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Tracking TrkA's Trafficking: NGF Receptor Trafficking Controls NGF Receptor Signaling

T. Moises · A. Dreier · S. Flohr · M. Esser · E. Brauers · K. Reiss · D. Merken · J. Weis · A. Krüttgen

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Abstract Growth factors such as the neurotrophins promote neuronal survival and shape neuronal morphology. Neurotrophin receptors are located on the surface of axons and dendrites and must convey their signal retrogradely to the nucleus to influence transcription of target genes. The distance between the site of receptor activation and the nucleus is tremendous. How is the retrograde transmission of survival signals being achieved? Recent work showed that signaling endosomes containing neurotrophin receptors and associated downstream kinases undergo retrograde vesicular transport along microtubules, propelled by the molecular motor dynein. The next objective in the "neurotrophin receptor trafficking meets signal transduction field" will be to elucidate the traffic control mechanisms governing the directed movement of signaling endosomes. Much is already known on the trafficking of the receptor for epidermal growth factor, EGFR. We will summarize the known traffic control mechanisms for EGFR and hypothesize whether EGFR-relevant traffic control mechanisms might also be relevant for neurotrophin receptor traffic control. Moreover, we speculate about potential implications of neurotrophin receptor traffic jams for neurodegenerative diseases.

Keywords Nerve growth factor · BDNF · TrkA · p75NTR · EGFR · Neurotrophin · Neurodegeneration · Endosomal · Traffic · Ubiquitin

T. Moises · A. Dreier · S. Flohr · M. Esser · E. Brauers · K. Reiss · D. Merken · J. Weis · A. Krüttgen (✉)
Institute for Neuropathology, University Hospital,
RWTH Aachen University,
Pauwelsstr. 30,
52074 Aachen, Germany
e-mail: akruettgen@ukaachen.de

Neurotrophins and their Receptors

Neurons have a high degree of morphological complexity. A neuron possesses one axon (which might be 1 m or longer in humans) and many dendrites that are in charge of the electrical communication with other neurons. The complex morphology of neurons is regulated by growth factors that not only promote neuronal survival but also direct the outgrowth of axons and dendrites. Nerve growth factor (NGF) is the prototypical and best characterized growth factor in the nervous system. Besides NGF, the NGF family of neurotrophins consists of three additional members, BDNF, NT3, and NT4/5 [1]. NGF binds to two different receptors, the receptor tyrosine kinase TrkA and the still enigmatic p75NTR. BDNF and NT4/5 bind to TrkB and p75NTR, whereas NT3 preferentially binds to TrkC and p75NTR [2]. Trk receptors mediate their signal transduction via kinases such as Erks and Akt and transcription factors such as CREB [3].

How do Neurotrophins Convey their Retrograde Signals?

Although neurotrophin receptors are localized to synapses at the surface of axons and dendrites, their mode of action involves transcriptional changes in the nucleus. How is the signal being conveyed from synapse to nucleus to bridge such a long distance? As first shown by Hendry et al. [4], iodinated NGF undergoes retrograde transport within sympathetic axons in vivo at a rate of 2.5 mm per hour. Also, NGF receptors are internalized into endosomes and transported retrogradely in nerves [5, 6]. Trk containing endosomes undergo retrograde axonal transport in vitro at a velocity of 1.4 mm per hour [7], similar to the velocity of

retrograde transport of NGF in vivo. Thus, retrogradely transported endosomes containing NGF and its receptors, so called "signaling endosomes" (Fig. 1), are an intriguing possibility to resolve the problem of retrograde signal transduction. Indeed, there is extensive experimental support for the "signaling endosome model", which has been extensively reviewed recently [8–10].

