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Dysregulation of Neurotrophin Signaling in the Pathogenesis of Alzheimer Disease and of Alzheimer Disease in Down Syndrome

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Abstract

Neurotrophic factors, including the members of the neurotrophin family, play important roles in the development and maintenance of the nervous system. Trophic factor signals must be transmitted over long distances from axons and dendrites to the cell bodies of neurons. A mode of signaling well suited to the challenge of robust long distance signaling is the signaling endosome. We review the biology of signaling endosomes and the “signaling endosome hypothesis”. Evidence for disruption of signaling endosome function in disorders of the nervous system is also reviewed. Changes in endosome structure in Alzheimer disease (AD) and Down syndrome (DS) are present early in these disorders. Data for the APP products responsible are reviewed and the consequent changes in signaling from endosomes discussed. We conclude by pointing to the need for additional studies to explore the biology of signaling endosomes in normal neurons and to elucidate their role in the pathogenesis of neurodegeneration.

Graphical abstract

Keywords

Neurotrophin; Signaling Endosome; Down Syndrome; Alzheimer's Disease; Endosome Enlargement; Axonal Transport Deficit; Degeneration

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1. Introduction

Alzheimer's disease (AD) is a fatal neurodegenerative disorder, characterized by progressive memory loss and cognitive decline with dementia [1, 2]. Down syndrome (DS), the most common genetic cause of AD, is due to trisomy for all or part of a third copy of chromosome 21. Almost all adults with DS develop AD-like neuropathology by the age of 40, a disorder termed AD in DS (AD-DS) [3, 4]. The evidence is compelling that increased gene dose for APP is necessary for AD-DS [5–7], but the underlying mechanism that links APP gene dose to neurodegeneration is unknown.

Our studies point to a role for increased levels of APP gene products in disrupting the formation and trafficking of signaling endosomes [8]. The signaling endosome is a recently discovered organelle in which a neurotrophin, bound to its activated receptor, signals robustly to the cytosol. Importantly, signaling endosomes are transported within neurons to carry neurotrophic signals from axons and dendrites to distant cell bodies, in so doing supporting developing and mature neurons [9–11]. In this article, we review the biology of the neurotrophic signaling endosome and highlight evidence pointing to impairment of neurotrophic signaling from endosomes as contributing to pathogenesis in AD and AD-DS. We suggest future directions for research to explore the potential mechanisms and significance of disruption of neurotrophic signaling in AD and AD-DS.

2. Neurotrophic Factors: Essential for the Developing and Mature Nervous System

Growth factors (also called trophic factors) are polypeptides that bind to specific cell membrane surface receptors to initiate signaling pathways that regulate diverse processes, including proliferation, survival, migration and differentiation. The neurotrophic factors constitute a class of trophic factors that act on cells of the peripheral and central nervous system (CNS). Prominent neurotrophic factors include the members of the neurotrophin (NT) family, the glial cell-line derived neurotrophic factor family and the ciliary neurotrophic factor family. Neurotrophic factors are best characterized with respect to their regulation of neuronal functions in the developing and mature nervous system [12–16].

The NT family consists of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4 (NT4). Each binds and activates a tropomyosin receptor kinase(s) (Trks) (NGF to TrkA, BDNF and NT4 to TrkB, NT3 to TrkC and to TrkA), to exert trophic effects [12, 14, 17]. All the NTs also bind to the p75 neurotrophin receptor (p75), to mediate cellular responses largely distinct from the classical trophic effects registered for Trk activation [12, 14, 17]. Activation of NT receptors initiates several downstream signaling pathways, including the mitogen-activated kinase (MAPK) pathway, the phosphoinositol-3-kinase (PI3 K) pathway and the phospholipase C- γ (PLC γ) pathway [18, 19]. Activation of these pathways, and integration of their signals, is responsible for the diverse and potent effects of NTs on neuron structure and function [18, 19].

3. The Biology of Long Distance Signaling in Neurons

NT-induced signaling in neurons is carried out in the context of a complex cellular anatomy in which cell bodies receive signals from elaborate dendritic networks as well as from axons projecting to distant synaptic partners. This architecture poses formidable challenges for delivering NT signals generated at cell surface receptors distant from the cell body. Methods must exist to support transmission of signals robustly and faithfully over long distances. One mechanism for transmitting the signals generated by NT/Trk complexes is via endosomes that carry the active signaling complex [9–11]. Following NT release from target cells and tissues, an NT binds and activates its cognate Trk receptor at the cell surface. The internalization of the activated complex via endocytosis creates the “signaling endosome”, an organelle that can then be trafficked to the cell body via dynein-based transport (Fig. 1) [11]. Though the signaling endosome is not uniquely found in neurons, its discovery was facilitated by recognizing the need for means by which to convey over long distances the NT signaling required for establishing and maintaining communication between geographically distant neuronal compartments.

