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TRK RECEPTORS: ROLES IN NEURONAL SIGNAL TRANSDUCTION*

Eric J. Huang[1] and Louis F. Reichardt[2]
[1]*Department of Pathology, University of California Veterans Administration Medical Center, San Francisco, California 94143; email: ejhuang@itsa.ucsf.edu*
[2]*Department of Physiology, Howard Hughes Medical Institute at the University of California, San Francisco, California 94143; email: lfr@cgl.ucsf.edu*

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■ **Abstract** Trk receptors are a family of three receptor tyrosine kinases, each of which can be activated by one or more of four neurotrophins—nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophins 3 and 4 (NT3 and NT4). Neurotrophin signaling through these receptors regulates cell survival, proliferation, the fate of neural precursors, axon and dendrite growth and patterning, and the expression and activity of functionally important proteins, such as ion channels and neurotransmitter receptors. In the adult nervous system, the Trk receptors regulate synaptic strength and plasticity. The cytoplasmic domains of Trk receptors contain several sites of tyrosine phosphorylation that recruit intermediates in intracellular signaling cascades. As a result, Trk receptor signaling activates several small G proteins, including Ras, Rap-1, and the Cdc-42-Rac-Rho family, as well as pathways regulated by MAP kinase, PI 3-kinase and phospholipase-C-γ (PLC-γ). Trk receptor activation has different consequences in different cells, and the specificity of downstream Trk receptor-mediated signaling is controlled through expression of intermediates in these signaling pathways and membrane trafficking that regulates localization of different signaling constituents. Perhaps the most fascinating aspect of Trk receptor-mediated signaling is its interplay with signaling promoted by the pan-neurotrophin receptor $p75^{NTR}$. $p75^{NTR}$ activates a distinct set of signaling pathways within cells that are in some instances synergistic and in other instances antagonistic to those activated by Trk receptors. Several of these are proapoptotic but are suppressed by Trk receptor-initiated signaling. $p75^{NTR}$ also influences the conformations of Trk receptors; this modifies ligand-binding specificity and affinity with important developmental consequences.

CONTENTS

INTRODUCTION

The Trk family of receptor tyrosine kinases derives its name from the oncogene that resulted in its discovery (1). This oncogene was isolated in gene transfer assays from a carcinoma and, when cloned, was found to consist of the first seven of eight exons of nonmuscle tropomyosin fused to the transmembrane and cytoplasmic domains of a novel tyrosine kinase. Consequently, the proto-oncogene was named tropomyosin-related kinase (trk) and is now commonly referred to as trkA. The *trkB and trkC* genes were identified because of their high homology to *trkA*. Comparisons of their sequences to those of other transmembrane tyrosine kinases indicated that they constitute a novel family of cell surface receptor tyrosine kinases. Specific patterns of expression within the nervous system suggested roles in neuronal development and function, but the Trk receptors were only a small percentage of the large number of orphan tyrosine kinases with high expression in the nervous system. In 1991, though, two groups independently presented convincing evidence that nerve growth factor bound to and activated the tyrosine kinase activity of TrkA (2, 3). Subsequently, TrkB and TrkC were shown to be receptors for other members of the neurotrophin family: BDNF and NT4 activated TrkB, and NT3 activated TrkC (4, 5). Subsequent work has shown that NT3 is able to activate each of the Trk receptors in some cell types.

The discovery of the first neurotrophin, NGF, preceded by several decades the identification of Trk receptors and was a seminal advance in developmental

neurobiology (6). Ablation and transplantation studies had previously indicated that targets of innervation secrete limiting amounts of survival factors that ensure a balance between target tissue size and innervation. NGF was the first protein identified that fulfilled this role. The availability of NGF made it possible to identify mechanisms of intercellular communication (7). For example, NGF was shown to be internalized by a receptor-dependent process and to be transported for long distances in small vesicles within axons by an energy- and microtubule-dependent process. NGF was shown to have both local and nuclear actions, which regulate, respectively, growth cone motility and expression of genes encoding the biosynthetic enzymes for neurotransmitters. Without receptors in hand, though, it was not possible to understand the molecular bases for these actions. The discovery of the Trk receptors had a revolutionary impact on this field, because it provided essential tools for pursuing the signaling pathways controlled by neurotrophins. In addition, the literature on other tyrosine kinases suggested that neurotrophins might have much more extensive roles in the nervous system and implicated a number of tyrosine kinase-regulated pathways, such as those activated by Ras, PI 3-kinase, and the Cdc-42-Rac-Rho family, that might mediate these functions. More recent studies on the signaling mechanisms and functions of tyrosine kinases in other systems continue to provide guidance cues for neuroscientists.

The pathways regulated by neurotrophin-mediated activation of Trk receptors include proliferation and survival; axonal and dendritic growth and remodeling; assembly and remodeling of the cytoskeleton; membrane trafficking and fusion; and synapse formation, function, and plasticity (Figure 1). Because of space constraints, comparatively little of the biology of neurotrophins and their receptors themselves are critically examined in this review, which focuses instead on the molecular interactions and pathways regulated by the Trk receptors. Interested readers are referred to many excellent reviews on the biological actions of neurotrophins (4, 5, 7–14).

CONTROL OF NEUROTROPHIN RESPONSIVENESS BY TRK

In general, the repertoire of endogenous Trk receptors expressed by a neuron predicts the set of neurotrophins able to promote a neuron's survival and differentiation. The presence of TrkA, TrkB, or TrkC confers responsiveness, respectively, to NGF, BDNF, and NT4, or to NT3. Ectopic expression of a Trk receptor has been shown to confer responsiveness to the neurotrophins able to activate that receptor in most but not all neurons (15). Differential splicing of the mRNAs encoding each of the Trk receptors, however, makes this generalization an oversimplification (Figure 2). The presence or absence of short sequences of

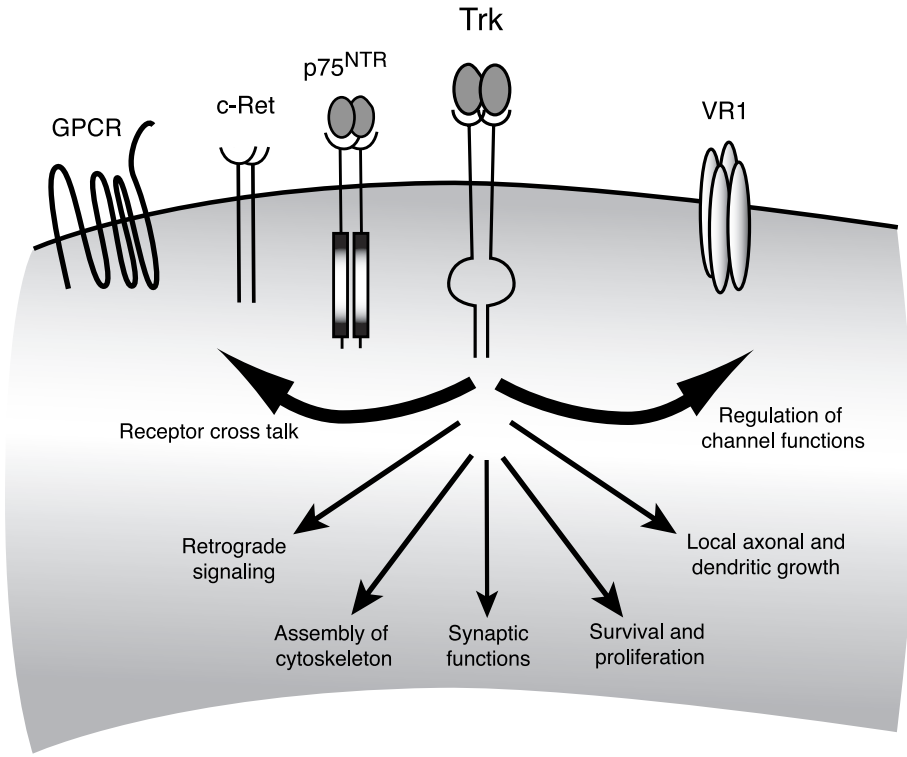


Figure 1 Major functions of Trk receptors. Neurotrophin-mediated activation of Trk receptors leads to a variety of biological responses, which include proliferation and survival, axonal and dendritic growth and remodeling, assembly and remodeling of cytoskeleton, membrane trafficking, and modifications of synaptic functions. In addition, cross talk has been reported between Trk receptors and other membrane receptors such as $p75^{NTR}$, G protein-coupled receptors (GPCRs), vannilloid receptor (VR1), and c-Ret.

amino acids in the juxtamembrane region of each Trk receptor has been shown to regulate the specificity of Trk receptor responsiveness. For example, an isoform of TrkA lacking this short insert is activated efficiently only by NGF, but the presence of this insert increases activation of TrkA by NT3 without affecting its activation by NGF (16). A TrkB isoform lacking a similar short insert can be activated only by BDNF, whereas TrkB containing the insert is also activatable by NT3 and NT4 (17, 18). TrkB with and without the insert is expressed differentially in subpopulations of sensory neurons, which suggests that regulation of splicing of the exon encoding this insert is important for normal neuronal development or function in vivo (18). The short polypeptide sequence in TrkA is immediately adjacent to the major ligand-binding domain in the membrane proximal Ig-like domain (19, 20). These residues were not included in the

