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TRAFFICKING OF α-SECRETASE ADAM10: NEW INSIGHTS INTO THE MOLECULAR MECHANISM OF MODULATION

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Lo scienziato non è l'uomo che fornisce le vere risposte
ma quello che pone le vere domande.

Claude Lévi Strauss

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INTRODUCTION

1. Intracellular trafficking

Eukaryotic cells transport newly synthesized proteins destined for the extracellular space, the plasma membrane, or the endocytic/lysosomal system through a series of functionally distinct, membrane-bound compartments, including the endoplasmic reticulum (ER), Golgi apparatus, and vesicular transport intermediates. This is the secretory membrane system (**Fig. 1**), which allows eukaryotic cells to perform three major functions: (1) distribute proteins and lipids synthesized in the ER to the cell surface and other cellular sites, (2) modify and/or store protein and lipid molecules after their export from the ER, and (3) generate and maintain the unique identity and function of the ER, Golgi apparatus, and plasma membrane. The secretory membrane system uses membrane-enclosed transport carriers to move thousands of diverse macromolecules—including proteins, proteoglycans, and glycoproteins—efficiently and precisely among different membrane-bound compartments (i.e., the ER, Golgi apparatus, and plasma membrane). Within the large cytoplasmic volume of the eukaryotic cell, this is essential for coordinating cellular needs in response to the constantly changing environment and organismal physiology. Newly synthesized transmembrane and lumenal proteins transported through the secretory system are called cargo. These include lumenal proteins destined to be stored within a compartment or secreted to the cell exterior, as well as transmembrane proteins that are retained in a particular compartment (e.g., Golgi processing enzymes), delivered to the plasma membrane, or recycled among compartments (e.g., transport machinery). Transfer of cargo molecules through the secretory system begins with their cotranslational insertion into or across the ER bilayer. The cargo molecules are next folded and assembled into forms that can be sorted and concentrated within membrane-bound transport intermediates destined for the Golgi apparatus. Once packaged into and transported by such a carrier, cargo enters the Golgi apparatus, which serves as the central processing and sorting station in the secretory membrane system. Within the Golgi apparatus, numerous enzymes modify the cargo molecules by trimming or elongating the cargo's glycan side chains or cleaving its polypeptides. Processed cargo is then sorted into membrane-bound carriers that bud out from the Golgi apparatus and move to the plasma membrane, to the endosome/lysosomal system, or back to the ER. In specialized cell types, the Golgi apparatus can sort certain classes of cargo into secretory granules (for storage and later release to the cell exterior in response to specific stimuli) or give rise to transport carriers that target to different polarized plasma membrane domains. Membrane-enclosed carriers mediate transport within the secretory membrane system.

Carriers are shaped as tubules, vesicles, or larger structures. The carriers are too large to diffuse freely in the crowded cytoplasm but are transported over long distances along microtubules or actin filaments by molecular motor proteins. Each carrier selects certain types of cargo before budding from a donor compartment and fuses only with an appropriate target membrane. Molecular markers on the cytoplasmic surface of the carrier, as well as on the acceptor membrane, steer the carrier through the cytoplasm and ensure that it fuses only with the correct target compartment. The carriers continuously shuttle among ER, Golgi apparatus, and plasma membranes, enabling cargo to be distributed to its appropriate target organelle. Sorting of cargo into transport carriers is facilitated by the presence of specialized lipids in the donor organelle membrane (such as sphingomyelin, glycosphingolipids, and phosphoinositides in the Golgi apparatus) and by the recruitment of protein-based sorting and transport machinery (e.g., coat proteins and tethering/fusion factors). Together, the specialized lipids and protein-sorting machinery generate membrane microdomains that concentrate or exclude cargo. The domains then pinch off the membrane bilayer as membrane-enclosed carriers and travel to target membranes. During transport of a carrier, the relative orientation of lipid and protein in the membrane bilayer, established during synthesis in the ER, is maintained. Hence, one side of the membrane always faces the cytoplasm. The other side initially faces the lumen of the ER. This side remains inside each membrane compartment along the secretory pathway but is exposed on the cell surface if the carrier fuses with the plasma membrane. Selection of proteins and lipids by a carrier, budding of the carrier, and subsequent fusion of the carrier with an acceptor compartment all also occur without leakage of contents from the carrier or the donor and target compartments. The flow of cargo and lipid forward through the secretory system toward the plasma membrane (anterograde traffic) is balanced by selective retrograde traffic of cargo and lipids back toward the ER **(Fig. 1).** Retrograde traffic allows proteins and lipids involved in membrane transport and fusion to be retrieved for repeated use. Retrograde traffic also returns proteins that have been inadvertently carried forward through the secretory system so they can be redirected to their proper destination. Both anterograde and retrograde flows of membrane within the secretory system are necessary for the ER, Golgi apparatus, and plasma membrane to generate and maintain their distinct functional and morphologic identities.

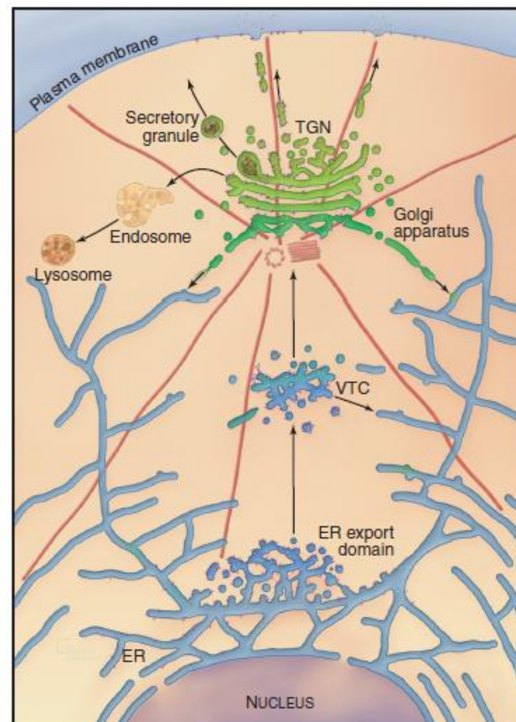


Figure 1. Overview of the secretory membrane system. *The three principal organelles of the secretory pathway—the ER, Golgi apparatus, and plasma membrane—communicate with one another and the cell exterior by way of transport carriers. The carriers (either small vesicles or larger vesicle-tubule elements) move along cytoskeletal elements (red lines) to transfer newly synthesized proteins, called cargo, from the ER to the Golgi, and from the Golgi to the plasma membrane (or to the endosome/lysosomal system). Carriers form from the ER at specialized regions, called ER export domains, producing pre-Golgi structures called vesicular tubular carriers (VTCs) that move to the Golgi. Retrograde transport carriers bud off from the VTC or Golgi apparatus to retrieve proteins and lipids back to the ER for repeated use and to balance the anterograde flow of membrane to the plasma membrane. The luminal spaces enclosed by the carriers and organelles of the secretory membrane system are all topologically equivalent to the outside of the cell.*

The secretory membrane system, found in all eukaryotic cells, offers numerous advantages over the simpler secretory process in prokaryotic cells, which involves insertion of newly synthesized proteins directly into or across the plasma membrane. First, synthesizing, folding, and processing membrane and secretory proteins within a series of distinct compartments provides a protective environment for cells to modify proteins before they are exposed on the cell surface. Newly synthesized proteins within the ER, for example, can fold into complex shapes and assemble into multisubunit complexes. Within the Golgi apparatus, the cargo molecules can be further modified by glycan processing and proteolytic cleavage. The resulting repertoire of protein structures that are expressed at the cell surface is significantly larger and capable of performing more diverse functions than that found in prokaryotes. A second advantage is the capacity of the secretory membrane system to regulate protein secretion and expression at the cell surface. Eukaryotic cells can store proteins in membrane compartments before releasing them at the cell

surface in response to internal or external signals. By exploiting these capabilities, eukaryotic cells have evolved elaborate ways to control the types of proteins located on or secreted from the cell surface. A third advantage relates to the differentiation of the plasma membrane. Prokaryotic cells synthesize their proteins at the plasma membrane, so they must keep this surface enriched in loosely packed glycerophospholipids that are pliable enough that newly synthesized proteins can enter into and fold in a hydrophobic environment. Consequently, prokaryotic cells secrete a rigid cell wall as a protective barrier to the outside. In eukaryotes, concentrating protein synthesis in the ER frees the plasma membrane to become enriched in lipids such as cholesterol and sphingolipids that can arrange into highly ordered, flexible arrays. The ordered, flexible arrays of cholesterol and sphingolipids in the plasma membrane provide mechanical stability and an impermeable barrier to water-soluble molecules. As a consequence, eukaryotic cells do not require a cell wall to survive (although some eukaryotes, such as plant and fungal cells, make cell walls) and can employ their plasma membrane in a wide range of functions, such as membrane protrusion for engulfing large extracellular objects and for crawling.

Building and Maintaining the Secretory Membrane System. A conserved feature of the secretory membrane system is the differential distribution of various classes of lipids along the pathway. These classes of lipids include glycerophospholipids (phosphoglycerides), sphingolipids (e.g., sphingomyelin and glycosphingolipids), and cholesterol. These lipids play a major role in the sorting of proteins within the secretory membrane system because of their immiscibility (i.e., the property of not mixing) in membranes with different lipid compositions. By not mixing with some lipids while mixing with others, these lipid classes form lateral lipid assemblies, termed microdomains, that can concentrate or exclude specific membrane proteins. Sphingolipids (e.g., glycosphingolipids and sphingomyelin) are synthesized in the Golgi apparatus, while the ER produces cholesterol and glycerophospholipids. Synthesis of these lipids at two different sites, combined with the self-organizing capacity of sphingolipids, cholesterol, and glycerophospholipids, gives rise to a pattern of lipid circulation within the secretory system that plays important roles in membrane sorting. Newly synthesized cholesterol is continually removed from the ER and redistributed to the Golgi apparatus, where high affinity interactions with sphingolipids prevent it from returning to the ER. The association of cholesterol with sphingolipids in the Golgi apparatus, in turn, triggers the lateral differentiation of domains enriched in these lipids. Through the additional activity of protein-based sorting and trafficking

machinery, these domains bud off the Golgi apparatus and move to the plasma membrane, redistributing sphingolipids and cholesterol to the cell surface. The forward flow of cholesterol, sphingolipids, and glycerophospholipids toward the plasma membrane is balanced by selective retrograde flow. Glycerophospholipids transferred from the ER to the Golgi apparatus are recycled back to the ER. Similarly, sphingolipids delivered to the plasma membrane from the Golgi apparatus are returned to the Golgi apparatus. Cholesterol, in contrast, is not returned through these retrograde pathways to either the ER or the Golgi apparatus but enters and circulates within the endocytic pathway leading to lysosomes. This pattern of lipid circulation creates a gradient of cholesterol, sphingolipids, and glycerophospholipids across the secretory membrane system. Within this gradient, the ER has a low concentration of cholesterol (e.g., sterols) and sphingolipids, the Golgi apparatus has an intermediate concentration, and the plasma membrane has a high concentration. The lipid gradient serves two important functions. First, it generates different lipid environments in the ER, Golgi apparatus, and plasma membrane compatible with their distinct functions. Second it promotes sorting of transmembrane proteins within the secretory system.

Sorting and transporting proteins within the secretory membrane system depend on several types of proteins (**Fig. 2**): Specialized “coats” help to generate both small and large transport carriers and sort proteins into them; motor proteins move carriers along the cytoskeleton; “tethering factors” attach carriers to the cytoskeleton and to their destination organelles prior to fusion; and fusion proteins mediate fusion of the carrier with an acceptor membrane. These components also associate with specific organelles, providing organelles with an identity that is both unique and dynamic. Many of the components are peripheral membrane proteins that lack transmembrane domains, so they must be recruited to the cytoplasmic surface of appropriate membranes by binding to either specific lipids, such as phosphoinositides, or to activated GTPases. Cells regulate the distributions of these organelle-specific lipids and GTPases. When infectious agents or stressful conditions disrupt these targeting molecules, secretory membrane trafficking can be disorganized and/or inhibited. The following sections describe the six major protein-based mechanisms that are used for sorting, transport, and fusion in the secretory membrane system.

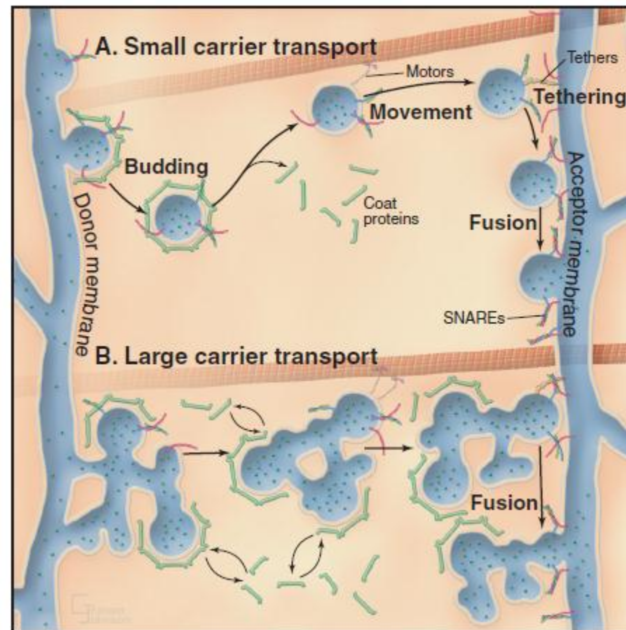


Figure 2. Protein machinery for secretory transport. *Coat proteins that cluster into polymerized arrays help to sort soluble cargo and transmembrane proteins into a coated bud that pinches off a donor membrane as a coated vesicle **(A)** or larger vesicular tubular carriers **(B).** The carriers move by motor proteins along either microtubules or actin. Tethering factors, including long coiled-coil proteins or multimeric tethering complexes, tether the carriers to an acceptor membrane. SNARE proteins on the carrier and acceptor membrane then form a complex that drives membrane fusion, leading to delivery of the carrier's content to the acceptor membrane.*

Arf family of GTPases includes Sar1, Arf1-6 and several distantly related Arf-like GTPases. These small GTPases mediate the association of a wide variety of protein effectors with specific membranes, which, in turn, leads to the differentiation of membrane domains that give rise to transport carriers and create compartmental identity.

The COPII coat complex is essential for sorting and trafficking secretory cargo out of the ER. It consists of Sar1p GTPase, the Sec23p•Sec24p subcomplex, and the Sec13p•Sec31p subcomplex. These components self-assemble into a polymeric, two-dimensional scaffold (called a coat) that then collects specific types of cargo. The intrinsic curvature of the coat promotes the formation of membrane buds that are capable of pinching off the membrane as coated vesicles.

The COPI coat complex is found on the cytoplasmic face of pre-Golgi (also called vesicular tubular carrier [VTC]) and Golgi compartments and helps to mediate protein sorting and retrograde transport from these structures back to the ER.

The Rab family of GTPases are the molecular switches that control the protein-protein interactions between transport carriers and docking complexes on target membranes. These complexes recruit motor proteins that transport carriers on actin filaments or microtubules and then tether carrier vesicles to an organelle prior to fusion.

Tethering factors are rod-shaped proteins that extend about 15 nm from membranes into the cytoplasm. They tether membrane carriers to target organelles prior to fusion and play structural roles as components of a Golgi matrix or scaffold for the assembly of other factors important for fusion and/or cargo sorting.

The SNAP receptor (SNARE) family of proteins participates in the fusion of carriers with their appropriate acceptor compartment. Most SNAREs are transmembrane proteins with their functional N-terminal domains in the cytoplasm and their C-termini anchored to the bilayer. Each contains a heptad repeat (i.e., "SNARE motif") of 60 to 70 amino acids that can form a coiled-coil. Multiple SNAREs assemble a SNARE complex consisting of a bundle of α-helices. Members of the SNARE protein family were originally grouped according to whether they were v-SNAREs or t-SNAREs, referring to whether they conferred function to the vesicle (v-SNARE) or target (t-SNARE) compartment.

Secretory Transport from the Endoplasmic Reticulum to the Golgi Apparatus. Transport of newly synthesized proteins out of the ER takes place in specialized areas called ER export domains**.** These structures are approximately 1 to 2 µm in diameter and appear in fluorescent images as dispersed, punctate structures that are scattered over the surface of the ER. An individual ER export domain is organized into two zones. One is a region of smooth ER membrane studded with COPII-coated buds and uncoated tubules. The other is a central cluster of vesicles and tubules with the capacity to detach and traffic to the Golgi apparatus. The ER membrane is continuous between these two zones until the vesicle-tubule cluster and its associated cargo detach from the ER and move to the Golgi apparatus as a transport intermediate, called vesicular tubular carrier (VTC). Cargo proteins are actively sorted into ER export domains through binding of signal motifs within their cytoplasmic tails to the COPII coat, and/or by lateral partitioning into the specialized lipid environment of this region. Sar1 GTPase initiates ER export domain formation through COPII-mediated sorting of specific integral membrane proteins (including the p24 family proteins and SNAREs) and the formation of coated buds. The presence of coated buds and specialized cargo in this region, together with the membrane tension produced by the coated buds, leads to changes in bilayer lipid composition. This, in turn, promotes partitioning of other transmembrane proteins into the ER export domain. Additional cytoplasmic proteins are then also recruited to the ER exit. Together, these molecules stimulate the membrane budding and fusion events that differentiate the ER export domain and VTC. Detachment of the VTC from the ER export domain and its maturation and delivery to the

Golgi apparatus are the next steps in protein trafficking from ER to Golgi apparatus. After VTCs have clustered by movement inward along microtubules, they undergo fusion with the Golgi apparatus. This occurs at the *cis* or entry face of the Golgi apparatus, also called the *cis*-Golgi network (CGN) because of its elaborate tubular appearance. The membrane fusion releases cargo proteins and lipids of the VTC into the Golgi system for processing by enzymes that modify the cargo's oligosaccharide side chains. Exactly how biosynthetic cargo is then transferred through the Golgi apparatus system has not been clarified experimentally, but three mechanisms are likely to contribute. The first mechanism uses vesicular transport to transfer cargo between the distinct cisternal elements that make up the Golgi apparatus. Vesicles derived from one cisternum transfer cargo to a neighboring cisternum. In a second mechanism, cargo is conveyed across the Golgi system by directed maturation of cisternal elements. A third mechanism involves diffusion and/or lateral partitioning of cargo within the membrane or lumenal spaces between interconnected cisternal Golgi elements. The contributions of each mechanism are still unclear and may vary depending on the cargo being transported through the Golgi system. (**Fig.3**).

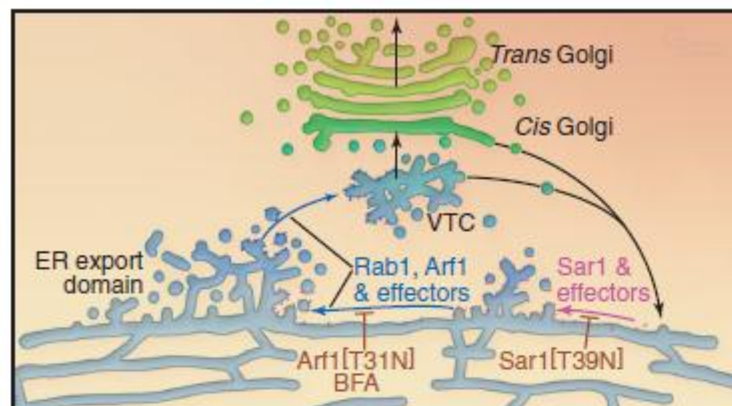


Figure 3. Transport from the endoplasmic reticulum to the Golgi apparatus. *ER to Golgi transport is orchestrated by the combined activities of many molecules. Sar1 and its effectors initiate COPII-coated bud formation and clustering of cargo at regions called ER export domains. This induces p115 and Rab1 to bind to these regions, which in turn recruits GBF1, the GEF for Arf1. Subsequent recruitment of Arf1 and its effectors further differentiates the ER export domain into a VTC. The VTC detaches from the ER and targets the Golgi apparatus, where it fuses with the cis face of the Golgi. The cargo in the VTC is then released into the Golgi and moves to the trans Golgi (where it will exit from the TGN). Expression of a constitutively inactive Sar1 mutant, Sar1[T39N], blocks COPII recruitment, and no ER exit sites form. Expression of an inactive Arf1 mutant, Arf1[T31N], or BFA treatment blocks recruitment of Arf1 effectors, which prevents ER exit sites from differentiating into VTCs. This causes the shrinkage and disappearance of the Golgi apparatus because new membrane from the ER cannot be delivered to the Golgi.*

Sorting from the *Trans*-Golgi Network. After transport through the Golgi system, cargo leaves the *trans* or exit face of the Golgi apparatus (**Fig. 4**). The exit region is called the *trans*-Golgi

network (TGN) because of its tubular network organization. This organization is characteristic of other sorting compartments, such as that of the VTC, the *cis* Golgi, and sorting endosomes. Depending on the cell type, the cargo that arrives in the TGN can be distributed, via distinct transport carriers, to several different intracellular locations, including the plasma membrane or cell exterior, the endosome/lysosomal system, or specialized secretory organelles or granules. The intracellular route taken by each protein depends on sorting properties that are encoded in the polypeptide chain.

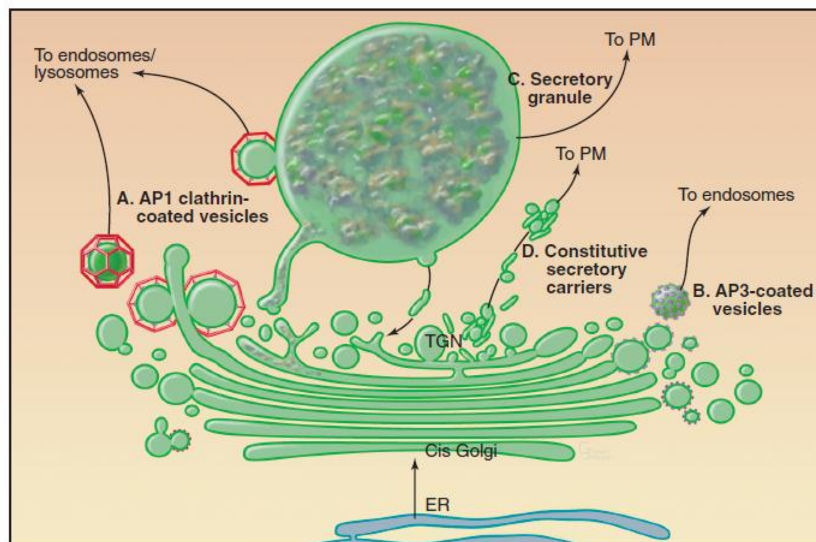


Figure 4. Divergence of biosynthetic/exocytic cargoes at the trans-Golgi network. A–D*, Cargo destined for secretion or distinct intracellular locations is sorted and packaged into distinct transport carriers. The tubular/vesicular geometry of the TGN plays an important role in protein sorting. PM, plasma membrane.*

- Constitutive Transport of Cargo to the Plasma Membrane or Cell Exterior. A steady stream of both proteins and lipids from the TGN to the cell surface occurs constitutively through tubular transport carriers that bud out from the TGN. No known coat proteins function in the formation of these structures. Instead, cargo proteins conveyed to the plasma membrane by these structures have transmembrane segments that partition into lipid domains containing sphingolipids and cholesterol. In mammalian cells, motor proteins such as kinesins move the constitutive membrane carriers outward from the Golgi apparatus along microtubules. Fusion of the carriers with the plasma membrane releases cargo within the lumen of the carrier vesicle into the extracellular space. After fusion, membrane lipids and proteins redistribute laterally by diffusion in the plane of the plasma membrane.

- Sorting to the Endosome/Lysosomal System. Proteins that are sorted into the endosome/lysosomal system include a large and diverse class of hydrolytic enzymes contained within lysosomes, the digestive centres of the cell.
- Secretory Granule Formation and Transport. An additional sorting pathway from the TGN occurs in specialized endocrine, exocrine, or neuronal cells that concentrate and package selected proteins in storage granules for eventual mobilization and discharge from the cell in response to hormonal or neural stimulation. This is the so-called regulated secretory pathway, which is used for discharging most of the body's polypeptide hormones, enzymes used in the digestive tract, and many other products that are needed intermittently rather than continuously.

Trafficking to the Plasma Membrane in Polarized Cells. In contrast to nonpolarized cells, polarized cells have functionally (and thus compositionally) distinct apical and basolateral domains separated by tight junctions that cement neighbouring cells together and prevent diffusion between the domains. As expected, the trafficking complexity increases as destination options increase and three distinct mechanisms for the polarized sorting of plasma membrane proteins have been revealed. One mechanism involves selective packaging of apically or basolaterally destined proteins into distinct carrier vesicles at the TGN for delivery to the appropriate surface. A second mechanism involves the random delivery of newly synthesized proteins to both surfaces, followed by selective retention or depletion so that, at steady state, they become differentially abundant because they are more stable at one surface than at the other. A third mechanism involves delivery of newly synthesized proteins to the basolateral surface, followed by selective internalization, sorting in the endosomal compartment, and delivery to the apical surface in a process termed transcytosis. Most epithelial cells use different combinations of these three mechanisms to generate and maintain cell polarity. Direct targeting uses basolateral targeting signals in the cytoplasmic domains of proteins to sort these molecules during secretory transport or during endocytosis by recycling the proteins from endosomes back to the appropriate membrane domain. Examples include receptors for low-density lipoprotein, transferrin, MPRs, and polymeric immunoglobulin receptor. Alternatively, direct targeting occurs by lateral partitioning of proteins into sphingomyelin- and cholesterol-rich subdomains (called lipid rafts) formed in the TGN or at the plasma membrane. GPI-anchored proteins or other integral membrane proteins that directly associate with these lipid rafts based on physical properties of their transmembrane domains are selectively targeted to the apical surface. The

unique physical properties of these lipid subdomains render them resistant to detergent solubilisation. The second sorting mechanism—random delivery followed by selective rearrangements—is particularly relevant to establishing polarity during cellular differentiation. In this case, uniformly distributed proteins that preexist on a non-polarized cell will redistribute themselves in a polarized fashion in response to cell-cell contacts that initiate polarization. Often, this occurs by the selective retention of a specific protein at the appropriate surface through intracellular (cytoskeletal) or extracellular (cell-cell or cell-matrix) interactions, or both. Proteins that are not actively retained on the other cell surface are internalized and degraded in lysosomes. Examples of proteins that are polarized in this way include Na^+K^+-ATPase and the cell adhesion molecule uvomorulin, an immunoglobulin-like cell adhesion molecule.

So the trafficking in non-neuronal cells can be summarized in this way, the central organelles involved in the secretory pathway are the ER, the Golgi apparatus, and the TGN. Proteins destined for the plasma membrane enter the lumen of the ER as they are translated by ER-associated ribosomes, where they fold into their proper three-dimensional structure assisted by chaperone proteins such as BiP, calnexin, and calreticulin (Kleizen and Braakman, 2004). Posttranslational modifications, including N-glycosylation and disulfide bond formation, also occur in the ER. Modified cargo that is ready to advance to the next step in the secretory pathway is concentrated at specific exit sites and leaves the ER in COPII-coated vesicles. These vesicles merge with the Golgi apparatus, where further protein modifications, including glycosylation and proteolysis occur. Finally, cargo is sorted at the TGN for transport to other endomembrane compartments or to the plasma membrane.

1.1. Neuronal trafficking machinery

Neurons reside at a pinnacle of cellular specialization. With their long extended axon and elaborate dendritic arbor, neurons establish the circuitry that detects, stores, and transmits information that is essential to the function of all complex organisms. Although neurons come in many shapes and sizes, they all polarize into discrete functional domains. The broadest level is between the axon and the somatodendritic compartment (Craig and Banker, 1994; Winckler and Mellman, 1999). Dendrites themselves can be further polarized (e.g., apical versus basolateral dendrites), and individual dendritic segments may have distinct molecular compositions and functional properties suggesting localized “polarity.” Neuronal polarity is established early on in development as neurons differentiate and extend processes and must be subsequently

maintained over a life span comprising decades. Both the maintenance and establishment of neuronal polarity involve coordinated and widespread regulation of the cytoskeleton and membrane trafficking machinery (da Silva and Dotti, 2002; Foletti et al., 1999). Given that neurons are cells with surface areas and cytoplasmic volumes 10,000 times greater than most eukaryotic cells, these regulatory mechanisms must be writ large.

Neurons are among the largest and most complex cells in the body. Functionally, protein trafficking to and from the postsynaptic membrane has emerged as a key mechanism underlying various forms of synaptic plasticity. All cells face the challenge of trafficking integral membrane proteins, secreted factors, and lipids to the appropriate subcellular location in the right amounts at the right time. For neurons, this task is especially daunting given their immense size and complex architecture. Although membrane-trafficking organelles were observed in neurons more than a century ago (Golgi, 1989), we are only now beginning to understand the mechanisms governing membrane transport to diverse neuronal functional domains and the role such transport plays in neuronal development, signalling, morphology, and plasticity. Many fundamental eukaryotic trafficking mechanisms are conserved in neurons, but neurons have evolved distinct modes of trafficking to accommodate their unique morphology. Neurons are highly polarized cells, with one axon housing the molecular machinery necessary for action potential propagation and neurotransmitter release and several dendrites containing receptors and signalling components that respond to neurotransmitter. At most excitatory synapses in the brain, presynaptic terminals directly appose membranous dendritic protrusions called spines, which are located along the entire length of dendrites and harbour the postsynaptic density (PSD), a multiprotein complex responsible for anchoring neurotransmitter receptors near sites of neurotransmitter release (Sheng, 2001). This layout requires that neurons traffic postsynaptic proteins over long distances, up to several hundred microns, through geometrically complex dendritic branches to satisfy the requirements of the most distal spines. Once delivered to spines, many synaptic components are subject to a new set of local trafficking rules, which determine whether the components are inserted into or removed from the plasma membrane and whether they are recycled or degraded. In some cases, protein trafficking is coupled to neural activity. For example, high-frequency afferent stimulation often leads to neurotransmitter receptor addition to the postsynaptic membrane, whereas low-frequency stimulation triggers receptor removal (Carroll et al., 1999; Hayashi et al., 2000; Shi et al., 1999). Given that the number and density of neurotransmitter receptors are critical determinants of synaptic strength, spine membrane protein trafficking has emerged as a key postsynaptic mechanism underlying

various forms of synaptic plasticity, including long-term potentiation (LTP) and depression (LTD) (Bredt and Nicoll, 2003; Malenka and Bear, 2004). Because individual dendritic spines are decorated with only tens to hundreds of surface glutamate receptors, the addition or subtraction of just a few receptors to the spine surface can, in principle, alter neurotransmission (Matsuzaki et al., 2001; Momiyama et al., 2003; Tanaka et al., 2005). Thus, the factors that govern postsynaptic receptor trafficking must be subject to stringent regulation. This regulation appears to have very precise spatial parameters considering that adjacent spines on a dendrite (often separated by only a few microns) can have strikingly different steady-state levels of synaptic proteins (Bagal et al., 2005; Matsuzaki et al., 2004). Recent work has begun unravelling the complex cell biology of neuronal membrane trafficking.

1.1.1. Dendritic organelles

Endoplasmic Reticulum, Golgi and the Neuronal Secretory Pathway. In most eukaryotic cells, the ER extends throughout the cell, whereas the Golgi network is located near the cell nucleus and the microtubule organizing center (Levine and Rabouille, 2005). In neurons, this arrangement is quite different (Horton and Ehlers, 2004). In dendrites isolated from the neuronal cell body, enzymatic activities associated with the Golgi network, such as protein glycosylation, persist (Torre and Steward, 1996). This observation, along with the demonstration that mRNAs for several integral membrane proteins are translated and secreted to the dendritic plasma membrane (Ju et al., 2004; Kacharmina et al., 2000), suggests that dendritic processes harbour all the necessary machinery for protein secretion. Indeed, electron microscopy studies have documented the presence of an extensive endomembrane network (including ER) that extends deep into dendritic processes (Gardiol et al., 1999; Spacek and Harris, 1997). Protein markers for Golgi membranes, including α-mannosidase II, giantin, and Rab6, have been found in the dendrites of some neurons (Gardiol et al., 1999; Pierce et al., 2001; Spacek and Harris, 1997). The dendritic localization of these organelles suggests that "satellite" protein secretion can occur at sites far from the nucleus. Investigators recently showed this by imaging live hippocampal neurons transfected with a temperature-sensitive variant of the vesicular stomatitis viral glycoprotein (VSVGtsO45), a transmembrane protein that is retained in the ER at 39.5◦C but released when the temperature is reduced to 32◦C (Bergmann, 1989; Presley et al., 1997). Upon synchronous release from the ER, a fraction of pre-Golgi carriers harbouring VSVGtsO45 merged with dendritic compartments positive for the Golgi markers galactosyltransferase and GM130,

demonstrating that the Golgi network found in dendrites is functional and that membrane protein processing and secretion likely occur at sites distant from the cell body (Horton and Ehlers, 2003a). At 20◦C, a temperature at which vesicle budding from Golgi is blocked, newly released VSVGtsO45 frequently accumulated at Golgi outposts located at dendritic branch points (Horton et al., 2005) (**Fig. 5** (Kennedy and Ehlers, 2006)).

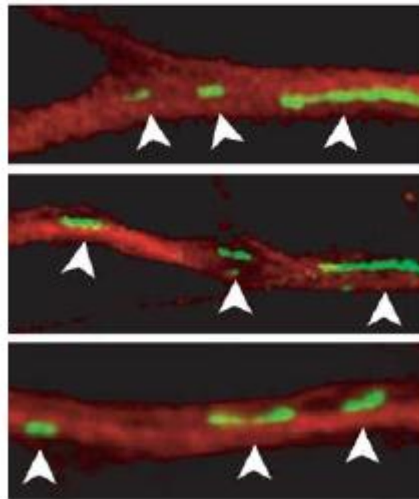


Figure 5. Dendritic Golgi outposts. *Labelling with GM130 (green) demonstrates the presence of local Golgi outposts (arrowheads) in dendritic shafts from three different cultured hippocampal neurons.*

Cargo destined for the distal ends of dendrites passes through several dendritic branch points on its journey, raising the question of what controls the flow of cargo to each branch? Golgi outposts situated at dendritic intersections engage in ongoing post-Golgi trafficking (Horton et al., 2005) and are properly positioned to regulate the identity or the amount of cargo that is trafficked to each branch. Despite the presence of functional Golgi outposts in some hippocampal dendrites, not all dendrites possess detectable Golgi. Moreover, even in those dendrites containing Golgi outposts, most ER-to-Golgi carriers originating in the dendrite are trafficked all the way back to the somatic Golgi (Horton and Ehlers, 2003a). Thus, dual modes of early secretory trafficking exist in dendrites. In fact, the major mode of ER-to-Golgi trafficking is directed long distances to the Golgi apparatus in the soma. This appears to be the exclusive mode of early secretory trafficking in those dendrites lacking Golgi outposts. A second, minor mode of ER-to-Golgi trafficking occurs locally in the dendrite, which may be specialized for localized control of dendritic secretion and dendritic membrane composition (Horton and Ehlers, 2003a, 2004). These experiments highlight important differences in the spatial organization of the secretory pathway between neurons and most other eukaryotic cells, whose Golgi compartments are generally confined to the perinuclear region. In addition to their crucial roles

in secreting integral membrane proteins, the ER and Golgi network are the primary sites of lipid biosynthesis. This is particularly important for neurons, which add an immense amount of plasma membrane during neurite outgrowth, allowing them to achieve surface areas up to 10,000 times greater than typical mammalian cells (Horton and Ehlers, 2003b). Consistent with a requirement for membrane trafficking through the secretory pathway in dendrite growth, disrupting Golgi function with brefeldin A in developing hippocampal neurons resulted in neurons with fewer and shorter dendritic processes (Horton et al., 2005). Subjecting mature neurons to the same treatment caused a dramatic simplification of dendrites and a ∼30% loss in total dendrite length over 24 h, demonstrating that even after neuronal architecture is established, membrane flux through the secretory pathway continues and is required for maintaining dendritic size and geometry (Horton et al., 2005). Somatic Golgi is nearly always oriented toward the apical dendrite. As a result, the majority of post-Golgi carriers are directed to the apical dendrite, which suggests that Golgi geometry may determine dendritic asymmetry. This idea is supported by the observation that somatic Golgi is asymmetrically distributed prior to specification of the apical dendrite. Furthermore, disrupting Golgi polarization by expressing GRASP65, a Golgi membrane protein required for cisternal stacking, disrupted Golgi polarization and blocked specification of the apical dendrite (Horton et al., 2005). In contrast with another recent study (de Anda et al., 2005) Horton et al. (2005) observed that somatic Golgi organization showed no relationship to axon position. Additionally, disrupting the secretory pathway by overexpressing a kinase-dead form of protein kinase D1, which prevents cargo budding from the TGN, resulted in cessation of dendritic growth, whereas axonal growth persisted for a period of days, indicating that a distinct pathway governs membrane addition to the growing axon (Horton et al., 2005).

The spine apparatus. **Smooth endoplasmic reticulum.** Whereas Golgi elements in dendrites appear to be localized to the dendritic shaft, the smooth endoplasmic reticulum (SER) extends through the neck of many spines, providing a direct conduit to the synapse. The presence of smooth vesicles near the tip of the spine apparatus raises the intriguing possibility that this organelle could supply membrane for spine growth and possibly traffic important synaptic proteins, such as AMPA and NMDA receptors, which have been localized to the spine apparatus (Nusser et al., 1998; Racca et al., 2000; Spacek and Harris, 1997).

Dendritic endosomes. Endosomes are intracellular, membrane-bound structures that accept endocytic vesicles from the plasma membrane and sort newly internalized membrane proteins for degradation or transport back to the cell surface. The endosomal network is composed of early/sorting endosomes, recycling endosomes, and lysosomes. Newly internalized vesicles shed their clathrin coats before fusing with one another and with sorting endosomes, which have a tubular-vesicular morphology. Sorting endosomes mature into late endosomes as they become more acidic and acquire acid hydrolase activity (Maxfield and McGraw, 2004). Before this occurs, molecules destined for reinsertion into the plasma membrane exit the sorting endosome on vesicles pinched off from small diameter tubules and are either trafficked directly to the cell surface or to recycling endosomes (Dunn et al., 1989; Mayor et al., 1993). The remaining contents of late endosomes are degraded in lysosomes (Maxfield and McGraw, 2004). Internal membranous compartments resembling endosomal structures have been observed in dendrites. These include coated and uncoated vesicles, tubular structures, and multivesicular bodies (Cooney et al., 2002). Approximately 70% of the endosome-like structures were situated within or at the base of dendritic spines.

Dendritic Mitochondria. Dendrites also harbour mitochondria, which are located mainly in the dendritic shaft but are occasionally found associated with spines (Adams and Jones, 1982; Cameron et al., 1991; Popov et al., 2005). A recent study demonstrated that mitochondria mobility in dendrites is controlled by synaptic activity. Synaptic stimulation decreased mitochondrial mobility and increased the association of mitochondria with dendritic spines (Li et al., 2004).

Microtubule transport. Intracellular transport mechanisms are required to deliver organelles and other cargo important for growth, function, and maintenance to axons and dendrites. To this end, neurons contain an elaborate network of microtubules radiating from the soma into dendritic and axonal processes. Axons contain microtubules with their plus ends pointed away from the cell body, whereas dendrites harbour microtubules in either orientation (Baas et al., 1988). The kinesin family of motor proteins travel along microtubule filaments, acting as intracellular couriers, shuttling soluble proteins, mRNA, and cellular organelles along the microtubule network (Vale and Fletterick, 1997). Kinesin was originally discovered as the molecular motor responsible for fast axonal transport (Brady, 1985; Vale et al., 1985).

To summarize, in **Fig. 6** (Horton and Ehlers, 2003a), it was reported dual modes of early secretory traffic in neurons. **Fig. 6 A** (1) In all neurons, cargo buds from the ER at specialized ER exit sites, located in both the soma and dendrites, and traffics to the neuronal Golgi. (2) In a subset of neurons, Golgi is present only in the neuronal soma, necessitating long-range inward trafficking of pre-Golgi carriers (long-range ER-to- Golgi transport). (3) Other neurons have, in addition to somatic Golgi, dendritic Golgi structures that function in secretory trafficking (local ER-to-Golgi transport). Dendritic ER exit sites and Golgi compartments function in secretory trafficking in parallel with the somatic Golgi, and are thus positioned to serve particular dendritic regions or sets of synapses.

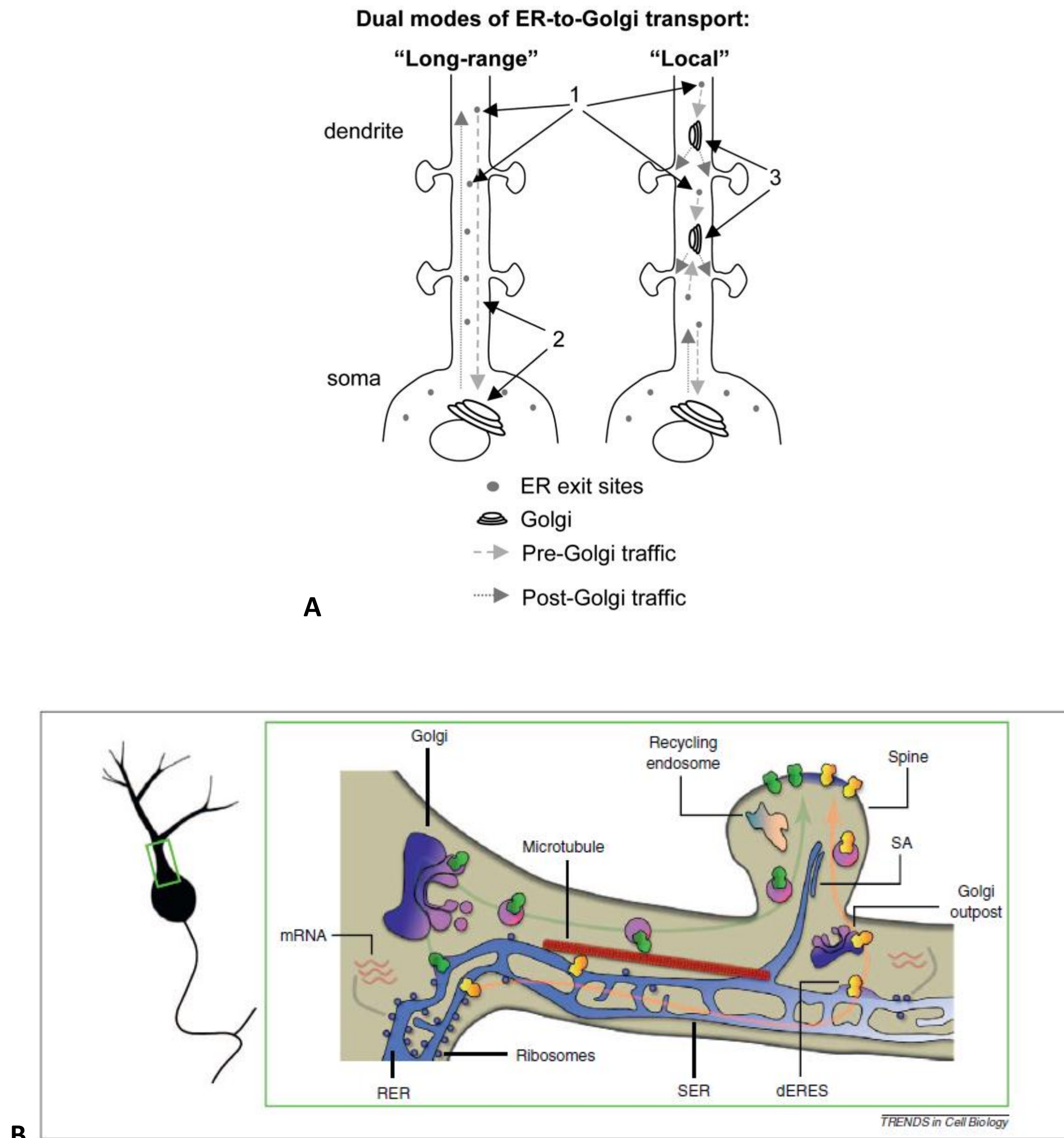


Figure 6. Proposed model of ER-to-Golgi trafficking in neuronal dendrites. (A). *An illustration of the dual organization of early secretory pathway components in neuronal dendrites.* ***(B)*** *(Ramirez and Couve, 2011)****.*** *Two modalities for nascent protein trafficking in dendrites. Membrane proteins using a canonical pathway (green receptors and green arrow) are synthesized and exported from the somatic ER, traverse a centralized Golgi compartment, and are sorted by means of long-range post-Golgi vesicles that travel assisted by molecular motors before plasma membrane delivery. Trafficking of membrane proteins through a non-canonical route (orange receptors and orange arrow) utilizes the dendritic ER, that allows protein synthesis or long-distance transport through the network, and dERES that control ER export along dendrites. Cargo exits the ER distally and travels across Golgi outposts before inserting into the plasma membrane. dERES, dendritic ER exit site; RER, rough ER; SER, smooth ER; SA, spine apparatus.*

Dendritic Golgi outposts as novel components of the secretory pathway.

A principal finding from the study of Horton and Ehlers (2003a) is the observation that many neurons possess both somatic Golgi and discrete, discontinuous Golgi-type structures located far into the dendrites. These dendritic Golgi outposts possess both matrix and enzyme components, and function similarly to the somatic Golgi. In other mammalian cells, the Golgi consists of a series of interconnected, flattened cisternae that are located in the perinuclear region (Rambourg and Clermont, 1990). In Horton and Ehlers, 2003a it was shown that this core organization is present in neurons, but, in addition, many neurons possess discrete packets of Golgi throughout neuronal dendrites. This distributed network of secretory organelles may allow for more localized control over protein sorting and membrane composition than would be conferred if Golgi were concentrated solely in the cell body. Dendritic Golgi outposts are much smaller, at 1–4 µm across, than somatic Golgi, which may have a surface area of tens of micrometers. It is possible that, given their small size, dendritic outposts are specialized to process only a subset of neuronal proteins. However, as only a minute fraction of Golgi is necessary to restore full secretory capability in transfected cytoplasts (Pelletier et al., 2000), it seems likely that dendritic Golgi outposts provide dendrites with substantial secretory capacity. Elaboration of a dendritic arbor and extension of an axon define neuronal shape and are the key morphological features of neuronal maturation (Bradke and Dotti, 2000; Jan and Jan, 2001; Scott and Luo, 2001). Both processes involve large increases in the surface area of the plasma membrane that require selective targeting of integral membrane proteins (Burack et al., 2000; Higgins et al., 1997; Sampo et al., 2003) as well as massive production of lipid membranes and surface incorporation via exocytosis (Bradke and Dotti, 2000; Martinez-Arca et al., 2001). These findings indicate that this period of process extension and surface-area expansion is accompanied by marked growth of the neuronal Golgi and fragmentation into dendritic Golgi outposts. Moreover, expansion and dispersal of secretory organelles may regulate neuronal morphology. In axons, new membrane is added at the tips of extending processes (Craig et al., 1995; Zakharenko and Popov, 1998). In growing dendrites, the points of membrane addition are uncertain, but the highly branched nature of dendrites suggests a requirement for multiple independently regulated points of membrane addition (Bradke and Dotti, 2000), a requirement ideally suited by autonomous dendritic secretory systems. It was shown that dendritic Golgi outposts appear during early periods of neuronal differentiation. Golgi outposts may form as fragments derived from somatic Golgi that expand into dendrites as the dendrites themselves grow. Alternatively, Golgi outposts may form *de novo* from transitional ER elements. In either

case, it seems quite likely that specific signals locally control the formation, distribution, and abundance of dendritic Golgi.

The remarkable feature is that, although many neurons possess components of the secretory pathway allowing dendritic ER-to-Golgi trafficking autonomous of the neuronal soma, other neurons have a Golgi compartment that is entirely somatic. In these neurons, all cargo exiting the ER in the dendrites must traffic in a manner retrograde to the somatic Golgi. Indeed, even in neurons possessing dendritic Golgi outposts, the bulk of ER-to-Golgi traffic is long-range toward the soma. This raises important questions about protein targeting in these neurons and suggests that pre-Golgi carriers retain specific identities over long distances. Furthermore, it was found that, although some dendritic pre-Golgi carriers merge with dendritic Golgi outposts, others bypass these outposts en route to the somatic Golgi, suggesting that pre-Golgi carriers themselves exist in distinct populations.

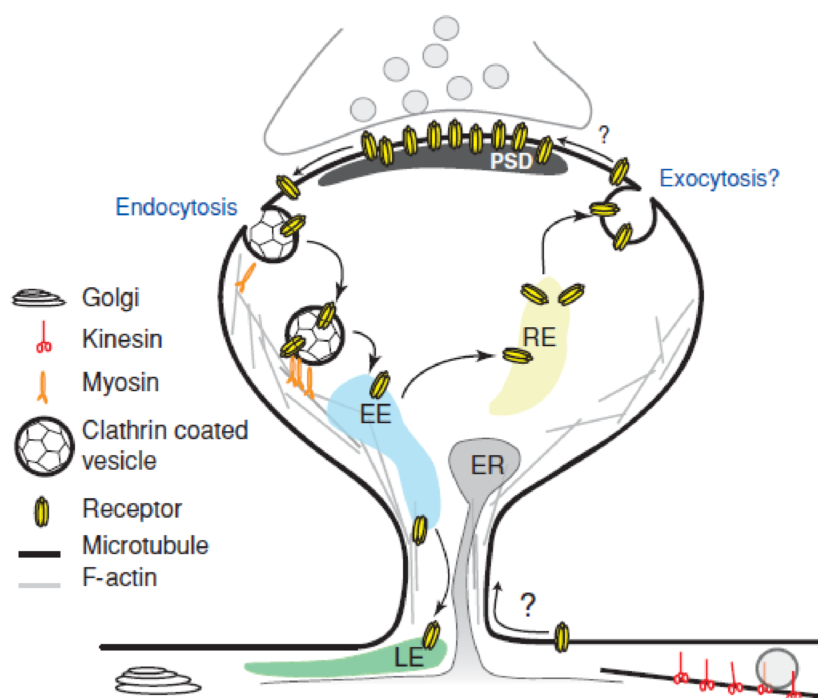


Figure 7. Model for local postsynaptic trafficking *(Kennedy and Ehlers, 2006)**.** Components of the synapse either diffuse from the synapse or are actively transported to endocytic zones surrounding the PSD, where they are internalized and trafficked to early endosomes (EE) and sorted either to late endosomes (LE) for degradation or to recycling endosomes (RE) for return to the spine surface. Cargo destined for the synapse is exocytosed to the cell surface at an unknown location, perhaps at the plasma membrane of the spine head. Alternatively, synaptic components could be exocytosed to the surface of the dendritic shaft and diffused through the spine neck to the synapse.*

2. A Disintegrin And Metalloprotease (ADAM) Family

Proteins belonging to the ADAM family have metalloprotase, integrin-binding, cell adhesion and intracellular signalling functions, thus ADAMs are well positioned to coordinate various cellular processes that are required for neuronal development, plasticity and repair (Wolfsberg et al., 1995a). In fact, ADAMs are central players of a biological process called regulated intramembrane proteolysis. ADAMs mediate the proteolytic cleavage of transmembrane proteins in their juxtamembrane region, causing their shedding, i.e. the release of their extracellular domain in a soluble form. In addition, through the intracellularly retained stubs, ADAMs can initiate the activation of intracellular signaling cascades.

In light of the above, ADAMs have an important role in cell-adhesion and cell-cell fusion, the latter playing a crucial role in vesicular trafficking and fertilisation (Evans, 2001). Other important roles have been identified in cell signalling (Wolfsberg and White, 1996) and in proteolysis of a wide range of cellular substrates, including: Amyloid Precursor Protein (APP), PrP, Notch, insulin-like growth factor-binding proteins (IGFBPs) and N-Cadherin. ADAMs cleavage often activates the substrate protein either by releasing of the active extracellular peptide (e.g., TNFα) or by enabling intracellular activation or further processing of the transmembrane protein (e.g., Notch). Because of this ectodomain shedding activity, the ADAMs are also known as sheddases. ADAMs may also play a role in cleavage-dependent inactivation of proteins (e.g., ADAM10 inactivating the ephrin/Eph complex by shedding ephrins (Hattori et al., 2000; Janes et al., 2005)) or extracellular matrix molecules (e.g., in Xenopus where ADAM13 regulates neural crest cell migration by cleaving fibronectin (Alfandari et al., 1997)).

From a structural point of view (**Fig. 8**) they are type I transmembrane proteins and possess an N-terminal signal peptide, a propeptide domain containing a cysteine switch, a cleavage site for the pro-hormone convertases, a metalloprotease domain, a cysteine-rich region, a disintegrin domain, an epidermal growth factor (EGF)-like sequence, a transmembrane domain and a short cytoplasmic tail. ADAMs are synthesized as inactive precursors and the removal of the propeptide by a furin-like endopeptidase (Loechel et al., 1998) leads to the activation of the metalloprotease domain. Most ADAMs contain the catalytic protease domain (HEXGHXXGXXHD) whose activity is dependent on the presence of zinc ions.

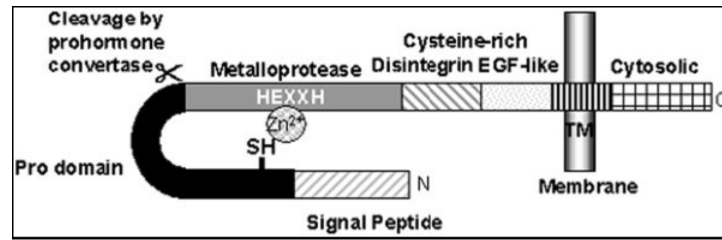


***Figure 8. Schematic representation of ADAMs structure*.**

The ADAMs are members of the extended family metazincins which also includes matrix metalloproteases (MMPs) and “ADAMs with ThromboSpondin motifs” (ADAMTS). ADAMs differ from most of the MMP and ADAMTS proteins because they have an integrin-binding (disintegrin) domain and because their shedding acitivity MMPs and ADAMTS proteins are more involved in digesting and inactivating proteins in the extracellular matrix (e.g., aggrecan, collagen) thus leading to structural changes necessary for cell function (Apte, 2004; Dzwonek et al., 2004).
Many of the ADAMs have an ECD motif (functionally equivalent to the classical RGD) in the disintegrin loop which can bind to various integrins (Bigler et al., 2000). However, almost nothing is known about the potential of ADAMs to activate intracellular signaling by integrins.
The ADAMs may also have classical cell adhesion properties because of the interaction between their cystein-rich domain to other proteins such as syndecan (Iba et al., 2000) and fibronectin (Gaultier et al., 2002), and because of dimerization with other ADAMs (Cho et al., 2000).
The cytoplasmic tail of some of the ADAMs contains SH3-binding sites which can interact with and potentially activate SH3 domains containing intracellular signaling molecules such as src, grb or SAP97 (Howard et al., 1999; Huang et al., 2002; Marcello et al., 2007; Suzuki et al., 2000). ADAM activities can be regulated by several mechanisms, such as gene expression, intracytoplasmic and pericellular regulation, zymogen activation and inhibition. Nevertheless the regulation mechanisms are not completely understood. Among the 33 known mammalian ADAMs, at least 17 have been observed in the nervous system (Alfandari et al., 1997; Gunn et al., 2002; Karkkainen et al., 2000; Perry et al., 1995; Yang et al., 2005; Yoshinaka et al., 2002). Among the 17 mammalian ADAMs expressed in the CNS, 10 ADAMs, including 1, 8–10, 12, 15, 17, 19, 21 and 33, are thought to be catalytically active metalloproteases capable of performing the dual functions of integrin-binding and protein cleavage (Primakoff and Myles, 2000; White, 2003; Wolfsberg et al., 1995a).

2.1. ADAM10

Members of the ADAM family are implicated in the processing of many single transmembrane-bound proteins ranging from cell surface receptors to growth factors and cytokines. Because of the biological significance of these processing events, a recurring theme in studying ADAM biology is that they are involved in physiological processes that can go awry and lead to disease states (Moss and Bartsch, 2004). A Disintegrin And Metalloprotease 10, ADAM10, was first isolated from bovine brain as an enzyme capable of degrading myelin basic protein (Chantry et al., 1989).

Subsequent isolation and sequencing of its cDNA revealed that it was a member of the ADAMs family and the enzyme was referred to as MADM (mammalian disintegrin-metalloproteinase) (Howard et al., 1996). Further studies revealed that ADAM10 is expressed in a wide variety of tissue either in Bos Taurus (Howard et al., 1996) and, more interestingly, in distinct areas of the human brain (Karkkainen et al., 2000; Marcinkiewicz and Seidah, 2000) and peripheral structures (Dallas et al., 1999; McKie et al., 1997). In adult brain, ADAM10 is widely distributed and expressed in astrocytes, microglial cells and neurons.

Initially, ADAM10 was suggested to be an enzyme, shaping the extracellular matrix by cleavage of collagen type IV, or to be a tumor necrosis factor α, (TNF-α) convertase. In a relatively short time, a wide variety of additional substrates (with APP, probably being the most prominent) have been identified and the search is still ongoing.

2.2. ADAM10 and its substrates in the central nervous system

In the central nervous system (CNS), ADAM10 “good” substrates can be found that participate in neuroprotection and regeneration and include: prion protein (PrP), N-Cadherin, neuroregulin, ephrins, L1 adhesion molecule, transmembrane chemokines, Notch and its ligand Delta and APP. By cleavage of these substrates, ADAM10 is thought to play critical roles in protective processes in neurodegenerative diseases and in regenerative events after CNS injury.

PrP. The cellular prion protein PrP is essential for the pathogenesis and transmission of prion diseases. Whereas the majority of PrP is bound to the cell membrane via a glycosylphosphatidylinositol (GPI) anchor, a secreted form of the protein has been identified. PrP can be released into the medium by both protease- and phospholipase-mediated mechanisms (Parkin et al., 2004). PrP undergoes constitutive cleavage involving ADAM10 and phorbol ester-

regulated proteolytic cleavage mediated by ADAM17 (Vincent, 2004). Therefore, activation of ADAM10 and ADAM17 could be seen as putative therapeutic strategy aiming at increasing normal PrP breakdown and thereby depleting cells of the putative toxic domain of PrP (Vincent, 2004).

N-Cadherin. Cadherins are Ca^{2+} dependent adhesion molecules expressed virtually by all cells that form solid tissue, during development as well as in adult life. N-Cadherin, the most abundant Cadherin in the CNS belongs to type I classical Cadherins. The release of its extracellular domain, which contains the homophilic binding site, is functionally of major importance for the regulation of cell adhesion, cell migration and neurite outgrowth (Nakagawa and Takeichi, 1998; Paradies and Grunwald, 1993). ADAM10 is responsible for the initial for the initial proteolytic event of N-Cadherin, leading to the release of the extracellular soluble domain and the production of the membrane-bound carboxy-terminal fragment CTF1 (Reiss et al., 2005). The coordinated interaction of ADAM10 and N-Cadherin may be significant for the coordinated interplay between cell-cell adhesion, cell detachment, cell proliferation and cell survival during embryogenic development, in wound healing and during tumor invasion.

