ways that we cannot yet imagine. The article by Seeley et al. highlights the critical importance of neural connectivity for any principled investigation of brain function and dysfunction. It would be a great service to this field if funding were specifically targeted for an international collaboration of cognitive neuroscientists, neuroimagers, and neuroanatomists so that the real connectivity of the human brain could be explored effectively.

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A p75^{NTR} Pivoting Paradigm Propels Perspicacity

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The p75 neurotrophin receptor (p75^{NTR}) is involved in numerous neuronal signaling paths but its fundamental signaling mechanisms are unknown. In this issue of *Neuron*, Vilar et al. show that p75NTR functions as a covalently crosslinked dimer to transduce NGF-induced signaling events.

The 75 kDa neurotrophin receptor (p75^{NTR}) is an important neuronal signaling protein that interacts with numerous ligands and coreceptors to regulate cellular survival and apoptosis, neurite outgrowth and repulsion, myelin formation and long-term depression. The long list of functions ascribed to this one receptor would be hard to believe were it not for the compelling in vivo data that demonstrates its participation in these activities. In the face of this biological reality, at some point or another, almost all cellular neurobiologists will eventually find themselves working a problem involving p75^{NTR}. So in this sense, we all have a stake in deciphering its mechanism of action.

p75^{NTR} was the founding member of the tumor necrosis family receptor superfamily, a group that is characterized by the presence of tandem arrays of cysteine-rich domains (CRDs) in their extracellular regions, which function as ligand binding domains. TNFRS members typically bind trimeric ligands of the TNF family, whereas p75^{NTR} binds dimeric ligands of the neurotrophin family. The molecular details of how neurotrophins transduce signals via p75^{NTR} have been uncertain, but in this issue of *Neuron*, Ibanez and colleagues provide new insights into the mechanisms of p75^{NTR} signal transduction (Vilar et al., 2009).

The authors show that p75^{NTR} exists as a covalently associated dimer in sympathetic neurons and PC12 cells, in cortex, hippocampus, and cerebellum, and when overexpressed in heterologous cells. The oligomer is lost in the presence of reducing agents, indicating that a disulfide linkage mediates this bimolecular association. Each of the cysteines in the p75^{NTR} extracellular domain exist as intramolecular pairs that maintain the receptors' extended structure and its intracellular cysteines exist in a reducing environment unable to support disulfides. So where in p75^{NTR} is the relevant cysteine?

Vilar et al. (2009) identify a cysteine residue within the p75^{NTR} transmembrane domain as the locus for disulfide formation between the p75^{NTR} chains. Introduction of a C257A mutation into the otherwise intact receptor blocks the formation of the covalently linked dimer. However, wild-type p75^{NTR} and p75^{NTRC257A} form cell-surface dimers with equal frequency, indicating that other mechanisms drive p75^{NTR} oligomerization. The p75^{NTR} transmembrane domain also contains an AxxxG motif at position 262–266. This motif is present in self-associating transmembrane domains within integrins and glycophorin A (Kubatzky et al., 2001) and by using the bacterial ToxCAT system and mammalian cell overexpression, Ibanez and colleagues show that the p75^{NTR} transmembrane domain is similarly self-associating. Comparison of the mammalian and reptilian receptor with p75^{NTR} orthologs identified in primitive deuterostomes such as sea urchin and acorn