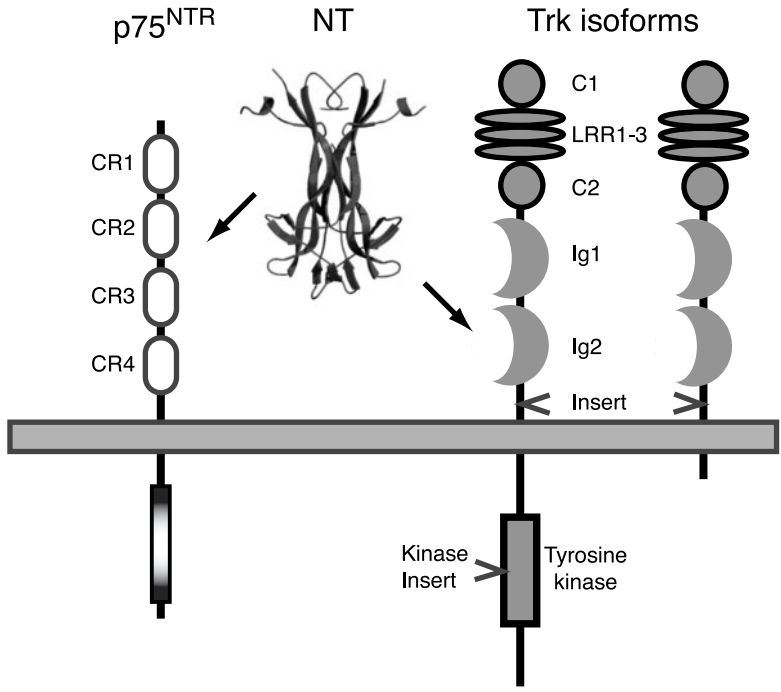


Figure 2 Interactions of neurotrophins (NT) with Trk and $p75^{NTR}$ receptors. The domain structures of the Trk receptors and of $p75^{NTR}$ are schematized in this figure. The locations in the Trk receptor extracellular domains of cysteine clusters (C), leucine-rich repeats (LRR), and immunoglobin-like domains (Ig) are depicted. The locations of the alternatively spliced juxtamembrane inserts that affect the ligand-binding specificity of Trk receptors are also depicted. Each Trk has a single transmembrane domain and a single cytoplasmic tyrosine kinase domain. An isoform of TrkC has been identified with a kinase insert domain. In addition, several truncated isoforms of TrkB and TrkC that lack the tyrosine kinase domain have been identified, and one of these is depicted on the far right. The second immunoglobin-like domain (Ig2) of TrkA and TrkB is the major ligand-binding interface. The domain structure of $p75^{NTR}$ is depicted on the far left. The extracellular domain of $p75^{NTR}$ consists of four cysteine-repeat domains (CR). Both CR2 and CR3 have been implicated in neurotrophin-binding interactions. $p75^{NTR}$ has single transmembrane and cytoplasmic domains. The latter contains a "death domain" similar to those identified in TNF receptors.

NGF-TrkA ligand binding domain complex that was solved at atomic resolution, but the organization of the interface between NGF and TrkA was considered compatible with an additional contribution to binding by these amino acids (19). Organization of the interface between NT4 and TrkB is very similar to that between NGF and TrkA and also appears compatible with a direct contribution

to binding by the amino acids in the differentially spliced TrkB insert (21). In addition to the structural data, mutational analysis of the TrkB insert indicates that there are interactions between negatively-charged amino acids in this insert with positively charged residues in NT3 (18).

The pan-neurotrophin receptor, $p75^{NTR}$, also regulates the responsiveness of Trk receptors to neurotrophins. In the presence of $p75^{NTR}$, NT3 is much less effective at activating TrkA, and NT3 and NT4 are much less effective at activating TrkB. In other words, presence of $p75^{NTR}$ enhances the specificity of TrkA and TrkB for their primary ligands, NGF and BDNF, respectively (16, 22–26). Thus, the specificity of neuronal responses to neurotrophins can be modulated by the type of receptor, differential splicing, and the absence or presence of $p75^{NTR}$. Not all isoforms of TrkB and TrkC contain their tyrosine kinase domains. Differential splicing results in expression of truncated receptors lacking the kinase domain (27–29). The functions of these truncated isoforms of TrkB and TrkC are poorly understood. Despite some evidence suggesting that truncated receptors alone can affect intracellular signaling directly (30, 31), tyrosine kinase activity is essential for the vast majority of Trk receptor-mediated responses to neurotrophins. When expressed in *trans*, e.g., on nonneural cells, it has been suggested that the truncated receptors can raise the local effective neurotrophin concentration by capturing and presenting neurotrophins to neurons expressing full-length Trk receptors. When expressed in *cis*, e.g., on the same neuron as a full-length Trk receptor, experiments indicate that truncated receptors inhibit activation of Trk kinases by forming nonproductive heterodimers (32). Consistent with this model, transgenic mice overexpressing a truncated TrkC receptor show neuronal loss in sensory ganglia and cardiac defects similar to those observed in mice lacking NT3 (33). Recent evidence also indicates that truncated isoforms help regulate the surface expression of full-length TrkB (34). Finally, another isoform of TrkC contains an amino acid insert within the kinase domain. The presence of this insert clearly modifies the substrate specificity of the TrkC kinase and alters cellular responses to its activation (35–37).

For a neuron to be responsive to a neurotrophin requires that a Trk receptor be expressed on the surface of the cell. In some cultured CNS neurons, Trk receptors are localized to intracellular vesicles in the absence of signals. Electrical activity, cAMP, and Ca^{2+} stimulate Trk insertion into the cell surface by exocytosis of cytoplasmic membrane vesicles containing Trk (38, 39). Thus, interactions with neighboring cells clearly affect the ability of these neurons to respond to neurotrophins. In summary, most survival-promoting and differentiation-promoting responses to neurotrophins require the presence of a Trk receptor on a neuron, but the competence of a Trk receptor to convey appropriate signals to the interior of the cell is regulated by additional factors, which include the proportions of truncated or insert-containing receptors produced by differential splicing, the presence or absence of $p75^{NTR}$, and second messengers that promote vesicle-mediated receptor insertion into the plasma membrane.

TRK RECEPTOR STRUCTURE AND LIGAND INTERACTIONS

Trk receptors share a common structural organization of their extracellular domains that clearly distinguishes them from other receptor tyrosine kinases (Figure 2) (40). Immediately following the cleaved signal sequence is an array of three leucine-rich 24-residue motifs flanked by two cysteine clusters. Two C2-type immunoglobulin-like domains are adjacent to these structures, which are followed by a single transmembrane domain and a cytoplasmic domain that contains a tyrosine kinase domain plus several tyrosine-containing motifs similar to those present in other receptor tyrosine kinases. Like other receptor tyrosine kinases, phosphorylation of cytoplasmic tyrosines in Trk receptors regulates tyrosine kinase activity and provides phosphorylation-dependent recruitment sites for adaptor molecules and enzymes that mediate initiation of intracellular signaling cascades (4, 5, 9–11).

The unique structural organization of the extracellular domain of Trk receptors suggests that they might mediate adhesive interactions in addition to neurotrophin signaling, but subsequent work has provided no support for this proposal. The major ligand-binding domains of the Trk receptors have been localized to the membrane-proximal Ig-C2-like domain (Ig2) (41). Structures of each of these domains have been solved (42), as have those of several neurotrophins (43–45). In addition, as mentioned earlier, the structures of NGF bound to the TrkA Ig-C2 domain and of NT4 associated with the TrkB Ig-C2 domain have been determined to high resolution. Comparisons between these structures have provided detailed descriptions of the interactions that regulate the specificity and strength of ligand binding to Trk receptors (19, 21). Ligand binding is promoted partly through a set of relatively conserved contacts, shared among the receptor family, while a second set of contacts is responsible for further promoting binding to cognate ligands and for selecting against binding to inappropriate neurotrophins. The N termini of neurotrophins are important in controlling specificity, and the structure of this region is reorganized upon binding to a Trk receptor. Interactions with Trk receptors also alter neurotrophin structures in other regions. This deformability appears important for permitting some neurotrophins to activate more than one type of Trk receptor. To achieve a definitive understanding of this flexibility will require solutions of structures of each of the Trk receptor ligand-binding domains with NT3, the most promiscuous of the neurotrophins.

Although the membrane proximal Ig domain (Ig-2) is the major interface for neurotrophin binding by Trk receptors, other regions in the Trk extracellular domains are also important for ligand binding, either contributing to binding directly or indirectly through effects on conformation of the ligand binding site. For example, mutation of a conserved cysteine in the membrane distal Ig-1 domain of TrkA abolishes NGF binding, suggesting that this domain may also be involved in ligand engagement (46). Studies using a series of TrkA-TrkB

chimeras also indicate that the Ig-1 domain of TrkB is required in addition to the TrkB Ig-2 domain for BDNF or NT3-dependent receptor activation (47). Several studies indicate that the cysteine-rich and leucine-rich domains of Trk receptors also participate in ligand binding, at least in some circumstances. For example, high-affinity binding of NT3 to TrkA is not observed in cells expressing a truncated TrkA lacking these regions (48). Mutation of the linker region between the leucine repeats and the Ig-1 domain actually increases the binding affinity of TrkA for NGF, which also implicates this region (46). A series of analyses of TrkA-TrkB chimeras has revealed that the TrkA Ig-2 domain is essential for NGF binding and receptor activation; but in the additional presence of $p75^{NTR}$, this Ig domain is no longer essential to observe ligand-mediated receptor activation (47). The presence of the first cysteine-rich domain of TrkA in a TrkA-TrkB chimera is sufficient to permit effective NGF-dependent activation of a chimeric receptor in the presence of $p75^{NTR}$.

In addition to regulating ligand binding, the different regions in the extracellular domains of Trk receptors also control ligand-independent Trk receptor dimerization. Deletion of Ig-1, Ig-2, or both domains of TrkA increases spontaneous receptor dimerization and activation, which suggests that these domains inhibit spontaneous dimerization in the absence of ligand (49). In contrast, analysis of a mutation in the leucine-rich repeat indicates that it may promote dimerization of ligand-engaged receptor without increasing the affinity of NGF binding (46, 49).

The picture that emerges from these studies suggests that each of the extracellular domains of Trk receptors helps to modulate ligand binding, either by directly interacting with neurotrophins or by modulating conformational changes in the ligand-binding Ig-2 domains of these receptors. Each of the extracellular subdomains also modulates receptor dimerization through interactions that are poorly understood. Despite the impressive three-dimensional crystal structures of neurotrophins complexed to Trk Ig-2 domains, much additional effort will be necessary to characterize the allosteric conformations and ligand-binding interactions of these receptors.

