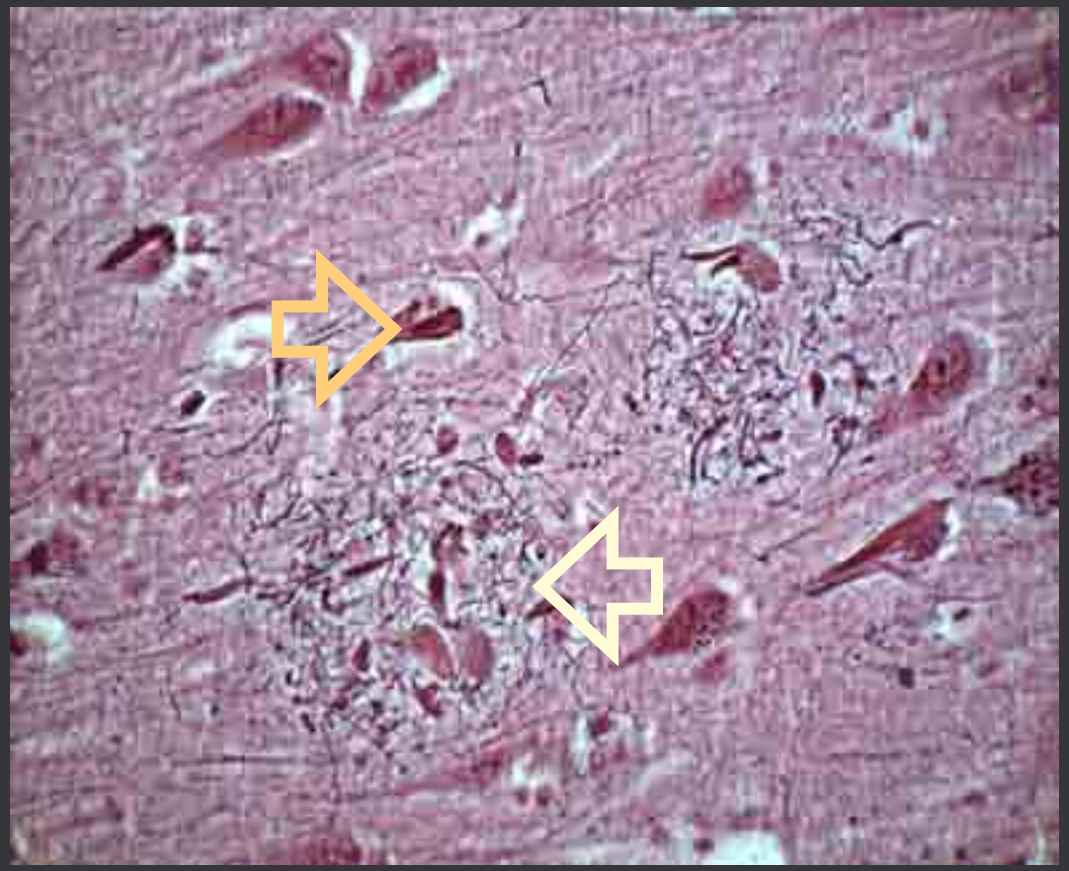
Therapeutic strategies exploiting amyloid-β generation mechanisms

Jean-Noël OCTAVE

Prague, March 10, 2009

Alzheimer disease : characteristic lesions

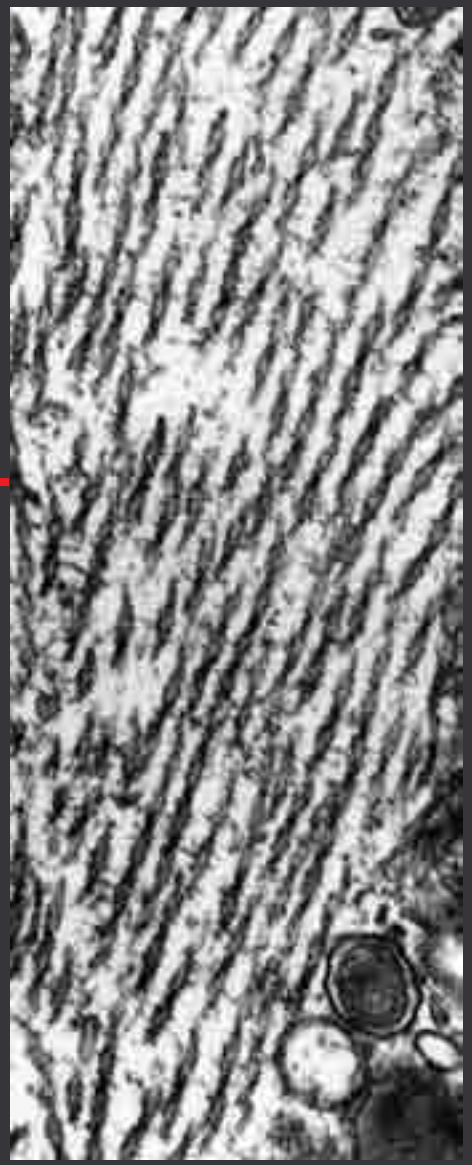
Neurofibrillary tangles



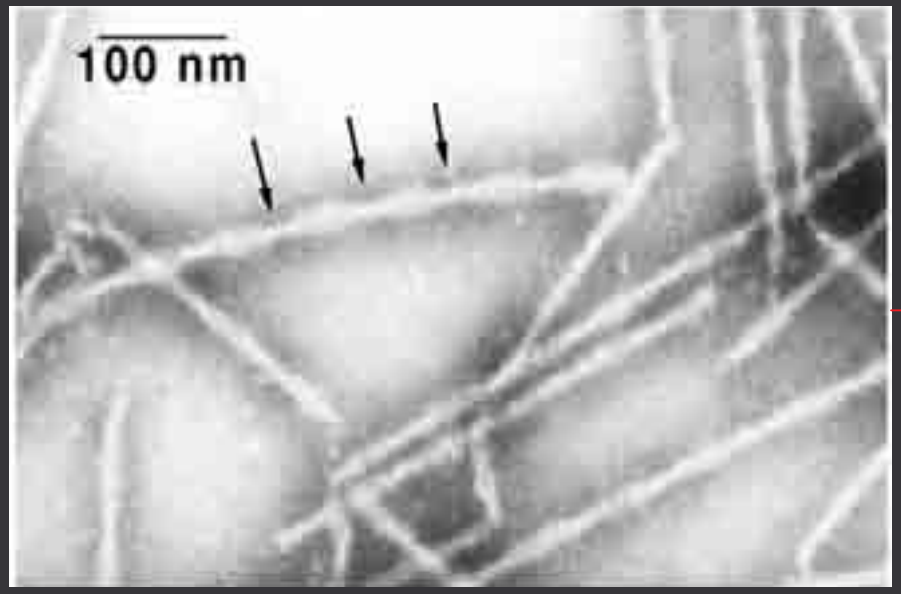
Senile plaques

Neurofibrillary tangles

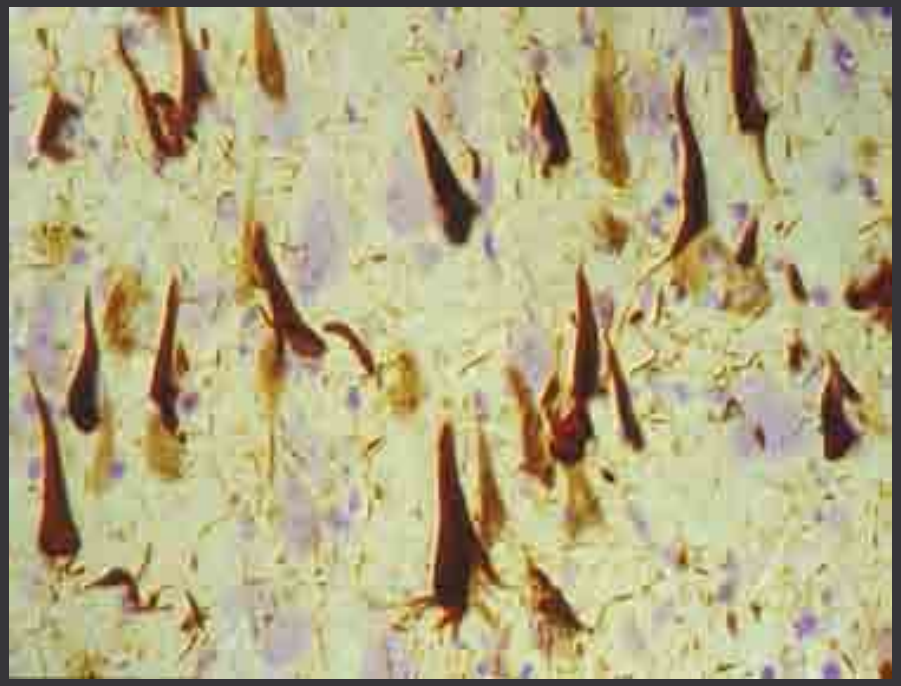
Paired Helical Filaments (PHF)



Composition of paired helical filaments



Protein tau



Labeling of neurofibrillary tangles by anti-tau antibodies

Tau: a Microtubule Associated Protein (MAP)

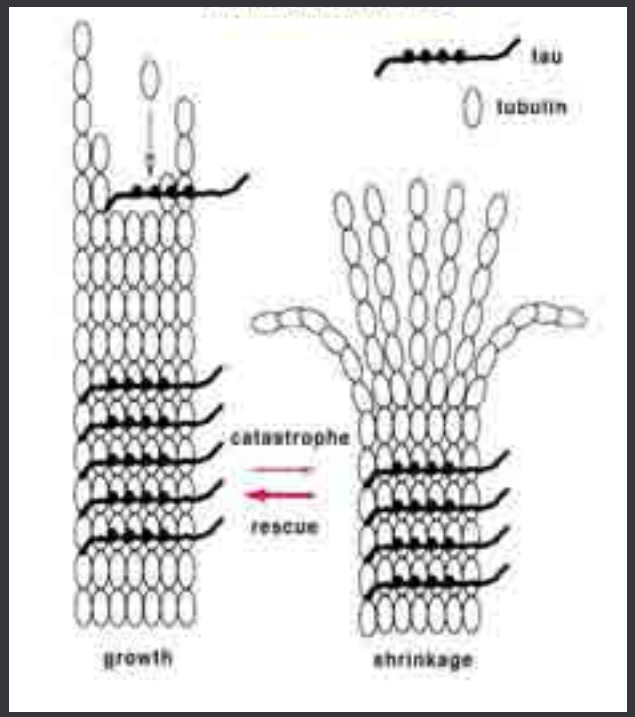
Protein tau

N

C

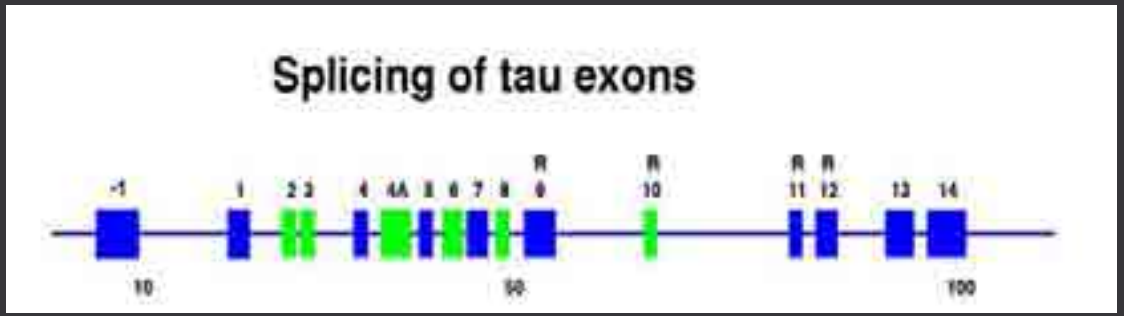
Tubulin binding domain

Microtubules stabilisation by tau



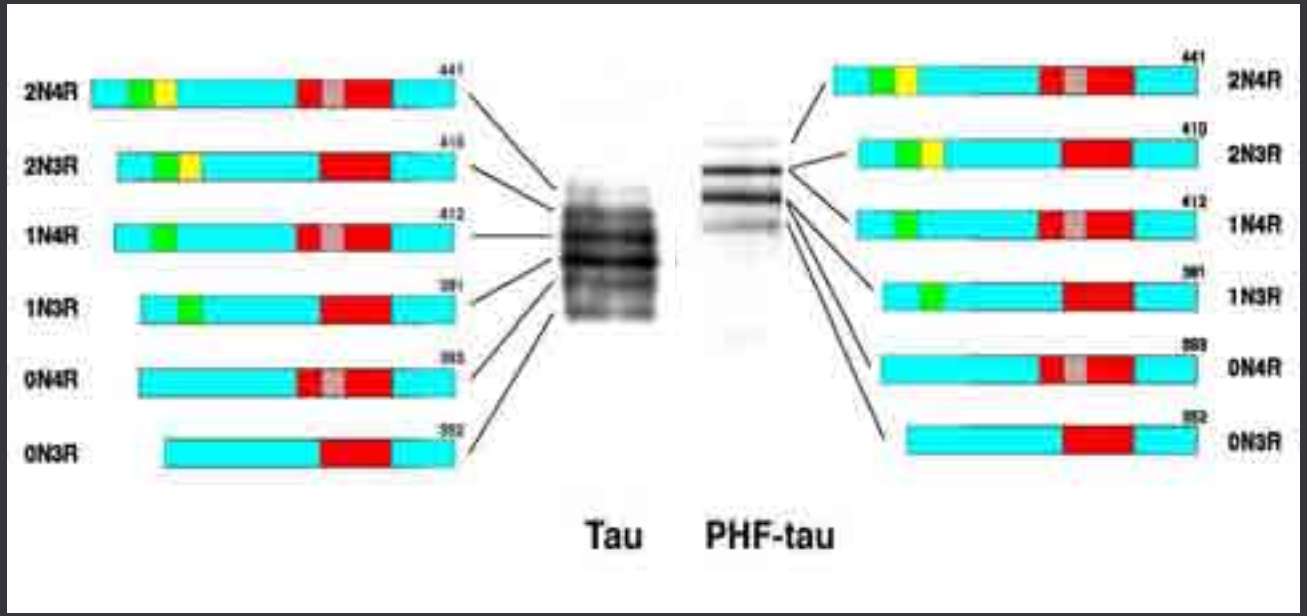
Tau proteins : gene and isoforms

Tau gene
(chr 17)

