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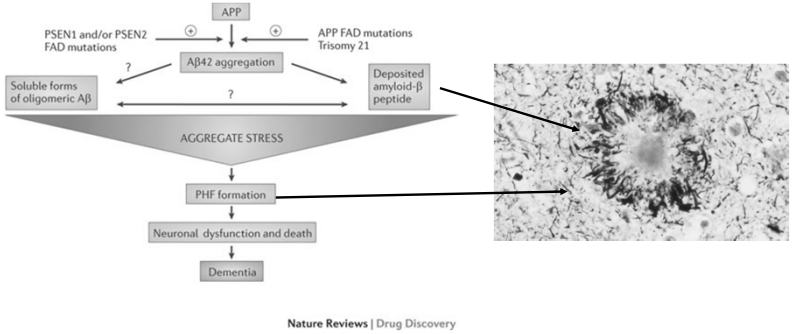
The role of N-terminal truncated Aβ peptides in Alzheimer's Disease Development

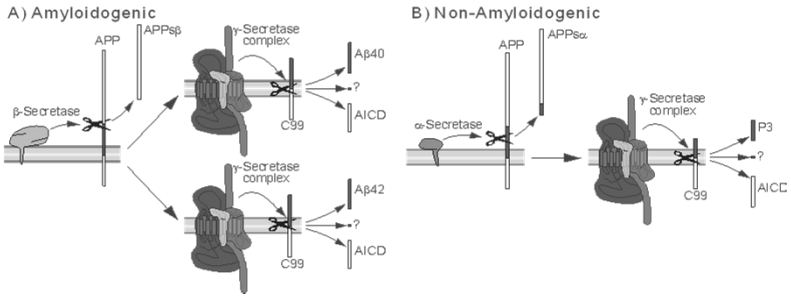
Claus U. Pietrzik, PhD
Professor for Pathobiochemistry
University Medical Center of the
Johannes Gutenberg-University Mainz

Alzheimer's Disease and amyloid hypothesis



From: Eric Karran, Marc Mercken & Bart De Strooper
Nature Reviews Drug Discovery 10, 698-712 (September 2011)

APP Processing



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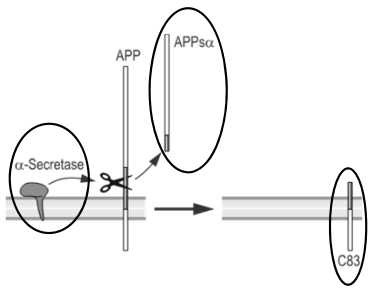

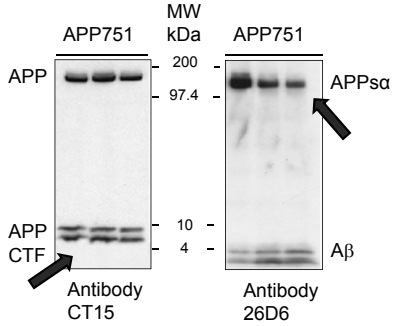

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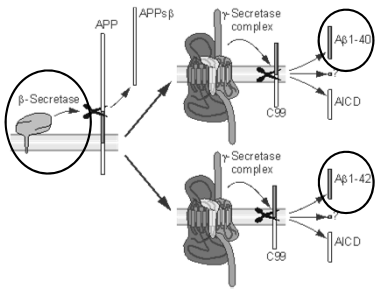
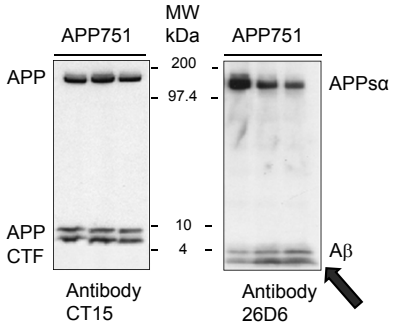
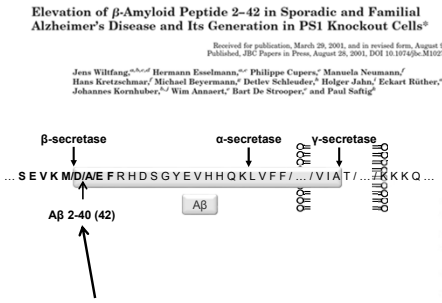
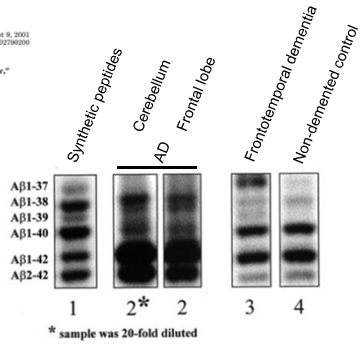




