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Title: Amyloid β production along the neuronal secretory pathway: Dangerous liaisons in the Golgi?

Running title: The Golgi and Amyloid β production

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Synopsis

APP and the β-secretase, BACE1, are segregated into distinct transport pathways in the secretory pathway, which regulates the level of amyloid β production. The Golgi undergoes changes in morphology in Alzheimer's disease which are likely to contribute to disease pathology. Here we hypothesize that perturbations in segregation of APP and BACE1 during Golgi transit, mediated by a variety of different processes, result in enhanced Aβ production and neurotoxicity.

Abstract (up to 200 words)

β-amyloid peptides (Aβ) are generated in intracellular compartments of neurons and secreted to form cytotoxic fibrils and plaques. Dysfunctional membrane trafficking contributes to aberrant Aβ production and Alzheimer's disease. Endosomes represent one of the major sites for Aβ production and recently the Golgi has re-emerged also as a major location for amyloid precursor protein (APP) processing and Aβ production. Based on recent findings, here we propose that APP processing in the Golgi is finely tuned by segregating newly-synthesised APP and the β-secretase BACE1 within the Golgi and into distinct *trans*-Golgi network transport pathways. We hypothesize that there are multiple mechanisms responsible for segregating APP and BACE1 during transit through the Golgi, and that perturbation in Golgi morphology associated with Alzheimer's disease, and or changes in cholesterol metabolism associated with Alzheimer's disease risk factors, may lead to a loss of partitioning and enhanced Aβ production.

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Keywords (two to six)

Golgi apparatus, neuronal membrane trafficking, APP, BACE1, amyloid β, membrane lipids, Alzheimer's disease

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Introduction: Role of amyloid peptides in the aetiology of Alzheimer's disease

Alzheimer's disease (AD) is characterised by the pathological hallmarks of extracellular amyloid plaque formation, neurofibrillary tangles, neuroinflammation and neuronal loss. Amyloid plaques and neurofibrillary tangles consist of aggregated amyloid β (Aβ) and the microtubule associated protein, tau, respectively. Amyloid deposition is a central event in the aetiology of AD [1]. The emerging view proposes that the initial production of Aβ is an early event in AD which promotes microglia-induced inflammation [2] leading to tau pathology and enhanced Aβ associated with an increase in inflammatory cytokines [3]. Hence, a vicious cycle is maintained driven by Aβ and neuroinflammation.

Aβ is derived from the intracellular proteolytic processing of the membrane protein, amyloid precursor protein (APP), by the membrane-bound secretases, BACE1 and γ-secretase, via the amyloidogenic pathway (**see below)** The hydrophobic Aβ is prone to aggregation to form oligomers and fibrils [4]. Soluble monomeric and oligomeric Aβ have been reported to promote a plethora of aberrant activities including brain hyperactivation, perturbation of cytoskeletal dynamics, induction of neuronal death, inflammation, and perturbation of lipid metabolism which impacts on lipid homeostasis and membrane composition [5,6]. The progressive accumulation of Aβ in neurons is neuro toxic, synaptotoxic [4,7] and alters neuronal trafficking [8].

There is considerable evidence based on AD susceptibility genes and experimental animal models that dysfunctional membrane trafficking contributes to aberrant Aβ production, synaptic dysfunction and AD [8-11]. A key issue in understanding the regulation and dysregulation of APP processing is the identification of the intracellular sites of convergence of APP and its secretases. For BACE1-mediated APP cleavage to take place, BACE1 and APP need to converge within an acidic compartment given the pH requirements for BACE1 activity [12]; the *trans*-Golgi network (TGN) and early/late endosomes are compatible locations [13]. Moreover, BACE1 and APP have to be incorporated into the same membrane subdomain of a given compartment for physical interaction and catalysis to occur. Hence, the regulation of membrane trafficking as well as membrane lipid domain organization of intracellular compartments are relevant to the control of Aβ production.

