

**Review:**

**"The role of endoproteolytic processing in neurodegeneration"**

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**Abstract:**

Endoproteolysis is a normal post-translational process in the eukaryotic cell and has 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, the amplification of cell signals etc. Not surprisingly, disruption or alternation of endoproteolytic cleavage maybe the root cause of many human diseases such as neurodegeneration including 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 by genetic mutations of the associated-proteases or in the target substrate. Endoproteolytic cleavage resulting in protein truncation has significantly consequences 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. A process accelerated through mainly the action of proteases. The physiological function of proteolysis can provide nutrients for the organism or be a means to control the activity of a protein after protein synthesis. This process is important for cellular homeostasis allowing the regulation of cellular processes as well as removing the build up of unwanted protein products that would 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. For this review, we will 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 clinically defined by the loss of neurons 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 the irreversible loss of neurons. The effect has a profound consequence on the affected individual with the loss of intellectual abilities especially the memory and the means to reason hindering the person's normal life activities and cognitive function.

### **APP processing**

Amyloid- $\beta$  protein ( $A\beta$ ) is mainly found in senile plaques.  $A\beta$  is generated from its precursor called amyloid precursor protein (APP) via sequential cleavage by the  $\beta$ - and  $\gamma$ -secretases [2] (Figure 1). Notably, genetic mutations observed in patients of

mainly the inherited form of familial AD resulted in either an increase of production and/or A $\beta$  aggregation, indicating that A $\beta$  is a seed of AD pathogenesis. Specifically, the C-terminal region of A $\beta$  shows diversity and the longer A $\beta$  peptide is principally deposited in AD patient brains [3]. Therefore, the  $\gamma$ -secretase has become a major molecular target in drug discovery of AD. Nevertheless, the putative APP  $\gamma$ -cleavage sites relate to amino acid residues located to the transmembrane domain (TMD), and thus it has taken more than a decade of research to advance our current understanding of the  $\gamma$ -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 were likely to change  $\gamma$ -secretase cleavage of APP resulting in elevated production of aggregation-prone A $\beta$ -form (A $\beta$ 42) as compared with the other main A $\beta$  species, A $\beta$ 40 [6, 7]. Secondly, the ablation of presenilin eliminated cleavage by the  $\gamma$ -secretase cleavage of APP [8, 9] and the intra-membrane aspartates found in PS TMD-6 and TMD-7 are necessary for A $\beta$  production [10]. A number of cross-linking approaches showed that  $\gamma$ -secretase inhibitors (GSIs) directly implicated and targeted the PS proteins [11, 12]. Lastly, the perturbed function of the clathrin assembly lymphoid myeloid leukaemia gene protein (CALM) that is encoded by the phosphatidylinositol binding clathrin assembly protein (PICALM), altered the lysosomal localisation of the  $\gamma$ -secretase and in turn the production of A $\beta$ 42 [13, 14]. These studies demonstrate that PS possesses the catalytic activity resulting in  $\gamma$ -secretase intramembrane cleavage of APP and thus affects 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 nature of the membrane active site amino acid residues. The aspartate IMPs are located to 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 for 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, expression of PS alone is not sufficient to reconstitute the  $\gamma$ -secretase activity. A number of genetic and biochemical studies followed that 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  $\gamma$ -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  $\gamma$ -secretase activity.[22, 23]. The activity of the  $\gamma$ -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  $\gamma$ -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  $\gamma$ -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. Evidence is growing to indicate that the soluble form of tau does 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 to the 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 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 alternative splicing of exon 10 yielding to tau isoforms containing 3 (3R) or 4 (4R) imperfect MT-binding repeats in the C-terminal domain, which are both found in the tangles of tauopathy patient brains [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 a role in signal transduction pathways through its interaction with proteins such as Phosphoinositide phospholipase C- $\gamma$  (PLC- $\gamma$ ) 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 directly is 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 providing a role for 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  $\alpha$ - and  $\beta$ -tubulin proteins. The nature of MT is highly dynamic but they present with 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 of MTs [39] and hence their stability 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, 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 that implicate and corroborate that tau enhances the stabilization and assembly MTs on one hand, and on the other, tau mutations alter the association of tau with MTs. 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], at the same time 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]), 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 [55]. In fact, tau cleavage site at D421 has already been observed in AD patient brains [55-57]. Furthermore, truncation of tau at D421 site accelerates tau aggregation compared to tau full length form [55] and this could act as a seed to initiating the process of aggregation of wild-type tau, as a mechanism prior to tangle formation [58, 59]. Tau cleavage by caspase does not impede hyperphosphorylation since both tau phosphorylation and truncation modifications are found in the brain of AD patients [55]. Furthermore, the D421-cleaved tau form can be phosphorylated by GSK3 $\beta$  [58].

