

Neurotrophin signaling endosomes: biogenesis, regulation, and functions

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In the nervous system, communication between neurons and their post-synaptic target cells is critical for the formation, refinement and maintenance of functional neuronal connections. Diffusible signals secreted by target tissues, exemplified by the family of neurotrophins, impinge on nerve terminals to influence diverse developmental events including neuronal survival and axonal growth. Key mechanisms of action of target-derived neurotrophins include the cell biological processes of endocytosis and retrograde trafficking of their Trk receptors from growth cones to cell bodies. In this review, we summarize the molecular mechanisms underlying this endosome-mediated signaling, focusing on the instructive role of neurotrophin signaling itself in directing its own trafficking. Recent studies have linked impaired neurotrophin trafficking to neurodevelopmental disorders, highlighting the relevance of neurotrophin endosomes in human health.

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Introduction

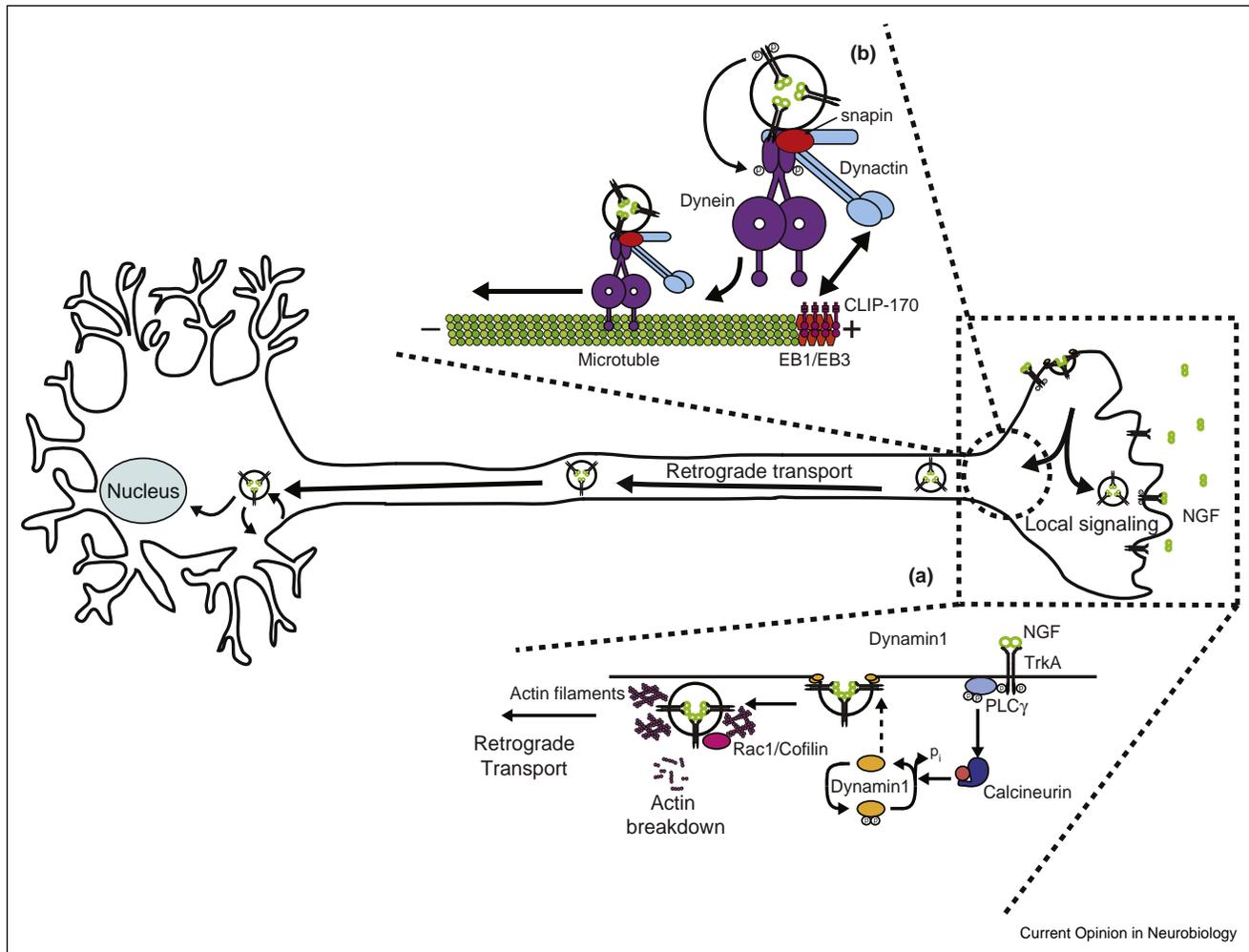
Long distance communication between axon terminals and neuronal cell bodies is a critical determinant in the establishment and refinement of neuronal circuits. The family of neurotrophins provides one of the best-known examples of soluble growth factors secreted by post-synaptic target tissues that retrogradely control neuronal survival, axon growth and synapse formation. Neurotrophins, which include nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), neurotrophin 3 (NT3), and NT4 (also known as NT5), initiate signals at axonal growth cones by binding specific Trk receptors, with TrkA responding primarily to NGF, TrkB to BDNF and NT4/5, and TrkC to NT-3 [1]. Neurotrophin-bound Trk receptors signal from axonal surfaces and are also

