domain structure of the MT-binding site.

Several cellular factors have been reported to affect tau aggregation, such as post-translational modifications including phosphorylation and acetylation (see review for further information [54, 55]), and proteolysis, including the molecular chaperone machinery. Tau proteolytic cleavage could play an important role in tau aggregation. Caspases-3 and -6 can cleave tau at amino residue sites D421 and D348 but caspase 3 has a greater efficiency [56]. In fact, tau cleavage site at D421 has already been observed in AD patient brains [56-58]. Furthermore, truncation of tau at D421 site accelerates tau aggregation compared to tau full length form [56] and this could act as a seed to initiating the process of aggregation of wild-type tau, as a mechanism prior to tangle formation [59, 60]. Tau cleavage by caspase does not impede hyperphosphorylation since both tau phosphorylation and truncation modifications are found in the brain of AD patients [56]. Tau phosphorylation slows

its proteolytic processing and conversely its de-phosphorylation facilitates its clearance [61]. Furthermore, the D421-cleaved tau form can be phosphorylated by GSK3β [59].

In addition to caspase cleavage, tau can be cleaved by other types of proteases including cathepsins, calpains, and thrombin [60, 62-64]. Pre-aggregated Aβ treatment results in tau truncation by calpain 1 generating a tau fragment 45 – 230 amino acids (aa) [60] that is commonly found in many tauopathies [65]. This fragment (45-230 aa) of tau is particularly neurotoxic whilst other fragments generated by the calpain 2 protease are neuroprotective [60, 66] through their ability to form aggregates when arachodonic acid is present, thus perturbing aggregation of tau full length [65]. Recent studies have shown that tau could be cleaved by another type of protease called asparagine endopeptidase (AEP) also known as legumain (LGMN). AEP is a cysteine protease located in the lysosomes, which cleaves its substrates within the C-terminal region of the asparagine [67, 68]. The AEP protease is auto-activated and involves the removal of both C- and N-terminal pro-peptides at varying pH levels [69]. Tau protein undergoes cleavage by AEP at positions N255 and N368 [70]. This cleavage gives rise to several fragments that are unable to bind to MTs but can efficiently aggregate through the exposure of aggregation-prone tau residues i.e. 275-280 and 306-311 [70]. These tau AEP-cleaved fragments are readily hyperphosphorylated [70]. Altogether, these studies demonstrate that tau can be cleaved by many proteases and the fragments generated may promote aggregation and toxicity, thus contributing to the pathological events in AD.

Huntington's disease (HD)

Huntington's disease (HD) is a progressive inherited neurodegenerative disease which results in motor, cognitive and psychiatric symptoms [71]. HD is triggered by the expansion of the CAG repeat on chromosome 4 that encodes glutamine in the N-terminal of huntingtin (Htt) [72]. The normal Htt gene has less than 36 CAG repeats whilst the abnormal Htt gene has more than 36 CAG repeats. For HD, the disease starts in the striatum in medium-spiny neurons and later penetrates deep into the cortex [71]. Htt protein is widely expressed in the CNS and in other non-neuronal tissues [73] and dispersed throughout the intracellular compartments [74-76]. The human Htt protein is a large protein with a molecular weight of 350 kDa and with 3144 aa. It is made up of several domains including the HEAT (Htt, elongation factor 3, the PR65/A subunit of protein phosphatase 2A and the lipid kinase Tor) domains that are 50 aa in length and consist of 2 anti-parallel α-helices that form a hairpin that is important for protein–protein interactions (Fig. 3). Approximately, 16 HEAT repeat domains have been found in Htt gene, which are arranged into clusters of 4 [77, 78]. The role and function of the HEAT domains for Htt is unknown. In addition, Htt contains 4 PEST sequences where the name is derived from P (proline), E (glutamic acid), S (serine) and T (threonine) serving as signals for protein degradation by the proteasome [79].