4. Structure and Biology of Signaling Endosomes: the Signaling Endosome Hypothesis

The signaling endosome contains not only the internalized and activated NT/Trk complex, but also carries on its cytosolic surface the activated isoforms of many of the proteins responsible for signaling through the MAPK, Akt and PLC γ pathways [15]. Justifying its name, the signaling endosome has been shown to signal to downstream substrates. Several lines of evidence support this. First, following NGF treatment, isolated clathrin-coated vesicles contain NGF bound to TrkA receptor together with activated components of the MAPK pathway [20–22]. Second, NGF/TrkA containing endosomes and active kinases of the MAPK signaling pathway are present in the axons and cell bodies of dorsal root ganglion (DRG) neurons where they colocalize with an early endosome marker [23]. Third, cellular fractionation of endosomes formed *in vivo* showed cofractionation with early endosomes of the activated TrkA receptor and components of the MAPK pathway [23]. Finally, isolated endosomes were able to transmit the MAPK signal in an *in vitro* kinase assay [23]. These data combine to demonstrate that continued signaling arises from the NT/Trk endosome during its transport from neuronal processes to the cell body [20, 23, 24]. Although there are additional models for long distance NT signaling, including possibly ligand-independent NT signal transduction [25], a large and compelling body of evidence strongly support the “signaling endosome” hypothesis [20, 23, 24, 26]. Excellent reviews speak to the formation of signaling endosomes, their composition and loading onto motor proteins, and initiation and regulation of transport [9, 27].

Once created the signaling endosome is loaded onto dynein motors for transport to the cell body where the NT/Trk signaling induces changes in both the cytosol and nucleus to support many aspects of neuronal structure and function [9–11, 15]. Given evidence of persistent signaling from NT/Trk containing endosomes, the possibility exists that signaling from endosomes informs all of the compartments through which it moves in transit to the cell

body. This facet of signaling endosome biology is yet to be adequately explored but serves as an important target of ongoing studies aimed at determining the local impact of NT/Trk signaling. The extent to which signaling endosomes support signaling from ligand/receptor complexes other than NT/Trk complexes is less well studied, but data support the view that this mode of signaling is widely used. Thus, endosome-based signaling also appears to define an important common platform for signaling transduction from receptor tyrosine kinases other than the Trks, including the receptors for epidermal growth factor and transforming growth factor. Remarkably, other types of receptors and protein complexes may also signal from endosomes, including G-protein-coupled receptors and ion channel [28–30].

5. Diversity in Signaling Endosomes

Both clathrin-dependent [20] and independent endocytosis, including Pincher-mediated endocytosis [31], have been shown to mediate internalization of the NT/Trk complex into endosomes. The process is regulated and coordinated by various Rab small GTPases and other regulatory proteins. After NT-provoked endocytosis into Rab5-positive early endosome, the NT/Trk complex is either sorted into: 1) Rab4, Rab11-positive recycling endosomes to return back to the cell membrane, or 2) Rab7-positive late endosomes and then into multivesicular bodies for finally delivery to lysosomes [9, 10]. Diverse organelles may thus act to carry the NT/Trk complex and thereby serve as signaling endosomes [23, 32].

There is controversy as to whether long distance axonal transport is via the Rab5-positive early endosomes or Rab7-positive late endosomes. Studies in vivo and in vitro in our lab and others used immunocytochemistry, cell biological methods and single-molecule studies to show that Rab5-positive early endosome were the principal carrier for the NT/Trk signaling endosome [23, 26, 33, 34]. The Rab7-positive late endosome has also been identified as the principal long-distance carrier for endosomal trafficking of NT signals [32, 35]. The controversy is yet to be resolved, but it is noteworthy that Rab5 and Rab7 are both present in an intermediate compartment in which early endosomes mature into late endosomes [36]. The dynamics of this process suggest that different Rab proteins collaborate and possibly synergize to drive the long-range transport of signaling endosomes [35]. Moreover, Rab5 and Rab7 activity and their regulatory actions may well be informed by the signal(s) generated within the endosome. Possibly relevant also, therefore, is the ligand/receptor complex being moved as well as the neuron type. Finally, transport of NT/Trk complexes in signaling endosomes may differ in different neuronal processes. Perhaps most important is that whether or not the compartment containing the NT/Trk complex is competent to signal. In this respect both the early endosome and late endosome can serve, in spite of the fact that in the latter the pH is relatively low [37, 38].

6. The Biological Significance of Signaling from Endosomes: Long Distance and Local

Signaling endosomes are transported within axons for delivery to the cell body, there regulating gene expression essential for neuronal differentiation and survival [39–41] in

developing neurons, and the maintenance of neuron structure and function in mature neurons [9]. Though best studied and appreciated, axonal transport of signaling endosomes to the neuronal soma is but one mode for signaling from these organelles. Thus, local NT actions in axons are supported, including axon formation and elongation [27] and post translational modification of proteins present in the axonal cytosol, including CREB [42]. Given the diversity of local events in axons, and the presence within the axon of a large set of mRNAs whose products contribute to axonal and synaptic function, an exciting area for research is how NT signaling could modify axonal structure and function through local effects on protein synthesis [43].

