

the basics of Alzheimer's disease (AD)

- an adult-onset neurodegenerative dementia characterized by the intracellular and/or extracellular accumulation of proteins which assemble into β -sheet fibrils
- patients present with a constellation of symptoms that reflect dysfunction and degeneration of neural cells in the cerebral cortex and hippocampus (neural specificity)
 - > progressive defects in memory and higher cognitive functions such as complex learned motor tasks and reasoning
- estimated 3-4 million people in US with probable AD
- most common progressive neurodegenerative disorder (Parkinson's is second)

amyloid β -peptide ($A\beta$) and Alzheimer's

- $A\beta$ is a sticky peptide that constitutes the predominant molecule in brain plaques characteristic of Alzheimer's disease
 - peptide is generated by processing of APP, β -amyloid precursor protein
- normal functions of the APP protein*
- kinesin receptor involved in the axonal transport of vesicles
 - involved in neuronal growth and neurite outgrowth
 - scaffold for several protein networks and signaling pathways
- mouse knockout of APP does not lead to any overt phenotypes (gain-of-function)

missense mutations in several proteins are associated with early-onset forms of familial Alzheimer disease

- β APP (β -amyloid precursor protein)
- PS1 and PS2 (presenilins)

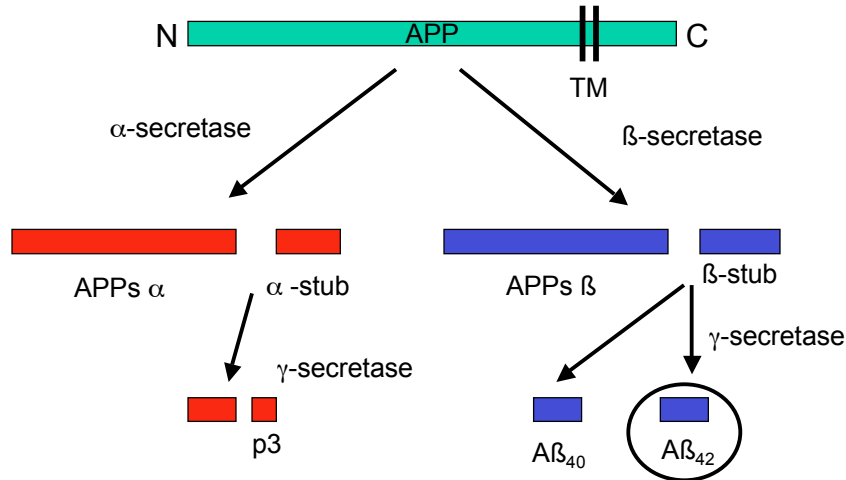
* mutations in these genes result in altered processing of β APP and the relative overproduction of either all forms of the β -amyloid peptide or specific overproduction of the A β 42 peptide

Alzheimer's and Downs syndrome

further support for genetic basis of Alzheimer's disease came from observation that all patients with trisomy 21 (Down's syndrome) develop the disease

- APP localizes to chromosome 21; this suggested that its overproduction (due to higher gene dose) is involved in pathology of AD

processing of β -amyloid precursor protein by secretases



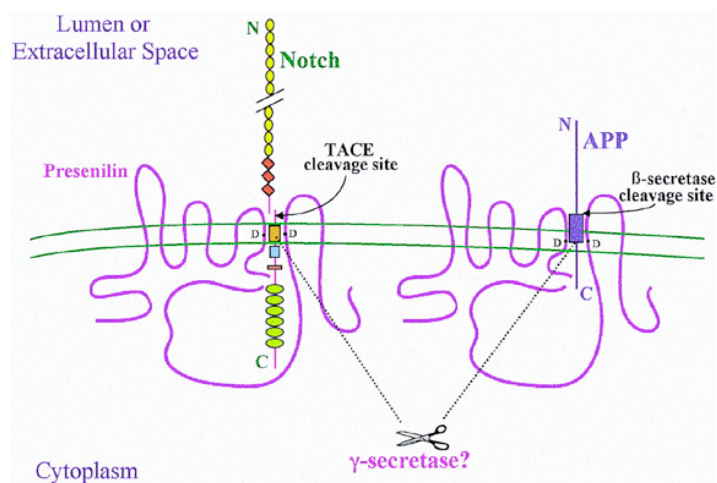
cleavage by α -secretase prevents $A\beta$ formation due to that fact that it cuts between two residues within the C-terminal portion of APP that form the $A\beta$ fragment

