Published in final edited form as:
Int Rev Cell Mol Biol. 2015 ; 314: 239–257. doi:10.1016/bs.ircmb.2014.10.002.

Biogenesis and Function of the NGF/TrkA Signaling Endosome

M. Caleb Marlin and **Guangpu Li***
Department of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

Abstract

Target-derived neurotrophin nerve growth factor (NGF) and its receptor TrkA are well known for retrograde signaling to promote survival and innervation of sympathetic and sensory neurons. In recent years, the signaling endosome model has been used to describe the sustained NGF/TrkA retrograde signaling as a process of endocytosis and retrograde transport of NGF/TrkA-containing endosomes from the axon terminal to the cell body for activation of NGF-inducible gene expression responsible for neuronal survival and development. Here, we review the biogenesis and function of NGF, TrkA, and the signaling endosome and discuss possible roles of Rab GTPases in the biogenesis and trafficking of signaling endosomes.

1. INTRODUCTION

Neurons in both peripheral and central nervous systems are comprised of a cell body, the soma, and long projections, axons and dendrites. In the context of the mammalian body, these neurons are paramount to motor function, pain reception, cognitive processing, etc. The overall health of the whole organism is dependent upon proper differentiation, growth, development, and function of these and auxiliary cells associated with the peripheral and central nervous systems. The neuron itself is highly dependent upon proper neuron-related growth factor, or neurotrophin, stimulation and subsequent signal transduction for growth and development (Chao, 2003; Huang and Reichardt, 2003). The neuron also poses great challenges not seen in any other cell type due to axons that can exceed the length of one meter. Neurotrophic signaling begins at the distal axon terminal and must be trafficked to the soma for regulation of target gene expression (Harrington and Ginty, 2013). Therefore, prolonged trafficking of a liganded/ activated receptor along an extended axon presents a fascinating and complex issue to address.

The signaling endosome hypothesis has been proposed to explain the prolonged receptor activation and trafficking events (Howe and Mobley, 2004). In short, the signaling endosome is a long-lived endocytic compartment that contains neurotrophin-activated receptors and traverses the axon in order to promote appropriate spatial and temporal signaling events (Grimes et al., 1996). The discussion that follows examines both well-known and novel findings in the field that aim to highlight major advances in our

*Corresponding author: Guangpu-li@ouhsc.edu.

understanding of signaling endosomes, as well as work that must be continued in order to elucidate specific mechanisms. Though multiple neurotrophins exist, this review focuses on nerve growth factor (NGF) and its high affinity receptor TrkA.

The overall model begins with NGF binding to TrkA, causing its dimerization and activation. TrkA is present on the plasma membrane and binds to dimerized NGF in the extracellular environment. Signaling then begins across the plasma membrane and into the intracellular cytoplasm, through major signaling cascades including phospholipase C-γ (PLC-γ), mitogenactivated protein kinase (MAPK), and phosphatidylinositol 3-kinase (PI3K) pathways. The NGF/TrkA complex is internalized by either classical clathrin-mediated endocytosis (Beattie et al., 2000; Howe et al., 2001) or pincher-mediated macropinocytosis (Philippidou et al., 2011; Shao et al., 2002; Valdez et al., 2005). Endocytosis can occur at the soma or the tip of an axon. In the latter case, the signaling endosome containing NGF/TrkA and associated signaling molecules must traffic down the axon, which may be exceptionally long, in order to reach the soma and dendrites in a process called retrograde transport (Figure 1), which requires association of motor proteins that move along microtubules (Heerssen et al., 2004) as well as prevention of endosome maturation in order to prolong the liganded state of TrkA. Therefore, the biogenesis and trafficking of signaling endosomes is a complex and highly regulated process.

2. NGF

The family of neurotrophins consists of four growth factors that bind to $p75^{NTR}$ with low affinity and to their respective high affinity tropomyosin-related kinase (Trk) receptors: NGF to TrkA, BDNF and NT-3 to TrkB, and NT-4 to TrkC (Chao, 2003; Huang and Reichardt, 2003). Upon binding of neurotrophin, the cognate Trk is activated by tyrosine phosphorylation in the cytoplasmic domain and consequently recruits and activates downstream signaling molecules to trigger various physiological responses. Surprisingly, however, neurotrophins and Trks do not exist in invertebrates such as *Drosophila melanogaster* or *Caenorhabditis elegans*, suggesting that they function in higher-order neuronal activities but are not essential for basic neuron network development (Chao, 2003). Although they all have physiological importance in their own right, NGF is arguably the best characterized and this review focuses on the interaction of NGF and TrkA and their incorporation into signaling endosomes.

