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Review

The $Y_{682}ENPTY_{687}$ motif of APP: Progress and insights toward a targeted therapy for Alzheimer's disease patients

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ABSTRACT

Alzheimer's disease (AD) is a devastating neurodegenerative disorder for which no curative treatments, disease modifying strategies or effective symptomatic therapies exist. Current pharmacologic treatments for AD can only decelerate the progression of the disease for a short time, often at the cost of severe side effects. Therefore, there is an urgent need for biomarkers able to diagnose AD at its earliest stages, to conclusively track disease progression, and to accelerate the clinical development of innovative therapies.

Scientific research and economic efforts for the development of pharmacotherapies have recently homed in on the hypothesis that neurotoxic β-amyloid (Aβ) peptides in their oligomeric or fibrillary forms are primarily responsible for the cognitive impairment and neuronal death seen in AD. As such, modern pharmacologic approaches are largely based on reducing production by inhibiting β and γ secretase cleavage of the amyloid precursor protein (APP) or on dissolving existing cerebral Aβ plaques or to favor Aβ clearance from the brain.

The following short review aims to persuade the reader of the idea that APP plays a much larger role in AD pathogenesis. APP plays a greater role in AD pathogenesis than its role as the precursor for Aβ peptides: both the abnormal cleavage of APP leading to Aβ peptide accumulation and the disruption of APP physiological functions contribute to AD pathogenesis.

We summarize our recent results on the role played by the C-terminal APP motif -the $Y_{682}ENPTY_{68}$ motif- in APP function and dysfunction, and we provide insights into targeting the Tyr_{682} residue of APP as putative novel strategy in AD.

1. Introduction

1.1. The scientific problem

Alzheimer's disease (AD) is the single most common form of dementia, which currently results in death for all diagnosed patients given that there is no cure and no effective treatments. Over 30 million people suffer from AD worldwide, with a projected rate of diagnosis of 1 in 85 by 2050 (https://www.alz.org/alzheimers-dementia/facts-figures).

The amyloidogenic hypothesis posits that amyloid β (Aβ) peptides, which are protease cleavage products of the type I transmembrane amyloid precursor protein (APP) by β-site APP-cleaving enzyme 1 (BACE1; also known as β-secretase) and γ-secretase, are causative of AD (Esteras-Chopo et al., 2005; Hardy and Selkoe, 2002; Karran and De Strooper, 2016; Makin, 2018; Selkoe et al., 2012). Aβ peptides, detectable in senile plaques in AD patient brains as well as in highly neurotoxic small oligomeric Aβ-aggregates, are considered to be central to AD etiology (Selkoe, 2004). Mutations in the genes encoding APP or presenilins (PSEN1 and 2), the alternative catalytic subunits of the γ secretase complex, cause autosomal dominance and early-onset familial AD (FAD) by increasing Aβ production (Karch et al., 2014; Gómez-Isla et al., 1999; Van Cauwenberghe et al., 2016). Conversely, the APP mutation (A673 T) that prevents β-secretase-mediated cleavage of APP and, consequently, reduces Aβ levels, protects against the risk of late-onset sporadic AD (Jonsson et al., 2012; Maloney et al., 2014).

The tau microtubule associated protein, considered the other critical component for AD development, forms neurotoxic hyperphosphorylated neurofibrillary tangles that purportedly function downstream of Aβ, thus exacerbating Aβ neurotoxicity (Selkoe et al., 2012).

To date, there have been over completed or in-process 1500 clinical trials for AD treatments targeting Aβ and tau neurotoxicity. Unfortunately, none of these trials have demonstrated clinical effects in

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Table 1
Results of PubMed database search using key words such as “APP and YENPTY” and “APP and Tyr/Tyrosine 682 phosphorylation”.