internalized into endosomal vesicles that propagate a trophic signal to cell bodies. In this review, we discuss the regulatory role of neurotrophin-mediated signaling itself in directing the formation and retrograde transport of neurotrophin-harboring signaling endosomes. We also discuss the functional consequences of retrograde neurotrophin trafficking to somatodendritic compartments, as well as recent evidence implicating dysregulated endosomal signaling in neurodevelopmental disorders.

Formation of neurotrophin endosomes in growth cones

Retrograde neurotrophin signaling starts at axonal growth cones with ligand binding, Trk receptor dimerization and phosphorylation on specific tyrosine residues, and initiation of signaling cascades, which includes activation of phosphatidylinositol-3 kinase (PI-3K)-, Ras/MAPK-, and phospholipase C γ (PLC γ)-mediated pathways [2]. Currently, numerous lines of evidence support a model in which neurotrophin-bound Trk receptors are also internalized into ‘signaling endosomes’ that include activated components of these downstream effector pathways [3–5]. Signaling endosomes acutely regulate growth events in axons, and also undergo long-distance transport to somatodendritic compartments to activate transcriptional programs and modulate synapse assembly, reviewed in [3–5]. The predominant mode of Trk receptor internalization in growth cones has been debated, with evidence to support both clathrin-dependent endocytosis and Pincher-mediated macropinocytosis, a process that involves plasma membrane ruffling and actions of actin-regulatory small GTPases [6–8]. Although the molecular mechanisms that underlie Trk receptor internalization are still poorly understood, it is clear that receptor-mediated signaling events regulate the endocytic process. TrkA kinase activity is necessary for association of clathrin with the plasma membrane in PC12 cells [9], suggesting that TrkA signaling regulates the formation or maturation of clathrin-coated pits to initiate receptor internalization. In sympathetic neurons, PLC γ is a key TrkA effector that controls the membrane recruitment of components of the endocytic machinery [10^{*}]. TrkA phosphorylation at tyrosine 794 (in rats, Y785 in human TrkA) drives receptor internalization by recruiting and activating PLC γ , which then stimulates calcineurin-dependent dephosphorylation of neuron-specific splicing isoforms of dynamin-1 [10^{*}] (Figure 1a). Dynamin-1 dephosphorylation might underlie its recruitment to the plasma membrane. Once internalized, TrkA-mediated endosomal signaling controls its own vesicular trafficking by promoting actin breakdown in

