CMLS, Cell. Mol. Life Sci. 58 (2001) 1021–1035
1420-682X/01/081021-15 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Receptor binding, internalization, and retrograde transport of neurotrophic factors

K. E. Neet[a,*] and R. B. Campenot[b]

[a] Department of Biochemistry and Molecular Biology, Finch UHS/Chicago Medical School, North Chicago (Illinois 60064, USA), Fax: +1847 578 3240, e-mail: neetk@finchcms.edu
[b] Department of Cell Biology, University of Alberta, Edmonton, AB, TGG 2H7 (Canada)

Abstract. This review deals with the receptor interactions of neurotrophic factors, focusing on the neurotrophins of the nerve growth factor (NGF) family, the glial cell derived neurotrophic factor (GDNF) family, and the ciliary neurotrophic factor (CNTF) family. The finding that two proteins, $p75^{NTR}$ and Trk, act as receptors for NGF in neurons generated the discovery of other neurotrophic factors/receptor families and has enhanced our understanding of the development, survival, regeneration, and degeneration of the nervous system. The kinetics of binding, the structure of the ligand-receptor complex, and the mechanism of retrograde transport of the neurotrophins are discussed in detail and compared to information available on the GDNF and CNTF families. Each neurotrophic factor family, i.e., NGF, GDNF, and CNTF, has a set of receptors with specificity for individual members of the family and a common receptor without member specificity that, in some families, generates the cellular signal and retrograde transport.

Key words. Neurotrophin; neurotrophic factor; nerve growth factor (NGF); glial cell-derived neurotrophic factor (GDNF); ciliary neurotrophic factor (CNTF); retrograde transport; receptor; Trk; cRet.

The neurotrophins and their receptors

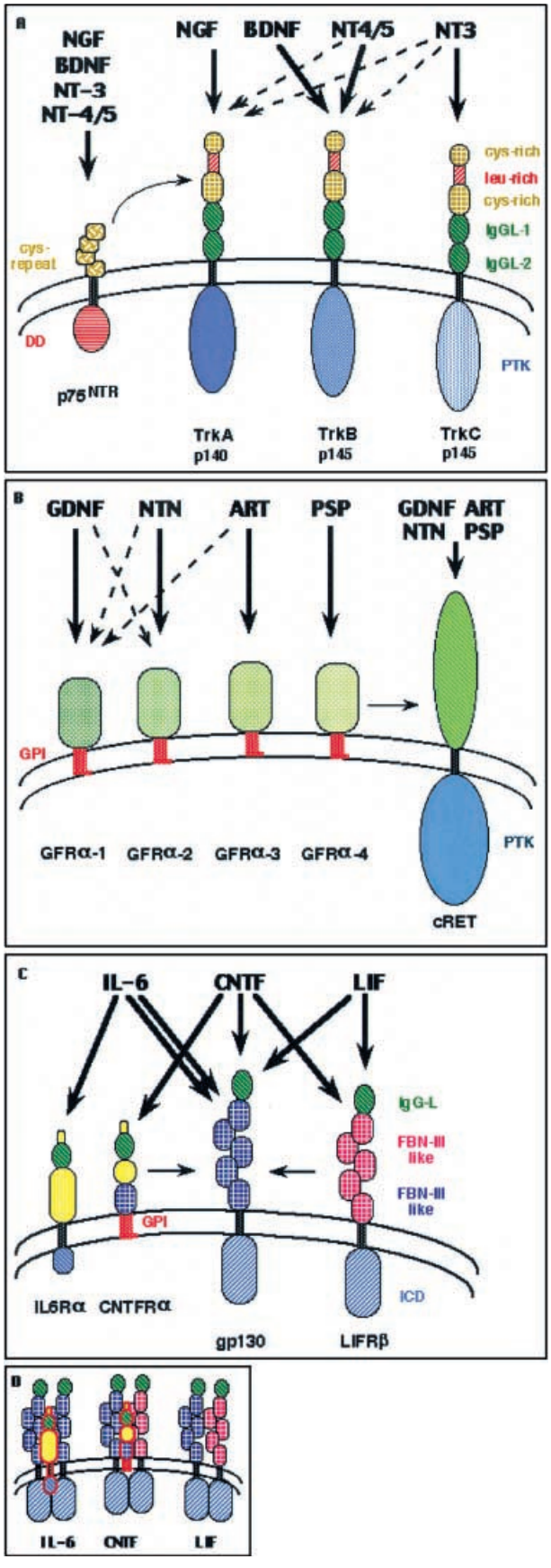
The protein tyrosine kinase product of the trk proto-oncogene is the receptor for nerve growth factor (NGF) that undergoes NGF-induced autophosphorylation and initiates a kinase cascade for signaling to neuronal differentiation [1–3]. NGF binds the TrkA (originally called Trk or $p140^{trk}$) receptor only, brain-derived neurotrophic factor (BDNF) binds the TrkB receptor only, but neurotrophin (NT)-3 can bind to all three Trk (A, B, C) receptors with a preference for TrkC [4–8], and NT-4/5 can bind both TrkA and TrkB [9, 10] (fig. 1A). However, the cell context of the receptor influences these specificities [11–13] (see below).

The 'low-affinity' neurotrophin receptor, $p75^{NTR}$, is a 75-kDa transmembrane glycoprotein that has no significant homology in the cytoplasmic domain to tyrosine or serine/threonine kinases [14, 15] and binds all neurotrophins (NGF, BDNF, NT-3, NT-4/5). The $p75^{NTR}$ receptor now appears to be essential for certain biological responses, such as apoptosis [16–19], and can modulate the response of Trk activation at the signaling level [20–22], but does not mediate other biological responses [23, 24].

* Corresponding author.

Binding to endogenous receptors in cells: Trk and $p75^{NTR}$

Early studies of sensory [25] and sympathetic [26] neurons clearly showed two classes of binding sites for NGF, with a high affinity less than 100 pM and a lower affinity near 1 nM. Note that what is termed 'low affinity' in this system still represents a quite high affinity in physiological terms. Similar findings were made with BDNF [27]. Subsequent studies on the pre-neuronal PC12 cell model system also revealed complex kinetic binding phenomena [28–30] and outlined the receptor internalization process [28, 31–34]. Discussion initially centered around whether these binding classes represented two separate receptors, negative co-operativity, one receptor in two environments, or one receptor undergoing a change in



structure [25, 28–30]. Cloning, isolation, and study of the two recombinant receptor classes have clarified their contributions to binding and signaling, but have not completely resolved some of the issues of high-affinity binding.

The accumulated results from 13 reports on 'standard' cellular preparations where the separate affinities have been analyzed and tabulated are available at www.finchcms.edu/biochem/neet.html. The K_d for 'low-affinity' binding ranges from about 0.2 to about 4 nM with a mean of 1.3 and a median of 0.9 nM, with no significant difference between 4 neuronal and 9 PC12 cell preparations. 'High-affinity' K_d values range from 10 to 23 pM for neurons (n=4) with a mean of 17 pM; K_d values with PC12 cells tend to be somewhat higher with a mean of 136 pM, a median of about 100 pM, and a range of 35–350 (n=6). No consistent trend in K_d with temperature has been observed, although temperature does affect rates of dissociation and internalization (see below). The low- and high-affinity binding have been associated with a rate of dissociation that is fast and slow, respectively [35]. From five studies, the 'fast' rates are in the range of 10^{-2}–10^{-3} M^{-1} s^{-1} and the 'slow' rates are about 10^{-3}–10^{-4} M^{-1} s^{-1} at 37 °C (see www.finchcms.edu/biochem/neet.html). The rate of the slow dissociation decreases by about ten fold upon lowering the temperature to 0 °C, thereby providing a convenient way to distinguish binding between the two classes of site [30]. These values for both rates and equilibrium binding are surprisingly consistent considering the range of laboratories, techniques, and preparations utilized. More limited studies with the other neurotrophins and their specific neurons suggest a similar pattern of binding.

Figure 1. Receptor binding patterns of three neurotrophic factor families. (*A*) The NGF neurotrophin family of NGF, BDNF, NT-3, and NT-4/5. The Trk receptor extracellular domain consists of two cysteine-rich domains, three leucine-rich motifs, and two IgG-like (IgGL) domains; the intracellular domain is a protein tyrosine kinase (PTK). The common $p75^{NTR}$ receptor has four cysteine repeats in the extracellular domain and one or two intracellular death domains. (*B*) The GDNF family of GDNF, NTN, ART, and PSP. The GFRα receptors are membrane attached with a glycosyl inositol phosphate (GPI) anchor. The common cRet (cRET) receptor has an intracellular PTK domain. (*C*) The IL-6/CNTF family of IL-6, CNTF, LIF. The other related cytokines (oncostatin M, granulocyte colony-stimulating factor, IL-11, and cardiotrophin-1] (not shown) utilize the common gp130 receptor subunit but have different co-receptor requirements, some of which are not yet well defined. The gp130 and LIFRβ receptor extracellular domains have one IgGL and five fibronectin (FBN) III-like domains; the intracellular domain (ICD) binds STAT and/or JAK. The IL6Rα and CNTFRα receptor extracellular domains have an IgGL and/or an FBN-like domain. CNTFRα is GPI anchored whereas IL6Rα has a transmembrane domain and a small ICD. (*D*) Composition of the signaling complexes for IL-6, CNTF, and LIF. (In all cases, higher-order receptor aggregates, i.e., heterotetramers or heterohexamers, may occur with the ligand [144].)

Binding to ectopically expressed receptors in cells: Trk and p75^{NTR}

Binding of NGF to cellular surfaces that express only the TrkA receptor have been reported to have a 'low affinity' of about 1 nM [36–40]. This value supports the contention that 'high-affinity' binding requires the presence of p75^{NTR} (see below). However, when TrkA is over-expressed by itself at high levels, about 2% of the binding is 'high affinity' (10 pM) and the remainder is 'low affinity' [2, 41]. This discrepancy may be because TrkA can dimerize more easily at these high membrane concentrations even in the absence of p75^{NTR}, or is perhaps due to other differences in binding methodology, cellular preparations, or analysis [42]. Indeed, studies with antibodies to p75^{NTR} suggested the presence of two types of high-affinity binding sites, one containing p75^{NTR} and one not [43].

Expression of the p75^{NTR} receptor by itself, both ectopically and endogenously, has demonstrated the low-affinity binding to this receptor. Ectopic expression of p75^{NTR} in 3T3 cells has demonstrated equilibrium dissociation constants of 1.2–1.6 nM [36, 41, 44], in agreement with values obtained with endogenous expression of p75^{NTR} in PC12nnr5 and A875 melanoma cells. In contrast to the specificity displayed by the Trk family for the neurotrophins, binding data demonstrate that the four neurotrophins (NGF, BDNF, NT-3, NT-4/5) can bind to p75^{NTR} with similar affinities, at a K_d of about 1–1.5 nM [5, 44–48]. Interestingly, the *rates* of association and dissociation differ by greater than 14-fold [44, 45], compensating in a parallel fashion to maintain the similar equilibrium binding. The molecular basis for this rate compensation and the biological relevance, if any, of the difference in rates have provoked considerable speculation.

