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Review

Nerve growth factor: structure and function

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Abstract. Neurotrophins are critical for the development and maintenance of the peripheral and central nervous system. These highly homologous, homodimeric growth factors control cell survival, differentiation, growth cessation, and apoptosis of sensory neurons. The biological functions of the neurotrophins are mediated through two classes of cell surface receptors, the Trk receptors and the p75 neurotrophin receptor ($p75^{NTR}$). Nerve growth factor (NGF), the best characterized member of the neurotrophin family, sends its survival signals through activation of TrkA and can induce cell death by binding to $p75^{NTR}$. Recent domain deletion and mutagenesis studies have identified the membrane-proximal domain of the Trks as necessary and sufficient for ligand binding. Crystal structures of this domain of TrkA, TrkB, and TrkC, and an alanine scanning analysis of this domain of TrkA and TrkC have allowed identification of the ligand-binding site. The recent crystal structure of the complex between NGF and the ligand-binding domain of TrkA defines the orientation of NGF in the signaling complex, and eludicates the structural basis for binding and specificity in the family. Further structural work on NGF-TrkA-$p7S^{NTR}$ complexes will be necessary to address the many remaining questions in this complex signaling system.

Key words. Nerve growth factor; neurotrophin; Trk; p75 neurotrophin receptor; mutagenesis; structure.

Introduction

Nerve growth factor (NGF), discovered almost half a century ago [1], is the founding and best-characterized member of the neurotrophin family [2]. The biological function of the neutrophins is the maintenance and survival of the peripheral and central nervous systems [3], which makes them of great therapeutic interest for the treatment of a number of neurodegenerative diseases. Beside the pivotal role of NGF in the development of neuronal cells, it also has important functions on non-neuronal cells. For example, it is an autocrine survival factor for memory B lymphocytes [4]. The neurotrophins represent a family of structurally and functionally related, homodimeric proteins, including NGF, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), neurotrophin-4/5 (NT-4/5), and neurotrophin-6 [5–8]. All neurotrophins share a pair-wise sequence identity of approximately 50% and are responsible for a variety of signaling events on distinct but overlapping sets of neuronal populations.

Neurotrophins mediate their signal through binding to two distinct classes of cell surface receptors: the shared p75 neurotrophin receptor ($p75^{NTR}$) and the Trk receptors. $p75^{NTR}$, which is also referred to as the low-affinity neurotrophin receptor, belongs to the tumor necrosis factor receptor family and binds to all neurotrophins with similar, nanomolar affinities [reviewed in refs 9, 10]. Despite this promiscuity, activation of $p75^{NTR}$ can only be achieved through NGF. Receptor activation then initiates apoptosis in the context of TrkA-negative neurons, but promotes survival even at low concentrations of NGF if

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TrkA is co-expressed on the cell surface [reviewed in refs 10–13].

The second class of neurotrophin receptors, the Trks, are receptor tyrosine kinases that dimerize upon ligand binding to their extracellular portions. This dimerization event leads to autophosphorylation of several tyrosine residues in the cytoplasmic domain of the receptors, triggering the intracellular signal transduction cascade. Three different Trk receptors are known in vertebrates. They bind to their ligands with high, picomolar affinity and display a high degree of specificity. TrkA is the receptor for NGF, TrkB binds to BDNF and NT-4/5, and TrkC is the preferred receptor for NT-3. NT-3 appears to be somewhat promiscuous, since it is also capable of binding to and signaling through TrkA and TrkB [14–20].

Over the past decade, a wealth of structural and functional information has been gathered on the neurotrophin signaling system [reviewed in refs 10, 21, 22]. For the ligands, determination of the crystal structures of NGF and several other neurotrophins sparked a number of mutagenesis studies to map the binding epitopes for $p75^{NTR}$ as well as the Trk receptors. The results of these earlier studies were successfully applied in the creation of artificial neurotrophins with novel receptor specificity profiles. In this review, we focus on new insights into the structural basis for affinity and specificity among the neurotrophins, their Trk receptors, and $p75^{NTR}$, derived from recent crystal structures, mutagenesis experiments, and modeling studies. For the Trks, deletion and mutagenesis analyses of the ectodomains have identified a necessary and sufficient ligand-binding fragment as well as individual residues important for affinity and specificity. The crystal structures of the ligand-binding domain of all three Trks now provide the structural background for the interpretation of these results. Furthermore, the crystal structure of the complex between NGF and the ligand-binding domain of TrkA unambiguously defines the orientation of NGF with respect to the membrane and elucidates specificity and binding for the entire family. Finally, for $p75^{NTR}$, a comprehensive modeling study has

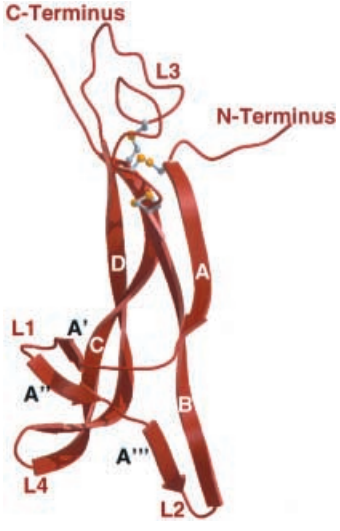

Figure 1. Ribbon diagram depicting the structure of the NGF monomer (PDB code 1BFT). The secondary structure elements are labeled and depicted according to McDonald et al. [23]. The termini as well as the loop regions, L1-L4, are labeled in red, and the cysteine residues forming the cysteine-knot motif near the top of the molecule are shown in gray and yellow in ball-and-stick rendering. Figures 1, 2, 4, and 6–8 were made using programs Molscript 2 [83] and Raste 3D [84].

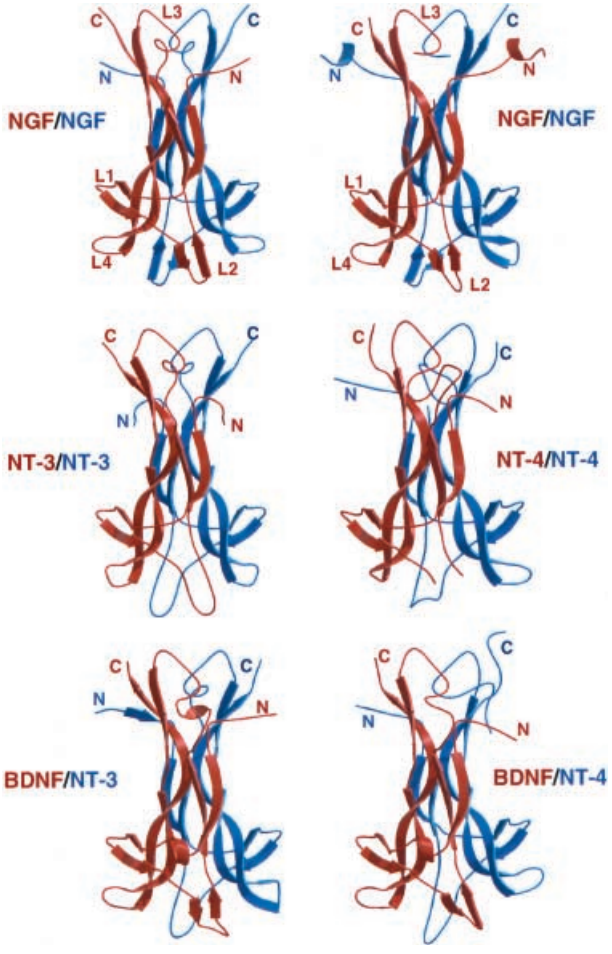

Figure 2. Ribbon diagrams of experimentally determined neurotrophin dimers. The secondary structure elements are depicted as determined with option 'molauto' in program Molscript 2 [83]. All N and C termini are labeled; unlabeled ends arc due to disordered segments in the crystal structures. In all cases, the monomers dimerize in parallel fashion, positioning the N and C termini at the same end of the molecule (top in this figure). Loops important for $p75^{NTR}$ binding (L1, L3, L4) or Trk specificity (L2, L4) are labeled. Upper row: NGF dimers (left: unbound NGF, PDB access code 1BET; right: NGF from the complex with the second Ig-like domain of its TrkA receptor, PDB access code 1WWW). Center row: dimers of NT-3 (left: PDB access code 1NT3) and NT-4 (right: PDB access code 1B98). Lower row: heterodimers between BDNF (in red) and NT3 (left: PDB access code 1BND), and between BNDF (in red) and NT4 (right: PDB access code 1B8M).

provided a possible view of its interactions with NGF, herein updated to take into account the most recent experimental structural information. It is hoped that these new results will inspire further studies aimed at answering the many questions that still remain unanswered.

