Assembly and Activation of Neurotrophic Factor Receptor Complexes

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ABSTRACT: **Neurotrophic factors play important roles in the development and function of both neuronal and glial elements of the central and peripheral nervous systems. Their functional diversity is in part based on their ability to interact with alternative complexes of receptor molecules. This review focuses on our current understanding of the mechanisms that govern the assembly and activation of neurotrophic factor receptor complexes. The realization that many, if not the majority, of these complexes exist in a preassembled form at the plasma membrane has forced the revision of classical ligand-mediated oligomerization models, and led to the discovery of novel mechanisms of receptor activation and generation of signaling diversity which are likely to be shared by many different classes of receptors.** © 2010 Wiley Periodicals, Inc. Develop Neurobiol 70: 323–331, 2010

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INTRODUCTION

Neurotrophic factors can operationally be defined as growth factors capable of promoting survival and differentiation of distinct cell subpopulations of neural origin. (The reader is directed to several of the articles appearing elsewhere in this issue for a deeper discussion of neurotrophic activities.) Growth factors with these functional characteristics appear among several families of structurally related polypeptide molecules. In this review, we will concentrate primarily on the receptor systems of only a few representative families, drawing examples from the neurotrophins, the glial cell line-derived neurotrophic factor (GDNF) ligand family and the neurokine ciliary neurotrophic factor (CNTF). Several of the principles discussed here are likely to have broad applicability across different classes of growth factor receptors. We have chosen to focus on two main issues concerning neurotrophic receptor function. The first one emanates from the realization that such receptors are often, if not always, multisubunit complexes formed by combinations of different types of membrane-associated proteins. The second concerns the mechanisms by which receptor complexes are activated upon ligand binding and how information is transmitted between extracellular and intracellular receptor domains.

MULTISUBUNIT RECEPTOR COMPLEXES FOR NEUROTROPHIC FACTORS

Mature neurotrophins are homodimers of roughly 100 amino acid residue-long polypeptide chains. They interact with two classes of plasma membrane receptors: (i) members of the Trk family of receptor tyrosine kinases (RTKs), and (ii) the p75 neurotrophin receptor (p75^{NTR}), a noncatalytic receptor related to members of the tumor necrosis factor receptor (TNFR) superfamily. These two types of receptors can act either independently or together to mediate the biological effects of neurotrophins. Multimerization of neurotrophin receptors can appear at various

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levels: homomerization, heteromerization, and interaction with other membrane-associated proteins. Chemical cross-linking studies (Klein et al., 1991; Rydén et al., 1997)—beautifully confirmed by more recent high-resolution structural analyses (Wiesmann et al., 1999; Wehrman et al., 2007; Gong et al., 2008)—indicate that both Trk and $p75^{NTR}$ receptors interact as homodimers with the mature form of neurotrophins [Fig. 1(A,B)]. Although there is broad consensus on the existence of functional interactions between these two types of receptors, it is still controversial whether $p75^{NTR}$ and Trks associate directly at the cell membrane (Jing et al., 1992; Bibel et al., 1999; Wehrman et al., 2007). Mutagenesis and structural studies indicate that $p75^{NTR}$ and Trks are unlikely to engage the same neurotrophin molecule simultaneously due to both binding site overlap (Ibáñez et al., 1992, 1993) and steric hindrance (Wehrman et al., 2007). However, those studies have not yet ruled out the possibility that $p75^{NTR}$ and Trks may form higher order complexes either prior or following neurotrophin binding, and there is experimental evidence indicating that the transmembrane regions of $p75^{NTR}$ and Trk can mediate interactions between the two receptors (Esposito et al., 2001).