Binding to recombinant purified Trk receptor domains and subdomains

Measurement of the binding of NGF to the purified recombinant extracellular domain of the TrkA receptor by surface plasma resonance found a dissociation constant of 2.9 nM [49], consistent with several of the reports discussed above with cells that only express TrkA. The association and dissociation rate constants were comparable to those expected for a 'slow on' rate (5.6×10^5 $M^{-1}\,s^{-1}$) and a 'fast off' rate ($10^{-3}\,s^{-1}$). On the other hand, when the TrkA extracellular subdomain is expressed as an Fc fusion protein, the resulting affinities for the immunoadhesion fusion proteins with NGF measured by solid phase plate assays are 47–62 pM [50, 51]. Similar high affinities were found for TrkB and TrkC with their cognate neurotrophin ligands (tabulated and compared at www.finchcms.edu/biochem/neet.html). In these cases, dimerization may be forced, because of the inherent dimerization capabilities of the IgG Fc region to which the Trk extracellular domains are fused, producing a high-affinity binding site for the neurotrophin on the Trk-Fc chimeric dimer (the so-called avidity effect).

As with other receptors, dimerization of Trk on the cell surface appears to be essential for transautophosphorylation of the cytosolic domains, with some cellular data directly supporting such a model [41, 52]. Neurotrophins can dimerize the extracellular domain of their respective Trks in solution [49, 53], although the association constant has not been demonstrated to be sufficient to account for receptor dimerization at physiological concentrations.

The Trk receptor: chimeras and mutagenesis

Recent literature contains seemingly definitive, but conflicting, reports on which *subdomain(s)* of the extracellular domain of Trk is (are) responsible for neurotrophin binding. However, different methodologies were used in each of these studies. The Trk receptor extracellular domain consists of the following five subdomains: Cys-rich$_1$ (d1), the Leu-rich motif (LRM) triad (d2), Cys-rich$_2$ (d3); IgGL$_1$ (d4), and IgGL$_2$ (d5), where IgGL refers to the immunoglobulin-like C2 folding motif (fig. 1A).

The IgGL subdomains

One or both IgGL subdomains have been reported as being most important for neurotrophin binding. The specificity of neurotrophin binding to full-length TrkA/TrkB chimeras expressed in 293 cells was determined by binding radiolabeled NGF by the sedimentation assay with intact cells [37]. TrkB chimeras containing both TrkA IgGL$_1$ (d4) and IgGL$_2$ (d5) subdomains could bind NGF with the expected affinity (1.3 nM), whereas chimeras containing *only* the IgGL$_1$ or the IgGL$_2$ TrkA subdomain did not have this binding capability. This study did not explicitly examine the LRM; however, the LRM was varied simultaneously with the second cysteine-rich subdomain and the LRM region did not appear to contribute to neurotrophin binding specificity.

Urfer et al. [50] reported that the IgGL$_2$ subdomain was necessary and sufficient for the binding and specificity of Trk interaction with the neurotrophins. TrkC chimeras with subdomains of TrkB and TrkA and deletions mutants of TrkC or TrkA were expressed as immunoadhesins with the antibody Fc region; binding was measured by radioimmunoassay on plates. Constructs containing the IgGL$_2$ subdomain bound the neurotrophin. Furthermore, the IgGL$_2$ subdomain from TrkC or TrkA, by itself, bound NT-3 or NGF, respectively, albeit with a three fold reduced affinity. Autophosphorylation of the Trk constructs expressed in 3T3 cells supported these findings. Interestingly, the combination of IgGL$_1$ with IgGL$_2$ slightly enhanced the binding or Trk phosphorylation found with

only the $IgGL_2$ subdomain [50]. Various TrkA constructs were expressed in PC12nnr5 cells (lacking endogenous TrkA) and the $IgGL_2$ domain was found to be essential for NGF or NT-3 binding and differentiation [54]. Mapping of the NGF/TrkA and NT-3/TrkC binding sites by alanine scanning mutagenesis with immunoadhesion fusion proteins on plates [55] has implicated the loops between the E and F β strands in the $IgGL_2$ as the primary affinity determinant in both pairs, but recent studies of Trk chimeras suggest that interactions in the $IgGL_1$ subdomain may dictate the specificity for cognate ligand-receptor pairs [56]. Finally, a TrkA $IgGL_1$-$IgGL_2$ subdomain construct that had been purified after expression in *Escherichia coli* bound NGF [57] and was subsequently used as the basis of crystallization studies of the TrkA $IgGL_2$ subdomain with and without NGF [58, 59]. This crystal structure of the ligand-receptor subdomain complex confirmed the contribution of the $IgGL_2$ loops between the E and F β strands as well as the face of the ABED β sheet to the NGF binding site. The authors argue from this structure that the $IgGL_1$ subdomain is unlikely to be close enough to bind NGF [59].

Chimeras have also recently been studied in the presence and absence of $p75^{NTR}$ as co-receptor (H.U. Saragovi, personal communication) with the interesting finding that domain utilization for neurotrophin binding to Trk receptors might be regulated by the presence of $p75^{NTR}$. For example, in the presence of $p75^{NTR}$, NT-3 prefers to bind the $IgGL_2$ subdomain of TrkA over that of TrkB, whereas this preference does not appear in the absence of $p75^{NTR}$; the binding of NGF to $IgGL_2$ is not influenced by $p75^{NTR}$. This effect of the co-receptor is consistent with cellular studies that show a differential effect of NGF and NT-3 on TrkA sympathetic neurons that contain $p75^{NTR}$ [13] and in vivo studies that show an influence of $p75^{NTR}$ on NT-3 specificity for TrkA binding to sympathetic neurons [12].

The LRM subdomains

In contrast, Windisch et al. [60–62] have implicated one sequence in the triad of the LRM repeats as a neurotrophin-binding region. Various Trk constructs fused to the maltose-binding protein (MBP) were expressed in *E. coli*. The kinetics and affinities were measured by immobilization of MBP-Trk on amylose columns, and bound and free radiolabeled neurotrophin were separated by centrifugation. These reports conclude that a 24-amino acid sequence in the second of the three LRM repeats accounts entirely for the affinity and specificity, with a dissociation constant of 1.3 nM [61, 62]. However, the lack of glycosylation in the bacterial recombinant MBP fusion proteins [60–62], may lead to false negatives with regard to binding of neurotrophin to IgGL subdomains and nonspecific binding to exposed hydrophobic patches on LRM subdomains with no carbohydrate attached. The LRM region of the Trk receptors has also been implicated in determining the specificity between NGF and NT-3 binding as well as having effects on secondary aspects of cellular response [54]. Finally, normal variants of TrkB have been found in sensory neurons with deletions in the LRM region; these mutations also abrogate binding of any neurotrophin [63].

Summary of Trk-ligand interactions

The evidence supports at least one, and perhaps two IgG subdomains as *mainly* responsible for neurotrophin specificity and affinity. The data for the LRM as the *sole* determinant are less compelling, but suggest that it may contribute some binding interaction. Part of the problem with these studies lies in the lack of distinction between residues that contribute to specificity (as determined by homology-swapped chimeras) and energetic contributions (determined by deletions and/or point substitutions) [55]. The most compelling, visual, evidence of course, is the crystal structure of NGF with the $IgGL_2$ (d5) subdomain of TrkA that has recently been determined at 2.2 Å resolution [59]. However, this complex, itself, is the product of proteolytic cleavage of the two subdomains, with cleavage occurring between the $IgGL_1$-$IgGL_2$ linker resulting in $IgGL_2$ in the final complex. However compelling this structure is, its existence does not prove that additional subdomains do not also contribute to formation of the receptor complex.

The interaction of neurotrophins with Trk receptors does not appear to be localized to a small surface area of the ligand protein [64, 65] [see the contribution by M. Butte to this multi-author review], in contrast to 'hot spots' that have been observed with hormone receptors such as growth hormone [66] and erythropoietin [67] receptors. The evidence from mutational analysis of NGF, BDNF, and NT-3 leads to the notion that a large proportion of the entire neurotrophin surface is capable of interacting with the receptor. Therefore, multiple receptor subdomains may also be needed to satisfy the large binding surface area of the neurotrophin. A single small subdomain (particularly the 24-residue LRM) would not be able to cover the entire NGF molecule; thus, the complex of the $IgGL_2$ subdomain with NGF may not adequately explain all the mutation data. The $IgGL_1$, $IgGL_2$, and LRM subdomains could each contribute to interactions with the neurotrophin and resolve some of the conflicting data cited. Such a complex with NGF 'wrapped' by the TrkA receptor might also account for the slow kinetics of dissociation discussed below. Further characterization and extension of crystal structures with larger portions of the Trk receptor are needed to resolve this conundrum.

The $p75^{NTR}$ receptor: subdomains and mutagenesis

Structural aspects of the $p75^{NTR}$ receptor binding site(s) for neurotrophins have been studied by subdomain dele-

tion [68–70], amino acid insertion [71], proteolysis [72], and insertion of glycosylation sites [73]. Although the data are somewhat conflicting, the consensus appears to support the second Cys-rich subdomain of $p75^{NTR}$ as being the most important for neurotrophin binding, with the first subdomain being the least important. Two computer models for $p75^{NTR}$ have been proposed [74, 75], based on a comparison with the homologous tumor necrosis factor (TNF) receptor p55 extracellular domain crystal structure. The later model [75] focuses on interactions with the second (and third) Cys-rich subdomains and, therefore, appears to more accurately represent the experimental evidence.

Interactions between the $p75^{NTR}$ and TrkA receptors

'High-affinity' binding

The role of the two receptors, $p75^{NTR}$ and TrkA, in NGF binding to cells that possess both receptors has been controversial [for reviews see refs 35, 42, 76–79]. Full development of high-affinity binding (10–100 pM), or 'slow dissociation,' appears to require co-expression of both $p75^{NTR}$ and TrkA [1, 3, 36, 80–82]. The most definitive evidence in favor of a $p75^{NTR}$ interaction with Trk generating a 'high-affinity' site is a careful study of the binding kinetics of transfected cells that demonstrates a clear effect of $p75^{NTR}$ on the rates of association, and hence the equilibrium, of NGF with TrkA [36]. In this study, 'low- affinity' binding ($K_d \sim 0.1–1$ nM) was observed for each of the receptors by themselves, but the combination of $p75^{NTR}$ and TrkA produced a higher affinity ($K_d < 10$ pM), as deduced from the kinetic constants. This view appears to be the prevalent one in the field, currently. However, others have reported a small percentage of high-affinity binding [2, 41] or slow dissociation [83] with over-expression of TrkA in the absence of $p75^{NTR}$ in cell lines (see above). Conversely, high-affinity binding ($K_d = 60$ pM) for NT-3 with the p75NTR receptor alone has been reported in embryonic chick sympathetic neurons, i.e., a context-dependent, high-affinity conformation of $p75^{NTR}$ [84].