NGF and the other neurotrophins

When the structure of NGF was determined by X-ray crystallography in 1991 [23], it represented a novel protein fold (fig. 1). The structure revealed that the NGF monomer has an elongated shape with the central part of the moleculc formed by two pairs of twisted, antiparallel β-strands. There are three hairpin loops on one end, and the other end carries a cysteine-knot motif that stabilizes the fold and locks the molecules in their conformation. In the biologically active form, two monomers are arranged in a parallel manner to form a close-packed homodimer (fig. 2). Since then, the structures of BDNF, NT-3, and NT-4, as well as two heterodimers, have also been determined [24-27] and shown to be very close structural homologues of NGF (fig. 2).

The cysteine-knot motif, observed for the first time in NGF, is formed by three disulfide bonds. Two of them, together with the protein backbone that connects the cysteines, form a closed ring, which is penetrated by the third disulfide bridge. This very stable, close-packed motif has since been found in the structures of a number of dimeric growth factors that share no other sequence homology with the neurotrophins. Four families are recognized to date, exemplified by NGF, platelet-derived growth factor, transforming growth factor-β, and human chorionic gonadotropin. Interestingly, these molecules share a similar overall structural scaffold, consisting of two pairs of two-stranded β strands in the center with the cystine-knot motif on one end and loops of varying lengths on the other. The characteristic feature distinguishing the four families is their dimerization mode. In contrast to the other families, NGF (like the other neurotrophins) forms parallel dimers with both monomers assembled around a central twofold axis. The long axis of the dimer coincides with the twofold axis, giving NGF an overall dumbbell-like shape. The two central β sheets (AB and CD; figs 1, 2) of the two monomers pack against each other and form the 'handle' of the dumbbell. Residues from these four β strands are responsible for the majority of interactions that stabilize the dimer. The cysteine-knot motif, the N and C termini, and the third loop connecting strands B and C (L3; figs 1, 2) form one end of the dumbbell, now shown by the crystal structure of the NGF-TrkA domain 5 complex [28] to be pointing away from the membrane (see below). The other, membrane-facing end contains three hairpin loops L1, L2, and L4, as well as four short β strands arranged in two antiparallel β sheets (figs 1, 2).

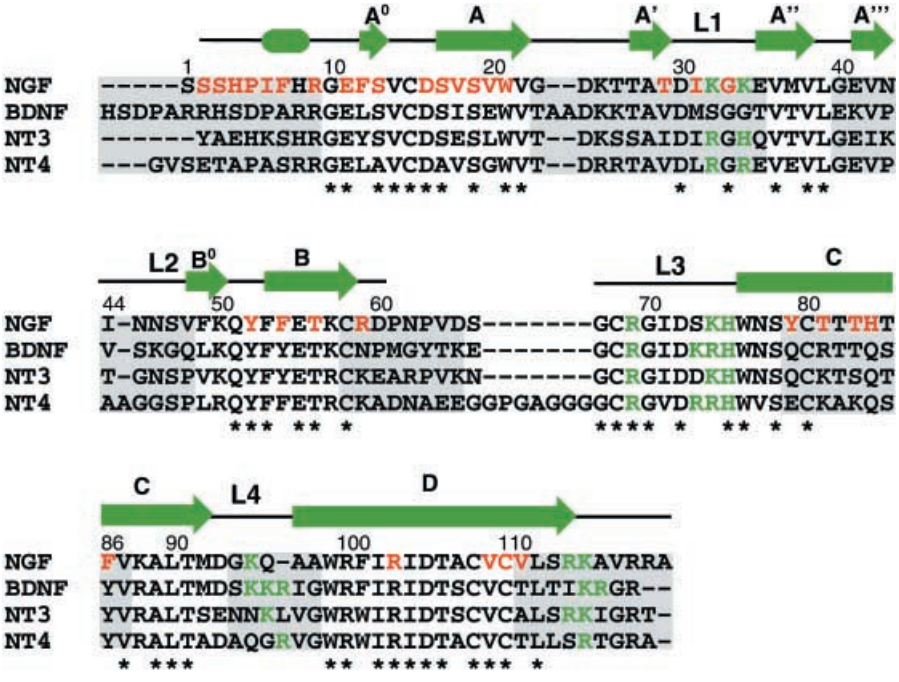

Figure 3. Sequence alignment of the neurotrophins. Secondary structure elements are depicted as defined in Wiesmann et al. [28]; numbering refers to the sequence of mature human NGF. Conserved residues are marked with an asterisk and regions with low sequence homology as defined by Ibáñez et al. [56] are shaded. NGF residues in contact with TrkA-d5 are shown in red, and residues important for $p75^{NTR}$ binding are shown in green.

Sequence alignments of the neurotrophins show that the overall sequence identity of about 50% is unevenly distributed along the primary structures. Four distinct segments are highly homologous (fig. 3). These segments map onto strands A, B, and D, and also include residues from loop L3 at the 'top' of the neurotrophin dimers. Many of these conserved residues are involved in the dimer interface, allowing the formation of certain heterodimeric neurotrophin species. For example, the BDNF monomer is especially promiscuous about its dimerization partner, and crystal structures of the BDNF/NT-3 and BDNF/NT-4 heterodimers have been reported [25, 26]. However, whether such heterodimeric species havc any biological relevance remains unclear.

7S NGF

NGF can be obtained in large quantities from the mouse submandibular salivary gland [29], where it is found in complex with α-NGF and γ-NGF, two related serine proteases of the kallikrein family (in this complex, NGF proper is denoted β-NGF). NGF is expressed in the form of a prepropeptide, containing a signal peptide comprising 18 residues as well as a propeptide comprising an additional 103 residues. While γ-NGF is a highly specific, active protease, capable of processing the pre-NGF to its mature form [30, 31], α-NGF is inactive and has been described as a 'locked zymogen' [32]. The crystal structure of the ternary complex has been solved at 3.15 Å resolution [32]. The complex contains two copies of each component, yielding an $\alpha_2\beta_2\gamma_2$-stoichiometry symmetrically arranged around a two-fold symmetry axis relating the two NGF molecules (fig. 4). The complex is stabilized by two zinc ions that bind in the interfaces between NGF and γ-NGF.