Authors	Experimental Model	Main Findings
(Koo and Squazzo, 1994)	*CHO cell line*	The NPTY motif of APP controls APP endocytosis and influences production and release of Aβ.
(Fiore et al., 1995)	*PC12 cell line*	The C-terminal region of Fe65 protein binds the YENPTY motif of APP.
(Lai et al., 1995)	*CHO cell line*	The two tyrosines on the YENPTY cytoplasmic tail of APP function as internalization signals and mediate APP trafficking from the cell surface toward the endocytic pathway.
(Aplin et al., 1996)	*In vitro assay*	Glycogen synthase mediates the phosphorylation of APP cytoplasmic domain.
(Borg et al., 1996)	*HEK293 and HER14 cell lines*	X11 protein binds the YENPTY motif of APP
(Bressler et al., 1996)	*In vitro assay*	
(McLoughlin and Miller, 1996)	*In vitro assay*	Binding analysis between proteins containing phosphotyrosine-binding domain (PTB) and the YENPTY motif of APP.
(Borg et al., 1998)	*HEK293 cell line*	X11-a factor binds the YENPTY motif, regulates APP processing and reduces Ab production and secretion.
(Duilio et al., 1998)	*HEK293 and HER14 cell lines*	Fe65L2 -a member of the Fe65 protein family- interacts with the intracellular YENPTY motif of APP.
(Sastre et al., 1998)	*N2a and HEK 293 cell lines*	X11 interacts with the YENPTY motif ofAPP and affects APP processing and Ab production.
(Ando et al., 1999)	*PC12 cell line*	APP phosphorylation on Thr668 residue triggers neuronal differentiation of PC12 cell line.
(Guénette et al., 1999)	*HEK293*	hFE65L influences APP maturation and secretion
(Perez et al., 1999)	*CHO cell line*	YENPTY motif of APP influences endocytosis, turnover, and generation of secreted Ab fragments
(Watanabe et al., 1999)	*Brain tissues*	UV-DDB protein binds to the cytoplasmic domain of APP
(Iijima et al., 2000)	*Rat hippocampal neuronal cultures*	Cyclin-dependent kinase 5 phosphorylates neuronal APP on the YENPTY motif
(Mueller et al., 2000)	*HEK293 cell line*	X11alpha and Mint-1 adaptor proteins modulate APP metabolism by interacting with the YENPTY motif.
(Ando et al., 2001)	*HEK293 cell line*	APP-Fe65 interaction results in the phosphorylation of APP on the YENPTY motifand affects A production
(Cao and Südhof, 2001)	*COS7, HEK293, PC12 cell lines*	Tip60 forms a complex with Fe65 and the YENPTY motif ofAPP.
(Cupers et al., 2001)	*Knockout mice tissues; Primary neuronal cultures*	The cytoplasmic APP fragments are degraded rapidly and they are partially located in the nuclear fraction.
(Kimberly et al., 2001)	*Cos7cell line*	Fe65 stabilizes the cytoplasmic APP fragment and favors APP translocation to the nucleus.
(Matsuda et al., 2001)	*COS7 cells and in vitro assays*	JNK-interacting protein-1 (JIP-1b) and its human homolog IB1 bind the cytoplasmic APP fragment
(Minopoli et al., 2001)	*Cos7 and PC12 cell lines*	The phosphorylation of the cytoplasmic APP fragment prevents Fe65 translocation to the nucleus
(Nunan et al., 2001)	*Transgenic mice tissues; Primary neuronal culture*	The C-terminal fragment of APP is degraded by a proteasome-dependent mechanism
(Ramelot and Nicholson, 2001)	*In vitro assay*	NMR analysis of phosphorylation-induced structural changes in the APP cytoplasmic tail
(Zambrano et al., 2001)	*Cos7cell line*	Abl protoncogene phosphorylates APP on tyrosine residues.
(Biederer et al., 2002)	*HEK 293 and HER14 cell line*	Differential transcription functions of Mint isoforms on APP gene.
(Edbauer et al., 2002)	*HEK 293 cell line*	Insulin-degrading enzyme is responsible for the rapid degradation of the APP intracellular domain (AICD)
(Roncarati et al., 2002)	*HEK 293, HeLa cell lines; Primary cultures of cortical neurons*	The C-terminal fragment of APP binds Numb and inhibits Notch signaling
(Russo et al., 2002)	*Primary cultures of rat cortical neurons and astrocytes; Human brain tissues*	Tyrosine-phosphorylated APP C-terminal fragments interact with Shc/Grb2 adaptor proteins.
(Tarr et al., 2002b)	*HEK293T, COS7 and N2a cell lines*	Tyrosine phosphorylation of APP cytoplasmic tail promotes interaction with Shc.
(Tarr et al., 2002a)	*HEK293 cell line*	NGF promotes tyrosine phosphorylation and processing of APP via TrkA activation.
(Taru et al., 2002)	*CHO cell lines*	JIP1b and JIP2 bind the cytosolic tail of APP.
(Bergman et al., 2003)	*CHOPro5 and HEK293 cell lines*	Mutations on the presenilin gene (FAD mutation) do not influence the production of APP C-terminal peptides.
(Hill et al., 2003)	*HeLA cell line*	Mint adaptor proteins bind the YENPTY motif of APP and enhances the APP amyloidogenic processing.
(Kim et al., 2003)	*HEK293 cell line*	The overexpression of the C-terminal fragments of APP increases the levels and the promoter activity of GSK-3β protein.
(King et al., 2003)	*HEK293 cell line*	X11a binds the YENPTY motif and regulates the secretory, endocytic trafficking and metabolism of APP
(Nunan et al., 2003)	*CHO cell line*	The proteasomal cleavage of the cytosolic domain of APP at the YENPTY motif prevents the g secretase processing, and consequently reduces Ab production.
(Scheinfeld et al., 2003b)	*HEK293T and N2a cell lines*	JNK-interacting protein-1 increases transcription of APP but not APP-like protein, following a mechanism different than that of Fe65
(King and Scott Turner, 2004)	*Review article*	Review discussing the role of adaptor protein interactions in modulating amyloid precursor protein metabolism and Alzheimer's disease.
(Zambrano et al., 2004)	*HEK293, HeLa, and HeLaAG cell lines*	Fe65 and Shc bind the YENPTY motif of APP and influence APP processing by two different mechanisms: Fe65 induces the caspase-dependent cleavage of APP, whereas Shc triggers the PDGF-mediated APP processing.
(Fassa et al., 2005)	*HEK293 cell line*	Notch 1 interacts with the YENPTY motif of APP in a Numb-independent manner
(Kerr and Small, 2005)	*Review article*	Review discussing the role of the cytoplasmic domain of the beta-amyloid protein precursor in the function, regulation of proteolysis, and implications for drug development of Alzheimer's disease.
(Russo et al., 2005)	*Review article*	Review discussing the physiological and pathological implications of the amyloid precursor protein and its network of interacting proteins.