In addition to caspase cleavage, tau can be cleaved by other types of proteases including cathepsins, calpains, and thrombin [59-62]. Pre-aggregated A $\beta$  treatment results in tau truncation by calpain 1 generating a tau fragment 45 – 230 aa [59] that is commonly found in many tauopathies [63]. This fragment (45-230aa) of tau is particularly neurotoxic whilst other fragments generated by the calpain 2 protease is neuroprotective [59, 64] through their ability to form aggregates when arachidonic acid is present and thus perturbs aggregation of tau full length [63]. 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 to the lysosomes, which cleaves its substrates within the C-terminal region of the asparagine [65, 66]. The AEP protease is auto-activated and involves the removal of both C- and N-terminal pro-peptides at varying pH levels [67]. Tau protein undergoes cleavage by AEP at positions N255 and N368 [68]. 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 [68]. These tau AEP-cleaved fragments are readily hyperphosphorylated [68]. 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 [69]. HD is triggered by the expansion of the CAG repeat on chromosome 4 that encodes glutamine in the N-terminal of huntingtin (Htt) [70]. 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 [69]. Htt protein is widely expressed in the CNS and in other non-neuronal tissues [71] and dispersed throughout the intracellular compartments [72-74]. 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  $\alpha$ -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 [75, 76]. 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).

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 [77]. Interestingly, Htt has been shown to elevate the production of brain-derived neurotrophic factor (BDNF), a growth factor produced by striatal neurons [78].

### **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 [79, 80] (Figure 3). Htt can be cleaved by several caspases such as caspase 3 at positions 513 and 552 aa [81, 82], caspase 2 at 552 aa [83] and by caspase 6 at 586 aa [82]. The matrix metalloproteinase, MMP-10 cleaves the Htt protein at 402 aa and is active in mice with HD [84]. 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 [81, 85, 86]. 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 the N-terminal fragments are more prone to aggregation and promote toxicity.

These N-terminal fragments have been demonstrated to be present on both mouse HD models and in the brains of HD patients [80, 87, 88]. 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 [82, 85]. 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 the proteases and the effect of the N-terminal truncated fragments of Htt in HD progression and the 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 ( $\text{PrP}^{\text{C}}$ ) converted into a structurally different  $\beta$ -sheet-rich pathogenic isoform ( $\text{PrP}^{\text{Sc}}$ ) that's partially resistant to proteinase-K treatment and tend to aggregate [89]. The  $\text{PrP}^{\text{C}}$  has a bipartite structure, N-terminal domain is disordered whilst the C-terminal domain is more structured containing 3  $\alpha$ -helices and 2  $\beta$ -strands [90]. The function of  $\text{PrP}^{\text{C}}$  is not clear but most studies indicate that  $\text{PrP}^{\text{C}}$  could provide neuroprotection and it's a neurotrophic protein [91].  $\text{PrP}^{\text{C}}$  neuroprotective function has been demonstrated in many *in vitro* and *in vivo* studies [92, 93]. These studies showed that the expression of  $\text{PrP}^{\text{C}}$  was sufficient to slow down cell death and in other expression studies,  $\text{PrP}^{\text{C}}$  could prevent cell death in stress conditions of serum deprivation of neurons [94]. Follow-on studies provided much evidence for  $\text{PrP}^{\text{C}}$  implication in cellular survival as

well as regulation of cell growth, proliferation, differentiation and stem cell expansion [95, 96].

### **Proteolysis in Prion diseases**

PrP<sup>C</sup> 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 the signal peptidase followed by a C-terminal peptide before the attachment of a GPI anchor to the C-terminus. PrP<sup>C</sup> undergoes at least three proteolytic cleavage events after trafficking through the secretory pathway to the plasma membrane [97]. At the plasma membrane or in the endosomes, PrP<sup>C</sup> can be cleaved by an  $\alpha$ -cleavage at position 110-111 and 112 aa to yield two products PrP<sup>C1</sup>, a product of 17kDa containing the GPI-anchor C-terminal fragment, and a 11kDa N-terminal fragment that is released from the cell into the extracellular space (Figure 4).  $\alpha$ -cleavage was first shown for the chicken PrP [98] and then in later studies shown in samples from humans [99]. The protease responsible for the  $\alpha$ -cleavage of PrP<sup>C</sup> is still unknown but is currently known as the  $\alpha$ -PrPase [100]. However, the ADAM family could be involved in  $\alpha$ -cleavage of PrP. ADAM10 and ADAM17 (also known as TACE) are implicated [101]. Also, ADAM9 [102] and ADAM8 [103] found in muscle could also be implicated in  $\alpha$ -cleavage [104]. There still remains some controversy of which protease is directly involved and how this process is regulated [104]. It is thought that the cleavage event occurs in the endosome [105] or the late endocytic pathway [106].  $\alpha$ -cleavage occurs in normal brains with approximately 50% of C1 present compared to full length PrP [99]. PrP<sup>C</sup> can also undergo  $\beta$ -cleavage by a calpain protease at position 89-90 aa to produce