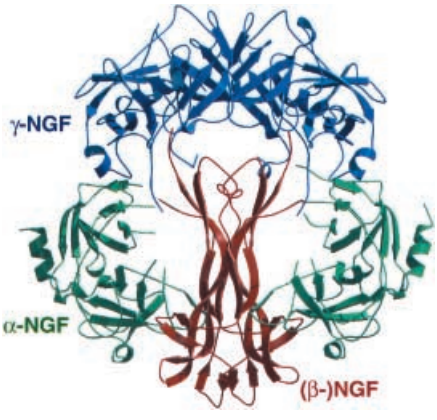

Figure 4. Ribbon diagram showing the structure of the 7S NGF complex (PDB access code I SGF). The dimer of (β-)NGF is depicited in red at the center, two copies of α-NGF are shown in green, and two copies of γ-NGF in blue. Both the Trk and the $p75^{NTR}$ binding sites are blocked by the α and γ chains (compare fig. 8).

The NGF dimer is in contact with both copies of the α- and γ-NGF molecules [32]. The α-NGF molecules are bound to the central portion of NGF, partially overlapping the 'conserved patch' of the Trk receptor-binding site (see below). This interface buries about 2150 $Å^2$ compared to approximately 2400 $Å^2$ of buried surface between NGF and γ-NGF. The contacts to γ-NGF involve residues at the 'top' of the NGF dimer, including the solvent-exposed loop L3 and a number of residues from the C terminus. Interestingly, the NGF C terminus is bound in the active site of the protease. While there are no contacts between the two α-NGF molecules, the two γ-NGF molecules have an extensive interface with each other. This interface is over 2500 $Å^2$ in area and likely to contribute significant stabilization to the overall complex. However, this interaction is not sufficient to stabilize γ-NGF dimers in the absence of NGF, since free γ-NGF is monomeric in solution [33].

Trk receptors

The neurotrophin receptors TrkA, TrkB, and TrkC share a common architecture, with their extracellular portion consisting of a cysteine-rich cluster (domain 1), three leucine-rich repeats (domain 2), a second cysteine-rich cluster (domain 3), and two immunoglobulin(Ig)-like domains (domains 4 and 5) [34]. The extracellular portion is linked by a single putative transmembrane helix to the intracellular tyrosine kinase domain. The kinase domains are highly homologous, with sequence identities greater than 75% [reviewed in ref 2], while the extracellular portions are more divergent as seen in sequence similarities of about 50–55% [35]. The most highly conserved segment in the extracellular region of the three receptors is a segment in domain 5 with 9 of 11 residues identical among the three Trks (fig. 5). The crystal structures of this domain of TrkA, TrkB, and TrkC [36] show that this segment includes the EF loop, and the structure of the complex between NGF and domain 5 of TrkA [28] reveals the function of these residues (see below).

The complex and modular nature of the extracellular portion of the Trk receptors raises the question of the function of the individual domains, and numerous studies have been aimed at identifying the ligand-binding domain. A number of studies have focused on the Ig-like domains (domains 4 and 5), and the preponderance of evidence supports a direct role of domain 5 in ligand binding. Early studies primarily involved the generation of truncated or chimeric versions of the receptors. Pérez et al. [37] constructed a series of chimeras using fragments from TrkA and TrkB, and showed that a tandem construct of both Ig-like domains is sufficient for neurotrophin binding. Consistent with these findings, Holden et al. [38] showed that a similar construct of TrkA ex-

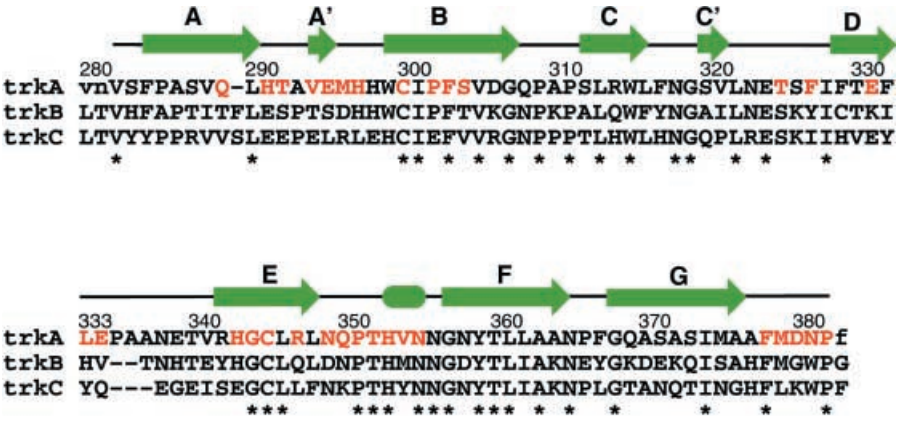

Figure 5. Sequence alignment of domain 5 of the Trk receptors. Secondary structure elements and numbering refer to TrkA-d5 [28]. TrkA residues that are in contact with NGF in the NGF-TrkA-d5 complex are colored red. Residues that are identical among all three Trks are marked with an asterisk.

pressed in *Escherichia coli* bound to NGF and inhibited NGF activity both in vitro and in vivo. Furthermore, deletion of only small portions within domain 5 of TrkA (TrkA-d5) abolished neurotrophin binding [39]. Urfer et al. [40], using chimeras and truncated versions from all three Trks, showed that ligand specificity is determined primarily by domain 5 (Trk-d5). A different approach was taken by Haniu et al. [41]. Using partial proteolytic digestion, they identified two TrkB fragments with significant binding affinity for BDNF. One of those fragments contained parts of the leucine-rich repeats (domain 2), the other comprised domain 5. Finally, the Ig-like domains of TrkA and TrkB have been proposed to be involved in a cell surface recognition process inhibiting neurite outgrowth [42].

The importance of individual amino acid residues for ligand binding and specificity was probed in an extensive alanine-scanning analysis of domain 5 of TrkA and TrkC [43]. Since no experimental structures of Trk receptor domains were available at the time, a model of domain 5 based on the crystal structure of VCAM was used to identify potential solvent-exposed surface residues as candidates for mutation to alanine. A total of about 50 residues were replaced individually in both TrkA and TrkC. Of these, 17 resulted in a greater than five fold reduction for NGF binding to TrkA, while only 4 alanine mutants had a similar effect on NT-3 binding to TrkC. These four residues are located in the EF loop (see below), the region with the highest sequence conservation in the entire ectodomain of the Trk receptors. The crystal structure of the NGF-TrkA-d5 complex shows that three of these four residues are intimately involved in ligand-receptor contacts (see below).

The crystal structures of domain 5 of TrkA, TrkB, and TrkC [36] provided the first structural information on any portion of the Trk receptors (fig. 6). As predicted [34], these domains adopt an Ig-like fold; the experimental structures further identified them as members of the I-set of Ig-like domains [36]. The overall structure shows two β sheets packed on top of each other in a β sandwich arrangement. Each of the sheets comprises four strands, with strands A, B, E, and D in one, and strands G, F, C, and C′ in the other sheet. The AB, EF and CC′ loops are located at the C-terminal pole of the domain (at the left-hand side in fig. 6), while the BC, DE, and FG loops comprise the opposite end. Interestingly, even though domain 5 of each Trk possesses two cysteine residues, these are not buried inside the β sandwich, as is common in Ig-like domains, but instead located at the surface, where they form a solvent-exposed disulfide bridge connecting strands B and E. Another unusual feature involves strand A. In domains of the I-set variety, the first strand can usually be divided into two pieces, A and A′, with A′ taking part in the GFCC′ sheet. In contrast, in Trk-d5, this strand continues to hydrogen-bond within the ABED sheet. It is noteworthy that these two very regions had been identified as involved in ligand binding in the alanine-scanning experiments of TrkA and TrkC [43], which was subsequently confirmed by the crystal structure of the NGF-TrkA-d5 complex (below).

