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p75NTR Is Positively Promiscuous: Novel Partners and New Insights

Minireview

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Although identified almost 20 years ago, the precise physiological role of the p75 neurotrophin receptor (p75NTR) has remained elusive. Recent studies have revealed that p75NTR is a component of three distinct receptor platforms that bind different ligands and that, under differing circumstances, facilitate cell survival, cell death, or growth inhibition. These recent developments provide new insights into the functions of this enigmatic receptor.

The neurotrophins exert a wide array of effects in the central and peripheral nervous system. Originally characterized for their survival promoting activity, the four mammalian neurotrophins are now implicated in functions that include neuronal growth, apoptosis, and synaptic modulation. At first glance, the receptor system that mediates these effects seems simple enough, consisting of the three Trk receptors and the p75 neurotrophin receptor (p75NTR). By and large, the Trk receptors behave as typical receptor tyrosine kinases, and details of the signaling pathways and cell biology surrounding their actions have steadily accumulated over the last several years. In contrast, the precise physiological roles of the p75NTR have been slow to emerge. However, recent studies have shown that p75NTR contributes to several different signaling platforms and suggest that its biological activities have been underestimated (Figure 1).

When p75NTR was cloned in 1986, it represented a novel receptor class that contained tandem arrays of cysteine-rich domains (CRDs) in its extracellular portion. The CRDs in p75NTR are required for neurotrophin binding and were subsequently recognized as the defining characteristic of the tumor necrosis receptor (TNFR) superfamily. TNFR superfamily members typically bind homotrimeric ligands that are produced as type II transmembrane proteins, and most act as independent signaling units. In contrast, p75NTR binds soluble dimeric ligands and often requires (or acts as) a coreceptor to activate biological activity. Some TNFR family members, including p75NTR, contain an ~80 amino acid death domain; most use the death domain as a protein binding module to interact with adaptor proteins that aggregate and activate Caspase-8 and thereby initiate apoptosis, but the death domain in p75NTR is structurally distinct from that in other TNFR superfamily members, and signaling properties of p75NTR are distinct from its TNFR breathren. Indeed, p75NTR can be considered the black sheep in the TNFR family.

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p75NTR and the Trks—Old but Not Forgotten

Trks are often termed high-affinity NGF receptors, but their neurotrophin binding affinity is actually similar to that of p75NTR, with a Kd of about 1–10 nM. However, when the receptors are coexpressed, p75NTR enhances the ability of Trk receptors to bind and respond to neurotrophins and sharpens the discrimination of Trks for their preferred neurotrophin ligands. Peripheral tissues produce low concentrations of neurotrophins to maintain appropriate levels of neuronal survival and innervation, and p75NTR appears to act as a coreceptor that allows the Trks to respond to limiting neurotrophin levels. Indeed, the loss of sympathetic and sensory neurons and the progressive peripheral neuropathy observed in adult p75NTR null mice (Lee et al., 1992; von Schack et al., 2001) likely reflect reduced Trk activation in peripheral neurons.

p75NTR and Sortilin—Partners in Crime

Over the last decade, numerous studies have shown that p75NTR can act as an apoptotic receptor during development and following injury, but there has been controversy about the precise ligand requirements for these effects. Mature neurotrophins are not effective activators of p75NTR-induced apoptosis, and high nonphysiological concentrations are often required to induce even modest levels of cell death. This led to speculation that there may be other mammalian ligands for p75NTR, and several labs initiated searches to try to find them. Although one p75NTR binding factor was identified in invertebrates (Fainzilber et al., 1996), it had no apparent mammalian homolog, and with this exception, these searches were uniformly unsuccessful. The discovery that proneurotrophins (proNTs), which had been under our collective noses all along, were the long-sought ligands for p75NTR was an important breakthrough that was rich in irony.

Neurotrophins are synthesized as precursors that can be cleaved by furin and proconvertases to produce mature NGF. However, in some tissues, a substantial proportion of proNGF and proBDNF eludes cleavage, raising the possibility that these uncleaved forms of the neurotrophin may have biological functions. Using a furin-resistant form of proNGF, Hempstead and colleagues found that proNGF binds p75NTR with high affinity and is a potent inducer of p75NTR-dependent apoptosis in sympathetic neurons, oligodendrocytes, and in a vascular smooth muscle cell line (Lee et al., 2001; Nykjaer et al., 2004). These investigators also showed that proNGF does not bind TrkA and suggested that proNGF is an apoptotic ligand that is specific for p75NTR.

