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## Amyloid- $\beta$ Precursor Protein: Multiple fragments, numerous transport routes and mechanisms

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### Abstract

This review provides insight into the intraneuronal transport of the Amyloid- $\beta$  Precursor Protein (APP), the prototype of an extensively posttranslationally modified and proteolytically cleaved transmembrane protein. Uncovering the intricacies of APP transport proves to be a challenging endeavor of cell biology research, deserving increased priority, since APP is at the core of the pathogenic process in Alzheimer's disease. After being synthesized in the endoplasmic reticulum in the neuronal soma, APP enters the intracellular transport along the secretory, endocytic, and recycling routes. Along these routes, APP undergoes cleavage into defined sets of fragments, which themselves are transported – mostly independently – to distinct sites in neurons, where they exert their functions. We review the currently known routes and mechanisms of transport of full-length APP, and of APP fragments, commenting largely on the experimental challenges posed by studying transport of extensively cleaved proteins. The review emphasizes the interrelationships between the proteolytic and posttranslational modifications, the intracellular transport, and the functions of the APP species. A goal remaining to be addressed in the future is the incorporation of the various views on APP transport into a coherent picture. In this review, the disease context is only marginally addressed; the focus is on the basic biology of APP transport in normal conditions. As shown, the studies of APP transport uncovered numerous mechanisms of transport, some of them conventional, and others, novel, awaiting exploration.

### Keywords

Amyloid- $\beta$  Precursor Protein; intracellular transport; microtubule motors; kinesin-1; phosphorylation; secretase cleavage

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## Introduction, or why should we care about APP transport

It is not uncommon for today's biomedical research that the function of a protein at the center of a major disease is poorly understood. Amyloid- $\beta$  Precursor Protein (APP), the protein viewed at the core of the pathogenesis of Alzheimer's disease (AD) makes one good example. APP was discovered more than 25 years ago [1], and became one of the most studied proteins as soon as it was associated with AD. Multiple functions have been proposed for APP [2], but none of them is clearly demonstrated (Sam Sisodia of the University of Chicago once referred to APP as the All Purpose Protein – seminar delivered ~10 years ago to an audience at Case Western Reserve University). There are several explanations for this situation: (1) In mammals, APP is one of three related proteins, which may have overlapping functions; (2) Mice deficient in the *APP* gene, show a plethora of phenotypic changes – none essential for survival – that remain mechanistically unexplained [2]; (3) APP has complex biology, and is the precursor protein for Amyloid- $\beta$  ( $A\beta$ ), and several other polypeptides, which are generated from APP by successive cleavages operated by numerous proteases [3]. These polypeptides could have their own functions, independent of the parent protein, thus increasing the complexity of APP functions. Moreover, APP is extensively posttranslationally modified by glycosylation – both in the ecto- and endo-domain – and by phosphorylation at several residues within its short, cytoplasmic domain [4]. This domain interacts with multiple cytoplasmic proteins, including molecular motors that carry APP to different destinations [5]. In neurons, APP is transported along secretory, endocytic, and recycling routes that are currently being elucidated [6]. The cleavage of APP into fragments occurs along these routes, certainly at more than one cellular location.

APP is subjected to successive proteolytic cleavages by two of three endoproteases, called secretases, which operate along two mutually exclusive pathways [3]. Depending on its intracellular location, APP is cleaved by either  $\alpha$ - and  $\gamma$ -secretase (non-amyloidogenic pathway) or  $\beta$ - and  $\gamma$ -secretase (amyloidogenic pathway) (Fig. 1A). Although the first and second cleavages may occur in the same subcellular compartment, they usually are temporarily and spatially separated. These two proteolytic pathways produce mostly distinct, but topologically similar, sets of protein fragments (Fig. 1A). It is the amyloidogenic pathway, which generates the potentially toxic  $A\beta$  peptide that is most relevant to AD. Other proteases, including caspases, also cleave APP, but these proteolytic pathways are less investigated. Obviously, the transport and cleavage of APP are intimately related processes, essential for both the physiology and pathology of the neuron, and cannot be dissociated from each other.

This short review provides a glimpse into the transport of APP in relation to its processing; due to space limitations, it is not a comprehensive list of all identified APP transport routes, and their regulation. We aim to reveal the complexity of APP transport, which comprises the transport of the full-length APP, and of its derived fragments; we will also discuss the experimental challenges encountered when trying to track down what is, in fact, transported: full-length APP, or APP fragments. We will limit our analysis to a few examples, focusing on neurons. For the most part, we will avoid the beaten path, and stress on novelty.