Demonstration of direct interactions between the $p75^{NTR}$ and TrkA receptors

Naturally, a diligent search for interactions between the two neurotrophin receptors was made, primarily with cross-linking or immunoprecipitation. This search has met with a notable lack of success for many years [35, 41], hinting at the subtle and, perhaps, weak nature of the interactions. Only relatively recently have better methods been utilized and physical interaction between the two receptors clearly demonstrated. Now, convincing evidence has been presented from both cross-linking and immunoprecipitation that a complex between $p75^{NTR}$ and Trk can/does exist [22, 85, 86]

In a different approach using fluorescent methods, Ross, Wolf and colleagues have shown that $p75^{NTR}$ and TrkA interact on the cell surface. Studies using fluorescence recovery after photobleaching have shown that $p75^{NTR}$ mobility in the plasma membrane is reduced in the presence of TrkA, but to a lesser extent with TrkB, and that immobilization via a putative complex can occur in the absence of NGF [87]. In contrast, addition of NGF promotes the immobilization of TrkA in the absence of $p75^{NTR}$ but requires an active tyrosine kinase, since the process is inhibitable by the TrkA tyrosine kinase inhibitor K252a [88]. However, in the presence of $p75^{NTR}$, K252a does not inhibit the NGF enhancement of TrkA immobilization; in other words, $p75^{NTR}$ shifts the NGF-induced immobilization of TrkA from being TrkA kinase dependent to kinase independent. Furthermore, co-patching of TrkA and $p75^{NTR}$ using fluorescent antibodies, cross-linking, and confocal microscopy demonstrated the cell surface co-localization of $p75^{NTR}$ with TrkA, but not TrkB [89]. These studies suggested that the extracellular domain of the receptors is sufficient for complex formation, but that the intracellular domain and even the transmembrane region make significant contributions to receptor-receptor interactions. In agreement with these studies, co-immunoprecipitation studies of various $p75^{NTR}$ and epitope-tagged Trk chimeric constructs demonstrated that a complex existed between $p75^{NTR}$ and each Trk species without addition of neurotrophin, that both the intracellular and extracellular domains of TrkB contributed to the interaction with $p75^{NTR}$, and that complex formation was dependent on an active Trk kinase [22]. However, one study [22] was able to demonstrate interaction between $p75^{NTR}$ and TrkB, whereas an other study [87, 89] could not; the reason for the discrepancy is unclear, but may be attributable to different sensitivities of the two techniques. Interestingly, these studies corroborate earlier studies, using chimeras between the epidermal growth factor receptor and $p75^{NTR}$, that suggested that the transmembrane domain was involved in the interaction between $p75^{NTR}$ and TrkA and generation of differentiation signals [90].

The role(s) of $p75^{NTR}$

The question remains as to the roles of $p75^{NTR}$ in neurotrophin action. The $p75^{NTR}$ receptor clearly has a Trk-independent signaling function [17–19, 21], interacts at the signaling level with Trk [20–22], and affects the specificity of neurotrophin binding to the Trk family [12, 13, 22, 81, 91]. However, the mechanism by which $p75^{NTR}$ influences Trk *affinity of binding* to neurotrophins has remained controversial [6, 35 ,42, 76, 77, 79]. Three main theories have been prominent. The first alternative, that $p75^{NTR}$ plays no direct role and that high-affinity binding is due solely to Trk, can be reasonably discounted based on the evidence discussed above. Second, the recruit-

ment/presentation model proposed that $p75^{NTR}$ affected association kinetics by a membrane proximity effect [92] in which $p75^{NTR}$ played a subsidiary role of recruitment or presentation of neurotrophin to the Trk receptor. Although this model cannot be ruled out entirely, no strong support exists at this stage. Third, a conceptually simple physical interaction between $p75^{NTR}$ and Trk was originally thought to explain high-affinity binding [1, 3, 6, 93]. With compelling evidence that Trk and $p75^{NTR}$ interact directly and that high- affinity binding requires $p75^{NTR}$ (see above), this direct-interaction model appears to be gaining support. However, a true structure of a ternary complex between $p75^{NTR}$, TrkA, and NGF by X-ray crystallographic analysis, cryoelectron microscopy, or atomic force microscopy may be required to completely convince proponents of other models.

NGF/TrkA/$p75^{NTR}$ complex structure

Can a neurotrophin concurrently bind Trk and $p75^{NTR}$ based on structural considerations? A thorough analysis of the conserved and mutationally sensitive sites were mapped on the three-dimensional structures of all four neurotrophins [64]. The conclusion was made that Trk and $p75^{NTR}$ binding was either mutually exclusive or the two receptors would have to 'tightly abut' each other to bind simultaneously. This conclusion is in agreement with an earlier analysis of the mutations [65]. Based on the crystal structure of NGF with the $IgGL_2$ domain of TrkA and the perceived lack of overlap with the sites on NGF that define the $p75^{NTR}$-binding site by mutation, Wiesmann et al. [59] concluded that NGF could bind both receptors simultaneously. However, the model of $p75^{NTR}$ with NGF [75] suggests that the $p75^{NTR}$- and TrkA-binding sites on NGF severely overlap, particularly at residues Arg103, His84, Ile31, and perhaps Trp21, which would lead to exclusive binding. Furthermore, the evidence cited above for direct interactions of $p75^{NTR}$ and TrkA through multiple domains would indicate that structural adaptability of the complex might occur to produce a neurotrophin-binding site in the full-length receptor in a cellular context that is somewhat different from that in the individual complexes, thus accommodating a ternary complex (or multiples thereof).

A model in which an NGF dimer bound $p75^{NTR}$ on one side and TrkA on the other subunit is possible, in principle, but would not seem to lead to a productive signaling complex, i.e., it would be unable to transautophosphorylate on the cytosolic kinase domains. However, such a heterodimeric complex has been suggested to serve as an intermediate in the formation of a final Trk homodimer that could then transautophosphorylate [41, 42, 92]. Interestingly, however, comparison of the $p75^{NTR}$ binding model with the TrkA X-ray structure reveals an opposite orientation with respect to the membrane, i.e. if each C terminus direction of the receptor extracellular domains was inserted in the membrane, the N and C termini of the elongated NGF molecule would be oriented away from the membrane in the case of the TrkA complex and oriented toward the membrane in the case of $p75^{NTR}$. If true, this would raise interesting speculations about mechanisms. Clearly, an X-ray structure of the NGF-$p75^{NTR}$ complex is needed to confirm the model building. Alternatively, $p75^{NTR}$ and TrkA may interact within the active complex, binding only a single NGF dimer to either TrkA or $p75^{NTR}$ but not both at the same time.

Finally, the TrkA-d5 subdomains have been noted to bind to the 'waist' of the parallel NGF dimer [59], relatively close together (20 Å) [94]. Two other members of the cysteine knot growth factor family, vascular endothelial growth factor (VEGF) and bone morphogenic protein (BMP), bind a subdomain of Flt-1 receptor or BRIA receptor, respectively, at the ends of the antiparallel molecule considerably farther apart (up to 65 Å). The separation of these receptor subdomains may be related to the distance of the ligand-binding subdomain in the receptor to the membrane surface [94]. More structures of growth factor-receptor complexes will be needed to confirm such a relationship.

Cellular internalization of NGF

Internalization, down-regulation, and intracellular signaling have been studied with the neurotrophins and their receptors in the context of axonal retrograde transport, and in intact cells without processes. Whether the latter is a good model for the former is not clear. Several methods have been used to estimate the internalization (or receptor-mediated endocytosis) in cultured cells, including acid stripping, Triton insolubility, electron microscopy, and inhibition of endosome trafficking. Early studies with PC12 cells indicated that the internalized labeled NGF was associated with high-affinity binding [31–34] and was partially in a cytoskeletal pool [28, 33, 34]. Subsequent studies with either the neuronal-like PC12 cells [95, 96] or nonneuronal cells that had been transfected with TrkA [97] showed that internalization of NGF was mediated by the presence of TrkA.

Studies with PC12 cells have indicated that TrkA and $p75^{NTR}$ are highly localized to caveolin-enriched membranes isolated on sucrose gradients [150]. This suggests that TrkA and $p75^{NTR}$ are localized to caveolae, plasma membrane regions where signaling molecules concentrate. The evidence also suggests that caveolin may play a role in regulating TrkA phosphorylation [151]. The relationship of caveolae to sites of NGF binding and internalization in neurons is unknown.

Retrograde transport and retrograde signaling by neurotrophic factors

The original in vivo retrograde model

Not long after the discovery of NGF and its dramatic neurotrophic effects, it was shown that [^{125}I] NGF injected into the anterior chamber of the mouse eye resulted in the transport of [^{125}I] to the ipsilateral superior cervical ganglion, peaking 12–16 h postinjection [98]. Autoradiography showed that the [^{125}I] was concentrated in neuronal cell bodies, a majority of the [^{125}I] was recovered by binding with anti-NGF antibodies, and subsequent studies confirmed by SDS-PAGE analysis and autoradiography that axonally transported [^{125}I] radioactivity co-migrated at 13 kDa with intact NGF [99]. By analogy with fast anterograde axonal transport and the retrograde transport of horseradish peroxidase, Hendry et al. [98] hypothesized that during retrograde transport, NGF is enclosed in vesicles that travel along axonal microtubules. Subsequent work led to the demonstration that NGF applied to axons produced increased expression of tyrosine hydroxylase resulting in increased norepinephrine synthesis. This effect is clearly mediated by mechanisms in the cell body, which led Hendry et al. [98] to speculate: 'NGF is able to exert its specific effects on the adrenergic neurons once it has been brought to the cell body by retrograde axonal transport.' Thus, much of the retrograde transport model of retrograde signaling by neurotrophic factors, i.e., uptake of NGF into vesicles and retrograde transport of the NGF-containing vesicles to the cell bodies where the NGF exerts its effects on survival and gene expression, entered the literature with the first descriptions of the retrograde transport of NGF. This hypothesis has had tremendous influence in guiding thinking and research in the neurotrophic factor field during the subsequent 25 years. The wealth of information about neurotrophic factor transport in the central nervous system has been recently reviewed [100]. Here we will focus only on the issues directly related to the mechanism of retrograde signaling.

The ligature model and retrograde transport of endogenous NGF

To establish a biological role for NGF, an important element was to establish that NGF produced within the organism under natural conditions is retrogradely transported. Detection of endogenous NGF in neuronal cell bodies by immunoassay does not reveal whether the protein got there by retrograde transport. Therefore, the principle that substances which are transported along axons accumulate when a ligature is encountered was used to demonstrate the retrograde transport of endogenous NGF. The superior postganglionic nerve was ligated in guinea pigs, and the accumulation of biologically active NGF distal to the ligature was demonstrated by anti-NGF immunohistochemistry and cell culture bioassay of nerve extracts [101].