Whereas this hypothesis of retrograde signaling endosomes containing neurotrophins and their activated receptors currently seems to be the predominant opinion in the field, it is important to note that also endosome-independent signaling pathways might exist. Using NGF immobilized to beads, the group of Campenot found evidence that NGF triggers retrograde signals without undergoing retrograde transport [11]. This might still allow for a retrograde transport of signaling endosomes containing activated Trk receptors but lacking ligand. However, this group also found that the propagation of retrograde phospho-tyrosine signaling occurs much faster than the transport of iodinated NGF [12]. Although the mechanisms responsible for this observation are not known, it seems possible that nature has also developed endosome-independent ways to transmit retrograde signals from processes to cell bodies. Intriguingly, fast retrograde signaling mechanism should be advantageous for neurons, as this would enable cells to react faster to remote stimuli. Similar mechanisms might be in place for the retrograde signaling of glutamate receptors from synapse to cell body [13]. As proposed by Berridge [14], Ca^{2+} entry at synapses might trigger a propagating wave of Ca^{2+} release from internal stores, extending from sites of Ca^{2+} entry on distal processes and moving retrogradely towards the nucleus. This might lead to nuclear CREB phosphorylation without retrograde transport of CREB or glutamate receptors. However, Howe and Mobley argued that a retrograde wave of calcium seems unlikely. Instead, they considered the possibility of a retrograde wave of IP3 signaling [8]. However, this hypothesis has not been addressed yet.

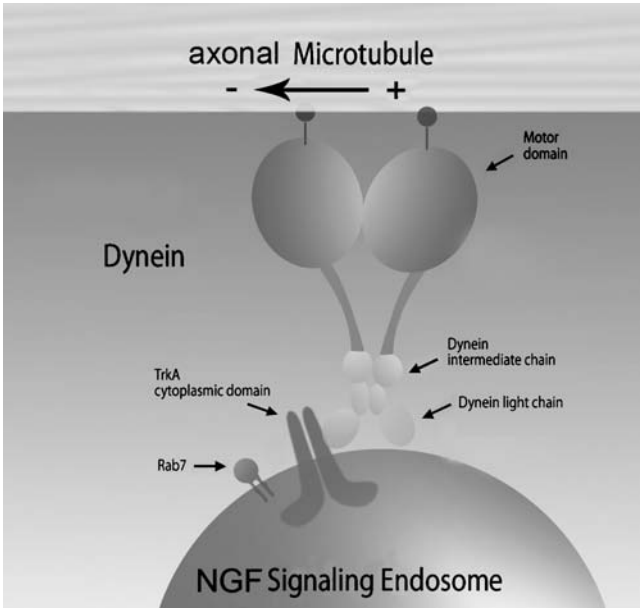

Fig. 1 Schematic on retrograde axonal transport of TrkA containing signaling endosomes. Retrograde transport of TrkA signaling endosomes depends on the molecular motor dynein. The juxtamembrane domain of TrkA binds directly to dynein light chain thereby linking the signaling endosome to the classical minus-end transport apparatus. Rab7, a small GTPase attached to the cytoplasmic surface of endosomes via lipid anchors, is found in a complex with TrkA and regulates the trafficking and signaling of this RTK

As transcription factors are present in neuronal processes [15], another potential avenue for retrograde neurotrophin signaling is suggested by recent work of Martin, Fainzilber and colleagues. These authors examined retrograde axonal signaling after axonal injury of peripheral sensory neurons [16] or retrograde dendritic signaling in hippocampal neurons during LTP-induction [17]. Hanz and colleagues showed that Erks are phosphorylated in sciatic nerve axoplasm upon nerve injury, concomitantly with the generation of soluble forms of the intermediate filament vimentin in axoplasm [16]. Vimentin binds phosphorylated Erks, thus linking these kinases to the dynein retrograde motor via direct binding of vimentin to importin. These complexes are subsequently transported in a retrograde way, independently of endosomes. In line with these findings, Thompson and coworkers showed that the classical nuclear importin pathway plays an important role in the synapse to nucleus signaling in hippocampal dendrites and axons [17]. Importins bind to transcription factors carrying a nuclear localization signal, assisting them to pass the nuclear pores. Important neuronal transcription factors carrying nuclear localization signals are, for instance, NF-kB [18] and NFAT [19], both known to be activated by neurotrophins [20, 21]. It has not been reported yet if importin-dependent mechanisms might play a role in neurotrophin nuclear signaling. Additional studies are necessary to elucidate this hypothesis.