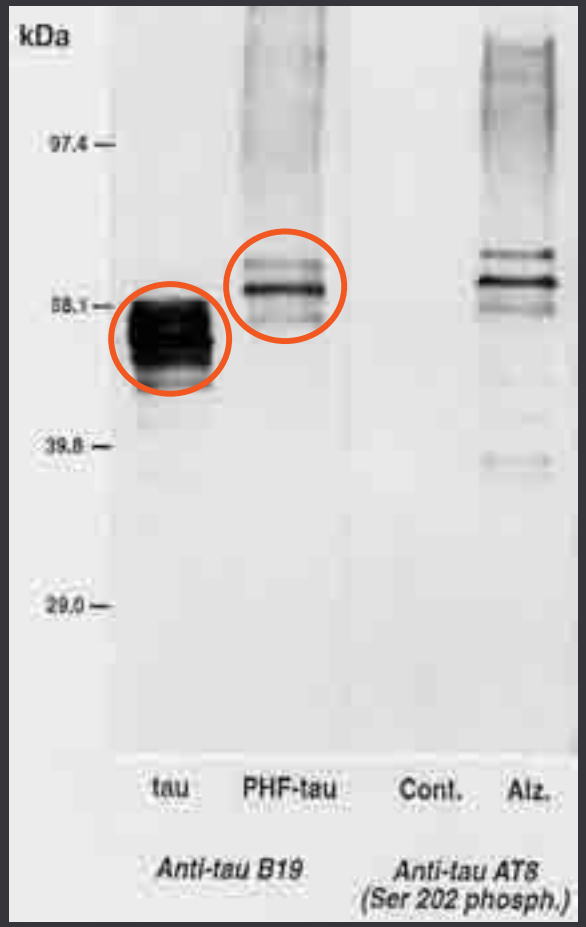


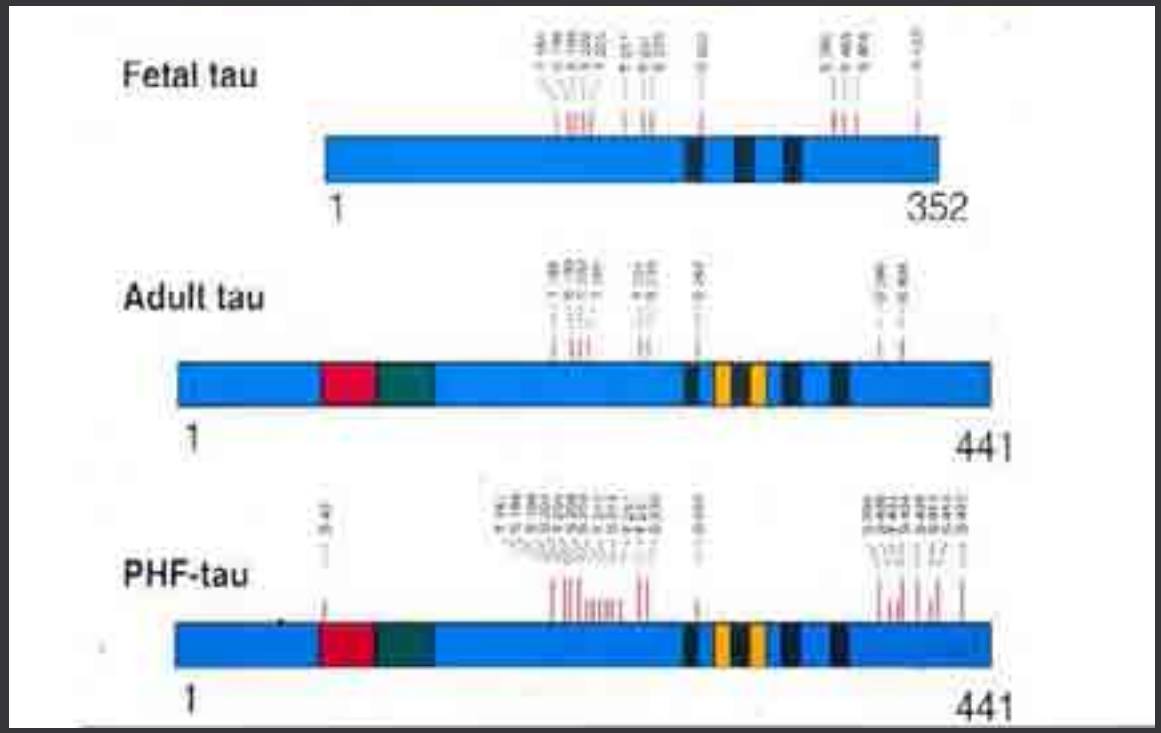
Tau proteins

PHF-tau proteins

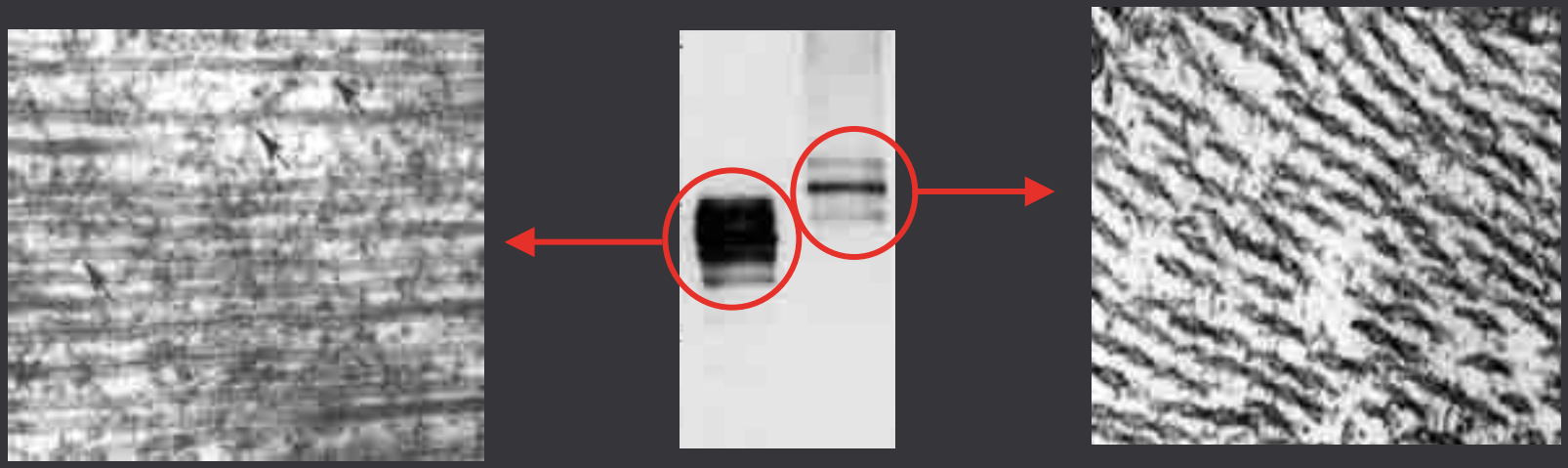


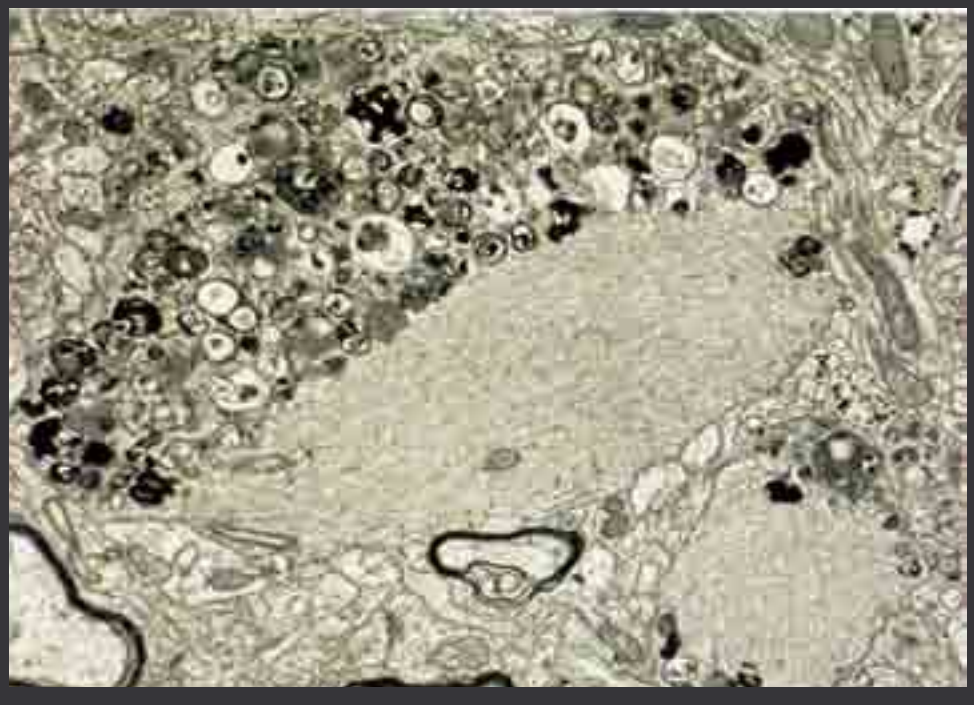
The protein tau in Alzheimer disease



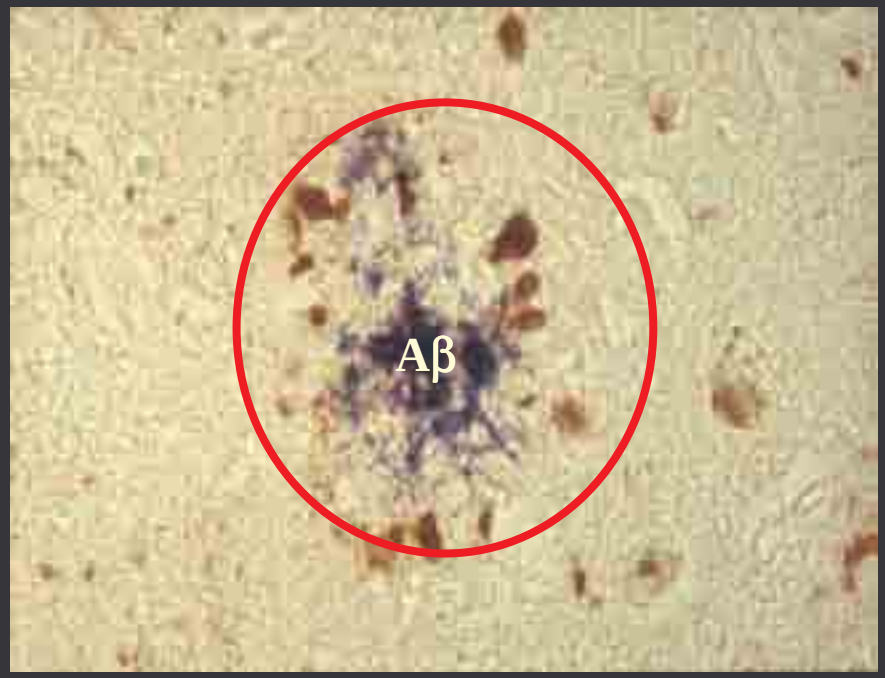


Alteration of axoplasmic flow in Alzheimer disease



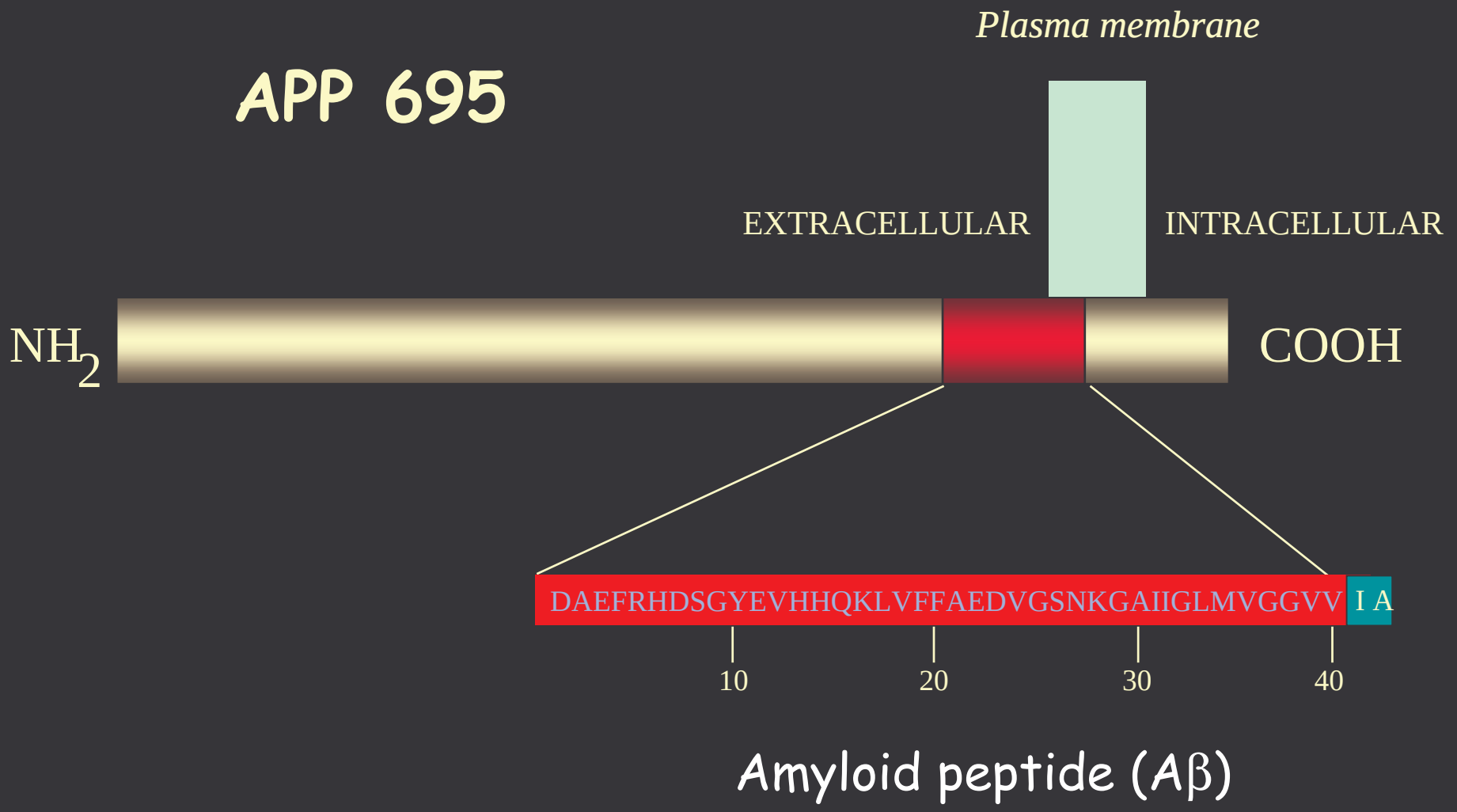


Senile plaques

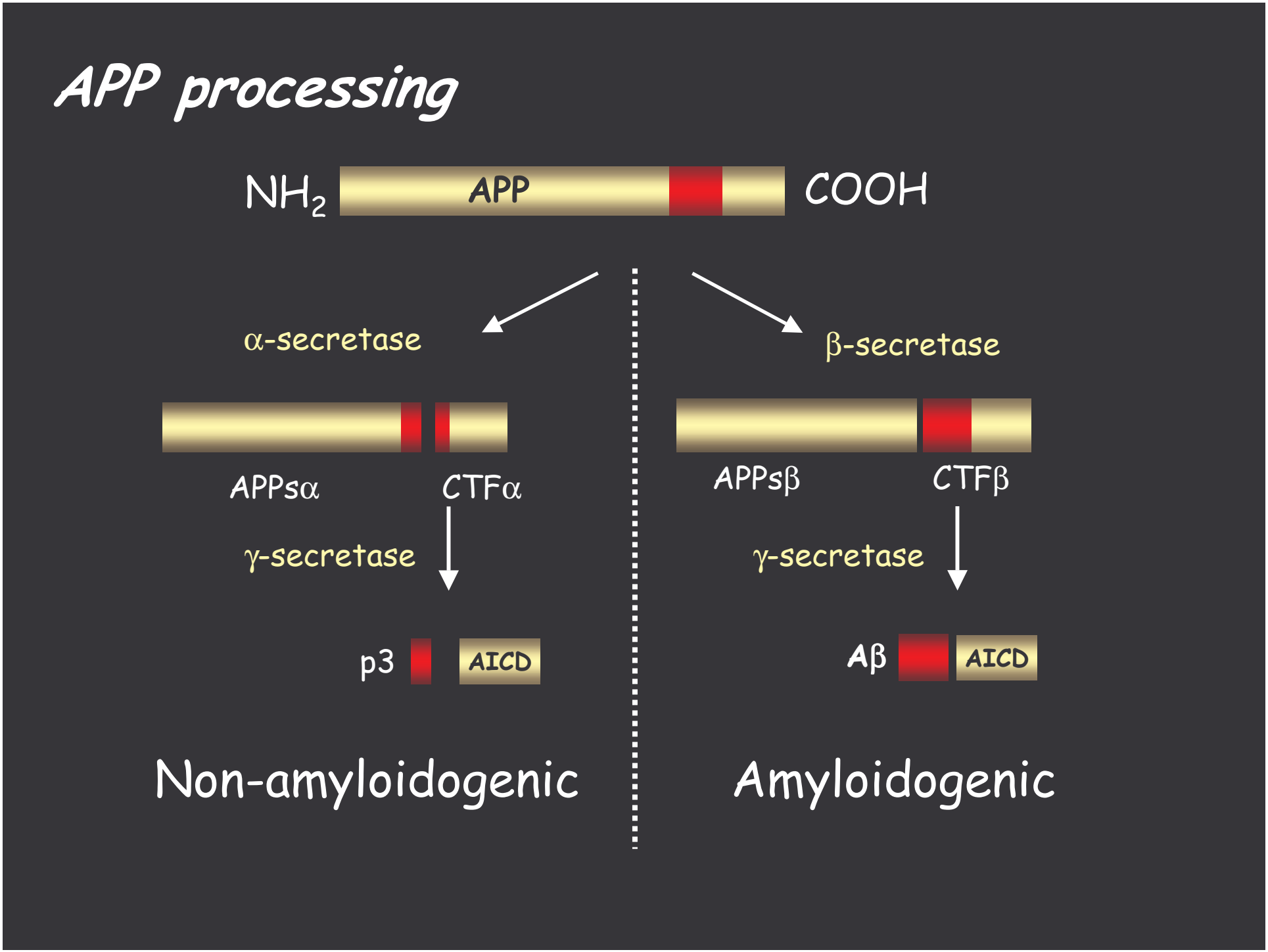


Senile plaques contain an amyloid core of Aβ peptide