Retrograde transport components and signaling

Transport vesicles and signaling endosomes

Initially, NGF arriving at the cell body was believed to be released into the cytosol and possibly enter the nucleus to produce its biological effects (e.g., ref 102). However, the ability of intracellular NGF to activate signaling mechanisms was soon dispelled by the observation that direct administration of NGF into PC12 cells via liposome fusion failed to induce neuronal differentiation, and administration of antibodies to NGF into the cytosol failed to block neuronal differentiation of PC12 cells in response to externally applied NGF [103]. Focus then firmly shifted to the hypothesis that NGF retrograde signaling is accomplished by NGF-containing vesicles in the cell body. Therefore, understanding endosomal trafficking in neurons is crucial to understanding neurotrophin retrograde signaling. A model of endosomal trafficking in neurons has been described based on work with cultured hippocampal neurons and analogy with epithelial cells [reviewed in ref. 104]. Neurotrophic factor is taken up by receptor-mediated endocytosis into clathrin-coated vesicles. These vesicles fuse with early endosomes in the axon terminals. Transport vesicles bud from the early endosomes, and travel retrograde along axonal microtubules using dynein motor proteins. Upon reaching the cell bodies, the transport vesicles fuse with late endosomes and finally to lysosomes where the neurotrophic factor is degraded. This useful model is simple, but contains little direct information about endosomal trafficking in neurons and has potential for considerable complexity.

Some information is available about the relationship of NGF and TrkA during axonal transport. According to the retrograde transport hypothesis of retrograde signaling, NGF-TrkA complexes remain intact after internalization in axon terminals and during transport to the cell bodies. Retrograde transport of TrkA has been demonstrated in the sciatic nerve ligature model. Analysis of material accumulating distal to a ligature with anti-pan Trk immunoprecipitation, followed by anti-TrkA or antiphosphotyrosine immunoblot analysis, suggests that TrkA accumulating distal to a ligature is tyrosine phosphorylated [105]. These data cannot resolve whether NGF and TrkA are colocalized in the same transport vesicle, how much of the retrogradely transported TrkA is tyrosine phosphorylated, and whether phosphorylated TrkA is bound to NGF. Experiments with compartmented cultures of sympathetic neurons have shown that surface biotinylated TrkA from distal axons is retrogradely transported to the cell bodies as an NGF-phosphoTrkA complex [106].

Vesicle fractions which contain internalized [^{125}I]NGF have been isolated from PC12 cells [107, 108]. A fraction of small vesicles (P3) accumulates when intact PC12 cells are subjected to 10 min of [^{125}I]NGF internalization followed by permeabilization and 15 min of in vitro reactions in the presence of ATP. Cross-linking followed by TrkA immunoprecipitation and autoradiography identified an [^{125}I]-labeled band migrating at a molecular weight consistent with a TrkA-NGF complex. Immunoblot analysis detected TrkA and phospholipase (PLC)γ in the P3 vesicle fraction from NGF-treated but not from untreated PC12 cells. Immunoprecipitation with anti-TrkA and anti-PLCγ followed by antiphosphotyrosine blot showed that the P3 vesicle fraction from NGF-treated cells contained tyrosine-phosphorylated TrkA and PLCγ. These results demonstrate that the P3 fraction includes vesicles containing TrkA-NGF complexes, vesicles containing tyrosine-phosphorylated TrkA, and vesicles containing tyrosine-phosphorylated PLCγ. These vesicles probably also contain the caveolae-like membranes discussed earlier [109]. The TrkA cross-linked to NGF is probably phosphorylated, but this has not been formally demonstrated. Thus, these data suggest that the P3 fraction contains vesicles with NGF in the lumen bound to activated TrkA in the membrane. Although one cannot conclude that the phosphorylated PLCγ co-localized on the same vesicles with NGF-TrkA complexes, this seems likely, especially since the phosphorylation of PLCγ was NGF dependent. The PLCγ and possibly other signaling molecules that bind to activated TrkA may also be carried on the transport vesicles. Evidence of retrograde transport of several downstream signaling molecules has been reported [110, 111]. Note that the P3 vesicles are derived from endocytosis on the cell bodies of unprimed PC12 cells that have no axons, no axonal transport occurs in these cells, and association of dynein motor proteins with the P3 vesicles has not yet been demonstrated. Furthermore, there is evidence that NGF is associated with multivesicular bodies during retrograde transport in cultured neurons [112]. Multivesicular bodies are larger than the P3 vesicles. Thus, while the evidence indicates that endocytosis of NGF in PC12 cells produces vesicles containing internalized NGF bound to TrkA, the relationship of these vesicles to transport vesicles is unknown.

The conclusion that transport vesicles contain NGF-TrkA complexes appears inconsistent with the idea that the transport vesicles could have a low lumenal pH that would promote dissociation of ligand and receptor. Overly and Hollenbeck [113] reported that over 80% of transported organelles derived from endocytosis in cultured chick sympathetic neurons have low lumenal pH, suggesting to them that only a small minority of neutral pH transport vesicles are potential carriers of NGF-TrkA complexes. However, the possibility that acidic transport vesicles could contain unbound TrkA in the membranes and unbound NGF in the lumens cannot be ruled out. Acidic NGF transport vesicles could possibly undergo trafficking events in the cell bodies that result in neutralization of the lumenal pH and binding of lumenal NGF to TrkA. In fact, if TrkA activity was too high in vesicles undergoing retrograde transport, this could possibly lead to stimulation of aberrant axon branching or other effects that are undesirable along the axons. Thus, reduction of TrkA signaling in NGF-containing endosomes undergoing transport may be beneficial.

Transport of [^{125}I]NGF in compartmented cultures

In vivo studies of the retrograde transport of [^{125}I]NGF do not allow quantitative estimates of the amount of NGF transported or the dynamics of transport and processing because the [^{125}I] label is lost to analysis once NGF is degraded in the cell bodies. Experiments using the ligature approach are limited because the ligature blocks anterograde transport as well, and without a resupply of TrkA and other components from the cell bodies, receptor-mediated endocytosis and retrograde transport will run down. Compartmented cultures of sympathetic neurons from newborn rats provide a useful approach [114, 115]. Axons elongating from sympathetic neurons of newborn rats plated in a proximal compartment extend axons left and right along collagen tracks, underneath Teflon partitions sealed to the dish surface with silicone grease, and into the separate fluid environments of distal compartments [116, 117]. Detectable [^{125}I] first appears in the cell bodies 1 h or more after application of [^{125}I]NGF to distal axons. Several hours later, [^{125}I] begins to appear in the medium bathing the cell bodies in the proximal compartments. SDS-PAGE analysis showed that most of the [^{125}I] accumulating in cell bodies/proximal axons was intact NGF whereas all of the [^{125}I] in the medium bathing the cell bodies/proximal axons ran with the dye front, showing that only small breakdown products were released from the cell bodies/proximal axons [115]. Results of experiments in multicompartment dishes, in which axons from cell bodies in proximal compartments extended across intermediate compartments and then into distal compartments, showed that little or no [^{125}I] is released into intermediate-compartment medium. These results indicate that NGF is not significantly degraded during its transport along axons. Observations of the time course of [^{125}I] release by cell bodies/proximal axons suggest that NGF has a half-life of about 3 h after reaching the cell bodies.

Retrograde phosphorylations in compartmented cultures

If retrogradely transported NGF is accompanied by phosphorylated TrkA, then the appearance of phosphorylated TrkA in cell bodies and proximal axons should have similar kinetics to the appearance of [^{125}I]NGF. However,

tyrosine-phosphorylated TrkA appeared in the cell bodies/proximal axons within 1 min of application of 200 ng/ml NGF to distal axons, and tyrosine phosphorylation of several other proteins occurred within 10–30 min [118]. Parallel experiments with the same NGF treatment confirmed that retrogradely transported [^{125}I]NGF does not appear for at least an hour after NGF treatment. Moreover, the estimated velocity of [^{125}I]NGF retrograde transport in compartmented cultures was 10–20 mm/h [114], suggesting that 3–6 min would be required for NGF bound to TrkA on an axon at its point of emergence into the distal compartments to be transported across the 1-mm barrier into the proximal axon just inside the proximal compartment. Moreover, the arrival of phosphorylated TrkA only from axonal regions adjacent to the barrier seems unlikely to be sufficient to be detected on immunoblots. Axons in the distal compartments are 5–10 mm long or longer. Consequently, most of the surface TrkA will be located several millimeters from the proximal compartment. Therefore, the minimum transport time for the arrival of most TrkA receptors on distal axons is likely to be much longer than 3 min.

Taken together, the kinetics of [^{125}I]NGF retrograde transport and retrograde TrkA phosphorylation suggest that the phosphorylated TrkA appearing in cell bodies/proximal axons may not represent the transport of TrkA-NGF complexes from the distal axons. Therefore, these results appear contradictory to the retrograde transport hypothesis of neurotrophic factor signaling. Application of NGF to distal axons may produce an unknown propagated signal that results in the phosphorylation of unbound TrkA already present in the cell bodies/proximal axons, as suggested [118]. Possible mechanisms are highly speculative, but a sequential phosphorylation of TrkA or calcium fluxes propagating along the axon could be candidates. Interestingly, biologically significant phosphorylation of unbound TrkA has recently been demonstrated, i.e., overexpression of gangliosides in PC12 cells results in NGF-independent TrkA phosphorylation and activation of the mitogen-activated protein kinase (MAPK) pathway [119]. A caveat does remain, though, since the possible existence of a faster component of retrograde transport that delivers a small (i.e., undetectable by [^{125}I]NGF analysis), but biologically significant, amount of NGF-phosphorylated TrkA complexes to the cell bodies/proximal axons within 1 min cannot be entirely ruled out.

In contrast, results of other studies of retrograde signaling in compartmented cultures of sympathetic neurons were interpreted as supporting the retrograde transport hypothesis of retrograde signaling [120]. NGF applied to distal axons produced the accumulation of phosphorylated cAMP response element binding protein transcription factor (P-CREB) in the nucleus within 20 min, but when NGF covalently bound to beads (to prevent internalization and retrograde transport) was applied to distal axons, nuclear accumulation of P-CREB did not occur. The interpretation that internalization and transport of NGF are required for retrograde signaling may not be straightforward, since the effectiveness of bead-bound NGF to produce phosphorylation of axonal TrkA receptors was not shown. Rather, these investigators demonstrated the effectiveness of bead-bound NGF in undifferentiated PC12 cells where the published blots clearly showed that maximal contact with bead-bound NGF produced much less, perhaps half, of the TrkA phosphorylation that was produced by treatment with 100 ng/ml soluble NGF. The greater effectiveness of soluble over bead-bound NGF could have been amplified in the retrograde signaling experiments where 200 ng/ml soluble NGF was used. Furthermore, axons are 1–2 μm in diameter which, by geometry alone, could prevent much of their surface from making direct contact with the surface of the 1-μm-diameter beads, and bundling of axons together could reduce direct bead contact even more. Bead-bound NGF applied directly to neuronal cell bodies did produce nuclear P-CREB accumulation, but cell body responses to direct application of NGF are generally much larger in magnitude than to the same concentration of NGF applied to axons [121]. Therefore, in these experiments, bead-bound NGF applied to distal axons probably produced much less TrkA phosphorylation than was produced by application of soluble NGF. This discrepancy raises the possibility that the reduced activation of axonal TrkA receptors, rather than prevention of retrograde transport, may have been responsible for the failure of bead-bound NGF to produce a detectable retrograde signal.