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Table 1 (*continued*)

Authors	Experimental Model	Main Findings
(Xie et al., 2005)	*H4 cell line*	X11alpha and X11beta bind the YENPTY motif of APP although they play a different role in APP processing and Ab production
(Ozaki et al., 2006)	*U2OS and H4 cell line*	The C-terminal domain of APP enhances p53-mediated apoptosis
(Nakaya and Suzuki, 2006)	*Neuro-2a (N2a) cells and mutant mice*	The phosphorylation of APPmodulates Fe65 intracellular localization and translocation to the nucleus with the consequent activation of AICD related genes.
(Pastorino et al., 2006)	*Knock out mice tissues*	The prolyl isomerase Pin1 binds the YENPTY motif of APP andregulates APP processing and Ab production
(Shaked et al., 2006)	*N2a and B103 cell lines*	Ab induces neuronal toxicity by interacting directly and specifically with membrane-bound APP and by facilitating the oligomerization of b-secretase cleaved APP C-terminal fragments. The YENPTY domain in the APP is critical for this cell death pathway.
(Parisiadou and Efthimiopoulos, 2007)	*HEK 293 and U251 cell lines*	mDab1 binds to the C-terminal tail of APP and prevents the APP amyloidogenic processing. X11alpha and mDab1 exert opposing effects on APP processing and Ab production.
(Ring et al., 2007)	*APP knock out mice and primary neuronal cultures.*	US" > Mice lacking of the YENPTY motif show reduced turnover of holoAPP, increased cell surface expression, and strongly reduced AUS" > bUS" > levels in brain.
(Yoon et al., 2007)	*US" > Primary rat cortical neuronal cultures*	US" > The okadaic acid-induced neurodegeneration results in the proteolytic processing of mint-1 and mint-2, and in the APP accumulation and phosphorylation.
(Xie et al., 2007)	*H4* neuroglioma *cell line*	US" > The lack of ShcC (but not ShcA) reduces APP-C-terminal and Ab levels. Fe65 absence increases APP-C-terminal levels, although Ab levels remain decreased.
(Venugopal et al., 2007)	*In vitro assay*	APP cytoplasmic fragments are degraded by insulysin (AbUS" > degrading enzyme)
(Beel et al., 2008)	*Cell line*	Cholesterol binds to APP-C-terminal (C99) fragment and influences APP trafficking and Ab production
(Hoe et al., 2008)	*COS7 and HEK293 cell lines*	Fyn phosphorylates Dab1 and enhances APP and ApoE receptor 2 processing
(McLoughlin and Miller, 2008)	*Review article*	Review discussing the role of FE65 proteins in Alzheimer's disease
(Müller et al., 2008)	*Review article*	Review discussing the amyloid precursor protein intracellular domain (AICD) as modulator of gene expression, apoptosis, and cytoskeletal dynamics in Alzheimer's disease
(Takahashi et al., 2008)	*HEK 293 cell lines*	Tyr687 phosphorylation on the the YENPTY motif of APP favors the non-amyloidogenic APP processing
(Waldron et al., 2008)	*CHO K1 and HEK 293 cell lines*	Increased AICD generation does not result in increased nuclear translocation or activation of target genes
(Austin et al., 2009)	*Endothelial cells*	Amyloid precursor protein mediates a tyrosine kinase-dependent activation response
(Ghosal et al., 2009)	*Transgenic mice tissues*	AICD overexpression induces hyperphosphorylation and aggregation of tau, neurodegeneration and working memory deficits and increased expression levels of GSK-3b.
(Shaked et al., 2009)	*B103 cell line; Human brain tissues*	Interactions between YENPTY motif of APP and G proteins influence calcium dysregulation and Ab toxicity
(Tamayev et al., 2009)	*Mammalian cell line*	Tyr682 and Thr668 are crucial for APP interactome shapingUS" >
(Zhou et al., 2009)	*HEK293 cell line*	Tyrosine phosphorylation on the YENPTY motif of APP inhibits Fe65-APP binding and Fe65 signaling
(Barbagallo et al., 2010)	*Knock-in mice brain tissues and primary neuronal culture*	US" > Mutation of Tyr682 in the intracellular domain of APP prevents amyloidogenic APP processing
(Buoso et al., 2010)	*Review article*	Review discussing the beta-Amyloid precursor protein metabolism on the functions and degradation of its intracellular domain
(Schettini et al., 2010)	*Review article*	Review discussing the phosphorylation of CTF-AICD domains and the interaction with adaptor proteins as signal transduction and/or transcriptional mediator of APP activity
(Barbagallo et al., 2011)	*Knock-in mice brain tissues; Primary neuronal culture*	Mutant knock-in mice carrying mutation on Tyr682 residue on the YENPTY motif show defect in development
(Matrone et al., 2011)	*US" > Knock-in mice tissues; Primary neuronal culture*	Phosphorylation of Tyr682 residue on the YENPTY motif is crucial for NGF signaling
(Xu et al., 2011)	*SK–N-SH, SK–N–AS and HeLA cell lines*	MED12/Mediator binds to AICD and activates the expression of AICD target genes
(Beyer et al., 2012)	*NEURO-2A and SH-SY5Y cell lines; Primary rat hippocampal neurons; Human brain tissues*	GULP1 interacts with the YENPTY motif of APP and alters trafficking and processing of APP
(Beckett et al., 2012)	*Review article*	Review discussing the AICD “enigma” in respect of APP nuclear signaling and transcriptional activity
(Matrone et al., 2012)	*Knock-in mice brain tissues; Primary neuronal culture*	Tyr682 residue on the YENPTY motif regulates synaptic connectivity, cholinergic function, and cognitive performance
(Xie et al., 2012)	*H4 cell line*	Lack of Dab and Numb -YENPTY adaptor proteins- reduce the γ-secretase mediated APP processing and prevent Aβ production.
(Caster and Kahn, 2013)	*HeLA cell line*	Mint3 binds the Tyr682 on the YENPTY motif and regulates APP trafficking from the Golgi to lysosomal compartments.
(Matrone, 2013)	*Review article*	A review discussing the role of Tyr682 residue of amyloid precursor protein in age-related neurodegeneration
(Riese et al., 2013)	*HEK293 cell line*	Bimolecular fluorescence complementation procedure used to visulize AICD-Fe65 and Tip60 interactions in the nucleus.
(Milosch et al., 2014)	*Knock-out mice and Primary hippocampal neurons*	sAPPα neuroprotection via G-protein-coupled activation of the Akt pathway does not require YENPTY motif.
(Klevanski et al., 2015)	*Knock-out mice*	The C-terminus domain of APP is essential for neuromuscular morphology and functions as well as for normal nervous system activities such as synaptic plasticity, spatial learning, and memory.
(Nhan et al., 2015)	*Review article*	A review discussing “friends and foes” of amyloid precursor protein and its proteolytic fragments.
(Poulsen et al., 2015)	*Knock-in mice brain tissues; Primary neuronal culture*	Tyr682 residue of APP is essential for the Clathrin and Adaptor Protein 2 binding to the YENPTY motif.