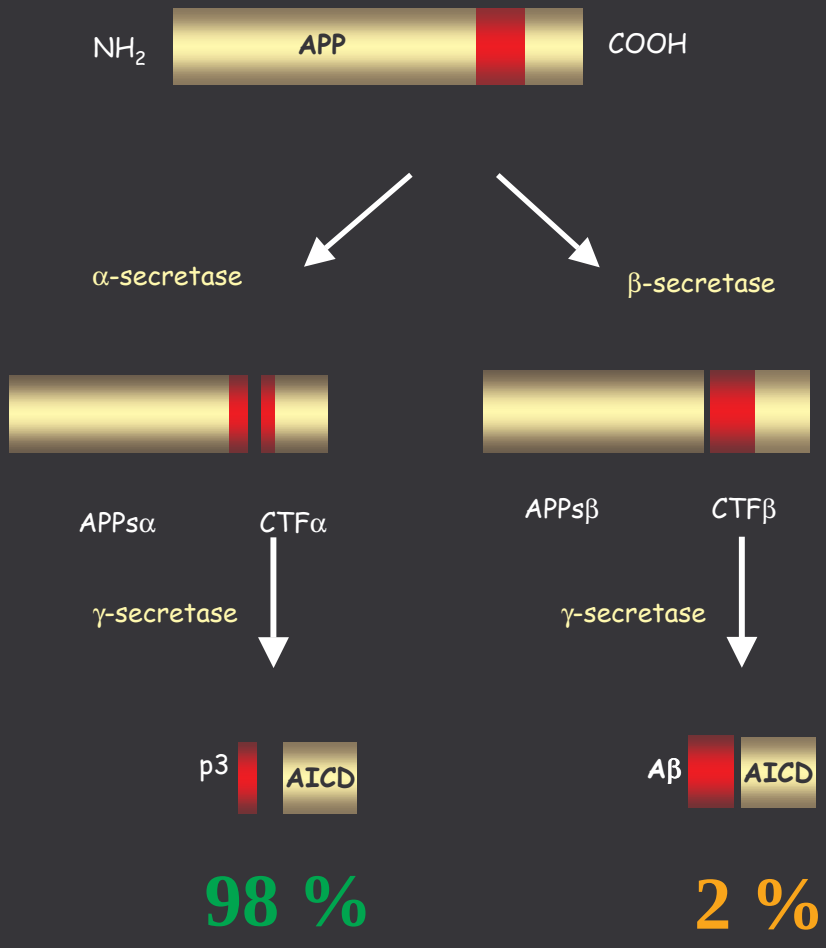
The Amyloid Precursor Protein : APP

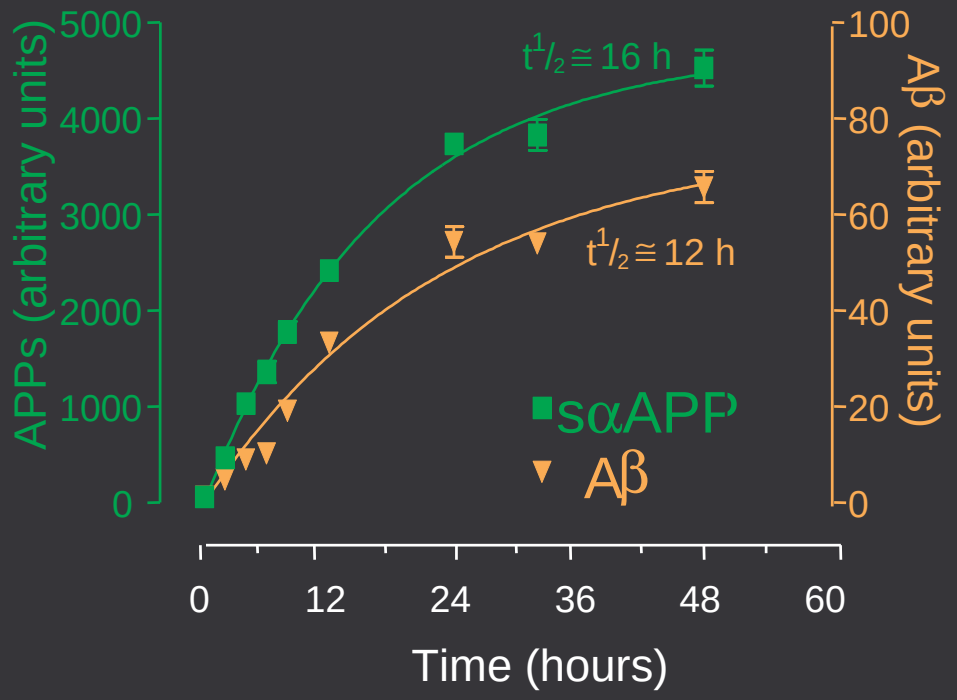


APP processing



APP processing in CHO cells





APP processing in CHO cells

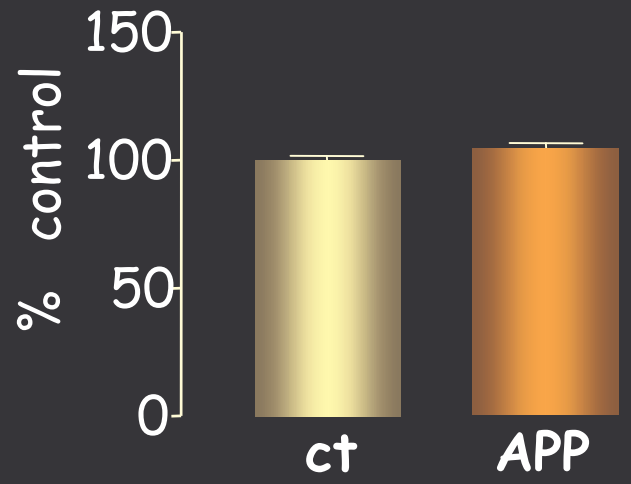
Expression of APP

Production of Aβ

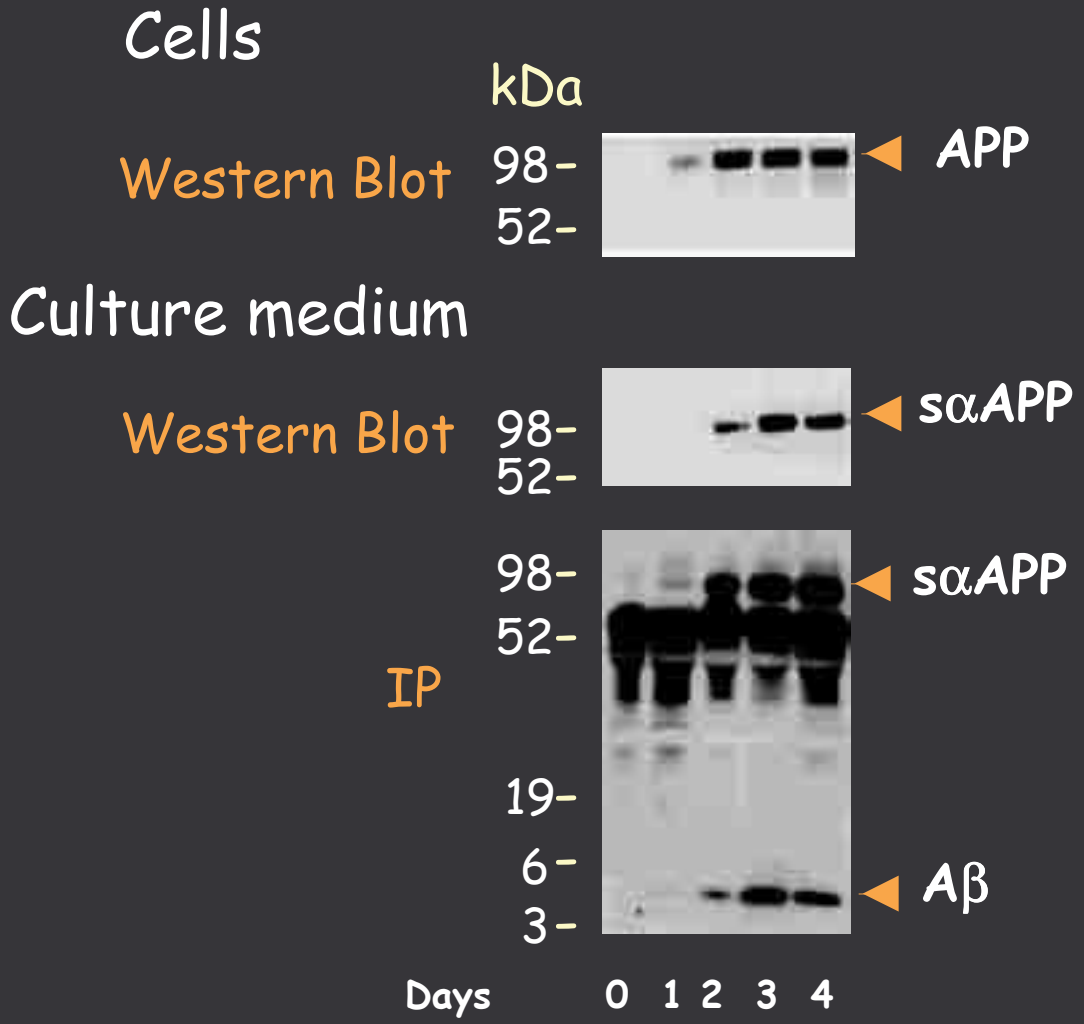
ELISA (pg/ml)

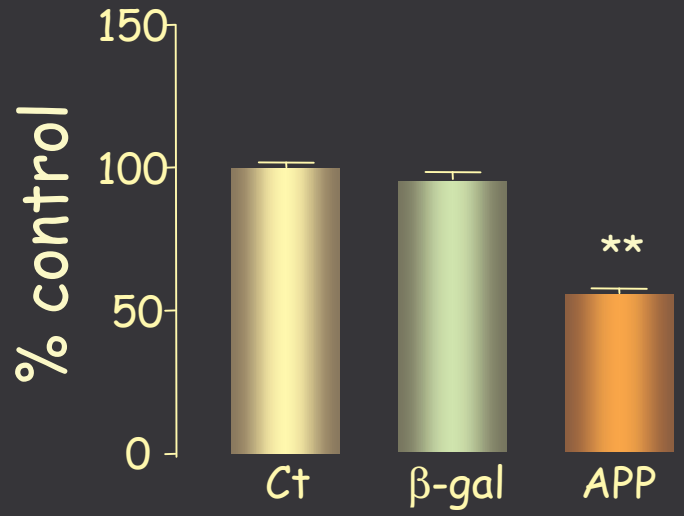
	extracellular		*intracellular*
Aβ 40	4711 ± 703	Aβ 40	non detect.
Aβ 42	427 ± 47	Aβ 42	non detect.

Survival test



APP processing in Neurons