Htt interacts with a number of proteins that play a role in apoptosis, vesicle transport, endocytosis, morphogenesis, cell signalling and transcriptional regulation. The function of Htt is believed to act as a scaffolding protein i.e. a bridge bringing its interacting partners in close proximity to allow signals to be transferred [80]. Interestingly, Htt has been shown to elevate the production of brain-derived

neurotrophic factor (BDNF), a growth factor produced by striatal neurons [81].

Proteolysis in Huntington's disease (HD)

Htt protein is proteolytically cleaved at many sites generating several N-terminal fragments. Htt can be cleaved by several proteases including calpain, caspases, AEPs and matrix metalloproteases. Htt contains 2 calpain sites located at positions 469 and 536 aa [82, 83] (Figure 3). Htt can be cleaved by several caspases such as caspase 3 at positions 513 and 552 aa [84, 85], caspase 2 at 552 aa [86] and by caspase 6 at 586 aa [85]. The matrix metalloproteinase MMP-10 cleaves the Htt protein at 402 aa and is active in mice with HD [87]. The presence of the Htt fragments, where the cleavage sites have been mutated, promote lower toxicity of Htt indicating that proteolytic processing of Htt could contribute to HD pathogenesis [84, 88, 89]. Hence the proteolytic processing of Htt resulting in the generation of N-terminal fragments that contain polyglutamine tract could be a necessary stage in HD progression. This is due to N-terminal fragments which are more prone to aggregation and promote toxicity. These N-terminal fragments have been demonstrated to be present both in the mouse HD models and in the brains of HD human patients [83, 90, 91]. HD progression in HD mouse model have shown to be perturbed through the development of protease inhibitors or by mutating specific sequences important in Htt protease cleavage [85, 88]. However, the task to prevent all the proteolytic cleavages of Htt is difficult through the use of protease inhibitors and represents a therapeutic challenge for drug discovery. Future efforts should be targeted to identify the specific roles of proteases and the effects of N-terminal truncated fragments of Htt on HD progression and their implications in the pathological process.

Prion diseases

Prion diseases such as Creutzfeldt-Jakob disease in humans, scrapie in sheep and bovine spongiform encephalopathy in cattle, are all infectious and potentially fatal neurological conditions as a result of misfolding of cellular prion protein (PrP^{C}) converted into a structurally different β-sheet-rich pathogenic isoform (PrP^{Sc}) that is partially resistant to proteinase-K treatment and tend to aggregate [92]. The PrP^{C} has a bipartite structure, N-terminal domain is disordered whilst the C-terminal domain is more structured containing 3 α-helices and 2 β-strands [93]. The function of PrP^{C} is not clear but most studies indicate that PrP^{C} could provide neuroprotection and it is a neurotrophic protein [94]. Neuroprotective function of PrP^{C} has been demonstrated in many *in vitro* and *in vivo* studies [95, 96]. These studies showed that the expression of PrP^{C} was sufficient to slow down cell death and, in several other expression studies, PrP^{C} was shown to prevent cell death in stress conditions of serum deprivation of neurons [97]. Follow up studies have provided much evidence for PrP^{C} implication in cellular survival as well as regulation of cell growth, proliferation, differentiation and stem cell expansion [98, 99].

Proteolysis in Prion diseases

PrP^{C} associates with the plasma membrane through a glycosyl-phosphatidylinositol (GPI) anchor. Early on after translocation into the endoplasmic reticulum, the N-terminal signal peptide is removed by signal peptidase followed by cleavage of the C-terminal hydrophobic domain (residues 231-253) before the attachment of a GPI anchor to the C-terminus. PrP^{C} undergoes at least three proteolytic cleavage events