Presenilins (PS1 and PS2)

- mutations in PS1 and PS2 represent the most common cause of early-onset, autosomal dominant AD
- PS1 expression is required for proper mammalian embryogenesis and survival but mutant human PS1 can convey these developmental functions
- PS1 and PS2 mutations linked to familial AD seem to lead to a gain of toxic function: the dysregulation of gamma-secretase in a way that selectively enhances the proteolysis of β APP to $A\beta_{42}$
- deletion of PS1 in mice dramatically reduces gamma-secretase activity and knockout of PS1 and PS2 results in complete loss of activity
- some studies suggest that presenilins have proteolytic activity as well

- presenilins are large membrane-spanning proteins required for the gamma-secretase cleavage of type I transmembrane proteins such as Notch and β -amyloid precursor protein
- proteolysis is unique in that it occurs intramembrane (termed RIP; regulated intramembrane proteolysis)
- presenilin-dependent intramembrane proteolysis of proteins can produce intracellular molecules that are transcriptionally active (new signaling paradigm)
- exists in a high molecular-weight complex with gamma-secretase, Aph-1, Pen-2, and nicastrin

Presenilin-dependent cleavage of Notch and APP

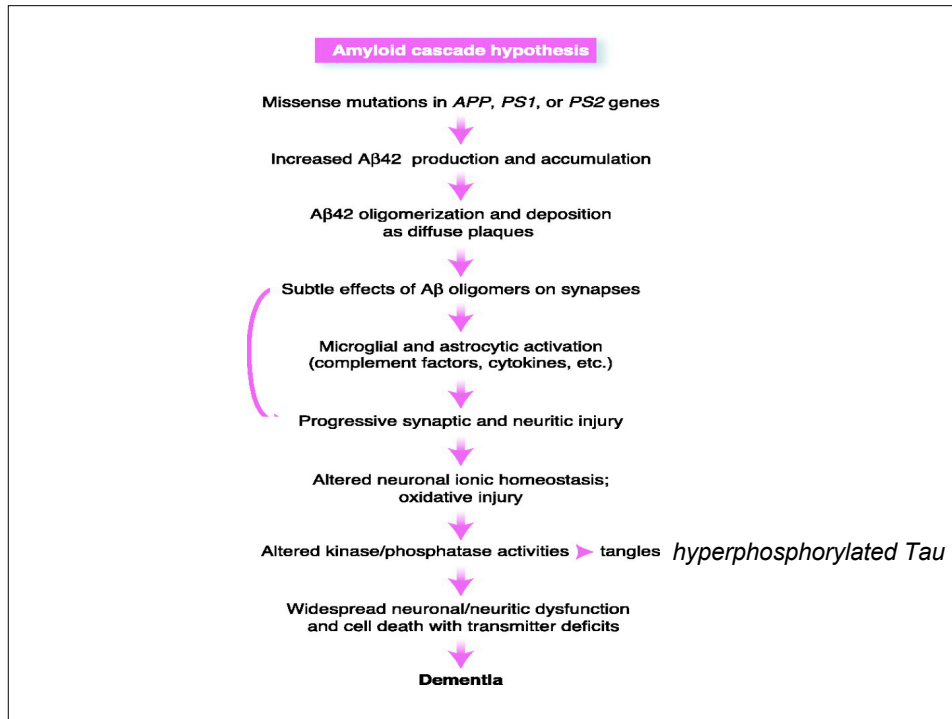


ϵ 4 allele of apolipoprotein (ApoE) is a major genetic risk factor for late-onset Alzheimer's disease

- ApoE binds to A β and may influence its aggregation
- ApoE4 does not contain cysteine residues and, therefore, cannot undergo intramolecular or intermolecular disulfide crosslinking
- ApoE-deficient, APP-transgenic mice have much lower amyloid formation
- subjects with two ϵ 4 alleles have a significantly higher # and density of A β deposits in the brain
- mechanism unclear but ApoE4 may be less effective at retarding A β aggregation into fibrils than ApoE2 or 3
- ApoE4 may also permit Tau protein to dissociate from microtubules and participate in fibril formation

