S-3 [11:20-11:50]

A role for presenilin 1 in regulating the delivery of amyloid precursor protein and the maturation of nicastrin

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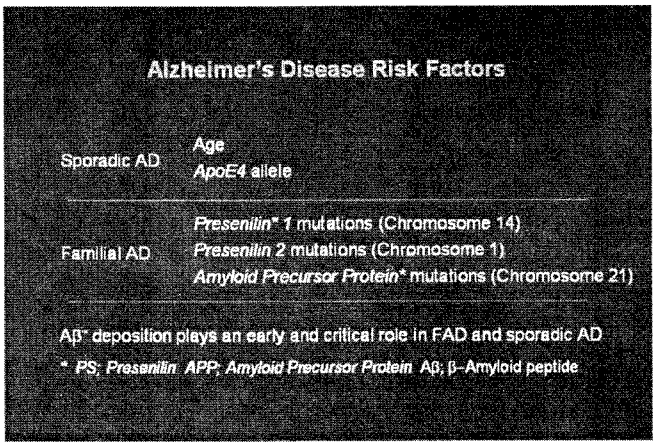
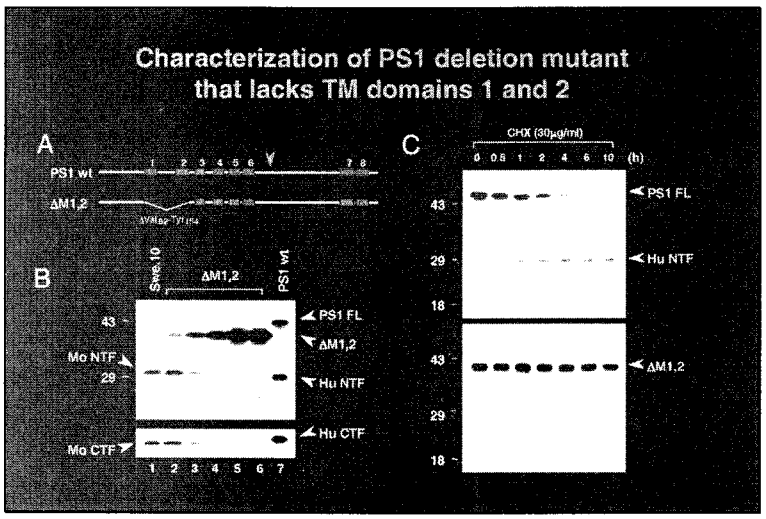
1. A role for presenilin 1 in regulating the delivery of amyloid precursor protein to the cell surface.

Mutations in genes encoding presenilin 1 (PS1) and presenilin 2 (PS2) are causative in the majority of cases of early onset familial Alzheimer's disease (FAD).

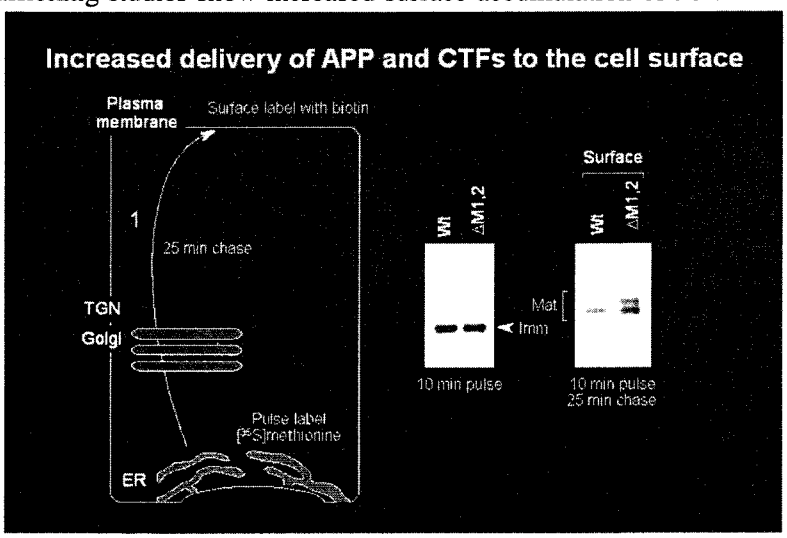


PS1 and PS2 play a critical role in the gamma-secretase processing of amyloid precursor protein (APP) and Notch1. We investigate maturation and intracellular