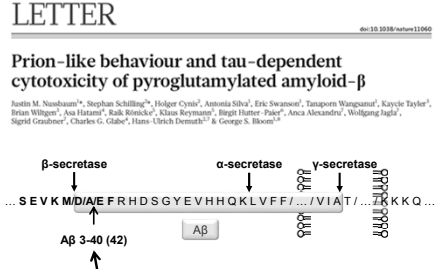
Aβ peptide generation in AD patients

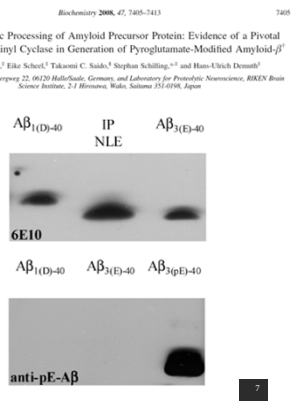




- BACE 1 can not cleave at position D/A !
- Maybe a different enzyme?

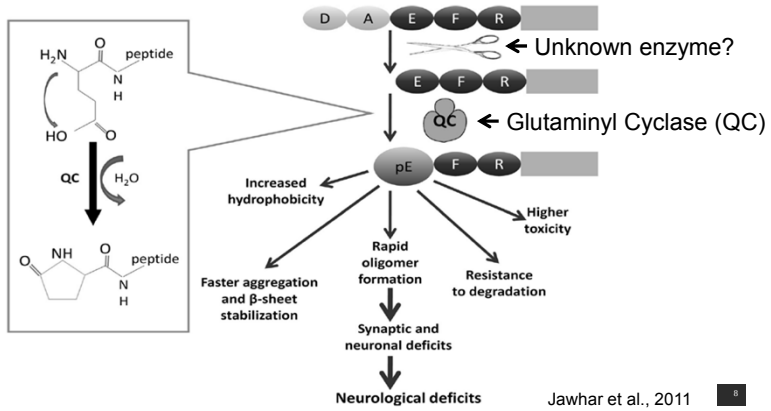
Aβ peptide generation at position 3





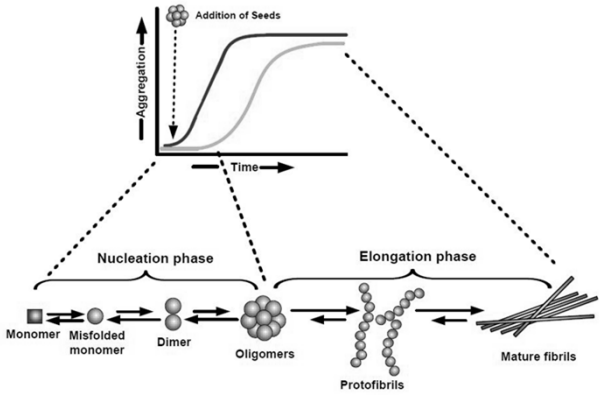
- BACE 1 can not cleave at position A/E !
- Maybe a different enzyme?

Pyroglutamate Aβ (pE-Aβ)