PrP<sup>C2</sup>, a 20kDa fragment containing the GPI-anchor, and also a smaller 8kDa fragment known as PrPN2.  $\beta$ -cleavage of PrP<sup>C</sup> 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<sup>C</sup> is shed constitutively at the plasma membrane by the zinc metalloprotease called ADAM10 [97]. Most evidence indicates that  $\alpha$ -cleavage of PrP has a neuroprotective function as opposed to contributing to prion disease. Whilst  $\beta$ -cleavage by contrast to generating the C2 fragment is sufficient to support prion disease and appears to be abundant in the brains of prion-diseased individuals than in healthy ones [99], indicating that  $\beta$ -cleavage could contribute to prion disease. However, it is likely that both types of cleavage have a role in prion disease but  $\alpha$ -cleavage prevents PrP from converting into PrP<sup>Sc</sup>.

## **Summary**

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. This number of individuals with NDs is increasing rapidly, due to no effective therapy that is available. 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. Current evidence emerging shows that protein truncation is not only the driving force behind ND, but

also serves as a seed for regeneration of novel truncated forms that finally speeds up the aggregation process leading to oligomeric assemblies of Htt and PrP, amyloid plaque, neurofibrillary tangle 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 to show the substrates and their endoproteases associated to human neurodegeneration.

### **Figure 1 APP processing**

(A) The 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  $\alpha$ -secretase pathway (no plaque formation) or the amyloidogenic  $\beta$ -secretase pathway (plaque formation). The non-amyloidogenic pathway results in the  $\alpha$ -secretase cleaving  $\beta$ -amyloid ( $A\beta$ ) in the centre to release a soluble fragment of APP called sAPP- $\alpha$ . Following this cleavage the APP C-terminal fragment 83 (APP-CTF83) is cleaved further by the  $\gamma$ -secretase to release two fragments, the APP intracellular domain (AICD) and the P3 fragment. The amyloidogenic pathway results in the  $\beta$ -secretase cleaving APP to generate a soluble fragment called the sAPP- $\beta$ . The CTF $\beta$  is then cleaved by the  $\gamma$ -secretase to generate  $A\beta_{40}$ ,  $A\beta_{42}$  and AICD.

### **Figure 2 Tau truncation**

Schematic representation of the 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 3144 amino acids, with a molecular weight of 350kDa. The N-terminal region contains 17 amino acid (N17) contains 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 contains a signal peptide and an octameric repeat region. The C-terminus is structurally ordered consisting of three  $\alpha$ -helixes and two  $\beta$ -strands, a disulphide 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.



## References:

1. Holtzman, D.M., et al., *Mapping the road forward in Alzheimer's disease*. Sci Transl Med, 2011. **3**(114): p. 114ps48.
2. De Strooper, B., R. Vassar, and T. Golde, *The secretases: enzymes with therapeutic potential in Alzheimer disease*. Nat Rev Neurol, 2010. **6**(2): p. 99-107.
3. Iwatsubo, T., et al., *Visualization of A beta 42(43) and A beta 40 in senile plaques with end-specific A beta monoclonals: evidence that an initially deposited species is A beta 42(43)*. Neuron, 1994. **13**(1): p. 45-53.
4. Tomita, T., *Molecular mechanism of intramembrane proteolysis by gamma-secretase*. J Biochem, 2014. **156**(4): p. 195-201.
5. Sherrington, R., et al., *Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease*. Nature, 1995. **375**(6534): p. 754-60.
6. Borchelt, D.R., et al., *Familial Alzheimer's disease-linked presenilin 1 variants elevate Abeta1-42/1-40 ratio in vitro and in vivo*. Neuron, 1996. **17**(5): p. 1005-13.
7. Tomita, T., et al., *The presenilin 2 mutation (N141I) linked to familial Alzheimer disease (Volga German families) increases the secretion of amyloid beta protein ending at the 42nd (or 43rd) residue*. Proc Natl Acad Sci U S A, 1997. **94**(5): p. 2025-30.
8. Wong, P.C., et al., *Presenilin 1 is required for Notch1 and DIII expression in the paraxial mesoderm*. Nature, 1997. **387**(6630): p. 288-92.
9. Shen, J., et al., *Skeletal and CNS defects in Presenilin-1-deficient mice*. Cell, 1997. **89**(4): p. 629-39.
10. Wolfe, M.S., et al., *Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and gamma-secretase activity*. Nature, 1999. **398**(6727): p. 513-7.
11. Esler, W.P., et al., *Transition-state analogue inhibitors of gamma-secretase bind directly to presenilin-1*. Nat Cell Biol, 2000. **2**(7): p. 428-34.
12. Li, Y.M., et al., *Photoactivated gamma-secretase inhibitors directed to the active site covalently label presenilin 1*. Nature, 2000. **405**(6787): p. 689-94.
13. Harold, D., et al., *Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease*. Nat Genet, 2009. **41**(10): p. 1088-93.
14. Kanatsu, K., et al., *Decreased CALM expression reduces Abeta42 to total Abeta ratio through clathrin-mediated endocytosis of gamma-secretase*. Nat Commun, 2014. **5**: p. 3386.
15. Lichtenthaler, S.F., C. Haass, and H. Steiner, *Regulated intramembrane proteolysis--lessons from amyloid precursor protein processing*. J Neurochem, 2011. **117**(5): p. 779-96.
16. Wolfe, M.S., *Processive proteolysis by gamma-secretase and the mechanism of Alzheimer's disease*. Biol Chem, 2012. **393**(9): p. 899-905.
17. Haass, C. and H. Steiner, *Alzheimer disease gamma-secretase: a complex story of GxGD-type presenilin proteases*. Trends Cell Biol, 2002. **12**(12): p. 556-62.
18. Arutyunova, E., et al., *Allosteric regulation of rhomboid intramembrane proteolysis*. EMBO J, 2014. **33**(17): p. 1869-81.

