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REVIEW

The physiological role of the amyloid precursor protein as an adhesion molecule in the developing nervous system

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Abstract

The amyloid precursor protein (APP) is a type I transmembrane glycoprotein better known for its participation in the physiopathology of Alzheimer disease as the source of the beta amyloid fragment. However, the physiological functions of the full length protein and its proteolytic fragments have remained elusive. APP was first described as a cell-surface receptor; nevertheless, increasing evidence highlighted APP as a cell adhesion molecule. In this review, we will focus on the current knowledge of the physiological role of APP as a cell adhesion molecule and its involvement in key events of neuronal development, such as migration, neurite outgrowth, growth cone pathfinding, and synaptogenesis. Finally, since APP is over-expressed in Down syndrome individuals because of the extra copy of chromosome 21, in the last section of the review, we discuss the potential contribution of APP to the neuronal and synaptic defects described in this genetic condition.

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Keywords: adhesion molecule, APP, connectopathy, neurodegeneration, neurodevelopment, plasticity.

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This review attempts to shed light about the participation of amyloid precursor protein (APP) as a cell adhesion molecule (CAM) during different steps of neurodevelopment. The development of the nervous system requires that neurons transit through a series of complex overlapping stages in order to build and integrate the neural network and reach functional maturity. In early stages, APP together with other cell adhesion molecules (CAMs) will be crucial for the migration of newborn neurons from the progenitor-rich ventricular zones to the appropriate layer of the cortical plate (Valiente & Marin 2010; Cooper 2013). They will also collaborate to generate the axon with a growth cone that will sense the environment in order to find a correct target to form synapses (Gupta *et al.* 2002; Lowery & Van Vactor 2009; Cooper 2013). CAMs are involved in contact guidance by engaging and coupling the neuronal cytoskeleton with other CAMs expressed in neighbor cells and with extracellular

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Abbreviations used: AD, Alzheimer disease; APP, amyloid precursor protein; CAM, cell adhesion molecule; CNS, central nervous system; DS, Down syndrome; ECM, extracellular matrix; GCs, growth cones.

matrix molecules (ECM) allowing neurons to navigate the complex environment of the CNS (Marin *et al.* 2010; Kolodkin & Tessier-Lavigne 2011; Sosa *et al.* 2013). This provided the necessary force and traction to drive the growth cone and the cell forward (clutch model) (Jessell 1988; Giannone *et al.* 2009; Parsons *et al.* 2010). This model implies a fine-tune between adhesion and motility which are mutually dependent and has to be coordinated with all neuronal subdomains. Either excessive or insufficient adhesion will break this mutual relationship and might cause altered motility and morphology, leading to abnormal organization of the neuronal network (DiMilla *et al.* 1991; Gupton & Waterman-Storer 2006; Schwartz & Horwitz 2006). In the following sections of this manuscript, we will intend to highlight the participation of APP as a cell adhesion molecule particularly implicated in the functional organization of highly dynamic cellular subdomains such as the growth cone (GC) and the dendritic spines. Finally, Down syndrome individuals carry an extra copy of chromosome 21 and thus over-expressed APP. In this context, we will discuss the implications of the dosage imbalance of APP on DS neurodevelopment.

Structural features of APP as a cell adhesion molecule

APP is a type I transmembrane glycoprotein first described as a putative orphan cell-surface receptor (Kang *et al.* 1987). It is composed of a large extracellular domain, a single transmembrane segment, and a short cytoplasmic tail that belongs to an evolutionarily conserved family of proteins (Fig. 1a). Mammalian homologs of APP include other two proteins, the APP-like proteins APLP1 and APLP2 (Wasco *et al.* 1992, 1993; Coulson *et al.* 2000). These three homologous proteins share conserved aminoacid sequences and protein subdomains, particularly in the extracellular and cytoplasmic regions. The conserved structure of the proteins appears also to extend to their physiological role, since functional redundancy was described in knockout animals (Wasco *et al*. 1992, 1993; Coulson *et al*. 2000). Different isoforms of APP exist as a result of alternative splicing; in neurons the APP695 isoform, which is the most prominent, is devoid of the Kunitz protease inhibitor domain (Kang & Muller-Hill 1990; Rohan de Silva *et al.* 1997). APP presents important CAM features at both the extracellular and cytoplasmic domains. The extracellular domain is capable of self-dimerization (Soba *et al.* 2005; Deyts *et al.* 2016b; Muller *et al.* 2017) and binding to multiple proteins of the ECM (Deyts *et al*. 2016b; Muller *et al*. 2017), whereas its cytoplasmic domain interacts with cytoskeleton-associated scaffold proteins (van der Kant & Goldstein 2015; Deyts *et al*. 2016b; Muller *et al*. 2017). These features suggest a role for APP as a CAM capable of mechanically linking the extracellular environment with the cellular cytoskeleton via scaffold protein(s), similar to other adhesion proteins such as cadherins, integrin β1, and L1 (Ooashi & Kamiguchi 2009; Parsons *et al*. 2010; Bignante *et al.* 2013; Deyts *et al*. 2016b). However, contrary to cadherins that concentrate and form adherent junctions to hold cells tightly together, APP is enriched in highly motile subdomains of the neuron such as the GC and dendritic spines. Moreover, APP is concentrated in the peripheral leading edge of the GC (Sabo *et al.* 2003; Sosa *et al*. 2013) where the nascent adhesions are coordinated with highly dynamic lamellipodia and filopodia of the cortical actin cytoskeleton. Therefore, by interacting with diverse components of the extracellular matrix and signaling through scaffolding proteins, APP might orchestrate the plastic remodeling of cell adhesions and the dynamic organization of the subcortical cytoskeleton. These processes are critical for allowing the axonal GC to crawl searching for its appropriate target and to let the synaptic contacts to be remodeled for adaptive mnesic processing (Lowery & Van Vactor 2009). In the following section, the structural aspects of APP as a CAM involved in plastic cell adhesion will be analyzed in more detail.

APP extracellular domain

APP presents a large ectodomain which consists of four basic subdomains, namely the globular E1 domain, the acidic domain, and the helix-rich domain (E2) followed by the juxtamembrane sequence of beta amyloid that further extends into the transmembrane domain (Fig. 1a). The E1 domain could be further divided into two regions, the heparin-binding domain and the copper/metal-binding domain. The heparin-binding domain has been implicated in neurite outgrowth (Small *et al.* 1994) and uncovers a highly positively charged surface capable of interaction with glycosaminoglycans. Adjacent to this region lies a hydrophobic pocket surface that might function in protein-protein interaction and dimerization of APP (Rossjohn *et al.* 1999). In the vicinity of this region, the copper/metal-binding domain is found (Bush *et al.* 1993). The E2 domain has a heparin-binding site and metal-binding sites which may sustain the E2 domain in a rigid conformation (Multhaup *et al.* 1994; Dahms *et al.* 2012). The E2 domain also contains the REMRS amino acid sequence motif which has been described to promote cell growth and neurite extension and seems to bind membrane-anchored heparan sulfate proteoglycans (HSPGs) (Jin *et al.* 1994; Reinhard *et al.* 2013) (Fig. 1a).

APP has been described to bind several ECM molecules playing fundamental roles in the formation and maintenance of brain architecture during development and also in adulthood (Dityatev *et al.* 2010; Mouw *et al.* 2014) (Fig. 1a). Also APP have the capacity to self-dimerize and binds other cell-surface receptors (Soba *et al*. 2005; Deyts