Recently, the mechanisms of retrograde signaling were approached using compartmented cultures of rat dorsal root ganglion (DRG) neurons [122]. A 20-min application of BDNF in combination with NGF to distal axons increased the phosphorylation of Trk receptors and CREB transcription factor in the cell bodies by 30–50%. To determine if the phosphorylated Trk receptors in the cell bodies originated from the distal axons, BDNF/NGF was applied to distal axons of neurons expressing TrkB fused to green fluorescent protein (GFP). After 10 min, TrkB-GFP fluorescence observed with confocal microscopy in the cell bodies was 12–16% above untreated controls, which these investigators attributed to the retrograde transport of TrkB-GFP from the distal axons. In similar experiments, 90% of the TrkB-GFP in the cell bodies was photobleached immediately prior to the application of BDNF/NGF to distal axons. After prebleaching, the arrival of the same absolute amount of unbleached TrkB-GFP from the distal axons should produce a tenfold greater increase in cell body fluorescence. However, the observed increase was only 8% over untreated controls, similar to the increase observed without prebleaching. This result could be accounted for if photobleaching

of the cell bodies somehow reduced the amount of TrkB-GFP arriving from the distal axons by an order of magnitude, but this seems unlikely and possible mechanisms are obscure. A more likely explanation is that the TrkB-GFP arriving at the measurement site in the cell bodies was prebleached and, therefore, could not have originated from the distal axons. One can speculate how this could have occurred. Since the fluorescence measurements were made with confocal microscopy, a specific plane through the cell body was probably involved, not the whole cell body. BDNF/NGF applied to distal axons could have produced a rapid retrograde signal that stimulated the dispersal of prebleached TrkB receptors from the endoplasmic reticulum-Golgi to the measurement site. Other speculative scenarios are, of course, possible. While the authors also demonstrated that the NGF/BDNF-induced increase in cell body fluorescence was blocked by a 1-h pretreatment of distal axons with colchicine [122], this result does not convincingly demonstrate that the TrkB-GFP originated from the distal axons. Disruption of axonal microtubules could have caused consequent damage to many axonal mechanisms within 1 h. Moreover, the effect of colchicine alone without NGF/BDNF treatment was not tested, so the small decrease in cell body TrkB-GFP fluorescence it produced may not have arisen from blocking the effects of NGF/BDNF. In conclusion, these experiments do not constitute compelling evidence that TrkB-GFP was transported from distal axons to cell bodies within 10 min of application of NGF/BDNF to distal axons.

The role of $p75^{NTR}$ in retrograde transport

Studies of the role of $p75^{NTR}$ in retrograde transport in peripheral nerve have shown that anti-$p75^{NTR}$ antibody or a soluble recombinant $p75^{NTR}$ extracellular domain inhibited the retrograde transport of NT-4 and BDNF by sensory neurons in vivo [123]. However, these treatments produced little or no effect on the transport of NGF in sensory sympathetic neurons. In $p75^{NTR}$ knockout mice, NT-4 and BDNF transport were reduced, but the transport of NGF was unaffected [123]. These results indicate that $p75^{NTR}$ can play a role in internalization/retrograde transport of neurotrophins, but it does not appear to be required for retrograde transport of NGF by peripheral neurons.

The mechanism of retrograde signaling

Although the retrograde transport theory of neurotrophic factor signaling was first proposed about 25 years ago, the evidence is still not completely convincing. Moreover, even if retrograde transport vesicles carrying NGF bound to activated TrkA can activate signaling systems in the cell bodies and produce biological effects, other possible mechanisms of retrograde signaling could also exist simultaneously. Elucidation of the mechanisms of retrograde signaling is extremely important, since the retrograde support of neuronal survival and the retrograde regulation of gene expression are fundamental to the development of a properly connected and functioning nervous system. Understanding the mechanisms of retrograde signaling will also help us to understand how neuronal survival and growth can be promoted in nervous systems afflicted by degenerative diseases or neurotrauma.

Other neurotrophic factors

Receptor binding

Glial cell-derived neurotrophic factor

Glial cell-derived neurotrophic factor (GDNF) and its related family members, neurturin (NTN), artemin (ART), and persephin (PSP), belong to the same superfamily of cysteine knot motif growth factors as the NGF-type neurotrophins but are more distantly related in sequence [for reviews see refs. 124, 125]. The action of these neurotrophic factors on neurons is similar to that of the NGF family but with distinct differences. Again, a common receptor binds all growth factors in the subfamily and a 'specificity' receptor exists that distinguishes factors within the subfamily. In this case, however, the common receptor, cRet, is a tyrosine kinase receptor, whereas the specificity receptor, (GDNF family receptor α) (GFRα) is a glycosyl phosphatidyl inositol (GPI)-anchored receptor. In general, GFRα1 binds GDNF, GFRα2 binds NTN, GFRα3 binds ART, and GFRα4 binds PSP [126–129] (fig. 1B), although apparently some cross-over occurs under certain conditions, and the strictness of the binding may depend upon the presence of cRet [126, 128, 130–132]. Signaling occurs through cRet tyrosine kinase activity in a complex with the neurotrophic factor, GFRα, and cRet, but evidence suggests that certain signals may also be initiated by ligand binding to the GFRα receptor alone [133, 134]. The high affinity binding, 2–10 pM, appears to require both GFRα and cRet while low affinity binding, 300–500 pM, occurs with GFRα alone [127, 129, 132].

Ciliary neurotrophic factor

Ciliary neurotrophic factor (CNTF) acts on many of the same neuron types as NGF and GDNF but, in contrast, belongs to an entirely distinct structural family of four-α-helical bundle cytokines that includes interleukin (IL)-6, leukemia inhibitory factor (LIF, or cholinergic differentiation factor), oncostatin M, granulocyte colony-stimulating factor (G-CSF), IL-11, and cardiotrophin-1 [for reviews see refs 135, 136]. Although CNTF supports survival or differentiation of a wide variety of central and peripheral neurons, including sympathetic, embryonic

motor, hippocampal, and ciliary ganglionic neurons, CNTF can also act on glial and skeletal muscle cells. Other members of the CNTF cytokine family (sometimes called neuropoietins) act on numerous immunological and hematopoietic cells of nonneuronal origin. The ligands in this family have structural similarity to other four-helix bundle cytokines, such as growth hormone, but the IL-6-type receptors are distinctly different and more complex in composition. In contrast to the NGF and GDNF families, the CNTF receptor family is coupled to and signals through the JAK/STAT pathway, rather than via the direct tyrosine kinase activity of NGF and GDNF receptors.

One reason for the diverse sites of action of these α-helical cytokines is the common utilization of one or more widely distributed receptor subunits. The high-affinity signal-transducing (or converter) subunit, gp130, was originally known as part of the IL-6 receptor [137] and an additional signaling subunit, LIFRβ, had been known as the LIF cytokine receptor [138]. A low-affinity, lower-molecular-weight specificity subunit, the Rα subunit, is associated with the final receptor complex and helps determine the response to ligand [139] (fig. 1C). Thus, IL-6 requires IL6Rα and a homodimer of gp130 for activity, LIF requires gp130 and LIFRβ in a heterodimer, and CNTF requires CNTFRα, gp130, and LIFRβ [138, 140–143]. In the case of CNTF, the specificity subunit CNTFRα is membrane anchored with GPI and no cytosolic domain [139]. In contrast, IL6Rα has a standard transmembrane domain with a small cytosolic domain and, remarkably, LIF does not even require a third α type of receptor in the signaling complex [135]. Figure 1D best describes the final receptor complexes involved. Interaction of two gp130 or LIFRβ polypeptide chains is necessary for transautophosphorylation of intracellular proteins, perhaps in a higher association state [144]. In this case, the transmembrane receptor chain is not, itself, a protein tyrosine kinase, in contrast to the Trk and cRet receptors, but associates with members of the JAK family kinases to initiate intracellular phosphorylation of STAT and further signaling. Interestingly, the *soluble* extracellular domain of CNTFRα, cleaved from its GPI anchor by phospholipase, can itself act with the gp130/LIFRβ complex to initiate signaling upon CNTF binding [145, 146].

Comparison of GDNF and CNTF receptor binding with NGF

Thus, three distinct styles of receptor complexes are utilized by nature for signaling in neuronal systems. The NGF family uses specificity in a tyrosine kinase receptor (Trk) and a common receptor with apoptotic functions. The GDNF family uses a common tyrosine kinase receptor (cRet) with specificity determined by a GPI-anchored receptor that may also generate signals that are ill-defined at this point. Finally, the CNTF family utilizes a common receptor (gp130) with a combination of one or two other receptor proteins in the ultimate complex. In such situations, the use of sobriquets for individual polypeptide chains, such as 'receptor' and 'co-receptor', is clearly misleading.

The general theme with these neurotrophic receptors is that the highest affinity and specificity are generated by the full complex of two or more membrane-bound polypeptide chains. This situation is analogous to that found in the immunological system, e.g. the T-cell receptor, but distinct from that found for some receptors in the hematopoietic, e.g., erythropoietin, and hormonal, e.g., growth hormone and insulin, systems. The signaling and biological aspects of these receptor complexes is certainly interesting and important. Equally intriguing, from the perspective of this article, are the protein-protein interactions that give rise to the structured receptor complex and the resultant formation of the binding site(s) for the neurotrophic ligand. Finally, resolving how such structures have evolved over eons of time to produce the modern receptor system by utilization of component parts from different places will be a problem for the future.

Retrograde transport of GDNF and CNTF

By analogy with the neurotrophins, neurotrophic factors of the GDNF and CNTF families would be expected to be internalized by distal axons and transported to the cell bodies. Recent work has demonstrated that [^{125}I]GDNF and [^{125}I]NTN injected at a crush site in the sciatic nerve are selectively transported by subsets of DRG neurons [147]. Motoneurons transported GDNF to a much greater degree than NTN, and sympathetic neurons transported neither. Although the neurons contained cRET, the specificity of transport was attributed to the GFRα co-receptor. Sympathetic neurons in compartmented cultures retrogradely transport [^{125}I]LIF, and LIF applied to distal axons can induce cholinergic neurotransmitter expression [115]. [^{125}I]CNTF or [^{125}I]LIF injected into the sciatic nerve are retrogradely transported by DRG neurons and motoneurons, and the magnitude of transport is increased by nerve lesion [148, 149]. Thus, receptor-mediated internalization and retrograde transport are general characteristics of neurotrophic factors, not just the NGF family of neurotrophins. Little or no information is available yet about the mechanisms of retrograde signaling used by GDNF and CNTF families of neurotrophic factors.

Acknowledgements. We would like to acknowledge Laura Dvorak and Sang B. Woo for helpful discussions and critical reading of portions of this manuscript. K. E. Neet is supported by USPHS NIH grants NS24380 and NS36700. R. B. Campenot is supported by the Alberta Heritage Foundation for Medical Research, Canadian Institutes of Health Research, Alberta Paraplegic Foundation, Rick Hansen Man in Motion Foundation and NeuroScience Canada Foundation.