19. Yu, G., et al., *Nicastrin modulates presenilin-mediated notch/glp-1 signal transduction and betaAPP processing*. Nature, 2000. **407**(6800): p. 48-54.
20. Francis, R., et al., *aph-1 and pen-2 are required for Notch pathway signaling, gamma-secretase cleavage of betaAPP, and presenilin protein accumulation*. Dev Cell, 2002. **3**(1): p. 85-97.
21. Sato, T., et al., *Active gamma-secretase complexes contain only one of each component*. J Biol Chem, 2007. **282**(47): p. 33985-93.
22. Edbauer, D., et al., *Reconstitution of gamma-secretase activity*. Nat Cell Biol, 2003. **5**(5): p. 486-8.
23. Takasugi, N., et al., *The role of presenilin cofactors in the gamma-secretase complex*. Nature, 2003. **422**(6930): p. 438-41.
24. Thinakaran, G., et al., *Endoproteolysis of presenilin 1 and accumulation of processed derivatives in vivo*. Neuron, 1996. **17**(1): p. 181-90.
25. Ahn, K., et al., *Activation and intrinsic gamma-secretase activity of presenilin 1*. Proc Natl Acad Sci U S A, 2010. **107**(50): p. 21435-40.
26. Wilson, C.M., et al., *DC2 and keratinocyte-associated protein 2 (KCP2), subunits of the oligosaccharyltransferase complex, are regulators of the gamma-secretase-directed processing of amyloid precursor protein (APP)*. J Biol Chem, 2011. **286**(36): p. 31080-91.
27. Brunden, K.R., J.Q. Trojanowski, and V.M. Lee, *Evidence that non-fibrillar tau causes pathology linked to neurodegeneration and behavioral impairments*. J Alzheimers Dis, 2008. **14**(4): p. 393-9.
28. Sydow, A., et al., *Tau-induced defects in synaptic plasticity, learning, and memory are reversible in transgenic mice after switching off the toxic Tau mutant*. J Neurosci, 2011. **31**(7): p. 2511-25.
29. Neve, R.L., et al., *Identification of cDNA clones for the human microtubule-associated protein tau and chromosomal localization of the genes for tau and microtubule-associated protein 2*. Brain Res, 1986. **387**(3): p. 271-80.
30. Majounie, E., et al., *Variation in tau isoform expression in different brain regions and disease states*. Neurobiol Aging, 2013. **34**(7): p. 1922 e7-1922 e12.
31. Andreadis, A., *Tau gene alternative splicing: expression patterns, regulation and modulation of function in normal brain and neurodegenerative diseases*. Biochim Biophys Acta, 2005. **1739**(2-3): p. 91-103.
32. Andreadis, A., W.M. Brown, and K.S. Kosik, *Structure and novel exons of the human tau gene*. Biochemistry, 1992. **31**(43): p. 10626-33.
33. Barghorn, S. and E. Mandelkow, *Toward a unified scheme for the aggregation of tau into Alzheimer paired helical filaments*. Biochemistry, 2002. **41**(50): p. 14885-96.
34. Goedert, M., et al., *Tau proteins of Alzheimer paired helical filaments: abnormal phosphorylation of all six brain isoforms*. Neuron, 1992. **8**(1): p. 159-68.
35. Goedert, M., et al., *Multiple isoforms of human microtubule-associated protein tau: sequences and localization in neurofibrillary tangles of Alzheimer's disease*. Neuron, 1989. **3**(4): p. 519-26.
36. Mietelska-Porowska, A., et al., *Tau protein modifications and interactions: their role in function and dysfunction*. Int J Mol Sci, 2014. **15**(3): p. 4671-713.
37. Yoshida, H., R.A. Crowther, and M. Goedert, *Functional effects of tau gene mutations deltaN296 and N296H*. J Neurochem, 2002. **80**(3): p. 548-51.

