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Sorting through the Cell Biology of Alzheimer's Disease: Intracellular Pathways to Pathogenesis

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Abstract

During the first 100 years of Alzheimer's disease research, this devastating and intractable disorder has been characterized at the clinical, histological, and molecular levels. Nevertheless, many key mechanistic questions remain unanswered. Here we will emphasize the importance of the cell biology of Alzheimer's disease, reviewing the relevant literature that has expanded our mechanistic understanding, with a particular focus on pathways regulating protein sorting. Accumulated evidence indicates that sorting pathways may be uniquely vulnerable to disease pathogenesis, and recent studies have begun to reveal disease-related defects in the regulation of protein sorting.

One hundred years ago, Alois Alzheimer examined the brain of Auguste D., a demented woman who began developing cognitive deficits in her late 40s, and thereby established that dementia can be associated with neurofibrillary tangles and amyloid plaques (Alzheimer et al., 1995 [an English translation of 1907 article by Alzheimer et al.]). In 1910, Emile Kraepelin defined “Alzheimer's disease” as a distinct dementing illness, which, he maintained, provided evidence that dementia can have an “organic” etiology (Schorer, 1985). Yet, for decades thereafter, the major dementia of late life was either dismissed as the inevitable endstage of normal aging or else attributed to “cerebral atherosclerosis” (Katzman, 1986). Over fifty years would pass before investigators began appreciating that late-onset (“senile”) dementia and early-onset (“pre-senile”) Alzheimer's disease are phenotypically indistinguishable under the microscope, prompting the unification of the disorders under the same name (Amaducci et al., 1986).

Nevertheless, despite a shared name and overlapping features, the early-onset and late-onset forms of Alzheimer's disease are epidemiologically and etiologically distinct (Mayeux, 2003; Bertram and Tanzi, 2005). The early-onset form is extremely rare and is caused by monogenic defects that follow an autosomal-dominant pattern (Mayeux, 2003; Bertram and Tanzi, 2005). Like other autosomal-dominant diseases, early-onset Alzheimer's disease has proven amenable to linkage analysis, and many pathogenic mutations have been isolated. In contrast, late-onset Alzheimer's disease, which typically manifests after the sixth decade,

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accounts for over 95% of all cases and has a complex etiology (Mayeux, 2003; Bertram and Tanzi, 2005). Like other complex disorders, the cause of late-onset Alzheimer's disease has been difficult to pinpoint. Indeed, the late-onset form has historically been called “sporadic” Alzheimer's disease to reflect the elusiveness of its etiology. Not only is late-onset disease the more common form of Alzheimer's disease, but with increased longevity, late-onset Alzheimer's disease has emerged as one of the most common disorders of the brain (Small and Mayeux, 2000).

Whereas the 20th century began with the histological description of Alzheimer's disease, the century ended with its genetic and molecular characterization. This era began in 1984 when amyloid-β (Aβ) was isolated as the core peptide of amyloid plaques (Glenner and Wong, 1984; Masters et al., 1985) and entered full swing when, in 1986, the gene encoding the peptide's parent protein, the amyloid precursor protein (APP), was identified (Goldgaber et al.,1987; Kang et al., 1987; Robakis et al., 1987; Tanzi et al., 1987). APP turned out to be a type-I transmembrane protein (Kang et al., 1987), and fine chromosomal mapping of APP revealed its localization to the Down's syndrome region of chromosome 21 (Tanzi et al., 1987), a particularly intriguing discovery since trisomy 21 (Down's syndrome) invariably causes early-onset Alzheimer's disease (Wisniewski et al., 1978). Then, in 1990 and 1991, linkage analyses revealed that mutations in APP can cause either early-onset Alzheimer's disease (Goate et al., 1991) or hereditary cerebrovascular amyloidosis (Dutch type) (Levy et al., 1990), and missense mutations were localized within or adjacent to the Aβ domain of APP (Levy et al., 1990). Although disease-causing mutations in APP occur in only a handful of families, these findings provided the strongest evidence that the disease can be initiated by abnormalities in APP processing and the accumulation of Aβ peptide (reviewed in Hardy and Allsop, 1991; Hardy and Higgins, 1992; Haass and Selkoe, 1993; and Tanzi and Bertram, 2005).

Further reaffirming the relevance of APP processing to the disease, in 1995, additional mutations causing early-onset Alzheimer's disease were isolated in two unknown genes encoding complex, polytopic proteins (Sherrington et al., 1995; Rogaev et al., 1995; Levy-Lahad et al., 1995a, 1995b). Since these genes cause early-onset, “pre-senile,” Alzheimer's disease, they were named presenilin 1 and presenilin 2, and they were localized to chromosome 14 and chromosome 1, respectively. Mutations in presenilins 1 and 2 modify APP processing by shifting the relative generation of Aβ species so as to increase the Aβ42/Aβ40 ratio (where the number indicates the amino-acid length of each peptide species) (Borchelt et al., 1996; Duff et al., 1996; Scheuner et al., 1996; Citron et al., 1997). Aβ42 oligomerizes more rapidly than other forms of Aβ (Hilbich et al., 1991; Burdick et al., 1992; Jarrett et al., 1993), and Aβ oligomers appear particularly toxic to neurons (reviewed in Klein et al., 2001; see also Lesne et al., 2006). Finally, in 1999–2000 this productive era was capped off with the isolation of β-site APP cleaving enzyme (BACE), a transmembrane protease that governs the first enzymatic step in APP processing, known as β-secretase cleavage or simply β-cleavage (Vassar et al., 1999; Sinha et al., 1999; Lin et al., 2000).

When the knowledge of the pathogenic mutations for early-onset disease were taken together with other discoveries related to APP metabolism, the basic biochemical formula for Aβ production was established (Figure 1A) (for review, see Gandy, 2005). The Aβ

domain is located within APP at the junction between the intralumenal and transmembrane domains. Two enzymatic steps liberate Aβ from APP. In the first, "β-cleavage" step, BACE cleaves APP at or near the N terminus of the Aβ peptide; then, in the second, or "γ-cleavage" step, the membrane-bound C-terminal APP fragment (CTF) generated by BACE goes on to be cleaved by the γ-secretase, a multimeric complex thought to be made up of an essential quartet of transmembrane proteins—presenilin 1 (or 2), nicastrin, APH1, and PEN2 (Edbauer et al., 2003; Kimberly et al., 2003; Takasugi et al., 2003). Recent evidence indicates that a fifth molecule, TMP21, is also associated with the γ-secretase complex and plays a regulatory role in γ-cleavage (Chen et al., 2006).

Knowing the biochemical formula for Aβ production is sufficient to explain the Aβ accumulation observed in early-onset Alzheimer's disease. Nevertheless, these molecular defects do not exist in late-onset Alzheimer's disease and therefore cannot explain the accumulation of both Aβ40 and Aβ42 that characterize the common form of the disease. In reality, of course, Aβ production occurs in living neurons, not in a cell-free biochemical reaction. Pursuing the cell biology of how and where in the cell APP is processed has expanded the list of possible mechanisms that regulate Aβ production and has suggested pathogenic possibilities that extend beyond simple molecular defects in APP or the secretases. In this regard, it is notable that APP and the secretases are all integral transmembrane proteins; that they are dynamically sorted through the plasma membrane and the membranes of intracellular organelles; and that the liberation of Aβ involves a transmembrane secretase enzyme acting on a transmembrane APP CTF substrate. Thus, from a cell biology perspective, sorting mechanisms that cause APP and the secretases to colocalize in the same membranous compartment would be expected to play important roles in the regulation of Aβ production.