1 Kaplan D. R., Martin-Zanca D. and Parada L. F. (1991) Tyrosine phosphorylation and tyrosine kinase activity of the trk proto-oncogene product induced by NGF. Nature **350:** 158–160
2 Klein R., Jing S. Q., Nanduri V., O'Rourke E. and Barbacid M. (1991) The trk proto-oncogene encodes a receptor for nerve growth factor. Cell **65:** 189–197
3 Kaplan D. R., Hempstead B. L., Martin-Zanca D., Chao, M. V. and Parada L. F. (1991) The trk proto-oncogene product: a signal transducing receptor for nerve growth factor. Science **252:** 554–558
4 Klein R., Nanduri V., Jing S. A., Lamballe F., Tapley P., Bryant, S. et al. (1991) The trkB tyrosine protein kinase is a receptor for brain-derived neurotrophic factor and neurotrophin-3. Cell **66:** 395–403
5 Squinto S. P., Stitt T. N., Aldrich T. H., Davis S., Bianco S. M., Radziejewski C. et al. (1991) trkB encodes a functional receptor for brain-derived neurotrophic factor and neurotrophin-3 but not nerve growth factor. Cell **65:** 885–893
6 Bothwell M. (1991) Keeping track of neurotrophin receptors. Cell **65:** 915–918
7 Lamballe F., Klein R. and Barbacid M. (1991) trkC, a new member of the trk family of tyrosine protein kinases, is a receptor for neurotrophin-3. Cell **66:** 967–979
8 Soppet D., Escandon E., Maragos J., Middlemas D. S., Reid S. W., Blair J. et al. (1991) The neurotrophic factors brain-derived neurotrophic factor and neurotrophin-3 are ligands for the trkB tyrosine kinase receptor. Cell **65:** 895–903
9 Ip N. Y., Ibanez C. F., Nye S. H., McClain J., Jones P. F., Gies D. R. et al. (1992) Mammalian neurotrophin-4: structure, chromosomal localization, tissue distribution, and receptor specificity. Proc. Natl. Acad. Sci. USA **89:** 3060–3064
10 Klein R., Lamballe F., Bryant S. and Barbacid M. (1992) The trkB tyrosine protein kinase is a receptor for neurotrophin-4. Neuron **8:** 947–956
11 Ip N. Y., Stitt T. N., Tapley P., Klein R., Glass D.J., Fandl J. et al. (1993) Similarities and differences in the way neurotrophins interact with the Trk receptors in neuronal and nonneuronal cells. Neuron **10:** 137–149
12 Brennan C., Rivas-Plata K. and Landis S. C. (1999) The p75 neurotrophin receptor influences NT-3 responsiveness of sympathetic neurons in vivo. Nat. Neurosci. **2:** 699–705
13 Belliveau D. J., Krivko I., Kohn J., Lachance C., Pozniak, C, Rusakov, D. et al. (1997) NGF and neurotrophin-3 both activate TrkA on sympathetic neurons but differentially regulate survival and neuritogenesis. J Cell Biol **136:** 375–388
14 Chao M.V., Bothwell M.A., Ross A.H., Koprowski H., Lanahan A. A., Buck C. R. et al. (1986) Gene transfer and molecular cloning of the human NGF receptor. Science **232:** 518–521
15 Radeke M. J., Misko T. P., Hsu C., Herzenberg L. A. and Shooter E. M. (1987) Gene transfer and molecular cloning of the rat nerve growth factor receptor. Nature **325:** 593–597
16 Rabizadeh S., Oh J., Zhong L. T., Yang J., Bitler, C. M., Butcher L.L. et al. (1993) Induction of apoptosis by the low-affinity NGF receptor. Science **261:** 345–348
17 Bothwell M. (1996) p75^{NTR}: a receptor after all. Science **272:** 506–507
18 Bredesen D. E. and Rabizadeh S. (1997) p75^{NTR} and apoptosis: Trk-dependent and Trk-independent effects. Trends Neurosci. **20:** 287–290
19 Frade J. M. and Barde Y. A. (1998) Nerve growth factor: two receptors, multiple functions. Bioessays **20:** 137X–145
20 Hantzopoulos P. A., Suri C., Glass D. J., Goldfarb M. P. and Yancopoulos G. D. (1994) The low affinity NGF receptor, p75, can collaborate with each of the Trks to potentiate functional responses to the neurotrophins. Neuron **13:** 187–201
21 Friedman W. J. and Greene L. A. (1999) Neurotrophin signaling via Trks and p75. Exp. Cell. Res. **253:** 131–142
22 Bibel M., Hoppe E. and Barde Y. A. (1999) Biochemical and functional interactions between the neurotrophin receptors trk and p75^{NTR}. EMBO J. **18:** 616–622
23 Pleasure S. J., Reddy U. R., Venkatakrishnan G., Roy A. K., Chen J., Ross, A. H. et al. (1990) Introduction of nerve growth factor (NGF) receptors into a medulloblastoma cell line results in expression of high- and low-affinity NGF receptors but not NGF-mediated differentiation. Proc. Natl. Acad. Sci. USA **87:** 8496–8500
24 Hempstead B. L., Schleifer L. S. and Chao M. V. (1989) Expression of functional nerve growth factor receptors after gene transfer. Science **243:** 373–375
25 Sutter A., Riopelle R. J., Harris-Warrick R. M. and Shooter E. M. (1979) Nerve growth factor receptors: characterization of two distinct classes of binding sites on chick embryo sensory ganglia cells. J. Biol. Chem. **254:** 5972–5982
26 Godfrey E. W. and Shooter E. M. (1986) Nerve growth factor receptors on chick sympathetic embryonic ganglion cells: Binding characteristics and development. J. Neurosci. **6:** 2543–2550
27 Rodriguez-Tebar A., Dechant G. and Barde Y. A. (1988) Binding characteristics of brain-derived neurotrophic factor to its receptors on neurons from the chick embryo. J.Neurosci. **8:** 3337–3342
28 Schechter A. L. and Bothwell M. A. (1981) Nerve growth factor receptors on PC12 cells: evidence for two receptor classes with differing cytoskeletal association. Cell **24:** 867–874
29 Woodruff N. R. and Neet K. E. (1986) Beta nerve growth factor binding to PC12 cells. Association kinetics and cooperative interactions. Biochemistry **25:** 7956–7966
30 Landreth G. E. and Shooter E. M. (1980) Nerve growth factor receptors on PC12 cells: ligand-induced conversion from low- to high-affinity states. Proc. Natl. Acad. Sci. USA **77:** 4751–4755
31 Bernd P. and Greene L. A. (1984) Association of 125I-nerve growth factor with PC12 pheochromocytoma cells. Evidence for internalization via high-affinity receptors only and for long-term regulation by nerve growth factor of both high- and low-affinity receptors. J. Biol. Chem. **259:** 15509–15516
32 Hosang M. and Shooter E. M. (1987) The internalization of nerve growth factor by high-affinity receptors on pheochromocytoma PC12 cells. EMBO J. **6:** 1197–1202
33 Kasaian M. T. and Neet K. E. (1988) Internalization of nerve growth factor by PC12 cells. A description of cellular pools. J. Biol. Chem. **263:** 5083–5090
34 Eveleth D. D. and Bradshaw R. A. (1988) Internalization and cycling of nerve growth factor in PC12 cells: interconversion of type II (fast) and type I (slow) nerve growth factor receptors. Neuron **1:** 929–936
35 Meakin S. O. and Shooter E. M. (1992) The nerve growth factor family of receptors. Trends Neurosci. **15:** 323–331
36 Mahadeo D., Kaplan L., Chao M. V. and Hempstead B. L. (1994) High affinity nerve growth factor binding displays a faster rate of association than p140trk binding. Implications for multi-subunit polypeptide receptors. J. Biol. Chem **269:** 6884–6891
37 Perez P., Coll P. M., Hempstead B. L., Martin-Zanca D. and Chao M. V. (1995) NGF binding to the trk tyrosine kinase receptor requires the extracellular immunoglobulin-like domains. Mol.Cell Neurosci. **6:** 97–105
38 Guo M., Meyer S. L., Kaur H., Gao J. J. and Neet K. E. (1996) Mutational studies of conserved residues in the dimer interface of nerve growth factor. Protein Sci. **5:** 447–455
39 Woo S. B. and Neet K. E. (1996) Characterization of histidine residues essential for receptor binding and activity of nerve growth factor. J. Biol. Chem. **271:** 24433–24441
40 Woo S. B., Timm D. E. and Neet K. E. (1995) Alteration of NH2-terminal residues of nerve growth factor affects activity and Trk binding without affecting stability or conformation. J.Biol.Chem **270:** 6278–6285

41 Jing S., Tapley P. and Barbacid M. (1992) Nerve growth factor mediates signal transduction through trk homodimer receptors. Neuron **9:** 1067–1079

42 Bothwell M. (1995) Functional interactions of neurotrophins and neurotrophin receptors. Annu. Rev. Neurosci. **18:** 223–253

43 Weskamp G. and Reichard, L. F. (1991) Evidence that biological activity of NGF is mediated through a novel subclass of high affinity receptors. Neuron **6:** 649–663

44 Rodriguez-Tebar A., Dechant G. and Barde Y. A. (1990) Binding of brain-derived neurotrophic factor to the nerve growth factor receptor. Neuron **4:** 487–492

45 Rodriguez-Tebar A., Dechant G., Gotz R. and Barde Y. A. (1992) Binding of neurotrophin-3 to its neuronal receptors and interactions with nerve growth factor and brain-derived neurotrophic factor. EMBO J. **11:** 917X–922

46 Hallbook F., Ibanez C. F. and Persson H. (1991) Evolutionary studies of the nerve growth factor family reveal a novel member abundantly expressed in Xenopus ovary. Neuron **6:** 845–858

47 Ernfors P., Ibanez C. F., Ebendal T., Olson L. and Persson H. (1990) Molecular cloning and neurotrophic activities of a protein with structural similarities to nerve growth factor: developmental and topographical expression in the brain. Proc. Natl. Acad. Sci. USA **87:** 5454–5458

48 Timm D. E., Ross A. H. and Neet K. E. (1994) Circular dichroism and crosslinking studies of the interaction between four neurotrophins and the extracellular domain of the low-affinity neurotrophin receptor. Protein Sci. **3:** 451–458

49 Woo S. B., Whalen C. and Neet K. E. (1998) Characterization of the recombinant extracellular domain of the neurotrophin receptor TrkA and its interaction with nerve growth factor (NGF). Protein Sci. **7:** 1006–1016

50 Urfer R., Tsoulfas P., O'Connell L., Shelton D. L., Parada L. F. and Presta L. G. (1995) An immunoglobulin-like domain determines the specificity of neurotrophin receptors. EMBO J. **14:** 2795–2805

51 Shelton D. L., Sutherland J., Gripp J., Camerato T., Armanini M. P., Phillips H. S. et al. (1995) Human trks: molecular cloning, tissue distribution, and expression of extracellular domain immunoadhesins. J. Neurosci. **15:** 477–491

52 Hartman D. S., McCormack M., Schubenel R. and Hertel C (1992) Multiple trkA proteins in PC12 cells bind NGF with a slow association rate. J. Biol. Chem **267:** 24516–24522