Figure 1



Trk-mediated signaling mechanisms underlying receptor internalization and retrograde transport initiation in distal axons. (a) In growth cones, TrkA signaling promotes receptor internalization by inducing recruitment of PLC γ 1, which activates calcineurin. Calcineurin dephosphorylates dynamin to promote TrkA endocytosis. TrkA-containing endosomes recruit actin-modulatory proteins, including Rac1 and cofilin, to promote actin disassembly in nerve terminals, necessary to overcome a dense actin meshwork in nerve terminals to travel retrogradely. **(b)** Interactions between the dynactin complex and microtubule plus end binding proteins (EBs) and a linker protein, CLIP-170 are required for exit of neurotrophin endosomes from distal axons. Trk-mediated phosphorylation of dynein intermediate chains may underlie recruitment of dynein motors to signaling endosomes. Trk receptors may bind directly with dynein motors or *via* adaptors such as snapin.

axon terminals [11^{*}] (Figure 1a). TrkA-containing signaling endosomes are intimately associated with the actin cytoskeleton, and TrkA signaling within endosomes recruits actin-modulatory proteins, including Rac1 and cofilin, to promote the severing of a dense actin meshwork in axon terminals to facilitate the entry of signaling endosomes into the axons for retrograde transport [11^{*}]. The activity of PI-3K, another TrkA effector, is necessary for early steps in the retrograde transport process. Inhibition of PI-3K activity in distal axons prevents the retrograde accumulation of NGF-TrkA endosomes in the cell bodies of sympathetic neurons [12], but does not affect receptor internalization (Kuruvilla and Ginty, unpublished results), suggesting a role for PI-3K at a post-endocytic sorting step in distal axons.

Retrograde transport may not be the only fate of Trk receptors that are internalized in axon terminals. In compartmentalized sympathetic cultures, only a minor fraction (approximately 2–2.5%) of activated Trk receptors in axon terminals undergo retrograde transport [13,14]. Thus, the majority of internalized Trk receptors may undergo recycling or proteolytic degradation in growth cones. Recycling of neurotrophin receptors may enhance the probability of interactions with the ligand, which is in limiting concentrations at the target. Alternatively, re-distribution of receptors to specific membrane domains may facilitate spatially restricted signaling in growth cones. Currently little is known about the sorting mechanisms that distinguish retrograde-bound receptors from those destined for recycling/degradation. However,

Nedd4-2, an E3 ubiquitin ligase that promotes the ubiquitination and down-regulation of TrkA [15], may be involved in diverting internalized receptors to a degradative pathway at the expense of the recycling and retrograde routes [16]. The mode of receptor internalization may also dictate the post-endocytic trafficking route(s). For Epidermal Growth Factor Receptor (EGFR), clathrin-mediated endocytosis appears to bias internalized receptors toward the recycling pathway, whereas clathrin-independent endocytosis preferentially targets receptors for degradation [17]. Finally, a critical determinant of the intracellular trafficking fate may be the nature of the ligand–receptor interactions within the acidic environment of endosomes. The related neurotrophins, NGF and NT-3, can both bind and activate TrkA receptors in sympathetic neurons [18,19]. However, NT-3 has reduced affinity for TrkA and is thought to dissociate readily from TrkA within endosomes, resulting in endosomes that are incapable of actin disassembly in growth cones [11*], a prerequisite step for retrograde transport. Thus, NT-3-TrkA-containing endosomes may recycle back to axonal surfaces or undergo degradation in axon terminals. Thus, molecular factors that affect endosomal pH, the stability of neurotrophin-Trk receptor complexes in endosomes, and sustained signaling within endosomes, may influence sorting decisions in growth cones.

