Targeting the Role of the Endosome in the Pathophysiology of Alzheimer's Disease: A Strategy for Treatment

Barbara A. Tate and Paul M. Mathews

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Membrane-bound endosomal vesicles play an integral role in multiple cellular events, including protein processing and turnover, and often critically regulate the cell-surface availability of receptors and other plasma membrane proteins in many different cell types. Neurons are no exception, being dependent on endosomal function for housekeeping and synaptic events. Growing evidence suggests a link between neuronal endosomal function and Alzheimer's disease (AD) pathophysiology. Endosomal abnormalities invariably occur within neurons in AD brains, and endocytic compartments are one likely site for the production of the pathogenic β-amyloid peptide (Aβ), which accumulates within the brain during the disease and is generated by proteolytic processing of the amyloid precursor protein (APP). The enzymes and events involved in APP processing are appealing targets for therapeutic agents aimed at slowing or reversing the pathogenesis of AD. The neuronal endosome may well prove to be the intracellular site of action for inhibitors of β-amyloidogenic APP processing. We present here the view that knowledge of the endosomal system in the disease can guide drug discovery of AD therapeutic agents.

A Brief Overview of Endocytosis

A discussion of the role of endocytic compartments in the pathogenesis of Alzheimer's disease (AD; see http://sageke.sciencemag.org/cgi/content/full/2001/1/dn2) is well served by a brief overview of the endocytic system. The endocytic system consists of a number of acidic, dynamically interacting vesicular compartments that regulate the movement of substances within the cell as well as between cells and the extracellular environment (*1*, *2*). The process by which the plasma membrane is invaginated is termed endocytosis, and the small, membrane-bound vesicle that is formed is termed an endosome. Once formed, endosomes can undergo a maturation process, adding and exchanging material through various fusion events while transporting, and in some cases recycling, their luminal and membrane contents (*1*, *2*). Each maturational stage of the endosome is characterized by the presence of specific functional and regulator proteins, which are often uniquely associated with a particular endocytic compartment (*1*, *3*, *4*). For example, the initial compartment in the endocytic pathway, the early endosome, is associated with the small GTPase rab5, which participates in the formation of the early endosome itself (*5-11*). Rab5 and its partners have been frequently used as markers of early endosomes in cells and human brain tissue (*12-16*). Once formed, the early endosome can contribute membrane and contents to late endosomes. Alternatively, material can be recycled back to the plasma membrane, making early endosomes unique in that they are in rapid communication with both the plasma membrane and distal compartments in the endosomal-lysosomal pathway (*1-3*, *17*). In addition to content from the early endosome, the late endosome receives material from the trans-Golgi network (TGN). Much of this TGN-derived material consists of newly synthesized acid hydrolases ultimately destined for the lysosome (*18*, *19*). Like early endosomes, late endosomes have their own unique constituents and associated regulatory proteins, which can be used to identify them. The terminal compartment in this system is the highly acidic lysosome, where final degradation of vesicle contents delivered from the late endosome occurs. The robust hydrolysis within the lysosome rapidly degrades its luminal contents, producing amino acids, carbohydrates, and lipids that can then be reused by the cell.

Endocytosis plays an important role in a number of cellular processes, including (i) bringing extracellular material into the cell, (ii) turning over cell-surface receptors and other membrane-associated proteins (*17*), (iii) internalizing and metabolizing lipids (*20*), and, in neurons, (iv) recycling of synaptic vesicles (*21*). Although not nearly as proteolytically active as the lysosome, there is a growing appreciation of the role of limited proteolysis within the endosome in membrane protein processing and signal transduction events (*17*). For instance, endocytosis has been shown to play a role in the cleavage of the Notch protein (http://sageke.sciencemag.org/cgi/content/full/2003/48/pe34), leading to downstream signaling (*22*), processing events long thought to occur solely at the cell surface. The amyloid precursor protein (APP; see http://sageke.sciencemag.org/cgi/genedata/sagekeGdbGene;197) is also likely to be processed within endosomes, which we discuss in the following section.

The Role of the Endosomal System in APP Processing and Aβ Production

APP is a ubiquitously expressed transmembrane protein localized both to the cell surface and to intracellular compartments, including the TGN and endosomes (*23*, *24*). After their synthesis, APP molecules can traffic from the TGN to the plasma membrane, where at least a portion of the APP pool is internalized again into early endosomes (*23, 25, 26*) and can be subsequently processed into the Aβ peptide (*27*), which is believed to play a central role in AD pathophysiology (*28*) (see "Detangling Alzheimer's Disease" at http://sageke.sciencemag.org/cgi/content/full/2003/43/oa2). Although the function of APP is still

The authors are at CNS Discovery, Global Research and Development, Pfizer Inc., Groton, CT 06234, USA (B.A.T.) and the Department of Psychiatry at the New York University School of Medicine, Center for Dementia Research, Nathan Kline Institute for Psychiatric Research, Orangeburg, NY 10962, USA (P.M.M). E-mail: barbara.tate@pfizer.com (B.A.T.), mathews@nki.rfmh.org (P.M.M.)

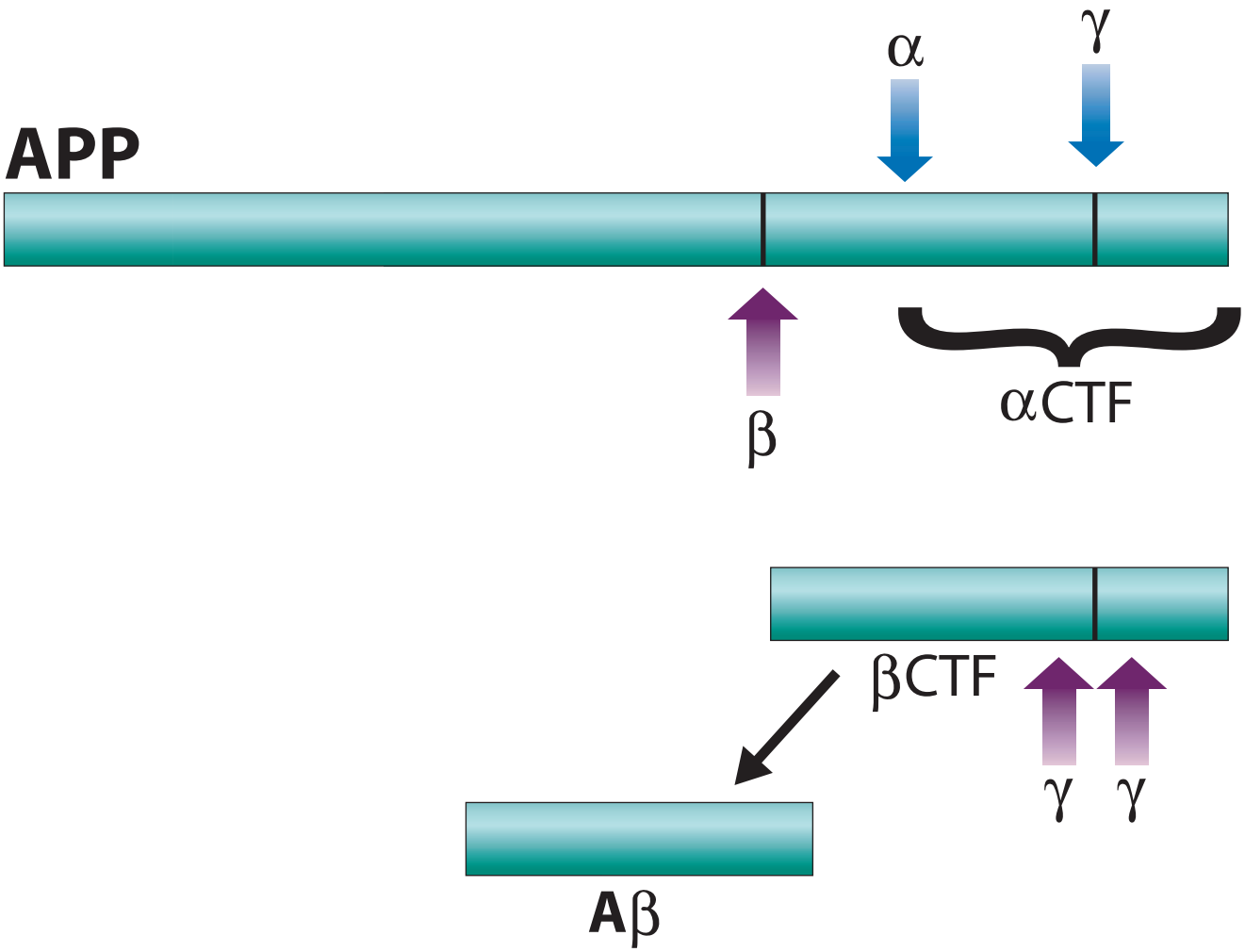

Fig. 1. A schematic representation of the sequence of enzymatic events that result in the production of the Aβ peptide from APP. α, α-secretase; β, β-secretase; γ, γ-secretase.