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Table 1 (*continued*)

Authors	Experimental Model	Main Findings
(Yáñez et al., 2016)	*Mutant and knock-out mice CHO cell line*	Tyr682 is crucial for the binding of c-Abl to APP. The inhibition of c-Abl prevents Aβ production and reduces levels of Aβ oligomers and of the carboxy-terminal fragment βCTF
(Guénette et al., 2017)	*Review article*	"APP Protein Family Signaling at the Synapse: Insights from Intracellular APP-Binding Proteins"
(Poulsen et al., 2017)	*Human neural stem cells from AD patients and healthy controls*	Excessive phosphorylation of APP Tyr residue(s) impairs the APP binding to the Clathrin endocytic complex and affects the APP trafficking in neurons from AD patients.
(Krasinski et al., 2018)	*In vitro assay*	The C-terminal APP fragment AICD57 forms micelle-like assemblies that are proteolyzed by insulin-degrading enzyme (IDE) particularly at level of the YENPTY motif.
(Cheng et al., 2019)	*Transgenic mice brain tissues; Primary neuronal culture*	Cofilin induces phosphorylation of APP C-terminal fragments and facilitates their nuclear translocation.

reversing, slowing or stopping cognitive decline and neuronal deficits in patients, thus raising skepticism among scientists with regards to the influence of Aβ as well as tau protein as necessary components AD etiology (Ghosal et al., 2009; Hardy and Selkoe, 2002; Karran and De Strooper, 2016; Nalivaeva and Turner, 2013). This concern seems to be heightened by the frequent lack of correspondence between the occurrence of brain amyloid deposits and cognitive and memory deficits in patients (Rabinovici, 2015; Armstrong, 2014).

Indeed, what seems plausible, considering the abundance of unsuccessful trials, is that Aβ plaques form within the brain at a later stage in AD development, suggesting that there is a point at which the disease becomes irreversible; that is, when reducing Aβ production and Aβ accumulation is not enough to rescue neuronal functions and the neurodegenerative processes progress independently from Aβ production, patients become unresponsive to any type of intervention.

It is therefore essential to prevent the pathology long before patients present with any clinical symptoms, certainly prior to the detection of Aβ plaques or neurofibrillary tangles. This means that it is necessary to find biomarkers to use as a screening tool that can detect the earliest biochemical signs of AD in people and thereby provide evidence of the disease prior to the presentation of obvious clinical symptoms, which is difficult when considering that a conclusive and exhaustive diagnosis of AD is only possible after a neuropathological examination of brain autopsy and that the symptoms of AD both start out very mildly and progress slowly, making it difficult for families and clinicians to detect any deterioration until well after the disease has progressed.

The ability to find biomarkers is even more challenging because of the lack of an animal model that accurately mimics all the features of AD neuropathology, such as Aβ deposition, synapse loss, inflammation, tau hyperphosphorylation, and the presence of neurofibrillary tangles. The currently available animal models of AD harbor mutations in genes encoding APP, PSEN1-2 or tau protein, which collectively account for < 5% of disease cases, thus prioritizing early-onset and familial forms of human AD and disregarding the vast majority of AD cases that are multifactorial and sporadic. This leads to data that are unequivocally difficult to translate to patients, which potentially explains why promising therapies in animal models frequently fail to replicate the same results in human clinical trials. Therefore, the role of APP in the onset or progression of AD requires a better understanding of its physiological function the malfunction thereof in AD, including a clear comprehension of the structure of the entire APP and its structural interactions with other proteins.