Survival test

Extraneuronal Aβ is not neurotoxic

* Culture medium of neurons
* Culture medium of CHO

→ Treatment of control neurons → Survival test

APP products from neurons

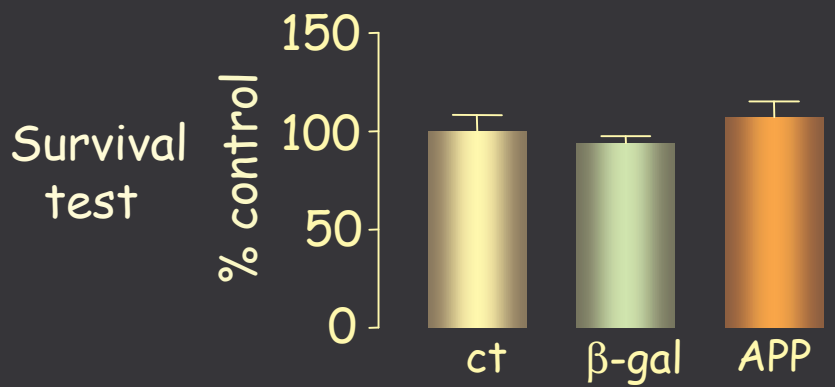
Ct β-gal APP

Western Blot

sαAPP

ELISA (pg/ml)	
Aβ 40	153 ± 32
Aβ 42	non detect

Survival test



APP products from CHO cells

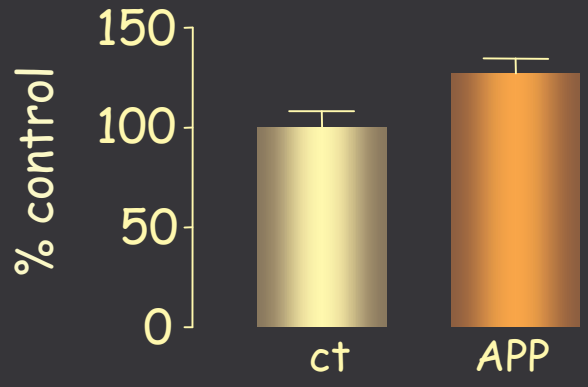
Ct APP

Western Blot

sαAPP

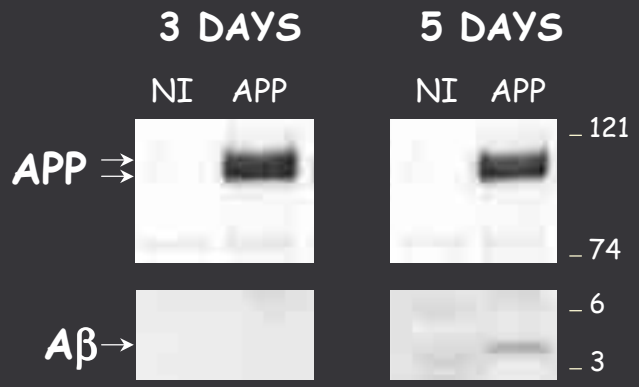
ELISA (pg/ml)	
Aβ 40	4711 ± 703
Aβ 42	427 ± 47