Over 30% of all proteins are transmembrane proteins (Cobbold et al., 2003), and most are typically sorted via the secretory and endocytic pathways (Figure 1B) (Le Borgne and Hoflack, 1998; Harter and Reinhard, 2000). During the last two decades, great strides have been made in understanding the molecular mechanisms that govern transmembrane protein sorting. The *trans*-Golgi network (TGN) and the endosome have emerged as especially key "waystation" organelles, where the complex movement of transmembrane proteins is orchestrated, particularly in polarized cells such as neurons (Ponnambalam and Baldwin, 2003; Gleeson et al., 2004; Rodriguez-Boulan and Musch, 2005). Contributing to their importance, a bidirectional pathway exists between these two organelles, providing the only direct intracellular link between the secretory and endocytic pathways (Figure 1) (Le Borgne and Hoflack, 1998). (Note: the "endosome" is, of course, not a singular organelle, but rather is made up of multiple subtypes—including the early endosome, the sorting endosome, the recycling endosome, the late endosome, and the multivesicular body. Nevertheless, for purposes of clarity, unless otherwise specified, we will use the term "endosome" to encompass all subtypes.)

Because of the importance of the trafficking pathways interconnecting the TGN, cell surface, and the endosome, investigators have sought to elucidate many of the basic mechanisms governing protein transport through this itinerary (for review, see Bonifacino and Rojas, 2006). In particular, the sorting signals contained within transmembrane proteins

and the specific coat complexes that initiate transport through each leg of this sorting itinerary have been characterized (Kreis and Pepperkok, 1994). The discovery of key findings about the cell biology of protein sorting has chronologically tracked with molecular discoveries related to Alzheimer's disease. For example, in 1990, the asparagiNe-Proline-any-tYrosine (N-P-X-Y) motif in the cytoplasmic tail of type-I transmembrane proteins was identified as an evolutionarily conserved sorting signal that targets proteins for clathrin pit localization and transport via clathrin-coated vesicles from the cell surface to the endosome (Chen et al., 1990) (Figure 2). APP, cloned 4 years earlier, was found to contain this motif, immediately generating specific predictions about its sorting itinerary. In a similar fashion, the sorting of BACE was informed by later reports showing that the D-X-X-L-L dileucine motif, present in the cytoplasmic tail of BACE, is a separate type of sorting signal involved with clathrin-coat transport between the TGN and endosome (He et al., 2005; Wahle et al., 2005). Most recently, at the turn of the century and about the same time that BACE was cloned, cell biological studies characterized a novel coat complex, the retromer, involved primarily in retrograde transport from the endosome (Seaman, 2004; Bonifacino and Rojas, 2006), and the retromer has recently been implicated in APP and BACE sorting (Small and Kim, 2003; Small et al., 2003, 2005; He et al., 2005).

The dovetailing of studies elucidating the general mechanisms that regulate transmembrane protein sorting with studies isolating the specific transmembrane proteins related to Alzheimer's disease has suggested potential pathogenic pathways and forms the basis of this review. We will first attempt to reconcile sometimes conflicting findings and pinpoint the membranous compartments in which neuronal APP is most commonly cleaved to generate discrete fragments. Next, we will review the known or postulated sorting mechanisms that regulate the transport of APP and the transmembrane secretase enzymes through relevant pathways of the neuron. In so doing, we will generate a list of sorting molecules that play roles in Aβ biogenesis and are therefore potential points for disease origins. Finally, we will end by reviewing recent findings showing that defects in some of these sorting pathways exist in late-onset Alzheimer's disease and may contribute to its pathogenesis.

The Membranous Compartments in which APP Is Cleaved

The First Enzymatic Step in the Amyloidogenic Pathway: *β*-Cleavage

The earliest studies of β-cleavage were conducted in the early 1990s, when β-cleavage and Aβ generation were discovered to accompany normal cellular metabolism (Shoji et al., 1992; Seubert et al., 1992, 1993; Haass et al., 1992). Both APP and BACE are sorted through the secretory and endocytic pathways and reside, at least transiently, in almost all membranous compartments of these pathways. A number of early cell biology studies suggested that the endocytic process and the endosomal/lysosomal pathways contributed significantly to Aβ production (Caporaso et al., 1992a; Gandy et al., 1992; Nordstedt et al., 1993; Caporaso et al., 1994; Perez et al., 1996; Ono et al., 1997; Marquez-Sterling et al., 1997; Soriano et al., 1999).

Madin-Darby canine kidney (MDCK)-based systems were applied to the study of polarization of APP trafficking and sorting, as models for similar events that go on in neuronal cells (Haass et al., 1994, 1995a; Capell et al., 2002). In MDCK cells, apical sorting

is often associated with proteins destined for axonal transport, while basolateral sorting is often associated with somatodendritic destinations (Ahn et al., 1996). In the MDCK system, there is selective basolateral secretion of sAPP and Aβ, both of which are generated intracellularly (Ahn et al., 1996). BACE, however, is apically sorted, where Aβ generation is limited (Capell et al., 2002). When a relative excess of APP is provided to the apical surface, a substantial fraction of that APP is converted to Aβ (Capell et al., 2002). This situation was considered potentially relevant to neurons since APP was known to be highly targeted for axonal transport (Koo et al., 1990). These data from MDCK cells tended to predict that, since APP and BACE may be targeted for axonal transport to nerve terminals, the presynaptic nerve terminal might be an especially efficient site for Aβ generation. The identity of the particular β-cleavage-competent organelle most relevant to the pathogenesis of Alzheimer's disease has been a matter of debate. Kindling this debate were apparently discrepant findings in studies reporting that Aβ production could be demonstrated in the cultured cell to occur, alternately, in the ER, in the TGN, or in the endosome (Haass et al., 1995b; Chyung et al., 1997; Cook et al., 1997; Greenfield et al., 1999; Skovronsky et al., 2000a; Petanceska et al., 2000).

Many factors contributed to the difficulty in resolving which of these compartments was most likely responsible for generation of the Aβ that causes pathology. Since BACE was only isolated and cloned in 1999, the task of localizing where BACE activity resides in the cell in studies from the 1990s could only be inferred based on indirect evidence. An example is the chemical poisoning of specific organelles with compounds such as brefeldin A and monensin and then determining how this manipulation affected the profile of intracellular and secreted APP fragments (Caporaso et al., 1992a, 1992b, 1994).

More generally, however, since they are both transmembrane proteins, APP and BACE do in fact reside, at least temporarily, in multiple organelles. It is important to emphasize that the mechanisms that sort transmembrane proteins are very much dependent on specific experimental conditions, and thus methodological differences across studies might shift the sites where APP and BACE interact. Methodological differences, therefore, probably account for some apparently discrepant findings in early cell culture studies investigating the cell biology of APP processing. One important source of variability is that studies often differed in whether wild-type or Swedish (K670N/M671L) mutant APP was transfected into cells under investigation. Since the Swedish mutation in particular enhances the efficiency of BACE cleavage of APP (Citron et al., 1992), metabolism of this mutant yields particularly robust generation of Aβ. For this reason, many investigators, past and present, have studied the cell biology of Swedish mutant APP. However, as compared to wild-type APP, Swedish APP is cleaved by BACE in more proximal compartments that are probably not identical, at least on a quantitative stoichiometric basis, to those where wild-type APP is cleaved by BACE (Haass et al., 1995b; Perez et al., 1996, 1999; Sodhi et al., 2004). Thus, while there was early agreement that most Swedish APP is β-cleaved in the TGN, there remained uncertainty about where most wild-type APP was β-cleaved. Furthermore, there was always the concern that artificially overexpressing APP or BACE might artifactually shift the locus of APP processing.