after trafficking through the secretory pathway to the plasma membrane [100]. At the plasma membrane or in the endosomes, PrP^{C} can be cleaved by α-cleavage at position 110-111 and 112 aa to yield two products: (i) PrP^{C1}, a product of 17kDa containing the GPI-anchor C-terminal fragment, and (ii) a 11kDa N-terminal fragment that is released from the cell into the extracellular space (Figure 4). α-cleavage was first shown for the chicken PrP [101] and then in later studies shown in samples from humans [102]. The protease responsible for the α-cleavage of PrP^{C} is still unknown but is currently known as the α-PrPase [103]. However, the ADAM (A Disintegrin And Metalloprotease) family could be involved in the α-cleavage of PrP. ADAM10 and ADAM17 (also known as TACE) are implicated [104] in the α-cleavage of PrP. Also, ADAM9 [105] and ADAM8 [106] found in muscle could also be involved in α-cleavage [107]. There still remains some controversy as to which protease is directly involved and how this process is regulated [107]. It is thought that the cleavage event occurs in the endosome [108] or the late endocytic pathway [109]. α-cleavage occurs in normal brains with approximately 50% of C1 present compared to full length PrP [102]. PrP^{C} can also undergo β-cleavage by a calpain protease at position 89-90 aa to produce PrP^{C2}, a 20kDa fragment containing the GPI-anchor and also a smaller 8kDa fragment known as PrPN2. β-cleavage of PrP^{C} is a pathological event occurring at the plasma membrane in prion diseases which is promoted during conditions of oxidative stress such as the presence of reactive oxygen species. Also, some of PrP^{C} is shed constitutively at the plasma membrane by the zinc metalloprotease called ADAM10 [100]. Most evidence indicates that α-cleavage of PrP has a neuroprotective function as opposed to contributing to prion diseases. In contrast, β-cleavage generates the PrP^{C2} fragment that is sufficient to support prion disease and appears to be abundant in the brains of prion-diseased individuals than in healthy ones [102], indicating that

β-cleavage could contribute to prion diseases. However, it is likely that both types of cleavage have a role in prion disease but α-cleavage prevents PrP from converting into PrPSc.

Concluding remarks

Human NDs such as AD, HD and prion diseases are characterized by neurodegeneration that leads to a progressive functional decline. More than 20 million people worldwide are suffering from ND each year. The number of individuals with NDs is increasing rapidly due to lack of effective therapies. Thus, novel efficient therapy for ND is foremost unmet health need. NDs such as AD, HD and prion diseases greatly differ in respect of their clinical manifestation, duration, onset, etc. Altogether, these human diseases share numerous common features such as altered protein cleavage, protein aggregation, oxidative stress, etc. The major pathological event that is crucial and shared by many NDs such as AD, HD and prion diseases is proteolytic cleavage of disease-modifying proteins. Emerging current evidence suggests that protein truncation not only acts as the driving force behind ND but also serves as a seed for regeneration of novel truncated forms that finally speed up the aggregation process leading to oligomeric assemblies of Htt and PrP, amyloid plaque, and neurofibrillary tangles formation. To this end, we need to concentrate our efforts towards understanding the endoproteolytic processes and how to perturb these proteases without affecting cellular homeostasis.

Figure legends

Table 1: List of endoproteases in ND

Table showing the substrates and their endoproteases associated with human neurodegeneration.

Figure 1: APP processing

(A) Schematic representation showing the domains of amyloid precursor protein (APP) and sites of endoproteolytic cleavage. (B) The amyloid precursor protein (APP) processing pathways. The APP protein transmembrane can be processed via two pathways: the non-amyloidogenic α-secretase pathway (no plaque formation) or the amyloidogenic β-secretase pathway (plaque formation). The non-amyloidogenic pathway results in the α-secretase cleaving β-amyloid (Aβ) in the centre to release a soluble fragment of APP called sAPP-α. Following this cleavage, the APP C-terminal fragment 83 (APP-CTF83) is cleaved further by the γ-secretase to release two fragments, the APP intracellular domain (AICD) and the P3 fragment. The amyloidogenic pathway results in the β-secretase cleaving APP to generate a soluble fragment called the sAPP-β. The CTFβ is then cleaved by the γ-secretase to generate Aβ40, Aβ42 and AICD.