Ephrin family. Ephrins are neuronal guidance molecules that bind to receptor tyrosine kinases of the Eph family. Ephrin cleavage has been shown fascinating. Ephrin ligands, presented on one cell surface, associate with their receptors on the surface of a juxtaposed cell, leading to cell-cell repulsion Ephrin ligand can be proteolytically released from its membrane tether by a complex on the opposing cell composed of the ephrin receptor and ADAM10. While ADAM10 constitutively associates with EphA3, the formation of a functional EphA3/ephrin-A5 complex creates a new molecular recognition motif for the ADAM10 cysteine-rich domain that positions the proteinase domain for effective ephrin-A5 cleavage. Surprisingly, the cleavage occurs in trans, with ADAM10 and its substrate being on the membranes of opposing cells, suggesting a simple mechanism for regulating ADAM10-mediated ephrin proteolysis, which ensures that only Eph bound ephrins are recognized and cleaved (Janes et al., 2005).
In particular, when the growth cone of a neuron that expresses Eph receptors encounters ephrin ligands on the surface of another cell, this initiates bidirectional signalling cascades that regulate cell adhesion, axonal guidance and neuronal plasticity. The growth cone then overcomes these adhesive forces and breaks away from the ephrin surface by proteolytic shedding of ephrin. A cleavage-inhibiting mutation within ephrin A2 delays axon detachment, suggesting that shedding

is critical for axon guidance in the CNS (Hattori et al., 2000). ADAM10 can cleave ephrin A5 bound to EphA3 and thus terminate binding via ephrin (Janes et al., 2005).

Notably, the ephrin receptor EphB2 also undergoes calcium-influx and n-methyl-d-aspartate (NMDA)- induced cleavage that is sensitive to ADAM10 inhibition (Janes et al., 2005). Thus, by cleavage of ephrins as well as their receptors, ADAM10 can be regarded as promoter of axon guidance and extension in the CNS.

Notch. The receptor Notch and its ligand Delta 1 are required for neuroepithelial development during embryogenesis, but also function in neuronal stem cell maintenance and self-renewal of the adult CNS. ADAM10 cleaves the extracellular domain of Notch (Brou et al., 2000; Hartmann et al., 2002) which is then directly followed by an intramembranous cleavage of the remaining cell-associated Notch molecule via γ-secretase. This results in the generation of a cytoplasmic cleavage fragment which can translocate into the nucleus and function as transcription factor (De Strooper et al., 1999). Thus ADAM10 is critically involved in the transcriptional signalling pathway of Notch and is required for its functions in neurogenesis even in the adult CNS. Other than N-Cadherin, at least two classes of cell-adhesion molecules are cleaved by ADAM10, γ-protocadherins and L1 adhesion molecule.

γ-Protocadherins (Pcdh-γ). Pcdh-γ are abundantly expressed in the nervous system. They are enriched at synapses and involved in synapse formation, specification and maintenance. Pcdh-γ C3 and Pcdh-γ B4 are constitutively cleaved within their ectodomains by ADAM10 inhibiting cell aggregation (Reiss et al., 2006).

L1 molecule adhesion. The immunoglobulin superfamily recognition molecule L1 promotes neuronal migration, neuronal survival and neurite out-growth (see (Kamiguchi, 2003)). L1 undergoes constitutive cleavage at the cell surface which can be enhanced by stimulation with PMA, cholesterol depletion or NMDA-treatment (Maretzky et al., 2005; Mechtersheimer et al., 2001). Constitutive and NMDA-induced shedding of L1 is mediated by ADAM10 while PMA stimulation or cholesterol depletion leads to ADAM17-mediated L1 cleavage (Maretzky et al., 2005). ADAM10-mediated release was found to occur in intracellular vesicles that are subsequently released while cleavage of L1 in response to PMA occurred at the cell surface (Stoeck et al., 2006). Soluble L1 has been shown to stimulate cellular migration, neurite out-growth and recovery after spinal cord injury (Doherty et al., 1995; Roonprapunt et al., 2003).

APP. Generation of Amyloid peptide (Aβ) from APP is at the beginning of a cascade that leads to Alzheimer's disease (AD). The shedding process is mediated by α- or β-secretases, while the cleavage of the membrane retained stubs is due to γ-secretase (Haass and Selkoe, 1993).
β- and γ-secretases are the principal players involved in Aβ production, while α-secretase cleavage on APP prevents Aβ deposition. Three members of the family of ADAMs have been shown to exert α-secretase activity on the Amyloid Precursor Protein (APP): ADAM10, ADAM17, which is also known as TACE, i.e., Tumor Necrosis Factor-alpha Converting Enzyme, and ADAM9, also referred to as MDC9, standing for metalloprotease/disintegrin/cysteine-rich protein (Allinson et al., 2003; Buxbaum et al., 1998; Hotoda et al., 2002; Lammich et al., 1999).
Two recent studies finally demonstrated that the constitutively cleaving α-secretase activity in neurons is selectively mediated by ADAM10 (Jorissen et al., 2010; Kuhn et al., 2010). ADAM10-mediated non amyloidogenic pathway on APP releases one soluble, neurotrophic fragment called sAPPα and one membrane associated stub, called CTF83, which can then be cleaved by the γ-secretase complex, liberating extracellular p3 and the AICD.
As regards ADAM10 involvement in AD pathogenesis, ADAM10 protein levels were found to be reduced significantly in platelets of sporadic AD patients and sAPPα levels in platelets and cerebrospinal fluid (CSF) of AD patients were also found to be decreased (Colciaghi et al., 2002). Complementary to these findings is the observation that α-secretase activity was reduced in temporal cortex homogenates from AD patients (Tyler et al., 2002). In contrast, ADAM10 mRNA levels were found to be two-fold increased in hippocampal and cerebellar sections of AD patients (Gatta et al., 2002). These results were obtained from the brains of severe AD patients and it is possible that, in later stages of the disease, ADAM10 expression is increased as a defense mechanism or as a secondary effect of inflammation and reactive gliosis.

2.3. ADAM10 gene structure

Using in situ hybridization, the human ADAM10 gene was located on chromosome 15 at position 15q21.3-q23 (Yamazaki et al., 1997b) and mouse ADAM10 gene on chromosome 9 (Yamazaki et al., 1997a).
The human ADAM10 coding region within the ADAM10 gene locus consists of 16 exons and comprises ~154 kb. Exon 1 contains the 5′-untranslated region and the coding region of the signal peptide. The prodomain is encoded by exons 2, 3, 4, and by a part of exon 5. The

metalloproteinase domain is encoded by exons 5-10 and the disintegrin domain by exons 10-12. A part of exon 12, exons 13-14 and a section of exon 15 code for the cysteine-rich domain, followed by the sequence encoding the transmembrane domain (exons 15 and 16). The translation stop codon TAA is located in exon 16, which also contains the sequence of the cytoplasmatic domain. With exception of exon 16, which is 764 bp in length, all other exons are relatively small, ranging in size from 93 to 499 bp. Intron sizes range between 482 and 35262 bp with the largest introns located primarily at the 5'-end of the gene. The mouse ADAM10 gene also consists of 16 exons interrupted by 15 introns, and the sizes of introns and exons are very similar between both species. The encoded human and mouse ADAM10 proteins do not differ in length, share 90% amino acid identity and 95% similarity. The GC content of the first 600 bp upstream of the ATG is 67% (Prinzen et al., 2005). Furthermore, this region is embedded in a GC-rich domain that extends from nucleotide -700 through the first exon into the first intron to +200, and nine CpG islands (Bird, 1986; Larsen et al., 1992) were found. The presence of these islands suggests that ADAM10 expression may be influenced by DNA methylation. A classical CAAT-box is located at -480 bp, and two Sp1 binding sites, (G/T)GGGCGG(G/A)(G/A)(C/A), are located at -521 bp and -366 bp, showing that regulatory elements that are required to initiate gene transcription are present. Further analysis identified two potential binding sites for the nervous system specific factor Brn-2 (-1972 and -1737) with the consensus sequence (He et al., 1989; Li et al., 1993). Four putative, but not highly conserved binding sites for sterol regulatory element binding protein (SREBP) (Yokoyama et al., 1993) are located at -1801 bp, -1623 bp, -1377 bp, and -1240 bp within the human ADAM10 5'-flanking region. The putative promoter also has potential binding sites for ubiquitously expressed transcription factors such as Oct-1 (-780) (Sturm et al., 1988). The 5'-flanking region of the human ADAM10 coding sequence also contains two tandem repeats of the dinucleotide CA (-1307/-1285 and -1172/-1138). This repetitive sequence was found multiple times in human and mouse genomes (Rietveld et al., 2003). The comparison of human, mouse, and rat ADAM10 5'-flanking regions revealed a remarkable sequence identity of 78% within 500 bp upstream of each translational start site. Multiple conserved motifs are present: Sp1-binding sites (-521 and -366), the CAAT-box (-479) and potential binding sites for the upstream stimulatory factor (USF) at position -317, Maz (-491 and -278), MZF-1 (-237), RXR (-302 and -203), NFκB (-198) and the CAAT displacement protein CDPCR3HD (-57). The maintenance of potential transcription factor binding sites suggests a biological relevance for the -500 bp region. More distant to the ATG, the resemblance decreases significantly indicating that these 5' upstream sequences may not participate in regulation of

ADAM10 expression (Prinzen et al., 2005). Because ADAM10 is ubiquitously expressed (Howard et al., 1996; Wolfsberg et al., 1995b), Prinzen and co-workers examined whether the putative human ADAM10 promoter is active in different cell lines such as HEK293, HepG2, SH-SY5Y, and IMR32 cells, which are representative for kidney, liver, and neural cells. As a negative control, the pGL3-basic vector, containing the promoterless luciferase gene, was used in parallel transfection experiments. For normalization of transfection efficiencies, the plasmid phRL-SV40, carrying a renilla luciferase cDNA under control of a SV40 promoter, was used additionally. After transfection the ADAM10 5′-flanking region in pCP53AB.1 induced a 68-fold increase in relative luciferase activity in SH-SY5Y cells, a 37- to 44-fold increase in HEK293 and IMR32 and a 20-fold increase in HepG2 cells. Thus the region -2179 to -1 upstream of the ADAM10 translation initiation site possesses functional promoter activity in the cells examined. To determine the 5′-flanking sequence, which is essential for transcription of the human ADAM10 gene and to identify regions involved in regulation of ADAM10 transcription, different sequentially deleted 5′-flanking regions were generated and cloned into the reporter plasmid pGL3-basic. After transient transfection of HEK293 cells, the promoter activities induced by these constructs were measured by using the dual luciferase reporter assay. The results obtained with the deletion mutants demonstrate the presence of activators of ADAM10 transcription between -508 and -300, which are critical for basal promoter activity. In this region a potential Maz binding site (-491), a CAAT-box (-479), a Sp1 binding site (-366) and an USF element (-317) are present, and the removal of some of these putative transcription factor binding sites in pCP49.2 (-300/-1) strongly decreased the promoter activity. Site-directed mutagenesis of a conserved USF element pointed to a central role of USF binding proteins in ADAM10 transcription. Site directed mutagenesis within the CAAT box and Sp1 sites did not influence promoter activity. USF is a transcriptional activator (Potter et al., 1991) which cooperates with TFII-I (Roy et al., 1991) and TFIID (Sawadogo, 1988) and facilitates formation of preinitiation complexes (Workman et al., 1990). Transcription initiation complexes are stabilized by interaction between Sp1 and TAFs (Kaufmann and Smale, 1994; Martinez et al., 1994). Deletion of Brn-2, SREBP, Oct-1 and CREB1/cJun consensus sequences did not significantly affect promoter activity, suggesting that these elements are not functionally important for constitutive ADAM10 promoter activity. This result does not exclude a role of the corresponding transcription factors in regulating the expression of ADAM10 in response to certain stimuli in other cellular backgrounds. Some of the potential transcription factor binding sites found in the human ADAM10 gene are also present in promoters of other genes involved in AD, including APP and presenilin genes: Sp1 sites have

been reported for APP (Izumi et al., 1992), presenilin-1 and (Mitsuda et al., 1997) presenilin-2 (Pennypacker et al., 1998) promoters. In vitro transcription and cotransfection studies showed that USF activates transcription from the human APP promoter (Kovacs et al., 1995).
Genetic studies revealed that Kuzbanian, the *Drosophila* orthologue of ADAM10, is involved in neurogenesis and axonal extension (Fambrough et al., 1996; Rooke et al., 1996). A physiological substrate for Kuzbanian is the cell surface Notch receptor (Pan and Rubin, 1997), which is involved in a number of signalling events during development (Artavanis-Tsakonas et al., 1995).

2.3.1. Regulation of ADAM10 gene expression by retinoic acid

Since enhancing the ADAM10 gene expression might be a promising approach for the treatment of AD, the human ADAM10 gene was functionally analysed (Prinzen et al., 2005). Two potential retinoic acid responsive elements (RAREs) are located in the ADAM10 promoter region −302 and −203 bp upstream of the translation start site of the ADAM10 gene. Accordingly, human ADAM10 promoter reporter assays showed a significant increase in transcriptional activity by treatment of neuroblastoma cells with the major vitamin A metabolite all-trans retinoic acid (atRA). Moreover, mRNA and protein levels of ADAM10 were increased by atRA, which was accompanied by increased sAPPα secretion in cells (Endres et al., 2005; Holback et al., 2005). Plasma levels of antioxidants, including retinol (vitamin A) are depleted in AD patients and subjects with mild cognitive impairment (MCI), a precursor phase of AD (Rinaldi et al., 2003). Furthermore, vitamin A acid is essential for hippocampal long-term synaptic plasticity (Chiang et al., 1998; Misner et al., 2001) and neurogenesis (Takahashi et al., 1999). Genetic, metabolic, and dietary evidence has been provided for a defective retinoid metabolism in AD: serum levels of the retinoic acid precursors, vitamin A and carotenoids, are decreased in AD patients (Jimenez-Jimenez et al., 1999; Zaman et al., 1992). The activity of the retinoic acid-generating enzyme retinaldehyde dehydrogenase (RALDH) is increased in the hippocampus and parietal cortex in AD brains (Connor and Sidell, 1997). Several genes of proteins that are linked to AD pathology, such as choline acetyltransferase (ChAT), insulin degrading enzyme (IDE), or apolipoprotein E (ApoE), are retinoid regulated (Cedazo-Minguez et al., 2001; Kobayashi et al., 1994; Melino et al., 1996). Moreover, genetic linkages can be found to AD for markers close to chromosomal loci coding for genes of retinoid receptors, retinol dehydrogenases, and cellular retinoic acid-binding proteins (Goodman and Pardee, 2003). Recently, retinoid hyposignaling was shown to result in aging-related decline of the hippocampal function in mice (Mingaud et al., 2008).

Response to retinoic acid is mediated by the retinoid X receptors (RXRs), which can bind to the DNA of target genes as RXR/RXR homodimers but also as heterodimers. Appropriate binding partners for RXRs are a second class of retinoid receptors—the retinoid acid receptors (RARs)—or a wide variety of other nuclear receptors, such as vitamin D receptor (VDR), thyroid hormone receptor (TR), peroxisome proliferator-activated receptor γ (PPARγ), and liver X receptor (LXR) (Aranda and Pascual, 2001) (**Fig. 9** (Tippmann et al., 2009)).

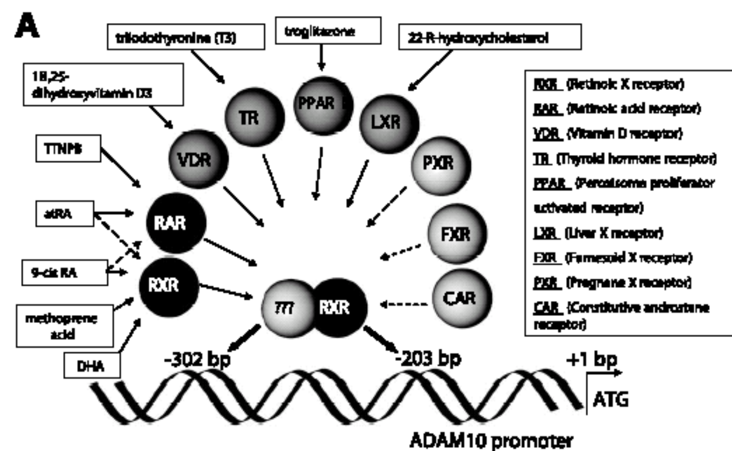


Figure 9. *RXR heterodimer binding partners and their ligands, which might play a role in the transcriptional activation of ADAM10. ADAM10 promoter contains two putative retinoic acid binding sites, at positions −203 and −302 bp upstream of the translation start site (Prinzen et al., 2005). While ligands of the retinoid receptors RAR and RXR (dark gray) stimulate ADAM10 promoter and α-secretase activity, ligands of the potential RXR dimerization partner VDR, TR, PPARγ, and LXR (gray) may also be involved in the regulation of ADAM10. Because other RXR dimer partners such as PXR, FXR, and CAR (light gray) are not predominantly expressed in the human brain, they were excluded from further investigations.*

Some of them might play a role in AD pathogenesis; the ligand-based PPARγ activation, for example, has been shown to decrease Aβ production by repression of the BACE1 promoter (Sastre et al., 2006). Agonists of LXR have been shown to enhance the expression of the cholesterol efflux transporter (ABCA1), which led to a decreased Aβ-production *"in vivo"* (Koldamova et al., 2003; Koldamova et al., 2005). Also, triiodothyronine, the biological active ligand for TR, negatively regulates the transcriptional activity of the APP gene (Belandia et al., 1998). The ω-3 polyunsaturated fatty acid docosahexaeonic acid (DHA) was suggested to be a physiological RXR ligand (Lengqvist et al., 2004). Epidemiologic studies provided evidence that increased intake of ω-3 fatty acids may be beneficial in reducing the risk for AD (Morris et al., 2003).

2.3.2. 5′-UTR ADAM10 gene expression regulation

ADAM10 has a 444-nucleotide-long, very GC-rich (69%) 5′-untranslated region (5′-UTR) with two upstream open reading frames. It is highly similar to a predicted ADAM10 5′-UTR of chimpanzee and of rhesus monkey, which may indicate that the 5′-UTR of ADAM10 might have a physiological function. Similar properties of 5′-UTRs are found in transcripts of many genes, which are regulated by translational control mechanisms. Sequence analysis revealed that the ADAM10 mRNA contains a long 5′-UTR with similar properties as the 5′-UTR of BACE1, which was shown to be involved in translational repression of BACE1 (De Pietri Tonelli et al., 2004; Lammich et al., 2004; Mihailovich et al., 2007; Rogers et al., 2004; Zhou and Song, 2006). It was demonstrated that the 5′-UTR of ADAM10 suppresses translation of ADAM10 "*in vitro*" and "*in vivo"*. Repression of ADAM10 expression is mediated by a very strong inhibitory element within the first 259 nucleotides of the ADAM10 5′-UTR (Lammich et al., 2010).

To investigate whether the 5′-UTR may affect expression of ADAM10, Lammich and co-workers transfected HEK293 cells with C-terminally V5-tagged ADAM10 cDNA constructs with or without the 5′-UTR. 24 h after transfection, they observed a significantly lower ADAM10 expression in the presence of the 5′-UTR. They detected the immature form of ADAM10 upon overexpression (Bech-Serra et al., 2006; Borroto et al., 2003; Lammich et al., 1999; Villanueva de la Torre et al., 2004). Quantification of ADAM10 levels revealed a 3-fold increase of ADAM10 protein in cells transfected with the cDNA construct lacking the 5′-UTR compared with the construct with the 5′-UTR of ADAM10. Significantly, the 5′-UTR had no effect on the respective mRNA levels, demonstrating that the 5′-UTR did not alter ADAM10 transcription. A similar repression of ADAM10 expression caused by the presence of the 5′-UTR was detected in African green monkey COS7 cells and in the human neuroblastoma cell line SH-SY5Y, demonstrating that the translational repression is not cell type-specific. To further provide evidence that the 5′-UTR of ADAM10 is involved in lowering ADAM10 protein expression by reducing ADAM10 mRNA translation, they investigated whether the 5′-UTR of ADAM10 could inhibit the expression of a heterologous ORF. They cloned the ADAM10 5′-UTR in front of a firefly luciferase reporter construct and transfected HEK293 cells with this construct or with luciferase lacking the 5′-UTR. Firefly luciferase activity was measured in cell lysates and normalized to "*Renilla"* luciferase, which was used as a transfection control. They could detect approximately four times more luciferase activity in cells transfected with luciferase lacking the 5′-UTR compared with the construct with the 5′-UTR of ADAM10, indicating that the 5′-UTR represses translation. To

provide further evidence, they performed an *"in vitro"* translation assay with equal amounts of "*in vitro"* transcribed luciferase mRNA with and without the 5′-UTR of ADAM10. They detected robust levels of luciferase protein only in the reaction mixture containing luciferase mRNA without the 5′-UTR of ADAM10. Taken together, these results demonstrate that the 5′-UTR of ADAM10 efficiently represses translation of ADAM10. Lammich and co-workers demonstrated also that the two uORFs do not contribute significantly to the translational repression mediated by the 5′-UTR of ADAM10. Instead, other features of the 5′-UTR such as the high GC content, which favors the formation of complex secondary structures, might be responsible for translational repression of ADAM10.

To elucidate whether increased ADAM10 expression levels go along with increased α-secretase activity, they transfected HEK293 cells overexpressing APP with ADAM10 cDNA containing the wild-type 5′-UTR or the 5′-UTR lacking the first 259 nucleotides, where is located the inhibitory element of ADAM10 expression. To allow for a sufficient ADAM10 maturation, they analyzed cell lysates for ADAM10 and APP and the corresponding conditioned medium for APP processing products 48 h after transfection. They detected a tremendous increase in ADAM10 expression, similar to that observed 24 h after transfection. Importantly, after 48 h they also detected small amounts of mature ADAM10 in cells transfected with the 5′-UTR ADAM10 construct lacking the first 259 nucleotides. As a consequence, they observed a 2-fold increase of APPsα, a mild reduction of APPsβ, and a significant decrease of secreted Aβ in the conditioned medium of these cells compared with mock transfected cells. However, after transfection of the 5′-UTR ADAM10 wild-type construct, no significant change in Aβ and APPsβ levels were observed, although they detected a mild increase in APPsα secretion in these cells compared with mock transfected cells. These data indicate that the 5′-UTR could have an important physiological role for post-transcriptional regulation of ADAM10 expression and consequently Aβ production.

2.4. ADAM10 protein structure

ADAM10 is a 748 amino acid type I membrane glycoprotein, which is ubiquitously expressed (Howard et al., 1996; Wolfsberg et al., 1995a). The enzyme belongs to the subgroup of metzincins within the zinc proteinases family. Members of the ADAM family are characterized by a defined domain structure, including a signal sequence, a N-terminal prodomain, followed by a catalytic proteinase domain containing a zinc-binding motif, a disintegrin, cysteine-rich transmembrane, and a cytoplasmatic domain (Weskamp et al., 1996; Wolfsberg et al., 1995a)

(**Fig. 8**). The nascent protein itself is not functional and is produced as a zymogene. ADAM10 is predominantly found as proenzyme intracellularly in the Golgi, presumably in an inactive form (Lammich et al., 1999). After cleavage of the signal sequence, ADAM10 enters the secretory pathway to be processed and thereby activated by the proprotein convertases furin or PC7 (Anders et al., 2001), as demonstrated for the prodomains of several ADAMs (Loechel et al., 1998; Lum et al., 1998; Roghani et al., 1999). Furin and PC7 are calcium-dependent endoproteases responsible for proteolytic cleavage of cellular and viral proteins transported via the constitutive secretory pathway. Cleavage occurs at the C-terminus of basic amino acid sequences, such as R-X-K/R-R and R-X-X-R. Furin was found predominantly in the trans-Golgi network (TGN), but also in clathrin-coated vesicles dispatched from the TGN, on the plasma membrane as an integral membrane protein and in the medium as an anchorless enzyme (Schafer et al., 1995). ADAM10 prodomain exhibits a dual function: the separately expressed prodomain is capable of inactivating endogenous ADAM10 in cell cultures while overexpressed ADAM10 without its prodomain is inactive (Anders et al., 2001). By contrast, coexpression of the prodomain in trans rescues the activity of the deletion mutant of ADAM10 without the intracellular prodomain (Anders et al., 2001). In addition, the recombinant murine prodomain purified from Escherichia coli acts as a potent and selective competitive inhibitor in experiments performed in vitro (Moss et al., 2007). This implicates that the prodomain of ADAM10 act not only as a transient inhibitor, but also as an internal chaperone in the maturation of the enzyme. The catalytic domain of ADAM10 contains a typical zinc-binding consensus motif (HEXGHXX GXXHD); the mutation E384A, compromising this motif, leads to a substantial decrease in sAPPα secretion (Fahrenholz et al., 2000; Postina et al., 2004). Although the removal of the disintegrin domain of ADAM10 did not grossly affect shedding of APP in cell cultures (Fahrenholz et al., 2000), cleavage of some substrates molecule is likely to be influenced by non-catalytic domains. For example, epidermal growth factor (EGF) cleavage is at least partially impaired in ADAM10$^{-/-}$ cells overexpressing a cytoplasmic domain deletion mutant of ADAM10 (Horiuchi et al., 2007). During transport through the secretory pathway, ADAM10 is complex N-glycosylated resulting in the active protease, which mediates proteolysis in the late compartments of the secretory pathway and at the plasma membrane. Cell-surface biotinylation experiments demonstrated that the proteolytically activated form of ADAM10 is localized mainly in the plasma membrane (Lammich et al., 1999).

2.5. Regulation of ADAM10 activity

ADAM10 activity is regulated at different levels (gene expression, maturation and trafficking, **Fig. 10**) and by multiple signalling pathways, such as ADAM10 shedding mediated by ADAM9 (Parkin and Harris, 2009), nonamyloidogenic α-secretase pathway activation by low levels of cholesterol (Kojro et al., 2001), stimulation of ADAM10 activity by different receptors, PACAP, NMDA, muscarinic recepetors, $P2Y_2$ receptors (Camden et al., 2005; Kim et al., 2010; Kojro et al., 2006; Marcello et al., 2007; Zimmermann et al., 2004). Stimulation of endogenously expressed PAC1 receptors with PACAP in human neuroblastoma cells increased sAPPα secretion and the MAP-kinase pathway [including extracellular-regulated kinase (ERK) 1 and ERK2] and phosphatidylinositol 3-kinase mediate the PACAP-induced α-secretase activation (Kojro et al., 2006). Another pathway involves NMDA receptors activation, since in neuronal cells it promotes ADAM10 cleavage of N-cadherin (Reiss et al., 2005). In particular, ectodomain shedding of N-cadherin by ADAM10 is a primary regulatory step in response to calcium influx, and that it is required for the subsequent PS1/ϒ-secretase- mediated ε-cleavage of N-cadherin (Uemura et al., 2006). The role of calcium is fundamental in ADAM10 activity control. Indeed in human 1321N1 astrocytoma cells, $P2Y_2$ receptor-mediated sAPPα release was dependent on extracellular calcium but was not affected by 1,2-bis(2-aminophenoxy)ethane-N,N,N,-trimethylammonium salt, an intracellular calcium chelator, indicating that $P2Y_2$R-stimulated intracellular calcium mobilization was not involved (Camden et al., 2005). CD44-mediated cell-matrix adhesion is cleaved by two independent ADAM family metalloproteinases, ADAM10 and ADAM17, differentially regulated in response to those stimuli. Ca^{2+} influx activates ADAM10 by regulating the association between calmodulin and ADAM10, leading to CD44 ectodomain cleavage. Depletion of ADAM10 strongly inhibits the Ca^{2+} influx-induced cell detachment from matrix. On the other hand, phorbol ester stimulation activates ADAM17 through the activation of PKC and small GTPase Rac, inducing proteolysis of CD44 (Nagano et al., 2004).

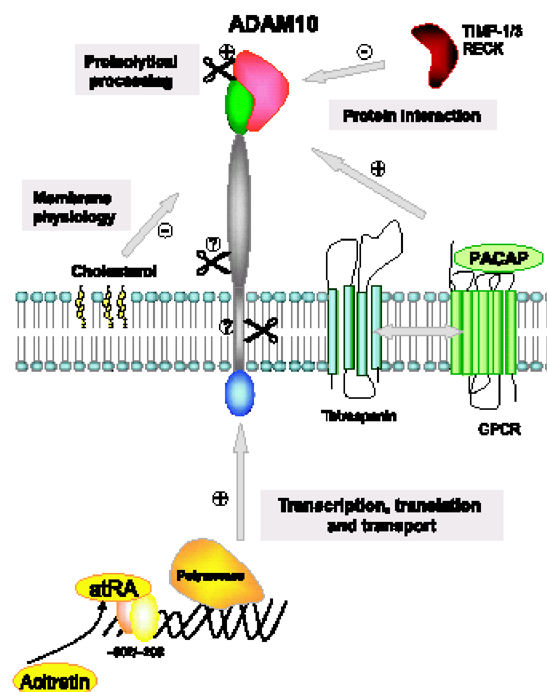


Figure 10. ***Different mechanisms regulating ADAM10 activity.***

2.5.1. ADAM10 trafficking

Since ADAM10 and its substrates are all integral transmembrane proteins, trafficking mechanisms could modulate ADAM10 shedding activity. As regards APP processing, endocytic APP trafficking is a prime modulator of α-secretase cleavage (Lichtenthaler, 2006). This could be explained by the cellular localization of the secretases' activity. α-Secretase cleavage occurs at the plasma membrane, whereas β-secretase cleavage mostly occurs in endosomes. A reduction of APP endocytosis increases APP levels at the cell surface, resulting in enhanced APP cleavage by α-secretase and reduced Aβ levels. Conversely, enlarged endosomes, which are consistent with an increased APP endocytosis and β-secretase cleavage, are associated with early neuropathological changes observed in AD brains (Grbovic et al., 2003; Nixon, 2005). Interestingly, a reduction of plasma membrane cholesterol, which increases α-secretase cleavage also reduces APP endocytosis (Kojro et al., 2001).

Very little is known about the mechanisms regulating ADAM10 trafficking. A recent study has identified tetraspanin 12 (TSPAN12) as a novel interaction partner of ADAM10. TSPAN12 is an integral membrane protein and was found to facilitate α-secretase cleavage of APP (Xu et al., 2009). TSPAN12 predominantly interacted with the mature form of ADAM10 and additionally promoted ADAM10 maturation, suggesting that it increases ADAM10 transport through the

secretory pathway to the plasma membrane, where APP α-secretase cleavage takes place. Other tetraspanins were reported to affect the proteolytic activity of γ-secretase and its interaction with substrates, potentially by localizing γ-secretase to tetraspanin-containing membrane microdomains (Wakabayashi et al., 2009). It will be interesting to see whether distinct tetraspanins interact with different ADAM proteases and thereby allow their selective activation. Another protein implicated in ADAM10 trafficking and APP α-secretase cleavage is nardilysin (N-arginine dibasic convertase) (Hiraoka et al., 2007). Although nardilysin is a metalloprotease, mutational studies demonstrated that the α-secretase enhancing activity did not require the proteolytic activity of nardilysin, suggesting that it acts as a scaffolding or transport protein. Further studies are needed to fully elucidate the exact molecular mechanism of action. As nardilysin binds different ADAM proteases and even the β-secretase BACE1, it does not only affect APP processing but also the shedding of additional membrane proteins (Hiraoka et al., 2008; Nishi et al., 2006; Ohno et al., 2009).

In neurons ADAM10 trafficking can be modulated by the interaction with specific partners and activation of signalling pathways. For example, short-term activation of the NMDA receptor in primary neurons activates APP α-secretase cleavage. The underlying mechanism involves synapse associated protein-97 (SAP97). With its SH3 domain, SAP97 binds to the proline-rich sequences in the cytosolic domain of ADAM10, thereby driving the protease to the post-synaptic membrane (Marcello et al., 2007) and increasing APP α-secretase cleavage.

A recent work has showed a novel mechanism regulating the intracellular trafficking and membrane delivery of ADAM10. ADAM10 cytoplasmic tail contains an ER retention signal. Sequential deletion/mutagenesis analyses showed that an arginine-rich sequence was responsible for the retention of ADAM10 in the ER and its inefficient surface trafficking. As SAP97 binds ADAM10 at its cytoplasmic tail and facilitates forward ADAM10 trafficking in neurons, the authors tested whether SAP97 could modulate ER export. However, SAP97 expression in heterologous cells or hippocampal neurons were sufficient to allow the ER exit of ADAM10, suggesting that other signaling pathways or alternative binding partners are responsible for ADAM10 ER exit (Marcello et al., 2010).

Taken together, these recent studies show an increasing number of trafficking and adaptor proteins which control α-secretase cleavage by altering the trafficking of ADAM10. The example of SAP97 mentioned above demonstrates that the trafficking of ADAM10 and, as a consequence, its activity can be regulated by signaling pathways. Thus, it is probable that other previously

known signaling pathways may activate α-secretase cleavage by promoting ADAM10 trafficking or its access to APP.

2.5.2. ADAM9-mediated ectodomain shedding of ADAM10

It was demonstrated that ADAM10 itself is subject to ectodomain shedding via a mechanism which was inhibited by a known ADAM inhibitor (GW4023) (Hussain et al., 2003) and stimulated by phorbol ester treatment of cells. The treatment of cells with GW4023 caused a reciprocal accumulation of membrane-associated mature ADAM10 in both cell lysates and extracellular membrane vesicles. Using a glycosylphosphatidylinositol (GPI)-anchored ADAM10 (GPI-AD10-FLAG) construct lacking the cytosolic and transmembrane regions of the wild-type (WT) protein, along with the a-helical juxtamembrane region, was shown that these regions of the protein are not prerequisites for the ADAM-mediated shedding of ADAM10. A truncated soluble construct of ADAM10 lacking the transmembrane and cytosolic domains, although correctly post-translationally processed and catalytically active with respect to a synthetic peptide substrate, was incapable of shedding cell-associated APP. It was demonstrated that ADAM9 is, at least in part, responsible for the phenomenon of ADAM10 shedding. This is a new mechanism by which levels of ADAM10 are regulated and may have implications in a range of human diseases including AD (Parkin and Harris, 2009). A previous study suggested that ADAM9 might be responsible for shedding an ADAM10-like activity capable of cleaving synthetic peptide substrates in conditioned cell culture media (Cisse et al., 2005). The identification of ADAM9 as an ADAM10 sheddase in HEK293 cells would seem to contradict the fact that the shed form of ADAM10 is unable to cleave APP at the cell surface. The release of an inactive (with respect to APP) form of ADAM10 from the cell surface would be expected to lead to a decrease (or at best no change) in sAPPα shedding. However, ADAM9 has been shown to enhance ADAM10-dependent shedding of sAPPα from mouse embryonic fibroblasts (Cisse et al., 2005) and the same report demonstrated an ADAM9-dependent increase in sAPPα shedding from HEK293 cells. One possible explanation for this apparent paradox might be that ADAM9, whilst capable of shedding ADAM10, might also promote the physical association of membrane bound ADAM10 with APP thereby enhancing sAPPα generation only in the presence of the former protein. It was demonstrated a molecular mechanism by which both cell-associated and extracellular levels of ADAM10 can be regulated and unequivocally demonstrated that ADAM9 is involved in regulating this mechanism (Parkin and Harris, 2009).

2.5.3. ADAM10 and cholesterol

Biochemical, epidemiological, and genetic findings demonstrate a link between cholesterol levels, processing of APP, and AD (Dartigues and Letenneur, 2000). The α-secretase ADAM10 was identified as a major target of the cholesterol effects on APP metabolism (Kojro et al., 2001). The ε4 allele of apolipoprotein E, which increases the risk for early onset of the disease, is associated with higher plasma cholesterol levels (Sing and Davignon, 1985). Several *in vitro* studies have demonstrated that high cholesterol concentrations in the medium of cultured cells inhibit the secretion of soluble APP (Bodovitz and Klein, 1996; Galbete et al., 2000; Racchi et al., 1997). Aβ production in APP transfected hippocampal cells was decreased after cellular cholesterol depletion (Simons et al., 1998). In a transgenic mouse model, high dietary cholesterol accelerates AD-related pathologies, including Aβ deposition (Refolo et al., 2000). Different mechanisms and target secretases have been discussed to explain the relationship between cholesterol and APP proteolysis. The main questions were if the removal of cellular cholesterol leads to a substantial increase of secreted sAPPα, in particular, if the α-secretase activity of ADAM 10 is regulated by cholesterol. What are the molecular mechanisms of α-secretase activity regulation by cholesterol? Is the increase of α-secretase activity after reduction of cellular cholesterol accompanied by a decrease of β-secretase activity and a reduction of Aβ production? To answer these questions, Kojro and co-workers reduced cellular cholesterol by treatment with either methyl-β-cyclodextrin (MβCD) or lovastatin in several peripheral and neural cell lines, including human embryonic kidney (HEK) 293 cells overexpressing the α-secretase ADAM10. The effect on APP processing, especially on α-secretase activity, was measured, and the influence of cholesterol depletion on membrane fluidity and APP internalization was determined. Furthermore, they examined the effect of cholesterol depletion on the localization of ADAM10 in lipid rafts and on the level of its expression. Treatment of various peripheral and neural cell lines with either MβCD or lovastatin resulted in a drastic increase of secreted sAPPα. In HEK cells, where ADAM10 has been identified as the major enzyme responsible for α-secretase activity (Lammich et al., 1999), a 3- to 6-fold increase in generation of sAPPα was observed after cholesterol depletion. This strong stimulatory effect of cholesterol depletion is in the range obtained with phorbol esters (Lammich et al., 1999). Overexpression of ADAM10 led to a several-fold enhanced α-secretase activity, which was further increased after cholesterol depletion. A lower increase of α-secretase activity was observed in cells overexpressing APP. The high concentration of substrate leads to high basal α-secretase activity, probably because of enzyme saturation, and thus limits an additional increase of α-secretase activity. It can be

concluded that an increase of APP holoprotein does not contribute to the increase of α-secretase activity by cholesterol depletion (Bodovitz and Klein, 1996; Simons et al., 1998). The regulatory role of cholesterol for α-secretase activity can be attributed to the following mechanisms: a) the MβCD-mediated removal of cholesterol from the plasma membranes significantly increased the membrane fluidity. Cholesterol depletion below a critical concentration (about 60% of the initial quantity) caused a significantly enhanced α-secretase activity along with increased membrane fluidity. This could increase the lateral movement of APP and the α-secretase within the membrane; b) APP as a transmembrane protein may reside at the cell surface (Shivers et al., 1988) and is reinternalized via clathrin-coated pits (Nordstedt et al., 1993; Yamazaki et al., 1996) to the endosomal–lysosomal pathway (Haass et al., 1992a). Several reports support a principally cell surface localization for α-secretase activity (Haass et al., 1992a; Parvathy et al., 1999). By two different approaches, it was demonstrated an inhibition of APP endocytosis and an increased amount of APP on the cell surface after cholesterol depletion with MβCD. This treatment resulted in an increased α-secretase cleavage of APP on the cell surface. c) Treatment of peripheral and neural cells with lovastatin, an inhibitor of hydroxymethyl glutaryl-CoA reductase, the ratelimiting enzyme in cholesterol synthesis, also resulted in a strong increase of the α-secretase activity. Secretion of sAPPα was prevented by complementation of cells with cholesterol, which suggests that the effect of lovastatin on α-secretase activity was because of its reduction of cholesterol synthesis. Because the membrane-impermeable cyclodextrin removes cell-surface cholesterol, alternative or additional mechanisms might be responsible for the effect of lovastatin on the α-secretase activity. So it was not found an influence of lovastatin on membrane fluidity. After 20-h treatment with lovastatin, it was observed an increase in the expression of the mature form and the proform of ADAM10.

Lipid rafts have been proposed as the site for the proteolytic processing of APP. Currently there are conflicting reports as to whether APP is present in lipid rafts (Bouillot et al., 1996; Lee et al., 1998; Parkin and Harris, 2009; Simons et al., 1998). Studies with peripheral cell lines led to the conclusion that α-secretase cleavage occurs at the cell surface and that cholesterol-rich caveolae microdomains may play a role in the α-secretase mediated proteolysis of APP *in vivo* (Ikezu et al., 1998). Because a fraction of the proform of ADAM10 is localized in rafts, cholesterol-rich domains might play a role for maturation and transport of ADAM10 in the secretory pathway. For APP, it has been shown that exposure of neural cells to cholesterol interfered with its glycosylation and decreased its secretion (Galbete et al., 2000). In cells overexpressing APP, it

was demonstrated that the increase of α-secretase activity after cholesterol reduction resulted in a decreased secretion of Aβ peptides. The decreased β-secretase activity after cholesterol depletion could be a result of the reduced amount of the substrate available for β-secretase cleavage. The β-secretase BACE was found to circulate between the cell membrane and endosomes (Huse et al., 2000). Thus cholesterol depletion leads to an increased nonamyloidogenic α-secretase cleavage accompanied by a decreased concentration of Aβ peptides. An inhibition of Aβ production after cholesterol depletion of hippocampal neurons overexpressing APP has also been demonstrated (Simons et al., 1998). Several potential mechanisms for the effect of ApoE4 in increasing Aβ deposition have been published, e.g., decreased inhibition of Aβ fibrilliogenesis by ApoE4 vs. ApoE3 (Weisgraber and Mahley, 1996). A recent study has shown that apolipoprotein E promotes the efflux of cholesterol and phosphatidylcholine from both astrocytes and neurons. The order of potency of the isoforms as lipid acceptors was ApoE2 > ApoE3 > ApoE4 in neurons (Michikawa et al., 2000). The conclusion is that the lower cholesterol efflux mediated by ApoE4 contributes to a reduction of α-secretase activity and to pathogenesis of AD.

2.5.4. ADAM10 and receptors

PACAP. The neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP) has neurotrophic as well as anti-apoptotic properties and is involved in learning and memory processes. Its specific G protein-coupled receptor PAC1 is expressed in several CNS regions, including the hippocampal formation. The pituitary adenylate cyclase-activating polypeptide (PACAP) is the most ancient and most conserved member of the secretin/glucagon/vasoactive intestinal peptide (VIP) family in terms of length and sequence identity of coding nucleotides and amino acids (Sherwood et al., 2000). PACAP exists as a 38-residue form (PACAP-38) and as a shorter form corresponding to the N-terminal 27 amino acids of PACAP-38 (PACAP-27). The major form in tissues is PACAP-38, with the highest concentration found in the hypothalamus, cerebral cortex, hippocampus, posterior pituitary, testes, and adrenal gland (Arimura, 1998). PACAP acts as a hypothalamic hormone, neurotransmitter, neuromodulator, and neurotrophic factor that may play an important role during brain development. In several in vitro and in vivo models of cerebral ischemia, PACAP-38 has potent neurotrophic and neuroprotective effects (Reglodi et al., 2000; Uchida et al., 1996; Vaudry et al., 2000). In *Drosophila*, a neuropeptide homologous to mammalian PACAP was implicated to be essential for associative learning and for

memory processes (Feany and Quinn, 1995). Furthermore, it was demonstrated that PACAP-38, administered intracerebroventricularly at very low dosages, improved memory in rat (Sacchetti et al., 2001). Both forms of PACAP bind to and activate several G protein-coupled receptors such as PAC1, VPAC1, and VPAC2 receptors. Activation of multiple receptors by PACAP or VIP has broad physiological effects (Vaudry et al., 2000). A clinical application of these neuropeptides would require selective activation of a particular receptor to minimize potential side effects mediated by other receptor subtypes. The PAC1 receptor that specifically and selectively binds PACAP peptides (with 1000-fold higher affinity than the related peptide VIP) is mainly localized in the CNS. High concentrations of PAC1 receptors occur in various hypothalamic structures, the cerebellar cortex, the hippocampal formation, and in various nuclei (Cauvin et al., 1991; Joo et al., 2004; Masuo et al., 1992; Shivers et al., 1991; Suda et al., 1991). It was suggested that presynaptic PAC1 receptor-mediated signaling at the mossy fiber synapse is involved in associative learning (Otto et al., 2001).

Since PACAP peptides and the PAC1 receptors are localized in areas affected by AD, it was shown that PAC1 receptor activation may influence the processing of APP and it was shown a possible stimulating role of PACAP on the α-secretase activity, which might contribute to the neurotrophic and neuroprotective properties of PACAP (Kojro et al., 2006).

The activation of the PAC1 receptor by its natural agonists PACAP-38 and PACAP-27 strongly increases the α-secretase activity. The activation of the α-secretase activity in cells endogenously expressing PAC1 receptor and its inhibition by a PAC1 receptor antagonist indicates that physiological receptor levels are sufficient to mediate this response. The PACAP-induced release of sAPPα was completely inhibited by two hydroxamate zinc metalloproteinase inhibitors, a broad spectrum inhibitor and an inhibitor that has been shown in vitro to be more specific for the α-secretase ADAM10 (Ludwig et al., 2005). Therefore, PACAP acts by stimulating α-secretase activity and not by enhancing the release of preformed sAPPα. The results furthermore provide evidence for a major role of ADAM 10 not only in the constitutive but also in PACAP-induced APP shedding. On the other hand, these studies support findings for a major involvement of other ADAMs like ADAM 17 in the PMA-stimulated shedding of APP (Buxbaum et al., 1998). After treatment of PAC1 cells with PACAP, it was shown an increased α-like cleavage of the APLP-2. Secreted APLP-2 has been shown to stimulate neurite outgrowth (Cappai et al., 1999). Furthermore, these results do not exclude an increased shedding of other putative ADAM10 substrates upon PACAP treatment. The neuropeptide PACAP stimulates several different signaling cascades, leading to activation of the adenylate cyclase/PKA system, phospholipase

C/PKC activation connected with the mobilization of calcium, and the mitogen-activated protein kinase signaling system (Arimura, 1998; Basille et al., 1995; Vaudry et al., 2000). In HEK cells overexpressing the PAC1 receptor, the direct stimulation of PKA with forskolin or with dibutyryl-cAMP had no effect on APPsα secretion (Kojro et al., 2006). The role of PKA in APP processing is until now not clear and the data about PKA-mediated effects on secretory processing of APP vary between studies and cell lines. Forskolin and other activators of adenylate cyclase inhibited both the constitutive and phorbol ester-stimulated sAPPα secretion in a glioma cell line (Efthimiopoulos et al., 1996) and in rat cortical astrocytes (Lee and Wurtman, 1997). Conversely, it was shown that forskolin and 8-bromo-cAMP increase the constitutive secretion of sAPPα from PC12 cells (Xu et al., 1996). It was not found stimulation of sAPPα production with forskolin and dibutyryl-cAMP and the PKA inhibitor H89 had no significant effect on PACAP-induced sAPPα production. In this respect, it is interesting to note that the PACAP-38 induced neurite outgrowth (in PC 12 cells) is dependent on PKC and ERKs but not on PKA activation (Lazarovici et al., 1998). PAC1 receptor activation stimulates ERK1/2 MAP kinase phosphorylation (Kojro et al., 2006). PKC inhibitor did not abolish the PACAP-induced phosphorylation of ERKs, so PACAP can stimulate MAPK in a PKC-independent manner (Kojro et al., 2006). In conclusion, the activation of the PAC1 receptor leads to the activation of three signaling cascades connected among each other with a central role of the MAP kinase pathway. PACAP treatment had no influence on expression level of α-secretases (ADAM 10, ADAM 17, and ADAM 9) and on the substrate APP. In spite of no changes in expression levels, this results in stimulation of the α-secretase activity with a major involvement of ADAM10.

NMDA. Recently, a close relationship between Aβ production and NMDA receptor activity in cultured neurons has been put forward (Snyder et al., 2005). In Marcello et al., 2007 it was demonstrated that the glutamatergic synapse can intrinsically express a mechanism, driven by NMDA activation, at a postsynaptic level, that is responsible for shifting APP metabolism toward a non-amyloidogenic pathway. Thus, this represents a potential intrinsic additional mechanism of regulation of pathogenesis eventually driven by Aβ. It was reported that SAP97, a key element responsible for the correct assembly of the glutamatergic synapses, which works as a cargo protein involved in trafficking iGluRs to the postsynaptic density (Gardoni et al., 2003; Mauceri et al., 2004), is responsible for driving ADAM10 to the postsynaptic membrane. NMDA receptor activation mediates this event and positively modulates α-secretase activity.

In Kim et al., 2010 it was reported that NMDA receptor activation, but not stimulation of AMPA or metabotropic glutamate receptors, resulted in robust α- and γ-secretase cleavage of nectin-1 in mature cortical neurons. Cleavage of nectin-1 required influx of Ca^{2+} through the NMDA receptor, and activation of calmodulin, but was not dependent on calcium/calmodulin-dependent protein kinase II (CaMKII) activation. It was found that ADAM10 is the major secretase responsible for nectin-1 ectodomain cleavage in neurons and the brain. Cultured neurons from nectin-1 KO mice show altered dendritic spine morphology as compared with wild type; hence nectin-1 is likely an important determinant of spine shape and structure (Togashi et al., 2006). Dendritic spine shape is continuously modulated by excitatory stimulation. This correlation has been suggested to be the cellular basis of memory formation. The spine head swells transiently in response to NMDA receptor stimulation and calmodulin activation, long-lasting spine enlargement on the other hand requires CaMKII, and is associated with an increase in AMPA receptor-mediated currents (Dell'Acqua et al., 2006; Miyamoto, 2006).

Muscarinic receptors. It was reported a cell-surface neurotransmitter receptor-mediated mechanism for the stimulation of rapid release of soluble NH_2-terminal APP derivatives. This mechanism presumably is mediated by receptor-coupled activation of PKC. This is the first report describing the effect of neurotransmitter receptor activation on APP processing, which appears to be changed in the brains of individuals with AD. These results lead to the hypothesis that abnormal APP processing in AD could in part be due to altered neurotransmitter receptor control of APP-producing cholinergic target cells expressing m1 and m3 mAChR (Nitsch et al., 1992). Nitsch et al. (1992) originally reported that the release of sAPPα is stimulated by an activation of muscarinic acetylcholine receptors. In addition, it has been shown that AChEIs can act through the cholinergic pathway, i.e. the increase in ACh caused by a decreased hydrolase activity increases the release of soluble APP (Buxbaum et al., 1994) through a muscarinic-dependent activation of PKC (Pakaski et al., 2001; Xu et al., 1995) and a concomitant decrease of Aβ release (DeLapp et al., 1998; Hung et al., 1993; Qiu et al., 2003). Numerous cells possess a basal level of α-secretase activity, and proteolysis of APP by this enzyme is enhanced by activation of intracellular pathways such as PKC (Buxbaum et al., 1998). Accordingly, the activation of membrane receptors coupled to PKC leads to an increase in APP α-secretase cleavage (Nitsch et al., 1992). Several compounds have been shown to stimulate α-secretase APP cleavage. Among them, donepezil (1-benzyl-4-[(5,6-dimethoxy-1-oxoindan-2-yl) methyl]piperidine hydrochloride) is a specific and potent acetylcholinesterase inhibitor (AChEI),

which is associated with significant improvements in cognitive function in mild to moderate AD patients (Borroni et al., 2001; Bryson and Benfield, 1997; Cardozo et al., 1992; Homma et al., 2000; Krall et al., 1999; Rogers et al., 1998; Trinh et al., 2003). So far, several mechanisms of action of AChEIs have been found, in particular, they have been shown to promote a feedback mechanism leading to raised AChE (Kaufer et al., 1998; Nitsch et al., 1998). These drugs also shift splicing from the primary mRNA products encoding the synaptic membrane AChE (AChE-S) protein to the rare 'readthrough' AChE-R transcript (Meshorer et al., 2002); this transcript interacts, intraneuronally, with the scaffold protein RACK1 and through this, with its target PKCβII (Birikh et al., 2003). It was shown that donepezil exerts its effect not only by enhancing metabolism of membrane APP towards the non-pathogenic pathway through ACh receptors (Pakaski and Kasa, 2003; Racchi et al., 2001), but additionally by promoting trafficking of ADAM 10, as well as APP, to the membranes, thus further enhancing α-secretase activity. The differentiated neuroblastoma cell line SH-SY5Y expressing both muscarinic receptors and AChE exposed to donepezil showed an increased release of sAPPα paralleled by a decreased release of Aβ, respectively, in the absence of an increase of total APP; an increase of ADAM 10 in cellular membranes; an increase in both ADAM 10 and APP levels in the cell membrane (Zimmermann et al., 2004). It has been shown that AChEIs can act through the cholinergic pathway, i.e. the increase in ACh, caused by enzyme inhibition, can affect the increase in soluble APP release via muscarinic receptors (Buxbaum et al., 1994; Nitsch et al., 1992). Therefore, blocking these receptors would result in a significant decrease in sAPPα release.

P2Y$_2$ receptors. P2 nucleotide receptors modulate a wide range of physiological responses following their activation by extracellular nucleotides (Ralevic and Burnstock, 1998; Schwiebert and Zsembery, 2003). The G protein-coupled P2Y$_2$ receptor (P2Y$_2$R) subtype is fully activated by equivalent concentrations of ATP or UTP (Lustig et al., 1992; Parr et al., 1994) and is up-regulated in salivary gland models of stress and disease (Ahn et al., 2000; Schrader et al., 2005; Turner et al., 1997) as well as in blood vessels after balloon angioplasty and in collared carotid arteries, where it induces intimal hyperplasia and inflammation by increasing smooth muscle cell proliferation and leukocyte infiltration (Seye et al., 1997; Seye et al., 2002). Moreover, nucleotides are released from damaged cells of all tissues and from excited neurons, aggregating platelets, and contracting smooth muscle under physiological conditions (Lazarowski and Boucher, 2001; Schwiebert and Zsembery, 2003). The diversity of cellular responses mediated by P2Y$_2$Rs is due in part to unique structural features that enable these receptors to stimulate a

variety of signal transduction pathways. In addition to the classical stimulation of $G\alpha_q$-dependent phospholipase C (Boarder et al., 1995; Weisman et al., 1996), the $P2Y_2R$ contains two Src homology 3 binding sites (P*XX*P motifs) in its intracellular C terminus that interact directly with Src to transactivate epidermal growth factor (EGF), platelet-derived growth factor, and vascular epidermal growth factor receptor 2 receptors (Liu et al., 2004; Seye et al., 2004). The $P2Y_2R$ also contains an Arg-Gly-Asp sequence in the first extracellular loop, which has been shown to interact with $\alpha_v\beta_3/\beta_5$ integrins (Erb et al., 2001) that regulate cell chemokinesis and chemotaxis (Jones et al., 1996; Kaido et al., 2004; Weerasinghe et al., 1998), responses associated with cell-mediated inflammation (Murdoch and Finn, 2000). Activation of the $P2Y_2R$ on endothelial cells and astrocytes also up-regulates the expression of cell surface adhesion molecules that play a role in monocyte-mediated inflammation (Seye et al., 2003) and reactive astrogliosis, respectively. A recent study describes a novel function for the $P2Y_2R$ in enhancing the α-secretase-dependent cleavage of the APP to generate a neuroprotective peptide rather than neurodegenerative Aβ, a finding that has relevance to AD (Camden et al., 2005).

It was demonstrated that activation of the G protein-coupled $P2Y_2$ nucleotide receptor subtype stimulates the α-secretase-dependent processing of APP (Camden et al., 2005). Utilizing the human 1321N1 astrocytoma cell line that lacks endogenous P2 receptor expression, it was demonstrated that stable transfection with $P2Y_2R$ cDNA promoted UTP-induced release of sAPPα in a time- and dose-dependent manner, similar to sAPPα release mediated by muscarinic, glutamate, bradykinin, and serotonergic receptors (Jolly-Tornetta et al., 1998; Lee et al., 1995; Nitsch et al., 1997; Nitsch et al., 1996; Nitsch et al., 1993; Nitsch et al., 1992). G protein-coupled receptors have been shown to stimulate α-secretase-dependent APP processing via PKC-dependent and PKC-independent pathways (Checler, 1995; Nitsch et al., 1994; Nitsch et al., 1995; Racchi et al., 1999). Nonetheless, stimulation of either pathway can lead to activation of the same proteolytic enzyme (Racchi et al., 1999). It was shown that $P2Y_2R$-mediated sAPPα release occurred independently of PKC activation because the PKC inhibitor GF109203 or PKC downregulation had no effect on UTP-induced APP processing, although direct activation of PKC with the phorbol ester PMA increased sAPPα release and was inhibited by GF109203 or PKC down-regulation (Camden et al., 2005). Although $P2Y_2Rs$ in 1321N1-P2Y2 cells are known to activate PKC (Ralevic and Burnstock, 1998) these data indicate that activation of PKC is not necessary for $P2Y_2R$-mediated increases in APP processing, suggesting the involvement of other cellular signalling pathways coupled to the $P2Y_2R$ in UTP-induced sAPPα release. It is well known that activation of the $P2Y_2R$ causes an increase in $[Ca^{2+}]_i$ through both the PLC-dependent release

of calcium from intracellular stores and the entry of extracellular calcium through ion-activated calcium channels (Ralevic and Burnstock, 1998; Weisman et al., 1996). Several physiological responses resulting from $P2Y_2R$ activation are dependent on the rise of $[Ca^{2+}]_i$ (*e.g.* ion secretion, fluid secretion, and vascular cell adhesion molecule-1 expression) (Blaug et al., 2003; Seye et al., 2003; Turner et al., 1998); however it was shown that $P2Y_2R$-mediated increases in $[Ca^{2+}]_i$ by either calcium released from intracellular stores or calcium influx were not required for sAPPα release, because chelation of intracellular calcium with BAPTA had no effect on UTP-induced sAPPα release. Furthermore, incubation with ionomycin or thapsigargin, agents that increase $[Ca^{2+}]_i$, did not promote sAPPα release, confirming that APP processing in 1321N1-$P2Y_2$ cells is not a result of an increase of cytosolic calcium. However, the absence of extracellular calcium does inhibit UTP-induced but not PMA-induced sAPPα release, suggesting the presence of an extracellular calcium-dependent protein that regulates $P2Y_2R$-mediated α-secretase activity. Because PKC-regulated α-secretase is found to be localized to the *trans*-Golgi network and not at the cell surface (Skovronsky et al., 2000), it is possible that the PKC-independent α-secretase expressed in 1321N1-P2Y2 cells is localized to the cell surface where its activity could be modulated by the extracellular calcium concentration. UTP-stimulated sAPPα release in 1321N1-$P2Y_2$ cells is partially dependent on the activation of ERK1/2. Activation of GPCRs, including the $P2Y_2R$, has been shown to stimulate ERK1/2 activity either directly through G protein signaling or by transactivation of EGFR via $P2Y_2R$ interaction with Src (Liu et al., 2004; Soltoff, 1998). Because EGFR activation has been reported to promote sAPPα release (Slack et al., 1997), it was investigated whether Src-dependent transactivation of the EGFR was involved in UTP-induced sAPPα release. However, inhibition of EGFR tyrosine phosphorylation by AG1478 or inhibition of Src by the pyrazole pyrimidine-type 2 inhibitor did not affect UTPinduced sAPPα release, suggesting that Src-mediated EGFR transactivation is not involved. Similar to the $P2Y_2R$, sAPPα release due to activation of M1 (Desdouits-Magnen et al., 1998) or M3 muscarinic receptors (Buxbaum et al., 1994; Slack et al., 1997) occurs primarily by MAPK- and PKC-independent pathways. These data suggest that $P2Y_2$ and muscarinic receptor-mediated stimulation of sAPPα release are coupled to an as yet unidentified signaling pathway, which highlights the mechanistic diversity of GPCR-mediated APP processing. It was shown that inhibition of the expression of ADAM10 or ADAM17/TACE with siRNAs results in a decrease in both UTP-stimulated sAPPα release and PMA-stimulated sAPPα release, whereas co-suppression of ADAM10 and ADAM17/TACE completely prevents UTP- or PMA-stimulated sAPPα release, implicating ADAM10 and ADAM17/TACE as the primary α-secretases involved (Camden et al., 2005).