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The early and late endosomes are recognised as a major site for Aβ production, and there have been many recent reviews focused on the role of endosomes in APP processing [8,9,11]. Late onset AD (LOAD), or sporadic, risk factors include components of the endocytic machinery which supports the relevance of membrane trafficking in the regulation of Aβ production. Although not yet studied, dysfunctional sorting in early endosomes could also affect retrograde transport between the early endosomes and the TGN and result in perturbations of the Golgi. For example, some of the LOAD risk factors, such as sortilin related receptor and VPS35 are integral components of the retromer-mediated transport to the TGN [14,15]. Schekman and colleagues have demonstrated that retromer mediated recycling of APP to the TGN was required for efficient Aβ production and secretion and moreover, retention of APP in the early endosomes reduced Aβ production, indicating that Aβ is generated mainly in the TGN in a transfected HEK cell system [16]. Retrograde transport between the early endosomes and the TGN is associated with substantial membrane flux between the two compartments [17,18], and defects in these pathways are associated with downstream perturbations in Golgi architecture [19,20]. Also, it remains unclear how much of the Aβ produced in the early endosomes is secreted from the cell, especially as the majority of the soluble contents of the endosomal pathway is transported to the lysosomes for degradation. Studies in the 1990s first identified Aβ production in the secretory pathway [21,22]. Recent studies have also highlighted a role for the secretory pathway and the Golgi apparatus as a major location for APP processing and Aβ production and secretion [23-26]. The Golgi/TGN is now recognised to be a major site for the regulation and contribution of membranes for autophagosome biogenesis [27], and there is evidence for a role of autophagy in the degradation and exosome secretion of Aβ [28]. These findings are particularly pertinent as Golgi morphology is altered in AD, and perturbation in Golgi architecture is likely to impact on APP processing [29]. In this commentary we will focus on APP processing and Aβ production in the secretory pathway, as the potential relevance of the secretory pathway has been often overlooked in the recent literature.

Modification of BACE1 and/or APP trafficking in non-neuronal cells can lead to an increase of Aβ production in the secretory pathway [26,30]. A key question is how APP is protected from unwanted processing along the neuronal secretory pathway given that newly synthesised APP, and the secretases, share the same pathway? Of note, recent studies have shown that BACE1 and APP are segregated into distinct post-Golgi transport pathways in neurons,

providing a mechanism to regulate levels of Aβ [23,25]. Multiple sorting mechanisms are likely to be responsible for the segregation of these two cargoes, as discussed in the following sections. Here we propose that these Golgi sorting mechanisms are fundamental for fine tuning the regulation of APP processing and Aβ generation along the secretory pathway, and that fragmentation of the Golgi in neurological diseases, including AD, perturbs these Golgi sorting processes.

Processing APP: the good and the bad.

Several proteases cleave APP following two distinct pathways: the non-amyloidogenic and the amyloidogenic pathway (**Fig. 1**).

In the **non-amyloidogenic pathway**, APP is initially cleaved by α-secretase to generate soluble APPα and the membrane associated α-CTF (C83) which is then cleaved by γ-secretase to generate p3 and APP intracellular domain (AICD) fragments. APPα protects neuronal outgrowth. In the **amyloidogenic pathway**, APP is first cleaved by BACE1 (β-site amyloid precursor protein cleaving enzyme) to generate soluble APP-β and β-CTF (C99) fragments. C99 is then cleaved by γ-secretase leading to the generation of Aβ peptides and AICD fragments. Monomers of Aβ, which have potential physiological properties that merits further investigation, aggregate to form neurotoxic oligomers. BACE1 expression and activity are increased in the AD brain and the downregulation of BACE1 in mice inhibits Aβ generation, illustrating the relevance of BACE1 cleavage of APP in AD (see reviews [3,10]. Additional APP processing pathways have recently been described (summarised in [13]) increasing the complexity of APP processing.

APP-processing- cell biology in neurons

APP is ubiquitously expressed in neurons and is detected in both the somato-dendritic and axonal compartments [31-34]. By confocal microscopy using organelle markers, endogenous APP was predominately localized to the Golgi complex and late endosomes [35] and overexpressed APP was detected in the TGN of mouse neurons [36]. Hence, although studies analysing the location of APP in neurons are limited, the TGN appears likely to be a major site for the intracellular location of APP under steady state conditions.

Endogenous BACE1 in neurons has been detected mainly in the recycling endosomes and early endosome under steady state conditions, with minor levels in the Golgi and late

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endosomes/lysosomes [25]. Similarly, the majority of the exogenously expressed BACE1-GFP was found in recycling endosomes with 10-30% co-localised with Golgi markers in mouse primary hippocampal neurons [37]. γ-secretase is present in both the secretory and endocytic compartments. The common view is that γ-secretase activity is present in the ER, Golgi, post-Golgi compartments and endosomes and plasma membrane [38,39]. There are conflicting reports on the detection of the γ-secretase complex in the TGN of neurons [40,41]. However, the finding that the products of APP cleavage by BACE1 in the TGN of mouse neurons are very rapidly processed by γ-secretase to Aβ peptides, suggests that γ-secretase activity is present in the TGN [25,42].