Similar to the neurotrophins, GDNF family members are also homodimers. The functional GDNF receptor is formed by a complex of two subunits, a binding receptor and a signaling receptor. Four different glycosyl phosphatidylinositol (GPI)-linked proteins—termed GFRα 1 to 4—function as dedicated ligand-binding subunits for each of the four members of the GDNF family. Most of the effects reported for GDNF and related proteins require the Ret RTK as signaling subunit of the receptor complex. Unlike the GFRαs, Ret cannot bind GDNF on its own, although chemical cross-linking experiments have indicted that it does make direct contact with the ligand (Trupp et al., 1996, 1998). Similar to other RTKs, Ret functions as a homodimer. In agreement with the two-fold symmetry of the ligands (Eigenbrot and Gerber, 1997), crosslinking and high-resolution structural studies have shown that GFRαs also interact with GDNF proteins as homodimers (Jing et al., 1996; Trupp et al., 1998; Wang et al., 2006; Parkash et al., 2008). Association between Ret and GFRαs has also been detected (Sanicola et al., 1997; Eketjäll et al., 1999; Cik et al., 2000), so the functional receptor complex for GDNF ligands is therefore likely to conform to a 2:2:2 stoichiometry [Fig. 1(C)], an assumption that awaits confirmation by resolution of the 3D structure of the full complex.

Unlike neurotrophins and GDNF ligands, CNTF is monomeric and interacts with a complex formed by three different receptor subunits: a GPI-anchored protein known as CNTF-Rα and two structurally related transmembrane components: gp130 and Leukemia Inhibitory Factor Receptor (LIF-R). CNTF has been shown to interact directly with all three receptors to form an asymmetric 1:1:1:1 quaternary complex (Skiniotis et al., 2008) [Fig. 1(D)].

ALTERNATIVE RECEPTOR COMPLEXES ALLOW DIVERSIFICATION OF NEUROTROPHIC FACTOR FUNCTION

Trks and $p75^{NTR}$ have very different intracellular domains and signaling capabilities. Alternative complements of $p75^{NTR}$ and Trk receptors could therefore allow cells to mount different types of responses to neurotrophins. $p75^{NTR}$ is also known to associate with the sorting receptor Sortilin (Nykjaer et al., 2004), an interaction that allows the formation of a receptor for the unprocessed form of neurotrophins. Through $p75^{NTR}$ and Sortilin, pro-neurotrophins have been reported to induce biological effects that in many ways are opposite to those elicited by their mature counterparts, such as cell death and depression of synaptic function (Lu et al., 2005). Neurons carrying both $p75^{NTR}$ and Trk receptors are not uncommon in the peripheral nervous system, but appear mostly restricted to basal forebrain cholinergic neurons in the brain. Although these cells could in principle be able to activate both signaling pathways simultaneously, it has been observed that sometimes either one of the two is prevalent. Earlier studies in peripheral neurons have established a positive effect of $p75^{NTR}$ on neurotrophin binding to Trk receptors, enhancing both ligand affinity and selectivity, and thereby strengthening survival responses (Lee et al., 1994; Rydén et al., 1997). In basal forebrain cholinergic neurons, however, simultaneous engagement of $p75^{NTR}$ and Trk receptors through concomitant stimulation with pro- and mature neurotrophin ligands resulted in death, not survival, of these neurons (Volosin et al., 2006), indicating that $p75^{NTR}$ signaling was dominant in this case. It would be interesting to test whether pro-neurotrophins can kill peripheral neurons in the absence or presence of their mature counterparts. In addition, $p75^{NTR}$ is able to associate with a number of other cell surface proteins to generate complexes that mediate responses to ligands that are entirely different from the neurotrophins. For example, $p75^{NTR}$ can associate with Nogo receptor (NgR) and Lingo-1 to form a receptor complex for several components of peripheral and central myelin, including Nogo, myelin associated glycoprotein