Figure 2: Tau truncation

Schematic representation of functional domains of the longest tau isoform (441 amino acids). Tau protein can be divided into two domains: the N-terminal projection domain, including an acidic and a proline-rich region and a C-terminal microtubule binding domain (MBD). The MBD contains three or four repeats (R1-R4) with a flanking N-terminal proline-rich region extending into the projection domain that contains most of the Ser/Thr-Pro motifs. The endoproteolytic cleavage sites are indicated.

Figure 3: Htt cleavage sites

Schematic representation of the Htt protein. The full-length Htt protein consist of 3,144 amino acids, with a molecular weight of 350 kDa. The N-terminal region contains 17 amino acids (N17) comprising of many post-translational modifications including sites for phosphorylation and ubiquitination. Immediately after the N17 region are the polyglutamine (polyQ) and polyproline (polyP) regions. The region between 400 to 600 amino acids contains many endoproteolytic sites for proteases such as caspases 2, 3, 6, calpain, and matrix metalloproteinase 10 (MMP-10). Approximately, 16 HEAT repeats have been identified in Htt with unknown function. The C-terminal region consists of a leucine-rich nuclear export signal (NES), which could target the full-length Htt to the cytoplasm.

Figure 4: PrP cleavage sites

Schematic representation of the prion protein. The N-terminus is structurally disordered containing a signal peptide and an octameric repeat region. The C-terminus is structurally ordered consisting of three α-helixes, two β-strands, and a disulfide bond (-S-S-). A glycosylphosphatidylinositol (GPI) anchor that anchors the prion protein at the cell surface is found at the C-terminus. The endoproteolytic cleavage sites are indicated.

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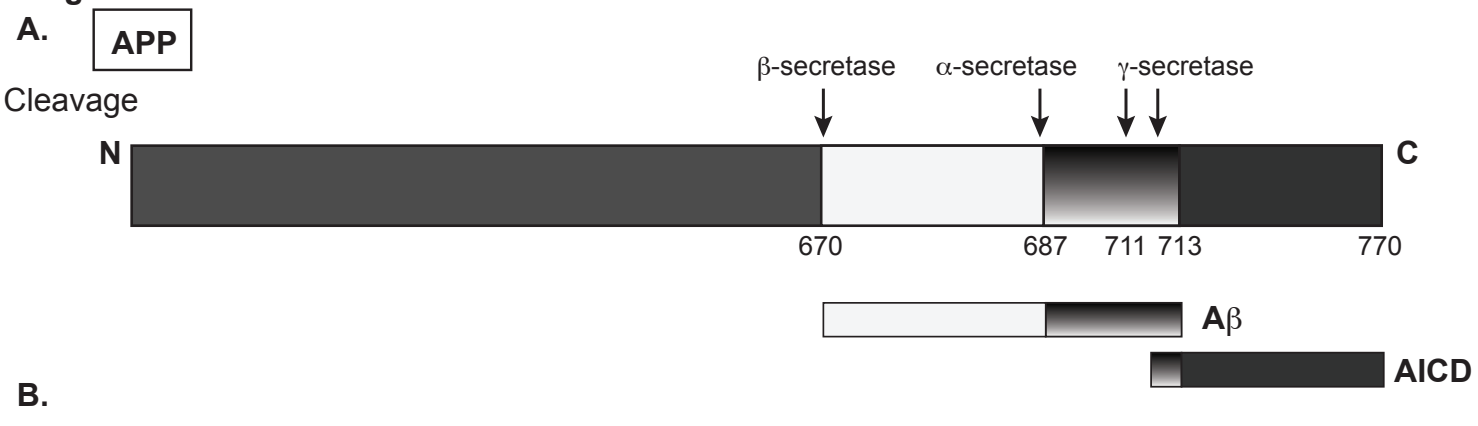
Figure 1