TRK RECEPTOR ACTIVATION MECHANISMS

Binding by neurotrophins provides the primary mechanism for activation of Trk receptors, but the affinity and specificity of Trk receptor activation by neurotrophins is regulated by the pan-neurotrophin receptor $p75^{NTR}$ (Figure 2). The presence of $p75^{NTR}$ is required to observe high-affinity binding of NGF to TrkA (16, 23, 24, 50, 51). Kinetic characterization of NGF interactions with TrkA and with $p75^{NTR}$ demonstrated that, although dissociation constants for each receptor are very similar, the kinetics are quite different. NGF associates with and dissociates from $p75^{NTR}$ much more rapidly than from TrkA, and the presence of $p75^{NTR}$ increases the rate of NGF association with TrkA (52). Recent data have

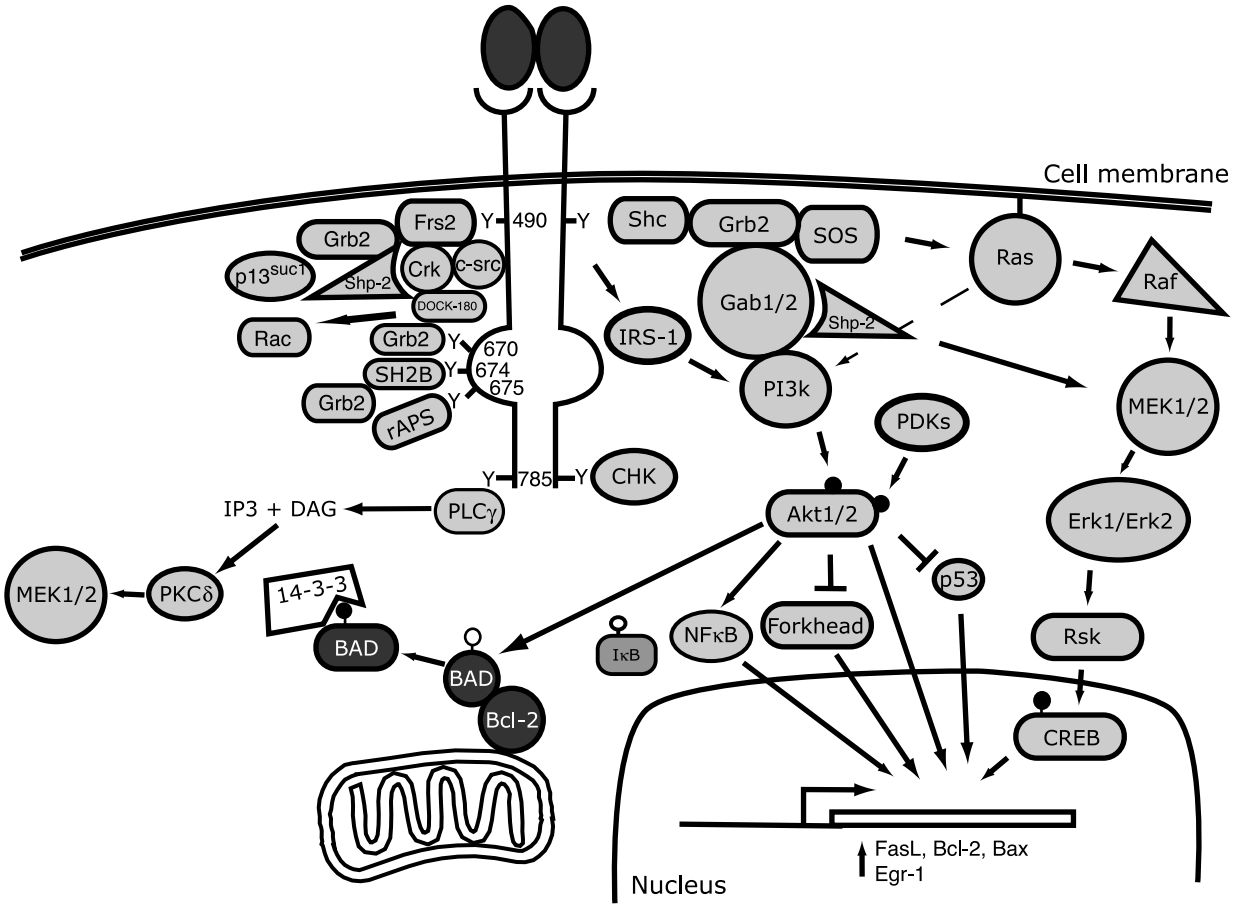
shown that mutations of the cytoplasmic or transmembrane domains of either TrkA or $p75^{NTR}$ prevent the formation of high-affinity binding sites on TrkA (53). Surprisingly, an intact ligand-binding site in $p75^{NTR}$ is not essential for it to promote high-affinity binding. Thus these data argue persuasively that the presence of $p75^{NTR}$ favors a conformation of TrkA that has a high-affinity binding site for NGF. In the presence but not in the absence of $p75^{NTR}$, the presence of only the first cysteine-rich domain of TrkA in an TrkA-TrkB chimera is able to mediate NGF-dependent receptor activation (47), which also supports an allosteric model. Inhibition by $p75^{NTR}$ of TrkA interactions with NT3 also appears to be caused by induction of a conformational change in TrkA (25). Although the extracellular domain of $p75^{NTR}$ must be present to observe inhibition, NT3 binding to $p75^{NTR}$ is not required.

Exciting recent data demonstrate that, similar to the EGF receptor (54, 55), Trk receptors can also be activated by at least two G protein–coupled receptors, the adenosineA_{2a} and PAC-1 receptors (56, 57) (Figure 1). Trk receptor activation through these G protein-coupled receptors has very different kinetics and requirements than activation of the same receptors by neurotrophins. G protein receptor-mediated activation occurs with exceedingly slow kinetics (hours, not minutes) and is prevented by chelators of internal Ca^{2+} and by an inhibitor of Src-family tyrosine kinases, but not by inhibitors of protein kinase C or protein kinase A (57). Compared to neurotrophin action, TrkA activation by pituitary adenylate cyclase-activating peptide (PACAP) results in preferential stimulation of Akt versus Erk1 and Erk2 (58). Potentially facilitating G protein-coupled receptor-mediated activation of Trk receptors, TrkA has been shown to be associated via a PDZ-containing linker protein named GIPC with GAIP (59). GAIP is a protein that contains a regulator of G protein signaling (RGS) domain.

Other receptor tyrosine kinases, such as the EGF receptor, can be activated by "lateral propagation" initiated by local receptor activation or by cell adhesion (60, 61). While propagated Trk receptor activation is a particularly attractive means to explain the rapid kinetics of retrograde signaling by Trk receptors from the axonal tip to the cell body in neurons (12, 62, 63), no experiments directly demonstrate that Trk receptors are activated through this mechanism in vitro or in vivo. There is also no evidence that adhesive interactions activate Trk receptors, although there are almost certainly synergistic interactions with integrins (64).

TRK RECEPTOR INTERACTIONS WITH CYTOPLASMIC ADAPTOR PROTEINS

Trk receptor activation results in phosphorylation of several of ten evolutionarily conserved tyrosines present in the cytoplasmic domains of each receptor (65, 66) (Figure 3). Three of these—Y670, Y674, and Y675 in human TrkA—are in the activation loop of the kinase domain. Phosphorylation of these residues poten-



tiates tyrosine kinase activity by pairing these negatively charged phosphotyrosine residues with basic residues in their vicinity (67). Phosphorylation of additional tyrosines creates docking sites for proteins containing PTB or SH2 domains. Intracellular signaling events activated by these adaptor proteins include Ras-Raf-Erk, PI3 kinase-Akt, PLC-γ-Ca^{2+}, NFκB, and atypical protein kinase C pathways (11, 68, 69). Research has focused on interactions mediated by two phosphorylated tyrosines. Y490 and Y785 are the major phosphorylated tyrosine residues that are not in the kinase activation domain (65). Phosphotyrosine-490 interacts with Shc, fibroblast growth factor receptor substrate 2 (Frs2), and other adaptors, which provide mechanisms for activation of ras and PI3 kinase. Phosphotyrosine-785 recruits PLC-γ1. It should be noted that single mutations of three of the remaining five tyrosines have been shown to inhibit NGF-dependent neurite outgrowth by PC12 cells (66). Thus at least eight tyrosines in the cytoplasmic domain contribute to Trk-mediated signaling, but the details of how several of them contribute are not very clear.

Although most work has focused on pathways controlled by Shc, Frs2, and PLC-γ1, several additional adaptors and signaling complexes have been identified that interact with activated Trk receptors, some of which depend on transport of Trk receptors to intracellular membrane compartments (70–74). In addition to interactions with cytoplasmic adaptors, Trk receptors interact with a number of other membrane proteins, including $p75^{NTR}$, either directly or indirectly (75, 76). Association of $p75^{NTR}$ and Trk undoubtedly facilitates the synergistic and antagonistic interactions between them.

Almost all models that describe the details of Trk receptor signaling focus on the activation of survival and differentiation pathways activated by the adaptors, Frs-2 and Shc, that interact with phospho-Y490 in TrkA and similarly positioned phosphotyrosine residues in TrkB and TrkC. Nothing illustrates the limitations of these models more clearly than the surprisingly modest phenotypes of mice homozygous for a Y-to-F mutation at this site in TrkB or TrkC (77, 78). In each case, major populations of neurons survive that are lost in mice homozygous for a kinase-deletion mutant in TrkB or TrkC. Thus, phospho-Y490-independent interactions must be capable of promoting neuronal survival.