The role of proNGF in p75NTR-dependent apoptosis in vivo has now been examined in two injury paradigms. Beattie et al. (2002) have shown that oligodendrocyte apoptosis that occurs after spinal cord trauma correlates with the synthesis of bioactive proNGF, indicating that proNGF is in the right place at the right time to induce cell death in vivo. Corticospinal neurons (CSN) undergo p75NTR-dependent apoptosis following lesion, and Harrington et al. (2004) used two loss-of-function

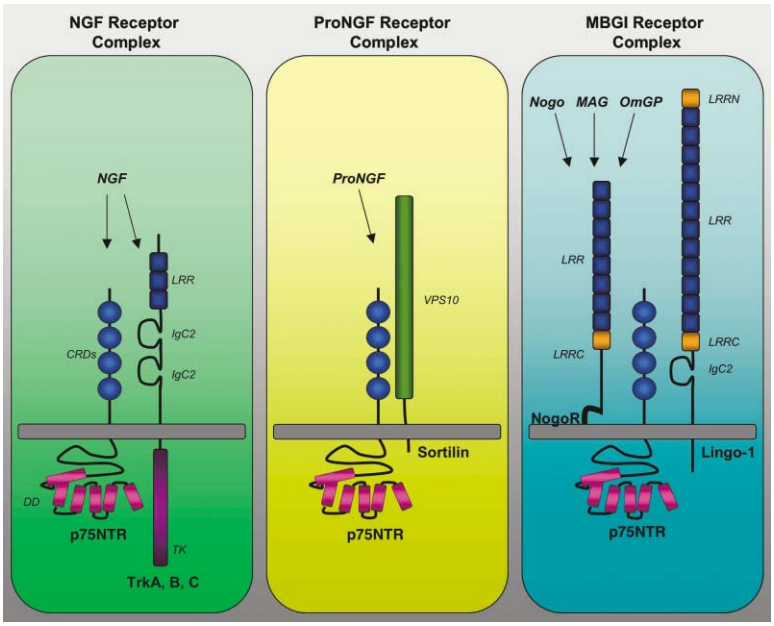

Figure 1. p75NTR Signaling Modules

p75NTR physically interacts with the TrkA receptor and enhances the ability of TrkA to respond to NGF and discriminate between preferred and nonpreferred neurotrophin ligands. A second signaling complex consisting of p75NTR and Sortilin mediates proapoptotic signals in response to proNGF binding. Finally, p75NTR may form a tripartite complex with the NogoR and with Lingo-1 that results in growth inhibitory signals to be transduced in response to Nogo, MAG, or OMgP. CRD, cysteine-rich domain; DD, death domain; LRR, leucine-rich repeat; IgC2, IgC2 domain; TK, tyrosine kinase domain; VPS10, VPS10 domain; LRRN, N-terminal leucine-rich repeat domain; LRRC, C-terminal leucine-rich repeat domain.

approaches to establish a role for proNGF in this system. In the first, antibodies directed against either mature NGF or against the prodomain of NGF were shown to reduce CSN loss, and in the second, significant protection of CSNs was observed in mice containing only a single NGF allele. Importantly, the investigators demonstrated that p75NTR immunoprecipitated from lesioned tissue is bound to proNGF and showed that infusion of antibodies directed against proNGF reduces this association. This well-crafted study thus provides direct and definitive evidence for the participation of proNGF in p75NTR-dependent cell death in vivo.

How does p75NTR bind and mediate the effects of proNGF? Studies that address this question are just beginning, but the identification of Sortilin as a p75NTR coreceptor that is necessary for proNGF-induced cell death is a major breakthrough (Nykjaer et al., 2004). Sortilin is a type I transmembrane protein expressed in a wide variety of tissues but is most abundant in the central nervous system during development and in adults. Nykjaer and colleagues (2004) recently found that the NGF prodomain directly binds the extracellular domain of Sortilin and, through crosslinking studies, established that p75NTR and Sortilin form a receptor complex that binds proNGF at the cell surface. Both receptors appear to be required to transduce the apoptotic effects of proNGF. Blocking the interaction of proNGF with Sortilin inhibits proNGF-mediated apoptosis, whereas expression of exogenous Sortilin in Schwann cells, which normally express only p75NTR, renders these cells sensitive to the apoptotic effect of proNGF (Nykjaer et al., 2004). Interestingly, although p75NTR and Sortilin are required for proNGF-induced apoptosis, their coexpression does not invariably result in proNGF-induced killing; for example, proNGF does not induce apoptosis of melanoma cells but instead enhances migratory activity (Shonukan et al., 2003).