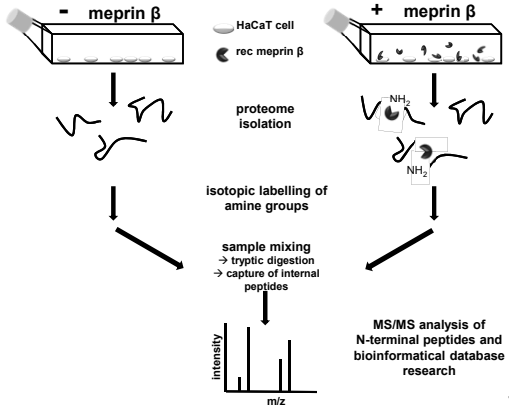


Jawhar et al., 2011

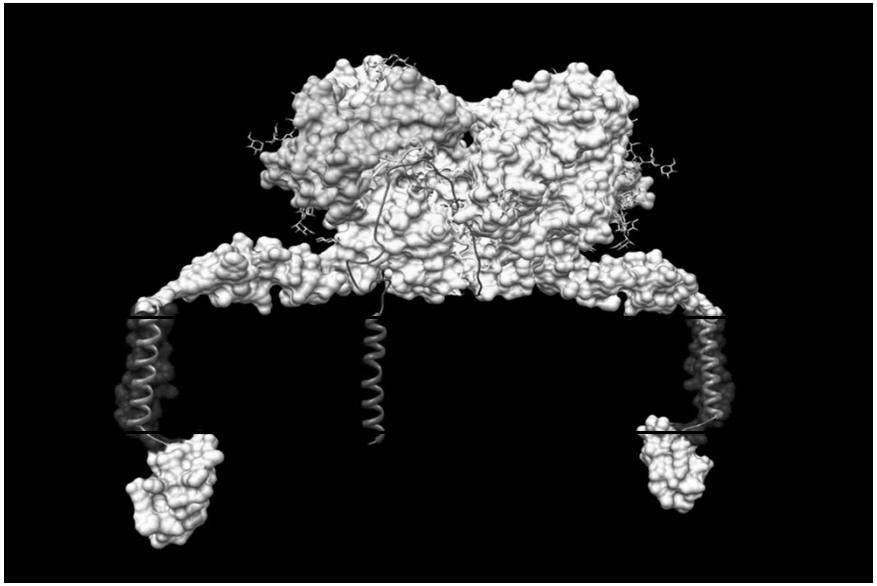
Pyroglutamate Aβ (pE-Aβ) aggregates faster



TAILS (Terminal Amine Isotopic Labeling of Substrates)



Jefferson et al. 2012 CMLS



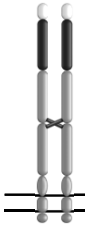
Meprin (metalloprotease from renal tissue)

meprin β

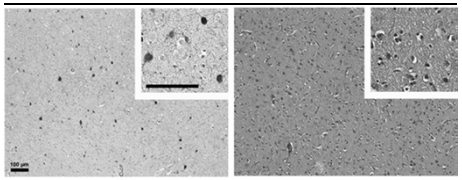
- Astacin family, metzincin superfamily
- Zinc-binding motif
- Complex glycosylated type 1 membrane protein
- Meprins exclusively in vertebrates
- Surface expression as dimer

Meprin β expression in human brain

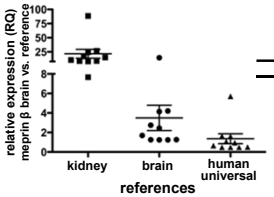
meprin β



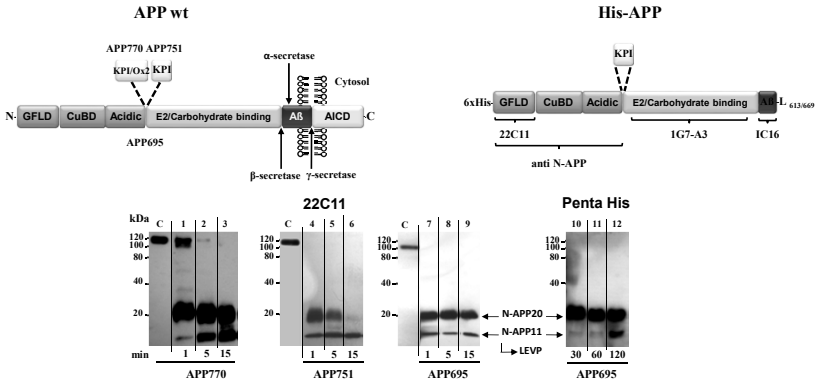
Human brain slices



Anti-meprin β stain Hematoxylin and eosin stain



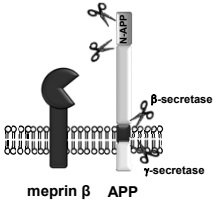
Recombinant APP processing by meprin β

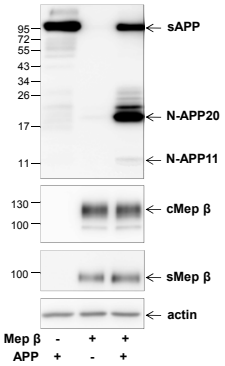


Jefferson et al. 2011 J Biol Chem.