The biological roles of the other domains, especially of domain 4, are less well established. A number of studies suggest that the presence of domain 4 is required for efficient expression and proper folding of the ligand-binding domain, domain 5, at least in vitro [38, 40]. Indirect support for this notion comes from the crystal structures of the various Trk-d5 constructs, where these domains appeared to be folded improperly [36]. All three structures formed dimers in which strand A of one domain had swapped places with the same strand of a neighboring molecule and vice versa. Although such ‘domain swapping’ has been proposed to have biological significance in some cases [reviewed in ref. 44], it was concluded that the Trk dimers are artificial, since they are

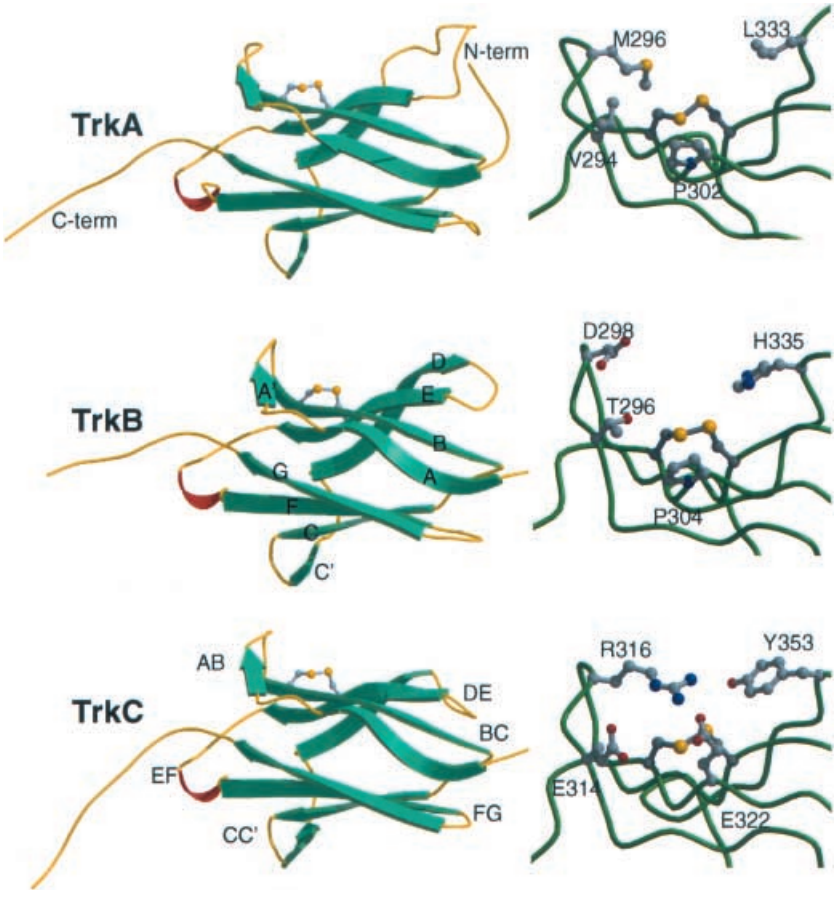

Figure 6. Models of domain 5 of the Trk receptors (left) and close-up view of the environment around the solvent-exposed disulfide bridges (right), all shown in the same orientation. The models depicted here are based on the artificial dimers observed in the crystal structures of the domains of TrkA (top), TrkB (center), and TrkC (bottom) (PDB access codes 1WWA, 1WWB, and 1WWC, respectively). The termini are labeled for TrkA, the secondary structure elements are labeled for TrkB, and various loops are labeled for TrkC. The β sandwich core of the molecules is comprised of β sheets ABED and CC′FG. The exposed disulfide bridges connect strands B and E. Note that the environment of the disulfide differs greatly among the Trks: an open, hydrophobic pocket in TrkA, a highly hydrophilic pocket in TrkB, and a pocket closed off and filled by Arg316 and Tyr353 in TrkC.

incapable of binding their respective ligands. Receptors that lack either or both of the Ig-like domains have been shown to induce ligand-independent neurite outgrowth in TrkA-deficient PC12 cells; therefore, the Ig-like domains may also function to prevent formation of receptor dimers with concomitant signal transduction in the absence of ligand [45].

The precise role of the leucine-rich repeats (domains 2) is still controversial. They have been suggested to be important for ligand binding by TrkA and TrkB [46, 47]; other experiments have indicated that they may have an indirect role in modulating neurotrophin-induced activities [39, 41]. In another study, these domains were found to be important for high-affinity binding of NT-3 to TrkC, but not for NGF binding to TrkA [39]. The recent discovery in the snail *Lymnaea stagnalis* of Ltrk, a Trk-like receptor, also supports a role in ligand-binding for domain 2 of TrkC. The ectodomain of Ltrk has an additional N-terminal extension when compared with the Trk receptors from vertebrates, but lacks both Ig-like domains. This receptor has the highest sequence identity to TrkC, and displays the same binding specificity [48].

Neurotrophin-Trk receptor interactions

The importance of NGF and other neurotrophins in a number of neurodegenerative diseases has led to great interest in these molecules as potential drug targets [see for example refs 21, 49]. Biological activity and binding data for a large number of mutants of NGF, BDNF, and NT-3, in conjunction with the structural information now available, provide detailed insights into receptor recognition by the neurotrophins, including the structural basis for affinity and specificity.

The crystal structure of the NGF-TrkA-d5 complex [28] shows the elongated, dumbbell-shaped NGF dimer binding through its central β sheet region to two copies of

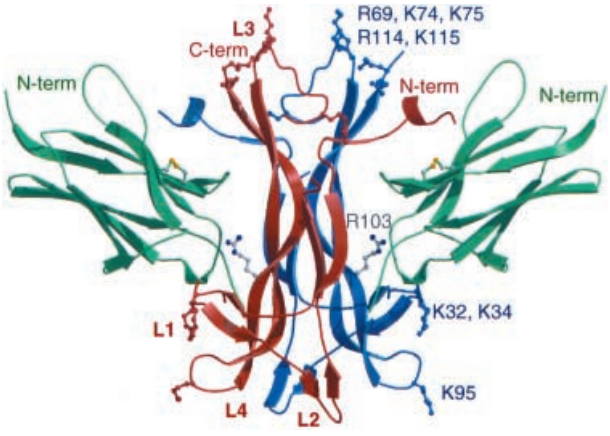

Figure 7. Ribbon diagram representing the crystal structure of the complex between NCF and domain 5 of TrkA (PDB access code 1WWW). The NGF monomers are depicted in red and blue, and the two copies of TrkA-d5 are shown in green. The TrkA-d5 N termini, the N and C terminus of the red NGF monomer, and the loops of the blue NGF monomer are labeled. Most of the NGF residues shown to be important for $p75^{NTR}$ binding are positively charged and located on loops Ll, L3, and L4; these residues are depicted in ball-and-stick representation in red and blue and are labeled. Arg103 of NGF is also shown in ball-and-stick rendering with gray carbon atoms. The solvent-exposed disulfide bridge at the surface of TrkA-d5, which is in contact with the N-terminal helix of NGF, is shown in green and yellow.