NGF signaling and its importance in the life of NGF responsive neurons has been the subject of intense study for over 60 years. The discovery of NGF and its role in embryologic development by the life-long work of late Rita Levi-Montalcini has defined the field of developmental neurophysiology (Abbott, 2009). While working with chick embryos in Victor Hamburger's lab at Washington University, Rita Levi-Montelvini grafted tissue of a mouse sarcoma onto a chick embryo whose wing buds had been extirpated, and observed that a soluble factor was released from the sarcoma and promoted the innervation of nearby sensory and sympathetic ganglia (Hamburger and Levi-Montalcini, 1949). NGF was subsequently isolated with the aid of Stanley Cohen (Cohen et al., 1954). The original isolation and a host of subsequent studies determined that NGF is a secreted growth factor from auxiliary cell types which stimulates and promotes neurite outgrowth and innervation.

Though this led to a great understanding of NGF's role in neuron physiology, the mechanisms of action, in terms of signaling and trafficking, have been poorly understood until recently.

2.1 Biogenesis

The NGF gene expresses two splice variants (Edwards et al., 1986), which are translated to produce precursor NGF (pro-NGF) that is translocated into the lumen of the ER, transported through the exocytic pathway, and converted to the biologically active mature form by proteolytic cleavages. The structure of secreted mature NGF has been resolved (McDonald et al., 1991) and shows NGF as a small homodimer with extensive hydrophobic interactions that hold the 13 kDa monomers together.

NGF is expressed by peripheral tissues that are innervated by sensory and sympathetic neuronal projections, as well as by cells within the central and periphery nervous system and even within the immune system (Levi-Montalcini et al., 1996). Tissues expressing NGF recruit nerve innervation during development and maintain nerve cell integrity in adulthood. It is primarily appreciated for its role in the survival and growth of neurons during embryonic development. In adults, however, NGF also plays an important role in neural protection after traumatic brain injury (Zhou et al., 2003), and in generation of pain sensation (Hefti et al., 2006; Zhang et al., 2005; Zhu et al., 2004). NGF and its receptors are dynamically regulated in response to tissue damage and a multitude of other pathologies in immune and inflammation systems. Though the mechanisms of these different roles of NGF are not fully understood, the vast importance of NGF in human physiology under homeostatic and stressed conditions is undisputed.

2.2 Physiological Importance

Since its discovery, NGF has been found to be involved in not just homeostatic growth in development, but also in pathologies across major systems in the human body. NGF has been used as potential therapy in a host of clinical trials for treating diseases including Alzheimer's disease, Parkinson's disease, Diabetes, Glioma, brain injuries, advanced optic nerve atrophy, HIV-associated peripheral neuropathy, ulcers throughout the body, retinopathy, and glaucoma (Aloe et al., 2012). Limitations exist, however, primarily in deleterious side effects in the sensory and autonomic systems, as well as the high doses needed for positive disease outcomes. In addition, anti-NGF antibodies are tested in clinical trials for chronic pain syndromes (Kumar and Mahal, 2012).Therefore, understanding NGF's specific mode of action and the importance of signaling endosomes in NGF signaling is a highly translational endeavor with benefit extending far beyond the lab bench.

3. TrkA

Trks are a family of three receptor tyrosine kinases (RTK) that are expressed in a host of cells throughout the mammalian body including neurons (Huang and Reichardt, 2003). TrkA in particular is shown to be a specific receptor for NGF and is activated upon NGF binding (Kaplan et al., 1991; Klein et al., 1991). Activation of TrkA at the cell surface leads to phosphorylation of tyrosine residues in the cytoplasmic domain, which in turn recruits

signaling molecules and activates multiple signaling pathways involving PLC-γ1, MAPK, and PI3K. The NGF-bound TrkA, together with associated signaling intermediates, is internalized into cells via classical clathrin-mediated endocytosis (Beattie et al., 2000; Howe et al., 2001) or pincher-mediated macropinocytosis (Shao et al., 2002). The fate of endocytosed NGF/TrkA is complex and it may be sorted into recycling endosomes or long-lived signaling endosomes that undergo retrograde transport along the axon to the somatodendritic area and propagate the survival and differentiation signals. Elucidation of the membrane trafficking pathways of TrkA should better understand its life cycle and function in NGF signal transduction.