Furthermore, the inhibition of sAPPα release by the furin inhibitor CMK supports the conclusion that furin-dependent metalloproteases/disintegrins are responsible for α-secretase dependent APP processing in 1321N1-P2Y2 cells. In conclusion, this study provides the first evidence for the regulation of APP processing by any P2 nucleotide receptor subtype (Camden et al., 2005).

2.5.5 ADAM10 and kinases

Activation of the PKC family of phospholipid-dependent serine/threonine kinases, which also promote cancer progression (Acevedo-Duncan et al., 2002; Koivunen et al., 2006; Nakashima, 2002), can induce MMP-mediated cell surface protein cleavage (Covington et al., 2006; Reiss et al., 2005). Activation of PKC is known to regulate the secretion (via proteolytic cleavage) or internalization of a number of membrane proteins (Beguinot et al., 1985; Ehlers and Riordan, 1991; Lin et al., 1986; Pandiella and Massague, 1991). Several reports suggest a pivotal role of PKC in routing APP between the two different pathways (reviewed in (Checler, 1995)): phorbol esters activation of PKC has been shown to increase α-secretase mediated sAPPα secretion and reduce cellular secretion of Aβ peptide (Checler, 1995; Hung et al., 1993; Jacobsen et al., 1994). A role for PKC in promoting cell surface protein cleavage events has been proposed in the context of AD, in which patients have been reported to have lower levels of PKC activity, leading to decreased cleavage of APP by α-secretases such as ADAM10 (Benussi et al., 1998). In AD brain both PKC activity and amount are reduced (Saitoh et al., 1991; Wang et al., 1994): these data suggested the hypothesis that the observed impairment of PKC signal transduction pathway may participate in the dysregulation of APP processing in AD patients, leading to the deposition of Aβ peptide. Numerous independent works demonstrated that PKC agonists consistently increased the recovery of secreted sAPPα in various cell lines (Caporaso et al., 1992; Dyrks et al., 1994; Efthimiopoulos et al., 1994; Fukushima et al., 1993; Gillespie et al., 1992; Loffler and Huber, 1993; Slack et al., 1993). PKC regulates both basal and phorbol ester-induced protein cleavage by ADAM10 (Kohutek et al., 2009). In addition, it was shown that PKC activity may be related to its ability to regulate the subcellular localization of ADAM10 (Kohutek et al., 2009). Pharmacological inhibition of the kinase activity of PKC blocked ADAM10-mediated N-cadherin cleavage and ADAM-10 membrane translocation (Kohutek et al., 2009). The enhanced membrane trafficking of ADAM 10 which is reminiscent of PKC-induced effect as noted also by Birikh et al. 2003.

3. ALZHEIMER'S DISEASE

AD is a prevalent, adult-onset, neurodegenerative disease clinically characterized by progressive impairments in cognition and memory.

It was first described in 1906 by a bavarian psychiatrist, Alois Alzheimer, who reported several cardinal features of the disorder that are currently detected in AD patients: progressive memory impairment, cognitive deficits, altered behavior including paranoia, delusions, loss of social appropriateness and a progressive decline in language function.

The disease's classification proposes two categories based on the age of onset.

Early-onset AD (diagnosed before the age of 65) is extremely rare, accounting for only ~2% of all cases and more than half of them are genetic. It is heterogeneous and complex, displaying no single or simple mode of inheritance even if there is a strong Mendelian inheritance pattern (Rossor et al., 1996).

Late-onset AD is the most common form of the disease. There is a genetic predisposition: it appears to involve several gene polymorphisms, some still unknown, that individually or in combination increase the risk for AD. Since the age of AD onset is significantly more variable for concordant non-identical twins than concordant identical twins, the genetic background strongly influences the timing of the disease. Genetic factors increasing the rick for AD are difficult to identify because their inheritance does not cause the disease phenotype, but rather modulates the age of onset.

The disease progression is similar for both early- and late-onset AD and is arbitrarily split into three overlapping stages: early/mild, moderate and severe. Neurodegeneration is estimated to start 20-30 years before clinical onset (Davies et al., 1988). During this pre-clinical phase, neuropathological lesions load increases and, at a certain threshold, the first symptoms appear. This clinical phase is referred to as Mild Cognitive Impairment (MCI) (Winblad et al., 2004) and is a transitional stage between normal aging and dementia. As, at the moment, complete drug-induced reversal of AD symptoms seems unlikely, researchers are now focusing on this early stage of AD when a therapeutic intervention is likely to realize the greatest impact. Recently neuroimaging has received significant scientific consideration as a promising *in vivo* disease-tracking modality that can evaluate *pre-mortem* AD-associated brain changes, as global atrophy with an early predilection for the hippocampal region and the tempo-parietal cortical areas.

Post-mortem analyses of AD brains revealed lesions in hippocampus, amygdala and in the association cortices of frontal, temporal and parietal lobes (Fox et al., 2001) that will be better described in the "Alzheimer's as a synaptic failure" section of this Introduction.

3.1. Alzheimer's disease histological features

At autopsy, the brains of all AD patients are characterized by two differing protein aggregates: intracellular deposition of neurofibrillary tangles (NFTs) and extracellular deposits known as β-amyloid in the form of senile plaques and blood vessel deposits, both prerequisites for a confirmed diagnosis of AD (**Fig. 11)** (Price et al., 1998). Although variable in form, density and location, these deposits, in combination, are generally accepted to disrupt essential functions in regions of the CNS.

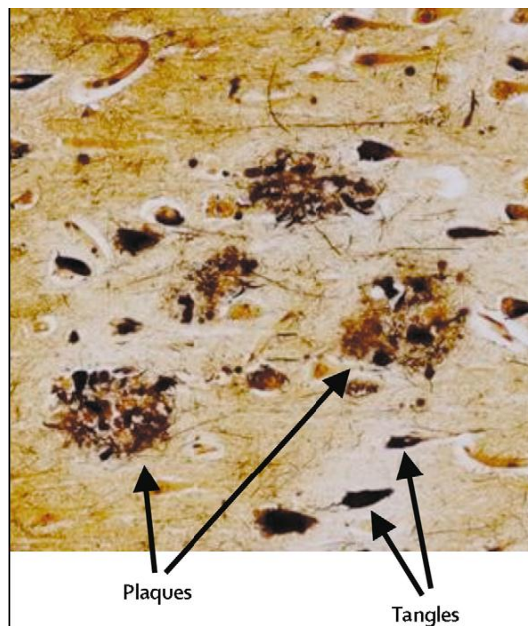


Figure 11. Plaques and tangles in AD cerebral cortex.

3.1.1. Senile Plaques

Senile plaques are microscopic foci of extracellular Aβ deposition generally found in large numbers in the limbic and association cortices (Dickson, 1997). The cross-sectional diameter of neuritic plaques in microscopic brain sections varies widely from 0.1 to 0.12 µm. The molecular composition of these protein aggregates is complex and could be the direct or indirect consequence of a pathogenic gene mutation, be the result of cell degeneration or reflect the acquisition of new substances by diffusion and molecular binding to existing proteins.

The major protein component of plaques is the polypeptide Amyloid-β (Aβ) that derives from APP, as described in section 2.5, and that occurs principally in a filamentous form, i.e., as star-shaped masses of amyloid fibrils. In senile plaques, the most common form of the peptide is

$A\beta_{42/43}$, whereas the more soluble $A\beta_{40}$ is found in association with blood vessels (Miller et al., 1993; Roher et al., 1993) and may develop later in the disease (Delacourte et al., 2002). $A\beta_{42/43}$ is more hydrophobic and is particularly prone to aggregation.

In addition to Aβ, senile plaques have a variety of "secondary" constituents including acute-phase proteins such as α-antichymotrypsin and α_2-macroglobulin (Verga et al., 1989), intercellular adhesion molecules such as cell-adhesion molecule 1 (CAM1) (Eikelenboom et al., 1994), apolipoprotein E (apoE) (Yamaguchi et al., 1994) and D (apoD) (Desai et al., 2005), the heterodimeric glycoprotein clusterin, vibronectin, the complement proteins C1q, C4 and C3 (Verga et al., 1989), blood proteins such as amyloid-P and the sulfated glycosaminoglycans such as heparan sulfate proteoglycan (HSPG). Many other senile plaques constituents could be a consequence of structural degeneration. Approximately 40% of diffuse plaques contain degenerating neuronal perikarya (Mochizuki et al., 1996) and many contain the processes of astrocytes. Acetylcholinesterase rich neurites have been found in plaques and may be the degenerating axonal terminals of neurons originating in the Nucleus Basalis of Meyner. Cholinergic neurites, and neurites positive for somatostatin, γ-amino butyric acid (GABA), neuropeptide Y and the catecholamines have all been recorded in plaques. The presence of chromagranin A, a soluble protein in dense-core synaptic vesicles within the dystrophic neurites of the "coronas" of classic plaques, may also be the result of cellular degeneration.

Recently, it has been shown that plaques form extraordinarily quickly, over 24 h. Within 1–2 days of a new plaque's appearance, microglia are activated and recruited to the site. Progressive neuritic changes ensue, leading to increasingly dysmorphic neurites over the next days to weeks. Therefore, plaques are widely considered as a critical mediator of neuritic pathology (Meyer-Luehmann et al., 2008).

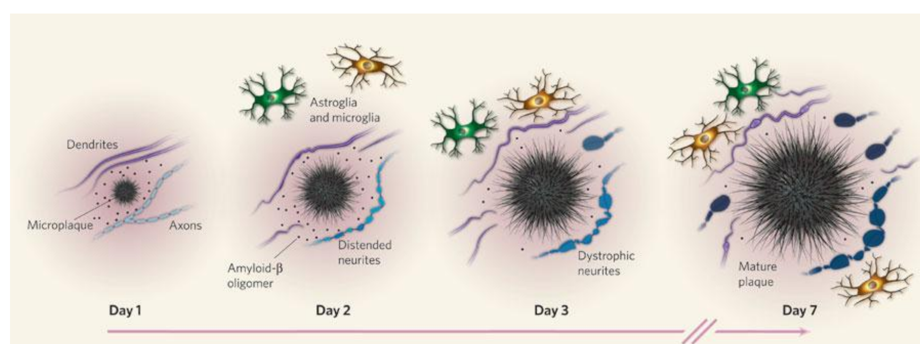


Figure 12. Senile plaque formation.

3.1.2. Neurofibrillary tangles

NFTs are intraneuronal hallmark of more than 20 neurodegenerative diseases, suggesting that neurofibrillary degeneration is a unique consequence of different types of etiological factors (Delacourte, 2005). However, tangles have a morphological and biochemical signature which is disease-specific: they are made up of different types of filaments such as paired helical filaments (PHFs) in AD or straight filaments in progressive supranuclear palsy.

The basic component involved in tangle formation is the microtubule-associated tau protein (Brion et al., 1985; Grundke-Iqbal et al., 1986; Nukina and Ihara, 1986).

In AD, NFT are large, non membrane-bound bundles of abnormal fibers consisting of pairs of 10 nm filaments wound into helices of 160 nm of period and therefore called PHFs. In PHFs, tau is hyperphosphorylated. A variety of kinases has been shown to be capable of phosphorylating tau *in vitro* at various sites (Illenberger et al., 1998). Nevertheless, it is not clear whether one or more kinases are principally responsible for initiating the hyperphosphorylation of tau *in vivo* which leads to its apparent dissociation from microtubules and aggregation into insoluble PHFs.

There is growing evidence that the formation of NFT in AD represents one of the several cytological responses by neurons to the gradual accumulation of Aβ (Hardy and Selkoe, 2002).

The answer to the question of the mechanisms underlying NFT toxicity is not simply. On the one hand, tau aggregation causes loss of physiological functions of tau and produces intermediate tau aggregates that may gain pathological functions. On the other hand, the mature tangles may be relatively inert or even may represent a cytoprotective effort by protein quality control machineries when facing the challenge of accumulating toxic tau species. It is possible that tau tangles are dynamic in such a way that individual tau molecules or aggregated intermediate tau comes on and off tangles depending on the equilibrium. Thus, tau tangles could serve as a reservoir for toxic tau species at certain stages of disease progression (Ding and Johnson, 2008).

4. Amyloid Precursor Protein

4.1. APP structure

The purification and partial sequencing of the Aβ protein from meningovascular amyloid deposits in AD and Down syndrome (Glenner and Wong, 1984) and the subsequent observation that Aβ was also the main component of senile plaques (Gorevic et al., 1986; Masters et al., 1985; Selkoe et al., 1986) led to the cloning of the gene encoding APP located on chromosome 21 (Kang et al., 1987).

APP has been identified in many vertebrate species and is a member of an evolutionarily conserved protein family (Collin et al., 2004; Coulson et al., 2000).

Searches for genes implicated in familiar AD led to the identification of the mammalian homologues, APLP1 (amyloid precursor-like protein 1) (Wasco et al., 1992) and APLP2 (Wasco et al., 1993). APP-like proteins have also been identified in *Drosophila melanogaster* (Rosen et al., 1989) and *Caenorhabditis elegans* (Daigle and Li, 1993). All of the members of the APP-like gene family display impressive amino acid and domain homology to APP as well as to each other.

The APP gene encodes an alternatively spliced transcript that, in its longest isoform, codes for a single membrane-spanning polypeptide of 770 aa (Goldgaber et al., 1987; Kang et al., 1987; Tanzi et al., 1987a). The APP gene contains 19 exons of which exon 7, 8 and 15 can be alternatively spliced. All possible variants have been detected by RT-PCR in tissues. Alternative splicing of exons 7 and 8 results in polypeptides of 695 aa (APP_{695}, expressed predominantly in brain) and 751 aa (APP_{751}) (Kitaguchi et al., 1988). The splicing of exon 15 creates a chondroitin-sulphate-glycosaminoglycan-attachment site used in astrocytes but not in neurons. The APP protein products are typical type 1, integral membrane proteins that contain a 17-residues amino-terminal signal peptide and a large ectodomain with sites for *N*-glycosylation, corresponding to about 88% of the total protein mass of the neuronal isoform. Two alternatively spliced exons of 56 and 19 aa are inserted at residue 289: the first contains a serine protease inhibitor domain of the Kunitz type. In human platelets, the Kunitz-type serine protease inhibitor serves as inhibitor of factor XIa, a serine protease of the coagulation cascade (Smith et al., 1990). A single membrane-spanning helix follows at aa 700-723 and then there is a short cytoplasmic domain (Kang et al., 1987).

In cultured mammalian cells, APP matures through the constitutive secretory pathway and is modified by the addition of both N- and O-linked carbohydrates and sulphate moieties before being transported to the cell surface (Citron et al., 1992; Hung and Selkoe, 1994; Sisodia, 1992). The APP ectoplasmic and cytoplasmic domains are phosphorylated (Walter et al., 1997), but the functional implications of these processes are unknown.

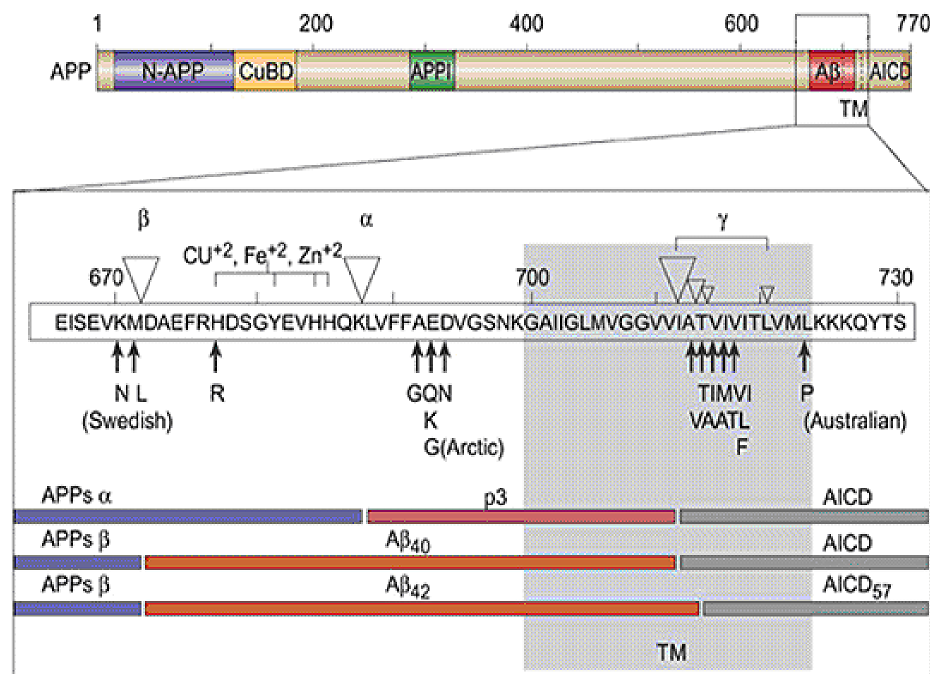


Figure 13. APP and Aβ structure.

4.2. APP functions

The function of APP is unknown.

Knockout (KO) of the mouse APP gene is not very illuminating, because it leads to only subtle phenotypes, including minor weight loss, decreased locomotor activity, abnormal forelimb motor activity and minor nonspecific reactive gliosis in the cortex (Zheng et al., 1996). Although the phenotypes of the single KO mice are relatively mild, *in vivo* and *in vitro* studies suggest that APP, APLP1 and APLP2 function to promote neurite outgrowth, neural cell migration and copper homoeostasis (Heber et al., 2000; Muller et al., 1994; Zheng et al., 1995) and that the rather benign phenotypes seen in KO mice may result from functional redundancy between APP, APLP1 and APLP2. In contrast, APLP2$^{-/-}$/APP$^{-/-}$ mice and APLP2$^{-/-}$/APLP1$^{-/-}$ mice each show a lethal phenotype (postnatal day 1), whereas APLP1$^{-/-}$/APP$^{-/-}$ mice are apparently normal (Heber et al., 2000). These results provide genetic evidence for at least some distinct physiological roles for

APP and APLP2 and suggest that APLP2 has the key physiological role among the family members. Curiously, no detectable gross or histopathological abnormalities were observed in any of these lines, whereas in triple KO mice (APLP2$^{-/-}$/APP$^{-/-}$/APLP1$^{-/-}$), which die *in utero*, 81% of animals showed cranial defects (Herms et al., 2004).

The main function hypothesized for APP is as receptor.

The idea that APP functions as a receptor was bolstered by the discovery that the Notch receptor signals through proteolytic processing that is remarkably similar to that of APP (Annaert and De Strooper, 1999; Selkoe and Kopan, 2003) and by the notion that APP may have extracellular ligands. However, APP proteolysis may also be a mechanism for turning off normal APP functions. In this regard, the proteases are simply degradative, a concept that is especially true for γ-secretase, which cleaves many type I integral membrane proteins and has even been likened to a proteasome of the membrane (Kopan and Ilagan, 2004).

Other studies have indicated that APP might be able to associate with heterotrimeric GTP-binding proteins to contribute to signal transduction (Nishimoto et al., 1993).

To further speculate on APP physiological roles, it is fundamental to consider the various domains composing the molecules and their capability to bind several different ligands intra- and extracellularly.

The search for APP extracellular ligands has not been especially fruitful. F-spondin, a secreted neuronal protein purportedly involved in cell-cell interactions, was identified by affinity isolation and coexpression of this protein prevents shedding of the APP ectodomain by β-secretase and reduces Aβ production (Ho and Sudhof, 2004). However, it is unclear whether soluble F-spondin added to cultures has the same effect or whether the F-spondin-APP interaction occurs when the two proteins are expressed in different cells. Reelin, an extracellular matrix protein essential for cortical development that shares homology with F-spondin, was shown to increase binding of the reelin signaling mediator Dab1 to APP (Hoe et al., 2006a). Furthermore, fewer reelin-expressing pyramidal neurons are observed in the entorhinal cortex of APP-transgenic mice and AD brains (Chin et al., 2007). The Nogo-66 receptor, implicated in axonal sprouting in the adult CNS, has also been reported to interact with the APP ectodomain and to inhibit Aβ production (Park et al., 2006). LRP and SORL1 (SorLA, LR11) also bind to the APP ectodomain and influence Aβ production (Andersen et al., 2005; Bu et al., 2006).

Another possible role of APP is as adhesion molecule: it binds to extracellular matrix proteins such as heparin and collagen (Beher et al., 1996; Multhaup, 1994). Moreover, homo- and heterodimerization between the APP family members in adjacent cells has been suggested to

promote intercellular adhesion (Soba et al., 2005). Such a mechanism would be analogous to that of known cell adhesion molecules such as cadherins and nectins.

Numerous proteins that interact with the intracellular tail of APP have been identified. These include proteins that are known to play a role in vesicular or protein trafficking: X11 (Mint1) and X11L (Mint2) involved in vesicle exocytosis; Jun N-terminal-kinase-interacting protein 1 (JIP-1), a scaffold protein that binds kinesin light chain 1 and coordinates transport of phosphorylated APP into neurites; kinesin 1; Pat1a, a microtubule-interacting protein that plays a role in anterograde transport of APP and APLPs (Kamal et al., 2001; King et al., 2004; Kuan et al., 2006; Muresan and Muresan, 2005; Zheng et al., 1998). APP-binding proteins are also involved in brain development: the Fe65 protein transmits an APP-dependent signal important for neuronal positioning in the developing cortex; mDab1 plays a key role in reelin signaling in the developing cortex and Numb is a scaffold protein important for Notch signaling (Guenette et al., 2006; Hoe et al., 2006b; Roncarati et al., 2002). Other APP-tail-binding proteins are implicated in regulating cell cycle progression: these include G_o, PAK3 and APP-BP1 (Chen et al., 2007; Chen et al., 2003; Giambarella et al., 1997; McPhie et al., 2003). The APP-interacting protein that has generated the most interest is Fe65, because a ternary complex consisting of Fe65, APP and the histone acetyltransferase Tip60 has been shown to activate transcription (Baek et al., 2002; Cao and Sudhof, 2001).

More recently, evidence has emerged for two other possible roles of APP.

First, it might serve as a receptor for Kinesin1 during the fast axoplasmic transport of vesicles that contain β-site APP-cleaving enzyme (BACE) and presenilins (Kamal et al., 2001). Second, in addition to generating Aβ, the proteolytic cleavage of APP generates a labile intracellular carboxy-terminal stub, AICD. This stub is transported to the nucleus, where it might function as a signal-transduction molecule (Cao and Sudhof, 2001; Cupers et al., 2001; Sastre et al., 2001). The translocation of this cytoplasmic carboxy-terminal domain to the nucleus is analogous to the fate of the Notch intracellular domain (NICD), which is generated by a similar proteolytic cleavage of the Notch receptor.

Apart from AICD, other products of APP metabolism have physiological functions: sAPPα released from the membrane after α–secretase cleavage, may serve as a signaling molecule. Evidence suggests that this shed ectodomain plays a role in the growth of fibroblasts in culture (Park et al., 2006). sAPPα was found to be neuroprotective for primary neurons in culture, preventing elevations in intracellular Ca^{2+} levels caused by glucose deprivation and raising the excitotoxic threshold of glutamate (Mattson et al., 1993), as well as mediating axonal and

dendritic growth (Perez et al., 1997). *In vitro* studies in cultured cells indicate that secreted APP can function as an autocrine factor to stimulate cell proliferation and cell adhesion.

On the whole, APP could function as a receptor, even if no clear extracellular ligands have been identified and no downstream targets genes have been confirmed; as an adhesion molecule, through dimerization or as a signaling molecule, because of its binding to Fe65, but much work still needs to be done to close the large gaps in our knowledge about its biology.

4.3. Trafficking and processing of the Amyloid Precursor Protein

As reported above in section 2.5, the APP is an integral membrane protein processed by several different proteases called secretases. β-secretase generates the NH_2-terminus of Aβ (Seubert et al., 1993), cleaving APP to produce a soluble version of APP (sAPPβ) and a 99-residue COOH-terminal fragment (CTF99) that remains membrane bound. In contrast, α-secretase cuts within the Aβ region (between residues Lys16 and Leu17 of Aβ) to produce sAPPα and an 83-residue COOH-terminal fragment (CTF83) (Esch et al., 1990; Sisodia, 1992).

Both CTF99 and CTF83 are substrates for γ-secretase, which performs an unusual proteolysis in the middle of the transmembrane domain to produce the 4-kD Aβ from CTF99 and a 3-kD peptide called p3 from CTF83 (Haass et al., 1992b; Haass and Selkoe, 1993). Proteolysis by γ-secretase is heterogeneous: most of the full-length Aβ species produced is a 40- residue peptide ($Aβ_{40}$), whereas a small proportion is a 42-residue COOH-terminal variant ($Aβ_{42}$) (Esler and Wolfe, 2001). During its transit from the ER to the plasma membrane through the constitutive secretory pathway (**Fig. 14**), nascent APP undergoes post-translational modification by *N*- and *O*-glycosylation, phosphorylation, and tyrosine sulfation. In cultured cells, it is estimated that only about 10% of nascent APP molecules are successfully delivered to the plasma membrane. APP can be proteolytically processed at the cell surface primarily by α-secretase, resulting in the shedding of sAPPα ectodomain (Sisodia, 1992).

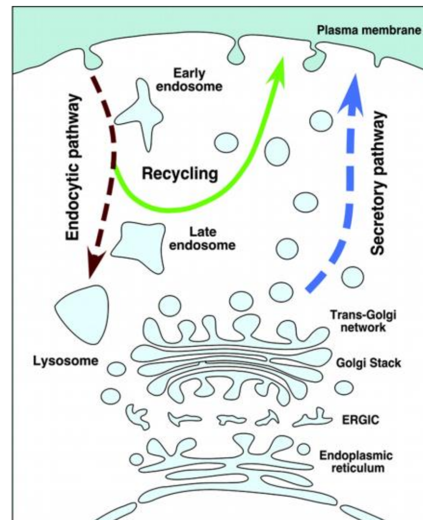


Figure 14. APP intracellular trafficking.

Activation of PKC increases sAPPα-secretion by mechanisms involving the formation and release of secretory vesicles from the TGN, thus enhancing APP (and possibly the α-secretase) trafficking to the cell surface.

Unlike many cell surface receptors, full-length APP does not reside for considerable length of time at the cell surface. Approximately 70% of surface bound APP is internalized within minutes of arriving at the plasma membrane. A "YENPTY" internalization motif located near the C-terminus of APP is responsible for this efficient internalization.

Following endocytosis, APP is delivered to late endosomes and a fraction of endocytosed molecules is recycled to the cell surface. Measurable amounts of internalized APP also undergo degradation within the lysosome.

β-secretase is predominantly localized in late Golgi/TGN and endosomes and cleaves wild-type APP during endocytic/recycling steps (Koo and Squazzo, 1994), releasing sAPPβ and generating CTF99.

Available data indicate the presence of γ-secretase complex and enzyme activity in multiple compartments including the ER, late-Golgi/TGN, endosomes and plasma membrane (Cook et al., 1997; Greenfield et al., 1999; Kaether et al., 2002; Takahashi et al., 2002; Xu et al., 1997). Recent studies estimated that only 6% of γ-secretase activity at the cell-surface (Chyung et al., 2005).

Aβ is mainly generated in TGN as APP is trafficked through the secretory and recycling pathways. Consequently, mutations within the YENPTY motif inhibit the internalization of APP and decrease Aβ generation (Perez et al., 1999).

Several cytosolic adaptors with phosphotyrosine-binding domains, including Fe65, Mint 1, Mint 2, and Mint3, bind to the APP cytoplasmic tail at or near the YENPTY motif, and regulate APP

trafficking and processing (King and Scott Turner, 2004). Interestingly, FE65 acts as a functional linker between APP and LRP in modulating endocytic APP trafficking and amyloidogenic processing (Pietrzik et al., 2004). In addition, phosphorylation of Thr-668 within the cytosolic domain of APP seems to modulate amyloidogenic processing by facilitating cleavage by β-secretase (Allinson et al., 2003). Despite the elaborate regulatory mechanisms that modulate cell surface transport and endocytic trafficking of APP, transit through these compartments is not essential for generation of Aβ as shown by amyloidogenic processing of APP in cells expressing syntaxin 1A mutants defective in exocytosis (Khvotchev and Sudhof, 2004).

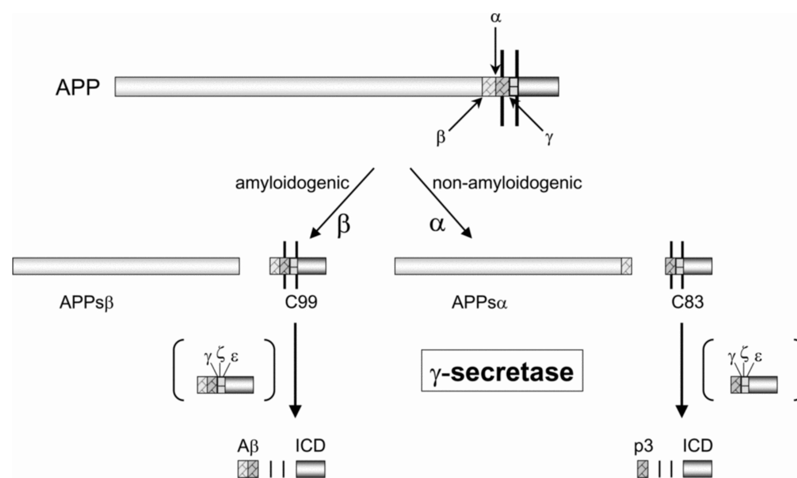


Figure 15. APP processing.

4.3.1. α-secretase

In the non-amyloidogenic pathway, APP is cleaved within the Aβ domain, at the Lys16-Leu17 bond, by α-secretase, thus precluding the formation of intact Aβ peptide. The principal determinants of APP cleavage by α-secretase appear to be the distance of the hydrolyzed bond from the membrane (12 or 13 residues) and a local helical conformation (Sisodia, 1992).

Within a range of class-specific proteinase inhibitors examined, only the zinc-chelating agent 1,10-phenanthroline was found to cause significant inhibition of sAPPα production (Roberts et al., 1994).

Several groups then reported that hydroxamic acid-based, active site-directed compounds, such as batimastat (BB94) and TAPI-2, inhibited the appearance of the α-secretase product sAPPα in the medium of a variety of neuronal and non-neuronal cell lines (Arribas et al., 1996; Parvathy et al., 1998; Racchi et al., 1999). In contrast, neither the level of full-length APP nor its cleavage by β-secretase was affected (Parvathy et al., 1998).

Interestingly, the constitutive release of sAPPα and of the blood pressure-regulating enzyme, angiotensinconverting enzyme, display an almost identical inhibition profile with a range of hydroxamic acid-based compounds (Parkin et al., 2002; Parvathy et al., 1998), suggesting that the shedding of these two proteins may be mediated by the same, or very closely related, zinc metalloproteinase(s) (Hooper and Turner, 2000).

In almost all cells in culture, sAPPα is secreted constitutively into the medium; however, activating second messenger cascades increases the proportion of APP cleaved by α-secretase. For example, activation of muscarinic and epidermal growth factor receptors enhances the production of sAPPα by pathways involving PKC, tyrosine kinases, the MAPKs, and extracellular signal-regulated kinase (Checler, 1995; Mills and Reiner, 1999).

Direct activation of PKC with phorbol esters also upregulates sAPPα secretion, and stimulating the α-secretase cleavage of APP leads to a significant decrease in Aβ formation (Buxbaum et al., 1993; Caporaso et al., 1992; Gabuzda et al., 1993; Hung et al., 1993; Jacobsen et al., 1994). It seems that the PKC ε isoform is responsible for the observed effect of phorbol esters on the reduction of Aβ and activation of α-secretase (Zhu et al., 2001).

Moreover, activation of receptors that work through PKC can augment α-secretase cleavage of APP with concomitant reduction in β-secretase processing. For instance, agonists of the metabotropic glutamate receptors can lower Aβ by shunting APP toward the α-secretase pathway (Lee et al., 1995). Muscarinic agonists (M1 and M3) can likewise decrease Aβ production, and this effect has been observed *in vitro* as well as *in vivo* (Haring et al., 1994; Hung et al., 1993; Lin et al., 1999; Nitsch et al., 1992; Wolf et al., 1995). Because of this effect on Aβ production, M1 and M3 agonists might be useful agents for treating AD.

Although both the carbachol-stimulated and constitutive α-secretase activities in the human neuroblastoma SH-SY5Y cell line were blocked completely by batimastat and showed a remarkably similar inhibition profile with a range of hydroxamic acid-based compounds (Parkin et al., 2002; Parvathy et al., 1998), another hydroxamic acid-based compound KD-IX-73-4 was able to distinguish between these two activities (Racchi et al., 1999). These data imply that at least two different but closely related zinc metalloproteinases are involved in the constitutive and regulated α-secretase cleavages of APP.

In the last few years, several data confirmed that various members of the ADAMs family of proteins (Black and White, 1998; Primakoff and Myles, 2000; Schlondorff and Blobel, 1999) act as α-secretases. Nevertheless, as described in section X, two recent studies finally demonstrated

that the constitutively cleaving α-secretase activity in neurons is selectively mediated by ADAM10 (Jorissen et al., 2010; Kuhn et al., 2010).

4.3.2. β-secretase

β-secretase has recently been identified as the aspartyl protease BACE, a type I membrane glycoprotein that cuts APP at its β-site (Hussain et al., 1999; Sinha et al., 1999; Vassar et al., 1999; Yan et al., 1999).

BACE belongs to the pepsin family of aspartyl proteases, but defines a novel subgroup of membrane-associated hydrolases.

BACE is 501 amino acids in length with two catalytic aspartic acid residues present in the diagnostic DT/SG sequence motif, D^{93}TS and D^{289}SG. It also has three disulfide bonds characteristic of mammalian enzymes. The crystal structure reveals that the protein has the same general polypeptide fold seen in other members of this family (Hong et al., 2000) despite an unusual disulfide bonding pattern, which initially led to speculation that the enzyme might reveal novel substrate specificities (Haniu et al., 2000).

BACE mediates the primary amyloidogenic cleavage of APP and generates a membrane-bound APP C-terminal fragment (CTF99), which is the immediate precursor for the intramembraneous γ-secretase cleavage. BACE is clearly the only protease with a well-defined β-secretase activity. This was unambiguously shown by the homozygous knockout of the BACE gene, which does not allow any Aβ generation (Cai et al., 2001; Luo et al., 2001).

A close homolog of BACE, BACE-2, was identified as well (Acquati et al., 2000); however, BACE-2 exhibits a α-secretase-like activity, which cleaves APP in the middle of the Aβ domain at aa 19 and 20 (Farzan et al., 2000). Thus, BACE-2 does not contribute to the amyloidogenic processing of APP, which is consistent with the complete lack of Aβ generation in a BACE knockout.

BACE is generated as a preproenzyme. Upon removal of the signal peptide, the prodomain is cleaved by a furin-like protease (Bennett et al., 2000). BACE is then targeted through the secretory pathway to the plasma membrane and clusters within lipid rafts (Ehehalt et al., 2003). APP processing by BACE also occurs preferentially within lipid rafts (Ehehalt et al., 2003). Interestingly, as described above in section 2.5.3, ADAM10 α-secretase activity can also be promoted by cholesterol reduction manifesting itself in increased levels of sAPPα (Kojro et al., 2001). Indeed, recent evidence suggests that amyloidogenic APP processing may preferentially occur in the cholesterol-rich regions of membranes known as lipid rafts, and that changes in

cholesterol levels could exert their effects by altering the distribution of APP-cleaving enzymes within the membrane: APP inside raft clusters seems to be cleaved by β-secretase, APP outside rafts undergoes cleavage by α-secretase. Thus, access of α- and β-secretase to APP, and therefore Aβ generation, may be determined by dynamic interactions of APP with lipid rafts (Ehehalt et al., 2003). Consistent with the finding of BACE in lipid rafts, BACE is sorted apically in polarized cells (Capell et al., 2002), the side where rafts are thought to accumulate. At the plasma membrane, BACE (like APP) can be internalized to endosomes (Huse et al., 2000; Walter et al., 2001). Thus BACE and APP follow similar trafficking routes and meet within endosomes, which may be the preferential site of BACE activity due to its acidic pH optimum (Vassar, 2001). Little is known about the physiological substrates of BACE. As described above, BACE has only limited access to basolaterally sorted APP in polarized cells, thus suggesting that APP is not the main substrate. This is supported by the finding that APP does not bear the optimal cleavage site for BACE (Liu et al., 2002). Besides APP, additional candidate BACE substrates were identified recently; these include P-selectin glycoprotein ligand-1 (Lichtenthaler et al., 2003) and the sialyl-transferase ST6GalI (Kitazume et al., 2001). However, the biological function of BACE remains unclear, and may be difficult to address since no phenotype is observed upon removal of BACE from the mouse genome.

Most interestingly, evidence exists that BACE expression is significantly enhanced in brains derived from patients with sporadic AD (Yang et al., 2003). The reasons for the enhanced BACE expression are currently unclear. However, in this regard, it is tempting to speculate that AD-associated stressors such as oxidative stress, radicals, unfolded proteins, head trauma, and others may induce BACE transcription and/or expression/activity during aging.

Therapeutic inhibition of BACE may be free of mechanism-based side effects, since BACE knockout mice lack Aβ and are phenotypically normal (Luo et al., 2003). BACE null mice engineered to overexpress human APP (BACE(-/-).Tg2576(+)) are rescued from Aβ-dependent hippocampal memory deficits. Moreover, impaired hippocampal cholinergic regulation of neuronal excitability found in the Tg2576 AD model is ameliorated in BACE(-/-).Tg2576(+) bigenic mice. The behavioral and electrophysiological rescue of deficits in BACE(-/-).Tg2576(+) mice is correlated with a dramatic reduction of cerebral $Aβ_{40}$ and $Aβ_{42}$ levels and occurs before amyloid deposition in Tg2576 mice. Our gene-based approach demonstrates that lower Aβ levels are beneficial for AD-associated memory impairments, validating BACE as a therapeutic target for AD (Ohno et al., 2004).

4.3.3. γ-secretase

γ-secretase is the name for a proteolytic activity that, in combination with β-secretase, is able to cleave the Aβ peptide from APP (Haass and Selkoe, 1993). This is a conserved and omnipresent membrane protein complex required for life in all multicellular animals. γ-secretase cleaves with remarkable relaxed sequence specificity transmembrane domains of many proteins. The only requirements are apparently a type I conformation of the transmembrane domain (N-terminus oriented to the extra-cellular side of the membrane) and a short (<50 AA) ectodomain (Lichtenthaler et al., 1999; Struhl and Greenwald, 1999). Therefore, γ-secretase proteolyses a variety of membrane-associated fragments derived from type I integral membrane proteins, including APP, involved in AD, and the Notch receptor, critical for cellular differentiation. This protease is composed of four integral membrane proteins: presenilin (PS), nicastrin, Aph-1 and Pen-2. When all four components were expressed together in Saccharomyces Cerevisiae, an organism that lacks any endogenous γ-secretase activity, fully active γ-secretase was reconstituted (Edbauer et al., 2003). Assembly of these four components leads to PS autoproteolysis into two subunits, each of which contributes one aspartate to the active site of an aspartyl protease.

The biochemical roles of the various components of the γ-secretase complex are only partially understood. How γ-secretase accomplishes hydrolysis within the hydrophobic environment of the lipid bilayer is also an intriguing biochemical question. Because the active site contains water and two aspartates, it is likely sequestered from the hydrophobic lipids (Wolfe et al., 1999a). PS apparently contains an initial docking site for the transmembrane region of the substrate that is distinct from the active site. Endogenous APP substrate can be copurified with the γ-secretase complex using an affinity column with immobilized transition-state analog inhibitor (Esler et al., 2000). Because the active site should be occupied by the immobilized inhibitor, the transmembrane domain of the substrate is presumably bound to an exosite on the protease complex. Helical peptides designed to mimic the APP transmembrane domain are potent inhibitors of γ-secretase (Das et al., 2003), with IC_{50} values as low as 140 pmol/L in biochemical assays (Bihel et al., 2004) and these peptides interact with a site distinct from that of transition-state analog inhibitors (Kornilova et al., 2003). Affinity labeling with helical peptides identified both presenilin N-Terminal Fragment (NTF) and CTF as contributors to the binding site; the other three components of γ-secretase were not labeled at all (Kornilova et al., 2003). These findings suggest that the docking site, like the active site, is at the interface between the two presenilin

subunits and implies that substrate passes, in whole or in part, between these subunits to access the internal active site.

Nicastrin plays a role in substrate recognition: its ectodomain resembles an aminopeptidase but lacking key catalytic residues and this domain can interact with the N-terminus of γ-secretase substrates. The shedding of the substrate's ectodomain (e.g. by either α- or β-secretase) allows the free N-terminus of the membrane-retained stub to interact with Nicastrin.

Aph-1 is thought to function as a scaffold for the γ-secretase complex, assembling first with Nicastrin and then with PS and Pen-2.

Pen-2 serves as a trigger for endoproteolysis of the presenilin holoprotein into the active heterodimer, although it is independently required for γ-secretase activity as well.

These findings may suggest some insight into the assembly of the γ-secretase complex.

Since Nicastrin is fairly stable in the ER, it is likely that it may be the scaffold for a second binding partner. APH-1 can then be recruited to Nicastrin, as suggested by the identification of a putative Nicastrin/APH-1 precomplex. Binding of PS to the precomplex then results in the formation of a trimeric complex. Finally PEN-2 joins the complex, thus allowing PS endoproteolysis and γ-secretase activity.

5. The amyloid hypothesis

The "amyloid hypothesis" was first proposed from research conducted in the middle of the 1980s showing that senile plaques found in AD brain tissue were composed mainly of a sticky Aβ peptide (Masters et al., 1985). This hypothesis was formalised by Hardy and Higgins (1992) who stated that Aβ "precipitates to form amyloid and, in turn, causes NFTs and cell death" (Hardy and Higgins, 1992). Up to now, most investigators believe that the production and cerebral deposition of amyloid plaques composed of the 38 to 42 aa Aβ peptide is central to the development of AD (Selkoe, 2000). According to the amyloid hypothesis, deposition and accumulation of Aβ in the brain is the primary factor driving AD pathogenesis (Hardy and Selkoe, 2002; Selkoe, 1991). In animal models Aβ deposition has also been observed to develop prior to the tangle pathology (Oddo et al., 2003).

Therefore, the basic biochemical formula for Aβ production was investigated in minute details to determine the aetiology of the disease.

5.1. The amyloid cascade as the primary event

The cloning of the gene encoding APP and its localization to chromosome 21 (Goldgaber et al., 1987; Kang et al., 1987; Robakis et al., 1987; Tanzi et al., 1987b), coupled with the earlier recognition that trisomy 21 (Downs syndrome) leads invariably to the neuropathology of AD (Olson and Shaw, 1969), set the stage for the proposal that Aβ accumulation is the primary event in AD pathogenesis. In addition, the identification of mutations in the APP gene that cause hereditary cerebral haemorrhage with amyloidosis (Dutch type) showed that APP mutations could cause Aβ deposition, albeit largely outside the brain parenchyma (Levy et al., 1990; Van Broeckhoven et al., 1990).

Soon, the first genetic mutations causing AD were discovered in the APP gene (Goate et al., 1991; Hardy, 1992; Hendriks et al., 1992; Mullan et al., 1992). The contemporaneous discovery that Aβ was a normal product of APP metabolism throughout life and could be measured in culture medium, cerebrospinal fluid, and plasma (Haass et al., 1992b; Seubert et al., 1992; Shoji et al., 1992) allowed scientists to quickly establish the biochemical abnormalities caused by APP mutations. The majority of the mutations cluster at or very near the sites within APP that are normally cleaved by secretases. In accordance with this, these mutations promote generation of Aβ by favouring proteolytic processing of APP by β- or γ-secretase or increase the relative

production of Aβ42 compared to Aβ40 (Cai et al., 1993; Citron et al., 1992; Suzuki et al., 1994). Furthermore, APP mutations internal to the Aβ sequence heighten the self-aggregation of Aβ into amyloid fibrils (Wisniewski et al., 1991).

These exciting developments provided the genetic framework for the emerging amyloid hypothesis (Hardy and Higgins, 1992; Selkoe, 1991).

In the past years, bolstered particularly by the cloning of PSs proteins (Levy-Lahad et al., 1995; Sherrington et al., 1995) and the demonstration that AD-causing mutations in PS1 and PS2 also enhance the processing of APP to form Aβ (Scheuner et al., 1996), the amyloid hypothesis has become the focus of AD research.

In addition to the cloning of PS1 and PS2 and the discovery that they alter APP metabolism (Borchelt et al., 1996; Citron et al., 1997; Duff et al., 1996) through a direct effect on the γ-secretase protease (De Strooper et al., 1998; Wolfe et al., 1999b), there have been four conceptually important observations that strongly support the amyloid hypothesis.

First, mutations in the gene encoding the tau protein, the main component of NFTs, cause frontotemporal dementia with parkinsonism (Hutton et al., 1998; Poorkaj et al., 1998; Spillantini et al., 1998).

This neurodegenerative disorder is characterized by severe deposition of tau in NFTs in the brain, but no deposition of amyloid. The clear implication is that even the most severe consequences of tau alteration - profound NFTs formation leading to fatal neurodegeneration - are not sufficient to induce the amyloid plaques characteristic of AD. Thus, the NFTs of wild-type tau seen in AD brains are likely to have been deposited after changes in Aβ metabolism and initial plaque formation, rather than before (Hardy et al., 1998).

Second, transgenic mice overexpressing both mutant human APP and mutant human tau undergo increased formation of tau-positive tangles (as compared with mice overexpressing tau alone), whereas the structure and number of their amyloid plaques are essentially unaltered (Lewis et al., 2001). This finding suggests that altered APP processing occurs before tau alterations in the pathogenic cascade of AD, a notion bolstered by the recent observation that in mouse hippocampal primary neuronal cultures, Aβ toxicity is tau dependent (Rapoport et al., 2002).

Third, crossing APP transgenic mice with ApoE–deficient mice markedly reduces cerebral Aβ deposition in the offspring (Bales et al., 1997), providing strong evidence that the pathogenic role of genetic variability at the human ApoE locus (Corder et al., 1993) is very likely to involve Aβ metabolism. And fourth, growing evidence indicates that genetic variability in Aβ catabolism

and clearance may contribute to the risk of late-onset AD (Bertram et al., 2000; Ertekin-Taner et al., 2000; Myers et al., 2000; Olson et al., 2001; Wavrant-DeVrieze et al., 1999).
Taken together, these findings are consistent with the notion that cerebral Aβ accumulation is the primary mechanism of AD pathogenesis and that the rest of the disease process, including tau tangle formation, results from an imbalance between Aβ production and Aβ clearance.

5.2. Updating the amyloid hypothesis

As the above-described amyloid cascade hypothesis of AD, as initially formulated, proposed that the hallmark progressive deposition of insoluble fibrillar Aβ in plaques triggered neurodegeneration which, in turn, caused the insidious escalation of debilitating symptoms, including progression through the different stages of clinical dementia. Support for this proposal came from the discovery that application of fibril containing Aβ to cultured neurons was highly toxic in vitro (Lorenzo and Yankner, 1996) and that intracerebral injection of fibril-containing Aβ caused a neurodegeneration-associated disruption of performance of cognitive tasks in animals (Maurice et al., 1996; McDonald et al., 1994; Nitta et al., 1994; Stephan et al., 2001). However, the relatively poor correlation between the severity of clinical dementia at the time of death of patients with AD and either the magnitude of fibrillar Aβ load or the extent of neuron loss in the brain provided a major challenge for the original amyloid cascade hypothesis (Terry, 1996).
In fact, many studies demonstrated that the best statistical correlation occurs between measures of synaptic density and degree of dementia (DeKosky and Scheff, 1990; Terry et al., 1991). Data obtained by electron microscopy (Davies et al., 1987; Scheff et al., 1990; Scheff et al., 2007; Scheff et al., 2006; Seabrook et al., 1999), immunocytochemical and biochemical analyses on synaptic marker proteins in AD biopsies and autopsies (Dickson et al., 1995; Honer et al., 1992; Terry et al., 1991) indicate that synaptic loss in the hippocampus and neocortex is an early event (Masliah et al., 1994) and the major structural correlate to cognitive dysfunction (Bertoni-Freddari et al., 1990; DeKosky and Scheff, 1990; Gibson, 1983; Hamos et al., 1989). Not NFTs, senile plaques, nor even neuronal loss show such a strong statistical correlation with dementia (Masliah and Terry, 1993; Terry et al., 1991).
Moreover, the decrease in synapse number and density seems disproportionate to the loss of neuronal cell bodies (Bertoni-Freddari et al., 1996; Davies et al., 1987; DeKosky and Scheff, 1990), suggesting that pruning of synaptic endings may precede the demise of the neuron in the disease process. Furthermore, some changes in the brains of AD patients and APP transgenic

mice suggest that synaptic function is compromised prior to the physical deterioration of neuronal structures (Oddo et al., 2003; Palop et al., 2003; Westphalen et al., 2003; Yao et al., 2003).

This evidence, coupled with the fact that large fibrillar plaques present much less Aβ surface area to neuronal membranes than do a multitude of small oligomers that can diffuse into synaptic clefts, indicates that such soluble assembly forms are better candidates for inducing neuronal and/or synaptic dysfunction than plaques, *per se*. Indeed, human Aβ can exist in diverse assembly states, including monomers, dimers, trimers, tetramers, dodecamers, higher-order oligomers and protofibrils, as well as mature fibrils, which can form microscopically visible amyloid plaques in brain tissues (Glabe, 2008).

Therefore, a new understanding of the amyloid cascade hypothesis proposes an alternative mechanism for memory loss based on the impact of small, soluble Aβ oligomers (Hardy and Selkoe, 2002; Klein et al., 2001). Indeed, different soluble molecular species that are generated at very early stages of the disease and that only at more advanced stages are deposited in an aggregated form could be involved in synaptic failure. It has, thus, been suggested that soluble assembly states of Aβ peptides can cause cognitive problems by disrupting synaptic function in the absence of significant neurodegeneration.

Therefore, current research investigates the relative importance of these various soluble Aβ assemblies in causing synaptic dysfunction and cognitive deficits.

6. The Post-Synaptic Density

Cell-cell communication in the CNS is mainly achieved by specialized contact zones between neurons called chemical synapses. The term “synapse” was introduced by Sherrington in 1897, who described, in “A text book of physiology”, that this close connection between two nerve cells is simply a contact without the continuity of substance. In the 1950s, electron microscopy substantiated this view, when synapses were described as polarized neuronal cell junctions with specific membrane specializations at transmitter-releasing presynaptic site and with, in close apposition, the afferent postsynaptic part. On the postsynaptic side of a synapse, especially in excitatory synapses, the membrane appears thickened and more electron-dense than other neuronal substructures. Therefore the afferent part of excitatory synapses was either called the post synaptic thickening or the post synaptic density (PSD). High resolution electron microscopy and elaborated cryo-fixation techniques have revealed that a synaptic contact zone is ultrastructurally characterized by:

- a presynaptic active zone: a cluster of dense granular presynaptic particles, fibres and synaptic vesicles (40 nm diameter), some of which are closely associated with a thickening of the presynaptic plasma membrane, called the active zone, where exocytosis occurs (Sudhof, 2004);
- a synaptic cleft with a width of 23.8 nm filled with an organized 8.2 nm transcleft complex composed of a wide variety of cell-adhesion molecules that hold pre- and postsynaptic membranes together in register and at the appropriate separation (Scheiffele, 2003);
- a PSD: an electron-dense thickening of the post-synaptic membrane, directly opposed to the active zone and perfectly matched with it in size and shape. PSD might reflect assembled proline-rich synapse-associated protein/SH3 and ankyrin repeat-containing protein (ProSAP/Shank) (Baron, 2006; Zuber et al., 2005). Together this protein network is visible by electron microscopy as an irregular structure, often disk-like, of~ 200-800 nm (mean 300-400 nm) width and~ 30-50 nm thickness (Carlin et al., 1980). The presence of a prominent PSD is characteristic of glutamatergic synapses (hence they are termed asymmetric), in contrast to inhibitory synapses, called symmetric (Colonnier, 1968; Gray, 1959).

The PSD can be divided into the PSD itself, as well as perisynaptic and extrasynaptic regions. Extrasynaptic regions have specialized postsynaptic functions and are enriched for a distinctive set of proteins, such as metabotropic glutamate receptors (Baude et al., 1993) and proteins involved in endocytosis (Racz et al., 2004). Over the last 30 years, the main approach used to identify PSD protein was biochemical, because PSD is abundant in the brain and can be enriched by a few centrifugation steps. PSD are typically purified by mammalian brain by differential

centrifugation and sucrose gradient sedimentation to obtain synaptosomes, followed by detergent extraction with nonionic detergent, as Triton-X which does not solubilize the PSD (Carlin et al., 1980). Then, several groups separated PSD proteins by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) or two-dimensional gel electrophoresis and sequenced major bands to identify abundant PSD components (Cho et al., 1992), or employed library screening with antibodies directed against PSD preparations or identified many proteins indirectly as binding partners of known postsynaptic proteins by the two-hybrid system (Kim and Sheng, 2004). Proteomic analysis of PSD is prone to include in the PSD many contaminants or false positive, such as mitochondrial proteins or glial proteins. It is also prone to miss *bona fide* components (false negative) that are in low abundance or only transiently associated with the PSD. More recently, mass spectrometry (MS) methods, such as matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) MS and liquid chromatography coupled with tandem MS, have detected large numbers of proteins in the PSD fraction (Cheng et al., 2006; Walikonis et al., 2000). An advantage of MS is that it can identify non-abundant PSD proteins and can reveal post-translational modifications of proteins. Current views of the molecular organization of the PSD are based largely on specific protein-protein interactions inferred from yeast two-hybrid, coimmunoprecipitation and *in vitro* studies (Kennedy et al., 2005; Kim and Sheng, 2004; McGee and Bredt, 2003). However, better functional insight into postsynaptic molecular architecture requires an understanding of the stoichiometry and 3D structure of individual PSD proteins. Recently, quantitative information on the stoichiometry of proteins in the PSD has come to the forefront, on the basis of several approaches, as EM combined with quantitative immunoblotting (Chen et al., 2005), quantitative MS (Cheng et al., 2006) and green fluorescent protein-based quantitative fluorescence calibration (Sugiyama et al., 2005).

What is known at the moment is that excitatory synapses are especially characterized by their different glutamate receptors (GluRs), such as α-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA) receptors (AMPARs), NMDA receptors (NMDARs) and group I metabotropic GluRs (mGluRs), which are specifically targeted and clustered at the PSD (Mayer and Armstrong, 2004). All these receptors are components of individual, tightly associated, multiprotein complexes that regulate synaptic targeting or removal from synaptic sites, local expression, signal transduction and clustering (Kim and Sheng, 2004). With increasing knowledge about protein composition, it became even more pressing to understand the spatial organization of molecules within the PSD.

The PSD of excitatory synapses seems to be arranged in a clear-cut laminar hierarchical structure (Zuber et al., 2005). This hierarchy appears to be determined by the targeting and binding

characteristic of the individual proteins. At the membrane, receptors are organized as distinct subcompartments that are then combined into larger protein units, which are finally attached to the local cytoskeleton. As a consequence, PSD proteins can be divided in different groups: membrane bound receptors and channels, i.e. GluRs; scaffolding proteins, i.e. members of the MAGUK family; signalling and regulatory proteins, i.e. Calcium-calmodulin-dependent Kinase II (CaMKII); cell adhesion proteins; cytoskeletal.

6.1. Glutamate receptors

Glutamate is generally acknowledged as the most important neurotransmitter for normal brain function. It is released from vesicles from pre-synaptic sites and interacts with its receptors on the post-synaptic site. There are two major classes of GluRs: ionotropic receptors (iGluR) or ligand-gated ion channels, that give rise to fast postsynaptic responses that last typically a few seconds and metabotropic or G-protein coupled receptors, which produce slower postsynaptic effects that can endure longer. Several types of iGluRs have been identified: AMPARs, NMDARs and kainate receptors (Hollmann and Heinemann, 1994). They are named after the agonists they are activated by: N-methyl-D-aspartate (NMDA); α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) and kainic acid. The cloning of iGluRs immediately led to relate their aa sequence to function and mechanism. Against initial expectations, iGluRs proved to have only three transmembrane domains (M1, M3 and M4) plus a cytoplasm-facing re-entrant membrane loop (M2). Thus the N-terminus is located extracellularly and the C-terminus intracellularly.

6.1.1. AMPA receptors

AMPARs are heterotetramers composed of a combination of GluRs 1 to 4 (GluR1-4) subunits that assemble in a combinatorial fashion to form a functional receptor channel (Nakanishi et al., 1990). They are activated by glutamate and antagonized by 6-ciano-7-nitroquinoxaline-2,3-dione (CNQX) and 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(f)quinoxaline (NBQX). These receptors are the major mediator of fast glutamatergic excitatory synaptic transmission in the CNS. Structural studies suggest that tetrameric assembly of AMPARs are achieved in a dimer-of-dimers fashion (Madden, 2002). Different subunits of AMPARs display distinct spatial and temporal pattern of expression and endow specific properties to AMPAR complexes: AMPARs containing GluR2 subunits show lower permeability to Ca^{2+} while receptors assembled from GluR1, GluR3 and

GluR4 are highly permeable to Ca^{2+} (Burnashev et al., 1992). Alternative splicing and RNA editing provide further variability of the functional receptor characteristics. GluR2 subunit, for example, undergoes RNA editing which prevents Ca^{2+} permeability, contributes to linear I-V relationship of current flux and confers insensitivity to intracellular spermine (Boulter et al., 1990; Keller et al., 1992; Washburn et al., 1997).

