Emerging themes in structural biology of neurotrophic factors

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Neurotrophic factors control the survival, differentiation and maintenance of neurons in the peripheral and central nervous systems. Their discovery and characterization have been instrumental to our understanding of a wide range of phenomena in the development, plasticity and repair of the nervous system. Their potential importance in the development of therapeutic agents against neurodegenerative disorders and nerve injury has led to a flurry of activity towards understanding their structure, function and signaling mechanisms. This knowledge has increased dramatically in recent years, in particular due to the elucidation of three-dimensional structures, the discovery of families of structurally related neurotrophic factors and the characterization of receptors and downstream signaling components. Common themes are emerging from these recent studies that allow us to make new insights and predictions as to the function and possible clinical utility of these molecules.

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NEUROTROPHIC FACTORS, like all polypeptide hormones, deliver their message to the cell interior via interaction with cell surface receptors. More often than not, neurotrophic factors interact with multicomponent receptors consisting of several distinct protein subunits. In some cases, such as the receptor complexes for ciliary neurotrophic factor (CNTF) and glial cell line-derived neurotrophic factor (GDNF), the tasks of ligand binding and downstream signaling are segregated among the different subunits[1–4] (Fig. 1). In a simplified model of receptor activation, a ligand-binding component, the α subunit, first associates with the hormone. This complex is subsequently recognized by the signaling receptor, the β subunit, which thereby becomes activated. A number of variations of this basic model are known to exist. The α subunits of the GDNF and CNTF receptor complexes are shed to some extent after cleavage of their glycosylphosphatidyl inositol (GPI) anchors. Soluble forms of α-receptor components are believed to capture ligand from the extracellular environment for subsequent assembly into a functional receptor complex on the membrane of cells carrying signaling subunits[4–6]. In the case of GDNF, receptor binding and crosslinking studies indicate that GDNF contacts both the α subunit, GFRα-1, and the signaling subunit, the receptor tyrosine kinase RET (Refs 3,4,7). In addition, GFRα-1 has some intrinsic affinity for RET in the absence of ligand[8,9], suggesting that pre-formed complexes of α and β subunits are also present at the cell surface. GFRα-1 has also been shown to modulate negatively the constitutive levels of RET tyrosine phosphorylation in the absence of ligand[9]. Thus, at least in this system, GFRα receptors could also provide a gain control mechanism to increase the signal-to-noise ratio of the response to ligand.

For other neurotrophic factors, however, ligand binding and signaling are not segregated into different receptor polypeptide chains. The neurotrophin nerve growth factor (NGF) binds to two different receptors, the p75 neurotrophin receptor ($p75^{NTR}$) and the receptor tyrosine kinase TrkA, each with distinct signaling capabilities[10–13] (Fig. 1). Although multimeric receptor complexes and functional interactions between both receptors have been observed, it is clear that NGF can bind to and elicit biological actions through each of these two receptors independently.

Most neurotrophic factors belong to families of structurally and functionally related molecules. This raises the issue of the specificity of the interaction of structurally similar but distinct ligands with their corresponding receptors. This specificity problem has been solved in different ways during the diversification of the different families of neurotrophic factors (Fig. 2). Subunits of the α receptor can help in the discrimination of related ligands. Thus, for example, although CNTF and another structurally related member of the neurokine family, leukemia inhibitory factor (LIF), utilize the same signaling receptor subunits (that is, LIFR-β and gp130), CNTF, but not LIF, can only activate these receptors via prior binding to CNTFRα (Ref. 23). Similarly, both GDNF and the related factor neurturin (NTN) utilize RET as a signaling receptor subunit. However, while GDNF binds preferentially to GFRα-1 (previously called GDNFR-α), NTN shows binding specificity for GFRα-2 (Refs 14,15; but see also Refs 8,16), the second member of an expanding family of structurally related GPI-linked receptors. The ligand specificities of two recently discovered members of this family, GFRα-3 (Refs 9,17–20) and GFRα-4 (Ref. 21) are unknown. Interestingly, although GFRα-3 does not bind GDNF directly, it is able to interact with GDNF in the presence of RET (Ref. 9).

In contrast to the neurokine and GDNF families, specificity in the neurotrophin family is achieved through interaction of the different ligands with distinct members of the Trk family of receptor tyrosine kinases