NT signaling in dendrites may also be mediated by signaling endosomes. An increasing focus on dendritic responses to NTs is driven in part by the realization that for some neuronal circuits NTs are delivered by anterograde transport for release at presynaptic sites [44–47]. The expectation is that activation of postsynaptic receptors would result in endocytosis of NT/Trk complexes for transport to cell bodies. Recent studies provide support for the assertion that signaling endosomes created in dendrites are moved retrogradely to neuron cell bodies. Holzbaur and colleagues added Quantum dot-labeled BDNF to cultured hippocampal neurons. This resulted in endocytosis into a TrkB-positive compartment in dendrites that then trafficked to the cell body. Inhibiting dynein reduced, but did not eliminate transport, pointing to an important role for dynein-based transport of signaling endosomes in dendrites [48]. Interestingly, while microtubules in axons are uniformly oriented with their minus ends projecting toward the cell body, in dendrites the orientation of microtubules is mixed. Nevertheless, in proximal dendrites there is an increase in the relative number of microtubules whose minus ends are oriented toward the cell body, a finding consistent with the increase in the number of dynein motors moving toward the cell body. Thus, dynein-based transport of signaling endosomes applies to dendrites as well as to axons [48].

Additional support for the diverse roles played by signaling endosomes in dendrites are studies showing that via transcytosis, axon-derived retrograde NGF-bearing signaling endosomes can enter the dendritic compartment of sympathetic neurons to regulate synapse assembly [49, 50]. In dendrites, BDNF/TrkB also modulates dendritic arborization, a process requiring Rab11 activity [51]. Endosomes also mediate activity-dependent BDNF release at postsynaptic sites of primary hippocampal neurons [52]. As for axons, it will be important to explore further the biological events induced by dendritically-derived signaling endosomes acting locally as well as in the cell body. Though only a speculation, it is an exciting possibility that signaling from dendritically-derived signaling endosomes may differ from that from signaling endosomes formed in axons. Signaling events tailored to support NT actions in dendrites can be predicted.

7. Implications of the Signaling Endosome Hypothesis for Disorders of the Nervous System

Though there is much to learn about the biology of signaling endosomes, it is evident that complex cellular events, and a host of regulatory factors, mediate their formation and

function. Correspondingly, disruption of these cellular processes could compromise the function of signaling endosomes resulting in significant deficits in neuron structure and function. There is evidence to support this view that points to defective formation and trafficking of signaling endosomes as contributing to neurodegenerative diseases [53].

Mutations in the genes for the NTs and their receptors would obviously impact the formation of signaling endosomes. NGF synthesized in the targets of DRG nociceptors and sympathetic neurons [54] is released to bind and activate TrkA receptors on distal axons prior to forming NGF/TrkA signaling endosomes that are then transported to neuron cell bodies. Deficits in NGF signaling would be expected to compromise the structure and function of these neurons. Mutations in the TrkA receptor for NGF result in an inherited neuropathy called congenital insensitivity to pain with anhidrosis (CIPA) [55, 56]; it is classified as Hereditary Sensory and Autonomic Neuropathy type IV (HSAN IV) [57]. More than 15 mutations have been identified [57, 58] as responsible for this disorder of childhood onset with decreased pain and temperature sensation, autonomic dysfunction, fractures, arthropathy, and mental retardation [59]. Mutations in NGF result in disorders of the peripheral nervous system classified as HSAN V [59]. In one, Norrbottnian neuropathy, substitution of tryptophan for arginine at residue 100 in mature NGF leads in homozygotes to a length dependent sensory neuropathy of childhood onset characterized by reduced pain and temperature sensation without anhidrosis and variable autonomic symptoms, together with fractures and arthropathy [59, 60]. There is no mental retardation. Heterozygotes show a later age of onset and reduced disease severity [59]. Although classified as HSAN V, a rare frameshift NGF mutation (V232fs) causes an autosomal recessive syndrome in Arab families that resembles that due to TrkA mutation, including the presence of mental retardation [61]. Thus, signaling endosome defects due to both NGF and TrkA mutation affect neurons in both the periphery and CNS.

Far more prevalent are disorders in which dysregulation of NT signaling is secondary to disruption of cellular events that impact signaling endosomes. The growing list features neurodegenerative disorders including DS [8, 62] and AD [62–64] to be discussed below. In a Huntington (HD)'s disease model, defective anterograde delivery to striatal neurons of BDNF synthesized in cortical neurons resulted in striatal neuron atrophy [65, 66]. These findings define a mechanism by which mutant Htt acts through compromised BDNF transport and release to impact pathogenesis [65, 66]. In addition, defective retrograde transport of BDNF/TrkB in striatal dendrites and in cortical axons has been demonstrated in HD models [65, 67]. Interestingly, expression of individual chaperonin subunits enhanced degradation of mutant Htt, normalized both anterograde and retrograde BDNF transport, and prevented atrophy of striatal neurons [65]. These findings build on the role(s) played by wild type Htt in transport of vesicular cargoes, including those containing BDNF and BDNF/TrkB complexes, and disruption of their transport by mutant Htt [65, 67]. That mutant Htt interrupts the ability of wild type Htt to interact with both anterograde and retrograde motor proteins provides a compelling perspective for understanding these findings [66–70]. It will be important to further explore the mechanism(s) by which mutant Htt disrupts normal trafficking of NTs and signaling endosomes.