Endosomal Trafficking Pathways: A Ticket to Enter a Cell

Recently, much effort has been directed towards elucidating which specific endosomes carry internalized NGF receptors. According to cell biology textbooks, many types of receptors are internalized via clathrin-coated pits, which then progress to form clathrin-coated vesicles and early endosomes also known as sorting endosomes (Fig. 2). In these organelles, receptors are either sorted to recycling endosomes, which return to the cell surface, or alternatively are sorted to late endosomes, also called multivesicular bodies (MVBs) according to their characteristic appearance

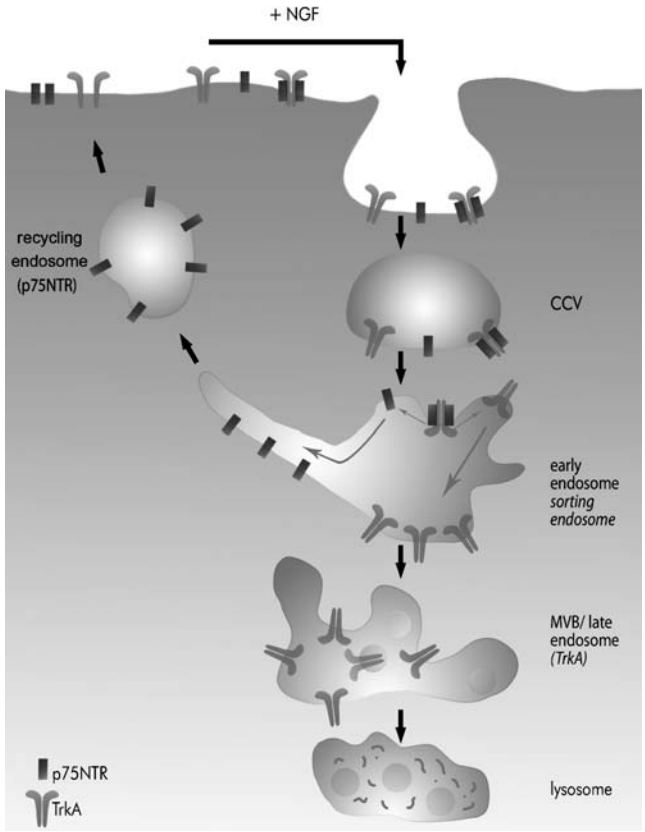

Fig. 2 Schematic on the sorting of internalized NGF receptors in PC12 cells. NGF induces the internalization of its two receptors into clathin-coated vesicles (CCV) and early endosomes (also called sorting endosomes), the classical sorting organelle for all incoming endosomes. p75NTR moves from early endosomes to recycling endosomes and returns back to the surface, whereas TrkA moves from early endosomes to MVBs/late endosomes, and eventually, lysosomes. *Circles* within MVBs/late endosomes and lysosomes represent internal vesicles, characteristic for these multivesicular organelles. The absolute levels of both NGF receptors on the surface or within endosomes are not considered in this figure

in electron microscopy pictures. From late endosomes, receptors are transported to lysosomes where they undergo degradation [22]. Recent studies also point towards the existence of two different types of early endosomes [23] and a fast and a slow recycling pathway [24].

Besides well-characterized clathrin-dependent pathways, also alternative internalization pathways are emerging. Viruses turned out to be very instructive for the study of endocytosis in mammalian cells, as mammalian viruses are experts in hijacking the cellular internalization machineries to enter and to infect their host cells. Adenoviruses are a good example, as they manipulate the endocytosis apparatus to trigger macropinocytosis during infection [25]. Besides Adenoviruses, also simian virus 40 (SV40) was very important in studying endosomal trafficking. Tracing this virus, Pelkmans and coworkers characterized caveosomes as novel endocytic entry ports, clearly distinct from known clathrin-dependent pathways [26]. These cholesterol-rich structures are characterized by the presence of the scaffold protein caveolin-1. Caveosomes undergo microtubule-dependent, long-range movement that can serve as fixed containers through vesicle traffic. It has not been examined yet if signaling receptors are using these trafficking pathways as a ticket to enter cells.