## Where does APP localize in neurons? Short answer: Almost everywhere

APP, a type I transmembrane protein, is synthesized at the endoplasmic reticulum, and enters the intracellular transport along the secretory, endocytic, and recycling routes, in the soma and neuronal processes. With immunocytochemistry at light or electron microscopy level, APP was detected at the ER, the ER-Golgi Intermediate Compartment (ERGIC), in all subcompartments of the Golgi apparatus, the trans-Golgi network (TGN), post-Golgi secretory vesicles, the plasma membrane, in the early, sorting/recycling, and late endosomes, lysosomes, and autophagosomes. APP is transported to both axons and dendrites, but the fates of axonally- and dendritically-localized APP likely differ [7]. APP – full length and/or fragments – was also found in unexpected places, such as mitochondria [8], the nucleus [9], the ciliary rootlet of the retinal photoreceptor cells [10], and even – apparently free – in the cytoplasm [11]. In some of these locations, cleaved fragments were predominant. Still, some anomalies are obvious: the detection of A $\beta$  epitope in the cytoplasm is not explainable, unless intracellular degradation of membrane-bounded compartments, retrotranslocation from the membrane-bounded organelles, or some other – yet to be explained – phenomenon, reverses the topology of the polypeptide. Also, the extent of APP localization to mitochondria needs to be carefully assessed, because ER-localized APP is enriched at ER-mitochondria contact sites [12]. The localization to the nucleus of APP represents the nuclear targeting of the cytoplasmic fragment of APP (the APP Intracellular Domain, AICD), presumed to enter the nucleus alone, or in association with the APP binding protein, Fe65 [9].

Exogenously expressed APP, usually C-terminally tagged with YFP [13], also localizes to many intraneuronal compartments. While the resolution of detection of the tag varies among reports, expressed, tagged APP usually shows fewer details, being often localized throughout the neuronal soma and processes, unless the expression levels are kept low [14]. Little justification is found in expressing APP-derived polypeptides with incorrect topology, such as the expression, or injection, of A $\beta$  in the cytoplasm, without demonstrating that such situation really occurs under specific physiological or pathological conditions.

## APP detection with antibodies in situ versus tagged APP

Since APP is extensively proteolytically processed, it is uncertain whether the immunostaining with antibodies reflects the full-length APP, or an APP fragment containing the cognate epitope(s). Therefore, the studies relying on only one antibody to detect endogenous APP provide results that are difficult to interpret. Dual immunolabeling of C- and epitopes of APP in cultured primary neurons and neuronal cells shows both colocalization, consistent with labeling of full-length APP, and non-overlapping staining, consistent with labeling of the cleaved fragments with distinct cellular targeting [14]. Nonoverlapping distributions of APP C- and N-terminal epitopes are clearly detected both in the neuronal soma and processes. However, the data of immunocytochemistry can be misleading, because of steric hindrance or epitope masking by proteins bound to APP. This particularly applies to epitopes from the APP cytoplasmic domain, which interacts with numerous proteins, including those that contain a phosphotyrosine binding (PTB) domain [15]. A solution to this problem is provided by the expression of dual tagged APP

constructs, with distinct tags introduced into the C- and the N-terminal regions. Although largely used in the study of APP transport, the single tag reporters do not solve the problem, since they do not indicate whether the tag represents full-length, or a cleaved APP fragment. Dual tagged APP, where one fluorescent tag is attached to the extreme C-terminus of APP, and the other either next to the signal sequence or inserted into a more internal region of the ectodomain, have been generated recently [16–18]. The expressed, fluorescent, dual tagged APP appears to be correctly processed by the secretases, generating the expected, tagged C- and N-terminal fragments (CTFs and NTFs) [16, 18]. However, the immunoblot detection of the tagged APP fragments only indicates that the dual tagged APP can be cleaved, not that it is cleaved at the same rate, and at the same location as endogenous APP. Two-channel imaging of the two fluorescent tags revealed some separation of the tags, especially within the neurites, but mostly showed colocalization. Colocalization was interpreted as indicating the presence of full-length APP, although it could also indicate colocalization of cleaved NTFs and CTFs. A quantitative analysis of the published data clearly shows that the intensity ratio of the two fluorescent tags varies largely between the labeled structures in the neurites [16, 18]; this indicates that those compartments contain mixtures of full-length APP and APP fragments. Interestingly, the degree of colocalization of the tags increases with increasing the levels of expression of dual tagged APP, which indicates that at high levels of expression the cleavage rate drops artificially, or that the sorting and transport machinery of the fragments are saturated. It is likely that the voluminous, fluorescent tags attached to, or inserted into, APP hinder the interaction of APP with normal binding partners. For instance, the YFP molecule immediately following the C-terminus of APP – as it is in a widely used APP-YFP construct [13] – likely interferes with the binding of proteins that normally attach to the C-terminal region of APP. Thus, it is likely that the YFP-tagged APP species will be transported within neurons in a manner that differs from that of endogenous APP. The intraneuronal distribution of APP tagged with small tags at its N- and C-termini showed a better resemblance to the distribution of endogenous APP epitopes compared to the dual tagged APP containing large, fluorescent tags of the GFP type [18]. Presently, the solution to the problem of studying APP localization and transport in neurons could be found in optimizing the placement of the tags, and undertaking combined investigation of APP localization and transport using both immunocytochemistry of endogenous APP, and exogenous expression of tagged APP.

