



A new molecular explanation for age-related neurodegeneration: The Tyr682 residue of amyloid precursor protein

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Emerging evidence supports the role for the intracellular domains of amyloid precursor protein (APP) in the physiology and function of APP. In this short report, I discuss the hypothesis that mutation of Tyr682 on the Y₆₈₂ENPTY₆₈₇ C-terminal motif of APP may be directly or indirectly associated with alterations in APP functioning and activity, leading to neuronal defects and deficits. Mutation of Tyr682 induces an early and progressive age-dependent cognitive and locomotor decline that is associated with a loss of synaptic connections, a decrease in cholinergic tone, and defects in NGF signaling. These findings support a model in which APP-C-terminal domain exerts a pathogenic function in neuronal development and decline, and suggest that Tyr682 potentially could modulate the properties of APP metabolites in humans.

Keywords:

■ abeta peptides; adaptor proteins; APP; neurodegeneration; NGF; Tyr682; YENPTY domain

Introduction

Amyloid precursor protein (APP) is a member of a family of conserved type 1-membrane proteins, which includes the following members, in mammals: APP-like protein (APLP) 1 and 2; in *Caenorhabditis elegans*: APL-1; and in *Drosophila*: the APP-like (APPL) [1, 2]. Structurally, APP resembles a cell surface receptor with a large ectodo-

main, a single transmembrane domain and a short cytoplasmic tail [1, 2]. In humans, APP is encoded by a gene located on chromosome 21 [1]. Alternative splicing of the APP transcript generates eight isoforms, including the following three predominant isoforms: a 695-residue form expressed primarily in the central nervous system (CNS) and 751- and 770-residue forms that are ubiquitously expressed [1, 3].

Whereas members of the APP family share conserved regions within the ectodomain (E1 and E2) and intracellular tail, the extracellular juxtamembrane region, containing A β sequence, is highly divergent [3]. In particular, the short cytoplasmic tail of APP, Y₆₈₂ENPTY₆₈₇ (residues 682–687 of the APP695 isoform), contains phosphorylation sites and functional motifs that play important roles in the regulation of APP trafficking, metabolism, and function [3]. Tyr682 and Tyr687 are the only APP residues that are phosphorylated in vitro [4]. Tyr682 phosphorylation seems to be critical for APP signaling and activity [5–9] because of its ability to profoundly regulate the APP interaction according to its phosphorylation state. Interestingly, the GY₆₈₂ENPTY₆₈₇ motif is 100% conserved from *C. elegans* to humans (Fig. 1), underlining and reiterating the importance of this sequence for APP, and it functions as an internalization signal for the rapid degradation of APP in lysosomal compartments [10, 11].

Here, I provide compelling evidence for the role of Tyr682 on Y₆₈₂ENPTY₆₈₇ in modulating APP signaling and activity. I discuss findings from the laboratories in which I worked that suggest a striking interplay between APP and nerve growth factor (NGF) in neurons. I suggest that Tyr682 has a crucial role in APP physiology, and I present the attractive hypothesis that alteration of Tyr682 phosphorylation and its consequent binding to target proteins may lead to premature neuronal decline and dementia in humans.

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Abbreviations:

AD, Alzheimer's disease; **CTF**, C-terminal fragment of APP; **Gly**, glycine; **NGF**, nerve growth factor; **TrkA**, NGF/tyrosin kinase receptor; **Tyr**, tyrosine.