Parkinson's disease biology may also implicate defective axonal transport of signaling endosomes. In a recent report, overexpression of α -synuclein resulted in decreased retrograde transport of BDNF and neuronal atrophy together with increased activity of Rab5 and Rab7 [71]. How α -synuclein dysregulates endocytic pathways leading to axonal dysfunction will be an important topic for further study. Charcot-Marie-Tooth disease type 2B, a disorder characterized by axonal dysfunction and degeneration, is caused by mutations in Rab7 [72]. In addition, a mutation in endosomal Na^+/H^+ exchanger 6 has been linked to an autism-related disorder, possibly through dysfunction of BDNF/TrkB signaling [73]. Importantly, several mutations in components of dynein-dynactin complex, responsible for retrograde transport of signaling endosomes, have been linked to neurodegenerative disorders, including those of motor neurons [74–76]. Taken together, these data point to a compelling role for the endocytic pathway in regulating the function and survival of neurons. In so doing, they emphasize the importance of studies to explore disease-linked changes in the biology of signaling endosomes.

8. AD and AD-DS: Endosomal Pathology

AD is a progressive fatal neurodegenerative disorder causing memory loss and cognitive decline with dementia [1, 2]. The neuropathological hallmarks include extracellular amyloid-containing neuritic plaques and intracellular neurofibrillary tangles [1, 2]. Down syndrome, the most common genetic cause of AD, is due to trisomy for all or part of a third copy of chromosome 21. Essentially all adults with DS demonstrate AD-like neuropathology by age 40 [4]. The majority suffer dementia; mean age of onset is in the mid-50s. In those older than 70 the prevalence reaches 80% [4]. In the affected DS elderly, extensive similarities with AD in clinical presentation, neuropathological findings and participation of genetic factors justifies the designation of AD in DS (AD-DS) [3, 4].

In addition to plaques and tangles, changes in Rab5-positive early endosomes are found in AD and AD-DS. Rab5 mediates the endocytosis of surface proteins and fusion of early endosomes [77]. Enlargement of early endosomes, a phenotype consistent with excessive activation of Rab5 [78], is found in neurons in the brains of those with both sporadic and familial AD [79–81] as well as in DS [80]. The phenotype impacts many neurons in the AD and AD-DS brain, including basal forebrain cholinergic neurons (BFCN), a population selectively vulnerable in AD [82] and DS [83] whose trophic status and possibly survival are linked to endosome-mediated signaling [8].

Enlargement of early endosomes serves as a very early marker for AD and DS pathogenesis [84], preceding emergence of the classical pathological hallmarks, amyloid plaques and neurofibrillary tangles [80, 84]. Remarkably, in DS early endosomal enlargement is present as early as 28 weeks gestation – i.e. four decades before presence of mature AD neuropathology [80].

Because the early endosome is linked to other endocytic compartments [85–87], dysregulation of early endosomes may impact the structure and function of downstream elements of the endolysosomal system. Indeed, in the AD and DS brain enlargement of Rab5-positive endosomes was present together with increased size of Rab7-positive

endosomes [88]; the AD brain showed increased levels of Rab4 [80]. Possibly linked to increased Rab5 activation, lysosomal biogenesis is up-regulated in brain during the early stages of AD [84]. A recent report examining iPSC-derived neurons expressing familial AD (FAD) mutations in Presenilin or APP showed defective endocytosis of APP in neuronal cell bodies and reduced transcytosis of APP from cell bodies to axons. Changes in the distribution of Rab11-positive compartments mirrored those for APP and decreasing Rab11 induced the observed changes in APP [89] suggesting a link between FAD mutations and the function of this regulator of endocytosis. The dysregulation of the endocytic pathway in AD and in AD in DS predicts changes in signaling endosomes that could compromise the trophic state and survival of neurons.

9. Animal and cellular models displaying endosomal deficits

Relatively few studies have explored endosomal pathology in mouse models of AD. Nevertheless, enlarged early endosomes have been documented in vivo in the APP transgenic mouse model (APP23) expressing the human APP cDNA harboring the Swedish K670N/M671L double mutation [90] as well as in the Line 41 mouse model expressing the human cDNA containing both the Swedish and London mutations [91]. The most used model of DS, the Ts65Dn mouse, shows enlarged early endosomes in a number of neuronal populations in vivo [8]. Primary cultures of BFCN neurons from the Ts65Dn mouse also show increased size of early endosomes [62] as demonstrated by immunostaining for Rab5. In preliminary studies, the enlarged early endosome phenotype is also evident in the Dp(16)1Yey/+ model of DS, which like the Ts65Dn mouse carries three copies of the mouse App gene [92].