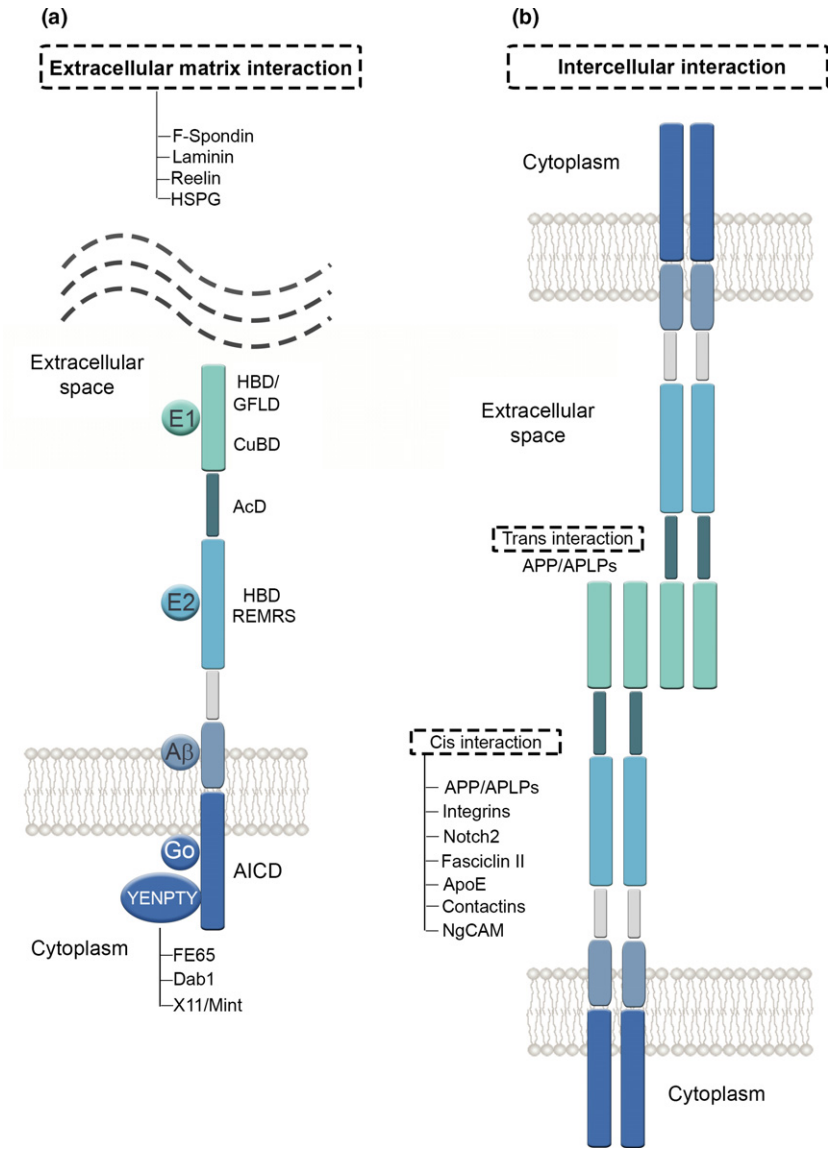

Fig. 1 Schematic diagram of amyloid precursor protein (APP) molecule, consisting of a large extracellular domain, a transmembrane domain, and a short cytoplasmic carboxyl terminus. (a) The extracellular region of APP is capable of binding different components of the extracellular matrix (ECM) and (b) interact in *cis*/*trans* with other cell adhesion molecules. The intracellular domain includes several domains capable of binding scaffold proteins associated with the dynamics of actin cytoskeleton. Abbreviations: Heparin-binding domain (HBD), growth factor-like domain (GFLD), acidic domain (AcD), APP intracellular domain (AICD).

et al. 2016b; Muller *et al*. 2017) (Fig. 1b). The potential of APP to bind different ECM might allow migrating neurons to dynamically adapt to the complex neurodevelopmental environment (Marin *et al*. 2010; Parsons *et al*. 2010; Cooper 2013). In the following section we will describe the interaction of APP with some of these ECM.

Reelin is an extracellular protein secreted by Cajal-Retzius cells involved in neuronal migration during cortical development. The interaction of Reelin with the extracellular domain of APP has been demonstrated in mouse brain lysate and primary cortical cultures by immunoprecipitation and by co-localization of immunostained hippocampal neurons (Hoe *et al.* 2009). Interestingly, it was also shown that Reelin increases the surface levels of APP, reducing APP endocytosis and beta secretase processing (Hoe *et al*. 2009). Also, using wild type and APP-transgenic mice uncovered a synergistic effect between APP, Reelin, and integrins that promotes neurite outgrowth and dendritic arborization (Hoe *et al.* 2009).

APP also binds F-Spondin, which is a protein that participates in cell–cell connection, axonal outgrowth, and pathfinding (Klar *et al*. 1992; Tzarfati-Majar *et al.* 2001; Ho & Sudhof 2004).Experiments made in cell lines and using affinity chromatography and immunoblotting from rat brain extracts, showed that the interaction of APP with F-Spondin impairs the α and β secretase cleavage of APP (Ho & Sudhof 2004). Also, APP-F-Spondin interaction has been suggested to mediate inhibition of cell adhesion favoring interaction with other substrates (Debby-Brafman *et al.* 1999; Tzarfati-Majar *et al*. 2001). Inhibition of cell adhesion at the floor

plate might be important to allow newborn neurons to initiate its migration to the cortical plate; this possibility requires further experimental support.

Laminin is another important ECM molecule present in brain tissue that also binds APP during neurodevelopment. Biochemical analysis from rat brain demonstrated that APP is present and enriched in GC adhesion on laminin together with integrin, CD81, and focal adhesion kinase (Sosa *et al*. 2013). This result is consistent with the extensive co-localization of APP and integrins in the axonal GC of mice hippocampal neurons, especially in the lamellipodia and filopodia where the dynamic actin cytoskeleton is found (Sosa *et al*. 2013). Furthermore, in cultured hippocampal neurons growing on laminin APP, dosage affects adhesion and axonal outgrowth (Kibbey *et al.* 1993; Sosa *et al.* 2013).

Heparan sulfate proteoglycans (HSPGs) are also components of the ECM. *In vitro* studies performed in chick sympathetic and mouse hippocampal neurons showed that APP is capable of binding with different types of HSPGs expressed during the neurodevelopment having distinct neurite outgrowth-promoting effect (Small *et al*. 1994).

Aside from the ability of APP to bind diverse components of the extracellular matrix, studies performed in cell lines and mouse brains extracts with a variety of experimental techniques demonstrated that APP is capable of interacting in *cis* – to form lateral homo and heterodimeric complexes with other APP family members (Soba *et al*. 2005) (Fig. 1b). The *cis* complexes are found at the surface of the membrane and promote cell–cell adhesion via *trans* cellular interaction (Soba *et al*. 2005) (Fig. 1b). The E1 domain of APP seems to be the principal region required for *trans* homo- and heterotypic interactions, while the E2 domain participates with lower affinity in the formation of the complex (Soba *et al*. 2005; Baumkotter *et al.* 2012; Coburger *et al.* 2014; Deyts *et al*. 2016b). Protein dimerization has been extensively described for cadherins, nectins, and integrins as a fundamental property of CAMs (Takai *et al.* 2003). Cell-surface dimerization of APP as well as its binding to extracellular matrix components appears also to modulate APP proteolytic processing by the secretases (see below), suggesting that generation of APP extracellular and intracellular fragments contributes to the control of the dynamics of APP-dependent adhesive contacts. Interestingly, it was also reported that proteolytic fragments of APP, including sAPP and Aβ, interact with holo-APP modulating neuritogenesis and synaptic function, further suggesting that APP can physiologically modulate plastic remodeling of the nervous system both during development and adulthood (Allinquant *et al.* 1995; Perez *et al.* 1997; Fogel *et al.* 2014). Interestingly, the pathological fibrillary form of Aβ that characterize Alzheimer disease also binds holo-APP, induces its multimerization, and triggers neuronal dystrophy and degeneration (Lorenzo *et al*. 2000; Van Nostrand *et al.* 2002; Shaked *et al.* 2006; Sola Vigo *et al.* 2009) suggesting that Aβ aggregates are pathologic APP ligands that trigger maladaptive plasticity and degeneration in Alzheimer disease.

It has also been reported that APP interact with other CAMs such as integrin (Fig. 1b). APP and integrin are enriched in the same fraction isolated from GCs of fetal rat brain by density gradients (Sosa *et al*. 2013). APP and integrins co-localize in the leading edge of the growth cone and dendrites of primary cortical and hippocampal neurons (Yamazaki *et al.* 1997; Hoe *et al*. 2009; Sosa *et al*. 2013). It is unclear yet whether APP and integrins work as a co-receptor for cell adhesion (Yamazaki *et al.* 1997; Young-Pearse *et al.* 2008; Hoe *et al*. 2009). For example, in one study performed in mice primary neurons, it was shown that the effect of Reelin and APP on neurite outgrowth requires integrins (Hoe *et al*. 2009). On the other hand, our studies performed on APP-specific substrate established that APP is capable of attaching and sustaining GC outgrowth independently of others CAM (Sosa *et al*. 2013). The apparent discrepancies of these results might rely on different substrates and cell types utilized in each study.