The isolation and cloning of BACE provided a much-needed experimental handle with which the cell biology of its interaction with APP could be investigated. A detailed analysis of the sorting itinerary of BACE provided clear evidence that mature BACE is found predominantly in the endosome, with relatively lower levels distributed among the cell surface and the TGN (Huse et al., 2000). Virtually no mature BACE is found in the lysosome or in the endoplasmic reticulum (Huse et al., 2000). The relative absence of BACE in the lysosome was important, since biochemical studies suggested that BACE activity is maximized in an acidic environment. Thus, the possibility that the lysosome was an important site for β-cleavage was excluded.

Based on the cellular localization of BACE alone, the site of β-cleavage was narrowed down significantly, leaving open the possibility that important β-cleavage occurs in the TGN, the cell surface, the endosome, or in the sorting pathways that connect these sites. Among the remaining possibilities, the endosome and the endocytic pathway are the most acidic, making these sites best suited for BACE activity. Nevertheless, the relatively subtle pH difference between endosomes and the TGN does not by itself provide sufficient evidence to resolve this important issue. In support of earlier findings, recent studies have shown that blocking endocytosis reduces Aβ production (Carey et al., 2005), whereas enhancing endocytosis favors its production (Grbovic et al., 2003), thus confirming that the endocytic pathway can play an important role in APP processing. However, these studies do not rule out the importance of the TGN and the secretory pathway for β-cleavage.

Only by explicitly comparing each of the three membrane compartments can a more definitive answer be provided. Indeed, the results of a number of recent studies seem to converge on the endocytic pathway as the one in which most β-cleavage of wild-type APP is most likely to occur. A recent study that directly tested for sites of BACE-APP interactions employed fluorescence resonance energy transfer (FRET) analysis, arguably the best technique for studying protein-protein interactions in living cells (Kinoshita et al., 2003). This study showed that wild-type APP binds BACE with greatest efficiency in the endosome, less so on the cell surface, and to a negligible degree in the TGN and secretory pathway (Kinoshita et al., 2003). Interestingly, an increase of APP:BACE binding was observed in the TGN when the Swedish mutant form of APP was investigated, agreeing with the earlier evidence that β-cleavage of Swedish mutant APP occurs preferentially within the TGN (Haass et al., 1995b).

In summary, although the interaction of APP with BACE can, in principle, occur in the ER, in the TGN, on the cell surface, in the endosome, or along the sorting pathways interconnecting them, the preponderance of circumstantial and direct evidence suggests that, for wild-type APP, β-cleavage probably occurs most commonly in the endosome and the endocytic pathway.

The Second Enzymatic Step in the Amyloidogenic Pathway: γ-Cleavage

Identifying the membranous site in which γ-secretase cleaves the carboxyl-terminal fragment of APP, the final step in liberating the Aβ peptide, has been even more controversial than localizing the cleavage site for β-cleavage. As discussed above, the fact that β-cleavage turned out to be governed by a monomeric protein greatly simplified the

search for its site of action. In contrast, not only is the γ-secretase a multimeric complex, made up of multiple proteins or protein fragments, but there is still debate about whether its full complement of component elements has been established. Although most studies agree that the presenilins are components of the γ-secretase, there are a number of experimental and biological reasons to explain why mapping the cellular location of the presenilins might not necessarily indicate the site of γ-cleavage. First, compared to endogenous patterns, overexpressing exogenous presenilins appears to shift their subcellular location (Thinakaran et al., 1996; Lee et al., 1997; Lah et al., 1997). Second, there is coordinated regulation of trafficking of various γ-secretase components (Zhang et al., 2005; Niimura et al., 2005). As a result, expressing presenilins with and without the other putative elements of the γ-secretase complex (nicastrin, APH1, PEN2) also shifts the membranous compartment in which presenilins or the other γ-secretase components are concentrated (Zhang et al., 2005; Niimura et al., 2005). Third, γ-secretase activity is dependent on membrane-bound cleaved fragments of presenilin, and presenilin fragments may exist in different compartments compared to the holoprotein (Petanceska et al., 2000), which is itself a zymogen and therefore is inactive until it is cleaved.

Thus, since simple protein localization studies are difficult to interpret, localizing γ-secretase *activity*, while indirect, may be more informative in identifying sites in which γ-cleavage occurs. In this regard, it is notable that a number of studies have established that γ-cleavage can occur in the presynaptic terminal of the neuron (Kamenetz et al., 2003). Components of the TGN are specifically excluded from the axon and axon hillock (Caporaso et al., 1994). Therefore, among the organelles implicated in γ-cleavage, the endosome is the only key Aβ-generating intracellular organelle that exists in the presynaptic terminal, therefore suggesting that endosomes, or the transport pathways into endosomes, contain the molecular machinery necessary for both β- and γ-cleavage (Koo et al., 1990; Ikin et al., 1996; Marquez-Sterling et al., 1997; Lah et al., 1997; Buxbaum et al., 1998a). Pathogenically, a convergence of findings suggests that Aβ biogenesis in the nerve terminal is the processing site of APP that is most relevant to the pathophysiology of Alzheimer's disease in patients (Arnold et al., 1991; Beach and McGeer, 1992; Rogers and Morrison, 1985) and in mouse models of disease (Buxbaum et al., 1998a; Lazarov et al., 2002; Sheng et al., 2002; Lee et al., 2005). Thus, based on the site within the neuron in which both β- and γ-cleavage occurs, it can be inferred that the γ-secretase is active, in a pathogenically relevant manner, in the endosome or endocytic pathway (Lah and Levey, 2000; Fukumori et al., 2006). Indeed, recent studies using a reporter system that couples γ-secretase cleavage to generation of a fluorescent signal shows unequivocally that, in cultured cells, most γ-cleavage, and hence, most Aβ generation, occurs in endosomes (Kaether et al., 2006).

Lipids appear to play important roles in APP processing. Lipid rafts, perhaps within endosomal membranes, may act by coordinating the proximity of APP, BACE, and γ-secretase (Riddell et al., 2001; Ehehalt et al., 2003; Cordy et al., 2003; Vetrivel et al., 2004, 2005). The role of cholesterol in APP metabolism has received much attention, and a proper review of that literature would occupy far more space than is allotted here. Suffice it to say that there are many important observations relating cholesterol to APP metabolism, but elucidation of the basis for that link has been challenging and frustrating. Polymorphisms in the major cholesterol transport protein, apolipoprotein E, specify risk for Alzheimer's in as

many as 25%–30% of cases of the common form of Alzheimer's (Strittmatter et al., 1993; Corder et al., 1993; Schmechel et al., 1993). Cholesterol-lowering drugs decrease Aβ levels released from cultured neurons (Simons et al., 1998) and attenuate Aβ accumulation in the brains of plaque-forming transgenic mice (Refolo et al., 2000, 2001). Surprisingly, however, the use of genetic manipulation to lower circulating cholesterol levels has no effect on brain Aβ (Fagan et al., 2004). Clearly, additional investigation will be required before one can develop a satisfactory and parsimonious model for the relationship between cholesterol and Aβ. From a clinical perspective, the link between cholesterol and Alzheimer's is being tested in a large, placebo-controlled, double-blind clinical trial in which the potential for the cholesterol-lowering drug simvastatin to slow the progression of Alzheimer's is being evaluated. Certainly, if the simvastatin trial is positive, this will provide enormous impetus for a redoubled effort toward explaining the “Alzheimer-cholesterol connection.”