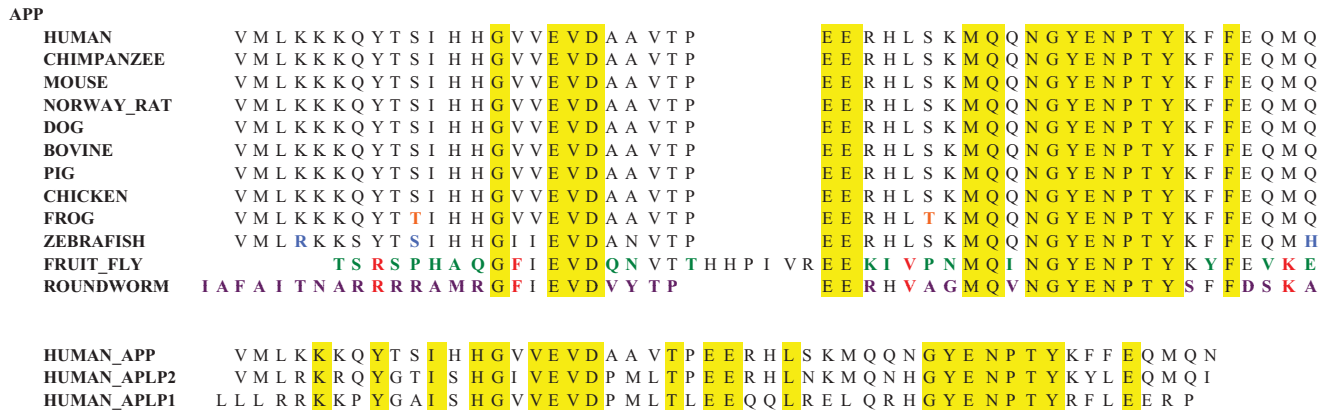


Figure 1. Alignments of (top) the endoplasmic tails of APP homologues from various species and of (bottom) human APP, APLP1, and APLP2.

Phosphorylation of Tyr682 affects binding to the Y₆₈₂ENPTY₆₈₇ domain

The importance of the Y₆₈₂ENPTY₆₈₇ domain in neurons is related to its ability to bind many adaptor proteins.

Phosphorylation of Tyr682 in this domain creates a docking site for some cytosolic proteins containing a Src homology 2 domain, such as Shc, Grb2, and Grb7 [12–15]. Conversely, it completely abolishes binding of other APP-interacting proteins, such as Fe65, Fe65L1, and Fe65L2 [16]. Proteins containing a phosphotyrosine-binding domain (PTB) bind this region of APP in the absence of phosphorylation. These proteins include mammalian disabled mDAB-1 [17], JNK-interacting proteins (JIPs) [18, 19], Munc-18 interacting proteins, Mint (X11) family members (mint1, mint2, and mint3) [20, 21], Fe65 and family members Fe65L1 and L2 [16], the Notch inhibitor Numb [22], Amyloid intracellular domain adaptor-1 (AIDA-1) [23, 24], and the autosomal recessive hypercholesterolemia protein ARH [25]. Essentially the phosphorylation of APP represents a “biochemical switch” that drastically changes the APP “interactome”, abolishing binding of some interactors and creating docking sites for others [14] (Fig. 2).

These interactions activate downstream signaling and regulate APP trafficking and processing. For example, Mint (X11) family members affect APP

processing by stabilizing cellular APP and by modifying both sAPP α and A β generation and secretion [20, 21]. mDAB-1 decreases the production of both β CTF and secreted A β [17]. Jip-1 and Fe65 may mediate amyloid intracellular domain (AID-1) transcriptional activity [26]. JIP-1 functions as an adaptor protein in APP axonal trafficking by bridging APP to kinesin, and this interaction regulates APP-dependent axonal transport of synaptic vesicles [27]. Interestingly, JIP1 is heavily

phosphorylated [27] and facilitates phosphorylation of APP on Thr668 (14 amino acids proximal to the Y₆₈₂ENPTY₆₈₇ motif) through JNK activation [28]. Thr668 phosphorylation also regulates the intracellular interactome of APP. It decreases the binding of Fe65 [29, 30] and creates a docking site for the peptidyl-prolyl cis/trans isomerase, Pin1 [30]. Pin1 regulates APP processing and Pin1^{-/-} mice show Tau and A β -related pathologies in an age-dependent manner, resembling