unclear, much progress has been made toward understanding its proteolytic processing, both the processing that leads to Aβ generation and the competing pathways that prevent the production of the Aβ peptide from a given APP molecule (Fig. 1). In addition to the bulk degradation of APP in lysosomes (*29*), an important pathway that prevents Aβ production is the so-called α-secretory pathway. A metalloprotease member of the ADAM family cleaves APP (*30-33*) within the Aβ sequence, preventing the generation of Aβ and releasing an extracellular, soluble fragment (sAPPα); a C-terminal fragment (CTF) remains associated with the membrane (*34*). Although this α-secretase-cleaved CTF (αCTF) is subsequently cleaved by the γ-secretase complex (which also functions to generate Aβ; see below), the small fragment released (termed P3) is not amyloidogenic and appears to have no role in AD pathogenesis. α-Secretase cleavage is believed to occur predominantly at the cell surface, although other compartments in the secretory pathway, such as the TGN, may contribute to α-secretase cleavage of APP.

An alternate series of cleavage events generates the disease-associated Aβ peptides. In this situation, cleavage of APP by the β-secretase [BACE (*35*, *36*)] generates another extracellular, soluble fragment (sAPPβ) that differs from the α-secretase fragment by only 16 amino acid residues. β-Secretase cleavage also produces a membrane-associated CTF (the βCTF); β-secretase cleavage generates the N terminus of Aβ, so the βCTF contains the whole Aβ peptide sequence. The βCTF is the immediate precursor of Aβ, and β-secretase cleavage is generally thought to be the rate-limiting step in Aβ production (*23*, *37*). Like the αCTF, the βCTF is subsequently cleaved within the membrane by the γ-secretase complex; however, in this case, γ-secretase cleavage releases the pathogenic Aβ peptide (*23*, *24*). Aβ is secreted from the cell into the extracellular space, where it aggregates and accumulates as the pathological hallmark of AD, the β-amyloid plaque. This suggests that Aβ itself is generated at the plasma membrane or within the lumen of vesicular compartments that are in communication with the extracellular space, such as endosomes.

The BACE null mouse appears generally normal and makes no Aβ (*38-40*); thus, BACE activity is a very attractive target for AD therapeutic agents (*41*). After conversion of the proenzyme to an active form by a furin-like protease (*42-44*), BACE localizes to multiple subcellular compartments, including the Golgi network, the cell surface, and within endosomes (*35*, *42*, *45-52*), but not within the lysosome where it appears to be de-

graded (*53*). This complex intracellular distribution of both the substrate, APP, and the protease, BACE, suggests that BACE cleavage could occur in multiple compartments. However, several lines of evidence suggest that endocytic compartments are likely candidate sites for at least some β-secretase cleavage of APP. First, βCTFs have been shown to colocalize with endocytic markers (*15*, *54*). However, the intracellular trafficking of βCTFs may be complex (*54*), and they are clearly found in other compartments, such as the TGN (*15*). The pH optimum for BACE suggests that it may directly function in an endoctytic compartment. BACE enzymatic activity is maximal at an acidic pH of 5, and little activity remains at a neutral pH (*55*). The acidic pH of endocytic compartments, although not as acidic at the pH optimum for BACE, is a reasonably hospitable environment for its enzymatic activity.

Interestingly, BACE may interact with APP at the plasma membrane where the complex may then be endocytosed, as suggested by studies using exogenously applied BACE (*56*). BACE-mediated cleavage of APP in the endocytic system dovetails well with previous work that has identified, through various trafficking mutants of APP, the early endosome as an important site for Aβ generation (*25-27*, *57*). The cytoplasmic domain of APP can be phosphorylated at multiple sites, and various phosphorylation events appear to alter APP localization and thus APP processing, in some cases promoting cell-surface localization and α-secretase cleavage (*58*). Important to this discussion, APP phosphorylated at Thr^{668} within the cytoplasmic domain has been shown to colocalize with BACE in endocytic compartments (*59*). Removing the phosphorylation site through mutagenesis decreased APP internalization and decreased Aβ production, additional support that Aβ production is subsequent to the endocytosis of APP. The trafficking of BACE also appears to be regulated by its phosphorylation. Blocking phosphorylation of BACE at residue Ser^{498} by mutagenesis results in retention of BACE within early endosomes (*45*, *47*), whereas phosphocompetent BACE was found both within the Golgi and within endosomes. Thus, the current evidence suggests that endosomes are likely to play an important role in the β-secretase cleavage of APP, although other compartments within the cell may well contribute to some βCTF generation.

Experimental evidence suggests that, like BACE, the γ-secretase is found in multiple vesicular compartments within the cell. Resolving the subcellular localization of the γ-secretase has been facilitated by the finding that the functional enzyme consists of a complex of four transmembrane proteins [presenilin, nicastrin, APH-1, and PEN-2 (*60, 61*) (see Wolfe Perspective at http://sageke.sciencemag.org/cgi/content/full/2003/11/pe7)], which must assemble for the complex to become active (*61, 62*) and to exit the endoplasmic reticulum (*63-66*). The γ-secretase complex has been localized to the Golgi (*67*), the plasma membrane (*68-71*), endocytic compartments (*72, 73*), lysosomes (*74*), and autophagic vacuoles (*75*), degradative compartments upstream from the lysosome that are in close communication with endocytic compartments (see Cuervo Perspective at http://sageke.sciencemag.org/cgi/content/full/2003/36/pe25). Although various proteolytic functions of the γ-secretase complex have been assigned to the plasma membrane [for example, Notch signaling (*76, 77*) and E-cadherin cleavage (*78*)], the site of γ-secretase cleavage of the βCTF remains unresolved. Nevertheless, the identification of the γ-secretase complex within endocytic and related compartments (autophagic vacuoles and lysosomes) suggest that these compartments should have the capacity to produce Aβ (*79, 80*).

The Neuronal Endocytic System in AD and Related Disorders

Over the past decade, the field's understanding of the proteases responsible for β-amyloidogenic APP processing and the probable subcellular compartmentalization of these events has combined with other lines of evidence showing endocytic alterations in AD and AD-relevant diseases to suggest that endosomes and endocytic activity may be central to early events in AD pathophysiology, perhaps even leading to overproduction of Aβ by neurons (*80*, *81*). In brain tissue derived from AD patients with Aβ deposition restricted to the entorhinal cortex, abnormally large early endosomes are found not only in neurons in the temporal cortices but also in frontal-cortical neurons (*13*, *14*). These individuals are thought to represent a patient population in the earliest stages of the disease; this finding suggests that neuronal endocytic changes occur during the pathogenesis of AD before extracellular β-amyloid deposition within a given brain region. This idea—that endocytic alterations are not a consequence of Aβ accumulation—derives further support from the finding that early-onset AD patients harboring AD-associated familial mutations in presenilin (http://sageke.sciencemag.org/cgi/genedata/sagekeGdbGene;198), patients whose brains contain abundant β-amyloid plaques, do not show swollen neuronal endosomes (*12*). Given that the mechanism driving Aβ deposition in such patients is dependent on the overproduction of the highly amyloidogenic Aβ42 (a version of Aβ that is 42 residues in length) as a result of alterations in presenilin-mediated γ-cleavage of APP [(*82-84*); reviewed in (*85*)], it is not unexpected that the mechanism(s) of β-amyloidosis and the involvement of neuronal endocytic compartments are different in late-onset, sporadic AD. Thus, increases in extracellular amyloid deposition reflect increased production of amyloidogenic Aβ species, and several mechanisms, including genetic mutations in proteins that make up the enzymatic machinery, can drive overproduction. In familial AD, the mechanism appears to be alterations in enzyme-substrate affinities and activities. In sporadic AD, the early endosomal abnormalities suggest enzyme-substrate compartmentalization that ultimately results in enhanced Aβ production.