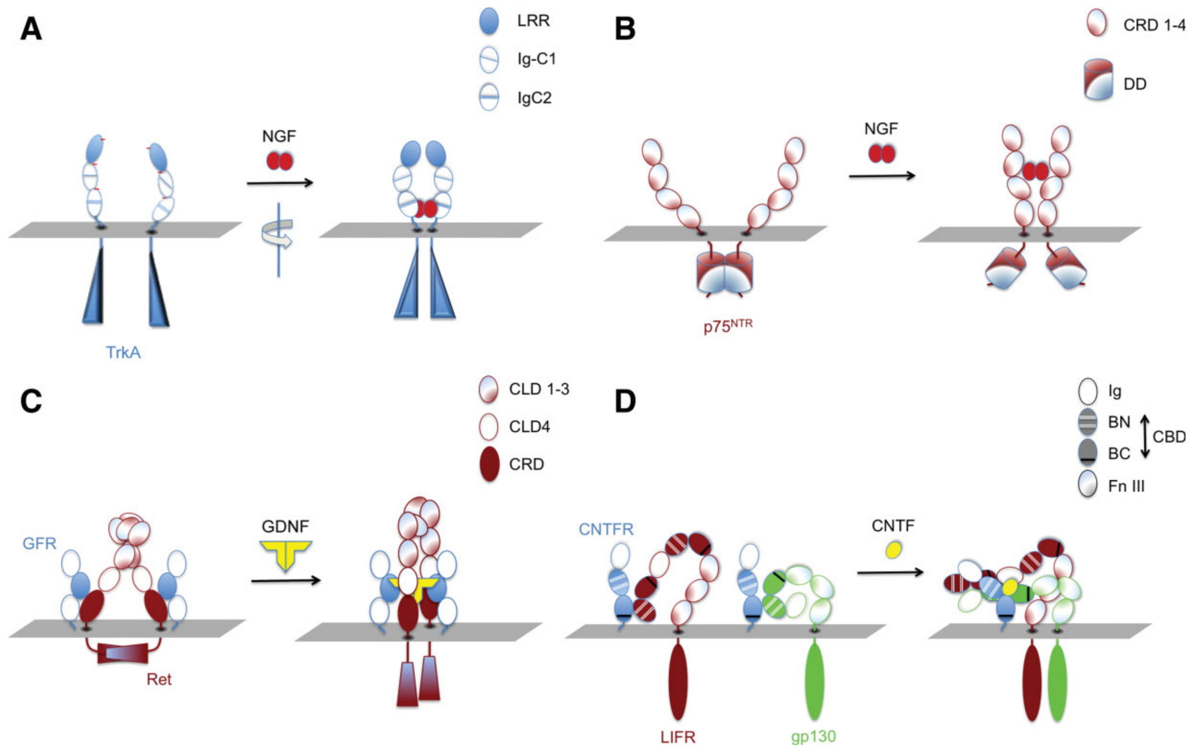

Figure 1 Hypothetical models of receptor subunit movements after ligand activation. Interactions between the different subunits may already exist in the absence of ligand but are not permissive to signaling. (A) TrkA residues in the linker region between LRR and the first Ig-like domains may prevent dimerization or keep a preformed dimer in inactive conformation. NGF binding to the second Ig-like domain may change the proximity and/or orientation of the intracellular kinase domains in the TrkA dimer allowing transphosphorylation. Autoinhibitory interactions are marked as minus (–). The arrow denotes a hypothetical rotation of the two receptor subunits that may result in activation by abolishing autoinhibition (this may be assisted by co-receptors such as p75^{NTR}; see text). LRR: Leucine-repeat region; Ig-C: C-terminal immunoglobulin ligand-binding domain. (B) The snail-tong model for p75^{NTR} activation postulates a separation of the death domains in a p75^{NTR}-preformed dimer induced by the closing of the two ECDs around the neurotrophin dimer. CRD: cystein-rich domain; DD: death domain. (C) Both the kinase and CRD domains of Ret seem to contain intrinsic inhibitory signals keeping Ret kinase activity at bay; a preformed complex with GFR may enhance this inhibitory conformation in the absence of GDNF. When GDNF forces the proximity of the II-domains in the GFR dimer, then the Ret dimer assumes an active conformation with its kinase domains in parallel. CLD: Cadherin-like domain; CRD: cystein-rich domain. (D) LIFR and gp130 compete for binding to CNTFR in the absence of ligand. CNTF binds not only CNTFR, but establishes contacts with both LIFR and gp130 thus stabilizing the oligomeric complex liable for signal transmission. Note that, although the proposed model illustrated here displays a 1:1:1:1 heterotetramer, this may only be a simplified view of a signaling complex which could easily consist of several such heterotetramers in 2:2:2:2 or even higher oligomeric states. Ig: immunogobulin-like domain; BN: N-terminal FnIII module; BC: C-terminal FnIII module; CBD: cytokine binding domain; FnIII: Fibronectin-type III domain. Generic cartoon representations of domains common to all three receptors are shown in gray.