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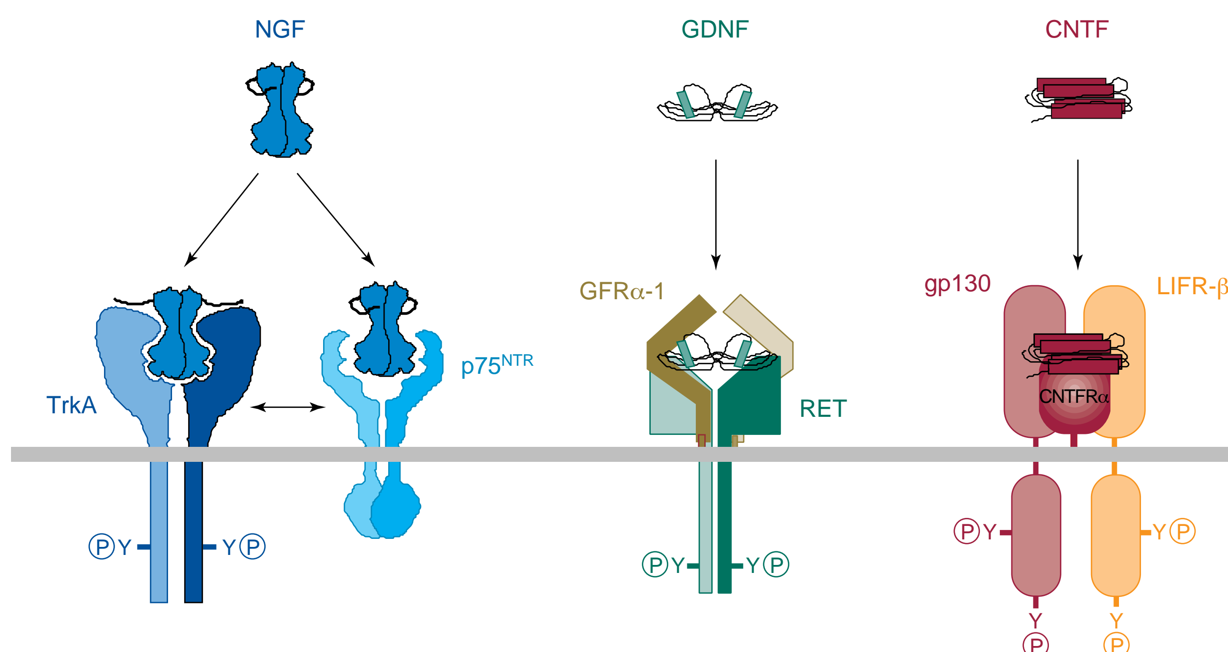

Fig. 1. ***Multicomponent receptor systems of neurotrophic factors.*** *Nerve growth factor (NGF) binds separately to the p75 neurotrophin receptor (p75^{NTR}) and to TrkA. Interactions between ligand-bound TrkA and p75^{NTR} complexes have been proposed. Glial cell line-derived neurotrophic factor (GDNF) binds to a complex formed by GDNF family receptor α-1 (GFRα-1) and the receptor tyrosine kinase RET. In this complex, the GPI-linked α component (GFRα-1) is essential for ligand binding. Ciliary neurotrophic factor (CNTF) interacts with a tripartite receptor complex formed by a ligand binding α-component (CNTFRα) and two signal-transducing β-components, LIFR-β and gp130. Binding of neurotrophic factors to their receptors leads to receptor tyrosine phosphorylation by either intrinsic (TrkA and RET) or associated cytoplamsic (gp130 and LIFR-β) tyrosine kinase activities.*

(Fig. 2). All neurotrophins, on the other hand, are able to bind to p75^{NTR} with similar affinities. Interestingly, however, recent evidence indicates that p75^{NTR} is in fact able to distinguish among the different neurotrophins[13]. The notion that ligand discrimination by p75^{NTR} may be of functional importance is underlined by the fact that NGF, but not BDNF or NT-3, is able to activate downstream signaling pathways through this receptor in Schwann cells and oligodendrocytes[24,25]. Thus, at least two different mechanisms appear to be at work to maintain ligand-specific responses in the neurotrophin family.

Receptor binding specificities in the neurotrophin and GDNF ligand families show a remarkable parallel to the evolutionary histories of cognate ligands and receptors. There is a similarity between the topology of the evolutionary trees of the ligands and their corresponding receptors, as well as an excellent correspondence between homologous branches and cognate pairs of ligands and receptors (Fig. 3). This suggests a strong degree of ligand–receptor co-evolution in families of neurotrophic factors, by which binding specificity is maintained or amplified, or both, during evolution by complementary changes in cognate ligand–receptor pairs[26]. In fact, these evolutionary relationships could be used to predict ligand–receptor interactions quite well. For example, GFRα-4 can be predicted to be a receptor for persephin (PSP) (Fig. 3B).

Knowledge of the molecular interactions underlying the assembly of these ligand–receptor complexes is of fundamental importance if we are to comprehend fully the molecular basis of signal transduction by neurotrophic factors. In terms of understanding protein–protein interactions, this implies the identification of the different sites of interaction with each of its receptors on any given trophic factor molecule, as well as of the individual chemical groups involved in receptor contact. It also means elucidating the molecular mechanisms of receptor binding specificity, that is, to identify the determinants that allow the discrimination of structurally similar neurotrophic factor homologues and, conversely, the functional epitopes shared among distinct factors that interact with a common set of receptor subunits. From a pharmacological point of view, we would like to use this knowledge to obtain molecules with novel binding profiles, such as antagonists, super-agonists and multispecific neurotrophic factors.