Several predictions can be made based on a model in which early-disease changes in endocytic function would promote greater neuronal Aβ production from a given pool of APP molecules. First, given sufficient sensitivity, Aβ should be detectable in endocytic compartments within neurons, which has now been reported by a number of groups using immunolabeling of human brain samples at the light and electron microscopic levels (*86-89*). Indeed, intracellular Aβ has been directly colocalized with rab5-immunoreactive early endosomes in early-stage AD cases (*89*). Concentrations of soluble Aβ (in other words, Aβ that is not deposited into extracellular β-amyloid plaques), were high in these brains (*89*), suggesting that the earliest evidence of an increase in Aβ in the AD brain corresponds with changes in the endosomal system. Another prediction is that modeling these endocytic alterations in cells should result in an increase in β-amyloidogenic APP processing; likewise, finding them in other disease states should also be associated with such an increase. In AD neurons, the pattern and intensity of immunolabeling for a number of regulators of early endocytic events strongly suggests that endocytosis itself is increased in the disease (*13*, *14*, *81*). Overexpression of rab5, which leads to

increased endocytosis and swollen early endosomes reminiscent of those seen in AD, has been shown to (i) increase endocytosis of APP, (ii) increase βCTF concentrations, and (iii) enhance Aβ production in transfected cells (*15*).

Down syndrome

Additional evidence that AD-like endosomal alterations play a role in increased Aβ production comes from two other disorders of the central nervous system (CNS). Individuals with Down syndrome (DS, caused by trisomy 21) inevitably develop accumulations of Aβ and AD (*90*-*92*) (see Devenny Case Study at http://sageke.sciencemag.org/cgi/content/full/2005/14/dn1). The *APP* gene is on human chromosome 21, and it has long been thought that the predisposition of DS patients to AD is due to overexpression of *APP* resulting from the increased gene dosage. Although a single additional copy of *APP* is sufficient to cause AD in humans (*93*), the situation in DS may be more complex and pernicious. Neurons in the DS brain, like those in the AD brain, have abnormal early endosomes that contain Aβ (detected by immunolabeling) (*89*), and intracellular Aβ has been detected before β-amyloid pathology develops in young DS individuals (*94*). Endosomal abnormalities (and likely increases in production of Aβ peptide) precede the inevitable Aβ extracellular deposition in DS patients and are present even in prenatal DS brain (*89*), suggesting that abnormalities in the endocytic machinery in DS are both very early markers of neuronal dysfunction and may play a role in increased Aβ production and later β-amyloid deposition. Similar early endosomal abnormalities occur in Ts65Dn mice, a mouse model that has triplication of many of the genes on human chromosome 21 and has morphological and development defects similar to DS (*95*). Using this mouse model, Cataldo and colleagues (*16*) have shown that the relation between APP and the early endosome may be more complex than endocytosis simply affecting APP processing. In this study, Ts65Dn mice were crossed with an *APP* knockout mouse to restore *APP* gene copy number to the normal diploid level while leaving the other genes in the trisomic region triplicated. This mouse does not have early endocytic abnormalities, suggesting that APP overexpression plays a role in the development of the endosomal dysfunction in DS. This finding suggests the potential for a bidirectional interaction between normal endosomal function in neurons and APP protein concentrations and processing. The Ts65Dn mouse and other DS mouse models offer excellent experimental systems to directly examine the impact of AD-like endocytic abnormalities in vivo on APP metabolism and Aβ production in the brain. A comparison of subcellular trafficking and APP processing in the DS mouse model and mouse models that produce disease-associated variants of APP and presenilin (APP×PS1 models) would also be very informative, as the transgenics that only produce disease-associated variants of presenilin appear to model the familial forms of AD and do not show the endocytic abnormalities characteristic of sporadic AD and DS (*96*).

Niemann-Pick Type C disease

One additional disease that has particular bearing to AD-relevant endocytic abnormalities is Niemann-Pick Type C (NPC) (*97*), a lysosomal storage disorder that causes progressive and ultimately fatal neurodegeneration in affected children (*20*). NPC is a rare autosomal recessive disease caused by mutations in one of two genetic loci, *Npc1* or *Npc2*, with the overwhelming majority of cases caused by mutations in *Npc1*. This gene encodes for a protein that localizes to the late endosomal membrane but is transiently associated with lysosomes and the TGN (*98*). In NPC, free cholesterol and sphingolipids accumulate in many tissues, including within neurons. Cholesterol appears to become trapped in late endosomes and early lysosomes, leading to the hypothesis that the NPC1 protein is involved in intracellular trafficking of cholesterol (*98*, *99*). Failure of efficient lysosomal degradation in this storage disorder appears to result from the cholesterol and sphingolipid accumulation in late endosomes, which (i) disrupts endosomal to lysosomal transport, (ii) results in endosomal swelling, and (iii) traps many proteins within endocytic compartments (*97*, *100*). Using mutant NPC mouse models, Burns *et al.* (*101*) demonstrated that this disrupted cholesterol transport led to an increase in Aβ, which they attributed to an increase in the early endosomal compartmentalization of the γ-secretase, although another report suggested that increasing cholesterol concentrations within endosomes results in the accumulation of the γ-secretase in late endosomes (*73*). The idea that NPC leads to endosomal alterations that increase endosomal Aβ production is further supported by additional work demonstrating colocalization of Aβ with markers of early endosomes in human NPC brain tissue (*102*) and in late endosomes in cells (*103*). Thus, a genetic mutation that alters cholesterol trafficking and thereby modulates endosomal trafficking and maturation substantially influences Aβ production.

The Importance of AD-Associated Endocytic Alterations to Drug Development

Aβ accumulation in the brain is an invariant feature of AD and appears to play a primary role in AD pathophysiology (*28*). Ultimately, the accumulation of Aβ in the brain parenchyma in a subset of aged individuals must result from a misbalance of Aβ production and Aβ clearance. The findings we have focused on in this position piece support the hypothesis that disruption of normal endosomal function may be one event important in the cascade that leads to development of the pathological features of AD. Although the disease is caused by numerous and in all likelihood synergistically compounding events that all concurrently interact—examples include (i) a compromise of Aβ clearance, (ii) the nucleation of Aβ oligomers, (iii) the interaction of Aβ with cofactors, (iv) direct neurotoxicity of various Aβ moieties, (v) neuroinflammation (see McGeer Review at http://sageke.sciencemag.org/cgi/content/full/2002/29/re3), and (vi) hyperphosphorylation of the microtubule-associated protein tau—increased production of Aβ subsequent to endocytic alterations could well contribute to the initial stages of the disease's pathophysiology. If this situation were indeed the case, we might use this knowledge of the role of endocytic compartments in early disease events to more effectively target therapeutic or potentially preventive treatments.

What about targeting the endocytic system directly? Although experimental "proof of concept" paradigms can be imagined [for example, altering rab5 activity by expressing dominant-negative, endocytosis-inhibiting mutant versions of the protein (*6*, *7*) in neurons], the essential, carefully balanced nature of endocytosis in all cells suggests that direct manipulation of endocytosis will not prove to be a practical approach for therapeutic intervention. Events as fundamental as synaptic vesicle recycling require endocytosis (*21*), so any broad inter-