Sorting Mechanisms in Alzheimer's Disease

Delivering transmembrane proteins to their precise intracellular destinations is of critical importance to the biology of the cell, and it is therefore not surprising that a growing number of sorting mechanisms are being described, dedicated to the faithful execution of this complex process (van Vliet et al., 2003). Defects in the sorting protein sorCS1 have recently been associated with increasing risk for another complex disorder, type-2 diabetes mellitus (Clee et al., 2006). For the next section of this review, we will be guided by the sorting itinerary proven important for APP processing. As a result, we will restrict our focus to the sorting mechanisms governing transport among the TGN, cell surface, and endosome. Specifically, since they are monomeric proteins, the sorting of APP and BACE is best understood. Therefore, we will review known or postulated sorting mechanisms involved in the transport of AP and BACE.

Vesicular Transport of APP and BACE

Coat complexes play critical roles in the budding of transport vesicles off donor membrane compartments that initiates the process of transmembrane protein sorting. Based on the sorting motifs expressed in APP and BACE, these proteins were predicted to be transported by the clathrin coat complex (Bonifacino and Traub, 2003). Clathrin proteins are constructed from three heavy chains and three light chains (Hirst and Robinson, 1998). Clathrin does not bind directly to its cargo, but rather, the globular tail of each clathrin heavy chain binds “adaptor” proteins, which, in turn, bind to the cytoplasmic tails of the transmembrane protein cargo (Hirst and Robinson, 1998). Clathrin coats are involved in two main sorting routes—the endocytic pathway connecting the cell surface to the endosome, and the pathway connecting the TGN to the endosome (Traub, 2005) (Figure 1). While identical clathrin proteins are involved in different sorting pathways and in sorting different transmembrane proteins, it is the variety of adaptor proteins that is believed to be responsible for imposing sorting specificity at the stage of cargo selection (Hirst and Robinson, 1998).

As mentioned above, the cytoplasmic tail of APP contains the NPXY amino acid motif, a motif that is evolutionarily conserved and which governs the targeting of cargo to clathrin-coated vesicles (Chen et al., 1990) (Figure 2). Motifs such as this are important for generating predictions about sorting events. However, direct experimental proof of these

predictions is required before these sorting itineraries are established. Indeed, mutagenesis studies have been used to assess and confirm that each of the amino acids within the NPXY motif plays some role in the process of transporting APP from the cell surface to the endosome (Chen et al., 1990). A number of families of adaptor proteins have been found to mediate the binding of the NPXY motif to clathrin coats. Accordingly, a growing number of clathrin adaptor proteins have been found to bind APP's NPXY motif and therefore serve as regulators of APP endocytosis. These adaptor proteins include the autosomal-recessive hypercholesterolemia (ARH) protein (Noviello et al., 2003), disabled family member (Dab) 1 (Homayouni et al., 1999), c-Jun N-terminal kinase interaction (Jip1b) protein (Inomata et al., 2003; Matsuda et al., 2001; Scheinfeld et al., 2002), members of the Fe65 family (including Fe65, Fe65L1, and Fe65L2) (Fiore et al., 1995; Borg et al., 1996; Bressler et al., 1996; Guenette et al., 1996; Zambrano et al., 1997; Duilio et al., 1998; Tanahashi and Tabira, 1999), and members of the X11 family of proteins, in particular X11a (Borg et al., 1996; McLoughlin et al., 1999; Tomita et al., 1999). In many instances, these adaptors reduce generation of Aβ by causing APP to spend more residence time at the plasma membrane or within the ER (Watanabe et al., 1999; Sabo et al., 1999).

In contrast to the NPXY signal within the APP cytoplasmic tail, BACE expresses a dileucine sorting motif in the corresponding domain (Rapoport et al., 1998). This sorting motif also binds the clathrin coat complex; however, transmembrane proteins that express the dileucine motif are typically transported through the pathway that directly connects the TGN and the endocytic system (Figure 2) (Boman, 2001; Bonifacino, 2004). The reason for this different sorting itinerary is attributed to the Golgi-localized γ-ear-containing ARF-binding (GGA) family of adaptor proteins, including GGA1, GGA2, and GGA3 (Boman, 2001; Bonifacino, 2004). GGA1 appears to be the GGA subtype that controls BACE transport from the endosome to the TGN. GGA proteins express a VPS-27, Hrs, and STAM (VHS) domain that binds the dileucine motif of BACE (Boman, 2001; Bonifacino, 2004). Localized mainly in the TGN and less so in endosomes, GGAs are involved in sorting transmembrane proteins away from the TGN and toward the endosome. Somewhat surprisingly, some, though not all, studies suggest that BACE is transported from the endosome to the TGN through GGA-mediated sorting (He et al., 2005).

The retromer is the second coat complex implicated in the transport of BACE (Figure 2) and perhaps APP as well (Small and Kim, 2003; Small et al., 2003, 2005; He et al., 2005) (Figure 2). First described in yeast, the retromer is a newly characterized coat complex, made up of VPS35, VPS26, VPS29, VPS5, and VPS17 (VPS = Vacuolar Protein Sorting), which, in yeast, serves to sort the type-I transmembrane protein VPS10 from the endosome back to the TGN (Seaman et al., 1998; Edgar and Polak, 2000; Haft et al., 2000; Nothwehr et al., 2000; Pfeffer, 2001; Reddy and Seaman, 2001; Seaman and Williams, 2002). The name “retromer” is derived from the role of this coat in retrograde sorting itineraries. VPS35 serves as the core of the retromer complex, binding not only the other retromer elements, but also binding the cytoplasmic tail of the transmembrane protein cargo that is being sorted (Figure 2) (Reddy and Seaman, 2001).

Except for VPS17, mammalian (including human) homologs of retromer molecules have been identified (Haft et al., 2000) and are highly expressed in the brain. VPS35, VPS26, and

VPS29 have single mammalian homologs, which are known by the same names (Haft et al., 2000). Recent studies suggest that VPS26 has two isoforms, termed VPS26a and VPS26b (Kerr et al., 2005). VPS5 has two mammalian homologs, known as sorting nexin 1 and sorting nexin 2 (Haft et al., 2000). Because of these homologies, the retromer was predicted to mediate the sorting of type-I transmembrane proteins in mammals. In 2004, this prediction was confirmed when a number of groups established that the retromer is involved in transporting type-I transmembrane proteins such as the cationic-independent mannose-6-phosphate receptor (Arighi et al., 2004; Seaman, 2004) and the polymeric immunoglobulin G receptor (Verges et al., 2004). Furthermore, these studies suggested that another molecule, known as sortilin, is also transported by the mammalian retromer (Seaman, 2004). This observation is particularly interesting since sortilin is one of the five known type-I transmembrane proteins that express VPS10, the primary cargo of the yeast retromer (Hampe et al., 2001), suggesting functional homologies between the yeast and mammalian retromer. The other members of this family are known as sorLA (also known as LR11), sorCS1, sorCS2, and sorCS3 (Hampe et al., 2001).