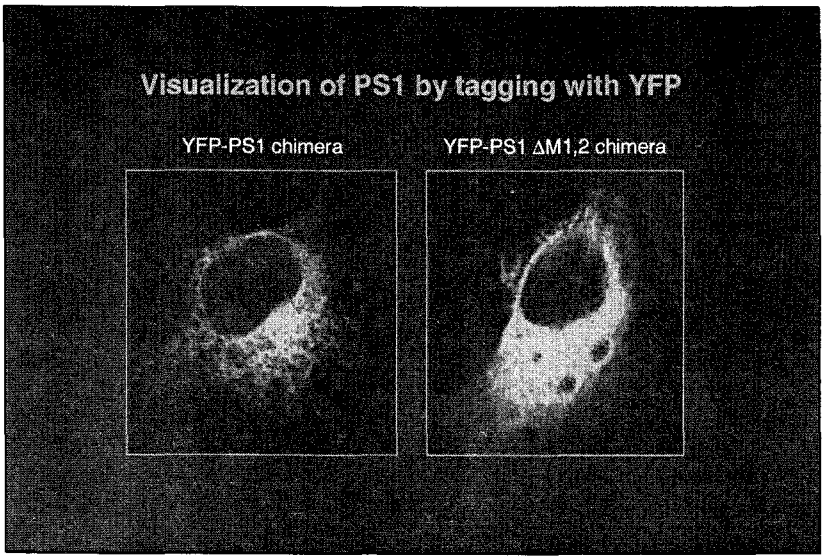
trafficking of APP and other membrane proteins in cells expressing an experimental PS1 deletion mutant (deltaM1,2). Stable expression of deltaM1,2 impairs gamma-secretase processing of Notch1 and delays Abeta (beta-amyloid) peptide secretion.

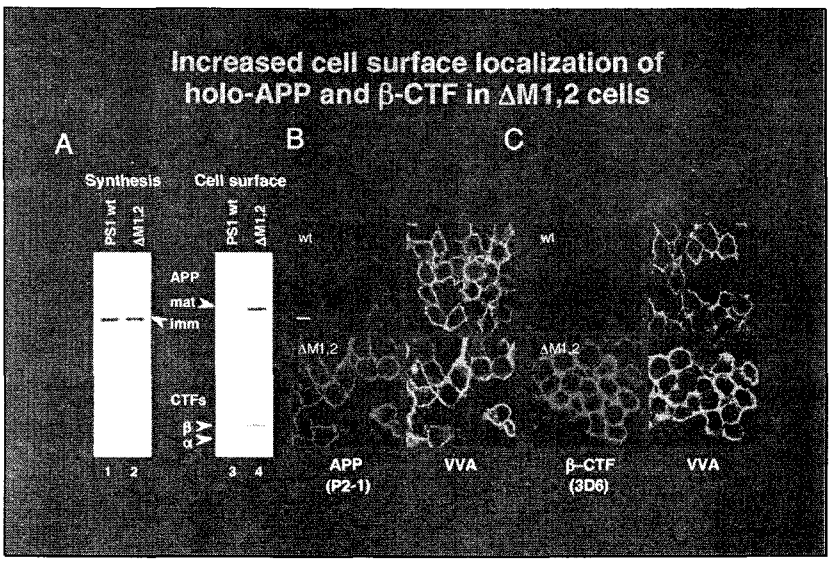


Kinetic studies show enhanced *O*-glycosylation and sialylation of holo-APP and marked accumulation of APP COOH-terminal fragments (CTFs). Surface biotinylation, live staining, and trafficking studies show increased surface accumulation of holo-APP and CTFs in deltaM1,2 cells resulting from enhanced surface delivery of newly synthesized APP.



Expression of a loss-of-function PS1 mutant (D385A) or incubation of cells with gamma-secretase inhibitors also increases surface levels of holo-APP and CTFs. In contrast to APP, glycosylation and surface accumulation of another type I membrane protein, nicastrin, are markedly reduced in deltaM1,2 cells.