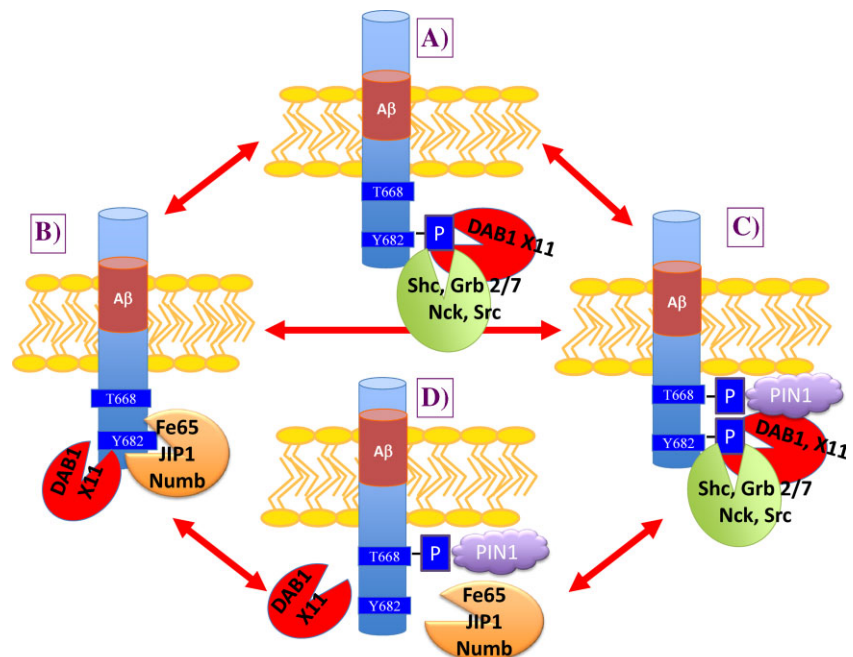


Figure 2. Schematic model representing how adaptor proteins can bind the intracellular APP Y₆₈₂ENPTY₆₈₇ motif. Some proteins, such as for Grb2/7, Shc, Src and Nck interact with APP only when Tyr682 is phosphorylated (A); others, such as Fe65, Jip1, and Numb only when this tyrosine is not phosphorylated (B). DAB1 and X11 bind APP and potentiate its phosphorylation and processing (A–D). Phosphorylation of Thr668 of APP impairs Fe65 interaction while it promotes Pin1 binding (C). Conversely, a further increase in Grb2/7, Shc and Nck binding to Tyr682 occurs when both Tyr682 and Thr668 are phosphorylated (D).

many aspects of human AD, which regulates APP processing [30, 31]. Phosphorylation of Tyr682 and Thr668 are consequential. Phosphorylation of Thr668 of APP impairs Fe65 interaction [30] while it promotes Pin1 binding [31]. Thr668 is followed by a Pro [14], which generates a consensus site for phosphorylation, in APP family members and in other species, except for APLP1 and *Drosophila* APP ortholog [14].

Among all these interacting proteins, only ShcA and Grb2 appear to require the specific tyrosine phosphorylation for binding to APP [32]. CTF, ShcA, and Grb2 amounts are upregulated in the brains of patients with Alzheimer's disease (AD), compared with age-matched non-demented controls [33]. β CTF, but not α CTF, appear to be phosphorylated on Tyr residues from AD brains [15]. These findings may support a new attractive model in which Tyr682 phosphorylation and its binding to adaptors may prevent A β accumulation and deposition in humans. Consistent with this hypothesis, a Tyr682 mutation introduced at the endogenous APP locus by knock-in (KI; Y682G mice) leads to a marked shift toward the non-amyloidogenic pathway in the brain with increased levels of sAPP α and α CTF fragments, unaltered β CTF, and reduced sAPP β and A β 40 levels [7].

NGF defects impact the APP pathway

Recent evidence implicates TrkA, the high affinity NGF receptor, as a crucial molecular actor in the APP pathway [6, 12]. NGF phosphorylates APP on Tyr residue(s) and directly binds APP under physiological conditions. When Tyr682 (Y) is replaced by Gly (G) in Y682G mice, NGF fails to phosphorylate TrkA and does not bind APP. Confocal microscopy indicates that mutation of Tyr682 induces a strong redistribution of APP and TrkA within neurons, with these proteins accumulating in the intracellular and perinuclear compartments [6]. These results indicate a common fate for these two proteins in Y682G mice and highlight the possibility of a consistent interplay in their signaling and activities. Dorsal root ganglia (DRG)

from Y682G mice require NGF to grow and differentiate and mostly die in vitro within a few days after plating [6]. These findings indicate that mutation of Tyr682 also affects NGF-dependent neuronal differentiation and survival. Similarly, the lack of APP and TrkA phosphorylation and the consequent reduction in their binding appear to be related to an age-dependent decrease in the expression levels of TrkA in Y682G mice and to the degeneration of cholinergic fibers with an associated decline in cognitive and neuromuscular performance [9].

It was previously suggested that the concerted phosphorylation of APP on Tyr682 or Tyr687 residues might be relevant to delivering APP to different subcellular compartments and to influencing post-translational modification and sorting [4]. This suggestion is very important considering the role that NGF may play in this mechanism. NGF phosphorylation on Tyr682 may influence interactions between Y₆₈₂ENPTY₆₈₇ and adaptor proteins and may trigger yet unknown signals involved in cellular physiopathology. This relevant crosstalk between NGF and APP fits into a more complex scenario in which an imbalance in NGF/proNGF levels and an alteration in NGF receptor signaling appear to be strictly related to amyloidogenic processing of APP, A β accumulation, and neuronal decline [34–39]. When these observations are translated to animal models of NGF or NGF signaling deficits, the result is an AD-like pathology that reproduces most of the features of AD in humans [40]. We demonstrated that A β accumulation resulting from a deficit in NGF (or from the exogenous addition of synthetic A β peptides) in hippocampal primary cultures induced anomalous NGF-independent TrkA phosphorylation [37]. TrkA switches from pro-survival to pro-death activity in the presence of high A β concentrations, leading to neuronal decline and death [37, 38]. Taken together, these findings suggest a role for TrkA as an effector of APP signaling that also can directly bind APP and modulate its activity via Tyr phosphorylation. These results may have therapeutic implications for AD.