Like p75NTR, Sortilin leads a complicated life. Sortilin is a member of the VPS10 family, named for the yeast gene that traffics cargo from the trans golgi network (TGN) to the vacuole. In mammalian cells, Sortilin has been shown to play an important role in TGN-to-endosome and TGN-to-lysosome trafficking events, and greater than 90% of the sortilin pool is retained in intracellular compartments (Nielsen et al., 2001). It is not known if p75NTR and Sortilin affect each other's subcellular distribution, but cell surface levels of Sortilin can be increased in response to insulin exposure (Morris et al., 1998). The regulated insertion of neurotrophin receptors has been previously documented, and it is plausible that regulated insertion of p75NTR-Sortilin complexes may also occur, perhaps in response to stress or neuronal injury.

ProNGF directly binds Sortilin, even in the absence of p75NTR. Since Sortilin plays a role in TGN-to-endosome trafficking, it is possible that Sortilin functions not only as a cell surface proNT receptor but also directs intracellular movement of newly synthesized proNTs. BDNF can be sorted to both constitutive and regulated secretory vesicles, and its prodomain has been shown to play an important role in these trafficking decisions. In humans, a polymorphism that results in a single substitution in the prodomain of BDNF (V66M) blocks trafficking of BDNF from TGN to secretory granules and is associated with deficits in episodic memory (reviewed in Lu, 2003). The precise molecular mechanism that leads to this secretory defect is unknown, but one possibility is that the V66M substitution increases the affinity of BDNF for Sortilin and thereby prevents its trafficking to regulated secretory vesicles. The functional roles of the other VPS10 proteins expressed in humans (Figure 2) are largely uncharacterized, and it may be worthwhile to determine if any of these physically or functionally interact with p75NTR or the proNTs.

Most studies that have examined p75NTR-induced apoptosis suggest that p75NTR induces an intrinsic apoptotic cascade that involves Rac1 and JNK activation, release of cytochrome C and SMAC from mitochon-

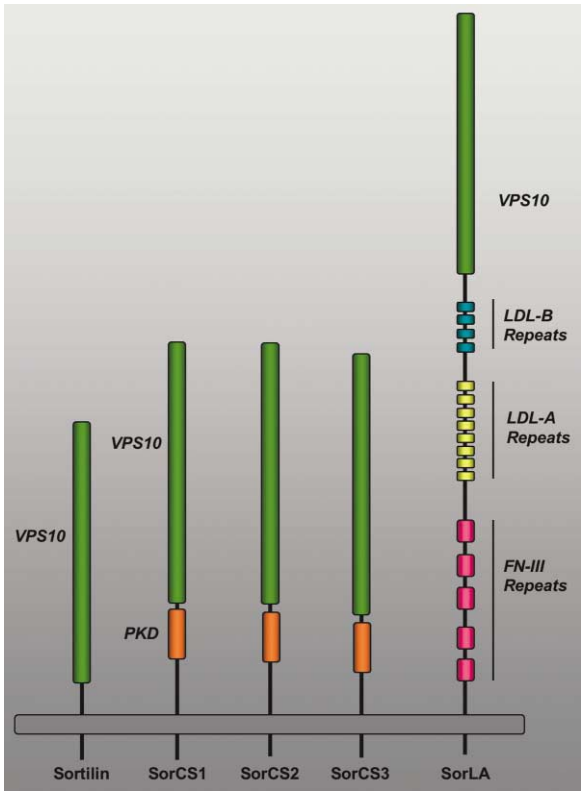

Figure 2. The Mammalian VPS10 Family

Domain structures of each of the receptors are indicated. The VPS10 domains extend into the TGN lumen or, in the small percentage of protein that reaches the cell surface, are extracellular. PKD domains define an Ig-like region, and LDL-A/LDL-B and FN-III repeats are conserved motifs originally identified in the low-density lipoprotein receptor and in fibronectin, respectively.

dria, and activation of caspase 9, 6, and 3 (Figure 2). The link between p75NTR-induced JNK activation and the mitochondrial effects appears to involve direct JNK-dependent phosphorylation of Bad, a BH3 domain-only protein, but proximal aspects of this pathway are not well understood (Bhakar et al., 2003). The identification of proNGF as a p75NTR ligand and the characterization of the p75NTR-Sortilin signaling complex will no doubt usher in a new phase in the analysis of p75NTR signaling.