APP processing by meprin β *in vitro*

in vitro

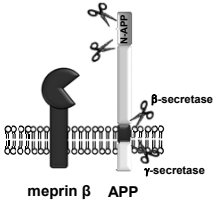


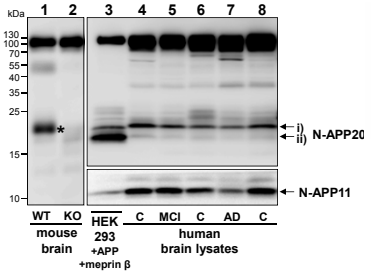


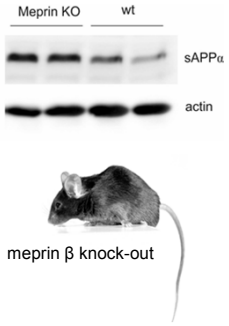
Jefferson et al. 2011 J Biol Chem. 286(31):27741-50

APP processing by meprin β *in vivo*

Endogenous protein expression



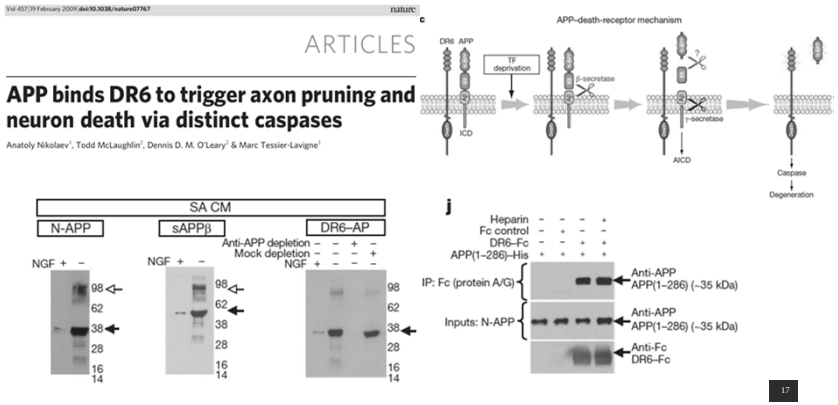




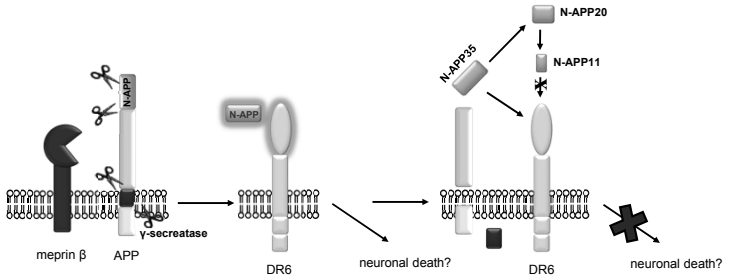
Bien et al. JBC 2012

Schoenherr et al. 2014; in preparation

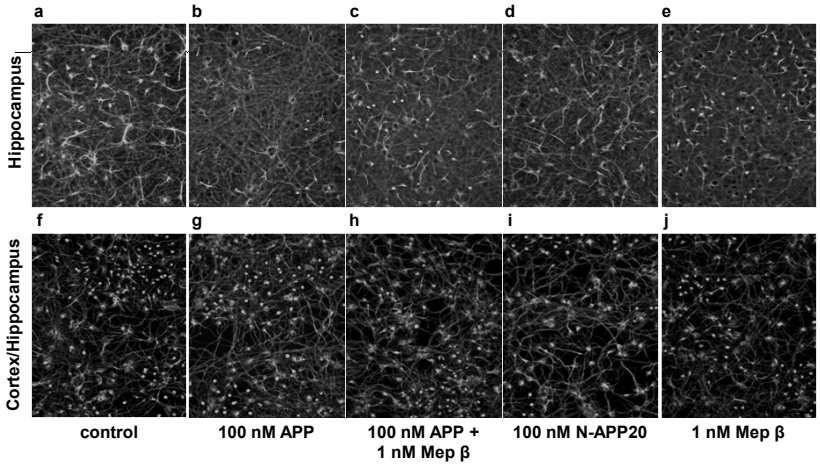
APP binds to DR6 and promotes cell death?



