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Significance of transcytosis in Alzheimer's disease: BACE1 takes the scenic route to axons

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Abstract

Neurons have developed elaborate mechanisms for sorting of proteins to their destination in dendrites and axons as well as dynamic local trafficking. Recent evidence suggests that polarized axonal sorting of β -site converting enzyme 1 (BACE1), a type I transmembrane aspartyl protease involved in Alzheimer's disease (AD) pathogenesis, entails an unusual journey. In hippocampal neurons, BACE1 internalized from dendrites is conveyed in recycling endosomes *via* unidirectional retrograde transport towards the soma and sorted to axons where BACE1 becomes enriched. In comparison to other transmembrane proteins that undergo transcytosis or elimination in somatodendritic compartment, vectorial transport of internalized BACE1 in dendrites is unique and intriguing. Dysfunction of protein transport contributes to pathogenesis of AD and other neurodegenerative diseases. Therefore, characterization of BACE1 transcytosis is an important addition to the multiple lines of evidence that highlight the crucial role played by endosomal trafficking pathway as well as axonal sorting mechanisms in AD pathogenesis.

Introduction

Alzheimer's disease (AD) causes memory impairments and a massive neuronal loss in aging individuals. Although AD affects millions of people worldwide, becoming an ever-increasing burden for the health care system and economy, there is currently no cure for this devastating neurodegenerative disease. One of the main pathological hallmarks of the disease is the extracellular deposition of β -amyloid peptides ($A\beta$) in so-called "senile plaques" in the brains of sufferers. Soluble oligomeric forms of $A\beta$ cause synaptic dysfunction and likely contribute to neuronal loss in AD [1]. Therefore, significant effort has been invested in developing and clinical testing of therapeutic strategies for AD, aimed at reducing cerebral amyloid burden. $A\beta$ is generated by the sequential cleavage of its precursor amyloid precursor protein (APP) by two proteases, BACE1 (also referred to as β -secretase) and γ -secretase [2,3]. BACE1 is expressed at high levels in neurons, and processing of APP by BACE1 is a pivotal step in initiating $A\beta$ production. The γ -secretase complex is ubiquitously expressed, and is responsible for intramembrane proteolysis of BACE1-cleaved APP C-terminal fragment to release $A\beta$. Approaches to inhibit the activities

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either enzyme are still being evaluated in preclinical and clinical trials, despite severe setbacks in the past due to lack of efficacy or mechanism-based toxicity in humans.

BACE1 is a type I transmembrane aspartyl protease that undergoes trafficking through the secretory and endocytic recycling pathways [3,4]. BACE1 has a short cytosolic tail of 23 amino acids within which it undergoes post-translational *S*-palmitoylation, serine phosphorylation, and ubiquitinylation. In addition, it harbors an “acidic di-leucine motif” capable of binding to AP2 as well as GGA proteins and the retromer complex [4]. Many details of BACE1 trafficking became apparent from studies conducted in cultured cell lines [4]. Notably, di-leucine motif-mediated association of BACE1 with AP2, retromer complex, and GGA proteins, as well as ubiquitinylation, are important for BACE1 internalization and subsequent sorting to either recycling endosomes for trafficking to the plasma membrane or directing to lysosomes for degradation. Serine phosphorylation also limits the extent of BACE1 recycling to the cell surface [3]. Several important insights on APP processing in endosomes, and how subcellular localization of BACE1 could influence the efficiency of A β production, have emerged from these studies. However, little is known about the regulation of BACE1 transport in neurons, and how dynamic subcellular localization of BACE1 in neurons might affect the efficiency of APP processing in somatodendritic and axonal compartments. Thus, crucial questions such as whether A β is mainly generated and released in the somato-dendritic domains or axons, and in which organelles APP undergoes amyloidogenic processing in neurons, remain unanswered.

Recently, we investigated the regulation of endosomal BACE1 trafficking in cultured hippocampal neurons [5]. Here, we highlight a novel facet of BACE1 biology that became apparent in our investigation of BACE1 trafficking using a video microscopy approach that permitted selective visualization of the fate of internalized BACE1 in primary hippocampal neurons. We also discuss how dynamic subcellular localization of BACE1 might affect the efficiency of amyloidogenic APP processing of APP and speculate on the importance of trafficking regulation of BACE1 for neuropathology in AD.