An unresolved question is the relative amounts of Aβ generated in the soma, dendrites and axons of neurons. Using a fluorescence complementation assay, exogenous APP and BACE1 have been shown to physically interact in the soma as well as dendrites and axons [36]. In addition, APP processing and Aβ production was detected in the axonal compartment in transgenic mice [43] and local processing of APP detected in the dendrites was observed after APP overexpression [34,44]. Interestingly, small Golgi units, called Golgi outposts (GOs), which include the TGN compartment, are scattered throughout the dendrites and function as a component of a local secretory network [45] and may potentiate APP processing.

Sites of convergence of newly synthesized APP and active secretases along the secretory pathway

Newly synthetised APP and BACE1 are transported from the endoplasmic reticulum (ER) along the secretory pathway (**Fig. 2**). The co-transport of these newly synthesized membrane proteins at first sight appears to represent a dilemma with a potential opportunity for APP processing and Aβ production [21,46]. However, consideration of the features of the secretory pathway helps to understand how Aβ production is tightly regulated along the anterograde pathway in healthy neurons. Firstly, BACE1 is synthesised as an inactive zymogen and is converted to the active secretase in the late Golgi by cleavage of the pro-domain (reviewed in [47]). In addition, BACE1 activity is optimal in an acidic environment [12,48] and its highest activity is detected in endosomes and TGN [49,50]. Given these constraints, the TGN is probably the earliest compatible location for substantial BACE1 cleavage of APP along the secretory pathway.

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Studies using both neuronal and non-neuronal cells have provided evidence that the TGN is one of the major sites for Aβ production [16,23,26,51]. Redirection of the BACE1 recycling route to the TGN in HeLa cells results in an increased Aβ production [30]. In addition, retention of endogenous BACE1 or APP in the TGN in primary mouse neurons results in an increase in APP cleavage by BACE1 and increased production and secretion of both Aβ40 and Aβ42 [25]. The production and secretion of the cytotoxic Aβ42 species from the secretory pathway is particularly relevant as it represents the major amyloid species of amyloid plaques [1]. These studies demonstrate that when BACE1 or APP levels increase in the TGN, the amyloidogenic processing pathway is enhanced due to an increased convergence between BACE1 and APP.

The TGN has a distinct morphology, with extensive tubular domains and budding vesicles which can form a highly dynamic extended network. The TGN is a traffic hub which receives cargo along the secretory pathway and also from the endocytic pathways. Cargoes are segregated into distinct membrane subdomains generated by the recruitment of adaptors, golgins, small G proteins, lipid binding proteins and kinases from the cytosol [52,53]. These distinct membrane subdomains were highlighted by the mapping of >15 *trans*-Golgi/TGN proteins using super-resolution microscopy [54]. Although APP and BACE1 were not analysed in this study, these findings add another important consideration for APP processing; APP and BACE1 need to be located not only within the same compartment but also within the same membrane sub-domain so physical contact of the two membrane proteins can occur.

Proposed model of partitioning of APP and BACE1 in neurons

The TGN generates a number of distinct transport carriers, which likely arise from the different membrane domains. The TGN exit of cargoes is selectively driven by the recognition of specific cargo motifs by the adaptor proteins of the transport carriers (review [53]. Specific sequences in the cytoplasmic tail of the cargoes, such as the tyrosine based (YXXΦ) and dileucine based (DEXXXLLI) motifs, are specifically recognised by adaptor proteins [55,56]. Recent studies have revealed that BACE1 and APP have different TGN sorting motifs recognised by different machinery and exit the TGN by independent pathways which are conserved across neuronal and non-neuronal cells [25,26]. The export of BACE1 from the Golgi to the plasma membrane (PM) is regulated by its DDISLL motif and the adaptor protein AP-1 and ARF1 and ARF4 small Golgi proteins [25]. On the other hand, the trafficking of APP from the TGN is regulated by a YKFFEE sequence within its cytoplasmic tail which

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is recognised by AP-4/Arl5b [23,26]. Overexpressed BACE1 and APP have been detected in different transport carriers in primary neurons after their TGN exit [57], consistent with independent transport pathways for each cargo. The anterograde pathways for BACE1 and APP in neurons, and the sites of APP processing, are illustrated in **Fig. 2.**