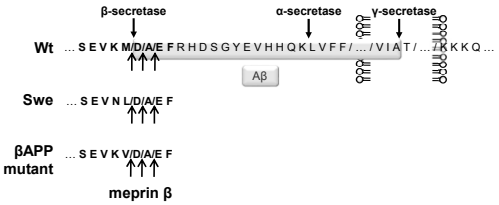
APP processing might lead to cell death

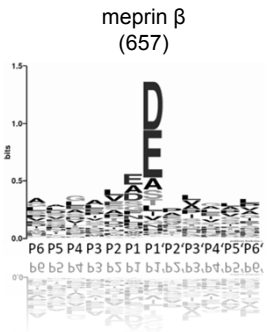


Nikolaev et al. 2009 Nature. Feb 19;457(7232):981-9



Cleveage specificity of meprin β

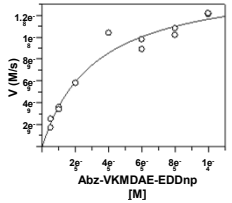




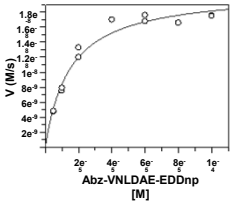
- MALDI-TOF analysis revealed a preference for aspartate (D) in p1 correlates with the BACE1 cleavage site.
- Meprin β cleaved a peptide bond with alanine (A) in p2 within all three peptides.
- Additionally meprin β cleaves the peptide bond (E) residue in p3.

Proteolytic efficiency of meprin β for APP

Aβ wt



Aβ swe

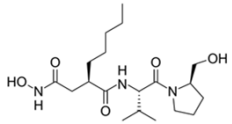


kinetic parameters	Wt VKMDAE	Swe VNLDAE
K_m (M)	3.2×10^{-5}	1.5×10^{-5}
Std. error K_m	5.87×10^{-6}	1.93×10^{-6}
V_{max} (M)	1.53×10^{-8}	2.1×10^{-8}
Std. error V_{max}	1.1×10^{-9}	8.0×10^{-10}
K_{cat} (s^{-1})	15.3	20.9
K_{cat}/K_m ($M^{-1}s^{-1}$)	4.8×10^{5}	1.41×10^{6}

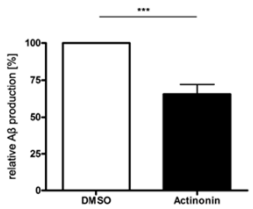
- APPwt and APPswe fluorogenic substrates.
- MEF wt, MEF BACE1/2 KO - and MEF BACE1/2 KO cells stably transfected with meprin β.
- ~50% increased activity of the meprin β overexpressing cells.

Actinonin:

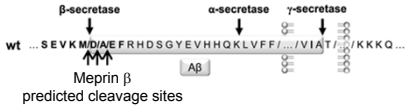
- Actinonin is a bioactive peptide and antibiotic.
- Actinonin is a potent inhibitor of CD13 and Aminopeptidase (leucine aminopeptidase).
- Actinonin has observed to be an inhibitor of PDF (peptide deformylase), meprin α, and thermolysin.

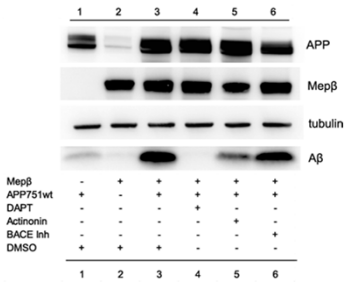


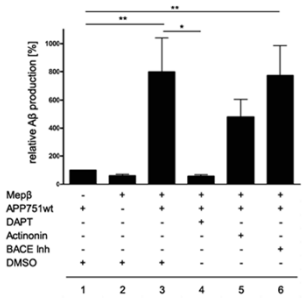
- Inhibition of endogenous meprin β expression reduces Aβ secretion.