(MAG) and oligodendrocyte myelin glycoprotein (OMgp), mediating inhibition of axonal growth (Fournier et al., 2001; Mi et al., 2004). Other members of the TNFR superfamily, such as Troy (Park et al., 2005), can replace p75^{NTR} in this complex, which is believed to mediate growth cone collapse and axon repulsion.

In addition to Ret, the GDNF/GFRα subcomplex can also team up with the neural cell adhesion molecule NCAM to mediate neurite outgrowth and cell migration (Paratcha et al., 2003). As a member of the immunoglobulin superfamily of cell adhesion molecules, NCAM modulates the growth, guidance, and stabilization of neurites in the developing CNS, by

direct interactions with both cell surface molecules (adhesion) as well as intracellular proteins (signaling). Unlike Ret, however, NCAM is able to bind GDNF on its own, although signal transduction appears to require the higher affinity afforded in the presence of the GFRα1 subunit (Paratcha et al., 2003). NCAM biding to GDNF requires a distinct determinant in the third Ig domain of the NCAM molecule, and NCAM point mutants have been generated that interfere with GDNF binding without affecting cell adhesion (Sjöstrand et al., 2007; Nielsen, 2009). The NCAM complexes involved in cell–cell adhesion are believed to be also dimeric, in which a cis-dimer is formed through Ig domains I and II bending over each other, and this structure would then interact in trans with another dimer forming a rather compact cluster. The conformation of the NCAM dimer bound to the GDNF-GFRα1 complex is however more open, which may explain why GFRα1-NCAM signaling competes with cell adhesion. NCAM has also been shown to directly interact with GFRα1, even in the absence of GDNF, through its fourth Ig domain (Sjöstrand and Ibáñez, 2008). Molecular modeling studies have suggested a 2:2:2 stoichiometry for the GDNF/GFRα1/NCAM complex (Sjöstrand et al., 2007). The observation that dimeric factors interact with receptor dimers raises two important but sometimes overlooked questions: (i) are receptor homodimers induced upon ligand binding or already preformed prior to ligand engagement?, and (ii) is homodimerization required for receptor activation and downstream signaling?

RECEPTOR COMPLEX ASSEMBLY: INDUCED OR PREFORMED?

Perhaps the most influential paradigm of receptor complex assembly is the concept of ligand-induced receptor dimerization. This model—originally proposed by Schlessinger and coworker to explain ligand-mediated RTK activation (Weiss and Schlessinger, 1998; Schlessinger, 2002)—postulates that receptors are initially present as monomers in the plasma membrane, and only dimerize as a consequence of ligand binding. Receptor dimerization brings intracellular domains in close proximity, and in the case of RTKs, this is followed by intermolecular autophosphorylation of key tyrosine residues in the activation loop of catalytic domains resulting in stimulation of kinase activity. Intuitive, sensible and straight-forward, this model also nicely accounts for the lack of activity of unliganded receptor chains. Although originally derived from studies on the receptor for epidermal growth factor (EGFR)—by far the most extensively studied RTK—this model is often accepted to be valid for all RTKs, almost as a default and without further considerations. Upon deeper scrutiny, however, several aspects of this concept do not seem to add up. The idea that a ligand would first bind to a single receptor chain and then recruit a second one to the complex, although simple to visualize, does not appear to be an efficient way to evoke a rapid response, particularly given the relatively low abundance of individual receptor subunits in the crowded environment of the plasma membrane. In thermodynamic terms, such a two-step event would be expected to result in positive cooperativity of ligand–receptor interaction: binding to the first receptor subunit should facilitate binding to the second. Experimentally, however, the opposite has been observed: EGF binding to cell surface receptors shows negative cooperativity (Lemmon, 2009). Negative cooperativity has also been seen in GDNF binding (Cik et al., 2000). A key prediction of negative cooperativity in ligand binding to cell surface receptors is that a significant proportion of receptors should already be present in oligomerized form in the absence of the ligand. This notion has been supported by a now rather voluminous literature which demonstrates the presence of preformed, inactive receptor dimers at the plasma membrane in the absence of ligand for several classes of RTKs as well as noncatalytic receptors (Jiang and Hunter, 1999; Moriki et al., 2001; Gerber et al., 2004; Lemmon, 2009). Dimerization of Trk receptors has been studied using co-immunoprecipitation experiments (Jing et al., 1992), which unfortunately are too stringent to allow the detection of cis interactions between plasma membrane components. In the case of Ret, the transmembrane domain has been shown to self-associate and facilitate oncogenic activation by mutations that alter the pattern of disulphide bridges in the Ret extracellular domain (Kjaer et al., 2006). Thus, preformed dimers, although more efficiently activated by ligand, do come with a cost in the form of a higher propensity to oncogenic activation.

