Spatiotemporal Intracellular Dynamics of Neurotrophin and Its Receptors. Implications for Neurotrophin Signaling and Neuronal Function

F.C. Bronfman, O.M. Lazo, C. Flores, and C.A. Escudero

Abstract

Neurons possess a polarized morphology specialized to contribute to neuronal networks, and this morphology imposes an important challenge for neuronal signaling and communication. The physiology of the network is regulated by neurotrophic factors that are secreted in an activity-dependent manner modulating neuronal connectivity. Neurotrophins are a well-known family of neurotrophic factors that, together with their cognate receptors, the Trks and the p75 neurotrophin receptor, regulate neuronal plasticity and survival and determine the neuronal phenotype in healthy and regenerating neurons. Is it now becoming clear that neurotrophin signaling and vesicular transport are coordinated to modify neuronal function because disturbances of vesicular transport mechanisms lead to disturbed neurotrophin signaling and to diseases of the nervous system. This chapter summarizes our current understanding of how the regulated secretion of neurotrophin, the distribution of neurotrophin receptors in different locations of neurons, and the intracellular transport of neurotrophin-induced signaling in distal processes are achieved to allow coordinated neurotrophin signaling in the cell body and axons.

Keywords

Neurotrophins • Trks • p75 • Endosomes • Rab GTPases • Molecular motors • Retrograde signaling

The nervous system is a highly wired structure formed by neurons and glial cells, which together sculpt neuronal networks. The physiology of the network is

F.C. Bronfman (✉) • O.M. Lazo • C. Flores • C.A. Escudero
Physiology Department, Pontificia Universidad Católica de Chile, Santiago, Chile

Millennium Nucleus in Regenerative Biology (MINREB), Faculty of Biological Sciences, Pontificia Universidad Católica de Chile, Santiago, Chile
e-mail: fbronfman@bio.puc.cl

G.R. Lewin and B.D. Carter (eds.), *Neurotrophic Factors*, Handbook of Experimental Pharmacology 220, DOI 10.1007/978-3-642-45106-5_3,

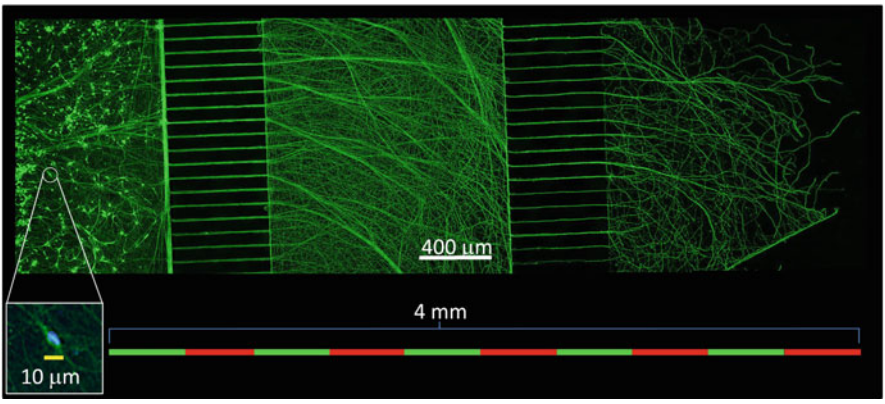

Fig. 1 Hippocampal axons can be as long as 400 times the diameter of the cell body. Hippocampal neurons were cultured in microfluidic chambers for 10 days in the presence of BDNF in the axonal compartment. The neurons were loaded with the fluorescent probe Calcien-AM (shown in *green*) to label the neuronal morphology. Nucleus was labeled with Hoechst staining (shown in *blue*)

regulated by neurotrophic factors that are secreted in an activity-dependent manner, modulating neuronal connectivity. Neurons exhibit a polarized morphology with two different compartments: the somatodendritic arbor and the axon, which are functionally differentiated to form and participate in neuronal networks. The neuronal soma acquires a particular morphology with branched prolongations specialized to form and receive synaptic contacts. The axon is a single prolongation that is specialized to transmit information from and back to the cell body, and it can be as long as 400 times the diameter of the neuronal soma in the case of rat hippocampal neurons (Fig. 1) or human lumbar motor neurons that can have axons longer than 10,000 times the diameter of the cell body. This special morphology imposes an important challenge for neuronal signaling and communication (Horton and Ehlers 2003b; Ibanez 2007).

Neurotrophins (NGF, BDNF, NT3, and NT4) are a well-known family of neurotrophic factors that, together with their cognate receptors, the Trks (TrkA, TrkB, and TrkC) and the p75 neurotrophin receptor (p75), regulate the development of neuronal networks by participating in the growth of neuronal processes, synaptic development and plasticity, neuronal survival, differentiation, and myelination. In the mature NS, neurotrophins determine the neuronal phenotype participate in neuronal plasticity and survival and in healthy and regenerating neurons. While each neurotrophin has a preferred Trk (NGF/NT3 binds TrkA; BDNF/NT4 binds TrkB; and NT3 binds TrkC), all neurotrophins bind p75 with a similar affinity. Additionally, two co-receptors for p75 have been described as participating in p75 signaling events: the neurotensin-3 receptor sortilin and the Nogo receptor (NogoR) for myelin-associated glycoproteins (Barker 2004; Greenberg et al. 2009; Huang and Reichardt 2001; Lu et al. 2005). In contrast to the Trks, p75 is capable of inducing opposing biological outcomes depending on its expression level,

association with different co-receptors at the plasma membrane, and the type of ligand (Bronfman and Fainzilber 2004; Gentry et al. 2004). p75 alone potentiates TrkA survival pathways and, in association with sortilin, induces cell death. Furthermore, it potentiates neurite outgrowth when acting alone or induced growth cone collapse in the presence of NogoR (Barker 2004; Higuchi et al. 2003).

Considering the particular morphology of the neuron, it is important to understand how the distribution of neurotrophin receptors in different locations of neurons, the regulated secretion of neurotrophin, and the intracellular transport of neurotrophin-induced signaling in distal processes are achieved to allow coordinated neurotrophin signaling in the cell body and axons. It is it now becoming clear that neurotrophin signaling and vesicular transport are coordinated to modify neuronal functioning because disturbances of vesicular transport mechanisms lead to disturbed neurotrophin signaling and to diseases of the nervous system (Bronfman et al. 2007; Perlson et al. 2010; Salinas et al. 2010). In this chapter, we will emphasize the role of key proteins that regulate vesicle transport and, thus, signaling, including Rab GTPases and molecular motors. Rab GTPases comprise a large family of small GTPases that control membrane identity and vesicle dynamics through the recruitment of different effector proteins (Stenmark 2009). There are two main classes of molecular motors that coordinate the transport of cargoes to the minus and plus ends of microtubules. The kinesins are a large gene family (KIFs, for kinesin superfamily proteins) that coordinates the transport of vesicles, macromolecular complexes, and organelles to the plus end of microtubules, thereby moving materials in an anterograde manner to the distal process of neurons, whereas cytoplasmic dynein is a protein complex that moves cargoes to the minus end of microtubules, thus moving materials in a retrograde fashion from the neuronal distal process to the cell body (Hirokawa et al. 2009; Kardon and Vale 2009).

1 Secretion and Anterograde Transport of Neurotrophins and Their Receptors

1.1 Neurotrophin Discovery and Biological Sources

Neurotrophins were first described as target-derived growth factors regulating the survival and differentiation of neurons from the peripheral nervous system (PNS, sensory and sympathetic neurons). The neurotrophic hypothesis, postulated by Rita Levi-Montalcini and Viktor Hamburger, stated that factors secreted in limiting amounts by tissues and target organs would ensure the correct number of neurons and their target fields, explaining the massive cell death of neurons during development in the PNS (Huang and Reichardt 2001; Korsching 1993; Levi-Montalcini 1966, 1987). It was later shown that neurotrophins have multiple functions in the central nervous system (CNS), including the regulation of synaptic plasticity and neuronal morphology. Most target tissues in the CNS also secrete neurotrophins that exert their effects by signaling back to the cell body (Bibel and Barde 2000;

Bilsland et al. 2010; Holzbaur 2004; Huang and Reichardt 2001; Lu et al. 2005; Mufson et al. 1999). Over the years, it has been shown that neurotrophins can be secreted not only by target tissues, which can be postsynaptic neurons or other types of cells, such as muscle, but also by presynaptic neurons, astrocytes, microglia, and glial cells, such as Schwann cells and oligodendrocytes, having paracrine and autocrine actions on neurons and other cell types (Bagayogo and Dreyfus 2009; Bessis et al. 2007; Cao et al. 2007; Dai et al. 2001; Lessmann et al. 2003; Matsuoka et al. 1991; Ohta et al. 2010; Schinder and Poo 2000; Verderio et al. 2006; Yune et al. 2007).

1.2 Coordination of Neurotrophin Processing, Local Translation, and Postsynaptic Secretion

Neurotrophins are homodimeric proteins synthesized as precursors (proneurotrophins) and are secreted to the extracellular space in a constitutive and regulated manner. As for many secreted proteins, after cleavage of the signal peptide in the endoplasmic reticulum, the homodimer transits through the Golgi, where it is subjected to glycosylation in its prodomain. The homodimers accumulate in vesicles in the *trans*-Golgi network (TGN), where the prodomains are cleaved by Furin and pro-convertases (PCs) to be secreted as non-glycosylated mature neurotrophins. The efficiency of cleavage varies according to neuronal and cell type. In hippocampal neurons, in the case of BDNF, proBDNF is secreted in an activity-dependent manner to the extracellular space, where it can be cleaved by the tissue plasminogen activator to be converted to mature BDNF (Lessmann et al. 2003; Nagappan et al. 2009; Yang et al. 2009). The regulation of neurotrophin secretion has been best studied in the case of BDNF. Although no studies have been reported that address how neurotrophins are sorted to either constitutive or regulated pathways, it is known that a polymorphism in the prodomain region (BDNF val to met) reduces the activity-dependent secretion of BDNF (Chen et al. 2004). This region of the BDNF prodomain has been shown to bind sortilin, a Vps10p domain protein that is known to bind the prodomain of proNGF to induce neuronal cell death by forming a complex with p75 (Nykjaer et al. 2004). In the TGN, sortilin has been associated with the proper intracellular trafficking of proteins in and out of the Golgi. The majority of sortilin resides in intracellular membranes that correspond to the TGN, endosomes, and secretory granules and vesicles in dendrites and axons (Willnow et al. 2008). Therefore, it is likely that this transmembrane protein plays a major role in targeting other soluble proteins and receptors out of the TGN to other cellular compartments. Thus, through interaction with sortilin, the prodomain of BDNF (not proNT4) regulates the sorting of BDNF to the regulated secretory pathway, and truncated versions of sortilin cause missorting of BDNF to the constitutive pathway, without affecting NT4-regulated secretion in hippocampal neurons, pointing to a neurotrophin-specific sorting mechanism to the regulated secretory pathway (Chen et al. 2005b).

The postsynaptic secretion of neurotrophins, particularly of BDNF, has been well documented (Cohen-Cory et al. 2010; Lessmann et al. 2003). Evidence has shown that BDNF and NT3 are more efficiently targeted to dendritic secretory granules in hippocampal neurons than are NT4 and NGF. Although secretion of neurotrophin is slower than neurotransmitter release, neurotrophin secretion co-localizes with PDZ-95, a postsynaptic marker of glutamatergic synapses. Regulated secretion of BDNF in glutamatergic synapses is tightly regulated, such that it will occur only in active synapses. For these phenomena to take place, there must be coordination of the local translation of BDNF messages and the secretion of vesicles in dendrites (Brigadski et al. 2005; Cohen-Cory et al. 2010).

The *BDNF* gene is characterized by complex transcriptional regulation; it can be transcribed from at least eight different promoters and can be polyadenylated on at least at two different sites, leading to the production of mRNA with a short 3′ untranslated region (UTR) or a long 3′UTR (Aid et al. 2007). The functional significance of the more than 16 transcripts that can be produced is unknown, but it has been suggested that this serves as a mechanism to add different layers of complexity to the regulation of the transcription and local translation of the *BDNF* gene (Greenberg et al. 2009). Dendritic transport of mRNA depends on specific dendritic targeting elements (DTEs) or cis-acting elements that are usually located in the 3′UTR; these sequences then target a specific mRNA for microtubular transport toward distal dendrites. BDNF mRNAs appear to share a common mechanism of transport with the mRNAs for calcium/calmodulin-dependent protein kinase II (alpha-CaMKII) and arc protein (Falley et al. 2009; Hirokawa 2006; Raju et al. 2011). These messages are transported in RNA granules in machinery that includes the trans-activating elements staufen and Pur-α and the kinesin-5 (KIF5) subfamily of molecular motors, in addition to the RNA-associated protein CArG box binding factor A (CBF-A). For *BDNF* specifically, an mRNA with a long 3′UTR seems to be more efficiently transported to dendrites compared to an mRNA with a short 3′UTR. A knockout mouse specific for this particular transcript exhibits abnormal pruning and enlargement of dendritic spines, as well as selective impairment of long-term potentiation in dendrites, but not the soma of hippocampal neurons (An et al. 2008).