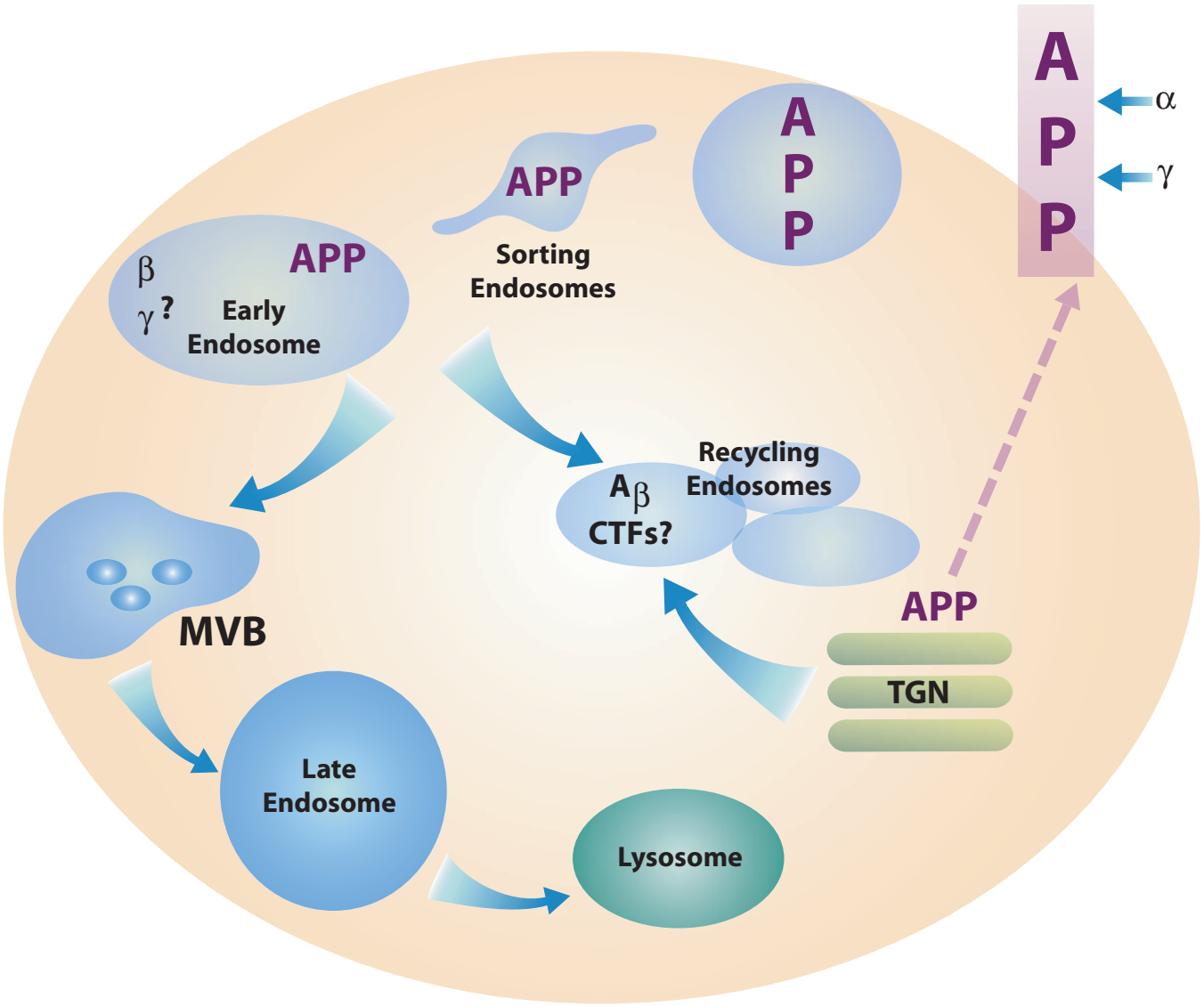

Fig. 2. A schematic representation of the endocytic system. The proposed localization of the secretases and APP are indicated. Arrows indicates movement and/or maturation of vesicles. α, α-secretase; β, β-secretase; γ, γ-secretase; MVB, multivesicular body.

ference with endocytic activity is likely to be disruptive of many key neuronal functions.

However, endocytosis appears to be altered in neurons early in AD. Identifying the upstream cause(s) of this alteration might afford one the ability to target the change in endocytosis, not normal, essential endocytic events (Fig. 2). Experimental data and even human genetic data now suggest at least one likely culprit in abnormal endocytosis/endocytic function: cholesterol. Analysis of NPC suggests that gross mismetabolism of cholesterol in the endocytic pathway can result in alterations of APP processing, leading to increased Aβ production. What about cholesterol and APP metabolism in less extreme states? APP, Aβ, and β-amyloidogenic pathway enzymes have been reported to be partially associated with cholesterol-rich membrane "raft" domains [(*72*, *104-108*); for review, see (*109*)], and BACE and APP might initially interact in such a domain (*107*). Indeed, directly targeting BACE by a glycosyl-phosphatidylinositol membrane anchor to cholesterol-rich domains of the membrane increases Aβ production (*110*). In vivo, rabbits and APP transgenic mice fed a high cholesterol diet have more Aβ in the brain and, in the case of the APP transgenic mice, develop significantly more β-amyloid plaques than those on a normal diet (*111*, *112*). In contrast, lowering cholesterol concentrations by treatment with β-methylcyclodextran and statins reduces Aβ concentrations in vitro (*113*, *114*), whereas statins and similar cholesterol-lowering drugs have been shown to reduce Aβ concentrations in vivo (*113*, *115*). Cholesterol metabolism in vivo may also be directly linked to alterations in neuronal early endosomes through the *Apolipoprotein E* (*ApoE*) gene product, an abundant lipid-carrier protein found in both the peripheral nervous system and the CNS. Common allelic variation in *ApoE* confers substantial risk for late-onset AD, with carriers of the *ApoE4* allele at increased risk for the disease (*116*). Interestingly, Cataldo and colleagues (*117*) have shown that the severity of

the early endocytic abnormalities seen in AD is increased in individuals with an *ApoE4* allele, compared to those without. Although not a direct demonstration that cholesterol can modulate endocytic changes in AD, this finding links a known genetic risk factor for AD, which also happens to be a cholesterol-carrying protein, with greater endocytic abnormalities in neurons. It may prove that lowering serum cholesterol and lipid concentrations will have a positive impact on the endocytic changes seen in the AD brain, perhaps retarding the onset or slowing the progression of the disease [while, not incidentally, having other important positive cardiovascular effects; reviewed in (*118*, *119*)]. Two clinical trials (www.clinicaltrials.gov) are currently under way examining the efficacy of statins as therapeutic agents for AD. Additional experimental evidence linking cholesterol with endocytic dysfunction in AD and related systems, such as the DS mouse models, would add further impetus to managing AD-related endocytic alterations by managing cholesterol concentrations.

Understanding the role of the endosomal system in the pathophysiology of AD may also provide new opportunities for rational drug development (Fig. 2). One approach to improving drug efficacy and safety is to target drug delivery to specific subcellular locations. For instance, small molecule inhibitors of β- or γ-secretase activity may be significantly more efficacious while also being safer if delivery of these inhibitors could be targeted directly to endosomal sites of APP processing. In the case of γ-secretase inhibitors, which have known toxicity issues resulting from the role of the γ-secretase in the processing and subsequent signaling of many important cell receptors [in particular, Notch (*77*, *120*)], endosomal targeting might result in a more selective inhibition of Aβ production within the endosomes. Such a strategy might spare the processing of other γ-secretase substrates that undergo the majority of their processing in other subcellular compartments (*76*). In the case of Notch, γ-secretase processing appears to occur at the plasma membrane (*76*, *77*) but possibly additionally within endosomes (*22*), so the success of this strategy may depend on the relative importance of these two compartments to Notch signaling. The field of cancer therapy has attempted to develop such tools and strategies to deliver drugs to specific intracellular compartments using methods to lessen the toxic effects of these compounds while enhancing their efficacy (*121*, *122*). Binding high molecular weight carrier molecules to small molecule drugs allows for their uptake by endocytosis and thus targets them to the endosomal system. The design of β-secretase inhibitors might particularly benefit from an endosome-directed approach relying on endocytic uptake. The active-site cleft within BACE is unusually large, which has made the development of high-affinity, high-specificity small compound inhibitors difficult (*123*, *124*). One reason that small compounds can be useful as drugs is that they can cross the cell membrane. Using endocytosis to deliver a larger, but highly specific, inhibitor from the extracellular space to the lumen of the endosome, where the active site of BACE would be located, obviates the need for the compound to cross the cell membrane. Small compounds have other advantages, such as the ability to be administered orally and to cross the blood-brain barrier; thus, this strategy is not without potential pitfalls. Additionally, "smart" polymer carrier molecules have been developed that enhance the cellular uptake of therapeutic proteins and nucleotides and allow their release via pH-sensitive bonds that are activated (cleaved) in the acidic environment of the endosome (*125*). However, the efficacy of these approaches has been demonstrated with systemic targets, including lymphatic tissues and peripheral tumors. Efficacy at CNS targets will undoubtedly be more difficult to achieve, because high molecular weight carriers may not cross the blood-brain barrier. However, alternative routes of administration (for example, intranasal dosing) may help to overcome this limitation.

Notwithstanding these potential hurdles, as we expand our understanding of the role of neuronal endocytosis in the pathophysiology of AD, we are likely to uncover new and potentially better therapeutic targets for this devastating disease. Hopefully, the growing appreciation of mouse models showing AD-relevant endocytic abnormalities will permit the field to examine these endosomal changes, their consequences, and their modulation directly within the CNS. Clearly, the more nuanced our understanding of AD becomes, the more successful our treatments are likely to be; knowledge of the role of the endosomal system in AD pathophysiology may well contribute to the development of successful treatment strategies.