It seems likely that phospho-Y490-independent neuronal differentiation and survival reflect the presence of additional adaptors that have not been incorporated into current models. For example, two additional adaptors, rAPS and

Figure 3 Schematic diagram of Trk receptor-mediated signal transduction pathways. Binding of neurotrophins to Trk receptors leads to the recruitment of proteins that interact with specific phosphotyrosine residues in the cytoplasmic domains of Trk receptors These interactions trigger the activation of signaling pathways, such as the Ras, PI 3-kinase, and PLCγ pathways and ultimately result in activation of gene expression, neuronal survival, and neurite outgrowth. The nomenclature for tyrosine residues in the cytoplasmic domains of Trk receptors is based on the human sequence for TrkA.

SH2-B, are similar to PH- and SH2-domain-containing proteins with tyrosines that are phosphorylated following Trk activation (73, 74, 79). They are recruited by phosphorylated tyrosines in the activation loops of each Trk receptor and may also interact with additional phosphorylated tyrosines elsewhere in the cytoplasmic domains of these receptors. Both rAPS and SH2-B form both homo- and heterodimers and, after Trk activation, associate with Grb2, which provides a potential link through SOS to Ras signaling and through both Ras and the Gab adaptor proteins to PI3-kinase. A dominant-interfering construct of SH2-B and antibodies to SH2-B interfere with NGF-dependent survival, Erk activation, and axonal outgrowth by sympathetic neurons (74). Insulin receptor substrate-1 and -2 are adaptors that are tyrosine phosphorylated, following which they promote sustained association with and activation of PI3-kinase in cortical neurons after BDNF application (80). Ligand engagement of TrkA in PC12 cells does not result in insulin receptor substrate-1 or 2 phosphorylation. This indicates that an adaptor protein must be missing in this cell line. CHK, a homolog of the cytoplasmic tyrosine kinase CSK (control of Src kinase), interacts with activated TrkA in PC12 cells and enhances MAP kinase signaling and neurite outgrowth by these cells (81). The mechanism by which it acts is not understood. Finally, a transmembrane protein with three extracellular Ig domains and four tyrosines in the cytoplasmic domain is phosphorylated after BDNF application to cortical neurons and provides a docking site for the protein tyrosine phosphatase, Src-homology phosphatase (Shp2) (82). Overexpression of this protein enhances BDNF-dependent activation of PI3-kinase and survival of cortical neurons.

Providing additional potential complexity, the presence of more than one gene and differential splicing result in expression of more cytoplasmic adaptor and signaling proteins than are considered in simplified signaling models. For example, Shc is expressed at much higher levels in PC12 cells than in neurons, where expression of two closely related homologs, ShcB and ShcC, predominates. Similar to Shc, ShcB and ShcC interact with and are activated by neurotrophin binding to Trk receptors, but they then interact differentially with the repertoire of signaling proteins and exhibit differences in signal transmission (83, 84). Absence of both ShcB and ShcC results in apoptosis of sympathetic and sensory neuron populations whose survival is dependent upon Trk receptor-mediated signaling (85). Despite their potential importance, ShcB and ShcC have been much less intensely studied than Shc itself.

Evidence also indicates that not all interactions with Trk receptors depend upon phosphorylation of tyrosine. c-Abl, a cytoplasmic tyrosine kinase, interacts with the juxtamembrane domain of TrkA, whether or not tyrosines in this region are phosphorylated (86). Deletion of five conserved amino acids in this region blocks differentiation of PC12 cells without preventing phosphorylation of Shc or Frs2 (87). Because c-Abl is involved in many aspects of differentiation, it may well prove to have a role in Trk receptor-mediated signaling that is prevented by this deletion. Recent experiments also indicate that Trk receptors interact with components of the dynein motor complex, which suggests a role for these

interactions in Trk receptor trafficking (88). Later in this review, we review compelling evidence implicating membrane trafficking in regulation of Trk receptor-mediated signaling.

In summary, there is far more complexity to Trk receptor-mediated interactions with cytoplasmic adaptor proteins than is currently either appreciated or understood. Different adaptors almost certainly compete with each other for binding to activated Trk receptors. Different neurons clearly may have different subsets of these adaptors. Many adaptors have been identified in studies of other neuronal tyrosine kinases that may also prove to function in Trk receptor-mediated signaling (89). In the future, it will be important to characterize signaling interactions using discrete, well-identified populations of neurons. Almost certainly, this will require technological advances to permit both spatial and temporal examination of protein-protein interactions in single neurons or small populations of identical neurons purified from the complex mixture of neurons present in the nervous system (90).

TRK RECEPTOR EFFECTOR MECHANISMS

PLC-γ1 Signaling

When phosphorylated, Y785 on TrkA and analogous sites on TrkB and TrkC bind PLC-γ1, which is then activated through phosphorylation by the Trk receptor kinase (11) (Figure 3). Activated PLC-γ1 hydrolyses PtdIns(4,5)P_2 to generate inositol tris-phosphate (IP3) and diacylglycerol (DAG). IP3 promotes release of Ca^{2+} from internal stores, which results in activation of enzymes, such as Ca^{2+}-regulated isoforms of protein kinase C and Ca^{2+}-calmodulin-regulated protein kinases. DAG stimulates DAG-regulated protein kinase C isoforms. In PC12 cells, one of these, PKCδ, is required for NGF-promoted neurite outgrowth and for activation of Erk1 and Erk2 (91). PKCδ appears to act between Raf and MEK in this cascade because inhibition of PKCδ reduces activation of MEK, but not of c-Raf.

Not surprisingly, the signaling pathways activated in neuronal cells by Trk-mediated activation of PLC-γ1 extend to the nucleus. Of particular interest, a brief pulse of NGF has been shown to activate a sequence of transcriptional events that results in long-term induction of a sodium channel gene (92). Recently, use of site-specific phosphotyrosine antibodies demonstrated that a brief exposure of PC12 cells to NGF resulted in prolonged phosphorylation of Y785 lasting for several hours (93). Resistance of this site to protein phosphatases provides a likely explanation for the unexpectedly long duration of sodium channel gene induction. This observation illustrates the critical role that protein tyrosine phosphatases play in controlling Trk receptor signaling. It will be exceedingly useful to identify and characterize the expression patterns of the phosphatases responsible for dephosphorylation of each of the cytoplasmic phosphotyrosine residues in Trk receptors.

The physiological functions of TrkB-mediated PLCγ signaling pathways have been tested in vivo by mutating the recruitment site, Y816, to phenylalanine (94). Mice homozygous for the Y816F mutation (*trkB*$^{PLC\text{-}/PLC\text{-}}$) have a normal life span but are hyperactive compared with control littermates. Electrophysiological experiments show that the *trkB*$^{PLC\text{-}/PLC\text{-}}$ mutants have significant deficiencies in the induction of both the early and late phases of hippocampal CA1 long-term potentiation (LTP). Results are similar to those observed in animals in which a floxed *trkB* allele was deleted in the postnatal forebrain using Cre recombinase expressed under control of the Ca^{2+}-CaMKII promoter. Surprisingly, while the Erk-MAPK pathways have been implicated in late phase hippocampal LTP (95, 96), BDNF-dependent phosphorylation of Erk and the distribution of phospho-Erk appear to be unaffected in cortical neurons of *trkB*$^{PLC\text{-}/PLC\text{-}}$ mutants. In contrast, phosphorylation of CREB, CaMKII, and CaMKIV are severely impaired in these neurons. Interestingly, expression of the zinc finger transcription factor Egr-1 (Krox24, Zif268), which is a downstream target of both Ras-Erk and CREB signaling and has been shown to be important for hippocampal LTP, is markedly reduced in the hippocampus of *trkB*$^{PLC\text{-}/PLC\text{-}}$ mutants. Taken together, these data indicate that signaling initiated at the PLCγ1 docking site on TrkB is important for the initiation and maintenance of hippocampal LTP. Future analyses of *trkB*$^{PLC\text{-}/PLC\text{-}}$ mutants in other parts of the nervous system will probably reveal additional roles for PLCγ1-initiated signaling. In the future, it will be important to determine whether the entire phenotype of this site mutation is caused by deficiencies in PLCγ1 signaling or also reflects contributions from other proteins that are normally recruited to this same site, such as the cytoplasmic tyrosine kinase CHK (81).

Ras-MAP Kinase Signaling

Activation of the Ras-MAPK/Erk signaling cascade is essential for neurotrophin-promoted differentiation of neurons and PC12 cells. In the response of PC12 cells to neurotrophins, transient versus prolonged activation of Erk signaling is closely but not absolutely associated, respectively, with mitogenic-promoting and differentiation-promoting outcomes (97, 97a).

Several pathways lead from Trk receptors to activation of Ras; most of these appear to involve phosphorylation at Y490. For example, phosphorylated Y490 provides a recruitment site for binding of the PTB domain of the adaptor protein, Shc. After its own phosphorylation, Shc recruits the adaptor protein, Grb2, complexed with SOS, an exchange factor for Ras (and Rac) (98). The presence of activated Ras stimulates signaling through several downstream pathways, which include those mediated by Class I PI3-kinases, Raf, and p38MAP kinase (99, 99a). Activation of Erk1 and Erk2 requires sequential phosphorylation by Raf of MEK1 and/or MEK2 and then phosphorylation of Erk1 and Erk2 by MEK1 or MEK2 (100) (Figure 3). Ras-GTP probably activates p38MAP kinase through a pathway involving sequential activation of RalGDS, Ral, and Src

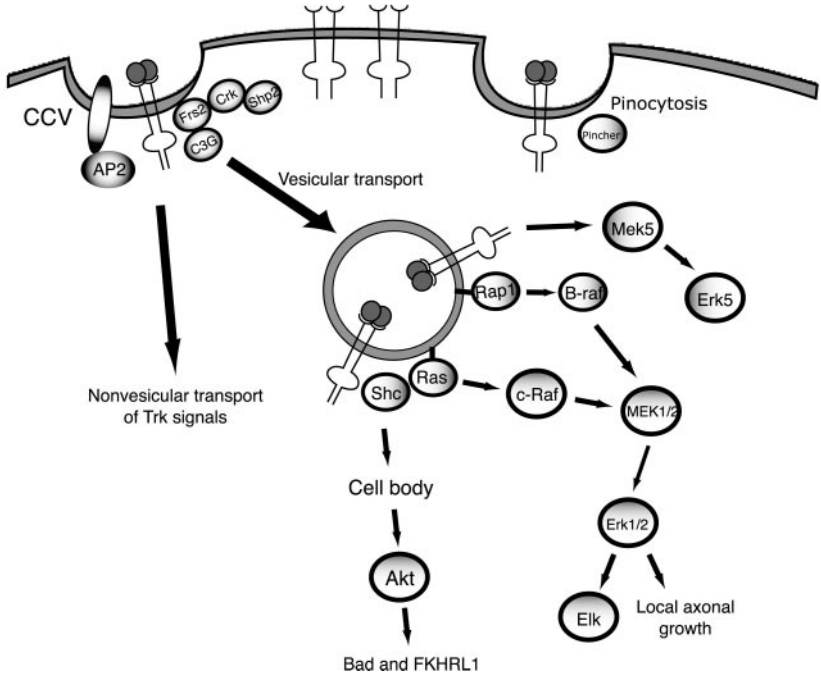


Figure 4 Signaling of Trk receptors by vesicle-mediated transport and vesicle-independent propagation of signals. This diagram illustrates the two distinct pathways that have been shown to propagate signals transmitted upon the activation of Trk receptors. Vesicular transport can be mediated through the clathrin-coated endosomal vesicles and/or by pinocytosis, which can be enhanced by Pincher, a novel protein induced by NGF in PC12 cells.