In the cerebral cortex, promoter IV-dependent transcription of *BDNF* accounts for the majority of activity-dependent BDNF transcription (Hong et al. 2008). This transcript is apparently related to translation and secretion in the cell body, as it has been reported that the transcript mainly localizes to the cell soma, and exon II and VI *BDNF*-containing transcripts are more efficiently targeted to neurites (Chiaruttini et al. 2009). This phenomenon is presumed to be independent of DTEs in the 3′UTR of *BDNF* transcripts. The study performed by Chiaruttini and collaborators also indicated that there is a sequence coding for the prodomain of the *BDNF* transcript that binds translin, an RNA-binding protein involved in RNA transport. This is the same sequence used by sortilin to bind proBDNF. Interestingly, the val66met polymorphism in the BDNF gene causes translin to lose its ability to bind to *BDNF* transcripts and impairs its transport to dendrites. Thus, the reduced hippocampal dendritic complexity, memory deficits, and susceptibility to

mood disorders caused by this BDNF polymorphism may be due to the effects of deficits in the regulated secretion of BDNF mediated by the interaction of the BDNF prodomain with sortilin and to the inhibition of *BDNF* transcript transport to dendrites that is mediated in part by Translin (Bath and Lee 2006; Chen et al. 2006; Pezawas et al. 2004). Additional studies are needed to clarify the molecular mechanisms implicated in the activity-dependent targeting of specific *BDNF* mRNAs to dendrites. However, it can be proposed that the local synthesis and secretion of BDNF in active synapses is carried out through a mechanism that involves the transport of specific *BDNF* transcripts in a KIF5-dependent fashion and the coordination of BDNF synthesis in endoplasmic reticulum membranes and secretion from Golgi outposts localized to distal dendrites. Thus, postsynaptically secreted BDNF activates postsynaptic TrkB receptors, resulting in autocrine regulation of synaptic potentiation, or presynaptic TrkB potentiation of neurotransmitter release and regulation of target innervation (see below) (Fig. 2) (Cohen-Cory et al. 2010; Horton and Ehlers 2003a).

1.3 Anterograde Transport of Neurotrophin and Its Receptors

There is good evidence that BDNF-regulated secretion can occur in the presynaptic terminal. For this phenomenon to happen, dense core vesicles (DCV) derived from the Golgi apparatus in the neuronal soma have to undergo anterograde travel in a kinesin-dependent fashion to the synaptic terminal. It has been shown that BDNF and NT3 undergo anterograde transport and accumulate in DCV in synapses. Additionally, it has recently been found that BDNF anterograde transport in DCV is dependent on KIF1A (a member of a subfamily of kinesin 3) (Lo et al. 2011), suggesting that targeting to anterograde transport is regulated in part during the targeting of BDNF-DCV to a specific kinesin subfamily. In the presynaptic terminal BDNF is secreted in an activity-dependent manner similarly to other neuropeptides, exerting postsynaptic effects that regulate the development and maintenance of neuronal networks (Altar and DiStefano 1998; Lessmann et al. 2003; Matsumoto et al. 2008; Shinoda et al. 2011) (Fig. 2). In sensory neurons, the anterograde transport and release of BDNF in the axon enhance myelination, pointing to a potential role for anterograde-transported BDNF during development and regeneration (Ng et al. 2007). An unexpected finding reported by Butowt and von Bartheld (2001) was that in chick retinal ganglion cells, endocytosed NT-3 is sorted to the Golgi in a kinase-dependent manner, after which it undergoes anterograde transport to the presynaptic terminal in a process that depends on p75 anterograde transport (Butowt and von Bartheld 2001). This result implies that the anterograde transport of neurotrophins can be receptor mediated in a cellular pathway including ligand/receptor endocytosis in the cell body and posterior sorting to the anterograde transport pathway. Thus, there are at least two different forms of neurotrophins sorted to the anterograde pathway: one is non-receptor mediated and the other is receptor mediated.

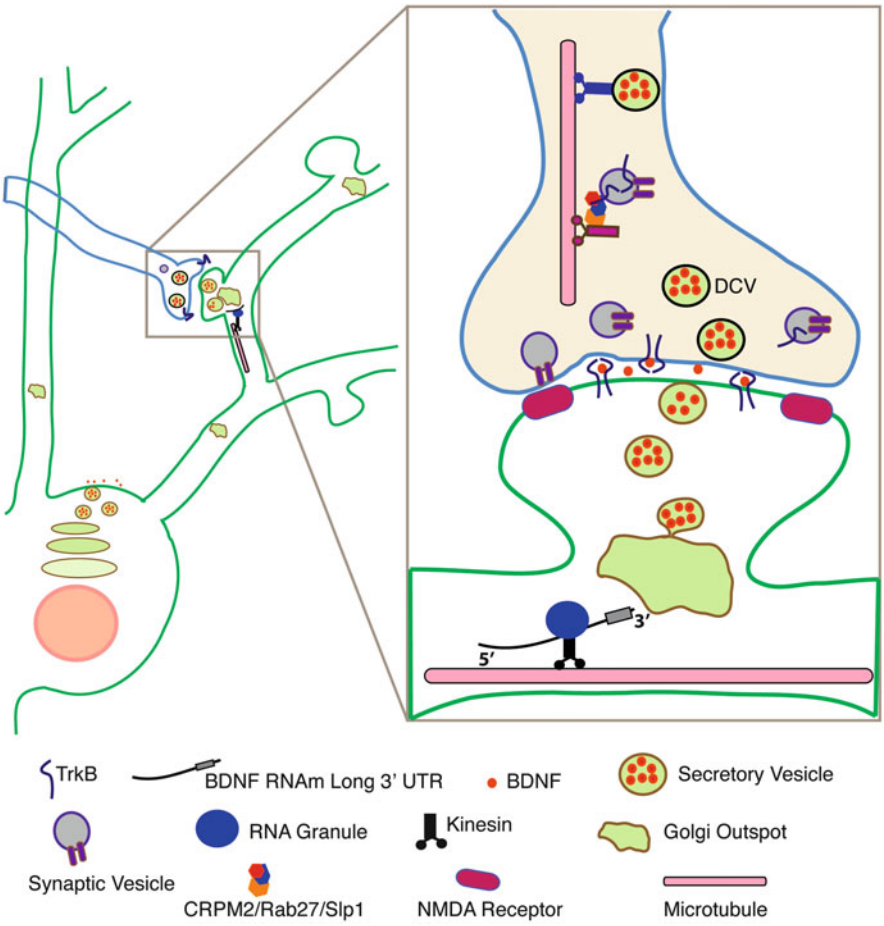

Fig. 2 Schematic diagram of a glutamatergic synapse in the central nervous system. In the presynaptic terminal, delineated in *blue*, it is illustrated how dense core vesicles (DCV) and synaptic vesicle precursors are transported to the terminal by different KIF members. DCV are filled with BDNF and they fuse with the plasma membrane in response to increase calcium concentration. The anterograde transport of synaptic vesicle precursors carrying TrkB receptors is regulated by the monomeric GTPase Rab27. There is a coordination of local translation and secretion of BDNF in the postsynaptic neuron that is delineated in *green*. The mRNA for BDNF is transported to the dendritic spine also by a KIF member. TrkB receptors are located at both pre- and postsynaptic membranes

As indicated above, sortilin is involved in regulating the secretion of BDNF in neurons. It was recently reported that in sensory neurons, sortilin facilitates the anterograde transport of Trks from the cell body along the axon (Vaegter et al. 2011). Determining whether the anterograde transport of BDNF and other neurotrophins also depends on sortilin, as has been shown for the regulated

secretion of BDNF and the anterograde transport of Trks, is a matter that will require further research.

Of note, in hippocampal neurons, anterograde-transported TrkB-positive vesicles are co-transported with VAMP2, a synaptic vesicle-associated protein, indicating that the final destination of these receptors is the presynaptic terminal (Gomes et al. 2006). The anterograde transport of TrkB is specifically mediated by conventional kinesin (kinesin-1) and the complex CRPM2/Slp1/Rab27a (Arimura et al. 2009). The CRPM2 protein associates with kinesin to bind microtubules, and through the Rab27a effector Slp1, which binds the TrkB cytosolic tail, Rab27a and CRPM2 engage TrkB in the anterograde axonal pathway (Fig. 2). Thus, upstream signaling pathways regulating CRPM2 activity will increase the transport of TrkB to the presynaptic terminal. Rab27a has been shown to be involved in the regulated secretion of TGN-derived secretory granules in many cellular models and may, therefore, play a key role in the targeting and insertion of TGN-derived TrkB in the presynaptic terminal (Fukuda 2008). Another monomeric GTPase of the Rab family involved in the anterograde transport of Trks is Rab11. Rab11 regulates the dynamics of the recycling endosome in many cells, and in sympathetic neurons, after TrkA endocytosis, Rab11 regulates the transcytosis and anterograde transport of TrkA to the sympathetic growth cone, where it enhances NGF sensitivity (Ascano et al. 2009). The kinesin associated with Rab11 vesicular trafficking is kinesin 2, through interaction with the Rab11 effector FIP5 (Schonteich et al. 2008). Although it has not been demonstrated that this complex regulates Rab11-dependent TrkA transcytosis, it is likely that different molecular motors and associated complexes regulate TGN-derived anterograde Trk vesicles *versus* transcytosis from the cell body of endocytosed Trks to the presynaptic terminal.

2 Internalization and Retrograde Signaling of Neurotrophin Receptors

After secretion, neurotrophins bind their cognate receptors, which can be located along the axon, in the neuronal cell body, or at the synapse (pre- or postsynaptic). After ligation, the neurotrophin/receptor complex rapidly activates signaling pathways in the plasma membrane and undergoes internalization. For quite some time, endocytosis of the neurotrophin/receptor complex was considered to be a mechanism solely involved in the downregulation of signal transduction. However, it is now well established that the internalization and post-endocytic trafficking of receptors are essential for signaling and neuronal function. After internalization, growth factor receptors continue signaling from endosomes, where they are associated with different signaling adaptors than in the plasma membrane. Additionally, the efficiency of endocytosis and the recycling of the receptors back to the cell surface is a mechanism that regulates the availability of the receptors for initiating signaling. Finally, the efficiency of ligand/receptor degradation in late endocytic pathways determines the duration of signaling inside the cell, thus having an important impact on cellular function (Fig. 3) (Bronfman et al. 2007;

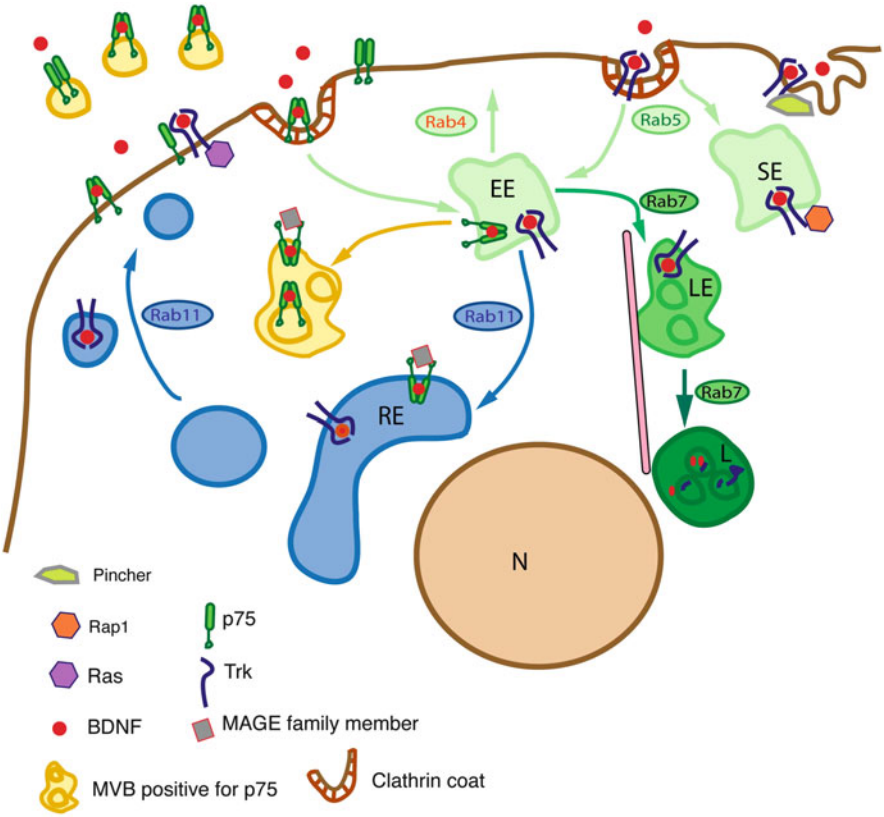

Fig. 3 Schematic diagram of the intracellular trafficking dynamic of the p75 and Trks receptors. In the plasma membrane p75 is found as a dimmer and the neurotrophin (Nt) binding triggers a conformational change that induces signaling adaptors binding (Vilar et al. 2009). p75 receptor is internalized through clathrin-coated pits and is found in early endosomes (EE) positive for Rab5 and recycling endosomes (RE) positive for Rab11. However, the majority of the receptor is accumulated in a multivesicular body (MVB) that is negative for late endocytic markers such as Rab7. The Trks are internalized through clathrin-coated pits and also by a mechanism that involves the formation of membrane ruffles, the actin cytoskeleton and the chaperone pincher. After internalization, Trks associates with signaling endosomes (SE) where activation of the GTPase Rap1 triggers the long-lasting activation of ERK1/2 and cellular differentiation. Trks are also found in the recycling pathways regulated by Rab11 and Rab4. Finally, downregulation of Trks signaling is initiated in its transit thought the late endosome (LE) and is achieved, in part, by degradation of the receptor in the lysosomes (L), process that is regulated by Rab7. Maturation from early endosomes to lysosomes needs the transport of endosomes from the cell periphery to the perinuclear region using microtubules and the molecular motor dynein

Miaczynska et al. 2004b; Platta and Stenmark 2011; Sorkin and Von Zastrow 2002).

The first evidence that neurotrophin receptors continue signaling inside the cell came from the groups of Mobley for the Trks and Fainzilber for p75, using PC12

cells as a model. Regarding TrkA, it was found that the internalized and activated TrkA receptor, together with NGF and PLC-γ1, was associated with intracellular vesicles. Later, it was reported that p75 internalizes more slowly than TrkA, accumulating in different vesicles, where the receptor is associated with NGF and signaling adaptors of the MAGE family (Bronfman et al. 2003; Grimes et al. 1996, 1997; Tcherpakov et al. 2002). The first description of the intracellular signaling of neurotrophin receptors gathered a great deal of interest related to understanding the mechanism of the internalization and trafficking of neurotrophin receptors because it was clear that it would shed new light on how neurons and neuronal networks interpret neurotrophin signaling. A summary of the more compelling findings on this topic will be presented below.