### **APP destinations within the neuronal soma, axons, and dendrites. How does APP reach these destinations?**

After exiting the ER, APP reaches multiple intracellular compartments in neurons; some of these are only obligate landmarks along the itinerary, while others are the sites where APP is cleaved into fragments, sorted for targeting to specific intracellular sites, or represent the “final destination” where APP species reside to fulfill specific functions, before their decay. The first destination harboring APP’s activity is the plasma membrane, where APP ectodomain is detectable throughout the surface of the soma and processes, particularly in non-permeabilized neurons. There, APP could function as receptor for extracellular ligands to regulate cell adhesion, survival, growth, path finding [2]. The plasma membrane is also a major site of  $\alpha$ -secretase cleavage [19], and shedding of sAPP $\alpha$ . It is also a transit station

before reentry of APP into the cell along the endocytic pathway [6], carrying APP – via the sorting/recycling endosome – back to the plasma membrane, to the TGN/Golgi, late endosome/lysosome, or to the ER/nuclear envelope. The function of APP along the retrograde/recycling trafficking route could be related to signaling – via signaling endosomes, for example. In the nucleus, APP participates in the regulation of gene expression via its AICD fragment [9]. The presence of CTFs in the nuclear membrane is also likely. Thus, the neuronal nucleus is a destination for APP. At the ER, the site of APP synthesis, APP could act as sensor of impeded axonal transport [20]. In this respect, it was proposed that phosphorylation and amyloidogenic processing of APP, triggered by cargo accumulation in the soma, initiates a cascade of events aimed at restoring normal transport. A destination of APP, frequently ignored, is the ER-mitochondria contact site, where APP and/or APP fragments accumulate [21]. As already mentioned, mitochondria themselves may contain APP and APP fragments [8].

Initially considered to be primarily transported into axons, APP is now known to be targeted into dendrites as well. Full-length APP localizes at the pre- and post-synaptic sites, where one proposed function is to form membrane tethers across the synapse [22]. APP fragments, including the N-terminal sAPP, concentrate at process endings [14], and may play a role in synaptic function [23]. While the APP transported into neurites was proposed to be proteolytically processed at the synapse, there is evidence that the NTFs and CTFs are transported into the processes, and reach the terminals independently, where they localize to distinct regions of the growth cone [14, 18]. In cultured neuronal cells, APP – mostly fragments – enters the fine, filopodia-like processes, another destination of APP [14]. Phosphorylated APP species preferentially accumulate in filopodia [14], and it is likely that posttranslational modifications in addition to phosphorylation, such as modification by the peptidyl-prolyl cis-trans isomerase, Pin1, or cytoplasmic glycosylation by *O*-GlcNAcase control the trafficking and the targeting/anchoring to specific destinations of APP. Thus, it is likely that APP reaches its destinations in neurites both as full-length APP, and as fragments cleaved elsewhere in the neuron (Fig. 1C).

### What is transported: full-length APP, or APP fragments?

There is evidence that APP is cleaved both in the neuronal soma, and in the neurites. The prevailing view is that APP is initially transported to the plasma membrane as full-length protein [6]; cleavage of APP then occurs locally, along endocytic/recycling pathways, upon reuptake of cell surface localized APP. In this scenario, the APP fragments generated in the soma are retained and degraded in the soma; consequently, all APP transported into the neurites would be full-length APP, with cleavage into fragments occurring mainly at synaptic regions. However, as described above, APP-derived fragments are detected separately in the processes (both with immunocytochemistry and dual tagged APP constructs), and accumulate at distinct sites at the terminals (Fig. 1B). In some cases, bona fide sAPP and A $\beta$  were detected along the neurites with C-terminal-end-specific antibodies to sAPP and A $\beta$ 40/42, unequivocally identifying these fragments [14, 18]. Moreover, A $\beta$  and N-truncated pyroglutamate A $\beta$  was detected in dense-core vesicles [24]; only the A $\beta$  peptide, not full-length APP, is modified by glutaminyl cyclase. Supporting independent transport of APP NTFs and CTFs, a study showed that APP deletion mutants, lacking either

the N- or C-terminus, are correctly transported to the axonal and dendritic compartments [25].

With regard to the plethora of fragments that are transported into neurites, their exact nature is not easily determined. It appears that the complexity of the proteolytic processing of APP is much higher than anticipated. In view that both full-length APP and APP fragments are transported in neurites, the precise site of processing, the site(s) of sorting of the generated fragments, and the directionality of their transport relative to soma remain to be established.