Tau protein and Alzheimer's

- mutations in Tau protein lead to another neurodegenerative disorder called FTD (frontotemporal dementia)
- Tau is deposited in structures referred to as NFT (neurofibrillary tangles) in FTD as well as AD
- NFTs of wild-type Tau protein are seen in AD and thought to be deposited after changes in A β metabolism and initial plaque formation
- Tau is often hyperphosphorylated in NFTs and this may contribute to its ability to form aggregates



Balancing Aβ production and breakdown

- Aβ accumulation is normally counterbalanced by its elimination via multiple mechanisms:

- 1) proteolytic degradation
- 2) cell-mediated clearance
- 3) active and passive transport out of the brain
- 4) deposition into non-toxic insoluble aggregates

- current evidence suggests that proteolytic degradation is a critical regulator of cerebral Aβ levels and AD pathogenesis

- defects in enzymes that are responsible for the breakdown of Aβ (not production) may represent the majority of the non-familial cases of AD

A β -degrading proteases

TABLE 1
Subcellular localizations of selected A β -degrading proteases
ER, endoplasmic reticulum.

Protease	Class	Location					
		Extracellular	ER/Golgi	Lysosomes	Cytosol	Mitochondria	Peroxisomes
NEP	Metallo	+	+				
ECE-1	Metallo	+	+				
ECE-2	Metallo	+	+				
MMP-2	Metallo	+	+				
MMP-9	Metallo	+	+				
IDE	Metallo	+			+	+	+
PreP	Metallo					+	
Plasmin	Serine	+					
CatB	Cysteine	+		+			

- these proteases may have different activity towards different oligomeric or aggregated forms of A β
- in addition, changes in the expression of their endogenous inhibitors may also influence the progression of AD

Mouse knockouts of A β -degrading proteases in APP transgenic mice is widely-regarded as the best method for assessing the role of a given protease in A β degradation and AD pathogenesis *in vivo*

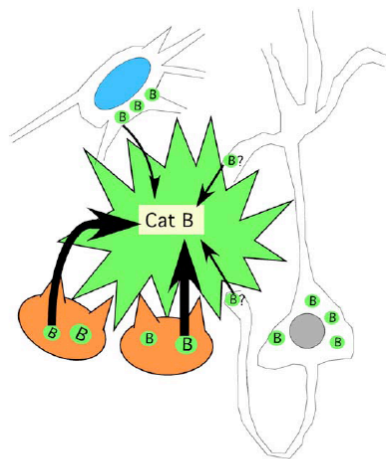


Figure 1. Schematic Diagram Showing the Role of Cathepsin B in Amyloid Clearance

Mueller-Stein et al. demonstrate that cathepsin B (CatB) can clear fibrillar amyloid- β . CatB can be secreted (arrows) into plaques (green star) and promote the degradation of extracellular amyloid- β . A major potential source of CatB in plaques may be microglia (orange), which secrete avidly *in vitro*, but other cell types (astrocytes, blue top left) and neurons (gray, right) may also contribute CatB to plaques. The green circles with "B" represent endosomes or lysosomes containing CatB, where it may degrade intracellular A β . It is unclear whether CatB secreted from neurons is primarily dendritic or nerve terminal in origin.

as with other diseases, the neurotoxic form of A β may not be the monomer or the large insoluble amyloid fibril but rather a small, diffusible oligomeric assembly

large aggregates may represent an inactive reservoir of these small putatively neurotoxic assemblies

Broad therapeutic strategies for Alzheimer's disease

1) active immunization with A β peptide takes advantage of the immune system to generate antibodies that can decrease A β -related pathology in mouse models of AD (likely to prevent oligomerization)

- passive immunization involves direct administration of anti-A β antibodies, thereby bypassing the need for an active immune response

2) certain NSAIDs (non-steroidal anti-inflammatory drugs) have been shown to decrease A β production possibly by binding directly to the gamma-secretase complex