The segregation of cargoes in the TGN is in part mediated by adaptors which shuttle different cargoes into specific transport carriers destined for different transport routes. As mentioned above, the silencing of AP-4 and the associated retention of APP in the TGN resulted in an increase in APP processing by β-secretase and α-secretase [23,26,42], however the increase was relatively modest implying that partitioning of APP and secretases was partially maintained in the absence of the adaptor. This finding suggests there are additional mechanisms for the partitioning of cargoes. Moreover, evidence is emerging that membrane cargoes can also be sorted and segregated prior to arrival at the TGN [58-61]. For example, membrane cargoes are sorted from membrane glycosyltransferases during passage through the Golgi stack, indicating mechanisms to sort different membrane proteins in the stack [62]. In non-neuronal cells, there is evidence that glycoprotein cargoes destined for the apical and basolateral surfaces are partitioned into different lipid environments and differ in glycosylation patterns indicating exposure to different sets of glycosyltransferases throughout the Golgi stack [61]. Furthermore, Bonifacino and colleagues has demonstrated in non-neuronal cells that two membrane cargoes are segregated into different membrane subdomains in the *cis-medial* Golgi and both the transmembrane and luminal domains contribute to this sorting process [63].

Given these collective findings, we propose that BACE1 and APP are partitioned from each other as they pass from the *cis*-Golgi to the TGN where they exit into two independent transport pathways (**Fig. 3A**). Based on this model, the partitioning of APP and BACE1 will involve multiple mechanisms, including lipid mediated sorting of transmembrane domain, specific interactions involving the luminal domains, and sorting motifs on the cytoplasmic domain that interact with adaptors at the TGN. Collectively, these mechanisms limit APP processing with only a low level of Aβ production under physiological conditions. Perturbation in any one of these mechanisms, either by mutations of the cargo sequences, perturbations of Golgi membrane composition and organization, extension of residence time of cargos in the Golgi, or deficiencies in sorting adaptors could result in reduced partitioning of the cargoes and an enhancement of the processing of APP by BACE1 in the TGN.

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Mutations and post-translational modifications of APP or BACE1 sequences can modify their intracellular trafficking, their steady-state localisation and directly affect the production of Aβ [50,64,65]. Notably, a number of mutations in APP are associated with an increased risk of early onset familial AD [66 67] (https://www.alzforum.org/mutations/app). Aβ production is increased in human iPSC-derived neurons bearing familial APP disease mutations [68]. More than half of the familial APP mutations are found in the transmembrane domain (https://www.alzforum.org/mutations/app), either close to or distant from the secretase cleavage sites. For some APP mutations increased Aβ levels may be due more efficient enzymatic cleavage of the APP substrate by BACE1 [69]. However, the molecular basis for the enhanced APP processing of most of these familial APP mutations is not known. Familial APP mutations in the transmembrane domain could affect Golgi lipid-mediated sorting mechanisms. Mutations in transmembrane domains of other cargoes have been shown to dramatically impact on membrane sorting processes in the Golgi, ER and endosomes [70-72]. Given the importance of the transmembrane and luminal domains in segregation of membrane cargoes in general [70,73,74], we predict that some AD causing APP mutations will affect membrane partitioning and result in increased clustering of APP and BACE1 during transport through the Golgi to the TGN (**Fig. 3B**). The increased clustering of APP and BACE1 could apply to the somatic Golgi and also the dendritic Golgi outposts. In addition, some APP mutations may also affect the transport kinetics of APP through the Golgi, resulting in increased Golgi residency time and elevated processing; increased APP processing has been observed for wild-type APP when the kinetics of Golgi exit is delayed [25].

Impact of fragmentation of the Golgi on Aβ production and neurodegeneration

In mammalian cells, individual Golgi stacks are joined into a twisted ribbon, typically found close to the nucleus. Neurons are unique with both a Golgi ribbon in the soma (cell body) and individual mini-stacks (outposts) in dendrites [75] (**Fig. 2**). Golgi morphology is highly dynamic and regulated by scaffold molecules which link Golgi membranes to the microtubule and actin cytoskeletons [76 77]. Moreover, perturbation of the Golgi ribbon modulates a number of signalling pathways [78,79].