Also, APP might function as a co-receptor for other CAMs, including Fasciclin II, contactins, and NgCAM (Wolfe & Guenette 2007; Deyts *et al*. 2016b; Muller *et al*. 2017). In the case of the interaction of Fasciclin II, studies were developed in *Drosophila*, which demonstrated that beta amyloid protein precursor-like, APPL (the insect ortholog of APP) is required in a complex together with dX11 in order to generate the appropriate signaling that allow synaptic bouton formation and growth (Ashley *et al*. 2005). Regarding the interaction of APP with contactin 4 and NgCAM, studies developed in the chick retinotectal system in cultured retinal ganglion cells demonstrated that APP and contactin 4 modulate the axonal outgrowth dependent of NgCAM (Osterfield *et al*. 2008). The mentioned co-receptor function of APP is not completely understood but it might serve as an adaptive strategy that ensures appropriate adhesive control in highly complex environment.

The data discussed above exhibit an intriguing property of APP as a CAM, which is its ability for binding many different ligands. It is unlikely that APP would simultaneously interact with all of them (Fig. 1), rather it appears more plausible that its 'promiscuity' offers a mechanism to control adaptive adhesion by modulating APP subcellular distribution, metabolism processing, and signaling.

For example, some ECM molecules are expressed in different regions and/or stages of brain development (Dityatev *et al*. 2010; Mouw *et al*. 2014) which imply that the migrating neuron is exposed to a selected range of contact guidance clues at each time (Tessier-Lavigne *et al.* 1988; Mouw *et al*. 2014). APP as a CAM might also be exposed to this changing environment but some ligands (such as F-Spondin) will promote inhibition of cell adhesion when binding to APP, thus favoring its interaction with other ligands located in adjacent areas (Debby-Brafman *et al.* 1999; Tzarfati-Majar *et al*.

2001). Also, controlling APP subcellular distribution and surface exposure will affect its adhesive function. APP subcellular localization and processing is regulated in a cell-type-specific and neuronal compartment-specific manner (Brunholz *et al.* 2012; Deyts *et al*. 2016b; Brady & Morfini 2017). In this context, targeting of APP at specific plasma membrane domains such as the GC relies on particular trafficking and sorting mechanisms characteristic of different population of neurons (Brunholz *et al*. 2012; Brady & Morfini 2017). APP transits the secretory pathway and is enriched in highly plastic subcellular domains of the neuron such as the axonal GC. In order to reach the dynamic lamellipodia of the axonal GC, APP is transported in vesicles together with other pre-synaptic molecules by Kinesin-1C, also known as KIF5C, in anterograde fast axonal transport (FAT) (Koo *et al.* 1990; Szodorai *et al.* 2009). Intriguingly, it has been found that a human mutation in KIF1C is associated with aberrant cortical development (Poirier *et al.* 2013). Subsequently, APP containing vesicles must be delivered at specific neuronal domains, a process that is mediated by phosphorylation-dependent mechanisms that regulate the molecular motors (Morfini *et al.* 2002a, 2009; Brady & Morfini 2017). Interestingly, it has been reported that aberrant regulation of molecular motors by phosphorylation-dependent mechanisms contributes to the loss of synaptic connectivity (Pigino *et al.* 2009; Brady & Morfini 2017). Besides the regulation of APP traffic, the processing of APP by secretase might also modulate its adhesion properties in a similar way to other CAMs such as cadherins and L1 (member of the immunoglobulin superfamily of proteins) (Maretzky *et al.* 2005; Reiss *et al.* 2005). In this regard, it has been demonstrated that APP processing is regulated by cell-type and subcellular distribution-dependent manner (Brunholz *et al*. 2012; Deyts *et al*. 2016b; Brady & Morfini 2017; Muller *et al*. 2017). APP surface localization favors its cleveage by α-secretase, whereas its endocytic localization promotes the action of β-secretase (Deyts *et al*. 2016b; Muller *et al*. 2017). Furthermore, binding of APP to different external ligands differentially affects its processing by the secretases (Ho & Sudhof 2004; Hoe *et al*. 2009; Rice *et al.* 2012). Additionally, APP-secreted fragments also control APP surface distribution and dimerization (Young-Pearse *et al*. 2008; Gralle *et al.* 2009), reflecting multiple and complex mechanism of controlling the CAM function of APP. In this view, the trafficking and processing of APP might control its function as a cell adhesion molecule in many ways. The cleavage of APP at the cell-surface might release APP from its ligand and allow the detachment and turnover of the adhesion. On the other hand, the soluble fragments of APP interact with the ectodomain of APP that is exposed at the cell surface, enhancing APP multimerization and signaling (Young-Pearse *et al*. 2008; Deyts *et al*. 2016b; Muller *et al*. 2017). Also, the intracellular fragments of APP affect the signaling, trafficking, and nuclear activity of the cell (Deyts *et al*. 2016b; Muller *et al*. 2017). In this context, it has been demonstrated in isolated squid axoplasms that intracellular oligomeric amyloid β (Aβ) cause inhibition in FAT as consequence of abnormal kinase activity (Pigino *et al*. 2009). Additionally, it has been described that Alzheimer disease (AD)-related mutations in APP that affect its processing by secretases also altered the activity of focal adhesion kinase and glycogen synthase kinase 3 and impaired cell adhesion and migration in N2a cells (Sheng *et al.* 2009). Furthermore, aberrant activation of glycogen synthase kinase 3 can modify the traffic, cellular distribution, and processing of APP (Morfini *et al.* 2002b). Nevertheless, it has to be experimentally demonstrated – the physiological mechanism that regulate APP surface expression and binding with ECM molecules during development and/or plastic remodeling of the nervous system in mammals. Also still remain elusive, the physiological role of the complex cleavage of APP during neurodevelopment and its implication in cell adhesion and neurodegenerative diseases.

APP intracellular domain

Similar to other CAMs, APP has no kinase activity by itself, and therefore it requires the interaction with scaffolding proteins and or signaling molecules in order to articulate with the actin cytoskeleton (Parsons *et al*. 2010). The cytoplasmic tail of APP presents the YENPTY amino acid sequence motif that is recognized by adaptor proteins capable to link with the actin cytoskeleton (Fig. 1a). This YENPTY is a highly conserved motif present in APP family members and also found in other tyrosine receptor kinase and adhesion proteins, such as integrin β1. This domain is capable of binding adaptor proteins containing the phosphotyrosine-binding domain (PTB) which are related to cell adhesion, migration, and synaptogenesis, including Fe65, Mint/X11, and Dab1 (Wolfe & Guenette 2007; van der Kant & Goldstein 2015; Deyts *et al*. 2016b; Muller *et al*. 2017). Analysis of the interactome based on the Human Protein Reference DataBase, points to Apbb1 (Fe65) as a particularly prominent actin linker candidate (Bai *et al.* 2008). Fe65 co-localizes with APP and Mena proteins in GC filopodia and lamellipodia and is linked to Mena for actin cytoskeleton remodeling and cell motility. Fe65 also might regulate the surface expression and localization of APP in growth cones and at sites of dynamic adhesion (focal complex) (Sabo *et al.* 2001, 2003). Interestingly, Fe65-null brains exhibit aberrant neuronal migration and wiring (Krause *et al.* 2003; Sabo *et al*. 2003; Guenette *et al.* 2006).

Dab1 is another docking protein that binds the YENPTY motif of APP, ApoER2, and VLDLR receptors playing a key role in the signal transduction pathway drive by Reelin and APP during neuronal migration (see neuronal migration section below) (Rice *et al.* 1998; Howell *et al.* 1999; Young-Pearse *et al.* 2007). The YENPTY motif of APP also binds Mint/X11 family proteins, which are involved in synaptogenesis and vesicle exocytosis. It has been shown that

X11 also regulates the traffic and processing of APP (Borg *et al.* 1996; Ashley *et al*. 2005; Rogelj *et al.* 2006; Ho *et al.* 2008; Sullivan *et al.* 2014).

Eb41 (band 4.1, a FERM protein) is another protein that was highlighted in the proteomics study as an APP-interacting protein candidate (Bai *et al.* 2008). Epb41 has a well-known role as a component of the cortical cytoskeleton, it directly interact with α-actin and other cytoskeletal proteins. Additionally, a second APP interactome study also identified Epb41I3 as an APP-binding protein and found spectrin α1 (which binds to Epb41 members), together with actin β and F65, in APP immunoprecipitates (Cottrell *et al.* 2005). Interestingly, Epb41 knockout mice exhibit neurobehavioral deficits (Arpin *et al.* 1994; Walensky *et al.* 1998; Calinisan *et al.* 2006). Despite the aforementioned evidence, an experimental demonstration of the mechanism that links APP, the scaffold proteins, and the cytoskeleton during development and/or plastic remodeling of cell adhesions is still required.