A.
APP
Cleavage
β-secretase
α-secretase
γ-secretase
N
C
670
687
711
713
770
Aβ
AICD

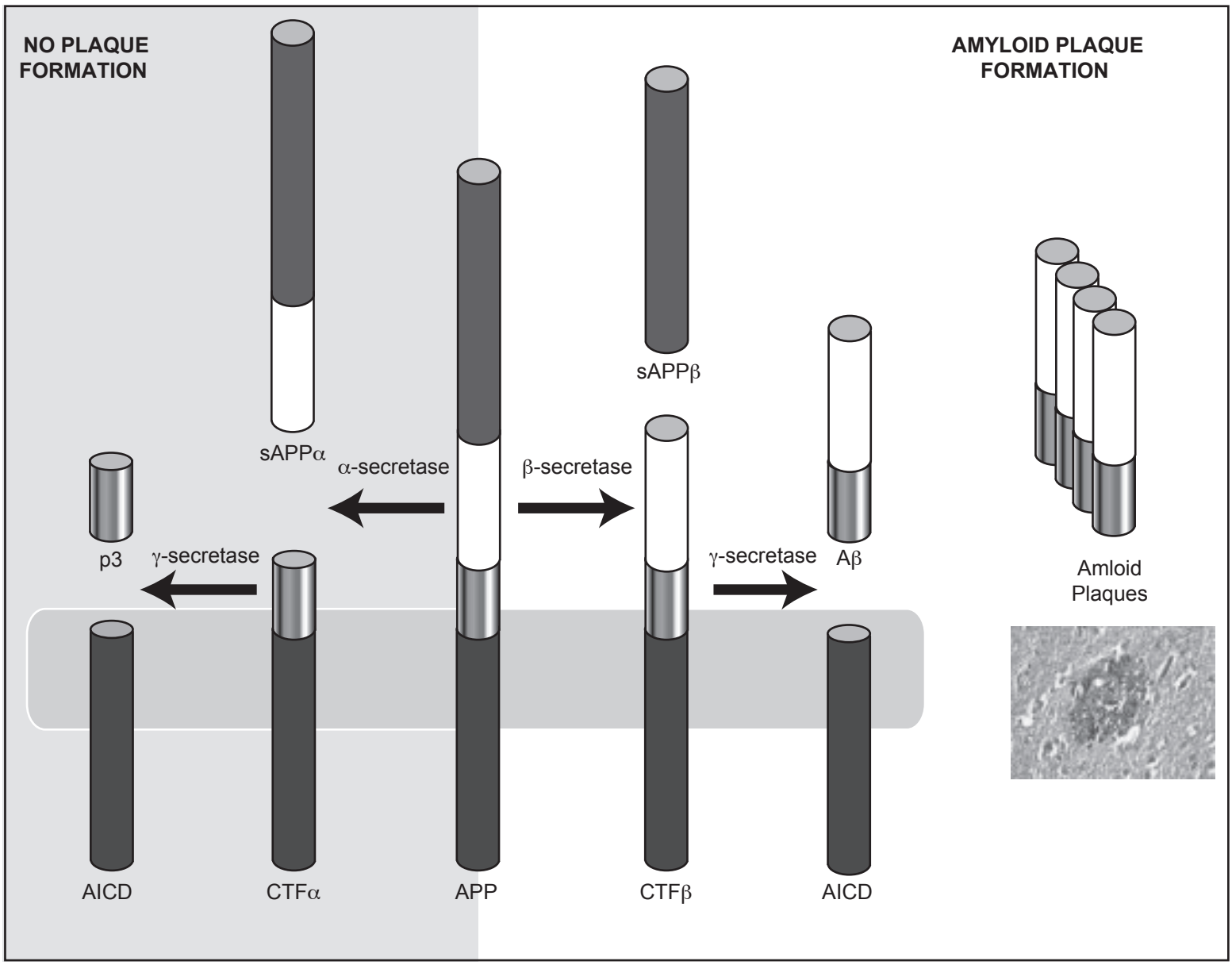

B.
NO PLAQUE FORMATION
AMYLOID PLAQUE FORMATION
sAPPα
α-secretase
β-secretase
sAPPβ
p3
γ-secretase
γ-secretase
Aβ
Amloid Plaques
AICD
CTFα
APP
CTFβ
AICD