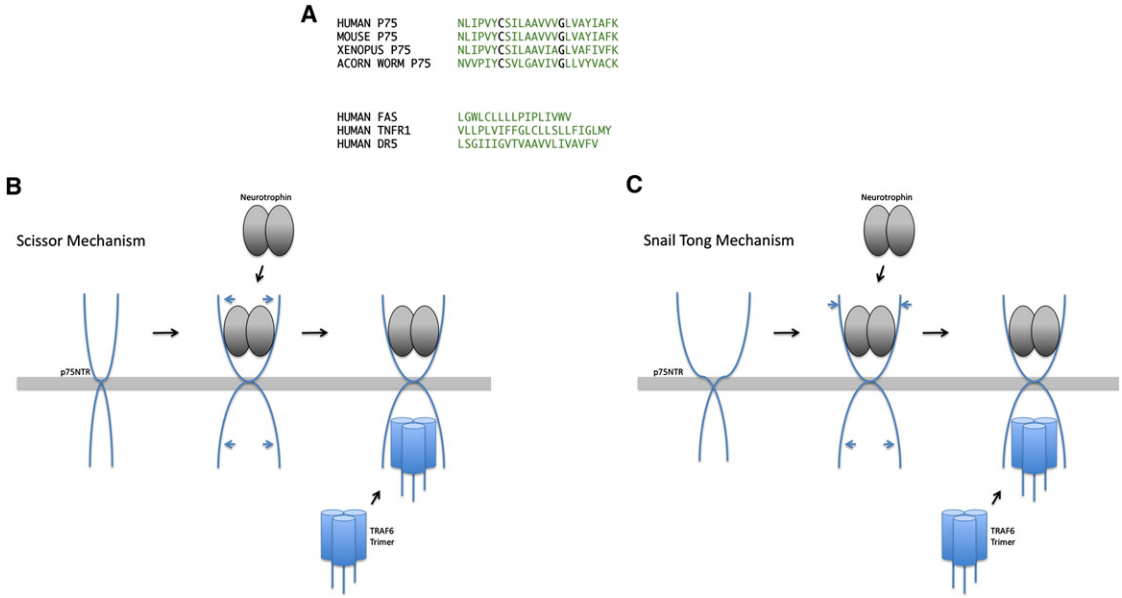

Figure 1. Transmembrane Associations in p75^{NTR} Signaling

(A) The p75^{NTR} transmembrane domain is conserved from vertebrates to deuterostomes such as the sea acorn (Bothwell, 2006). Transmembrane sequences are indicated in green except for the transmembrane cysteine and glycine required for disulfide formation and transmembrane association, respectively, which are indicated in black.

(B) In a "scissor" mechanism, neurotrophin binding to preformed p75^{NTR} dimers forces a separation of the extracellular domains and thereby exposes interactor binding surfaces in the juxtamembrane and death domain region of p75^{NTR}.

(C) In the snail-tong mechanism, ligand binding drives p75^{NTR} extracellular domains closer and causes p75^{NTR} intracellular domains to move apart. Both models allow receptors to engage the outer rim of TRAF6 oligomers.

worm, reveal not only that the receptor's transmembrane domain is the most well conserved region within p75^{NTR} (Bothwell, 2006) but also that residues C257 and G266 are almost invariably conserved (Figure 1). Vilar et al. (2009) show that a G266I substitution prevents transmembrane domain oligomerization and, therefore, transmembrane domain association and disulfide crosslinking may represent ancient features of p75^{NTR} crucial for its function. It seems likely that these biochemical effects are functionally linked such that dimerization enabled by the AxxxG motif is a prerequisite for disulfide formation via C257.

The concept that trimeric TNF receptor superfamily members exist as unliganded oligomers is well entrenched in the field. In some of these "preligand assembly complexes," the receptor region that facilitates oligomerization is the amino-terminal cysteine rich domain (CRD1) present in the extracellular domain. This region, which has been termed the preligand assembly domain or PLAD, plays a critical role in producing oligomers of TNFRS members such as TNFR1 and DR5 (Chan et al., 2000; Papoff et al., 1999). CRD2, CRD3, and CRD4 and the cleft between CRD1 and CRD2 contain the binding surfaces that allow p75^{NTR} to bind neurotrophin. To date, a functional role has not been ascribed directly to the CRD1 in p75^{NTR}. Previous studies have shown that the transmembrane receptor forms oligomers in the absence of ligand (Wehrman et al., 2007), and the notion that extracellular motifs, perhaps CRD1, can promote p75^{NTR} oligomerization is supported by data showing that isolated soluable extracellular domain of p75^{NTR} can oligomerize (He and Garcia, 2004). However, a more recent study has found that p75^{NTR} extracellular domains do not self-associate (Gong et al., 2008), and Vilar et al. (2009) have found that p75^{NTR} containing C257A and G266I substitutions does not show ligand-independent dimerization when expressed in heterologous cells. Thus, the CRD1 in p75^{NTR} may be unlikely to function as a PLAD, at least under physiological conditions.