An important question is how changes in endosome size could impact the structure and function of neurons. Of note, our studies in the Ts65Dn mouse pointed two defects due to increased gene dose in the Ts65Dn mouse [8]. The first was the necessary role for increased App gene dose for BFCN degeneration. The second was that increased App gene dose resulted in defective retrograde axonal transport of signaling endosomes containing NGF [8]. To explore the latter, ¹²⁵I-NGF was used to examine axonal transport from hippocampus to BFCN neurons. There was a marked decrease in Ts65Dn mice that was largely prevented by reducing App gene copy number to two [8]. Next, we attempted to understand whether the defect in transport was linked to neuronal loss. The question posed was whether there was defective transport of NGF signaling endosomes. Evidence that this was the case were data showing that cholinergic terminals in Ts65Dn mice harbored enlarged early endosomes that contained NGF, App, and App-CTF [8]. Moreover, early endosomes in BFCN axons contained NGF specifically bound to its receptors [8]. These data pointed to decreased transport of NGF signaling endosomes and supported the idea that this loss of trophic support contributed to dysfunction and loss of BFCNs. Defective transport of signaling endosomes appears to affect other neuronal populations in mice that model AD-DS [93]. In a more recent study examining BDNF signaling endosomes in the Ts65Dn cortex we noted a significant increase in both TrkB and pTrkB in synaptosomal membranes together with increased colocalization of pTrkB with Rab5 positive endosomes; however, we also noted that the distribution of pTrkB deviated from normal in that while TrkB activation was increased at synapses in Ts65Dn neurons it was decreased in neuron cell bodies [93]. These

findings, like those for NGF in BFCNs, point to a failure in retrograde axonal transport of signaling endosomes from axon terminals to cell bodies. Together, the data demonstrate that increased APP gene dose disrupts both TrkA and TrkB signaling in endosomes with changes in endosomal size as well as trafficking (Fig. 1). We cannot rule out other changes in neurotrophin signaling from enlarged endosomes, but such changes can be envisioned, as will be discussed below.

Changes in early endosomes are detected not only in neurons in many brain regions [94] but also in non-neuronal cells including endothelial cells [81], fibroblasts [88, 95], and blood mononuclear cells [96]. Furthermore, PC12 cells and N2a cells with ectopic expression of APP or APP metabolites also display enlarged early endosome [62, 97], in which GTP-Rab5 pull-down assay [62] and fluorescence recovery after photobleaching [97] experiments have confirmed Rab5 overactivation, respectively. In addition, in DS fibroblasts, increases were noted in the size of Rab5- and Rab7-positive endosomes as well as increased levels of Rab5, Rab4 and Rab7 [88].

10. Genes and Endosomal Phenotypes in AD and AD-DS

The genetics of AD has provided insights essential for deciphering pathogenesis. Autosomal dominant forms of AD are due to mutations in the amyloid precursor protein (APP) [98, 99], the protein from which the A β peptide present in amyloid plaques is derived, increased copy number for APP, and mutations in Presenilin 1 and 2, whose protein products regulate processing of APP [98, 99]. APP, a type 1 transmembrane protein, is processed by sequential cleavage via either β - or α -secretase to produce the C-terminal fragments, β -CTF (or C99) or α -CTF (or C83), respectively. β -CTF is then cleaved by γ -secretase to yield the APP intracellular domain (AICD) and A β peptides; α -CTF cleavage yields the AICD and the P3 peptide [100–102]. An immense body of evidence points to a pathogenic role for APP and its products in AD [1, 103]. In particular, under the amyloid hypothesis [103], A β peptides (A β 40/42) act to induce synaptic dysfunction and neurodegeneration. A growing body of data point to soluble A β as the toxic species; toxic oligomers are thought to consist of 8–24 mers [104].

The APP gene is present on chromosome 21 and is therefore triplicated in DS [105, 106]. Correspondingly, the vast majority of adults with DS show increased expression of the gene with increases in the levels of the full length APP protein (Fl-APP) and all of its products [3, 4, 6, 7]. Importantly, data are compelling that increased gene dose for APP is necessary for AD-DS [4–7]. In one report, a 78 y.o. woman with DS partially trisomic for HSA21, with only two copies of *APP*, was neither demented nor showed AD pathology [7]. In a second case of partial trisomy without a third copy of *APP*, a 72 y.o. man without dementia and a negative amyloid PET scan showed no neuritic plaques at postmortem [5]. Finally, in a study of 30 partially trisomic for HSA21, increased dose of *APP* alone was necessary for AD-DS [6] with no necessary role for other genes.

Studies in mouse models of DS confirm the necessity for increased APP gene dose for age-related degeneration of neurons that also degenerate in AD-DS [8, 107]. Model systems that

recapitulate increased APP gene dose also show that it is necessary for the early endosome phenotype present in the DS brain [62].

Further supports for the view that AD and AD-DS share pathogenetic mechanisms are commonalities in the impact of genetic risk factors. The most compelling risk factor for both is the E4 allele of the ApoE gene [108]. In DS, ApoE4 increases the age-related risk of dementia [109–115] and deposition of A β [109–115]. How ApoE4 mediates its effects is yet to be fully defined, but a role for ApoE4 in the endocytic pathway is supported by evidence that ApoE4, but not ApoE2 or 3, increased the size of early endosomes in neurons of patients expressing the APP Swedish mutation [81]. ApoE4 also increased early endosome size in early stage sporadic AD [80]. Furthermore, ApoE binding to the ApoE receptor 2 (ApoER2) increased endocytosis of APP together with β -secretase, leading to increased production of A β [116]. ApoE4 was more effective than ApoE2 or 3 in increasing A β [116]. To understand the links between endosomal changes and AD, it will be important to explore further the roles for ApoE isoforms in regulating APP endocytosis, processing and clearance.