2.1 Trks Internalization and Intracellular Trafficking

In general terms, activated receptors in the plasma membrane can be internalized via clathrin-mediated or clathrin-independent routes. The clathrin-independent routes include at least eight different mechanisms, including caveolar-type endocytosis, macropinocytosis, Arf6-dependent endocytosis, and cholesterol-dependent and caveolin- and clathrin-independent pathways. All clathrin-mediated or clathrin-independent pathways of internalization are thought to converge on peripheral early endosomes (also referred to as sorting endosomes) (Doherty and McMahon 2009; Mayor and Pagano 2007). From there, some components are either rapidly recycled back to the plasma membrane or more slowly recycled through the recycling endosome (or pericentriolar endosome). From the early endosome, receptors are also sorted to late endosomes and lysosomes, where proteins are degraded (Di Fiore and De Camilli 2001; Miaczynska et al. 2004b; Sorkin and Von Zastrow 2002; Stenmark 2009) (Fig. 3).

The dynamics of intracellular trafficking, including through the endo-lysosomal system, are coordinated by Rab GTPases, which are a large family of small GTPases that control membrane identity and vesicle budding, uncoating, motility, and fusion through the recruitment of different and diverse effector proteins (Stenmark 2009). For example, Rab5 is a key regulator of early endosomal trafficking (Sonnichsen et al. 2000); Rab11 and Rab4 regulate transport through the recycling pathway; and Rab7 regulates transit from early endosomes to late endosomes and from late endosomes to lysosomes (Fig. 3) (Bucci et al. 1992, 2000; Cavalli et al. 2001; Somsel Rodman and Wandinger-Ness 2000).

Two different major routes of Trk internalization have been suggested in the literature: one is clathrin and dynamin dependent, and the other involves a macropinocytic process that depends on the new chaperone Pincher and is Rac and actin dependent, but dynamin independent. Dynamin is a GTPase that causes the pinching and scission of vesicles from the plasma membrane, and Rac is a small GTPase from the Rho family that regulates the dynamics of the actin cytoskeleton. In PC12 cells, NGF increases the association of clathrin with membranes, and TrkA is recovered in fractions containing clathrin-coated vesicles, together with signaling

components of the ERK1/2 pathway. Additionally, TrkA and TrkB internalization is inhibited by monodansylcadaverine, a drug that inhibits clathrin-mediated internalization. In support of a role for clathrin-coated pits in TrkA internalization, TrkA mutants with a truncated carboxyl-terminal domain that therefore lack potential clathrin-mediated internalization motifs exhibit dramatically decreased NGF internalization (Fig. 3) (Beattie et al. 2000; Doherty and McMahon 2009; Howe et al. 2001; Joset et al. 2010; Jullien et al. 2003; Mayor and Pagano 2007; Shao et al. 2002; Zhang et al. 2000; Zheng et al. 2008). Both types of internalization appear to take place in the cell body, as well as in axons because both dynamin and Pincher dominant negative mutants inhibit the internalization and retrograde transport of activated Trk receptors in sympathetic neurons (Valdez et al. 2005; Ye et al. 2003). Although Trks have been found in caveolae-like domains and lipid rafts (cholesterol and sphingolipid-rich membrane domains) upon ligand stimulation, there is no evidence that Trks are internalized through a clathrin-independent but cholesterol-dependent pathway. Similar to what happens to other receptor tyrosine kinases (RTKs), such as the epidermal growth factor (EGF) receptor (EGFR), localization to lipid rafts is necessary for signaling. However, in contrast to what is seen for EGFR, the Trks exhibit increased localization to lipid rafts after ligand binding. It is of note that for TrkB, translocation to lipid rafts in cortical and hippocampal neurons is necessary for synaptic modulation and requires TrkB receptor phosphorylation and internalization, suggesting that association with lipid rafts occurs on intracellular membranes (Assaife-Lopes et al. 2010; Huang et al. 1999; Limpert et al. 2007; Nishio et al. 2004; Pereira and Chao 2007; Zwang and Yarden 2009).

In general, the most important pathway for RTK internalization is clathrin-mediated internalization. Macropinocytosis is associated with areas where plasma membrane spreading and ruffling take place, which is a process, regulated by actin dynamics (Cavalli et al. 2001; Kirkham and Parton 2005) (Fig. 3). Although EGFR has also been shown to use this internalization mechanism, it has been suggested that this route is utilized only when there is an excess of ligand (Zwang and Yarden 2009). Most studies related to the Pincher-mediated macropinocytosis of Trks have been performed in cells overexpressing the Trk receptor or a chimeric version of it, and different concentrations of ligands have not been tested; therefore, the physiological relevance of this process has to be viewed with caution (Philippidou et al. 2011; Shao et al. 2002; Valdez et al. 2005).

Cargo recognition during clathrin-mediated endocytosis is mediated by different adaptors that possess a phospholipid-interacting motif and may also interact with transmembrane receptors. These adaptors interact with clathrin, increasing its affinity for the plasma membrane and for the cytosolic motif present in the cytoplasmic tails of transmembrane receptors. The best studied adaptor protein functioning at the plasma membrane is the AP-2 complex, which recognizes the YXXϕ sequence (where ϕ is a hydrophobic residue, and X is a variable residue) and dileucine sequences [DE]XXXL[LI] (where the second leucine can be isoleucine, and X is a variable residue that can be followed by an asparagine and lysine). These sequences are found in classic endocytic receptors, such as the transferrin receptor.

There are also other adaptors such as Epsin and EPS15 that can associate directly with clathrin, recognizing mono- or polyubiquitins in the cytoplasmic tail of receptors (Bonifacino and Traub 2003; Hawryluk et al. 2006; Marmor and Yarden 2004). EGFR activation induces the binding of the c-Cbl ubiquitin ligase, which adds multiple monoubiquitins to the receptor, inducing its internalization through the EPS15 and Epsin adaptors and clathrin (de Melker et al. 2001; Marmor and Yarden 2004). Other clathrin adaptors that work differently than AP2 are AP180 and Dab2. AP180 is specifically expressed in the nervous system and induces the internalization of synaptic vesicle-associated proteins, and DAB2 binds to NPXY sequences (whereas X is a variable residue) found in the lipoprotein receptors LDLR and ApoER2, inducing their internalization from the apical domain of epithelial cells (which is proposed to be equivalent to the presynaptic terminal of neurons) (Cuitino et al. 2005; Morris et al. 2002; Rodriguez-Boulan and Powell 1992; Slepnev and De Camilli 2000; Sorkin 2004).

There is little information about which clathrin adaptors mediate the clathrin-dependent internalization of Trks. There is one report that AP-2 mediates the internalization of TrkB in hippocampal neurons. Both TrkA and TrkB possess AP-2 and DAB2 consensus sequences; however, it is not known whether they serve as binding sequences for AP2 or DAB2 (Fig. 4). It would be of interest to evaluate whether DAB2 and AP180 participate in the clathrin-dependent endocytosis of Trks in the synaptic terminal to mediate Trk presynaptic local effects or retrograde signaling (see below). Additionally, similar to EGFR, the Trks are multimonoubiquitinated, and TrkA, in particular, is multimonoubiquitinated or polyubiquitinated in a Nedd4-2-dependent or TRAF6-dependent manner, respectively. However, through reducing TRAF6-mediated ubiquitination alone, the internalization of the receptor is diminished, and it is possible that Nedd4 ubiquitination mediates sorting to lysosomes and not internalization (see below). To date, there have been no studies reported indicating whether the Trks are recognized by clathrin adaptors such as EPS15 and Epsin that specifically recognize ubiquitinated cargoes (Arevalo et al. 2006; Geetha et al. 2005).

The neuroendocrine PC12 cell line is a frequently used model of NGF signaling expressing both p75 and TrkA receptors. When treated with NGF, PC12 cells differentiate to a sympathetic neuron-like phenotype, extending neurites and increasing the expression of different neurotransmitters (Greene and Tischler 1976). Numerous studies on neurotrophin signaling and trafficking have been performed in this neuronal model system. Many lines of research support the idea that intracellular trafficking of neurotrophin receptors regulates neurotrophin-signaling outcomes. In PC12 cells, inhibition of dynamin and TRAF6-dependent internalization inhibits the neurite extension induced by NGF (Geetha et al. 2005; Zhang et al. 2000). This is consistent with the fact that internalization of TrkA is necessary for sustained activation of the ERK1/2 pathways that are required for PC12 cell differentiation. Different publications indicate that this is achieved by increasing the activation of Rap1 that is mainly associated with endosomes (Fig. 3) (Kao et al. 2001; Mochizuki et al. 2001; Nomura et al. 2004; Wu et al. 2001; York et al. 2000). Interestingly, endosomal signaling mediated by TrkA and Rap1 and

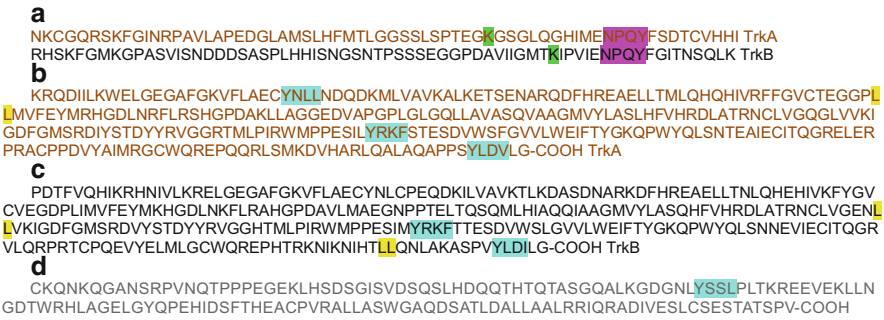

Fig. 4 Potential sequence of neurotrophin receptors regulating its internalization. The juxtamembrane portion of the TrkA and TrkB receptor is shown in **a**, the sequence NPXY is labeled in *pink*, and it is a potential binding site for the clathrin adaptor Dab2. NPXY is also the binding site for signaling adaptors such as Shc and Grb2 leading to activation of ERK1/2 and IP3K (Huang and Reichardt 2003). The lysine (K) labeled in *green* is ubiquitinated by TRAF6 in TrkA and regulates its internalization (Geetha et al. 2005). It is not known whether this lysine is also ubiquitinated in TrkB. In **b** and **c** is shown the rest of the intracellular domain of TrkA (**b**) or TrkB (**c**) and the intracellular domain of p75 (**d**). In *blue* are labeled the YXXϕ, which is a potential binding site for the clathrin adaptor AP2. In *yellow* are shown the dileucine motifs in the context of [DE]XXXL[LI], which are also a potential binding site for the clathrin adaptor AP2 (Bonifacino and Traub 2003)

sustained ERK1/2 activation require dynein retrograde transport of endosomes from the cell periphery to the perinuclear region of PC12 cells and sensory neurons. This indicates that during microtubular and dynein-dependent transport, TrkA-positive endosomes mature and acquire the adaptors and signaling molecules necessary for TrkA signaling to Rap1 and ERK1/2 (Wu et al. 2007).

Although TrkA and the EGFR are both targeted to the degradative pathway, EGFR is more efficiently targeted to the lysosomal pathway, whereas the TrkA receptor continues signaling in Rab5-positive endosomes for a longer period of time. Which molecular interaction might account for this difference in trafficking kinetics and signaling? There are at least two different reports in the literature regarding this point. The first is related to the association of Trks with the ankyrin-rich transmembrane protein ARMS, which does not associate with EGFR. ARMS is rapidly tyrosine phosphorylated after the binding of neurotrophins to Trk receptors and provides a docking site for the CrkL–C3G complex, resulting in sustained Rap1-dependent ERK activation (Arevalo et al. 2004). The other report is related to the association of endocytosed TrkA with RabGAP5, a protein that downregulates Rab5 activity to facilitate neurite outgrowth and differentiation. Downregulation of Rab5 activity delays the maturation of early endosomes into late endosomes and lysosomes, precluding TrkA degradation. Consistently, overexpression of a dominant negative form of Rab7 induced endosomal accumulation of TrkA and potentiated Erk1/2 phosphorylation and neurite outgrowth (Liu et al. 2007; Saxena et al. 2005a). Another factor contributing to different signaling outcomes between EGFR and TrkA is the efficiency of lysosomal targeting by monoubiquitination.

Compared to TrkA, the EGFR rapidly targets to the degradative pathway through monoubiquitination by the ubiquitin ligase c-Cbl, whereas TrkA is monoubiquitinated by the E3 ubiquitin ligase Nedd4-2, which targets the receptor for endosomal degradation. However, TrkA monoubiquitination does not impair signaling but rather appears to potentiate sustained ERK1/2 activation, suggesting that the TrkA receptor may continue signaling even in the late endosomal pathway (Arevalo et al. 2004; Georgieva et al. 2011; Haglund et al. 2002; Marmor and Yarden 2004; Saxena et al. 2005b).

Another molecular adaptor that might function downstream of the TrkA internalization to achieve sustained signaling and differentiation is the APPL1 cytosolic protein. Under certain conditions, APPL1 associates with Rab5 and defines a subpopulation of Rab5-positive endosomes. With respect to EGF signaling, it has been shown that after downregulation of Rab5 activity in early endosomes, APPL1 is released from the endosomal membrane and translocates to the nucleus, where it associates with components of the nucleosome remodeling and histone deacetylation machinery. In the case of TrkA, APPL1 associates with TrkA through two different means: indirectly via GIPC1 (a PDZ protein) and directly through the APPL1 phosphotyrosine-binding domain. Cell fractionation studies have demonstrated that APPL1, GIPC1, and phosphorylated TrkA are present in the same endosomal fractions and that both GIPC1 and APPL1 are recruited to TrkA-positive endosomes upon ligand stimulation. Additionally, both the APPL1 and GIPC1 proteins are required for NGF-induced ERK1/2 and Akt activation and neurite outgrowth. Although it has not been demonstrated that APPL1 is released from endosomes and translocates to the nucleus after binding TrkA in endosomes, these results are consistent with a potential role for APPL1 in NGF-dependent transcription to induce neuronal differentiation (Lin et al. 2006; Miaczynska et al. 2004a; Varsano et al. 2006).