References

1. E. Perret, A. Lakkaraju, S. Deborde, R. Schreiner, E. Rodriguez-Boulan, Evolving endosomes: How many varieties and why? *Curr. Opin. Cell Biol.* **17**, 423-434 (2005).
2. N. E. Bishop, Dynamics of endosomal sorting. *Int. Rev. Cytol.* **232**, 1-57 (2003).
3. J. Gruenberg, The endocytic pathway: A mosaic of domains. *Nat. Rev. Mol. Cell Biol.* **2**, 721-730 (2001).
4. S. Pfeffer, Membrane domains in the secretory and endocytic pathways. *Cell* **112**, 507-517 (2003).
5. M. Deneka, P. van der Sluijs, 'Rab'ing up endosomal membrane transport. *Nat. Cell Biol.* **4**, E33-E35 (2002).
6. J. P. Gorvel, P. Chavrier, M. Zerial, J. Gruenberg, rab5 controls early endosome fusion in vitro. *Cell* **64**, 915-925 (1991).
7. C. Bucci, R. G. Parton, I. H. Mather, H. Stunnenberg, K. Simons, B. Hoflack, M. Zerial, The small GTPase rab5 functions as a regulatory factor in the early endocytic pathway. *Cell* **70**, 715-728 (1992).
8. M. J. de Hoop, L. A. Huber, H. Stenmark, E. Williamson, M. Zerial, R. G. Parton, C. G. Dotti, The involvement of the small GTP-binding protein Rab5a in neuronal endocytosis. *Neuron* **13**, 11-22 (1994).
9. H. Stenmark, R. G. Parton, O. Steele-Mortimer, A. Lutcke, J. Gruenberg, M. Zerial, Inhibition of rab5 GTPase activity stimulates membrane fusion in endocytosis. *EMBO J.* **13**, 1287-1296 (1994).
10. M. A. Barbieri, R. L. Roberts, A. Mukhopadhyay, P. D. Stahl, Rab5 regulates the dynamics of early endosome fusion. *Biocell* **20**, 331-338 (1996).
11. G. Li, Rab5 GTPase and endocytosis. *Biocell* **20**, 325-330 (1996).
12. A. Cataldo, G. W. Rebeck, B. Ghetri, C. Hulette, C. Lippa, C. Van Broeckhoven, C. van Duijn, P. Cras, N. Bogdanovic, T. Bird *et al.*, Endocytic disturbances distinguish among subtypes of Alzheimer's disease and related disorders. *Ann. Neurol.* **50**, 661-665 (2001).
13. A. M. Cataldo, C. M. Peterhoff, J. C. Troncoso, T. Gomez-Isla, B. T. Hyman, R. A. Nixon, Endocytic pathway abnormalities precede amyloid beta deposition in sporadic Alzheimer's disease and Down syndrome: Differential effects of APOE genotype and presenilin mutations. *Am. J. Pathol.* **157**, 277-286 (2000).
14. A. M. Cataldo, J. L. Barnett, C. Pieroni, R. A. Nixon, Increased neuronal endocytosis and protease delivery to early endosomes in sporadic Alzheimer's disease: Neuropathologic evidence for a mechanism of increased beta-amyloidogenesis. *J. Neurosci.* **17**, 6142-6151 (1997).
15. O. M. Grbovic, P. M. Mathews, Y. Jiang, S. D. Schmidt, R. Dinakar, N. B. Summers-Terio, B. P. Ceresa, R. A. Nixon, A. M. Cataldo, 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 (2003).
16. A. M. Cataldo, S. Petanceska, C. M. Peterhoff, N. B. Terio, C. J. Epstein, A. Villar, E. J. Carlson, M. Staufenbiel, R. A. Nixon, App gene dosage modulates endosomal abnormalities of Alzheimer's disease in a segmental trisomy 16 mouse model of down syndrome. *J. Neurosci.* **23**, 6788-6792 (2003).
17. C. S. Pillay, E. Elliott, C. Dennison, Endolysosomal proteolysis and its regulation. *Biochem. J.* **363**, 417-429 (2002).
18. N. M. Dahms, P. Lobel, S. Kornfeld, Mannose 6-phosphate receptors and lysosomal enzyme targeting. *J. Biol. Chem.* **264**, 12115-12118 (1989).