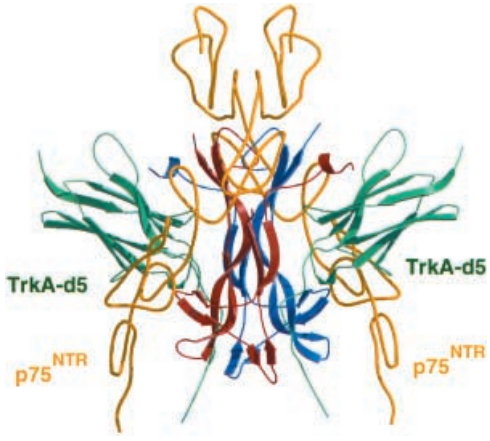

Figure 8. Qualitative model of a ternary complex between NGF (in red and blue), domain 5 of the TrkA receptor (shown in green), and $p75^{NTR}$ (depicted as yellow coil). In this figure, the membrane would be located at the bottom of the page. The $p75^{NTR}$ receptor was modeled based on the structures of the tumor necrosis factor p75 receptor and of death receptor-5 (PDB access codes 1TNR and ID0G, respectively), and manually docked on the two patches of NGF shown to be important for $p75^{NTR}$ binding. All four cysteine-rich repeats Of $p75^{NTR}$ contribute to the complex formation, with the majonty of the interactions formed by the second cysteine-rich repeat. The C-terminal linker between TrkA-d5 and the membrane, most of which is absent in the crystal structure of the NGF-TrkA-d5 complex, was modeled to interact with the L2 and L4 loops of NGF, which are known to be important for specificity [56, 58]. This TrkA linker and the C terminus of $p75^{NTR}$ both point in the same direction (down toward where the membrane would be).

domain 5 of TrkA (fig. 7). The orientation of the complex with respect to the membrane is defined by the position of the C terminus of TrkA-d5 (pointing down in fig. 7; hence the membrane would be at the bottom of the figure). TrkA-d5 is oriented with its 'C-terminal pole' in contact with NGF, and the beginning of the C-terminal segment connecting this domain to the membrane-spanning helix points towards loops L2 and L4 of NGF (most of the linker segment was not present in the crystal). In this orientation, the cysteine knot and N and C termini of NGF are near the top of the molecule. The residues most important for $p75^{NTR}$ binding are all at least partially exposed in this complex, with the common charged patch at the top of the complex. Thus, the NGF-TrkA-d5 complex is consistent with the notion that NGF can simultaneously bind to both TrkA and $p75^{NTR}$ (a model of such a complex is shown in fig. 8).

Each symmetrical NGF-TrkA-d5 interface buries about 2220 $Å^2$ of solvent-accessible surface. Within each interface, two distinct patches can be distinguished. The smaller of these two patches ('specificity patch') comprises the N terminus of NGF in contact with the surface of the ABED sheet of TrkA-d5. Residues in this interaction possess a very low degree of sequence conservation among the neurotrophins on the one hand and the Trks on the other, and the interactions observed in this region are likely to be unique for this complex and responsible for the specificity profile of NGF. The second patch ('conserved patch') is formed by residues from the central β sheet of NGF and loops AB, C′D, and EF of TrkA-d5. Since most of the residues in this patch are homologous in the other neurotrophins and Trk receptors, similar interactions are likely to be present in the other complexes in this family.

The 'specificity patch'

The NGF residues that are involved in the 'specificity patch' were disordered in all previous crystal structures of NGF in its unbound form [23, 24]. It is noteworthy that in the crystal structures of BDNF, NT-3, and NT-4, the equivalent residues are disordered as well, adopting no preferred conformation in the absence of receptor. However, in the complex with TrkA, the N-terminal NGF residues 6–9 form a short segment of helix which packs closely against the 'top' of the receptor (fig. 7). In this patch, Ile6 is the central hydrophobic residue, with its side chain penetrating into a hydrophobic pocket on the surface of the ABED sheet. The bottom of this pocket is formed by the unusual disulfide bridge that is solvent exposed in the free domain. While this disulfide bond is conserved among the Trks, the residues surrounding the pocket are highly variable (fig. 6). In TrkA, the pocket is lined by Val294, Met296, Pro302, and Leu333, resulting in a very hydrophobic environment. In contrast, the analogous

positions are occupied by Thr296, Asp298, Pro302, and His335 in TrkB, giving rise to a much more hydrophilic cavity unsuitable for hydrophobic side chains such as Ile6 of NGF. Finally, the pocket is absent altogether in TrkC, since here the side chains of Glu314, Arg316, Glu322, and Tyr353 block access [36].

The crystal structure of the complex reveals at least two additional NGF residues of interest in this specificity patch. Phe7 participates in the hydrophobic interaction, and His4 forms a hydrogen bond to the protein backbone of TrkA. All these interactions are in excellent agreement with the binding data obtained on a number of neurotrophins with altered N termini. For example, shortening NGF by ten residues using proteolytic cleavage decreases the affinity for TrkA about 300-fold [50], and even a truncation of only the first five residues has a 9-fold effect [51]. The single exchange of His4 against an aspartic acid causes a dramatic loss in binding affinity, resulting in 1000-fold weaker binding to TrkA compared to native NGF. That this interaction is unique for NGF binding to TrkA is shown by the observation that similar mutations in NT-3 barely affect the affinily towards its receptor, TrkC [52]. Moreover, introducing the N-terminal residues from NGF into NT-3 results in a molecule that elicits NGF-like activities [52].

The 'conserved patch'

The second binding patch consists of residues from the central portion of NGF in contact with the AB, C′D, and EF loops at the C-terminal pole of TrkA-d5. In contrast to the specificity patch, where all contacts were formed by residues from a single NGF monomer, this second binding site involves residues protruding from the two central sheets of both subunits. It is noteworthy that most of the residues involved in this binding patch are highly homologous among the neurotrophins and the Trks, and the crystal structure of the NGF-TrkA-d5 complex in conjunction with results from a number of mutagenesis experiments suggest that the hallmarks of this interaction are conserved in the entire neurotrophin system.

A large portion of this patch is formed by Trp 21, Ile31, Phe54, and Phe86 on the surface of NGF. The side chains of these residues pack against TrkA residues protruding from the EF loop and the C-terminal end. Since most mutagenesis studies have focused on the differences between the neurotrophins, thus aiming at the identification of residues that are important for specificity rather than affinity, these conserved hydrophobic residues have not been extensively studied. However, from the published data not all of them appear to be important for binding. The exchange of Trp21 to phenylalanine or leucine, for example, did not affect the biological activity of NGF [53, 54]; similarly, mutation of Phe86 to alanine yielded nearly fully active protein [55]. In contrast, the importance of Ile31 for high-affinity binding is well established, since mutation of this residue to alanine reduces TrkA binding to NGF by 4-fold [56]. A large effect was observed when Phe54 was changed to alanine. This mutant, albeit being expressed at very low levels, had a 40-fold lower biological activity than wild-type NGF [55]. Interestingly, the presence of this hydrophobic cluster has been noted [52] and indeed predicted to form a typical protein-protein interaction site [26] before the crystal structure of the NGF-TrkA-d5 complex was available.

One residue that was identified to be important for receptor binding of NT-3 is Arg103. This residue is strictly conserved among all neurotrophins, but whereas mutation to alanine or even Iysine results in a dramatically decreased binding affinity of NT-3 towards TrkC [52], mutation of this arginine was reported not to affect NGF binding to TrkA [55]. The structure of the NGF-TrkA-d5 complex reveals that Arg103 is almost completely buried in the interface between NGF and TrkA, forming a hydrogen bond to a main-chain carbonyl oxygen of the equally well-conserved EF loop of TrkA-d5.

A third patch important for specificity?