Any therapy aimed at re-establishing the correct balance between NGF defects and the amyloidogenic pathway

may impact AD in humans. The clinical applications of NGF in AD are debatable, and several NGF-mimetic compounds have failed in clinical applications because of difficulties achieving a pharmacologically adequate concentration of NGF in target brain areas while preventing adverse pain effects. Some new insights have emerged with the use of an engineered hNGF (human NGF) molecule with a mutation on residue R100, inspired by the human genetic disease, HSAN V (Hereditary Sensory Autonomic Neuropathy Type V) [41, 42]. This molecule has the potential to reach brain areas at a higher concentration than previous NGF-mimetics without triggering pain. Moreover, hNGF can counteract the AD phenotype in an animal model of AD [41, 42]. Relevantly, a second promising candidate non-peptidic compound with NGF-mimetic properties (MT2) has recently been characterized in an in vitro neuronal model of AD [43]. Further investigations will be necessary to evaluate the clinical relevance of these compounds in human pathology.

The APPY682G mouse model

Insights into the in vivo role of the Y₆₈₂ENPTY₆₈₇ motif in APP physiology came from APP KI mice, in which the Tyr682 residue was replaced by a Gly (APPY682G) [7]. Mutation of Tyr682 affects development and aging in mice and induces synaptic loss, neuronal degeneration, and cognitive and learning deficits in the absence of A β deposition and accumulation [7, 9]. Five-month-old APPY682G mice exhibit synaptic failure and impaired physical resistance in a treadmill test that appear to be consistently associated with deficits in neuromuscular activity, as evaluated by endplate potential. A strong early loss in dendritic spine density was detected in hippocampi from 2-month-old APPY682G mice, suggesting that synaptic impairment precedes behavioral deficits [9] (Fig. 3).

When the Y682G point mutation is introduced into an APLP2^{-/-} background [8], APPY682G/APLP2^{-/-} mutant mice exhibit neuromuscular synapse deficits and early lethality