Nogo + Lingo = Don't Grow?

For many years after it was cloned, p75NTR was searching for a function, but now the receptor seems to be making up for lost time. In addition to its roles as a Trk coreceptor and regulator of apoptosis, p75NTR has recently emerged as a key player in the regulation of neuronal growth. Definitive data linking p75NTR to this function came from studies of Barde and colleagues who demonstrated that unliganded p75NTR was a potent activator of RhoA and showed that neurotrophins could suppress this effect (Yamashita et al., 1999). In adults, RhoA activation mediates the effects of CNS-derived myelin-based growth inhibitors (MBGIs) that include Nogo, myelin-associated glycoprotein (MAG), and oligodendrocyte myelin glycoprotein (OMgP). The discovery that RhoA could be regulated by p75NTR prompted Yamashita and colleagues to determine if p75NTR played a role in MBGI-induced growth inhibition. Consistent with this, they found that MAG-induced growth inhibition and RhoA activation were attenuated in sensory and cerebellar granule neurons (CGNs) derived from p75NTR null mice (Yamashita et al., 2002). The MBGIs bind to the Nogo receptor (NogoR), a GPI-linked protein with no intracellular signaling capability, and the discovery that p75NTR played a role in MAG signaling suggested that p75NTR may collaborate with the NogoR to mediate responses to MBGIs. Two groups have independently confirmed this prediction and shown that a complex containing NogoR and p75NTR can be identified in cells coexpressing the two receptors (Wang et al., 2002; Wong et al., 2002).

The precise signaling mechanisms that are activated by the p75NTR-NogoR complex are now the subject of intense scrutiny. Initial studies indicated that p75NTR directly binds and regulates RhoA, but more recent analyses have shown that p75NTR regulates RhoA by directly binding Rho GDP dissociation inhibitor α (Rho-GDIα). Binding of MBGIs to the p75NTR-NogoR complex appears to enhance the association of Rho-GDIα with p75NTR, whereas NGF binding abolishes the p75NTR-RhoGDIα interaction (Yamashita and Tohyama, 2003). In a clever set of experiments, Yamashita and Tohyama (2003) took advantage of a p75NTR death domain binding peptide that binds the p75NTR death domain. This peptide blocks the association of Rho-GDIα with p75NTR in vitro and was shown to antagonize Nogo- and MAG-induced growth inhibition when delivered to primary sensory neurons. Thus, the association of MBGIs with the p75NTR-NogoR complex causes Rho-GDIα to bind the p75NTR death domain and causes RhoA-GDP to be released from Rho-GDIα. RhoA is then able to exchange bound GDP for GTP and thus gain its active conformation (Figure 3). Earlier work of Yamashita and colleagues that showed that unliganded p75NTR was a potent activator of RhoA could be explained if the association of Rho-GDIα with p75NTR is attenuated by NogoR coexpression, but this has not yet been demonstrated experimentally.

In the latest development, LINGO-1 (also known as LERN1) has been identified as an essential component of the p75NTR-NogoR receptor complex that is required for MBGI signaling. Mi et al. (2004) found that the extracellular domain of LINGO-1 binds both NogoR and p75NTR and showed that full-length LINGO-1 can be immunoprecipitated with either NogoR or p75NTR (Mi et al., 2004). In COS cells, coexpression of all three receptors was required for RhoA activation in response to OMgP, MAG, or NOGO, indicating that, at least in this cell type, LINGO-1 is an essential cofactor required for MBGI signaling. Interestingly, mutant LINGO-1 lacking its 38 amino acid intracellular domain inhibits MBGI-induced growth inhibition when expressed in CGNs, indicating that this domain may play an important role in the assembly, cell surface expression, and/or signaling of the MBGI receptor complex.