11. Endosomal Dysregulation by APP and its Products in AD and AD-DS

An important question is which APP product(s) are implicated in signaling endosome deficits in DS. A role for the β -CTF is established (Fig. 2) [62, 97]. Consistently, overexpression of β -CTF acted through increased Rab5 activity to enlarge early endosomes, markedly reduced NGF-induced ERK activation and reduced neurite outgrowth in PC12 cells [62]. Overexpression of β -CTF also induced activation of Rab5 and enlargement of early endosomes in BFCNs; these changes were correlated with decreased retrograde axonal transport of NGF together with atrophy of BFCNs [62]. Note, also that β -CTF is increased in cholinergic terminals and colocalized with Rab5-positive early endosome in Ts65Dn mice [8].

Whether or not Fl-APP overexpression plays a role in dysregulation of Rab5 activity has also been examined. Transfection of APP^{M596V}, an APP protein mutated to prevent β -CTF production, resulted in increased levels of the full length protein. As for wild type APP, APP^{M596V} overexpression induced increased activation of Rab5, enlargement of early endosomes and reduced neurite outgrowth in PC12 cells (Fig. 2) [62]. APP also induced activation of Rab5 in BFCNs and APP and APP^{M596V} overexpression increased early endosome size in these neurons [62]. Finally, APP overexpression was correlated with atrophy of BFCNs [62]. We note, however, that these findings differ from those of Nixon and colleagues [95, 97]. While they found that β -CTF increased Rab5 activation and enlarged early endosomes, in their studies APP^{M596V} overexpression had no effect on Rab5 activity or on the size of early endosomes. These contradictory results may be explained by the use of different experimental systems. Further studies of APP^{M596V}, including the use of knock-in mice, will be needed to resolve the issue.

How APP and its products act to dysregulate endosomal structure and function is yet to be determined. A recent study provided evidence that β -CTF recruits APPL1 (adaptor protein containing pleckstrin homology domain, phosphotyrosine binding (PTB) domain and

leucine zipper motif) to Rab5-positive early endosomes; APPL1 then stabilizes the active form of Rab5, Rab5-GTP, leading to pathological enlargement of the early endosome (Fig. 2) [97]. Another report documented a role for the APP binding protein APP-BP1 in mediating the interaction of Fl-APP and Rab5, with resulting endosome enlargement [117].

Fewer published observations document a role for A β 42 in endosomal disruption, but a recent study in the Ts65Dn mouse model of a vaccine that targeted A β showed relative preservation of BFCNs [118]. Though it is intriguing to suggest a role for A β 42 in inducing changes in endosomal structure and function, further studies are needed to address this possibility. Moreover, it will be important to address the molecular basis for how Fl-APP and its β -CTF induce endosomal dysregulation.

Because of the important role played by the early endosomes in protein sorting, its function is subject to many regulatory factors [119]. The possibility exists that more than one pathway may mediate the effects of increased levels of Fl-APP and β -CTF in inducing increased activation of Rab5.

APP processing and A β production are carried out, at least in part, in endosomes [100]. Enlarged early endosomes in DS have been shown to contain A β well before the presence of amyloid plaques and tangles [94], pointing to the endosomal compartment not only as an early marker of pathogenesis but also as a site from which APP gene dose-linked pathogenesis may emerge [94]. It is possible that APP overexpression results in changes in processing that favor dysregulation of endosome structure and axonal transport. Interestingly, overexpression of Rab5, via stimulation of endocytosis, has also been shown to increase both β -CTF and A β production [120]. Future studies to explore links between increased levels of APP and its products on the biology of the early endosome will be essential for exploring the pathogenesis of AD and AD-DS.

Increased expression of the genes for Rab5 and other Rab GTPases, at either the transcription or translation levels, could also contribute to endosomal abnormalities in AD and AD-DS [80, 88, 121–123]. Interestingly, upregulation of gene expression for Rab5, 4 and 7 correlated with cognitive decline during AD progression and with down-regulation of expression of the genes for TrkB, TrkC and possibly TrkA [121]. Whether or not changes in gene expression of these Rabs are mechanistically linked to changes in cognition and NT receptor levels is unknown. One possibility, given the negative impact of upregulation of Rab5 on retrograde transport of NT receptors [62], is that the changes represent a compensation for decreased retrograde trafficking of NT signals. Given the possible positive feedback between NT signaling and NT receptor gene expression [124], the decrease in Trk gene expression might be explained in the same way. In any event, changes in gene expression for the Rab5 and for the Trks are unlikely to account for early events in pathogenesis because they appear to follow the increase in early endosome size. Thus, early endosomal changes are likely due to increased Rab5 activation rather than increased Rab5 gene expression.

12. The Biological Significance of Increased Activation of Rab5

Rab5 regulates transmission of information between neurons. In part this is achieved by controlling the levels of surface receptors for the neurotransmitters used to convey neuronal activity. APP endocytosis is also increased by increased activation of Rab5 [120]. This review has focused on another important Rab5 function, its link to the receptors for neurotrophic factors, including the NTs and their Trk receptors. Our findings for increased internalization of surface receptors, including Trks, in the setting of increased activation of Rab5 suggest that Rab5 plays a role in the creation of signaling endosomes [93]. Moreover, they link increased activation of Rab5 to decreased retrograde transport of NT/TrkA signaling endosomes to neuron cell bodies [62, 93]. Given the important roles for Rab5, it is not surprising that its activity is tightly regulated.