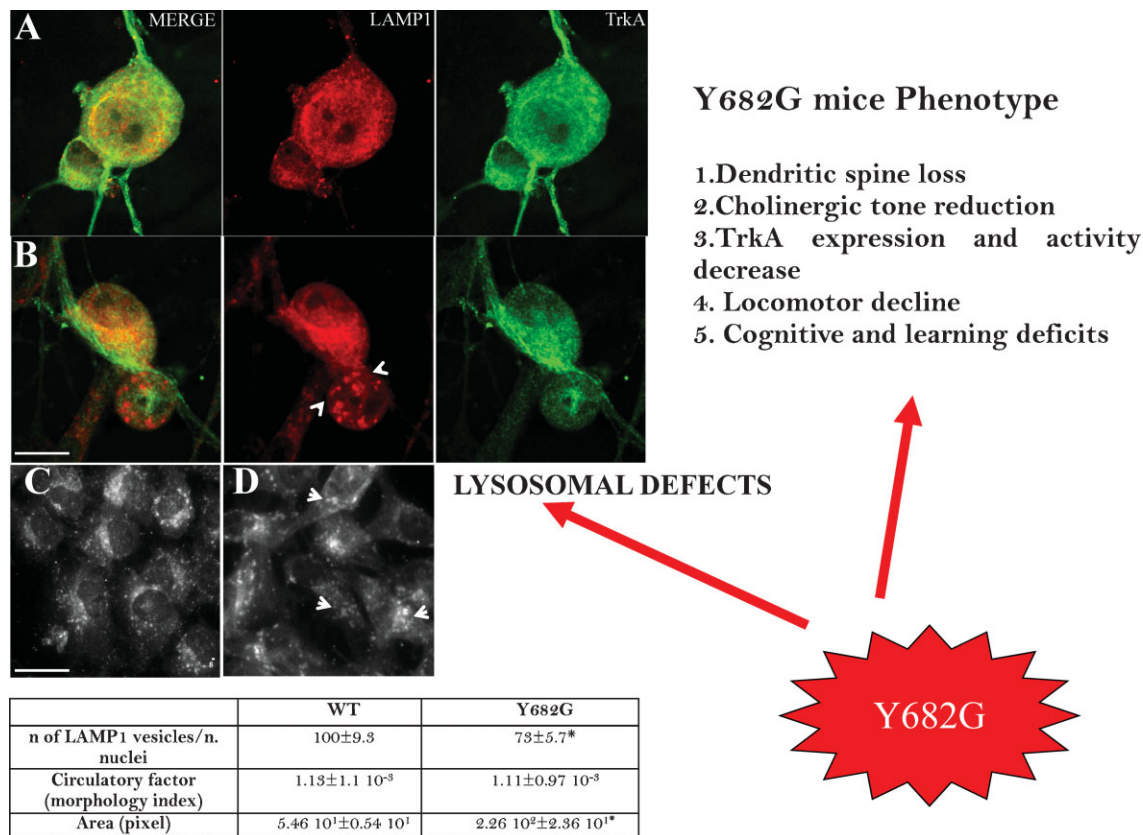


Figure 3. Depiction of AD-like features exhibited by Y682G mice. Confocal microscopy analysis for Lamp1 (red) positive vesicles and TrkA (green) from **A:** WT and **B:** Y682G DRG neurons. Panel C and D report contrast microscopy analysis of LAMP1 from **C:** WT and **D:** Y682G fibroblasts. Table below shows high content analysis (Olympus scan[^]R 2.4 high content screening software) of Lamp1 positive vesicles from hippocampal neurons. The number of Lamp1 positive vesicles are normalized on the basis of the number of DAPI positive nuclei and expressed as % of the correspondent control samples (WT). Circulatory factor is a measure of the Lamp1 vesicle morphology; the area of the vesicles is expressed in pixel. Arrows mark Lamp1-positive vesicles with an increased area. Pictures are representative of six different experiments. Values in the Table are the mean of six different experiments performed in triplicate. * $p < 0.05$. Scale bar: A,B 5 μ M. C,D 8 μ M.

similar to APP/APLP2 double knock-out mice [9]. Similarly, Thr668 (T668A)-mutated KI mice crossed with APLP2^{-/-} do not display any relevant defects during development or in the formation of neuromuscular synapses [44], strongly supporting a specific role of Y682 in such events.

I hypothesized that mutation of Tyr682 might induce a redistribution of endogenous APP into the neurons, leading to a dysfunctional neuronal network. Our findings indicate that APP is distributed in an anomalous pattern in Y682G neurons. APP staining was decreased along the neuritis with aberrant accumulation in the perinuclear and intracellular compartments [6].

I also assessed the morphology, number, and area of Lamp1-positive vesicles to detect defects in the lysosomal system of Y682G neurons. I identified an irregular and unusual increase in the area and a decrease in the number of Lamp1-positive vesicles, suggesting their engulfment or a defect in the normal processing of target proteins (Table 1 in Fig. 3). These data suggest a sorting function that may be regulated by Tyr682 phosphorylation in APP. According to this hypothesis, mutation of Tyr682 may contribute to the persistence of APP in lysosomes. As a result, APP may undergo alternative proteolytic processing, leading to the accumulation of its C-terminal peptides within neurons.

Sorting implies the docking of proteins to specific intracellular trafficking machineries and their adaptors. Mutation of the Tyr682 residue in APP prevents its phosphorylation and may affect the binding of APP to adaptor proteins. This hypothesis underscores the potential significance of the selective regulation of Tyr682 phosphorylation, which may impact APP signaling and processing. Future investigations should aim to identify the proteins involved in these events.

Testing the hypothesis

The results reported here shed new light on the role of Tyr682 in intracellular trafficking, and on the proteolytic processing of APP. It is likely that adaptor proteins, which fail to bind the YENPTY domain, could induce a diversion of APP from its normal transport route and influence APP processing. Consequently, a large-scale screening for adaptor proteins that may be involved in the perturbation of APP trafficking in the Y682G mice model, can assume a crucial clinical relevance.

Recent emerging evidence points to SorLA (SORL1 or LR11), a neuronal sorting protein for APP, and low-density lipoprotein receptor-related protein (LRP) as possible adaptors involved in the APP pathway. SorLA function is to re-route internalized APP molecules from early endosomes back to the Golgi, bypassing delivery of the precursor protein to late endosomes where β -secretase resides [45]. Similarly, LRP binds to APP through Fe65 proteins [46] and mediates A β clearance [47, 48]. Lack of LRP expression causes reduced APP internalization and A β secretion [48–50]. Thus, an abnormal function of these proteins may affect APP trafficking leading to the defects reported in Y682G mice. Accordingly, other adaptor proteins that regulate various steps in the APP route from the cell surface to the intracellular compartments could similarly impact the processing of APP. This is the case with Fe65 (16), Mint1/X11 (20), JIP-1 (18), and others previously discussed.