The crystal structure of the NGF-TrkA-d5 complex did not include most of the linker segment that connects TrkA-d5 with the putative transmembrane helix. This linker segment is known to be important for NGF binding [39], and alanine-scanning experiments have further shown that some of the linker residues influence the specificity of ligand binding by the Trks [43, 57]. Based on the crystal structure and these mutagenesis studies, it appears very likely that some of the linker residues pack against the loops at the membrane-facing end of NGF. The structure shows the last visible TrkA residue packing against the 'bottom' end of the central NGF β sheet (fig. 7), with the C terminus projecting toward a groove formed by loops 2 and 4 of NGF. Furthermore, these two loops display a high degree of sequence diversity among the neurotrophins, and swapping experiments in which loops or individual residues were exchanged between NGF and BDNF [53] or between NGF and NT-3 [58] demonstrated that they carry important specificity determinants for ligand-receptor recognition. Therefore, a third patch, not observed in the crystal structure of the NGF-TrkA-d5 complex and consisting of the linker segment of the receptor interacting with loops L2 and L4 of NGF, is likely to complete the interface between NGF, and intact TrkA.

The p75 neurotrophin receptor

In addition to its role as a trophic factor, NGF is able to initiate apoptosis in certain cell types through signaling

via the p75 neurotrophin receptor, $p75^{NTR}$ [59, 60]. In comparison with the three Trk receptors, relatively little is know about $p75^{NTR}$ [for a review, see ref. 11]. For example, the mode of receptor activation or the ligand-binding sites have not yet been fully elucidated. In contrast to the Trk receptors, which show a high degree of specificity for their individual ligands, $p75^{NTR}$ binds to all neurotrophins with relatively weak, nanomolar affiniy [7, 10, 21, 61]. The biological activity mediated by this receptor depends on which neurotrophin is the triggering ligand, as well as on the context of Trk-receptor expression on the cell surface [reviewed in ref. 62]. In the context of TrkA-negative cells, NGF binding to $p75^{NTR}$ will lead to apoptosis [63], whereas in the presence of TrkA, $p75^{NTR}$ enhances the NGF-binding affinity of TrkA, creating 'high-affinity' binding sites, and promotes survival [for a recent review, see ref. 64].

The $p75^{NTR}$ receptor is a transmembrane glycoprotein. Its small cytoplasmic domain contains a death domain, a structural motif that has been found in many other apoptosis-inducing receptors such as the Fas and p75 TNF receptors. The struclure of the $p75^{NTR}$ death domain has been determined by nuclear magnetic resonance. The structure has the typical death domain fold consisting of six α helices arranged in a compact globular domain [65]. In contrast to the cytoplasmic domain, no experimental structure of the extracellular portion of $p75^{NTR}$ has been reported. However, the structures of the homologous receptors for TNF and Apo2L/TRAIL are known [65–71]. These receptors have a common elongated, rod-like shape consisting of a variable number of cysteine-rich repeats, each containing three disulfide bridges. In their ligand complexes, they bind along a groove formed by two of the subunits of their homotrimeric ligands [for a review, see ref. 72]. The ectodomain of $p75^{NTR}$ is comprised of four cysteine-rich repeats [73-75], and a reliable model of its structure has been proposed [76].

Domain deletion studies on $p75^{NTR}$ show that its four cysteine-rich repeats are all required for full-affinity NGF binding, while affinity was unaffected by deletion of the linker region connecting the cysteine-rich domains to the transmembrane region [74]. Mutagenesis studies in which glycosylation sites were introduced at various solvent-exposed positions have further identified the second cysteine-rich domain as being the most critical for NGF affinity [75]

$p75^{NTR}$ binding site on NGF

The $p75^{NTR}$ binding site on NGF has been elucidated using neurotrophin mutants and chimeric variants [52, 77–79]. Two spatially distinct regions on the surface of NGF were identified to be important for $p75^{NTR}$ binding and activation (similar separation into two patches has been observed in the ligands for the related TNF and Apo2L/TRAIL receptors). One of these regions includes residues from the solvent-exposed and highly conserved L3 loop together with residues from the C-terminal tail. Mapped on the surface of NGF, the patch is located at the 'top' of the molecule (fig. 7) and is primarily comprised of positively charged residues. Its identification resulted from alanine-scanning experiments on NT-3, where the mutation Arg68 (corresponding to Arg69 in NGF) reduced $p75^{NTR}$ binding affinity by almost two orders of magnitude [52]. A similar decrease in binding was observed for a double mutant in which Arg114 and Lys115 were substituted by alanine. Additional important positively charged residues in this same patch are Lys73 in NT-3 [52] and the analogous residue Lys74 as well as Lys75 in NGF [80]. It is noteworthy that these residues are also conserved in BDNF, suggesting that this first patch may be a common $p75^{NTR}$-binding motif for all neurotrophins.

The second patch important for $p75^{NTR}$ binding is located near the 'bottom' of NGF and includes positively charged residues from loops L1 and L4 (fig. 7). Mutation to alanine of Lys32, Lys34, or Lys95 greatly decreased the ability of NGF to bind to $p75^{NTR}$ [81], and the triple alanine mutant of Lys32, Lys34, and Glu35 showed a 70-fold reduction in binding with no loss in binding affinity to TrkA [79]. In contrast, mutation to alanine of the analogous residues in NT-3, Arg31, His33, and Lys95, resulted in only a modest reduction in binding. Moreover, the positively charged residues in positions 32 and 34 in L1 (NGF numbering) are not conserved in BDNF. Instead, the L4 loop of BDNF contains three consecutive positively charged residues (Lys95, Lys96, and Arg97) compared to only a single such residue in NGF and NT-3. The additional charge in L4 of BDNF presumably compensates for the lack of positive charge at positions 32 and 34 [77]. Finally, two hydrophobic residues nearby have also been identified as being important for p75 binding. These are Ile31 [81] and Trp21 [53, 54]. In the crystal structure of the complex between NGF and TrkA-d5 [28], these residues are located at the edge of the conserved patch in contact with TrkA. However, their side chains are still partially exposed to solvent and their importance for both TrkA and $p75^{NTR}$ binding therefore does not preclude the possible formation of a ternary complex.

To date, two different structural models for the NGF-$p75^{NTR}$ complex have been reported [76, 82]. The model of Chapman and Kuntz [76] orients the receptor with its C terminus near loops L1 and L4 of NGF; the position of the $p75^{NTR}$ C terminus would therefore be consistent with simultaneous binding of $p75^{NTR}$ and TrkA. However, the proposed contacts to NGF are inconsistent with the results from Baldwin and Shooter [75] in suggesting that the second cysteine-rich domain not be

involved in NGF binding. The model of Shamovsky et al. [82] shows how the second and third cysteine-rich domains might be in contact with NGF, and is consistent with the available mutagenesis data in this respect. However, this model would place the N and C termini of NGF proximal to the membrane, and thus opposite to what is observed in the NGF-TrkA-d5 complex (see fig. 7). Therefore, if NGF can simultaneously bind to $p75^{NTR}$ and TrkA, the orientation proposed in the model is incorrect. The alternative, taking the NGF-TrkA-d5 complex as the starting point, is presented in fig. 8.