Meprin β overexpression increases Aβ production

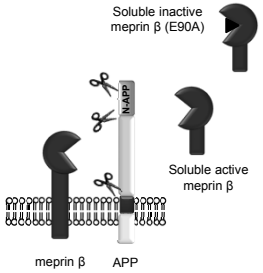


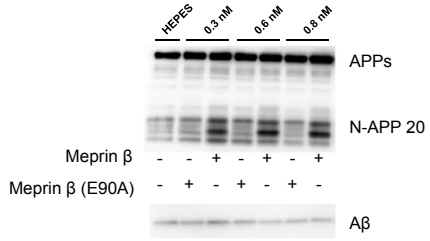




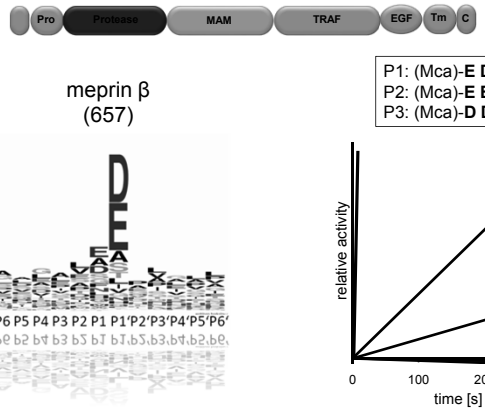
Bien et al. JBC 2012

Cell-bound Meprin β is responsible for Aβ generation

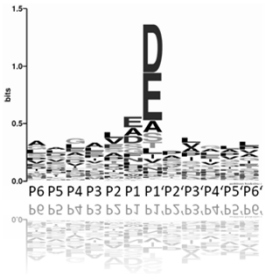




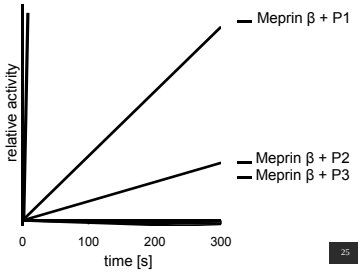
Meprin β cleaves acidic peptides



meprin β
(657)

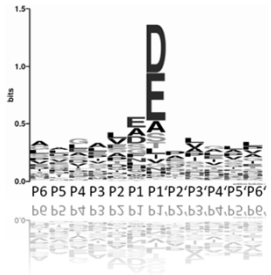


P1: (Mca)-**E D E D E D**-(K-ε-dnp)
P2: (Mca)-**E E E E E E**-(K-ε-dnp)
P3: (Mca)-**D D D D D D**-(K-ε-dnp)

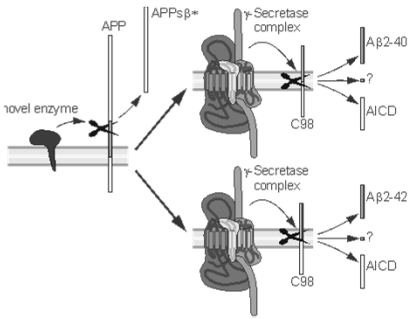


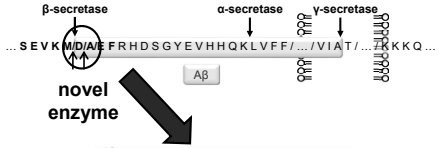
APP may be an ideal target for meprin β

meprin β
(657)

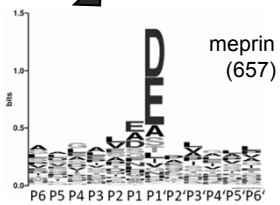


APP processing by a novel enzyme

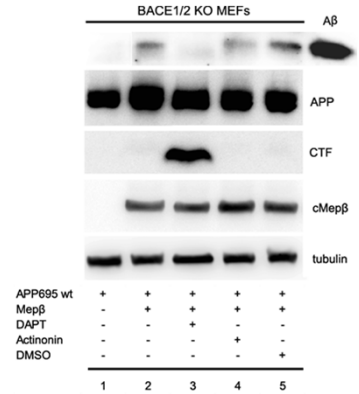


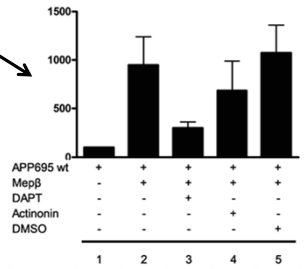


meprin β
(657)



Meprin β generates Aβ-petides in the absence of BACE1

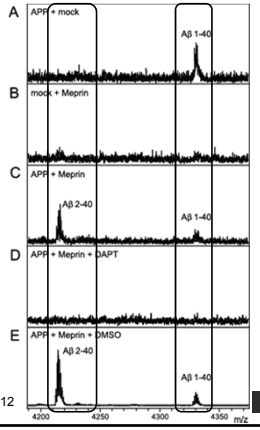




Bien et al. JBC 2012

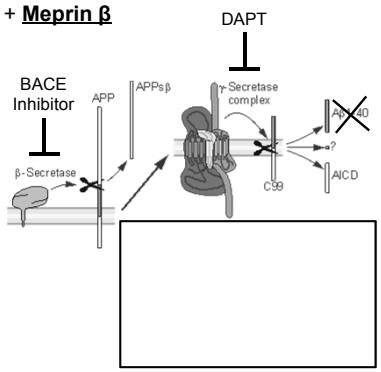
Meprin β generates N-terminal truncated $A\beta_{2\text{-}40}$