Conclusion and outlook

Here, I present emerging evidence suggesting a role for the Y₆₈₂ENPTY₆₈₇ motif of APP in the onset of dementia and neuronal degeneration. Results from the laboratories in which I worked suggest that the Tyr682 residue exerts a positive role on the functions of APP and is associated with memory formation and neurotrophic activity.

Remarkably, the features we observed in APPY682G mice have previously been described in mouse models of AD. However, unlike AD models, APPY682G mice exhibit memory, cognitive, and molecular deficits in the absence of A β deposition and accumulation.

These findings highlight an attractive scenario in which Tyr682 may be a crucial actor in APP/A β signaling such that a disruption of APP binding to adaptors, mediated by a Tyr682 mutation or altered phosphorylation status, may lead to neuronal defects and decline. I suggest that alterations in the phosphorylation status and activity of the Y₆₈₂ENPTY₆₈₇ motif deserve more attention and may provide tools to understand the pathogenic mechanisms of dementia in humans.

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References

1. Bayer TA, Cappai R, Masters CL, Beyreuther K, et al. 1999. It all sticks together—the APP-related family of proteins and Alzheimer's disease. *Mol Psychiatry* **4**: 524–8.
2. Breteler MM, Claus JJ, van Duijn CM, Launer LJ, et al. 1992. Epidemiology of Alzheimer's disease. *Epidemiol Rev* **14**: 59–82.
3. Thinakaran G, Koo EH. 2008. Amyloid precursor protein trafficking, processing, and function. *J Biol Chem* **283**: 29615–9.
4. Schettini G, Govoni S, Racchi M, Rodriguez G. 2010. Phosphorylation of APP-CTF-AICD domains and interaction with adaptor proteins: signal transduction and/or transcriptional role—relevance for Alzheimer pathology. *J Neurochem* **115**: 1299–308.
5. Perez RG, Soriano S, Hayes JD, Ostaszewski B, et al. 1999. Mutagenesis identifies new signals for beta-amyloid precursor protein endocytosis, turnover, and the generation of secreted fragments, including Abeta42. *J Biol Chem* **274**: 18851–6.
6. Matrone C, Barbagallo AP, La Rosa LR, Florenzano F, et al. 2011. APP is phosphorylated by TrkA and regulates NGF/TrkA signaling. *J Neurosci* **31**: 11756–61.
7. Barbagallo AP, Weldon R, Tamayev R, Zhou D, et al. 2010. Tyr(682) in the intracellular domain of APP regulates amyloidogenic APP processing in vivo. *PLoS One* **5**: e15503.
8. Barbagallo AP, Wang Z, Zheng H, D'Adamio L. 2011. A single tyrosine residue in the amyloid precursor protein intracellular domain is essential for developmental function. *J Biol Chem* **286**: 8717–21.
9. Matrone C, Luvisetto S, La Rosa LR, Tamayev R, et al. 2012. Tyr682 in the A β -precursor protein intracellular domain regulates synaptic connectivity, cholinergic function, and cognitive performance. *Aging Cell* **11**: 1084–93.
10. Weidemann A, Eggert S, Reinhard FB, Vogel M, et al. 2002. A novel epsilon-cleavage within the transmembrane domain of the Alzheimer amyloid precursor protein demonstrates homology with Notch processing. *Biochemistry* **41**: 2825–35.
11. Lai A, Sisodia SS, Trowbridge IS. 1995. Characterization of sorting signals in the beta-amyloid precursor protein cytoplasmic domain. *J Biol Chem* **270**: 3565–73.
12. Tarr PE, Roncarati R, Pelicci G, Pelicci PG, et al. 2002. Tyrosine phosphorylation of the beta-amyloid precursor protein cytoplasmic

tail promotes interaction with Shc. *J Biol Chem* **277**: 16798–804.