38. Mitchison, T. and M. Kirschner, *Dynamic instability of microtubule growth*. Nature, 1984. **312**(5991): p. 237-42.
39. Weingarten, M.D., et al., *A protein factor essential for microtubule assembly*. Proc Natl Acad Sci U S A, 1975. **72**(5): p. 1858-62.
40. Cleveland, D.W., S.Y. Hwo, and M.W. Kirschner, *Purification of tau, a microtubule-associated protein that induces assembly of microtubules from purified tubulin*. J Mol Biol, 1977. **116**(2): p. 207-25.
41. Drubin, D.G. and M.W. Kirschner, *Tau protein function in living cells*. J Cell Biol, 1986. **103**(6 Pt 2): p. 2739-46.
42. Goode, B.L., et al., *Functional interactions between the proline-rich and repeat regions of tau enhance microtubule binding and assembly*. Mol Biol Cell, 1997. **8**(2): p. 353-65.
43. Breuzard, G., et al., *Molecular mechanisms of Tau binding to microtubules and its role in microtubule dynamics in live cells*. J Cell Sci, 2013. **126**(Pt 13): p. 2810-9.
44. Bunker, J.M., et al., *Modulation of microtubule dynamics by tau in living cells: implications for development and neurodegeneration*. Mol Biol Cell, 2004. **15**(6): p. 2720-8.
45. Choi, M.C., et al., *Human microtubule-associated-protein tau regulates the number of protofilaments in microtubules: a synchrotron x-ray scattering study*. Biophys J, 2009. **97**(2): p. 519-27.
46. Panda, D., et al., *Differential regulation of microtubule dynamics by three- and four-repeat tau: implications for the onset of neurodegenerative disease*. Proc Natl Acad Sci U S A, 2003. **100**(16): p. 9548-53.
47. Barghorn, S., et al., *Structure, microtubule interactions, and paired helical filament aggregation by tau mutants of frontotemporal dementias*. Biochemistry, 2000. **39**(38): p. 11714-21.
48. Fischer, D., et al., *Structural and microtubule binding properties of tau mutants of frontotemporal dementias*. Biochemistry, 2007. **46**(10): p. 2574-82.
49. Hasegawa, M., M.J. Smith, and M. Goedert, *Tau proteins with FTDP-17 mutations have a reduced ability to promote microtubule assembly*. FEBS Lett, 1998. **437**(3): p. 207-10.
50. Neumann, M., et al., *Novel G335V mutation in the tau gene associated with early onset familial frontotemporal dementia*. Neurogenetics, 2005. **6**(2): p. 91-5.
51. van Herpen, E., et al., *Variable phenotypic expression and extensive tau pathology in two families with the novel tau mutation L315R*. Ann Neurol, 2003. **54**(5): p. 573-81.
52. Han, D. and H.K. Paudel, *FTDP-17 missense mutations site-specifically inhibit as well as promote dephosphorylation of microtubule-associated protein tau by protein phosphatases of HEK-293 cell extract*. Neurochem Int, 2009. **54**(1): p. 14-27.
53. Han, D., et al., *Familial FTDP-17 missense mutations inhibit microtubule assembly-promoting activity of tau by increasing phosphorylation at Ser202 in vitro*. J Biol Chem, 2009. **284**(20): p. 13422-33.
54. Martin, L., et al., *Tau protein kinases: involvement in Alzheimer's disease*. Ageing Res Rev, 2013. **12**(1): p. 289-309.
55. Rissman, R.A., et al., *Caspase-cleavage of tau is an early event in Alzheimer disease tangle pathology*. J Clin Invest, 2004. **114**(1): p. 121-30.