The actin cytoskeleton could be also controlled through transduction pathways mediated by heterotrimeric G protein-coupled receptor (GPCRs) reviewed by (Sah *et al.* 2000). In this regard, cumulative evidence indicates that APP functions as an unconventional heterotrimeric GPCRs, similar to many other single pass membrane receptors (Patel 2004; Hawkes *et al.* 2007; Copenhaver & Kogel 2017). Following the initial identification of a 20 amino acid peptide (His657-Lys676; numbering in APP695) within the intracellular domain of APP that could directly bind and activate heterotrimeric Go protein in reconstituted membranes (Nishimoto *et al.* 1993), several other groups provided compelling experimental evidence supporting the role of APP as a bona fide GPCR. The functional interaction of holo-APP with Goα, but not Giα or Gsα family proteins, was documented in a variety of biological samples including cultured cell lines, mice primary hippocampal cultures, and brain tissue from mice and humans by employing multiple experimental approaches including high resolution fluorescence microscopy and fluorescence resonance energy transfer, co-immunoprecipitation assays and by genetic manipulations of APP and Goα (Ikezu *et al.* 1996; Brouillet *et al.* 1999; Shaked *et al.* 2009; Sola Vigo *et al*. 2009; Ramaker *et al.* 2013; Fogel *et al*. 2014). Coincidental with these observations, the over-expression of familial Alzheimer disease-forms of APP promotes constitutive Go activation leading to cellular toxicity (Okamoto *et al.* 1995, 1996; Yamatsuji *et al.* 1996a, 1996b; Niikura *et al.* 2004). Similarly, promoting cell-surface multimerization of APP with either, antibodies to APP-ectodomain or pathologic assemblies of Amyloid β also triggers Go activation and neuronal degeneration (Rohn *et al.* 2000; Sudo *et al.* 2000; Shaked *et al*. 2009; Sola Vigo *et al*. 2009; Xu *et al.* 2009). Although these observations provide compelling evidence that binding of pathogenic holo-APP ligands triggers Go activation and neurodegeneration in Alzheimer disease, the potential role of APP-Go signaling in the normal development and plasticity of the nervous system has received much less attention. Recently, in a refined fluorescence resonance energy transfer-based study, it was shown that physiological forms of Aβ (monomers and dimmers) binds holo-APP activating Go signaling in the pre-synaptic terminals leading to enhanced neurotransmitter release in excitatory synapses (Fogel *et al*. 2014). Also, using *in vitro* preparations of mammalian neurons, it was shown that the neuroprotective effect of soluble sAPPα reduces cell-surface APP dimerization (Gralle *et al.* 2009) and activates the PI3K/Akt pathway through a Gαo/i-dependent signaling (Milosch *et al.* 2014). These observations further support the observation that APP is a bona fide receptor of Aβ and other metabolic fragments of APP (Lorenzo *et al*. 2000). Intriguingly, it was also shown that the C-terminal fragments of APP can also interact with Gαs promoting neurite outgrowth via adenylyl cyclase/protein kinase A (PKA)-dependent pathways (Deyts *et al.* 2012, 2016a). These observations open the fascinating possibility that interaction and signaling of APP through different heterotrimeric G (Go and/or Gs) proteins might be regulated by both, the type of extracellular APP ligands and APP metabolic fragments generated by secretases (α, β, and γ) activity. Therefore, neuronal cell-type-specific and/or compartment-specific processing of APP in particular neuronal populations might render these neurons differentially dependent to APP-G protein signaling for survival and or plastic remodeling. Compelling evidence for the evolutionary conserved signaling of APP and Go in neuronal development and plasticity arose from studying the role of APPL (the insect ortholog of APP) in neurodevelopment of *Drosophila* and *Manduca sexta* (tobacco hornworm). In *Drosophila*, it was shown that the formation and maturation of neuromuscular synapse depends on APPL and Go signaling (Torroja *et al.* 1996, 1999). Furthermore, the ability of Fasciclin, a transmembrane CAM of the Ig superfamily, to promote new synapse formation also requires an APPL-dependent transduction cascade as was previously mentioned (Ashley *et al*. 2005). The interaction of APPL and Goα within photoreceptors and developing synapses in *Drosophila* was revealed *in vivo* by bimolecular fluorescence complementation in genetic fly lines that co-express the fusion constructs of APPL and Gαo (Ramaker *et al*. 2013). It was also shown that during the formation of the enteric nervous system of the *Manduca* embryo, APPL co-localizes within the leading processes and growing axons of migratory neurons (Swanson *et al.* 2005), similar to the co-localization of APP and Gαo in cultured mammalian neurons (Ramaker *et al*. 2013). More importantly, it was shown that APPL-Gαo signaling plays an important role in regulating neuronal motile behaviors. Inhibition of either APPL expression or Gαo activity increased neuronal migration and promoted ectopic axonal growth; conversely, enhanced stimulation of the APPL-Gαo

pathway induced collapse-stall responses (Ramaker *et al*. 2013). More recently, it was also shown that *Manduca* Contactin (which is expressed by adjacent glial cells) is a candidate ligand for APPL that induces activation of APPL-Gαo signaling in the migratory neurons to induce local retraction responses, avoiding ectopic outgrowth (Ramaker *et al.* 2016). Collectively, these experiments provide compelling evidence that APPL (and likely other APP family members) regulates multiple aspects of neuronal morphogenesis and plasticity through the activation of Go/Gs signaling. Nevertheless, it has to be experimentally demonstrated that these signaling mechanisms are physiologically activated during development and/or plastic remodeling of the nervous system in mammals and also how this is connected to the scaffold proteins and the cytoskeleton.

The role of APP in neuronal migration and cortex development

Adhesion molecules play crucial roles in cortical development by shaping morphological maturation and coordinating migration of neuronal cells from progenitor-rich ventricular zones to the cortical plate. In migrating neurons, the cell body, the leading process (future dendrite) and the trailing process (future axon) contain different adhesion complexes, whose activities need to be coordinated with specific instructive long-distance and short-distance signaling molecules present in the surrounding environment. Altered expression of cell adhesion and guidance molecules is associated with disorders in neuronal migration and morphology leading to aberrant brain architecture (Lauffenburger & Horwitz 1996; Marin & Rubenstein 2003; Marin *et al.* 2006; Ayala *et al.* 2007; Kawauchi *et al.* 2010; Valiente & Marin 2010; Solecki 2012; Cooper 2013; Famulski & Solecki 2013). In this context, cortical dysplasia defects have been described in KO mice models of CAMs like β1 integrin (Graus-Porta *et al.* 2001), α6 integrin (Georges-Labouesse *et al.* 1998), and related proteins, such as MARCKS (Stumpo *et al.* 1995; Blackshear *et al.* 1997; Weimer *et al.* 2009) and focal adhesion kinase (Beggs *et al.* 2003).

It is therefore worth noting that APP is highly expressed in the developing cortex from early embryonic days along with differentiation and migration of cortical neurons (Lorent *et al.* 1995; Young-Pearse *et al*. 2007) suggesting an important role in neuronal development. This idea is supported by a recent work that described a human homozygous nonsense mutations in the APP gene associated with microcephaly, hypotonia, developmental delay, thinning of the corpus callosum, and seizures (Klein *et al.* 2016). Surprisingly, APP knockout (KO) mice displayed a mild phenotype (Muller *et al.* 1994; Zheng *et al.* 1995). Moreover, similar mild phenotypes were observed in double KO mice for APLP1 and APLP2 (Heber *et al.* 2000) bringing forward the existence of functional redundancy among these proteins. On the other hand, studies with triple KO mice ablating all three APP family members showed that 80% of the animals exhibited brain aberrations. These abnormalities included regions of focal dysplasia with the presence of ectopic clusters of neuroblasts migrating beyond the basal lamina and via membrane, accumulating within the marginal zone (MZ) and the subarachnoid space of the forebrain (Fig. 2). In these triple KO mice, the marginal zone displayed partial loss and dysfunction of Cajal-Retzius cells, which may contribute to the over migration of cortical neurons (Herms *et al.* 2004; Aydin *et al.* 2012; Guo *et al.* 2012; Muller & Zheng 2012). The phenotype described above resembles the human type II (cobblestone) lissencephaly (*smooth brain)*, which is a rare brain disorder caused by defective neuronal migration (Gleeson & Walsh 2000; Kerjan & Gleeson 2007; Valiente & Marin 2010). Interestingly, KO mice for both Fe65 and Fe65L proteins or Mena, a Fe65-downstream effector protein, also displayed neuronal migration and axonal projection defects, similar to those found in mice lacking APP family members (Lanier *et al.* 1999; Guenette *et al*. 2006), suggesting that these proteins are involved in APP signaling during neuronal migration. This over migration abnormality has also been described in *Manduca sexta* after inhibiting APPL expression (the insect APP hortolog) *(*Ramaker *et al*. 2016*)*.