13. Zhou D, Noviello C, D'Ambrosio C, Scaloni A, et al. 2004. Growth factor receptor-bound protein 2 interaction with the tyrosine-phosphorylated tail of amyloid beta precursor protein is mediated by its Src homology 2 domain. *J Biol Chem* **279**: 25374–80.
14. Tamayev R, Zhou D, D'Adamio L. 2009. The interactome of the amyloid beta precursor protein family members is shaped by phosphorylation of their intracellular domains. *Mol Neurodegener* **4**: 28–35.
15. Russo C, Dolcini V, Salis S, Venezia V, et al. 2002. Signal transduction through tyrosine-phosphorylated C-terminal fragments of amyloid precursor protein via an enhanced interaction with Shc/Grb2 adaptor proteins in reactive astrocytes of Alzheimer's disease brain. *J Biol Chem* **277**: 35282–8.
16. Zhou D, Zambrano N, Russo T, D'Adamio L. 2009. Phosphorylation of a tyrosine in the amyloid-beta protein precursor intracellular domain inhibits Fe65 binding and signaling. *J Alzheimer's Dis* **16**: 301–7.
17. Hoe HS, Tran TS, Matsuoka Y, Howell BW, et al. 2006. DAB1 and Reelin effects on amyloid precursor protein and ApoE receptor 2 trafficking and processing. *J Biol Chem* **281**: 35176–85.
18. Scheinfeld MH, Ghersi E, Davies P, D'Adamio L. 2003. Amyloid beta protein precursor is phosphorylated by JNK-1 independent of, yet facilitated by, JNK-interacting protein (JIP)-1. *J Biol Chem* **278**: 42058–63.
19. Matsuda S, Matsuda Y, D'Adamio L. 2003. Amyloid beta protein precursor (AbetaPP), but not AbetaPP-like protein 2, is bridged to the kinesin light chain by the scaffold protein JNK-interacting protein 1. *J Biol Chem* **278**: 38601–6.
20. Borg JP, Yang Y, De Taddéo-Borg M, Margolis B, et al. 1998. The X11alpha protein slows cellular amyloid precursor protein processing and reduces Abeta40 and Abeta42 secretion. *J Biol Chem* **273**: 14761–6.
21. Sastre M, Turner RS, Levy E. 1998. X11 interaction with beta-amyloid precursor protein modulates its cellular stabilization and reduces amyloid beta-protein secretion. *J Biol Chem* **273**: 22351–7.
22. Roncarati R, Sestan N, Scheinfeld MH, Berechid BE, et al. 2002. The gamma-secretase-generated intracellular domain of beta-amyloid precursor protein binds Numb and inhibits Notch signaling. *Proc Natl Acad Sci USA* **99**: 7102–7.
23. Ghersi E, Noviello C, D'Adamio L. 2004. Amyloid-beta protein precursor (AbetaPP) intracellular domain-associated protein-1 proteins bind to AbetaPP and modulate its processing in an isoform-specific manner. *J Biol Chem* **279**: 49105–12.
24. Ghersi E, Vito P, Lopez P, Abdallah M, et al. 2004. The intracellular localization of amyloid beta protein precursor (AbetaPP) intracellular domain associated protein-1 (AIDA-1) is regulated by AbetaPP and alternative splicing. *J Alzheimer's Dis* **6**: 67–78.
25. Noviello C, Vito P, Lopez P, Abdallah M, et al. 2003. Autosomal recessive hypercholesterolemia protein interacts with and regulates the cell surface level of Alzheimer's amyloid beta precursor protein. *J Biol Chem* **278**: 31843–7.