Trafficking of EGFR as a Paradigm for other RTKs

Much is already known about the traffic control of the EGFR, and this prototypical RTK may serve as a paradigm for the signaling and trafficking of other RTKs such as the Trk receptors. We first want to review our knowledge on EGFR trafficking before summarizing the current knowledge on Trk trafficking. Finally, we will discuss whether EGFR relevant mechanisms might also be relevant for Trks.

Hopkins and coworkers provided the first ultrastructural examination of the endosomal trafficking of the EGFR in EGF-stimulated fibroblasts [27]. By immuno-EM, it was established that internalized EGFR is sorted from clathrin-coated pits and CCVs to late endosomes/MVBs and lysosomes. Moreover, these authors also established the first traffic control mechanism for RTKs by demonstrating that the kinase activity of EGFR is important for diverting the EGFR from the recycling pathway, which since then has been considered to be a default sorting pathway in the absence of specific sorting signals such as kinase activity. The activity of PI3-kinases, known to be downsteam of RTKs, is of great importance for their sorting from early endosomes to the late endosomal/MVB trafficking pathway as inhibition of PI3Ks with Wortmannin leads to accumulation of EGFR within early endosomes [28].

Also, GTPases play a major role in EGFR traffic control. Seminal studies by Schmid and coworkers revealed that the large GTPase dynamin controls the endocytosis of EGFR into clathrin-coated vesicles by triggering membrane fission leading to clathrin-coated vesicle release from the plasma membrane [29]. In addition, the small GTPase Rab5 activity controls early endosome dynamics, whereas Rab7 controls late endosomal mobility along microtubules and fusion of late endosomes with lysosomes [30]. Interesting studies using dominant-negative (DN) or constitutive-active (CA) variants of these GTPases showed that Rab5 controls the surface levels of EGFR by regulating its internalization [31, 32], whereas Rab7 activity controls the late endosomal trafficking of EGFR and its lysosomal degradation [33].

Most recently, ubiquitination emerged as a major traffic control mechanism for EGFR. Ubiquitin is a 76 amino acid peptide that is covalently attached to proteins serving as a sorting signal: whereas polyubiquitination (which generally

requires a polyubiquitin chain that is at least four subunits long) marks cytoplasmic proteins for proteasomal destruction, mono-ubiquitination is an important endosomal sorting signal for transmembrane proteins. The ubiquitin ligase c-Cbl has been identified as responsible for EGFR mono-ubiquitination, serving as a traffic signal for sorting from early endosomes to late endosomes/MVBs [34]. How is this being achieved? Many proteins involved in the late endosomal sorting machinery carry so-called ubiquitin interacting motif (UIM) domains that bind to mono-ubiquitin [35]. Thus, mono-ubiquitin covalently attached to endosome-based internalized receptors serves as a lattice around endosomes linking receptors to traffic control proteins. Which traffic-controlling proteins bind to mono-ubiquitin? An important ubiquitin binding protein involved in the trafficking of the EGFR is Hrs (hepatocyte growth factor-regulated tyrosine kinase substrate), discovered by Kitamura and coworkers [36]. Hrs is phosphorylated in response to EGF and mediates sorting of EGFR from early endosomes to late endosomes/MVBs [37]. This was shown by over-expression of wild-type Hrs or a mutant variant defective in EGF-dependent phosphorylation; both proteins decrease EGF receptor (EGFR) degradation by preventing EGFR incorporation into the lumenal vesicles of late endosomes/MVBs [38].