Concluding remarks

Recent structural and mutagenesis studies have made important contnbutions to our understanding of binding and specificity of the neurotrophins for the Trks as well as $p75^{NTR}$. The structures of the ligands and the ligand-binding domain of the Trks are now known, the NGF-TrkA-d5 complex reveals the details of the interactions and defines the orientation of the complex with respect to the membrane. Despite this progress, however, the mechanism by which the neurotrophins mediate their functions remains difficult to understand. Even with the crystal structure of the NGF-TrkA-d5 complex in hand, the functional role of the leucine-rich repeats of the Trk receptors remains ambiguous. Also unclear is how $p75^{NTR}$ binding to the various neutrotrophins contributes to the formation of high-affinity binding sites. The $p75^{NTR}$ receptor and the Trks can bind independently to the neurotrophins, and various mutagenesis studies, together with the crystal structure of the NGF-TrkA-d5 complex, indicate that the binding sites do not significantly overlap. However, it is unclear whether both types of receptor are in contact with each other in the signaling complex, and whether conformational changes between $p75^{NTR}$ and the Trk receptors are responsible for the formation of high-affinity complexes. It further remains unclear if and how the intracellular portions of the various receptors interact with each other. Is the potency of NGF in the presence of $p75^{NTR}$ only increased through the formation of high-affinity binding sites or are the cytoplasmic domains in contact? Another interesting questions is why the two $p75^{NTR}$ molecules in the signaling complex appear to be sufficient to signal apoptosis, while in all other apoptosis-inducing receptors, formation of trimers appears to be required for activity. Further structural characterization of the neurotrophin system will help answer many of these questions. For example, the structure of the full-length TrkA extracellular domain in complex with NGF would be invaluable for defining the role of the TrkA leucine-rich domains and C-terminal linker segment. And ultimately, a crystal structure combining all of the components in a single complex between NGF, TrkA, and $p75^{NTR}$ would greatly enhance our understanding of how the neurotrophins decide between life and death.

1 Levi-Montalcini R. (1987) Nerve growth factor 35 years later. Science **237:** 1154–1161
2 Chao M. V. (1992) Neurotrophin receptors: a window into neuronal differentiation. Neuron **9:** 583–593
3 Snider W. D. (1994) Functions of the neurotrophins during nervous system development: what the knockouts are teaching us. Cell **77:** 627–638
4 Torcia M., Bracci-Laudiero L., Lucibello M., Nencioni L., Labardi D., Rubartelli A. et al. (1996) Nerve growth factor is an autocrine survival factor for memory B Iymphocytes. Cell **85:** 345–356
5 Barde Y. A., Edgar D. and Thoenen H. (1982) Purification of a new neurotrophic factor from mammalian brain. EMBO J. **1:** 549–553
6 Ernfors P., Ibáñez 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 **187:** 5454–5458
7 Hallböök F., Ibáñez C. F. and Persson H. (1991) Evolutionary studies of the nerve growth factor family reveal a novel member abllndantly expressed in *Xenopus* ovary. Neuron **6:** 845–858
8 Götz R., Köster R., Winkler C., Raulf F., Lottspeich F., Schartl M. et al. (1994) Neurotrophin-6 is a new member of the nerve growth factor family. Nature **372:** 266–269
9 Bothwell M. (1995) Functional interactions of neurotrophins and neurotrophin receptors. Annu. Rev. Neurosci. **18:** 223–253
10 Yano H. and Chao M. (2000) Neurotrophin receptor structure and interactions. Pharm. Acta. Helv. **74:** 253–260
11 Dechant G. and Barde Y. A. (1997) Signalling through the neurotrophin receptor $p75^{NTR}$ Curr. Opin. Neurobiol. **7:** 413–418
12 Casaccia-Bonnefil P., Kong H. and Chao M. V. (1998) Neurotrophins: the biological paradox of survival factors eliciting apoptosis. Cell Death Differ. **5:** 357–364
13 Kaplan D. R. and Miller F. D. (2000) Neurotrophin signal transduction in the nervous system. Curr. Opin. Neurobiol. **10:** 381–391
14 Cordon-Cado C., Tapley P., Jing S. Q., Nanduri V., O'Rourke E., Lamballe F. et al. (1991) The trk tyrosine protein kinase mediates the mitogenic properties of nerve growth factor and neurotrophin-3. Cell **66:** 173–183
15 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
16 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
17 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
18 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
19 Berkemeier L. R., Winslow J. W., Kaplan D. R., Nikolics K., Goeddel D. V. and Rosenthal A. (1991) Neurotrophin-5: a novel neurotrophic factor that activates trk and trkB. Neuron **7:** 857–866
20 Ip N. Y., Ibáñez C. F., Nye S. H., McClain J., Jones P. F., Gies D. R. et al. (1992) Mammalian neurotrophin-4: structure, chro-

mosomal localization, tissue distribution, and receptor specificity. Proc. Natl. Acad. Sci. USA **89:** 3060–3064
21 Ibáñez C. F. (1995) Neurotrophic factors: from structure-function relationships to designing efficient therapeutics. Trends Biotechnol. **13:** 217–227
22 Ibáñez C. F. (1998) Emerging themes in structural biology of neurotrophic factors. Trends Neurosci. **21:** 438–444
23 McDonald N. Q., Lapatto R., Murray-Rust J., Gunning J., Wlodawer A. and Blundell T. L. (1991) New protein fold revealed by a 2.3-A resolution crystal structure of nerve growth factor. Nature **354:** 411–414
24 Holland D. R., Cousens L. S., Meng W. and Matthews B. W. (1994) Nerve growth factor in different crystal forms displays structural flexibility and reveals zinc binding sites. J. Mol. Biol. **239:** 385–400
25 Robinson R. C., Radziejewski C., Stuart D. I. and Jones E. Y. (1995) Structure of the brain-derived neurotrophic factor/neurotrophin 3 heterodimer. Biochemistry **34:** 4139–4146
26 Robinson R. C., 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/neurotrophin4 heterodimer reveal a common Trk-binding site. Protein Sci. **8:** 2589–2597
27 Butte M. J., Hwang P. K., Mobley W. C. and Fletterick R. J. (1998) Crystal structure of neurotrophin-3 homodimer shows distinct regions are used to bind its receptors. Biochemistry **37:** 16846–16852
28 Wiesmann C., Ultsch M. H., Bass S. H. and Vos A. M. de (1999) Crystal structure of NGF in complex with the ligand-binding domain of the TrkA receptor. Nature **401:** 184–188
29 Angeletti R. H., Bradshaw R. A. and Wade R. D. (1971) Subunit structure and amino acid composition of mouse submaxillary gland nerve growth factor. Biochemistry **2:** 10463–10469
30 Edwards R. H., Selby M. J., Garcia P. D. and Rutter W. J. (1988) Processing of the native nerve growth factor precursor to form biologically active nerve growth factor. J. Biol. Chem. **263:** 6810–6815
31 Jongstra-Bile N. J., Coblentz L. and Shooter E. M. (1989) The in vitro processing of the NGF precursors by the gamma-subunit of the 7S NGF complex. Brain Res. Mol. Brain Res. **5:** 159–169
32 Bax B., Blundell T. L., Murray-Rust J. and McDonald N. Q. (1997) Structure of mouse 7S NGF: a complex of nerve growth factor with four binding proteins. Structure **5:** 1275–1285
33 Silverman R. E. and Bradshaw R. A. (1982) Nerve growth factor: subunit interactions in the mouse submaxillary gland 7S complex. J. Neurosci. Res. **8:** 127–136
34 Schneider R. and Schweiger M. (1991) A novel modular mosaic of cell adhesion motifs in the extracellular domains of the neurogenic trk and trkB tyrosine kinase receptors. Oncogene **6:** 1807–1811
35 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
36 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
37 Pérez 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 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
39 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
40 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
41 Haniu M., Montestruque S., Bures E. J., Talvenheimo J., Toso R., Lewis-Sandy S. et al. (1997) Interactions between brain-derived neurotrophic factor and the TRKB receptor: identification of two ligand-binding domains in soluble TRKB by affinity separation and chemical crosslinking. J. Biol. Chem. **272:** 25296–25303
42 Tannahill L., Klein R. and Schachner M. (1995) The neurotrophin receptors TrkA and TrkB are inhibitory for neurite outgrowth. Eur. J. Neurosci. **7:** 1424–1428
43 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
44 Schlunegger M. P., Bennett M. J. and Eisenberg D. (1997) Oligomer formation by 3D domain swapping: a model for protein assembly and misassembly. Adv. Protein Chem. **50:** 61–122
45 Arevalo J. C., Conde B., Hempstead B. L., Chao M. V., Martin-Zanca D. and Pérez P. (2000) TrkA immunoglobulin-like ligand binding domains inhibit spontaneous activation of the receptor. Mol. Cell Biol. **20:** 5908–5916
46 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
47 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
48 Van Kesteren R. E., Fainzilber M., Vreugdenhil E., Smit A. B., Ibáñez C., Geraerts W. P. M. et al. (1998) Early evolutionary origin of the neurotrophin receptor family. EMBO J. **17:** 2534–2542
49 Saragovi H. U. and Gehring K. (2000) Development of pharmacological agents for targeting neurotrophins and their receptors. Trends Pharmacol. Sci. **21:** 93–98
50 Kahle P., Burton L. E., Schmelzer C. H. and Hertel C. (1992) The amino terminus of nerve growth factor is involved in the interaction with the receptor tyrosine kinase p 140trkA. J. Biol. Chem. **267:** 22707–22710
51 Shih A., Laramee G. R., Schmelzer C. H., Burton L. E. and Winslow J. W. (1994) Mutagenesis identifies amino-terminal residues of nerve growth factor necessary for Trk receptor binding and biological activity. J. Biol. Chem. **269:** 27679–27686
52 Urfer R., Tsoulfas P., Soppet D., Escandon E., Parada L. F. and Presta L. G. (1994) The binding epitopes of neurotrophin-3 to its receptors trkC and gp75 and the design of a multifunctional human neurotrophin. EMBO J. **13:** 5896–5909
53 Ibáñez C. F., Hallbook F., Ebendal T. and Persson H. (1990) Structure-function studies of nerve growth factor: functional importance of highly conserved amino acid residues. EMBO J. **9:** 1477–1483
54 Drinkwater C. C., Suter U., Angst C. and Shooter E. M. (1991) Mutation of tryptophan-21 in mouse nerve growth factor (NGF) affects binding to the fast NGF receptor but not induction of neurites on PC12 cells. Proc. R. Soc. Lond. B **246:** 307–313
55 Guo M., Meyer S. L., Kaur H., Gao J-J. and Neet K. (1996) Mutational studies of conserved residues in the dimer interface of nerve growth factor. Protein Sci. **5:** 447–455
56 Ibáñez C. F., Ilag L. L., Murray-Rust J. and Persson H. (1993) An extended surface of binding to Trk tyrosine kinase receptors in NGF and BDNF allows the engineenng of a multifunctional pan-neurotrophin. EMBO J. **12:** 2281–2293