26. **Scheinfeld MH, Roncarati R, Vito P, Lopez PA**, et al. 2002. Jun NH2-terminal kinase (JNK) interacting protein 1 (JIP1) binds the cytoplasmic domain of the Alzheimer's beta-amyloid precursor protein (APP). *J Biol Chem* **277**: 3767–75.
27. **D'Ambrosio C, Arena S, Fulcoli G, Scheinfeld MH**, et al. 2006. Hyperphosphorylation of JNK-interacting protein 1, a protein associated with Alzheimer disease. *Mol Cell Proteomics* **5**: 97–113.
28. **Inomata H, Nakamura Y, Hayakawa A, Takata H**, et al. 2003. A scaffold protein JIP-1b enhances amyloid precursor protein phosphorylation by JNK and its association with kinesin light chain 1. *J Biol Chem* **278**: 22946–55.
29. **Fiore F, Zambrano N, Minopoli G, Donini V**, et al. 1995. The regions of the Fe65 protein homologous to the phosphotyrosine interaction/phosphotyrosine binding domain of Shc bind the intracellular domain of the Alzheimer's amyloid precursor protein. *J Biol Chem* **270**: 30853–6.
30. **Pastorino L, Sun A, Lu PJ, Zhou XZ**, et al. 2006. The prolyl isomerase Pin1 regulates amyloid precursor protein processing and amyloid-beta production. *Nature* **440**: 528–34.
31. **Balastik M, Lim J, Pastorino L, Lu KP**. 2007. Pin1 in Alzheimer's disease: multiple substrates, one regulatory mechanism? *Biochim Biophys Acta* **1772**: 422–9.
32. **Russo C, Venezia V, Repetto E, Nizzari M**, et al. 2005. The amyloid precursor protein and its network of interacting proteins: physiological and pathological implications. *Brain Res Brain Res Rev* **48**: 257–64.
33. **Russo C, Salis S, Dolcini V, Venezia V**, et al. 2001. Amino-terminal modification and tyrosine phosphorylation of [corrected] carboxy-terminal fragments of the amyloid precursor protein in Alzheimer's disease and Down's syndrome brain. *Neurobiol Dis* **8**: 540–8.
34. **Capsoni S, Brandi R, Arisi I, D'Onofrio M**, et al. 2011. A dual mechanism linking NGF/proNGF imbalance and early inflammation to Alzheimer's disease neurodegeneration in the AD11 anti-NGF mouse model. *CNS Neurol Disord Drug Targets* **10**: 635–47.
35. **Matrone C, Di Luzio A, Meli G, D'Aguzzano S**, et al. 2008. Activation of the amyloidogenic route by NGF deprivation induces apoptotic death in PC12 cells. *J Alzheimer's Dis* **13**: 81–96.
36. **Matrone C, Ciotti MT, Mercanti D, Marolda R**, et al. 2008. NGF and BDNF signaling control amyloidogenic route and Abeta production in hippocampal neurons. *Proc Natl Acad Sci USA* **105**: 13139–44.
37. **Matrone C, Marolda R, Ciafrè S, Ciotti MT**, et al. 2009. Tyrosine kinase nerve growth factor receptor switches from prosurvival to proapoptotic activity via Abeta-mediated phosphorylation. *Proc Natl Acad Sci USA* **106**: 11358–63.
38. **Calissano P, Amadoro G, Matrone C, Ciafrè S**, et al. 2010. Does the term 'trophic' actually mean anti-amyloidogenic? The case of NGF. *Cell Death Differ* **17**: 1126–33.
39. **Cattaneo A, Calissano P**. 2012. Nerve growth factor and Alzheimer's disease: new facts for an old hypothesis. *Mol Neurobiol* **46**: 588–604.
40. **Capsoni S, Cattaneo A**. 2006. On the molecular basis linking nerve growth factor (NGF) to Alzheimer's disease. *Cell Mol Neurobiol* **26**: 619–33.
41. **Capsoni S, Marinelli S, Ceci M, Vignone D**, et al. 2012. Intranasal "painless" human Nerve Growth Factors slows amyloid neurodegeneration and prevents memory deficits in App X PS1 mice. *PLoS One* **7**: e37555.
42. **Capsoni S, Covaceuszach S, Marinelli S, Ceci M**, et al. 2011. Taking pain out of NGF: a "painless" NGF mutant, linked to hereditary sensory autonomic neuropathy type V, with full neurotrophic activity. *PLoS One* **28**: e17321.
43. **Scarpi D, Cirelli D, Matrone C, Castronovo G**, et al. 2012. Low molecular weight, non-peptidic agonists of TrkA receptor with NGF-mimetic activity. *Cell Death Dis* **3**: e339.
44. **Barbagallo AP, Wang Z, Zheng H, D'Adamo L**. 2011. The intracellular threonine of amyloid precursor protein that is essential for docking of Pin1 is dispensable for developmental function. *PLoS One* **6**: e18006.
45. **Fjorback AW, Andersen OM**. 2013. SorLA is a molecular link for retromer-dependent sorting of the amyloid precursor protein. *Commun Integr Biol* **5**: 616–9.
46. **Trommsdorff M, Borg JP, Margolis B, Herz J**. 1998. Interaction of cytosolic adaptor proteins with neuronal apolipoprotein E receptors and the amyloid precursor protein. *J Biol Chem* **273**: 33556–60.
47. **Narita M, Holtzman DM, Schwartz AL, Bu G**. 1997. Alpha2-macroglobulin complexes with and mediates the endocytosis of beta-amyloid peptide via cell surface low-density lipoprotein receptor-related protein. *J Neurochem* **69**: 1904–11.
48. **Deane R, Wu Z, Sagare A, Davis J**, et al. 2004. LRP/amyloid beta-peptide interaction mediates differential brain efflux of Abeta isoforms. *Neuron* **43**: 333–44.
49. **Ulery PG, Beers J, Mikhailenko I, Tanzi RE**, et al. 2000. Modulation of beta-amyloid precursor protein processing by the low density lipoprotein receptor-related protein (LRP). Evidence that LRP contributes to the pathogenesis of Alzheimer's disease. *J Biol Chem* **275**: 7410–5.
50. **Pietrzik CU, Busse T, Merriam DE, Weggen S**, et al. 2002. The cytoplasmic domain of the LDL receptor-related protein regulates multiple steps in APP processing. *EMBO J* **21**: 5691–700.