The functional role of APP in neuronal migration was also studied modulating APP expression by *in utero* electroporation (IUE) (Young-Pearse *et al*. 2007). APP over-expression induced by electroporating human cDNA resulted in accelerated neuronal migration into the cortex (Fig. 2). Conversely, IUE with APP-shRNA resulted in acute APP down-regulation and accumulation of multipolar microtubule-associated protein 2 (MAP2)-positive neurons just below the cortical plate (Fig. 2). This phenotype was rescued by over-expression of the full length human APP or the other APP family members, APLP1 or APLP2, indicating functional redundancy of these proteins. Significantly, rescue failed when APP-shRNA were co-electroporated with truncated forms of human APP or APP with a targeted mutation in the YENPTY motif required for the interaction with scaffold proteins such as Fe65 and Dab1 (Young-Pearse *et al*. 2007).

Although these experiments further emphasize the functional role of APP in neuronal migration during cortical development, the data appear to contradict the no apparent migration phenotype observed in single APP-KO and double APP-APLP1 KO mice and the over migration phenotype described in triple APP-APLP-APLP2 KO mice (see above). It is likely that mosaicism and/or compensatory protein expression might explain these phenotypic differences. In KO mice, complete protein ablation is observed in all cell types, including neurons and glia while random electroporation generates a mosaic in which APP expression remains normal in non-electroporated cells and is down-regulated in electroporated cells. In addition, in KO mice, the chronic

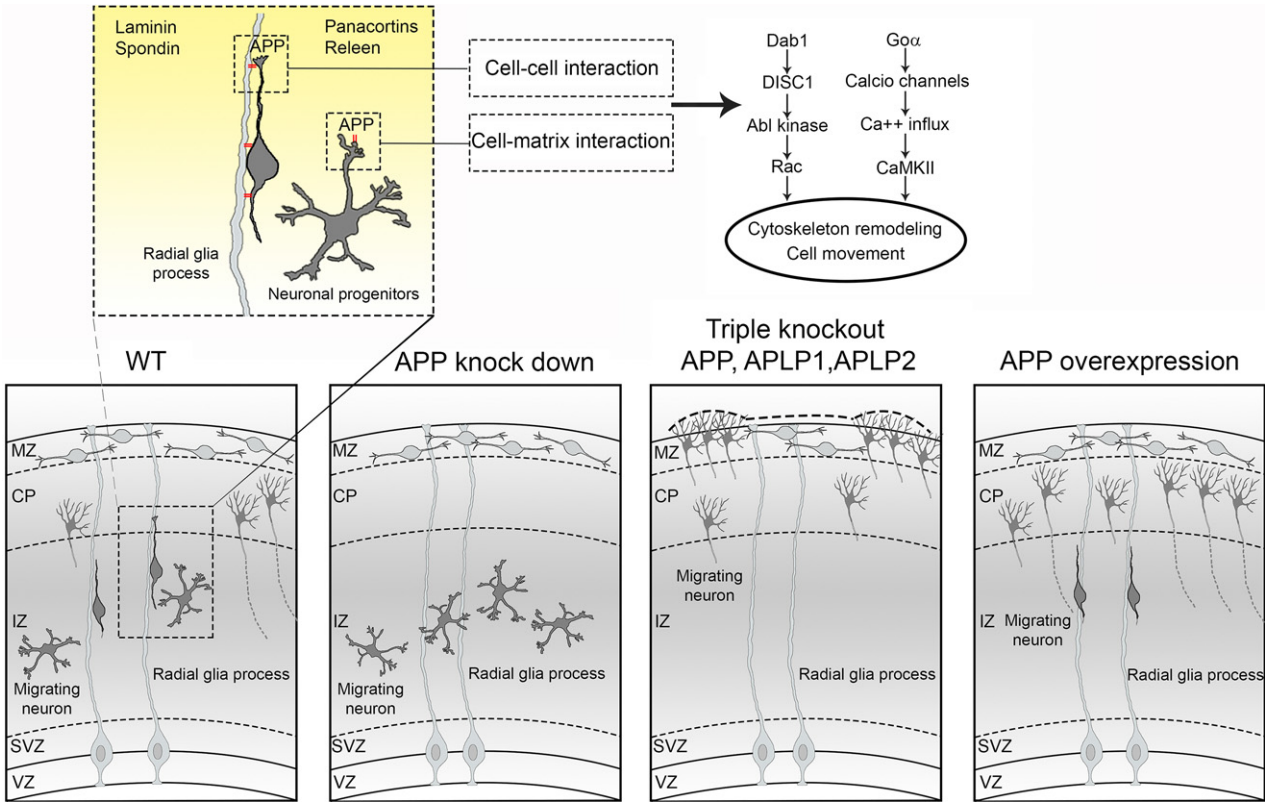

Fig. 2 Schematic diagram of the involvement of amyloid precursor protein (APP) on neuronal migration through its interaction with the extracellular matrix (ECM) and cell adhesion molecules placed on the radial glia cells and scaffold proteins implicated in the signaling and connection with the actin cytoskeleton. APP misexpression caused aberrant neuronal migration and development of the cerebral cortex. When APP is knocked down, neurons fail to enter in the cortical plate, being arrested in the intermediate zone, whereas over-expression of APP caused an acceleration of the neuronal migration into the cortical plate. The triple knockdown neurons for all APP family proteins present focal dysplasia regions of neuroblast, placed within the marginal zone. Abbreviation: ventricular zone (VZ), subventricular zone (SVZ), intermediate zone (IZ), cortical plate (CP), marginal zone (MZ).

protein ablation allows compensatory responses, likely by other APP family members, while acute APP knockdown by IUE occurs in the absence of compensation. It is also important to consider that the cerebral cortex is built with a number of different neuronal types (Molyneaux *et al.* 2007). Concomitantly, it is likely that APP in each neuronal type might have particular characteristics of APP expression, intracellular trafficking, secretases processing, and cell-surface expression. These cell-type differences might also determine variability in the relevance of APP as a CAM in neuronal migration. In this context, different pattern of expression and processing of APP during cortical neuronal maturation and differentiation (Bergstrom *et al.* 2016) has been described. Also, a variety of studies have reported different subcellular distribution and processing of APP between neuronal and non-neuronal cells (reviewed by (Brunholz *et al*. 2012). The surface expression of APP in radial glia cell might work like the steps of a ladder, allowing the homotypic recognition and the migration of the neurons to its final place. These possibilities will have to be experimentally analyzed.

IUE experiments also raised the interesting observation that Dab1 over-expression partially rescued the migration-arrested phenotype of APP knock down (Young-Pearse *et al*. 2007) (Fig. 2). Strikingly, one of the proteins associated with schizophrenia, the disrupted-in-schizophrenia 1 protein appears also to interact downstream of APP and Dab1 promoting neuronal migration (Young-Pearse *et al.* 2010). Dab1 also recognized the YENPTY motif of ApoER2 and VLDLR receptors involved in the signaling of the extracellular matrix protein Reelin during neuronal migration. Furthermore, it was described that the external domain of APP interacts with Reelin (Hoe *et al*. 2009), suggesting that APP could be a receptor for Reelin. Intriguingly, the phenotypes described in the cortex of the triple KO mice for all three APP family members are different from the phenotype exhibited by Reelin KO mice that displays an abnormal inside-out arrangement of cortical layers

(D'Arcangelo *et al.* 1999a, 1999b; Tissir & Goffinet 2003), likely suggesting that APP may act dissimilar to the ApoER2 and VLDLR receptors during cortical migration.

Pancortins have also been described as extracellular cues that can interact with APP and regulate the migration of cortical neurons with different outcomes depending on the Pancortin isoform. Although BMZ isoform promotes the entry of neurons into the cortical plate, the AMY isoform has the opposite effect (Rice *et al.* 2012). It is unclear yet if the opposite effects of the Pancortin isoforms are because of the ability of each isoform to activate different APP-dependent signaling pathways, perhaps by binding to different APP extracellular domains and or engaging different co-receptors.

Finally, it is worth mentioning that during the formation of the enteric nervous system of the Manduca larvae a neuronal population migrates to form the nerve plexus. In a series of well conducted experiments, it was demonstrated that a mechanism that involves activation Go signaling by APPL (the insect ortholog of the mammalian APP) is required for the correct migration of these neurons (Ramaker *et al*. 2013) (Fig. 2). Curiously, the potential involvement of APP-Go signaling in neuronal migration in mammals has not yet been explored.