Survival test



Intraneuronal Aβ 42 is neurotoxic

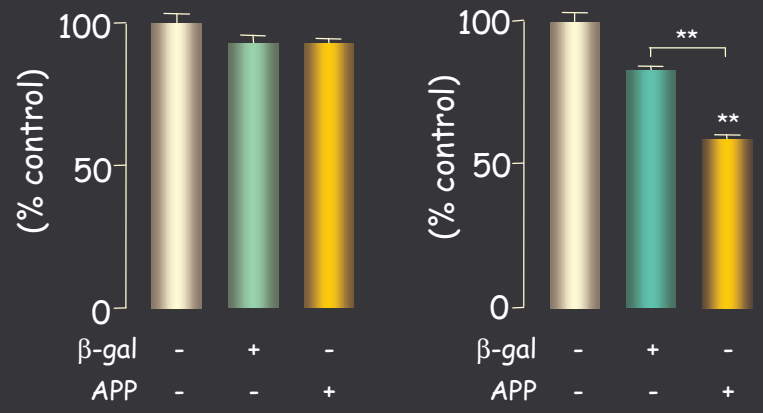
Expression of APP



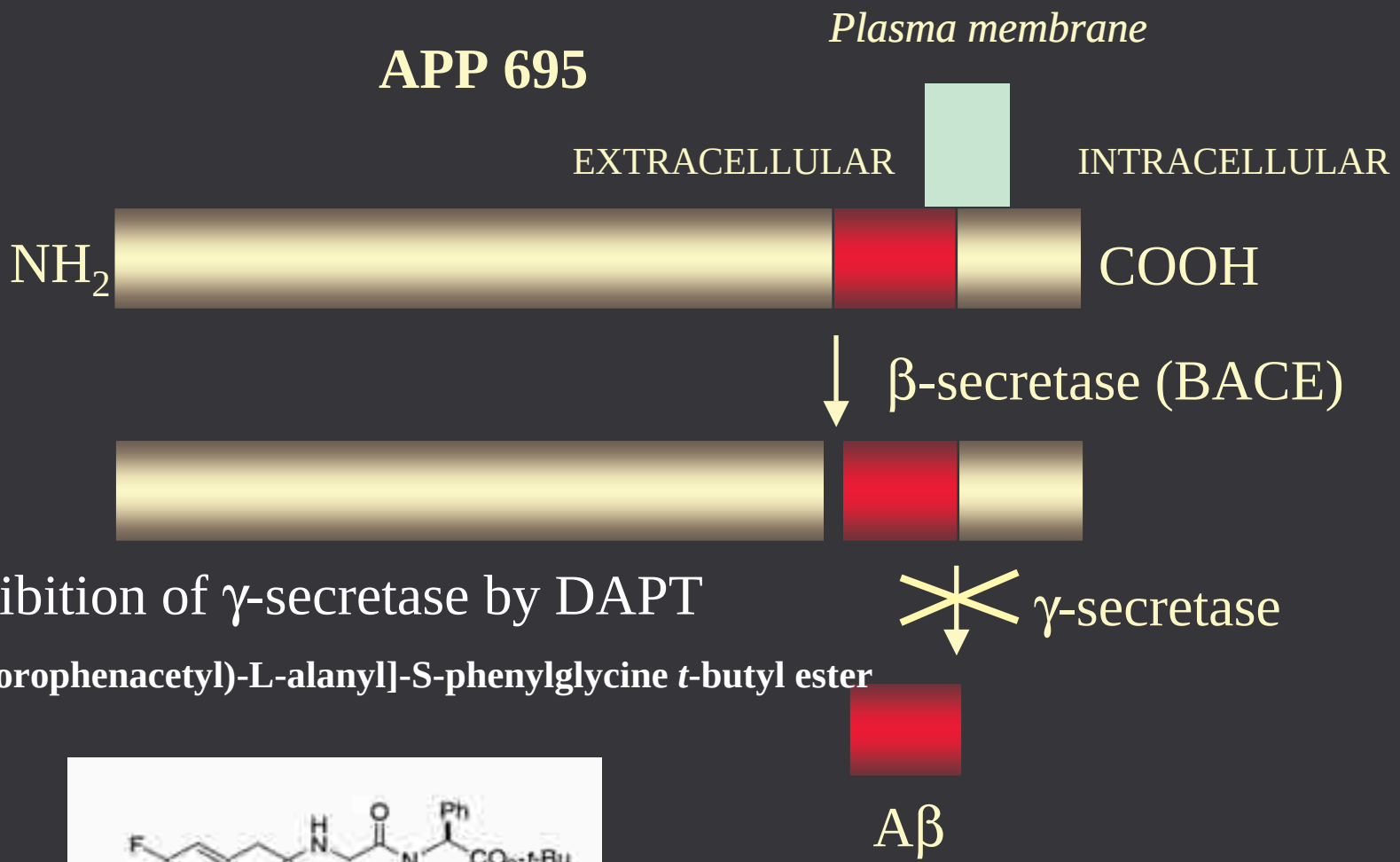
Intraneuronal Aβ

	NI	APP	NI	APP
Aβ1-42 (pg/mg)	-	-	-	115±24

Cell survival

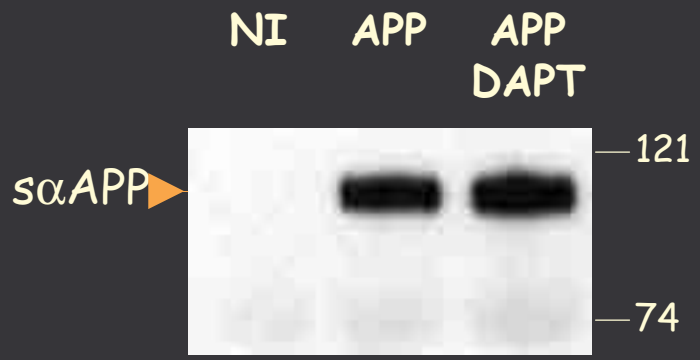


Inhibition of Aβ production



Intraneuronal Aβ 42 is neurotoxic

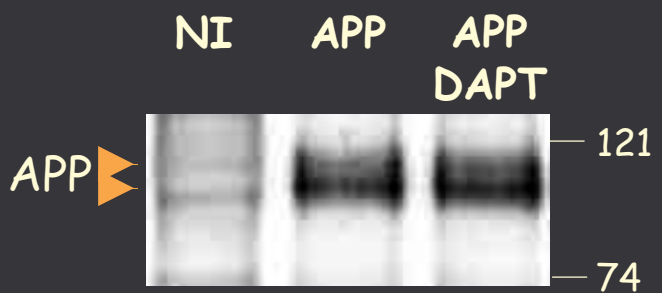
Extracellular APP



Extracellular Aβ

	NI	APP	APP DAPT
Aβ1-40 (pg/ml)	-	61±2	-

Intraneuronal APP

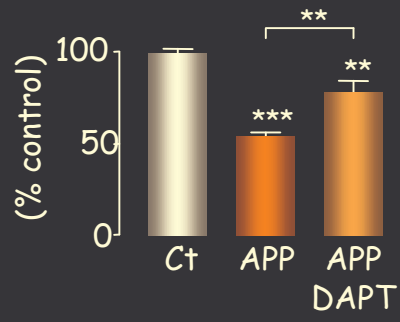


Intraneuronal Aβ

	NI	APP	APP DAPT
Aβ1-42 pg/mg	-	115 ±23	49 ±34

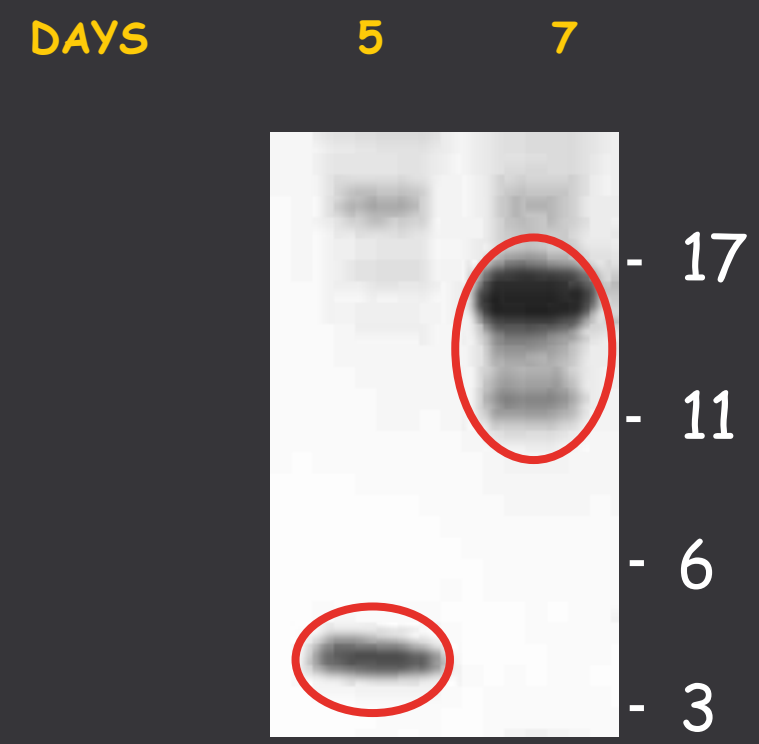
- 57 % $A\beta 1\text{-}42_{int}$

Cell survival



+ 52 % survival

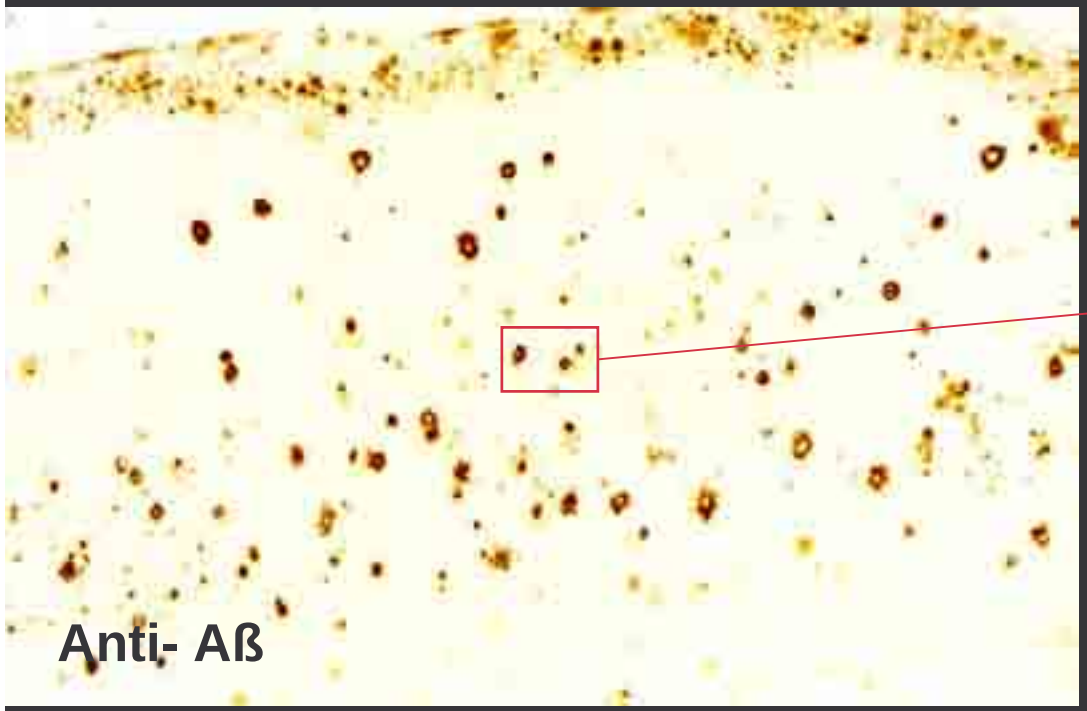
Intraneuronal oligomerization of Aβ 1-42