Retrograde transport along the axon

Active transport of Trk-containing signaling endosomes along axons is dependent on the microtubule network and interactions with the retrograde motor dynein and its obligate co-factor, the dynactin multi-protein complex. Disruption of dynein-mediated functions by over-expression of the dynactin subunit, dynamitin, impairs the retrograde propagation of a neurotrophin survival signal in sensory neurons [20]. The exit of retrograde-bound neurotrophin receptors from distal axons depends, in part, on a conserved CAP-Gly domain in dynactin, that works in concert with the microtubule plus end binding proteins, EB1 and EB3, and the cytoplasmic linker protein CLIP-170, to coordinate the efficient initiation of retrograde transport [21*] (Figure 1b). Since phosphorylation events may underlie the ordered recruitment of some of these molecular components to microtubule plus ends [22], this presents an attractive mechanism by which neurotrophins can exert control over initiation of retrograde transport.

Only a single dynein heavy chain gene product serves as the motor domain protein for retrograde transport, while more than 45 kinesin gene products are responsible for anterograde transport. However, specificity in terms of cargo recognition may arise from the existence of different dynein complexes in cells due to diverse intermediate, light intermediate, and light chain gene products that associate with dynein heavy chain [23]. Although neurons express at least six different splicing isoforms of the

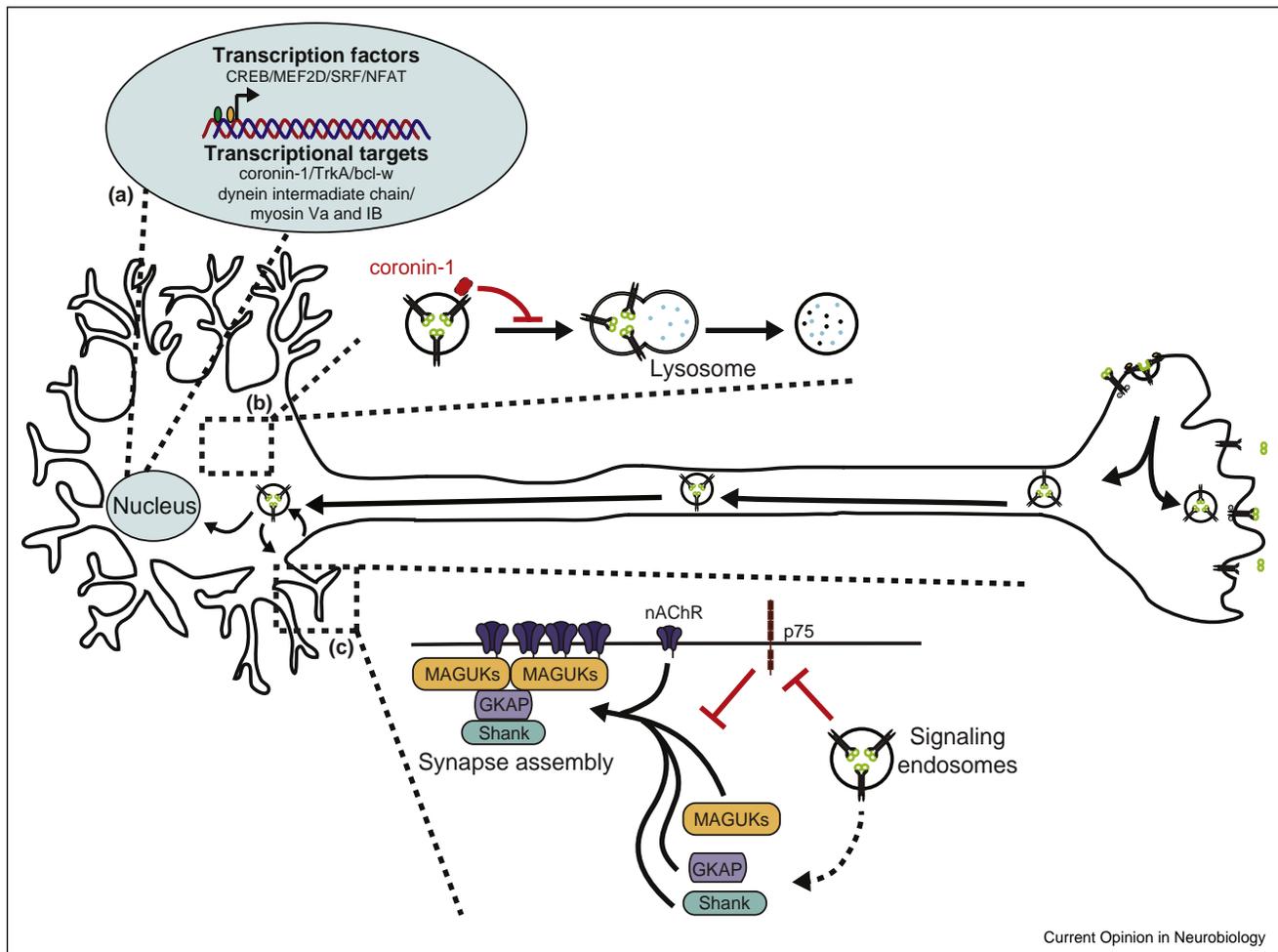
dynein intermediate chain (ICs), dynein complexes containing a specific variant, IC-1B mediate the retrograde transport of TrkB endosomes [24]. Recruitment of the dynein motor to Trk-containing signaling endosomes may occur *via* direct interactions of Trk receptors with dynein light chains [25], or *via* binding of dynein adaptors such as snapin to dynein intermediate chains (ICs) [26]. Deletion of *snapin* or disrupting snapin-dynein interactions impaired retrograde axonal transport of TrkB-containing endosomes and BDNF-mediated trophic signaling [26]. An additional mechanism by which neurotrophin signaling governs its own retrograde transport is by phosphorylation of dynein intermediate chains. Dynein ICs are phosphorylated on a conserved serine residue, which underlies the recruitment of dynein to Trk-containing endosomes [27*].