53 Philo J., Talvenheimo J., Wen J., Rosenfeld R., Welcher A. and Arakawa T. (1994) Interactions of neurotrophin-3 (NT-3), brain-derived neurotrophic factor (BDNF), and the NT-3.BDNF heterodimer with the extracellular domains of the TrkB and TrkC receptors. J.Biol.Chem **269:** 27840–27846

54 MacDonald J. I. and Meakin S. O. (1996) Deletions in the extracellular domain of rat trkA lead to an altered differentiative phenotype in neurotrophin responsive cells. Mol. Cell. Neurosci. **7:** 371–390

55 Urfer R., Tsoulfas P., O'Connell L., Hongo J. A., Zhao W. and Presta L. G. (1998) High resolution mapping of the binding site of TrkA for nerve growth factor and TrkC for neurotrophin-3 on the second immunoglobulin-like domain of the Trk receptors. J. Biol. Chem **273:** 5829–5840

56 O'Connell L., Hongo J.A., Presta L. G. and Tsoulfas P. (2000) TrkA amino acids controlling specificity for nerve growth factor. J. Biol. Chem. **275:** 7870–7877

57 Holden P. H., Asopa V., Robertson A. G., Clarke A. R., Tyler S., Bennett G. S. et al. (1997) Immunoglobulin-like domains define the nerve growth factor binding site of the TrkA receptor. Nat. Biotechnol. **15:** 668–672

58 Ultsch M. H., Wiesmann C., Simmons L. C., Henrich J., Yang M., Reilly D. et al. (1999) Crystal structures of the neurotrophin-binding domain of TrkA, TrkB and TrkC. J. Mol. Biol. **290:** 149–159

59 Wiesmann C., Ultsch M. H., Bass S. H. and Vos A. M. de (1999) Crystal structure of nerve growth factor in complex with the ligand- binding domain of the TrkA receptor. Nature **401:** 184–188

60 Windisch J. M., Auer B., Marksteiner R., Lang M. E. and Schneider R. (1995) Specific neurotrophin binding to leucine-rich motif peptides of TrkA and TrkB. FEBS Lett. **374:** 125–129

61 Windisch J. M., Marksteiner R., Lang M. E., Auer B. and Schneider, R (1995) Brain-derived neurotrophic factor, neurotrophin-3, and neurotrophin-4 bind to a single leucine-rich motif of TrkB. Biochemistry **34:** 11256–11263

62 Windisch J. M., Marksteiner R. and Schneider R. (1995) Nerve growth factor binding site on TrkA mapped to a single 24-amino acid leucine-rich motif. J. Biol. Chem **270:** 28133–28138

63 Ninkina N., Grashchuck M., Buchman V. L. and Davies A. M. (1997) TrkB variants with deletions in the leucine-rich motifs of the extracellular domain. J. Biol. Chem. **272:** 13019–13025

64 Robinson R., Radziejewski C., Spraggon G., Greenwald J., Kostura M. R., Burtnick L. D. et al. (1999) The structures of the neurotrophin-4 homodimer and the brain-derived neurotrophic factor/neurotrophin-4 heterodimer reveal a common Trk-binding site. Protein Sci. **8:** 2589–2597

65 Ibanez C. F. (1994) Structure-function relationships in the neurotrophin family. J. Neurobiol. **25:** 1349–1361

66 Wells J. A. (1996) Binding in the growth hormone receptor complex. Proc. Natl. Acad. Sci. USA **93:** 1–6

67 Livnah O., Stura E. A., Middleton S. A., Johnson D. L., Jolliffe L. K. and Wilson I. A. (1999) Crystallographic evidence for preformed dimers of erythropoietin receptor before ligand activation. Science **283:** 987–990

68 Sehgal A., Bothwell M. and Chao M. (1989) Gene transfer of truncated NGF receptor clones leads to cell surface expression in mouse fibroblasts. Nucleic Acids Res. **17:** 5623–5632

69 Welcher A. A., Bitler C. M., Radeke M. J. and Shooter E. M. (1991) Nerve growth factor binding domain of the nerve growth factor receptor. Proc. Natl. Acad. Sci. USA **88:** 159–163

70 Baldwin A. N., Bitler C. M., Welcher A. A. and Shooter E. M. (1992) Studies on the structure and binding properties of the cysteine-rich domain of rat low affinity nerve growth factor receptor ($p75^{NGFR}$). J. Biol. Chem. **267:** 8352–8359

71 Yan H. and Chao M. V. (1991) Disruption of cysteine-rich repeats of the p75 nerve growth factor receptor leads to loss of ligand binding. J. Biol. Chem. **266:** 12099–12104

72 Vissavajjhala P., Leszyk J. D., Lin-Goerke J. and Ross A. H. (1992) Structural domains of the extracellular domain of human nerve growth factor receptor detected by partial proteolysis. Arch. Biochem. Biophys. **294:** 244–252

73 Baldwin A. N. and Shooter E. M. (1995) Zone mapping of the binding domain of the rat low affinity nerve growth factor receptor by the introduction of novel N-glycosylation sites. J. Biol. Chem. **270:** 4594–4602

74 Chapman B. S. and Kuntz I. D. (1995) Modeled structure of the 75-kDa neurotrophin receptor. Protein Sci. **4:** 1696–1707

75 Shamovsky I. L., Ross G. M., Riopelle R. J. and Weaver D. F. (1999) The interaction of neurotrophins with the $p75^{NTR}$ common neurotrophin receptor: a comprehensive molecular modeling study. Protein Sci **8:** 2223X–2233

76 Barbacid M. (1993) Nerve growth factor: a tale of two receptors. Oncogene. **8:** 2033–2042

77 Glass D. J. and Yancopoulos G. D. (1993) The neurotrophins and their receptors. Trends Cell Biol. 3: 262–268

78 Raffioni S., Bradshaw R. A. and Buxser S. E. (1993) The receptors for nerve growth factor and other neurotrophins. Annu. Rev. Biochem. **62:** 823–850

79 Barbacid M. (1994) The Trk family of neurotrophin receptors. J. Neurobiol. **25:** 1386–1403

80 Battleman D. S., Geller A. I. and Chao M. V. (1993) HSV-1 vector-mediated gene transfer of the human nerve growth factor receptor $p75^{hNGFR}$ defines high-affinity NGF binding. J. Neurosci. **13:** 941–951

81 Benedetti M., Levi A. and Chao M. (1993) Differential expression of NGF receptors leads to altered binding affinity and neurotrophin responsiveness. Proc. Natl. Acad. Sci.USA **90:** 7859–7863

82 Hempstead B. L., Martin-Zanca D., Kaplan D. R., Parada L. F. and Chao M. V. (1991) High-affinity NGF binding requires coexpression of the trk proto-oncogene and the low-affinity NGF receptor. Nature **350:** 678–683

83 Meakin S. O., Suter U., Drinkwater C. C., Welcher A. A. and Shooter E. M. (1992) The rat trk protooncogene product exhibits properties characteristic of the slow nerve growth factor receptor. Proc. Natl. Acad. Sci. USA **89:** 2374–2378

84 Dechant G., Tsoulfas P., Parada L. F. and Barde Y. A. (1997) The neurotrophin receptor p75 binds neurotrophin-3 on sympathetic neurons with high affinity and specificity. J. Neurosci. **17:** 5281–5287

85 Huber L. J. and Chao M. V. (1995) A potential interaction of p75 and trkA NGF receptors revealed by affinity crosslinking and immunoprecipitation. J. Neurosci. Res. **40:** 557–563

86 Ross G. M., Shamovsky I. L., Lawrance G., Solc M., Dostaler S, M,, Weaver D. F. and Riopelle R. J. (1998) Reciprocal modulation of TrkA and $p75^{NTR}$ affinity states is mediated by direct receptor interactions. Eur. J. Neurosci. **10:** 890–898

87 Wolf D. E., McKinnon C. A., Daou M. C., Stephens R. M., Kaplan D. R. and Ross A. H. (1995) Interaction with TrkA immobilizes gp75 in the high affinity nerve growth factor receptor complex. J. Biol. Chem **270:** 2133–2138

88 Wolf D. E., McKinnon-Thompson C., Daou M. C., Stephens R. M., Kapla, D. R. and Ross A. H. (1998) Mobility of TrkA is regulated by phosphorylation and interactions with the low-affinity NGF receptor. Biochemistry **37:** 3178–3186

89 Ross A. H., Daou M. C., McKinnon C. A., Condon P. J., Lachyankar M. B., Stephens R. M. et al. (1996) The neurotrophin receptor, gp75, forms a complex with the receptor tyrosine kinase TrkA. J. Cell. Biol. **132:** 945–953

90 Yan H., Schlessinger J. and Chao M. V. (1991) Chimeric NGF-EGF receptors define domains responsible for neuronal differentiation. Science **252:** 561–563

91 Clary D. O. and Reichardt L. F. (1994) An alternatively spliced form of the nerve growth factor receptor TrkA confers an enhanced response to neurotrophin 3. Proc. Natl. Acad. Sci. USA **91:** 11133–11137

92 Barker P. A. and Shooter E. M. (1994) Disruption of NGF binding to the low affinity neurotrophin receptor $p75^{LNTR}$ reduces NGF binding to TrkA on PC12 cells. Neuron. **13:** 203–215

93 Chao M. V. (1992) Neurotrophin receptors: a window into neuronal differentiation. Neuron. **9:** 583–593

94 Wiesmann C. and Vos A. M. de (2000) Variations on ligand-receptor complexes. Nature Struct. Biol. **7:** 440–442

95 Loeb D. M and Greene L. A. (1993) Transfection with trk restores „slow" NGF binding, efficient NGF uptake, and multiple NGF responses to NGF-nonresponsive PC12 cell mutants. J.Neurosci. **13:** 2919–2929

96 Eveleth D. D. and Bradshaw R. A. (1992) Nerve growth factor nonresponsive pheochromocytoma cells: altered internalization results in signaling dysfunction. J.Cell Biol. **117:** 291–299

97 Zapf-Colby A. and Olefsky J. M. (1998) Nerve growth factor processing and trafficking events following TrkA- mediated endocytosis. Endocrinology **139:** 3232–3240

98 Hendry I. A., Stockel K., Thoenen H. and Iversen L. L. (1974) The retrograde axonal transport of nerve growth factor. Brain Res. **68:** 103–121

99 Stoeckel K., Guroff G., Schwab M. and Thoenen H. (1976) The significance of retrograde axonal transport for the accumulation of systemically administered nerve growth factor (NGF) in the rat superior cervical ganglion. Brain Res. **109:** 271–284

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

101 Palmatier M. A., Hartman B. K. and Johnson E. M. Jr (1984) Demonstration of retrogradely transported endogenous nerve growth factor in axons of sympathetic neurons. J Neurosci **4:** 751–756

102 Johnson E. M. Jr, Andres R. Y. and Bradshaw R. A. (1978) Characterization of the retrograde transport of nerve growth factor (NGF) using high specific activity [125-I] NGF. Brain Res. **150:** 319–331

103 Heumann R., Schwab M. and Thoenen H. (1981) A second messenger required for nerve growth factor biological activity? Nature **292:** 838–840