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

58 Kullander K. and Ebendal T. (1994) Neurotrophin-3 acquires NGF-like activity after exchange to five NGF amino acid residues: molecular analysis of the sites in NGF mediating the specific interaction with the NGF high affinity receptor. J. Neurosci. Res. **39:** 195–210

59 Johnson D., Lanahan A., Buck C. R., Sehgal A., Morgan C., Mercer E. et al. (1986) Expression and structure of the human NGF receptor. Cell **47:** 545–554

60 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

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

62 Chao M., Casaccia-Bonnefil P., Carter B., Chittka A., Kong H. and Yoon S. O. (1998) Neurotrophin receptors: mediators of life and death. Brain Res. Brain Res. Rev. **26:** 295–301

63 Frade J. M., Rodriguez-Tébar A. and Barde Y. A. (1996) Induction of cell death by endogenous nerve growth factor through its p75 receptor. Nature **383:** 166–168

64 Meldolesi J., Sciorati C. and Clementi E. (2000) The p75 receptor: first insights into the transduction mechanisms leading to either cell death or survival. Trends Pharmacol. Sci. **21:** 242–243

65 Liepinsh E., Ilag L. L., Otting G. and Ibáñez C. (1997) NMR structure of the death domain of the p75 neurotrophin receptor EMBO J. **16:** 4999–5005

66 Banner D. W., D'Arcy A., Janes W., Gentz R., Schoenfeld H. J., Broger C. et al. (1993) Crystal structure of the soluble human 55 kd TNF receptor-human TNF beta complex: implications for TNF receptor activation. Cell **73:** 431–445

67 Naismith J. H., Devine T. Q., Brandhuber B. J. and Sprang S. R. (1995) Crystallographic evidence for dimerization of unliganded tumor necrosis factor receptor. J. Biol. Chem. **270:** 13303–13307

68 Naismith J. H., Devine T. Q., Kohono T. and Sprang S. R. (1996) Structures of the extracellular domain of the type I tumor necrosis factor receptor. Structure **4:** 1251–1262

69 Hymowitz S. G., Christillger H. W., Fuh G., Ullsch M. H., O'Connell M. P., Kelley R. F. et al. (1999) Triggering cell death: crystal structure of Apo2L/TRAIL in complex with death receptor 5. Mol. Cell **4:** 563–571

70 Mongkolsapaya J., Grimes J. M., Chen N., Xu X. N., Stuart D. I., Jones E. Y. et al. (1999) Structure of the TRAIL-DR5 complex reveals mechanisms conferring specificity in apoptotic initiation. Nat. Struct. Biol. **6:** 1048–1053

71 Cha S. S., Sung B. J., Kim Y. A., Song Y. L., Kim H. J., Kim S. et al. (2000) Crystal structure of TRAIL-DR5 complex identifies a critical role of the unique frame insertion in conferring recognition specificity. J. Biol. Chem. **275:** 31171–31177

72 Naismith J. H. and Sprang S. R. (1998) Modularity in the TNF-receptor family. Trends Biochem. Sci. **23:** 74–79

73 Yan H., Schlessinger J. and Chao M. V. (1991) Chimeric NGF-EGF receptors define domains responsible for neuronal differentiation. Science **252:** 561–356

74 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

75 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

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

77 Rydén M., Murray-Rust J., Glass D., Ilag L. L., Trupp M., Yancopoulos G. D. et al. (1995) Functional analysis of mutant neurotrophins deficient in low-affinity binding reveals a role for p75LNGFR in NT-4 signalling. EMBO J. **14:** 1979–1990

78 Rydén M. and Ibáñez C. F. (1996) Binding of neurotrophin-3 to p75LNGFR, TrkA and TrkB mediated by a single functional epitope distinct from that recognized by TrkC. J. Biol. Chem. **271:** 5623–5637

79 Rydén M., Hempstead B. and Ibáñez C. F. (1997) Differential modulation of neuron survival during devclopmcnt by nerve growth factor binding to the p75 neurotrophin receptor. J. Biol. Chem. **272:** 16322–16328

80 Rydén M. and Ibáñez C. F. (1997) A second determinant of binding to the p75 neurotrophin receptor revealed by alanin-scanning mutagenesis of a conserved loop in nerve growth factor. J. Biol. Chem. **272:** 33085–33091

81 Ibáñez C. F., Ebendal T., Barbany G., Murray-Rust J., Blundell T. L. and Persson H. (1992) Disruption of the low affinity receptor-binding site in NGF allows neuronal survival and differentiation by binding to the trk gene product. Cell **69:** 329–341

82 Shamovsky I. L., Ross G. M., Riopelle R. J. and Weaver D. F. (1999) The interaction of neurotrophins with the p75NTR common neurotrophin receptor: a comprehensive molecular modeling study. Protein Sci. **8:** 2223–2233

83 Kraulis P. J. (1991) MOLSCRIPT: a program to produce both detailed and schematic plots of protein structures. J. Appl. Cryst. Allogr. **24:** 940–950

84 Merritt E. A. and Bacon D. J. (1997) Raster3D: photorealistic molecular graphics. Methods Enzymol. **277:** 505–524