Protein quantification study on the purified PSD fraction reported the ratio among different GluR subunits suggesting that the major AMPARs in the forebrain are likely to be GluR1- and GluR2-containing (Cheng et al., 2006).

All four subunits of AMPARs have several identified phosphorylation sites on their intracellular C-termini that regulate their function and that seem to be involved in the regulation of various form of synaptic plasticity.

GluR1 is one of the most abundantly expressed subunit of AMPARs in hippocampal and neocortical neurons (Martin et al., 1993). Most of the phosphorylation on GluR1 occurs on serine and threonine residues (Blackstone et al., 1994), but tyrosine phoshorylation has also been observed under certain circumstances (Moss et al., 1993). Ser831 and Ser845 have been implicated to mediate the expression of LTP and LTD (Barria et al., 1997). Phosphorylation on GluR1-Ser831 is mediated by PKC and CaMKII (Roche et al., 1996), while GluR1-Ser845 is phosphorylated by protein kinase A (PKA) (Roche et al., 1996). Phosphorylation on both of these residues can enhance current through AMPARs, albeit via distinct mechanisms (Derkach et al., 1999).

GluR2 is a key subunit which renders AMPARs channel impermeable to Ca^{2+} and confers specific biophysical properties (Tanaka et al., 2000). There are several serine phosphorylation sites (Ser863 and Ser880) mapped on the intracellular C-terminal of GluR2 that are phosphorylated by PKC (Matsuda et al., 1999). Among these, Ser880 has been implicated to play a role in synaptic plasticity. When unphosphorylated at Ser880, GluR2 can interact with PDZ domains of glutamate receptor interacting protein GRIP/ABP (AMPARs Binding Protein) or protein interacting with PICK-1 (Matsuda et al., 1999). Interaction between GluR2 and GRIP family of proteins has been shown to stabilize AMPARs at synaptic locations (Dong et al., 1997) or intracellular pools (Daw et al., 2000). On the other hand, phosphorylated Ser880 allows preferential binding to PICK-1. Interaction of Ser880 phosphorylated GluR2 to PICK-1 has been demonstrated either to promote endocytosis (Chung et al., 2000) or allow trafficking of receptors to the plasma membrane (Daw et al., 2000).

GluR3 and GluR4 are not only highly expressed in the adult forebrain structures as compared to GluR1 and GluR2 subunits (Petralia and Wenthold, 1992). However they contain homologous phosphorylation sites corresponding to those on GluR1 and GluR2, which have been implicated in synaptic plasticity mechanisms. Expression of GluR4 in the hippocampus is limited during early post-natal period development and it can be inserted into synapses by spontaneous activity (Zhu et al., 2000).

6.1.2. NMDA receptors

NMDARs are heteromeric assembly of different subunits that form a non-selective cation channel and allow the entry of Ca^{2+} in addition to monovalent cations, as Na^{+} and K^{+}. Functional importance of NMDARs derives from the potential of these receptors to act as coincidence detectors of pre- and post-synaptic activity. Due to a voltage-dependent relieve of a Mg^{2+} block of the ion channel (Mayer et al., 1984), NMDARs are activated only when presynaptic glutamate release coincides with sufficient post-synaptic depolarization. Extracellular Mg^{2+} exerts a voltage-dependent block of the open ion channel (Nowak et al., 1984). In contrast, activation of NMDARs requires the simultaneous binding of two different agonists: in addition to the conventional agonist-binding site typically occupied by glutamate, the binding of glycine appears to be necessary for receptor activation (Kleckner and Dingledine, 1988). At hyperpolarized membrane potentials, more negative than -70 mV, the concentration of Mg^{2+} in the extracellular fluid is sufficient to virtually abolish ion flux through NMDARs channels even in the presence of glutamate and glycine. A positive change in transmembrane potential will increase the probability of the exit of Mg^{2+}. NMDARs are composed of multiple subunits proteins (NR1, NR2A-D, NR3) that assemble in heteropentameric or heterotetrameric structures. These structures exhibit distinct properties depending on the subunit composition. Whereas NR1 is essential for NMDAR function, NR2A-D and NR3A subunits may regulate this function (Dingledine et al., 1999). The various isoforms of NR2 subunits contain the glutamate-binding site (Hirai et al., 1996) while glycine-binding site appears to be located on the NR1 subunit.

The NR1 subunit is abundantly expressed in all brain regions and is mandatory for the assembly of functional NMDARs. NR1 undergoes alternative splicing to generate eight receptor variants (Zukin and Bennett, 1995) that add molecular diversity to NMDARs.

Unlike NR1, NR2 subunits are differentially expressed across various cell types and control the biophysical, pharmacological and electrophysiological properties of NMDARs. The NR2A subunit

confers a lower affinity for glutamate, distinctly faster kinetics, greater channel open probability and more prominent Ca^{2+} dependent desensitization if compared to NR2B. The NR2D and NR2C subunits are characterized by low conductance openings and reduced sensitivity to Mg^{2+} block. NR3-containing receptors are more predominant in a narrow time window during development. The NR3A/NR3B containing receptors are considered a distinct class of nonconventional NMDARs since NR3 shows limited sequence homology to NR1 and NR2 and since it confers unique properties to the channel.

NMDARs have been shown to contain consensus sequences for phosphorylation by protein kinases (Meddows et al., 2001). CaMKII can associate to NR1, NR2A and NR2B but they can be phosphorylated also by other kinases as PKC or PKA. In the brain, between 10 and 70% of NR1 and NR2 subunits seem to be phosphorylated by PKA or PKC (Leonard and Hell, 1997) thus increasing the heterogeneity of NMDARs.

6.2. The MAGUK family

In the last decade, the increasing knowledge of the structure and function of the excitatory glutamatergic PSD lead to the identification of key protein families that play fundamental roles in governing GluRs' localization at synapse and, consequently, GluRs' functions.

One of these families is the Membrane Associated Guanylate Kinase (MAGUK) family, a super family of multi-domain proteins related by the presence of a shared set of structural domains. Members of this family can be seen as "master organizing" molecules of the PSD (Sheng and Kim, 2000) as they are able to build up large sheets within the PSD (Baron, 2006) and can interact with a growing number of different proteins, large protein complexes, membrane-spanning, signaling and cytoskeletal proteins (Gundelfinger et al., 2006).

According to their prominent role within the organization of the PSD, MAGUK proteins are early components of PSD specialization during synaptogenesis (Boeckers et al., 1999). They are efficiently targeted to synaptic sites and are equipped with a huge set of protein-protein interaction domains. They contain three PDZ (PSD-95/DLG/ZO1) domains, a Src homology 3 (SH3) region and a Guanylate Kinase (GK)-like sequence.

Four vertebrate homologues of DLG have been described, synapse associated protein 90 (SAP90/PSD-95), SAP102, SAP97/hDlg and Chapsyn-110/PSD-93.

They are all localized in the CNS (Garner et al., 2000) but each member is distributed differently in brain cell compartments. PSD-95 and PSD-93 are highly enriched in the PSD, especially due to

their high palmytoilation degree (El-Husseini et al., 2000) while SAP97 and SAP102 have been found in dendrites and axons and are abundant in the cytoplasm as well as at synapses.

Three main roles for the MAGUK family have been recognized: targeted distribution of receptors and channel proteins within specialized domains of plasma membrane; scaffolding of functional molecules and modulation of ion channel activity.

PDZ domain scaffolds have been shown by genetic, electrophysiological and morphological studies to be essential for controlling the structure, strength and plasticity of synapses. Since these properties are related to the capability of MAGUK proteins to interact with other PSD components, it is important to understand the structural characteristics of their domains.

PDZ domains are protein-interaction domains of approximately 90 aa, often found in multi-domain scaffolding proteins, important for the organization of membrane proteins, particularly at cell-cell junctions, including synapses. They can bind to the C-termini of proteins or can form dimers with other PDZ domains and are classified on the basis of the consensus sequence they recognize:

- CLASS I: X-S/T-X-V or X-S/T-X-L
- CLASS II: X-ϕ-X- ϕ
- CLASS III: X-D-X-V

Where ϕ is a hydrophobic aa and X is an unspecified aa.

SH3 modules are 55–70 aa, non-catalytic protein domains that mediate critical protein–protein interactions. They were originally discovered as an homologous region present in the tyrosine kinase product of the v-Src oncogene (Dalgarno et al., 1997). SH3 ligands are, at minimum, seven residues in length and contain PXXP sequences. However, an intramolecular interaction between the SH3 and the GK regions of PSD-95 has been found. The GK module lacks the polyproline motifs that specifically bind to SH3 and instead the SH3/GK binding appears as a bi-domain interaction requiring both intact regions (McGee and Bredt, 1999). The functional significance of this interaction is still unclear.

GK module is homologous to the enzyme that catalyzes the conversion of GMP to GDP at the expense of ATP. The aa responsible for nucleotide binding and catalysis have been identified in yeast and they appear to be differentially conserved within the MAGUK protein family. The MAGUK proteins are predicted to bind neither GMP nor ATP and are, therefore, assumed to be enzymatically inactive (Kistner et al., 1995). The GK module in certain proteins mediates protein–protein interactions independently of its predicted enzymatic activity (Kim et al., 1997).

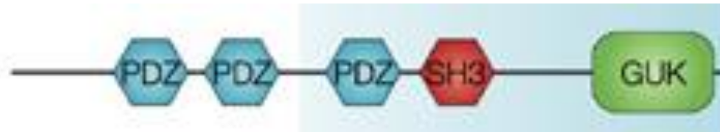


Figure 16. MAGUK's domains.

Despite sharing a common domain structure, MAGUKs are particularly distinct in their N-terminal aa sequence.

In addition, alternative splicing generates splice variants of PSD-95, PSD-93 and SAP97 that contain an **L27** domain at the N-terminus (Chetkovich et al., 2002). This domain mediates protein multimerization and regulates excitatory synaptic strength (Nakagawa et al., 2004).

Through these domains MAGUK proteins organize heterogeneous ensembles of proteins, the composition of which changes at different locations in the cell, both during development and in response to neuronal activity.

Moreover, MAKUG themselves can be mobile within neurons and their activity and expression levels are regulated by phosphorylation, lipid modification and ubiquitynation. There is widespread evidence that PDZ-ligand interactions are disrupted by phosphorylation, typically on the C-terminal peptide of the ligand (Hu et al., 2002). MAGUK-peptide interactions can also be regulated by phosphorylation of the PDZ domain: CaMKII-dependent phosphorylation of the PDZ1 of SAP97 disrupts its interaction with NR2A subunit of NMDAR (Gardoni et al., 2003).

6.2.1. SAP97: a MAGUK shuttling to the synapse

SAP97/hDlg is a member of the MAGUK family broadly expressed throughout the body, not just in CNS, but also in epithelial cells (Muller et al., 1995), that exerts several different functions.

It has been found to be essential for the assembly of septate junctions and epithelial tumor suppression (Woods and Bryant, 1991), as well as for the assembly of larval neuromuscular junctions (Budnik et al., 1996). In the CNS, it is important for the trafficking of molecules to the PSD and for synaptic activity.

SAP97 versatility is due to the existence of at least seven different isoforms (McLaughlin et al., 2002), produced by alternative splicing that insert short peptidic sequences either in the N-terminus or in the HOOK region. The insertion at the N-terminus of two different proline-rich sequences (I1A and I1B) allows the binding of SH3 domains of three different kinases: Lck (Hanada et al., 2003), Crk and Abl (McLaughlin et al., 2002) and induces homo-multimerization of SAP97 molecules containing these inserts. Between the commonly shared SH3 and GK domains,

SAP97 has a HOOK domain, which contains four alternatively spliced insertions, named I2, I3, I4 and I5 (Ruiz-Canada et al., 2002). I3 encodes for the actin/spectrin associated protein, 4.1 N (Thomas et al., 2000), that facilitates the synaptic recruitment of SAP97 and its association with the actin cytoskeleton within dendritic spines (Rumbaugh et al., 2003). Moreover, the HOOK region has a specific site for calmodulin binding which is Ca^{2+}-dependent and could regulate the intramolecular interaction between SH3, HOOK and GK of SAP97 (Paarmann et al., 2002).

SAP97, as PALS1 and PSD-95β (a splicing variant of PSD-95), contains alternative N-terminal domains expressing either double cysteines that are normally palmytoilated (α-isoforms) or an L27 domain (β-isoforms). Whereas α-isoform influences AMPAR-mediated synaptic strength independently of activity, the effects of β-isoform are regulated by CaMKII activity (Schluter et al., 2006). As regards SAP97, Li and co-workers performed parallel functional and imaging studies that revealed that N-terminal α and β splice variants of SAP97 differentially regulate the synaptic localization of AMPARs and NMDARs, resulting in major changes in the ability of neurons to express synaptic plasticity. These data show that αSAP97 is important for localizing AMPARs in the PSD and increases the synaptic strength, but that these receptors are readily removed from the synapse in response to LTD. In contrast, βSAP97 appears to be a negative regulator of synaptic potentiation, favouring a shift towards synaptic depression by sequestering both AMPARs and NMDARs at extrasynaptic sites. As a result βSAP97 can block and/or modulate both the induction and the expression of synaptic plasticity. Together these data reveal that synaptic SAP97 isoforms can play functionally distinct roles in regulating glutamate receptor levels at synapses and as such influence synaptic plasticity mechanisms (Li et al., 2011).

The L27 domain gets its name from a pair of *C.Elegans* proteins: LIN-2, a MAGUK protein, and LIN-27, a small PDZ protein, which have this region near their N-termini (Doerks et al., 2000). Individual L27 domains are unfolded, while upon heteromultimerization, the domains assume rigid confirmations and helical folds. L27 can bind specifically to the L27 domain of CASK. SAP97/CASK interaction permits their polymerization and allows forming supramolecular complexes in polarized cells. More importantly, these complexes are free to recruit additional proteins through other protein-protein interaction modules and thereby augment their networking capacity.

There is wide evidence that SAP97 regulates cell-to-cell adhesion and has a role in organizing membrane specializations, as PSD.

In contrast to PSD-95, which is homogenously distributed throughout the PSD, SAP97 is concentrated near edges of cleft sides of the PSDs and in small clumps on the cytoplasmic sides.

The distribution of SAP97 confirms that this protein is actually an integral component of the PSD and suggests that it may have a role in inserting or stabilizing its binding partners at the edge of the PSD. Moreover, since SAP97 is a member of the MAGUK family, it shares the common domains responsible for protein-protein interactions. The ability to form clusters of proteins and the peripheral organization of SAP97 at the PSD is compatible with its trafficking role.

SAP97 is known to bind to the GluR1 subunit of AMPAR (Leonard and Hell, 1997) and there is evidence that it may be associated with the AMPARs outside the PSD that are recycling onto or have just left the PSD. AMPARs appear to be concentrated at edges of PSD, so the relatively high concentration of SAP97 at edges may reflect its recent arrival in the company of AMPARs and its clusters on the cytoplasmic surfaces of PSDs may represent the residuals of AMPARs-SAP97 complexes that have been inserted into the PSD. This interaction occurs early in the secretory pathway, while the receptors are in the ER or *cis*-Golgi. In contrast, few synaptic receptors are associated with SAP97, suggesting that it dissociates from the receptors once at the plasma membrane.

Synaptic trafficking of SAP97 itself is modulated by CaMKII-dependent phosphorylation in cultured hippocampal neurons. CaMKII activation leads to increased targeting of SAP97 into dendritic spines, whereas CaMKII inhibition is responsible for SAP97 high colocalization in the cell soma (Mauceri et al., 2004). CaMKII-dependent SAP97-Ser39 phosphorylation regulates the association of SAP97 with the postsynaptic complex, thus providing a fine molecular mechanism responsible for the synaptic delivery of SAP97 interacting proteins.

Apart from being involved in AMPARs trafficking to the PSD, SAP97 seems to be responsible for NMDARs delivery to the membrane. SAP97 is able to bind to the C-terminal tail of NR2A NMDARs' subunit and again this interaction is regulated by CaMKII phosphorylation on Ser32 residue in SAP97-PDZ1 (Gardoni et al., 2003). SAP97 PDZ1 and PDZ2 can also bind NR2B subunit of NMDARs, with higher affinity for PDZ2 (Wang et al., 2005).

Trafficking NMDARs and AMPARs to the PSD, SAP97 has a pivotal role in synaptic plasticity and synapse development.

Its overexpression drives GluR1 to synapse leading to a higher concentration of synaptic AMPARs, to spine enlargement, to increased miniature EPSC frequency and to LTP occlusion. This potentiation of synaptic transmission depends on SAP97 multimerization through its L27 (Nakagawa et al., 2004).

SAP97 has also presynaptic effects on synaptic transmission since it recruits complexes of proteins that enhance presynaptic terminal growth and function (Regalado et al., 2006).

Since SAP97 is involved in events controlling synaptic transmission, its role in neurodegenerative diseases has been largely addressed and alterations have been identified in several disorders, especially in early stages.

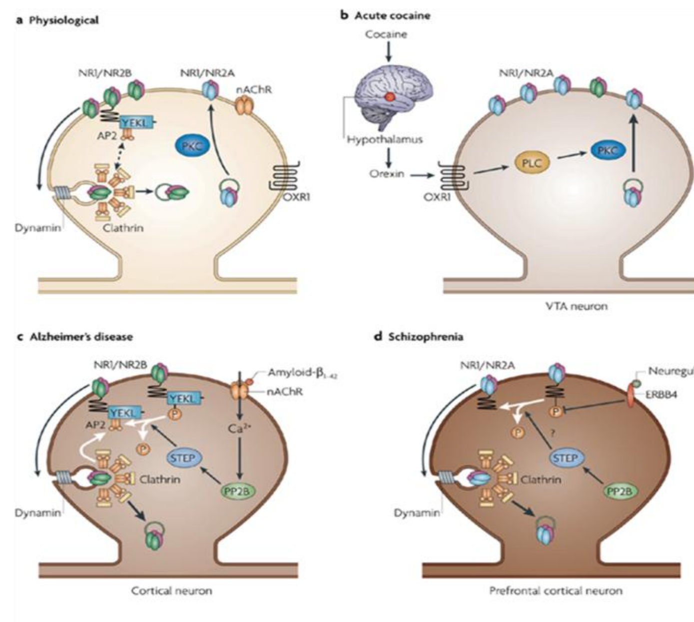


Figure 17. SAP97 involvement in different diseases.

In schizophrenic patients, for example, SAP97 was shown to be dramatically reduced in pre-frontal cortex if compared to healthy controls (Toyooka et al., 2002). Moreover, experimental Parkinsonism in rats appears to be associated to decreased synaptic membrane localization and increased vesicular localization of PSD-95 and SAP97 (Nash et al., 2005). Post L-DOPA treatment, PSD-95 and SAP97 levels raise because the two proteins are redistributed toward synaptic membrane from vesicular compartments (Nash et al., 2005). Most important for this thesis is the involvement of SAP97 in AD. A disturbance of SAP97/GluR1 interaction has been reported (Wakabayashi et al., 1999a) in *post mortem* tissues from AD patients, suggesting a defect in trafficking and function of SAP97 in the course of the disease. Recent data have shown that SAP97 is responsible for driving ADAM10, the most accredited candidate for α-secretase activity, to the post synaptic membrane where the enzyme exerts its anti-amyloidogenic cleavage on APP (Marcello et al., 2007). SAP97 also binds to the cytoplasmic tail of TACE, another α-secretase candidate and this interaction might have functional implications for TACE shedding activity (Peiretti et al., 2003).

Thus, alterations of SAP97 can be considered key elements of the pathogenesis of several neurodegenerative disorders.

AIM

An attempt to clarify the correlation between events of the amyloid cascade and synaptic failure has been recently made by Marcello *et al.* who have described the existence of a molecular bridge between ADAM10, one of the actors involved in the amyloid cascade, and SAP97, a cargo protein of the glutamatergic synapse (Marcello et al., 2007). SAP97, a scaffolding protein belonging to the MAGUK family, is responsible for the correct assembly of the glutamatergic synapses and has been shown to be involved in the trafficking to the post-synaptic membrane of ADAM10, the major α-secretase candidate. This event is mandatory for ADAM10 activity on APP, because the enzyme can cleave APP only when the two proteins colocalize either at the plasma membrane or in late secretory vesicles (Lammich et al., 1999). Interfering with this mechanism by means of the cell permeable peptide Tat-Pro highlighted the importance of SAP97-mediated intracellular trafficking of ADAM10. This peptide mimics the ADAM10 proline rich region, which is responsible for the binding to the SH3 domain of SAP97 and, consequently, competes with ADAM10 for the binding to SAP97. Treatments of primary hippocampal neurons or acute intraperitoneal administration of the peptide to mice result in the dissociation of ADAM10/SAP97 complex, in a reduction of ADAM10 localization to the post-synaptic membrane and in a shift of APP metabolism towards amyloidogenesis.

These data suggest a possible pathogenic involvement of deficits of ADAM10/SAP97 complex in AD.

In this frame, the specific aim of this thesis was to decipher the involvement of ADAM10/SAP97 complex in the pathogenesis of AD in order to identify molecular mechanisms capable of balancing ADAM10/SAP97 complex defects in AD.

In particular, specific aims were:

1- to investigate possible alterations of both ADAM10 synaptic localization and ADAM10/SAP97 complex in the earlier phases of AD pathogenesis, when APP metabolism is shifted toward amyloidogenesis but the loss of synaptic structure is not prominent. We analysed autoptic brain tissues obtained from 6 late onset AD patients, fulfilling criteria for Braak 4 stage, and 6 aged-matched control subjects. We focused on the hippocampus as an area strongly affected by the disease and on the superior frontal gyrus, as control area since no significant plaques deposition is detectable at this stage of

the disease. Moreover, we evaluated the synaptic protein composition and association of SAP97 to ionotropic glutamate receptor subunits, i.e. GluR1 and NR2A.

2- to investigate the intracellular pathways, which control SAP97-mediated ADAM10 trafficking and its enzymatic activity. The comprehension of these mechanisms could provide new tools to induce ADAM10 activity/trafficking in AD. Moreover, the modulation of the intracellular trafficking of ADAM10 could constitute an innovative therapeutic strategy to finely tune its shedding activity towards APP. In particular we focused on the mechanisms underlying PKC-induced ADAM10 activity.

MATERIAL AND METHODS

Characteristics of the subjects

The hippocampus (Hp) and the superior frontal gyrus (SFG) from late onset/sporadic AD patients (AD; *n*=6) and healthy controls (HC; *n*=6) were obtained from the Netherlands Brain Bank. Stringent criteria were used in the case selection of human brain tissues employed in this study. HC have no history of psychiatric or neurological disease and no evidence of significant age-related neurodegeneration.

Braak and Braak criteria were used to categorize AD tissues in accordance with established guidelines (Braak and Braak, 1991). As previously reported, these criteria are based on the distribution of neurofibrillary tangles (NFTs), which are seen first in the transentorhinal area (stage 1), spread to the entorhinal region (stage 2), extend to the hippocampus proper (stage 3), increase in number there (stage 4), and involve the association neocortex (stage 5), and finally the primary cortex (stage 6). These stages are divided into the entorhinal (stage 1–2), limbic (stage 3–4), and neocortical (stage 5–6) phase corresponding, respectively, to cognitively normal, cognitively impaired, and demented patients. Demographic and clinical characteristics of AD patients and HC are shown in **Table 1.** AD patients fulfilled Braak 4 stage. Accordingly, in AD cases there were tangles and neuritic plaques in Hp, whereas the amount of plaques in SFG closely resembled HC. All procedures were in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory human tissue and were approved by the Ethics Committee of the University of Milan, Italy.

Triton insoluble synaptic membrane (TIF), soluble fraction (S2) and P3 microsomal fraction preparation

For human samples Triton Insoluble Fraction (TIF), a fraction highly enriched in all categories of postsynaptic density proteins (i.e., receptor, signaling, scaffolding, and cytoskeletal elements) absent of presynaptic markers, and soluble fraction S2, were isolated. In order to avoid protein degradation, AD samples were paired to HC samples and processed at the same time. The relative values for each case were replicated in duplicate experiments, considering for each paired sample the ratio AD/HC. To obtain the TIF fractions, samples of human brain were homogenized at 4°C in an ice-cold buffer with protease inhibitors (*Complete™,* GE Healthcare, Mannheim, Germany), Ser/Thr and Tyr phosphatase inhibitors (*Sigma-Aldrich),* 0.32 M Sucrose, 1 mM Hepes, 1 mM NaF, 0.1 mM PMSF, 1 mM $MgCl_2$ using a glass-teflon homogenizer. An

aliquot of homogenate (Homo) was kept for Western Blot (WB) analysis. Homo were then centrifuged at 1000*g* for 5 min at 4°C, to remove nuclear contamination and white matter. The supernatant was collected and centrifuged at 13000*g* for 15 min at 4°C. The resulting pellet (crude membrane) was resuspended in resuspension buffer (1 mM Hepes with protease inhibitors (*Complete™,* GE Healthcare)) and then centrifuged at 100000*g* for 1 h at 4°C. Triton-X extraction of the resulting pellet was carried out at 4°C for 20 min in an extraction buffer (1% Triton-X, 75 mM KCl and protease inhibitors (*Complete™,* GE Healthcare)). After extraction, the samples were centrifuged at 100000*g* for 1 h at 4°C and the TIFs obtained were resuspended in 20 mM HEPES with protease inhibitors (*Complete™,* GE Healthcare). To get the soluble fraction (S2), samples of human brain were homogenized at 4°C in an ice-cold buffer with protease inhibitors (*Complete™,* GE Healthcare), Ser/Thr and Tyr phosphatase inhibitors (*Sigma-Aldrich),* 0.32 M Sucrose, 1 mM Hepes, 2 mM EDTA, 0.1 mM PMSF, 1 mM EGTA using a hand-held glass-teflon homogenizer. In this case, aliquots of Homo were kept to perform immunoprecipitation (ip) assay. Homo were then centrifuged at 1000*g* for 10 min at 4°C, to remove nuclear contamination and white matter. The supernatants were collected and centrifuged at 100000*g* for 1h at 4°C. The resulting pellet (crude membrane) was discarted and the supernatant obtained corresponds to the S2 fraction.

For rat acute hippocampal slices, TIF fraction and P3 microsomal fraction, a fraction enriched of endoplasmic reticulum and Golgi apparatus proteins, were isolated. Rat acute hippocampal slices were homogenized at 4°C in an ice-cold buffer with protease inhibitors (*Complete™,* GE Healthcare), Ser/Thr and Tyr phosphatase inhibitors (*Sigma-Aldrich),* 0.32 M Sucrose, 1 mM Hepes, 0.1 mM PMSF, 1 mM $MgCl_2$ using a glass-teflon homogenizer. An aliquot of Homo was kept for WB analysis. Homo were then centrifuged at 10000*g* for 20 min at 4°C. The resulting pellet was used to purify TIF while the supernatant was used to obtain P3 microsomal fraction. Triton-X extraction of the resulting pellet was carried out at 4°C for 20 min in an extraction buffer (0.5% Triton-X, 150 mM KCl and protease inhibitors (*Complete™,* GE Healthcare)). After extraction, the samples were centrifuged at 100000*g* for 1 h at 4°C and the TIFs obtained were resuspended in 20 mM Hepes with protease inhibitors (*Complete™,* GE Healthcare). The Triton Soluble Fraction (TSF) was kept for WB analysis. The supernatant was centrifuged at 100000*g* for 2h at 4°C and the microsomal pellets were resuspended in the same buffer of the homo (Gurd et al., 1974).

Immunoprecipitation (i.p.)

Aliquots of 50 µg (for ADAM10/SAP97 and GluR1/SAP97 immunoprecipitation [i.p.]) or of 70 µg (for SAP97/NR2A i.p.) of homogenate obtained from the S2 purification from Hp and SFG of HC and AD were incubated overnight at 4 °C in RIA buffer containing 200 mM NaCl, 10 mM ethylene-diaminetetra-acetic acid (EDTA), 10 mM Na_2HPO_4, 0.5% NP-40, 0.1% sodium dodecyl sulphate (SDS) in a final volume of 150 µl with an antibody (Ab) against ADAM10, GluR1, or SAP97. Protein A-sepharose beads (Sigma-Aldrich) were added and incubation was continued for 2 hours, at room temperature, with shaking. Beads were collected by centrifugation and washed three times with RIA buffer before adding sample buffer for SDS-PAGE and boiling for 5 minutes. Beads were collected by centrifugation, all supernatants were applied onto 6% SDS-PAGE and revealed by either anti-SAP97 or anti-NR2A.

Western blotting (WB)

30 µg of proteins from human homogenate and TIF and 30 µg of proteins from rat acute hippocampal slices homogenate, triton soluble fraction (TSF), TIF and P3 microsomal pellet were separated on 6% or 7% SDS-PAGE, transferred to a nitrocellulose membrane and probed with the corresponding primary Ab, followed by incubation with horseradish peroxidase-conjugated secondary Ab. To analyze APP Cterminal fragments (CTFs), TIF proteins were separated on a 15% Tris-Tricine SDS-PAGE and WB analysis was performed with 4G8 Ab. Membranes were developed using electrochemiluminescence reagent (Amersham Biosciences, Buckinghamshire, UK).

Cloning, expression, and purification of glutathione S-transferase (GST) fusion protein

Glutathione S-transferase (GST)-ADAM10 C-terminal domain (Ct) fusion protein contains the cytoplasmic domain of ADAM10 (695–749) and the GST-SAP97-SH3 (Marcello et al., 2007), GST-NR2A the sequence of NR2A C-terminal domain from amino acid 1349–1464 (Gardoni et al., 1999). GST-ADAM10 C-terminal S741-A, GST-ADAM10 C-terminal 734Δ, GST-ADAM10 C-terminal 721Δ and GST-SAP97-GKΔ using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA), following the manufacturer's instructions. All constructs were verified by sequencing. GST-GluR1 C-terminal domain (Ct) was kindly provided by M. Passafaro (DTI Dulbecco Telethon Institute, Milano, Italy) and includes the sequence of GluR1 cytoplasmic tail. GST-SAP97 full

length (rat SAP97, isoform i1b, i3) was kindly provided by Kari Keinanen (University of Helsinki). GST fusion proteins were expressed in *Escherichia coli* and purified on glutathione agarose beads (Sigma Aldrich, St. Louis, MO, USA) as previously described (Gardoni et al., 2001).

In vitro fusion protein phosphorylation

GST-SAP97 full length, the mutant GST-SAP97-GKΔ, GST-SAP97-N-terminal, GST-SAP97-PDZ1, GST-SAP97-PDZ3, GST-SAP97-SH3, GST-ADAM10 C-terminal, the mutants GST-ADAM10 C-terminal S741-A, GST-ADAM10 C-terminal 734Δ, GST-ADAM10 C-terminal 721Δ and GST-NR2A C-terminal domain (1349-1464) purified fusion proteins were incubated with 50 units of purified protein kinase (PKC) for 30 min at 37°C, in presence of 10 mM Tris-HCl pH 7.4, 10 mM $MgCl_2$, 0.5 mM $CaCl_2$, and 10 µM ATP ([γ-^{32}P]ATP 2 µCi/tube; 3000 Ci/mmol; PerkinElmer). Proteins were separeted by SDS PAGE and phosphoproteins revealed by autoradiograph.

Pull-down assay

Aliquots of homogenate from either Hp or SFG of AD patients and HC subjects were diluted with Tris Buffered Saline (TBS, 10 mM Tris and 150 mM NaCl) to a final volume of 1 mL and incubated 2 hours with 40 µl of GST saturated with the C-terminal domain of ADAM10 or the C-terminal domain of NR2A or the C-terminal domain of GluR1 or GST alone. Aliquots of rat homogenate were diluted with TBS to a final volume of 1 mL and incubated 2 hours with 26 µl of cold-phosphorylated GST SAP97 full length, GST SAP97-SH3 GST ADAM10 C-terminal, GST ADAM10 C-terminal S-A and GST alone. After incubation, beads were washed five times with TBS and 0.1% Triton X-100. Bound proteins were resolved by SDS-PAGE and subjected to immunoblot analysis with monoclonal SAP97 Ab or polyclonal ADAM10 Ab and monoclonal GST Ab.

Antibodies, reagents and cell-permeable peptides

The following antibodies were used: monoclonal antibody (mAb) 22C11 (raised against N-terminal domain of APP, 66-81 aa), mAb 4G8 (against 17-24 aa of Aβ), mAb anti-α-calcium/calmodulin-dependent kinase II (α-CaMKII), polyclonal antibody (pAb) anti-AMPA GluR1 and mAb 6E10, raised against 1-17 aa of Aβ, were purchased from Millipore (Billenca, MA,USA); pAb anti-ADAM10 and pAb 2072 (against 1-10 aa of Aβ) were purchased from Abcam (Cambridge, MA, USA), mAb anti-pan-Shank, anti-GST and anti-PSD95 were purchased from

NeuroMab (University of California Davis, Davis, CA, USA); mAb anti-SAP97 was purchased from Stressgen (Victoria, Canada); pAb anti-SAP97 and mAb DSI were purchased from ABR-Thermoscientific (Rockford, IL USA); pAb anti-Actin and mAb anti-α-Tubulin were purchased from Sigma-Aldrich (St. Louis, MO); mAb against NR2A was purchased from Zymed (San Francisco, CA, USA); anti-synaptophysin and anti-SNAP25 from Synaptic Systems (Göttingen, Germany); mAb GM130 was purchased from BD Biosciences (NJ, USA). Peroxidase-conjugated secondary anti-mouse Ab was purchased from Pierce (Rockford, IL, USA) while peroxidase-conjugated secondary anti-rabbit Ab was purchased from Bio-Rad (Hercules, CA, USA). AlexaFluor secondary Abs were purchased from Invitrogen (Carlsbad, CA).

Phorbol 12,13-dibutyrate (PDBu), Brefeldin A (BFA) and Adenosine 5'-Triphosphate (ATP) were purchased from Sigma-Aldrich (St. Louis, MO); PKC and PKC lipid activator were purchased from Millipore (Billenca, MA,USA); [γ-^{32}P] ATP was purchased from PerkinElmer (USA).

The cell permeable peptide Tat-Pro ADAM10 $^{709\text{-}729}$ was obtained linking the 11 amino acids human immunodeficiency virus Tat transporter sequence (Aarts et al., 2002) to the 21 amino acids sequence corresponding to ADAM10 proline-rich domains (NH_2-YGRKKRRQRRR-PKLPPPKPLPGTLKRRRPPQP-COOH). Tat-Ala ADAM10 $^{709–729}$ peptide, in which all proline residues were mutated into alanine (NH_2-YGRKKRRQRRR-AKLAAAKALAGTLKRRRAAQA-COOH), was used as the control (Marcello et al., 2007). All peptides were synthesized by XigenPharma (Lausanne, Switzerland).

Acute hippocampal slices, neuronal cultures preparation, transfection and treatment

Acute hippocampal slices were prepared as previously described (Gardoni et al., 2001; Gardoni et al., 2003). Briefly, brains were removed and placed into chilled (4°C) oxygenated Krebs' buffer. After removal of meninges, hippocampal slices were prepared quickly with a McIlwain tissue chopper and placed in custom-made chambers equilibrated continuously with O_2 95%-CO_2 5% (v/v). Slices were then equilibrated at 37°C (O_2 95%-CO_2 5%) for 60 minutes. After the equilibration period, slices were incubated with pharmacological treatments. After pharmacological treatments slices were quickly frozen at -80°C. Slices were than thawed on ice and subcellular fractionation was performed.

Hippocampal neuronal primary cultures were prepared from embryonic day 18-19 (E18-E19) rat hippocampi as previously described (Piccoli et al., 2007). Neurons were transfected at DIV7 with

ds RED ER construct using calcium-phosphate method. Neurons were treated at DIV14, fixed and then immunostained.

Immunocytochemistry

For colocalization studies, hippocampal neurons treated with PDBu (100 nM, 30 minutes) or with both Tat-Pro and Tat-Ala peptide (1 µM, 30 minutes) or with PDBu and Tat-Ala or Tat-Pro were fixed 10 minutes in 4% paraformaldehyde plus 4% sucrose at 4°C and immunostained for ADAM10 or SAP97 and GM130, as Golgi apparatus marker, or 15 minutes in methanol at -20°C and immunostained for ADAM10 or SAP97 and PSD95, as postsynaptic marker; primary and secondary antibodies were applied in GDB buffer (Sala et al., 2001) (30 mM phosphate buffer, pH 7.4, containing 0.2% gelatin, 0.5% Triton X-100 and 0.8 M NaCl). Cells were chosen randomly for quantification from different coverslips. Fluorescence images were acquired by using Zeiss Confocal LSM510 Meta system with 63x objective and a sequential acquisition setting at 1024x1024 pixels resolution; colocalization analysis was performed with the Zeiss Confocal Software.

Quantification and statistical analysis

Quantification of WB analysis was performed by means of computer-assisted imaging (ImageJ). The levels of the proteins were expressed as relative optical density (OD) measurements and normalized on actin or tubulin. Data obtained by pull-down assays were normalized on GST OD, corresponding to the amount of GST fusion protein. Levels and values were expressed as mean ± standard error of the mean (SEM). The values for AD patients were expressed as percentage of HC in the same blot ± SEM. For the analysis of CTFs, the ratio between the OD of CTF83 and CTF99 was measured. Colocalization analysis was performed using Zeiss AIM 4.2 software. Statistical evaluations were performed by Student *t* test or Pearson correlations or, as appropriate, by one-way ANOVA followed by Bonferroni's as a *post hoc* test.

Table 1. Demographic and neuropathological characteristics of AD and HC cases.

Subjects	gender	age at death (years)	PMD (hours)	pH CSF	brain weight (g)	cause of death	Braak level
1 (AD)	F	91	3.45	6.36	1011	general deterioration	4
2 (AD)	F	91	4.15	6.27	1202	dehydration	4
3 (AD)	F	86	4.10	6.34	1083	sepsis	4
4 (AD)	F	86	5.05	6.62	998	general deterioration	4
5 (AD)	M	81	4.05	6.42	1253	dehydration	4
6 (AD)	F	86	5.55	6.85	950	cachexia	4
1 (HC)	F	84	4.45	6.26	1179	heart failure	1
2 (HC)	F	81	6.40	7.16	1164	eutanasia	1
3 (HC)	M	80	7.15	5.8	1376	cachexia	0
4 (HC)	M	84	7.05	5.9	1385	chronic pulmunary disease	1
5 (HC)	F	85	5.00	6.72	1257	abdominal aneurysm	1
6 (HC)	F	85	4.40	6.71	1165	dehydration	2

AD: Alzheimer's disease patients; HC: Healthy controls; m: male; f: female: PMD: Post Mortem delay; CSF: Cerebrospinal Fluid.

RESULTS

1. SAP97-mediated local trafficking is altered in Alzheimer disease patients' hippocampus

1.1. Experimental design: characterising the system

Although current available animal models have been extremely informative about key pathogenic pathways of AD, to assess the relevance of SAP97 complexes with its synaptic partners in AD pathogenesis we exploited autoptic brain tissues obtained from 6 late onset AD patients, fulfilling criteria for Braak 4 stage, and 6 aged-matched control subjects (HC) (**Table 1**).

Our experimental design implied the study of the synaptic role of SAP97 in the earliest phase of AD pathogenesis, when APP metabolism is shifted toward amyloidogenesis but the loss of synaptic structure is not prominent. Thus we focused only on Hp, the first brain area affected by the disease, and SFG, a less affected area used as negative control because no significant plaque deposition was detectable at this stage, as revealed by neuropathology reports.

To validate our experimental design and to support neuropathological data with biochemical ones, we characterized APP metabolism, postsynaptic structural composition, and presynaptic proteins in both areas of HC and AD patients' groups.

Firstly, in Hp and in SFG, we measured release of APP ectodomain generated by α-secretase (sAPPα) in the soluble fraction. Immunoblot experiments, performed with 6E10 antibody, raised against 1-17 aminoacids of Aβ, showed a reduction in sAPPα levels in Hp of AD compared with HC (n=6; −38.8 ± 12.7%, p=0.028, AD *vs* HC; **Fig. 1A**), but not in SFG (n=6; +33.7 ± 56.4%, p>0.05, AD *vs* HC; **Fig. 1B**). To strengthen these results, APP C-terminal fragments (CTFs) CTF99 for β-cleavage and CTF83 for α-cleavage were evaluated in the Triton Insoluble Fraction (TIF), which is enriched in postsynaptic density (PSD) proteins (Adlard et al., 2005; Kamenetz et al., 2003; Zimmermann et al., 2004). TIF proteins were separated on a 15% Tris-Tricine SDS-PAGE and immunoblot analysis was performed with 4G8 antibody. CTF83/CTF99 ratio was significantly decreased in Hp of AD compared with HC (n=6; −25.85 ± 8.5%, p=0.029 AD *vs* HC, **Fig. 1C**), but not in SFG (n=6; +23.7 ± 25.2%,p>0.05, AD *vs* HC, **Fig. 1D**). These data confirm that, at this stage of pathogenesis, APP metabolism is shifted toward amyloidogenesis only in Hp and not in SFG of AD patients.

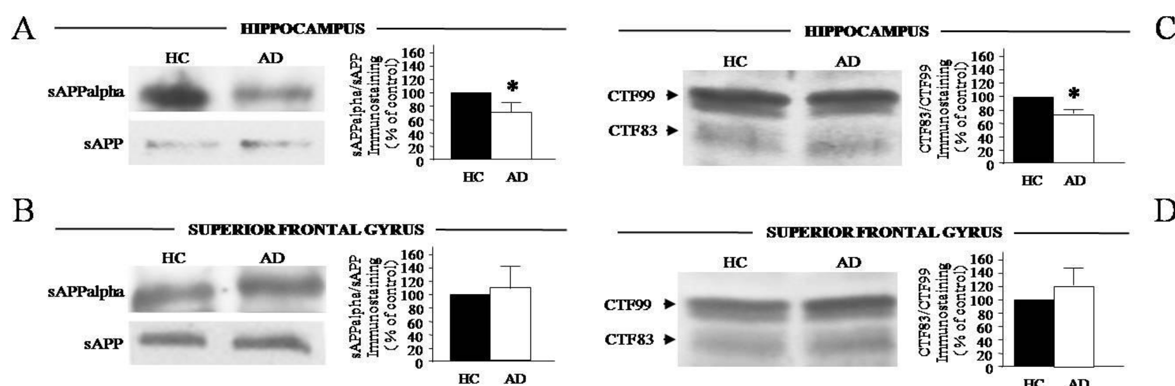


Figure 1. ADAM10/SAP97 modification affects α-secretase activity. *WB analysis was performed on the soluble fraction, obtained from the Hp* ***(A)*** *and the SFG* ***(B)*** *of HC and AD patients. The ratio sAPPα/sAPP is reduced in the Hp but not in the SFG of AD patients. Quantitative analysis of immunostaining is shown as percentage of HC (p=0.028). WB analysis was performed on the TIF fraction, obtained from the Hp* ***(C)*** *and the SFG* ***(D)*** *of HC and AD patients, to reveal CTFs****.*** *The ratio CTF83/CTF99 is reduced in the Hp but not in the SFG of AD patients. Quantitative analysis of immunostaining is shown as percentage of HC (p=0.029).*

AD is also characterized by an impairment of excitatory synapses. AD-induced synaptic alterations are not the broad consequence of wholescale neuronal deterioration but derive from a highly specific attachment to spines of excitatory synapses and this cross-talk occurs primarily at the post-synaptic membrane.

So, we evaluated possible alterations in the postsynaptic structure occurring at this stage of the disease.

We assessed biochemically the levels and synaptic localization of the three major PSD components, i.e., Shank, PSD-95, and α-CaMKII (Sheng and Hoogenraad, 2007). Quantitative analyses showed no significant modifications of Shank, PSD-95 and α-CaMKII in total homogenate (Homo) and TIF fraction of both Hp and SFG of AD and HC, suggesting that postsynaptic structure is preserved in our experimental groups (Hp (Homo), n=6; α-CaMKII = −1.8 ± 11.9%, PSD-95 = −5.2 ± 19.8%, Shank = −6.9 ± 9.6%; SFG (Homo), n=6; α-CaMKII = −9.12 ± 9.8%, PSD-95 = −7.8 ± 9.1%, Shank = −22.8 ± 12.8%; overall: *p*>0.05; Hp (TIF), n=6; α-CaMKII = −2.9 ± 4.5%, PSD-95 = +20.8 ± 15.5%, Shank = +80.8 ± 41.4%; SFG (TIF), n=6; α-CaMKII = −5.8 ± 7.8%, PSD-95 = +3.9 ± 14.1%, Shank = +41.1 ± 25.7%, overall: *p*> 0.05; AD *vs* HC; **Fig. 2A-B**).

Moreover, we evaluated the levels of the presynaptic proteins synaptophysin and SNAP25. Quantitative analyses revealed no significant alterations of synaptophysin and SNAP25 in total homogenate of both Hp and SFG of AD and HC, suggesting that the presynapse is still not affected in our experimental conditions (n=6, Hp, synaptophysin = −4.8 ± 9.8%, SNAP25 = +7.9 ±

4.1%; SFG, synaptophysin = −13.1 ± 28%; SNAP25 = −9.5 ± 11.1%, overall: $p > 0.05$; AD *vs* HC; **Fig. 2C**).

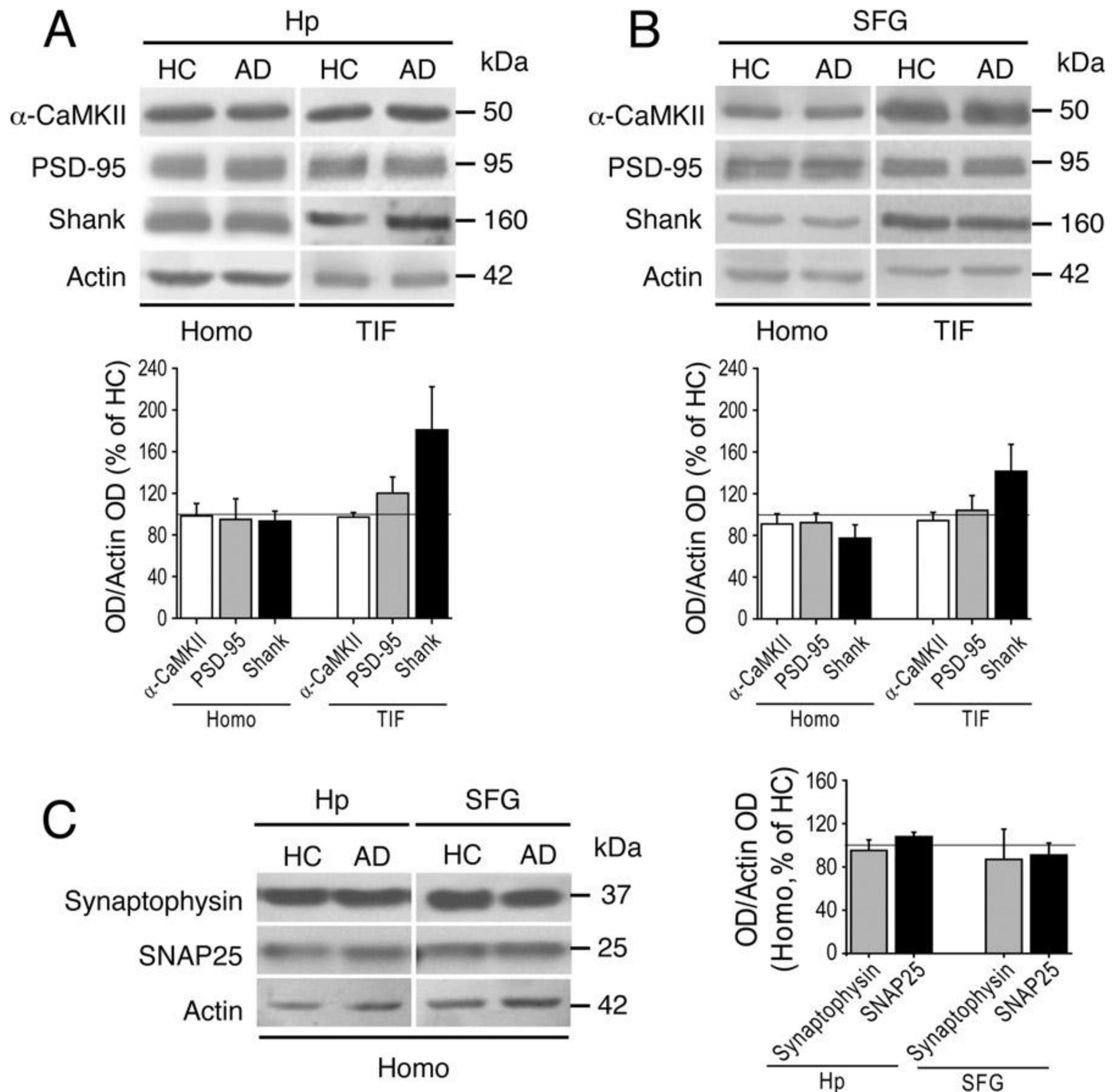


Figure 2. (A). *WB of homogenate and triton insoluble fraction (TIF) obtained from Hp of AD patients and healthy controls (HC) performed with anti-α-CaMKII, anti-PSD-95 and anti-Shank antibodies. No alterations were found in the levels of these proteins in total homogenate and in TIF.* ***(B).*** *WB of homogenate and TIF obtained from SFG of AD patients and HC performed with anti-α-CaMKII, anti-PSD-95 and anti-Shank antibodies. No alterations were found in the levels of these proteins in total homogenate and in TIF.* ***(C).*** *WB of homogenate obtained from SFG of AD patients and HC performed with anti-synaptophysin and anti-SNAP25 antibodies. No alterations were found in the levels of these proteins in total homogenate.*

1.2. Synaptic levels of ADAM10 and GluR1, but not of NR2A, are changed in Hp of AD patients

We then evaluated possible alterations of synaptic localization of SAP97 and its key partners, i.e., ADAM10, GluR1, and NR2A. The levels of these proteins have been assessed in both total homogenate and TIF fraction.

SAP97 levels in both total homogenate and TIF of Hp (n=6; Homo, +31.4 ± 35.1%; TIF, +24.8 ± 25.6%, $p > 0.05$; AD *vs* HC; **Fig. 3A**) and SFG (n=6; Homo, +9.3 ± 18.5%; TIF, −8.5 ± 5.6%, $p > 0.05$; AD vs HC; **Fig. 3B**) were not affected.

Western blot analyses of TIF showed a significant reduction of ADAM10 levels in Hp of AD when compared with HC (n=6; −32.6 ± 5.6%, $p = 0.002$, AD *vs* HC; **Fig. 3A**) but not in SFG (n=6; +18 ± 37.1%, $p > 0.05$; AD *vs* HC; **Fig. 3B**). ADAM10 levels in total homogenate were unaltered in both areas (n=6; Hp, −7.7 ± 11.7%; SFG, +19.5 ± 13.2%, $p > 0.05$; AD *vs* HC; **Fig. 3A-B**).

GluR1 levels were significantly increased in TIF membranes of Hp of AD patients when compared with HC subjects (n=6; +43.0 ± 16.6%, $p = 0.048$, AD vs HC; **Fig. 3A**) but not in SFG (n=6; +7.9 ± 22.4%, $p > 0.05$; AD vs HC; **Fig. 3B**). Western blot analyses of homogenates revealed no alterations in GluR1 total levels both in Hp (n=6; −7.5 ± 27.7%, $p > 0.05$, AD *vs* HC; **Fig. 3A**) and in SFG (n=6; +18.5 ± 13.3%, $p > 0.05$, AD *vs* HC; **Fig. 3B**).

Immunoblot experiments detected no significant alterations of NR2A levels both in total homogenate (n=6; −6.6% ± 19.7%, $p > 0.05$, AD *vs* HC; **Fig. 3A**) and in TIF fraction of AD Hp (n=6; +34.1 ± 15.1%, $p > 0.05$, AD *vs* HC; **Fig. 3A**) compared with HC. In SFG of AD patients compared with HC, NR2A levels were concomitantly reduced in both homogenate and TIF (n=6; Homo, −39.9 ± 8%, $p = 0.004$, TIF, −32.3 ± 8.8%, $p = 0.015$; AD *vs* HC; **Fig. 3B**).

These data showed an altered synaptic localization of ADAM10 and GluR1, but not of NR2A, in Hp of AD patients.

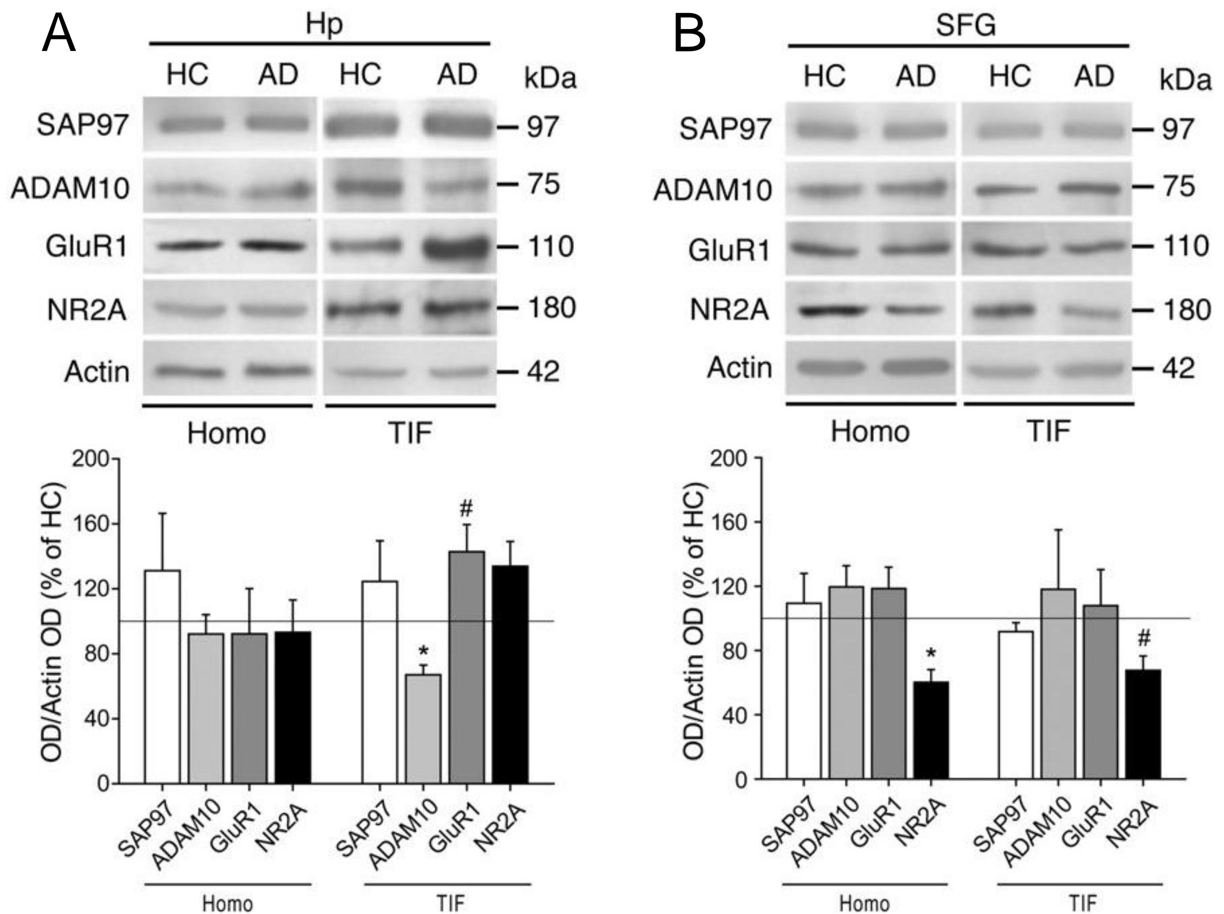


Figure 3. (A). *WB of homogenate and TIF obtained from Hp of AD patients and HC performed with anti-SAP97, anti-ADAM10, anti-GluR1, and anti-NR2A antibodies. No alterations were found in the levels of these proteins in total homogenate. A significant reduction of ADAM10 levels and an increase of GluR1 levels in TIF were observed in AD compared with HC (ADAM10: * p=0.002, GluR1: # p=0.048).* ***(B).*** *WB of homogenate and TIF obtained from SFG of AD patients and HC performed with anti-SAP97, anti-ADAM10, anti-GluR1, and anti-NR2A antibodies. No alterations of SAP97, ADAM10, and GluR1 were found in the levels of these proteins in total homogenate and in TIF. NR2A was significantly reduced in both homogenate and TIF (Homo: * p=0.004, TIF: # p=0.015). All data were normalized using actin. For all experiments quantitative analysis of immunostaining is shown as percentage of HC in the same experiment.*

1.3. SAP97 capability to associate to both ADAM10 and GluR1, is altered in AD patients' Hp

Because binding to SAP97 is pivotal for these synaptic proteins' trafficking, the ability of SAP97 to effectively bind its partners in AD cases and control subjects was then evaluated. The capability of SAP97 to bind ADAM10, through the SH3 domain of SAP97, and glutamate receptor subunits, i.e., GluR1 (Leonard et al., 1998) and NR2A (Bassand et al., 1999), via its PDZ domains,

was analyzed by coimmunoprecipitation experiments. In Hp of AD cases compared with HC, ADAM10/SAP97 coimmunoprecipitation was reduced (n=6; −33.7 ± 4.9%, *p*=0.001; AD *vs* HC; **Fig. 4A**). No differences were detectable between the two groups in SFG (n=6; +26.9 ± 21.0, *p*>0.05; AD *vs* HC; **Fig. 4A**). Coimmunoprecipitation experiments revealed a significant increase of GluR1/SAP97 coprecipitation in Hp homogenates of AD patients, when compared with HC (n=6; +90.3 ± 34.2%, *p*=0.04; AD *vs* HC; **Fig. 4B**). In the SFG of AD cases compared with HC, no alterations were observed (n=6; +21.1 ± 18%, *p*>0.05; AD *vs* HC; **Fig. 4B**). Western blot analyses of NR2A/SAP97 immunocomplex showed no significant differences between AD and HC subjects in Hp (n=6; −28.3 ± 21.5%, *p*>0.05; AD *vs* HC; **Fig. 4C**). Because NR2A expression levels were reduced in SFG (**Fig. 3B**) coimmunoprecipitation assays were performed precipitating the unchanged protein, i.e., SAP97. Coimmunoprecipitation assays of NR2A/SAP97 complex were performed from SFG homogenates and no significant alterations were found (n=6; +17.3 ± 9.8%, *p*>0.05; AD vs HC; **Fig. 4C**). These data point to an alteration of SAP97 capability to bind ADAM10 and GluR1, but not NR2A, in AD Hp.