Rab5 belongs to the Ras superfamily of small Rab GTPases. Active Rab5 localizes to both the plasma membrane and early endosomes [77, 125, 126]. Through plasma membrane localization it is poised to regulate endocytosis. Endosomal localization of Rab5 regulates intracellular trafficking of endosomes. Rab5 cycles between GTP-bound and GDP-bound states. Only Rab5-GTP is active. The conformational switch from the Rab5-GDP and Rab5-GTP states enables the biological functions of Rab5 during endocytosis and fusion of endosomal membranes [77, 125]. Cellular processes that impact the location of Rab5 and the ratio of GTP versus GDP binding to Rab5 constitute a regulatory circuit. While GTP-bound Rab5 is membrane-associated, GDP-bound Rab5 localizes in cytosol through association with a Rab GDP-dissociation inhibitor (Rab-GDI) [77, 125]. Guanine-nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs) catalyze nucleotide exchange and hydrolysis, respectively, thus defining the Rab5 GTP/GDP cycle [77, 125]. As for other Rab GTPases, GTP-bound Rab5 binds effector proteins that instruct downstream events. Also, Rab5 is functionally connected with downstream GTPases of the Rab family as well as others, including the Rho family [127, 128].

Rab5 promotes endocytosis and modulates the formation of early endosomes [126, 129]; endosome fusion is mediated by Rab5 recruitment of cytosolic components of the fusion apparatus [126, 130]. Rab5 retains its association with endocytic membranes via early steps of early endosome maturation, and participates in conversion of the early to late endosome [36]. Furthermore, Rab5 has been shown to regulate early endosome motility towards the minus ends of microtubules [131]. The key role played by Rab5 in the sorting and transport of intracellular cargoes was revealed in studies showing that Rab5 was necessary for the biogenesis of the endolysosomal system [132].

Though it is uncertain as to how increased activation of Rab5 is implicated in the pathogenesis of AD and AD-DS, at least three suggestions can be offered. First, increased activation of Rab5 may increase endocytosis of surface receptors and protein complexes located on surface membranes. Overexpressing Rab5 or a constitutively active form of Rab5 does increase endocytosis of both receptors and fluid phase markers [120, 133]. In so doing, the ability of the neuron to respond to cues, both cell autonomous as well as non-autonomous, could be compromised. Of note, Rab5 plays an important role in endosome trafficking in synapse and synaptic function [134, 135]. It participates in the process of the

long-term potentiation and depression of excitatory synaptic transmission [136, 137], mechanisms important for learning and memory [138]. Thus, increased activation of Rab5 may impair synaptic function and cell-cell communication. Second, via enhanced fusion of early endosomal membranes, increased Rab5 activity could change the structure of early endosomes. Expressing a constitutively active form of Rab5 greatly increases the size of early endosomes [62, 139]. Whether the protein constituents of enlarged endosomes differ from normal is unknown, but this possibility can be envisioned. If so, the enlarged endosome may abnormally interact with proteins at endocytic sites that support local signaling. Finally, studies in which overexpression of β -CTF was used to increase Rab5 activity showed that there was decreased axonal transport of signaling endosomes together with neuronal atrophy; atrophy was eliminated if we reduced Rab5 activation by co-expressing a dominant negative isoform of Rab5 [62]. These findings point to the important possibility that failed delivery of endosomes that signal to support neuron structure and function are responsible for degeneration. The underlying mechanism(s) for reduced transport is unknown. We speculate that differences in scaffolding proteins, motor proteins, in the activity of Rab5 and other Rab GTPases could each participate. It is an interesting possibility that enlarged size alone may contribute to transport deficits. Studies to elucidate mechanisms will be important for understanding how increased activation of Rab5 contributes to the pathogenesis of AD and AD-DS.

13. Endosomal Dysregulation Beyond APP in AD-DS

While increased APP gene dose plays a necessary role in dysregulation of early endosomes, other genes present in three copies in DS may contribute. A recent study provides evidence that triplication of synaptojanin 1, a regulator of clathrin-mediated endocytosis, contributes to endosome changes in DS [96]. Dyrk1A, another gene on chromosome 21, may upregulate the activity of synaptojanin, raising the possibility that Dyrk1a contributes to endosomal dysfunction in DS [140]. Triplication in DS of the gene for RCAN1, an inhibitor of the phosphatase calcineurin, has been shown to interrupt TrkA receptor endocytosis and retrograde neurotrophic signaling, leading to developmental abnormalities of the sympathetic nervous system [141]. Whether RCAN1 regulates the phenotype of early endosome in CNS neurons is an important topic for study. Other chromosome 21 genes are yet to be evaluated for effects on endosomal structure and function. Moreover, genes not on chromosome 21 may mediate changes in endosomes. It is noteworthy that among AD risk factors, Rin3 and Bin1 have been shown to modulate endosome function [142, 143]; other AD risk alleles linked to genes whose products impact endosomes may also contribute [144].