3) partial inhibition of either β -secretase or γ -secretase that generate A β from APP (full inhibition might interfere with signaling by Notch proteins)

4) modulation of cholesterol homeostasis: chronic use of cholesterol-lowering drugs such as statins associated with a lower incidence of AD; conversely, high-cholesterol diets have been shown to increase A β pathology (occurs through direct effects on APP processing)

5) chelation of copper and zinc ions that are required for A β aggregation can prevent A β deposition in APP transgenic mice

6) prevention of synaptotoxic and neurodegenerative effects triggered by A β accumulation (damage control)

many of these approaches have candidates in clinical trials but only drugs clinically validated for AD treatment simply treat the symptoms such as behavioral disturbances

Huntington's disease

- autosomal, dominantly inherited neurodegenerative disorder
- characterized clinically by abnormal involuntary movements (chorea), intellectual impairment, changes in memory and mood
- as with other neurodegenerative disorders, selective neuronal loss is observed
- disease gene, huntingtin, encodes a cytoplasmic protein of unknown function (thought to play a role in axonal protein transport)
- strikes at about age 40

Huntington's disease is a polyglutamine (polyQ) disease

- expansion of a polymorphic trinucleotide tract (the sequence CAG that codes for glutamine) to a length that exceeds 40 repeat units in exon 1 of the huntingtin (Htt) gene correlates with onset and progression of disease

- wild-type, non-pathogenic allele of Htt contains fewer than 35 repeats

 - 35-39 repeats: may or may not develop disease

 - 40-60 repeats: develop HD as adults

 - > 60 repeats: onset before age of 20

HD shows genetic anticipation; onset of disease gets earlier with successive generations

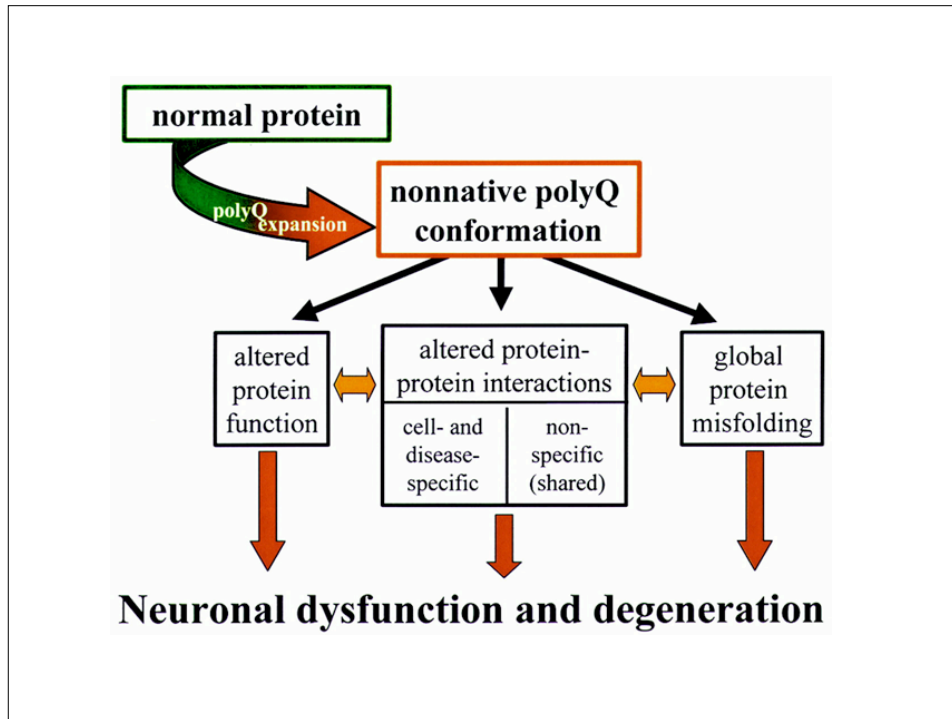
mutant huntingtin represents a gain-of-toxic function not a loss of function

- complete loss of Htt in Htt-deficient knockout mice leads to early embryonic death

- Htt containing expanded polyQ tract leads to protein aggregation and neuronal death