Internalized BACE1 undergoes unidirectional retrograde transport in dendrites

In neurons, endosomes are dispersed throughout the somatodendritic compartment and axons, yet internalization and recycling of proteins in dendritic spines and axon terminals differ markedly in terms of kinetics and molecular machinery (Box 1). In order to follow the fate of BACE1 after its internalization from the cell surface in neurons, we employed a strategy developed to study receptor internalization [6]. In this approach, introduction of a 13-residue α -bungarotoxin binding site near the N-terminus of BACE1 allowed robust labeling of surface-resident BACE1 in transfected neurons by incubation with fluorescently labeled α -bungarotoxin (Figure 1A). In dendrites, the dynamic transport of endosomes, visualized by live-cell imaging of fluorescently tagged endosomal-resident proteins, occurs in both directions: away (anterograde) or toward the cell body (retrograde) (Box 2). By live-cell imaging of BACE1, we made the unexpected discovery that after internalization from the surface of dendrites, surface-labeled BACE1 (visualized by fluorescent α -bungarotoxin) was transported in endosomes exclusively toward the neuronal soma. However, when nascent secretory and internalized BACE1 molecules were observed

together (visualized by YFP fluorescence) bidirectional BACE1 transport was apparent in the same dendrites, with no obvious bias in directionality (Figure 1B). Surprisingly, unidirectional retrograde transport of internalized BACE1 was observed only in dendrites, whereas vesicles containing internalized BACE1 were transported in both retrograde and anterograde direction in axons.

Is BACE1 delivered to axons by transcytosis?

The function of two members of the epidermal growth factor receptor substrate 15 homology domain containing proteins, EHD1 and EHD3, was required for unidirectional retrograde transport of internalized BACE1 in dendrites [5]. EHDs are “dynamin-like” ATPases, which function in endosome membrane tubulation and vesicle fission. In non-neuronal cells and in neurons EHDs are part of a complex machinery that coordinates membrane sorting, as well as vesicular transport in early and recycling endosomes along with Rab GTPases [7-9]. The way in which the function of EHD in endosomal carrier formation is coupled to unidirectional retrograde transport of internalized BACE1 cargo-selective vesicles in dendrites remains unknown. Interestingly, compromising EHD activity not only affected retrograde vesicular transport of internalized BACE1 in dendrites, but also significantly decreased BACE1 axonal targeting [5]. These findings raised the possibility that unidirectional retrograde transport might be coupled to the delivery of BACE1 to the axonal compartment. On the contrary, impairing the activity of Rab11 GTPase, which indirectly interacts with EHD protein via its effector Rab11 family interacting protein 2, attenuated BACE1 axonal sorting without having a discernible effect on the retrograde transport of internalized BACE1 in dendrites (unpublished data) [10,11]. Upon expression of dominant negative Rab11, there was a concomitant increase in the levels of BACE1 in the soma, suggesting that Rab11-dependent regulation of BACE1 transport succeeded EHD1 and EHD3 function. In the model we propose based on these findings, nascent BACE1 molecules reach the dendritic plasma membrane through secretory transport, and are then endocytosed and retrieved to the soma in an EHD1/EHD3-dependent unidirectional retrograde transport. Subsequent sorting of endosomal vesicles containing BACE1 from the soma to the axon requires Rab11 activity (Figure 2).

BACE1 undergoes dynamic sorting and axonal transport in endosomes

The model for axonal delivery of BACE1 through transcytosis proposed above predicts that BACE1 should be enriched in axons, and that axonal sorting of BACE1 involves transport in endosomes. As described below, *in vitro* and *in vivo* observations support this model. Indeed, polarized sorting of BACE1 through transcytosis is evident in longitudinal analysis of BACE1 distribution in transfected hippocampal neurons where transgene-derived BACE1-YFP initially distributes to both dendrites and axons, but gets preferentially targeted to axons over time [11]. Furthermore, polarized axonal sorting of BACE1 *in vivo* can also be deduced from the prominent localization of BACE1 in the mouse brain in the *stratum lucidum* of the hippocampus, in presynaptic terminals of mossy fibers from dentate gyrus granule cells. Notably, BACE1 is only weakly detected in dendrites or cell bodies of granule cells, indicative of preferential and efficient axonal sorting [5,12-14]. BACE1 is not the only protein that appears to show discrepancy in protein localization between *in vivo* localization (mainly in axons) and in transfected neurons (dendrites and axons). Like BACE1, kainate-

type glutamate receptor subunits GluK2 and GluK3 localize predominantly in axons and presynaptic terminals of the hippocampal mossy fibers in the mouse brain but are more prominent in dendrites of cultured neurons after transfection [15,16].