14. Summary and Perspective

Existing observations allow for the conclusion that signaling from endosomes does play an important role in the development and maintenance of the nervous system and is almost certainly implicated in pathogenesis. But there is much to learn about the biology of signaling endosomes in normal neurons and in those impacted by neurodegenerative disorders. Studies of signaling endosomes are yet to provide a comprehensive view of their actions. As one example, the context for signaling from endosomes has only recently been

examined. Understanding the meaning of the signals generated from endosomes will almost certainly need to define the signaling context; indeed, context may be as important for defining biological meaning as the signals generated. With respect to context it will be important to explore at least these elements: the cellular compartments in which endosomes signal, the composition of signaling endosomes in these compartments, the stage of neuronal maturation in which signaling occurs, and whether or not the neuron is impacted by a degenerative process. These data will be essential for deciphering the biological significance of signaling from endosomes and, in turn, what changes in meaning are imposed by pathogenesis. Though technical challenges must be addressed to define the local signaling events, recent advances encourage the view that this will be possible. For example, it is possible to examine quantitatively and in real time the transit of individual NT molecules contained in endosomes [34]. It will be important to create and use new technologies that make possible real-time observations of local signaling from defined endosomal compartments.

We are also at an early stage of understanding what role signaling endosomes play in the pathogenesis of AD and AD-DS. Nevertheless, the data are compelling that these disorders manifest changes in the structure of early endosomes. Studies in models of these disorders demonstrate disruptions in the structure and signaling from early endosomes correlated with degenerative phenotypes. The necessity for increased APP gene dose for AD-DS in humans and animal models has enhanced the focus on APP and its products in inducing endosomal changes. Recent findings document the participation of Fl-APP and β -CTF. The stage is now set to explore how these APP products act, possibly in concert with the products of other genes present in increased copy number in AD-DS, to cause neurodegeneration. It is an exciting possibility that studies focused on dysregulation of signaling endosome may identify much needed novel therapeutic targets for preventing AD-DS.

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Highlights

- Neurotrophic signaling from endosomes plays an important role in both developing and mature nervous system
- Increased APP gene dose is necessary for AD and AD-DS
- Studies in vitro and in vivo provide evidence that increased levels of APP and its products induce defects in neurotrophin signaling from endosomes
- Defects in the formation and trafficking of neurotrophin signaling endosomes appear to contribute to neuronal dysfunction in AD and AD-DS

Fig. 1. Endosomal dysfunction in DS compromises signaling of neurotrophic factors

Neurons derive an important source of neurotrophic support from postsynaptic neurons. The latter synthesize and release neurotrophin molecules which then diffuse to presynaptic neurons where they bind to specific cognate receptors on surface membranes. Signaling endosomes are formed when the neurotrophin/receptor complex undergoes endocytosis. This remarkable organelle carries on its cytosolic surface activated isoforms of the downstream signaling pathways activated by the neurotrophin/receptor complex. Once created, signaling endosomes convey signaling to all parts of the presynaptic neuron, including the synapse, the axon, the cell body, and in some cases also dendrites. In normal, healthy neurons, there is a steady flow of signaling endosomes from synapses to cell bodies via dynein-mediated retrograde transport in axons. Long-distance axonal transport allows for the postsynaptic neuron to influence both somal and nuclear events in the presynaptic neuron that serve to ensure the structure and function of the presynaptic neuron, including its ability to synaptically partner with the postsynaptic neuron. Due to increased activation of Rab5, in AD and AD-DS there is enlargement of early endosomes, a change that is correlated with changes in trafficking of signaling endosomes. Though the mechanisms by which APP and its products act are yet to be defined, existing data point to two consequences of disrupted trafficking of signaling endosomes: increased neurotrophin signaling from endosomes in distal axons and deficient transport of signaling endosomes to cell bodies. Studies to explore these changes will be important for understanding their biological significance. Existing data support the view that decreased transport of signaling endosomes to cell bodies results in decreased trophic signaling with resulting atrophy, a hallmark of neurodegeneration in AD and AD-DS. As yet undefined are which signaling pathways are impacted by reduced delivery of signaling endosomes to cell bodies. Nevertheless, that such deficits are present is data showing that p-CREB levels were reduced in neurons overexpressing C99 (62).

Fig. 2. Schematic illustrating proposed effects of C99 and FI-APP on regulation of Rab5 activity and early endosome size

The left panel portrays an early endosome from cells expressing normal levels of C99 (above the dotted line), or FI-APP (below the dotted line). Increased levels of C99 and FI-APP result from increases in APP gene dose in DS, as well as in FAD due to APP duplication. Increased C99 is also seen in certain other FADs. Increases in C99 and FI-APP induce activation of Rab5. The right panel portrays the result of increased levels of C99 (above the dotted line) or FI-APP (below the dotted line). C99 recruits APPL1 to early endosome to stabilize activated Rab5 and induce enlargement of early endosomes. Independent of C99, FI-APP also induces activation of Rab5 and increases the size of early endosomes. The proteins that link FI-APP to Rab5 are less well defined. Evidence supports involvement of APP-BP1, but other adaptors may play a role, including APPL1. It is unclear to what extent Rab5 activity is regulated by C99 and FI-APP in cells that do not overexpress APP, but a gene dose effect of APP has been shown (62).