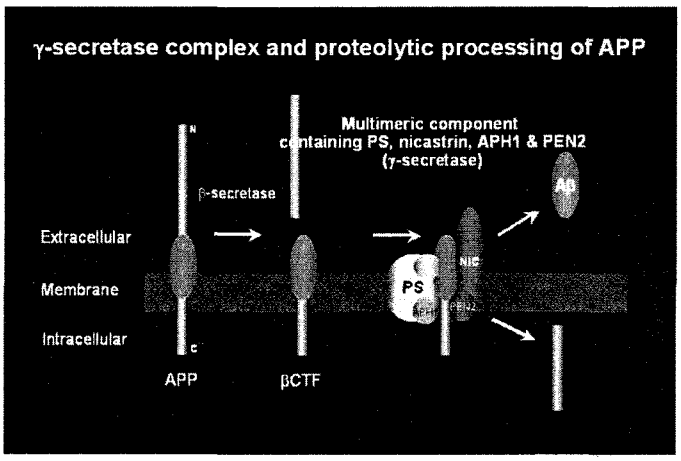




Finally, expression of deltaM1,2 results in the increased assembly and surface expression of nicotinic acetylcholine receptors, illustrating that PS1's influence on protein trafficking extends beyond APP and other type I membrane the glycosylation and intracellular trafficking of APP and select membrane proteins.

2. Presenilin 1 is required for maturation and cell surface accumulation of nicastrin.

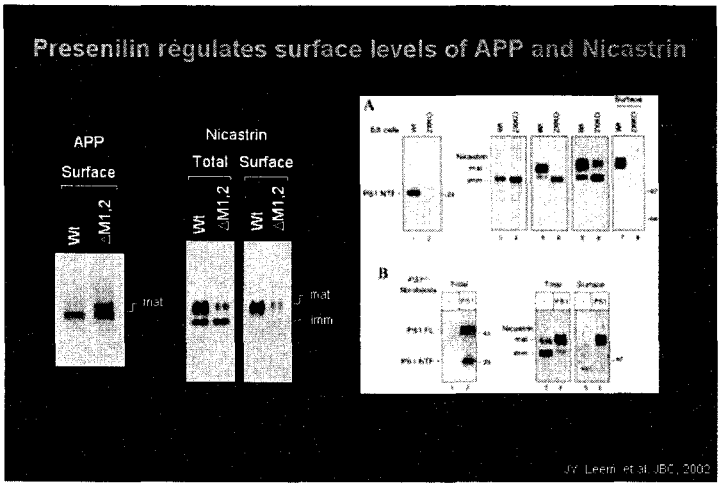
Biochemical and pharmacological evidence support a catalytic or accessory role for PS1 in gamma-secretase cleavage, as well as a regulatory role in select membrane protein trafficking. We demonstrate that PS1 is required for maturation and cell surface accumulation of nicastrin, an integral component of the multimeric gamma-secretase complex.

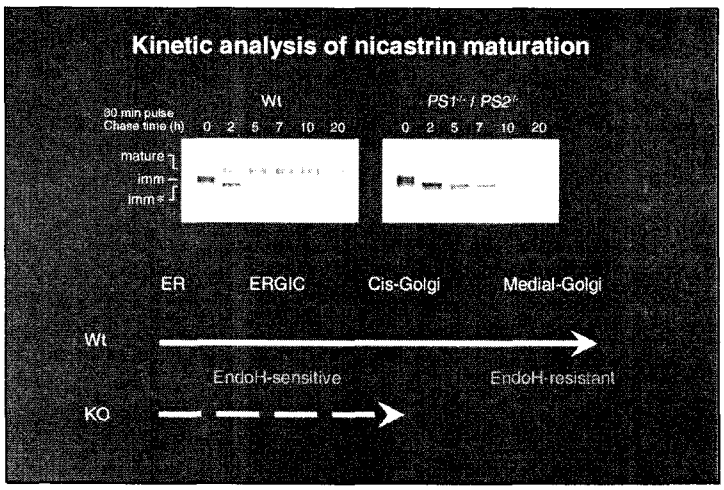


Using kinetic labeling studies we show that in PS1(-/-)/PS2(-/-) cells nicastrin fails to reach the medial Golgi compartment, and as a consequence, is incompletely

glycosylated. Stable expression of human PS1 restores these deficiencies in PS1(-/-) fibroblasts. Moreover, membrane fractionation studies show co-localization of PS1 fragments with mature nicastrin.

These results indicate a novel chaperone-type role for PS1 and PS2 in facilitating nicastrin maturation and transport in the early biosynthetic compartments.





Our findings are consistent with PS1 influencing gamma-secretase processing at

multiple steps, including maturation and intracellular trafficking of substrates and component(s) of the gamma-secretase complex.