Although the retromer is highly expressed in the brain, until recently, the type-I transmembrane proteins transported by the neuronal retromer were unknown. The first clues about which type-I transmembrane proteins might be sorted by the neuronal retromer came from a study exploring microarray data generated from human brain tissue (Small and Kim, 2003; Small et al., 2003). Among a list of possible retromer cargo molecules, this study demonstrated that sorLA and BACE were among the type-I transmembrane molecules whose expression levels cross-correlated most strongly with levels of neuronal VPS35 (Small and Kim, 2003; Small et al., 2003). Although this cross-correlation approach has been successfully used in microarray studies to identify molecules that are candidates for interacting with one another (Bhardwaj and Lu, 2005), these predictions can, of course, only be used as a screen whose results require confirmation by direct experimental investigation. Direct evidence that the retromer plays a role in BACE sorting has recently emerged from a study that used siRNA to knock down VPS26 in cell culture (He et al., 2005). This manipulation, which was originally used in the studies implicating the retromer in mannose-6-phosphate receptor sorting, reduces both VPS26 and VPS35 levels, causing retromer dysfunction (Arighi et al., 2004; Seaman, 2004; Verges et al., 2004). By knocking down VPS26, retromer dysfunction was shown to missort BACE as manifested by an increase in endosomal BACE (He et al., 2005). Experimental confirmation regarding interaction of VPS35 with sorLA has not yet been provided. Nevertheless, since sorLA has close homologies to sortilin, its VPS10-containing family member, one would plausibly speculate that sorLA is also sorted by the neuronal retromer. Interestingly, a number of studies have shown that sorLA binds APP and BACE (Andersen et al., 2005; Offe et al., 2006). Therefore, sorLA and perhaps other VPS10-containing molecules might function as the equivalent of an adaptor protein, binding the retromer complex to cargo proteins such as APP (Figure 2). Of note, although some studies suggest that GGAs transport BACE along the same sorting pathway—from the endosome to the TGN—there is no evidence that GGA and retromer sorting interact or compete with each other.

Whereas coat complexes begin the process of transmembrane sorting by forming transport vesicles at the donor membrane compartment, the process ends, of course, when the cargo

travels over its route and successfully arrives at its destination membrane (Bonifacino and Glick, 2004). It is worth noting that transport vesicles typically travel over microtubule-supported routes, and, therefore, any process that interrupts the integrity of microtubules might affect transmembrane protein sorting. One major neuropathological feature of Alzheimer's disease that is not discussed in detail here involves accumulation of intracellular precipitates of the cytoskeleton and formation of structures known as neurofibrillary tangles (Alzheimer et al., 1995). While a discussion of neurofibrillary tangle pathogenesis is beyond the scope of this review, the fact that the major tangle protein, tau, is a microtubule-binding protein merits mention in this review of protein sorting (see Geschwind, 2003, for review).

The fusion of a transport vesicle to the membrane of the targeted organelle completes the process of transmembrane protein sorting, and this fusion step is governed by three main families of proteins—soluble N-ethylmaleimide sensitive factor-attachment protein receptor (SNARE) proteins (Rothman, 1994), Rab (a subfamily of the Ras superfamily of low-molecular-mass GTPases) proteins (Novick and Zerial, 1997), and Sec1/Munc18 (SM) proteins (Toonen and Verhage, 2003). Membrane fusion and cargo delivery occur when SNAREs expressed in the membranes of the transport vesicle (e.g., syntaxins and vesicle-associated membrane proteins [VAMPs]) interact with a single SNARE protein expressed in the membrane of the recipient or-ganelles, while the Rabs and SM proteins play important roles in mediating this interaction. The fidelity of vesicular transport, in which vesicles are differentially delivered to their proper destinations, is governed by the specific combination of SNARES, Rabs, and SM proteins found in the receptor membranes. Although among these families of proteins, the specific combinations have not yet been identified for the transport of APP and BACE through the cell surface, endosome, and TGN, a select group of these fusing proteins have been implicating in this sorting itinerary. This list includes syntaxins 5, 6, 7, 8, 13, and 16 (Prekeris et al., 1999); VAMPs 5, 7, and 8 (Prekeris et al., 1999); Rabs 5 and 11 (Pfeffer, 1999); the SM protein VPS45, Munc 13, and Munc 18 (Toonen and Verhage, 2003; Rossner et al., 2004).

Phosphorylation-State-Specific Sorting of APP and BACE

The phosphorylation states of substrate proteins and lipids also play roles in APP and BACE transport. The phosphorylation states of membrane proteins such as APP or BACE, and/or the phosphorylation states of their specific interacting proteins, provide for dynamic regulation of signal transduction and protein sorting on a moment-to-moment basis, thereby integrating protein sorting and neurotransmission (Mostov and Cardone, 1995; Clague and Urbe, 2001; Bonifacino and Traub, 2003). A striking example is that of regulated ectodomain shedding of APP (Buxbaum et al., 1990, 1992; Caporaso et al., 1992b; Nitsch et al., 1992; Gillespie et al., 1992; Pedrini et al., 2005). During regulated shedding, first messengers such as neurotransmitters and hormones (Buxbaum et al., 1992; Nitsch et al., 1992; Jaffe et al., 1994; Xu et al., 1998; Qin et al., 2006) impinge upon neurons and direct APP toward the cell surface and away from the TGN and endocytic pathways (Xu et al., 1995), and hence away from BACE. At the cell surface, APP can be processed by a nonamyloidogenic pathway, known as the α-secretase pathway and defined by the metallo-proteinases ADAM-9, ADAM-10, and ADAM-17 (Buxbaum et al., 1998b; Esler and Wolfe, 2001; Allinson et al., 2003; Postina et al., 2004; Kojro and Fahrenholz, 2005). ADAM is an

acronym derived from "a disintegrin and metalloproteinase." The molecular mechanism of regulated shedding remains to be fully elucidated but appears to involve phosphorylation of components of the TGN vesicle biogenesis machinery (thereby increasing APP delivery to the cell surface; Xu et al., 1995) as well as phosphorylation of protein components of the endocytic system (thereby blocking APP internalization; Chyung and Selkoe, 2003; Carey et al., 2005). The phosphorylation states of APP and BACE do not appear to be involved in this process (Gandy et al., 1988; Oishi et al., 1997; da Cruz e Silva et al., 1993; Jacobsen et al., 1994; Pastorino et al., 2002). With regard to Aβ generation, this phenomenon is noteworthy because hyperactivation of the α pathway (e.g., with a combination of simultaneous protein kinase activation and protein phosphatase inhibition) can lead to relatively greater cleavage of APP by α-secretase(s) (Caporaso et al., 1992b; Gillespie et al., 1992), thereby reducing or completely abolishing Aβ generation (Buxbaum et al., 1993; Gabuzda et al., 1993; Hung et al., 1993). Interest in this phenomenon has recently been revived with the demonstration that microdialysis techniques can be used to demonstrate and quantify regulated shedding and regulated Aβ generation in the brains of living experimental animals (Cirrito et al., 2005; Selkoe, 2006).

Recent evidence suggests that axonal transport of APP (Lee et al., 2003) and perhaps also prolyl isomerization might be modulated by the state of phosphorylation of the APP cytoplasmic tail at threonine-668 (Pastorino et al., 2006). APP is axonally transported in holoprotein form (Koo et al., 1990; Buxbaum et al., 1998a); hence, the phosphorylation of threonine-668 was proposed to serve as a "tag," targeting phospho-forms of APP for delivery to the nerve terminal (Lee et al., 2003). However, recent evidence calls into question the proposal that the phosphorylation state of threonine-668 plays a major physiological role in APP localization or Aβ generation, since threonine-to-alanine-668 knock-in mice show normal levels and subcellular distributions of APP and its metabolites, including Aβ (Sano et al., 2006). There is compelling evidence, however, that, once at the nerve terminal, APP is processed, generating Aβ locally at the terminal and releasing Aβ at, near, or into the synapse (Kamenetz et al., 2003).

