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Review

# Local versus long-range neurotrophin receptor signalling: Endosomes are not just carriers for axonal transport

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ABSTRACT

Neurotrophins play a critical role in neuronal development and survival, as well as maintenance of the adult nervous system. Neurotrophins can mediate their effects by signalling locally at the nerve terminal, or signalling retrogradely from the axonal terminal to the cell soma to regulate gene expression. Given that the axon terminals of many nerve cells can be up to a metre away from their soma, neurons have evolved specialized long-range signalling platforms that depend on a highly regulated network of intracellular membrane compartments termed "signalling endosomes". Endosomal trafficking of activated receptors controls not only the axonal retrograde signals but also local receptor recycling and degradation. Endosomal trafficking involving the sorting and compartmentalizing of different signals, which are subsequently distributed to the appropriate cellular destination, can at least partially explain how neurotrophins generate a diverse array of signalling outcomes. Although signalling endosomes provide a useful model for understanding how different cell surface receptor-mediated signals are generated and transported, the precise role, identity and functional definition of a signalling endosome remains unclear. In this review we will discuss the regulation of local versus long-range neurotrophin signalling, with a specific focus on recent developments in the role of endosomes in regulating the fate of Trk receptors.


**Contents**



## 1. Introduction

Initially identified as target-derived growth factors, neurotrophins mediate a multitude of biological functions including survival, differentiation, cell cycle arrest, neurodegeneration, neuroprotection, apoptosis and modulation of synaptic plasticity [1]. Neurotrophins comprise a family of structurally and functionally related immature (pro-) and mature proteins including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3) and neurotrophin 4 (NT-4) [2], which elicit their functions by binding to transmembrane receptors. All neurotrophins bind the p75 neurotrophin receptor ($p75^{NTR}$), which acts

*Abbreviations:* AP-2, adaptor protein complex 2; BDNF, brain-derived neurotrophic factor; CREB, cAMP response element-binding protein; EEA1, early endosomal antigen 1; Erk1/2, extracellular signal-regulated kinase 1/2; Erk5, extracellular signal-regulated kinase 5; GIPC, G-alpha interacting protein-C-terminus; GSK3, glycogen synthase kinase 3; MAPK, mitogen-activated protein kinase; MVB, multi-vesicular body; NGF, nerve growth factor; NHE5, neuron-specific $Na^+/H^+$ exchanger NHE5 isoform; NT-3, neurotrophin 3; NT-4, neurotrophin 4; PI3K, phosphatidylinositide 3-kinase; PLCγ, phospholipase C gamma; p75NTR, p75 neurotrophin receptor; RAC1, Ras-related C3 botulinum toxin substrate 1; Trk, tropomyosin receptor kinase; V-ATPase, vacuolar-type $H^+$-ATPase.

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predominantly to promote neuronal degeneration or growth cone collapse, or to inhibit axonal regeneration [2–4]. In contrast, the binding of mature neurotrophins to tyrosine receptor-like kinases Trk A, B or C is highly specific: NGF binds preferentially to TrkA, BDNF and NT4 bind to TrkB, and NT3 binds to TrkC [5], leading to subsequent activation of specific well characterized signalling pathways that are predominantly neurotrophic [6]. In addition, NT3 binds with low affinity to TrkA and TrkB, activating a different physiological response from that elicited by the binding of the Trk receptor to its preferred ligand (potentiating neurite outgrowth but not neuronal survival) [1,7].

Studies investigating NGF signalling in sympathetic neurons and the PC12 sympathetic neuronal model demonstrated that Trk receptors which are activated at the axonal terminals are endocytosed and, together with any recruited downstream signalling molecules, can be retrogradely transported along the axon to the cell body by the dynein-microtubule system [8–10]. Upon arrival at the cell body, nuclear transcription factors such as cAMP response element-binding protein (CREB) are activated, resulting in their translocation to the nucleus where they regulate gene expression [11,12]. The axonal retrograde trafficking of signalling endosomes is necessary for neurotrophin-mediated survival [11,13]. Indeed, the significance of axonal retrograde transport in signalling endosomes containing activated neurotrophin receptors is highlighted by increasing evidence showing that numerous neurodegenerative diseases are directly or indirectly underpinned by impaired neurotrophin trafficking, resulting in reduced trophic signalling [14,15].