Insights into neurotrophic factor function from structural analyses

A three-dimensional structure, as determined by X-ray crystallographic or nuclear magnetic resonance (NMR) methods, provides information on the structural environment of each residue in the protein, whether buried or surface accessible, hydrogen bonded or making van der Waal contacts, and identifies which residues are spatially close together. Such information can be helpful in predicting the location of functional sites on the protein surface, features that may guide subsequent mutagenesis experiments.

The structure of NGF revealed a novel tertiary fold dominated by two central pairs of anti-parallel β-strands that define the elongated shape of the molecule[27,28] (Fig. 4A). These β-strands are connected by a number of highly flexible hairpin loops, in which most of the sequence variability among the different neurotrophins is located. The three disulphide bridges of the molecule are clustered, with two disulphide bridges and their connecting residues forming a ring structure through which the third disulphide bridge passes to form a 'cystine knot' motif[30,31]. In the NGF dimer, the two protomers are arranged in a head-to-head conformation and stabilized by a large hydrophobic

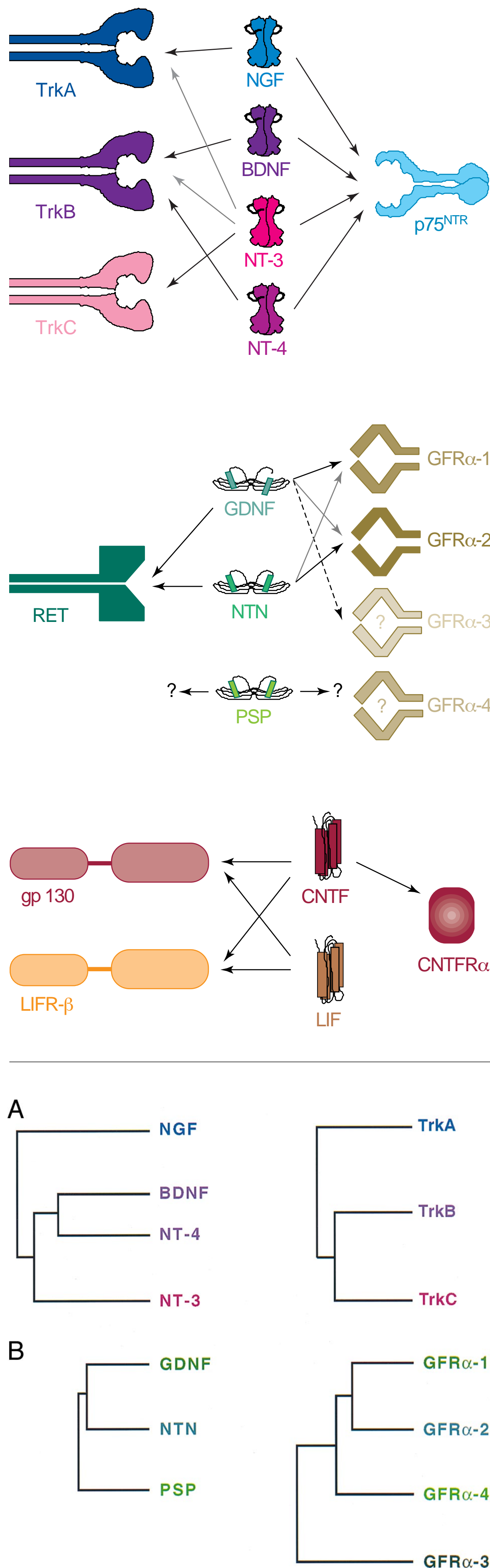

Fig. 2. ***Specificity and cross-talk in families of neurotrophic factors and receptors.*** *Ligand receptor interactions in the neurotrophin* **(top)**, *glial cell line-derived neurotrophic factor (GDNF)* **(center)** *and neurokine* **(bottom)** *families. In the neurotrophins NGF, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4 (NT-4), specificity is determined by ligand binding to different members of the Trk receptor tyrosine kinase family. Cross-talk is also apparent in these interactions as more than one neurotrophin may bind to the same Trk receptor, and more than one Trk may bind to the same neurotrophin. Although all members of the neurotrophin family interact with* $p75^{NTR}$*, this receptor is also capable of detecting differences between the different neurotrophins (see text for details). In the GDNF ligand family, GDNF and neurturin (NTN) use the same signaling receptor subunit, RET. Differential binding to members of the GFRα receptor family has been proposed as a mechanism of specificity[14,15]; however, substantial cross-talk also exists between GDNF ligands and GFRα receptors[8,16]. GFRα-3 (Refs 9,17–20) and GFRα-4 (Ref. 21) are two recently discovered members of the GFRα receptor family. Although GFRα-3 does not bind GDNF directly, it can associate with GDNF in the presence of RET (Ref. 9). This association, however, does not result in efficient RET tyrosine phosphorylation, suggesting the existence of cognate GFRα-3 ligand(s) distinct from GDNF. The receptor binding specificities of persephin (PSP), the third member of the GDNF ligand family, have not been investigated in detail, although it appears not to interact with GFRα-1 or GFRα-2 (Ref. 22). In the neurokine family, ciliary neurotrophic factor (CNTF) and leukemia inhibitory factor (LIF) utilize the same signaling receptor subunits, that is, LIFR-β and gp130. However, CNTF, but not LIF, can only activate these receptors via prior binding to CNTFRα (Ref. 23). Solid lines, major interactions; broken lines, minor or lower-affinity interactions.*