3.1 Biogenesis and Localization

Like other type I transmembrane proteins, newly synthesized TrkA is inserted into the ER membrane with the N-terminus facing the ER lumen and the C-terminus in the cytoplasm. After cleavage of the signal peptide, TrkA is transported from the ER to the Golgi complex and then the cell surface, along the exocytic pathway where TrkA undergoes post-translational N-glycosylation and matures from a 110-kDa precursor to a 140-kDa mature form (Jullien et al., 2002; Martin-Zanca et al., 1989; Zhou et al., 1995). In polarized neurons with dendritic and axonal cell surfaces, TrkA undergoes anterograde transport and concentrates at the distal axonal growth cone where it binds to the target-derived ligand, NGF, at the synapse for initiation of survival and growth signaling.

The anterograde transport of TrkA and other axonal membrane proteins may follow different membrane trafficking pathways to the axon terminal (Ascano et al., 2009; Sampo et al., 2003). TrkA-containing post-Golgi transport vesicles may directly follow the exocytic pathway and move on the microtubules along the axon to the growth cone (Horton and Ehlers, 2003; Segal, 2003; Vaegter et al., 2011) (Figure 1). However, a recent study showed that newly synthesized TrkA is initially targeted to the somatodendritic plasma membrane, followed by transcytosis to the distal axon terminal (Ascano et al., 2009) (Figure 1). The transcytosis of TrkA is dependent on Rab11 recycling endosomes and is promoted by NGF signaling itself at the axon terminal, establishing a positive feedback network to replenish TrkA at the axonal growth cone and amplify NGF signal transduction.

3.2 Signaling

TrkA signaling starts at the axon terminal upon NGF binding to the extracellular Ig-C2 domain of TrkA (Perez et al., 1995), leading to conformational changes and activation of the tyrosine kinase activity in the cytoplasmic domain of TrkA (Figure 2). Single-molecule tracking shows that NGF binding induces TrkA dimerization or oligomerization and reduces its mobility on the plasma membrane to facilitate recruitment of signaling intermediates and signal transduction (Marchetti et al., 2013). The TrkA activation process may be regulated by $p75^{NTR}$ (Benedetti et al., 1993; Davies et al., 1993; Hempstead et al., 1991; Mahadeo et al., 1994) and G protein-coupled receptors (Lee and Chao, 2001; Lee et al., 2002). There are 10 conserved tyrosine residues in the cytoplasmic domain of TrkA. Phosphorylation in the activation loop of the kinase domain on Y670, Y674, and Y675 enhances the kinase activity (Cunningham and Greene, 1998; Stephens et al., 1994) (Figure 2). Outside the kinase domain, phosphorylation of Y490 and Y785 is most extensively characterized, but other

tyrosine residues may also contribute to TrkA signaling (Biarc et al., 2013; Inagaki et al., 1995). Phosphorylated Y490 recruits adaptors Shc or Frs2 for activation of the MAPK and PI3K pathways, while phosphorylated Y785 recruits PLC-γ1 (Obermeier et al., 1993; Stephens et al., 1994) (Figure 2). The MAPKs activated by TrkA include ERK1/ERK2, and ERK5, which phosphorylate and activate downstream transcription factors CREB, Elk-1, and MEF2, respectively, to regulate target gene expression contributing to neuronal differentiation and survival (Pearson et al., 2001; Riccio et al., 1999). The activation of PI3K also plays an important role in cell survival by activation of Akt and phosphorylation and inhibition of apoptosis-promoting proteins such as Bad and GSK3β (Datta et al., 1999; Hetman et al., 2000) (Figure 2). PLC-γ1 phosphorylation and activation by TrkA leads to production of IP_3 and DAG, which in turn promote Ca^{2+} mobilization and activation of a number of protein kinase C isoforms (Kaplan and Miller, 2000). The PLC-γ1 activation is sustained and induces target gene expression such as the PN1 sodium channel gene (Choi et al., 2001). Taken together, these NGF/TrkA signaling pathways are essential for the survival, axon and dendritic growth, specification and synapse formation of sympathetic and sensory neurons (Harrington and Ginty, 2013).