The early endosomal protein Hrs interacts with the late endosomal protein Tsg101 (tumor susceptibility gene 101), an important component of the large ESCRT (endosomal sorting complex required for transport) machinery discovered by Katzmann, et al. [39]. Perturbation of Tsg101/Hrs interaction prevented the trafficking of EGFR to late endosomes and leads to the cellular accumulation of ubiquitinated EGFR in early endosomes [40]. The action of Tsg101 seems to be very important for growth factor signal transduction, as loss of function of Tsg101 is embryonically lethal in mice [41].

Endosomal Traffic Controls the Signaling of EGFR

What is the functional significance of growth factor receptor trafficking? Initially, internalization of receptor tyrosine kinases was solely regarded to fulfill the purpose of down-regulating these receptors by ensuring their lysosomal degradation. This view has changed dramatically in recent years. It is now commonly accepted that there is an inseparable partnership between the trafficking and signaling of growth factor receptors [42]. Most evidence regarding the importance of receptor trafficking on receptor signaling was gathered using EGFR as a model, using dominant-negative or constitutive-active variants of known traffic-control proteins described in the previous chapter. Initially, it was demonstrated that a dominant-negative dynamin isoform, which inhibits the internalization of the EGFR, exerts an impressive effect on EGFR signaling: EGF dependent activation of Erk1/2 and PI3K were suppressed when endocytosis of EGFR was inhibited [29]. In contrast, the important signaling intermediate Shc was less phosphorylated under the same conditions. Further studies showed that expression of dominant negative or constitutive active Rab5 exerts interesting effects on EGFR signaling. Overexpression of DN-Rab5 selectively blocks EGF activation of the Erk1/2 kinase pathway without affecting the activity of the JNK pathway [31]. In contrast, overexpression of a CA-Rab5 variant downregulates “empty” (EGFR not bound by its ligand EGF) EGFR from the surface, and thus, diminishes EGFR responsiveness [43]. Also, the effect of the early endosomal traffic control protein Hrs on EGFR signaling is interesting. Overexpression of Hrs reduced EGFR levels and EGF-mediated Stat3 activation [44]. Taken together, these studies clearly demonstrated the importance of traffic regulatory proteins in controlling the signal transduction of the EGFR [45].

Endosomal Trafficking of NGF Receptors: Late Endosomes/MVBs as TrkA Signaling Endosomes?

Endosomes play an important role in the retrograde axonal trafficking of NGF receptors. Which organelles serve as NGF receptor trafficking platforms? Pharmacological studies indicated sorting of TrkA in NGF stimulated PC12 cells to the late endosomal degradation pathway, as lysosomal inhibitors inhibited degradation of NGF and TrkA [46, 47]. Accordingly, cell fractionation studies and imaging experiments demonstrated that endogenous TrkA receptors in neuronal cells and primary neurons are sorted to clathrin-coated vesicles (CCVs) [48, 49], early endosomes [47, 50], and late endosomes/MVBs [47, 51]. Most importantly, also phosphorylated TrkA was discovered in fractions corresponding to CCVs, early endosomes, late endosomes/MVBs, implicating these organelles as important retrograde NGF signaling platforms (Fig. 2). Which of these organelles is the sought-after signaling endosome undergoing retrograde axonal transport? Our lab advanced the hypothesis of late endosomes/MVBs as the major retrograde transport organelle for neurotrophin receptors [47, 52]. What data supports this hypothesis? Parton, Simons and Dotti were the first to examine endosomal trafficking in axons and dendrites of hippocampal neurons [53]. In axons, early endosomes were confined to presynaptic terminals and to varicosities, whereas MVBs/late endosomes were shown to predominantly mediate the retrograde axonal transport of endocytosed markers between nerve terminals and the neuronal cell body. Studies by Campenot and coworkers [54] demonstrated that MVBs are the major endosomal population labeled by endocytosed iodinated NGF in cell bodies of sympathetic neurons. As