The cytoplasmic tail of BACE also undergoes reversible phosphorylation, and that event appears to specify its recycling (von Arnim et al., 2004; He et al., 2005). In cell lines, the dephospho- and phospho-forms of BACE appear to perform with similar efficiencies in generating Aβ40 and Aβ42 (Pastorino et al., 2002), but this has not been evaluated in primary neuronal cultures. This failure of Aβ generation to be regulated by BACE recycling is somewhat unexpected since, as reviewed above, most Aβ is believed to arise from the endocytic pathway. Hence, one would expect that increasing BACE concentration in the endocytic pathway would increase generation of Aβ. One explanation for this unexpected result is that substrate may be limiting in post-TGN compartments, and therefore increased levels of BACE are unable to raise Aβ generation. This notion agrees with the proposal mentioned above that regulated shedding acts at the TGN to divert APP molecules toward the plasma membrane as a means of lower generation of Aβ at least in part because a limited pool of APP is transported out of the TGN (Buxbaum et al., 1993; Skovronsky et al., 2000b). Indeed, in some neuronal-like cell types, over 80% of the newly synthesized moles of APP are degraded without generating obvious discrete metabolic fragments (Caporaso et al., 1992b).

Clathrin-independent endocytosis of transmembrane proteins is regulated by protein phosphorylation (Robertson et al., 2006). Further, two components of the endocytosis machinery, dynamin and amphiphysin, control clathrin-mediated endocytosis in a fashion that is sensitive to their direct phosphorylation by the protein kinase cdk5 (Tomizawa et al., 2003; Nguyen and Bibb, 2003). Retromer function is regulated by a separate complex of molecules known as “complex II” (Burda et al., 2002). Complex II includes several catalytic functions that direct retromer action. The phosphoinositide kinase VPS34 binds the protein kinase VPS15, and then, secondarily, VPS30 and VPS38 are recruited and the four molecules comprise the complete complex II (Burda et al., 2002). Thus, complex II action is modulated not only by protein phosphorylation but also by lipid phosphorylation (Stack et al., 1995). Some investigators have proposed that the PI 3-kinase component of complex II directs synthesis of a specific pool of endosomal PI3, which, in turn, activates or stimulates assembly of the retromer complex, thereby ensuring efficient endosome-to-Golgi retrograde transport (Stack et al., 1995). These regulatory mechanisms may have implications for Aβ generation, but such a connection, if one exists, remains to be elucidated. Clearly, much remains to be studied about the intricacies of retromer function and dysfunction.

Presenilins and APP Sorting

Soon after the discovery of presenilins, gene targeting experiments were performed in mice in order to investigate the essential bioactivities of these complex, polytopic, molecules, especially presenilin 1 (PS1) (Wong et al., 1997; Naruse et al., 1998). In cells from PS1-deficient mice, delivery of multiple type-I proteins to the cell surface was observed to be disturbed; APP and the p75 neurotrophin receptor were among those missorted proteins (Naruse et al., 1998). This work was somewhat overshadowed, however, when cells from PS1-deficient mice were demonstrated to be incapable of generating Aβ (De Strooper et al., 1998). This observation placed APP and PS1 on a common metabolic pathway for the first time and was rapidly followed by demonstration that PS1 did, indeed, contain the catalytic site of γ-secretase, as established by cross-linking of γ-secretase inhibitors to PS1 (Li et al., 2000a, 2000b).

The unusual intramembranous localization of two aspartate residues led to the postulation that these amino acids were forming the active site of an aspartyl proteinase (Wolfe et al., 1999). This dovetailed with the apparent fact that APP CTFs were cleaved by regulated intramembranous proteolysis (RIP), and when the aspartates were mutated to alanines, γ-secretase activity was abolished (Wolfe et al., 1999). RIP was, at the time, a relatively recently recognized phenomenon, and conventional wisdom up to that point had held that the hydrophobicity of membranes would preclude the entry of water into the lipid bilayer to enable hydrolysis of peptide bonds. Even to this day, the mechanism that provides the capability for surmounting that energy barrier is poorly understood. The popular formulation at that point was that PS1 was a proteinase, and the notion that PS1 was a trafficking factor was underemphasized. The possibility was also raised that perhaps aberrant trafficking in PS1-deficient cells was due to the inability of some unidentified PS1 substrate trafficking factor to function properly in its uncleaved state since its cognate protease (PS1) was absent.

Beginning in the last few years, experiments in cultured cells and cell-free assays have begun to yield compelling evidence that PS1 serves a trafficking function in addition to its catalytic function. However, these recent experiments have a common potential alternative interpretation: i.e., the exact same processing effects would be predicted in the event that key trafficking proteins were important substrates for cleavage by PS1. Therefore, when PS1 is deficient, post-TGN trafficking of membrane protein cargos would become abnormal due to deficient processing of the trafficking factor/PS substrate (Kaether et al., 2002; Wang et al., 2004; Wood et al., 2005; Rechards et al., 2006).

Most PS1-deficient mice and cells are highly compromised and resemble Notch-deficient mice and cells (Wong et al., 1997). This is not entirely unexpected since Notch is a substrate for cleavage by γ-secretase as are another several dozen type-I transmembrane proteins, including cadherin, erb-b4, and the p75 NGF receptor (De Strooper et al., 1999; Struhl and Greenwald, 1999; for review, see Fortini, 2002). Therefore, PS1 deficiency can lead to dysfunction of a host of proteins whose physiological function requires cleavage by RIP in order to release their cytoplasmic domains. In many examples, the cytoplasmic domain released by γ-secretase appears to diffuse rapidly to the nucleus, where these intracellular domains (ICDs) such as Notch intracellular domain (NICD) modulate gene transcription (Cupers et al., 2001; Fortini, 2002; Cao and Sudhof, 2001).

PS1-mediated trafficking appears to localize to post-TGN steps of trafficking of type-I transmembrane proteins (Annaert et al., 1999; Kaether et al., 2002; Cai et al., 2003; Wang et al., 2004, 2006; Wood et al., 2005; Zhang et al., 2006; Cai et al., 2006a, 2006b; S.G. et al., unpublished data). This role for PS1 in regulation of APP trafficking has been implicated in both cell culture and cell-free in vitro reconstitution studies (Annaert et al., 1999; Kaether et al., 2002; Cai et al., 2003; Wang et al., 2004, 2006; Wood et al., 2005; Zhang et al., 2006; Cai et al., 2006a, 2006b; S.G. et al., unpublished data). Pathogenic PS1 mutations retard egress of APP from the TGN by a mechanism that appears to involve phospholipase D (Cai et al., 2006a, 2006b), a known TGN budding modulator (Kahn et al., 1993). It is clear that the mutations that have been tested so far increase the residence time at the TGN while also increasing the Aβ42/40 ratio (Kahn et al., 1993). Recent data suggest that TGN retention per se can increase generation of Aβ42/40 in cerebral neurons in vivo, indicating that abnormal post-TGN trafficking of APP might be sufficient to initiate Aβ accumulation (S.G. et al., unpublished data).

The pathogenic PS1 defect can be corrected in cell culture and in cell-free systems following supplementation of the budding factor phospholipase D (PLD) (Cai et al., 2003, 2006a, 2006b). The molecular details of how PS1 and PLD are connected remain obscure; however, as cargos other than APP are found to be missorted, including, e.g., tyrosinase (Wang et al., 2006), the notion that PS1 has a protein trafficking function has become more widely appreciated and accepted. Now, the challenge is to identify at the molecular level those factors that selectively favor cleavage at the Aβ42-43 scissile bond.

PS1 has also been implicated in trafficking of APP and perhaps its carboxyl-terminal fragments out of the endosome (Zhang et al., 2006). Thus, PS1 dysfunction could also result in retention of APP and CTFs within the endocytic compartment, which, in turn, would

favor Aβ generation. Thus, accumulating evidence implicates PS1 in the regulation of APP trafficking. The possibility exists that the local environment within the TGN or the endocytic system contributes to misalignment of mutant PS1 and APP carboxyl-terminal fragments, thereby favoring generation of Aβ42. Such a mechanism has been implicated in other diseases (e.g., cystic fibrosis) that are also caused by missense mutations in polytopic proteins (Gentzsch et al., 2004).