Colocalization of BACE1 with EHD1 and EHD3 in the dentate gyrus granule cell axons and presynaptic terminals in mouse brain indicates steady-state residence of axonal BACE1 in endosomes [5]. When analyzed by live-cell imaging, bidirectional axonal BACE1 transport in highly dynamic tubular and vesicular carriers can be observed in acute hippocampal slices from BACE1-YFP transgenic mice and in mature hippocampal neurons transfected with BACE1-YFP. In both instances, axonal BACE1 localizes to a highly dynamic pool of vesicles, as well as larger stationary structures, which corresponded to presynaptic sites [11]. The dynamic characteristics of BACE1 vesicular transport - alternating rapid movement in both anterograde or retrograde directions and pausing behavior - are consistent with the motility of endosomes along microtubules, and highly reminiscent of the dynamics of syntaxin 13, a marker of recycling endosomes [17,18]. Indeed, in neurons BACE1 localizes predominantly to endosomes populated by syntaxin 13, as well as by transferrin receptor and Rab11 GTPase [11,19]. Two-color live-cell imaging studies further confirmed dynamic co-transport of BACE1 and Rab11 in recycling endosomes in both dendrites and axons [11].

How can internalized BACE1 achieve unidirectional dendritic retrograde transport - oneway traffic on a two-way street?

Although a few neuronal proteins have been characterized as undergoing transcytosis, unidirectional dendritic retrograde transport of BACE1 discussed above is unique, as this mode of vectorial transport in dendrites has never been reported in the literature for any transmembrane protein cargo. What could be the basis for this unidirectional dendritic transport? Long-range vesicular transport in dendrites and axons are mediated by microtubule-based transport. Two families of motor proteins, namely the kinesins and dynein, facilitate the transport of vesicles toward the microtubule plus-end or minus-end, respectively [20]. Cargo delivery is achieved through a complex adaptor protein machinery that links vesicles to the appropriate molecular motor protein, thus allowing their movement toward the plus-end (kinesin) or minus-end (dynein) of the microtubule [20]. The bidirectional and saltatory nature of BACE1 transport in neurons is characteristic of microtubule-based vesicular transport. Microtubules in mature neurons have a uniform plus-end-out orientation in axons and in distal dendritic segments, but in proximal dendrites they have a mixed orientation [21] (Box 2 and Figure 3). Therefore, even though vesicles containing internalized BACE1 in dendrites could acquire the ability to uniquely recruit retrograde motor to support unidirectional movement toward the minus-end, it is expected that the mixed polarity of the microtubules will lead to bidirectional movement in proximal dendritic segments (Figure 4). However, new *in vitro* evidence suggests that the spacing of the microtubule bundles and the presence of select microtubule-associated proteins could affect cargo transport by influencing the ability of motors to reverse direction, as well as affect the velocity and run length of processive motor proteins [22]. Still, the vectorial transport of internalized BACE1 is remarkable because not only do the transport vesicles containing internalized BACE1 need to distinguish between the plus-end and minus-end of microtubules in the near vicinity, they must also employ long-range guidance cues in order

to navigate through dendritic branch points in a unidirectional manner as they progressively move toward the soma. Finally, it is important to note that recycling endosomes in dendrites, visualized by fluorescently-tagged Rab-11, EHD1, EHD3, as well as transferrin receptor or internalized transferrin, are known to undergo bidirectional transport [23]. The majority of internalized BACE1-containing vesicles were positive for Rab11, EHD1, and EHD3 [5,11]; nevertheless, these vesicles underwent unidirectional transport in dendrites. This raises the possibility that internalized BACE1 itself might, directly or indirectly (*via* associated cytosolic adaptor proteins), have an active role in guiding the vectorial vesicular transport of recycling endosomes in dendrites (Figure 4).

How does BACE1 transcytosis compare with other transcytotic transport?

Nearly three decades ago, it was discovered that apical sorting of certain proteins in polarized epithelial cells involves biosynthetic delivery to basolateral surface, followed by internalization and trafficking in endosomes to the apical cell surface [24]. Transcytosis of proteins, as well as the signaling motifs and adaptors involved in polarized sorting, were originally characterized using cultured epithelial cells [25,26]. Apical sorting of epithelial proteins *via* transcytosis shares a high degree of similarity with polarized sorting of proteins from the somatodendritic to axonal surface in neurons, as illustrated by transcytosis of polymeric Ig receptor and L1/NgCAM [27-30]. Thus far, only a few proteins have been characterized as undergoing somatodendritic-to-axonal transcytosis in neurons, and there is only limited information available on the machinery and the molecular players involved in transcytosis. Endocytic sorting of dendritic BACE1 prior to axonal targeting is similar to transcytosis of L1/NgCAM and TrkA, which are also type I transmembrane proteins that get axonally targeted following endocytosis from the somato-dendritic cell surface. However, it remains to be established by live-cell imaging whether these well-characterized proteins also undergo vectorial transport following internalization from the dendritic surface.