Another means of increasing the sustained activation of signaling molecules is through the recycling of activated receptors. Chen and collaborators found that TrkA is more efficiently recycled to the plasma membrane compared to TrkB in PC12 cells because it possesses a post-endocytic recycling signal in the juxtamembrane domain. Accordingly, in PC12 cells, TrkA causes sustained signaling of phosphatidylinositol 3-kinase/Akt, resulting in increased cell survival, whereas TrkB does not have this effect. Targeting of TrkA to the recycling pathways does not require the receptor to exhibit kinase activity, while targeting of the receptor to the degradative pathway does, suggesting that kinase activity modulates targeting of the receptor to the degradative or recycling pathway (Chen et al. 2005a; Saxena et al. 2005b). Although the TrkB receptor does not possess the recycling signal present in TrkA, it has been described (in hippocampal neurons) that there is a regulated recycling of TrkB that depends on its kinase activity and the adaptor Hrs. This regulated recycling pathway appears to be different than the constitutive recycling pathways used by transferrin and is required for sustained ERK1/2 signaling induced by TrkB (Huang et al. 2009). Highlighting the functional role of the recycling pathway in hippocampal neurons, we have found that TrkB recycling in dendrites is mainly mediated by Rab11, a RabGTPase regulating the

recycling of receptors back to the plasma membrane, and that inhibition of Rab11 activity reduces the dendritic ramifications induced by BDNF. Inhibition of Rab11 activity also reduced targeting of TrkB to dendrites. Additionally, we showed that TrkB activation increases Rab11 activity and changed Rab11 dynamics in dendrites. This is one of the first examples indicating that regulation of Rab11 activity by TrkB is necessary for structural plasticity induced by BDNF (Lazo et al. 2013). Consistent with the idea that post-endocytic trafficking of the TrkB receptor is necessary for signaling, inhibition of TrkB internalization reduces phosphatidylinositol 3-kinase/Akt signaling and neurite outgrowth of hippocampal neurons (Huang et al. 2009; Zheng et al. 2008).

2.2 p75 Internalization and Intracellular Trafficking

Regarding p75 internalization, we have previously shown that pharmacological inhibition of clathrin-mediated internalization completely blocks ligand-dependent p75 internalization in PC12 cells. Additionally, p75 is internalized with slower kinetics compared to transferrin and TrkA (Bronfman et al. 2003; Saxena et al. 2004). These different kinetics of internalization result in targeting p75 and TrkA to different types of endosomes (McCaffrey et al. 2009). In contrast to what is seen for the Trks, p75 is not targeted to the degradative pathway (i.e., late endosomes and lysosomes) within the time frame of these experiments. Initially, observations of partial colocalization with transferrin suggested that endocytosed p75 accumulates in recycling endosomes, where it continues signaling (Bronfman et al. 2003; Saxena et al. 2005b). However, recent studies by our group using quantitative confocal microscopy and deconvolution have indicated that after internalization, p75 evades Rab5-positive early endosomes and accumulates in two different organelles. One organelle is positive for Rab11, and another one positive for the tetraspanin CD63. CD63 labels multivesicular bodies for exosomal release and we found p75 in exosomes derived from PC12 cells and sympathetic neurons (Fig. 3) (Escudero et al. unpublished work). Additionally studies are needed to understand the particular trafficking features of p75 in different types of neurons. These studies are important because p75 continues signaling inside the cells and we have shown that endocyted p75 is proteolytically processed. Several lines of evidences have indicated that after proteolytic processing, p75-derived COOH-terminal fragments are important for signaling; therefore, internalized p75 may interact with signaling adaptors in endosomes or to be proteolytically processed to generate signaling fragments (Bronfman 2007; Bronfman et al. 2003; Kanning et al. 2003; Kenchappa et al. 2006; Urra et al. 2007).

Other mechanisms of internalization are apparent in neurons. For example, in motor neurons, p75 internalization is ligand independent and is inhibited by the expression of dominant negative forms of dynamin, but not of AP-2 or AP180 clathrin adaptors, suggesting that in the motor neuron cell body, p75 internalization is clathrin independent. However, in motor neuron axons, clathrin-dependent and independent pathways coexist. The clathrin-dependent route targets p75 for

retrograde transport in the axon (Deinhardt et al. 2007). Similar to what is seen in motor neurons, in sympathetic neurons that express TrkA and p75, BDNF internalization is partially inhibited by sucrose (pharmacological inhibition of clathrin-dependent pathways) and nystatin (an independent drug that disrupts lipid rafts), suggesting that there are also clathrin-dependent and clathrin-independent mechanisms for the internalization of p75 in sympathetic neurons (Hibbert et al. 2006). Similar to the Trks, p75 also associates with lipid rafts to carry out signaling; however, additional studies are needed to understand the role of lipid rafts in the regulation of internalization, signaling, and p75 stability in the plasma membrane (Fujitani et al. 2005; Huang et al. 1999; Nishio et al. 2004). We have analyzed p75 internalization kinetics in three different cells types. In hippocampal neurons, p75 is internalized rapidly, whereas p75 internalization in PC12 cells is slower than in hippocampal neurons, but twice as fast as in sympathetic neurons (Bronfman et al. 2003, 2007). We have found that in cultures, these three different cell types exhibit substantial differences in the cholesterol content of their membranes, the greater the content of cholesterol in the cell, the slower the internalization of the receptor with sympathetic neurons presenting the highest levels of cholesterol and hippocampal neurons the lowest. Of note, although sympathetic neurons exhibit the greatest content of cholesterol in the plasma membrane the mobility of p75 in the plasma membrane seems to be similar than in PC12 cells (Fig. 5). These observations suggest that the content of cholesterol in the plasma membrane plays a role in the time of residence of the p75 receptor in the plasma membrane after ligand binding and thus regulates its internalization kinetics.

2.3 Neurotrophin Trafficking and Neurodegenerative Diseases

The role of the internalization and intracellular trafficking of neurotrophin receptors in signaling outcomes is emphasized by the fact that mutations in trafficking proteins cause neurodegeneration in humans and alteration of neurotrophin signaling. For example, missense mutants in the late endosomal Rab7 GTPase cause the autosomal dominant peripheral neuropathy Charcot–Marie–Tooth disease type 2B (CMT2B). Mutant Rab7 acts as a constitutively active GTPase, increasing the activity of Rab7 and downregulating NGF-induced differentiation through abnormal ERK1/2 signaling. Additionally, loss of function of alsin, an activator of the Rac1 and Rab5 small GTPases, causes ALS2, an autosomal recessive motor neuron disease with juvenile onset and slow progression. Als2(−/−) mice exhibit a marked diminution of Rab5-dependent endosome fusion activity, together with disturbances in the endosomal transport of the insulin-like growth factor 1 (IGF1) and BDNF receptors (BasuRay et al. 2010; Cogli et al. 2010; Devon et al. 2006). We have analyzed the consequences of loss of function of the Niemann–Pick type C 1 (NPC1) protein for neurotrophin signaling. NPC1 is a transmembrane protein that controls the efflux of cholesterol from endocytic pathways and causes abnormal endocytic function and neurodegeneration. Our analysis indicated that NPC1 loss of

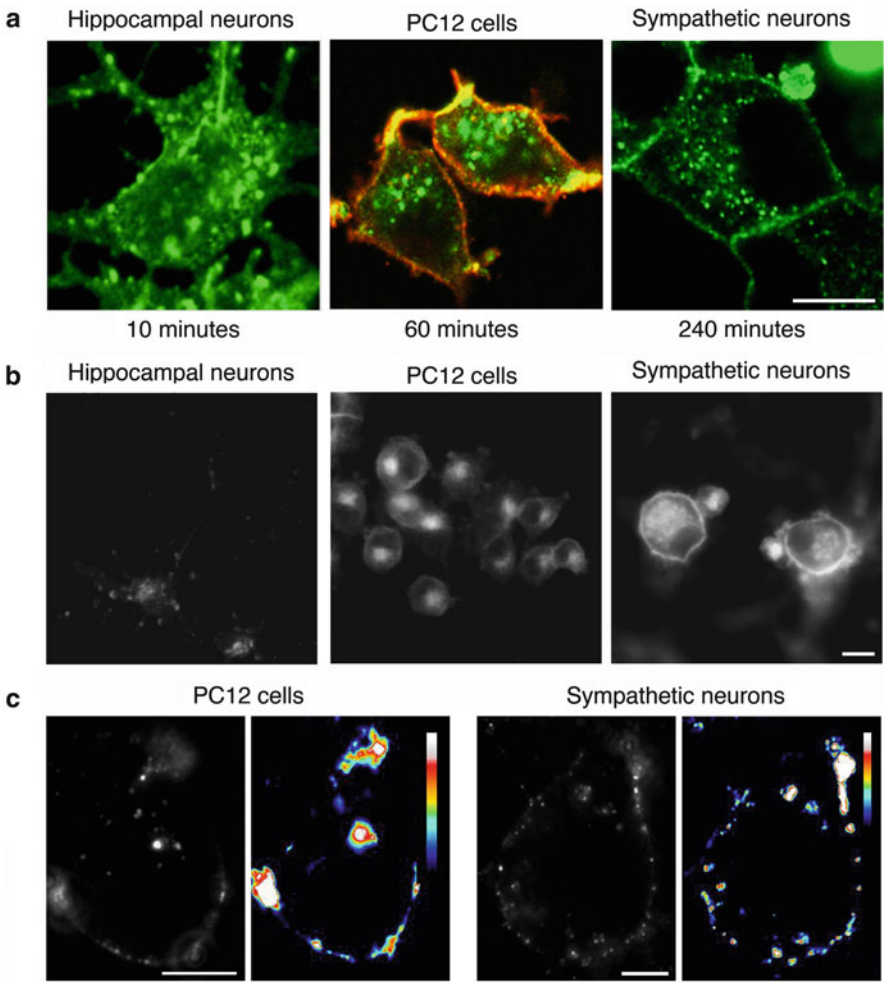

Fig. 5 Dynamics of p75 receptor in three different neuronal models. (**a**) p75 internalization in three different neuronal models. The peak of p75 internalization is observed at different time points in the three different models. p75 internalization was visualized as indicated in Bronfman et al. (2003, 2007). (**b**) Visualization of the levels of cellular cholesterol observed in three different neuronal models stained with filipin (a fluorescent drug that binds cholesterol). **a**, **b**, *scale bar*, 10 μm. (**c**) p75 localized in the plasma membrane of PC12 and sympathetic neurons was labeled with and antibody against the extracellular domain of p75 (MC192) labeled with Q-Dots. Movement of p75 in the plasma membrane was studied by real-time microscopy with a frequency of 1.5 frames/second (a total of 150 frames). The 150 frames were condensed to 1 and showed in a gray scale (*left panel*) or with segmentation of intensity ranges into a pseudo-colored scale (*right panel*). *Blue* indicates mobile p75. **c**, *scale bar*, 5 μm

function causes increased neurotrophin signaling and reduced recycling of TrkA in addition to increased pathological Tau phosphorylation (Cabeza et al. 2012). Other hereditary neurodegenerative diseases related to abnormal functioning of Rab

GTPases (including Rab5, Rab11) and alterations in BDNF and NGF transport are Huntington's disease, Alzheimer's disease, and Down syndrome (Gauthier et al. 2004; Ginsberg et al. 2010; Li et al. 2009; Pal et al. 2006; Salehi et al. 2006). A conclusion arising from these findings is that assembly of specific signaling complexes on specific endosomes provides a way to solve the problem of specificity in signal transduction; alteration of the trafficking properties of a neuron would alter this specificity, causing miss-regulation of signaling and contributing to diverse neurodegenerative diseases.