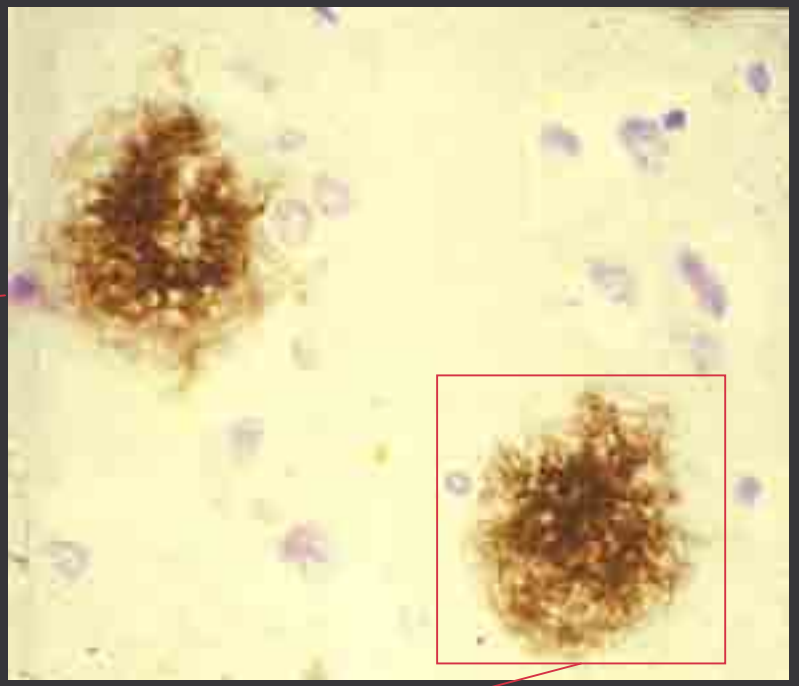


Intraneuronal Aβ

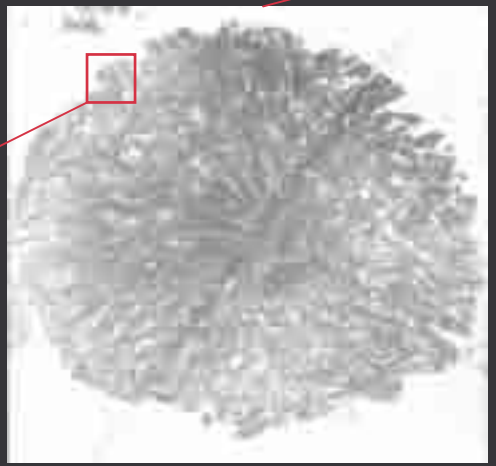
OLIGOMERIZATION

Senile plaques of Alzheimer disease

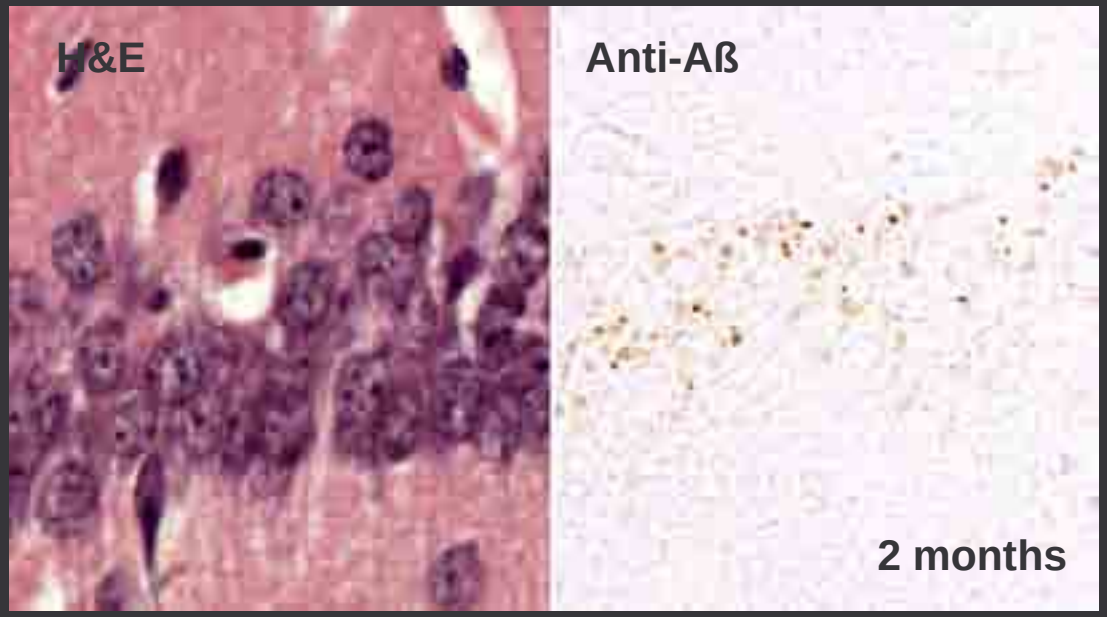




Aß amyloïd fibrils

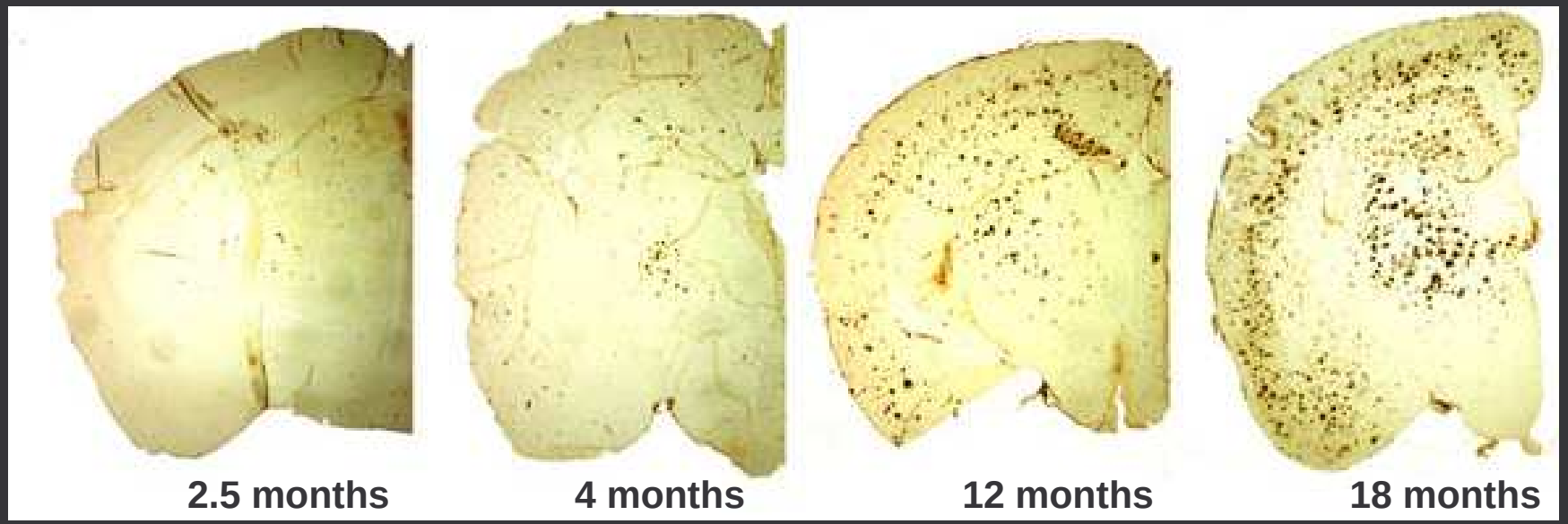


Aß deposits in transgenic mice



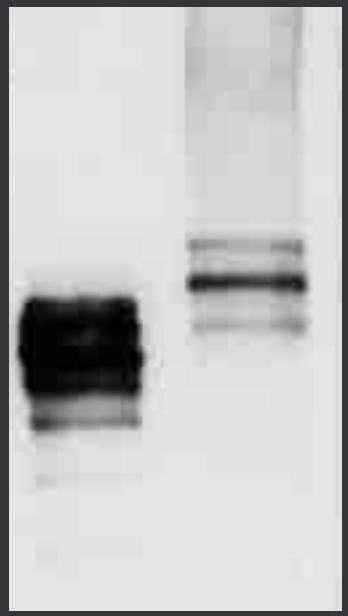
← Intraneuronal Aß

Extracellular Aß ↓



Alzheimer Disease

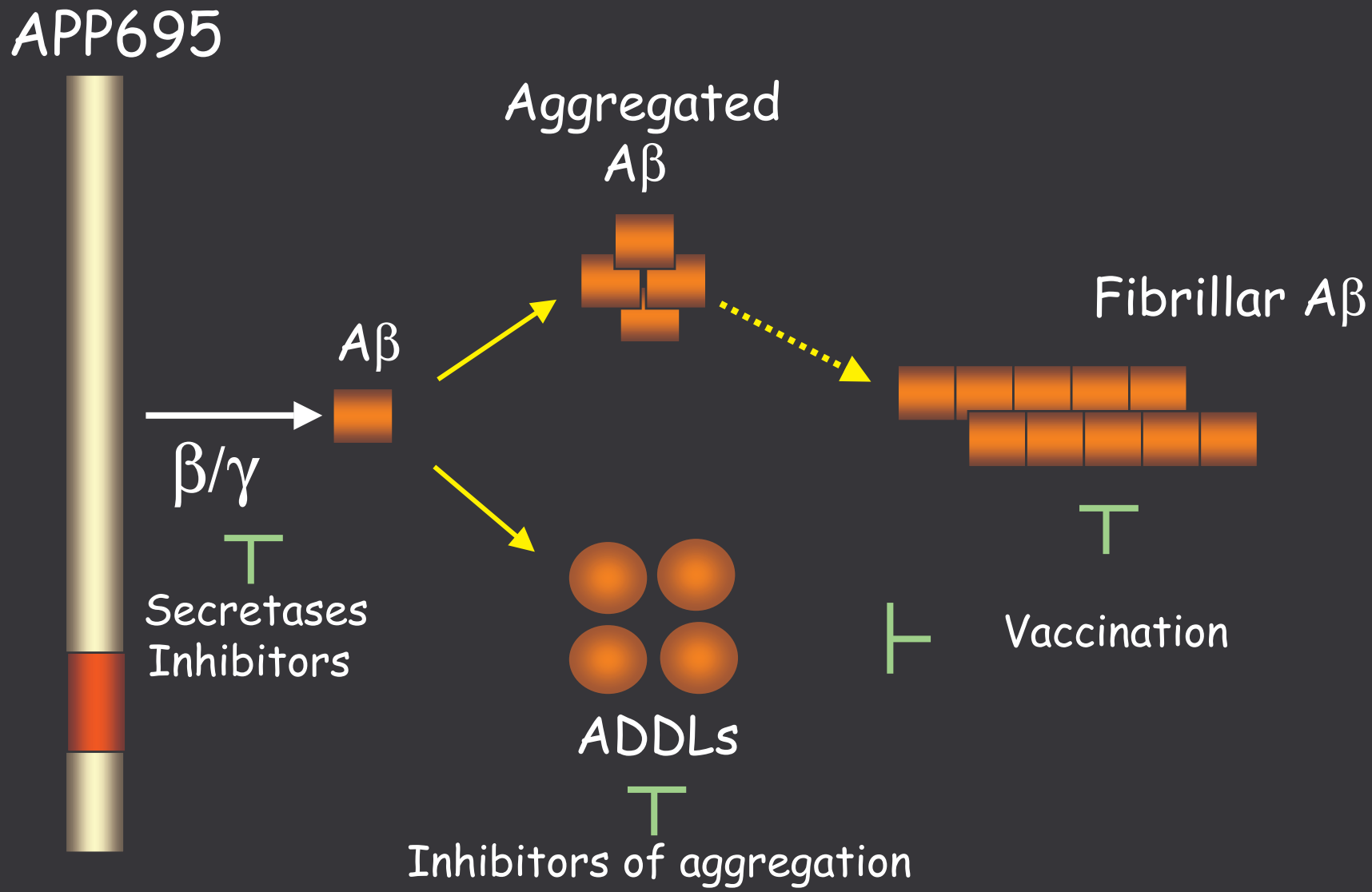
Biochemistry



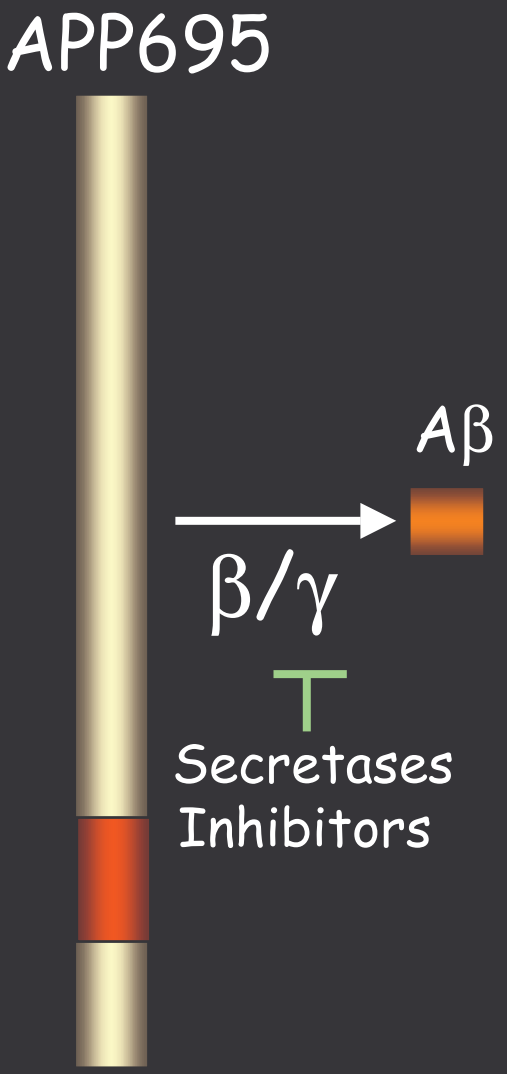
Intraneuronal accumulation of Aβ oligomers

Intraneuronal accumulation of P-tau in PHF

Amyloïd-β as a therapeutic target



Therapeutic strategies exploiting amyloid-β generation mechanisms



BUT

Bace 1 controls myelination

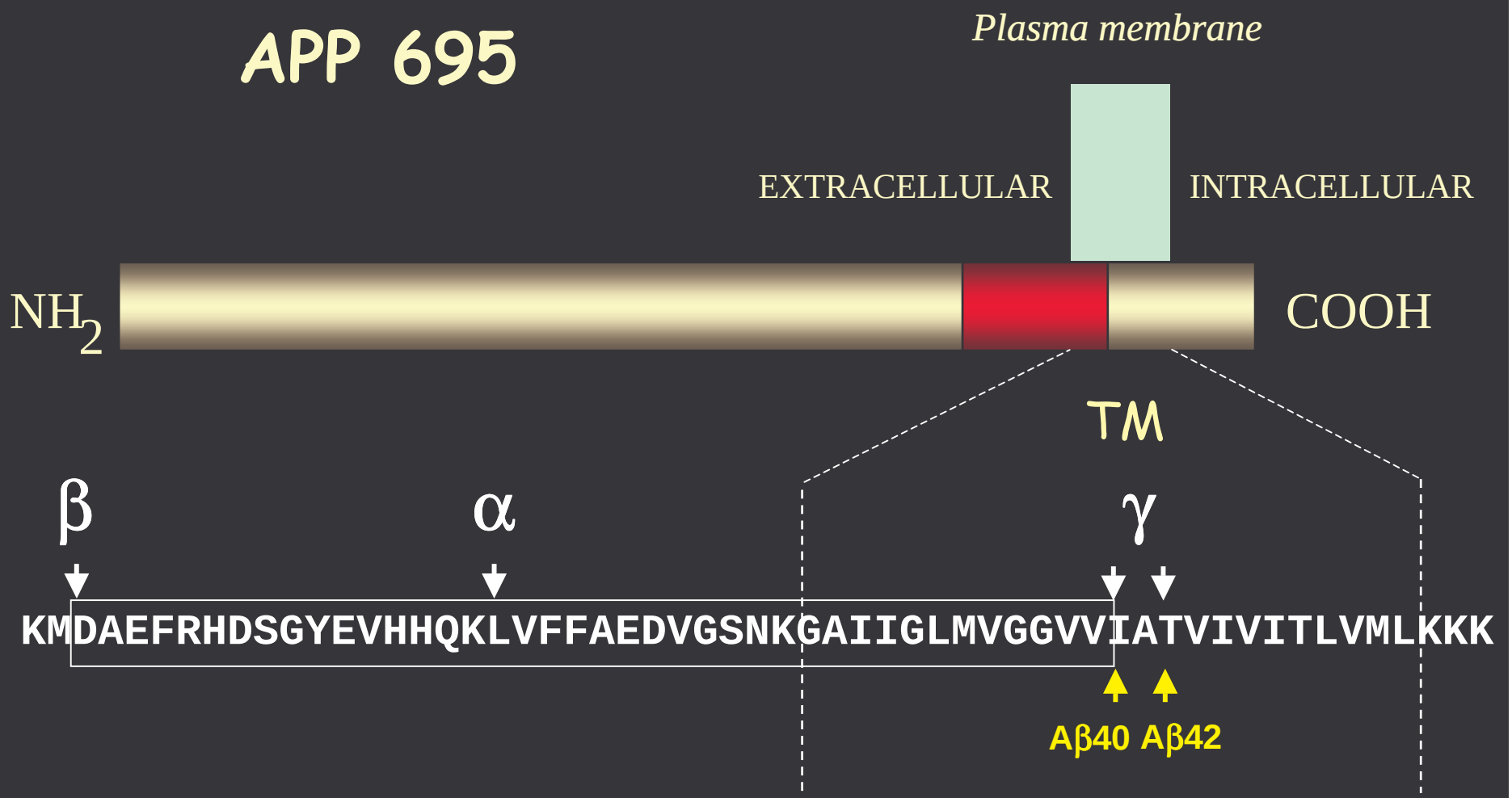
Willem M. et al. Science 2006
Hu X. et al. Nature Neurosci. 2006