Transcytosis of both NgCAM and BACE1 requires the function of EHD family proteins [5,7]; however, important differences exist in EHD-dependent axonal targeting of NgCAM and BACE1. First, axonal surface expression of NgCAM is prominent and highly polarized in transfected cultured neurons [31]. But, BACE1 is uniformly distributed on the surface of dendrites and axons one day after transfection of cultured neurons, and internalized BACE1 is also readily detected in both dendrites and axons [5,11]. Second, somatodendritic NgCAM is mainly sorted to NEEP21-positive endosomes prior to transcytosis, whereas, a large fraction of internalized BACE1 is found in recycling endosomes, with a relatively smaller fraction present in NEEP21-positive early endosomes. Third, whereas NgCAM endocytosis was markedly reduced by dominant-negative EHD1 expression in neurons [32], neither BACE1 endocytosis nor membrane sorting to recycling endosomes was affected by dominant-negative EHD1 or EHD3 expression. Rather, EHD function appears to be uniquely required for retrograde motility of vesicles containing internalized BACE1 [5]. Thus, it appears that NgCAM and BACE1 are subject to distinct endosomal sorting steps that precede transcytosis. On the other hand, axonal BACE1 sorting in recycling endosomes shares similarities with transcytosis of TrkA in Rab11-positive endosomes [33]. Expression of a dominant-negative Rab11 mutant caused accumulation of internalized BACE1 in the neuronal soma concomitant with a loss of BACE1 levels in the axons [11]. Retrograde

transport of internalized BACE1 in dendrites can still be observed in these neurons (unpublished data), suggesting that Rab11 is involved in axonal sorting of recycling endosomes. Although dominant-negative Rab11 expression attenuated axonal sorting of TrkA, whether Rab11 regulates a similar step in mobilizing or guiding TrkA-positive recycling endosomes from soma to axon is not known.

Polarized localization can be achieved through alternate mechanisms

Recent studies have shown that polarized delivery of transmembrane proteins to the somatodendritic domain is largely achieved by signal-mediated cargo sorting through the binding of the clathrin-associated adaptor protein 1 (AP1) and clathrin [26]. Binding of clathrin and AP1 to TGN-derived vesicles is required for exclusion of these cargos from axonal domains. In addition, AP1 may be involved in the retrieval of somatodendritic cargos from axonal transport carriers. On the other hand, the strategies for polarized axonal localization of transmembrane proteins are more complex in nature. Aside from dendrite-to-axon transcytosis (discussed above), compartmentalized distribution of certain axonal proteins is also achieved by somatodendritic endocytosis followed by compartment-specific elimination and selective retention in axons. For example, contactin-associated protein 2 (Caspr2) [a type I transmembrane domain protein member of the neurexin super family] localizes to juxtaparanodes of myelinated axons where it associates with K⁺ channels [34]. Axonal surface localization of Caspr2 is achieved by non-polarized biosynthetic delivery to dendritic and axonal plasma membrane followed by its compartment-specific endocytic elimination from the somatodendritic compartment [35]. In another example, Vesicle-Associated Membrane Protein 2 (VAMP2), a synaptic vesicle type II transmembrane protein is biosynthetically delivered to the surface of both axons and dendrites, but polarized axonal VAMP2 localization is achieved by preferential removal from the dendritic membrane via endocytosis and selective retention at the axonal surface prior to incorporation into synaptic vesicles [36]. Similarly, axonal enrichment of the neuronal sodium channel pore-forming α -subunit Na_v1.2 (a multi-pass transmembrane protein) is achieved by endocytosis and selective degradation from the somatodendritic compartment, as well as selective tethering at the axon initial segment [37,38]. Although details of the mechanisms involved in endocytic elimination are not available, it should be noted that retrograde transport to the soma is not a prerequisite for lysosomal degradation of internalized cargo proteins. Lysosomes are present throughout the soma and dendrites, and proteins destined for degradation undergo dynamic lysosomal sorting in dendrites. This is best illustrated by local lysosomal targeting and degradation of AMPA receptor subunits endocytosed in dendrites upon stimulation with AMPA or NMDA [39,40].

In the case of Na_v1.2, a nine-amino acid segment within the C-terminal cytoplasmic tail, which includes a di-leucine motif, is required for axonal compartmentalization. Mutation of the di-leucine domain impairs dendritic endocytosis as well as polarized distribution of Na_v1.2. Similarly, a di-leucine motif near the C-terminus is critical for BACE1 endocytosis [41]. Whether a mutation of the di-leucine motif will compromise polarized distribution of BACE1 has not been investigated. One possible complication in characterizing the role of the di-leucine motif by mutagenesis approach is the observation that di-leucine-containing motifs also mediate dendritic targeting of certain proteins [42]. Nevertheless, inhibition of

retrograde dendritic transport of BACE1 is accompanied by a concomitant decrease in the levels of total and internalized BACE1 in axons. Thus, unlike the case of Caspr2, VAMP2, and Na_v1.2, endocytic elimination does not appear to be the reason why BACE1 internalized from dendrites is transported to the soma as supported by the increase of internalized BACE1 in the soma when Rab11 function is impaired [11]. Rather, internalized BACE1 in dendrites reaches the soma and ultimately gets targeted to axons by transcytotic transport.