2.4 Mechanism of the Axonal Transport of Neurotrophin Signaling in Neurons

Neurotrophins were first discovered as target-derived factors essential for the survival and maturation of sensory neurons and sympathetic neurons of the peripheral nervous system (Glebova and Ginty 2005; Huang and Reichardt 2001; Korsching 1993; Levi-Montalcini 1966, 1987). The question then arose of how long-range projection neurons transmit the neurotrophic survival signal from the presynaptic terminal to the neuronal cell body to induce transcriptional changes? The first hint of an answer to this question came from the work of Hendry and colleagues, who showed that radiolabeled NGF was retrograde transported from adrenergic terminals in the mouse and rat iris to the cell body of sympathetic neurons in the superior cervical ganglia (SCGs). This transport was found to be sensitive to colchicine (a drug that destabilizes microtubules) and was inhibited by antibodies against NGF, indicating that the transport was specific and dependent on microtubules (Hendry et al. 1974a, b). Later, it was found that activated TrkA accumulated distally to a ligation site in the sciatic nerve, indicating that activated TrkA complexes are retrograde transported (Bhattacharyya et al. 1997; Ehlers et al. 1995). Additionally, Hendry and collaborators reported that the transport of radiolabeled NGF in sensory axons, mediated by the microtubule-associated molecular motor dynein, depends on signal transduction by different kinases, including TrkA and PI3-K (Reynolds et al. 1998). More details regarding the molecular mechanism of the transport of neurotrophin signaling came with the development of compartmentalized cultures of sensory and sympathetic neurons. In these cultures, neuronal cell bodies are located in a different compartment than axons. Thus, these cultures allow neurotrophin axonal stimulation without stimulation of the neuronal soma. Using them, Ginty, Segal, and collaborators reported that both the kinase activity and internalization of Trks are required for retrograde-transmitted nuclear responses, including the activation of transcription factors such as CREB and c-Fos. Furthermore, inhibition of dynein activity in the axons of compartmentalized sensory neuron cultures causes downregulation of the transport of activated TrkB, together with inhibition of the survival responses in the cell body. A biochemical explanation for these results was offered by detection of the direct interaction of Trks with the molecular motor dynein, as described by Chao

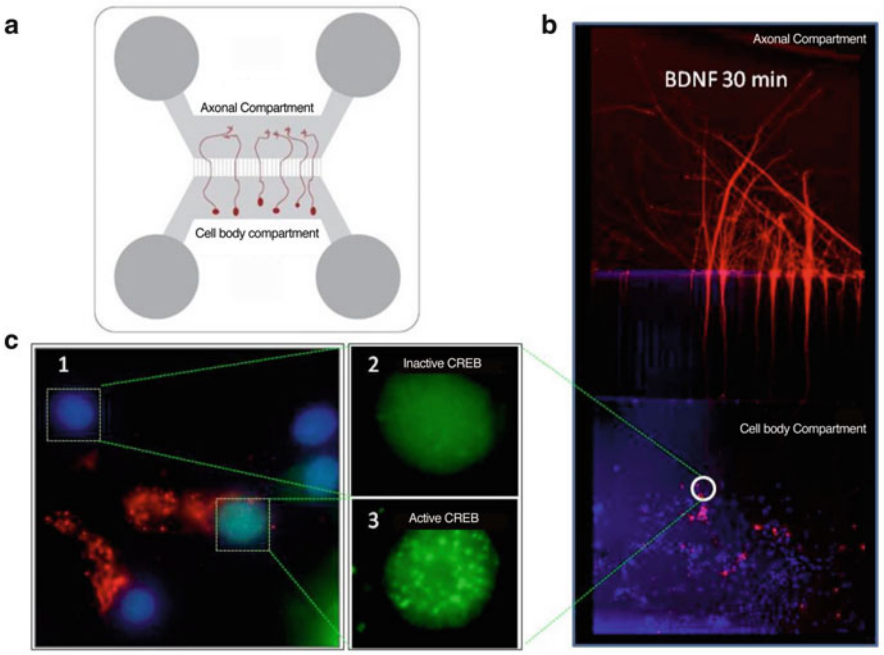

Fig. 6 Retrograde activation of CREB. (**a**) Scheme of a compartmentalized culture of cortical neurons. (**b**) Cortical neurons were cultured in microfluidic chambers and the axons were retrogradely labeled using a fluorescent (Alexa-555) subunit B of the cholera toxin (CTX). CTX was added only in the axonal compartment for 6 h, time that was enough to label only the somas of neurons that have crossed axons to the axonal compartment. Later, the axons were treated with BDNF for 30 min and the cultures were washed and fixed and the neuronal cell body compartment treated with an antibody to label pCREB. Neurons without axons in the axonal compartment are not labeled by CTX. Nucleus was labeled with Hoechst staining (shown in *blue*). (**c**) The image of neuronal cell bodies showed in **b** with a *circle* was magnified and the nuclear staining of pCREB is appreciated only in neurons retrogradely labeled with CTX

and coworkers (Heerssen et al. 2004; Riccio et al. 1997, 1999; Watson et al. 1999, 2001; Yano et al. 2001). These results, together with the isolation of endosomes derived from sciatic nerve axoplasm containing activated TrkA, p75, phospho-ERK1/2, PI3-K, phospho-p38, and Rap1 (Delcroix et al. 2003), led to the signaling endosome hypothesis, which postulated that after binding to neurotrophins in the synaptic terminal, activated Trks are internalized in endosomes that contain signaling molecules. These endosomes are retrograde transported back to the cell body in a dynein-dependent manner. Upon arrival to the cell body, signaling endosomes are expected to trigger nuclear responses (Heerssen and Segal 2002; Howe and Mobley 2004). Recent evidence has shown that, in central neurons, TrkB elicits a retrograde signaling that leads to CREB activation and an increase in dendritic ramification (Fig. 6) (Zhou et al. 2012). This retrograde response is mediated by the snapin

recruitment of dynein to TrkB signaling endosomes supporting the role of signaling endosomes in the retrograde signaling of peripheral and central neurons.

Other mechanisms have been postulated for the propagation of neurotrophin signaling along the axon, including the "wave propagation model" and the "retrograde effector model" (Bronfman and Kapon 2007; Howe and Mobley 2004). Additionally, Campenot's group has suggested that there might be signaling endosome-independent pathways of retrograde signaling because, using compartmentalized cultures of sympathetic neurons, they found that the addition of NGF to axonal terminals induces increased activation of TrkA in the cell body 1 min after NGF addition to axons and much earlier than the arrival of NGF-associated vesicles. However, these researchers have not yet provided a molecular mechanism for their findings, and a mechanism that has gained the most substantial support though experimental validation is the "signaling endosome model" (MacInnis and Campenot 2002; Senger and Campenot 1997; Ye et al. 2003). An interesting alternative to the signaling endosome hypothesis is that activated signaling complexes, such as ERKs, could undergo retrograde travel in an endosome-independent manner associated directly with dynein. Macromolecular complexes of ERK1/2 in association with locally synthesized importin, vimentin, and dynein have been described in sciatic nerves under injury conditions, supporting the existence of non-vesicular transport of activated signaling molecules (Hanz et al. 2003; Perlson et al. 2005).

Another controversial issue in this field is the nature of the transport organelle that carries retrograde neurotrophin signaling. Mobley and collaborators reported characterizing an early endosomal fraction (positive for Rab5 and EEA1) derived from sciatic nerve axoplasm where there are activated TrkA receptors and activated signaling molecules, such as Erk1/2, p38, and Akt. They have also provided evidence from electron microscopy and double immunostaining that the Rab5 GTPase co-localizes with activated TrkA and retrograde-transported NGF in axons. Another report also associates Rab5 with an axonal retrograde organelle formed by the Pincher chaperone. Intriguingly, the organelle is a multivesicular endosome/body (MVB) positive for Rab5 (Philippidou et al. 2011).

Multivesicular endosomes/bodies (MVBs) are organelles of the early-late endocytic pathway that sort endocytosed proteins to different destinations. Many lysosomally directed membrane proteins are sorted onto intraluminal vesicles, while recycling proteins remain on the perimeter membrane, from which they are removed via tubular extensions. Rab5–Rab7 conversion and the resulting change in the repertoire of Rab effector proteins on the endosome membrane mark the final progression of an MVB to a fusion-competent state, in which it can fuse with lysosomes. In the case of non-polarized cells in culture, MVBs are moved to the cell center during this maturation process by cytoplasmic dynein. An elegant study carried by Schiavo and collaborators provided evidence that the Rab5 GTPase is important for sorting to the retrograde transport pathway of vesicles. However, they found that it was the Rab7 GTPase, a classical marker of late endosomes, necessary for the retrograde transport of an organelle positive for p75 and TrkB in motor neurons. It is known that the Rab7 effector RIPL links Rab7-positive organelles to

dynein and to the subsequent movement of MBVs to the minus end of microtubules close to the perinuclear region of cells in culture. It is possible that Rab7 effectors are specifically distributed along the axon and that they play the role of linking transport carriers to dynein-mediated transport, rather than acting in late endosomes fusion. It has been described that there is a small proportion of NGF bound to axon terminals in primary cultures of sympathetic neurons that is actually retrograde transported and that a proportion of the NGF is recycled in the synaptic terminal (Deinhardt et al. 2006; Tsui-Pierchala and Ginty 1999; Ure and Campenot 1997; Weible et al. 2001). Thus, we can envision a model in which receptors are recycled in the synaptic terminal, and only a small proportion of the receptors are able to become active and recruit active Rab5. Rab5-tagged endosomes are sorted to retrograde pathways, and similar to the Rab5–Rab7 conversion that occurs in the cell body leading to maturation of late endosomes, there would be a conversion of Rab5 to Rab7 for retrograde transport. Therefore, it is possible that retrograde-transported vesicles are a heterogeneous group of vesicles with different degrees of Rab5/Rab7 loads that are not necessary multivesicular bodies since it has been described that MVBs are not frequently found in axons and that radiolabeled neurotrophins are often found with simple and small organelles more reminiscent than early endosomes (Fig. 7) (Altick et al. 2009).

It is of note that Rab5, Rab7, and Rab11 GTPases have all been functionally linked to dynein-mediated transport, and it is known that there are different dynein isoforms. Therefore, it is possible that each GTPase regulates the dynamics of different dynein isoforms. Additional research will be required to understand the heterogeneity of different retrograde-transported signaling organelles and the molecular machinery that generates them and regulates their transport (Horgan et al. 2010; Loubery et al. 2008; Satoh et al. 2008; Tan et al. 2011).

2.5 Retrograde Signaling and the Development of Proper Connectivity

Postganglionic sympathetic neurons have long served as a good model to study the molecular events underlying neuronal survival, axon growth, and the elaboration of dendrites in the PNS. These neurons express the TrkA and p75 receptors, and both NGF and NT3 are required for sympathetic nervous system development. It has been shown that during development of sympathetic axons, NT3 and NGF are required for proper axonal growth. While NT3 is required for the local growth of sympathetic axons through their local targets, such as the vasculature, NGF is required for final target innervation (for example, of the heart). This differential response is achieved by NT3 inducing a local response through the activation of TrkA in the growth cone, while NGF induces retrograde signaling that induces survival in the cell body, dependent on the activity of the CREB transcription factor. Different mechanisms have been developed by sympathetic neurons to increase their sensitivity to NGF once TrkA-NGF-mediated retrograde signaling has been initiated. NGF-TrkA retrograde signaling induces transcytosis of cell

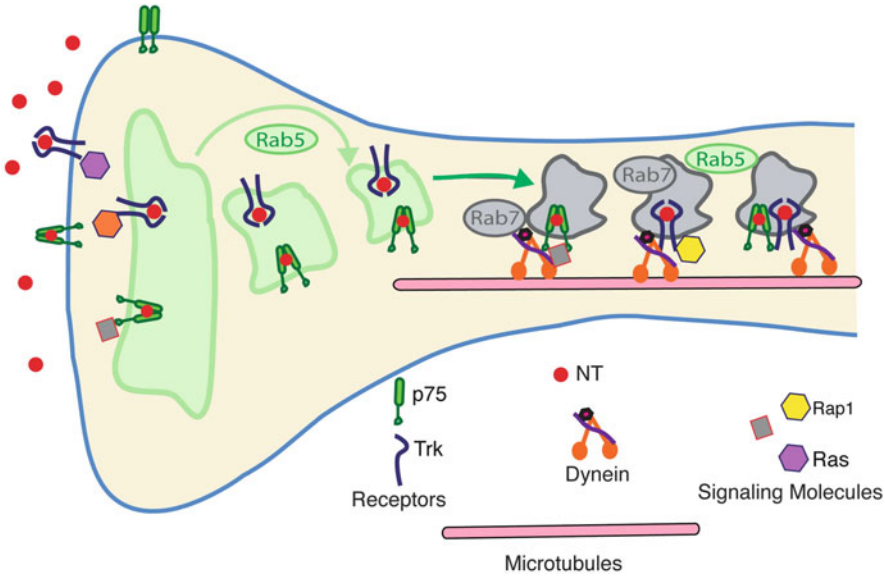

Fig. 7 Signaling endosomes in the axon. In response to neurotrophins (NTs) the neurotrophin receptors are internalized and a proportion of them are sorted to the dynein-dependent retrograde transport. Endosomes positive for p75, Trks only, or p75 and Trks are transported in the axon. The Rabs GTPases Rab5 and Rab7 are involved in the sorting and retrograde transport of potential signaling endosomes. Retrograde killing and survival signals are induced by p75 and Trks activation in distal axons, respectively. Several evidences indicate that these endosomes are associated with signaling molecules (see text for references)

body-associated TrkA receptors to the presynaptic terminal in a Rab11-dependent mechanism. The transcytosis of TrkA to axons results in increased sensitivity to NGF. Additionally, NGF-TrkA-mediated responses induce increased expression of TrkA and p75. p75 increases the specificity of TrkA binding to neurotrophins; thus, in the presence of p75, NT3 no longer binds TrkA. Another effect of NGF retrograde signaling is the increased expression of BDNF and NT4 that through the p75 receptor activate cell death signaling in neighboring neurons associated with low levels of TrkA-NGF signaling (Ascano et al. 2009; Deppmann et al. 2008; Kuruvilla et al. 2004). Therefore, there are at least three different positive feed-forward loops that increase sensitivity to NGF in sympathetic neurons and transform it into a "winner" in the competition for survival.

Contrary to what has been reported in PC12 cells regarding the p75 regulation of TrkA internalization (Geetha et al. 2005; Makkerh et al. 2005), p75 has no effect on the retrograde transport of TrkA signaling during neurotrophin-mediated target innervation of sympathetic neurons (Kuruvilla et al. 2004). However, it has been reported that p75 is internalized in the cell bodies and axons of sympathetic neurons through a mechanism that is partially dependent on clathrin coats and cholesterol. Additionally, internalized p75/BDNF vesicles appear more slowly than TrkA vesicles, suggesting (as observed in PC12 cells) that different populations of

vesicles are formed and transported in response to the binding of neurotrophins to p75 or TrkA (Bronfman et al. 2003; Hibbert et al. 2006). The significance of the retrograde transport of p75 for p75 signaling is largely unknown. It was recently reported that proNT3 is able to induce retrograde killing in sympathetic compartmentalized cultures when applied to axons (Yano et al. 2009), and we have found that BDNF is able to induce retrograde killing in compartmentalized cultures of sympathetic neurons in a dynein and c-Jun-amino-terminal kinase (JNK)-dependent manner (Escudero et al. unpoblished work). Therefore, it is possible that BDNF accumulates in DCVs in sympathetic synaptic terminals and it is secreted in an activity-dependent manner by the neurons that have established synaptic contacts and exhibit high levels of NGF-TrkA signaling. In the growth cone of neurons with low levels of TrkA-NGF signaling, BDNF through binding to p75 may induce retrograde killing of sympathetic neurons (Mok et al. 2009). A retrograde killing for neurotrophin withdrawal has also been recently described for sympathetic neurons. Determining whether this process is p75 dependent will require further research.