56. Basurto-Islas, G., et al., *Accumulation of aspartic acid421- and glutamic acid391-cleaved tau in neurofibrillary tangles correlates with progression in Alzheimer disease*. J Neuropathol Exp Neurol, 2008. **67**(5): p. 470-83.
57. Ugolini, G., A. Cattaneo, and M. Novak, *Co-localization of truncated tau and DNA fragmentation in Alzheimer's disease neurones*. Neuroreport, 1997. **8**(17): p. 3709-12.
58. Gamblin, T.C., et al., *Caspase cleavage of tau: linking amyloid and neurofibrillary tangles in Alzheimer's disease*. Proc Natl Acad Sci U S A, 2003. **100**(17): p. 10032-7.
59. Park, S.Y. and A. Ferreira, *The generation of a 17 kDa neurotoxic fragment: an alternative mechanism by which tau mediates beta-amyloid-induced neurodegeneration*. J Neurosci, 2005. **25**(22): p. 5365-75.
60. Arai, T., J.P. Guo, and P.L. McGeer, *Proteolysis of non-phosphorylated and phosphorylated tau by thrombin*. J Biol Chem, 2005. **280**(7): p. 5145-53.
61. Kenessey, A., et al., *Degradation of tau by lysosomal enzyme cathepsin D: implication for Alzheimer neurofibrillary degeneration*. J Neurochem, 1997. **69**(5): p. 2026-38.
62. Yang, L.S., W. Gordon-Krajcer, and H. Ksiezak-Reding, *Tau released from paired helical filaments with formic acid or guanidine is susceptible to calpain-mediated proteolysis*. J Neurochem, 1997. **69**(4): p. 1548-58.
63. Ferreira, A. and E.H. Bigio, *Calpain-mediated tau cleavage: a mechanism leading to neurodegeneration shared by multiple tauopathies*. Mol Med, 2011. **17**(7-8): p. 676-85.
64. Garg, S., et al., *Cleavage of Tau by calpain in Alzheimer's disease: the quest for the toxic 17 kD fragment*. Neurobiol Aging, 2011. **32**(1): p. 1-14.
65. Chen, J.M., et al., *Cloning and expression of mouse legumain, a lysosomal endopeptidase*. Biochem J, 1998. **335** ( Pt 1): p. 111-7.
66. Chen, J.M., et al., *Identification of the active site of legumain links it to caspases, clostripain and gingipains in a new clan of cysteine endopeptidases*. FEBS Lett, 1998. **441**(3): p. 361-5.
67. Li, D.N., et al., *Multistep autoactivation of asparaginyl endopeptidase in vitro and in vivo*. J Biol Chem, 2003. **278**(40): p. 38980-90.
68. Zhang, Z., et al., *Cleavage of tau by asparagine endopeptidase mediates the neurofibrillary pathology in Alzheimer's disease*. Nat Med, 2014. **20**(11): p. 1254-62.
69. Novak, M.J. and S.J. Tabrizi, *Huntington's disease*. BMJ, 2010. **340**: p. c3109.
70. Ross, C.A., et al., *Huntington disease: natural history, biomarkers and prospects for therapeutics*. Nat Rev Neurol, 2014. **10**(4): p. 204-16.
71. Strong, T.V., et al., *Widespread expression of the human and rat Huntington's disease gene in brain and nonneural tissues*. Nat Genet, 1993. **5**(3): p. 259-65.
72. De Rooij, K.E., et al., *Subcellular localization of the Huntington's disease gene product in cell lines by immunofluorescence and biochemical subcellular fractionation*. Hum Mol Genet, 1996. **5**(8): p. 1093-9.
73. DiFiglia, M., et al., *Huntingtin is a cytoplasmic protein associated with vesicles in human and rat brain neurons*. Neuron, 1995. **14**(5): p. 1075-81.
74. Saudou, F., et al., *Polyglutamine expansions and neurodegenerative diseases*. Cold Spring Harb Symp Quant Biol, 1996. **61**: p. 639-47.
75. Andrade, M.A. and P. Bork, *HEAT repeats in the Huntington's disease protein*. Nat Genet, 1995. **11**(2): p. 115-6.

76. Tartari, M., et al., *Phylogenetic comparison of huntingtin homologues reveals the appearance of a primitive polyQ in sea urchin*. Mol Biol Evol, 2008. **25**(2): p. 330-8.
77. Harjes, P. and E.E. Wanker, *The hunt for huntingtin function: interaction partners tell many different stories*. Trends Biochem Sci, 2003. **28**(8): p. 425-33.
78. Zuccato, C., et al., *Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease*. Science, 2001. **293**(5529): p. 493-8.
79. Gafni, J. and L.M. Ellerby, *Calpain activation in Huntington's disease*. J Neurosci, 2002. **22**(12): p. 4842-9.
80. Kim, Y.J., et al., *Caspase 3-cleaved N-terminal fragments of wild-type and mutant huntingtin are present in normal and Huntington's disease brains, associate with membranes, and undergo calpain-dependent proteolysis*. Proc Natl Acad Sci U S A, 2001. **98**(22): p. 12784-9.
81. Wellington, C.L., et al., *Caspase cleavage of gene products associated with triplet expansion disorders generates truncated fragments containing the polyglutamine tract*. J Biol Chem, 1998. **273**(15): p. 9158-67.
82. Wellington, C.L., et al., *Inhibiting caspase cleavage of huntingtin reduces toxicity and aggregate formation in neuronal and nonneuronal cells*. J Biol Chem, 2000. **275**(26): p. 19831-8.
83. Hermel, E., et al., *Specific caspase interactions and amplification are involved in selective neuronal vulnerability in Huntington's disease*. Cell Death Differ, 2004. **11**(4): p. 424-38.
84. Miller, J.P., et al., *Matrix metalloproteinases are modifiers of huntingtin proteolysis and toxicity in Huntington's disease*. Neuron, 2010. **67**(2): p. 199-212.
85. Graham, R.K., et al., *Cleavage at the caspase-6 site is required for neuronal dysfunction and degeneration due to mutant huntingtin*. Cell, 2006. **125**(6): p. 1179-91.
86. Gafni, J., et al., *Inhibition of calpain cleavage of huntingtin reduces toxicity: accumulation of calpain/caspase fragments in the nucleus*. J Biol Chem, 2004. **279**(19): p. 20211-20.
87. Landles, C., et al., *Proteolysis of mutant huntingtin produces an exon 1 fragment that accumulates as an aggregated protein in neuronal nuclei in Huntington disease*. J Biol Chem, 2010. **285**(12): p. 8808-23.
88. Wellington, C.L., et al., *Caspase cleavage of mutant huntingtin precedes neurodegeneration in Huntington's disease*. J Neurosci, 2002. **22**(18): p. 7862-72.
89. Geissen, M., et al., *Understanding the natural variability of prion diseases*. Vaccine, 2007. **25**(30): p. 5631-6.
90. Wuthrich, K. and R. Riek, *Three-dimensional structures of prion proteins*. Adv Protein Chem, 2001. **57**: p. 55-82.
91. Linden, R., et al., *Physiology of the prion protein*. Physiol Rev, 2008. **88**(2): p. 673-728.
92. Lo, R.Y., et al., *New molecular insights into cellular survival and stress responses: neuroprotective role of cellular prion protein (PrPC)*. Mol Neurobiol, 2007. **35**(3): p. 236-44.
93. Roucou, X. and A.C. LeBlanc, *Cellular prion protein neuroprotective function: implications in prion diseases*. J Mol Med (Berl), 2005. **83**(1): p. 3-11.