4. THE SIGNALING ENDOSOME

Although signal transduction is initiated at the plasma membrane upon binding of growth factors to their cognate receptors with recruitment and activation of local signaling molecules, it is now clear that the signaling continues on endosomes well after the growth factor and receptor complex is endocytosed into the cell. Signaling on the endosome was first reported for epidermal growth factor (EGF)-mediated signal transduction (Vieira et al., 1996) and was later found in other growth factor systems including NGF/TrkA-mediated signaling in neurons and PC12 cells where such an endosome was coined the signaling endosome (Howe and Mobley, 2004; Howe et al., 2001).

RTKs like TrkA and EGF receptor (EGFR) are endocytosed upon ligand binding and sorted into heterogeneous endosomal compartments including early endosomes destined for lysosomal degradation, recycling endosomes, or signaling endosomes. It is not well understood to what extent these receptors are sorted into each trafficking pathway and how the sorting process is regulated. In the case of TrkA in sensory and sympathetic neurons, target-derived NGF binds to TrkA at the axon terminal, followed by endocytosis into the aforementioned endosomal compartments serving different functions. Recent studies suggest that Nedd4-2-mediated ubiquitination of TrkA facilitates lysosomal degradation and downregulation of TrkA level (Georgieva et al., 2011; Yu et al., 2014, 2011), while those NGF/TrkA complexes on signaling endosomes undergo long-distance retrograde transport down the axon to the soma as well as the dendrites to amplify the signal and exert the various physiological functions of NGF (Harrington and Ginty, 2013; Sharma et al., 2010) (Figure 3). Once TrkA signaling endosomes reach the soma, an effector protein (Coronin-1) is induced and prevents fusion with lysosomes and degradation. Instead the TrkA signaling endosomes are recycled to the somatodendritic surface and reinternalized to sustain retrograde signaling for neuronal survival (Suo et al., 2014).

4.1 Biogenesis

NGF/TrkA is endocytosed via clathrin-dependent or -independent pathway such as pincher/EHD4-mediated macroendocytosis. The regulatory mechanism is unknown, but both processes require the GTPase dynamin function (Valdez et al., 2007; Ye et al., 2003; Zhang et al., 2000) and may be facilitated by activation of PLC-γ1 (Bodmer et al., 2011) and PI3K (Kuruvilla et al., 2000). In addition, the NGF/TrkA signaling endosome activates the Rac1-co?lin pathway to dissemble actin cytoskeleton and to promote the entry into axon for retrograde transport (Harrington et al., 2011). In contrast, another TrkA ligand, NT3, easily dissociates from TrkA in the acidic environment of endosomes and fails to recruit the Rac1-cofilin module to overcome the actin barrier. As a result, the NT3/TrkA endosome cannot support retrograde transport and survival signaling in neurons (Harrington et al., 2011).

The nature of NGF/TrkA signaling endosome has been intensively investigated in recent years, but remains to be firmly established. Different studies suggest the signaling endosome to be a Rab5-positive early endosome, a Rab7-positive late endosome, or a multivesicular body (MVB) (Harrington and Ginty, 2013). It is likely that they reflect sequential stages of the NGF/TrkA signaling endosome, e.g., newly formed NGF/TrkA endosomes at the early stage of retrograde transport may be Rab5-positive but may eventually mature and undergo conversion to Rab7-positive late endosomes near the destination of retrograde transport, the soma, even though the process may be delayed in neurons because of the longdistance trafficking in axon. Alternatively, Rab5-positive and Rab7-positive NGF/TrkA endosomes may represent parallel trafficking pathways for NGF/TrkA, with Rab5-positive endosomes specialized for sustained signaling and Rab7-positive endosomes destined for degradation and down-regulation. Consistent with this contention, a recent study shows that pincher-mediated NGF/TrkA signaling endosomes are Rab5-positive endosomes and MVBs that appear refractory to conversion to Rab7-positive endosomes (Philippidou et al., 2011) (Figure 3). Along this line, increased Rab7 activity is shown to increase anterograde transport of Rab7-positive endosomes from soma to axon and consequently the TrkA level is dramatically reduced, presumably due to degradation in these overactive Rab7 endosomes and/or downstream lysosomes, as in the case of expression of activated Rab7 mutants that cause the Charcot–Marie–Tooth type 2B neuropathy (Zhang et al., 2013). In addition, the more acidic environment of Rab7-positive late endosomes may cause dissociation of NGF and TrkA and make them less amenable to function as signaling endosomes.