2. Methods

2.1. Search strategy

Review articles discussing recent advances in the amyloidogenic hypothesis were selected using PubMed database (typing "the amyloidogenic hypothesis") and limiting the search to the last 5 years.

A computer-based search of PubMed database was performed to identify studies in which key words such as "APP and $Y_{682}ENPTY_{687}$" and "APP and Tyr/Tyrosine 682 phosphorylation" were typed. The search was performed without any specific restrictions with the exception of the language, as such only publications in English were included. All the papers have been listed in Table 1 and some of them have been mentioned and discussed along the text. Papers from reviews including the key words $Y_{682}ENPTY_{687}$ and Ty682 were also added to Table 1.

3. One possible solution: focus on the $Y_{682}ENPTY_{687}$ DOMAIN

APP belongs to an evolutionarily conserved family of type I transmembrane glycoproteins that includes the paralogs amyloid precursor-like proteins 1 and 2 (APLP1 and APLP2) (Gralle and Ferreira, 2007). These proteins share several conserved motifs, including the E1 and E2 domains in the extracellular region, and harbor a short cytoplasmic domain, the APP intracellular domain (AICD), which contains the $Y_{682}ENPTY_{687}$ motif patch with the highest homology (APP structure Fig. 1A) (Kaden et al., 2012). Indeed, that the $Y_{682}ENPTY_{687}$ motif is evolutionarily conserved among mammalian species and is present in either APP as APLP1 and APLP2 rather than in the non-conserved Aβ sequence, clearly suggests an essential role for the $Y_{682}ENPTY_{687}$ motif in APP function as well as its subsequent dysfunction.

Consistent with the importance of the $Y_{682}ENPTY_{687}$ motif in APP function and dysfunction, the proteolytic processing of APP either under physiologic (α- and γ-secretase cleavage producing a P3 fragment and COOH-terminal Aβ segment) or pathologic conditions (β- and γ-secretase cleavage releasing Aβ peptides) results in the production of the AICD intracellular fragment (Vassar et al., 1999; Selkoe, 2004), the significance of which remains unclear and controversial (Fig. 1B).

A large body of evidence (Table 1) points to a role for AICD in regulating the transcription of genes involved in downstream APP functions. AICD, when associated with Med 12, Fe65 protein, and the histone acetyltransferase Tip60, forms a stable transcription complex that can translocate into the nucleus to activate target genes (Cao and Südhof, 2001; Xu et al., 2011; Beckett et al., 2012). Examples of genes that have been linked with AICD include genes directly related to AD such as APP itself, β-secretase, and the neprilysin Aβ-degrading enzyme as well as GSK3β (Beckett et al., 2012; Alves da Costa et al., 2006; Belyaev et al., 2009).

In addition to the role of AICD in gene regulation, its conservative $Y_{682}ENPTY_{68}$ motif has been proposed to control APP trafficking and sorting via phosphorylation of the Tyr_{682} residue (Table 1).

We previously compared APP phosphorylation at Tyr_{682} to a "biochemical switch" that drastically changes the APP "interactome" thereby abolishing its binding to some proteins and creating docking sites for others (Matrone, 2013).

$Y_{682}ENPTY_{68}$ has been reported to interact with more than 20 adaptor protein partners, and this interaction largely depends on the