94. Kuwahara, C., et al., *Prions prevent neuronal cell-line death*. Nature, 1999. **400**(6741): p. 225-6.
95. Martins, V.R., et al., *Prion protein: orchestrating neurotrophic activities*. Curr Issues Mol Biol, 2010. **12**(2): p. 63-86.
96. Miranda, A., et al., *The role of prion protein in stem cell regulation*. Reproduction, 2013. **146**(3): p. R91-9.
97. Altmepfen, H.C., et al., *Proteolytic processing of the prion protein in health and disease*. Am J Neurodegener Dis, 2012. **1**(1): p. 15-31.
98. Harris, D.A., et al., *Processing of a cellular prion protein: identification of N- and C-terminal cleavage sites*. Biochemistry, 1993. **32**(4): p. 1009-16.
99. Chen, S.G., et al., *Truncated forms of the human prion protein in normal brain and in prion diseases*. J Biol Chem, 1995. **270**(32): p. 19173-80.
100. Oliveira-Martins, J.B., et al., *Unexpected tolerance of alpha-cleavage of the prion protein to sequence variations*. PLoS One, 2010. **5**(2): p. e9107.
101. Vincent, B., et al., *The disintegrins ADAM10 and TACE contribute to the constitutive and phorbol ester-regulated normal cleavage of the cellular prion protein*. J Biol Chem, 2001. **276**(41): p. 37743-6.
102. Cisse, M.A., et al., *The disintegrin ADAM9 indirectly contributes to the physiological processing of cellular prion by modulating ADAM10 activity*. J Biol Chem, 2005. **280**(49): p. 40624-31.
103. Liang, J., et al., *Cellular prion protein regulates its own alpha-cleavage through ADAM8 in skeletal muscle*. J Biol Chem, 2012. **287**(20): p. 16510-20.
104. Liang, J. and Q. Kong, *alpha-Cleavage of cellular prion protein*. Prion, 2012. **6**(5): p. 453-60.
105. Shyng, S.L., M.T. Huber, and D.A. Harris, *A prion protein cycles between the cell surface and an endocytic compartment in cultured neuroblastoma cells*. J Biol Chem, 1993. **268**(21): p. 15922-8.
106. Walmsley, A.R., et al., *alpha-cleavage of the prion protein occurs in a late compartment of the secretory pathway and is independent of lipid rafts*. Mol Cell Neurosci, 2009. **40**(2): p. 242-8.

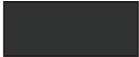
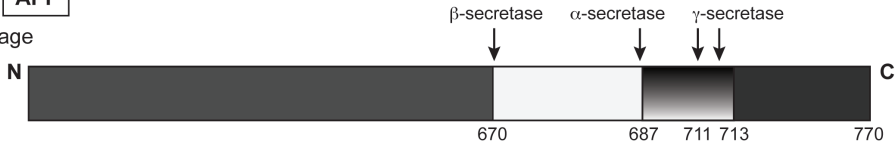


Figure 1

A. **APP**

Cleavage



B.