The loss of a compact Golgi ribbon and the appearance of dispersed Golgi structures in the cytoplasm of mammalian cells is commonly referred to as ‘Golgi fragmentation’. Dispersal

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of the compact Golgi associated with experimental and pathological conditions may reflect a variety of different morphological states of the Golgi, such as the conversion of the ribbon morphology into distinct Golgi mini-stacks or perturbation of the integrity of the Golgi stacks themselves (for reviews on this topic see [80,81]). The precise morphology of the 'fragmented Golgi' is relevant to understanding the functional status of the Golgi in these cells, in respect to alterations in membrane transport, glycosylation and signalling [76,80].

The relevance of Golgi architecture in neurons is emphasized by reports of a fragmented somatic Golgi in all the major neurodegenerative diseases, including AD [29,67,82,83]. Although the number of studies analysing the Golgi morphology of patients' neurons is limited there have been many studies in animal models of AD and cell models of neurodegeneration which suggest that changes in the Golgi morphology is a hallmark of AD [29,67,82,83]. Strikingly, restoration of a compact Golgi in animal models of AD protects from disease progression [84,85] implying that Golgi fragmentation contributes directly to the pathology of disease. Notably, Golgi fragmentation is associated with increased APP processing and Aβ production [29,84], acting as positive feedback loop. Indeed, Aβ promotes Golgi fragmentation by activating cyclin dependent cdk5, which phosphorylates GRASP65, a Golgi protein which has a key role in regulating the Golgi ribbon structure [84] and which has been proposed to be the major mechanisms for Golgi fragmentation in AD. Inflammation may also contribute to the perturbation of the Golgi in neurons in AD, as the TGN is disassembled from the Golgi stack to act as a scaffold to activate the NLRP3 inflammasome complex [86].

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Perturbation of the Golgi structure by pharmacological agents also have been shown to influence APP processing and Aβ production. Brefeldin A (BFA) treatment induces a re-absorption of the *cis* and *medial* Golgi proteins into the endoplasmic reticulum and blocks transport along the secretory pathway. BFA increases the level of mature full-length APP detected in cells, including neuronal cells, and reduces APP processing [24,87], indicating APP is processed in post-ER compartments. Similarly, accumulation of APP was observed in presence of BFA in primary rat neurons however, in this system APP was overexpressed and an increase in Aß production was detected [88]. Agents and treatments which affect transport through the Golgi, i.e monensin, or maturation and transport of vesicles from the TGN, i.e 20^{o}C temperature block, inhibited APP delivery to the PM in transfected COS cells but only partially inhibited the generation of Aß [89]. Collectively, these studies also demonstrate that APP processing and production of Aß can occur in the Golgi.

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Given the importance of the TGN in the sorting and partitioning of APP and BACE1, and APP processing and Aβ production, we propose that the loss and/or alteration of the Golgi ribbon architecture perturbs the organisation of the Golgi compartments and the efficiency of cargo partitioning, resulting in an increase in Aβ (**Fig. 3A, C**). Also, fragmentation of the Golgi ribbon is likely to result in breakdown of ER-TGN contact sites which are required for transfer of lipid molecules to maintain the correct lipid composition of Golgi membranes [90]. Cholesterol is known to modulate PI4P synthesis [91], a critical phosphoinositide at the TGN which regulates trafficking [92]. Alterations in cholesterol metabolism has been reported to be associated with elevated Aβ [93,94] which we propose is a consequence of cholesterol mediated disruption of the membrane organisation of the TGN. For example, sphingolipid- and cholesterol-rich membrane microdomains (or rafts) in the TGN are considered to be critical for sorting of apical proteins in polarized cells [95,96]. Modification of the cholesterol-rich membrane microdomains, arising from Aβ-mediated dysfunctional lipid homeostasis, is also like to impact on partitioning of APP and BACE1 and subsequent processing.

A*POE4*, the strongest genetic risk factor for LOAD, is known to raise levels of circulating cholesterol and enhance amyloid pathology. Moreover, increasing the cholesterol level in neuronal culture induces the early phenotypes of AD [97]. Although the mechanism by which ApoE4 enhances Aβ production is poorly understood, cholesterol influences the properties of membranes and impacts on protein sorting events in the secretory and endocytic pathways [98,99] which could result in enhanced clustering of APP and the secretases in membranes, particularly the TGN where distinct lipid domains have been identified [100-102], leading to increased APP processing and Aβ generation

In addition to lipid dysregulation, familial APP mutations can also influence the organization of the Golgi as a consequence of perturbing the regulation of APP processing. As an example, the APP Swedish mutation drives Golgi fragmentation in neurons through Aβ production and phosphorylation of GRASP65 [84]; Golgi fragmentation will in turn likely result in enhanced APP processing.