(99b). p38MAP kinase activates MAP kinase-activated protein kinase-2 (99). Ras activation also triggers a signaling cascade independent of Raf that results in activation of Erk5 through sequential activation of MEKK3 and MEK5 (101–103) (Figure 4). Trk receptor-mediated stimulation of Ras through Shc and Grb2/SOS promotes transient but not prolonged activation of Erk signaling (104). Termination of signaling through this pathway appears to be caused by Erk- and Rsk-mediated phosphorylation of SOS, which results in dissociation of the SOS-Grb2 complex (105).

In contrast to the transient activation of MAP kinase signaling promoted through Shc, Grb2/SOS, and Ras, prolonged Erk activation depends on a distinct signaling pathway involving the adapter protein Crk, the guanine nucleotide exchange factor C3G, the small G protein Rap1, the protein tyrosine phosphatase Shp2, and the serine threonine kinase B-Raf (72, 104, 105) (Figure 4). Trk signaling appears to initiate activation of this pathway by recruitment of an

adaptor fibroblast growth factor receptor substrate-2 (Frs2) to phosphorylated Y490 (106, 107). Trk activation results in Frs2 phosphorylation (Figure 3). Frs2 provides binding sites for numerous additional signaling proteins, including the adaptors Grb2 and Crk, the enzymes c-Src and Shp2, and the cyclin-dependent kinase substrate $p13^{suc1}$ (106). Trk signaling increases the association of Crk with Frs2 (105). Association with Crk results in activation of the guanyl nucleotide exchange factor C3G for Rap1(108). Rap-1-GTP stimulates B-Raf, which initiates the Erk cascade (Figures 3 and 4). In addition, Frs2 also contains several phosphorylation-dependent recruitment sites for Grb2; Grb2 provides a mechanism independent of Shc for activation of Ras through the Grb2/SOS exchange factor complex. As expected, overexpression of intermediates in this pathway promotes differentiation of PC12 cells (106, 109–111). Although associated with prolonged MAP kinase signaling, differentiation-promoting effects of Frs2 and Crk are almost certainly not mediated solely through this pathway. The activity of Shp2, which is associated with Frs2, is essential for NGF-dependent activation of the MAP kinase pathway and probably acts by inactivation of an inhibitor, such as Ras-GAP or MAP kinase phosphatase (112) (Figure 3). The recruitment of c-Src to Frs2 may well promote differentiation that synergizes with, but is not dependent upon Frs2-mediated promotion of MAP kinase signaling through B-raf, because activation of endogenous c-Src has been shown to promote neurite outgrowth without altering the kinetics of MAP kinase signaling in PC12 cells (97). Crk contains a binding site for DOCK-180, an exchange factor for Rac (113). Rac activation is also likely to promote differentiative responses through Jun kinase, the Arp2/3 complex, and other effectors that are not completely dependent upon MAP kinase signaling. Finally, despite a consensus on the importance of Crk-associated signaling proteins in mediating activation of MAP kinases by Trk receptors, different groups have reached quite different conclusions on the constituents and assembly kinetics of Crk-associated signaling complexes (72, 105). In particular, these groups have reached different conclusions about the presence of Frs2 in these complexes.

The various MAP kinases activated through Ras and Rap1 have different downstream targets that synergize with each other to mediate gene transcription and cell differentiation. Thus, Erk1, Erk2, and Erk5 substrates include the Rsk family of protein kinases (Figure 3). Rsks and MAP kinase-activated protein kinase-2 can each phosphorylate CREB. CREB has been shown to regulate genes whose products are essential for normal differentiation and prolonged survival of neurons in vitro and in vivo (114, 115). However, different MAPKs also have specific transcription factor targets. For example, Erk5, but not Erk1 or Erk2, activates MEF2 directly; whereas Erk1and Erk2, but not Erk5, activate Elk-1 (116). Recent results indicate that NGF signaling can activate Erk1- and Erk2-mediated signaling locally, but not at a distance (102), which may further contribute to specificity. NGF that is internalized at the growth cone and transported to the cell body appears to activate only Erk5 and not Erk1 or Erk2.

The transcriptional targets of NGF-regulated transcription factors are diverse. Of particular interest, recent work implicates the transcription factor Egr-1, which is the product of an immediate early response gene, in the signaling pathway leading to cell cycle withdrawal and neurite outgrowth in PC12 cells (117–119). NGF application induces transcription of *egr-1*, which in turn induces transcription of the *p35* gene, whose protein product activates Cdk5. Inhibition of either Egr-1 or Cdk5 suppresses NGF-stimulated but not cAMP-stimulated neurite outgrowth. Egr-1 acts, at least in part, through interaction with and activation of c-Jun and not by direct binding to DNA (118). Forced expression of a constitutively active MEK is reported to activate Erk, which results in transcription of the *egr-1* and *p35* genes and neurite outgrowth (119). Unfortunately, there is disagreement on whether MEK inhibitors block NGF-mediated induction of this interesting transcriptional and signaling network (117, 119). In any event, this work illustrates what will undoubtedly become a major effort in this field, namely to identify the intermediates between proximal Trk-induced signaling and the various differentiated phenotypes of neurons ultimately evoked by neurotrophins.

PI3-Kinase Signaling

Production of P3-phosphorylated phosphoinositides promotes survival of many populations of neurons. Phosphatidylinositides are generated by PI3-kinase and activate phosphatidylinositide-dependent protein kinase (PDK-1). Together with these 3-phosphoinositides, PDK-1 activates the protein kinase Akt (also known as PKB), which then phosphorylates several proteins important in promoting cell survival (Figure 3). Class I PI3-kinases are activated through Ras-dependent and independent pathways (99a, 120, 121). Direct activation by Ras of PI3-kinase is a major pathway through which Trk signaling promotes survival in many, but not all, neurons (122, 123). In addition, phosphorylated Grb2 recruits the adaptor proteins Gab1 and Gab2 (122–124). Subsequently, phosphorylated Gab proteins recruit and facilitate the activation of Class I PI3-kinases. In many neurons, but not in PC12 cells, Trk signaling has been shown to result in phosphorylation of insulin receptor substrate-1, which in turn recruits and activates Class I PI3-kinases (80).

In response to growth factor signaling, Gab proteins also nucleate formation of complexes that include the tyrosine phosphatase Shp2 (124). As discussed earlier, Shp2 enhances activation of MAP kinase signaling.

Substrates of Akt include proteins involved in several steps of cell death pathways (120, 121). One of these, Bad, is a Bcl-2 family member that promotes apoptosis through sequestration of Bcl-XL, which otherwise would inhibit Bax, a proapoptotic protein (125). 14-3-3 proteins bind to phosphorylated Bad; this interaction prevents Bad from promoting apoptosis. In addition, Akt-mediated phosphorylation of Bad at S136 has been shown to increase the accessibility of a conserved phosphorylation site (S155) in the BH3 domain of Bad to protein

kinases; these include protein kinase A (126). Phosphorylation of S155 directly prevents binding of Bad to Bcl-XL. 14-3-3 proteins, binding to phospho-S136, function as essential cofactors that facilitate phosphorylation of S155. Phosphorylation of both S136 and S155 is necessary to inhibit completely the proapoptotic activity of Bad. Activation of MAP kinase signaling also results in phosphorylation, probably by a RSK, of Bad at S112 (126a, 126b). Phospho-S112 may also promote binding to 14-3-3 proteins and increase the accessibility of S155 to survival-promoting kinases (126). Many additional proteins in the apoptotic pathway, including Bcl-2, Apaf-1, caspase inhibitors, and caspases, also have consensus sites for Akt-mediated phosphorylation, but they have not been shown to actually be substrates of this kinase (121).

The activity of glycogen synthase kinase 3-β (GSK3β) is also negatively regulated by Akt-mediated phosphorylation (127). In cultured neurons, elevated GSK3β promotes apoptosis, so inhibition through phosphorylation undoubtedly contributes to the prosurvival effects of Trk activation. The inhibitory binding partner for NFκB, IκB, is another substrate of Akt. Akt-mediated phosphorylation of IκB promotes its degradation, which results in liberation of active NFκB. NFκB-promoted gene transcription has been shown to promote neuronal survival. Activation of the Trk and $p75^{NTR}$ receptors both promote activation of NFκB (68, 76). Finally, the forkhead transcription factor, FKHRL1, is another substrate of Akt (128, 129). Phosphorylation of FKHRL1 within the nucleus is followed by formation of a complex of FKHRL1 and 14-3-3 (128). The complex is exported from the nucleus and sequestered in the cytoplasm. Because FKHRL1 promotes expression of several proapoptotic proteins, including the FasL (Figure 3), preventing its nuclear entry promotes survival.