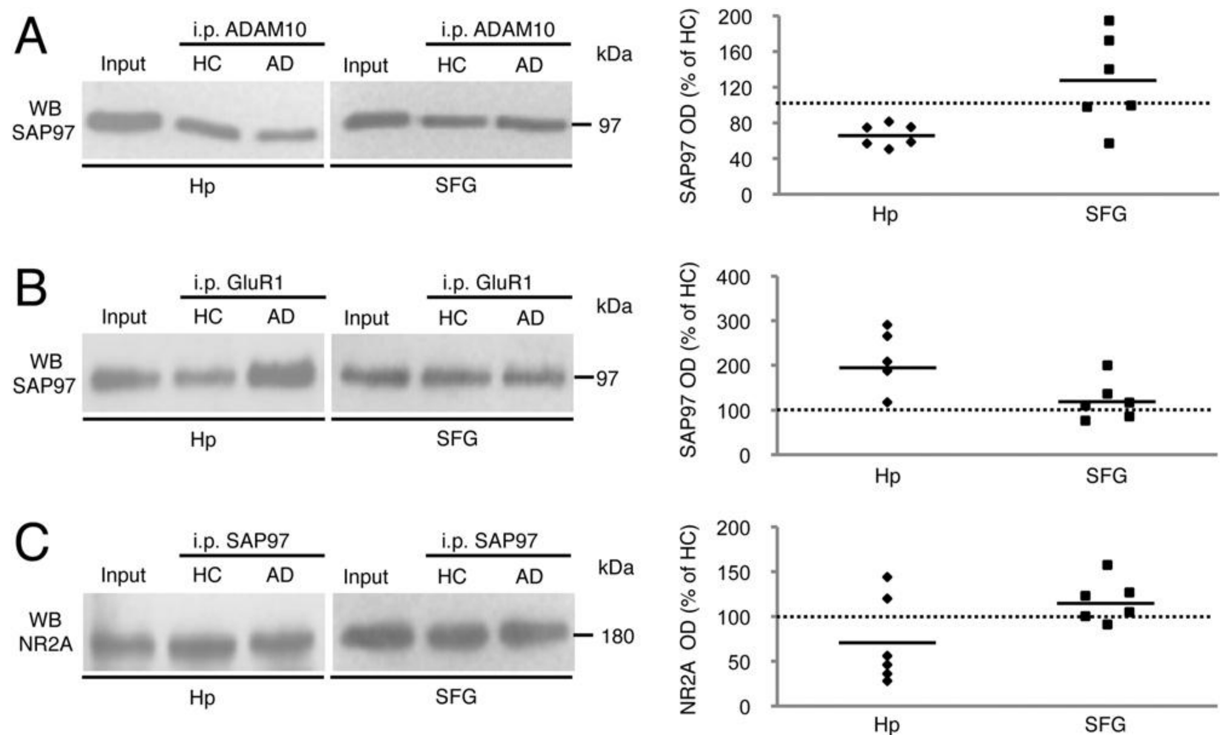


Figure 4. SAP97 capability to associate to both ADAM10 and GluR1 is changed in AD patients' Hp. (A). *Homogenate from Hp and SFG of HC and AD patients was immunoprecipitated with anti-ADAM10 antibody and SAP97 presence was evaluated. ADAM10/SAP97 association is reduced in AD patients compared with HC (p=0.001). No differences were detectable in SFG.* ***(B).*** *Homogenate from Hp and SFG of HC and AD patients was immunoprecipitated with anti-GluR1 antibody and SAP97 presence was evaluated. GluR1/SAP97 association is increased in AD patients compared with HC (p=0.04). No differences were detectable in SFG.* ***(C).*** *Homogenate from Hp and SFG of HC and AD patients was immunoprecipitated with anti-SAP97 antibody and NR2A presence was evaluated. SAP97/NR2A association is not modified in AD patients compared with HC. No differences were detectable in SFG. Quantitative analysis of immunostaining is shown as percentage of HC in the same experiment.*

1.4. SAP97 binding capacity is modified in AD patients' Hp

To further confirm these results and to assess SAP97 binding capacity in an in vitro system, pull-down experiments were carried out. For each binding partner, fusion proteins between GST and the domains of ADAM10, GluR1, and NR2A responsible for the interaction with SAP97 were generated, purified, and incubated with either Hp or SFG homogenate of AD and HC. Pull-down assays were performed and Western blot analysis revealed a band corresponding to SAP97, as expected. Quantitative analyses showed a significant increase of endogenous SAP97 binding to ADAM10 cytoplasmic tail, a significant reduction of GluR1 C-terminal tail capability to bind SAP97, and no alterations of SAP97 precipitation with GST-NR2A C-terminal tail (1349 –1464) in Hp of AD patients when compared with HC (ADAM10, +404.9 ± 124.6%, p=0.009; GluR1, −24.5 ± 9.9%, p=0.033; NR2A, +32.5 ± 22.5%, p>0.05; AD vs HC; **Fig. 5**). Pull-down experiments carried out from SFG samples did not reveal any significant modification of all proteins between the two groups (ADAM10, +69.1 ± 66.2%; GluR1 +14.4 ± 16.5%; NR2A, +30.9 ± 18.2%, p>0.05; **Fig. 5**). Pull-down assays data mirror the results obtained by immunoprecipitation analyses. In AD patients' Hp a reduction of ADAM10/SAP97 complex entails an increased availability of SAP97 to bind in vitro ADAM10 cytoplasmic tail, as demonstrated by the pull-down assay. On the other hand, in AD Hp, GluR1/SAP97 association is enhanced and consequently in vitro experiments showed a reduced binding of SAP97 to GluR1 tail. Correlation analysis of data obtained by pull-down experiments with Pearsons method, revealed that SAP97 binding to GluR1 correlates with ADAM10 binding in Hp (p=0.03, **Fig. 5D**), but not in SFG (p=0.336, **Fig. 5D**). These results suggest an interdependence between the alterations of ADAM10 and GluR1 binding to SAP97 in Hp of AD patients.

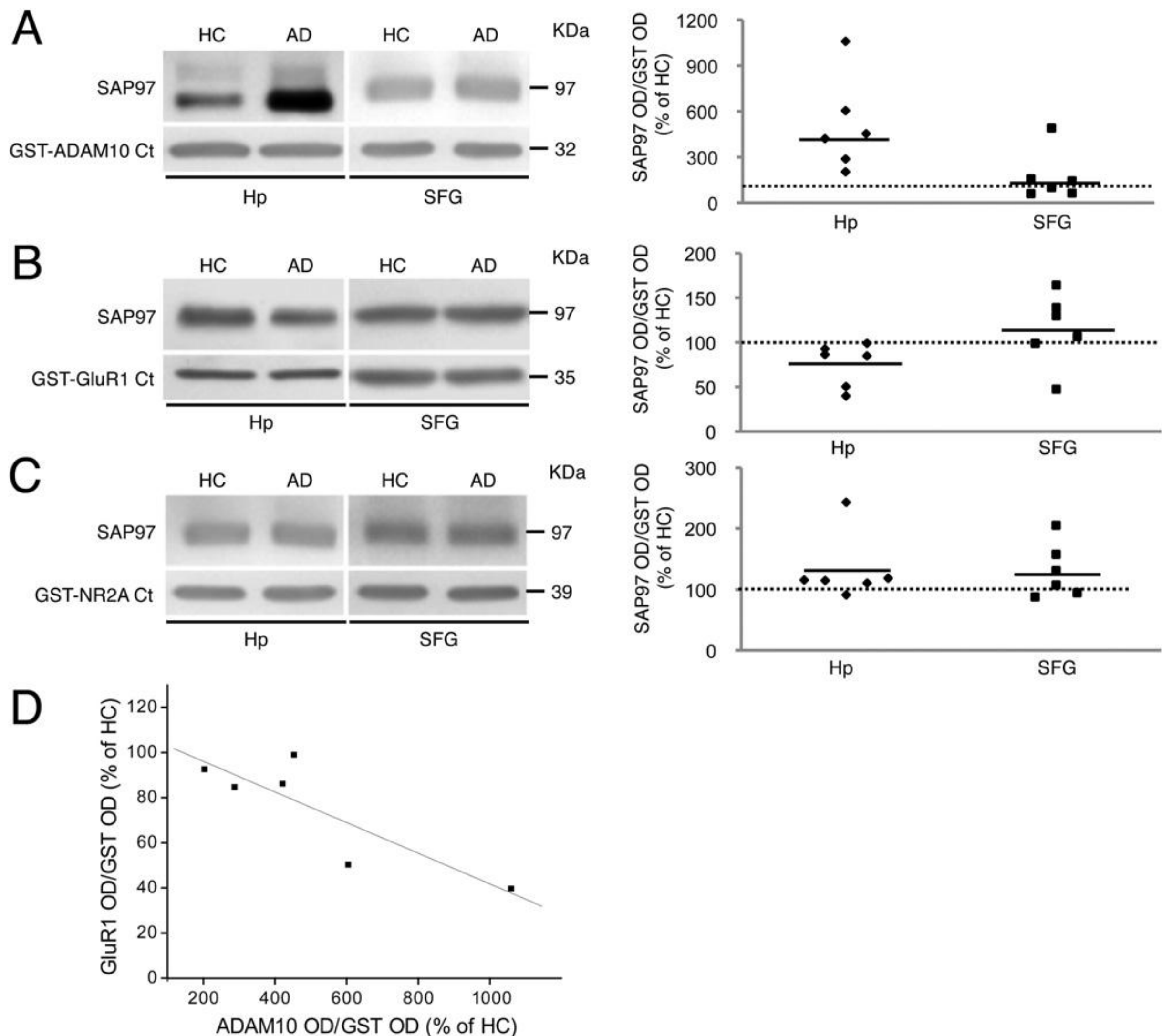


Figure 5. SAP97 capability to pull-down ADAM10 and GluR1, but not NR2A, is modified in Hp of AD patients. (A). *Glutathione S-transferase (GST)-ADAM10 C-terminal tail fusion protein was incubated in a pull-down assay with total homogenate from Hp and SFG of AD patients and HC. WB analysis was performed with anti-SAP97 antibody. SAP97 amount pulled down by GST–ADAM10 C-terminal fusion protein is significantly higher in AD Hp compared with HC (p=0.009) while no alterations are detectable in SFG.* ***(B).*** *GST-GluR1 C-terminal tail fusion protein was incubated, in a pull-down assay, with total homogenate from Hp and SFG of AD and HC. WB analysis was performed with anti-SAP97 antibody. SAP97 pulled down by GST–GluR1 C-terminal fusion protein is reduced in AD Hp compared with HC (p=0.033) while no alterations are detectable in SFG.* ***(C).*** *GST-NR2A C-terminal (1349 –1464) fusion protein was incubated in a pull-down assay with total homogenate from Hp and SFG of AD and HC. WB analysis was performed with anti-SAP97 antibody. No differences were detectable in both areas between AD patients and HC.* ***(D).*** *In Hp, the levels of SAP97 pulled down by GluR1 C-terminal tail negatively correlate to the amount of SAP97 precipitated by ADAM10 tail (correlation analysis with Pearson method: p=0.031). No correlation was present in SFG. All data were normalized on GST staining. For all experiments quantitative analysis of immunostaining is shown as percentage of HC in the same experiment.*

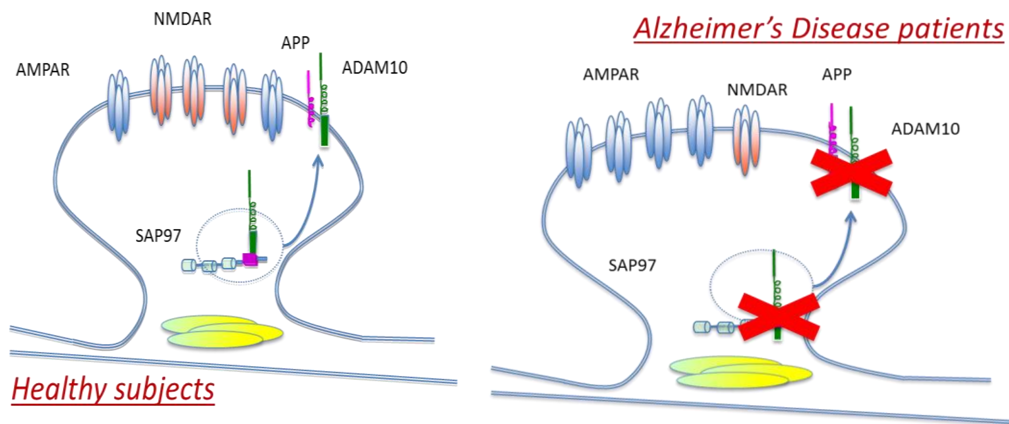


Figure 6. The glutamatergic synapse in healty subjects and in Alzheimer's Disease patients.

2. SAP97 mediates PKC-induced ADAM10 local trafficking

Our results showed an alteration of SAP97 capability to interact with its binding partners just in vulnerable brain areas of AD patients at initial stages, thus suggesting SAP97 cargo dysfunction as an early mechanism of pathogenesis.

Since SAP97 has been previously described as a determinant for the activity of the α-secretase enzyme ADAM10 (Epis et al., 2010; Marcello et al., 2007), the reduction of α-secretase activity in AD patients could be ascribed to a defect in ADAM10 trafficking rather than to an alteration of its expression. Indeed, it is known that ADAM10 can exert its catalytic activity both along the secretory system and at the plasma membrane.

In light of these considerations, we focused on the intracellular pathways which control ADAM10 trafficking and enzymatic activity. In fact, the modulation of the intracellular trafficking of ADAM10 could constitute an innovative therapeutic strategy to finely tune its shedding activity towards its substrates, i.e. APP.

Among the different intracellular pathways regulating ADAM10 activity, we drew our attention on PKC because it is known that the activation of PKC leads to a relative increase in utilization of the non-amyloidogenic α-secretase cleavage pathway for APP processing at the expense of other pathways (Gandy and Greengard, 1994), and treatment with a PKC activator increased ADAM10 substrates cleavage (Kohutek et al., 2009). In fact, phorbol esters activation of PKC has been shown to increase α-secretase mediated sAPP secretion and reduce cellular secretion of Aβ peptide (Checler, 1995; Hung et al., 1993; Jacobsen et al., 1994). In addition, the capability of PKC-α of regulating both basal and phorbol-ester-induced protein cleavage by ADAM10 may be related to its ability to modify the subcellular localization of ADAM-10 (Kohutek et al., 2009).

Therefore we wondered whether SAP97 could be involved in PKC-induced ADAM10 trafficking and activity.

2.1. PKC activation induces ADAM10 trafficking to the post synaptic compartment

To study ADAM10 trafficking, we set up a biochemical fractionation protocol to purify from hippocampal slices both TIF fraction and P3 microsomal fraction, a fraction enriched of the proteins of Golgi apparatus and endoplasmic reticulum (ER) (**Fig. 7A**). The biochemical fractionation was validated evaluating the enrichment of different markers: the Golgi marker GM130, the ER-resident protein DSI, and the post-synaptic density protein PSD95, (**Fig. 7B**). Then we analyzed ADAM10 and SAP97 localization, which are enriched in TIF fraction compared to P3 microsomal fraction, as shown in **Fig. 7B**.

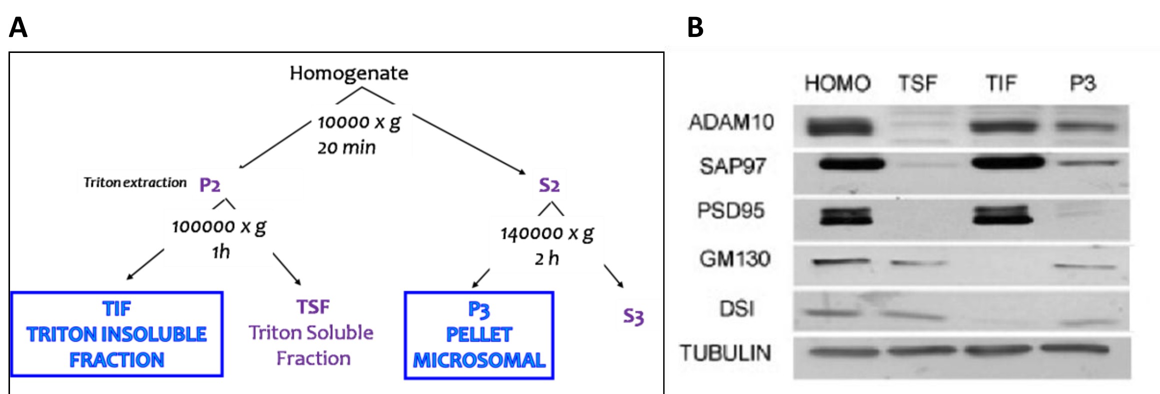


Figure 7. (A). *Microsomal fraction (P3) and Triton Insoluble Fraction (TIF) preparation from rat hippocampal slices.* ***(B).*** *ADAM10 and SAP97 localization in various subcellular compartments obtained from rat hippocampal slices preparation; representative WB for different proteins to validate the biochemical fractionation and to analyze ADAM10 and SAP97 localization. HOMO, Homogenate; TSF, Triton Soluble Fraction; TIF, Triton Insoluble Fraction; P3, microsomal fraction.*

Acute hippocampal slices were treated with Phorbol 12,13-Dibutyrate (PDBu), a phorbol ester activator of PKC (100 nM, 30 minutes). To check the activation of ADAM10, sAPPα release in the medium of incubation of the hippocampal slices was evaluated by WB analyses with Ab2072 antibody, raised against the N-terminus of Aβ, and 22C11 monoclonal antibody recognizing the N-terminal domain of APP. The ratio between sAPPα and total sAPP was measured and it was significantly increased after PDBu treatment compared to the control (data not shown). These data confirm that in our experimental conditions PDBu treatment can activate PKC and stimulate ADAM10 activity.

Then, the localization of ADAM10 and SAP97 was analyzed in total Homo, TIF and P3 fractions. Statistical analysis revealed a significant increase of ADAM10 levels in TIF fraction (+29.82 ±

7.6%, p=0.0079 PDBu *vs* CTRL; **Fig. 8A**) and a parallel reduction of the enzyme levels in the P3 fraction (-26.91 ± 6.93%, p=0.0081 PDBu *vs* CTRL; **Fig. 8A**) after PDBu treatment. A reduction of SAP97 levels was observed only in P3 fraction (-21.75 ± 7.13%, p=0.02 PDBU *vs* CTRL; **Fig. 8A**), but not in TIF fraction (-3 ± 6.12%, p>0.05 PDBU *vs* CTRL). No alterations were detected in Homo fraction both for ADAM10 levels (-9.75 ± 16.9%, p>0.05) and for SAP97 levels (+9.13 ± 11.2%, p>0.05). These data suggest that PKC activation induces ADAM10 trafficking from P3 fraction, ER and Golgi apparatus, to the post synaptic compartment where it can work as a sheddase. Moreover, PKC activation affects SAP97 intracellular localization.

Since both ADAM10 and SAP97 levels are reduced in P3 fraction, enriched of both ER and Golgi proteins, after PDBu treatment, we decided to interfere with ER–Golgi trafficking to investigate which intracellular compartment is involved in PKC-induced trafficking.

To this, acute hippocampal slices were pre-treated 20 minutes with Brefeldin-A (BFA, 10 µg/ml) that inhibits transport of proteins from ER to Golgi apparatus, and then stimulated with PDBu for 30 minutes (100 nM). After the treatment, hippocampal slices were processed and the localization of ADAM10 and SAP97 was analyzed in homo, TIF and P3 fraction. Statistical analysis revealed that disrupting ER–Golgi apparatus proteins transport blocks PKC-induced ADAM10 trafficking. In fact, pre-treatment with BFA prevents the reduction of ADAM10 levels in P3 fraction (BFA PDBU= -6.63 ± 1.62%; BFA= -47.02 ± 3.26%; PDBU= -28.48 ± 2.55%; overall p<0,05; **Fig. 8B**) and the correspondent increase in TIF fraction (BFA PDBU= -41.08 ± 7.44%; BFA= -46.55 ± 6.67%; PDBU= +52.8 ± 12.62%; overall p<0,05; **Fig. 8B**). Conversely, we found out that SAP97 levels were not affected by BFA pre-treatment both in TIF (BFA PDBU= -18.93 ± 6.35%; BFA= -20.53 ± 6.33%, p<0,05; PDBU= -18.01 ± 7.5%; **Fig. 8B**) and in P3 fraction (BFA PDBU= -30.94 ± 2.73%; BFA= -29.22 ± 5.26%, p<0,05; PDBU= -29.36 ± 2.4%, p<0,05; **Fig. 8B**). Thus, these results suggest that PKC-activation fosters ADAM10 transport from the ER, while PKC-induced SAP97 trafficking involves Golgi apparatus but not ER compartment.

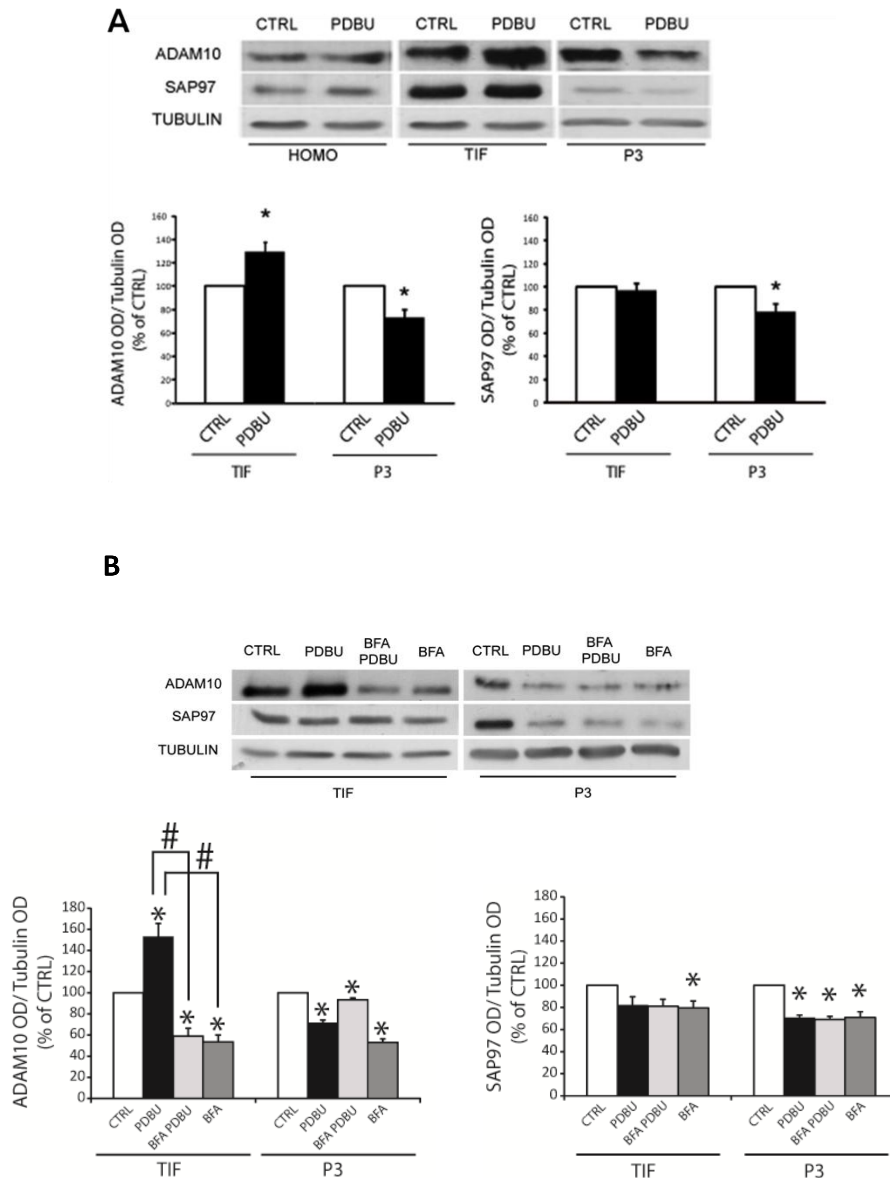


Figure 8. (A). *Representative Western Blot of ADAM10 and SAP97 localization after PKC activation in hippocampal slices (PDBu, 100 nM, 30 min). PKC activation induces an increase of ADAM10 levels in TIF fraction (+29.82 $\pm$ 7.6%, *p=0.0079 PDBU vs CTRL) and a parallel reduction of the enzyme levels in the P3 fraction (-26.91 $\pm$ 6.93%, *p=0.0081 PDBU vs CTRL). SAP97 levels were reduced only in P3 fraction (-21.75 $\pm$ 7.13%, *p=0.02 PDBU vs CTRL). Optical Density (OD) of ADAM10 and SAP97 is normalized on Tubulin OD. Data are expressed as percentage of CTRL.* ***(B).*** *Representative WB of ADAM10 and SAP97 localization in TIF and P3 of hippocampal slices pre-treated 20 min with Brefeldin A (BFA, 10 µg/ml), and exposed to PDBU (100 nM) for 30 min. Pre-treatment with BFA prevents ADAM10 trafficking from P3 fraction (BFA PDBU= -6.63 $\pm$ 1.62%; BFA= -47.02 $\pm$ 3.26%; PDBU= -28.48 $\pm$ 2.55%) to TIF fraction (BFA PDBU= -41.08 $\pm$ 7.44%; BFA= -46.55 $\pm$ 6.67%; PDBU= +52.8 $\pm$ 12.62%). The pre-treatment with BFA doesn't affect SAP97 levels in both TIF (BFAPDBU= -18.93 $\pm$ 6.35%; BFA= -20.53 $\pm$ 6.33%; PDBU= -18.01 $\pm$ 7.5%) and in P3 fraction (BFA PDBU= -30.94 $\pm$ 2.73%; BFA= -29.22 $\pm$ 5.26%; PDBU= -29.36 $\pm$ 2.4%). * = p < 0,05 PDBU, BFA PDBU, BFA vs CTRL; # = p < 0,05 BFA PDBU, BFA vs PDBU. OD of ADAM10 and SAP97 is normalized on Tubulin OD. Data are expressed as percentage of CTRL.*

To confirm these biochemical data and to characterize in details ADAM10 and SAP97 trafficking induced by PKC, we performed immunocytochemical labeling of ADAM10 and SAP97 in hippocampal neuronal primary cultures. At DIV14, hippocampal neurons were incubated, with PDBu (100 nM, 30 minutes). After treatment, cells were fixed and immunolabelled to analyze the colocalization of ADAM10 or SAP97 with different markers of the subcellular compartments. To detect ER we transfected dsRED-ER at DIV7, to stain the Golgi apparatus and the postsynaptic density we used an anti-GM130 antibody and an anti-PSD95 antibody respectively. After PDBu treatment we measured a significant decrease of both ADAM10–dsRED ER and ADAM10–GM130 colocalization in dendrites and soma (**Fig. 9A-B**), and a significant increase of ADAM10-PSD95 colocalization (**Fig. 9C**) when compared to CTRL (ADAM10/ dsRED ER: dendrites -29 ± 3.7%, p= 0.001, soma -29 ± 5.4%, p=0.009; PDBu vs CTRL; ADAM10/ GM130: dendrites -30.6 ± 1.2%, p=0.001, soma -28.4 ± 1.7%, p=0.0006; PDBu vs CTRL; ADAM10/PSD95: +78.77 ± 2,52%, p<0.005 PDBu vs CTRL). PDBu treatment significantly reduces SAP97-GM130 colocalization both in dendrites and in soma when compared to CTRL (**Fig. 10B**), but did not affect SAP97 colocalization with both dsRED ER and PSD-95 (**Fig. 10A-C**) (SAP97/ GM130: dendrites -42.78 ± 1.8%, p=0.0001, soma -39.94 ± 1.1, p=0.0006, PDBu vs CTRL; SAP97/ ds RED ER: dendrites -9.7 ± 4.7%, soma -6.8 ± 3.6% p>0.05, PDBu vs CTRL; SAP97/ PSD95: +12 ± 1.8%, p>0.05 PDBu vs CTRL). These data confirm the results obtained in hippocampal slices showing that PKC activation induces ADAM10 sorting from the ER whereas SAP97 trafficking begins from Golgi apparatus. Moreover, imaging analyses revealed that ADAM10 and SAP97 were trafficked through both somatic Golgi apparatus and dendritic Golgi outposts.

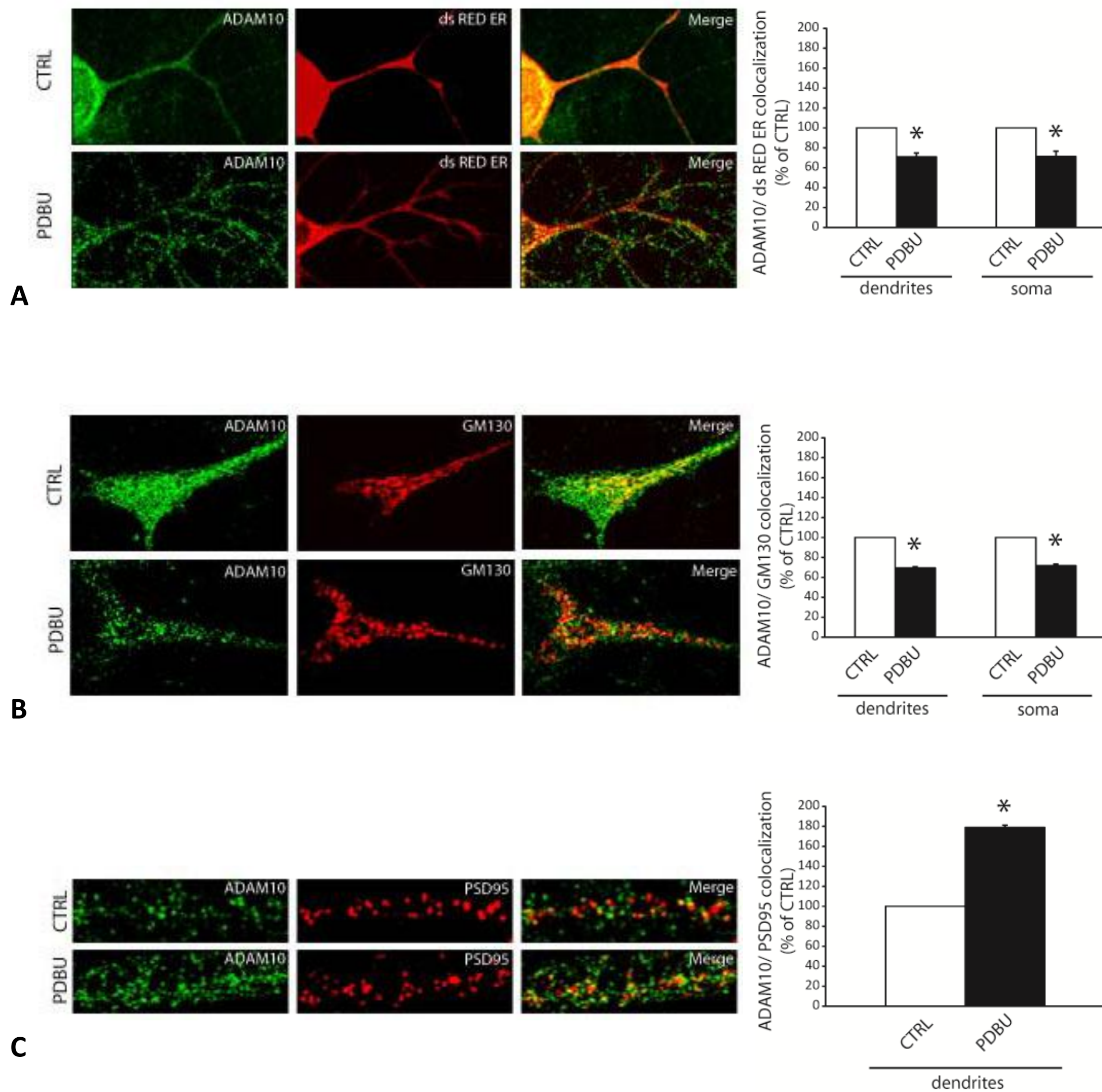


Figure 9. Representative immunostaining of ADAM10 localization in dendrites and soma of primary hippocampal neurons at DIV 14 after 30 min PDBu treatment (100 nM). (A-B). *30 min PDBu treatment induces a reduction of ADAM10/ds RED ER and ADAM10/GM130 colocalization both in dendrites and in soma (ADAM10/ds RED ER: dendrites -29 ± 3.7% *p= 0.001, soma -29 ± 5.4% *p=0.009 PDBu vs CTRL; ADAM10/GM130: dendrites -30.6 ± 1.2% *p=0.001, soma -28.4 ± 1.7% *p=0.0006 PDBu vs CTRL) and* ***(C)*** *leads to an increase of ADAM10/PSD95 colocalization (+78.77 ± 2,52% *p<0.005 PDBu vs CTRL). *= p<0.05 PDBu vs CTRL. Data are expressed as percentage of CTRL.*

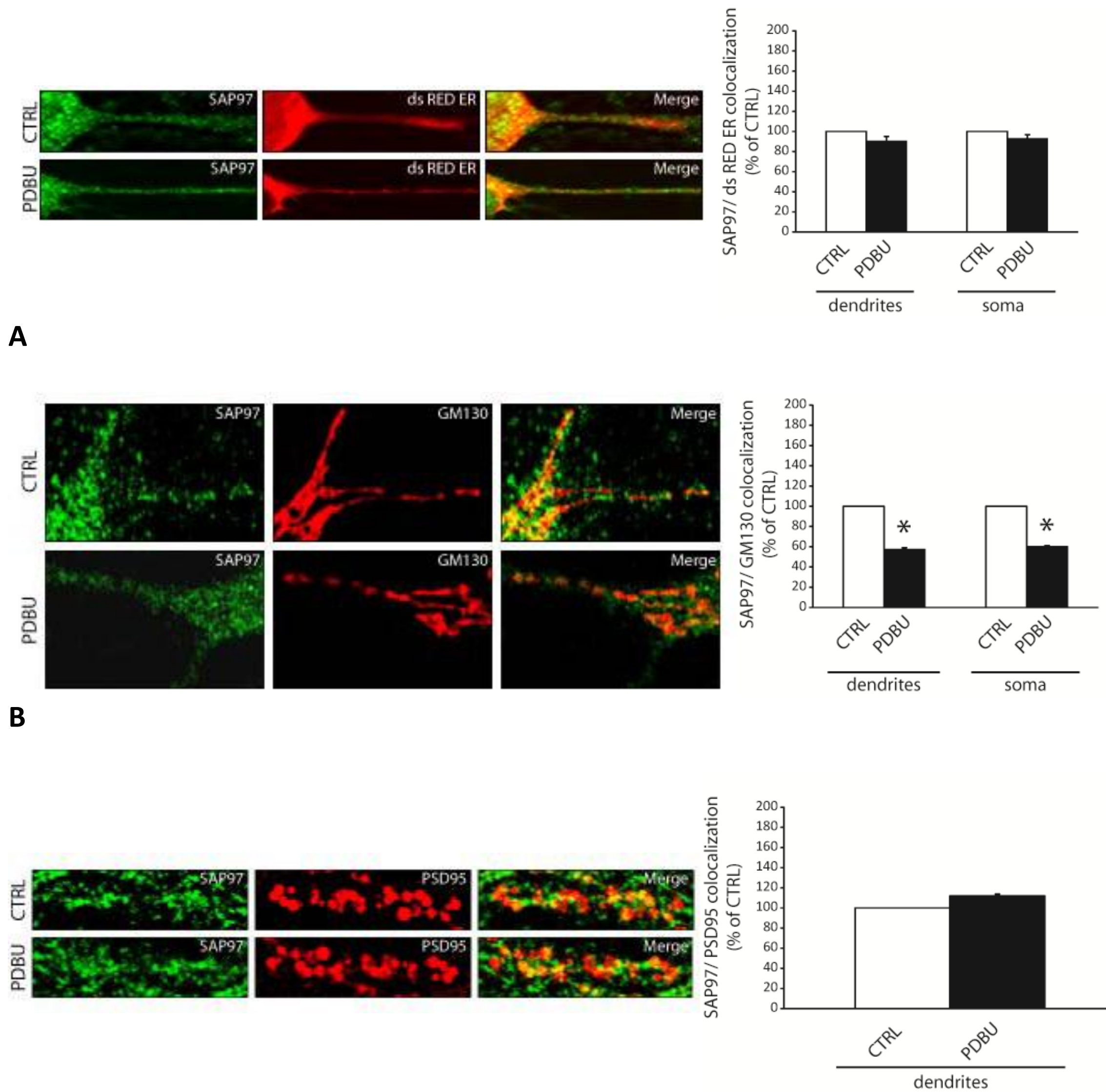


Figure 10. Representative immunostaining of SAP97 localization in dendrites and soma of primary hippocampal neurons at DIV 14 after 30 min PDBu treatment (100 nM). (B). *30 min PDBu treatment induces a reduction of SAP97 levels in the Golgi apparatus both in dendrites and in soma (SAP97/GM130 colocalization: dendrites -42.78 ± 1.8% *p=0.0001, soma -39.94 ± 1.1 *p=0.0006 PDBu vs CTRL) but* ***(A-C)*** *doesn't affect SAP97 localization in the ER (SAP97/ds RED ER dendrites -9.7 ± 4.7%, soma -6.8 ± 3.6% p>0.05 PDBu vs CTRL) and in the postynaptic fraction (SAP97/PSD95 +12 ± 1.8% p>0.05 PDBu vs CTRL). *= p<0.05 PDBu vs CTRL. Data are expressed as percentage of CTRL.*

2.2. PKC-induced ADAM10 trafficking is mediated by SAP97 and involves dendritic Golgi outposts

The interaction between SAP97 and ADAM10 involves SAP97 SH3 domain which binds ADAM10 C-terminal proline-rich domain (Marcello et al., 2007). This complex can be uncoupled by means of a cell-permeable peptide, Tat-Pro (Marcello et al., 2007). In this peptide the Tat domain of HIV is fused to the C-terminal proline-rich domain of ADAM10 (Tat-Pro ADAM10$^{709\text{-}729}$; **Fig. 11A**). Thus, Tat-Pro peptide competes with endogenous ADAM10 for the binding to the SH3 domain of SAP97 and disrupts endogenous ADAM10/SAP97 complex (Marcello et al., 2007).

As control, an analogous inactive peptide unable to bind SH3 of SAP97 because all proline residues were substituted by alanines (Tat-Ala ADAM10$^{709\text{-}729}$). Peptide capability of interfering with the association between SAP97 and ADAM10 was investigated by co-immunoprecipitation experiments in homogenates of the rat hippocampal slices. Tat-Pro treated slices showed a reduction of the co-immunoprecipitation of the two proteins, if compared to Tat-Ala treated slices, suggesting the capability of the Tat-Pro peptide to disrupt the endogenous complex.

We took advantage of this tool to determine in which subcellular compartment ADAM10/SAP97 complex formation takes place.

To dissect in minute details the involvement of SAP97 in ADAM10 trafficking we treated hippocampal neurons with Tat-Pro or Tat-Ala (1 μM, 30 minutes). After treatment, cells were fixed and immunolabelled to analyze the colocalization of ADAM10 or SAP97 with the markers of the subcellular compartments previously used. Treatment with Tat-Pro peptide induced a significant increase of ADAM10/GM130 colocalization in dendrites but not in the soma (dendrites: +52.9 ± 3.18%, $p=0.0006$ PRO vs ALA; soma: -0.6 ± 2.6% $p>0.05$ PRO vs ALA; **Fig. 11C**) and a concomitant significant reduction of ADAM10/PSD-95 colocalization (-28.4 ± 2.37%, $p=0.04$ PRO vs ALA; **Fig. 11D**) when compared to Tat-Ala peptide treatment. ADAM10/dsRED-ER colocalization was not affected in dendrites and soma (dendrites:-13.2 ± 5.6%; soma: -2 ± 6%, PRO vs ALA; **Fig. 11B**). Tat-Pro peptide did not modify SAP97 localization in ER, Golgi apparatus and postsynaptic density (data not shown).

These results suggest that ADAM10/SAP97 complex formation takes place in dendritic Golgi outposts, thus uncovering SAP97 role in post-Golgi trafficking of ADAM10.

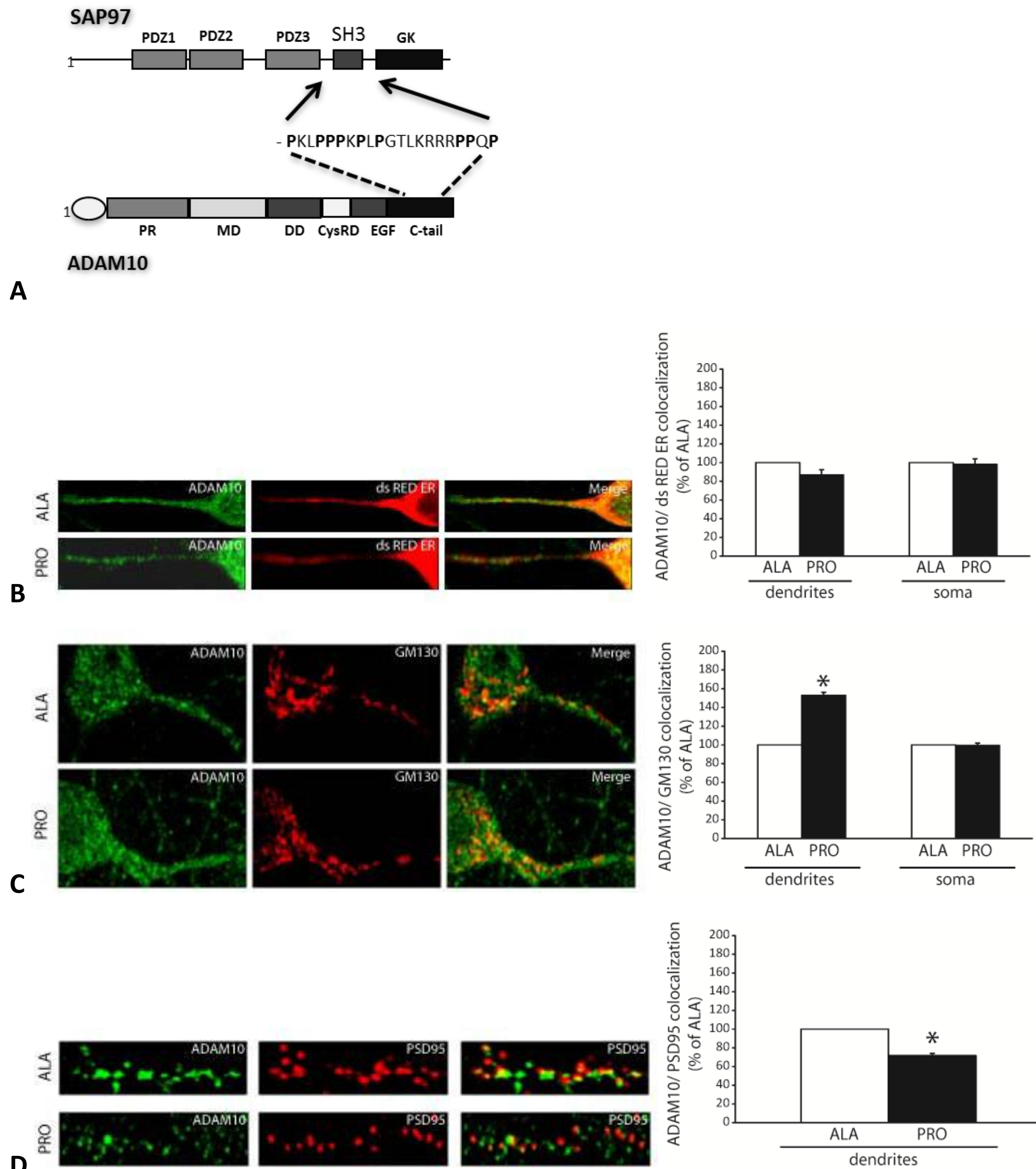


Figure 11. (A). *Schematic representation of SAP97, ADAM10 and of the amino acid sequence of cell-permeable Tat-Pro peptide corresponding to ADAM10 proline-rich domain.* ***(B-C-D).*** *Representative immunostaining of ADAM10 localization in dendrites and soma of primary hippocampal neurons at DIV 14 after 30 min Tat-Ala and Tat-Pro ADAM10 peptide treatment.* ***(B).*** *Tat-Pro peptide doesn't affect ADAM10 levels in the ER both in dendrites (ADAM10/ds RED ER colocalization -13.2 ± 5.6% PRO vs ALA) and in soma (ADAM10/ds RED ER colocalization -2 ± 6% PRO vs ALA).* ***(C).*** *Tat-Pro peptide increases ADAM10 levels in the dendritic Golgi apparatus (ADAM10/GM130 +52.9 ± 3.18% *p=0.0006 PRO vs ALA) but not in the somatic Golgi apparatus (ADAM10/GM130 colocalization -0.6 ± 2.6% PRO vs ALA).* ***(D).*** *Tat-Pro induces a reduction of ADAM10 levels at the postsynaptic density (ADAM10/PSD95 colocalization -28.4 ± 2.37% * p=0.04 PRO vs ALA). Data are expressed as percentage of ALA.*

In light of these results we wondered whether SAP97 could be involved in PKC induced ADAM10 trafficking.

To this, rat acute hippocampal slices were pre-treated 2 minutes with Tat-Pro or Tat-Ala peptide (1µM), to uncouple endogenous ADAM10/SAP97 complex, and then stimulated with PDBu 30 minutes (100 nM). After treatment the hippocampal slices were processed and the localization of ADAM10 and SAP97 was analyzed.

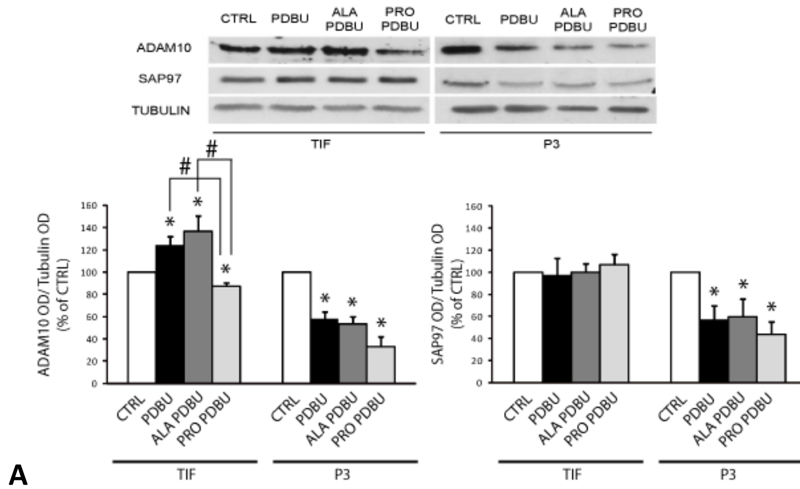
Tat-Pro peptide treatment prevented the increase of ADAM10 levels in TIF induced by PKC activation (PRO PDBU= -12.73 ± 2.76%; ALA PDBU= +36.43 ± 13.54%; PDBU= +24.05 ± 8.2%, overall vs CTRL $p<0{,}05$, **Fig.12A**) but not the reduction of ADAM10 in P3 fraction (PRO PDBU= -67.2 ± 8.33%; ALA PDBU= -46.36 ± 6.24%; PDBU= -41.94 ± 6.09%, overall vs CTRL $p<0{,}05$, **Fig.12A**). Tat-Pro peptide doesn't affect SAP97 levels in both TIF (PRO PDBU= +6.85 ± 8.84%; ALA PDBU= +0.11 ± 8.10%; PDBU= -2.61 ± 15.44%, **Fig.12A**) and P3 fraction (PRO PDBU= -56.34 ± 10.87%; ALA PDBU= -40.22 ± 15.95%; PDBU= -42.45 ± 12.46%, **Fig.12A**).

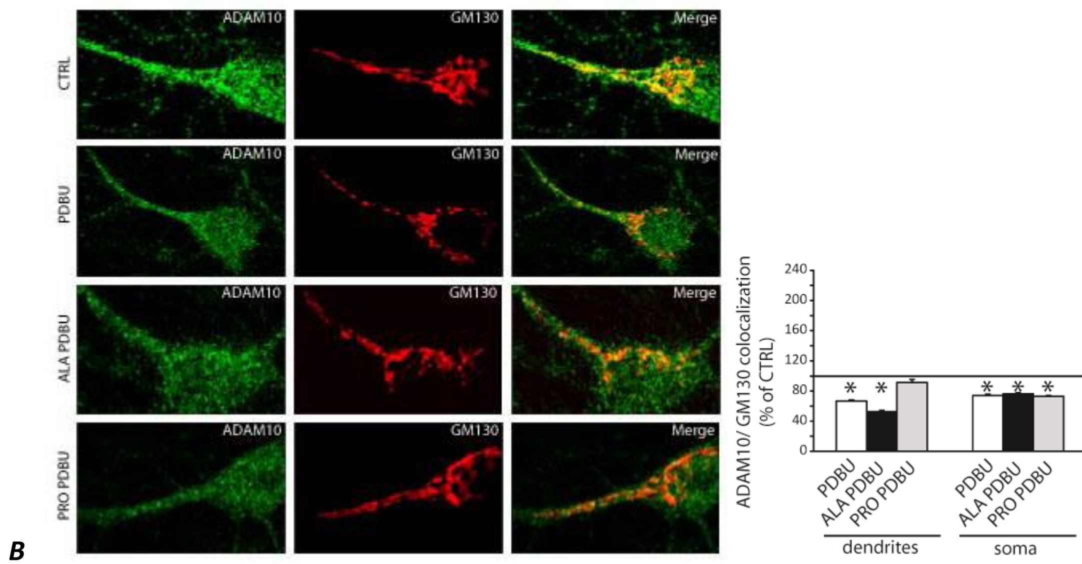
In order to confirm these results neuronal cultures were pre-treated with Tat-Pro or Tat-Ala peptide (1 µM, 30 minutes) and then stimulated with PDBu 30 minutes (100 nM). After treatments, cells were fixed and immunolabelled to analyze the colocalization of ADAM10 or SAP97 and the subcellular compartments.

The pre-treatment with Tat-Pro peptide blocked PKC induced ADAM10 trafficking from the dendritic Golgi, (dendrites: ADAM10-GM130, PRO PDBU -8.56 ± 3.92%, $p=0.5$; ALA PDBU -47.44 ± 1.8% and PDBU -33.3 ± 1.43 vs CTRL $p<0.05$, **Fig.12B**) to the post synaptic compartment (ADAM10/PSD-95, PRO PDBU -39.65 ± 0.75%, ALA PDBU +98.01 ± 2.22%, PDBU +86.72 ± 1.78%; overall vs CTRL $p<0.05$, **Fig.12C**).

On the other hand, Tat-Pro peptide treatment did not affect PKC-induced ADAM10 trafficking from somatic Golgi apparatus (PRO PDBU -26.9 ± 1.18%, ALA PDBU -23.77 ± 1.5%, PDBU -25.74 ± 1.67%; overall vs CTRL $p<0.05$, **Fig.12B**) and from ER (data not shown).

These results demonstrate that SAP97 is responsible for basal and PKC-induced ADAM10 trafficking from dendritic Golgi outposts.


CTRL
PDBU
ALA PDBU
PRO PDBU
ADAM10
SAP97
TUBULIN
TIF
P3
ADAM10 OD/ Tubulin OD (% of CTRL)
SAP97 OD/ Tubulin OD (% of CTRL)
A


ADAM10
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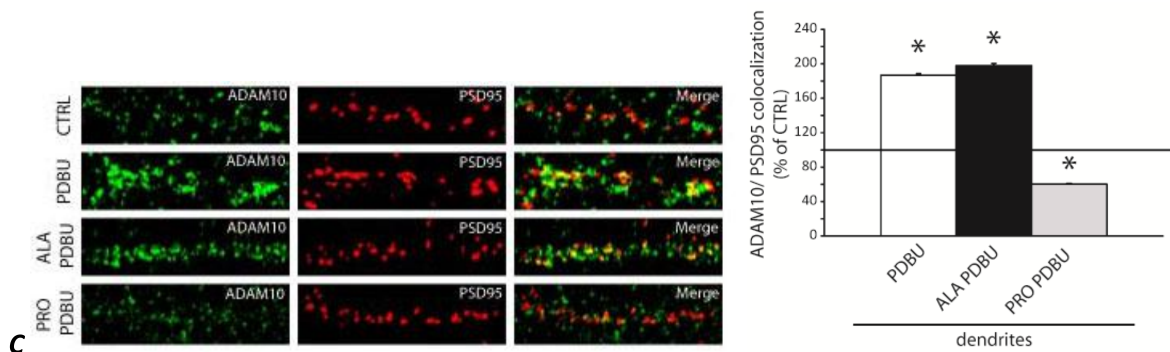
ADAM10
PSD95
Merge
CTRL
PDBU
ALA PDBU
PRO PDBU
ADAM10/ PSD95 colocalization (% of CTRL)
dendrites
C

Figure 12. (A). *Representative WB of ADAM10 and SAP97 localization in TIF and P3 of hippocampal slices pre-treated 2 min with both Tat-Ala and Tat-Pro peptide (1µM) and exposed to PDBU (100 nM) for 30 min. The treatment with Tat-Pro ADAM10 peptide prevents ADAM10 trafficking to TIF fraction (PRO PDBU= -12.73 ± 2.76%; ALA PDBU= +36.43 ± 13.54%; PDBU= +24.05 ± 8.2%) but not the trafficking from the P3 fraction (PRO PDBU= -67.2 ± 8.33%; ALA PDBU= -46.36 ± 6.24%; PDBU= -41.94 ± 6.09%). Tat-Pro peptide doesn't affect SAP97 levels in both TIF (PRO PDBU= +6.85 ± 8.84%; ALA PDBU= +0.11 ± 8.10%; PDBU= -2.61 ± 15.44%) and P3 fraction (PRO PDBU= -56.34 ± 10.87%; ALA PDBU= -40.22 ± 15.95%; PDBU= -42.45 ± 12.46%). * = p < 0,05 PDBU, ALA PDBU, PRO PDBU vs CTRL; # = p < 0,05 ALA PDBU, PRO PDBU vs PDBU. Optical Density (OD) of ADAM10 and SAP97 is normalized on Tubulin OD. Data are expressed as percentage of CTRL.*

(B-C). *Representative immunostaining of ADAM10 localization in dendrites and soma of primary hippocampal neurons at DIV 14 pre-treated 30 min with both Tat-Ala and Tat-Pro ADAM10 peptide (1 µm) and exposed 30 min to PDBU (100 nM).* ***(B).*** *The pre-treatment with Tat-Pro peptide prevents PDBU induced ADAM10 trafficking from dendritic Golgi outposts (PRO PDBU -8.56 ± 3.92% p=0.5; ALA PDBU -47.44 ± 1.8%, PDBU -33.3 ± 1.43; *p<0.05) but not from somatic Golgi apparatus (PRO PDBU -26.9 ± 1.18%, ALA PDBU -23.77 ± 1.5%, PDBU -25.74 ± 1.67%; *p<0.05) *=p<0.05 PDBU, ALA PDBU, PRO PDBU vs CTRL. Data are expressed as percentage of CTRL.* ***(C).*** *Tat-PRO treatment prevents PKC-induced ADAM10 trafficking towards the postsynaptic density (PRO PDBU -39.65 ± 0.75%, ALA PDBU +98.01 ± 2.22%, PDBU +86.72 ± 1.78%; *p<0.05).*

2.3. ADAM10 and SAP97 are phosphorylated by PKC

PKC is involved in controlling the function of other proteins through the phosphorylation of hydroxyl groups of serine and threonine amino acid residues on these proteins. Since PKC activation affects both SAP97 and ADAM10 localization, we first investigated which protein is a PKC substrate.

To this we performed an "*in vitro*" phosphorylation assay using different GST fusion proteins. To study SAP97 phosphorylation, we used GST-SAP97 full length, the mutant GST-SAP97-GKΔ lacking the GK domain and GST fusion proteins with different domain of SAP97 (N-terminal, PDZ1, PDZ3, SH3). To analyze ADAM10 we prepared GST fusion protein with the C-terminal domain of ADAM10 and a series of deletion mutants of GST-ADAM10 C-term (GST-734Δ and GST-721Δ lacking the last 15 aa and 28 aa of ADAM10 tail respectively).

The GST-NR2A C-terminal domain (1349-1464) fusion protein was used as PKC phosphorylation positive control (Gardoni et al., 2001). GST fusion proteins were incubated or not with purified PKC in presence of [γ-^{32}P]ATP as a phosphate donor. ADAM10 cytoplasmic tail, but not the deletion mutants GST-734Δ and GST-721Δ, is phosphorylated indicating that the phopshosite is in the last 15 amino acid of the tail (**Fig. 13A**). GST-SAP97 full length is phosphorylated as well as GST-SAP97-GKΔ suggesting that the GK domain of SAP97 is not relevant for PKC phosphorylation

(**Fig. 13C**). **Fig. 13B** shows a representative autoradiograph of "*in vitro"* PKC-dependent phosphorylation where a radioactive band corresponding to SAP97 SH3 is clearly visible confirming the presence of the PKC phosphosites into this domain. There are no phosphosites in the PDZ1, PDZ3 and N-terminal domain of SAP97 as shown in the autoradiograph.

We confirm the PKC phosphorylation of the GST-NR2A C-terminal domain (1349-1464) (**Fig. 13B**) (Gardoni et al., 2001). The phosphorylation signal shown is specific, since GST alone did not show any phospho-band (**Fig. 13A**, leftmost lane). No signal is detected when GST fusion proteins are incubated without PKC, (data not shown).