A lot of substrates for γ-secretase

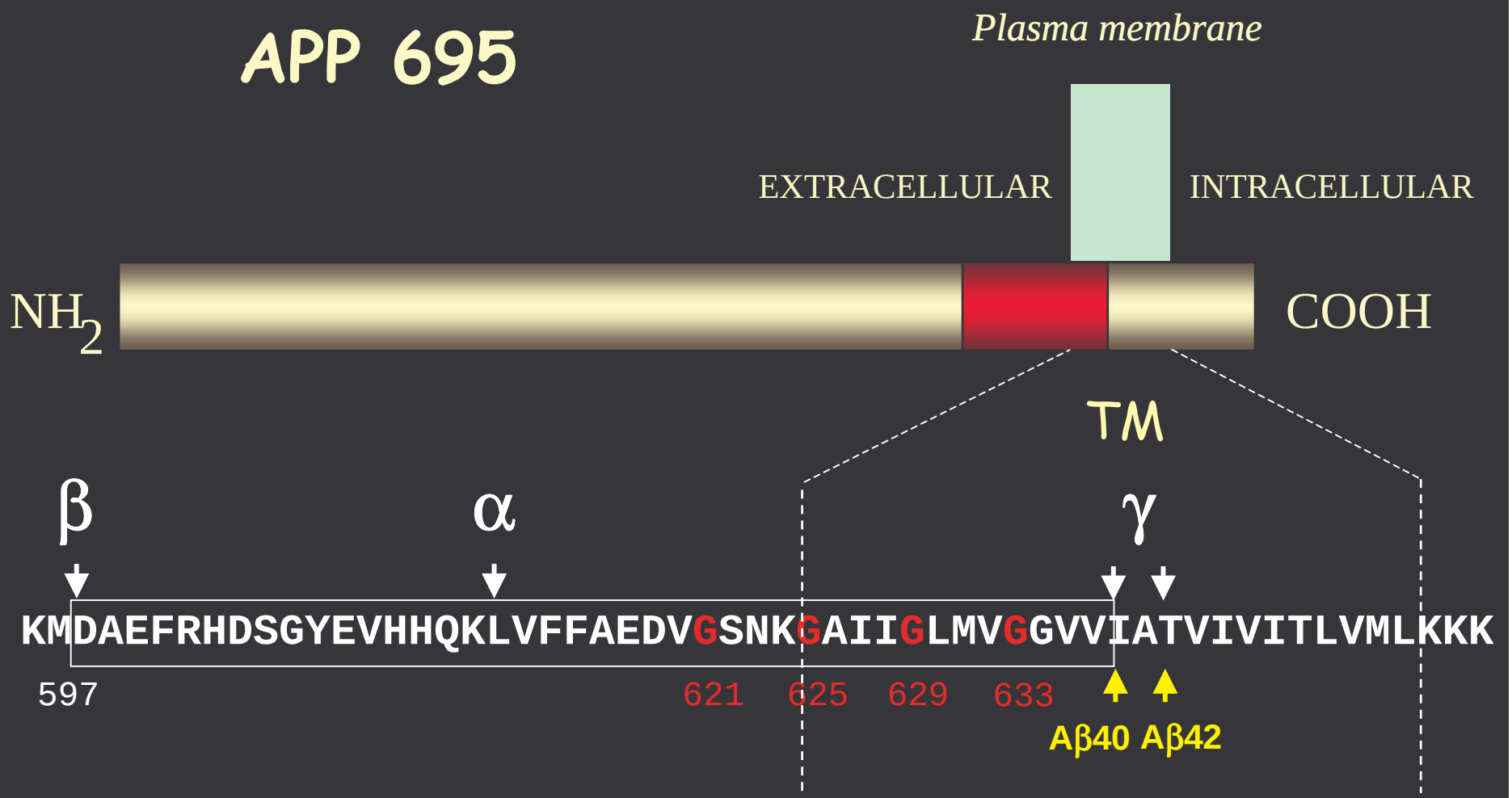
APP, APLP1, APLP2, CD44, DG, DNER
DSG2, Erb-4, E-cadherin, Gnt-V,
HLA, LDLR, NPR-C, Notch1, PLXDC2,
Synd-1, Synd-2, Vasorin ...

Nakahara S. et al. Faseb J. 2006
Hemming ML et al, 2008

What about the substrate APP

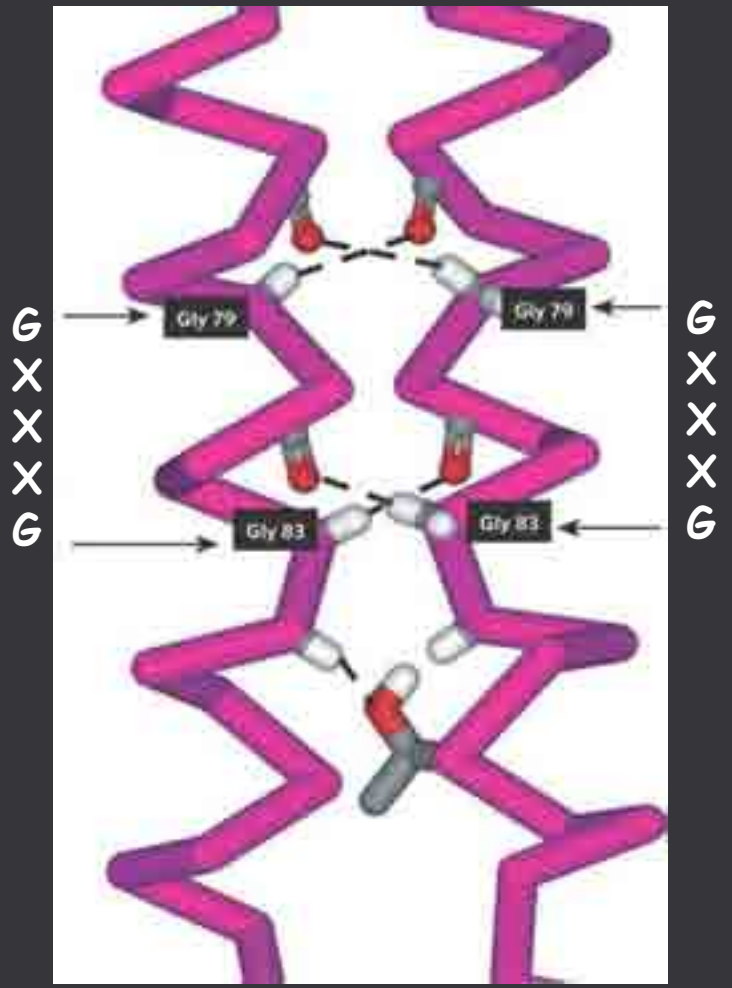


The Amyloid Precursor Protein : APP



GxxxG membrane motifs

From Glycophorin A (GpA)



Self-Association of TM helices

In APP

hAPP695

Extracellular TM

…AEDV**G**SNK**G**AII**G**LMV**G**GVVIATVIVITLVML**K**KK…

621 625 629 633 γ

Homodimerization?

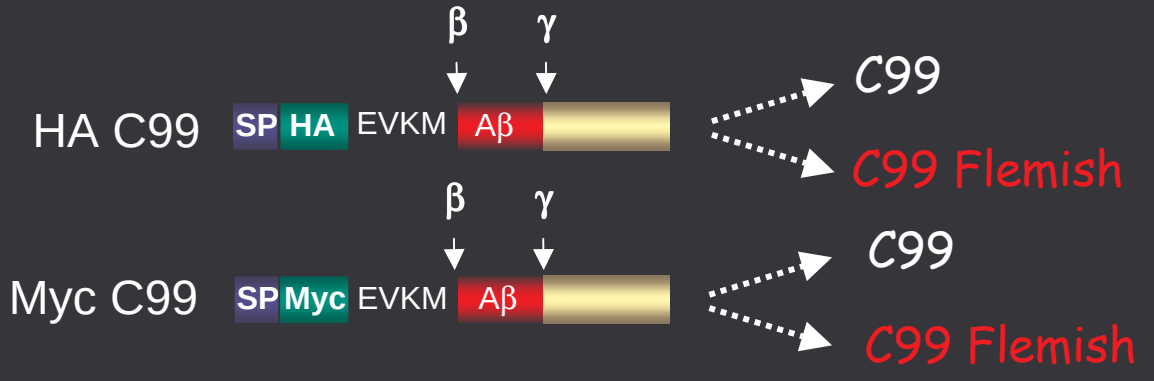
Metabolism

Low energy structure of an APP transmembrane homodimer

Additional GxxxG motif in Flemish APP mutant

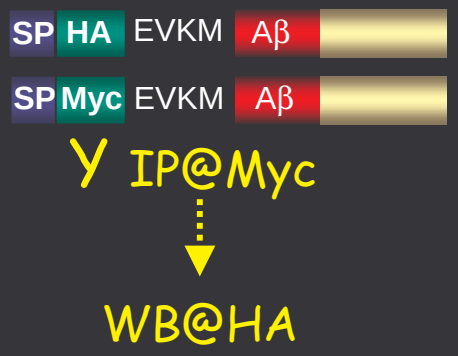
Flemish APP mut (A617G)

GxxxG motifs and C99 homodimerization



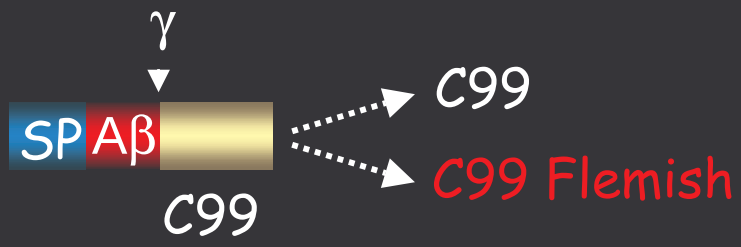
C99 tagged constructs

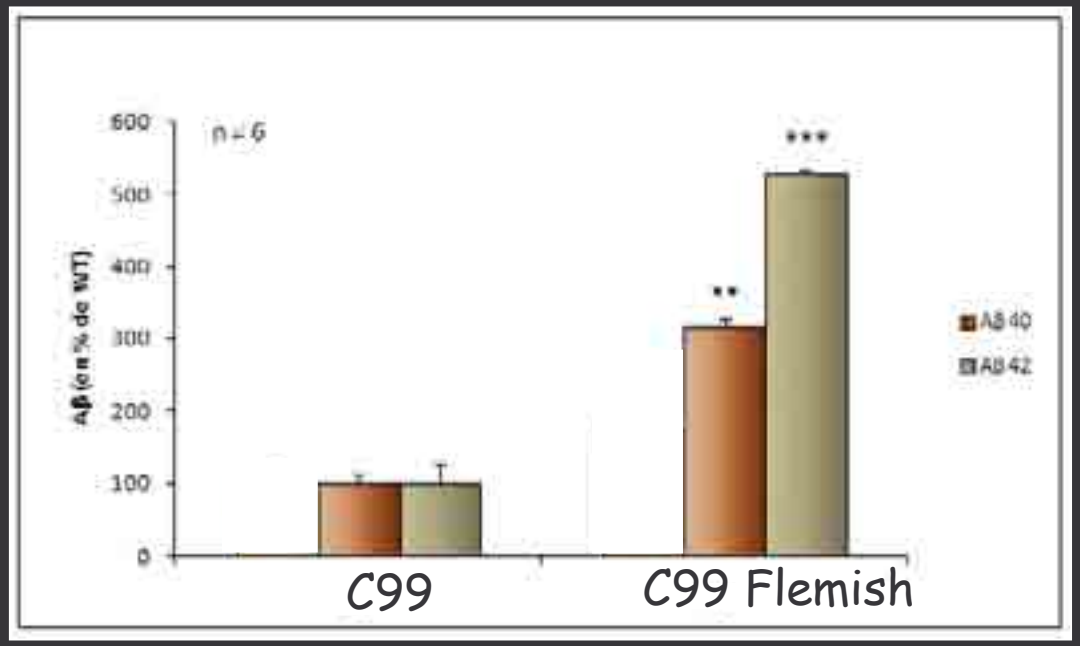
Co-Immunoprecipitations



- *GxxxG motifs promote homodimerization of C99*
- *Flemish mutation increases homodimerization*

GxxxG motifs and Aβ production





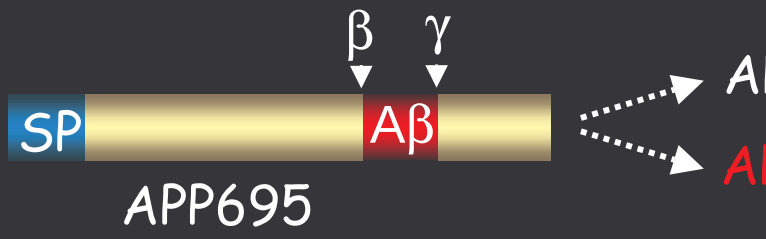
Aβ production

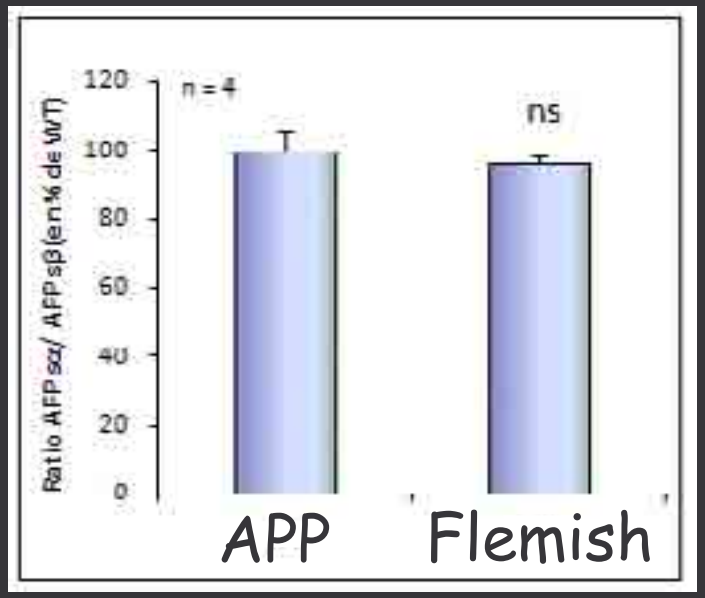
Aβ 40 : + 217 %

Aβ 42 : + 428 %

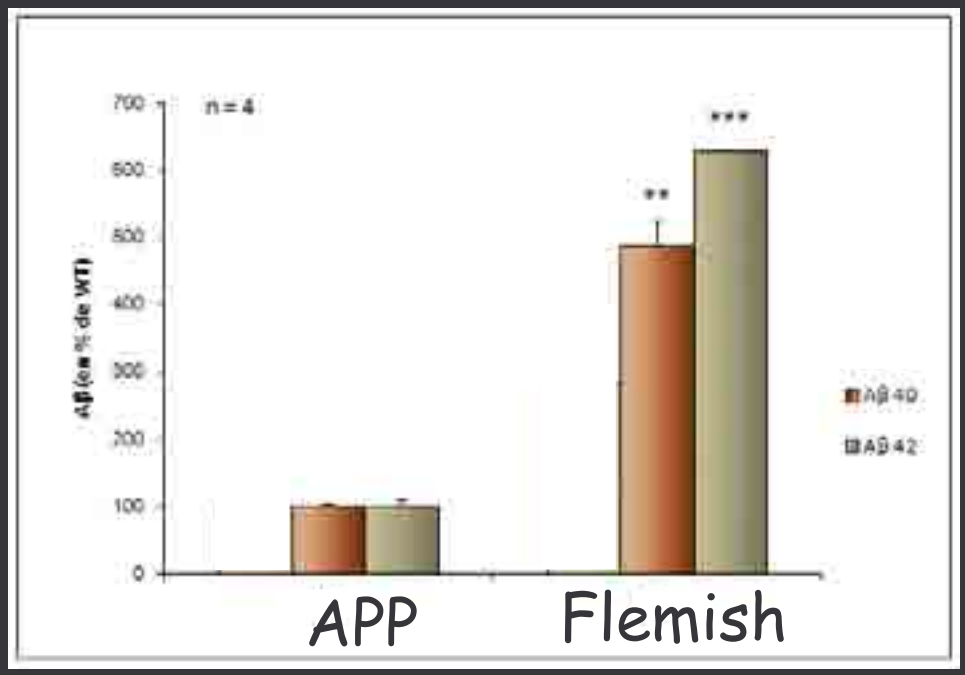
➢ *Flemish mutation increases Aβ production from C99*