19. K. von Figura, A. Hasilik, Lysosomal enzymes and their receptors. *Annu. Rev. Biochem.* **55**, 167-193 (1986).
20. S. U. Walkley, K. Suzuki, Consequences of NPC1 and NPC2 loss of function in mammalian neurons. *Biochim. Biophys. Acta* **1685**, 48-62 (2004).
21. V. N. Murthy, P. De Camilli, Cell biology of the presynaptic terminal. *Annu. Rev. Neurosci.* **26**, 701-728 (2003).
22. M. B. Wilkin, M. Baron, Endocytic regulation of Notch activation and down-regulation (review). *Mol. Membr. Biol.* **22**, 279-289 (2005).
23. K. S. Vetrivel, G. Thinakaran, Amyloidogenic processing of beta-amyloid precursor protein in intracellular compartments. *Neurology* **66**, S69-S73 (2006).
24. B. De Strooper, W. Annaert, Proteolytic processing and cell biological functions of the amyloid precursor protein. *J. Cell. Sci.* **113**, 1857-1870 (2000).
25. D. J. Selkoe, T. Yamazaki, M. Citron, M. B. Podlisny, E. H. Koo, D. B. Teplow, C. Haass, The role of APP processing and trafficking pathways in the formation of amyloid beta-protein. *Ann. N.Y. Acad. Sci.* **777**, 57-64 (1996).
26. R. G. Perez, S. Soriano, J. D. Hayes, B. Ostaszewski, W. Xia, D. J. Selkoe, X. Chen, G. B. Stokin, E. H. Koo, 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 (1999).
27. E. H. Koo, S. L. Squazzo, Evidence that production and release of amyloid beta-protein involves the endocytic pathway. *J. Biol. Chem.* **269**, 17386-17389 (1994).
28. J. Hardy, D. J. Selkoe, The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. *Science* **297**, 353-356 (2002).
29. C. Haass, E. H. Koo, A. Mellon, A. Y. Hung, D. J. Selkoe, Targeting of cell-surface beta-amyloid precursor protein to lysosomes: Alternative processing into amyloid-bearing fragments. *Nature* **357**, 500-503 (1992).
30. J. D. Buxbaum, K. N. Liu, Y. Luo, J. L. Slack, K. L. Stocking, J. J. Peschon, R. S. Johnson, B. J. Castner, D. P. Cerretti, R. A. Black, 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 (1998).
31. E. Lopez-Perez, Y. Zhang, S. J. Frank, J. Creemers, N. Seidah, F. Checler, Constitutive alpha-secretase cleavage of the beta-amyloid precursor protein in the furin-deficient LoVo cell line: Involvement of the pro-hormone convertase 7 and the disintegrin metalloprotease ADAM10. *J. Neurochem.* **76**, 1532-1539 (2001).
32. S. Lammich, E. Kojro, R. Postina, S. Gilbert, R. Pfeiffer, M. Jasionowski, C. Haass, F. Fahrenholz, 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 (1999).
33. S. Parvathy, I. Hussain, E. H. Karran, A. J. Turner, N. M. Hooper, Cleavage of Alzheimer's amyloid precursor protein by alpha-secretase occurs at the surface of neuronal cells. *Biochemistry* **38**, 9728-9734 (1999).
34. S. S. Sisodia, Beta-amyloid precursor protein cleavage by a membrane-bound protease. *Proc. Natl. Acad. Sci. U.S.A.* **89**, 6075-6079 (1992).
35. R. Vassar, B. D. Bennett, S. Babu-Khan, S. Kahn, E. A. Mendiaz, P. Denis, D. B. Teplow, S. Ross, P. Amarante, R. Loeloff *et al.*, Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science* **286**, 735-741 (1999).
36. X. Lin, G. Koelsch, S. Wu, D. Downs, A. Dashti, J. Tang, Human aspartic protease memapsin 2 cleaves the beta -secretase site of beta-amyloid precursor protein. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 1456-1460 (2000).
37. R. Vassar, The beta-secretase, BACE: A prime drug target for Alzheimer's disease. *J. Mol. Neurosci.* **17**, 157-170 (2001).
38. S. L. Roberds, J. Anderson, G. Basi, M. J. Bienkowski, D. G. Branstetter, K. S. Chen, S. B. Freedman, N. L. Frigon, D. Games, K. Hu *et al.*, BACE knockout mice are healthy despite lacking the primary beta-secretase activity in brain: Implications for Alzheimer's disease therapeutics. *Hum. Mol. Genet.* **10**, 1317-1324 (2001).
39. H. Cai, Y. Wang, D. McCarthy, H. Wen, D. R. Borchelt, D. L. Price, P. C. Wong, BACE1 is the major beta-secretase for generation of Abeta peptides by neurons. *Nat. Neurosci.* **4**, 233-234 (2001).
40. Y. Luo, B. Bolon, S. Kahn, B. D. Bennett, S. Babu-Khan, P. Denis, W. Fan, H. Kha, J. Zhang, Y. Gong *et al.*, Mice deficient in BACE1, the Alzheimer's beta-secretase, have normal phenotype and abolished beta-amyloid generation. *Nat. Neurosci.* **4**, 231-232 (2001).
41. A. K. Ghosh, L. Hong, J. Tang, Beta-secretase as a therapeutic target for inhibitor drugs. *Curr. Med. Chem.* **9**, 1135-1144 (2002).
42. A. Capell, H. Steiner, M. Willem, H. Kaiser, C. Meyer, J. Walter, S. Lammich, G. Multhaup, C. Haass, Maturation and pro-peptide cleavage of beta-secretase. *J. Biol. Chem.* **275**, 30849-30854 (2000).
43. B. D. Bennett, P. Denis, M. Haniu, D. B. Teplow, S. Kahn, J. C. Louis, M. Citron, R. Vassar, A furin-like convertase mediates propeptide cleavage of BACE, the Alzheimer's beta-secretase. *J. Biol. Chem.* **275**, 37712-37717 (2000).
44. J. W. Creemers, D. Ines Dominguez, E. Plets, L. Serneels, N. A. Taylor, G. Multhaup, K. Craessaerts, W. Annaert, B. De Strooper, Processing of beta-secretase (Bace) by furin and other members of the proprotein convertase family. *J. Biol. Chem.* **276**, 4211-4217 (2000).
45. J. Walter, R. Fluhrer, B. Hartung, M. Willem, C. Kaether, A. Capell, S. Lammich, G. Multhaup, C. Haass, Phosphorylation regulates intracellular trafficking of beta-secretase. *J. Biol. Chem.* **276**, 14634-14641 (2001).
46. R. Yan, P. Han, H. Miao, P. Greengard, H. Xu, The transmembrane domain of the Alzheimer's beta-secretase (BACE1) determines its late Golgi localization and access to APP substrate. *J. Biol. Chem.* **276**, 36788-36796, (2001).
47. X. He, F. Li, W. P. Chang, J. Tang, GGA proteins mediate the recycling pathway of memapsin 2 (BACE). *J. Biol. Chem.* **280**, 11696-11703 (2005).
48. T. Wahle, K. Prager, N. Raffler, C. Haass, M. Famulok, J. Walter, GGA proteins regulate retrograde transport of BACE1 from endosomes to the trans-Golgi network. *Mol. Cell. Neurosci.* **29**, 453-461 (2005).
49. J. T. Huse, K. Liu, D. S. Pijak, D. Carlin, V. M. Lee, R. W. Doms, Beta-secretase processing in the trans-Golgi network preferentially generates truncated amyloid species that accumulate in Alzheimer's disease brain. *J. Biol. Chem.* **277**, 16278-16284 (2002).
50. X. He, W. P. Chang, G. Koelsch, J. Tang, Memapsin 2 (beta-secretase) cytosolic domain binds to the VHS domains of GGA1 and GGA2: Implications on the endocytosis mechanism of memapsin 2. *FEBS Lett.* **524**, 183-187 (2002).
51. L. Pastorino, A. F. Ikin, A. C. Nairn, A. Pursnani, J. D. Buxbaum, The carboxyl-terminus of BACE contains a sorting signal that regulates BACE trafficking but not the formation of total A(beta). *Mol. Cell. Neurosci.* **19**, 175-185 (2002).
52. J. T. Huse, D. S. Pijak, G. J. Leslie, V. M. Lee, R. W. Doms, Maturation and endosomal targeting of beta-site amyloid precursor protein-cleaving enzyme. The Alzheimer's disease beta-secretase. *J. Biol. Chem.* **275**, 33729-33737 (2000).
53. Y. H. Koh, C. A. von Arnim, B. T. Hyman, R. E. Tanzi, G. Tesco, BACE is degraded via the lysosomal pathway. *J. Biol. Chem.* **280**, 32499-32504 (2005).
54. P. M. Mathews, Y. Jiang, S. D. Schmidt, O. M. Grbovic, M. Mercken, R. A. Nixon, Calpain activity regulates the cell surface distribution of amyloid precursor protein: Inhibition of calpains enhances endosomal generation of beta-cleaved C-terminal APP fragments. *J. Biol. Chem.* **277**, 36415-36424 (2002).
55. J. Ermolieff, J. A. Loy, G. Koelsch, J. Tang, Proteolytic activation of recombinant pro-memapsin 2 (pro-beta-secretase) studied with new fluorogenic substrates. *Biochemistry* **39**, 12450-12456 (2000).
56. X. P. Huang, W. P. Chang, G. Koelsch, R. T. Turner 3rd, F. Lupu, J. Tang, Internalization of exogenously added memapsin 2 (beta-secretase) ectodomain by cells is mediated by amyloid precursor protein. *J. Biol. Chem.* **279**, 37886-37894 (2004).
57. S. Soriano, A. S. Chyung, X. Chen, G. B. Stokin, V. M. Lee, E. H. Koo, Expression of beta-amyloid precursor protein-CD3gamma chimeras to demonstrate the selective generation of amyloid beta(1-40) and amyloid beta(1-42) peptides within secretory and endocytic compartments. *J. Biol. Chem.* **274**, 32295-32300 (1999).
58. S. Pedrini, T. L. Carter, G. Prendergast, S. Petanceska, M. E. Ehrlich, S. Gandy, Modulation of statin-activated shedding of Alzheimer APP ectodomain by ROCK. *PLoS Med.* **2**, e18 (2005).
59. M. S. Lee, S. C. Kao, C. A. Lemere, W. Xia, H. C. Tseng, Y. Zhou, R. Neve, M. K. Ahlijanian, L. H. Tsai, APP processing is regulated by cytoplasmic phosphorylation. *J. Cell Biol.* **163**, 83-95 (2003).
60. W. T. Kimberly, M. J. LaVoie, B. L. Ostaszewski, W. Ye, M. S. Wolfe, D. J. Selkoe, Gamma-secretase is a membrane protein complex comprised of presenilin, nicastrin, Aph-1, and Pen-2. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 6382-6387 (2003).
61. N. Takasugi, T. Tomita, I. Hayashi, M. Tsuruoka, M. Niimura, Y. Takahashi, G. Thinakaran, T. Iwatsubo, The role of presenilin cofactors in the gamma-secretase complex. *Nature* **422**, 438-441 (2003).
62. W. J. Luo, H. Wang, H. Li, B. S. Kim, S. Shah, H. J. Lee, G. Thinakaran, T. W. Kim, G. Yu, H. Xu, PEN-2 and APH-1 coordinately regulate proteolytic processing of presenilin 1. *J. Biol. Chem.* **278**, 7850-7854 (2003).
63. A. Herreman, G. Van Gassen, M. Bentahir, O. Nyabi, K. Craessaerts, U. Mueller, W. Annaert, B. De Strooper, gamma-Secretase activity requires the presenilin-dependent trafficking of nicastrin through the Golgi apparatus but not its complex glycosylation. *J. Cell Sci.* **116**, 1127-1136 (2003).
64. J. Y. Leem, S. Vijayan, P. Han, D. Cai, M. Machura, K. O. Lopes, M. L. Veselits, H. Xu, G. Thinakaran, Presenilin 1 is required for maturation and cell surface accumulation of nicastrin. *J. Biol. Chem.* **277**, 19236-19240 (2002).
65. D. S. Yang, A. Tandon, F. Chen, G. Yu, H. Yu, S. Arawaka, H. Hasegawa, M. Duthie, S. D. Schmidt, T. V. Ramabhadran *et al.*, Mature glycosylation and trafficking of nicastrin modulate its binding to presenilins. *J. Biol. Chem.* **277**, 28135-28142 (2002).