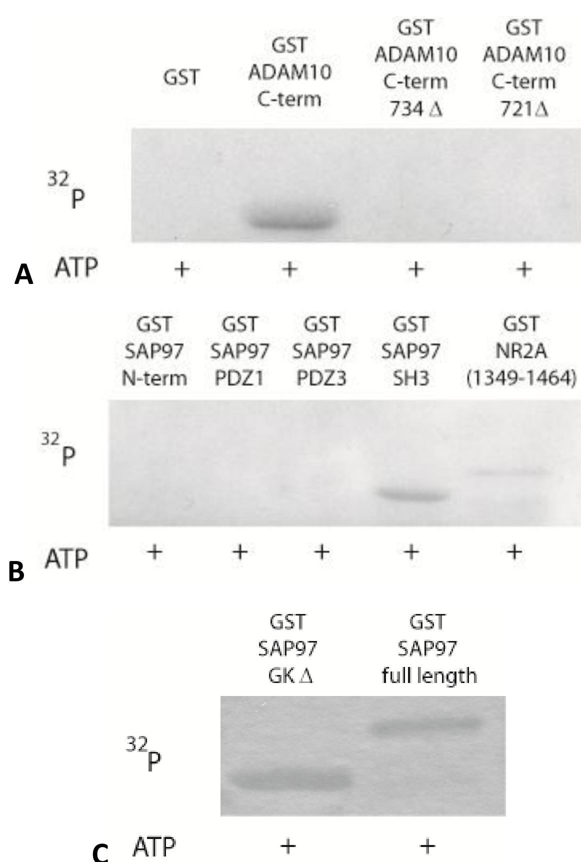

***Figure 13. In vitro phosphorylation of GST-ADAM10, GST-SAP97 and GST NR2A fusion proteins**. GST, GST-ADAM10 C terminal, GST-ADAM10 C-term 734Δ and 721, GST-SAP97 N terminal, GST-SAP97 PDZ1, GST-SAP97 PDZ3, GST-SAP97 SH3, GST-SAP97 full length, GST-SAP97-GKΔ and GST-NR2A (1349-1464) purified fusion proteins were incubated with 50 units of purified protein kinase (PKC) for 30 min at 37°C, in presence of 10 mM Tris-HCl pH 7.4, 10 mM $MgCl_2$, 0.5 mM $CaCl_2$, and 10 µM ATP ([γ-^{32} P] ATP as a phosphate donor (2 µCi/tube). Proteins were separated by SDS PAGE and phosphoproteins revealed by autoradiograph. GST-NR2A was used as positive control of PKC phosphorylation (Gardoni et al., 2001). The phosphorylation signal shown is specific, since GST alone did not show any phospho-band (leftmost lane).*

Since we found that both domains responsible for ADAM10/SAP97 association, i.e. SH3 domain of SAP97 and C-terminal tail of ADAM10, are phosphorylated by PKC we analysed their aminoacidic sequence searching for PKC consensus motifs (**Fig. 14A,** (Kennelly and Krebs, 1991)). As we shown in **Fig. 14B** we found four possible phosphosites in SH3 domain of SAP97, three serine and one threonine (S-582, 597, 642, T-629) and only one serine (S-741) in the last 15 aa of ADAM10 C-terminal domain.

We performed an "*in vitro*" phosphorylation assay with the mutant GST ADAM10 C-term S741-A. As shown in **Fig. 14C,** the mutation of serine 741 to alanine abolishes PKC phosphorylation, thus confirming that serine 741 is a PKC phosphosite.

Summary of consensus sequences most frequently recognized by protein kinases

This table represents a simplified version of the information in the text to allow rapid comparisons to be made. The reader is advised to refer to the text to gain more detailed information on the origin and predictive reliability of the sequences presented.

Protein kinase	Consensus sequence
cAMP-PK	R-R/K-X-S*/T* > R-X_2-S*/T* = R-X-S*/T*
cGMP-PK	$(R/K)_{2\text{-}3}$-X-S*/T*
PKC	(R/$K_{1\text{-}3}$, $X_{2\text{-}0}$)-S*/T*-($X_{2\text{-}0}$, R/$K_{1\text{-}3}$) > S*/T*-($X_{2\text{-}0}$, R/$K_{1\text{-}3}$) ≥ (R/$K_{1\text{-}3}$, $X_{2\text{-}0}$)-S*/T*
AMP-PK	ND[a]
CaM kinase II	R-X-X-S*/T*
$p34^{cdc2}$	S*/T*-P-X-R/K[b]
Phosphorylase kinase	ND
smMLCK	(K/R_2, X)-$X_{1\text{-}2}$-K/R_3-$X_{2\text{-}3}$-R-X_2-S*-N-V-F
skMLCK	(K/R_2, X)-$X_{1\text{-}2}$-K/R_3-$X_{2\text{-}3}$-R-X_2-S*-N-V-F > (K/R_2, X)-$X_{1\text{-}2}$-K/R_3-$X_{2\text{-}3}$-E-X_2-S*-N-V-F
MHCK I	R/$K_{1\text{-}2}$-$X_{-1\text{-}2}$-S*/T*-X-Y[c]
MHCK II	R-G-X-S*-X-R
GSK-3	S*-X_3-S(P)
CK I	S(P)-$X_{1\text{-}3}$-S*/T* ≫ (D/$E_{2\text{-}4}$, $X_{2\text{-}0}$)-X-S*/T*
CK II	S*/T*-(D/E/S$(P)_{1\text{-}3}$, $X_{2\text{-}0}$)

[a] ND, unable to predict consensus sequence due to lack of sufficient information and/or influence of other factors in influencing substrate specificity.
[b] Where X is polar.
[c] Where X +1 or −1 is a hydroxyl amino acid.

A

695- GFIKICSVHTPSNPKLPPPKPLPGTLKRRRPPQPIQQPPRQRPRE**S**YQMGHMRR- 749

ADAM10 C-TERM

580- KR**S**LYVRALFDYDKIKD**S**GLP**S**QGLNFKFGDILHVINA**S**DDEWWQARQV**T**PDGE**S**DEVGVIP**S**KRRVEKKE- 650

SAP97 SH3

B

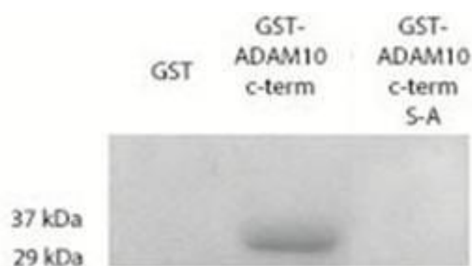

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Figure 14. (A). *Summary of consensus sequences most frequently recognized by protein kinases. In red PKC consensus sequence (Kennelly and Krebs, 1991).* ***(B).*** *ADAM10 C-terminal domain sequence and SAP97 SH3 domain sequence. In red the possible identified PKC phosphosites.* ***(C).*** *In vitro phosphorylation of GST-ADAM10 C-terminal domain and GST-ADAM10 C-terminal S-A fusion proteins. The fusion proteins were incubated with 50 units of purified protein kinase (PKC) for 30 min at 37°C, in presence of 10 mM Tris-HCl pH 7.4, 10 mM $MgCl_2$, 0.5 mM $CaCl_2$, and 10 µM ATP ([γ-^{32} P] ATP as a phosphate donor (2 µCi/tube). Proteins were separated by SDS PAGE and phosphoproteins revealed by autoradiograph. GST-NR2A was used as positive control of PKC phosphorylation (Gardoni et al., 2001). The phosphorylation signal shown is specific, since GST alone did not show any phospho-band (leftmost lane).*

2.4. SAP97 phosphorylation can modulate ADAM10/SAP97 interaction

In light of the above, we wondered whether PKC phosphorylation of SAP97 SH3 domain and ADAM10 C-terminal tail could modulate their interaction.

We performed pull-down assay with rat brain homogenate and GST SAP97 SH3, GST SAP97 full length, GST ADAM10 C-terminal and GST ADAM10 C-terminal S741-A fusion proteins cold-phosphorylated by PKC in the presence or absence of ATP. As shown in **Fig. 15A**, the phosphorylation of both SAP97 SH3 domain and SAP97 full length is able to significantly increase the interaction with ADAM10 (SH3, +32.88 ± 3.34%, p=0.01; SAP97 full length, +95 ± 4.86, p=0.002; phosphorylated *vs* non phosphorylated). On the other hand, PKC phosphorylation of either ADAM10 or the mutant S741-A does not affect the binding to SAP97 (GST-ADAM10 Cterm, +4.73 ± 5.35%; GST-ADAM10 Cterm S741-A, -21.73 ± 16.20%, p>0.05, phosphorylated *vs* non phosphorylated; **Fig. 15B**).

These results suggest that SAP97 phosphorylation modulates ADAM10/SAP97 interaction, while ADAM10 tail phosphorylation has another biological role. Therefore it would be relevant to characterize in details the phosphorylation of SAP97 SH3 domain by PKC.

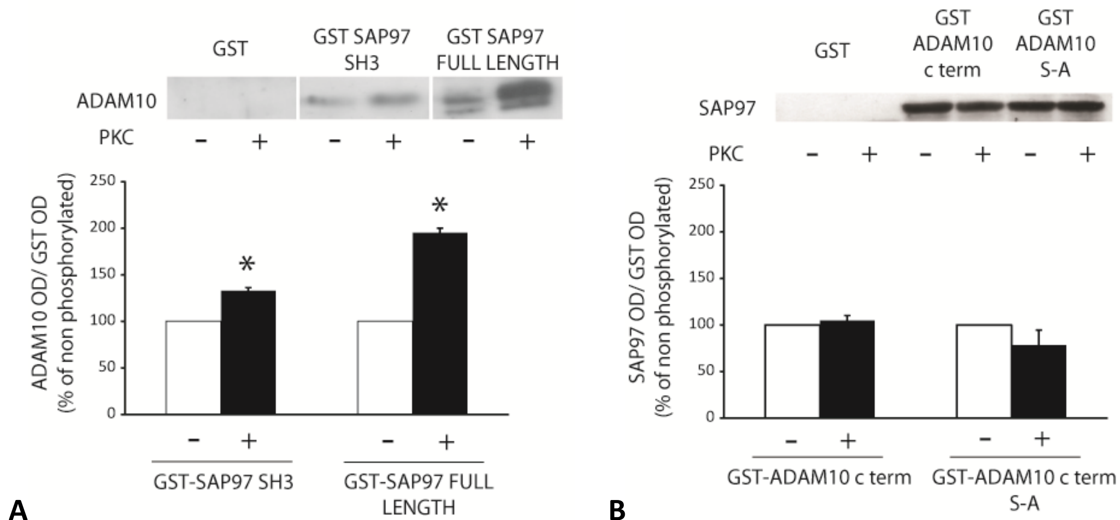

Figure 15. (A). *GST SAP97 SH3 and GST SAP97 full length were cold-phosphorylated in a PKC-dependent manner in the presence or absence of ATP and then incubated in a pull-down assay with rat brain homogenate. Following extensive washes, the bound proteins were eluted from the beads with SDS sample buffer, separated by SDS-PAGE. WB analysis was performed using anti-ADAM10 polyclonal antibody and GST monoclonal antibody to normalize. ADAM10 pulled down by GST-SAP97 SH3 fusion protein (+32.88 ± 3.34%, *p=0.01) and SAP97 full length fusion protein (+95 ± 4.86%, *p=0.002) is increased after PKC phosphorylation compared with non phosphorylated. All data were normalized on GST staining. For all experiments quantitative analysis of immunostaining is shown as percentage of non phosphorylated condition in the same experiment.* ***(B).*** *GST ADAM10 C-terminal and GST ADAM10 C-terminal S-A were cold-phosphorylated in a PKC-dependent manner in the presence or absence of ATP and then incubated in a pull-down assay with rat brain homogenate, WB analyses was performed using anti-SAP97 monoclonal antibody and GST monoclonal antibody to normalize. No alterations are detectable in SAP97 binding after phosphorylation of both ADAM10 C-terminal domain (+4.73 ± 5.35%, p>0.05) and ADAM10 C-terminal S-A (-21.73 ± 16.20%, p>0.05) compared with non phosphorylated. All data were normalized on GST staining. For all experiments quantitative analysis of immunostaining is shown as percentage of non phosphorylated in the same experiment.*

DISCUSSION

Although the aetiology of AD remains unclear, the leading hypothesis is that accumulation of Aβ peptide is the causative event of the pathogenesis (Hardy and Selkoe, 2002). Nevertheless, studies in patients failed to demonstrate a clear correlation between Aβ plaques and cognitive impairment. On a different ground, a higher correlation has been shown between the loss of synapses and decreased cognitive function (Terry et al., 1991). Lately, this led to the emerging concept that AD pathogenesis has to be considered the result of a complex interplay of crossing pathways involving amyloid cascade and synaptic dysfunction (Bossy-Wetzel et al., 2004). The complete comprehension of the mechanism(s) through which these two main components of AD pathogenesis reciprocally interact and influence each other turned out as more and more important to reach a complete picture of the disease's molecular pathogenesis. In the last few years, several studies aimed at understanding precisely how Aβ accumulation and assembly compromise synaptic structure and function of excitatory synapses, taking advantage of synthetic or cell-derived Aβ aggregates. This has become the centrepiece of therapeutically oriented research on the disease (Selkoe, 2008). In this study, we changed perspective and tried to address the question by studying possible intraneuronal pathways bridging synaptic function and amyloid production. We focused on SAP97, a cargo protein responsible not only for the trafficking of ionotropic glutamate receptor subunits, i.e., GluR1 (Leonard et al., 1998) and NR2A (Bassand et al., 1999), but also for the synaptic localization of α-secretase ADAM10 (Marcello et al., 2007). Therefore, SAP97 is involved in both functional organization of the glutamatergic synapse (Gardoni et al., 2009; Garner et al., 2000) and modulation of α-secretase activity (Marcello et al., 2007). Notably a dysfunction of SAP97 has been already reported in AD patients' brain (Wakabayashi et al., 1999b), as well as in other neurological disorders (Nash et al., 2005; Sato et al., 2008). The first aim of our study was to investigate possible alterations of SAP97 complexes with its partners in the earlier phases of AD pathogenesis, when APP metabolism is shifted toward amyloidogenesis but the loss of synaptic structure is not prominent. To this, we exploited autoptic Hp and SFG, respectively as an affected and a less affected area, obtained from late onset AD patients, fulfilling criteria for Braak 4 stage, and age-matched control subjects. We precisely defined our experimental design because results in the literature describing alterations of the glutamatergic synapses of AD patients at different stages of the disease are unfortunately very variable (Gylys et al., 2004; Masliah et al., 2001; Mukaetova-Ladinska et al., 2000). At present our knowledge is a mosaic of several different results obtained in different studies, not always obtained in comparable experimental conditions. In light of these considerations, first of all we characterized APP metabolism and synaptic structural composition.

As confirmed by the measurements of the amyloid cascade products, at this stage of disease APP metabolism is shifted toward amyloidogenesis only in Hp and not in SFG. In our experimental conditions, biochemical analyses of both presynaptic proteins and the major PSD components demonstrated that the presynapse and the postsynaptic structure are still not affected in both areas. Here we showed an alteration of SAP97 capability to interact with its binding partners just in vulnerable brain areas of AD patients at initial stages, thus suggesting SAP97 cargo dysfunction as an early mechanism of pathogenesis. In Hp of AD patients, levels of ADAM10 and GluR1 at synaptic membranes are respectively reduced and augmented, while NR2A localization is not affected. These alterations are specific because no modifications were found in SFG. Important insights may be gleaned from our findings, adding new pieces to the puzzle in understanding how Aβ formation is upregulated in sporadic AD. Our results suggest that at this stage of the disease the reduction of α-secretase activity could be ascribed to a defect in ADAM10 trafficking rather than to an alteration of its expression. Indeed, it turned out that α-secretase activity is reduced only in Hp of AD patients, the brain area where ADAM10 localization at postsynaptic membranes is impaired. Furthermore, membrane protein trafficking has a pivotal role not only in the process leading to Aβ formation, but also in the mechanisms regulating synaptic function. Therefore, the increased localization of GluR1 at the synaptic site in AD Hp could reflect an impairment of dynamic regulation of the number of synaptic receptors, thus probably being involved in the pathogenic processes responsible for synaptic dysfunction. The altered localization of ADAM10 and GluR1 could be ascribed to a failure of SAP97 to properly bind these partners. Both immunoprecipitation and pull-down assays demonstrated that SAP97 failed to correctly couple to ADAM10 and GluR1, but not to NR2A, in AD Hp. The observations that ADAM10/SAP97 complex is markedly reduced and that GluR1/SAP97 interaction is significantly increased in Hp of AD patients indicates alterations of SAP97 as a key element of molecular pathogenesis of the disease. Indeed, SAP97 has been previously described as a determinant for the activity at the synapse of the α-secretase enzyme ADAM10. A disruption of ADAM10/SAP97 complex in rodents led to a reduction of ADAM10 localization at postsynaptic sites and to a shift of APP metabolism toward the amyloidogenic pathway (Marcello et al., 2007). In addition, the altered binding affinity to cytoplasmic tails of ADAM10 and GluR1 in AD Hp is strictly interdependent. This negative correlation suggests that a modification of SAP97 function in AD pathogenesis could lead to a reduced ADAM10 delivery to the postsynaptic membrane, thus shifting APP metabolism toward amyloidogenesis, and to an increased localization of GluR1 which impairs synaptic function. So the first part of this study sheds light on an open question in

AD research concerning the mutual interaction between different pathways of pathogenesis, namely synaptic failure and amyloid cascade. In particular, we depict a new mechanism of pathogenesis which goes beyond both amyloid production/release in the extracellular fluids and deleterious effects of Aβ on neighbouring cells, but rather points to a dysfunction of a key intracellular process: local protein trafficking and synapse assembly. This new vision may help not only in indicating SAP97 as a point of convergence between amyloid cascade and synaptic function, but also in a different interpretation of AD which in this new context can be perceived as synaptic trafficking defect pathology.

In light of the above, we focused our attention on the intracellular pathways that control ADAM10 trafficking in order to identify new tools to balance ADAM10 trafficking defects in AD. The modulation of the intracellular trafficking of ADAM10 could constitute an innovative therapeutic strategy to finely tune its shedding activity towards its substrates, i.e. APP.

Among the different intracellular pathways regulating ADAM10 activity, we drew our attention on PKC because it is well known that the activation of PKC leads to a relative increase in utilization of the non-amyloidogenic α-secretase cleavage pathway for APP processing at the expense of other pathways (Gandy and Greengard, 1994), and treatment with a PKC activator increased ADAM10 substrates cleavage (Kohutek et al., 2009).

Therefore we wondered whether SAP97 could be involved in PKC-induced ADAM10 activity.

We analysed ADAM10 and SAP97 localization after a treatment with the phorbol ester PDBu in the rat acute hippocampal slices. In this model we demonstrated that PKC activation induces ADAM10 trafficking to the post synaptic density and affects SAP97 intracellular localization.

Since we found a reduction of both ADAM10 and SAP97 levels in a P3 fraction that is enriched of the proteins of ER and Golgi we decided to interfere with ER–Golgi trafficking to investigate which intracellular compartment is involved in PKC-induced trafficking. We used Brefeldin-A, that inhibits transport of proteins from ER to Golgi apparatus, and we found that PKC activation fosters ADAM10 transport from the ER, while PKC-induced SAP97 trafficking involves Golgi apparatus but not ER compartment. These data were confirmed by imaging analyses in hippocampal neuronal primary cultures. Moreover, these analyses revealed that ADAM10 and SAP97 were trafficked through both somatic Golgi apparatus and dendritic Golgi outposts.

The interaction between SAP97 and ADAM10 involves SAP97 SH3 domain which binds ADAM10 proline-rich domains (Marcello et al., 2007) and ADAM10/SAP97 complex levels can be reduced by means of a cell-permeable peptide, Tat-Pro (Marcello et al., 2007) interfering with the endogenous complex ADAM10/SAP97. In fact the peptide couples a Tat moiety to the proline

rich structure of ADAM10. We took advantage of this tool to determine in which subcellular compartment ADAM10/SAP97 complex formation takes place. Tat-Pro peptide treatment in hippocampal neurons suggests that ADAM10/SAP97 complex formation takes place in dendritic Golgi outpost, thus uncovering SAP97 role in post-Golgi trafficking of ADAM10. Moreover, with PKC activation after Tat-Pro peptide treatment we demonstrated that SAP97 is responsible for basal and PKC-induced ADAM10 trafficking from dendritic Golgi outposts.

PKC is involved in controlling the function of other proteins through the phosphorylation of hydroxyl groups of serine and threonine amino acid residues on these proteins. Since PKC activation affects both SAP97 and ADAM10 localization, we first investigated which protein is a PKC substrate. To this we performed an "*in vitro*" phosphorylation assay. It turned out that both domains responsible for ADAM10/SAP97 association, i.e. SH3 domain of SAP97 and C-terminal tail of ADAM10, are phosphorylated by PKC.

In light of the above, we wondered whether PKC phosphorylation of SAP97 SH3 domain and ADAM10 C-terminal tail could modulate their interaction. We found that SAP97 phosphorylation modulates ADAM10/SAP97 interaction, while ADAM10 tail phosphorylation probably has another biological role.

In conclusion, we demonstrated the involvement of ADAM10/SAP97 complex in AD pathogenesis, describing a defect of SAP97-mediated ADAM10 trafficking.

Therefore, we decided to search for a molecular mechanism able to rescue this alteration.

We demonstrated that PKC activation fosters ADAM10 trafficking and that SAP97 is responsible for its transport from Golgi outposts to the postsynaptic compartment (**Fig. 16**). Moreover, SAP97 SH3 domain is a PKC substrate and the phosphorylation increases the binding to ADAM10, thus highlighting the role of PKC in modulating ADAM10/SAP97 complex formation.

Therefore, the characterization of this cellular mechanism can be relevant for the development of new therapeutic strategies aimed at balancing ADAM10 trafficking defect in AD.

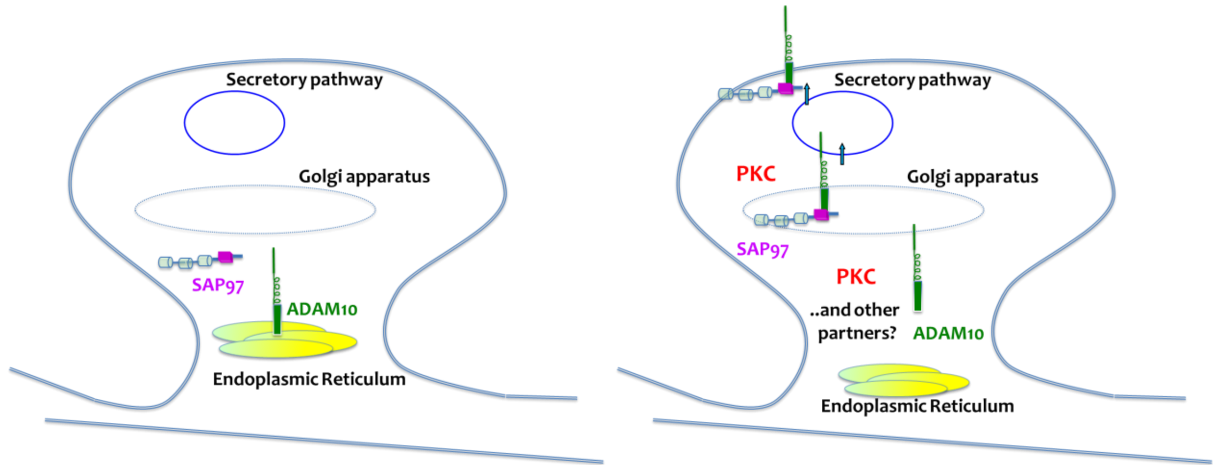

Figure 16. PKC-induced ADAM10 trafficking and SAP97 involvement in the glutamatergic synapse.

BIBLIOGRAPHY

Aarts, M., Liu, Y., Liu, L., Besshoh, S., Arundine, M., Gurd, J.W., Wang, Y.T., Salter, M.W., and Tymianski, M. (2002). Treatment of ischemic brain damage by perturbing NMDA receptor- PSD-95 protein interactions. Science *298*, 846-850.

Acevedo-Duncan, M., Patel, R., Whelan, S., and Bicaku, E. (2002). Human glioma PKC-iota and PKC-betaII phosphorylate cyclin-dependent kinase activating kinase during the cell cycle. Cell Prolif *35*, 23-36.

Acquati, F., Accarino, M., Nucci, C., Fumagalli, P., Jovine, L., Ottolenghi, S., and Taramelli, R. (2000). The gene encoding DRAP (BACE2), a glycosylated transmembrane protein of the aspartic protease family, maps to the down critical region. FEBS Lett *468*, 59-64.

Adams, I., and Jones, D.G. (1982). Quantitative ultrastructural changes in rat cortical synapses during early-, mid- and late-adulthood. Brain Res *239*, 349-363.

Adlard, P.A., Perreau, V.M., Pop, V., and Cotman, C.W. (2005). Voluntary exercise decreases amyloid load in a transgenic model of Alzheimer's disease. J Neurosci *25*, 4217-4221.

Ahn, J.S., Camden, J.M., Schrader, A.M., Redman, R.S., and Turner, J.T. (2000). Reversible regulation of P2Y(2) nucleotide receptor expression in the duct-ligated rat submandibular gland. Am J Physiol Cell Physiol *279*, C286-294.

Alfandari, D., Wolfsberg, T.G., White, J.M., and DeSimone, D.W. (1997). ADAM 13: a novel ADAM expressed in somitic mesoderm and neural crest cells during Xenopus laevis development. Dev Biol *182*, 314-330.

Allinson, T.M., Parkin, E.T., Turner, A.J., and Hooper, N.M. (2003). ADAMs family members as amyloid precursor protein alpha-secretases. J Neurosci Res *74*, 342-352.

Anders, A., Gilbert, S., Garten, W., Postina, R., and Fahrenholz, F. (2001). Regulation of the alpha-secretase ADAM10 by its prodomain and proprotein convertases. FASEB J *15*, 1837-1839.

Andersen, O.M., Reiche, J., Schmidt, V., Gotthardt, M., Spoelgen, R., Behlke, J., von Arnim, C.A., Breiderhoff, T., Jansen, P., Wu, X.*, et al.* (2005). Neuronal sorting protein-related receptor sorLA/LR11 regulates processing of the amyloid precursor protein. Proc Natl Acad Sci U S A *102*, 13461-13466.

Annaert, W., and De Strooper, B. (1999). Presenilins: molecular switches between proteolysis and signal transduction. Trends Neurosci *22*, 439-443.

Apte, S.S. (2004). A disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motifs: the ADAMTS family. Int J Biochem Cell Biol *36*, 981-985.

Aranda, A., and Pascual, A. (2001). Nuclear hormone receptors and gene expression. Physiol Rev *81*, 1269-1304.

Arimura, A. (1998). Perspectives on pituitary adenylate cyclase activating polypeptide (PACAP) in the neuroendocrine, endocrine, and nervous systems. Jpn J Physiol *48*, 301-331.

Arribas, J., Coodly, L., Vollmer, P., Kishimoto, T.K., Rose-John, S., and Massague, J. (1996). Diverse cell surface protein ectodomains are shed by a system sensitive to metalloprotease inhibitors. J Biol Chem *271*, 11376-11382.

Artavanis-Tsakonas, S., Matsuno, K., and Fortini, M.E. (1995). Notch signaling. Science *268*, 225-232.

Baas, P.W., Deitch, J.S., Black, M.M., and Banker, G.A. (1988). Polarity orientation of microtubules in hippocampal neurons: uniformity in the axon and nonuniformity in the dendrite. Proc Natl Acad Sci U S A *85*, 8335-8339.

Baek, S.H., Ohgi, K.A., Rose, D.W., Koo, E.H., Glass, C.K., and Rosenfeld, M.G. (2002). Exchange of N-CoR corepressor and Tip60 coactivator complexes links gene expression by NF-kappaB and beta-amyloid precursor protein. Cell *110*, 55-67.

Bagal, A.A., Kao, J.P., Tang, C.M., and Thompson, S.M. (2005). Long-term potentiation of exogenous glutamate responses at single dendritic spines. Proc Natl Acad Sci U S A *102*, 14434-14439.

Bales, K.R., Verina, T., Dodel, R.C., Du, Y., Altstiel, L., Bender, M., Hyslop, P., Johnstone, E.M., Little, S.P., Cummins, D.J.*, et al.* (1997). Lack of apolipoprotein E dramatically reduces amyloid beta-peptide deposition. Nat Genet *17*, 263-264.

Baron, M. (2006). Readers' responses and authors' reply to "medical marijuana: politics trumps science at the FDA". MedGenMed *8*, 79; author reply 79.

Barria, A., Muller, D., Derkach, V., Griffith, L.C., and Soderling, T.R. (1997). Regulatory phosphorylation of AMPA-type glutamate receptors by CaM-KII during long-term potentiation. Science *276*, 2042-2045.

Basille, M., Gonzalez, B.J., Desrues, L., Demas, M., Fournier, A., and Vaudry, H. (1995). Pituitary adenylate cyclase-activating polypeptide (PACAP) stimulates adenylyl cyclase and phospholipase C activity in rat cerebellar neuroblasts. J Neurochem *65*, 1318-1324.

Bassand, P., Bernard, A., Rafiki, A., Gayet, D., and Khrestchatisky, M. (1999). Differential interaction of the tSXV motifs of the NR1 and NR2A NMDA receptor subunits with PSD-95 and SAP97. Eur J Neurosci *11*, 2031-2043.

Baude, A., Nusser, Z., Roberts, J.D., Mulvihill, E., McIlhinney, R.A., and Somogyi, P. (1993). The metabotropic glutamate receptor (mGluR1 alpha) is concentrated at perisynaptic membrane of neuronal subpopulations as detected by immunogold reaction. Neuron *11*, 771-787.

Bech-Serra, J.J., Santiago-Josefat, B., Esselens, C., Saftig, P., Baselga, J., Arribas, J., and Canals, F. (2006). Proteomic identification of desmoglein 2 and activated leukocyte cell adhesion molecule as substrates of ADAM17 and ADAM10 by difference gel electrophoresis. Mol Cell Biol *26*, 5086-5095.

Beguinot, L., Hanover, J.A., Ito, S., Richert, N.D., Willingham, M.C., and Pastan, I. (1985). Phorbol esters induce transient internalization without degradation of unoccupied epidermal growth factor receptors. Proc Natl Acad Sci U S A *82*, 2774-2778.

Beher, D., Hesse, L., Masters, C.L., and Multhaup, G. (1996). Regulation of amyloid protein precursor (APP) binding to collagen and mapping of the binding sites on APP and collagen type I. J Biol Chem *271*, 1613-1620.

Belandia, B., Latasa, M.J., Villa, A., and Pascual, A. (1998). Thyroid hormone negatively regulates the transcriptional activity of the beta-amyloid precursor protein gene. J Biol Chem *273*, 30366-30371.

Bennett, B.D., Babu-Khan, S., Loeloff, R., Louis, J.C., Curran, E., Citron, M., and Vassar, R. (2000). Expression analysis of BACE2 in brain and peripheral tissues. J Biol Chem *275*, 20647-20651.

Benussi, L., Govoni, S., Gasparini, L., Binetti, G., Trabucchi, M., Bianchetti, A., and Racchi, M. (1998). Specific role for protein kinase C alpha in the constitutive and regulated secretion of amyloid precursor protein in human skin fibroblasts. Neurosci Lett *240*, 97-101.

Bergmann, J.E. (1989). Using temperature-sensitive mutants of VSV to study membrane protein biogenesis. Methods Cell Biol *32*, 85-110.

Bertoni-Freddari, C., Fattoretti, P., Casoli, T., Caselli, U., and Meier-Ruge, W. (1996). Deterioration threshold of synaptic morphology in aging and senile dementia of Alzheimer's type. Anal Quant Cytol Histol *18*, 209-213.

Bertoni-Freddari, C., Fattoretti, P., Casoli, T., Meier-Ruge, W., and Ulrich, J. (1990). Morphological adaptive response of the synaptic junctional zones in the human dentate gyrus during aging and Alzheimer's disease. Brain Res *517*, 69-75.

Bertram, L., Blacker, D., Mullin, K., Keeney, D., Jones, J., Basu, S., Yhu, S., McInnis, M.G., Go, R.C., Vekrellis, K., *et al.* (2000). Evidence for genetic linkage of Alzheimer's disease to chromosome 10q. Science *290*, 2302-2303.

Bigler, D., Takahashi, Y., Chen, M.S., Almeida, E.A., Osbourne, L., and White, J.M. (2000). Sequence-specific interaction between the disintegrin domain of mouse ADAM 2 (fertilin beta) and murine eggs. Role of the alpha(6) integrin subunit. J Biol Chem *275*, 11576-11584.

Bihel, F., Das, C., Bowman, M.J., and Wolfe, M.S. (2004). Discovery of a Subnanomolar helical D-tridecapeptide inhibitor of gamma-secretase. J Med Chem *47*, 3931-3933.

Bird, A.P. (1986). CpG-rich islands and the function of DNA methylation. Nature *321*, 209-213.

Birikh, K.R., Sklan, E.H., Shoham, S., and Soreq, H. (2003). Interaction of "readthrough" acetylcholinesterase with RACK1 and PKCbeta II correlates with intensified fear-induced conflict behavior. Proc Natl Acad Sci U S A *100*, 283-288.

Black, R.A., and White, J.M. (1998). ADAMs: focus on the protease domain. Curr Opin Cell Biol *10*, 654-659.

Blackstone, C., Murphy, T.H., Moss, S.J., Baraban, J.M., and Huganir, R.L. (1994). Cyclic AMP and synaptic activity-dependent phosphorylation of AMPA-preferring glutamate receptors. J Neurosci *14*, 7585-7593.

Blaug, S., Rymer, J., Jalickee, S., and Miller, S.S. (2003). P2 purinoceptors regulate calcium-activated chloride and fluid transport in 31EG4 mammary epithelia. Am J Physiol Cell Physiol *284*, C897-909.

Boarder, M.R., Weisman, G.A., Turner, J.T., and Wilkinson, G.F. (1995). G protein-coupled P2 purinoceptors: from molecular biology to functional responses. Trends Pharmacol Sci *16*, 133-139.

Bodovitz, S., and Klein, W.L. (1996). Cholesterol modulates alpha-secretase cleavage of amyloid precursor protein. J Biol Chem *271*, 4436-4440.

Boeckers, T.M., Kreutz, M.R., Winter, C., Zuschratter, W., Smalla, K.H., Sanmarti-Vila, L., Wex, H., Langnaese, K., Bockmann, J., Garner, C.C*., et al.* (1999). Proline-rich synapse-associated protein-1/cortactin binding protein 1 (ProSAP1/CortBP1) is a PDZ-domain protein highly enriched in the postsynaptic density. J Neurosci *19*, 6506-6518.

Borchelt, D.R., Thinakaran, G., Eckman, C.B., Lee, M.K., Davenport, F., Ratovitsky, T., Prada, C.M., Kim, G., Seekins, S., Yager, D*., et al.* (1996). Familial Alzheimer's disease-linked presenilin 1 variants elevate Abeta1-42/1-40 ratio in vitro and in vivo. Neuron *17*, 1005-1013.

Borroni, B., Colciaghi, F., Pastorino, L., Pettenati, C., Cottini, E., Rozzini, L., Monastero, R., Lenzi, G.L., Cattabeni, F., Di Luca, M*., et al.* (2001). Amyloid precursor protein in platelets of patients with Alzheimer disease: effect of acetylcholinesterase inhibitor treatment. Arch Neurol *58*, 442-446.

Borroto, A., Ruiz-Paz, S., de la Torre, T.V., Borrell-Pages, M., Merlos-Suarez, A., Pandiella, A., Blobel, C.P., Baselga, J., and Arribas, J. (2003). Impaired trafficking and activation of tumor necrosis factor-alpha-converting enzyme in cell mutants defective in protein ectodomain shedding. J Biol Chem *278*, 25933-25939.

Bossy-Wetzel, E., Schwarzenbacher, R., and Lipton, S.A. (2004). Molecular pathways to neurodegeneration. Nat Med *10 Suppl*, S2-9.

Bouillot, C., Prochiantz, A., Rougon, G., and Allinquant, B. (1996). Axonal amyloid precursor protein expressed by neurons in vitro is present in a membrane fraction with caveolae-like properties. J Biol Chem *271*, 7640-7644.

Boulter, J., Hollmann, M., O'Shea-Greenfield, A., Hartley, M., Deneris, E., Maron, C., and Heinemann, S. (1990). Molecular cloning and functional expression of glutamate receptor subunit genes. Science *249*, 1033-1037.

Braak, H., and Braak, E. (1991). Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol *82*, 239-259.

Bradke, F., and Dotti, C.G. (2000). Establishment of neuronal polarity: lessons from cultured hippocampal neurons. Curr Opin Neurobiol *10*, 574-581.

Brady, S.T. (1985). A novel brain ATPase with properties expected for the fast axonal transport motor. Nature *317*, 73-75.

Bredt, D.S., and Nicoll, R.A. (2003). AMPA receptor trafficking at excitatory synapses. Neuron *40*, 361-379.

Brion, J.P., Couck, A.M., Passareiro, E., and Flament-Durand, J. (1985). Neurofibrillary tangles of Alzheimer's disease: an immunohistochemical study. J Submicrosc Cytol *17*, 89-96.

Brou, C., Logeat, F., Gupta, N., Bessia, C., LeBail, O., Doedens, J.R., Cumano, A., Roux, P., Black, R.A., and Israel, A. (2000). A novel proteolytic cleavage involved in Notch signaling: the role of the disintegrin-metalloprotease TACE. Mol Cell *5*, 207-216.

Bryson, H.M., and Benfield, P. (1997). Donepezil. Drugs Aging *10*, 234-239; discussion 240-231.

Bu, G., Cam, J., and Zerbinatti, C. (2006). LRP in amyloid-beta production and metabolism. Ann N Y Acad Sci *1086*, 35-53.

Budnik, V., Koh, Y.H., Guan, B., Hartmann, B., Hough, C., Woods, D., and Gorczyca, M. (1996). Regulation of synapse structure and function by the Drosophila tumor suppressor gene dlg. Neuron *17*, 627-640.

Burack, M.A., Silverman, M.A., and Banker, G. (2000). The role of selective transport in neuronal protein sorting. Neuron *26*, 465-472.

Burnashev, N., Monyer, H., Seeburg, P.H., and Sakmann, B. (1992). Divalent ion permeability of AMPA receptor channels is dominated by the edited form of a single subunit. Neuron *8*, 189-198.

Buxbaum, J.D., Koo, E.H., and Greengard, P. (1993). Protein phosphorylation inhibits production of Alzheimer amyloid beta/A4 peptide. Proc Natl Acad Sci U S A *90*, 9195-9198.

Buxbaum, J.D., Liu, K.N., Luo, Y., Slack, J.L., Stocking, K.L., Peschon, J.J., Johnson, R.S., Castner, B.J., Cerretti, D.P., and Black, R.A. (1998). Evidence that tumor necrosis factor alpha converting enzyme is involved in regulated alpha-secretase cleavage of the Alzheimer amyloid protein precursor. J Biol Chem *273*, 27765-27767.

Buxbaum, J.D., Ruefli, A.A., Parker, C.A., Cypess, A.M., and Greengard, P. (1994). Calcium regulates processing of the Alzheimer amyloid protein precursor in a protein kinase C-independent manner. Proc Natl Acad Sci U S A *91*, 4489-4493.

Cai, H., Wang, Y., McCarthy, D., Wen, H., Borchelt, D.R., Price, D.L., and Wong, P.C. (2001). BACE1 is the major beta-secretase for generation of Abeta peptides by neurons. Nat Neurosci *4*, 233-234.

Cai, X.D., Golde, T.E., and Younkin, S.G. (1993). Release of excess amyloid beta protein from a mutant amyloid beta protein precursor. Science *259*, 514-516.

Camden, J.M., Schrader, A.M., Camden, R.E., Gonzalez, F.A., Erb, L., Seye, C.I., and Weisman, G.A. (2005). P2Y2 nucleotide receptors enhance alpha-secretase-dependent amyloid precursor protein processing. J Biol Chem *280*, 18696-18702.

Cameron, H.A., Kaliszewski, C.K., and Greer, C.A. (1991). Organization of mitochondria in olfactory bulb granule cell dendritic spines. Synapse *8*, 107-118.

Cao, X., and Sudhof, T.C. (2001). A transcriptionally [correction of transcriptively] active complex of APP with Fe65 and histone acetyltransferase Tip60. Science *293*, 115-120.

Capell, A., Meyn, L., Fluhrer, R., Teplow, D.B., Walter, J., and Haass, C. (2002). Apical sorting of beta-secretase limits amyloid beta-peptide production. J Biol Chem *277*, 5637-5643.

Caporaso, G.L., Gandy, S.E., Buxbaum, J.D., Ramabhadran, T.V., and Greengard, P. (1992). Protein phosphorylation regulates secretion of Alzheimer beta/A4 amyloid precursor protein. Proc Natl Acad Sci U S A *89*, 3055-3059.

Cappai, R., Mok, S.S., Galatis, D., Tucker, D.F., Henry, A., Beyreuther, K., Small, D.H., and Masters, C.L. (1999). Recombinant human amyloid precursor-like protein 2 (APLP2) expressed in the yeast Pichia pastoris can stimulate neurite outgrowth. FEBS Lett *442*, 95-98.

Cardozo, M.G., Kawai, T., Iimura, Y., Sugimoto, H., Yamanishi, Y., and Hopfinger, A.J. (1992). Conformational analyses and molecular-shape comparisons of a series of indanone-benzylpiperidine inhibitors of acetylcholinesterase. J Med Chem *35*, 590-601.

Carlin, R.K., Grab, D.J., Cohen, R.S., and Siekevitz, P. (1980). Isolation and characterization of postsynaptic densities from various brain regions: enrichment of different types of postsynaptic densities. J Cell Biol *86*, 831-845.

Carroll, R.C., Lissin, D.V., von Zastrow, M., Nicoll, R.A., and Malenka, R.C. (1999). Rapid redistribution of glutamate receptors contributes to long-term depression in hippocampal cultures. Nat Neurosci *2*, 454-460.

Cauvin, A., Robberecht, P., De Neef, P., Gourlet, P., Vandermeers, A., Vandermeers-Piret, M.C., and Christophe, J. (1991). Properties and distribution of receptors for pituitary adenylate cyclase activating peptide (PACAP) in rat brain and spinal cord. Regul Pept *35*, 161-173.

Cedazo-Minguez, A., Hamker, U., Meske, V., Veh, R.W., Hellweg, R., Jacobi, C., Albert, F., Cowburn, R.F., and Ohm, T.G. (2001). Regulation of apolipoprotein E secretion in rat primary hippocampal astrocyte cultures. Neuroscience *105*, 651-661.

Chantry, A., Gregson, N.A., and Glynn, P. (1989). A novel metalloproteinase associated with brain myelin membranes. Isolation and characterization. J Biol Chem *264*, 21603-21607.

Checler, F. (1995). Processing of the beta-amyloid precursor protein and its regulation in Alzheimer's disease. J Neurochem *65*, 1431-1444.

Chen, X., Vinade, L., Leapman, R.D., Petersen, J.D., Nakagawa, T., Phillips, T.M., Sheng, M., and Reese, T.S. (2005). Mass of the postsynaptic density and enumeration of three key molecules. Proc Natl Acad Sci U S A *102*, 11551-11556.

Chen, Y., Bodles, A.M., McPhie, D.L., Neve, R.L., Mrak, R.E., and Griffin, W.S. (2007). APP-BP1 inhibits Abeta42 levels by interacting with Presenilin-1. Mol Neurodegener *2*, 3.

Chen, Y., Liu, W., McPhie, D.L., Hassinger, L., and Neve, R.L. (2003). APP-BP1 mediates APP-induced apoptosis and DNA synthesis and is increased in Alzheimer's disease brain. J Cell Biol *163*, 27-33.

Cheng, D., Hoogenraad, C.C., Rush, J., Ramm, E., Schlager, M.A., Duong, D.M., Xu, P., Wijayawardana, S.R., Hanfelt, J., Nakagawa, T.*, et al.* (2006). Relative and absolute quantification of postsynaptic density proteome isolated from rat forebrain and cerebellum. Mol Cell Proteomics *5*, 1158-1170.

Chetkovich, D.M., Bunn, R.C., Kuo, S.H., Kawasaki, Y., Kohwi, M., and Bredt, D.S. (2002). Postsynaptic targeting of alternative postsynaptic density-95 isoforms by distinct mechanisms. J Neurosci *22*, 6415-6425.

Chiang, M.Y., Misner, D., Kempermann, G., Schikorski, T., Giguere, V., Sucov, H.M., Gage, F.H., Stevens, C.F., and Evans, R.M. (1998). An essential role for retinoid receptors RARbeta and RXRgamma in long-term potentiation and depression. Neuron *21*, 1353-1361.

Chin, J., Massaro, C.M., Palop, J.J., Thwin, M.T., Yu, G.Q., Bien-Ly, N., Bender, A., and Mucke, L. (2007). Reelin depletion in the entorhinal cortex of human amyloid precursor protein transgenic mice and humans with Alzheimer's disease. J Neurosci *27*, 2727-2733.

Cho, C., Ge, H., Branciforte, D., Primakoff, P., and Myles, D.G. (2000). Analysis of mouse fertilin in wild-type and fertilin beta(-/-) sperm: evidence for C-terminal modification, alpha/beta dimerization, and lack of essential role of fertilin alpha in sperm-egg fusion. Dev Biol *222*, 289-295.

Cho, K.O., Hunt, C.A., and Kennedy, M.B. (1992). The rat brain postsynaptic density fraction contains a homolog of the Drosophila discs-large tumor suppressor protein. Neuron *9*, 929-942.

Chung, H.J., Xia, J., Scannevin, R.H., Zhang, X., and Huganir, R.L. (2000). Phosphorylation of the AMPA receptor subunit GluR2 differentially regulates its interaction with PDZ domain-containing proteins. J Neurosci *20*, 7258-7267.

Chyung, J.H., Raper, D.M., and Selkoe, D.J. (2005). Gamma-secretase exists on the plasma membrane as an intact complex that accepts substrates and effects intramembrane cleavage. J Biol Chem *280*, 4383-4392.

Cisse, M.A., Sunyach, C., Lefranc-Jullien, S., Postina, R., Vincent, B., and Checler, F. (2005). The disintegrin ADAM9 indirectly contributes to the physiological processing of cellular prion by modulating ADAM10 activity. J Biol Chem *280*, 40624-40631.

Citron, M., Oltersdorf, T., Haass, C., McConlogue, L., Hung, A.Y., Seubert, P., Vigo-Pelfrey, C., Lieberburg, I., and Selkoe, D.J. (1992). Mutation of the beta-amyloid precursor protein in familial Alzheimer's disease increases beta-protein production. Nature *360*, 672-674.

Citron, M., Westaway, D., Xia, W., Carlson, G., Diehl, T., Levesque, G., Johnson-Wood, K., Lee, M., Seubert, P., Davis, A.*, et al.* (1997). Mutant presenilins of Alzheimer's disease increase production of 42-residue amyloid beta-protein in both transfected cells and transgenic mice. Nat Med *3*, 67-72.

Colciaghi, F., Borroni, B., Pastorino, L., Marcello, E., Zimmermann, M., Cattabeni, F., Padovani, A., and Di Luca, M. (2002). [alpha]-Secretase ADAM10 as well as [alpha]APPs is reduced in platelets and CSF of Alzheimer disease patients. Mol Med *8*, 67-74.

Collin, R.W., van Strien, D., Leunissen, J.A., and Martens, G.J. (2004). Identification and expression of the first nonmammalian amyloid-beta precursor-like protein APLP2 in the amphibian Xenopus laevis. Eur J Biochem *271*, 1906-1912.

Colonnier, M. (1968). Synaptic patterns on different cell types in the different laminae of the cat visual cortex. An electron microscope study. Brain Res *9*, 268-287.

Connor, M.J., and Sidell, N. (1997). Retinoic acid synthesis in normal and Alzheimer diseased brain and human neural cells. Mol Chem Neuropathol *30*, 239-252.

Cook, D.G., Forman, M.S., Sung, J.C., Leight, S., Kolson, D.L., Iwatsubo, T., Lee, V.M., and Doms, R.W. (1997). Alzheimer's A beta(1-42) is generated in the endoplasmic reticulum/intermediate compartment of NT2N cells. Nat Med *3*, 1021-1023.

Cooney, J.R., Hurlburt, J.L., Selig, D.K., Harris, K.M., and Fiala, J.C. (2002). Endosomal compartments serve multiple hippocampal dendritic spines from a widespread rather than a local store of recycling membrane. J Neurosci *22*, 2215-2224.

Corder, E.H., Saunders, A.M., Strittmatter, W.J., Schmechel, D.E., Gaskell, P.C., Small, G.W., Roses, A.D., Haines, J.L., and Pericak-Vance, M.A. (1993). Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science *261*, 921-923.

Coulson, E.J., Paliga, K., Beyreuther, K., and Masters, C.L. (2000). What the evolution of the amyloid protein precursor supergene family tells us about its function. Neurochem Int *36*, 175-184.

Covington, M.D., Burghardt, R.C., and Parrish, A.R. (2006). Ischemia-induced cleavage of cadherins in NRK cells requires MT1-MMP (MMP-14). Am J Physiol Renal Physiol *290*, F43-51.

Craig, A.M., and Banker, G. (1994). Neuronal polarity. Annu Rev Neurosci *17*, 267-310.

Craig, A.M., Wyborski, R.J., and Banker, G. (1995). Preferential addition of newly synthesized membrane protein at axonal growth cones. Nature *375*, 592-594.

Cupers, P., Orlans, I., Craessaerts, K., Annaert, W., and De Strooper, B. (2001). The amyloid precursor protein (APP)-cytoplasmic fragment generated by gamma-secretase is rapidly degraded but distributes partially in a nuclear fraction of neurones in culture. J Neurochem *78*, 1168-1178.

da Silva, J.S., and Dotti, C.G. (2002). Breaking the neuronal sphere: regulation of the actin cytoskeleton in neuritogenesis. Nat Rev Neurosci *3*, 694-704.

Daigle, I., and Li, C. (1993). apl-1, a Caenorhabditis elegans gene encoding a protein related to the human beta-amyloid protein precursor. Proc Natl Acad Sci U S A *90*, 12045-12049.

Dalgarno, D.C., Botfield, M.C., and Rickles, R.J. (1997). SH3 domains and drug design: ligands, structure, and biological function. Biopolymers *43*, 383-400.

Dallas, D.J., Genever, P.G., Patton, A.J., Millichip, M.I., McKie, N., and Skerry, T.M. (1999). Localization of ADAM10 and Notch receptors in bone. Bone *25*, 9-15.

Dartigues, J.F., and Letenneur, L. (2000). Genetic epidemiology of Alzheimer's disease. Curr Opin Neurol *13*, 385-389.

Das, C., Berezovska, O., Diehl, T.S., Genet, C., Buldyrev, I., Tsai, J.Y., Hyman, B.T., and Wolfe, M.S. (2003). Designed helical peptides inhibit an intramembrane protease. J Am Chem Soc *125*, 11794-11795.

Davies, C.A., Mann, D.M., Sumpter, P.Q., and Yates, P.O. (1987). A quantitative morphometric analysis of the neuronal and synaptic content of the frontal and temporal cortex in patients with Alzheimer's disease. J Neurol Sci *78*, 151-164.

Davies, L., Wolska, B., Hilbich, C., Multhaup, G., Martins, R., Simms, G., Beyreuther, K., and Masters, C.L. (1988). A4 amyloid protein deposition and the diagnosis of Alzheimer's disease: prevalence in aged brains determined by immunocytochemistry compared with conventional neuropathologic techniques. Neurology *38*, 1688-1693.

Daw, M.I., Chittajallu, R., Bortolotto, Z.A., Dev, K.K., Duprat, F., Henley, J.M., Collingridge, G.L., and Isaac, J.T. (2000). PDZ proteins interacting with C-terminal GluR2/3 are involved in a PKC-dependent regulation of AMPA receptors at hippocampal synapses. Neuron *28*, 873-886.

de Anda, F.C., Pollarolo, G., Da Silva, J.S., Camoletto, P.G., Feiguin, F., and Dotti, C.G. (2005). Centrosome localization determines neuronal polarity. Nature *436*, 704-708.

De Pietri Tonelli, D., Mihailovich, M., Di Cesare, A., Codazzi, F., Grohovaz, F., and Zacchetti, D. (2004). Translational regulation of BACE-1 expression in neuronal and non-neuronal cells. Nucleic Acids Res *32*, 1808-1817.

De Strooper, B., Annaert, W., Cupers, P., Saftig, P., Craessaerts, K., Mumm, J.S., Schroeter, E.H., Schrijvers, V., Wolfe, M.S., Ray, W.J.*, et al.* (1999). A presenilin-1-dependent gamma-secretase-like protease mediates release of Notch intracellular domain. Nature *398*, 518-522.

De Strooper, B., Saftig, P., Craessaerts, K., Vanderstichele, H., Guhde, G., Annaert, W., Von Figura, K., and Van Leuven, F. (1998). Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. Nature *391*, 387-390.

DeKosky, S.T., and Scheff, S.W. (1990). Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity. Ann Neurol *27*, 457-464.

Delacourte, A. (2005). Tauopathies: recent insights into old diseases. Folia Neuropathol *43*, 244-257.

Delacourte, A., Sergeant, N., Champain, D., Wattez, A., Maurage, C.A., Lebert, F., Pasquier, F., and David, J.P. (2002). Nonoverlapping but synergetic tau and APP pathologies in sporadic Alzheimer's disease. Neurology *59*, 398-407.

DeLapp, N., Wu, S., Belagaje, R., Johnstone, E., Little, S., Shannon, H., Bymaster, F., Calligaro, D., Mitch, C., Whitesitt, C.*, et al.* (1998). Effects of the M1 agonist xanomeline on processing of human beta-amyloid precursor protein (FAD, Swedish mutant) transfected into Chinese hamster ovary-m1 cells. Biochem Biophys Res Commun *244*, 156-160.

Dell'Acqua, M.L., Smith, K.E., Gorski, J.A., Horne, E.A., Gibson, E.S., and Gomez, L.L. (2006). Regulation of neuronal PKA signaling through AKAP targeting dynamics. Eur J Cell Biol *85*, 627-633.

Derkach, V., Barria, A., and Soderling, T.R. (1999). Ca2+/calmodulin-kinase II enhances channel conductance of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate type glutamate receptors. Proc Natl Acad Sci U S A *96*, 3269-3274.

Desai, P.P., Ikonomovic, M.D., Abrahamson, E.E., Hamilton, R.L., Isanski, B.A., Hope, C.E., Klunk, W.E., DeKosky, S.T., and Kamboh, M.I. (2005). Apolipoprotein D is a component of compact but not diffuse amyloid-beta plaques in Alzheimer's disease temporal cortex. Neurobiol Dis *20*, 574-582.

Desdouits-Magnen, J., Desdouits, F., Takeda, S., Syu, L.J., Saltiel, A.R., Buxbaum, J.D., Czernik, A.J., Nairn, A.C., and Greengard, P. (1998). Regulation of secretion of Alzheimer amyloid precursor protein by the mitogen-activated protein kinase cascade. J Neurochem *70*, 524-530.

Dickson, D.W. (1997). The pathogenesis of senile plaques. J Neuropathol Exp Neurol *56*, 321-339.

Dickson, D.W., Crystal, H.A., Bevona, C., Honer, W., Vincent, I., and Davies, P. (1995). Correlations of synaptic and pathological markers with cognition of the elderly. Neurobiol Aging *16*, 285-298; discussion 298-304.

Ding, H., and Johnson, G.V. (2008). The last tangle of tau. J Alzheimers Dis *14*, 441-447.

Dingledine, R., Borges, K., Bowie, D., and Traynelis, S.F. (1999). The glutamate receptor ion channels. Pharmacol Rev *51*, 7-61.

Doerks, T., Bork, P., Kamberov, E., Makarova, O., Muecke, S., and Margolis, B. (2000). L27, a novel heterodimerization domain in receptor targeting proteins Lin-2 and Lin-7. Trends Biochem Sci *25*, 317-318.

Doherty, P., Williams, E., and Walsh, F.S. (1995). A soluble chimeric form of the L1 glycoprotein stimulates neurite outgrowth. Neuron *14*, 57-66.

Dong, H., O'Brien, R.J., Fung, E.T., Lanahan, A.A., Worley, P.F., and Huganir, R.L. (1997). GRIP: a synaptic PDZ domain-containing protein that interacts with AMPA receptors. Nature *386*, 279-284.

Duff, K., Eckman, C., Zehr, C., Yu, X., Prada, C.M., Perez-tur, J., Hutton, M., Buee, L., Harigaya, Y., Yager, D., *et al.* (1996). Increased amyloid-beta42(43) in brains of mice expressing mutant presenilin 1. Nature *383*, 710-713.

Dunn, K.W., McGraw, T.E., and Maxfield, F.R. (1989). Iterative fractionation of recycling receptors from lysosomally destined ligands in an early sorting endosome. J Cell Biol *109*, 3303-3314.

Dyrks, T., Monning, U., Beyreuther, K., and Turner, J. (1994). Amyloid precursor protein secretion and beta A4 amyloid generation are not mutually exclusive. FEBS Lett *349*, 210-214.

Dzwonek, J., Rylski, M., and Kaczmarek, L. (2004). Matrix metalloproteinases and their endogenous inhibitors in neuronal physiology of the adult brain. FEBS Lett *567*, 129-135.

Edbauer, D., Winkler, E., Regula, J.T., Pesold, B., Steiner, H., and Haass, C. (2003). Reconstitution of gamma-secretase activity. Nat Cell Biol *5*, 486-488.

Efthimiopoulos, S., Felsenstein, K.M., Sambamurti, K., Robakis, N.K., and Refolo, L.M. (1994). Study of the phorbol ester effect on Alzheimer amyloid precursor processing: sequence requirements and involvement of a cholera toxin sensitive protein. J Neurosci Res *38*, 81-90.

Efthimiopoulos, S., Punj, S., Manolopoulos, V., Pangalos, M., Wang, G.P., Refolo, L.M., and Robakis, N.K. (1996). Intracellular cyclic AMP inhibits constitutive and phorbol ester-stimulated secretory cleavage of amyloid precursor protein. J Neurochem *67*, 872-875.

Ehehalt, R., Keller, P., Haass, C., Thiele, C., and Simons, K. (2003). Amyloidogenic processing of the Alzheimer beta-amyloid precursor protein depends on lipid rafts. J Cell Biol *160*, 113-123.

Ehlers, M.R., and Riordan, J.F. (1991). Membrane proteins with soluble counterparts: role of proteolysis in the release of transmembrane proteins. Biochemistry *30*, 10065-10074.

Eikelenboom, P., Zhan, S.S., van Gool, W.A., and Allsop, D. (1994). Inflammatory mechanisms in Alzheimer's disease. Trends Pharmacol Sci *15*, 447-450.

El-Husseini, A.E., Schnell, E., Chetkovich, D.M., Nicoll, R.A., and Bredt, D.S. (2000). PSD-95 involvement in maturation of excitatory synapses. Science *290*, 1364-1368.

Endres, K., Postina, R., Schroeder, A., Mueller, U., and Fahrenholz, F. (2005). Shedding of the amyloid precursor protein-like protein APLP2 by disintegrin-metalloproteinases. FEBS J *272*, 5808-5820.

Epis, R., Marcello, E., Gardoni, F., Vastagh, C., Malinverno, M., Balducci, C., Colombo, A., Borroni, B., Vara, H., Dell'Agli, M.*, et al.* (2010). Blocking ADAM10 synaptic trafficking generates a model of sporadic Alzheimer's disease. Brain *133*, 3323-3335.