NGF accumulates in the neuronal cell bodies and resides there for hours before being degraded [55], MVBs are likely to represent an important, stable endosomal platform for NGF signaling after retrograde transport. As mentioned above, we showed by subcellular fractionations of NGF-stimulated neuronal cells that fractions of MVBs/late endosomes contain the highest amounts of TrkA and phosphorylated TrkA [47]. As the cytoplasmic domains of RTKs located at the outer limiting membrane of MVBs/late endosomes face the cytoplasm [56], TrkA in MVBs/late endosomes should be signaling-competent as its signaling domain is exposed to the cytoplasm. Supporting this notion, an immuno-EM study found phosphorylated Trk immunoreactivity in MVBs of sciatic nerves [57]. TrkA activates the MAPKs ERK1/2 from endosomes. Interestingly, the MAPK scaffold p14 is localized to the outer limiting membrane of MVBs, and this localization is essential for appropriate RTK signaling [58]. In line with the contention that Trk receptors signal from MVBs, there are also reports supporting the notion that other RTKs signal from MVBs [59].

Interestingly, less well-characterized internalization pathways have also been reported for TrkA receptors. Halegoua and coworkers first described the internalization of TrkA receptors via clathrin-independent macroendosomes derived from plasma membrane ruffles and subsequent trafficking of these TrkA containing vesicles to late endosomes/MVBs [51]. Another avenue for the endosomal trafficking of NGF receptors is indicated by the fact that three groups reported the presence of TrkA receptors in caveolae [60–62]. As described above, these flask-like structures are internalized into caveosomes, whose trafficking and purpose is under intense investigation. As Trk receptors are known to localize to lipid rafts and to co-immunoprecipitate with caveolin-1 [60], TrkA receptors might be transported via caveosomes as well. It is currently unclear if TrkA receptors are also internalized from caveolae and sorted from caveosomes to early endosomes or whether NGF concentrations determine the trafficking pathways of TrkA receptors to clathrin-dependent or clathrin-independent internalization pathways [63].

Endosomal Trafficking of NGF Receptors: Recycling Endosomes as Signaling Organelles for p75NTR?

Recycling endosomes are abundantly present at synapses [53]. The relevance of recycling endosomes for neurotrophin trafficking at neuronal growth cones is supported by the observation that loading of iodinated NGF to the retrograde axonal transport apparatus of developing sympathetic axons is slow and inefficient and that large amounts of NGF remain associated with axons instead of undergoing rapid retrograde transport [55, 64]. Two groups reported that in PC12 cells, endogenous p75NTR is internalized via clathrin-coated pits and undergoes trafficking to early endosomes where p75NTR joins the recycling pathway and returns back to the cell surface [47, 65] (Fig. 2).

What might be the functional significance of p75NTR recycling? The classical function of p75NTR is to support TrkA signaling. Thus, one potential role for p75NTR as a recycling receptor at axon tips may be to sequester NGF for subsequent binding to TrkA [2]. Accordingly, we found that inhibition of p75NTR recycling inhibited its TrkA supporting function at low concentrations of NGF [47]. Potentially, NGF-driven recycling of p75NTR secures sufficiently high surface levels of p75NTR, which might be necessary for appropriate NGF binding to TrkA specifically at low concentrations of NGF [66]. This, in turn, might be important for efficient TrkA internalization at low concentrations of NGF.

Regarding other potential roles of p75NTR recycling, it also appears possible that recycling endosomes containing p75NTR might trigger TrkA-independent signaling pathways [65]. Indeed, in NGF stimulated PC12 cells, p75NTR trafficking diverges from TrkA trafficking, with p75NTR specifically being found in recycling endosomes (Fig. 2). In line with the contention of specific p75NTR “recycling signaling endosomes”, it has been reported that internalized insulin receptors signal from a special recycling compartment containing the marker protein GLUT4 [67].

In contrast to results obtained with cells expressing endogenous NGF receptors, a portion of overexpressed TrkA receptors in transfected cells undergoes recycling to the cell surface, instead of being sorted to late endosomes/MVBs [68, 69]. Potentially, this indicates a different endosomal sorting of TrkA receptors depending on TrkA expression levels. Similar observations have been made for overexpressed members of the EGFR family [70, 71].