PI3 kinase-mediated signaling does not simply promote cell survival. Akt has been shown to activate effectors not involved directly in the survival response, e.g., p70 and p85 S6 kinases, which are important for promoting translation of a subset of mRNAs (for example, those encoding some of the cyclins) (130). In addition, the 3-phosphoinositides generated by PI3 kinase recruit many signaling proteins to the membrane; these include regulators of the Cdc-42-Rac-Rho family of G proteins that are activated through ligand engagement of Trk receptors and control the behavior of the F-actin cytoskeleton (130a). Localized activation of PI3 kinase has been shown to result in localized activation of these G proteins, which permits directed cell motility (131, 132). As mentioned earlier, activated Ras has also been shown to activate an exchange factor activity of SOS for Rac through a mechanism dependent upon PI 3-kinase (98). Localized Trk-promoted activation of Ras and PI 3-kinase almost certainly accounts for the ability of neurotrophin gradients to steer growth cones (133, 134). Activation of Akt by Trk receptors also appears to have effects on axon diameter and branching that are distinct from those observed after MAP kinase activation (135). In the future, it will be very interesting to dissect the pathways responsible for this intriguing phenotype.

REGULATION OF SIGNALING THROUGH MEMBRANE TRANSPORT OF TRK RECEPTORS

Neurotrophin sources are frequently localized in tissues at substantial distances from the cell bodies of innervating neurons. Although neurotrophins have local effects on signaling at axon terminals that affect growth cone motility and exocytosis, it is well established that signaling in the cell soma and nucleus is essential to promote neuronal survival and differentiation (6, 7). During the past few years, there have been intensive studies aimed at identifying mechanisms of retrograde signal transduction, but many issues remain to be clarified.

On the cell surface, Trk receptors are enriched in caveoli-like areas of the plasma membrane (136). Ligand engagement stimulates internalization of Trk receptors through clathrin-coated pits and by macropinocytosis in cell surface ruffles (137, 138) (Figure 4). After internalization, neurotrophins are localized with Trk receptors in endosomes that also contain activated signaling intermediates, such as Shc and PLC-γ1 (139). Retrograde transport of these endosomes provides a conceptually attractive mechanism through which neurotrophin-mediated signals can be conveyed to the cell soma and nucleus.

While there is unambiguous evidence that TrkA is transported together with NGF to the cell soma and that TrkA activity within the soma is required for transmission of a neurotrophin-initiated signal (115), a recent report has demonstrated that neuronal survival can be supported by bead-coupled NGF application to distal axons with little or no internalization and transport of NGF (62). These results suggest the existence of a mechanism for propagation of neurotrophin signaling that does not require internalization and transport of the neurotrophin. Lateral propagation of Trk receptor activation, similar to the activation of ErbB1 observed after focal application of bead-attached EGF (90), provides a potential mechanism through which unliganded Trk receptors might be activated, internalized, and retrogradely transported. Alternatively, there may be retrograde transport of some of the signaling intermediates activated by Trk receptor action. It is unclear how the activation state of these receptors or signaling intermediates would be maintained in the absence of NGF, but it is conceivable that, after concentration in endocytotic vesicles, the basal activity of Trk receptors is sufficiently high to maintain activity. When overexpressed, Trk receptors have been shown to have relatively high levels of basal activation (63).

A second topic of contemporary interest is the role of endocytosis and subsequent membrane trafficking of Trk receptors in controlling Trk receptor-initiated signaling cascades. Recent evidence indicates that membrane sorting events help determine which pathways are activated by Trk receptors, probably because signaling intermediates are preferentially localized in different membrane compartments. For example, unusually rapid internalization of TrkA is induced by exposure to a complex of NGF and a monoclonal antibody (mAb) that does not interfere with receptor binding (70). This NGF-mAb complex promotes Shc phosphorylation, transient Erk activation, and survival of PC12

cells, but it does not promote phosphorylation of Frs2 or differentiation of PC12 cells. Frs2, which is N-myristoylated, is preferentially concentrated in lipid rafts on the cell surface (140). Perhaps because of this localization, it is only recruited by TrkA with comparatively slow kinetics, so that rapid internalization of TrkA prevents its recruitment. Future experiments will be essential to test this hypothesis. Counterintuitively, a thermosensitive dynamin that inhibits clathrin-mediated endocytosis at high temperature has an effect similar to that of the NGF-mAb complex, which acts to inhibit differentiation-promoting but not survival-promoting effects of Trk receptor signaling (141). As work described earlier has indicated that prolonged MAP kinase activation through a complex containing Crk, C3G, and Rap1 is required for differentiation-promoting responses of PC12 cells, the data suggest that Frs2 must be recruited by Trk on the cell surface, but Trk and Frs2-containing signaling complexes must then be internalized to efficiently activate Rap1. Examination of the differential distributions of Ras and Rap1 provide an attractive explanation for the data (71, 72). Ras is prominently expressed on the cell surface where it can be activated without the necessity of TrkA internalization. In contrast, Rap1 appears to be localized almost exclusively in small intracellular vesicles. Thus the data suggest that Trk must be internalized into intracellular vesicles that then fuse with endosomal vesicles containing Rap1 to permit sustained activation of the Erk pathway and normal differentiation of PC12 cells.

Two provocative recent papers have suggested that Trk signaling is regulated by additional, poorly understood mechanisms. In one contribution, local application of NGF to either the distal axons or cell bodies of dorsal root ganglion sensory neurons was shown to activate both the Erk1-Erk2 and Erk5 signaling cascades (102). Surprisingly, application of NGF to the distal axons resulted at the cell somas in activation of Erk5 in the cell soma, but not of Erk1 or Erk2. Activation of Erk5 at the cell somas required receptor internalization because it was inhibited by a thermosensitive dynamin. Local application of K252a, a relatively specific Trk inhibitor, at either the distal axon or cell body also prevented activation of Erk5 in the cell soma by NGF applied at the distal axon. Thus, the results strongly support a model in which active Trk receptors are internalized into signaling endosomes, but the mechanism through which distance affects the specificity of signaling is very uncertain.

One possibility, supported by some recent data, is that Trk receptor activation generates more than one population of signaling endosomes that differ in their potential for retrograde transport. In addition to clathrin-dependent endocytosis through coated pits, it has recently been shown that Trk receptors can also be internalized by pinocytosis (138). A novel protein, Pincher, has been identified that promotes pinocytosis, but not endocytosis, of TrkA together with NGF (138). In PC12 cells, overexpression of Pincher mutated in an ATP-binding site inhibits pinocytosis and prevents the intracellular accumulation of phospho-Erk5 without affecting that of phospho-Erk1 and -Erk2. This result suggests that Clathrin and Pincher may promote formation of endosomal vesicles that differ in

their signaling potential. If only the contents of the latter vesicles are transported efficiently in the retrograde direction, Erk5 might be preferentially activated through retrograde transport. To achieve a molecular understanding of these observations, it will be essential to confirm these results and to compare the molecular compositions of the vesicle populations formed by endocytosis and pinocytosis.

In conclusion, Trk receptor-mediated signaling is regulated by both the kinetics and specificity of membrane transport. Regulation of membrane transport provides many additional potential steps at which specificity can be imposed upon signaling.

ACTIVATION OF ION CHANNELS, RECEPTORS AND OTHER RECEPTOR TYROSINE KINASES

Activation of Trk receptors regulates the expression and activities of ion channels, neurotransmitter receptors, and other receptor tyrosine kinases. Trk receptor activation also modulates exocytosis and endocytosis of synaptic vesicles. Some of these effects are observed in seconds to minutes and clearly do not require protein synthesis, while other actions do involve regulation of gene expression through control of transcription factors, some of which have been described above. Work to date implicates regulation of activity through lipid metabolism and protein phosphorylation, localization of proteins and organelles, and local regulation of protein translation in addition to control of gene expression. Each of these mechanisms affects function of the synapse, and these mechanisms are attractive candidates to mediate the important roles that neurotrophins have in regulating synaptic plasticity in the hippocampus and elsewhere. Only a few of the most recent examples of short-term regulation can be cited here. This area is more extensively reviewed elsewhere (4, 14).

It has only recently been appreciated that Trk receptors control membrane properties on a time scale similar to the actions of classical neurotransmitters, and studies on these phenomena are consequently of intense current interest (75, 142–144). For example, low concentrations of BDNF and NT4 have been shown to activate a sodium ion conductance as rapidly as glutamate in slices of CA1 hippocampus, cortex, and cerebellum (144). Neurotrophin action is reversibly blocked by K252a, a reasonably selective Trk receptor inhibitor, which indicates that signaling occurs through TrkB. Until the Na^+ channel regulated so directly by Trk activation is identified, it will be difficult to pursue mechanistic studies, but the extremely rapid response time argues that this channel is very likely to physically associate with TrkB in these neurons. BDNF and NT4 also have been shown to activate postsynaptic Ca^{2+} currents in dentate granule cells. Activation of the Ca^{2+} channels may well be secondary to membrane depolarization resulting from the activation of the Na^+ channel described above. Interestingly, dendritic spines appear to be the exclusive site of rapid activation by BDNF of

these Ca^{2+} channels, which synergize with NMDA receptors to induce robust LTP. Not surprisingly, several groups studying different preparations have observed that neurotrophins also enhance intracellular Ca^{2+} levels and potentiate synaptic transmission through PLCγ1-mediated generation of IP3 that results in release of intracellular Ca^{2+} stores (145).