Figure 2

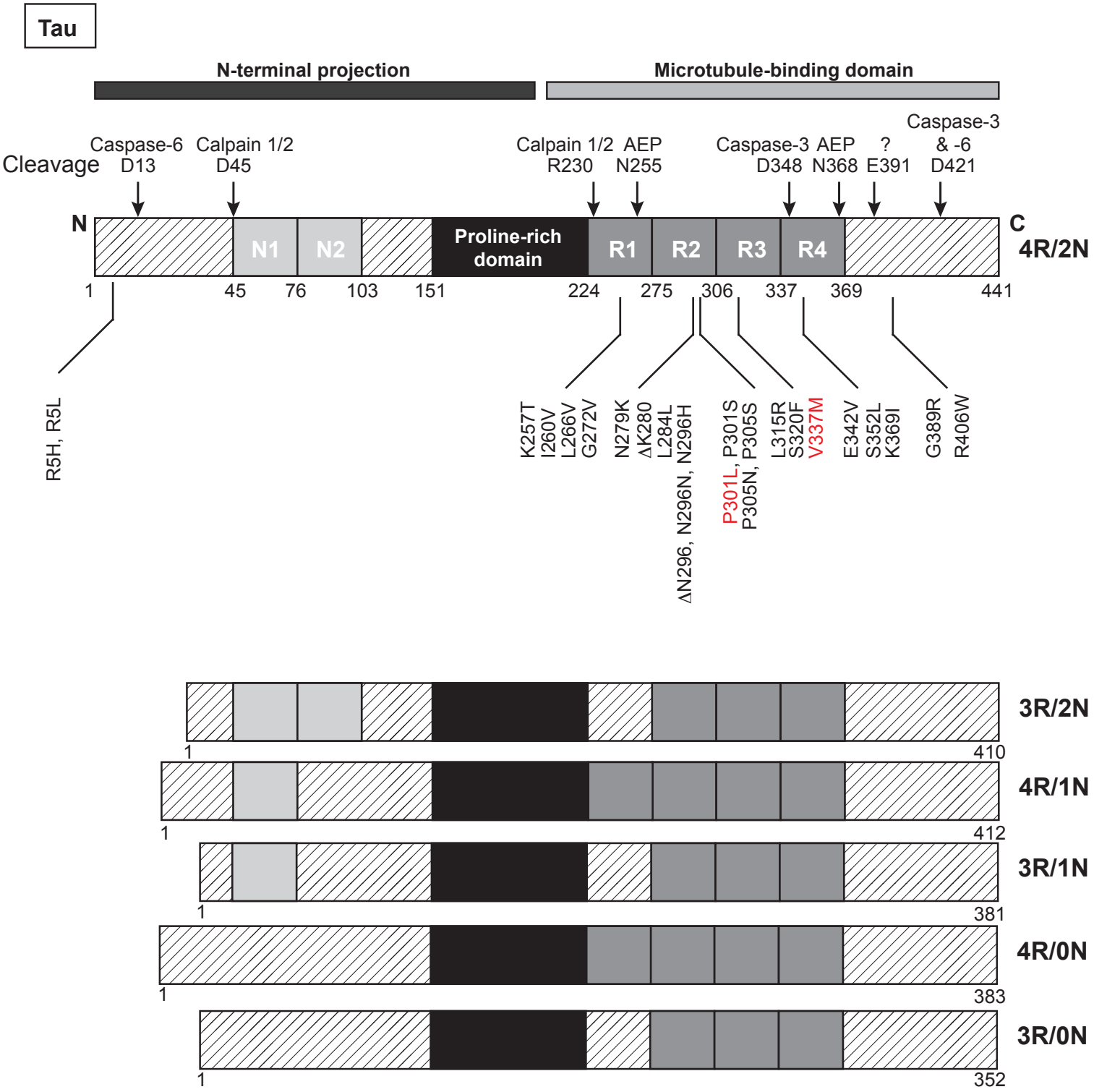

Tau
N-terminal projection
Microtubule-binding domain
Cleavage
Caspase-6 D13
Calpain 1/2 D45
Calpain 1/2 R230
AEP N255
Caspase-3 D348
AEP N368
? E391
Caspase-3 & -6 D421
N
C
N1
N2
Proline-rich domain
R1
R2
R3
R4
4R/2N
1
45
76
103
151
224
275
306
337
369
441
R5H, R5L
K257T
I260V
L266V
G272V
N279K
ΔK280
L284L
ΔN296, N296N, N296H
P301L, P301S
P305N, P305S
L315R
S320F