### Where is APP cleaved in physiological and pathological conditions?

It is largely considered that  $\beta$ -secretase cleavage of APP occurs in endosomes and late Golgi compartments, including the TGN [6]. Many studies indicate that  $\gamma$ -secretase cleavage occurs at the plasma membrane, and the endosomal/lysosomal system, including phagosomes and autophagosomes, both in the soma and the neurites [26–32]. Regions in the vicinity of synapses, both pre- and post-synaptic, are viewed as hot spots of  $A\beta$  production [7], mostly in response to synaptic activity. Synaptic activity and conditions of AD appear to facilitate convergence of APP with the secretases in acidic compartments [33]. In the soma, much of the APP is processed along the endocytic route, and the cleaved fragments – primarily the NTFs – are then transported into neurites [34, 35] (Fig. 2A). However, data accumulated over the years indicate that both  $\beta$ - and  $\gamma$ -secretase cleavage could also occur in early secretory compartments [20, 26, 36]: the ER, the ERGIC, and Golgi. The main argument against the possibility of APP processing in early secretory compartments is that neither BACE1 (the  $\beta$ -secretase in neurons), nor the  $\gamma$ -secretase complex, could be active here [6]. Yet, at steady-state BACE1 primarily localizes in the soma, including the ER. Moreover, among the major BACE1 interactors are the reticulons [37], which are largely restricted to the tubular ER [38]. Also, all subunits of the  $\gamma$ -secretase complex colocalize at the ER. With regard to the invoked necessity of posttranslational modifications of secretase subunits required for activity, known to occur in post ER compartments, we stress that there is robust bidirectional (i.e., anterograde and retrograde) trafficking both between the plasma membrane and the TGN, and between Golgi and ER. Active secretases could thus relocate to the ER, ERGIC, and cis-Golgi. While colocalization of APP with the secretases during transport into neurites under normal conditions is still debated, APP certainly colocalizes with the secretases in the soma, in early secretory compartments. Although their colocalization is not proof of cleavage, in conditions of dysregulated transport of APP and secretases, as likely occur in AD [5], significant active  $\beta$ - and  $\gamma$ -secretases could accumulate in the ER/ERGIC compartments. The argument that BACE1 would not be active at a pH higher than that of endosomes is also not realistic; maximal activity of the enzyme is not required for APP cleavage. In addition, phosphorylation of APP – which occurs to a large extent at the ER [20], and is increased in AD [39] and stress [40] – could favor APP cleavage at the slightly higher pH in the ER lumen. Recent studies reported that, in the aging brain, APP fragments accumulate in perinuclear compartments in the soma [41, 42]. In cultured neuronal cells, bona fide APP fragments are occasionally detected in a perinuclear compartment of the ER that associates with neurofilaments [43] (Fig. 2B). We conclude that APP cleavage is a dynamic, continuously changing process, which occurs at multiple sites within the neuron, not only in endocytic compartments.



## The motors and the roads for the transport of APP and APP fragments

It is assumed – although not always clearly demonstrated – that, within the soma, short distance transport of APP to and from the plasma membrane, and between various organelles, relies on the microtubule motors, kinesin-1 and cytoplasmic dynein. As for the long distance transport, studies indicate that anterograde transport of APP in axons is powered by kinesin-1 [13, 44, 45], moving along a mixed population of acetylated and nonacetylated microtubules [14, 18]. Retrograde transport uses cytoplasmic dynein [46, 47]. These motors appear to power transport of both full-length APP and APP fragments; the CTFs actually enter neurites with low efficiency [14, 18, 48] (see also Fig. 2A). There is less information on the transport of APP within the dendrites; however, it is likely that the same microtubule motors, kinesin-1 and cytoplasmic dynein, are used. Other kinesin motors could also deliver APP to the processes. If one assumes that APP itself recruits the motor (see below), there is at least one other kinesin that could bind to APP: the kinesin-2 member, KIF17, a primarily dendritic and ciliary motor, which could be indirectly recruited to APP via the APP binding protein, Mint1/X11 $\alpha$  [49–51].

The CTFs, mostly phosphorylated, have been detected at the growth cone, in filopodia, and along neuronal processes, in filiform extensions along the neurites of cultured neuronal cells [14]. These cellular structures are devoid of microtubules, but contain actin filaments. While APP could be recruited to transport entities penetrating in actin rich extensions in many ways, we mention that Myosin-X, a filopodial motor [52], contains an ERM domain capable of directly binding the consensus YENPTY- motif in the APP cytoplasmic domain. Thus, transport of APP to various sites in the neuron could use a several microtubule and actin motors.

### Is APP a motor-cargo linker, or only a cargo protein?

Two papers at the turn of the millennium proposed that APP serves as a cargo linker for the major anterograde motor, kinesin-1, by directly binding to the light chains, and thus recruiting the motor to a variety of cargo vesicles [29, 44]. Other studies support a slightly amended model where the binding of kinesin-1 to APP is not direct, but mediated by bridging proteins, such as JNK-interacting protein-1 (JIP-1) [53, 54]. We note that only a small fraction of APP (which is phosphorylated at Thr<sup>668</sup>) may do so under physiological conditions [45]. Although transport of APP can occur in the absence of JIP-1 [55], this scaffolding protein is involved in regulating bidirectional transport of APP [46]. While there is strong support for a role of APP in axonal transport [47, 56], likely as a motor-cargo linker [57], as described above, other mechanisms for motor recruitment to APP carrying vesicles have been reported. For example, it was shown that calsyntenin, a well-characterized kinesin-1 binding protein, recruits this motor to some APP-containing vesicles [58].