As the two NGF receptors might not only signal independently of each other but also collaborate in signal transduction at low ligand concentration, the existence of shared endosomes containing both receptors seems likely. These shared vesicles might be CCVs and early endosomes, as both receptors transit these organelles on their endosomal journey (Fig. 2) [47]. So, far the functional relevance of endosomes containing both p75NTR and TrkA has not been examined. However, it seems likely that these organelles are enucleating points for signaling pathways assigned to p75NTR:TrkA heteromers, as proposed by Neet and coworkers [72].

Taken together, the two NGF receptors p75NTR and TrkA have been localized to several types of endosomes, and the study of the functional significance of these organelles in neurotrophin physiology is an exciting area of current research.

Endosomal Traffic Controls the Signaling of Trk Receptors

What regulates the trafficking of NGF receptors? Moreover, does the trafficking of NGF receptors regulate the signal transduction of NGF? The answer to the second question is yes, as the three traffic regulators dynamin, Rab7, and pincher have been implicated in TrkA trafficking and TrkA signaling. In PC12 cells and primary neurons, DN-dynamin inhibited the endocytosis of TrkA and activation of Erk1/2 [73]. Importantly, these authors showed that Erk activation and neurite outgrowth signaling preferentially occurs on endosomes, whereas Akt activation and survival signaling preferentially occurs on the cell surface [73]. A similar dichotomy of Akt/ERK signaling was observed by our group, when PC12 cells expressing DN-Rab7 were stimulated with NGF [52]. Whereas Erk1/2 signaling and neurite outgrowth was potentiated, Akt signaling was unaltered. Also, functional interference with Pincher, a novel Trk-traffic specific protein controlling the formation of TrkA containing macro-endosomes, diminished endosomal TrkA signaling via Erks [51]. Taken together, as functional interference with TrkA traffic control proteins interferes with TrkA signal transduction, one can conclude that trafficking of TrkA controls TrkA signal transduction.

Ubiquitination and ubiquitin binding proteins play an important role in the trafficking of EGFR. Is there also a similar role in Trk trafficking? Recently, three groups showed that TrkA undergoes ubiquitination in response to NGF [74–76]. However, there are some apparent differences between these studies. It is well established that ubiquitination is a hallmark for the trafficking of receptor tyrosine kinases to late endosomes diverting them from the recycling pathway. Thus, the finding of Chao and coworkers of TrkA mono-ubiquitination [76] is in apparent disaccord with the observation of Lee and coworkers that TrkA is sorted to recycling endosomes in overexpressing cells [68]. Another unresolved finding relates to the identity of the ubiquitin-ligase responsible for TrkA ubiquitination. Whereas Wooten and coworkers described that TRAF6 ubiquitinates TrkA [75], Chao and coworkers found TrkA ubiquitination to be dependent on Nedd4-2 [76]. Another likely candidate as a TrkA ubiquitin-ligase is c-Cbl, given the interaction of TrkA and p75NTR [77] and the reported interaction of p75NTR and c-Cbl [78]. Thus, c-Cbl should be in close proximity to TrkA, implying a functional role of c-Cbl in TrkA ubiquitination. Taken together, all groups agree that TrkA is ubiquitinated. However, the exact mechanisms governing this process need to be examined in more detail. Moreover, it has not been examined yet whether Trk ubiquitination serves other functions such as shunting of newly synthesized TrkA receptors from the trans-Golgi network to late endosomes [79].

What are the similarities and differences between TrkA and EGFR trafficking? As described in detail earlier, both RTKs are sorted to CCVs, early endosomes, and late endosomes/MVBs, and clathrin plays a major role in the endocytosis of both receptors. Interestingly, both receptors are found in cholesterol-rich caveolae and interact with caveolin-1 indicating also non-clathrin-dependent trafficking pathways without known functional significance. So far, two traffic control proteins are shared between EGFR and TrkA, dynamin [29, 73], and Rab7 [33, 52]. Moreover, it seems likely that also Rab5 plays a functional role for the trafficking of both receptors, as Rab5 is associated with early NGF signaling endosomes [50]. Besides GTPases, mono-ubiquitination is an emerging tag for the sorting of both RTKs. A difference between EGFR and Trks seem to exist regarding their specific ubiquitin-ligases. Furthermore, Pincher plays an important role in TrkA internalization, but its role in EGFR trafficking has not been examined yet. On the other hand, the role of the key EGFR traffic control proteins Hrs and TSG101 in TrkA trafficking has not been reported.