Golgi fragmentation also increases autophagosome activity [103]. Autophagy, an intracellular degradation process essential for neuron survival, contributes to neurodegenerative diseases

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including AD [28]. Interestingly, autophagosome-lysosome fusion is regulated by GRASP55, a Golgi protein which also regulates Golgi morphology [104]. Moreover, the APP cargo adaptor protein for TGN exit (AP-4) has been also shown to regulate autophagosome formation [105]. Autophagosomes may be another potential Aß production site under pathological conditions [106], especially with their ability to secrete Aß [107]. Overexpression of ApoE4 in mice, a genetic risk factor for LOAD, results in dysfunctional autophagy, increase in Aβ42 in lysosomes, and neuronal death [108]. Altogether, these findings illustrate the potential synergy between the Golgi and autophagosomes and in APP processing and Aß production.

Taken together, we propose a positive feedback loop of increasing Aβ production driving Golgi fragmentation and alteration in membrane microdomains which in turn result in loss of partitioning of APP and BACE1 in the Golgi and increased APP processing in the secretory pathway (**Fig. 3**).

Outstanding Questions and Concluding Remarks

There are a number of outstanding questions that need to be resolved to address the hypothesise proposed here:

What is the contribution of local secretory pathway in dendrites and axons in APP processing and Aβ production? There is evidence of local secretory pathways in dendrites and also possibly axons, distant from the cell body of the neuron. A specialized set of Golgi ministacks known as Golgi outposts are located in dendrites, which are independent from the somatic Golgi [45,109]. However, it is unknown if APP and the secretases are synthesised locally in the dendrites and/or transported from the soma ER to the Golgi outposts. This is relevant to understanding the potential of the secretory organelles in dendrites to generate Aβ.

What is the impact of Golgi fragmentation in neurological diseases on TGN subdomain organisation, membrane lipids and exit of cargo? Fragmentation of the Golgi ribbon is associated with many neurological disorders and diseases. However, more information on the consequence of Golgi fragmentation in primary neurons on TGN function and APP processing is required. What is the consequence of Golgi fragmentation in primary neurons on the kinetics of Golgi exit of APP and processing in the TGN? How does Golgi fragmentation impact on ER-contact sites and lipid homeostasis? What is the impact of inflammasome activation on TGN function and APP processing? Does restoration of a

compact Golgi reduce levels of APP processing and Aβ production? Answers to these questions will provide a deeper understanding of the perturbations of sorting and trafficking events associated with AD pathogenesis.

In conclusion, both adaptor-mediated and lipid mediated sorting mechanisms are likely to play a critical role to segregate and partition APP from BACE1 in distinct membrane domains along the secretory pathway. Our model proposes that perturbations of these mechanisms will enhance APP processing and Aβ production. The Aβ generated in the secretory pathway in neurons, which includes the more cytotoxic Aβ42, can then be readily secreted via the normal exocytosis pathways. The framework we are proposing integrates the previous disparate findings in the AD field of disease-causing APP mutations, perturbations in Golgi morphology, and the effects of Aβ on lipid homeostasis and cholesterol. With the advent of human iPSC derived neurons, there is now the ability to directly examine these mechanisms in human neurons in detail from patients with AD.

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The authors declare no conflict of interest.

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Figure Legends

Figure 1: APP processing pathways

A schematic outlining the processing pathways of APP. In the non-amyloidogenic processing pathway (upper, olive background), APP is first processed by α-secretase (gold) within the amyloid-β (Aβ) domain (red) to generate the soluble APP ectodomain, sAPPα, and the C-terminal fragment, CTFα/C83. The subsequent cleavage of CTFα/C83 by γ-secretase (green) liberates p3 and AICD. In the amyloidogenic processing pathway (crimson background), the β- secretase, BACE1 (purple), cleaves APP at the interface of the Aβ domain and APP luminal domain to generate sAPPβ and CTFβ/C99. CTFβ/C99 is then cleaved by γ-secretase to release the Aβ peptide and AICD.

Figure 2: Anterograde trafficking of APP and BACE1 and APP processing hub in neurons.