In our view, there is not a unique way by which APP – and its fragments – are transported into the processes. Redundant mechanisms likely coexist, and many certainly have not yet been discovered. We would like to point to a caveat in the interpretation of data derived from the use of C-terminally-tagged APP, when the tag is a GFP variant. GFP tags could

alter the biology of APP, including transport, by interfering with protein binding to its cytoplasmic domain; as such, they bias against transport mechanisms where APP serves as an anchor for the motor, and favor transport mechanisms where APP is not involved in motor recruitment. Also, the level of expression of APP – if too high – alters normal recruitment of motors to APP, potentially altering the APP:motor ratio. In conclusion, APP containing transport entities are likely targeted to the neurites by mechanisms involving both APP-dependent and independent motor recruitment.

## Conventional and unconventional mechanisms of APP transport

Information about the pathways and mechanisms of APP transport have been obtained with three approaches: (1) Assessment of the progression of the radioactive wave of pulse-labeled APP along axonal tracts, such as the optic nerve and perphorant path [59, 60]; (2) Direct visualization of transport of fluorescently tagged APP in cultured, transfected neurons [13]; and (3) Extraction of information on transport from the localization of endogenous or exogenously expressed, tagged APP along its delivery routes [14, 18]. Transport of APP tagged with YFP at the extreme C-terminus, showed striking, unidirectional motility of elongated structures, moving at speeds up to 10  $\mu\text{m/s}$ , which are not characteristic for kinesin-1 motility [13]. This strange behavior of APP-YFP is not consistent with the results of immunodetection of endogenous APP with antibodies to its cytoplasmic domain, which show a discrete distribution of punctiform structures. On the other hand, the distribution of the small C- and N-terminal tags of a dual-tagged FLAG-APP-Myc is remarkably similar to the distribution of C- and N-terminal epitopes of endogenous APP in flat processes of neuronal cells in culture [14, 18]. Interestingly, these distributions are strikingly different: The labeling of the C-terminal region of APP shows discrete, vesicle-like distributions – typical for kinesin-1 transported vesicles – throughout the neurites, including the growth cone. By contrast, the N-terminal labeling – likely representing NTFs – shows a peculiar, filamentary distribution that extends from the soma to neurite terminals [18] (Figs. 1B and 2C, D). This distribution of the NTFs is not typical for kinesin-1 cargos, and suggests that the NTFs and CTFs use different mechanisms of transport in neurites. The distribution of the NTFs is reminiscent of extensive tubular networks, such as that of the ER (Fig. 2C), an organelle that extends protrusions deep into the neurites. Because mitochondria also contain APP (see above), these two organelles – the tubular ER and the mitochondria – likely play active roles in the transport of APP and fragments. The possibility that the tubular ER performs long distance transport is an area of intensive research.

## Factors that regulate the transport of APP also regulate its processing, and vice-versa

Transport of APP from its site of synthesis in the soma to its destinations throughout the neuron is regulated at each step: (1) sorting and recruitment of APP into the cargo; (2) recruitment of the molecular motor to the cargo, and its activation; (3) motile properties of the molecular motor; (4) availability of tracks (microtubules or actin filaments); (5) presence of “road blocks” that prevent free movement of cargo along the tracks; (6) availability of ATP; (7) level of expression of APP; (8) nature and degree of proteolytic processing of APP at the site of synthesis and at transit stations along the transport routes. The AD linked



mutations in *APP*, *PS1* and *PS2* (subunits of  $\gamma$ -secretase complex), or in other genes that increase the susceptibility to AD may act at many of these levels, and alter APP transport. Because APP fragments appear to be transported separately, the modifiers of APP processing are likely to alter APP's downstream transport. Of particular importance is the phosphorylation of APP at Thr<sup>668</sup> (numbering in APP695) [61, 62], since this modification facilitates the amyloidogenic processing of APP by both  $\beta$ - and  $\gamma$ -secretase [39, 63]. This phosphorylation also specifies the recruitment of kinesin-1 via JIP-1 [45], and possibly the recruitment of myosin motors, which is determinant for targeting pAPP to distal ends in the growth cone [14]. Phosphorylation of AICD could be required for targeting to the nucleus [64]. Overall, this phosphorylation alters the interactome of APP's cytoplasmic domain [15]. Most importantly, in mouse models of AD the phosphorylation of APP at Thr<sup>668</sup> is required for the development of neuronal pathology [63, 65–68]. The effects on APP transport of phosphorylation at other residues in the cytoplasmic domain of APP, of glycosylation by O-GlcNAcase, or isomeration of proline by Pin1, are little investigated.

A recent study found that a variant of *KLC1*, *KLC1vE*, confers increased susceptibility to amyloidogenic cleavage of APP, by an unknown mechanism [69]. The recruitment of kinesin-1 to APP via *KLC1vE* could alter trafficking of APP through the compartments responsible for amyloidogenic cleavage. Alternatively, *KLC1vE* could more efficiently recruit JNK to APP, and facilitate the phosphorylation conducive of secretase cleavage [70]. These scenarios highlight the numerous ways by which APP transport and processing are entangled.