Sorting Defects in Late-Onset Alzheimer's Disease

As reviewed, the sorting of APP and BACE among the membranous compartments of the TGN, cell surface, and the endosome is a dynamic and highly regulated process. Thus, even subtle sorting defects that would slow the trafficking in and out of any individual membranous compartment could, in principle, increase Aβ generation. Guided by the sorting mechanisms that transport APP and BACE from the surface to the endosome, and from the endosome back to the TGN, a list of candidate molecules can be generated in which an abnormality could, in theory, contribute to the accumulation of Aβ found in late-onset Alzheimer's disease (Table 1).

In principle, manipulating any molecule on this list might modulate Aβ generation. Indeed, this predicted effect has already been shown for many of the listed molecules (e.g., Ho et al., 2002; Hill et al., 2003). To date, however, only a few of these molecules have been found to be defective in brain tissue harvested from patients with late-onset Alzheimer's disease. VPS35, VPS26, and Beclin (the mammalian homolog of VPS30) were recently implicated in a study that relied on brain imaging to construct an a priori model predicting how pathogenic molecules should behave—spatially and over time (Small et al., 2005). This model was used as a guide, first in generating gene-expression profiles with microarray technology from areas of the brain most vulnerable (the entorhinal cortex) and relatively resistant (the dentate gyrus) to late-onset Alzheimer's disease, and second in analyzing the complex microarray dataset. As discussed in a recent review, imaging-guided microarray is an effective approach for addressing many of the analytic limitations inherent to profiling gene expression patterns generated from postmortem brains (Lewandowski and Small, 2005). sorLA (also known as LR11), a fourth molecule on the list, was implicated in a study that first used microarray technology to profile the gene expression patterns of lymphocytes of patients with late-onset Alzheimer's disease and then found that sorLA levels are reduced in affected brains (Scherzer et al., 2004). Subsequently, experimental evidence directly established a role for sorLA in APP trafficking and Aβ generation in endosomal compartments (Andersen et al., 2005; Offe et al., 2006).

Although some of these identified molecules serve multiple functions, they are all unified in playing a role in the retromer trafficking pathway. As reviewed, VPS35 and VPS26 are core molecules of the retromer complex itself (Seaman, 2005); VPS30 interacts with retromer-related phosphoinositide kinases, whose activity mediates the binding of the retromer complex to the endosomal membrane (Seaman, 2005); and, sorLA is a putative cargo of the neuronal retromer (Small and Kim, 2003; Small et al., 2003).

Taken together, these studies suggest that the retromer trafficking pathway might play a primary role in Aβ generation and the pathogenesis of late-onset Alzheimer's disease. Furthermore, since APP and/or BACE (directly or indirectly via sorLA) (Figure 2) might be trafficked by the retromer, this generates a specific hypothesis for how Aβ might be overproduced in the absence of defects in APP, BACE, or components of the γ-secretase. Namely, defects in the retromer trafficking pathway are predicted to increase the residence time of APP and/or BACE in the endosome, thereby increasing the β-cleavage step of APP processing (Figure 3). Indeed, a number of studies have suggested that β-cleavage is enhanced in late-onset disease (Fukumoto et al., 2002; Holsinger et al., 2002; Johnston et al., 2005; Li et al., 2004; Tyler et al., 2002; Yang et al., 2003).

Nevertheless, many questions remain about the retromer trafficking pathway and Alzheimer's disease. For example, further confirmation is required to establish whether the retromer affects the sorting of APP and/or BACE and whether it does so by directly binding either of these type-I transmembrane proteins. Also, although studies agree that sorLA modulates the production of Aβ and binds APP, there are discrepancies in the currently existing data as to whether sorLA downregulation missorts APP to the TGN or to the endosome (Offe et al., 2006; Spoelgen et al., 2006). Finally, the upstream mechanisms that cause the reductions in VPS35, VPS26, VPS30, and sorLA have not yet been established. In this regard, the reported mismatch between the levels of VPS35 mRNA and protein (Small et al., 2005) suggests that retromer elements might undergo accelerated degradation in the disease state. Although a recent report did not find disease-related polymorphisms for VPS26 (Riemenschneider et al., 2006), a pattern of accelerated degradation is consistent with a genetic defect, suggesting that polymorphisms in other retromer elements might exist (Lewandowski and Small, 2005).

More generally, beyond implicating a specific pathway, the recent convergence of evidence provides proof-of-principle that protein sorting is a cell biological mechanism that can contribute to late-onset Alzheimer's disease. As a complex disorder, many molecular defects are expected to contribute to late-onset Alzheimer's disease, and other sorting molecules on the list are predicted to contribute to disease pathogenesis.

Conclusions

Transmembrane protein sorting is a fundamental property of normal cellular function, and it is therefore not surprising that a growing number of diseases are found to be associated with, or directly caused by, defects in sorting molecules (Olkkonen and Ikonen, 2000; Cobbold et al., 2003; Clee et al., 2006). On theoretical grounds, because APP and its cleaving enzymes are transmembrane proteins, and because Aβ liberation is a membrane-dependent event, protein sorting is a cell biological process uniquely vulnerable to Alzheimer's disease pathogenesis. Specifically, the trafficking of APP and BACE among the TGN, cell surface, and endosome are predicted to be the sorting pathways most relevant to Alzheimer's disease. Indeed, recent studies have begun confirming this prediction, isolating disease-related defects in protein sorting, and future studies are expected to identify additional defects that regulate the transport of APP and its cleaving enzymes through its sorting itinerary (Table 1).

Elucidating the mechanisms that sort APP and the secretases through the TGN, cell surface, and endosome has significantly expanded the understanding of Alzheimer's disease cell biology. More importantly, isolating specific defects in protein sorting opens up unexplored therapeutic avenues, which optimistically, may accelerate the development of effective treatments for this devastating and intractable disease.

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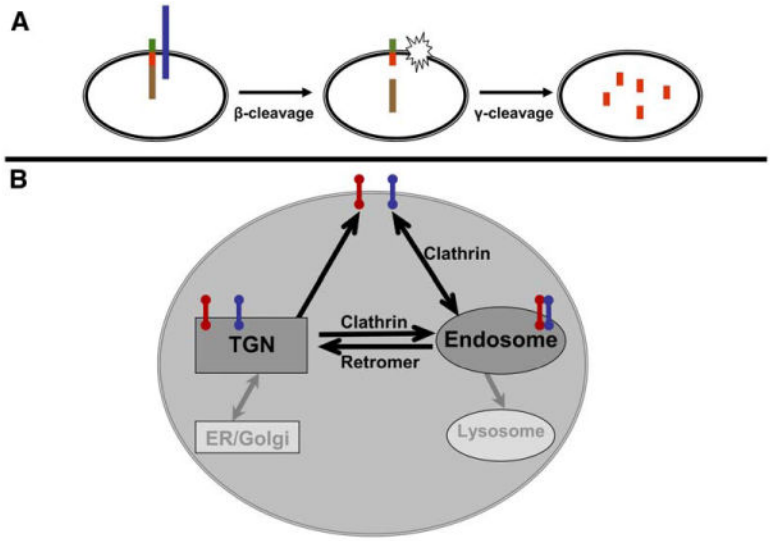

Figure 1.

Protein Sorting as a Key Mechanism in Alzheimer's Cell Biology, (A) Aβ (red bar) is liberated from its parent protein, APP (multicolor bar), in two enzymatic steps. In the first β-cleavage step, BACE (blue bar) splits full-length APP into an sAPPβ fragment (brown bar) and a C-terminal fragment (CTFβ, red/green bar). Then, in the γ-cleavage step, the γ-secretase (star) splits CTFβ into Aβ (red bar) and amyloid intracellular domain (AICD, green bar). β-cleavage is the committed step in APP processing and may be upregulated in late-onset Alzheimer's disease.