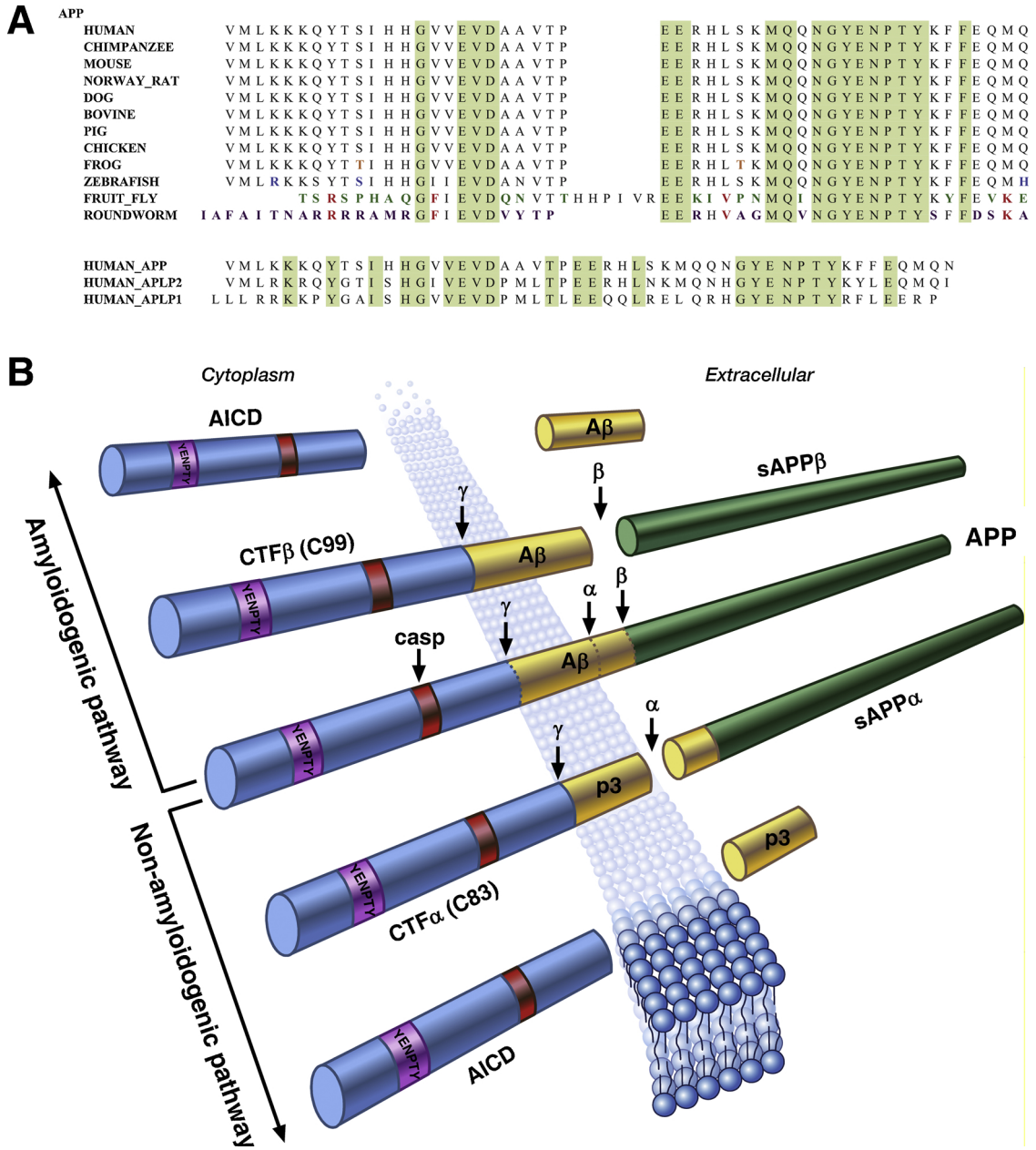

Fig. 1. Illustration of the primary sequence of APP (endoplasmic tails) and of the non-amyloidogenic and amyloidogenic APP pathway in neurons.
(A) In green are marked conservative domains from various species. Differences in residues among the species are marked in red, green and purple colors. Note that, human APP, APLP1, and APLP2 share conserved $Y_{682}ENPTY_{687}$ motif in the carboxyl terminus. (B) In the non-amyloidogenic pathway, α -secretase cleaves APP within the Aβ region. The CTF α (C83) peptide is then cut by γ-secretase, thus releasing the peptide P3 fragment. In the amyloidogenic pathway, APP protein is a substrate for enzymatic β secretase (BACE) cleavage, thus producing sAPPβ and the CTFβ (C99) fragment. The C99 fragment is further cleaved by the γ-secretase to generate Aβ. Note that the AICD peptide is produced both in the amyloidogenic and non-amyloidogenic pathway.
Abbreviations: APP amyloid-β precursor protein; Aβ amyloid beta; CTF carboxy-terminal fragment; AICD APP-intracellular domain; casp caspase; α, β γ secretasess.

phosphorylation status of the Tyr_{682} residue and, as such, acts as major regulator of APP fate (Matrone, 2013). Proteins interacting with the $Y_{682}ENPTY_{68}$ motif include the X11 and Fe65 family, which modulate cellular trafficking and the processing of APP (Pietrzik et al., 2004; Scheinfeld et al., 2003a); JIP1, a c-Jun N-terminal kinase JNK-signaling scaffold, which facilitates APP axonal trafficking (Scheinfeld et al., 2003a; Roncarati et al., 2002) and mediates transcription (Scheinfeld et al., 2003b); peptidyl-prolyl *cis/trans* isomerase Pin1, which regulates APP amyloidogenic processing (Pastorino et al., 2006); and Tip60, an histone acetyltransferase, and SET, a nucleosome assembly factor, which activates transcription of target genes (McLoughlin and Miller, 2008; Telese et al., 2005). The Shc family (ShcA and ShcB), mammalian disabled (mDab1), Numb, Abl, and Grb2 also interact with the $Y_{682}ENPTY_{687}$ domain, although the precise functions of these interactions require further elucidation (Klevanski et al., 2015; Müller and Zheng, 2012; Raychaudhuri and Mukhopadhyay, 2007).

We hypothesized that Tyr_{682} phosphorylation could induce a diversion of APP from its normal transport route, thus driving APP toward alternative neuronal compartments where it can be processed to generate Aβ (Matrone, 2013). Consistent with this hypothesis, we demonstrated that knock-in mice (YG mice) carrying a mutation in the Tyr_{682} residue (Barbagallo et al., 2010, 2011) show defects in APP trafficking and sorting (La Rosa et al., 2015).