What is the relevance of BACE1 transcytosis in endosomes for AD pathogenesis?

Endosomal dysfunction is thought to occur early in AD pathogenesis, contributing to A β overproduction and accumulation [43]. Enlarged endosomes containing both early and recycling endosome markers, as well as A β , have been reported in the brains of patients with AD [44]. Microarray analysis of differential gene expression in hippocampal CA1 neurons implicates early endosomal dysfunction in AD progression [45]. The expression of certain Rab GTPases that coordinate endosomal vesicular transport, such as Rab5 and Rab7, are upregulated in AD [46], further supporting the idea that loss of homeostatic control of endocytic transport may contribute to pathogenesis in AD. Indeed, perturbation of the endosomal membrane sorting by siRNA knock down of several Rab GTPases affect the efficiency of APP processing by BACE1 and A β secretion [47]. Our recent finding that BACE1 undergoes transcytosis in recycling endosomes in hippocampal neurons also adds significant novel insights into the cellular mechanisms involved in neuronal A β production. Knockdown of EHD1 or EHD3 expression in cultured neurons significantly reduced A β levels, suggesting that EHD-mediated BACE1 transcytosis is critical for A β production [5]. Another recent report revealed that axonal BACE1 undergoes retrograde transport in Rab7-positive vesicles for lysosomal degradation. Loss of expression of Snapin, a dynein motor adaptor for late endosomes, stabilizes BACE1 by compromising its retrograde axonal sorting to lysosomes in cultured neurons, and results in significantly elevated A β levels in mouse brain [48]. These findings implicate neuronal endosomal trafficking pathways in amyloidogenic processing of APP, and are consistent with the data from non-neuronal cells (discussed below). The highly dynamic nature of bidirectional endocytic membrane transport in neurons could be a reason why under physiological conditions only a subset of APP molecules undergoes processing by BACE1 and γ -secretase. Nevertheless, as the above examples illustrate, perturbations of endosomal sorting and transport mechanisms could have a profound influence on AD pathogenesis.

BACE1 and APP undergo differential sorting after internalization

Based on biochemical and genetic evidence, the initial BACE1-mediated cleavage of APP is considered to be a rate-limiting step in A β production [4]. Notably, coding mutations immediately adjacent to the site of BACE1 cleavage in APP can predispose individuals to early-onset familial AD disease [K670N/M671L; referred to as the “Swedish” double mutation], or protect against AD [A673T] by influencing the efficiency of BACE1 cleavage [3,49,50]. In which compartment or organelles within the neuron A β peptides are generated remains a question of considerable controversy. APP and the secretases undergo dynamic secretory and endocytic trafficking, and at steady-state are predominantly distributed between the Golgi, TGN, and endosomes [3]. Initial studies on the analysis of wild-type APP metabolism in neurons and non-neuronal cells suggested that APP cleavage by BACE1

and γ -secretase occurs in endosomes following internalization of APP [51]. Since then investigations using cell biology, genetics, and histopathology approaches have divulged the significant role played by endosomal transport in A β production.

Although BACE1 and APP are both internalized from the cell surface of cultured cell lines and neurons, considerable differences exist in the mode of endocytosis of these proteins and their fate following internalization. Whereas both APP and BACE1 utilize the clathrin-mediated pathway for endocytosis, BACE1 also appears to undergo clathrin-independent internalization via an ARF6-mediated pathway [2,41,52,53]. However, following internalization, the majority of BACE1 and APP follow distinct paths of membrane sorting - BACE1 to recycling endosomes, and APP to late endosomes/lysosomes [5,11,53]. Recycling to the cell surface appears to be the default sorting pathway for internalized BACE1; based on the long protein half-life and the rate of recycling it is estimated that each BACE1 molecule is capable of undergoing multiple rounds of endocytosis and recycling [54]. On the other hand, only a small fraction of APP is recycled to the cell surface as internalized APP is cleaved by BACE1 or sorted to endocytic degradation pathway [2,3]. Striking differences have also been reported in polarized sorting of these proteins in Madin-Darby canine kidney cells, where the majority of BACE1 is sorted to the apical surface whereas APP is predominantly trafficked to the basolateral surface [55-58]. Despite intense investigations over the years, our understanding of the intracellular pathways that regulate APP and BACE1 trafficking and membrane dynamics in neurons is far from complete. For example, chemical activation of synapses increases the endosomal co-localization of BACE1 and APP in dendrites of transfected neurons [19]. The biological relevance of this observation to axonal transport of either protein or A β production has yet to be confirmed using appropriate physiological synaptic stimuli and analyzing the localization of endogenous BACE1 and APP.