Several noncatalytic receptors have also been shown to exist as preassembled dimers at the plasma membrane in the absence of ligand. Many studies have focused on the growth hormone and erythropoietin (EPO) receptors, which are present as preformed homodimers despite the fact their ligands are actually monomeric (Seubert et al., 2003; Brown et al., 2005). Our group has recently discovered that $p75^{NTR}$ is present in homodimeric form at the plasma membrane prior to neurotrophin binding (Vilar et al., 2009). $p75^{NTR}$ dimers are held together by both cova-

lent and noncovalent interactions between transmembrane domains. An intramembrane cysteine residue links a proportion of $p75^{NTR}$ dimers at the cell surface through disulphide bridges. In the absence of this cysteine, however, $p75^{NTR}$ dimers are still held together through specific noncovalent interactions mediated by other residues in their transmembrane, and possibly also intracellular, domains (Vilar et al., 2009) [Fig. 1(B)]. The twofold symmetry of the complex formed between $p75^{NTR}$ and the neurotrophins would at first appear to set this receptor apart from other members of the TNFR superfamily, which have been shown to form threefold symmetry complexes with trimeric TNF ligands (Banner et al., 1993). However, crystallographic studies of full-length TNFR extracellular domains have revealed the formation of receptor dimers (Idriss and Naismith, 2000), suggesting that preassembled TNFR dimers can form trimers of dimers in a lattice type of arrangement that incorporates both twofold and threefold axes of symmetry (Chan, 2007).

Moving on to the assembly of heteromeric receptors, the idea that ligand binding drives the association of such complexes has also encountered difficulties. For GDNF receptor complexes, for example, the prevalent paradigm postulates that GDNF first interacts with the GFRα1 subunit (likely in dimeric form), and only then does this complex in turn recruit Ret receptor molecules. Evidence for this model came from co-immunoprecipitation studies which failed to recover an interaction between GFRα1 and Ret unless GDNF was added (Jing et al., 1996). However, GFRα1 does bind Ret in the absence of GDNF, as this is in fact one of the ways in which GFRα1 was first isolated, namely using the Ret protein as a screening probe (Sanicola et al., 1997). Binding and mutagenesis studies have subsequently provided additional evidence for the existence of preformed complexes of Ret and GFRα1 molcules at the plasma membrane in the absence of GDNF (Eketjäll et al., 1999; Cik et al., 2000) [Fig. 1(C)]. There is also experimental indications that co-expression of Ret with GFRα prevents spontaneous activation of the Ret kinase in the absence of ligand (Trupp et al., 1998), a mechanism that might also contribute to restrain the activity of a preformed Ret complex in the absence of ligand [Fig. 1(C)]. Preassembled receptor complexes are unlikely to survive dissolution of the plasma membrane, so co-immunoprecipitation is not always the best method to detect this type of interactions. In the case of NCAM, chemical crosslinking studies have indicated that GFRα1 and NCAM can and do interact in the absence of GDNF (Sjöstrand and Ibáñez, 2008). This interaction downregulates the ability of NCAM to function as a cell adhesion molecule and increases its affinity for GDNF (Paratcha et al., 2003), suggesting that the GFRα1/NCAM complex is also preassembled in the absence of GDNF.