Erb, L., Liu, J., Ockerhausen, J., Kong, Q., Garrad, R.C., Griffin, K., Neal, C., Krugh, B., Santiago-Perez, L.I., Gonzalez, F.A.*, et al.* (2001). An RGD sequence in the P2Y(2) receptor interacts with alpha(V)beta(3) integrins and is required for G(o)-mediated signal transduction. J Cell Biol *153*, 491-501.

Ertekin-Taner, N., Graff-Radford, N., Younkin, L.H., Eckman, C., Baker, M., Adamson, J., Ronald, J., Blangero, J., Hutton, M., and Younkin, S.G. (2000). Linkage of plasma Abeta42 to a quantitative locus on chromosome 10 in late-onset Alzheimer's disease pedigrees. Science *290*, 2303-2304.

Esch, F.S., Keim, P.S., Beattie, E.C., Blacher, R.W., Culwell, A.R., Oltersdorf, T., McClure, D., and Ward, P.J. (1990). Cleavage of amyloid beta peptide during constitutive processing of its precursor. Science *248*, 1122-1124.

Esler, W.P., Kimberly, W.T., Ostaszewski, B.L., Diehl, T.S., Moore, C.L., Tsai, J.Y., Rahmati, T., Xia, W., Selkoe, D.J., and Wolfe, M.S. (2000). Transition-state analogue inhibitors of gamma-secretase bind directly to presenilin-1. Nat Cell Biol *2*, 428-434.

Esler, W.P., and Wolfe, M.S. (2001). A portrait of Alzheimer secretases--new features and familiar faces. Science *293*, 1449-1454.

Evans, J.P. (2001). Fertilin beta and other ADAMs as integrin ligands: insights into cell adhesion and fertilization. Bioessays *23*, 628-639.

Fahrenholz, F., Gilbert, S., Kojro, E., Lammich, S., and Postina, R. (2000). Alpha-secretase activity of the disintegrin metalloprotease ADAM 10. Influences of domain structure. Ann N Y Acad Sci *920*, 215-222.

Fambrough, D., Pan, D., Rubin, G.M., and Goodman, C.S. (1996). The cell surface metalloprotease/disintegrin Kuzbanian is required for axonal extension in Drosophila. Proc Natl Acad Sci U S A *93*, 13233-13238.

Farzan, M., Schnitzler, C.E., Vasilieva, N., Leung, D., and Choe, H. (2000). BACE2, a beta -secretase homolog, cleaves at the beta site and within the amyloid-beta region of the amyloid-beta precursor protein. Proc Natl Acad Sci U S A *97*, 9712-9717.

Feany, M.B., and Quinn, W.G. (1995). A neuropeptide gene defined by the Drosophila memory mutant amnesiac. Science *268*, 869-873.

Foletti, D.L., Prekeris, R., and Scheller, R.H. (1999). Generation and maintenance of neuronal polarity: mechanisms of transport and targeting. Neuron *23*, 641-644.

Fox, N.C., Crum, W.R., Scahill, R.I., Stevens, J.M., Janssen, J.C., and Rossor, M.N. (2001). Imaging of onset and progression of Alzheimer's disease with voxel-compression mapping of serial magnetic resonance images. Lancet *358*, 201-205.

Fukushima, D., Konishi, M., Maruyama, K., Miyamoto, T., Ishiura, S., and Suzuki, K. (1993). Activation of the secretory pathway leads to a decrease in the intracellular amyloidogenic fragments generated from the amyloid protein precursor. Biochem Biophys Res Commun *194*, 202-207.

Gabuzda, D., Busciglio, J., and Yankner, B.A. (1993). Inhibition of beta-amyloid production by activation of protein kinase C. J Neurochem *61*, 2326-2329.

Galbete, J.L., Martin, T.R., Peressini, E., Modena, P., Bianchi, R., and Forloni, G. (2000). Cholesterol decreases secretion of the secreted form of amyloid precursor protein by interfering with glycosylation in the protein secretory pathway. Biochem J *348 Pt 2*, 307-313.

Gandy, S., and Greengard, P. (1994). Regulated cleavage of the Alzheimer amyloid precursor protein: molecular and cellular basis. Biochimie *76*, 300-303.

Gardiol, A., Racca, C., and Triller, A. (1999). Dendritic and postsynaptic protein synthetic machinery. J Neurosci *19*, 168-179.

Gardoni, F., Bellone, C., Cattabeni, F., and Di Luca, M. (2001). Protein kinase C activation modulates alpha-calmodulin kinase II binding to NR2A subunit of N-methyl-D-aspartate receptor complex. J Biol Chem *276*, 7609-7613.

Gardoni, F., Marcello, E., and Di Luca, M. (2009). Postsynaptic density-membrane associated guanylate kinase proteins (PSD-MAGUKs) and their role in CNS disorders. Neuroscience *158*, 324-333.

Gardoni, F., Mauceri, D., Fiorentini, C., Bellone, C., Missale, C., Cattabeni, F., and Di Luca, M. (2003). CaMKII-dependent phosphorylation regulates SAP97/NR2A interaction. J Biol Chem *278*, 44745-44752.

Gardoni, F., Schrama, L.H., van Dalen, J.J., Gispen, W.H., Cattabeni, F., and Di Luca, M. (1999). AlphaCaMKII binding to the C-terminal tail of NMDA receptor subunit NR2A and its modulation by autophosphorylation. FEBS Lett *456*, 394-398.

Garner, C.C., Nash, J., and Huganir, R.L. (2000). PDZ domains in synapse assembly and signalling. Trends Cell Biol *10*, 274-280.

Gatta, L.B., Albertini, A., Ravid, R., and Finazzi, D. (2002). Levels of beta-secretase BACE and alpha-secretase ADAM10 mRNAs in Alzheimer hippocampus. Neuroreport *13*, 2031-2033.

Gaultier, A., Cousin, H., Darribere, T., and Alfandari, D. (2002). ADAM13 disintegrin and cysteine-rich domains bind to the second heparin-binding domain of fibronectin. J Biol Chem *277*, 23336-23344.

Giambarella, U., Yamatsuji, T., Okamoto, T., Matsui, T., Ikezu, T., Murayama, Y., Levine, M.A., Katz, A., Gautam, N., and Nishimoto, I. (1997). G protein betagamma complex-mediated apoptosis by familial Alzheimer's disease mutant of APP. EMBO J *16*, 4897-4907.

Gibson, P.H. (1983). EM study of the numbers of cortical synapses in the brains of ageing people and people with Alzheimer-type dementia. Acta Neuropathol *62*, 127-133.

Gillespie, S.L., Golde, T.E., and Younkin, S.G. (1992). Secretory processing of the Alzheimer amyloid beta/A4 protein precursor is increased by protein phosphorylation. Biochem Biophys Res Commun *187*, 1285-1290.

Glabe, C.G. (2008). Structural classification of toxic amyloid oligomers. J Biol Chem *283*, 29639-29643.

Glenner, G.G., and Wong, C.W. (1984). Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem Biophys Res Commun *120*, 885-890.

Goate, A., Chartier-Harlin, M.C., Mullan, M., Brown, J., Crawford, F., Fidani, L., Giuffra, L., Haynes, A., Irving, N., James, L*., et al.* (1991). Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature *349*, 704-706.

Goldgaber, D., Lerman, M.I., McBride, O.W., Saffiotti, U., and Gajdusek, D.C. (1987). Characterization and chromosomal localization of a cDNA encoding brain amyloid of Alzheimer's disease. Science *235*, 877-880.

Golgi, C. (1989). On the structure of nerve cells. 1898. J Microsc *155*, 3-7.

Goodman, A.B., and Pardee, A.B. (2003). Evidence for defective retinoid transport and function in late onset Alzheimer's disease. Proc Natl Acad Sci U S A *100*, 2901-2905.

Gorevic, P.D., Goni, F., Pons-Estel, B., Alvarez, F., Peress, N.S., and Frangione, B. (1986). Isolation and partial characterization of neurofibrillary tangles and amyloid plaque core in Alzheimer's disease: immunohistological studies. J Neuropathol Exp Neurol *45*, 647-664.

Gray, E.G. (1959). Axo-somatic and axo-dendritic synapses of the cerebral cortex: an electron microscope study. J Anat *93*, 420-433.

Grbovic, O.M., Mathews, P.M., Jiang, Y., Schmidt, S.D., Dinakar, R., Summers-Terio, N.B., Ceresa, B.P., Nixon, R.A., and Cataldo, A.M. (2003). Rab5-stimulated up-regulation of the endocytic pathway increases intracellular beta-cleaved amyloid precursor protein carboxyl-terminal fragment levels and Abeta production. J Biol Chem *278*, 31261-31268.

Greenfield, J.P., Tsai, J., Gouras, G.K., Hai, B., Thinakaran, G., Checler, F., Sisodia, S.S., Greengard, P., and Xu, H. (1999). Endoplasmic reticulum and trans-Golgi network generate distinct populations of Alzheimer beta-amyloid peptides. Proc Natl Acad Sci U S A *96*, 742-747.

Grundke-Iqbal, I., Iqbal, K., Tung, Y.C., Quinlan, M., Wisniewski, H.M., and Binder, L.I. (1986). Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. Proc Natl Acad Sci U S A *83*, 4913-4917.

Guenette, S., Chang, Y., Hiesberger, T., Richardson, J.A., Eckman, C.B., Eckman, E.A., Hammer, R.E., and Herz, J. (2006). Essential roles for the FE65 amyloid precursor protein-interacting proteins in brain development. EMBO J *25*, 420-431.

Gundelfinger, E.D., Boeckers, T.M., Baron, M.K., and Bowie, J.U. (2006). A role for zinc in postsynaptic density asSAMbly and plasticity? Trends Biochem Sci *31*, 366-373.

Gunn, T.M., Azarani, A., Kim, P.H., Hyman, R.W., Davis, R.W., and Barsh, G.S. (2002). Identification and preliminary characterization of mouse Adam33. BMC Genet *3*, 2.

Gurd, J.W., Jones, L.R., Mahler, H.R., and Moore, W.J. (1974). Isolation and partial characterization of rat brain synaptic plasma membranes. J Neurochem *22*, 281-290.

Gylys, K.H., Fein, J.A., Yang, F., Wiley, D.J., Miller, C.A., and Cole, G.M. (2004). Synaptic changes in Alzheimer's disease: increased amyloid-beta and gliosis in surviving terminals is accompanied by decreased PSD-95 fluorescence. Am J Pathol *165*, 1809-1817.

Haass, C., Koo, E.H., Mellon, A., Hung, A.Y., and Selkoe, D.J. (1992a). Targeting of cell-surface beta-amyloid precursor protein to lysosomes: alternative processing into amyloid-bearing fragments. Nature *357*, 500-503.

Haass, C., Schlossmacher, M.G., Hung, A.Y., Vigo-Pelfrey, C., Mellon, A., Ostaszewski, B.L., Lieberburg, I., Koo, E.H., Schenk, D., Teplow, D.B., *et al.* (1992b). Amyloid beta-peptide is produced by cultured cells during normal metabolism. Nature *359*, 322-325.

Haass, C., and Selkoe, D.J. (1993). Cellular processing of beta-amyloid precursor protein and the genesis of amyloid beta-peptide. Cell *75*, 1039-1042.

Hamos, J.E., DeGennaro, L.J., and Drachman, D.A. (1989). Synaptic loss in Alzheimer's disease and other dementias. Neurology *39*, 355-361.

Hanada, T., Takeuchi, A., Sondarva, G., and Chishti, A.H. (2003). Protein 4.1-mediated membrane targeting of human discs large in epithelial cells. J Biol Chem *278*, 34445-34450.

Hardy, J. (1992). Framing beta-amyloid. Nat Genet *1*, 233-234.

Hardy, J., Duff, K., Hardy, K.G., Perez-Tur, J., and Hutton, M. (1998). Genetic dissection of Alzheimer's disease and related dementias: amyloid and its relationship to tau. Nat Neurosci *1*, 355-358.

Hardy, J., and Selkoe, D.J. (2002). The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science *297*, 353-356.

Hardy, J.A., and Higgins, G.A. (1992). Alzheimer's disease: the amyloid cascade hypothesis. Science *256*, 184-185.

Haring, R., Gurwitz, D., Barg, J., Pinkas-Kramarski, R., Heldman, E., Pittel, Z., Wengier, A., Meshulam, H., Marciano, D., Karton, Y., *et al.* (1994). Amyloid precursor protein secretion via muscarinic receptors: reduced desensitization using the M1-selective agonist AF102B. Biochem Biophys Res Commun *203*, 652-658.

Hartmann, D., de Strooper, B., Serneels, L., Craessaerts, K., Herreman, A., Annaert, W., Umans, L., Lubke, T., Lena Illert, A., von Figura, K., *et al.* (2002). The disintegrin/metalloprotease ADAM 10 is essential for Notch signalling but not for alpha-secretase activity in fibroblasts. Hum Mol Genet *11*, 2615-2624.

Hattori, M., Osterfield, M., and Flanagan, J.G. (2000). Regulated cleavage of a contact-mediated axon repellent. Science *289*, 1360-1365.

Hayashi, Y., Shi, S.H., Esteban, J.A., Piccini, A., Poncer, J.C., and Malinow, R. (2000). Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction. Science *287*, 2262-2267.

He, X., Treacy, M.N., Simmons, D.M., Ingraham, H.A., Swanson, L.W., and Rosenfeld, M.G. (1989). Expression of a large family of POU-domain regulatory genes in mammalian brain development. Nature *340*, 35-41.

Heber, S., Herms, J., Gajic, V., Hainfellner, J., Aguzzi, A., Rulicke, T., von Kretzschmar, H., von Koch, C., Sisodia, S., Tremml, P., *et al.* (2000). Mice with combined gene knock-outs reveal essential and partially redundant functions of amyloid precursor protein family members. J Neurosci *20*, 7951-7963.

Hendriks, L., van Duijn, C.M., Cras, P., Cruts, M., Van Hul, W., van Harskamp, F., Warren, A., McInnis, M.G., Antonarakis, S.E., Martin, J.J., *et al.* (1992). Presenile dementia and cerebral haemorrhage linked to a mutation at codon 692 of the beta-amyloid precursor protein gene. Nat Genet *1*, 218-221.

Herms, J., Anliker, B., Heber, S., Ring, S., Fuhrmann, M., Kretzschmar, H., Sisodia, S., and Muller, U. (2004). Cortical dysplasia resembling human type 2 lissencephaly in mice lacking all three APP family members. EMBO J *23*, 4106-4115.

Higgins, D., Burack, M., Lein, P., and Banker, G. (1997). Mechanisms of neuronal polarity. Curr Opin Neurobiol *7*, 599-604.

Hirai, H., Kirsch, J., Laube, B., Betz, H., and Kuhse, J. (1996). The glycine binding site of the N-methyl-D-aspartate receptor subunit NR1: identification of novel determinants of co-agonist potentiation in the extracellular M3-M4 loop region. Proc Natl Acad Sci U S A *93*, 6031-6036.

Hiraoka, Y., Ohno, M., Yoshida, K., Okawa, K., Tomimoto, H., Kita, T., and Nishi, E. (2007). Enhancement of alpha-secretase cleavage of amyloid precursor protein by a metalloendopeptidase nardilysin. J Neurochem *102*, 1595-1605.

Hiraoka, Y., Yoshida, K., Ohno, M., Matsuoka, T., Kita, T., and Nishi, E. (2008). Ectodomain shedding of TNF-alpha is enhanced by nardilysin via activation of ADAM proteases. Biochem Biophys Res Commun *370*, 154-158.

Ho, A., and Sudhof, T.C. (2004). Binding of F-spondin to amyloid-beta precursor protein: a candidate amyloid-beta precursor protein ligand that modulates amyloid-beta precursor protein cleavage. Proc Natl Acad Sci U S A *101*, 2548-2553.

Hoe, H.S., Magill, L.A., Guenette, S., Fu, Z., Vicini, S., and Rebeck, G.W. (2006a). FE65 interaction with the ApoE receptor ApoEr2. J Biol Chem *281*, 24521-24530.

Hoe, H.S., Tran, T.S., Matsuoka, Y., Howell, B.W., and Rebeck, G.W. (2006b). DAB1 and Reelin effects on amyloid precursor protein and ApoE receptor 2 trafficking and processing. J Biol Chem *281*, 35176-35185.

Holback, S., Adlerz, L., and Iverfeldt, K. (2005). Increased processing of APLP2 and APP with concomitant formation of APP intracellular domains in BDNF and retinoic acid-differentiated human neuroblastoma cells. J Neurochem *95*, 1059-1068.

Hollmann, M., and Heinemann, S. (1994). Cloned glutamate receptors. Annu Rev Neurosci *17*, 31-108.

Homma, A., Takeda, M., Imai, Y., Udaka, F., Hasegawa, K., Kameyama, M., and Nishimura, T. (2000). Clinical efficacy and safety of donepezil on cognitive and global function in patients with Alzheimer's disease. A 24-week, multicenter, double-blind, placebo-controlled study in Japan. E2020 Study Group. Dement Geriatr Cogn Disord *11*, 299-313.

Honer, W.G., Dickson, D.W., Gleeson, J., and Davies, P. (1992). Regional synaptic pathology in Alzheimer's disease. Neurobiol Aging *13*, 375-382.

Hong, L., Koelsch, G., Lin, X., Wu, S., Terzyan, S., Ghosh, A.K., Zhang, X.C., and Tang, J. (2000). Structure of the protease domain of memapsin 2 (beta-secretase) complexed with inhibitor. Science *290*, 150-153.

Hooper, N.M., and Turner, A.J. (2000). Protein processing mechanisms: from angiotensin-converting enzyme to Alzheimer's disease. Biochem Soc Trans *28*, 441-446.

Horiuchi, K., Le Gall, S., Schulte, M., Yamaguchi, T., Reiss, K., Murphy, G., Toyama, Y., Hartmann, D., Saftig, P., and Blobel, C.P. (2007). Substrate selectivity of epidermal growth factor-receptor ligand sheddases and their regulation by phorbol esters and calcium influx. Mol Biol Cell *18*, 176-188.

Horton, A.C., and Ehlers, M.D. (2003a). Dual modes of endoplasmic reticulum-to-Golgi transport in dendrites revealed by live-cell imaging. J Neurosci *23*, 6188-6199.

Horton, A.C., and Ehlers, M.D. (2003b). Neuronal polarity and trafficking. Neuron *40*, 277-295.

Horton, A.C., and Ehlers, M.D. (2004). Secretory trafficking in neuronal dendrites. Nat Cell Biol *6*, 585-591.

Horton, A.C., Racz, B., Monson, E.E., Lin, A.L., Weinberg, R.J., and Ehlers, M.D. (2005). Polarized secretory trafficking directs cargo for asymmetric dendrite growth and morphogenesis. Neuron *48*, 757-771.

Hotoda, N., Koike, H., Sasagawa, N., and Ishiura, S. (2002). A secreted form of human ADAM9 has an alpha-secretase activity for APP. Biochem Biophys Res Commun *293*, 800-805.

Howard, L., Lu, X., Mitchell, S., Griffiths, S., and Glynn, P. (1996). Molecular cloning of MADM: a catalytically active mammalian disintegrin-metalloprotease expressed in various cell types. Biochem J *317 (Pt 1)*, 45-50.

Howard, L., Nelson, K.K., Maciewicz, R.A., and Blobel, C.P. (1999). Interaction of the metalloprotease disintegrins MDC9 and MDC15 with two SH3 domain-containing proteins, endophilin I and SH3PX1. J Biol Chem *274*, 31693-31699.

Hu, L.A., Chen, W., Premont, R.T., Cong, M., and Lefkowitz, R.J. (2002). G protein-coupled receptor kinase 5 regulates beta 1-adrenergic receptor association with PSD-95. J Biol Chem *277*, 1607-1613.

Huang, L., Feng, L., Yang, L., Zhou, W., Zhao, S., and Li, C. (2002). Screen and identification of proteins interacting with ADAM19 cytoplasmic tail. Mol Biol Rep *29*, 317-323.

Hung, A.Y., Haass, C., Nitsch, R.M., Qiu, W.Q., Citron, M., Wurtman, R.J., Growdon, J.H., and Selkoe, D.J. (1993). Activation of protein kinase C inhibits cellular production of the amyloid beta-protein. J Biol Chem *268*, 22959-22962.

Hung, A.Y., and Selkoe, D.J. (1994). Selective ectodomain phosphorylation and regulated cleavage of beta-amyloid precursor protein. EMBO J *13*, 534-542.

Huse, J.T., Pijak, D.S., Leslie, G.J., Lee, V.M., and Doms, R.W. (2000). Maturation and endosomal targeting of beta-site amyloid precursor protein-cleaving enzyme. The Alzheimer's disease beta-secretase. J Biol Chem *275*, 33729-33737.

Hussain, I., Hawkins, J., Shikotra, A., Riddell, D.R., Faller, A., and Dingwall, C. (2003). Characterization of the ectodomain shedding of the beta-site amyloid precursor protein-cleaving enzyme 1 (BACE1). J Biol Chem *278*, 36264-36268.

Hussain, I., Powell, D., Howlett, D.R., Tew, D.G., Meek, T.D., Chapman, C., Gloger, I.S., Murphy, K.E., Southan, C.D., Ryan, D.M., *et al.* (1999). Identification of a novel aspartic protease (Asp 2) as beta-secretase. Mol Cell Neurosci *14*, 419-427.

Hutton, M., Lendon, C.L., Rizzu, P., Baker, M., Froelich, S., Houlden, H., Pickering-Brown, S., Chakraverty, S., Isaacs, A., Grover, A., *et al.* (1998). Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17. Nature *393*, 702-705.

Iba, K., Albrechtsen, R., Gilpin, B., Frohlich, C., Loechel, F., Zolkiewska, A., Ishiguro, K., Kojima, T., Liu, W., Langford, J.K., *et al.* (2000). The cysteine-rich domain of human ADAM 12 supports cell adhesion through syndecans and triggers signaling events that lead to beta1 integrin-dependent cell spreading. J Cell Biol *149*, 1143-1156.

Ikezu, T., Trapp, B.D., Song, K.S., Schlegel, A., Lisanti, M.P., and Okamoto, T. (1998). Caveolae, plasma membrane microdomains for alpha-secretase-mediated processing of the amyloid precursor protein. J Biol Chem *273*, 10485-10495.

Illenberger, S., Zheng-Fischhofer, Q., Preuss, U., Stamer, K., Baumann, K., Trinczek, B., Biernat, J., Godemann, R., Mandelkow, E.M., and Mandelkow, E. (1998). The endogenous and cell cycle-dependent phosphorylation of tau protein in living cells: implications for Alzheimer's disease. Mol Biol Cell *9*, 1495-1512.

Izumi, R., Yamada, T., Yoshikai, S., Sasaki, H., Hattori, M., and Sakaki, Y. (1992). Positive and negative regulatory elements for the expression of the Alzheimer's disease amyloid precursor-encoding gene in mouse. Gene *112*, 189-195.

Jacobsen, J.S., Spruyt, M.A., Brown, A.M., Sahasrabudhe, S.R., Blume, A.J., Vitek, M.P., Muenkel, H.A., and Sonnenberg-Reines, J. (1994). The release of Alzheimer's disease beta amyloid peptide is reduced by phorbol treatment. J Biol Chem *269*, 8376-8382.

Jan, Y.N., and Jan, L.Y. (2001). Dendrites. Genes Dev *15*, 2627-2641.

Janes, P.W., Saha, N., Barton, W.A., Kolev, M.V., Wimmer-Kleikamp, S.H., Nievergall, E., Blobel, C.P., Himanen, J.P., Lackmann, M., and Nikolov, D.B. (2005). Adam meets Eph: an ADAM substrate recognition module acts as a molecular switch for ephrin cleavage in trans. Cell *123*, 291-304.

Jimenez-Jimenez, F.J., Molina, J.A., de Bustos, F., Orti-Pareja, M., Benito-Leon, J., Tallon-Barranco, A., Gasalla, T., Porta, J., and Arenas, J. (1999). Serum levels of beta-carotene, alpha-carotene and vitamin A in patients with Alzheimer's disease. Eur J Neurol *6*, 495-497.

Jolly-Tornetta, C., Gao, Z.Y., Lee, V.M., and Wolf, B.A. (1998). Regulation of amyloid precursor protein secretion by glutamate receptors in human Ntera 2 neurons. J Biol Chem *273*, 14015-14021.

Jones, J.I., Prevette, T., Gockerman, A., and Clemmons, D.R. (1996). Ligand occupancy of the alpha-V-beta3 integrin is necessary for smooth muscle cells to migrate in response to insulin-like growth factor. Proc Natl Acad Sci U S A *93*, 2482-2487.

Joo, K.M., Chung, Y.H., Kim, M.K., Nam, R.H., Lee, B.L., Lee, K.H., and Cha, C.I. (2004). Distribution of vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide receptors (VPAC1, VPAC2, and PAC1 receptor) in the rat brain. J Comp Neurol *476*, 388-413.

Ju, W., Morishita, W., Tsui, J., Gaietta, G., Deerinck, T.J., Adams, S.R., Garner, C.C., Tsien, R.Y., Ellisman, M.H., and Malenka, R.C. (2004). Activity-dependent regulation of dendritic synthesis and trafficking of AMPA receptors. Nat Neurosci *7*, 244-253.

Kacharmina, J.E., Job, C., Crino, P., and Eberwine, J. (2000). Stimulation of glutamate receptor protein synthesis and membrane insertion within isolated neuronal dendrites. Proc Natl Acad Sci U S A *97*, 11545-11550.

Kaether, C., Lammich, S., Edbauer, D., Ertl, M., Rietdorf, J., Capell, A., Steiner, H., and Haass, C. (2002). Presenilin-1 affects trafficking and processing of betaAPP and is targeted in a complex with nicastrin to the plasma membrane. J Cell Biol *158*, 551-561.

Kaido, T., Perez, B., Yebra, M., Hill, J., Cirulli, V., Hayek, A., and Montgomery, A.M. (2004). Alphav-integrin utilization in human beta-cell adhesion, spreading, and motility. J Biol Chem *279*, 17731-17737.

Kamal, A., Almenar-Queralt, A., LeBlanc, J.F., Roberts, E.A., and Goldstein, L.S. (2001). Kinesin-mediated axonal transport of a membrane compartment containing beta-secretase and presenilin-1 requires APP. Nature *414*, 643-648.

Kamenetz, F., Tomita, T., Hsieh, H., Seabrook, G., Borchelt, D., Iwatsubo, T., Sisodia, S., and Malinow, R. (2003). APP processing and synaptic function. Neuron *37*, 925-937.

Kamiguchi, H. (2003). The mechanism of axon growth: what we have learned from the cell adhesion molecule L1. Mol Neurobiol *28*, 219-228.

Kang, J., Lemaire, H.G., Unterbeck, A., Salbaum, J.M., Masters, C.L., Grzeschik, K.H., Multhaup, G., Beyreuther, K., and Muller-Hill, B. (1987). The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. Nature *325*, 733-736.

Karkkainen, I., Rybnikova, E., Pelto-Huikko, M., and Huovila, A.P. (2000). Metalloprotease-disintegrin (ADAM) genes are widely and differentially expressed in the adult CNS. Mol Cell Neurosci *15*, 547-560.

Kaufer, D., Friedman, A., Seidman, S., and Soreq, H. (1998). Acute stress facilitates long-lasting changes in cholinergic gene expression. Nature *393*, 373-377.

Kaufmann, J., and Smale, S.T. (1994). Direct recognition of initiator elements by a component of the transcription factor IID complex. Genes Dev *8*, 821-829.

Keller, B.U., Hollmann, M., Heinemann, S., and Konnerth, A. (1992). Calcium influx through subunits GluR1/GluR3 of kainate/AMPA receptor channels is regulated by cAMP dependent protein kinase. EMBO J *11*, 891-896.

Kennedy, M.B., Beale, H.C., Carlisle, H.J., and Washburn, L.R. (2005). Integration of biochemical signalling in spines. Nat Rev Neurosci *6*, 423-434.

Kennedy, M.J., and Ehlers, M.D. (2006). Organelles and trafficking machinery for postsynaptic plasticity. Annu Rev Neurosci *29*, 325-362.

Kennelly, P.J., and Krebs, E.G. (1991). Consensus sequences as substrate specificity determinants for protein kinases and protein phosphatases. J Biol Chem *266*, 15555-15558.

Khvotchev, M., and Sudhof, T.C. (2004). Proteolytic processing of amyloid-beta precursor protein by secretases does not require cell surface transport. J Biol Chem *279*, 47101-47108.

Kim, E., Naisbitt, S., Hsueh, Y.P., Rao, A., Rothschild, A., Craig, A.M., and Sheng, M. (1997). GKAP, a novel synaptic protein that interacts with the guanylate kinase-like domain of the PSD-95/SAP90 family of channel clustering molecules. J Cell Biol *136*, 669-678.

Kim, E., and Sheng, M. (2004). PDZ domain proteins of synapses. Nat Rev Neurosci *5*, 771-781.

Kim, J., Lilliehook, C., Dudak, A., Prox, J., Saftig, P., Federoff, H.J., and Lim, S.T. (2010). Activity-dependent alpha-cleavage of nectin-1 is mediated by a disintegrin and metalloprotease 10 (ADAM10). J Biol Chem *285*, 22919-22926.

King, G.D., Cherian, K., and Turner, R.S. (2004). X11alpha impairs gamma- but not beta-cleavage of amyloid precursor protein. J Neurochem *88*, 971-982.

King, G.D., and Scott Turner, R. (2004). Adaptor protein interactions: modulators of amyloid precursor protein metabolism and Alzheimer's disease risk? Exp Neurol *185*, 208-219.

Kistner, U., Garner, C.C., and Linial, M. (1995). Nucleotide binding by the synapse associated protein SAP90. FEBS Lett *359*, 159-163.

Kitaguchi, N., Takahashi, Y., Tokushima, Y., Shiojiri, S., and Ito, H. (1988). Novel precursor of Alzheimer's disease amyloid protein shows protease inhibitory activity. Nature *331*, 530-532.

Kitazume, S., Tachida, Y., Oka, R., Shirotani, K., Saido, T.C., and Hashimoto, Y. (2001). Alzheimer's beta-secretase, beta-site amyloid precursor protein-cleaving enzyme, is responsible for cleavage secretion of a Golgi-resident sialyltransferase. Proc Natl Acad Sci U S A *98*, 13554-13559.

Kleckner, N.W., and Dingledine, R. (1988). Requirement for glycine in activation of NMDA-receptors expressed in Xenopus oocytes. Science *241*, 835-837.

Klein, W.L., Krafft, G.A., and Finch, C.E. (2001). Targeting small Abeta oligomers: the solution to an Alzheimer's disease conundrum? Trends Neurosci *24*, 219-224.

Kleizen, B., and Braakman, I. (2004). Protein folding and quality control in the endoplasmic reticulum. Curr Opin Cell Biol *16*, 343-349.

Kobayashi, M., Matsuoka, I., and Kurihara, K. (1994). Cholinergic differentiation of cultured sympathetic neurons induced by retinoic acid. Induction of choline acetyltransferase-mRNA and suppression of tyrosine hydroxylase-mRNA levels. FEBS Lett *337*, 259-264.

Kohutek, Z.A., diPierro, C.G., Redpath, G.T., and Hussaini, I.M. (2009). ADAM-10-mediated N-cadherin cleavage is protein kinase C-alpha dependent and promotes glioblastoma cell migration. J Neurosci *29*, 4605-4615.

Koivunen, J., Aaltonen, V., and Peltonen, J. (2006). Protein kinase C (PKC) family in cancer progression. Cancer Lett *235*, 1-10.

Kojro, E., Gimpl, G., Lammich, S., Marz, W., and Fahrenholz, F. (2001). Low cholesterol stimulates the nonamyloidogenic pathway by its effect on the alpha -secretase ADAM 10. Proc Natl Acad Sci U S A *98*, 5815-5820.

Kojro, E., Postina, R., Buro, C., Meiringer, C., Gehrig-Burger, K., and Fahrenholz, F. (2006). The neuropeptide PACAP promotes the alpha-secretase pathway for processing the Alzheimer amyloid precursor protein. FASEB J *20*, 512-514.

Koldamova, R.P., Lefterov, I.M., Ikonomovic, M.D., Skoko, J., Lefterov, P.I., Isanski, B.A., DeKosky, S.T., and Lazo, J.S. (2003). 22R-hydroxycholesterol and 9-cis-retinoic acid induce ATP-binding cassette transporter A1 expression and cholesterol efflux in brain cells and decrease amyloid beta secretion. J Biol Chem *278*, 13244-13256.

Koldamova, R.P., Lefterov, I.M., Staufenbiel, M., Wolfe, D., Huang, S., Glorioso, J.C., Walter, M., Roth, M.G., and Lazo, J.S. (2005). The liver X receptor ligand T0901317 decreases amyloid beta production in vitro and in a mouse model of Alzheimer's disease. J Biol Chem *280*, 4079-4088.

Koo, E.H., and Squazzo, S.L. (1994). Evidence that production and release of amyloid beta-protein involves the endocytic pathway. J Biol Chem *269*, 17386-17389.

Kopan, R., and Ilagan, M.X. (2004). Gamma-secretase: proteasome of the membrane? Nat Rev Mol Cell Biol *5*, 499-504.

Kornilova, A.Y., Das, C., and Wolfe, M.S. (2003). Differential effects of inhibitors on the gamma-secretase complex. Mechanistic implications. J Biol Chem *278*, 16470-16473.

Kovacs, D.M., Wasco, W., Witherby, J., Felsenstein, K.M., Brunel, F., Roeder, R.G., and Tanzi, R.E. (1995). The upstream stimulatory factor functionally interacts with the Alzheimer amyloid beta-protein precursor gene. Hum Mol Genet *4*, 1527-1533.

Krall, W.J., Sramek, J.J., and Cutler, N.R. (1999). Cholinesterase inhibitors: a therapeutic strategy for Alzheimer disease. Ann Pharmacother *33*, 441-450.

Kuan, Y.H., Gruebl, T., Soba, P., Eggert, S., Nesic, I., Back, S., Kirsch, J., Beyreuther, K., and Kins, S. (2006). PAT1a modulates intracellular transport and processing of amyloid precursor protein (APP), APLP1, and APLP2. J Biol Chem *281*, 40114-40123.

Lammich, S., Buell, D., Zilow, S., Ludwig, A.K., Nuscher, B., Lichtenthaler, S.F., Prinzen, C., Fahrenholz, F., and Haass, C. (2010). Expression of the anti-amyloidogenic secretase ADAM10 is suppressed by its 5'-untranslated region. J Biol Chem *285*, 15753-15760.

Lammich, S., Kojro, E., Postina, R., Gilbert, S., Pfeiffer, R., Jasionowski, M., Haass, C., and Fahrenholz, F. (1999). Constitutive and regulated alpha-secretase cleavage of Alzheimer's amyloid precursor protein by a disintegrin metalloprotease. Proc Natl Acad Sci U S A *96*, 3922-3927.

Lammich, S., Schobel, S., Zimmer, A.K., Lichtenthaler, S.F., and Haass, C. (2004). Expression of the Alzheimer protease BACE1 is suppressed via its 5'-untranslated region. EMBO Rep *5*, 620-625.

Larsen, F., Gundersen, G., Lopez, R., and Prydz, H. (1992). CpG islands as gene markers in the human genome. Genomics *13*, 1095-1107.

Lazarovici, P., Jiang, H., and Fink, D., Jr. (1998). The 38-amino-acid form of pituitary adenylate cyclase-activating polypeptide induces neurite outgrowth in PC12 cells that is dependent on protein kinase C and extracellular signal-regulated kinase but not on protein kinase A, nerve growth factor receptor tyrosine kinase, p21(ras) G protein, and pp60(c-src) cytoplasmic tyrosine kinase. Mol Pharmacol *54*, 547-558.

Lazarowski, E.R., and Boucher, R.C. (2001). UTP as an extracellular signaling molecule. News Physiol Sci *16*, 1-5.

Lee, R.K., and Wurtman, R.J. (1997). Metabotropic glutamate receptors increase amyloid precursor protein processing in astrocytes: inhibition by cyclic AMP. J Neurochem *68*, 1830-1835.

Lee, R.K., Wurtman, R.J., Cox, A.J., and Nitsch, R.M. (1995). Amyloid precursor protein processing is stimulated by metabotropic glutamate receptors. Proc Natl Acad Sci U S A *92*, 8083-8087.

Lee, S.J., Liyanage, U., Bickel, P.E., Xia, W., Lansbury, P.T., Jr., and Kosik, K.S. (1998). A detergent-insoluble membrane compartment contains A beta in vivo. Nat Med *4*, 730-734.

Lengqvist, J., Mata De Urquiza, A., Bergman, A.C., Willson, T.M., Sjovall, J., Perlmann, T., and Griffiths, W.J. (2004). Polyunsaturated fatty acids including docosahexaenoic and arachidonic acid bind to the retinoid X receptor alpha ligand-binding domain. Mol Cell Proteomics *3*, 692-703.

Leonard, A.S., Davare, M.A., Horne, M.C., Garner, C.C., and Hell, J.W. (1998). SAP97 is associated with the alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor GluR1 subunit. J Biol Chem *273*, 19518-19524.

Leonard, A.S., and Hell, J.W. (1997). Cyclic AMP-dependent protein kinase and protein kinase C phosphorylate N-methyl-D-aspartate receptors at different sites. J Biol Chem *272*, 12107-12115.

Levine, T., and Rabouille, C. (2005). Endoplasmic reticulum: one continuous network compartmentalized by extrinsic cues. Curr Opin Cell Biol *17*, 362-368.

Levy-Lahad, E., Wasco, W., Poorkaj, P., Romano, D.M., Oshima, J., Pettingell, W.H., Yu, C.E., Jondro, P.D., Schmidt, S.D., Wang, K.*, et al.* (1995). Candidate gene for the chromosome 1 familial Alzheimer's disease locus. Science *269*, 973-977.

Levy, E., Carman, M.D., Fernandez-Madrid, I.J., Power, M.D., Lieberburg, I., van Duinen, S.G., Bots, G.T., Luyendijk, W., and Frangione, B. (1990). Mutation of the Alzheimer's disease amyloid gene in hereditary cerebral hemorrhage, Dutch type. Science *248*, 1124-1126.

Lewis, J., Dickson, D.W., Lin, W.L., Chisholm, L., Corral, A., Jones, G., Yen, S.H., Sahara, N., Skipper, L., Yager, D.*, et al.* (2001). Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP. Science *293*, 1487-1491.

Li, D., Specht, C.G., Waites, C.L., Butler-Munro, C., Leal-Ortiz, S., Foote, J.W., Genoux, D., Garner, C.C., and Montgomery, J.M. (2011). SAP97 directs NMDA receptor spine targeting and synaptic plasticity. J Physiol *589*, 4491-4510.

Li, P., He, X., Gerrero, M.R., Mok, M., Aggarwal, A., and Rosenfeld, M.G. (1993). Spacing and orientation of bipartite DNA-binding motifs as potential functional determinants for POU domain factors. Genes Dev *7*, 2483-2496.

Li, Z., Okamoto, K., Hayashi, Y., and Sheng, M. (2004). The importance of dendritic mitochondria in the morphogenesis and plasticity of spines and synapses. Cell *119*, 873-887.

Lichtenthaler, S.F. (2006). Ectodomain shedding of the amyloid precursor protein: cellular control mechanisms and novel modifiers. Neurodegener Dis *3*, 262-269.

Lichtenthaler, S.F., Dominguez, D.I., Westmeyer, G.G., Reiss, K., Haass, C., Saftig, P., De Strooper, B., and Seed, B. (2003). The cell adhesion protein P-selectin glycoprotein ligand-1 is a substrate for the aspartyl protease BACE1. J Biol Chem *278*, 48713-48719.

Lichtenthaler, S.F., Wang, R., Grimm, H., Uljon, S.N., Masters, C.L., and Beyreuther, K. (1999). Mechanism of the cleavage specificity of Alzheimer's disease gamma-secretase identified by phenylalanine-scanning mutagenesis of the transmembrane domain of the amyloid precursor protein. Proc Natl Acad Sci U S A *96*, 3053-3058.

Lin, C.R., Chen, W.S., Lazar, C.S., Carpenter, C.D., Gill, G.N., Evans, R.M., and Rosenfeld, M.G. (1986). Protein kinase C phosphorylation at Thr 654 of the unoccupied EGF receptor and EGF binding regulate functional receptor loss by independent mechanisms. Cell *44*, 839-848.

Lin, L., Georgievska, B., Mattsson, A., and Isacson, O. (1999). Cognitive changes and modified processing of amyloid precursor protein in the cortical and hippocampal system after cholinergic synapse loss and muscarinic receptor activation. Proc Natl Acad Sci U S A *96*, 12108-12113.

Liu, J., Liao, Z., Camden, J., Griffin, K.D., Garrad, R.C., Santiago-Perez, L.I., Gonzalez, F.A., Seye, C.I., Weisman, G.A., and Erb, L. (2004). Src homology 3 binding sites in the P2Y2 nucleotide receptor interact with Src and regulate activities of Src, proline-rich tyrosine kinase 2, and growth factor receptors. J Biol Chem *279*, 8212-8218.

Liu, K., Doms, R.W., and Lee, V.M. (2002). Glu11 site cleavage and N-terminally truncated A beta production upon BACE overexpression. Biochemistry *41*, 3128-3136.

Loechel, F., Gilpin, B.J., Engvall, E., Albrechtsen, R., and Wewer, U.M. (1998). Human ADAM 12 (meltrin alpha) is an active metalloprotease. J Biol Chem *273*, 16993-16997.

Loffler, J., and Huber, G. (1993). Modulation of beta-amyloid precursor protein secretion in differentiated and nondifferentiated cells. Biochem Biophys Res Commun *195*, 97-103.

Lorenzo, A., and Yankner, B.A. (1996). Amyloid fibril toxicity in Alzheimer's disease and diabetes. Ann N Y Acad Sci *777*, 89-95.

Ludwig, A., Hundhausen, C., Lambert, M.H., Broadway, N., Andrews, R.C., Bickett, D.M., Leesnitzer, M.A., and Becherer, J.D. (2005). Metalloproteinase inhibitors for the disintegrin-like metalloproteinases ADAM10 and ADAM17 that differentially block constitutive and phorbol ester-inducible shedding of cell surface molecules. Comb Chem High Throughput Screen *8*, 161-171.

Lum, L., Reid, M.S., and Blobel, C.P. (1998). Intracellular maturation of the mouse metalloprotease disintegrin MDC15. J Biol Chem *273*, 26236-26247.

Luo, Y., Bolon, B., Damore, M.A., Fitzpatrick, D., Liu, H., Zhang, J., Yan, Q., Vassar, R., and Citron, M. (2003). BACE1 (beta-secretase) knockout mice do not acquire compensatory gene expression changes or develop neural lesions over time. Neurobiol Dis *14*, 81-88.

Luo, Y., Bolon, B., Kahn, S., Bennett, B.D., Babu-Khan, S., Denis, P., Fan, W., Kha, H., Zhang, J., Gong, Y.*, et al.* (2001). Mice deficient in BACE1, the Alzheimer's beta-secretase, have normal phenotype and abolished beta-amyloid generation. Nat Neurosci *4*, 231-232.

Lustig, K.D., Erb, L., Landis, D.M., Hicks-Taylor, C.S., Zhang, X., Sportiello, M.G., and Weisman, G.A. (1992). Mechanisms by which extracellular ATP and UTP stimulate the release of prostacyclin from bovine pulmonary artery endothelial cells. Biochim Biophys Acta *1134*, 61-72.

Madden, D.R. (2002). The structure and function of glutamate receptor ion channels. Nat Rev Neurosci *3*, 91-101.

Malenka, R.C., and Bear, M.F. (2004). LTP and LTD: an embarrassment of riches. Neuron *44*, 5-21.

Marcello, E., Gardoni, F., Di Luca, M., and Perez-Otano, I. (2010). An arginine stretch limits ADAM10 exit from the endoplasmic reticulum. J Biol Chem *285*, 10376-10384.

Marcello, E., Gardoni, F., Mauceri, D., Romorini, S., Jeromin, A., Epis, R., Borroni, B., Cattabeni, F., Sala, C., Padovani, A.*, et al.* (2007). Synapse-associated protein-97 mediates alpha-secretase ADAM10 trafficking and promotes its activity. J Neurosci *27*, 1682-1691.

Marcinkiewicz, M., and Seidah, N.G. (2000). Coordinated expression of beta-amyloid precursor protein and the putative beta-secretase BACE and alpha-secretase ADAM10 in mouse and human brain. J Neurochem *75*, 2133-2143.

Maretzky, T., Schulte, M., Ludwig, A., Rose-John, S., Blobel, C., Hartmann, D., Altevogt, P., Saftig, P., and Reiss, K. (2005). L1 is sequentially processed by two differently activated metalloproteases and presenilin/gamma-secretase and regulates neural cell adhesion, cell migration, and neurite outgrowth. Mol Cell Biol *25*, 9040-9053.

Martin, L.J., Blackstone, C.D., Levey, A.I., Huganir, R.L., and Price, D.L. (1993). AMPA glutamate receptor subunits are differentially distributed in rat brain. Neuroscience *53*, 327-358.

Martinez-Arca, S., Coco, S., Mainguy, G., Schenk, U., Alberts, P., Bouille, P., Mezzina, M., Prochiantz, A., Matteoli, M., Louvard, D.*, et al.* (2001). A common exocytotic mechanism mediates axonal and dendritic outgrowth. J Neurosci *21*, 3830-3838.

Martinez, E., Chiang, C.M., Ge, H., and Roeder, R.G. (1994). TATA-binding protein-associated factor(s) in TFIID function through the initiator to direct basal transcription from a TATA-less class II promoter. EMBO J *13*, 3115-3126.

Masliah, E., Mallory, M., Alford, M., DeTeresa, R., Hansen, L.A., McKeel, D.W., Jr., and Morris, J.C. (2001). Altered expression of synaptic proteins occurs early during progression of Alzheimer's disease. Neurology *56*, 127-129.

Masliah, E., Mallory, M., Hansen, L., DeTeresa, R., Alford, M., and Terry, R. (1994). Synaptic and neuritic alterations during the progression of Alzheimer's disease. Neurosci Lett *174*, 67-72.

Masliah, E., and Terry, R. (1993). The role of synaptic proteins in the pathogenesis of disorders of the central nervous system. Brain Pathol *3*, 77-85.

Masters, C.L., Simms, G., Weinman, N.A., Multhaup, G., McDonald, B.L., and Beyreuther, K. (1985). Amyloid plaque core protein in Alzheimer disease and Down syndrome. Proc Natl Acad Sci U S A *82*, 4245-4249.

Masuo, Y., Ohtaki, T., Masuda, Y., Tsuda, M., and Fujino, M. (1992). Binding sites for pituitary adenylate cyclase activating polypeptide (PACAP): comparison with vasoactive intestinal polypeptide (VIP) binding site localization in rat brain sections. Brain Res *575*, 113-123.

Matsuda, S., Mikawa, S., and Hirai, H. (1999). Phosphorylation of serine-880 in GluR2 by protein kinase C prevents its C terminus from binding with glutamate receptor-interacting protein. J Neurochem *73*, 1765-1768.

Matsuzaki, M., Ellis-Davies, G.C., Nemoto, T., Miyashita, Y., Iino, M., and Kasai, H. (2001). Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons. Nat Neurosci *4*, 1086-1092.

Matsuzaki, M., Honkura, N., Ellis-Davies, G.C., and Kasai, H. (2004). Structural basis of long-term potentiation in single dendritic spines. Nature *429*, 761-766.

Mattson, M.P., Cheng, B., Culwell, A.R., Esch, F.S., Lieberburg, I., and Rydel, R.E. (1993). Evidence for excitoprotective and intraneuronal calcium-regulating roles for secreted forms of the beta-amyloid precursor protein. Neuron *10*, 243-254.

Mauceri, D., Cattabeni, F., Di Luca, M., and Gardoni, F. (2004). Calcium/calmodulin-dependent protein kinase II phosphorylation drives synapse-associated protein 97 into spines. J Biol Chem *279*, 23813-23821.

Maurice, T., Lockhart, B.P., and Privat, A. (1996). Amnesia induced in mice by centrally administered beta-amyloid peptides involves cholinergic dysfunction. Brain Res *706*, 181-193.

Maxfield, F.R., and McGraw, T.E. (2004). Endocytic recycling. Nat Rev Mol Cell Biol *5*, 121-132.

Mayer, M.L., and Armstrong, N. (2004). Structure and function of glutamate receptor ion channels. Annu Rev Physiol *66*, 161-181.

Mayer, M.L., Westbrook, G.L., and Guthrie, P.B. (1984). Voltage-dependent block by Mg2+ of NMDA responses in spinal cord neurones. Nature *309*, 261-263.

Mayor, S., Presley, J.F., and Maxfield, F.R. (1993). Sorting of membrane components from endosomes and subsequent recycling to the cell surface occurs by a bulk flow process. J Cell Biol *121*, 1257-1269.

McDonald, M.P., Dahl, E.E., Overmier, J.B., Mantyh, P., and Cleary, J. (1994). Effects of an exogenous beta-amyloid peptide on retention for spatial learning. Behav Neural Biol *62*, 60-67.

McGee, A.W., and Bredt, D.S. (1999). Identification of an intramolecular interaction between the SH3 and guanylate kinase domains of PSD-95. J Biol Chem *274*, 17431-17436.

McGee, A.W., and Bredt, D.S. (2003). Assembly and plasticity of the glutamatergic postsynaptic specialization. Curr Opin Neurobiol *13*, 111-118.

McKie, N., Edwards, T., Dallas, D.J., Houghton, A., Stringer, B., Graham, R., Russell, G., and Croucher, P.I. (1997). Expression of members of a novel membrane linked metalloproteinase family (ADAM) in human articular chondrocytes. Biochem Biophys Res Commun *230*, 335-339.

McLaughlin, M., Hale, R., Ellston, D., Gaudet, S., Lue, R.A., and Viel, A. (2002). The distribution and function of alternatively spliced insertions in hDlg. J Biol Chem *277*, 6406-6412.

McPhie, D.L., Coopersmith, R., Hines-Peralta, A., Chen, Y., Ivins, K.J., Manly, S.P., Kozlowski, M.R., Neve, K.A., and Neve, R.L. (2003). DNA synthesis and neuronal apoptosis caused by familial Alzheimer disease mutants of the amyloid precursor protein are mediated by the p21 activated kinase PAK3. J Neurosci *23*, 6914-6927.

Mechtersheimer, S., Gutwein, P., Agmon-Levin, N., Stoeck, A., Oleszewski, M., Riedle, S., Postina, R., Fahrenholz, F., Fogel, M., Lemmon, V.*, et al.* (2001). Ectodomain shedding of L1 adhesion molecule promotes cell migration by autocrine binding to integrins. J Cell Biol *155*, 661-673.

Meddows, E., Le Bourdelles, B., Grimwood, S., Wafford, K., Sandhu, S., Whiting, P., and McIlhinney, R.A. (2001). Identification of molecular determinants that are important in the assembly of N-methyl-D-aspartate receptors. J Biol Chem *276*, 18795-18803.

Melino, G., Draoui, M., Bernardini, S., Bellincampi, L., Reichert, U., and Cohen, P. (1996). Regulation by retinoic acid of insulin-degrading enzyme and of a related endoprotease in human neuroblastoma cell lines. Cell Growth Differ *7*, 787-796.

Meshorer, E., Erb, C., Gazit, R., Pavlovsky, L., Kaufer, D., Friedman, A., Glick, D., Ben-Arie, N., and Soreq, H. (2002). Alternative splicing and neuritic mRNA translocation under long-term neuronal hypersensitivity. Science *295*, 508-512.

Meyer-Luehmann, M., Spires-Jones, T.L., Prada, C., Garcia-Alloza, M., de Calignon, A., Rozkalne, A., Koenigsknecht-Talboo, J., Holtzman, D.M., Bacskai, B.J., and Hyman, B.T. (2008). Rapid appearance and local toxicity of amyloid-beta plaques in a mouse model of Alzheimer's disease. Nature *451*, 720-724.

Michikawa, M., Fan, Q.W., Isobe, I., and Yanagisawa, K. (2000). Apolipoprotein E exhibits isoform-specific promotion of lipid efflux from astrocytes and neurons in culture. J Neurochem *74*, 1008-1016.

Mihailovich, M., Thermann, R., Grohovaz, F., Hentze, M.W., and Zacchetti, D. (2007). Complex translational regulation of BACE1 involves upstream AUGs and stimulatory elements within the 5' untranslated region. Nucleic Acids Res *35*, 2975-2985.

Miller, D.L., Papayannopoulos, I.A., Styles, J., Bobin, S.A., Lin, Y.Y., Biemann, K., and Iqbal, K. (1993). Peptide compositions of the cerebrovascular and senile plaque core amyloid deposits of Alzheimer's disease. Arch Biochem Biophys *301*, 41-52.

Mills, J., and Reiner, P.B. (1999). Mitogen-activated protein kinase is involved in N-methyl-D-aspartate receptor regulation of amyloid precursor protein cleavage. Neuroscience *94*, 1333-1338.

Mingaud, F., Mormede, C., Etchamendy, N., Mons, N., Niedergang, B., Wietrzych, M., Pallet, V., Jaffard, R., Krezel, W., Higueret, P.*, et al.* (2008). Retinoid hyposignaling contributes to aging-related decline in hippocampal function in short-term/working memory organization and long-term declarative memory encoding in mice. J Neurosci *28*, 279-291.

Misner, D.L., Jacobs, S., Shimizu, Y., de Urquiza, A.M., Solomin, L., Perlmann, T., De Luca, L.M., Stevens, C.F., and Evans, R.M. (2001). Vitamin A deprivation results in reversible loss of hippocampal long-term synaptic plasticity. Proc Natl Acad Sci U S A *98*, 11714-11719.

Mitsuda, N., Roses, A.D., and Vitek, M.P. (1997). Transcriptional regulation of the mouse presenilin-1 gene. J Biol Chem *272*, 23489-23497.

Miyamoto, E. (2006). Molecular mechanism of neuronal plasticity: induction and maintenance of long-term potentiation in the hippocampus. J Pharmacol Sci *100*, 433-442.

Mochizuki, A., Peterson, J.W., Mufson, E.J., and Trapp, B.D. (1996). Amyloid load and neural elements in Alzheimer's disease and nondemented individuals with high amyloid plaque density. Exp Neurol *142*, 89-102.

Momiyama, A., Silver, R.A., Hausser, M., Notomi, T., Wu, Y., Shigemoto, R., and Cull-Candy, S.G. (2003). The density of AMPA receptors activated by a transmitter quantum at the climbing fibre-Purkinje cell synapse in immature rats. J Physiol *549*, 75-92.

Morris, M.C., Evans, D.A., Bienias, J.L., Tangney, C.C., Bennett, D.A., Aggarwal, N., Schneider, J., and Wilson, R.S. (2003). Dietary fats and the risk of incident Alzheimer disease. Arch Neurol *60*, 194-200.

Moss, M.L., and Bartsch, J.W. (2004). Therapeutic benefits from targeting of ADAM family members. Biochemistry *43*, 7227-7235.

Moss, M.L., Bomar, M., Liu, Q., Sage, H., Dempsey, P., Lenhart, P.M., Gillispie, P.A., Stoeck, A., Wildeboer, D., Bartsch, J.W.*, et al.* (2007). The ADAM10 prodomain is a specific inhibitor of ADAM10 proteolytic activity and inhibits cellular shedding events. J Biol Chem *282*, 35712-35721.

Moss, S.J., Blackstone, C.D., and Huganir, R.L. (1993). Phosphorylation of recombinant non-NMDA glutamate receptors on serine and tyrosine residues. Neurochem Res *18*, 105-110.

Mukaetova-Ladinska, E.B., Garcia-Siera, F., Hurt, J., Gertz, H.J., Xuereb, J.H., Hills, R., Brayne, C., Huppert, F.A., Paykel, E.S., McGee, M.*, et al.* (2000). Staging of cytoskeletal and beta-amyloid changes in human isocortex reveals biphasic synaptic protein response during progression of Alzheimer's disease. Am J Pathol *157*, 623-636.

Mullan, M., Crawford, F., Axelman, K., Houlden, H., Lilius, L., Winblad, B., and Lannfelt, L. (1992). A pathogenic mutation for probable Alzheimer's disease in the APP gene at the N-terminus of beta-amyloid. Nat Genet *1*, 345-347.

Muller, B.M., Kistner, U., Veh, R.W., Cases-Langhoff, C., Becker, B., Gundelfinger, E.D., and Garner, C.C. (1995). Molecular characterization and spatial distribution of SAP97, a novel presynaptic protein homologous to SAP90 and the Drosophila discs-large tumor suppressor protein. J Neurosci *15*, 2354-2366.

Muller, U., Cristina, N., Li, Z.W., Wolfer, D.P., Lipp, H.P., Rulicke, T., Brandner, S., Aguzzi, A., and Weissmann, C. (1994). Behavioral and anatomical deficits in mice homozygous for a modified beta-amyloid precursor protein gene. Cell *79*, 755-765.

Multhaup, G. (1994). Identification and regulation of the high affinity binding site of the Alzheimer's disease amyloid protein precursor (APP) to glycosaminoglycans. Biochimie *76*, 304-311.

Murdoch, C., and Finn, A. (2000). Chemokine receptors and their role in inflammation and infectious diseases. Blood *95*, 3032-3043.

Muresan, Z., and Muresan, V. (2005). Coordinated transport of phosphorylated amyloid-beta precursor protein and c-Jun NH2-terminal kinase-interacting protein-1. J Cell Biol *171*, 615-625.

Myers, A., Holmans, P., Marshall, H., Kwon, J., Meyer, D., Ramic, D., Shears, S., Booth, J., DeVrieze, F.W., Crook, R., *et al.* (2000). Susceptibility locus for Alzheimer's disease on chromosome 10. Science *290*, 2304-2305.

Nagano, O., Murakami, D., Hartmann, D., De Strooper, B., Saftig, P., Iwatsubo, T., Nakajima, M., Shinohara, M., and Saya, H. (2004). Cell-matrix interaction via CD44 is independently regulated by different metalloproteinases activated in response to extracellular Ca(2+) influx and PKC activation. J Cell Biol *165*, 893-902.

Nakagawa, S., and Takeichi, M. (1998). Neural crest emigration from the neural tube depends on regulated cadherin expression. Development *125*, 2963-2971.

Nakagawa, T., Futai, K., Lashuel, H.A., Lo, I., Okamoto, K., Walz, T., Hayashi, Y., and Sheng, M. (2004). Quaternary structure, protein dynamics, and synaptic function of SAP97 controlled by L27 domain interactions. Neuron *44*, 453-467.