The role of APP in axonal outgrowth and contact guidance during neurodevelopment

The growth cone (GC) is an amoeboid structure crowning the tip of neuronal processes. Of particular interest is the axonal GC, the compartment where outgrowth takes place and APP is highly enriched (Sabo & McAllister 2003; Sabo *et al*. 2003; Sosa *et al*. 2013). The axonal GC helps to steer the axons toward their appropriate targets by sensing molecular cues across the complex environment of the CNS (Raper & Mason 2010; Kolodkin & Tessier-Lavigne 2011). In order to precisely achieve this goal, the GC integrates and tightly coordinates a variety of events including cytoskeleton dynamics, membrane expansion and cell adhesions balance (Conde & Caceres 2009; Lowery & Van Vactor 2009; Pfenninger 2009; Kolodkin & Tessier-Lavigne 2011). It is worth noting that APP binds a series of ECM molecules including laminin, collagen, spondin, heparin sulfate, glypican, and Reelin, suggesting an important function of APP as an adhesion molecule in the GC of developing neurons (Small *et al*. 1994; Beher *et al.* 1996; Williamson *et al.* 1996; Caceres & Brandan 1997; Salinero *et al.* 2000; Ho & Sudhof 2004; Hoe *et al*. 2009; Sosa *et al*. 2013) (Fig. 1). In fact, when cultured neurons are grown on laminin, APP co-localizes integrin β1 in dynamic adhesion complexes (Sabo *et al*. 2001, 2003; Hoe *et al*. 2009) and the extent of the adhesion area and initial rate of axonal outgrowth is related to APP dosage (Sosa *et al*. 2013). APP-suppression significantly reduces GC size, adhesive area, and axonal outgrowth, whereas APP over-expression has the opposite effect (Sosa *et al*. 2013) (Fig. 3a and b). To further characterize the specific contribution of APP to the GC dynamics, we used monospecific substrate capable of selectively recognizing for APP, β1 integrin, or L1 protein (which belong to the immunoglobulin superfamily). Thus, APP-KO and APP over-expressing neurons grown in substrates recognized only by β1 integrin or L1 protein showed indistinguishable GC morphology. On the contrary, compared to wild type neurons, APP-KO neurons cultured on the APP-specific substrate exhibited fusiform GCs with reduced adhesive area, while APP over-expressing neurons displayed enlarged growth cones with increased adhesion area (Sosa *et al*. 2013). The previous experiments indicate that APP can function as a cell autonomous protein independently of other CAMs, such as β1 integrin (which also binds laminin) and L1 protein. Surprisingly, compared to wild type neurons that express normal APP levels, the rate of axonal outgrowth was reduced in both APP-KO and APP over-expressing neurons grown on the APP-specific substrate. These observations highlight a relationship between APP dosage and cell adhesion at the GC, and suggest that a precise balance of APP expression is required for adhesions strength/stability and axonal outgrowth (Sosa *et al*. 2013). This interpretation is in line with others' experimental data and mathematical models that predict a biphasic relationship when motility is affected below and above an adhesion optimum/pinnacle point (DiMilla *et al*. 1991, 1993; Lemmon *et al.* 1992).

CAMs are also capable of recognizing and engaging with substrate-bound cues, thus contributing to growth cone pathfinding (Letourneau 1975; Raper & Mason 2010). In this context, it has been demonstrated using stripes assays (Knoll *et al.* 2007) that axonal GCs of APP over-expressing neurons grew preferentially over the mono-substrates that binds specifically APP, while the axonal GCs from APP-KO neurons actively avoided them. Notably, GCs from wild type neurons showed random growth in the same stripe pattern (Sosa *et al*. 2013) (Fig. 3c). Accordingly, It has been described that APP-mediated guidance of commissural axonal *in vivo* requires APP acting as co-receptor of the deleted in colorectal cancer protein (DCC) and the netrin-1 signaling (Rama *et al.* 2012). Validating the *in vitro* evidence, axonal growth defects have been described in mushroom body neurons from Appl null flies (Soldano *et al.* 2013) and in Zebrafish after knockdown of the APP hortolog, Appb (Song & Pimplikar 2012). Consistently with this, humans harboring truncating mutation in the APP gene, as well as single (APP) KO mice and the triple APP/APLP1/APLP2-KO mice exhibited various neurodevelopmental defects. Among others, these included an increased frequency of dysgenesis or agenesis of the corpus callosum and reduced size of the forebrain commissures, particularly the ventral hippocampal commissures (Muller *et al*. 1994; Zheng *et al*. 1995; Li *et al.* 1996; Magara *et al.* 1999;

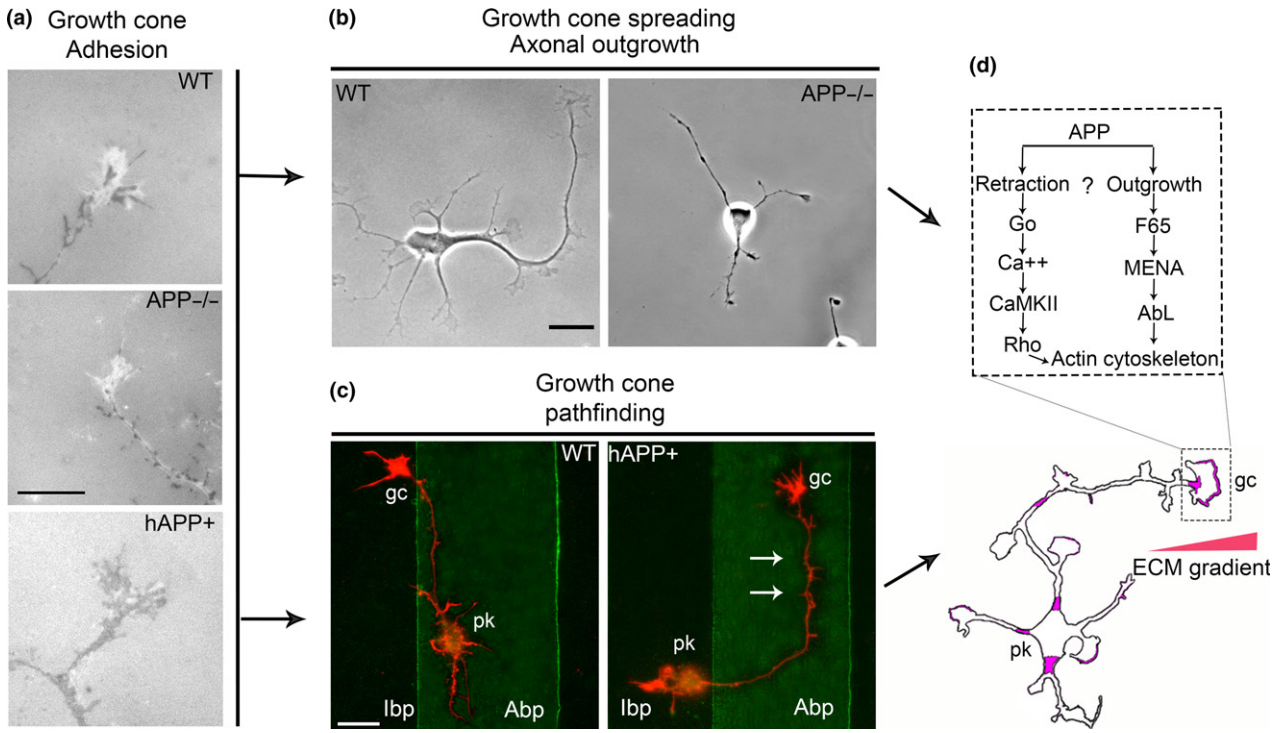

Fig. 3 Amyloid precursor protein (APP) Growth cone adhesion mediate axonal outgrowth and pathfinding. (a)Growth cone adhesions of live neurons from wild type (WT) and mutant mice on laminin imaged by reflection interference contrast microscopy (RICM). Note the reduced or extensive adhesion area (black) in the APP knockout and APP over-expressing mice, respectively, in comparison with WT growth cone. The growth cones (GCs) adhesion plays a critical role on axonal outgrowth and contact guidance (b and c). Scale bar 10 μm. (b)Phase contrast images of WT and APP knockout neurons on laminin after 24 h in culture. Note the reduced axonal extension on APP knockout neurons. Scale bar 20 μm. (c)Contact guidance assays of WT and hAPP+ over-expressing neurons mice. Neurons grown for 24 h on alternating lanes of the indicated synthetic substrate. Note that WT neurons growth without any preference, whereas GC from hAPP+ over-expressing mice prefer to growth on the Abp substrate (white arrows). Abbreviation: Perikaryon (pk); axonal growth cone (gc), amyloid binding peptide (Abp), Integrin binding peptide (Ibp). Scale bar 20 μm. (d) Schematic diagram of the possible pathways involved in axonal outgrowth and contact guidance. The APP-dependent adhesion sites are remarked on Fuchsia.