interface. All neurotrophins adopt a similar fold and dimer arrangement, as demonstrated by the crystal structure of a BDNF–NT-3 heterodimer[32].

The crystal structure of GDNF reflects, as expected, its structural similarities to transforming growth factor-β (TGF-β), predicted from the conserved pattern of cysteine residues in the primary sequences of these two factors[33]. TGF-β was the second protein after NGF shown to have a cystine knot motif[34,35], a feature conserved in the GDNF crystal structure. Like TGF-β, the structure of the GDNF monomer is characterized by two long fingers formed by pairs of anti-parallel β-strands connected by loops, and a helical region at the opposite end of the molecule[36] (Fig. 4A). In contrast to NGF, the two protomers in the GDNF dimer are arranged in a 'hand shake'-like, head-to-tail orientation. The amino acid sequences of the loops in the fingers of GDNF, NTN and PSP show high variability. Interestingly, in both the neurotrophins and the GDNF ligand family, variable hairpin loops are spatially close together, indicating possible determinants of receptor binding specificity.

The three-dimensional structure of CNTF revealed a four-helix bundle motif similar to that of LIF and other cytokines[37] (Fig. 4A). The four helices are arranged in a left-handed anti-parallel manner and are

Fig. 3. ***Co-evolution of ligand–receptor pairs.*** *In the neurotrophin* **(A)** *and glial cell line-derived neurotrophic factor (GDNF)* **(B)** *families the evolutionary histories of ligands (left) and receptors (right) correspond quite well with their respective binding specificities (compare with the relationships shown in Fig. 2), and could be used to predict additional interactions. Alignments were made with programs PILEUP and LINEUP (Genetics Computer Group, 1994) using amino acid sequences corresponding to the mature parts of each protein. For Trk receptors, only the extracellular, ligand-binding domains were used. Evolutionary trees were constructed with programs DISTANCES and GROWTREE (Genetics Computer Group, 1994).*

connected by two long cross-over loops and a shorter loop. The structure of CNTF unexpectedly showed it to be dimeric under the conditions used for crystallization. Studies of CNTF in solution confirmed that CNTF is dimeric at concentrations above 40 μM. However, the high potency and low extracellular concentrations of CNTF suggest that the monomeric form is physiologically relevant. Several features of the CNTF and LIF structures, including pronounced kinks that interrupt the regular main chain hydrogen bonding patterns in two of the helices, appear to be unique to the neurokine family. Remarkably, CNTF and LIF share very little or no sequence identity despite having very similar structures[38].

Although several efforts are on their way at various laboratories, no structural information is currently available on receptors for neurotrophic factors, the only exception so far being the intracellular domain of the p75 neurotrophin receptor (p75ICD) (Ref. 39). The p75ICD lacks catalytic activity but contains a motif similar to death domains found in the cytoplasmic regions of members of the tumor necrosis factor (TNF) receptor family and their downstream targets. The NMR structure of the 145-residue-long p75ICD consists of two perpendicular sets of three helices packed into a globular structure, with remarkable similarities to the Fas death-domain structure[40], despite low sequence homology. The polypeptide segment connecting the transmembrane and death domains as well as the serine/threonine-rich carboxy-terminal end are highly flexible in p75ICD. Unlike the death domains involved in signaling by the TNF receptor and Fas, p75ICD does not self-associate in solution, suggesting a different signaling mechanism for the neurotrophin receptor. A surface area devoid of charged residues in the p75ICD death domain has been proposed as a potential site of interaction with downstream targets[39].