LINGO-1 is a member of a four-protein family (the others are LINGO-2, -3, and -4), all of which are characterized by extracellular leucine-rich repeats (LRRs) and an IgC2 domain. The primary function of LRR and IgC2 domains is to mediate protein-protein interactions, but their role in LINGO-1 function is unknown. There are

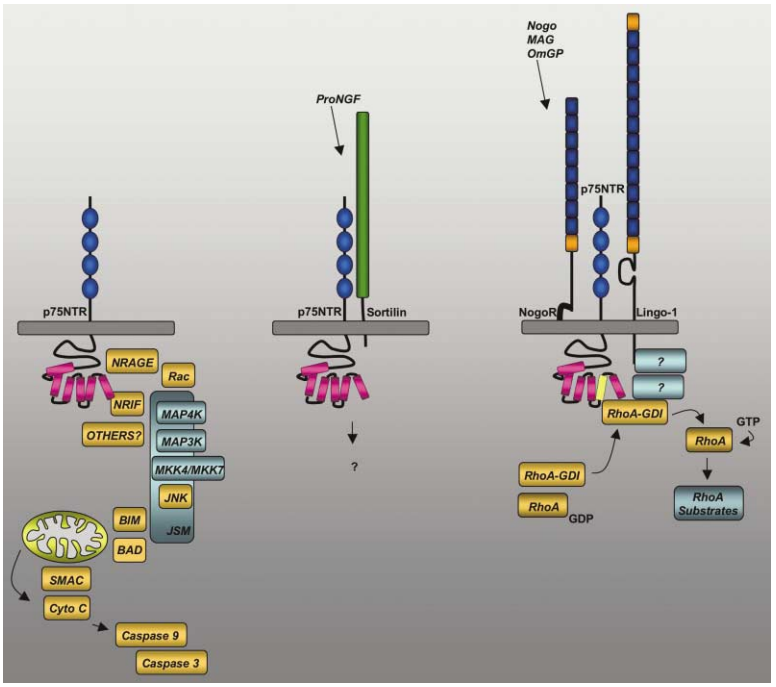

Figure 3. Putative p75NTR-Induced Signaling Cascades

The proapoptotic signaling events mediated by proNGF are not yet known (middle), but earlier studies using cleaved neurotrophins or p75NTR overexpression paradigms provide the outlines of a possible p75NTR apoptosis cascade (left). Upon activation, p75NTR assembles a signaling complex that may include NRAGE, NRIF, and other adaptors. Rac1 is activated and leads to activation of a JNK cascade that results in phosphorylation of Bad and perhaps other BH3 domain-only family members that release inhibition of Bax and Bak (data not shown). Subsequent release of mitochondrial components that include SMAC and cytochrome C facilitates caspase activation. p75NTR mediates RhoA activation via a direct interaction with Rho-GDIα (right). When the complex is in an unliganded state, Rho-GDIα is associated with inactive Rho-GDP in the cytosol. Binding of MBGIs to the complex produces a conformational shift in the complex that allows Rho-GDIα to bind to the fifth helix of the p75NTR death domain and thereby release RhoA. Once released from Rho-GDIα, RhoA is able to exchange GDP for GTP and achieve its active conformation and activate downstream substrates. Orange boxes indicate proteins implicated in p75NTR signaling through experimentation; blue boxes indicate potential participants.

relatively few proteins that contain both LRR and Ig-like domains in their extracellular region, and it is noteworthy that TrkA, TrkB, and TrkC belong to this group (Figure 1). The NogoR does not contain IgC2 domains but does contain multiple LRRs, and it may be more than a coincidence that this structural motif is present within three classes of transmembrane proteins that form physical complexes with p75NTR. Studies that define the structural basis for these interactions will likely be a top priority since they will provide insights into the function of these complexes, could lead to the identification of other p75NTR binding partners, and may reveal strategies that could result in the production of selective p75NTR antagonists.

Reconciling the recent in vitro data that demonstrate a role for p75NTR in MBGI signaling with in vivo findings on the role of p75NTR in neuronal growth remains a significant challenge. At the moment, there is considerable variance between in vivo studies, even when similar experimental paradigms are employed. For example, two studies of adult facial neuron regeneration in p75NTR null mice have revealed increased regeneration and enhanced functional recovery (see Boyd and Gordon, 2001), but others have found that the rate of axonal elongation was not altered (Gschwendtner et al., 2003). In the injured spinal cord, the initial phase of RhoA activation is dependent upon p75NTR expression (Dubreuil et al., 2003), yet p75NTR null mice do not show enhanced regeneration of lesioned corticospinal neurons or ascending sensory tracts after spinal cord damage (Song et al., 2004).