66. Y. W. Zhang, W. J. Luo, H. Wang, P. Lin, K. S. Vetrivel, F. Liao, F. Li, P. C. Wong, M. G. Farquhar, G. Thinakaran *et al.*, Nicastrin is critical for stability and trafficking but not association of other presenilin/gamma-secretase components. *J. Biol. Chem.* **280**, 17020-17026 (2005).
67. R. Siman, J. Velji, Localization of presenilin-nicastrin complexes and gamma-secretase activity to the trans-Golgi network. *J. Neurochem.* **84**, 1143-1153 (2003).
68. J. H. Chyung, D. M. Raper, D. J. Selkoe, Gamma-secretase exists on the plasma membrane as an intact complex that accepts substrates and effects intramembrane cleavage. *J. Biol. Chem.* **280**, 4383-4392 (2005).
69. K. Uemura, N. Kitagawa, R. Kohno, A. Kuzuya, T. Kageyama, K. Chonabayashi, H. Shibasaki, S. Shimohama, Presenilin 1 is involved in maturation and trafficking of N-cadherin to the plasma membrane. *J. Neurosci. Res.* **74**, 184-191 (2003).
70. C. Kaether, S. Lammich, D. Edbauer, M. Ertl, J. Rietdorf, A. Capell, H. Steiner, C. Haass, 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 (2002).
71. H. Wang, W. J. Luo, Y. W. Zhang, Y. M. Li, G. Thinakaran, P. Greengard, H. Xu, Presenilins and gamma-secretase inhibitors affect intracellular trafficking and cell surface localization of the gamma-secretase complex components. *J. Biol. Chem.* **279**, 40560-40566 (2004).
72. K. S. Vetrivel, H. Cheng, W. Lin, T. Sakurai, T. Li, N. Nukina, P. C. Wong, H. Xu, G. Thinakaran, Association of gamma-secretase with lipid rafts in post-Golgi and endosome membranes. *J. Biol. Chem.* **279**, 44945-44954 (2004).
73. H. Runz, J. Rietdorf, I. Tomic, M. de Bernard, K. Beyreuther, R. Pepperkok, T. Hartmann, Inhibition of intracellular cholesterol transport alters presenilin localization and amyloid precursor protein processing in neuronal cells. *J. Neurosci.* **22**, 1679-1689 (2002).
74. S. H. Pasternak, R. D. Bagshaw, M. Guiral, S. Zhang, C. A. Ackerley, B. J. Pak, J. W. Callahan, D. J. Mahuran, Presenilin-1, nicastrin, amyloid precursor protein, and gamma-secretase activity are co-localized in the lysosomal membrane. *J. Biol. Chem.* **278**, 26687-26694 (2003).
75. W. H. Yu, A. M. Cuervo, A. Kumar, C. M. Peterhoff, S. D. Schmidt, J. H. Lee, P. S. Mohan, M. Mercken, M. R. Farmery, L. O. Tjernberg *et al.*, Macroautophagy: A novel Beta-amyloid peptide-generating pathway activated in Alzheimer's disease. *J. Cell Biol.* **171**, 87-98 (2005).
76. L. Tarassishin, Y. I. Yin, B. Bassit, Y. M. Li, Processing of Notch and amyloid precursor protein by gamma-secretase is spatially distinct. *Proc. Natl. Acad. Sci. U.S.A.* **101**, 17050-17055 (2004).
77. D. Selkoe, R. Kopan, Notch and presenilin: Regulated intramembrane proteolysis links development and degeneration. *Annu. Rev. Neurosci.* **26**, 565-597 (2003).
78. P. Marambaud, J. Shioi, G. Serban, A. Georgakopoulos, S. Sarner, V. Nagy, L. Baki, P. Wen, S. Efthimiopoulos, Z. Shao *et al.*, A presenilin-1/gamma-secretase cleavage releases the E-cadherin intracellular domain and regulates disassembly of adherens junctions. *EMBO J.* **21**, 1948-1956 (2002).
79. S. H. Pasternak, J. W. Callahan, D. J. Mahuran, The role of the endosomal/lysosomal system in amyloid-beta production and the pathophysiology of Alzheimer's disease: Reexamining the spatial paradox from a lysosomal perspective. *J. Alzheimers Dis.* **6**, 53-65 (2004).
80. R. A. Nixon, Endosome function and dysfunction in Alzheimer's disease and other neurodegenerative diseases. *Neurobiol. Aging* **26**, 373-382 (2005).
81. R. A. Nixon, P. M. Mathews, A. M. Cataldo, The neuronal endosomal-lysosomal system in Alzheimer's disease. *J. Alzheimers Dis.* **3**, 97-107 (2001).
82. W. Xia, J. Zhang, D. Kholodenko, M. Citron, M. B. Podlisny, D. B. Teplow, C. Haass, P. Seubert, E. H. Koo, D. J. Selkoe, Enhanced production and oligomerization of the 42-residue amyloid beta- protein by Chinese hamster ovary cells stably expressing mutant presenilins. *J. Biol. Chem.* **272**, 7977-7982 (1997).
83. K. Duff, C. Eckman, C. Zehr, X. Yu, C. M. Prada, J. Perez-tur, M. Hutton, L. Buee, Y. Harigaya, D. Yager *et al.*, Increased amyloid-beta42(43) in brains of mice expressing mutant presenilin 1. *Nature* **383**, 710-713 (1996).
84. D. Scheuner, C. Eckman, M. Jensen, X. Song, M. Citron, N. Suzuki, T. D. Bird, J. Hardy, M. Hutton, W. Kukull *et al.*, 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 (1996).
85. A. L. Brunkan, A. M. Goate, Presenilin function and gamma-secretase activity. *J. Neurochem.* **93**, 769-792 (2005).
86. G. K. Gouras, J. Tsai, J. Naslund, B. Vincent, M. Edgar, F. Checler, J. P. Greenfield, V. Haroutunian, J. D. Buxbaum, H. Xu *et al.*, Intraneuronal Abeta42 accumulation in human brain. *Am. J. Pathol.* **156**, 15-20 (2000).
87. R. H. Takahashi, C. G. Almeida, P. F. Kearney, F. Yu, M. T. Lin, T. A. Milner, G. K. Gouras, Oligomerization of Alzheimer's beta-amyloid within processes and synapses of cultured neurons and brain. *J. Neurosci.* **24**, 3592-3599 (2004).
88. R. H. Takahashi, T. A. Milner, F. Li, E. E. Nam, M. A. Edgar, H. Yamaguchi, M. F. Beal, H. Xu, P. Greengard, G. K. Gouras, Intraneuronal Alzheimer abeta42 accumulates in multivesicular bodies and is associated with synaptic pathology. *Am. J. Pathol.* **161**, 1869-1879 (2002).
89. A. M. Cataldo, S. Petanceska, N. B. Terio, C. M. Peterhoff, R. Durham, M. Mercken, P. D. Mehta, J. Buxbaum, V. Haroutunian, R. A. Nixon, Aβ localization in abnormal endosomes: Association with earliest Aβ elevations in AD and Downs syndrome. *Neurobiol. Aging* **25**, 1263-1272 (2004).
90. G. G. Glenner, C. W. Wong, Alzheimer's disease and Down's syndrome: sharing of a unique cerebrovascular amyloid fibril protein. *Biochem. Biophys. Res. Commun.* **122**, 1131-1135 (1984).
91. C. L. Masters, G. Simms, N. A. Weinman, G. Multhaup, B. L. McDonald, K. Beyreuther, Amyloid plaque core protein in Alzheimer disease and Down syndrome. *Proc. Natl. Acad. Sci. U.S.A.* **82**, 4245-4249 (1985).
92. B. T. Hyman, Down syndrome and Alzheimer disease. *Prog. Clin. Biol. Res.* **379**, 123-142 (1992).
93. A. Rovelet-Lecrux, D. Hannequin, G. Raux, N. Le Meur, A. Laquerriere, A. Vital, C. Dumanchin, S. Feuillette, A. Brice, M. Vercelletto *et al.*, APP locus duplication causes autosomal dominant early-onset Alzheimer disease with cerebral amyloid angiopathy. *Nat. Genet.* **38**, 24-26 (2006).
94. K. A. Gyure, R. Durham, W. F. Stewart, J. E. Smialek, J. C. Troncoso, Intraneuronal abeta-amyloid precedes development of amyloid plaques in Down syndrome. *Arch. Pathol. Lab. Med.* **125**, 489-492 (2001).
95. R. H. Reeves, N. G. Irving, T. H. Moran, A. Wohn, C. Kitt, S. S. Sisodia, C. Schmidt, R. T. Bronson, M. T. Davisson, A mouse model for Down syndrome exhibits learning and behaviour deficits. *Nat. Genet.* **11**, 177-184 (1995).
96. D. Langui, N. Girardot, K. H. El Hachimi, B. Allinquant, V. Blanchard, L. Pradier, C. Duyckaerts, Subcellular topography of neuronal Abeta peptide in APP×PS1 transgenic mice. *Am. J. Pathol.* **165**, 1465-1477 (2004).
97. R. A. Nixon, Niemann-Pick type C disease and Alzheimer's disease: The APP-endosome connection fattens up. *Am. J. Pathol.* **164**, 757-761 (2004).
98. T. Y. Chang, P. C. Reid, S. Sugii, N. Ohgami, J. C. Cruz, C. C. Chang, Niemann-Pick type C disease and intracellular cholesterol trafficking. *J. Biol. Chem.* **280**, 20917-20920 (2005).
99. J. E. Vance, H. Hayashi, B. Karten, Cholesterol homeostasis in neurons and glial cells. *Semin. Cell Dev. Biol.* **16**, 193-212 (2005).
100. M. Zhang, N. K. Dwyer, D. C. Love, A. Cooney, M. Comly, E. Neufeld, P. G. Pentchev, E. J. Blanchette-Mackie, J. A. Hanover, Cessation of rapid late endosomal tubulovesicular trafficking in Niemann-Pick type C1 disease. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 4466-4471 (2001).
101. M. Burns, K. Gaynor, V. Olm, M. Mercken, J. LaFrancois, L. Wang, P. M. Mathews, W. Noble, Y. Matsuoka, K. Duff, Presenilin redistribution associated with aberrant cholesterol transport enhances beta-amyloid production in vivo. *J. Neurosci.* **23**, 5645-5649 (2003).
102. L. W. Jin, I. Maezawa, I. Vincent, T. Bird, Intracellular accumulation of amyloidogenic fragments of amyloid-beta precursor protein in neurons with Niemann-Pick type C defects is associated with endosomal abnormalities. *Am. J. Pathol.* **164**, 975-985 (2004).
103. T. Yamazaki, T. Y. Chang, C. Haass, Y. Ihara, Accumulation and aggregation of amyloid beta-protein in late endosomes of Niemann-Pick type C cells. *J. Biol. Chem.* **276**, 4454-4460 (2001).
104. L. M. Refolo, I. S. Wittenberg, V. L. Friedrich Jr., N. K. Robakis, The Alzheimer amyloid precursor is associated with the detergent-insoluble cytoskeleton. *J. Neurosci.* **11**, 3888-3897 (1991).
105. S. J. Lee, U. Liyanage, P. E. Bickel, W. Xia, P. T. Lansbury Jr., K. S. Kosik, A detergent-insoluble membrane compartment contains A beta in vivo. *Nat. Med.* **4**, 730-734 (1998).
106. D. R. Riddell, G. Christie, I. Hussain, C. Dingwall, Compartmentalization of beta-secretase (Asp2) into low-buoyant density, noncaveolar lipid rafts. *Curr. Biol.* **11**, 1288-1293 (2001).
107. R. Ehehalt, P. Keller, C. Haass, C. Thiele, K. Simons, Amyloidogenic processing of the Alzheimer beta-amyloid precursor protein depends on lipid rafts. *J. Cell Biol.* **160**, 113-123 (2003).
108. S. Wahrle, P. Das, A. C. Nyborg, C. McLendon, M. Shoji, T. Kawarabayashi, L. H. Younkin, S. G. Younkin, T. E. Golde, Cholesterol-dependent gamma-secretase activity in buoyant cholesterol-rich membrane microdomains. *Neurobiol. Dis.* **9**, 11-23 (2002).
109. B. Wolozin, Cholesterol and the biology of Alzheimer's disease. *Neuron* **41**, 7-10 (2004).
110. J. M. Cordy, I. Hussain, C. Dingwall, N. M. Hooper, A. J. Turner, Exclusively targeting beta-secretase to lipid rafts by GPI-anchor addition up-regulates beta-site processing of the amyloid precursor protein. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 11735-11740 (2003).
111. D. L. Sparks, S. W. Scheff, J. C. Hunsaker 3rd, H. Liu, T. Landers, D. R. Gross, Induction of Alzheimer-like beta-amyloid immunoreactivity in the brains of rabbits with dietary cholesterol. *Exp. Neurol.* **126**, 88-94 (1994).
112. L. M. Refolo, B. Malester, J. LaFrancois, T. Bryant-Thomas, R. Wang, G. S. Tint, K. Sambamurti, K. Duff, M. A. Pappolla, Hypercholesterolemia accelerates the Alzheimer's amyloid pathology in a transgenic mouse model. *Neurobiol. Dis.* **7**, 321-331 (2000).
113. K. Fassbender, M. Simons, C. Bergmann, M. Stroick, D. Lutjohann, P. Keller,