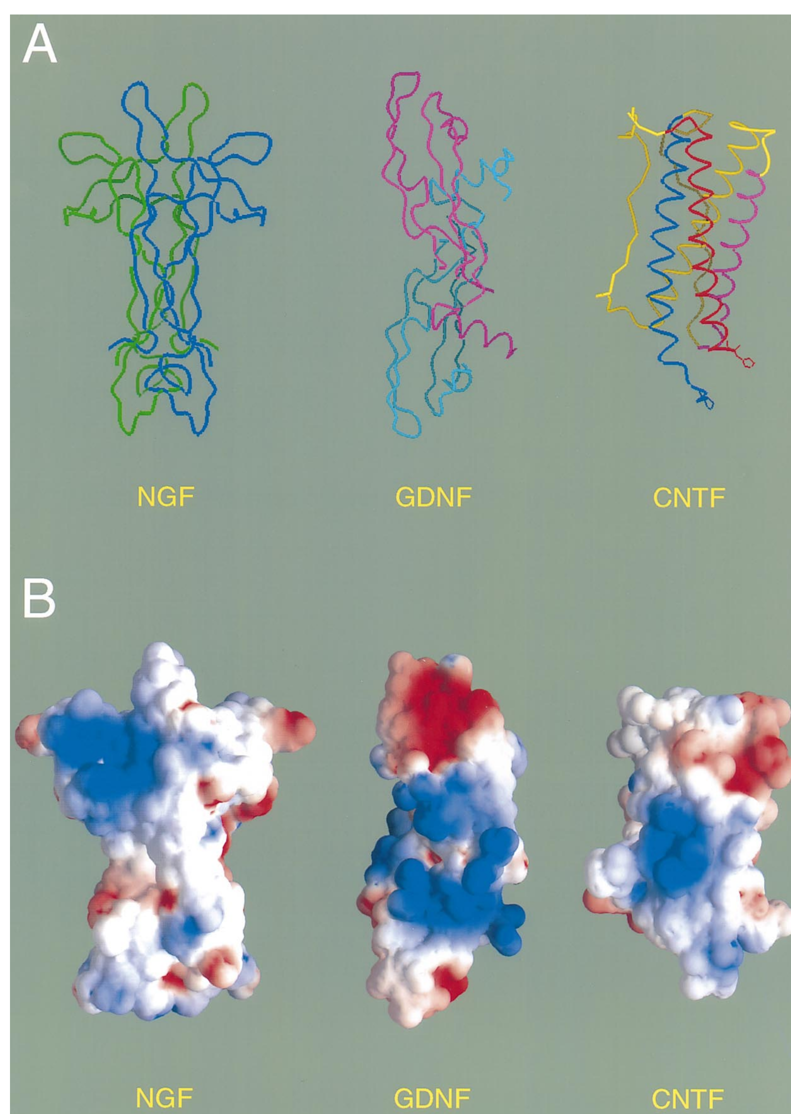

Fig. 4. ***Three-dimensional structures of nerve growth factor (NGF), glial cell line-derived neurotrophic factor (GDNF) and ciliary neurotrophic factor (CNTF).*** **(A)** *The Cα chains of NGF (left), GDNF (center) and CNTF (right) are shown. The two NGF protomers are colored blue and green, respectively, and are viewed perpendicular to a vertical symmetry axis. The two GDNF protomers are colored magenta and cyan, respectively, and are viewed perpendicular to a horizontal symmetry axis. In CNTF, helices A, B, C and D are colored red, yellow, magenta and blue, respectively. The two long loops are colored yellow. Images were generated with the program MacImdad (Molecular Applications Group).* **(B)** *Electrostatic potential on the surfaces of NGF, GDNF and CNTF. Negative potential is shown in red, positive in blue, and neutral in white. Images were generated with the program GRASP (Ref. 29).*

Importance of electrostatic forces for the interaction of neurotrophic factors with their receptors

Examination of the surface properties of a molecule can help to identify functionally important regions that may provide binding surfaces to interaction with receptors. These might be flexible loops, crevices, hydrophobic patches and charged clusters. The latter contribute electrostatic forces that guide ligands to receptors to bind or collide in a productive fashion. Electrostatic interactions are generally long range and are predicted to affect the association rate of binding. Use of long range electrostatic forces is likely to be important in recruiting the limiting amounts of neurotrophic factors available *in vivo* and localizing them at the cell membrane.

The charge distribution in the solvent-accessible surface of NGF reveals a positive electrostatic patch on each protomer formed primarily by three lysine residues from two of the hairpin loops[27] (Fig. 4B). Replacement of these lysine residues with alanine by site-directed mutagenesis severely compromises the binding of the mutants to $p75^{NTR}$, but not to TrkA, indicating that these positive charges are an important functional epitope for the interaction of NGF with this receptor[41]. Interestingly, the third and fourth cysteine-rich domains of $p75^{NTR}$, which are important for binding to NGF, contain a number of glutamic and aspartic acid residues that could be important in defining electrostatic interactions with positively charged residues in NGF (Ref. 42). Although all neurotrophins are capable of interacting with $p75^{NTR}$, the lysine residues involved in the binding of NGF to $p75^{NTR}$ are not conserved in other members of the family. However, modeling of BDNF, NT-3 and NT-4, confirms the preservation of a positively charged surface in this region in all the neurotrophins[43], suggesting a similar manner of engaging $p75^{NTR}$ but with differences in the precise positioning of the charged surface.