Do Neurotrophin Receptor Traffic Jams Drive the Initiation of Neurodegenerative Diseases?

As initially predicted by Appel in 1981 [80] and reinforced again by us in 2003 [81], there is tantalizing evidence that this is indeed the case. A landmark study by Mobley and coworkers [82] showed a decreased retrograde transport of NGF from the hippocampus to the basal forebrain in vivo in a mouse model of Down's syndrome (Trisomy 21). This paper has vast implications, as the neuropathology of Down's syndrome shows some parallels with the neuropathology observed in Alzheimer's disease. The gene for amyloid-precursor protein (APP) is located on Chromosome 21 in humans, and it has been argued that increased expression of APP might cause the classical neuropathological phenoptype featuring plaques and tangles that are observed in patients with Down's syndrome and in patients with Alzheimer's disease.

What is the evidence for NGF playing a role in the development of Alzheimer's disease and Down's Syndrome? Hefti and coworkers showed that NGF is a powerful survival factor for basal forebrain cholinergic neurons innervating the hippocampus [83]. These neurons degenerate in Alzheimer's disease. Moreover, knockout mice lacking one allele of NGF show atrophy of these neurons, and memory defects [84], and transgenic mice expressing a neutralizing anti-NGF antibody develop plaques, tangles, and memory defects [85]. In addition, gene therapy trials with NGF in eight patients with Alzheimer's disease showed a marked decrease in

cognitive decline in response to NGF [86]. Thus, there is some evidence pointing towards disturbed NGF signal transduction in the development of Alzheimer's disease. Could the diminished NGF signaling be caused by traffic jams? Intriguingly, a recent study showed that axonal transport defects are a hallmark of Alzheimer's disease [87]. Moreover, the mentioned study by Mobley and coworkers demonstrated decreased retrograde transport of NGF and TrkA in an animal model of Down's syndrome [88]. Thirdly, a widely discussed study by Goldstein and coworkers showed that APP is an adaptor protein linking the molecular motor kinesin to vesicles destined for anterograde transport [89]. These authors identified TrkA as one of the cargos of these APP/kinesin containing vesicles. This intriguing finding has imminent implications for the pathophysiology of Alzheimer's disease. However, this study also met resistance, as other authors could not reproduce the reported findings [90]. Therefore, studies by other independent groups on this topic are needed.

Besides Alzheimer's disease, disturbed neurotrophin transport has been implicated in two other important neurodegenerative diseases. First, Saudou and coworkers demonstrated a disturbed axonal transport of BDNF in neurons expressing a mutant form of Huntingtin, thus, implicating disturbed neurotrophin trafficking in Huntington's disease [91]. Second, Segal and coworkers found that functional interference with dynein, known to cause an amyotropic lateral sclerosis (ALS) like phenotype in certain inbred mouse strains, kills motoneurons by inhibiting the retrograde axonal transport and survival signaling of the BDNF receptor TrkB [7].

Summary

In recent years, we have witnessed a dramatic development in the field of growth factor receptor trafficking and signaling. These studies likely will have a significant impact on the field of molecular neurobiology. As neurons are very complex cells, appropriate trafficking of growth factor receptors seems to be of utmost importance. Disturbed trafficking of growth factor receptors thus seems to be a likely contributor to neurodegenerative diseases. Indeed, exciting work is starting to link disturbed trafficking of neurotrophin receptors to important human neurological diseases such as Down's Syndrome, Alzheimer's disease, Huntington's disease and ALS. Thus, correction of "traffic jams" will most likely be a mandatory aim for the development of drugs targeting neurodegenerative diseases in the future.

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