## What is transport telling us about the function of APP?

A fraction of APP is transported to the synapse as full-length protein, where it could mediate cell-to-cell interactions across the synapse, and thus regulate the function of the synapse. Full-length APP is required for this function. Other cellular processes may also rely only on full-length APP [71]. However, both the full-length APP and the cleavage fragments appear to be required for other functions of APP. For example, full-length APP serves as a sensor for impeded axonal transport, and elicits a stress response aimed at restoring transport. Yet, this response relies on the amyloidogenic cleavage of APP and the generation of fragments with defined functions. This homeostatic mechanism has relevance for LOAD [20]. Finally, the fact that APP fragments, in addition to full-length APP, are transported to remote locations in the neuron indicates that the fragments themselves could have functions that are independent of the parent protein [14]. The finding that the NTFs and CTFs are transported in neurites separately, using distinct mechanisms of transport [18], suggests that their functions are also independent of each other. Further, since the transport routes of pAPP differ from those of nonphosphorylated APP [14, 45], it is likely that pAPP species have functions distinct from those of their nonphosphorylated counterparts.

## Elucidating Transport of APP: The Challenges Ahead

In spite of extensive research, the pathways and the mechanisms of transport of APP in neurons are only beginning to be understood. APP trafficking is highly dynamic, and subjected to complex regulation by factors that vary according to the physiological

challenges of the neuron. As a consequence, APP transport differs between neighboring neurons, both in situ and in cell culture. Under normal conditions, the variability could arise – among others – from the cell-to-cell differences in the levels of expression of APP, degree of regulated proteolytic processing, and degradation. This situation is exacerbated when studying the transport of exogenously expressed APP, whose expression levels could vary by an order of magnitude among cells. The stress of all sorts, which becomes chronic at old age, affects transport and thus alters APP processing, which in turn modifies the transport of APP fragments. The real problem with the elucidation of APP transport routes and transport mechanisms is that intracellular APP is an intractable protein with the current methodology. What do antibodies detect? Full-length APP, or APP fragments? What fragments? What do the tags report: the presence of full-length APP, or presence of APP fragments? Does tagged APP faithfully reproduce the biology of APP, at least with regard to processing and transport? These questions are difficult to answer. Certainly, the generation of improved constructs, with the tags placed in ways that do not interfere with the complex biology of APP, is essential. Finally, is the primary neuron in culture the best system to study APP transport? How relevant are the neuronal cell lines to the normal physiology of the neuron in situ? Although the technology is rapidly improving, imaging APP transport in neurons in live animals still lacks sufficient resolution. Even in cell culture, the small soma and the thin processes of primary neurons limit the unambiguous identification of the transport pathways. Not much more than a moving particle – a dot or a line – can be detected in the neurons transfected with fluorescently tagged APP. Choosing a neuronal cell line that extends high caliber, flat neurites, where the microtubule tracks are clearly distinguished (e.g., the CAD cells) [72], could help the study of APP transport. The analysis of the movement of a fluorescent dot along the neurite, currently done with kymographs, provides general characteristics of motility: direction of movement, velocity, run length, frequency and duration of pauses. This tool becomes powerful in studying the mechanisms of motility only when combined with molecular biology approaches that target proteins suspected to regulate transport, hoping that they will alter the characteristics of transport. Such approaches work best when the research allows for more exploration rather than being limited to a few hypotheses.

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## Abbreviations

<b>APP</b>	Amyloid- $\beta$ Precursor Protein
<b>AD</b>	Alzheimer's disease
<b>A<math>\beta</math></b>	Amyloid- $\beta$
<b>ER</b>	endoplasmic reticulum
<b>ERGIC</b>	ER-Golgi Intermediate Compartment

<b>TGN</b>	trans-Golgi network
<b>AICD</b>	APP Intracellular Domain
<b>PTB</b>	phosphotyrosine binding
<b>JIP-1</b>	JNK-interacting protein-1
<b>APP<sub>C</sub> and APP<sub>N</sub></b>	APP C- and N-terminal epitopes
<b>NTF and CTF</b>	N- and C-terminal fragment
<b>RTN4</b>	Reticulon 4