A. A human neuron is represented. BACE1 and APP are synthetised in the endoplasmic reticulum (ER) and are transported through the cisternae of the somatic Golgi apparatus (GA) to the *trans*-Golgi network (TGN). From the TGN, BACE1 (in green) is transported to the plasma membrane (PM) whereas the majority of APP (in red) is transported to the early endosomes (EE). In the dendrites, BACE1 and APP may be transported from the somatic Golgi pool or from the ER-exit sites (ERES) of the dendrites, before trafficking through the Golgi outposts (GOs) to reach the dendritic PM. **B-C**. Zoom in the cell body (**B**) and in a dendrite (**C**) of a human neuron. APP and the inactive BACE1 are both indicated in the ER. The model proposes that immature BACE1 is converted to active BACE1 in the TGN and Aβ is generated in both the somatic Golgi (**B**) and the GOs (**C**). Following internalisation BACE1and APP are also distributed in EE, recycling endosomes (RE) late endosomes (LE) and lysosomes (LYS).

Figure 3: Influence of the loss of the Golgi ribbon and APP mutations on Aβ production in neurons.

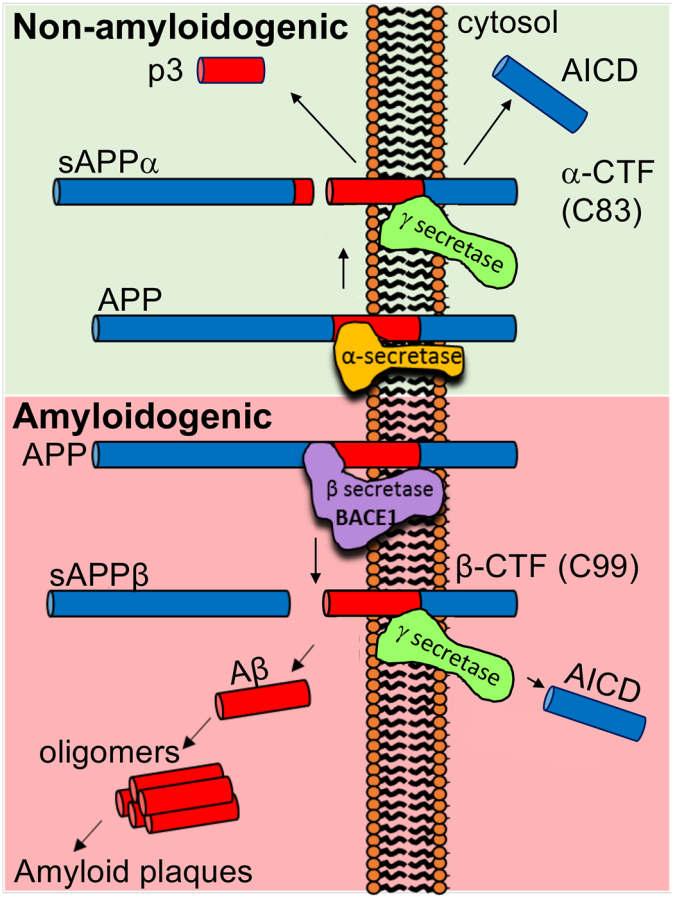
Schematic representation of the partitioning of APP and BACE1 in the Golgi apparatus in neurons.

A. WT neurons display a compact somatic Golgi ribbon. The segregation of APP (in red) and BACE1 (in green) increases during transit through the *cis*, *medial* and *trans* Golgi cisternae. BACE1 is concentrated in cholesterol rich lipid rafts (yellow). In the TGN, APP and BACE1

are well segregated and are packed into different transport carriers using specific adaptor proteins (AP-1 and AP-4). **B**. APP mutations (in purple) may affect its transport kinetics (and change the APP Golgi residency time), its segregation from BACE1 and its recognition by the AP. Many APP mutations lead to an increase in Aβ production which will induce Golgi fragmentation. **C.** The Golgi apparatus is "fragmented" in AD. Our model predicts that in the absence of the ribbon, segregation of APP and BACE1 in the Golgi in impaired, resulting in an increase of APP and BACE1 colocalization in the TGN and Aβ production. If APP and BACE1 segregation can still occur via AP interactions, then Aβ production is limited to the TGN. If the partitioning via the AP is affected by the loss of the ribbon, colocalization of APP and BACE1 will lead to enhanced Aβ production in the Golgi and the transport carriers. Elevated Aβ levels will then act to further increase Golgi fragmentation.