APP and BACE1 are conveyed to axons via divergent transport mechanisms

From multiple experimental systems it has been known for some time that APP undergoes kinesin-1-mediated fast anterograde transport, but there is some debate as to whether APP and BACE1 are co-transported in the same vesicles along the axons [59-62]. As discussed above, in transfected neurons transgene-derived BACE1 first appears in cell body and dendrites [5,19], and then BACE1 molecules internalized from somatodendritic compartment get targeted to axons via transcytosis in recycling endosomes [5,11]. In stark contrast, nascent APP molecules are first targeted to axonal cell surface, and subsequently delivered to dendrites via axon-to-dendrite transcytosis [63]. These findings are consistent with the notion that not all APP and BACE1 molecules undergoing axonal transport are conveyed in the same vesicles, and yet axonal APP can be processed by BACE1 in peripheral and central nervous system [60,62]. The preferential steady-state localization of BACE1 in axons is also in agreement with presynaptic BACE1 cleavage of APP and A β release at or near synapses, reported from several *in vivo* studies [64-66]. Multiple adaptor proteins including AP2, Arf6, GGA, sortilin/SORLA, CALM, the retromer complex, sorting nexins, have been implicated in APP and/or BACE1 trafficking in non-neuronal cells and neurons [67-69]. Despite the usage of distinct sorting and axonal routing mechanisms, how, and in which organelle(s), a subset of APP (axonally sorted in secretory vesicles) and

BACE1 (axonally sorted in recycling endosomes) come together to initiate amyloidogenic processing is a critical question that remains to be explored.

Axonal transport dysfunction is linked to AD pathogenesis

Dysfunction of axonal transport has been implicated in the pathogenesis of AD and other neurodegenerative diseases [70-75]. Axonal transport dysfunction has been linked to pathological changes in tau, a neuronal microtubule-associated protein that stabilizes microtubules. The levels of tau, hyperphosphorylated tau, and aggregated tau are elevated in the brains of individuals with AD [76]. Overexpression of tau in neurons inhibits kinesin-dependent transport of neurofilaments, mitochondria, peroxisomes, and Golgi-derived vesicles, thus rendering cells vulnerable to oxidative stress and contributing to neurodegeneration [77]. Moreover, overexpression of tau retarded trafficking of APP-YFP into distal neurites as well as axonal transport of APP-YFP in neurons. However, A β production was unaltered under these conditions [77]. Nevertheless, it remains to be examined whether pathological changes in tau affected BACE1 trafficking and axonal transport, thus contributing to neuronal dysfunction through aberrant processing of its other physiological substrates (see below). The link between axonal transport and AD pathogenesis was also demonstrated by studies where kinesin-1 light chain (KLC1) expression was perturbed by loss of one *KLC1* allele in mice. Anterograde transport of transfected APP-YFP was significantly reduced in hippocampal neurons cultured from hemizygous *KLC1* mice. Reduced KLC1 expression in APP transgenic mice significantly increased A β production, intraneuronal A β 42 accumulation, and amyloid deposition, illustrating that even subtle dysfunction of axonal transport could markedly influence AD pathogenesis [78].

Intriguingly, in the brains of patients affected by AD, BACE1 abnormally accumulates in dystrophic neurites (swollen structures that are positive for the presynaptic marker synaptophysin) surrounding amyloid plaques, a feature also observed in AD mouse models such as the 5XFAD or Tg2576 transgenic mice [13,14]. The local increase of BACE1 levels at the presynaptic terminals is thought to contribute to cerebral amyloid burden in AD by facilitating overproduction of A β . Since BACE1 undergoes endosomal sorting and bidirectional transport in axons [5,11,48], one could anticipate that defects in the fidelity of axonal sorting machinery would impact dynamic BACE1 transport and steady-state localization, thus contributing to its accretion in dystrophic neurites. However, it remains unknown whether defects in the axonal transport of BACE1 also contribute to dystrophic neurite morphology through A β overproduction and ensuing neuronal abnormalities. In this regard, endosomal dysfunction in AD might lead to the co-accumulation of APP and BACE1 at the presynaptic terminals, leading to local amyloid overproduction and presynaptic swelling. In the 5XFAD mouse model, although APP colocalizes with BACE1 in dystrophic neurites within organelles that are likely to be of endosomal in origin [14], further studies are needed for the unambiguous identification of these organelles.