- mutant Htt adopts a different conformation (distinct monoclonal antibodies can bind only mutant Htt) that leads to partial unfolding or abnormal folding

- forms a β -sheet rich fibrillar structure via a nucleation-dependent process



whereas normal Htt is localized to the cytoplasm, mutant Htt is also localized to the nucleus, especially an N-terminal fragment of Htt

this nuclear localization is thought to be important in the development of neuronal toxicity although the mechanism is not completely known

- the Q-expanded proteins or fragments thereof form nuclear inclusions that also contain ubiquitin, proteasomes and chaperones
- within the nucleus, mutant Htt might itself act as a transcription factor or act to sequester resident transcription factors such as TATA-binding protein (TBP) or CREB-binding protein (CBP)

altered transcription could provide the trigger for cell dysfunction and eventual death

neuronal toxicity in HD may also be due to formation of cytoplasmic inclusions or aggresomes that can inhibit the proteasomes and induce apoptosis

role of proteolysis

- most rapidly progressing mouse models of HD express transgenes that contain only N-terminal segments of mutant Htt suggesting that the molecule may only be pathogenic following cleavage by a protease
- caspases have been shown to be responsible for cleavage of Htt and may represent excellent targets for therapy
- upregulation of caspases to cleave mutant Htt, however, may further induce apoptosis

two general mechanisms of HD pathogenesis...

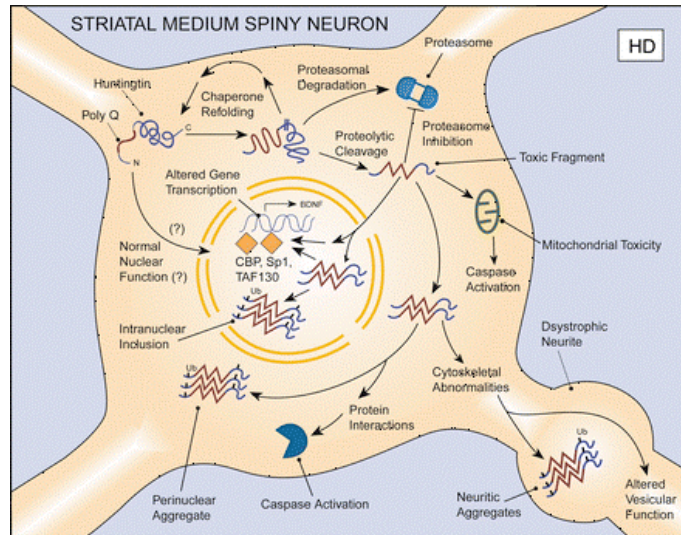
- 1) aggregation of mutant Htt or fragments of mutant Htt block normal proteasome function and sequester other proteins
- 2) proteolytic fragments of mutant Htt are cytotoxic themselves

paradox: are aggregates harmful or protective?

therapeutic targets...

- agents that block aggregation, the conformational change, proteolytic activation, nuclear import, and interaction with its cellular partners of mutant Htt,

Model for HD cellular pathogenesis



Why doesn't mutant Htt affect all cells?

- Htt is expressed in nearly all cells but affects only a subset of neurons, mostly the medium spiny striatal neurons (possibly only these cells express a protein partner crucial for pathology)
- although many interacting proteins have been identified, none stand out as specifically neuronal
- interestingly, neurons in the cortex contain much more inclusions than the striatum suggesting that Htt aggregates are not a good indicator of which cells are destined to die in HD

Neurodegenerative diseases

- abnormal protein deposits are often found in these disorders and the pathogenic process seems to evade immune surveillance

disease	CNS protein deposits	mutant gene in familial disease
Prion	PrP ^{Sc} , PrP amyloid plaques	PrP
Alzheimer's	A β , A β amyloid plaques, Tau, NFTs	APP, PS1, PS2, ApoE*
Parkinson	alpha-synuclein, Lewy bodies	α -synuclein, Parkin
Frontotemporal dementia (FTD)	Tau fibrils	Tau
Huntington	HD, nuclear HD aggregates	Huntingtin protein

* ApoE modulates the age of onset of both familial and sporadic Alzheimer's