Although axonal retrograde transport of NGF, later confirmed to be bound to TrkA, was demonstrated in 1974 [16], it was not until the mid 1990s that the field appreciated that receptor-mediated signalling not only occurred at the plasma membrane but could also continue following receptor internalization [17,18]. Up until that point, the widely accepted model for receptor signalling was a cascade of protein:protein interactions leading to the spread of an amplified wave of protein phosphorylation that ultimately led to nuclear transcription [19]. This cascading wave model depends inherently upon the notion that activated transcription factors or other signalling moieties diffuse through the cytoplasm, enter the nucleus, and promote transcriptional activation [19]. Although such models of signal transduction in neurons persist (reviewed elsewhere; [20,21]), these models did not fit well with the physical constraints of the neuronal architecture, and a significant body of work led to the formation of the "signalling endosome hypothesis". A "signalling endosome" packages a growth factor-induced signal into a discrete membrane-bound organelle that is transported along the length of the axon to the soma via a cytoskeleton-based transport process. The formation of a signalling endosome is further proposed to occur through a directed process of signal transduction, and overcomes the physical constraints of axonal distances and volumes [22]. Despite the overwhelming evidence that signalling endosomes can carry survival signals from the axon to the soma, the functional description of a signalling endosome remains poorly defined as it is unclear which endosomal compartments are involved in the signalling events and which molecular components of endosomes are necessary for that signalling. However there have been recent insights into this problem, as highlighted below.

## 2. Endosomes play an important role in spatio-temporal regulation of Trk signalling

It is now widely accepted that the activation of Trk receptors triggers their dimerization, kinase activation, and autophosphorylation, creating docking sites for adaptor proteins that couple the receptors to intracellular signalling cascades [23]. The three best characterized signalling pathways are the mitogen-activated protein kinase (MAPK) pathway, the phosphatidylinositide 3-kinase (PI3K) pathway, and the phospholipase C-gamma (PLCγ) pathway, resulting in axonal growth, neuronal survival, and target innervation [2]. The fact that neurotrophin-Trk receptor signalling is able to activate such a wide variety of physiological functions, via only a few heterogeneous signalling pathways, is its most intriguing feature.

It is equally well established that the outcomes of the signalling pathways activated by NGF acting on TrkA receptors at the cell surface are different from the signals generated by NGF-TrkA complexes found in endosomal compartments [12]. For example, NGF at the cell surface transiently activates the signalling effector Ras GTPase, whereas endocytosed NGF-TrkA complexes lead to the sustained activation of closely related GTPase Rap1 and MAPK pathways [24]. A number of cellular and molecular mechanisms have been proposed to explain this phenomenon. Firstly, receptor activation does not always result in the same subcellular location of the receptors, thereby mediating different signalling outcomes. Although NGF and NT-3 have different affinities for TrkA, they both phosphorylate surface TrkA to a similar extent [25]. These neurotrophins also act together to support the development of sympathetic neurons by activating TrkA-dependent axonal outgrowth. However, only NGF is able to promote the axonal retrograde transport of TrkA-containing endosomes that lead to survival [25,26]. The molecular mechanisms regulating the differential signalling of NGF and NT-3 through TrkA are beginning to emerge and will be the focus of this review. Second, differential signalling can be generated as a consequence of receptor internalization routes. For example, clathrin-dependent endocytosis is associated with activation of the MAPK Erk1/2, whereas macropinocytosis involving the endocytic chaperone protein Pincher preferentially activates the Erk5 kinase [15,27]. Third, the same signalling effectors have been shown to produce different physiological effects depending on their subcellular location. For example, PI3K and Erk1/2 have together been shown to be necessary for neurite outgrowth when activated by NGF binding to TrkA at the presynaptic nerve terminal, whereas activation of PI3K within the cell body prevents apoptosis by activation of the anti-apoptotic kinase Akt [7]. Survival signals can also be mediated by NGF-Trk-induced Erk5 activation in the cell body, which results in activation of the transcription factor CREB [7].

The current evidence suggests that although the specificity of neurotrophic signalling is regulated by multiple cellular and molecular mechanisms, for example, the recruitment of distinct signalling messenger complexes that determine the fate of the receptor, a major regulator of Trk receptor function is its internalization in various endosomes, including those with signalling capacity.

## 3. Insights into ligand binding and Trk receptor activation leading to endocytosis

TrkA can engage a number of ligands, specifically the pro- and mature forms of NGF, as well as NT-3, with each ligand producing a different physiological outcome. This indicates a ligand-specific mechanism of signal transduction. Trk receptors interact with their ligands through the second immunoglobulin-like domain, which consists of a conserved binding motif shared among all neurotrophins, and a site that is specific for the interaction between a particular ligand and its preferred receptor [28,29]. Although the dynamic steps involved in ligand binding to Trk receptors remain poorly defined, it is acknowledged that TrkA has a different affinity for NGF, proNGF and NT-3, and that the signalling outcome varies with each ligand [5,30,31]. However, the length of ligand–receptor

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