The $Y_{682}ENPTY_{687}$ motif also binds Clathrin/AP2 complex, and we

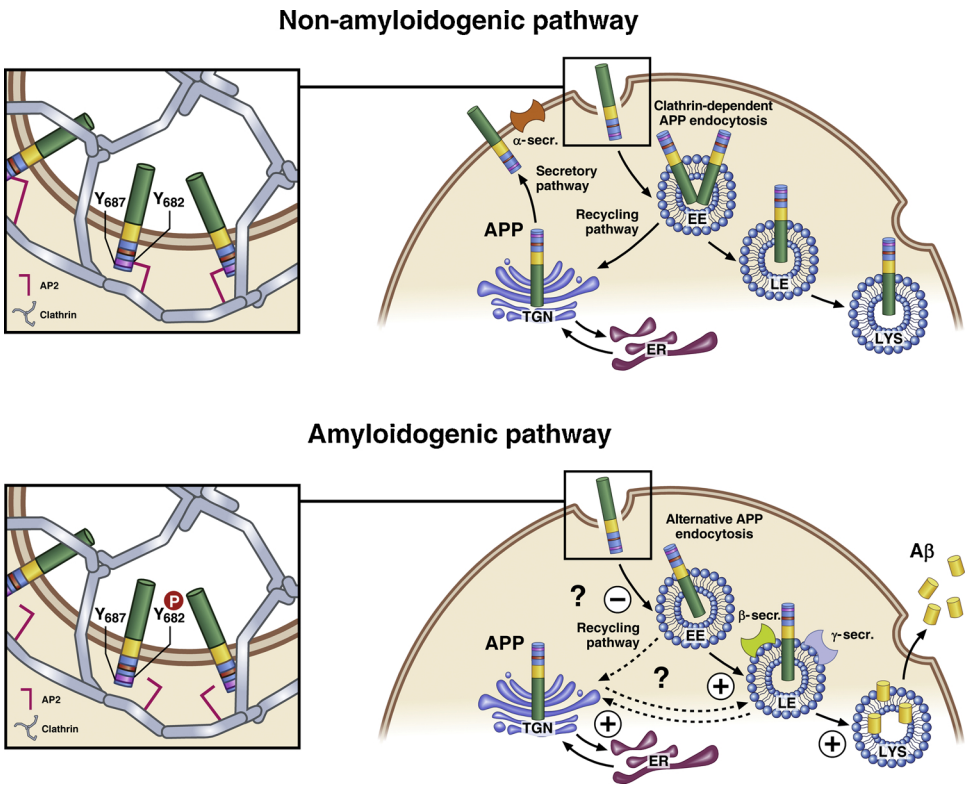

Fig. 2. Proposed model of APP endocytosis and trafficking before and after Tyr682 phosphorylation.
Nascent APP molecule after maturation through the constitutive secretory pathway reaches the plasma membrane where it is rapidly internalized through the binding of the $Y_{682}ENPTY_{687}$ motif to the Clathrin/AP2 complex (upper left panel). After Clathrin-dependent endocytosis, APP is trafficked through early endosome (EE) and Trans Golgi network (TGN) and recycled back to the cell surface (upper right panel). A small fraction also reaches late endosome (LE) and lysosome (LYS) where APP can be degraded. Non-amyloidogenic processing mainly occurs at the cell surface where α-secretase mediates APP cleavage (Haass et al., 2012).
In the amyloidogenic processing, APP transits through the acidic endocytic organelles LE and LYS, where β-secretase is mostly located and triggers amyloidogenic APP cleavage (lower right panel).
We propose a model in which the increased APP Tyr_{682} phosphorylation causes the loss of APP binding to AP2 and Clathrin endocytic complex (lower left panel), thus leading to an alternative APP trafficking toward acidic organelles where APP accumulates and it is ultimately processed to generate A*β*.

have reported that, in neurons from mutant knock-in mice, the lack of APP binding to the Clathrin-AP2 complex causes apparent defects in APP endocytosis (La Rosa et al., 2015; Poulsen et al., 2015).

We additionally demonstrated that the replacement of Tyr_{682} residue with Gly (YG) resulted in a gain-of-function phenotype that forced APP to be retained in an acidic neuronal compartment, such as the late endosome or the lysosome (La Rosa, 2015), where β secretase is more active and triggers amyloidogenic APP processing (Vassar et al., 1999; Domínguez et al., 2010). As a consequence, lysosomes appeared reduced in number and enlarged, forming massive cellular structures (Matrone, 2013). Additionally, cognitive and learning tests in these mice showed premature aging related deficits (Matrone et al., 2012) (Fig. 2).

In neurons differentiated from progenitor cells retrieved from AD patients that partially reflect the abnormalities reported in neurons seen in AD patients (such Aβ production and neuronal degeneration), we assessed increased extent levels of APP Tyr_{682} phosphorylation when compared to age matched healthy volunteers, which -as observed in neurons from mutant mice- causes a lack of APP binding to the Clathrin-AP2 complex and consequently affects APP internalization from the plasma membrane as well as its trafficking inside neurons (Poulsen et al., 2015, 2017). Additionally, an accumulation of Tyr_{682} hyperphosphorylated APP inside late endosome and lysosome was observed in these patient-derived cells.

Consistent with the idea that the prolonged permanence of APP inside lysosomes and endosomes promotes β secretase cleveage (Vassar et al., 1999; Domínguez et al., 2010), we found an increase in Aβ released into the media from AD neurons carrying mutations in the PSEN1 gene (Zollo et al., 2017) as well as in pigs harboring the same mutation (Jakobsen et al., 2016). Such an increase could be counteracted by Tyr kinase inhibitor exposures, concomitant to the reversion of the alterations in APP endocytosis and trafficking as well as the reduction of APP Tyr_{682} hyperphosphorylation levels (Poulsen et al., 2017).