For the CNTF receptor complex, however, it is still unclear whether CNTF, perhaps assisted by CNTF-Rα, induces the formation of the gp130/LIF-R signaling complex or, as it is now common in many other receptor systems, it rather alters the orientation of a preformed receptor complex to activate signaling. Interestingly, through its BC domain, CNTF-Rα can form dimers with either gp130 or LIF-R ligand-binding domains in the absence of ligand, with the two latter competing with each other for binding to CNTF-Rα (Man et al., 2003) [Fig. 1(D)]. Recently, on the basis of evidence from fluorescence cross-correlation spectroscopy studies, it has been hypothesized that CNTF-induced CNTF-Rα dimerization in living cells could reflect higher order association of tetrameric CNTF receptor complexes (Neugart et al., 2009). Together, these observations suggest that different pre-formed complexes of CNTF-Rα with its partner receptors may exist at the cell membrane.

In retrospect, it is somewhat surprising that the idea that RTKs and other receptors may preexist as dimers in the absence of ligand has taken such a long time to get hold, given the fact that some of these receptors have been known to exist as preformed homodimers for quite a while, a case in point being the insulin receptor, which is actually covalently cross-linked by a disulphide bond (Lemmon, 2009). Admittedly, this notion does raise some new questions, at least two of which are of importance for our discussion. The first is how receptor homodimers are kept inactive in the absence of ligand. Fortunately, there are now reports of many different mechanisms that can account for the low activity (or inactivity) of preformed dimers. Although the details are outside the scope of this review, several examples have been reported—some of which at high structural resolution (Jura et al., 2009)—of distinct receptor subdomains functioning to specifically block receptor activation in the absence of ligand. If receptor dimers are preassembled, the second question that arises concerns the mechanism(s) by which a preformed dimer becomes activated upon ligand binding.

ACTIVATION MECHANISMS OF NEUROTROPHIC FACTOR RECEPTORS

Mechanisms proposed for the activation of RTK dimers invoke allosteric changes that result in the displacement of intrinsic inhibitory domains which

normally block the kinase activity of the receptor (Jiang and Hunter, 1999). Importantly, the devil is in the details, and different RTKs employ distinct regions of the molecule to perform inhibitory functions. The crystal structure of both the phosphorylated and unphosphorylated forms of the Ret kinase have recently been solved, showing identical active kinase conformations with a preorganized activation loop independently of phosphorylation status (Knowles et al., 2006). Biochemical experiments showed that not only do both forms of the kinase show comparable activity but also the complete intracellular domain displays high kinase activity even in the unphosphorylated state (Knowles et al., 2006), indicating the absence of cis-inhibitory mechanisms, in contrast to the kinases present in other receptors. Interestingly, although monomeric in solution, the Ret kinase formed head-to-tail dimers in the crystal, suggesting a novel inhibitory mechanism by which two kinase domains may inhibit each other through interactions in trans (Knowles et al., 2006). Intriguingly, some oncogenic mutations that activate the Ret kinase map to the crystallographic interaction interface (Knowles et al., 2006), suggesting that receptor activation may involve separation––or reorientation––of kinase domains, a paradoxical departure from the old dimerization model.

Although structural studies of the TrkA kinase are still lacking, evidence pointing to the existence of inhibitory domains comes from studies on the ligand-independent activities of TrkA variants in tumors. For example, a 75 amino acid deletion in the TrkA extracellular domain found in patients with acute myeloid leukemia (Arevalo et al., 2000), and a TrkA splice variant lacking Ig-like and cysteine-rich domains found in neuroblastoma (Tacconelli et al., 2004) resulted in constitutive kinase activity. This suggests that the truncated receptor has the potential to dimerize in the absence of ligand and supports a role for the extracellular region of TrkA as an autoinhibitory domain [Fig. 1(A)]. The mechanisms by which NGF binding may disinhibit TrkA dimers remain unknown at present. Studies using chimeric receptors suggest that association with $p75^{NTR}$ may induce conformational changes in Trk, such as for example a relative rotation of the two receptor chains [Fig. 1(A)], that allow high-affinity ligand binding and activation (Esposito et al., 2001). The possibility that relative rotation of receptor chains may be part of the mechanism of Trk receptor activation warrants further investigation.