V337M
E342V
S352L
K369I
G389R
R406W
3R/2N
1
410
4R/1N
1
412
3R/1N
1
381
4R/0N
1
383
3R/0N
1
352

Figure 3

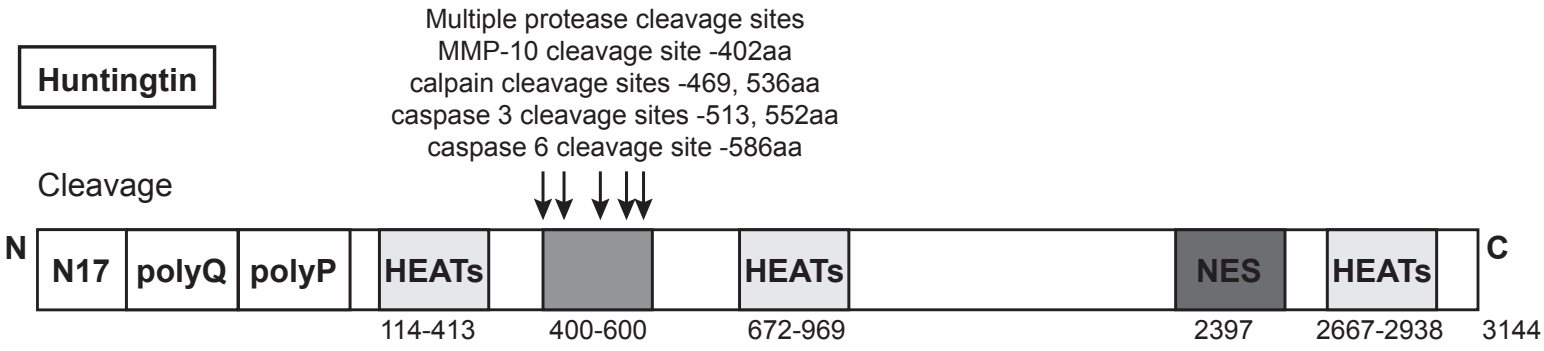

Huntingtin
Multiple protease cleavage sites
MMP-10 cleavage site -402aa
calpain cleavage sites -469, 536aa
caspase 3 cleavage sites -513, 552aa
caspase 6 cleavage site -586aa
Cleavage
N
N17
polyQ
polyP
HEATs
HEATs
NES
HEATs
C
114-413
400-600
672-969
2397
2667-2938
3144