A standing question has been why the activation of TrkA by NT3 only induces local axonal growth while NGF induces the internalization of TrkA and retrograde signaling. Kuruvilla and Ginty and collaborators have recently provided the answer to this question proposing two different, but not mutually exclusive, explanations; NGF, but not NT3, promotes the endocytosis of TrkA through the calcineurin-mediated dephosphorylation of the endocytic GTPase dynamin1. NGF is able to induce specific dephosphorylation of dynamin1, increasing the internalization of TrkA and, thus, the retrograde transport associated with survival signaling, while the interaction of NT3 with TrkA does not have the same effect. Consistently, conditional deletion of *calcineurin* in sympathetic neurons disrupts NGF-dependent innervation of peripheral target tissues (Bodmer et al. 2011). On the other hand, NGF and not NT3 is able to activate, in endosomes, the actin regulators Rac1-GTP-cofilin, enabling the NGF/TrkA signaling endosomes to "escape" the actin network for retrograde transport (Harrington et al. 2011).

Another less explored mechanism for neurotrophin-induced axonal elongation is control of the local translation of axonal mRNAs. A recent study from Riccio and collaborators combined compartmentalized cultures of rat sympathetic neurons and sequential analysis of gene expression (SAGE) to analyze the mRNA content of sympathetic axons. Their screen yielded more than 11,000 tags in axons that matched known transcripts. Bioinformatics analysis revealed that many transcripts were highly enriched in axons compared to cell bodies, indicating that the accumulation of specific mRNAs in axons depends on active transport. The most abundant transcript in axons was myo-inositol monophosphatase-1 (Impa1), a key enzyme that regulates the inositol cycle and the main target of lithium in neurons. A novel localization element within the 3′ untranslated region of Impa1 mRNA was found to specifically target Impa1 transcripts to sympathetic neuron axons and regulate local Impa1 translation in response to axonally supplied NGF. Reduction of NGF-induced Impa1 synthesis in axons was observed to decrease nuclear CREB activation and induce axonal degeneration. Related findings have been reported by

the Twiss group in regenerating axons of adult sensory neurons grown in compartmentalized cultures. In their system, neurotrophins have been found to selectively increase the transport of specific RNAs to axons (Andreassi et al. 2010; Willis et al. 2007). Thus, target-derived NGF induces axonal elongation by cytoskeleton remodeling and axonal translation of proteins. Additionally, NGF-TrkA retrograde signaling in the cell body changes the repertoire of mRNAs that are anterograde transported to the synaptic terminal to increase target innervation and, thus, neuronal survival.

One interesting concept has emerged from the groups of Ginty and Segal and collaborators. In addition to regulating the number of neuronal cells that survive, long-distance neurotrophin signaling might regulate the degree of connectivity with preganglionic sympathetic neurons located in the central nervous system. In sympathetic neurons, NGF–TrkA signaling endosomes travel from distal axons to cell bodies and dendrites, where they promote postsynaptic density (PSD) formation. The presence of p75 restricts PSD formation, suggesting an important role for antagonistic NGF–TrkA and p75 signaling pathways during retrograde control of synapse establishment. A similar model has been suggested for sensory neurons, in which BDNF and NGF retrograde signaling induce the activation of ERK5 in the axon and cell body and the transcription factor MEF2D. MEF2-dependent transcriptional programs, in addition to inducing survival through the upregulation of the anti-apoptotic bcl-2 family member bcl-w, are also critical for establishing synaptic morphology and for regulating synapse numbers (Flavell et al. 2006; Pazyra-Murphy et al. 2009; Shalizi et al. 2006; Sharma et al. 2010).

In conclusion, proper connectivity of the nervous system is achieved by coordinating signal transduction with intracellular processes, such as secretion, endocytosis, and molecular motor transport, together with the local translation of localized mRNA in distal neuronal processes. To understand the molecular mechanisms that regulate these events through extracellular molecules (i.e., neurotrophins) will be useful to understand how the nervous system works and will provide better insight into how perturbation of intracellular trafficking may lead to neurodegenerative diseases.

Acknowledgments The authors gratefully acknowledge financial support from CARE (CONICYT PFB12/2007), FONDECYT 1085273 and 1120146 (FB) FONDECYT 3100076 (CF), and Proyecto Núcleo Milenio (MINREB) P07/011-F.

References

Aid T, Kazantseva A, Piirsoo M, Palm K, Timmusk T (2007) Mouse and rat BDNF gene structure and expression revisited. J Neurosci Res 85:525–535

Altar CA, DiStefano PS (1998) Neurotrophin trafficking by anterograde transport. Trends Neurosci 21:433–437

Altick AL, Baryshnikova LM, Vu TQ, von Bartheld CS (2009) Quantitative analysis of multivesicular bodies (MVBs) in the hypoglossal nerve: evidence that neurotrophic factors do not use MVBs for retrograde axonal transport. J Comp Neurol 514:641–657

An JJ, Gharami K, Liao GY, Woo NH, Lau AG et al (2008) Distinct role of long 3′ UTR BDNF mRNA in spine morphology and synaptic plasticity in hippocampal neurons. Cell 134:175–187
Andreassi C, Zimmermann C, Mitter R, Fusco S, De Vita S et al (2010) An NGF-responsive element targets myo-inositol monophosphatase-1 mRNA to sympathetic neuron axons. Nat Neurosci 13:291–301
Arevalo JC, Yano H, Teng KK, Chao MV (2004) A unique pathway for sustained neurotrophin signaling through an ankyrin-rich membrane-spanning protein. EMBO J 23:2358–2368
Arevalo JC, Waite J, Rajagopal R, Beyna M, Chen ZY et al (2006) Cell survival through Trk neurotrophin receptors is differentially regulated by ubiquitination. Neuron 50:549–559
Arimura N, Kimura T, Nakamuta S, Taya S, Funahashi Y et al (2009) Anterograde transport of TrkB in axons is mediated by direct interaction with Slp1 and Rab27. Dev Cell 16:675–686
Ascano M, Richmond A, Borden P, Kuruvilla R (2009) Axonal targeting of Trk receptors via transcytosis regulates sensitivity to neurotrophin responses. J Neurosci 29:11674–11685
Assaife-Lopes N, Sousa VC, Pereira DB, Ribeiro JA, Chao MV, Sebastiao AM (2010) Activation of adenosine A2A receptors induces TrkB translocation and increases BDNF-mediated phospho-TrkB localization in lipid rafts: implications for neuromodulation. J Neurosci 30:8468–8480
Bagayogo IP, Dreyfus CF (2009) Regulated release of BDNF by cortical oligodendrocytes is mediated through metabotropic glutamate receptors and the PLC pathway. ASN Neuro 1(1): pii: e00001
Barker PA (2004) p75NTR is positively promiscuous: novel partners and new insights. Neuron 42:529–533
BasuRay S, Mukherjee S, Romero E, Wilson MC, Wandinger-Ness A (2010) Rab7 mutants associated with Charcot-Marie-Tooth disease exhibit enhanced NGF-stimulated signaling. PLoS One 5:e15351
Bath KG, Lee FS (2006) Variant BDNF (Val66Met) impact on brain structure and function. Cogn Affect Behav Neurosci 6:79–85
Beattie EC, Howe CL, Wilde A, Brodsky FM, Mobley WC (2000) NGF signals through TrkA to increase clathrin at the plasma membrane and enhance clathrin-mediated membrane trafficking. J Neurosci 20:7325–7333
Bessis A, Bechade C, Bernard D, Roumier A (2007) Microglial control of neuronal death and synaptic properties. Glia 55:233–238
Bhattacharyya A, Watson FL, Bradlee TA, Pomeroy SL, Stiles CD, Segal RA (1997) Trk receptors function as rapid retrograde signal carriers in the adult nervous system. J Neurosci 17:7007–7016
Bibel M, Barde YA (2000) Neurotrophins: key regulators of cell fate and cell shape in the vertebrate nervous system. Genes Dev 14:2919–2937
Bilsland LG, Sahai E, Kelly G, Golding M, Greensmith L, Schiavo G (2010) Deficits in axonal transport precede ALS symptoms in vivo. Proc Natl Acad Sci U S A 107:20523–20528
Bodmer D, Ascano M, Kuruvilla R (2011) Isoform-specific dephosphorylation of dynamin1 by calcineurin couples neurotrophin receptor endocytosis to axonal growth. Neuron 70:1085–1099
Bonifacino JS, Traub LM (2003) Signals for sorting of transmembrane proteins to endosomes and lysosomes. Annu Rev Biochem 72:395–447
Brigadski T, Hartmann M, Lessmann V (2005) Differential vesicular targeting and time course of synaptic secretion of the mammalian neurotrophins. J Neurosci 25:7601–7614
Bronfman FC (2007) Metalloproteases and gamma-secretase: new membrane partners regulating p75 neurotrophin receptor signaling? J Neurochem 103(Suppl 1):91–100
Bronfman FC, Fainzilber M (2004) Multi-tasking by the p75 neurotrophin receptor: sortilin things out? EMBO Rep 5:867–871
Bronfman FC, Kapon R (2007) Commuting within the cell-mind the GAPs. Workshop on Systems Dynamics of Intracellular Communication: overcoming Distance in Signalling Networks. EMBO Rep 8:1011–1015

Bronfman FC, Tcherpakov M, Jovin TM, Fainzilber M (2003) Ligand-induced internalization of the p75 neurotrophin receptor: a slow route to the signaling endosome. J Neurosci 23:3209–3220

Bronfman FC, Escudero CA, Weis J, Kruttgen A (2007) Endosomal transport of neurotrophins: roles in signaling and neurodegenerative diseases. Dev Neurobiol 67:1183–1203

Bucci C, Parton RG, Mather IH, Stunnenberg H, Simons K et al (1992) The small GTPase rab5 functions as a regulatory factor in the early endocytic pathway. Cell 70:715–728

Bucci C, Thomsen P, Nicoziani P, McCarthy J, van Deurs B (2000) Rab7: a key to lysosome biogenesis. Mol Biol Cell 11:467–480

Butowt R, von Bartheld CS (2001) Sorting of internalized neurotrophins into an endocytic transcytosis pathway via the Golgi system: ultrastructural analysis in retinal ganglion cells. J Neurosci 21:8915–8930

Cabeza C, Figueroa A, Lazo OM, Galleguillos C, Pissani C et al (2012) Cholinergic abnormalities, endosomal alterations and up-regulation of nerve growth factor signaling in Niemann-Pick type C disease. Mol Neurodegener 7:11

Cao L, Zhu YL, Su Z, Lv B, Huang Z et al (2007) Olfactory ensheathing cells promote migration of Schwann cells by secreted nerve growth factor. Glia 55:897–904

Cavalli V, Corti M, Gruenberg J (2001) Endocytosis and signaling cascades: a close encounter. FEBS Lett 498:190–196

Chen ZY, Patel PD, Sant G, Meng CX, Teng KK et al (2004) Variant brain-derived neurotrophic factor (BDNF) (Met66) alters the intracellular trafficking and activity-dependent secretion of wild-type BDNF in neurosecretory cells and cortical neurons. J Neurosci 24:4401–4411

Chen ZY, Ieraci A, Tanowitz M, Lee FS (2005a) A novel endocytic recycling signal distinguishes biological responses of Trk neurotrophin receptors. Mol Biol Cell 16:5761–5772

Chen ZY, Ieraci A, Teng H, Dall H, Meng CX et al (2005b) Sortilin controls intracellular sorting of brain-derived neurotrophic factor to the regulated secretory pathway. J Neurosci 25:6156–6166

Chen ZY, Jing D, Bath KG, Ieraci A, Khan T et al (2006) Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. Science 314:140–143

Chiaruttini C, Vicario A, Li Z, Baj G, Braiuca P et al (2009) Dendritic trafficking of BDNF mRNA is mediated by translin and blocked by the G196A (Val66Met) mutation. Proc Natl Acad Sci U S A 106:16481–16486

Cogli L, Progida C, Lecci R, Bramato R, Kruttgen A, Bucci C (2010) CMT2B-associated Rab7 mutants inhibit neurite outgrowth. Acta Neuropathol 120:491–501

Cohen-Cory S, Kidane AH, Shirkey NJ, Marshak S (2010) Brain-derived neurotrophic factor and the development of structural neuronal connectivity. Dev Neurobiol 70:271–288

Cuitino L, Matute R, Retamal C, Bu G, Inestrosa NC, Marzolo MP (2005) ApoER2 is endocytosed by a clathrin-mediated process involving the adaptor protein Dab2 independent of its Rafts' association. Traffic 6:820–838

Dai X, Qu P, Dreyfus CF (2001) Neuronal signals regulate neurotrophin expression in oligodendrocytes of the basal forebrain. Glia 34:234–239

de Melker AA, van der Horst G, Calafat J, Jansen H, Borst J (2001) c-Cbl ubiquitinates the EGF receptor at the plasma membrane and remains receptor associated throughout the endocytic route. J Cell Sci 114:2167–2178

Deinhardt K, Reversi A, Berninghausen O, Hopkins CR, Schiavo G (2007) Neurotrophins Redirect p75NTR from a clathrin-independent to a clathrin-dependent endocytic pathway coupled to axonal transport. Traffic 8:1736–1749

Deinhardt K, Salinas S, Verastegui C, Watson R, Worth D, Hanrahan S, Bucci C, Schiavo G. (2006) Rab5 and Rab7 control endocytic sorting along the retrograde transport pathway. Neuron 19;52(2):293–305

Delcroix JD, Valletta JS, Wu C, Hunt SJ, Kowal AS, Mobley WC (2003) NGF signaling in sensory neurons: evidence that early endosomes carry NGF retrograde signals. Neuron 39:69–84

Deppmann CD, Mihalas S, Sharma N, Lonze BE, Niebur E, Ginty DD (2008) A model for neuronal competition during development. Science 320:369–373