Rab5 controls early events of endocytosis such as early endosome fusion and concentration of endocytosed cargoes and receptors, and Rab5 activity is also critical for the maturation and conversion to Rab7-positive late endosomes by recruiting the Mon1 (SAND-1)/Ccz1 complex that in turn recruits and activates Rab7 (Kinchen and Ravichandran, 2010; Nordmann et al., 2010; Poteryaev et al., 2010). The Rab5-positive TrkA endosomes and MVBs refractory to conversion to Rab7 endosomes in the axon (Philippidou et al., 2011) suggest that the Rab5 activity/function is inhibited. Indeed, NGF/TrkA is known to reduce active Rab5-GTP level by recruitment of RabGAP5 that accelerates GTP hydrolysis by Rab5 in PC12 cells (Liu et al., 2007). Moreover, it may be necessary to suppress Rab5 function for the TrkA signaling endosomes to undergo retrograde transport on microtubules, since one of the Rab5 effectors is the plus-end kinesin-3 (KIF16B) motor (Hoepfner et al.,

2005) that would promote anterograde transport on microtubules in the axon towards the growth cone rather than retrograde transport to the soma. Both Rab5 and Rab7 are among the best characterized endosomal markers because of their housekeeping functions in endocytosis as well as abundant expression and ubiquitous tissue distribution including neurons. However, the focus on these two Rabs may overlook other endosomal Rabs that are expressed at lower levels and difficult to detect in screens for signaling endosome-associated proteins. One of such Rabs, Rab22, has recently been shown to associate with NGF/TrkA endosomes and promote NGF signaling-mediated neurite outgrowth in PC12 cells (Wang et al., 2011) (Figure 3). Rab22 is a member of the Rab5 subfamily and closely related to Rab5 in evolution. Like Rab5, Rab22 is ubiquitously expressed in all tissues despite at a much lower level (Rodrigues and Pereira-Leal, 2012). Unlike Rab5, however, Rab22 does not promote endosomal conversion to Rab7-positive late endosomes, instead it sorts both clathrin-dependent and clathrin-independent endocytic cargoes into a population of recycling endosomes in cell lines (Magadan et al., 2006; Weigert et al., 2004). In polarized cells like neurons, recycling endosomes may mediate transcytosis, as in the case of Rab11-dependent recycling endosomes for anterograde transport of newly synthesized TrkA from the somatodendritic plasma membrane to the axon terminal (Ascano et al., 2009). We speculate that Rab22-dependent recycling endosomes may mediate NGF/TrkA retrograde transport and signaling (Figure 3). In this regard, Rab22, like NGF/TrkA, does not exist in lower eukaryotes such as *D. melanogaster* or *C. elegans*.

4.2 trafficking

There are at least two types of TrkA-containing endosomes in axon depending on the direction they travel: the Rab11-dependent recycling endosomes undergoing anterograde transport of newly synthesized TrkA to the growth cone (Ascano et al., 2009) and the signaling endosomes undergoing retrograde transport of target-derived NGF and activated TrkA from the axon terminal to the soma (Harrington and Ginty, 2013) (Figure 3). These morphologically similar but functionally distinct TrkA-containing endosomes make it a challenging task for Identification and isolation of TrkA-signaling endosomes. The vesicle trafficking on the microtubule cytoskeleton in the axon depends on recruitment of microtubule motor proteins that determine the directionality. Since microtubule filaments are polarized in axons with the plus-end directed towards the axon terminal and growth cone (Burton and Paige, 1981; Heidemann et al., 1984, 1981), plus-end motors such as KIF16B are expected to facilitate anterograde transport while minus-end motors such as dynein facilitate retrograde transport. Indeed, NGF/TrkA signaling endosomes are shown to associate with and require dynein for retrograde transport and survival signaling (Heerssen et al., 2004; Wu et al., 2007; Yano et al., 2001) (Figure 3). Importantly, sustained activation of the MAPK pathway on Trk signaling endosomes can stimulate phosphorylation and recruitment of dynein for retrograde transport (Mitchell et al., 2012).