In addition to synaptic actions on Na^+ and Ca^{2+} currents, Trk receptor activation has also been shown to activate several members of the TRP family of cation channels. Many members of this family had previously been shown to be activated through PLC. In the CNS, TRPC3 is broadly expressed and is activated through TrkB-dependent activation of PLC (146). In nociceptive sensory neurons, activity of the heat-activated TRP channel, VR1 is potentiated by NGF signaling through TrkA resulting in hypersensitization to thermal and mechanical stimuli (75). In this instance, the channel is activated by depletion of phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2) following hydrolysis by PLC or antibody sequestration. TrkA and VR1 can be coimmunoprecipitated, which indicates that they exist in a macromolecular complex in the plasma membrane. It seems quite likely that relief from PtdIns(4,5)P_2-mediated repression will provide an explanation for the PLC-dependent activation of other TRP channels. It will also be interesting to determine whether other members of this family form complexes with Trk receptors. While it seems important to examine in the TrkB-PLCγ1 docking site mutant (94), the response to BDNF of the rapidly activated CNS Na^+ channel described in the previous paragraph (144), this channel appears to be activated far more rapidly than would be possible through recruitment and activation of PLCγ1. Trk receptors almost certainly activate this channel by a more direct mechanism.

Trk receptor signaling also controls the activity and localization of neurotransmitter receptors through protein phosphorylation. For example, BDNF activation of TrkB promotes the phosphorylation and dephosphorylation of the NMDA receptor subunit NR2B with phosphorylation increasing the open probability of the NMDA receptor ion channel and thereby rapidly enhancing synaptic transmission (147, 148). Trk signaling has been shown to be essential to maintain the integrity of the neuromuscular junction in vivo and clustering of neuronal acetylcholine receptors in vitro by signaling mechanisms that have not been further dissected (149, 150). TrkB-mediated signaling also promotes formation of synapses by inhibitory interneurons in the mouse cerebellum and by the axons of retinal ganglion cells in the optic tectum of *Xenopus laevis* (151, 152).

Rapid potentiation of synaptic transmission has been seen in many systems, including developing *Xenopus* neuromuscular synapses, hippocampal cultures, and brain synaptosomes. In the latter, potentiation by BDNF of transmitter release is mediated by an Erk-mediated phosphorylation of synapsin I (153). Potentiation is not observed in synaptosomes isolated from synapsin I-deficient mice and is prevented by inhibitors of the Erk cascade. Synapsins immobilize synaptic vesicles through attachment to the F-actin cytoskeleton and phosphor-

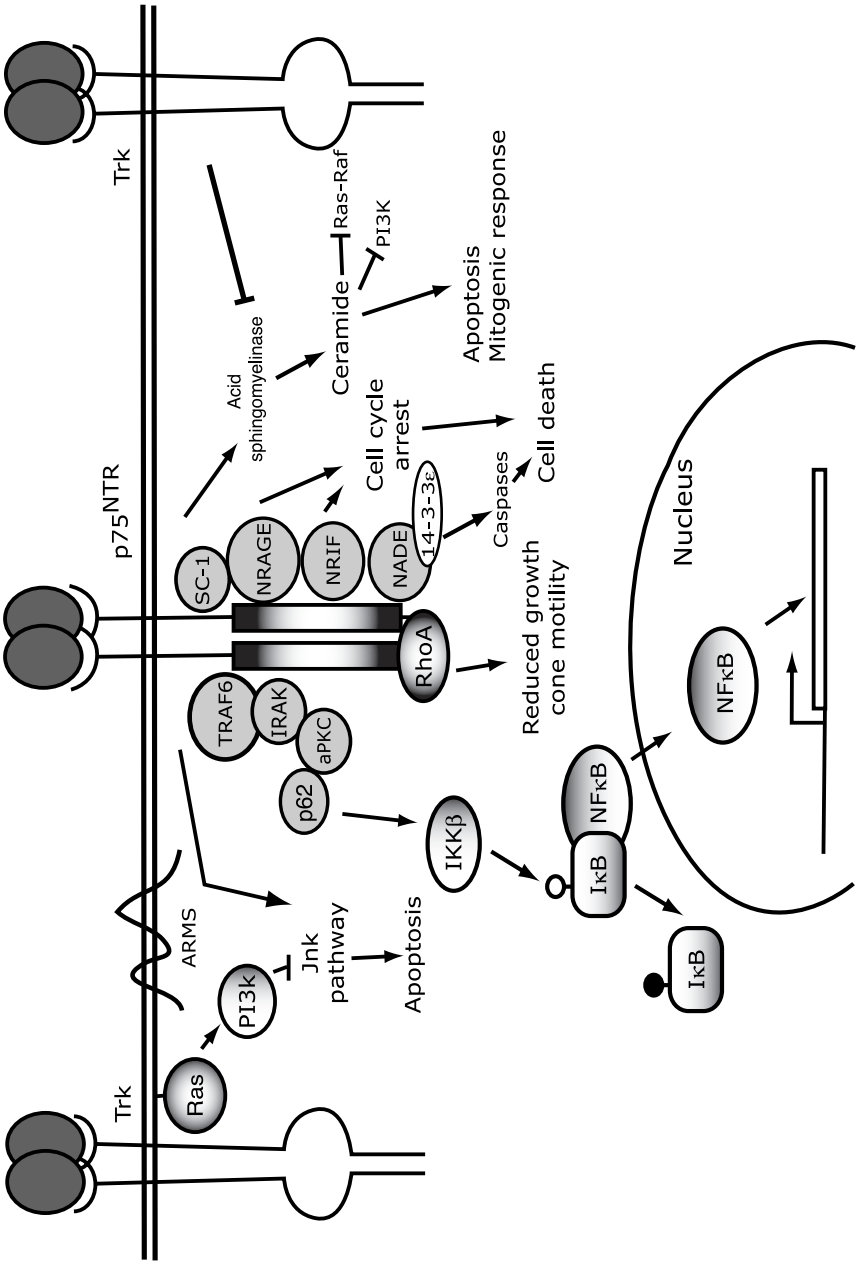
ylation by Erk inhibits synapsin interactions with F-actin. Thus Erk signaling in this system probably potentiates synaptic transmission by releasing synaptic vesicles from the cytoskeleton. In addition to these acute effects of Trk activation, additional deficits are seen in mice lacking normal TrkB-mediated signaling. These include reductions in vesicles docked at release sites and reduced expression of synaptic proteins (154).

While its functional significance has not yet been fully explored, BDNF has recently been shown to regulate local translation of a GFP reporter mRNA in dendrites (155). This reporter contained the 5′ and 3′ untranslated regions of the *Ca^{2+}-CAMKII-alpha* mRNA that confer dendritic localization and BDNF-controlled translational regulation. In a variety of previous studies, protein synthesis-dependent synaptic potentiation has been observed in response to neurotrophins (14). The mechanisms revealed through use of this GFP reporter seem almost certain to be contributors to these phenomena. In the future it will be exceedingly interesting to identify the repertoire of mRNAs whose translation is controlled locally by neurotrophins.

Finally, in postnatal sympathetic neurons, Trk receptors can also activate at least one additional receptor tyrosine kinase, the long-tailed Ret51 isoform of c-Ret (156). TrkA-mediated phosphorylation of Ret51 increases with postnatal age and does not involve the ligand-binding coreceptors required for c-Ret activation by GDNF and related trophic factors. Activation occurs with unusually extended kinetics and does not depend upon PI3 kinase or MAP kinase signaling. The presence of c-Ret is essential to observe full trophic responses of sympathetic neurons to NGF in vivo. Signaling mechanisms are not known but should be vigorously pursued in the future.

INTERACTIONS WITH P75^{NTR}- AND P75^{NTR}-REGULATED SIGNALING PATHWAYS

Each of the neurotrophins also binds to the pan-neurotrophin receptor p75^{NTR}, a member of the TNF receptor superfamily (5, 8). p75^{NTR} binds each of the neurotrophins with approximately equal affinity. Recent work has also shown that p75^{NTR} interacts with the Nogo receptor as a signal-transmitting subunit that mediates inhibitory effects on axon growth of three myelin-associated glycoproteins—Nogo, MAG, and Omgp (156a, 156b). Neurotrophin binding to p75^{NTR} promotes survival of some cells and apoptosis of others as well as affects axon outgrowth both in vivo and in vitro. p75^{NTR} exerts these diverse actions through a set of signaling pathways largely distinct from those activated by Trk receptors that can only be summarized in this review because of space constraints (8) (Figure 5). Prosurvival pathways activated by p75^{NTR} include NFκB and Akt (76, 157). Ligand binding to p75^{NTR} also stimulates several proapototic pathways, which include the Jun kinase signaling cascade, sphingolipid turnover, and association with several adaptors (e.g., NRAGE and NADE) that directly promote



cell cycle arrest and apoptosis (158–161). $p75^{NTR}$ also activates the small G proteins Rac and Rho that directly affect growth cone motility (162). Signaling by Trk receptors modulates signaling through many of these $p75^{NTR}$-mediated pathways, thereby altering the nature of the signals conveyed by neurotrophins to neurons. An important consequence is that in the absence of Trk receptor activation neurotrophins are much more effective at inducing apoptosis through $p75^{NTR}$.