Assessing the precise role of p75NTR in neuronal growth in vivo will be an interesting challenge, and several factors may slow progress in this area. First, p75NTR plays several functional roles, and in many cases, the link between p75NTR deletion and an observed phenotype will be difficult to establish. Second, compensatory mechanisms may complicate analyses of p75NTR loss-of-function strains. One interesting candidate for compensatory effects in null strains is neurotrophin receptor homolog 2 (NRH2), a recently identified type I transmembrane protein that lacks the CRDs but otherwise is remarkably similar to p75NTR (Kanning et al., 2003). NRH2 has recently been shown to physically interact with TrkA receptors (Murray et al., 2004), and it will be interesting to learn if this homolog can also bind Sortilin, NogoR, or LINGO-1 and participate in the effects of proNTs and MBGIs. A third factor affecting in vivo analyses is that there have been complications with the two strains of p75NTR null mice produced to date (Lee et al., 1992; von Schack et al., 2001). The strain in which exon 3 was targeted has been reported to express a p75NTR splice variant that lacks CRD2-CRD4 (von Schack et al., 2001). We have been unable to detect this protein in our analyses of the exon 3 mouse (Paul et al., 2004), but nonetheless, caution is warranted. Recent analyses have shown that the exon 4 targeted strain harbors a p75NTR fragment that contains the transmembrane and intracellular domain and is capable of activating p75NTR signaling cascades (Paul et al., 2004; von Schack et al., 2001). The presence of this product may explain some differences between the exon 3 and 4 strains. For example, the profound vascular phenotype that is observed in the exon 4 strain may represent a neomorphic gain-of-function defect resulting from expression of the aberrant p75NTR fragment. Although

much useful information has been obtained from the strains created to date, a complete understanding of the physiological effects of the receptor will likely require the creation of new conditional mutants and the generation of compound nulls.

Too Much of a Good Thing Is Wonderful

The past decade has seen the p75NTR receptor emerge as a key player in regulating Trk action, in the control of apoptosis, and in the neuronal growth response. p75NTR continues to turn up in surprising functions, and recent studies have identified roles for p75NTR in myelination (Cosgaya et al., 2002) and in the extension of cortical subplate neurons and establishment of thalamocortical tracts (McQuillen et al., 2002). An additional intriguing finding is that p75NTR undergoes cleavage by γ-secretase, which results in release of the cytoplasmic domain from its transmembrane tether and accumulation in the nucleus (Kanning et al., 2003). The function of this released fragment is not yet known, but by analogy with Notch and other receptors that undergo similar cleavage, it is conceivable that the p75NTR intracellular domain may act as a regulatory component of transcriptional complexes. Finally, in the most recent turn in the p75NTR tale, the crystal structure of mature NGF bound to p75NTR has been solved, with surprising results. He and Garcia (2004) have shown that binding of dimeric NGF to a single chain of p75NTR results in an allosteric change in the NGF dimer that actually prevents interactions with additional p75NTR molecules. Thus, the stoichiometry of NGF-p75NTR receptor complexes is 2:1, and NGF binding to p75NTR will therefore block, rather than facilitate, the formation of p75NTR dimers. This finding has broad implication for understanding the actions of p75NTR; for example, it suggests that p75NTR is a coreceptor capable of sharing or delivering neurotrophin ligand to Trk receptors or Sortilin and raises the possibility that RhoA activation induced by p75NTR requires the formation of a dimeric receptor complex.

All in all, not bad for a receptor that a decade ago was thought to be little more than biological flotsam. Moving forward, a top priority will be to refine the signaling functions of p75NTR and to begin to place the actions of the receptor in a cell biological context. Concurrently, the various activities of p75NTR that have been observed in vitro will need to be firmly linked to its physiological roles in vivo using appropriate gain- and loss-of-function models.

Sorting out the complexities of the actions of p75NTR remains a challenging task, but recent developments suggest that the paradox of p75NTR lies in its promiscuity. With the identification of new ligands and coreceptors for p75NTR, it is becoming increasingly difficult for the receptor to keep its secrets. Together, these findings mark the beginning of a new phase of neurotrophin receptor research that is certain to provide new insights into nervous system function.

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