4.3 Rab Function

The Rab family of GTPases represents the largest branch of Ras-like small molecular weight GTPases that govern multiple aspects of intracellular membrane trafficking, including vesicle formation, movement and fusion (Hutagalung and Novick, 2011; Li and Segev, 2012; Pfeffer, 2013). In human cells, there are 66 Rabs that target to distinct membrane

compartments along the endocytic, recycling, and exocytic pathways. Each Rab alternates between an inactive GDP-bound conformation and an active GTP-bound conformation, with the latter interacting with multiple effectors temporally and spatially to promote the membrane trafficking events, e.g., packaging cargoes into transport vesicles, recruitment of motor proteins for movement along actin or microtubule cytoskeleton, and recruitment of tethering factors for membrane docking and fusion with the target compartment. As such, Rab proteins may regulate the biogenesis and trafficking of NGF/TrkA signaling endosomes, especially the endocytic and recycling Rabs.

It is not surprising then that Rab5 or Rab7 is found to be associated with TrkA-containing endosomes in neurons, as discussed above. However, it is less clear how Rab5 or Rab7 may facilitate the retrograde transport in axon, and there is controversy. In motor neurons, it is shown that the endocytosis and retrograde transport of tetanus neurotoxin down the axon requires Rab5 and Rab7, respectively, and Rab5 is absent from the axonally transported endosomes (Deinhardt et al., 2006), in contrast to pincher-generated Trk-containing MVBs that retain Rab5 but not Rab7 in SCG neurons (Philippidou et al., 2011). In addition, detailed tracking of the speed and direction of TrkA-signaling endosomes and Rab7-positive endosomes in the axon of dorsal root ganglion neurons suggest that they normally do not overlap unless Rab7 is overactive upon expression of the activated Rab7 mutants such as those found in Charcot–Marie–Tooth type 2B neuropathy, which increases the anterograde transport of Rab7 endosomes into the axon leading to premature fusion with TrkA signaling endosomes and degradation of TrkA (Zhang et al., 2013).

Proteomic analysis of biochemically isolated TrkA endosomes from PC12 cells reveals multiple Rabs are associated with these TrkA-positive compartments, including exocytic Rab1b and Rab18, endocytic Rab7, and recycling Rab11b and Rab14 (Harrington et al., 2011). These Rabs do not normally co-localize in the same compartment and suggest that the TrkA-positive compartments represent a mixture of anterograde and retrograde transport vesicles, with the latter possible candidates for signaling endosomes. Of the two recycling Rabs, Rab14 is known to recruit the plus-end kinesin motor KIB16B for anterograde transport towards the plasma membrane (Ueno et al., 2011). Rab11 is also known for recruitment of plus-end kinesins (Schonteich et al., 2008; Simon and Prekeris, 2008) and it is shown to mediate anterograde transport of newly synthesized TrkA to the distal axon terminal (Ascano et al., 2009). Interestingly, Rab11 can also recruit dynein via a different effector (Horgan et al., 2010a,b) and is thus a potential candidate for mediating retrograde transport of TrkA signaling endosomes in the axon. Understanding the temporal and spatial regulation of Rab11-mediated recruitment of kinesin and dynein motors may provide further insight into the retrograde transport of TrkA signaling endosomes in neurons.

5. CONCLUDING REMARKS

Signaling endosomes have emerged as an important intracellular platform for signal transduction pathways by RTKs including the retrograde signaling of NGF/TrkA for neuronal survival. Target-derived NGF binds to TrkA on the plasma membrane at the axon terminal of sympathetic and sensory neurons, and activates TrkA signal transduction pathways. It also induces endocytosis to form NGF/TrkA signaling endosomes and initiate