Whether the hyperphosphorylation of APP Tyr_{682} residue actually plays a role in provoking AD remains to be demonstrated in patients. However, a variety of studies have offered intriguing results that support this evidence from human AD brains (Kametani, 2008; Gao and Pimplikar, 2001; Ghosal et al., 2009).

Further insights came from a liquid chromatography-tandem mass spectrometry analysis carried out in PSEN1 minipigs and in background-age-matched control minipigs. The results of this analysis identified Fyn Tyr kinase as a unique binding partner of the APP Tyr_{682} residue. Such an interaction was promoted by Tyr_{682} phosphorylation on the $Y_{682}ENPTY_{687}$ motif of APP and was increased in cortical brain tissues from PSEN1 minipigs as well as in AD patients carrying the same PSEN1 mutation (Poulsen et al., 2017).

Fyn kinase is a member of the Src family of non-receptor tyrosine kinases. Fyn has multiple isoforms, namely FynB, FynT, and FynD7, which arise from alternative splicing at exon 7. Fyn isoform 1, also known as FynB, is particularly enriched at synaptic structures and acts as a key regulator in synaptic transmission and plasticity (Bhaskar et al., 2005).

In the same neurons in which APP Tyr_{682} was hyperphosphorylated, we assessed increased Fyn Tyr kinase activity (assessed as increased Tyr_{420} phosphorylation levels) and increased interaction of Fyn kinase with the APP $Y_{682}ENPTY_{687}$ domain (Poulsen et al., 2017). Whether this interaction is instrumental for APP Tyr_{682} phosphorylation or is a downstream effect and whether other APP $Y_{682}ENPTY_{687}$ adaptors contribute to such interaction is still under evaluation by our group. However, it is notable that Tyr kinase inhibitor (TKI) exposure reduced the APP/Fyn interaction, reinstated proper APP compartmentalization (Poulsen et al., 2017), and prevented Aβ production in human AD neurons, thus suggesting that APP Tyr_{682} phosphorylation might be an upstream event and delineating a complex interplay among APP and Fyn proteins that clearly deserves further investigation.

Previous evidence has emphasized the clinical relevance of TKI in AD. Masitinib and Saracatinib have been demonstrated to be efficacious in treating AD symptoms by reducing Fyn Tyr kinase activity in experimental AD mouse models (Folch et al., 2015; Kaufman et al., 2015). Interestingly, Netzer et al., primed our results. They demonstrated that the TKI Imatinib renders APP less susceptible to proteolysis by β-

secretase, making it less available to β-secretase cleavage in acidic neuronal compartments (Netzer et al., 2017). We now can hypothesize that the altered APP trafficking that prolongs APP permanence in such acidic neuronal compartments and makes APP more accessible for β-secretase cleavage is largely due to the increased level of APP Tyr_{682} phosphorylation (La Rosa et al., 2015; Poulsen et al., 2017). Accordingly, by reducing APP Tyr_{682} phosphorylation levels, TKIs prevent APP retention in late endosomes and lysosomes, ultimately reducing Aβ production (Poulsen et al., 2017). Nonetheless, it remains well known that Aβ triggers downstream neurotoxic events, including the phosphorylation of Tyr residues of target proteins, such as Fyn and Tau (Nygaard et al., 2014; Nygaard, 2018; Babus et al., 2011).

In line with the above observations, we found that an intriguing target of Aβ is the NGF receptor TrkA (Matrone et al., 2009). We reported a paradoxical switch of TrkA from a pro-survival to pro-death function due to an increase in Aβ levels in an *in vitro* neuronal model lacking in NGF support (Matrone et al., 2008a; Basso and Matrone, 2013; Matrone et al., 2008b). Because of the increased Aβ production, TrkA activity resulted in hyperphosphorylation of Tyr residues despite the absence of NGF, thereby eliciting neuronal death. Notably, this pro-apoptotic switch of the TrkA receptor could be prevented by reducing Aβ production and accumulation (Babus et al., 2011), which was achieved by exposure to either anti-Aβ antibodies (as well as β and γ secretase inhibitors) or TKI (such as PP1) (Matrone et al., 2009), suggesting a complex progressive neurodetrimental mechanism in which Fyn Tyr kinases, APP Tyr_{682} hyperphosphorylation, β and γ secretases, Aβ and several downstream neurotoxic targets all appear to be implicated.

Together, these observations bring us back to the initial scientific question of this short review regarding the necessity to analyze AD before Aβ is produced, looking at dysfunction in the APP pathway rather that to the aberrant accumulation and deposition of Aβ.

In conclusion, we have provided new evidence that implicates the Tyr_{682} residue and the $Y_{682}ENPTY_{687}$ motif of APP in the onset and mostly likely progression of neurodegenerative processes in AD neurons. More detailed research is clearly needed to gain a more complete picture of the mechanisms responsible for increased APP Tyr_{682} phosphorylation and of the strategies that can be used to prevent amyloidogenic APP processing.

Further elucidation of the role played by $Y_{682}ENPTY_{687}$ in AD might provide a new target to identify a subclass of patients with increased phosphorylation levels of APP Tyr_{682} residue and to develop a personalized treatment for those AD patients.

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