A second region of interaction with $p75^{NTR}$ in NGF is formed by another pair of positively charged residues located in a highly conserved and exposed hydrophilic loop region (bottom loop in the views of NGF, Fig. 4). Mutation of these residues to alanine, alone or in combination, reduces NGF binding to $p75^{NTR}$ but not to TrkA (Ref. 44). Homologous residues in NT-3 have

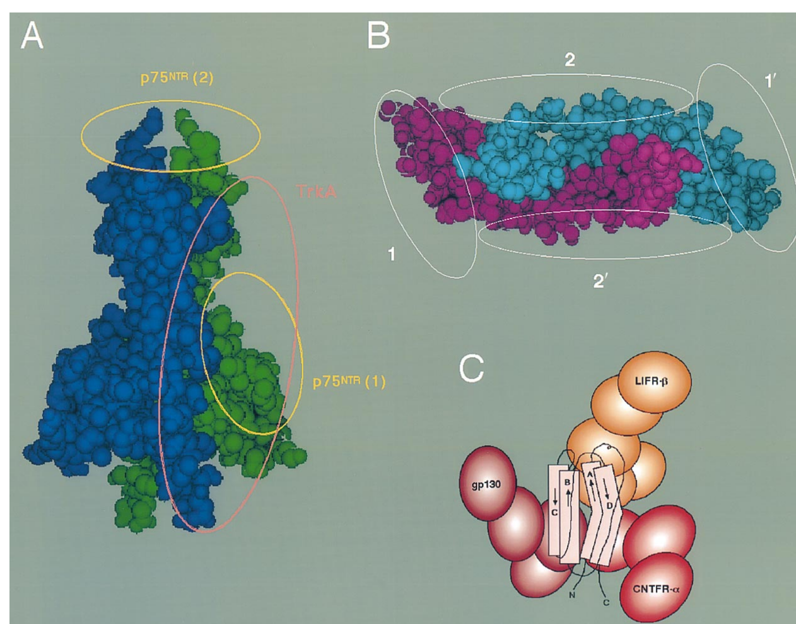

Fig. 5. ***Receptor binding surfaces in neurotrophic factors.*** **(A)** *A filled model of the nerve growth factor (NGF) dimer (upside down compared to the views shown in Fig. 4). The cell membrane is hypothesized to be at the bottom, perpendicular to the plane of the image. The binding surface to TrkA, and the two sites of interaction to $p75^{NTR}$ are indicated. Analogous surfaces in other members of the neurotrophin family are also predicted to be used for receptor binding. The image was generated with MacImdad.* **(B)** *Hypothetical receptor binding surfaces in glial cell line-derived neurotrophic factor (GDNF). A filled model of the GDNF dimer viewed down the two-fold symmetry axis (from the right-hand side of the views shown in Fig. 4). The cell membrane is hypothesized to be behind the molecule, parallel to the plane of the image. 1 and 1′, and 2 and 2′ denote symmetrically related, putative binding surfaces to GFRα-1 and RET receptors. The image was generated with MacImdad.* **(C)** *Model of the tripartite ciliary neurotrophic factor (CNTF) receptor viewed above the cell membrane.* Adapted, with permission, from Ref. 37. *The coloured elipsoids represent the extracellular domains of the receptors CNTFRα, LIFR-β and gp130. Helices A, B, C and D in CNTF are denoted by cylinders and are labeled.*

also been shown to form part of the binding epitope of this neurotrophin to $p75^{NTR}$ (Ref. 45), suggesting a similar role for this region in all neurotrophins. The fact that the two binding epitopes to $p75^{NTR}$ are located at the two opposite ends of the neurotrophin molecule (Fig. 5A) indicates that the interactions between the neurotrophins and $p75^{NTR}$ might be more extensive than previously thought.

In GDNF, negatively, positively and uncharged regions are well segregated (Fig. 4B). A continuous band of net positive charge forms along the side of the dimer including positively charged residues from both protomers. Another feature of the charge distribution in the solvent-accessible surface of GDNF is a pair of symmetric clusters of negatively charged residues one at each end of the elongated GDNF dimer (Fig. 4B). The picture that emerges from these analyses shows GDNF as an elongated, 'cigar-shaped' molecule with a central band of positive electrostatic potential flanked by regions of negative potential at the two ends of the protein (Fig. 4B). At the time of writing this manuscript, no information is yet available from mutagenesis analyses of GDNF. However, based on what has been learnt so far from studies on NGF, CNTF (see below) and other growth factors and cytokines, it is safe to speculate on the functional importance of the charged regions in GDNF. The evidence available supports a stoichiometry of $(GFR\alpha\text{-}1)_2(RET)_2$GDNF for the ligand–receptor complex[3]. The negatively charged loop regions in the fingers of GDNF could be sites of contact with GFRα receptors, as these show considerable sequence variability between GDNF, NTN and PSP. Although α-receptor components are the indispensable ligand binding subunit, GDNF also physically interacts with RET in this complex, as demonstrated by chemical cross-linking experiments[7]. Thus, in one possible scenario of complex assembly, two GFRα-1 molecules could clamp GDNF at the two ends of the molecule through contacts with negatively charged residues in the loops, thereby generating a composite surface formed by residues from GFRα-1 and the positively charged central region of GDNF available for interaction with RET (Fig. 5B). Alternative models are equally possible, as a number of variable residues are also present in the central region of GDNF family ligands. A definitive demonstration of the importance of the charged regions in GDNF awaits the results of ongoing site-directed mutagenesis studies.