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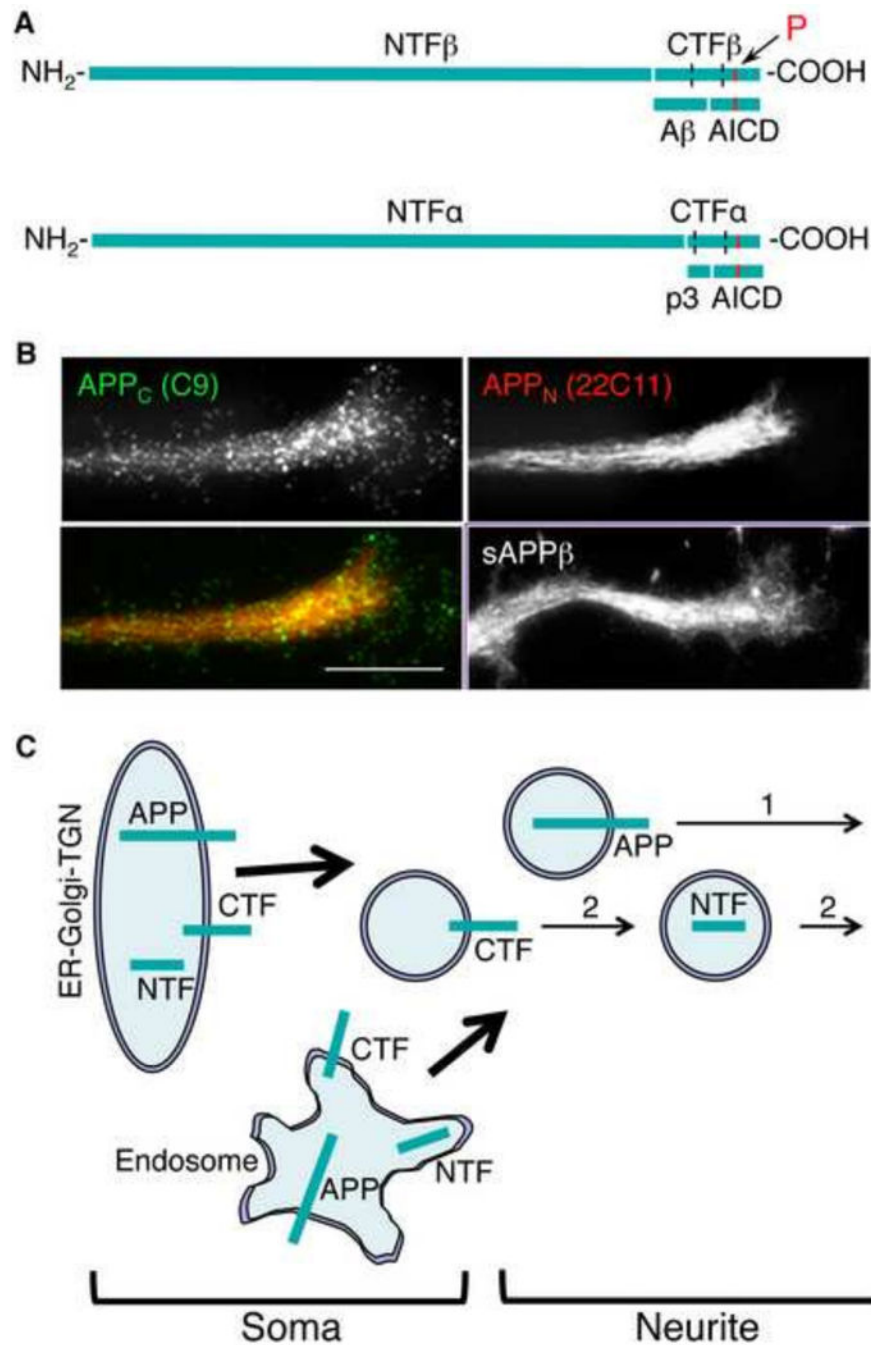
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### Highlights

- APP is cleaved throughout the neuron, including in early secretory compartments
- The APP fragments that accumulate in neurites are largely generated in the soma
- The APP N- and C-terminal fragments are independently transported to distinct destinations
- Post-Golgi and endosome-derived vesicles, and tubular endoplasmic reticulum transport APP in neuritis
- The mechanisms of transport of APP N- and C-terminal fragments are fundamentally different