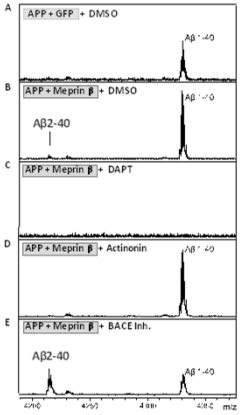
- A: APP695 + Mock → Aβ1-40 ↑
- B: Mock + Meprin β → Aβ ↓
- C: APP695 + Meprin β → Aβ1-40 ↑ + **Aβ2-40** ↑
- D: APP695 + Meprin β + DAPT→ Aβ ↓
- E: APP695 + Meprin β + DMSO → Aβ1-40 ↑ + **Aβ2-40** ↑



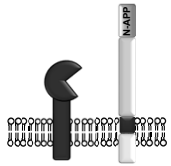
Bien et al. JBC 2012

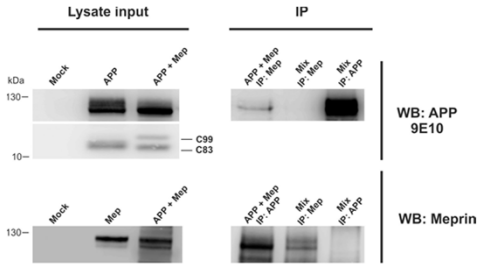
Meprin β generates N-terminal truncated $A\beta_{2\text{-}40}$





APP interacts with Meprin β





- Interaction between Meprin β and APP is specific within the same cell.

Schoenherr et al. 2014; in preparation

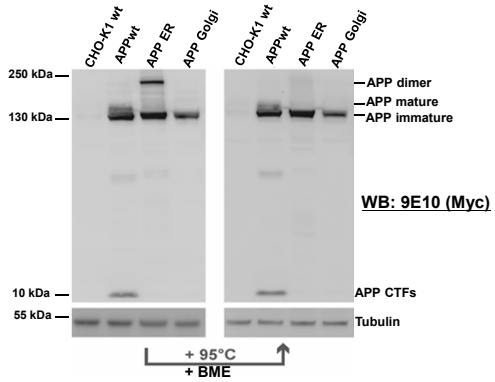
APP can form SDS-resistant dimers in the ER

→ PAGE on 4-12 % BisTris Acrylamide Gels

→ dimers do not dissociate by SDS

→ stable under standard-detergent-lysis conditions

→ suggesting presence of intermolecular S-S bonds generated in ER

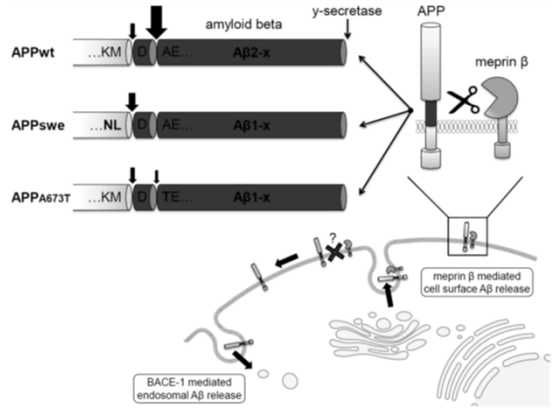


Isbert et al. 2012 Cell Mol Life Sci.

Summary

- Meprin β processes APP *in vivo*.
- Meprin β levels are increased in AD brain.
- Meprin β overexpression increases Aβ-production in the absence of BACE1.
- Meprin β interacts with APP and generates Aβ prior to endosomal compartments.
- Meprin β generates Aβ 2-40 peptides preferentially from APPwt.

Meprin β processes APP



Steffen Storck, Simone Isbert, Roswitha Nehrbaß, Erik Hameister, Johanna Wesselowski, Uta Herrmann, Sabrina Meister, Verena Wolf, Caroline Schönherr, Jessica Bien

- Sascha Weggen (University Düsseldorf)
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