More than one type of endosomal organelle may be responsible for the retrograde axonal transport of neurotrophin signals. Ultrastructural analyses show that, in sciatic nerves, retrogradely transported active TrkA receptors are localized in structurally distinct types of vesicles that range in size from 50 to 200 nm, and include both coated and uncoated vesicles as well as many multi-vesicular bodies [28]. NGF-TrkA containing vesicles, biochemically isolated from intact sciatic nerve preparations, were found to contain markers of early endosomes such as Rab5 and EEA1 [29]. Other studies have argued for Rab7-positive late endosomes being the long-distance carriers of the retrograde neurotrophin signal [30]. Together, these findings suggest that the structural identity of neurotrophin-containing endosomes is dynamic during the retrograde transport, with Trk-containing endosomes transitioning from early to late endosomes. Alternatively, it is possible that several distinct organelles, each with its own unique set of endosomal and signaling effectors simultaneously carry the retrograde signal. If so, this raises the intriguing possibility that specialized signaling endosomes may exist to exert specific control over the diverse biological functions of neurotrophins at cell bodies and dendrites.

Signaling endosome-mediated events in cell bodies and dendrites

On reaching cell bodies, Trk-harboring endosomes stimulate trophic signaling pathways that include the PI-3K and MAPK cascades [12,31]. Activation of transcription factors including CREB, MEF2D, SRF, and NFAT may mediate changes in gene expression necessary for neuronal survival and axon growth [32–35] (Figure 2a). Recent studies in compartmentalized cultures and genetic screens in neurotrophin-deficient peripheral neurons are elucidating transcriptional targets downstream of retrograde signaling, including genes encoding for neurotrophin receptors, guidance and adhesion transmembrane proteins, anti-apoptotic factors, metabolism-related proteins, and transcription factors [33,36,37]. Interestingly, in sympathetic neurons, several transcriptional targets identified downstream of NGF signaling have functions in