(B) Both APP (red bar) and BACE (blue bar) are type-I transmembrane proteins that are sorted through multiple membranous compartments of the cell. The sorting triangle that interconnects the *trans*-Golgi network (TGN), cell surface, and the endosome is critically important for APP and BACE sorting. As indicated, clathrin is the coat complex that regulates transport from the cell surface and the TGN to the endosome, while the retromer is the coat complex that regulates transport from the endosome back to the TGN. A convergence of findings suggests that APP and BACE are most likely to interact within the membranes of the endosomal system, initiating the potentially amyloidogenic pathway.

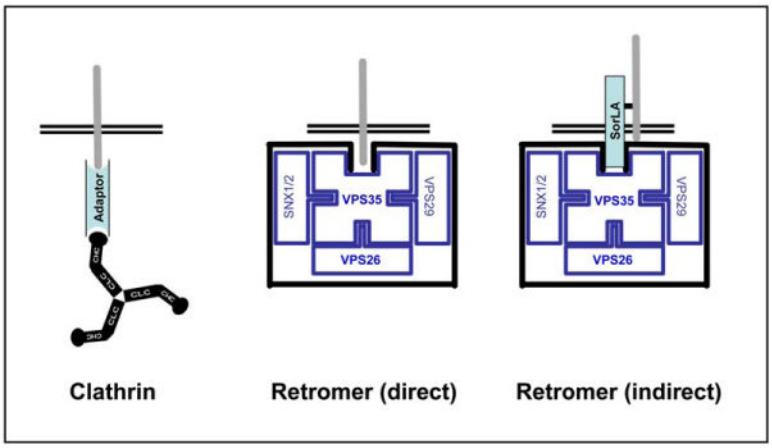

Figure 2.

Coat Complexes in Alzheimer's Disease, The clathrin coat (left). The clathrin coat complex transports type-I transmembrane proteins to the endosome from the TGN or from the cell surface. Each clathrin molecule is made up of three clathrin light chains (CLC) and three clathrin heavy chains (CHC). The terminal domains of CHC binds adaptor proteins (blue), which in turn bind the to-be-sorted transmembrane cargo proteins (gray bar). The adaptor proteins impose cargo and itinerary specificity. The dileucine motif found in BACE typically binds GGAs, a family of adaptor proteins engaged in clathrin-mediated transport from the TGN to the endosome. The NPXY motif found in APP binds a wide-range of adaptor proteins (as discussed in the text) engaged in clathrin-mediated transport from the cell surface to the endosome.

The retromer (middle and right panels). The retromer coat complex transports type-I transmembrane proteins from the endosome to the TGN. VPS35 is the core of the retromer and binds directly to the to-be-transported type-I transmembrane protein cargo (gray bar). VPS26, VPS29, and sortin nexins (SNX) 1 or 2 assemble onto VPS35 to generate the complete functional retromer complex. Recent findings suggest that the retromer sorts APP or BACE, either by direct binding to the retromer or by indirect binding to the transmembrane adaptor protein sorLA (blue).

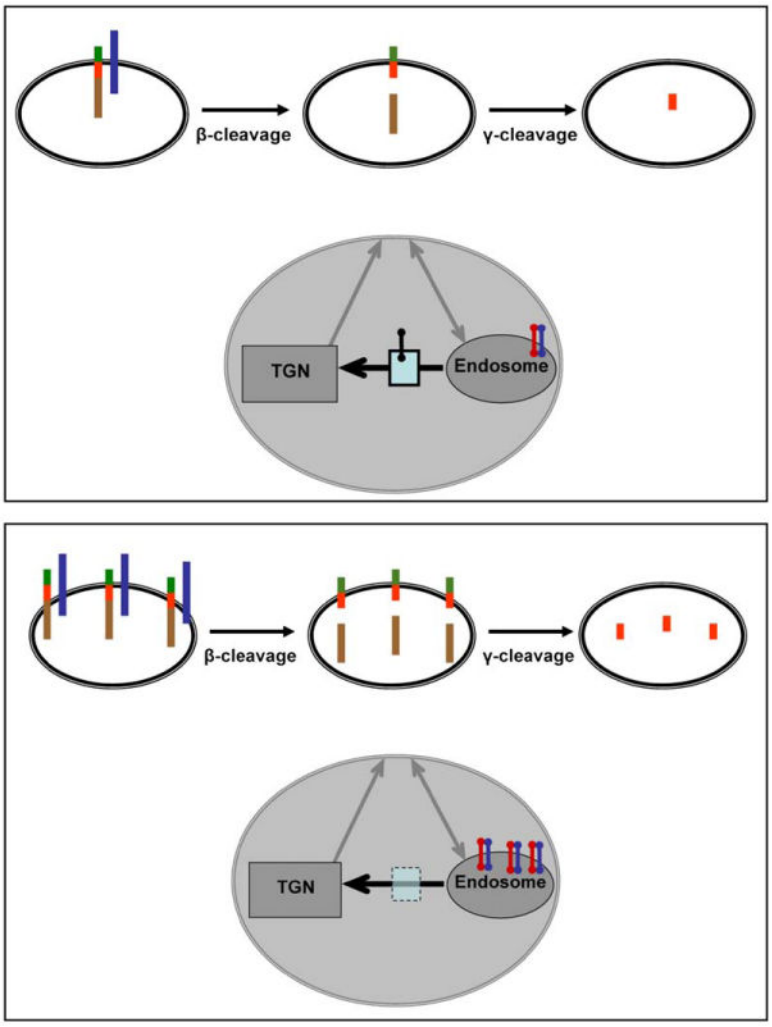

Figure 3.

Sorting Defects in Late-Onset Alzheimer's Disease, To date, a handful of sorting molecules involved in APP and BACE transport (see Table 1) have been found to be downregulated in the brains of patients with late-onset Alzheimer's disease. Although serving multiple functions, the identified molecules all play potential roles in retromer-mediated transport (blue box, retromer; black bar, type-I transmembrane cargo protein transported from the endosome to the TGN), suggesting a model whereby endosome-to-TGN transport is a sorting itinerary particularly vulnerable to disease. When compared to normal neurons (upper panel), neurons of Alzheimer's patients (lower panel) are predicted to have diminished transport of APP and/or BACE (black bar) out of the endosome, thereby increasing the colocalization of APP and BACE in endosomes. This, in turn, could lead to excess β-cleavage and excess generation of Aβ.

Table 1
Molecules that Participate in Sorting APP and/or BACE

Coat complexes	**clathrin heavy chains, clathrin light chains, VPS35, VPS26A, VPS26B, VPS29, sorting nexin 1, sorting nexin 2, ARF**
Coat adaptors	sorLA, sortilin, ARH, Numb, Dab1, Dab2, Jip1b, Fe65, Fe65L1, Fe65L2, X11α, GGA1, GGA2, GGA3
Factors for docking and/or fusion	syntaxin 5, 6, 7, 8, 13, 16; VAMP 5, 7, 8; Rab 5, 11; SM protein VPS45, Munc 13, 18
Protein or lipid kinases, phosphatases	PKA, PKC, ERK, cdk5, GSK3, ROCK1, PP1, PP2A, PI3K, Akt, VPS30 (beclin)-VPS34-PI3K
Other factors	presenilin 1, presenilin 2, nicastrin, APH1, PEN2, lipid rafts, caveolin, flotillin, cholesterol