retrograde transport and signaling along the axon to the soma, where the signaling is sustained for activation of target gene expression essential for neuronal survival. This signaling endosome concept is well documented, although the mechanisms of biogenesis and retrograde transport remain to be firmly established and are sometimes controversial. It remains to be clari?ed if both clathrin-mediated and pincher-mediated endocytosis are essential for NGF/TrkA endocytosis and retrograde signaling or if each mechanism generates a distinct population of NGF/ TrkA endosomes destined for different fates. In this regard, a study on EGF and EGFR shows that clathrin-mediated endocytosis supports recycling and sustained signaling while clathrin-independent endocytosis promotes sorting into late endosomes/lysosomes and degradation (Sigismund et al., 2008). The nature of NGF/TrkA signaling endosome also needs further clari?cation, since different studies have suggested early endosomes, recycling endosomes, late endosomes or MVBs for the role. The heterogeneity of TrkA-containing vesicles trafficking in both directions in the axon and at the growth cone makes it difficult to identify true retrogradely transported signaling endosomes and may have partly contributed to the confusion. The NGF/TrkA signaling endosome is long lived for long distance retrograde transport along the axon and for sustained signaling, as such it is expected to avoid maturation and fusion with late endosomes/lysosomes where the ligand and receptor tend to dissociate and undergo degradation by low pH and acid hydrolases. We speculate that upon endocytosis, NGF/TrkA complexes are sorted into various functional compartments for local recycling and signaling at the growth cone, for degradation and down-regulation, and for retrograde signaling and cell survival, which constitutes the fraction of signaling endosomes. The retrograde transport of signaling endosomes from the axon to the soma would resemble the transcytosis of recycling vesicles from apical to basolateral plasma membrane in polarized epithelial cells. In this regard, Rab proteins specify membrane trafficking pathways in the cell and identification of a recycling Rab(s) essential for the retrograde transport of NGF/TrkA signaling endosome should provide further insight into this important process for neuronal survival and development.

ACKNOWLEDGMENTS

Our own work was supported by the NIH/NIGMS grant R01 GM074692 to G.L.

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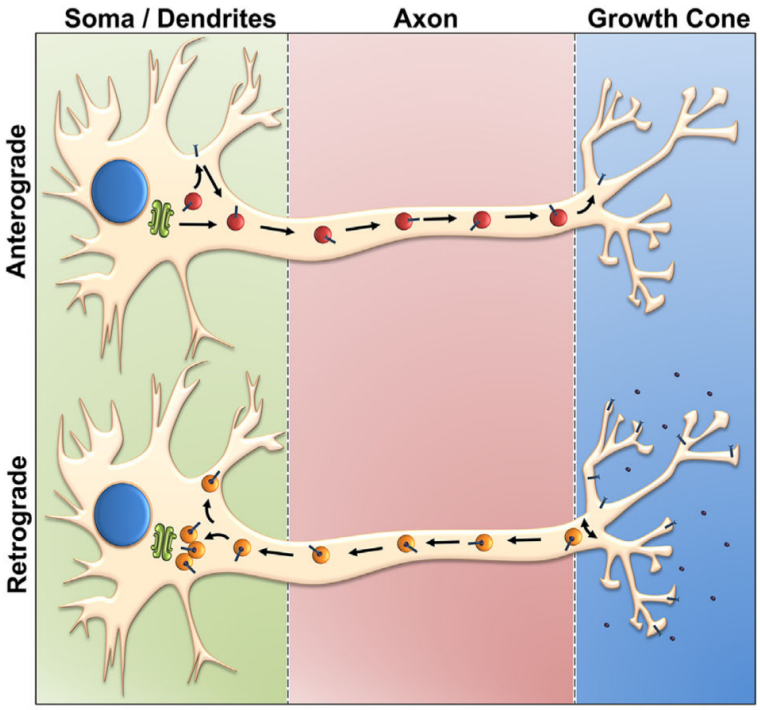

Figure 1.

Anterograde and retrograde trafficking of TrkA-containing endosomes in neurons. The upper panel illustrates anterograde transport of newly synthesized TrkA (blue bars) from the soma to the axonal growth cone, via exocytic or transcytotic pathway. Upon glycosylation and packaging into Golgi (Green)-derived transport vesicles (red), TrkA is either transported directly along the axon to the growth cone or trafficked locally to the somatodendritic plasma membrane first, followed by transcytosis to the axonal growth cone. The lower panel illustrates retrograde transport of TrkA upon binding and activation by target-derived NGF (purple spheres), from the axonal growth cone to the soma. NGF/TrkA is endocytosed and incorporated into endocytic vesicles (orange) to be trafficked locally for recycling or for retrograde transport to the soma. The signaling endosome hypothesis suggests that these long-lived retrograde endosomes remain liganded and continue signaling in the axon and at the soma for trafficking and activation of gene expression essential for neuronal survival and development. (For interpretation of the references to color in this figure legend, the reader is referred to the online version of this book.)