Neuronal BACE1 trafficking through transcytosis also has significant implications for neuronal dysfunction in AD through proteolytic processing of a broad range of additional physiological substrates that are important for synapse formation and neuronal activity

[4,79,80]. Details are beginning to emerge on BACE1 cleave of these diverse substrates that localize to the Golgi apparatus (ST3GAL-I sialyltransferase), lysosomes (Lysosomal membrane glycoprotein 1), surface and intracellular organelles in dendrites and axons (APP, APLP1, APLP2, and SEZ6 family proteins), axonal surface (L1, L1/NgCAM, contactin-2/TAG-1), presynaptic sites (neurexin and latrophilin family proteins), and post-synaptic sites (neuroligin family proteins) [79] (for details, see [4]). From these analyses, it is apparent that although APP processing is thought to largely occur in endosomes, BACE1 is capable of cleaving substrates that localize in the secretory pathway and other subcellular locations in neurons. It is notable that the intracellular itinerary of BACE1 is well suited to cleave diverse sets of substrates in the neuronal soma, dendrites, and axons, as it traverses through the secretory pathway to the neuronal surface and then undergoes dendrite-to-axon transcytosis in endosomes. How abnormalities in BACE1 transcytosis or axonal transport might impact on processing of its diverse substrates and consequently neuronal activity and function remains to be explored. For example, cleavage of Na_v β-subunits by BACE1 regulates the cell surface levels of Na_v1.1α, Na_v1.2α, and Na_v1.6α subunits and neuronal activity in mouse brain [81-83]. Similarly, BACE1 cleavage regulates the surface levels of contactin-2 in neurons [79], which is likely to influence homophilic trans interaction between contactin-2 located on the surface of axons and oligodendrocytes at the juxtaparanodal interface. This homophilic interaction as well as heterophilic cis interaction between contactin-2 and Caspr2 at the axonal surface has been implicated in the clustering of K⁺ channels at juxtaparanodes of myelinated axons [84]. Finally, BACE1 cleavage of neurexins and neuroligins, which are key synaptic cell adhesion molecules residing in the presynaptic and postsynaptic plasma membrane, could alter their relative stoichiometry on opposing synaptic membranes leading to an imbalance between excitatory and inhibitory neurotransmission [85]. BACE1 cleavage of neuronal proteins is an important area of investigation as it has significant implications for the successful outcome of therapeutic inhibition of BACE1 enzyme activity as a strategy to lower cerebral amyloid burden.

Conclusions and outlook

Advances in imaging and video microscopy have made it possible to study endosomal dynamics in greater detail and appreciate dynamic sorting of proteins between dendrites and axons, which is not possible to discern from steady-state analysis of endogenous proteins that have reached their preferred final destination. Progress in understanding the mechanisms involved in transcytosis such as polarized sorting of proteins through endosomal recycling or degradation pathways, and activity-dependent axonal transport, is now beginning to shed more light on how neurons achieve steady-state localization of proteins in the axonal compartment. Recent investigations have revealed key differences in endocytic sorting mechanisms used to transport BACE1 and APP. The discovery of BACE1 transcytosis in neurons provides important insights towards developing a better understanding of the sorting steps where APP and BACE1 trafficking intersect to initiate the production of pathogenic Aβ species. Novel therapeutic strategies that target APP secretases, in an effort to reduce cerebral Aβ burden, are undergoing testing in clinical trials. Progress in this area has been hampered because both BACE1 and γ-secretase have many other physiological neuronal transmembrane protein substrates [79,86], and total inhibition of their enzyme activities results in mechanism based toxicity [87-89]. Compartment-specific

inhibition of secretases remains a potentially interesting strategy for attenuation of APP processing [90]. A better understanding of the steady-state neuronal localization of endogenous APP and its secretases, their membrane sorting dynamics, as well as the trafficking pathways that convey these proteins to the different neuronal compartments will be critical for successful implementation of such strategies. Finally, it will be of interest to determine whether additional neuronal transmembrane proteins utilize the unusual unidirectional dendritic retrograde transport mode utilized by internalized BACE1 for axonal sorting, and if EHD proteins possess a general role in modulating this mode of transport of internalized cargo in neurons.

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Abbreviations

AD	Alzheimer's disease
Aβ	β -amyloid
APP	amyloid precursor protein
BACE1	β -site converting enzyme 1
BTX	α -bungarotoxin
EHD	EH-domain containing protein
KLC1	kinesin-1 light chain

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Box 1. Endosomal transport in neurons

The term “endosomes” refers to a mosaic of dynamic organelles made of highly motile vesicular and tubular carriers whose identity and function are tightly regulated by a complex machinery of proteins. The function of the endosomal membrane trafficking pathway is to sort and transport proteins and lipids internalized from the plasma membrane to organelles such as lysosomes and trans-Golgi network, or recycle them back to the cell surface.