Figure 2



Neurotrophin signaling endosome-mediated events in somatodendritic compartments. (a) Upon reaching cell bodies, Trk-harboring signaling endosomes activate transcriptional programs necessary for neuronal survival and axon growth, and for regulation of endocytic transport. (b) Coronin-1 is a transcriptional target of retrograde NGF signaling that is recruited to TrkA-containing endosomes and extends the duration of endosomal signaling by preventing endosome fusion with lysosomes. (c) Signaling endosomes are retrogradely transported long-distance from distal axons to the dendrites, where they regulate the clustering of nAChRs and pre-existing postsynaptic density components including MAGUK, GKAP and Shank. Signaling endosomes modulate the assembly of postsynaptic components, in part, by restricting the anti-synaptic actions of p75 signaling in dendrites.

endocytic trafficking [36]. One interesting example is coronin-1, an endosomal effector, which is expressed in an NGF-dependent manner [38*]. Coronin-1 is specifically recruited to TrkA endosomes in cell bodies and extends the duration of endosomal signaling by preventing endosome fusion with lysosomes [38*] (Figure 2b). Thus, NGF controls the persistence of its retrograde signal in cell bodies by transcriptional regulation and recruitment of coronin-1. One possible fate of Trk endosomes that evade lysosomal fusion may be to recycle back to the plasma membrane. Retrogradely transported TrkA receptors were found to shuttle between the plasma membrane and intracellular compartments in neuronal soma, and this recycling was attenuated in *coronin-1* mutant neurons [38*]. Factors such as Bicaudal-D1 (BICD1), best known

as a dynein adaptor, may also function in these sorting decisions in neuronal soma. *BICD1* depletion increased the plasma membrane recycling of neurotrophin receptors at the expense of lysosomal degradation [39].

The cell body is not the final destination of Trk endosomes that have been retrogradely transported from distal axons. A recent study provided evidence that TrkA endosomes originating from distal axons were transported all the way to dendrite arbors of sympathetic neurons to regulate synaptic connectivity with preganglionic sympathetic neurons [40*] (Figure 2c). In dendrites, signaling likely from internalized receptors, regulated the clustering of post-synaptic density components such as Membrane-Associated Guanylate Kinases (MAGUK), Shank,

Guanylate Kinase-Associated Protein (GKAP), and nicotinic acetylcholine receptors (nAChRs) [40^{*}]. These intriguing findings raise several key cell biological questions; how do Trk endosomes switch from axonal microtubules of uniform polarization to dendrite microtubules with mixed polarity? Are there additional functions for Trk endosomal signaling in dendrites and synapses, and what are the ultimate fates of these endosomes in dendrites? In central nervous system (CNS) neurons, BDNF-induced dendrite branching depends on local recycling of TrkB receptors *via* Rab11-positive recycling endosomes [41]. Notably, TrkB recycling in dendrites requires binding to a co-receptor, Slitrk5, a transmembrane protein with extracellular leucine-rich repeat (LRR) domains and intracellular Trk-like domains [42]. In peripheral neurons, similar mechanisms of local recycling of distal axon-derived Trk receptors within dendritic sub-compartments may allow for modulation of synaptic responses.

Defects in signaling endosomes in neurodevelopmental disorders

While the functional relevance of neurotrophin trafficking has been most appreciated during normal development, a corollary view is that dys-regulation of endocytic trafficking could be the basis for the loss of neurotrophin-dependent neurons in developmental disorders and progressive neurodegenerative diseases [3,43–45]. Two recent studies provide insights into how altered neurotrophin endosomal signaling might contribute to the pathogenesis of neurodevelopmental disorders. Patel and colleagues highlighted a link between impaired neurotrophin trafficking and neurodevelopmental defects in the peripheral nervous system in Down syndrome [46^{*}]. One of the genes that is triplicated in Down syndrome is *Regulator of Calcineurin1 (RCAN1)*, an endogenous calcineurin inhibitor. Overexpression of *RCAN1* impaired TrkA internalization and attenuated retrograde NGF signaling. *In vivo*, mice expressing three copies of *RCAN1* exhibited a developmental loss of sympathetic neurons and diminished innervation of peripheral targets, phenotypes that are recapitulated in mutant mice lacking NGF signaling. Furthermore, genetically correcting *RCAN1* levels in a mouse model of Down syndrome mice improved NGF-dependent receptor trafficking, neuronal survival and innervation. Since reducing *RCAN1* gene dosage did not fully rescue sympathetic neuronal deficits in Down syndrome mice, it is likely that *RCAN1* cooperates with other human chromosome 21 gene products such as amyloid precursor protein [47] and intersectin, a known endocytic regulator [48], to elicit sympathetic nervous system dysfunction in Down syndrome. The defects in retrograde NGF trafficking and developmental abnormalities in the sympathetic nervous system in Down syndrome mice are of interest because sympathetic innervation was also found to be diminished in peripheral organs from infants with Down syndrome [46^{*}]. Together, the findings suggest anatomical and cellular bases for