GxxxG motifs and Aβ production





α/β Cleavage



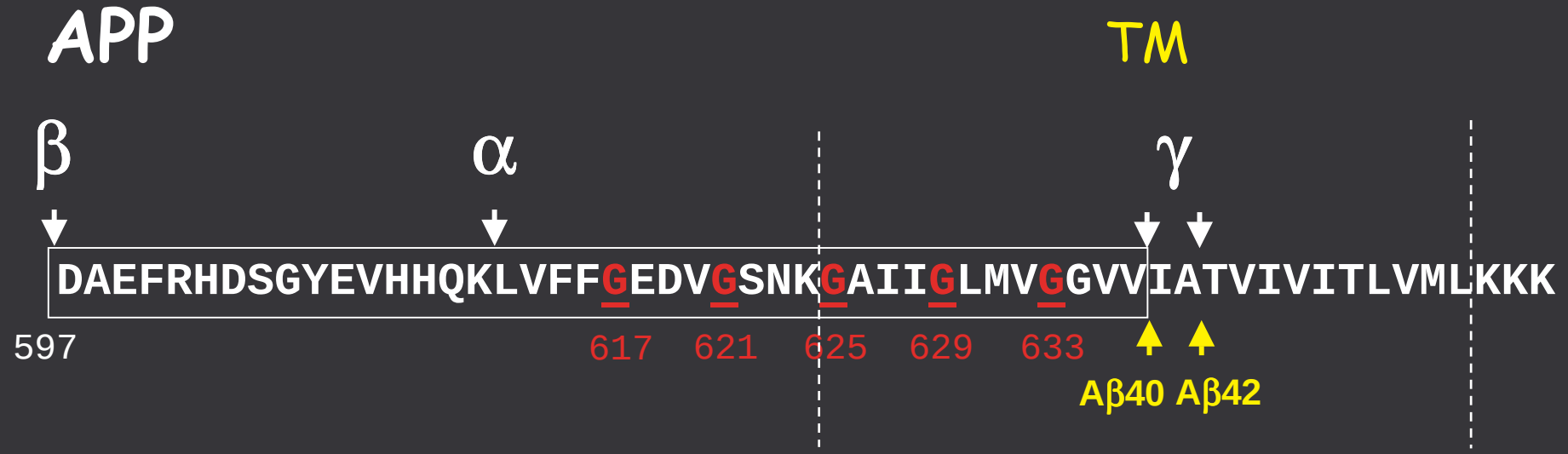
Aβ production

Aβ 40 : + 389 %

Aβ 42 : + 531 %

Flemish mutation increases amyloidogenic processing of APP

Additional GxxxG motif in Flemish APP mutant



GxxxG motifs promote homodimerization of C99
Flemish mutation increases homodimerization

Flemish mutation increases Aβ production from C99

Flemish mutation increases amyloidogenic processing of APP

Role of the GxxxG motifs in APP processing

TM

human APP695 EDVGSNKGAIIGLMVGGVVIATVIVITLVMLKKK

621 625 629 633

mut1 (GG 621/625 AA) EDVASNKAAIIGLMVGGV KKK

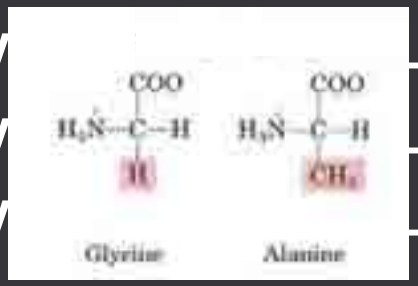
mut2 (GG 625/629 AA) EDVGSNKAAIIALMVGGV KKK

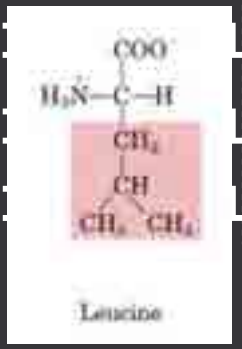
mut3 (GG 629/633 AA) EDVGSNKGAIIALMVAGV KKK

mut4 (GG 621/625 LL) EDVLSNKLAIIGLMVGGVVIATV KKK

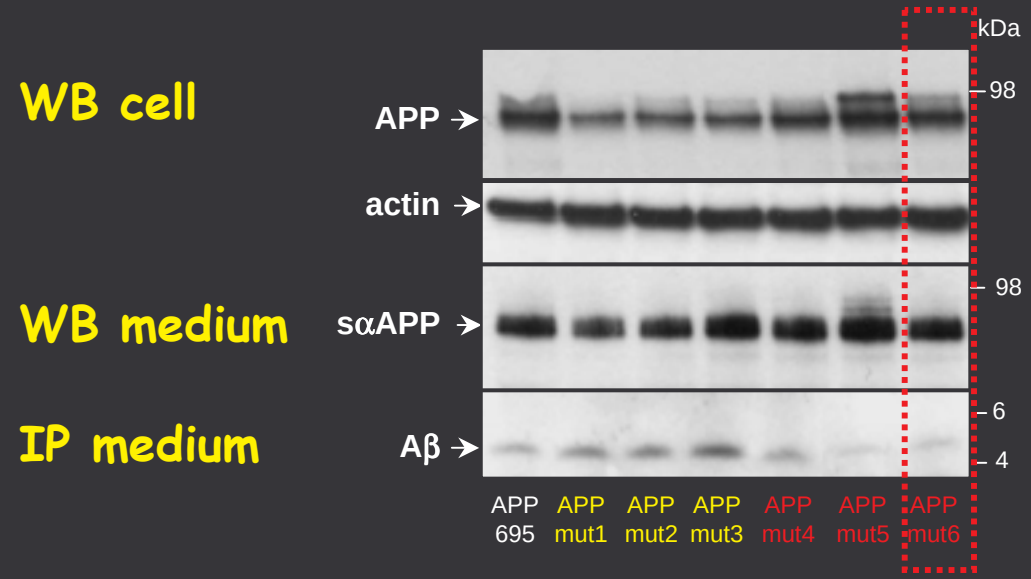
mut5 (GG 625/629 LL) EDVGSNKLAIILLMVGGVVIATV KKK

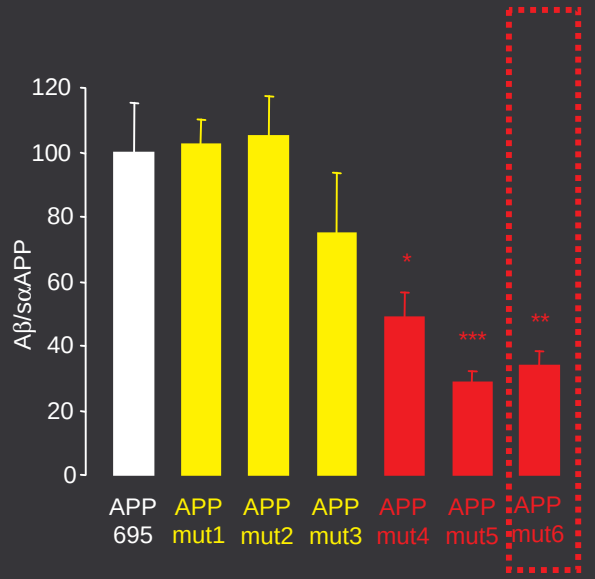
mut6 (GG 629/633 LL) EDVGSNKGAIILLMVLGVVIATV KKK





Role of the GxxxG motifs in APP processing





human APP695 EDVGSNKGAIIGLMVGGVVIATVIVITLVMLKKK

mut6 (GG 629/633 LL) EDVGSNKGAIILLMVLGVVIATVIVITLVMLKKK

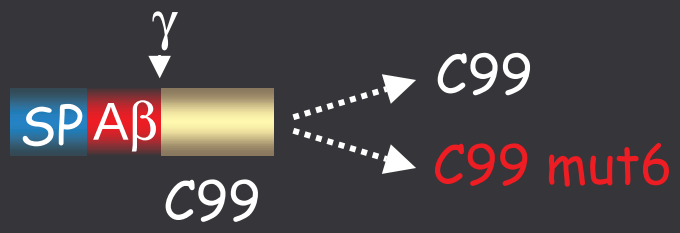
TM

No difference in soluble α APP

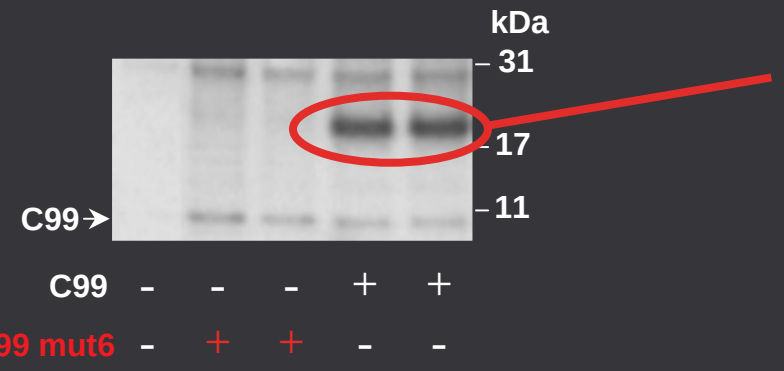
Strong decrease in Aβ

-> amyloidogenic processing

GxxxG motifs and C99 homodimerization



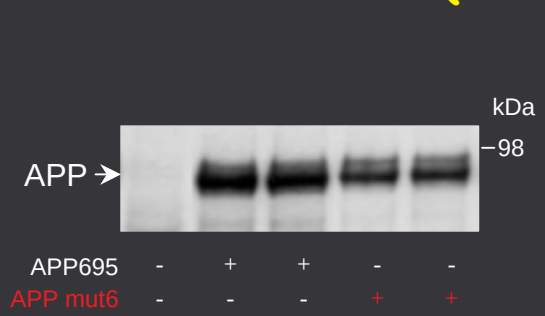
Western Blot (cells)



➢ *GxxxG mutation inhibits C99 homodimerization*

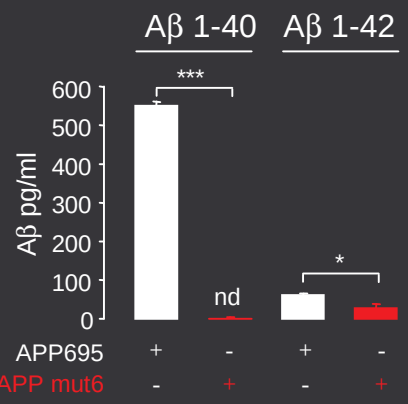
Role of the GxxxG motifs in γ-cleavage

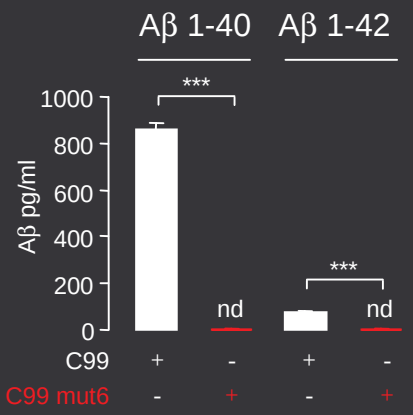
Western Blot (cells)



➢ *GxxxG mutation inhibits Aβ production (γ-cleavage)*

ELISA (Aβ)





Role of the GxxxG motifs in APP processing

GxxxG motifs promote homodimerization of C99

Flemish mutation increases C99 homodimerization

Flemish mutation increases Aβ production

Mutation of a GxxxG motif inhibits C99 homodimerization

Mutation of a GxxxG motif inhibits Aβ production

Conclusions

Neurotoxicity in Alzheimer disease

Intraneuronal accumulation of PHF and Aβ oligomers

Therapeutic strategies exploiting amyloid-β generation

Inhibition of β-secretase

Inhibition of γ-secretase

?

Inhibition of APP homodimerization.

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