H. Runz, S. Kuhl, T. Bertsch, K. von Bergmann *et al.*, Simvastatin strongly reduces levels of Alzheimer's disease beta-amyloid peptides Abeta 42 and Abeta 40 in vitro and in vivo. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 5856-5861 (2001).
114. M. Simons, P. Keller, B. De Strooper, K. Beyreuther, C. G. Dotti, K. Simons, Cholesterol depletion inhibits the generation of beta-amyloid in hippocampal neurons. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 6460-6464 (1998).
115. L. M. Refolo, M. A. Pappolla, J. LaFrancois, B. Malester, S. D. Schmidt, T. Thomas-Bryant, G. S. Tint, R. Wang, M. Mercken, S. S. Petanceska *et al.*, A cholesterol-lowering drug reduces beta-amyloid pathology in a transgenic mouse model of Alzheimer's disease. *Neurobiol. Dis.* **8**, 890-899 (2001).
116. E. H. Corder, A. M. Saunders, W. J. Strittmatter, D. E. Schmechel, P. C. Gaskell, G. W. Small, A. D. Roses, J. L. Haines, M. A. Pericak-Vance, Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* **261**, 921-923 (1993).
117. A. M. Cataldo, C. M. Peteroff, J. C. Troncoso, T. Gomez-Isla, B. T. Hyman, R. A. Nixon, Endocytic pathway abnormalities precede amyloid beta deposition in sporadic Alzheimer's disease and Down syndrome: Differential effects of APOE genotype and presenilin mutations. *Am. J. Pathol.* **157**, 277-286 (2000).
118. L. Puglielli, R. E. Tanzi, D. M. Kovacs, Alzheimer's disease: The cholesterol connection. *Nat. Neurosci.* **6**, 345-351 (2003).
119. S. S. Petanceska, S. DeRosa, V. Olm, N. Diaz, A. Sharma, T. Thomas-Bryant, K. Duff, M. Pappolla, L. M. Refolo, Statin therapy for Alzheimer's disease: Will it work? *J. Mol. Neurosci.* **19**, 155-161 (2002).
120. J. Shen, R. T. Bronson, D. F. Chen, W. Xia, D. J. Selkoe, S. Tonegawa, Skeletal and CNS defects in presenilin-1-deficient mice. *Cell* **89**, 629-639 (1997).
121. M. Dyba, N. I. Tarasova, C. J. Michejda, Small molecule toxins targeting tumor receptors. *Curr. Pharm. Des.* **10**, 2311-2334 (2004).
122. A. Nori, J. Kopecek, Intracellular targeting of polymer-bound drugs for cancer chemotherapy. *Adv. Drug Deliv. Rev.* **57**, 609-636 (2005).
123. L. Hong, G. Koelsch, X. Lin, S. Wu, S. Terzyan, A. K. Ghosh, X. C. Zhang, J. Tang, Structure of the protease domain of memapsin 2 (beta-secretase) complexed with inhibitor. *Science* **290**, 150-153 (2000).
124. J. Tang, X. He, X. Huang, L. Hong, Recent developments on the studies of human memapsin 2 (beta-secretase). *Curr. Alzheimer Res.* **2**, 261-264 (2005).
125. M. E. El-Sayed, A. S. Hoffman, P. S. Stayton, Smart polymeric carriers for enhanced intracellular delivery of therapeutic macromolecules. *Expert Opin. Biol. Ther.* **5**, 23-32 (2005).
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