Klein *et al*. 2016). Aberrations in corpus callosum and axonal projections tracts are also observed in mice deficient of other proteins involved in cell adhesion such as MARCKS, Pin1, and Fe65 (Stumpo *et al*. 1995; Guenette *et al*. 2006; Sosa *et al.* 2016). On the other hand, the over-expression of human APP and APPL in *Drosophila* neurons resulted in increased post-developmental axonal outgrowth and arborization, which depends critically on the conserved YENPTY motif of APP (Leyssen *et al.* 2005). Also, a mice line moderately over-expressing APP exhibit an increase in synaptic terminals and enhanced expression of GAP43, a typical GC component (Mucke *et al.* 1996). In the future, more experiments will be required in order to identify the signaling mechanisms activated during APP-dependent GC pathfinding (Fig. 3d). Further experiments are required in order to demonstrate if different expression levels of APP affect neuronal outgrowth, pathfinding, and synaptogenesis *in vivo* and whether this is a cell autonomous phenomenon caused by APP.

The role of APP in synaptogenesis and dendrite spine morphology

Adhesion molecules also play a fundamental role in the formation, maturation, and plastic remodeling of neuronal synapses (Dalva *et al.* 2007; Missler *et al.* 2012; Chia *et al.* 2013). Interestingly, APP expression increases during synaptogenesis (Loffler & Huber 1992; Wang *et al.* 2009). In addition, at the synapse, APP localizes in both pre- and post-synaptic membranes and can dimerize trans-synapticaly through *trans* interactions involving the E1-E2 domains (Fig. 4). Indeed the *trans* interaction/dimerization of the APP located in pre- and post-synaptic terminals play a fundamental role in synaptogenesis of the CNS and PNS and is also required for the functional and structural dynamics of the synapse (Soba *et al*. 2005; Wang *et al.* 2005, 2009; Priller *et al.* 2006; Baumkotter *et al*. 2012; Ludewig & Korte 2016). It has been described that part of the synaptogenic function of APP is carried out through its

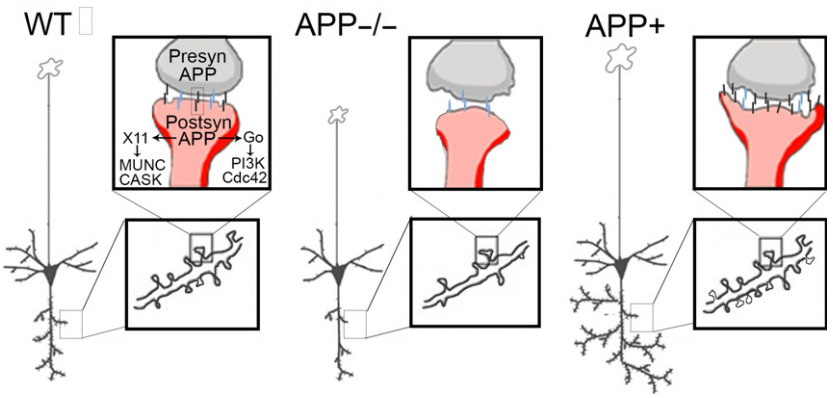

Fig. 4 Schematic diagram of the involvement of amyloid precursor protein (APP) in synaptogenesis. The *trans* interaction of APP located in the pre-synaptic and post-synaptic terminal play a fundamental role in the synaptogenesis and plasticity of the CNS, regulating functional and structural dynamics of the synapse through its interaction with X11/Mint and also Go. APP −/− neurons showed shorter dendritic spines as well as a reduction in the synapses numbers and spine density, whereas APP over-expressing mice present increased density of spines and post-developmental arborization.

interaction with the scaffold proteins X11/Mint which recognizes the YENPTY cytoplasmic domain of APP as well as of others synaptic adhesion molecules like Neurexins (Borg *et al*. 1996) (Fig. 4). In *Drosophila*, the interaction of APPL, fasciclin II, and dX11 (X11 homology) is required for the assembly of new synapses (Ashley *et al*. 2005). Also, APP interaction with Mint2, can recruit Munc18-1, a known regulator of synaptic vesicle exocytosis (Weyer *et al.* 2011).

Beside the YENPTY cytoplasmic domain of APP, studies performed in *Drosophila* demonstrated that APP-Go protein binding site is required for neuromuscular synaptic formation (Torroja *et al*. 1999) (Fig. 4). Interestingly, the interaction of APP with heterotrimeric Go protein mediated the increase in Ca^{2+} flux and synaptic vesicle release induced by Aβ (Fogel *et al*. 2014).

It is therefore not surprising that APP-suppressed hippocampal neurons showed a reduction in the size and number of dendritic spines as well as decreased presence of the synaptic proteins Bassoon (pre-synaptic) and PSD-95 (post-synaptic)(Lee *et al.* 2010). This phenotype is also observed in neurons that express APP with truncated domains. *In vivo* studies with APP-KO mice revealed the presence of abnormalities in the morphology of the dendritic tree. These abnormalities included shorter dendritic spines, as well as reduction in synapse number and spine density of neurons located in cortical layers II/III and hippocampal CA1 region (Fig. 4). In addition, double APP/APLP2 KO mice exhibits impaired synaptic structure, neuromuscular junction formation, and defective synaptic transmission (Seabrook *et al.* 1999; Wang *et al*. 2005; Lee *et al*. 2010; Tyan *et al.* 2012; Weyer *et al.* 2014). On the other hand, APP over-expressing neurons had significantly more spines and puncta density of the synaptic proteins PSD-95 and Bassoon (Lee *et al*. 2010). Also, *in vivo* studies of young mice over-expressing APP exhibit increased neurite outgrowth, dendritic complexity, and spines density of both cortical layer II/III and hippocampal CA1 (Hoe *et al*. 2009) (Fig. 4). Additional studies are required to determine the precise role of APP and its fragments during synaptogenesis and the plastic events of the adult brain.

The implication of APP in Down syndrome neurodevelopment

Down syndrome (DS) is the most common genetic cause of intellectual disability (Hassold & Jacobs 1984) and is originated by trisomy of the 21 chromosome (HSA21) (Lejeune *et al.* 1959). Although DS was first described by John Langdon Down in 1866, its pathophysiology is still poorly understood. In recent years, different theories have been proposed to explain this condition, such as the *dosage imbalance hypothesis* and the *amplified imbalance instability hypothesis* (Antonarakis *et al*. 2004; Roper & Reeves 2006; Dierssen 2012). Individuals with DS present a wide range of structural and functional defects in the CNS and also in others organs. Brain of DS individual exhibits disorganized cortical lamination, delayed myelination of axonal fibers, and reduced brain volume, especially in the frontal cortex, hippocampus, and cerebellum (Schapiro *et al.* 1992; Pinter *et al.* 2001; Antonarakis & Epstein 2006; Haydar & Reeves 2012). It was also reported that several regions of the DS brain, including the cortex, display reduced number and density of neurons. Moreover, neurons themselves present an aberrant morphology and orientation that in part may be caused by an abnormal adhesive behavior (Takashima *et al.* 1981; Larsen *et al.* 2008). Additionally, human DS neurons have been described with aberrant enlarged dendrites and spine abnormalities (Takashima *et al*. 1981, 1989; Becker

et al. 1986; Dierssen *et al.* 2003; Contestabile *et al.* 2010). Furthermore, data derived from positron emission tomography describe altered circuitry patterns in different brain regions implying defective neuronal connectivity (Schapiro *et al*. 1992). In this context, APP is one of the proteins encoded in chromosome 21 (Goldgaber *et al.* 1987; Tanzi *et al.* 1987) and is over-expressed in humans with DS (Busciglio *et al.* 2002), thus it may contribute to the DS phenotype and intellectual disability. Interestingly, individuals with DS develop an early onset form of Alzheimer disease, likely related to APP over-expression. In this context, individuals that present APP gene duplication and increased expression of wild type APP developed early onset of Alzheimer disease with cerebral amyloid angiopathy but none have been described with cognitive deficit before the onset of dementia. Furthermore, affected individuals did not show any clinical feature suggestive of DS (Rovelet-Lecrux *et al.* 2006). Interestingly, these individuals also present seizure which may suggest an altered neuronal circuitry; unfortunately, histological, cytological, and molecular studies are lacking prior to the onset of the disease precluding further speculations on the potential mechanism of this affection.