Devon RS, Orban PC, Gerrow K, Barbieri MA, Schwab C et al (2006) Als2-deficient mice exhibit disturbances in endosome trafficking associated with motor behavioral abnormalities. Proc Natl Acad Sci U S A 103:9595–9600

Di Fiore PP, De Camilli P (2001) Endocytosis and signaling. An inseparable partnership. Cell 106:1–4

Doherty GJ, McMahon HT (2009) Mechanisms of endocytosis. Annu Rev Biochem 78:857–902

Ehlers MD, Kaplan DR, Price DL, Koliatsos VE (1995) NGF-stimulated retrograde transport of trkA in the mammalian nervous system. J Cell Biol 130:149–156

Falley K, Schutt J, Iglauer P, Menke K, Maas C et al (2009) Shank1 mRNA: dendritic transport by kinesin and translational control by the 5′untranslated region. Traffic 10:844–857

Flavell SW, Cowan CW, Kim TK, Greer PL, Lin Y et al (2006) Activity-dependent regulation of MEF2 transcription factors suppresses excitatory synapse number. Science 311:1008–1012

Fujitani M, Kawai H, Proia RL, Kashiwagi A, Yasuda H, Yamashita T (2005) Binding of soluble myelin-associated glycoprotein to specific gangliosides induces the association of p75NTR to lipid rafts and signal transduction. J Neurochem 94:15–21

Fukuda M (2008) Regulation of secretory vesicle traffic by Rab small GTPases. Cell Mol Life Sci 65:2801–2813

Gauthier LR, Charrin BC, Borrell-Pages M, Dompierre JP, Rangone H et al (2004) Huntingtin controls neurotrophic support and survival of neurons by enhancing BDNF vesicular transport along microtubules. Cell 118:127–138

Geetha T, Jiang J, Wooten MW (2005) Lysine 63 polyubiquitination of the nerve growth factor receptor TrkA directs internalization and signaling. Mol Cell 20:301–312

Gentry JJ, Barker PA, Carter BD (2004) The p75 neurotrophin receptor: multiple interactors and numerous functions. Prog Brain Res 146:25–39

Georgieva MV, de Pablo Y, Sanchis D, Comella JX, Llovera M (2011) Ubiquitination of TrkA by Nedd4-2 regulates receptor lysosomal targeting and mediates receptor signaling. J Neurochem 117:479–493

Ginsberg SD, Alldred MJ, Counts SE, Cataldo AM, Neve RL et al (2010) Microarray analysis of hippocampal CA1 neurons implicates early endosomal dysfunction during Alzheimer's disease progression. Biol Psychiatry 68:885–893

Glebova NO, Ginty DD (2005) Growth and survival signals controlling sympathetic nervous system development. Annu Rev Neurosci 28:191–222

Gomes RA, Hampton C, El-Sabeawy F, Sabo SL, McAllister AK (2006) The dynamic distribution of TrkB receptors before, during, and after synapse formation between cortical neurons. J Neurosci 26:11487–11500

Greenberg ME, Xu B, Lu B, Hempstead BL (2009) New insights in the biology of BDNF synthesis and release: implications in CNS function. J Neurosci 29:12764–12767

Greene LA, Tischler AS (1976) Establishment of a noradrenergic clonal line of ral adrenal pheochromocytoma cells which respond to nerve growth factor. Proc Natl Acad Sci USA 73 (7):2424–2428

Grimes ML, Zhou J, Beattie EC, Yuen EC, Hall DE et al (1996) Endocytosis of activated TrkA: evidence that nerve growth factor induces formation of signaling endosomes. J Neurosci 16:7950–7964

Grimes ML, Beattie E, Mobley WC (1997) A signaling organelle containing the nerve growth factor-activated receptor tyrosine kinase, TrkA. Proc Natl Acad Sci U S A 94:9909–9914

Haglund K, Shimokawa N, Szymkiewicz I, Dikic I (2002) Cbl-directed monoubiquitination of CIN85 is involved in regulation of ligand-induced degradation of EGF receptors. Proc Natl Acad Sci U S A 99:12191–12196

Hanz S, Perlson E, Willis D, Zheng JQ, Massarwa R et al (2003) Axoplasmic importins enable retrograde injury signaling in lesioned nerve. Neuron 40:1095–1104

Harrington AW, St Hillaire C, Zweifel LS, Glebova NO, Philippidou P et al (2011) Recruitment of actin modifiers to TrkA endosomes governs retrograde NGF signaling and survival. Cell 146:421–434

Hawryluk MJ, Keyel PA, Mishra SK, Watkins SC, Heuser JE, Traub LM (2006) Epsin 1 is a polyubiquitin-selective clathrin-associated sorting protein. Traffic 7:262–281
Heerssen HM, Segal RA (2002) Location, location, location: a spatial view of neurotrophin signal transduction. Trends Neurosci 25:160–165
Heerssen HM, Pazyra MF, Segal RA (2004) Dynein motors transport activated Trks to promote survival of target-dependent neurons. Nat Neurosci 7:596–604
Hendry IA, Stach R, Herrup K (1974a) Characteristics of the retrograde axonal transport system for nerve growth factor in the sympathetic nervous system. Brain Res 82:117–128
Hendry IA, Stockel K, Thoenen H, Iversen LL (1974b) The retrograde axonal transport of nerve growth factor. Brain Res 68:103–121
Hibbert AP, Kramer BM, Miller FD, Kaplan DR (2006) The localization, trafficking and retrograde transport of BDNF bound to p75NTR in sympathetic neurons. Mol Cell Neurosci 32:387–402
Higuchi H, Yamashita T, Yoshikawa H, Tohyama M (2003) PKA phosphorylates the p75 receptor and regulates its localization to lipid rafts. EMBO J 22:1790–1800
Hirokawa N (2006) mRNA transport in dendrites: RNA granules, motors, and tracks. J Neurosci 26:7139–7142
Hirokawa N, Noda Y, Tanaka Y, Niwa S (2009) Kinesin superfamily motor proteins and intracellular transport. Nat Rev Mol Cell Biol 10:682–696
Holzbaur EL (2004) Motor neurons rely on motor proteins. Trends Cell Biol 14:233–240
Hong EJ, McCord AE, Greenberg ME (2008) A biological function for the neuronal activity-dependent component of Bdnf transcription in the development of cortical inhibition. Neuron 60:610–624
Horgan CP, Hanscom SR, Jolly RS, Futter CE, McCaffrey MW (2010) Rab11-FIP3 links the Rab11 GTPase and cytoplasmic dynein to mediate transport to the endosomal-recycling compartment. J Cell Sci 123:181–191
Horton AC, Ehlers MD (2003a) Dual modes of endoplasmic reticulum-to-Golgi transport in dendrites revealed by live-cell imaging. J Neurosci 23:6188–6199
Horton AC, Ehlers MD (2003b) Neuronal polarity and trafficking. Neuron 40:277–295
Howe CL, Mobley WC (2004) Signaling endosome hypothesis: a cellular mechanism for long distance communication. J Neurobiol 58:207–216
Howe CL, Valletta JS, Rusnak AS, Mobley WC (2001) NGF signaling from clathrin-coated vesicles: evidence that signaling endosomes serve as a platform for the Ras-MAPK pathway. Neuron 32:801–814
Huang EJ, Reichardt LF (2001) Neurotrophins: roles in neuronal development and function. Annu Rev Neurosci 24:677–736
Huang EJ, Reichardt LF (2003) Trk receptors: roles in neuronal signal transduction. Annu Rev Biochem 72:609–642
Huang CS, Zhou J, Feng AK, Lynch CC, Klumperman J et al (1999) Nerve growth factor signaling in caveolae-like domains at the plasma membrane. J Biol Chem 274:36707–36714
Huang SH, Zhao L, Sun ZP, Li XZ, Geng Z et al (2009) Essential role of Hrs in endocytic recycling of full-length TrkB receptor but not its isoform TrkB.T1. J Biol Chem 284:15126–15136
Ibanez CF (2007) Message in a bottle: long-range retrograde signaling in the nervous system. Trends Cell Biol 17:519–528
Joset A, Dodd DA, Halegoua S, Schwab ME (2010) Pincher-generated Nogo-A endosomes mediate growth cone collapse and retrograde signaling. J Cell Biol 188:271–285
Jullien J, Guili V, Derrington EA, Darlix JL, Reichardt LF, Rudkin BB (2003) Trafficking of TrkA-green fluorescent protein chimerae during nerve growth factor-induced differentiation. J Biol Chem 278:8706–8716
Kanning KC, Hudson M, Amieux PS, Wiley JC, Bothwell M, Schecterson LC (2003) Proteolytic processing of the p75 neurotrophin receptor and two homologs generates C-terminal fragments with signaling capability. J Neurosci 23:5425–5436

Kao S, Jaiswal RK, Kolch W, Landreth GE (2001) Identification of the mechanisms regulating the differential activation of the mapk cascade by epidermal growth factor and nerve growth factor in PC12 cells. J Biol Chem 276:18169–18177

Kardon JR, Vale RD (2009) Regulators of the cytoplasmic dynein motor. Nat Rev Mol Cell Biol 10:854–865

Kenchappa RS, Zampieri N, Chao MV, Barker PA, Teng HK et al (2006) Ligand-dependent cleavage of the P75 neurotrophin receptor is necessary for NRIF nuclear translocation and apoptosis in sympathetic neurons. Neuron 50:219–232

Kirkham M, Parton RG (2005) Clathrin-independent endocytosis: new insights into caveolae and non-caveolar lipid raft carriers. Biochim Biophys Acta 1745:273–286

Korsching S (1993) The neurotrophic factor concept: a reexamination. J Neurosci 13:2739–2748

Kuruvilla R, Zweifel LS, Glebova NO, Lonze BE, Valdez G et al (2004) A neurotrophin signaling cascade coordinates sympathetic neuron development through differential control of TrkA trafficking and retrograde signaling. Cell 118:243–255

Lazo OM, Gonzalez A, Ascaño M, Kuruvilla R, Couve A, Bronfman FC (2013) BDNF regulates Rab11-mediated recycling endosome dynamics to induce dendritic branching. J Neurosci 33 (14):6112–6122

Lessmann V, Gottmann K, Malcangio M (2003) Neurotrophin secretion: current facts and future prospects. Prog Neurobiol 69:341–374

Levi-Montalcini R (1966) The nerve growth factor: its mode of action on sensory and sympathetic nerve cells. Harvey Lect 60:217–259

Levi-Montalcini R (1987) The nerve growth factor 35 years later. Science 237:1154–1162

Li X, Standley C, Sapp E, Valencia A, Qin ZH et al (2009) Mutant huntingtin impairs vesicle formation from recycling endosomes by interfering with Rab11 activity. Mol Cell Biol 29:6106–6116

Limpert AS, Karlo JC, Landreth GE (2007) Nerve growth factor stimulates the concentration of TrkA within lipid rafts and extracellular signal-regulated kinase activation through c-Cbl-associated protein. Mol Cell Biol 27:5686–5698

Lin DC, Quevedo C, Brewer NE, Bell A, Testa JR et al (2006) APPL1 associates with TrkA and GIPC1 and is required for nerve growth factor-mediated signal transduction. Mol Cell Biol 26:8928–8941

Liu J, Lamb D, Chou MM, Liu YJ, Li G (2007) Nerve growth factor-mediated neurite outgrowth via regulation of Rab5. Mol Biol Cell 18:1375–1384

Lo KY, Kuzmin A, Unger SM, Petersen JD, Silverman MA (2011) KIF1A is the primary anterograde motor protein required for the axonal transport of dense-core vesicles in cultured hippocampal neurons. Neurosci Lett 491:168–173

Loubery S, Wilhelm C, Hurbain I, Neveu S, Louvard D, Coudrier E (2008) Different microtubule motors move early and late endocytic compartments. Traffic 9:492–509

Lu B, Pang PT, Woo NH (2005) The yin and yang of neurotrophin action. Nat Rev Neurosci 6:603–614

MacInnis BL, Campenot RB (2002) Retrograde support of neuronal survival without retrograde transport of nerve growth factor. Science 295:1536–1539

Makkerh JP, Ceni C, Auld DS, Vaillancourt F, Dorval G, Barker PA (2005) p75 neurotrophin receptor reduces ligand-induced Trk receptor ubiquitination and delays Trk receptor internalization and degradation. EMBO Rep 6:936–941

Marmor MD, Yarden Y (2004) Role of protein ubiquitylation in regulating endocytosis of receptor tyrosine kinases. Oncogene 23:2057–2070

Matsumoto T, Rauskolb S, Polack M, Klose J, Kolbeck R et al (2008) Biosynthesis and processing of endogenous BDNF: CNS neurons store and secrete BDNF, not pro-BDNF. Nat Neurosci 11:131–133

Matsuoka I, Meyer M, Thoenen H (1991) Cell-type-specific regulation of nerve growth factor (NGF) synthesis in non-neuronal cells: comparison of Schwann cells with other cell types. J Neurosci 11:3165–3177

Mayor S, Pagano RE (2007) Pathways of clathrin-independent endocytosis. Nat Rev Mol Cell Biol 8:603–612

McCaffrey G, Welker J, Scott J, der Salm L, Grimes ML (2009) High-resolution fractionation of signaling endosomes containing different receptors. Traffic 10:938–950

Miaczynska M, Christoforidis S, Giner A, Shevchenko A, Uttenweiler-Joseph S et al (2004a) APPL proteins link Rab5 to nuclear signal transduction via an endosomal compartment. Cell 116:445–456

Miaczynska M, Pelkmans L, Zerial M (2004b) Not just a sink: endosomes in control of signal transduction. Curr Opin Cell Biol 16:400–406

Mochizuki N, Yamashita S, Kurokawa K, Ohba Y, Nagai T et al (2001) Spatio-temporal images of growth-factor-induced activation of Ras and Rap1. Nature 411:1065–1068

Mok SA, Lund K, Campenot RB (2009) A retrograde apoptotic signal originating in NGF-deprived distal axons of rat sympathetic neurons in compartmented cultures. Cell Res 19:546–560