the reported autonomic dysfunction in the disease including impaired neural regulation of heart rate and blood pressure.

In another study, Ouyang and colleagues highlighted a role for endosomal pH as a critical regulator of neurotrophin signaling during neuronal circuit formation [49^{*}]. In humans, mutations in *NHE6*, an endosomal sodium proton exchanger, are commonly associated with Christianson's syndrome which presents with delayed development, microcephaly, intellectual disability, and several autism-related features. Genetic deletion of *NHE6* in mice resulted in perturbations in neuronal connectivity, including attenuated axonal and dendrite arborization, and reduced spine formation in the developing hippocampus and cortex. Ouyang and colleagues provided insight into the cell biological underpinnings of the morphological defects by showing that *NHE6* co-localized with the TrkB receptor for BDNF in early and late endosomes, and that BDNF signaling and TrkB levels were attenuated in *NHE6* mutant neurons, likely due to over-acidification of endosomes. These findings link disruptions in acidification of neurotrophin signaling endosomes with neuronal morphogenesis defects in an autism-related neurodevelopmental disorder.

Conclusions

Significant advances have been made in identifying functional roles for retrograde neurotrophin signaling in normal development, and recently toward understanding the detrimental consequences of aberrant receptor trafficking in disease states. Despite this progress, knowledge of the detailed sequence of events underlying receptor internalization and sorting in nerve terminals, long distance transport in axons, and signal termination in somato-dendritic compartments remains fragmentary. Importantly, to date, much of the information on cellular mechanisms of retrograde trafficking has been gleaned from compartmentalized cultures of peripheral neurons. Therefore, key directions for the future include delineating the functions and mechanisms of retrograde neurotrophin trafficking/signaling in central nervous system neurons, for example, TrkA-positive basal forebrain cholinergic neurons that have critical roles in attention and memory. Importantly, *in vivo* studies to elucidate functions of retrograde neurotrophin trafficking have been accomplished by genetic manipulation of endocytic effectors [10^{*},38^{*}], which could affect neurotrophin-independent functions in cells. Knowledge of intrinsic determinants, *i.e.* endocytic motifs, within Trk receptors, will allow for the generation of knock-in mice that will more precisely dissect the physiological relevance of receptor trafficking *in vivo*. Furthermore, comparative genome-wide analyses of trafficking of Trk receptors with other receptors that undergo ligand-mediated (EGFR) or constitutive endocytosis (transferrin) may reveal molecular components specific to neurotrophin trafficking. Recent *in silico* analyses have revealed gene networks required

for organelle functions including lysosomes [50]. Similar analyses can be exploited to screen available microarray data sets to gain insight into transcriptional programs that may control endocytosis in neurotrophin-responsive neuronal populations. Finally, recent advances in super-resolution imaging and single-particle tracking approaches provide unique opportunities to study dynamic processes underlying receptor internalization, recycling, and long-distance transport in neuronal sub-cellular compartments. We anticipate that advances in methodology for imaging, biochemical purification, and genome-wide analyses of endosomes will provide further fundamental biological insights into neurotrophin signaling endosomes in neural circuit formation and maintenance.

Conflict of interest statement

Nothing declared.

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