Neurons have a complex endosomal system where heterogeneous endosomes are distributed throughout the axon, dendrites, and cell body [91]. Bidirectional short- and long-range transport of endosomal carriers occurs along both axons and dendrites on microtubules, mediated by kinesin and dynein superfamily motor proteins [17,92]. At the presynaptic terminals, endosomal intermediates are involved in the sorting of synaptic proteins and synaptic vesicle exo-endocytosis cycling. In addition, actin cytoskeleton and myosin family motor protein mediates endocytic trafficking and local recycling of endosomes in dendritic spines. Endosomal abnormalities are among the earliest known neuropathological changes reported in Alzheimer's disease [93].

Box 2. Anterograde and retrograde vesicular transport in neurons

The polarity of microtubules differs in axons and dendrites of mature neurons. Microtubules in axons have uniform orientation, with their plus-ends oriented towards the axon tips. In dendrites, microtubules have a mixed orientation in proximal segments, with their plus-ends facing either toward or away from the soma, and plus-end-out orientation in distal segments [21]. Plus-end-directed kinesin family proteins mediate anterograde transport of organelles from the cell body to the synapses in axons, whereas minus-end-directed dynein motors are responsible for retrograde transport from the distal tips towards the soma [92]. Dyneins are also responsible for conveying vesicles from the cell body into dendrites by bidirectional transport along mixed microtubules [94].

Figure 1.

Unidirectional retrograde transport of endocytosed BACE1 in dendrites visualized by the α -bungarotoxin (BTX)-labeling strategy. **A:** The BTX-labeling strategy used to visualize endocytic BACE1 dynamics by live-cell imaging. BACE1 was modified by the introduction of a BTX-binding site (BBS) near the N-terminus (N-ter) and YFP at the C-terminus (C-ter) [5]. Neurons transfected with BBS-BACE1-YFP construct were incubated in the presence of fluorescently labeled BTX at 37°C followed by a wash to remove unbound BTX. Whereas all BACE1 molecules are visualized by YFP fluorescence, internalized BACE1 molecules can be selectively visualized by BTX fluorescence. EE: early endosomes; RE: recycling endosomes; PM: plasma membrane. **B:** Internalized BACE1 dynamics in a dendrite visualized using the BTX-labeling assay. Transfected neurons were observed by live-cell imaging and time-lapse series of YFP fluorescence and BTX fluorescence were acquired sequentially. A representative frame and the kymograph corresponding to a proximal dendritic segment are shown. The neuronal cell body is on the left side of the images. Note the unidirectional retrograde movement of the vesicles containing internalized BACE1 (adapted from Buggia-Prévot et al. [5]).

Figure 2.

BACE1 transcytosis in neurons. Following endocytosis from the dendritic cell surface, internalized BACE1 undergoes EHD1 and EHD3-dependent unidirectional transport towards the soma. The high degree of colocalization of endocytosed BACE1 with Rab11 and transferrin receptor in dendrites indicates that retrograde vesicles containing BACE1 are recycling endosomes. Rab11 GTPase activity is required for further sorting of endocytosed BACE1 from the soma to axons. Thus, we suggest that BACE1 undergoes dendrite to axon transcytosis in recycling endosomes, regulated by EHD1, EHD3 and Rab11. In axons, endocytosed BACE1 undergoes bidirectional transport in highly dynamic vesicles, and also gets enriched in presynaptic terminals. We hypothesize that membrane dynamics and transcytosis of BACE1 restricts its encounter with APP, and thus contributes to spatiotemporal regulation of APP processing and A β production in distinct subcellular compartments of neurons.

Figure 3.

Microtubule orientation and BACE1 dynamics after internalization in neurons. Microtubules in mature neurons have uniformly oriented microtubules in axons and in distal dendritic segments with the microtubule plus-end facing away from the soma (plus-end-out orientation), but in proximal dendrites they have a mixed orientation [21]. As shown in Figure 1B, internalized BACE1 is transported exclusively in the retrograde direction in dendrites toward the soma, despite the mixed orientation of the microtubules. In axons, vesicles containing internalized BACE1 undergo bidirectional transport, despite the uniform plus-end-out orientation of the microtubules.

Figure 4.

Model of BACE1 transport machinery in neurons. Molecular motors kinesins and dynein drive vesicular movement along microtubules toward the plus- or the minus-end, respectively. We hypothesize that Rab11 FIP forms a complex with EHD and Rab11 proteins, and tethers BACE1-containing vesicles to molecular motors. It remains to be established if BACE1 binds to either Rab11 or EHD1/3 directly or indirectly, and which molecular motor drives the unidirectional retrograde motility of vesicles containing internalized BACE1 in dendrites.