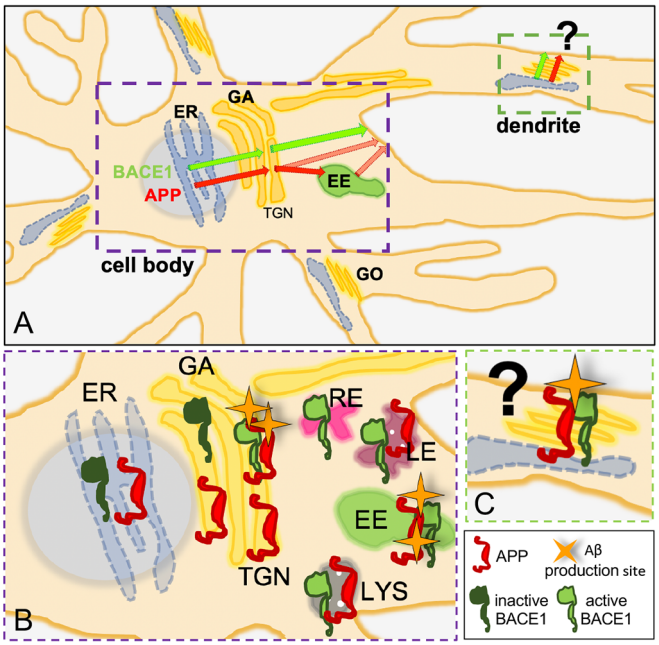
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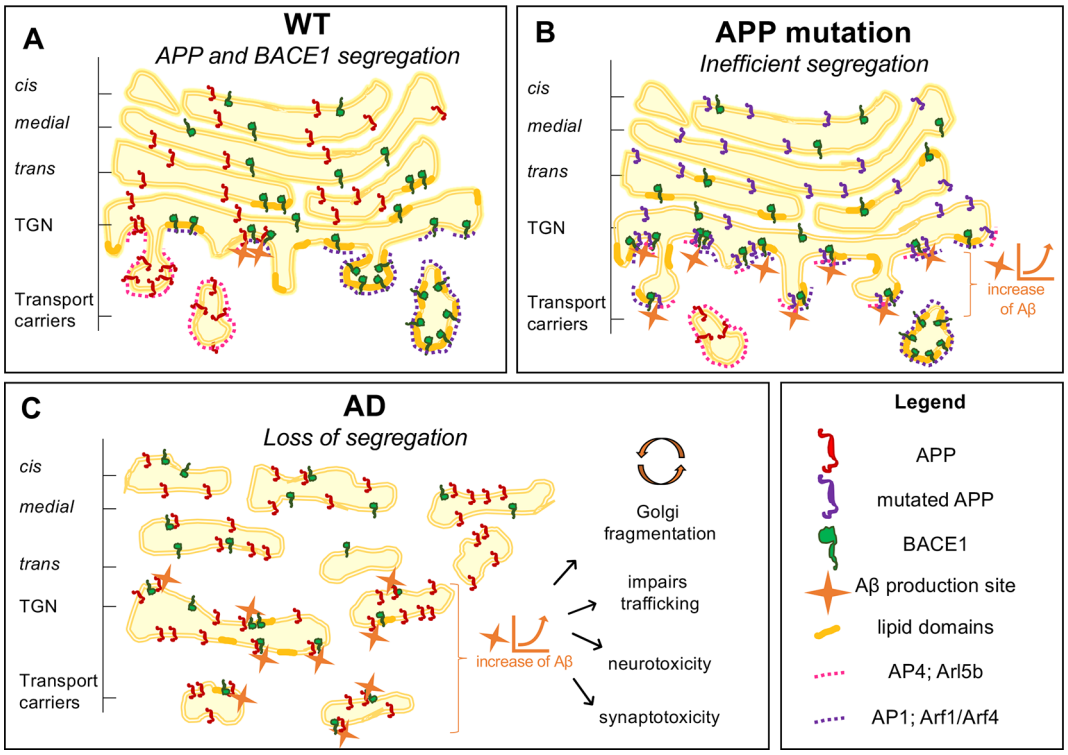
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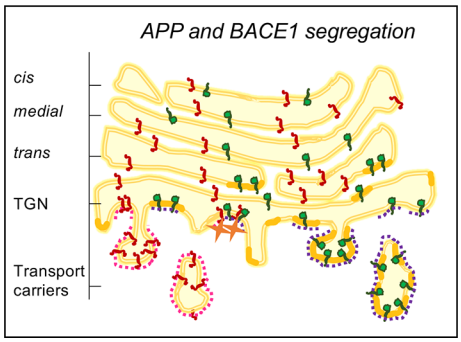
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