A similar analysis of the electrostatic surface of CNTF shows a cluster of three Arg residues that are solvent accessible and form a prominent patch of positive electrostatic potential on the surface of CNTF not found in other neurokines (Fig. 4B)[37]. Site-directed mutagenesis analyses have established the importance of these positively charged positions for the interaction of CNTF with the CNTFRα receptor. They do, however, seem to be dispensable for the interaction of CNTF with gp130 or LIFR-β (Ref. 46). Interestingly, substitutions that add a fourth positive charge to this cluster create a protein with greatly enhanced potency and affinity for CNTFRα (Ref. 46). Thus, not only do these observations underline the importance of electrostatic interactions for receptor binding, but they also point to a plausible strategy for the design of ligands with enhanced binding affinities and 'super-agonists'.

Site-directed mutagenesis and structure–activity relationships

Although analysis of the surface properties of a ligand gives information regarding the possible sites of interaction with receptors, the functional importance of individual residues needs to be assessed directly by site-directed mutagenesis. Moreover, predictions based on three-dimensional structures of ligand–receptor complexes (not yet available for neurotrophic factors) need to be confirmed by direct functional studies. Alanine-scanning mutagenesis, the systematic replacement of amino acid residues with alanine, is a widely used technique for obtaining a preliminary profile of the functionally important regions of a polypeptide. Homologue-scanning mutagenesis is another strategy that consists of the replacement of either single residues, parts of or whole variable regions with homologous sequences from another family member with a different receptor specificity, although it is only applicable to families of proteins.

Alanine- and homologue-scanning mutagenesis of variable regions of the neurotrophins have served to establish their importance for receptor binding and specificity (reviewed in Ref. 47). In NGF, determinants of binding to the TrkA receptor are distributed along the two-fold symmetry axis of the molecule, following the interface between the two protomers (Fig. 5A). Interestingly the N-terminus of NGF, which does not have enough electron density to be visualized in the crystal structure, is an important binding determinant as either truncation[48], deletion[49], point mutation[50] or replacement with BDNF sequences[51] results in decreased

binding to TrkA. Several residues in NGF can transfer TrkA binding activity to other members of the neurotrophin family. However, mutation of individual residues has little effect on activity, suggesting that all residues contribute synergistically to receptor binding. With the exception of the N-terminus, which is so far unique in its importance for NGF binding to TrkA, it appears that all neurotrophins make use of residues clustered along the dimer interface of the central β-strand bundle and loop regions for contact with Trk receptors[45,51–58].

Site-directed mutagenesis studies on CNTF have identified candidate regions for interaction with gp130 and LIFR-β. A region important for CNTF binding to gp130 is centered around Asp-30 in the first helix (helix A)[46]. Structure superposition and sequence alignments show that residues at homologous positions in growth hormone (GH)[59] and interleukin-6 (IL-6)[60] are also receptor binding epitopes, reinforcing the conservation of functionally important regions across distantly-related four-helix bundle cytokines. It has also been shown that residues in the so-called D1 motif in the N-terminus of helix-D form a binding epitope on CNTF to LIFR-β, as mutation of these positions specifically inhibited CNTF interaction with LIFR-β without affecting binding to CNTFRα or gp130 (Ref. 61). Together, the results from these analyses delineate three discrete and non-overlapping surfaces for interaction of CNTF with its tripartite receptor complex (Fig. 5C).

Ligands with altered receptor binding specificity offer new insights into neurotrophic factor function

Taking advantage of its TrkA-only binding profile, a NGF mutant deficient in the ability to bind to $p75^{NTR}$ with alanine replacements at positions 32, 34 and 35 (termed triNGF) has been used to probe for possible roles of $p75^{NTR}$ in the survival responses of primary embryonic neurons to NGF (Ref. 62). The mutant was found to be less potent than wild-type NGF at low ligand concentrations (similar to or lower than the high affinity dissociation binding constant). The reduced responsiveness to the mutant NGF was increasingly evident at later developmental stages; late embryonic neurons did not respond to concentrations of triNGF that were saturating at earlier developmental stages. These results indicate that $p75^{NTR}$ enhances responsiveness to ligand, particularly when this is present at limiting concentrations, and modulates responsiveness to NGF during development, so that binding to $p75^{NTR}$ becomes increasingly important for a biological response at later stages.