Recent experiments have identified adaptors that appear able to nucleate formation of multiprotein complexes including both $p75^{NTR}$ and Trk, which provide scaffolds for the reciprocal interactions between these two receptors. One of these, named ankyrin repeat-rich membrane spanning adaptors (ARMS), has been shown to interact with both receptors and to be phosphorylated following either Trk or Eph receptor activation (163) (Figure 5). Caveolin provides another platform that may mediate interplay between Trk and $p75^{NTR}$ signaling because it has been shown to interact with both of these receptors (164). A third platform is a multiprotein complex required for NFκB activation (76). Neurotrophins have been shown to promote NFκB activation and NFκB-promoted survival through $p75^{NTR}$ (165). NGF promotes the association of the adaptor protein TRAF6 with the juxtamembrane region of $p75^{NTR}$ (166). Interleukin 1 receptor-associated kinase (IRAK) is recruited to this complex leading to formation of a complex of TRAF6 and IRAK with atypical protein kinase C-ι (aPKC-ι) and the aPKC-interacting protein, p62 (167, 76). IκB kinase-β (IKKβ), a known substrate of aPKC, is recruited to the complex; this results in phosphorylation and degradation of IκB and leads to activation of NFκB. Activation of NFκB depends upon p62 and the kinase activity of IRAK. In addition to mediating NFκB activation, the p62-TRAF6-IRAK complex also can function as a scaffold for association of TrkA and $p75^{NTR}$ with TRAF6 binding to $p75^{NTR}$ and p62 to TrkA (76). NGF mediates assembly of this complex, which stimulates association of TRAF6 with both $p75^{NTR}$ and p62 prior to the recruitment of p62 to TrkA. Intriguingly, even though TrkA-mediated signaling in the absence of $p75^{NTR}$ stimulation results in activation of NFκB (68), probably through phosphorylation of IκB by Akt (121), activation of Trk in the presence of $p75^{NTR}$ stimulation actually suppresses NFκB activation and retards the degradation of IκB in PC12 cells (168). An attractive but unproven possibility is that interactions of TrkA with p62 prevent p62 from functioning as a scaffold to facilitate activation of IRAK, aPKC, or IKKβ. In neurons, at least, suppression can not be complete, because in the

Figure 5 Schematic diagram of $p75^{NTR}$-mediated signal transduction pathways. $p75^{NTR}$ interacts with proteins (TRAF6, RhoA, NRAGE, SC-1, and NRIF) and regulates gene expression, the cell cycle, apoptosis, mitogenic responses, and growth cone motility. Binding of neurotrophins to $p75^{NTR}$ has also been shown to activate the Jnk pathway, which can be inhibited by activation of the Ras-PI 3-kinase pathway by Trk receptors. See text for detailed descriptions.

presence of Trk signaling, activation of the NFκB cascade by $p75^{NTR}$ has been shown to make a synergistic contribution to survival (165).

Trk activation also affects $p75^{NTR}$-mediated signaling through other mechanisms. Activation of a Trk receptor completely suppresses the activation by $p75^{NTR}$ of acidic sphingomyelinase through association of activated PI3-kinase with acidic sphingomyelinase in caveoli-related domains (169, 170). Acidic sphingomyelinase activation results in generation of ceramide that promotes apoptosis and mitogenic responses in different cell types through control of many signaling pathways, which include Erk, PI3-kinase, and aPKC. For example, ceramide binds to Raf and may induce formation of inactive Ras-Raf complexes, thereby inhibiting Erk signaling (171). Ceramide also inhibits the activity of PI3-kinase, either by modifying the association of receptor tyrosine kinases and PI3-kinase with caveolin in lipid rafts or by directly inhibiting PI3-kinase activity (172, 173). Thus Trk receptor-mediated suppression of these $p75^{NTR}$-mediated signaling pathways prevents interference by $p75^{NTR}$ with the survival and differentiation-promoting actions of neurotrophins.

Trk receptor activation also suppresses activation of the Jnk cascade by $p75^{NTR}$-mediated signaling. In the absence of Trk receptor activation, the activated Jnk cascade promotes apoptosis through elevation of p53 (13). Both Akt and c-Raf have recently been shown to interact with and inactivate the protein kinase ASK-1, an upstream activator of the Jnk pathway (174, 175). Akt also inhibits Jnk signaling by binding to a Jnk-associated scaffolding protein Jip1 (176).

Although Trk receptors suppress $p75^{NTR}$-mediated signaling, Trk receptors are not always completely efficient at preventing $p75^{NTR}$-mediated apoptosis. NGF, for example, increases apoptosis of cultured motor neurons from wild-type, but not from $p75^{NTR-/-}$ embryos (177). In PC12 cells, $p75^{NTR}$ activation is reported to reduce NGF-dependent TrkA autophosphorylation (178).

The studies cited above indicate that proapoptotic signaling by $p75^{NTR}$ is strongly inhibited by Trk-mediated signaling through Raf and Akt. As described earlier, $p75^{NTR}$ also appears to refine the ligand-binding specificities of Trk receptors. This may result in apoptosis of neurons not exposed to their preferred trophic factor environment. Consistent with this role for $p75^{NTR}$, *p75*$^{NTR-/-}$ embryos exhibit less apoptosis in both the retina and spinal cord (179). Recent experiments indicate that in the embryonic chick retina coordinated signaling by TrkA and $p75^{NTR}$ functions to regulate the number of mature retinal ganglion cells (180). Differentiated retinal ganglion cells have been shown to express TrkA, $p75^{NTR}$, and NGF, while migrating precursors express only $p75^{NTR}$. Survival of the migrating precursors is impaired by the presence of the differentiated retinal ganglion cells and by NGF; this suggests that NGF secreted by mature retinal ganglion cells functions to promote apoptosis only in the immature cells that express $p75^{NTR}$, but not TrkA (180).

In contrast to these observations, the absence of $p75^{NTR}$ reduces the number of surviving sensory neurons (181). The presence of $p75^{NTR}$ has been shown to

increase retrograde transport of neurotrophins (182, 183). In addition, sensory axon outgrowth is slower than normal during development in the absence of $p75^{NTR}$, which possibly reflects the recent demonstration that $p75^{NTR}$ activates Rho in the absence but not in the presence of NGF (184). As a result, axons may not be exposed to adequate levels of neurotrophins in *$p75^{NTR-/-}$* animals. The recent demonstration that $p75^{NTR}$ interacts with the NOGO receptor to mediate the inhibitory effects of several myelin-associated glycoproteins on growth cone motility also raises that possibility that absence of $p75^{NTR}$ disrupts signaling pathways important in sensory neuron survival not related to neurotrophin function (156a, 156b). Determination of the mechanisms underlying the neuronal deficits observed in *$p75^{NTR-/-}$* animals is an important issue for future investigation. It will also be interesting to examine the effects of the presence of myelin-associated glycoproteins on the signaling events initiated by neurotrophin-binding to Trk and $p75^{NTR}$.

SPECIFICITY IN TRK RECEPTOR-MEDIATED SIGNALING

Although the proximal signaling mechanisms of Trk receptors appear very similar, the effects of signaling through these different receptors are occasionally quite different, and it is a major challenge to understand how these differences arise at the molecular level. As the best example, gradients of neurotrophins have long been known to steer growth cones in vitro (185). In these assays, each of the neurotrophins can be either a chemoattractant or chemorepellant, depending upon the second messenger levels within the neurons (186). When assayed using *Xenopus* motor neurons expressing an appropriate Trk receptor, for example, NGF and BDNF normally attract growth cones, but in the presence of cAMP inhibitors, these factors instead repel growth cones. Similarly, NT3 is a chemoattractant for these growth cones, but is a chemorepellant for the same growth cones when cultured in the presence of cGMP. Inhibitors of cAMP-mediated signaling have no effect on NT3-mediated chemotropism, and inhibitors of cGMP-mediated signaling have no effect on the chemotropic responses to NGF and BDNF. In addition, a uniformly high concentration of netrin, a guidance protein whose actions are also regulated by cAMP, extinguishes chemotropic responses to NGF and BDNF but does not affect responses to NT3. These neurotrophins have also been shown to differ in their effects on synaptic transmission at the neuromuscular junction where BDNF-mediated potentiation of neurotransmitter release requires extracellular Ca^{2+} influx, whereas NT3-mediated potentiation is independent of extracellular Ca^{2+} but requires Ca^{2+} stores and activation of Ca^{2+}-calmodulin-dependent kinase II and PI 3-kinase (14, 187). NT3 and BDNF also have strikingly different effects on the physiological properties and ion channel composition of spiral ganglion neurons (14, 188). Thus, despite their similarities in signaling, NGF and BDNF appear to

regulate axon guidance, synaptic function, and neuronal differentiation through signaling pathways that sometimes differ fundamentally from those utilized by NT3. Achieving a satisfactory understanding of the molecular underpinnings of these differences is one of the most interesting challenges facing workers in this field.

In a preliminary effort to examine differences between TrkB- and TrkC-mediated signaling, mice have been generated with Shc-site mutations in TrkB and TrkC (equivalent to Y490F in TrkA) (77, 78). As described earlier, phospho-Y490 is a major recruitment site for Shc and Frs2. In comparisons of mice with Shc-site mutations in TrkB and TrkC, surviving sensory neurons lost target contact in the former but not the latter animals (78). In biochemical assays using the Shc-site mutants, autophosphorylation of TrkB, but not of TrkC, was reduced compared to autophosphorylation of wild-type receptors. Thus these data represent a first attempt to understand specificity in Trk receptor-mediated signaling. It is uncertain whether these differences observed in biochemical assays are relevant for understanding the fundamental differences in the pathways utilized by BDNF and NT3 in axon guidance.

Intriguingly, the Shc-site mutation in TrkB resulted in losses of neurons dependent upon NT4, but with only modest affects on BDNF-dependent neurons (77). In culture, NT4-stimulated Erk activation was more severely compromised than BDNF-stimulated Erk activation in neurons homozygous for the Shc-site mutant. NT4-dependent neuronal survival was similarly impaired in vitro. Thus, these data argue that specificity in signaling through a receptor can be controlled by interactions with different ligands.

CONCLUSION

Neurotrophins are now known to have surprisingly diverse roles in development and function of the nervous system, considering that they were discovered as target-derived trophic factors that ensure during development a match between the number of neurons and the requirement for their function. Neurotrophins have been shown to regulate cell fate, axon growth and guidance, dendrite structure and pruning, synaptic function, and synaptic plasticity. The discovery of Trk receptors was a tremendous advance for these studies and made it possible to initiate studies of the signaling pathways involved in each of these phenotypes. Analyses of Trk receptor structures and signaling properties have been very productive but are still incomplete. While progress in mechanistic studies has been rapid using model cells, such as PC12, much less is known about the pathways utilized by subpopulations of neurons, and it has become clear that different neurons exhibit very different responses to the same ligand. A major challenge for the future will be to devise methodologies appropriate for studying these cell populations that differentiate and function in the context of an exceedingly complex nervous system.

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CONTENTS