104 Parton R. G. and Dotti C. G. (1993) Cell biology of neuronal endocytosis. J. Neurosci. Res. **36:** 1–9

105 Ehlers M. D., Kaplan D. R., Price D. L. and Koliatsos V. E. (1995) NGF-stimulated retrograde transport of trkA in the mammalian nervous system. J.Cell Biol. **130:** 149–156

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

107 Grimes M. L., Beattie E. and Mobley W. C. (1997) A signaling organelle containing the nerve growth factor-activated receptor tyrosine kinase, TrkA. Proc. Natl. Acad. Sci. USA **94:** 9909–9914

108 Grimes M. L., Zhou J., Beattie E. C., Yuen E. C., Hall D. E., Valletta J. S. et al. (1996) Endocytosis of activated TrkA: evidence that nerve growth factor induces formation of signaling endosomes. J. Neurosci. **16:** 7950–7964

109 Huang C. S., Zhou J., Feng A. K., Lynch C. C., Klumperman J., DeArmond S. J. et al. (1999) Nerve growth factor signaling in caveolae-like domains at the plasma membrane. J. Biol. Chem. **274:** 36707–36714

110 Hendry I. A. and Crouch M. F. (1991) Retrograde axonal transport of the GTP-binding protein Gi alpha: a potential neurotrophic intra-axonal messenger. Neurosci. Lett. **133:** 29–32

111 Johanson S. O., Crouch M. F. and Hendry I. A. (1995) Retrograde axonal transport of signal transduction proteins in rat sciatic nerve. Brain Res. **690:** 55–63

112 Claude P, Hawrot, E, Dunis, DA and Campenot, RB (1982) Binding, internalization, and retrograde transport of 125I-nerve growth factor in cultured rat sympathetic neurons. J. Neurosci. **2:** 431–442

113 Overly C. C. and Hollenbeck P. J. (1996) Dynamic organization of endocytic pathways in axons of cultured sympathetic neurons. J. Neurosci. **16:** 6056–6064

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

115 Ure D. R. and Campenot R. B. (1994) Leukemia inhibitory factor and nerve growth factor are retrogradely transported and processed by cultured rat sympathetic neurons. Dev. Biol. **162:** 339–347

116 Campenot R. B. (1992) Compartmented culture analysis of nerve growth. In: Cell-Cell Interactions: A Practical Approach pp. 275–298, Stevenson B., Paul D., and Gallin W. (eds), IRL Oxford

117 Campenot R. B. (1997) Construction and use of compartmented cultures. In: Protocols for Neural Cell culture pp. 107–116, Federoff S. and Richardson A. (eds), Humana, Totowa, N. J.

118 Senger D. L. and Campenot R. B. (1997) Rapid retrograde tyrosine phosphorylation of trkA and other proteins in rat sympathetic neurons in compartmented cultures. J. Cell. Biol. **138:** 411–421

119 Fukumoto S., Mutoh T., Hasegawa T., Miyazaki H., Okada M, Goto G. et al. (2000) GD3 synthase gene expression in PC12 cells results in the continuous activation of TrkA and ERK1/2 and enhanced proliferation. J. Biol. Chem. **275:** 5832–5838

120 Riccio A., Pierchala B. A., Ciarallo C. L. and Ginty D. D. (1997) An NGF-TrkA-mediated retrograde signal to transcription factor CREB in sympathetic neurons. Science **277:** 1097–1100

121 Toma J. G., Rogers D., Senger D. L., Campenot R. B. and Miller F. D. (1997) Spatial regulation of neuronal gene expression in response to nerve growth factor. Dev. Biol. **184:** 1–9

122 Watson F. L., Heerssen H. M., Moheban D. B., Lin M. Z., Sauvageot C. M., Bhattacharyya A. et al. (1999) Rapid nuclear responses to target-derived neurotrophins require retrograde transport of ligand-receptor complex. J. Neurosci. **19:** 7889–7900

123 Curtis R., Adryan K. M., Stark J. L., Park J. S., Compton D. L., Weskamp G. et al. (1995) Differential role of the low affinity neurotrophin receptor (p75) in retrograde axonal transport of the neurotrophins. Neuron **14:** 1201–1211

124 Airaksinen M. S, Titievsky A. and Saarma M. (1999) GDNF family neurotrophic factor signaling: four masters, one servant? Mol. Cell. Neurosci. **13:** 313–325

125 Baloh R. H., Enomoto H., Johnson E. M. Jr and Milbrandt J. (2000) The GDNF family ligands and receptors - implications for neural development. Curr. Opin. Neurobiol. **10:** 103–110

126 Baloh R. H., Gorodinsky A., Golden J. P., Tansey M. G., Keck C. L., Popescu N. C. et al. (1998) GFRalpha3 is an orphan member of the GDNF/neurturin/persephin receptor family. Proc. Natl. Acad. Sci. USA **95:** 5801–5806

127 Klein R. D., Sherman D., Ho W. H., Stone D., Bennett G. L., Moffat B. et al (1997) A GPI-linked protein that interacts with Ret to form a candidate neurturin receptor. Nature **387:** 717–721

128 Baloh R. H., Tansey M. G., Golden J. P., Creedon D. J., Heuckeroth R. O., Keck C. L. et al. (1997) TrnR2, a novel receptor that mediates neurturin and GDNF signaling through Ret. Neuron **18:** 793–802

129 Jing S., Yu Y., Fang M., Hu Z., Holst P. L., Boone T. et al. (1997) GFRalpha-2 and GFRalpha-3 are two new receptors for ligands of the GDNF family. J. Biol. Chem. **272:** 33111–33117

130 Sanicola M., Hession C., Worley D., Carmillo P., Ehrenfels C., Walus L. et al. (1997) Glial cell line-derived neurotrophic factor-dependent RET activation can be mediated by two different cell-surface accessory proteins. Proc. Natl. Acad. Sci. USA **94:** 6238–6243

131 Suvanto P., Wartiovaara K., Lindahl M., Arumae U., Moshnyakov M., Horelli-Kuitunen N. et al. (1997) Cloning, mRNA distribution and chromosomal localisation of the gene for glial cell line-derived neurotrophic factor receptor beta, a homologue to GDNFR-alpha. Hum. Mol. Genet. **6:** 1267–1273

132 Cik M., Masure S., Lesage A. S., Linden I. Van Der, Gompel P. Van, Pangalos M. N. et al. (2000) Binding of GDNF and neurturin to human GDNF family receptor {alpha} 1 and 2 (GFR{alpha}1-2); Influence of cRET and co-operative interactions. J. Biol. Chem. **275:** 25505–25512

133 Trupp M., Scott R., Whittemore S. R. and Ibanez C. F. (1999) Ret-dependent and -independent mechanisms of glial cell line-derived neurotrophic factor signaling in neuronal cells. J. Biol. Chem. **274:** 20885–28894

134 Poteryaev D., Titievsky A., Sun Y. F., Thomas-Crusells J., Lindahl M., Billaud M. et al. (1999) GDNF triggers a novel ret-independent Src kinase family-coupled signaling via a GPI-linked GDNF receptor alpha1. FEBS Lett **463:** 63–66

135 Ip N. Y. and Yancopoulos G. D. (1996) The neurotrophins and CNTF: two families of collaborative neurotrophic factors. Annu. Rev. Neurosci. **19:** 491–515

136 Heinrich P. C., Behrmann I., Muller-Newen G., Schaper F. and Graeve L. (1998) Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. Biochem. J. **334:** 297–314

137 Taga T., Hibi M., Hirata Y., Yamasaki K., Yasukaw, K., Matsuda T. Hirano T. et al. (1989) Interleukin-6 triggers the association of its receptor with a possible signal transducer, gp130. Cell **58:** 573–581

138 Gearing D. P., Thut C. J., VandeBos T., Gimpel S. D., Delaney P. B., King J. et al. (1991) Leukemia inhibitory factor receptor is structurally related to the IL-6 signal transducer, gp130. EMBO J. **10:** 2839–2848

139 Davis S., Aldrich T. H., Valenzuela D. M., Wong V. V., Furth M. E., Squinto S. P. et al. (1991) The receptor for ciliary neurotrophic factor. Science **253:** 59–63

140 Ip N. Y., Nye S. H., Boulton T. G., Davis S., Taga T., Li Y. et al. (1992) CNTF and LIF act on neuronal cells via shared signaling pathways that involve the IL-6 signal transducing receptor component gp130. Cell **69:** 1121–1132

141 Davis S., Aldrich T. H., Stahl N., Pan L., Taga T., Kishimoto T., Ip N. Y. and Yancopoulos G. D. (1993) LIFR beta and gp130 as heterodimerizing signal transducers of the tripartite CNTF receptor. Science **260:** 1805–1808

142 Murakami M., Hibi M., Nakagawa N., Nakagawa T., Yasukawa K., Yamanishi K. et al. (1993) IL-6-induced homodimerization of gp130 and associated activation of a tyrosine kinase. Science **260:** 1808–1810

143 Stahl N., Davis S., Wong V., Taga T., Kishimoto T., Ip N. Y. and Yancopoulos G. D. (1993) Cross-linking identifies leukemia inhibitory factor-binding protein as a ciliary neurotrophic factor receptor component. J. Biol. Chem. **268:** 7628–7631

144 Simpson R. J., Hammacher A., Smith D. K., Matthews J. M. and Ward, LD (1997) Interleukin-6: structure-function relationships. Protein Sci. **6:** 929–955

145 Davis S., Aldirch T. H., Ip N. Y., Stahl N., Scherer S., Farruggella T. et al. (1993) Released form of CNTF receptor alpha component as a soluble mediator of CNTF responses. Science **259:** 1736–1739

146 Marz P., Otten U. and Rose-John S. (1999) Neural activities of IL-6-type cytokines often depend on soluble cytokine receptors. Eur. J. Neurosci. **11:** 2995–3004

147 Leitner M. L., Molliver D. C., Osborne P. A., Vejsada R., Golden J. P., Lampe P. A. et al. (1999) Analysis of the retrograde transport of glial cell line-derived neurotrophic factor (GDNF), neurturin, and persephin suggests that in vivo signaling for the GDNF family is GFRalpha coreceptor-specific. J. Neurosci. **19:** 9322–9331

148 Curtis R., Adryan K. M., Zhu Y., Harkness P. J., Lindsay R. M. and DiStefano P. S. (1993) Retrograde axonal transport of ciliary neurotrophic factor is increased by peripheral nerve injury. Nature **365:** 253–255

149 Curtis R., Scherer S. S., Somogyi R., Adryan K. M., Ip N. Y., Zhu Y. et al. (1994) Retrograde axonal transport of LIF is increased by peripheral nerve injury: correlation with increased LIF expression in distal nerve. Neuron. **12:** 191–204

150 Bilderback T. R., Gazula V. R., Lisanti M. P. and Dobrowsky R. T. (1999) Caveolin interacts with Trk A and p75(NTR) and regulates neurotrophin signaling pathways. J. Biol. Chem **274:** 257–263

151 Huang C. S., Zhou J., Feng A. K., Lynch C. C., Klumperman J., DeArmond S. J. and Mobley W. C. (1999) Nerve growth factor signaling in caveolae-like domains at the plasma membrane. J. Biol. Chem. **274:** 36707–36714