In addition to ligands with a more restricted receptor binding specificity, neurotrophins with a broader spectrum of activities have also been engineered. Taking advantage of the extended binding surface to Trk receptors, as well as distinctive features of the binding determinants of individual neurotrophins, site-directed mutagenesis has been used to engineer multifunctional molecules combining portions of the binding sites of several neurotrophins. Using an NT-3 skeleton, molecules combining NGF, BDNF and NT-3 specific activities into a single polypeptide have been constructed[45,51]. Neurotrophin molecules with multiple specificities offer an alternative approach to combination therapy, and may be particularly useful for the treatment of peripheral sensory neuropathies in which the survival of neurons with different neurotrophic requirements is compromised. In this regard, some of the *in vivo* activities of multispecific neurotrophins characterized so far are promising, including axonal retrograde transport to different classes of neurons in rat dorsal root ganglia[63], and accelerated regeneration of sensory and motor axons after crush or transection of the sciatic nerve in a transgenic mouse model[64].

Random mutagenesis followed by selection using phage display is a very powerful method for the identification of high affinity binding variants of a ligand (super-agonists). This approach has been applied to select for high affinity CNTFRα ligands after random mutagenesis of the fourth helix (helix-D) in CNTF (Ref. 65). An increase in binding affinity of up to 20-fold was observed in some of the variants recovered, which correlated with enhanced biological effects, including survival of chicken ciliary neurons[65]. Taking advantage of this increased affinity, and with the knowledge of residues important for the interaction of CNTF with the LIFR-β signaling receptor, the same authors combined these mutations to generate a very potent competitive antagonist of CNTF (Ref. 61). This double CNTF mutant showed enhanced binding to CNTFRα but was not biologically active as it was incapable of interacting with LIFR-β. Moreover, the mutant inhibited the activity of wild-type CNTF as well as the CNTF super-agonist in several cell lines[61]. Because the pleiotropic activities of CNTF and related neurokines may be due, in part, to the sharing and cross-activation of receptor subunits, specific CNTF agonists and antagonists constitute new tools to probe for potential therapeutic uses of this molecule.

Concluding remarks

Understanding of structure–function relationships in families of neurotrophic factors and their receptors has not only furthered our knowledge of important aspects of the biology of these proteins, but it has also provided molecules with novel activity profiles, thereby expanding the repertoire of tools with which to explore the therapeutic applications of neurotrophic factors.

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Neurexin IV, caspr and paranodin – novel members of the neurexin family: encounters of axons and glia

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Axonal insulation is of key importance for the proper propagation of action potentials. In *Drosophila* and other invertebrates, it has recently been demonstrated that septate junctions play an essential role in axonal insulation or blood–brain-barrier formation. Neurexin IV, a molecular component of *Drosophila* septate junctions, has been shown to be essential for axonal insulation in the PNS in embryos and larvae. Interestingly, a vertebrate homolog of Neurexin IV, caspr – also named paranodin – has been shown to localize to septate-like junctional structures. These vertebrate junctions are localized to the paranodal region of the nodes of Ranvier, between axons and Schwann cells. Caspr/paranodin might play an important role in barrier formation, and link neuronal membrane components with the axonal cytoskeletal network.

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RAPID PROPAGATION of action potentials in neurons can be accomplished by keeping distances short, as seen in many insect species, by increasing the diameter of the axons, as exemplified in the squid giant axon, or by clustering of voltage-gated channels to unensheathed areas of axons flanked by glial sheaths, as seen in vertebrates (Fig. 1A,B). This organization is characterized by the presence of nodes of Ranvier and extensive myelination of axons by Schwann cells or oligodendrocytes. These cells provide insulation and promote Na^+ and K^+ channel clustering, allowing vertebrate axons to propagate action potentials rapidly through saltatory conduction (for review see Ref. 1). In the periphery, Schwann cells ensheath not only the large myelinated axons, but also the thin unmyelinated axons (PNS) (Fig. 1C). A group of fascicles of axons and their associated Schwann cells are surrounded by a perineurium, which is composed of specialized fibroblasts that are connected to each other with tight junctions, forming a protective barrier against the diffusion of substances into the peripheral nerve fascicle.

The mechanisms underlying axonal insulation in invertebrate species are less understood than those in vertebrates, as axons are not myelinated and the protein composition of the insulating glia is unknown. The axons also lack the typical nodes of Ranvier[1]. However, unmyelinated axons in *Aplysia* also exhibit clustering of Na^+ channels, suggesting that nodes of Ranvier are not a prerequisite for clustering, and that clustering is probably important to optimize action potential conduction in many species[6]. Invertebrate axons are insulated from their environment through a glial-dependent blood–brain barrier, which plays a crucial role in electrical and chemical insulation[5,7].

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