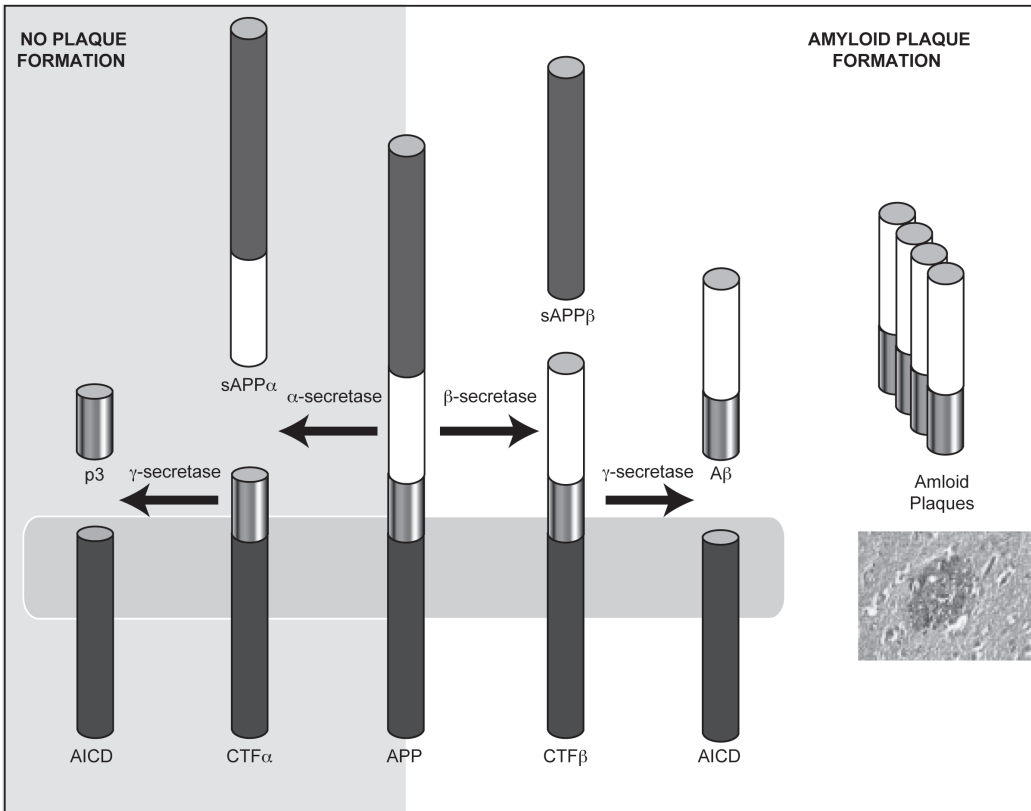


Figure 2

Tau

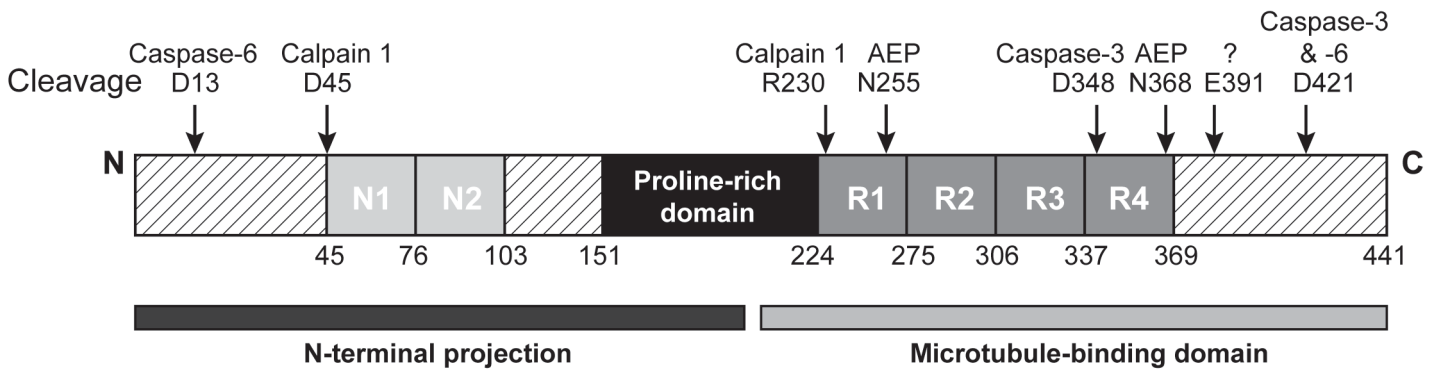
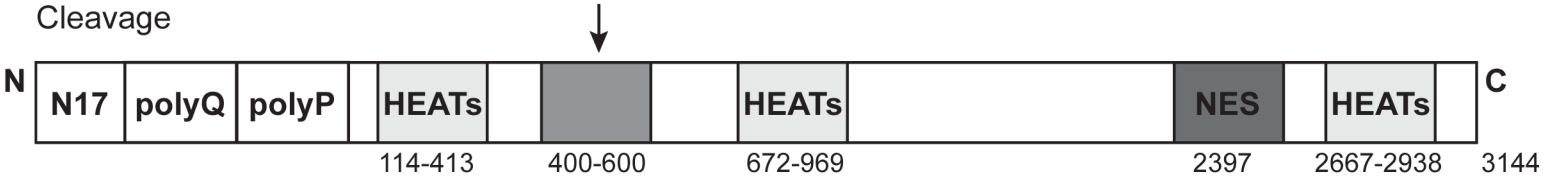




Figure 3



**Figure 4**

Cleavage