In the case of the studies performed on APP over-expressing mice, beside the abnormal features described previously in neurite and dendritic spine development, behavior and cognitive studies showed disturbed defects before and independent of Amyloid β plaque formation (Moechars *et al.* 1999; Simon *et al.* 2009). In this regard, studies developed in transgenic mice that over-express wild type human APP exhibit memory impairments and cytoskeletal pathology with barely detectable Aβ and high levels of APP intracellular domains (Simon *et al*. 2009).

Nevertheless, it has to be experimentally demonstrated if the cognitive defect found in mice that over-express APP is the consequence of a functional disturbance or the result of a neurodevelopmental problem.

Also, further studies are required in order to elucidate if APP gain of function affect neuronal wiring *in vivo* and if this phenotype responds to neuron-autonomous process. In humans, APP-promoter mutation is associated with early onset AD, likely because of increased expression of APP (Theuns *et al.* 2006) but neurodevelopmental and cognitive studies characterizing the preclinical stages of these patients were not performed. Therefore, it remains unknown how APP over-expression in humans might affect the neurodevelopment predisposing to AD later in life.

The Ts65Dn is one of the more extensively studies transgenic mice that develop a DS-like condition. This mice present a segmental trisomy of the chromosome 16, which contains orthologs of about half of human chromosome 21, and over-expressed APP together with other genes (Reeves *et al.* 1995). Analysis performed with Ts65Dn mice revealed enrichment of APP in the cortex and within neurons, in the axonal growth cone (around 1.7 fold) compared to WT control neurons (Reeves *et al.* 1995; Sosa *et al.* 2014). In this context, hippocampal pyramidal neurons derived from Ts65Dn mice and cultured on laminin substrate exhibit aberrant growth cones with large adhesive areas and abundant filopodia (Fig. 5a and b). Consistently, APP was

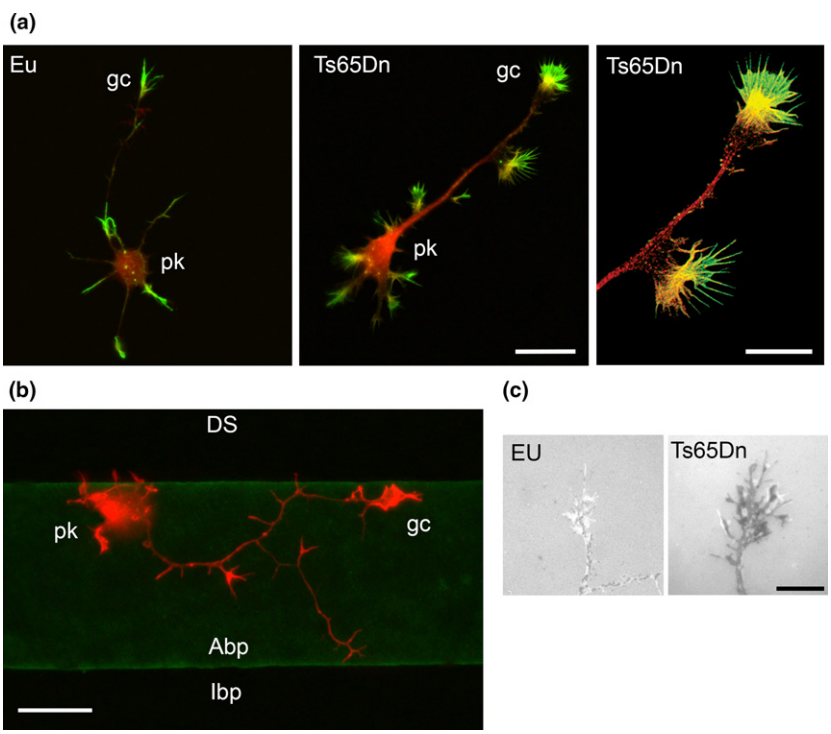

Fig. 5 (a) Hippocampal neurons and growth cones (GCs) from newborn Ts65Dn and euploid littermate's brains cultured on laminin and imaged by TIRF after labeling the actin filaments (green) and amyloid precursor protein (APP) (red). Scale bar 20 μm. (b) Contact guidance assays of Down syndrome human neurons grown for 24 h on alternating lanes of the indicated synthetic substrate. Note the preference of Down syndrome neurons to growth on the Abp substrate (white arrows). Scale bar 20 μm. (c) Growth cone adhesions imaged by reflection interference contrast microscopy (RICM) of live neurons from euploid (Eu) and Ts65DN mutant mice on laminin. Note the extensive adhesion area (black) in the Ts65Dn mice. Scale bar 10 μm. Abbreviation: Perikaryon (pk); axonal growth cone (gc), amyloid-binding peptide (Abp), Integrin-binding peptide (Ibp).

also enriched in the membrane surface of human DS cortical neurons cultured on laminin. GC of these neurons are large and grow faster than those derived of euploid human neurons (Sosa *et al*. 2014). Since several genes are over-expressed in DS, it becomes necessary to identify and differentiate the effects of APP from other CAMs on adhesion properties and the overall cell phenotype. In this regard, we have reported the culture of human DS neurons on laminin-derived peptides that selectively bind APP. Under these conditions, human DS neurons cultured on the mono specific substrate recognized only by APP exhibited very large growth cones along with increased adhesion and axonal outgrowth. In contrast, the same neurons present a phenotype similar to euploid neurons when plated on substrates that are recognized by integrin or L1 (Sosa *et al*. 2014). The dosage effect of APP in adhesions was confirmed when partial knockdown of APP in DS neurons restored growth cone adhesion and phenotype to a range similar to euploid neurons. Concomitantly, GC from neurons of human DS also presents aberrant contact guidance behavior, showing a preference for substrates recognized by APP (Fig. 5c). The partial knockdown of APP in DS neurons recovers the euploid cone behavior and axonal growth, which showed no preference for any substrate. These studies suggest that over-expression of APP affect GC pathfinding in DS neurons and hence might contribute to aberrant development of the brain circuitry. Furthermore, dendrites and spine abnormalities have been described in Ts65Dn and human DS neurons. Human DS neurons display increase number of growth cone filopodia and neurite-like processes together with endosomal abnormalities that are partially dependent on APP over-expression and might contribute to aberrant synaptogenesis at later stages (Cataldo *et al.* 2003; Sosa *et al*. 2014). Intriguingly, neocortical pyramidal neurons of adult DS human and Ts65Dn mice exhibit reduced dendritic complexity including reduced length, branching, and spine density, while young Ts65Dn mice and DS humans showed abnormally enlarged dendrites with increased branching. Additionally, these aberrantly enlarged dendrites also exhibited oversized dendritic spines, pre-synaptic and post-synaptic terminals, as well as synaptic clefts in both the cortex and hippocampus (Takashima *et al*. 1981, 1989; Becker *et al.* 1986; Dierssen *et al.* 2003; Belichenko *et al.* 2007; Contestabile *et al.* 2010). Together with previous evidence, it is tempting to speculate that the increase in the number of immature and abnormal synapses may precede exacerbated pruning and aberrant remodeling, which in turn may contribute to cognitive deficit and neurodegeneration in the DS brain (Olsen *et al.* 2014; Hong *et al.* 2016). In this context, the aberrant synapses and brain architecture previously described in DS would generate altered formation of neural circuits and connectopathies (Schapiro *et al*. 1992; Contestabile *et al*. 2010) which might contribute to the intellectual disability. Beside the neurodevelopmental defects described, the increased expression and processing of APP in DS individuals clearly stand as the main responsible for the elevated prevalence to develop AD. Furthermore, the increased expression of APP could also contribute to developing AD by affecting connectivities during development and compromising the target-derived trophic support leading to loss of synapsis and making neurons more vulnerable to neurodegeneration (Teipel & Hampel 2006; Ben-Ari 2008). Indeed, it has been described that in Ts65Dn, the increased dose of APP markedly decreased nerve growth factor (NGF) retrograde transport, causing degeneration of basal forebrain cholinergic neurons (Salehi *et al.* 2009) and impairment in the proliferation of neuronal precursor cells (Trazzi *et al.* 2011). Also, the increased levels of Aβ present in DS might cause inhibition of the FAT and predispose to distal axonopathies and dying back neuropathology (Pigino *et al*. 2009; Brady & Morfini 2017).

Finally, although DS is a complex genetic condition which remains poorly understood, the previously described consequence of APP dosage expression is clearly one of the variables that might contribute to this condition and it is likely that the dosage imbalance of multiples genes that characterize DS might further exacerbate the effects of APP.

Acknowledgments and conflict of interest disclosure

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