Nakanishi, N., Shneider, N.A., and Axel, R. (1990). A family of glutamate receptor genes: evidence for the formation of heteromultimeric receptors with distinct channel properties. Neuron *5*, 569-581.

Nakashima, S. (2002). Protein kinase C alpha (PKC alpha): regulation and biological function. J Biochem *132*, 669-675.

Nash, J.E., Johnston, T.H., Collingridge, G.L., Garner, C.C., and Brotchie, J.M. (2005). Subcellular redistribution of the synapse-associated proteins PSD-95 and SAP97 in animal models of Parkinson's disease and L-DOPA-induced dyskinesia. FASEB J *19*, 583-585.

Nishi, E., Hiraoka, Y., Yoshida, K., Okawa, K., and Kita, T. (2006). Nardilysin enhances ectodomain shedding of heparin-binding epidermal growth factor-like growth factor through activation of tumor necrosis factor-alpha-converting enzyme. J Biol Chem *281*, 31164-31172.

Nishimoto, I., Okamoto, T., Matsuura, Y., Takahashi, S., Murayama, Y., and Ogata, E. (1993). Alzheimer amyloid protein precursor complexes with brain GTP-binding protein G(o). Nature *362*, 75-79.

Nitsch, R.M., Deng, A., Wurtman, R.J., and Growdon, J.H. (1997). Metabotropic glutamate receptor subtype mGluR1alpha stimulates the secretion of the amyloid beta-protein precursor ectodomain. J Neurochem *69*, 704-712.

Nitsch, R.M., Deng, M., Growdon, J.H., and Wurtman, R.J. (1996). Serotonin 5-HT2a and 5-HT2c receptors stimulate amyloid precursor protein ectodomain secretion. J Biol Chem *271*, 4188-4194.

Nitsch, R.M., Rossner, S., Albrecht, C., Mayhaus, M., Enderich, J., Schliebs, R., Wegner, M., Arendt, T., and von der Kammer, H. (1998). Muscarinic acetylcholine receptors activate the acetylcholinesterase gene promoter. J Physiol Paris *92*, 257-264.

Nitsch, R.M., Slack, B.E., Farber, S.A., Borghesani, P.R., Schulz, J.G., Kim, C., Felder, C.C., Growdon, J.H., and Wurtman, R.J. (1993). Receptor-coupled amyloid precursor protein processing. Ann N Y Acad Sci *695*, 122-127.

Nitsch, R.M., Slack, B.E., Farber, S.A., Schulz, J.G., Deng, M., Kim, C., Borghesani, P.R., Korver, W., Wurtman, R.J., and Growdon, J.H. (1994). Regulation of proteolytic processing of the amyloid beta-protein precursor of Alzheimer's disease in transfected cell lines and in brain slices. J Neural Transm Suppl *44*, 21-27.

Nitsch, R.M., Slack, B.E., Wurtman, R.J., and Growdon, J.H. (1992). Release of Alzheimer amyloid precursor derivatives stimulated by activation of muscarinic acetylcholine receptors. Science *258*, 304-307.

Nitsch, R.M., Wurtman, R.J., and Growdon, J.H. (1995). Regulation of proteolytic processing of the amyloid beta-protein precursor by first messengers. A novel potential approach for the treatment of Alzheimer's disease. Arzneimittelforschung *45*, 435-438.

Nitta, A., Itoh, A., Hasegawa, T., and Nabeshima, T. (1994). beta-Amyloid protein-induced Alzheimer's disease animal model. Neurosci Lett *170*, 63-66.

Nixon, R.A. (2005). Endosome function and dysfunction in Alzheimer's disease and other neurodegenerative diseases. Neurobiol Aging *26*, 373-382.

Nordstedt, C., Caporaso, G.L., Thyberg, J., Gandy, S.E., and Greengard, P. (1993). Identification of the Alzheimer beta/A4 amyloid precursor protein in clathrin-coated vesicles purified from PC12 cells. J Biol Chem *268*, 608-612.

Nowak, L., Bregestovski, P., Ascher, P., Herbet, A., and Prochiantz, A. (1984). Magnesium gates glutamate-activated channels in mouse central neurones. Nature *307*, 462-465.

Nukina, N., and Ihara, Y. (1986). One of the antigenic determinants of paired helical filaments is related to tau protein. J Biochem *99*, 1541-1544.

Nusser, Z., Lujan, R., Laube, G., Roberts, J.D., Molnar, E., and Somogyi, P. (1998). Cell type and pathway dependence of synaptic AMPA receptor number and variability in the hippocampus. Neuron *21*, 545-559.

Oddo, S., Caccamo, A., Shepherd, J.D., Murphy, M.P., Golde, T.E., Kayed, R., Metherate, R., Mattson, M.P., Akbari, Y., and LaFerla, F.M. (2003). Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. Neuron *39*, 409-421.

Ohno, M., Hiraoka, Y., Matsuoka, T., Tomimoto, H., Takao, K., Miyakawa, T., Oshima, N., Kiyonari, H., Kimura, T., Kita, T.*, et al.* (2009). Nardilysin regulates axonal maturation and myelination in the central and peripheral nervous system. Nat Neurosci *12*, 1506-1513.

Ohno, M., Sametsky, E.A., Younkin, L.H., Oakley, H., Younkin, S.G., Citron, M., Vassar, R., and Disterhoft, J.F. (2004). BACE1 deficiency rescues memory deficits and cholinergic dysfunction in a mouse model of Alzheimer's disease. Neuron *41*, 27-33.

Olson, J.M., Goddard, K.A., and Dudek, D.M. (2001). The amyloid precursor protein locus and very-late-onset Alzheimer disease. Am J Hum Genet *69*, 895-899.

Olson, M.I., and Shaw, C.M. (1969). Presenile dementia and Alzheimer's disease in mongolism. Brain *92*, 147-156.

Otto, C., Kovalchuk, Y., Wolfer, D.P., Gass, P., Martin, M., Zuschratter, W., Grone, H.J., Kellendonk, C., Tronche, F., Maldonado, R.*, et al.* (2001). Impairment of mossy fiber long-term potentiation and associative learning in pituitary adenylate cyclase activating polypeptide type I receptor-deficient mice. J Neurosci *21*, 5520-5527.

Paarmann, I., Spangenberg, O., Lavie, A., and Konrad, M. (2002). Formation of complexes between Ca2+.calmodulin and the synapse-associated protein SAP97 requires the SH3 domain-guanylate kinase domain-connecting HOOK region. J Biol Chem *277*, 40832-40838.

Pakaski, M., and Kasa, P. (2003). Role of acetylcholinesterase inhibitors in the metabolism of amyloid precursor protein. Curr Drug Targets CNS Neurol Disord *2*, 163-171.

Pakaski, M., Rakonczay, Z., and Kasa, P. (2001). Reversible and irreversible acetylcholinesterase inhibitors cause changes in neuronal amyloid precursor protein processing and protein kinase C level in vitro. Neurochem Int *38*, 219-226.

Palop, J.J., Jones, B., Kekonius, L., Chin, J., Yu, G.Q., Raber, J., Masliah, E., and Mucke, L. (2003). Neuronal depletion of calcium-dependent proteins in the dentate gyrus is tightly linked to Alzheimer's disease-related cognitive deficits. Proc Natl Acad Sci U S A *100*, 9572-9577.

Pan, D., and Rubin, G.M. (1997). Kuzbanian controls proteolytic processing of Notch and mediates lateral inhibition during Drosophila and vertebrate neurogenesis. Cell *90*, 271-280.

Pandiella, A., and Massague, J. (1991). Cleavage of the membrane precursor for transforming growth factor alpha is a regulated process. Proc Natl Acad Sci U S A *88*, 1726-1730.

Paradies, N.E., and Grunwald, G.B. (1993). Purification and characterization of NCAD90, a soluble endogenous form of N-cadherin, which is generated by proteolysis during retinal development and retains adhesive and neurite-promoting function. J Neurosci Res *36*, 33-45.

Park, J.H., Gimbel, D.A., GrandPre, T., Lee, J.K., Kim, J.E., Li, W., Lee, D.H., and Strittmatter, S.M. (2006). Alzheimer precursor protein interaction with the Nogo-66 receptor reduces amyloid-beta plaque deposition. J Neurosci *26*, 1386-1395.

Parkin, E., and Harris, B. (2009). A disintegrin and metalloproteinase (ADAM)-mediated ectodomain shedding of ADAM10. J Neurochem *108*, 1464-1479.

Parkin, E.T., Trew, A., Christie, G., Faller, A., Mayer, R., Turner, A.J., and Hooper, N.M. (2002). Structure-activity relationship of hydroxamate-based inhibitors on the secretases that cleave the amyloid precursor protein, angiotensin converting enzyme, CD23, and pro-tumor necrosis factor-alpha. Biochemistry *41*, 4972-4981.

Parkin, E.T., Watt, N.T., Turner, A.J., and Hooper, N.M. (2004). Dual mechanisms for shedding of the cellular prion protein. J Biol Chem *279*, 11170-11178.

Parr, C.E., Sullivan, D.M., Paradiso, A.M., Lazarowski, E.R., Burch, L.H., Olsen, J.C., Erb, L., Weisman, G.A., Boucher, R.C., and Turner, J.T. (1994). Cloning and expression of a human P2U nucleotide receptor, a target for cystic fibrosis pharmacotherapy. Proc Natl Acad Sci U S A *91*, 13067.

Parvathy, S., Hussain, I., Karran, E.H., Turner, A.J., and Hooper, N.M. (1998). Alzheimer's amyloid precursor protein alpha-secretase is inhibited by hydroxamic acid-based zinc metalloprotease inhibitors: similarities to the angiotensin converting enzyme secretase. Biochemistry *37*, 1680-1685.

Parvathy, S., Hussain, I., Karran, E.H., Turner, A.J., and Hooper, N.M. (1999). Cleavage of Alzheimer's amyloid precursor protein by alpha-secretase occurs at the surface of neuronal cells. Biochemistry *38*, 9728-9734.

Peiretti, F., Deprez-Beauclair, P., Bonardo, B., Aubert, H., Juhan-Vague, I., and Nalbone, G. (2003). Identification of SAP97 as an intracellular binding partner of TACE. J Cell Sci *116*, 1949-1957.

Pelletier, L., Jokitalo, E., and Warren, G. (2000). The effect of Golgi depletion on exocytic transport. Nat Cell Biol *2*, 840-846.

Pennypacker, K.R., Fuldner, R., Xu, R., Hernandez, H., Dawbarn, D., Mehta, N., Perez-Tur, J., Baker, M., and Hutton, M. (1998). Cloning and characterization of the presenilin-2 gene promoter. Brain Res Mol Brain Res *56*, 57-65.

Perez, R.G., Soriano, S., Hayes, J.D., Ostaszewski, B., Xia, W., Selkoe, D.J., Chen, X., Stokin, G.B., and Koo, E.H. (1999). Mutagenesis identifies new signals for beta-amyloid precursor protein endocytosis, turnover, and the generation of secreted fragments, including Abeta42. J Biol Chem *274*, 18851-18856.

Perez, R.G., Zheng, H., Van der Ploeg, L.H., and Koo, E.H. (1997). The beta-amyloid precursor protein of Alzheimer's disease enhances neuron viability and modulates neuronal polarity. J Neurosci *17*, 9407-9414.

Perry, A.C., Jones, R., and Hall, L. (1995). Analysis of transcripts encoding novel members of the mammalian metalloprotease-like, disintegrin-like, cysteine-rich (MDC) protein family and their expression in reproductive and non-reproductive monkey tissues. Biochem J *312 (Pt 1)*, 239-244.

Petralia, R.S., and Wenthold, R.J. (1992). Light and electron immunocytochemical localization of AMPA-selective glutamate receptors in the rat brain. J Comp Neurol *318*, 329-354.

Piccoli, G., Verpelli, C., Tonna, N., Romorini, S., Alessio, M., Nairn, A.C., Bachi, A., and Sala, C. (2007). Proteomic analysis of activity-dependent synaptic plasticity in hippocampal neurons. J Proteome Res *6*, 3203-3215.

Pierce, J.P., Mayer, T., and McCarthy, J.B. (2001). Evidence for a satellite secretory pathway in neuronal dendritic spines. Curr Biol *11*, 351-355.

Pietrzik, C.U., Yoon, I.S., Jaeger, S., Busse, T., Weggen, S., and Koo, E.H. (2004). FE65 constitutes the functional link between the low-density lipoprotein receptor-related protein and the amyloid precursor protein. J Neurosci *24*, 4259-4265.

Poorkaj, P., Bird, T.D., Wijsman, E., Nemens, E., Garruto, R.M., Anderson, L., Andreadis, A., Wiederholt, W.C., Raskind, M., and Schellenberg, G.D. (1998). Tau is a candidate gene for chromosome 17 frontotemporal dementia. Ann Neurol *43*, 815-825.

Popov, V., Medvedev, N.I., Davies, H.A., and Stewart, M.G. (2005). Mitochondria form a filamentous reticular network in hippocampal dendrites but are present as discrete bodies in axons: a three-dimensional ultrastructural study. J Comp Neurol *492*, 50-65.

Postina, R., Schroeder, A., Dewachter, I., Bohl, J., Schmitt, U., Kojro, E., Prinzen, C., Endres, K., Hiemke, C., Blessing, M.*, et al.* (2004). A disintegrin-metalloproteinase prevents amyloid plaque formation and hippocampal defects in an Alzheimer disease mouse model. J Clin Invest *113*, 1456-1464.

Potter, J.J., Cheneval, D., Dang, C.V., Resar, L.M., Mezey, E., and Yang, V.W. (1991). The upstream stimulatory factor binds to and activates the promoter of the rat class I alcohol dehydrogenase gene. J Biol Chem *266*, 15457-15463.

Presley, J.F., Cole, N.B., Schroer, T.A., Hirschberg, K., Zaal, K.J., and Lippincott-Schwartz, J. (1997). ER-to-Golgi transport visualized in living cells. Nature *389*, 81-85.

Price, D.L., Tanzi, R.E., Borchelt, D.R., and Sisodia, S.S. (1998). Alzheimer's disease: genetic studies and transgenic models. Annu Rev Genet *32*, 461-493.

Primakoff, P., and Myles, D.G. (2000). The ADAM gene family: surface proteins with adhesion and protease activity. Trends Genet *16*, 83-87.

Prinzen, C., Muller, U., Endres, K., Fahrenholz, F., and Postina, R. (2005). Genomic structure and functional characterization of the human ADAM10 promoter. FASEB J *19*, 1522-1524.

Qiu, Y., Wu, X.J., and Chen, H.Z. (2003). Simultaneous changes in secretory amyloid precursor protein and beta-amyloid peptide release from rat hippocampus by activation of muscarinic receptors. Neurosci Lett *352*, 41-44.

Racca, C., Stephenson, F.A., Streit, P., Roberts, J.D., and Somogyi, P. (2000). NMDA receptor content of synapses in stratum radiatum of the hippocampal CA1 area. J Neurosci *20*, 2512-2522.

Racchi, M., Baetta, R., Salvietti, N., Ianna, P., Franceschini, G., Paoletti, R., Fumagalli, R., Govoni, S., Trabucchi, M., and Soma, M. (1997). Secretory processing of amyloid precursor protein is inhibited by increase in cellular cholesterol content. Biochem J *322 (Pt 3)*, 893-898.

Racchi, M., Sironi, M., Caprera, A., Konig, G., and Govoni, S. (2001). Short- and long-term effect of acetylcholinesterase inhibition on the expression and metabolism of the amyloid precursor protein. Mol Psychiatry *6*, 520-528.

Racchi, M., Solano, D.C., Sironi, M., and Govoni, S. (1999). Activity of alpha-secretase as the common final effector of protein kinase C-dependent and -independent modulation of amyloid precursor protein metabolism. J Neurochem *72*, 2464-2470.

Racz, B., Blanpied, T.A., Ehlers, M.D., and Weinberg, R.J. (2004). Lateral organization of endocytic machinery in dendritic spines. Nat Neurosci *7*, 917-918.

Ralevic, V., and Burnstock, G. (1998). Receptors for purines and pyrimidines. Pharmacol Rev *50*, 413-492.

Rambourg, A., and Clermont, Y. (1990). Three-dimensional electron microscopy: structure of the Golgi apparatus. Eur J Cell Biol *51*, 189-200.

Ramirez, O.A., and Couve, A. (2011). The endoplasmic reticulum and protein trafficking in dendrites and axons. Trends Cell Biol *21*, 219-227.

Rapoport, M., Dawson, H.N., Binder, L.I., Vitek, M.P., and Ferreira, A. (2002). Tau is essential to beta -amyloid-induced neurotoxicity. Proc Natl Acad Sci U S A *99*, 6364-6369.

Refolo, L.M., Malester, B., LaFrancois, J., Bryant-Thomas, T., Wang, R., Tint, G.S., Sambamurti, K., Duff, K., and Pappolla, M.A. (2000). Hypercholesterolemia accelerates the Alzheimer's amyloid pathology in a transgenic mouse model. Neurobiol Dis *7*, 321-331.

Regalado, M.P., Terry-Lorenzo, R.T., Waites, C.L., Garner, C.C., and Malenka, R.C. (2006). Transsynaptic signaling by postsynaptic synapse-associated protein 97. J Neurosci *26*, 2343-2357.

Reglodi, D., Somogyvari-Vigh, A., Vigh, S., Maderdrut, J.L., and Arimura, A. (2000). Neuroprotective effects of PACAP38 in a rat model of transient focal ischemia under various experimental conditions. Ann N Y Acad Sci *921*, 119-128.

Reiss, K., Maretzky, T., Haas, I.G., Schulte, M., Ludwig, A., Frank, M., and Saftig, P. (2006). Regulated ADAM10-dependent ectodomain shedding of gamma-protocadherin C3 modulates cell-cell adhesion. J Biol Chem *281*, 21735-21744.

Reiss, K., Maretzky, T., Ludwig, A., Tousseyn, T., de Strooper, B., Hartmann, D., and Saftig, P. (2005). ADAM10 cleavage of N-cadherin and regulation of cell-cell adhesion and beta-catenin nuclear signalling. EMBO J *24*, 742-752.

Rietveld, I., Janssen, J.A., Van Duijn, C.M., and Lamberts, S.W. (2003). A polymorphic CA repeat in the promoter region of the insulin-like growth factor-I (IGF-I) gene. Eur J Epidemiol *18*, 191-193.

Rinaldi, P., Polidori, M.C., Metastasio, A., Mariani, E., Mattioli, P., Cherubini, A., Catani, M., Cecchetti, R., Senin, U., and Mecocci, P. (2003). Plasma antioxidants are similarly depleted in mild cognitive impairment and in Alzheimer's disease. Neurobiol Aging *24*, 915-919.

Robakis, N.K., Ramakrishna, N., Wolfe, G., and Wisniewski, H.M. (1987). Molecular cloning and characterization of a cDNA encoding the cerebrovascular and the neuritic plaque amyloid peptides. Proc Natl Acad Sci U S A *84*, 4190-4194.

Roberts, S.B., Ripellino, J.A., Ingalls, K.M., Robakis, N.K., and Felsenstein, K.M. (1994). Non-amyloidogenic cleavage of the beta-amyloid precursor protein by an integral membrane metalloendopeptidase. J Biol Chem *269*, 3111-3116.

Roche, K.W., O'Brien, R.J., Mammen, A.L., Bernhardt, J., and Huganir, R.L. (1996). Characterization of multiple phosphorylation sites on the AMPA receptor GluR1 subunit. Neuron *16*, 1179-1188.

Rogers, G.W., Jr., Edelman, G.M., and Mauro, V.P. (2004). Differential utilization of upstream AUGs in the beta-secretase mRNA suggests that a shunting mechanism regulates translation. Proc Natl Acad Sci U S A *101*, 2794-2799.

Rogers, S.L., Doody, R.S., Mohs, R.C., and Friedhoff, L.T. (1998). Donepezil improves cognition and global function in Alzheimer disease: a 15-week, double-blind, placebo-controlled study. Donepezil Study Group. Arch Intern Med *158*, 1021-1031.

Roghani, M., Becherer, J.D., Moss, M.L., Atherton, R.E., Erdjument-Bromage, H., Arribas, J., Blackburn, R.K., Weskamp, G., Tempst, P., and Blobel, C.P. (1999). Metalloprotease-disintegrin MDC9: intracellular maturation and catalytic activity. J Biol Chem *274*, 3531-3540.

Roher, A.E., Lowenson, J.D., Clarke, S., Wolkow, C., Wang, R., Cotter, R.J., Reardon, I.M., Zurcher-Neely, H.A., Heinrikson, R.L., Ball, M.J.*, et al.* (1993). Structural alterations in the peptide backbone of beta-amyloid core protein may account for its deposition and stability in Alzheimer's disease. J Biol Chem *268*, 3072-3083.

Roncarati, R., Sestan, N., Scheinfeld, M.H., Berechid, B.E., Lopez, P.A., Meucci, O., McGlade, J.C., Rakic, P., and D'Adamio, L. (2002). The gamma-secretase-generated intracellular domain of beta-amyloid precursor protein binds Numb and inhibits Notch signaling. Proc Natl Acad Sci U S A *99*, 7102-7107.

Rooke, J., Pan, D., Xu, T., and Rubin, G.M. (1996). KUZ, a conserved metalloprotease-disintegrin protein with two roles in Drosophila neurogenesis. Science *273*, 1227-1231.

Roonprapunt, C., Huang, W., Grill, R., Friedlander, D., Grumet, M., Chen, S., Schachner, M., and Young, W. (2003). Soluble cell adhesion molecule L1-Fc promotes locomotor recovery in rats after spinal cord injury. J Neurotrauma *20*, 871-882.

Rosen, D.R., Martin-Morris, L., Luo, L.Q., and White, K. (1989). A Drosophila gene encoding a protein resembling the human beta-amyloid protein precursor. Proc Natl Acad Sci U S A *86*, 2478-2482.

Rossor, M.N., Fox, N.C., Freeborough, P.A., and Harvey, R.J. (1996). Clinical features of sporadic and familial Alzheimer's disease. Neurodegeneration *5*, 393-397.

Roy, A.L., Meisterernst, M., Pognonec, P., and Roeder, R.G. (1991). Cooperative interaction of an initiator-binding transcription initiation factor and the helix-loop-helix activator USF. Nature *354*, 245-248.

Ruiz-Canada, C., Koh, Y.H., Budnik, V., and Tejedor, F.J. (2002). DLG differentially localizes Shaker K+-channels in the central nervous system and retina of Drosophila. J Neurochem *82*, 1490-1501.

Rumbaugh, G., Sia, G.M., Garner, C.C., and Huganir, R.L. (2003). Synapse-associated protein-97 isoform-specific regulation of surface AMPA receptors and synaptic function in cultured neurons. J Neurosci *23*, 4567-4576.

Sacchetti, B., Lorenzini, C.A., Baldi, E., Bucherelli, C., Roberto, M., Tassoni, G., and Brunelli, M. (2001). Pituitary adenylate cyclase-activating polypeptide hormone (PACAP) at very low dosages improves memory in the rat. Neurobiol Learn Mem *76*, 1-6.

Saitoh, T., Masliah, E., Jin, L.W., Cole, G.M., Wieloch, T., and Shapiro, I.P. (1991). Protein kinases and phosphorylation in neurologic disorders and cell death. Lab Invest *64*, 596-616.

Sala, C., Piech, V., Wilson, N.R., Passafaro, M., Liu, G., and Sheng, M. (2001). Regulation of dendritic spine morphology and synaptic function by Shank and Homer. Neuron *31*, 115-130.

Sampo, B., Kaech, S., Kunz, S., and Banker, G. (2003). Two distinct mechanisms target membrane proteins to the axonal surface. Neuron *37*, 611-624.

Sastre, M., Dewachter, I., Rossner, S., Bogdanovic, N., Rosen, E., Borghgraef, P., Evert, B.O., Dumitrescu-Ozimek, L., Thal, D.R., Landreth, G.*, et al.* (2006). Nonsteroidal anti-inflammatory drugs repress beta-secretase gene promoter activity by the activation of PPARgamma. Proc Natl Acad Sci U S A *103*, 443-448.

Sastre, M., Steiner, H., Fuchs, K., Capell, A., Multhaup, G., Condron, M.M., Teplow, D.B., and Haass, C. (2001). Presenilin-dependent gamma-secretase processing of beta-amyloid precursor protein at a site corresponding to the S3 cleavage of Notch. EMBO Rep *2*, 835-841.

Sato, J., Shimazu, D., Yamamoto, N., and Nishikawa, T. (2008). An association analysis of synapse-associated protein 97 (SAP97) gene in schizophrenia. J Neural Transm *115*, 1355-1365.

Sawadogo, M. (1988). Multiple forms of the human gene-specific transcription factor USF. II. DNA binding properties and transcriptional activity of the purified HeLa USF. J Biol Chem *263*, 11994-12001.

Schafer, W., Stroh, A., Berghofer, S., Seiler, J., Vey, M., Kruse, M.L., Kern, H.F., Klenk, H.D., and Garten, W. (1995). Two independent targeting signals in the cytoplasmic domain determine trans-Golgi network localization and endosomal trafficking of the proprotein convertase furin. EMBO J *14*, 2424-2435.

Scheff, S.W., DeKosky, S.T., and Price, D.A. (1990). Quantitative assessment of cortical synaptic density in Alzheimer's disease. Neurobiol Aging *11*, 29-37.

Scheff, S.W., Price, D.A., Schmitt, F.A., DeKosky, S.T., and Mufson, E.J. (2007). Synaptic alterations in CA1 in mild Alzheimer disease and mild cognitive impairment. Neurology *68*, 1501-1508.

Scheff, S.W., Price, D.A., Schmitt, F.A., and Mufson, E.J. (2006). Hippocampal synaptic loss in early Alzheimer's disease and mild cognitive impairment. Neurobiol Aging *27*, 1372-1384.

Scheiffele, P. (2003). Cell-cell signaling during synapse formation in the CNS. Annu Rev Neurosci *26*, 485-508.

Scheuner, D., Eckman, C., Jensen, M., Song, X., Citron, M., Suzuki, N., Bird, T.D., Hardy, J., Hutton, M., Kukull, W.*, et al.* (1996). Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. Nat Med *2*, 864-870.

Schlondorff, J., and Blobel, C.P. (1999). Metalloprotease-disintegrins: modular proteins capable of promoting cell-cell interactions and triggering signals by protein-ectodomain shedding. J Cell Sci *112 (Pt 21)*, 3603-3617.

Schluter, O.M., Xu, W., and Malenka, R.C. (2006). Alternative N-terminal domains of PSD-95 and SAP97 govern activity-dependent regulation of synaptic AMPA receptor function. Neuron *51*, 99-111.

Schrader, A.M., Camden, J.M., and Weisman, G.A. (2005). P2Y2 nucleotide receptor up-regulation in submandibular gland cells from the NOD.B10 mouse model of Sjogren's syndrome. Arch Oral Biol *50*, 533-540.

Schwiebert, E.M., and Zsembery, A. (2003). Extracellular ATP as a signaling molecule for epithelial cells. Biochim Biophys Acta *1615*, 7-32.

Scott, E.K., and Luo, L. (2001). How do dendrites take their shape? Nat Neurosci *4*, 359-365.

Seabrook, G.R., Smith, D.W., Bowery, B.J., Easter, A., Reynolds, T., Fitzjohn, S.M., Morton, R.A., Zheng, H., Dawson, G.R., Sirinathsinghji, D.J.*, et al.* (1999). Mechanisms contributing to the deficits in hippocampal synaptic plasticity in mice lacking amyloid precursor protein. Neuropharmacology *38*, 349-359.

Selkoe, D., and Kopan, R. (2003). Notch and Presenilin: regulated intramembrane proteolysis links development and degeneration. Annu Rev Neurosci *26*, 565-597.

Selkoe, D.J. (1991). The molecular pathology of Alzheimer's disease. Neuron *6*, 487-498.

Selkoe, D.J. (2000). Toward a comprehensive theory for Alzheimer's disease. Hypothesis: Alzheimer's disease is caused by the cerebral accumulation and cytotoxicity of amyloid beta-protein. Ann N Y Acad Sci *924*, 17-25.

Selkoe, D.J. (2008). Soluble oligomers of the amyloid beta-protein impair synaptic plasticity and behavior. Behav Brain Res *192*, 106-113.

Selkoe, D.J., Abraham, C.R., Podlisny, M.B., and Duffy, L.K. (1986). Isolation of low-molecular-weight proteins from amyloid plaque fibers in Alzheimer's disease. J Neurochem *46*, 1820-1834.

Seubert, P., Oltersdorf, T., Lee, M.G., Barbour, R., Blomquist, C., Davis, D.L., Bryant, K., Fritz, L.C., Galasko, D., Thal, L.J.*, et al.* (1993). Secretion of beta-amyloid precursor protein cleaved at the amino terminus of the beta-amyloid peptide. Nature *361*, 260-263.

Seubert, P., Vigo-Pelfrey, C., Esch, F., Lee, M., Dovey, H., Davis, D., Sinha, S., Schlossmacher, M., Whaley, J., Swindlehurst, C.*, et al.* (1992). Isolation and quantification of soluble Alzheimer's beta-peptide from biological fluids. Nature *359*, 325-327.

Seye, C.I., Gadeau, A.P., Daret, D., Dupuch, F., Alzieu, P., Capron, L., and Desgranges, C. (1997). Overexpression of P2Y2 purinoceptor in intimal lesions of the rat aorta. Arterioscler Thromb Vasc Biol *17*, 3602-3610.

Seye, C.I., Kong, Q., Erb, L., Garrad, R.C., Krugh, B., Wang, M., Turner, J.T., Sturek, M., Gonzalez, F.A., and Weisman, G.A. (2002). Functional P2Y2 nucleotide receptors mediate uridine 5'-triphosphate-induced intimal hyperplasia in collared rabbit carotid arteries. Circulation *106*, 2720-2726.

Seye, C.I., Yu, N., Gonzalez, F.A., Erb, L., and Weisman, G.A. (2004). The P2Y2 nucleotide receptor mediates vascular cell adhesion molecule-1 expression through interaction with VEGF receptor-2 (KDR/Flk-1). J Biol Chem *279*, 35679-35686.

Seye, C.I., Yu, N., Jain, R., Kong, Q., Minor, T., Newton, J., Erb, L., Gonzalez, F.A., and Weisman, G.A. (2003). The P2Y2 nucleotide receptor mediates UTP-induced vascular cell adhesion molecule-1 expression in coronary artery endothelial cells. J Biol Chem *278*, 24960-24965.

Sheng, M. (2001). Molecular organization of the postsynaptic specialization. Proc Natl Acad Sci U S A *98*, 7058-7061.

Sheng, M., and Hoogenraad, C.C. (2007). The postsynaptic architecture of excitatory synapses: a more quantitative view. Annu Rev Biochem *76*, 823-847.

Sheng, M., and Kim, E. (2000). The Shank family of scaffold proteins. J Cell Sci *113 (Pt 11)*, 1851-1856.

Sherrington, R., Rogaev, E.I., Liang, Y., Rogaeva, E.A., Levesque, G., Ikeda, M., Chi, H., Lin, C., Li, G., Holman, K*., et al.* (1995). Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. Nature *375*, 754-760.

Sherwood, N.M., Krueckl, S.L., and McRory, J.E. (2000). The origin and function of the pituitary adenylate cyclase-activating polypeptide (PACAP)/glucagon superfamily. Endocr Rev *21*, 619-670.

Shi, S.H., Hayashi, Y., Petralia, R.S., Zaman, S.H., Wenthold, R.J., Svoboda, K., and Malinow, R. (1999). Rapid spine delivery and redistribution of AMPA receptors after synaptic NMDA receptor activation. Science *284*, 1811-1816.

Shivers, B.D., Gorcs, T.J., Gottschall, P.E., and Arimura, A. (1991). Two high affinity binding sites for pituitary adenylate cyclase-activating polypeptide have different tissue distributions. Endocrinology *128*, 3055-3065.

Shivers, B.D., Hilbich, C., Multhaup, G., Salbaum, M., Beyreuther, K., and Seeburg, P.H. (1988). Alzheimer's disease amyloidogenic glycoprotein: expression pattern in rat brain suggests a role in cell contact. EMBO J *7*, 1365-1370.

Shoji, M., Golde, T.E., Ghiso, J., Cheung, T.T., Estus, S., Shaffer, L.M., Cai, X.D., McKay, D.M., Tintner, R., Frangione, B*., et al.* (1992). Production of the Alzheimer amyloid beta protein by normal proteolytic processing. Science *258*, 126-129.

Simons, M., Keller, P., De Strooper, B., Beyreuther, K., Dotti, C.G., and Simons, K. (1998). Cholesterol depletion inhibits the generation of beta-amyloid in hippocampal neurons. Proc Natl Acad Sci U S A *95*, 6460-6464.

Sing, C.F., and Davignon, J. (1985). Role of the apolipoprotein E polymorphism in determining normal plasma lipid and lipoprotein variation. Am J Hum Genet *37*, 268-285.

Sinha, S., Anderson, J.P., Barbour, R., Basi, G.S., Caccavello, R., Davis, D., Doan, M., Dovey, H.F., Frigon, N., Hong, J*., et al.* (1999). Purification and cloning of amyloid precursor protein beta-secretase from human brain. Nature *402*, 537-540.

Sisodia, S.S. (1992). Beta-amyloid precursor protein cleavage by a membrane-bound protease. Proc Natl Acad Sci U S A *89*, 6075-6079.

Skovronsky, D.M., Moore, D.B., Milla, M.E., Doms, R.W., and Lee, V.M. (2000). Protein kinase C-dependent alpha-secretase competes with beta-secretase for cleavage of amyloid-beta precursor protein in the trans-golgi network. J Biol Chem *275*, 2568-2575.

Slack, B.E., Breu, J., Muchnicki, L., and Wurtman, R.J. (1997). Rapid stimulation of amyloid precursor protein release by epidermal growth factor: role of protein kinase C. Biochem J *327 (Pt 1)*, 245-249.

Slack, B.E., Nitsch, R.M., Livneh, E., Kunz, G.M., Jr., Breu, J., Eldar, H., and Wurtman, R.J. (1993). Regulation by phorbol esters of amyloid precursor protein release from Swiss 3T3 fibroblasts overexpressing protein kinase C alpha. J Biol Chem *268*, 21097-21101.

Smith, R.P., Higuchi, D.A., and Broze, G.J., Jr. (1990). Platelet coagulation factor XIa-inhibitor, a form of Alzheimer amyloid precursor protein. Science *248*, 1126-1128.

Snyder, E.M., Nong, Y., Almeida, C.G., Paul, S., Moran, T., Choi, E.Y., Nairn, A.C., Salter, M.W., Lombroso, P.J., Gouras, G.K*., et al.* (2005). Regulation of NMDA receptor trafficking by amyloid-beta. Nat Neurosci *8*, 1051-1058.

Soba, P., Eggert, S., Wagner, K., Zentgraf, H., Siehl, K., Kreger, S., Lower, A., Langer, A., Merdes, G., Paro, R., *et al.* (2005). Homo- and heterodimerization of APP family members promotes intercellular adhesion. EMBO J *24*, 3624-3634.

Soltoff, S.P. (1998). Related adhesion focal tyrosine kinase and the epidermal growth factor receptor mediate the stimulation of mitogen-activated protein kinase by the G-protein-coupled P2Y2 receptor. Phorbol ester or [Ca2+]i elevation can substitute for receptor activation. J Biol Chem *273*, 23110-23117.

Spacek, J., and Harris, K.M. (1997). Three-dimensional organization of smooth endoplasmic reticulum in hippocampal CA1 dendrites and dendritic spines of the immature and mature rat. J Neurosci *17*, 190-203.

Spillantini, M.G., Bird, T.D., and Ghetti, B. (1998). Frontotemporal dementia and Parkinsonism linked to chromosome 17: a new group of tauopathies. Brain Pathol *8*, 387-402.

Stephan, A., Laroche, S., and Davis, S. (2001). Generation of aggregated beta-amyloid in the rat hippocampus impairs synaptic transmission and plasticity and causes memory deficits. J Neurosci *21*, 5703-5714.

Stoeck, A., Keller, S., Riedle, S., Sanderson, M.P., Runz, S., Le Naour, F., Gutwein, P., Ludwig, A., Rubinstein, E., and Altevogt, P. (2006). A role for exosomes in the constitutive and stimulus-induced ectodomain cleavage of L1 and CD44. Biochem J *393*, 609-618.

Struhl, G., and Greenwald, I. (1999). Presenilin is required for activity and nuclear access of Notch in Drosophila. Nature *398*, 522-525.

Sturm, R.A., Das, G., and Herr, W. (1988). The ubiquitous octamer-binding protein Oct-1 contains a POU domain with a homeo box subdomain. Genes Dev *2*, 1582-1599.

Suda, K., Smith, D.M., Ghatei, M.A., Murphy, J.K., and Bloom, S.R. (1991). Investigation and characterization of receptors for pituitary adenylate cyclase-activating polypeptide in human brain by radioligand binding and chemical cross-linking. J Clin Endocrinol Metab *72*, 958-964.

Sudhof, T.C. (2004). The synaptic vesicle cycle. Annu Rev Neurosci *27*, 509-547.

Sugiyama, Y., Kawabata, I., Sobue, K., and Okabe, S. (2005). Determination of absolute protein numbers in single synapses by a GFP-based calibration technique. Nat Methods *2*, 677-684.

Suzuki, A., Hayashida, M., Ito, T., Kawano, H., Nakano, T., Miura, M., Akahane, K., and Shiraki, K. (2000). Survivin initiates cell cycle entry by the competitive interaction with Cdk4/p16(INK4a) and Cdk2/cyclin E complex activation. Oncogene *19*, 3225-3234.

Suzuki, N., Cheung, T.T., Cai, X.D., Odaka, A., Otvos, L., Jr., Eckman, C., Golde, T.E., and Younkin, S.G. (1994). An increased percentage of long amyloid beta protein secreted by familial amyloid beta protein precursor (beta APP717) mutants. Science *264*, 1336-1340.

Takahashi, J., Palmer, T.D., and Gage, F.H. (1999). Retinoic acid and neurotrophins collaborate to regulate neurogenesis in adult-derived neural stem cell cultures. J Neurobiol *38*, 65-81.

Takahashi, R.H., Milner, T.A., Li, F., Nam, E.E., Edgar, M.A., Yamaguchi, H., Beal, M.F., Xu, H., Greengard, P., and Gouras, G.K. (2002). Intraneuronal Alzheimer abeta42 accumulates in multivesicular bodies and is associated with synaptic pathology. Am J Pathol *161*, 1869-1879.

Tanaka, H., Grooms, S.Y., Bennett, M.V., and Zukin, R.S. (2000). The AMPAR subunit GluR2: still front and center-stage. Brain Res *886*, 190-207.

Tanaka, J., Matsuzaki, M., Tarusawa, E., Momiyama, A., Molnar, E., Kasai, H., and Shigemoto, R. (2005). Number and density of AMPA receptors in single synapses in immature cerebellum. J Neurosci *25*, 799-807.

Tanzi, R.E., Bird, E.D., Latt, S.A., and Neve, R.L. (1987a). The amyloid beta protein gene is not duplicated in brains from patients with Alzheimer's disease. Science *238*, 666-669.

Tanzi, R.E., Gusella, J.F., Watkins, P.C., Bruns, G.A., St George-Hyslop, P., Van Keuren, M.L., Patterson, D., Pagan, S., Kurnit, D.M., and Neve, R.L. (1987b). Amyloid beta protein gene: cDNA, mRNA distribution, and genetic linkage near the Alzheimer locus. Science *235*, 880-884.

Terry, R.D. (1996). The pathogenesis of Alzheimer disease: an alternative to the amyloid hypothesis. J Neuropathol Exp Neurol *55*, 1023-1025.

Terry, R.D., Masliah, E., Salmon, D.P., Butters, N., DeTeresa, R., Hill, R., Hansen, L.A., and Katzman, R. (1991). Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. Ann Neurol *30*, 572-580.

Thomas, U., Ebitsch, S., Gorczyca, M., Koh, Y.H., Hough, C.D., Woods, D., Gundelfinger, E.D., and Budnik, V. (2000). Synaptic targeting and localization of discs-large is a stepwise process controlled by different domains of the protein. Curr Biol *10*, 1108-1117.

Tippmann, F., Hundt, J., Schneider, A., Endres, K., and Fahrenholz, F. (2009). Up-regulation of the alpha-secretase ADAM10 by retinoic acid receptors and acitretin. FASEB J *23*, 1643-1654.

Togashi, H., Miyoshi, J., Honda, T., Sakisaka, T., Takai, Y., and Takeichi, M. (2006). Interneurite affinity is regulated by heterophilic nectin interactions in concert with the cadherin machinery. J Cell Biol *174*, 141-151.

Torre, E.R., and Steward, O. (1996). Protein synthesis within dendrites: glycosylation of newly synthesized proteins in dendrites of hippocampal neurons in culture. J Neurosci *16*, 5967-5978.

Toyooka, K., Iritani, S., Makifuchi, T., Shirakawa, O., Kitamura, N., Maeda, K., Nakamura, R., Niizato, K., Watanabe, M., Kakita, A.*, et al.* (2002). Selective reduction of a PDZ protein, SAP-97, in the prefrontal cortex of patients with chronic schizophrenia. J Neurochem *83*, 797-806.

Trinh, N.H., Hoblyn, J., Mohanty, S., and Yaffe, K. (2003). Efficacy of cholinesterase inhibitors in the treatment of neuropsychiatric symptoms and functional impairment in Alzheimer disease: a meta-analysis. JAMA *289*, 210-216.

Turner, J.T., Redman, R.S., Camden, J.M., Landon, L.A., and Quissell, D.O. (1998). A rat parotid gland cell line, Par-C10, exhibits neurotransmitter-regulated transepithelial anion secretion. Am J Physiol *275*, C367-374.

Turner, J.T., Weisman, G.A., and Camden, J.M. (1997). Upregulation of P2Y2 nucleotide receptors in rat salivary gland cells during short-term culture. Am J Physiol *273*, C1100-1107.

Tyler, S.J., Dawbarn, D., Wilcock, G.K., and Allen, S.J. (2002). alpha- and beta-secretase: profound changes in Alzheimer's disease. Biochem Biophys Res Commun *299*, 373-376.

Uchida, D., Arimura, A., Somogyvari-Vigh, A., Shioda, S., and Banks, W.A. (1996). Prevention of ischemia-induced death of hippocampal neurons by pituitary adenylate cyclase activating polypeptide. Brain Res *736*, 280-286.

Uemura, K., Kihara, T., Kuzuya, A., Okawa, K., Nishimoto, T., Ninomiya, H., Sugimoto, H., Kinoshita, A., and Shimohama, S. (2006). Characterization of sequential N-cadherin cleavage by ADAM10 and PS1. Neurosci Lett *402*, 278-283.

Vale, R.D., and Fletterick, R.J. (1997). The design plan of kinesin motors. Annu Rev Cell Dev Biol *13*, 745-777.

Vale, R.D., Reese, T.S., and Sheetz, M.P. (1985). Identification of a novel force-generating protein, kinesin, involved in microtubule-based motility. Cell *42*, 39-50.

Van Broeckhoven, C., Haan, J., Bakker, E., Hardy, J.A., Van Hul, W., Wehnert, A., Vegter-Van der Vlis, M., and Roos, R.A. (1990). Amyloid beta protein precursor gene and hereditary cerebral hemorrhage with amyloidosis (Dutch). Science *248*, 1120-1122.

Vassar, R. (2001). The beta-secretase, BACE: a prime drug target for Alzheimer's disease. J Mol Neurosci *17*, 157-170.

Vassar, R., Bennett, B.D., Babu-Khan, S., Kahn, S., Mendiaz, E.A., Denis, P., Teplow, D.B., Ross, S., Amarante, P., Loeloff, R.*, et al.* (1999). Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. Science *286*, 735-741.

Vaudry, D., Gonzalez, B.J., Basille, M., Yon, L., Fournier, A., and Vaudry, H. (2000). Pituitary adenylate cyclase-activating polypeptide and its receptors: from structure to functions. Pharmacol Rev *52*, 269-324.

Verga, L., Frangione, B., Tagliavini, F., Giaccone, G., Migheli, A., and Bugiani, O. (1989). Alzheimer patients and Down patients: cerebral preamyloid deposits differ ultrastructurally and histochemically from the amyloid of senile plaques. Neurosci Lett *105*, 294-299.

Villanueva de la Torre, T., Bech-Serra, J.J., Ruiz-Paz, S., Baselga, J., and Arribas, J. (2004). Inactivating mutations block the tumor necrosis factor-alpha-converting enzyme in the early secretory pathway. Biochem Biophys Res Commun *314*, 1028-1035.

Vincent, B. (2004). ADAM proteases: protective role in Alzheimer's and prion diseases? Curr Alzheimer Res *1*, 165-174.

Wakabayashi, K., Hayashi, S., Morita, T., Shibasaki, Y., Watanabe, Y., and Takahashi, H. (1999a). Neurofibrillary tangles in the peripheral sympathetic ganglia of non-Alzheimer elderly individuals. Clin Neuropathol *18*, 171-175.

Wakabayashi, K., Narisawa-Saito, M., Iwakura, Y., Arai, T., Ikeda, K., Takahashi, H., and Nawa, H. (1999b). Phenotypic down-regulation of glutamate receptor subunit GluR1 in Alzheimer's disease. Neurobiol Aging *20*, 287-295.

Wakabayashi, T., Craessaerts, K., Bammens, L., Bentahir, M., Borgions, F., Herdewijn, P., Staes, A., Timmerman, E., Vandekerckhove, J., Rubinstein, E.*, et al.* (2009). Analysis of the gamma-secretase interactome and validation of its association with tetraspanin-enriched microdomains. Nat Cell Biol *11*, 1340-1346.

Walikonis, R.S., Jensen, O.N., Mann, M., Provance, D.W., Jr., Mercer, J.A., and Kennedy, M.B. (2000). Identification of proteins in the postsynaptic density fraction by mass spectrometry. J Neurosci *20*, 4069-4080.

Walter, J., Capell, A., Hung, A.Y., Langen, H., Schnolzer, M., Thinakaran, G., Sisodia, S.S., Selkoe, D.J., and Haass, C. (1997). Ectodomain phosphorylation of beta-amyloid precursor protein at two distinct cellular locations. J Biol Chem *272*, 1896-1903.

Walter, J., Fluhrer, R., Hartung, B., Willem, M., Kaether, C., Capell, A., Lammich, S., Multhaup, G., and Haass, C. (2001). Phosphorylation regulates intracellular trafficking of beta-secretase. J Biol Chem *276*, 14634-14641.

Wang, H.Y., Pisano, M.R., and Friedman, E. (1994). Attenuated protein kinase C activity and translocation in Alzheimer's disease brain. Neurobiol Aging *15*, 293-298.

Wang, L., Piserchio, A., and Mierke, D.F. (2005). Structural characterization of the intermolecular interactions of synapse-associated protein-97 with the NR2B subunit of N-methyl-D-aspartate receptors. J Biol Chem *280*, 26992-26996.

Wasco, W., Bupp, K., Magendantz, M., Gusella, J.F., Tanzi, R.E., and Solomon, F. (1992). Identification of a mouse brain cDNA that encodes a protein related to the Alzheimer disease-associated amyloid beta protein precursor. Proc Natl Acad Sci U S A *89*, 10758-10762.

Wasco, W., Gurubhagavatula, S., Paradis, M.D., Romano, D.M., Sisodia, S.S., Hyman, B.T., Neve, R.L., and Tanzi, R.E. (1993). Isolation and characterization of APLP2 encoding a homologue of the Alzheimer's associated amyloid beta protein precursor. Nat Genet *5*, 95-100.

Washburn, M.S., Numberger, M., Zhang, S., and Dingledine, R. (1997). Differential dependence on GluR2 expression of three characteristic features of AMPA receptors. J Neurosci *17*, 9393-9406.

Wavrant-DeVrieze, F., Lambert, J.C., Stas, L., Crook, R., Cottel, D., Pasquier, F., Frigard, B., Lambrechts, M., Thiry, E., Amouyel, P.*, et al.* (1999). Association between coding variability in the LRP gene and the risk of late-onset Alzheimer's disease. Hum Genet *104*, 432-434.

Weerasinghe, D., McHugh, K.P., Ross, F.P., Brown, E.J., Gisler, R.H., and Imhof, B.A. (1998). A role for the alphavbeta3 integrin in the transmigration of monocytes. J Cell Biol *142*, 595-607.

Weisgraber, K.H., and Mahley, R.W. (1996). Human apolipoprotein E: the Alzheimer's disease connection. FASEB J *10*, 1485-1494.

Weisman, G.A., Turner, J.T., and Fedan, J.S. (1996). Structure and function of P2 purinocepters. J Pharmacol Exp Ther *277*, 1-9.

Weskamp, G., Kratzschmar, J., Reid, M.S., and Blobel, C.P. (1996). MDC9, a widely expressed cellular disintegrin containing cytoplasmic SH3 ligand domains. J Cell Biol *132*, 717-726.

Westphalen, R.I., Scott, H.L., and Dodd, P.R. (2003). Synaptic vesicle transport and synaptic membrane transporter sites in excitatory amino acid nerve terminals in Alzheimer disease. J Neural Transm *110*, 1013-1027.

White, J.M. (2003). ADAMs: modulators of cell-cell and cell-matrix interactions. Curr Opin Cell Biol *15*, 598-606.

Winblad, B., Palmer, K., Kivipelto, M., Jelic, V., Fratiglioni, L., Wahlund, L.O., Nordberg, A., Backman, L., Albert, M., Almkvist, O.*, et al.* (2004). Mild cognitive impairment--beyond controversies, towards a consensus: report of the International Working Group on Mild Cognitive Impairment. J Intern Med *256*, 240-246.

Winckler, B., and Mellman, I. (1999). Neuronal polarity: controlling the sorting and diffusion of membrane components. Neuron *23*, 637-640.

Wisniewski, T., Ghiso, J., and Frangione, B. (1991). Peptides homologous to the amyloid protein of Alzheimer's disease containing a glutamine for glutamic acid substitution have accelerated amyloid fibril formation. Biochem Biophys Res Commun *179*, 1247-1254.

Wolf, B.A., Wertkin, A.M., Jolly, Y.C., Yasuda, R.P., Wolfe, B.B., Konrad, R.J., Manning, D., Ravi, S., Williamson, J.R., and Lee, V.M. (1995). Muscarinic regulation of Alzheimer's disease amyloid precursor protein secretion and amyloid beta-protein production in human neuronal NT2N cells. J Biol Chem *270*, 4916-4922.

Wolfe, M.S., De Los Angeles, J., Miller, D.D., Xia, W., and Selkoe, D.J. (1999a). Are presenilins intramembrane-cleaving proteases? Implications for the molecular mechanism of Alzheimer's disease. Biochemistry *38*, 11223-11230.

Wolfe, M.S., Xia, W., Ostaszewski, B.L., Diehl, T.S., Kimberly, W.T., and Selkoe, D.J. (1999b). Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and gamma-secretase activity. Nature *398*, 513-517.

Wolfsberg, T.G., Primakoff, P., Myles, D.G., and White, J.M. (1995a). ADAM, a novel family of membrane proteins containing A Disintegrin And Metalloprotease domain: multipotential functions in cell-cell and cell-matrix interactions. J Cell Biol *131*, 275-278.

Wolfsberg, T.G., Straight, P.D., Gerena, R.L., Huovila, A.P., Primakoff, P., Myles, D.G., and White, J.M. (1995b). ADAM, a widely distributed and developmentally regulated gene family encoding membrane proteins with a disintegrin and metalloprotease domain. Dev Biol *169*, 378-383.

Wolfsberg, T.G., and White, J.M. (1996). ADAMs in fertilization and development. Dev Biol *180*, 389-401.

Woods, D.F., and Bryant, P.J. (1991). The discs-large tumor suppressor gene of Drosophila encodes a guanylate kinase homolog localized at septate junctions. Cell *66*, 451-464.

Workman, J.L., Roeder, R.G., and Kingston, R.E. (1990). An upstream transcription factor, USF (MLTF), facilitates the formation of preinitiation complexes during in vitro chromatin assembly. EMBO J *9*, 1299-1308.

Xu, D., Sharma, C., and Hemler, M.E. (2009). Tetraspanin12 regulates ADAM10-dependent cleavage of amyloid precursor protein. FASEB J *23*, 3674-3681.

Xu, H., Greengard, P., and Gandy, S. (1995). Regulated formation of Golgi secretory vesicles containing Alzheimer beta-amyloid precursor protein. J Biol Chem *270*, 23243-23245.

Xu, H., Sweeney, D., Greengard, P., and Gandy, S. (1996). Metabolism of Alzheimer beta-amyloid precursor protein: regulation by protein kinase A in intact cells and in a cell-free system. Proc Natl Acad Sci U S A *93*, 4081-4084.

Xu, H., Sweeney, D., Wang, R., Thinakaran, G., Lo, A.C., Sisodia, S.S., Greengard, P., and Gandy, S. (1997). Generation of Alzheimer beta-amyloid protein in the trans-Golgi network in the apparent absence of vesicle formation. Proc Natl Acad Sci U S A *94*, 3748-3752.

Yamaguchi, H., Ishiguro, K., Sugihara, S., Nakazato, Y., Kawarabayashi, T., Sun, X., and Hirai, S. (1994). Presence of apolipoprotein E on extracellular neurofibrillary tangles and on meningeal blood vessels precedes the Alzheimer beta-amyloid deposition. Acta Neuropathol *88*, 413-419.

Yamazaki, K., Mizui, Y., Sagane, K., and Tanaka, I. (1997a). Assignment of a disintegrin and metalloproteinase domain 10 (Adam10) gene to mouse chromosome 9. Genomics *46*, 528-529.

Yamazaki, K., Mizui, Y., and Tanaka, I. (1997b). Radiation hybrid mapping of human ADAM10 gene to chromosome 15. Genomics *45*, 457-459.

Yamazaki, T., Koo, E.H., and Selkoe, D.J. (1996). Trafficking of cell-surface amyloid beta-protein precursor. II. Endocytosis, recycling and lysosomal targeting detected by immunolocalization. J Cell Sci *109 (Pt 5)*, 999-1008.

Yan, R., Bienkowski, M.J., Shuck, M.E., Miao, H., Tory, M.C., Pauley, A.M., Brashier, J.R., Stratman, N.C., Mathews, W.R., Buhl, A.E.*, et al.* (1999). Membrane-anchored aspartyl protease with Alzheimer's disease beta-secretase activity. Nature *402*, 533-537.

Yang, L.B., Lindholm, K., Yan, R., Citron, M., Xia, W., Yang, X.L., Beach, T., Sue, L., Wong, P., Price, D.*, et al.* (2003). Elevated beta-secretase expression and enzymatic activity detected in sporadic Alzheimer disease. Nat Med *9*, 3-4.

Yang, P., Baker, K.A., and Hagg, T. (2005). A disintegrin and metalloprotease 21 (ADAM21) is associated with neurogenesis and axonal growth in developing and adult rodent CNS. J Comp Neurol *490*, 163-179.

Yao, P.J., Zhu, M., Pyun, E.I., Brooks, A.I., Therianos, S., Meyers, V.E., and Coleman, P.D. (2003). Defects in expression of genes related to synaptic vesicle trafficking in frontal cortex of Alzheimer's disease. Neurobiol Dis *12*, 97-109.

Yokoyama, C., Wang, X., Briggs, M.R., Admon, A., Wu, J., Hua, X., Goldstein, J.L., and Brown, M.S. (1993). SREBP-1, a basic-helix-loop-helix-leucine zipper protein that controls transcription of the low density lipoprotein receptor gene. Cell *75*, 187-197.

Yoshinaka, T., Nishii, K., Yamada, K., Sawada, H., Nishiwaki, E., Smith, K., Yoshino, K., Ishiguro, H., and Higashiyama, S. (2002). Identification and characterization of novel mouse and human ADAM33s with potential metalloprotease activity. Gene *282*, 227-236.

Zakharenko, S., and Popov, S. (1998). Dynamics of axonal microtubules regulate the topology of new membrane insertion into the growing neurites. J Cell Biol *143*, 1077-1086.

Zaman, Z., Roche, S., Fielden, P., Frost, P.G., Niriella, D.C., and Cayley, A.C. (1992). Plasma concentrations of vitamins A and E and carotenoids in Alzheimer's disease. Age Ageing *21*, 91-94.

Zheng, H., Jiang, M., Trumbauer, M.E., Hopkins, R., Sirinathsinghji, D.J., Stevens, K.A., Conner, M.W., Slunt, H.H., Sisodia, S.S., Chen, H.Y.*, et al.* (1996). Mice deficient for the amyloid precursor protein gene. Ann N Y Acad Sci *777*, 421-426.

Zheng, H., Jiang, M., Trumbauer, M.E., Sirinathsinghji, D.J., Hopkins, R., Smith, D.W., Heavens, R.P., Dawson, G.R., Boyce, S., Conner, M.W.*, et al.* (1995). beta-Amyloid precursor protein-deficient mice show reactive gliosis and decreased locomotor activity. Cell *81*, 525-531.

Zheng, P., Eastman, J., Vande Pol, S., and Pimplikar, S.W. (1998). PAT1, a microtubule-interacting protein, recognizes the basolateral sorting signal of amyloid precursor protein. Proc Natl Acad Sci U S A *95*, 14745-14750.

Zhou, W., and Song, W. (2006). Leaky scanning and reinitiation regulate BACE1 gene expression. Mol Cell Biol *26*, 3353-3364.

Zhu, G., Wang, D., Lin, Y.H., McMahon, T., Koo, E.H., and Messing, R.O. (2001). Protein kinase C epsilon suppresses Abeta production and promotes activation of alpha-secretase. Biochem Biophys Res Commun *285*, 997-1006.

Zhu, J.J., Esteban, J.A., Hayashi, Y., and Malinow, R. (2000). Postnatal synaptic potentiation: delivery of GluR4-containing AMPA receptors by spontaneous activity. Nat Neurosci *3*, 1098-1106.

Zimmermann, M., Gardoni, F., Marcello, E., Colciaghi, F., Borroni, B., Padovani, A., Cattabeni, F., and Di Luca, M. (2004). Acetylcholinesterase inhibitors increase ADAM10 activity by promoting its trafficking in neuroblastoma cell lines. J Neurochem *90*, 1489-1499.

Zuber, B., Nikonenko, I., Klauser, P., Muller, D., and Dubochet, J. (2005). The mammalian central nervous synaptic cleft contains a high density of periodically organized complexes. Proc Natl Acad Sci U S A *102*, 19192-19197.

Zukin, R.S., and Bennett, M.V. (1995). Alternatively spliced isoforms of the NMDARI receptor subunit. Trends Neurosci *18*, 306-313.