Morris SM, Tallquist MD, Rock CO, Cooper JA (2002) Dual roles for the Dab2 adaptor protein in embryonic development and kidney transport. EMBO J 21:1555–1564

Mufson EJ, Kroin JS, Sendera TJ, Sobreviela T (1999) Distribution and retrograde transport of trophic factors in the central nervous system: functional implications for the treatment of neurodegenerative diseases. Prog Neurobiol 57:451–484

Nagappan G, Zaitsev E, Senatorov VV Jr, Yang J, Hempstead BL, Lu B (2009) Control of extracellular cleavage of ProBDNF by high frequency neuronal activity. Proc Natl Acad Sci U S A 106:1267–1272

Ng BK, Chen L, Mandemakers W, Cosgaya JM, Chan JR (2007) Anterograde transport and secretion of brain-derived neurotrophic factor along sensory axons promote Schwann cell myelination. J Neurosci 27:7597–7603

Nishio M, Fukumoto S, Furukawa K, Ichimura A, Miyazaki H et al (2004) Overexpressed GM1 suppresses nerve growth factor (NGF) signals by modulating the intracellular localization of NGF receptors and membrane fluidity in PC12 cells. J Biol Chem 279:33368–33378

Nomura K, Kanemura H, Satoh T, Kataoka T (2004) Identification of a novel domain of Ras and Rap1 that directs their differential subcellular localizations. J Biol Chem 279:22664–22673

Nykjaer A, Lee R, Teng KK, Jansen P, Madsen P et al (2004) Sortilin is essential for proNGF-induced neuronal cell death. Nature 427:843–848

Ohta K, Kuno S, Inoue S, Ikeda E, Fujinami A, Ohta M (2010) The effect of dopamine agonists: the expression of GDNF, NGF, and BDNF in cultured mouse astrocytes. J Neurol Sci 291:12–16

Pal A, Severin F, Lommer B, Shevchenko A, Zerial M (2006) Huntingtin-HAP40 complex is a novel Rab5 effector that regulates early endosome motility and is up-regulated in Huntington's disease. J Cell Biol 172:605–618

Pazyra-Murphy MF, Hans A, Courchesne SL, Karch C, Cosker KE et al (2009) A retrograde neuronal survival response: target-derived neurotrophins regulate MEF2D and bcl-w. J Neurosci 29:6700–6709

Pereira DB, Chao MV (2007) The tyrosine kinase Fyn determines the localization of TrkB receptors in lipid rafts. J Neurosci 27:4859–4869

Perlson E, Hanz S, Ben-Yaakov K, Segal-Ruder Y, Seger R, Fainzilber M (2005) Vimentin-dependent spatial translocation of an activated MAP kinase in injured nerve. Neuron 45:715–726

Perlson E, Maday S, Fu MM, Moughamian AJ, Holzbaur EL (2010) Retrograde axonal transport: pathways to cell death? Trends Neurosci 33:335–344

Pezawas L, Verchinski BA, Mattay VS, Callicott JH, Kolachana BS et al (2004) The brain-derived neurotrophic factor val66met polymorphism and variation in human cortical morphology. J Neurosci 24:10099–10102

Philippidou P, Valdez G, Akmentin W, Bowers WJ, Federoff HJ, Halegoua S (2011) Trk retrograde signaling requires persistent, Pincher-directed endosomes. Proc Natl Acad Sci U S A 108:852–857

Platta HW, Stenmark H (2011) Endocytosis and signaling. Curr Opin Cell Biol 23(4):393–403

Raju CS, Fukuda N, Lopez-Iglesias C, Goritz C, Visa N, Percipalle P (2011) In neurons, activity-dependent association of dendritically transported mRNA transcripts with the transacting factor CBF-A is mediated by A2RE/RTS elements. Mol Biol Cell 22(11):1864–1877

Reynolds AJ, Bartlett SE, Hendry IA (1998) Signalling events regulating the retrograde axonal transport of 125I-beta nerve growth factor in vivo. Brain Res 798:67–74

Riccio A, Pierchala BA, Ciarallo CL, Ginty DD (1997) An NGF-TrkA-mediated retrograde signal to transcription factor CREB in sympathetic neurons. Science 277:1097–1100

Riccio A, Ahn S, Davenport CM, Blendy JA, Ginty DD (1999) Mediation by a CREB family transcription factor of NGF-dependent survival of sympathetic neurons. Science 286:2358–2361

Rodriguez-Boulan E, Powell SK (1992) Polarity of epithelial and neuronal cells. Annu Rev Cell Biol 8:395–427

Salehi A, Delcroix JD, Belichenko PV, Zhan K, Wu C et al (2006) Increased App expression in a mouse model of Down's syndrome disrupts NGF transport and causes cholinergic neuron degeneration. Neuron 51:29–42

Salinas S, Schiavo G, Kremer EJ (2010) A hitchhiker's guide to the nervous system: the complex journey of viruses and toxins. Nat Rev Microbiol 8:645–655

Satoh D, Sato D, Tsuyama T, Saito M, Ohkura H et al (2008) Spatial control of branching within dendritic arbors by dynein-dependent transport of Rab5-endosomes. Nat Cell Biol 10:1164–1171

Saxena S, Howe CL, Cosgaya JM, Hu M, Weis J, Kruttgen A (2004) Differences in the surface binding and endocytosis of neurotrophins by p75NTR. Mol Cell Neurosci 26:292–307

Saxena S, Bucci C, Weis J, Kruttgen A (2005a) The small GTPase Rab7 controls the endosomal trafficking and neuritogenic signaling of the nerve growth factor receptor TrkA. J Neurosci 25:10930–10940

Saxena S, Howe CL, Cosgaya JM, Steiner P, Hirling H et al (2005b) Differential endocytic sorting of p75NTR and TrkA in response to NGF: a role for late endosomes in TrkA trafficking. Mol Cell Neurosci 28:571–587

Schinder AF, Poo M (2000) The neurotrophin hypothesis for synaptic plasticity. Trends Neurosci 23:639–645

Schonteich E, Wilson GM, Burden J, Hopkins CR, Anderson K et al (2008) The Rip11/Rab11-FIP5 and kinesin II complex regulates endocytic protein recycling. J Cell Sci 121:3824–3833

Senger DL, Campenot RB (1997) Rapid retrograde tyrosine phosphorylation of trkA and other proteins in rat sympathetic neurons in compartmented cultures. J Cell Biol 138:411–421

Shalizi A, Gaudilliere B, Yuan Z, Stegmuller J, Shirogane T et al (2006) A calcium-regulated MEF2 sumoylation switch controls postsynaptic differentiation. Science 311:1012–1017

Shao Y, Akmentin W, Toledo-Aral JJ, Rosenbaum J, Valdez G et al (2002) Pincher, a pinocytic chaperone for nerve growth factor/TrkA signaling endosomes. J Cell Biol 157:679–691

Sharma N, Deppmann CD, Harrington AW, St Hillaire C, Chen ZY et al (2010) Long-distance control of synapse assembly by target-derived NGF. Neuron 67:422–434

Shinoda Y, Sadakata T, Nakao K, Katoh-Semba R, Kinameri E et al (2011) Calcium-dependent activator protein for secretion 2 (CAPS2) promotes BDNF secretion and is critical for the development of GABAergic interneuron network. Proc Natl Acad Sci U S A 108:373–378

Slepnev VI, De Camilli P (2000) Accessory factors in clathrin-dependent synaptic vesicle endocytosis. Nat Rev Neurosci 1:161–172

Somsel Rodman J, Wandinger-Ness A (2000) Rab GTPases coordinate endocytosis. J Cell Sci 113 (Pt 2):183–192

Sonnichsen B, De Renzis S, Nielsen E, Rietdorf J, Zerial M (2000) Distinct membrane domains on endosomes in the recycling pathway visualized by multicolor imaging of Rab4, Rab5, and Rab11. J Cell Biol 149:901–914

Sorkin A (2004) Cargo recognition during clathrin-mediated endocytosis: a team effort. Curr Opin Cell Biol 16:392–399

Sorkin A, Von Zastrow M (2002) Signal transduction and endocytosis: close encounters of many kinds. Nat Rev Mol Cell Biol 3:600–614

Stenmark H (2009) Rab GTPases as coordinators of vesicle traffic. Nat Rev Mol Cell Biol 10:513–525

Tan SC, Scherer J, Vallee RB (2011) Recruitment of dynein to late endosomes and lysosomes through light intermediate chains. Mol Biol Cell 22:467–477

Tcherpakov M, Bronfman FC, Conticello SG, Vaskovsky A, Levy Z et al (2002) The p75 neurotrophin receptor interacts with multiple MAGE proteins. J Biol Chem 277:49101–49104

Tsui-Pierchala BA, Ginty DD (1999) Characterization of an NGF-P-TrkA retrograde-signaling complex and age-dependent regulation of TrkA phosphorylation in sympathetic neurons. J Neurosci 19:8207–8218

Ure DR, Campenot RB (1997) Retrograde transport and steady-state distribution of 125I-nerve growth factor in rat sympathetic neurons in compartmented cultures. J Neurosci 17:1282–1290

Urra S, Escudero CA, Ramos P, Lisbona F, Allende E et al (2007) TrkA receptor activation by nerve growth factor induces shedding of the p75 neurotrophin receptor followed by endosomal gamma-secretase-mediated release of the p75 intracellular domain. J Biol Chem 282:7606–7615

Vaegter CB, Jansen P, Fjorback AW, Glerup S, Skeldal S et al (2011) Sortilin associates with Trk receptors to enhance anterograde transport and neurotrophin signaling. Nat Neurosci 14:54–61

Valdez G, Akmentin W, Philippidou P, Kuruvilla R, Ginty DD, Halegoua S (2005) Pincher-mediated macroendocytosis underlies retrograde signaling by neurotrophin receptors. J Neurosci 25:5236–5247

Varsano T, Dong MQ, Niesman I, Gacula H, Lou X et al (2006) GIPC is recruited by APPL to peripheral TrkA endosomes and regulates TrkA trafficking and signaling. Mol Cell Biol 26:8942–8952

Verderio C, Bianco F, Blanchard MP, Bergami M, Canossa M et al (2006) Cross talk between vestibular neurons and Schwann cells mediates BDNF release and neuronal regeneration. Brain Cell Biol 35:187–201

Vilar M, Charalampopoulos I, Kenchappa RS, Simi A, Karaca E et al (2009) Activation of the p75 neurotrophin receptor through conformational rearrangement of disulphide-linked receptor dimers. Neuron 62:72–83

Watson FL, Heerssen HM, Moheban DB, Lin MZ, Sauvageot CM et al (1999) Rapid nuclear responses to target-derived neurotrophins require retrograde transport of ligand-receptor complex. J Neurosci 19:7889–7900

Watson FL, Heerssen HM, Bhattacharyya A, Klesse L, Lin MZ, Segal RA (2001) Neurotrophins use the Erk5 pathway to mediate a retrograde survival response. Nat Neurosci 4:981–988

Weible MW 2nd, Bartlett SE, Reynolds AJ, Hendry IA (2001) Prolonged recycling of internalized neurotrophins in the nerve terminal. Cytometry 43:182–188

Willis DE, van Niekerk EA, Sasaki Y, Mesngon M, Merianda TT et al (2007) Extracellular stimuli specifically regulate localized levels of individual neuronal mRNAs. J Cell Biol 178:965–980

Willnow TE, Petersen CM, Nykjaer A (2008) VPS10P-domain receptors – regulators of neuronal viability and function. Nat Rev Neurosci 9:899–909

Wu C, Lai CF, Mobley WC (2001) Nerve growth factor activates persistent Rap1 signaling in endosomes. J Neurosci 21:5406–5416

Wu C, Ramirez A, Cui B, Ding J, Delcroix JD et al (2007) A functional dynein-microtubule network is required for NGF signaling through the Rap1/MAPK pathway. Traffic 8:1503–1520

Yang J, Siao CJ, Nagappan G, Marinic T, Jing D et al (2009) Neuronal release of proBDNF. Nat Neurosci 12:113–115

Yano H, Lee FS, Kong H, Chuang J, Arevalo J et al (2001) Association of Trk neurotrophin receptors with components of the cytoplasmic dynein motor. J Neurosci 21:RC125

Yano H, Torkin R, Martin LA, Chao MV, Teng KK (2009) Proneurotrophin-3 is a neuronal apoptotic ligand: evidence for retrograde-directed cell killing. J Neurosci 29:14790–14802

Ye H, Kuruvilla R, Zweifel LS, Ginty DD (2003) Evidence in support of signaling endosome-based retrograde survival of sympathetic neurons. Neuron 39:57–68

York RD, Molliver DC, Grewal SS, Stenberg PE, McCleskey EW, Stork PJ (2000) Role of phosphoinositide 3-kinase and endocytosis in nerve growth factor-induced extracellular signal-regulated kinase activation via Ras and Rap1. Mol Cell Biol 20:8069–8083

Yune TY, Lee JY, Jung GY, Kim SJ, Jiang MH et al (2007) Minocycline alleviates death of oligodendrocytes by inhibiting pro-nerve growth factor production in microglia after spinal cord injury. J Neurosci 27:7751–7761

Zhang Y, Moheban DB, Conway BR, Bhattacharyya A, Segal RA (2000) Cell surface Trk receptors mediate NGF-induced survival while internalized receptors regulate NGF-induced differentiation. J Neurosci 20:5671–5678

Zheng J, Shen WH, Lu TJ, Zhou Y, Chen Q et al (2008) Clathrin-dependent endocytosis is required for TrkB-dependent Akt-mediated neuronal protection and dendritic growth. J Biol Chem 283:13280–13288

Zhou B, Cai Q, Xie Y, Sheng ZH (2012) Snapin recruits dynein to BDNF-TrkB signaling endosomes for retrograde axonal transport and is essential for dendrite growth of cortical neurons. Cell Rep 2:42–51

Zwang Y, Yarden Y (2009) Systems biology of growth factor-induced receptor endocytosis. Traffic 10:349–363