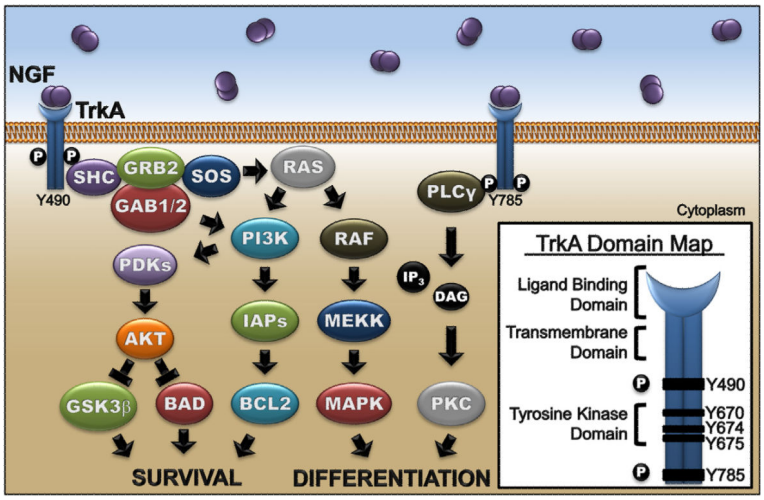

Figure 2.

NGF/TrkA signal transduction pathways in survival and differentiation of neurons. TrkA (blue bars) signaling is best characterized for activation of MAPK, PI3K and PLC γ pathways mediated mainly by the phosphorylation (black spheres) of two tyrosine residues in the cytoplasmic domain, Tyr490 (Y490) and Tyr785 (Y785). NGF dimers (purple spheres) bind to the extracellular ligand-binding domain of two TrkA monomers promoting homodimerization, activation of the tyrosine kinase domain in the activation loop, and *trans*-phosphorylation of Y490 and Y785. Phosphorylation of these residues recruits pro-differentiation and pro-survival adaptor and signaling molecules and activates the SHC-Ras-MAPK, PI3K-AKT, and PLCγ-PKC signaling pathways. (For interpretation of the references to color in this figure legend, the reader is referred to the online version of this book.)

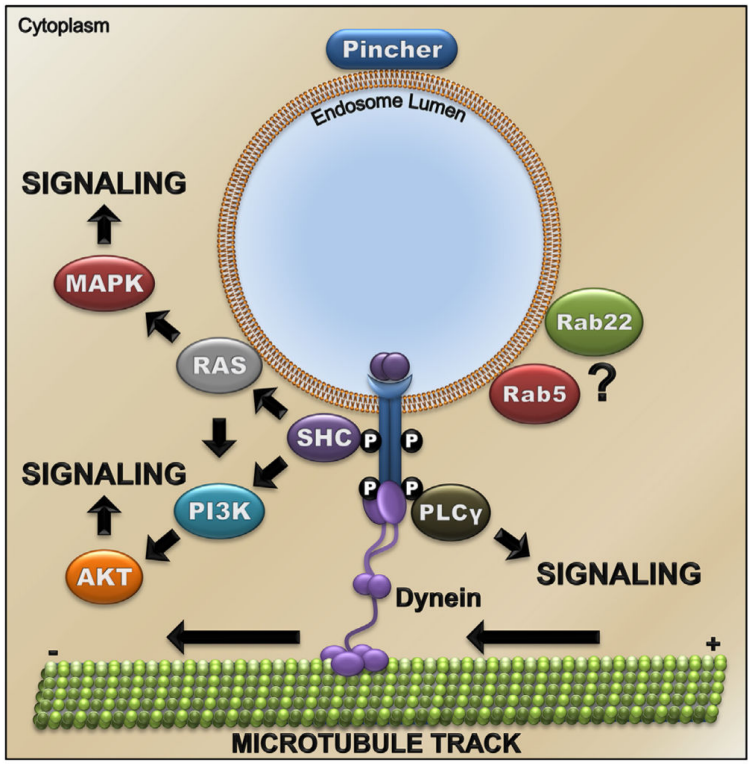

Figure 3.

A model for NGF/TrkA signal endosome. Pincher facilitates the biogenesis of NGF/TrkA signaling endosomes, which recruit the minus-end motor protein dynein to promote retrograde transport on microtubules in the axon. NGF remains bound to TrkA in the signaling endosome and keeps TrkA activated for sustained signaling that facilitates trafficking and leads to neuronal survival and differentiation. Rab5 or Rab22 or other Rabs may regulate the biogenesis and retrograde transport of NGF/TrkA signaling endosomes.