Four basic types of possible motions have been proposed to account for outside-inside signal transmission by transmembrane receptors: translation, piston, rotation parallel to the membrane (pivot), and rotation perpendicular to the membrane (Matthews et al., 2006). Among noncatalytic receptors, the mechanisms of activation of the growth hormone and EPO receptors are some of the most intensively investigated, and the prevalent model here proposes relative rotation of transmembrane domains within the plane of the membrane (Seubert et al., 2003; Brown et al., 2005). This work has also suggested that transphosphorylation of JAKs requires a precise positioning of the intracellular receptor chains, whereas binding of adaptors of the MAP kinase pathway may be less sensitive to orientation (Seubert et al., 2003). Structural observations made on the gp130/LIF-R complexes that mediate CNTF signaling suggest that, similar to the case of the EPO receptor, transmembrane and juxtamembrane segments are relatively rigid units able to relay structural perturbations generated by ligand binding to extracellular domains (Skiniotis et al., 2008). Whether such perturbations will also involve rotation of transmembrane regions, as in the case of the EPO receptor, remains to be determined.

Our laboratory has recently elucidated a novel mechanism for the activation of $p75^{NTR}$ dimers involving relative pivot-like rotation of receptor subunits with axis on the intramembrane Cys^{257} (Vilar et al., 2009). In this model, the role of Cys^{257} is akin to the pin in a pair of scissors: in its absence, relative movements at one end can not be propagated to the other. This model was based both on the requirement of Cys^{257} for $p75^{NTR}$ signaling in response to neurotrophins, and on FRET experiments showing that receptor intracellular domains distance from each other upon ligand binding (Vilar et al., 2009). Something that remained unclear from those experiments was whether such rearrangement was brought about by opening or closure of extracellular domains upon ligand binding. In more recent experiments, we have used a series of Cysteine substitution mutants to show that crosslinking of $p75^{NTR}$ dimers by disulphide-bonding of the juxtamembrane region of the extracellular domains mimics activation elicited by neurotrophin binding (Vilar et al., 2009). This suggests that, unlike a normal pair of scissors, intracellular separation of receptor domains is elicited by closure, not opening, of extracellular domains onto the ligand, a novel type of receptor activation which we have termed the "snail-tong" mechanism (Vilar et al., 2009a) [Fig. 1(B)]. Interestingly, the activity profiles of different cysteine substitution mutants were found to be similar but not identical (Vilar et al., 2009b), suggesting that different degrees of separation between intracellular domains may favor the recruitment or activation of different subsets of intracellular

effectors or downstream pathways. Neurotrophin binding to p75^{NTR} may induce an array of receptor configurations, each with its own particular functional bias, but which as a whole encompass the full repertoire of p75^{NTR} functions.

The snail-tong mechanism has interesting implications for the diversification of receptor signaling. Although crucially required for neurotrophin signaling via p75^{NTR}, Cys^{257} was dispensable for the ability of p75^{NTR} to transmit signals in response to MAG together with the NgR/Lingo-1 complex (Vilar et al., 2009). This indicates that one and the same receptor can have different activation mechanisms depending on the ligands and the nature of the complex that it forms together with other transmembrane proteins. In addition, many receptors in the TNFR superfamily bear intramembrane cysteines, so it is likely that they too play a role in receptor activation, perhaps—in analogy to the snail-tong model—by allowing the transmission of ligand-induced conformational changes from the extracellular to the intracellular domains of a multisubunit receptor complex.

CONCLUDING REMARKS

Receptors for neurotrophic factors are formed by multimeric complexes of receptor subunits. Although the details of the activation mechanisms vary between different receptors, these invariably involve relative movement of receptor subunits, either in a dimer or a multimeric complex. It is therefore clear that the oligomeric nature of these receptors is intrinsic to their mechanisms of activation and hence required for receptor function. Because in most instances such intersubunit movements have been inferred from indirect biophysical or biochemical observations, an important area for future research will be the actual visualization of receptor complex conformation changes at high resolution and at the single molecule level. Methods for specifically labeling extra and intracellular receptor domains with electron-dense tags of different sizes may allow direct visualization of conformational changes by electron microscopy and could thus represent a way to achieve this goal.

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