Figure 4

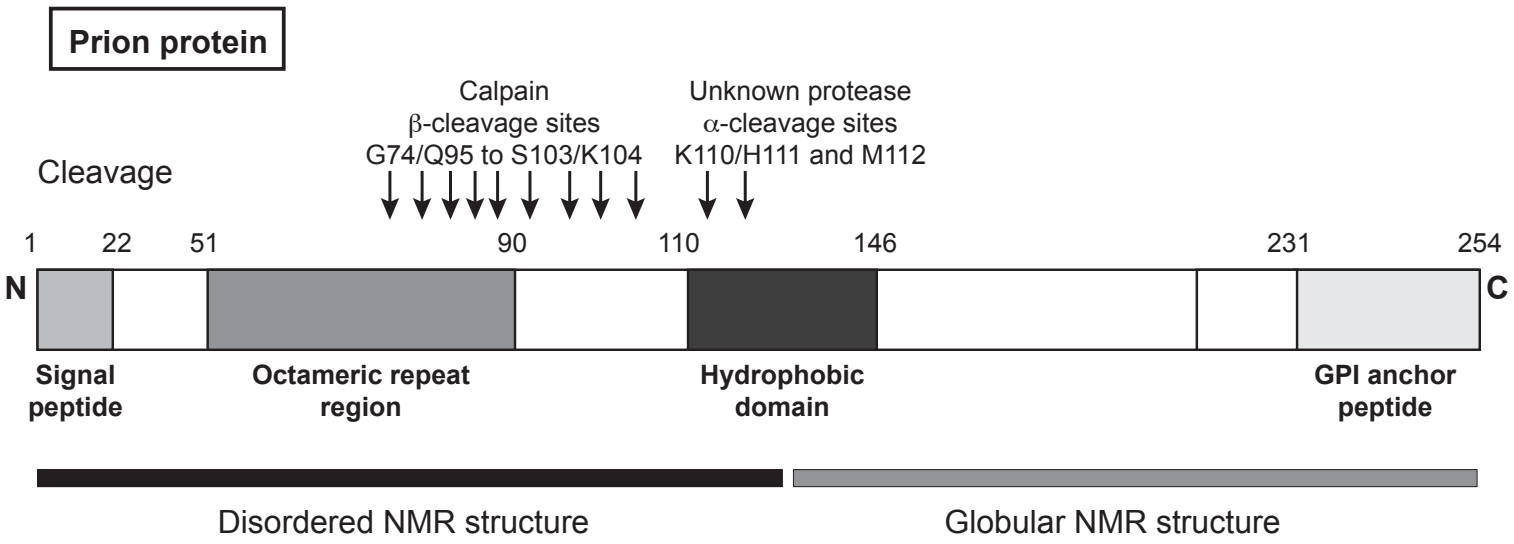

Prion protein
Calpain
β-cleavage sites
G74/Q95 to S103/K104
Unknown protease
α-cleavage sites
K110/H111 and M112
Cleavage
1
22
51
90
110
146
231
254
N
C
Signal peptide
Octameric repeat region
Hydrophobic domain
GPI anchor peptide
Disordered NMR structure
Globular NMR structure

Table 1

Substrate	Protease	Type of protease	Associated human disease	Refs
APP	β-secretase (BACE1)	Aspartic-acid protease	Alzheimer's Disease	2
APP	γ-secretase (PS1, APH-1, nicastrin and PEN2)	Intramembrane protease	Alzheimer's Disease	2, 4, 15, 16
Tau	Caspases-3 & -6	cysteine-aspartic acid protease	Alzheimer's Disease	55-59
Tau	Calpains 1 and 2	cysteine protease	Alzheimer's Disease	59, 63, 64
Tau	AEP	cysteine protease	Alzheimer's Disease	65-68
Htt	Calpain	cysteine protease	Huntington's disease	79, 80, 86
Htt	Caspase -2, -3 & -6	cysteine-aspartic proteases	Huntington's disease	81-83, 85
Htt	MMP-10		Huntington's disease	84
Prion	α-secretase	disintegrin and metalloprotease domain protease	Prion disease?	98, 99-106
Prion	β-secretase	Aspartic-acid protease	Prion disease?	97