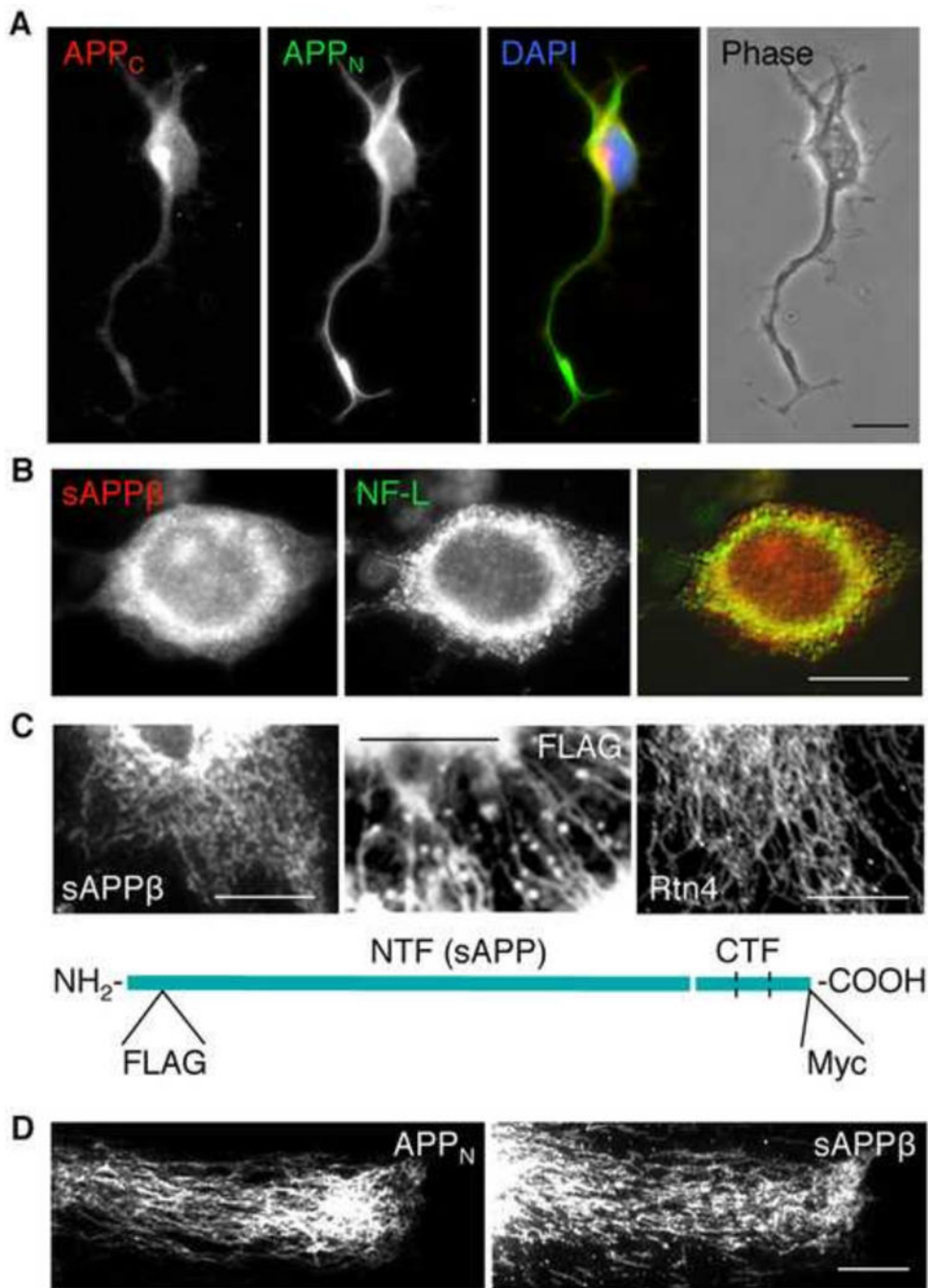


**Figure 1.**

Segregated transport of N- and C-terminal fragments of APP. (A) Processing of APP via the amyloidogenic (by  $\beta/\gamma$ -secretase; upper) and non-amyloidogenic (also called anti-amyloidogenic; by  $\alpha/\gamma$ -secretase; lower) pathways. The transmembrane domain of APP (dotted lines), and the APP-derived fragments are shown. The arrow indicates the location of phosphorylatable Thr<sup>668</sup> (red bar). Cleavage via the amyloidogenic pathway produces NTF $\beta$  (known as sAPP $\beta$ ) and CTF $\beta$ . The A $\beta$  (or p3) and the APP intracellular domain (AICD) are generated from CTF $\beta$  (or CTF $\alpha$ ) by cleavage by  $\gamma$ -secretase.

(B) Punctate, discrete versus filamentary distribution of APP C- and N-terminal epitopes (APP<sub>C</sub> and APP<sub>N</sub>) in extending neurites. Immunocytochemistry was carried out in CAD cells with antibodies C9 (kindly provided by Dr. Denis Selkoe) and 22C11 (Millipore). Note that APP<sub>N</sub> accumulates in the center of the growth cone. The antibodies recognizing the cleaved C-terminal end of sAPP $\beta$  show a labeling similar to antibodies recognizing APP<sub>N</sub>. Bar = 20  $\mu$ m.

(C) Two possible trafficking pathways of APP. In pathway (2), the cleaved C- and N-terminal fragments of APP are segregated in different vesicles, and transported independently into neurites. Cleavage and sorting into distinct transport carriers could occur in ER-Glogi-TGN or endosomes, in the soma.



**Figure 2.**

The APP NTFs are present in tubular structures extending from the soma to neurite terminals. (A) Accumulation of APP N-terminal (labeling with antibody 22C11), but not C-terminal epitopes (labeling with antibody Y188, Eptomics) is detected at neurite terminals of CAD cells. Bar = 20  $\mu$ m.

(B) In the soma, sAPP $\beta$ , detected with a C-terminal-end-specific antibody (IBL), colocalizes with neurofilaments (antibody to NF-L; Sigma). Bar = 20  $\mu$ m.

(C) Filamentous distribution of sAPP $\beta$  (detected with a C-terminal-end-specific antibody), and of FLAG-tagged APP NTFs, in the soma of CAD cells transfected with FLAG-APP-Myc. These distributions are similar to that of Rtn4, a bona fide constituent of the tubular ER. The diagram shows the positions of the tags in FLAG-APP-Myc. Bar = 20  $\mu$ m.

(D) Filamentous distribution of APP NTFs, detected in flat processes of CAD cells with antibodies to a N-terminal epitope (22C11; APP<sub>N</sub>) and to sAPP $\beta$ . A nexus of filaments, colocalizing with the ER marker, Rtn4, is often detected at terminals. Bar = 20  $\mu$ m.