The AxxxG motif that drives transmembrane domain oligomerization in p75^{NTR} is not present in these other TNFR family members (Figure 1), so it would appear that TNFRS members use different strategies to achieve oligomerization in the absence of ligand. An important issue that remains unresolved is whether formation of p75^{NTR} dimers is a prerequisite for efficient neurotrophin binding under physiological circumstances. Deletion of the PLAD in TNFR1, TNFR2, DR4, or DR5 prevents formation of preligand assembly complexes and in turn, severely compromises their ligand binding capabilities (Chan, 2007). It will therefore be interesting to test if transmembrane substitutions in p75^{NTR} that block dimerization (e.g., C257 + G266) alter neurotrophin binding.

p75^{NTR} activates an array of signaling paths and Vilar et al. (2009) show that the covalent association of p75^{NTR} chains via C257 plays a crucial role transducing extracellular signals into intracellular action. Comparison of wild-type p75^{NTR} with p75^{NTRC257A} showed that the latter was incapable of mediating neurotrophin-dependent NF-kB activation, caspase-3, cleavage or cell death. Further, p75^{NTRC257A} showed sharply reduced association with NRIF and TRAF6, cytosolic adaptor proteins required for p75^{NTR} signaling. Using a robust intramolecular

FRET approach, Vilar et al. (2009) demonstrate that NGF normally induces a shift in the relative positions of $p75^{NTR}$ intracellular domains that is lost with the $75^{NTRC257A}$ mutant. Thus, the covalent association of $p75^{NTR}$ via C257 appears to invoke structural constraints on receptor pairs necessary for the formation of surfaces that bind adaptor proteins.

$p75^{NTR}$ has been cocrystallized with neurotrophin in two recent studies but the results obtained have been starkly different. Wehrman et al. (2007) found that the interaction of a $p75^{NTR}$ monomer with an NGF dimer induces a structural change in the ligand that prevents association with a second $p75^{NTR}$ molecule, whereas Gong et al. (2008) found that neurotrophin dimers engage 2 chains of $p75^{NTR}$ to produce a 2:2 ligand-receptor stoichiometry. Although the former model might occur in some circumstances, the shift in the relative position of the intracellular domains that occur in wild-type but not the $p75^{NTRC257A}$ mutant seems more likely if neurotrophin and $p75^{NTR}$ bind with 2:2 ligand-receptor stoichiometry.

Taken together, these data suggest that the C257-C257 disulfide bond that links the $p75^{NTR}$ chains acts as a fulcrum around which rigid $p75^{NTR}$ structures pivot (Figure 1B). The specifics of how this could occur are not known but two possibilities are shown schematically in Figure 1. In a "scissor" mechanism, unliganded $p75^{NTR}$ dimers exist in a relatively closed conformation; neurotrophin binding then forces a separation of the extracellular domains and thereby exposes interactor binding surfaces in the juxtamembrane and death domain region of $p75^{NTR}$ (Figure 1B). In the snail-tong mechanism favored by Vilar et al. (2009), neurotrophin binding acts to pull $p75^{NTR}$ extracellular domains closer and this action pushes $p75^{NTR}$ intracellular domains apart (Figure 1C). Both of these models dovetail with earlier structural studies that show that TRAF proteins exist as mushroom-shaped trimers that contain receptor binding motifs on the outer rim of the TRAF oligomer (Ye et al., 2002). Precisely how dimeric $p75^{NTR}$ complexes might engage trimeric adaptor complexes and how co-receptors involved in neurotrophin signaling, such as sortilin or the Trks, fit into this structural picture are important issues for further study.

In addition to functioning as a neurotrophin receptor, $p75^{NTR}$ also participates as a coreceptor in cell-surface signaling complexes that regulate growth come collapse and axon guidance (Schecterson and Bothwell, 2008). In the former, myelin-based growth inhibitors such as MAG bind to the NgR1 receptor and rely on a tripartite complex of NgR1, $p75^{NTR}$, and LINGO-1 to transduce a growth inhibitory signal. Interestingly, the $p75^{NTRC257A}$ mutant is capable of supporting MAG-induced signaling events, indicating that mechanisms of $p75^{NTR}$ signaling in NGF- versus MAG-responsive signaling complexes differ significantly. Clearly, the downstream signaling actions of $p75^{NTR}$ are strongly determined by the company it keeps.

$p75^{NTR}$ has a surprisingly complex array of actions. It induces an array of neurotrophin-induced signaling events and is also a promiscuous receptor that collaborates with diverse cell surface proteins to impinge on a large number of neuronal functions and dysfunctions. This complexity will only be understood when the molecular details of $p75^{NTR}$'s dalliances are revealed. The work of Vilar et al. (2009) is an important step toward that goal and provides a framework that is certain to propel functional and structural studies that will yield further insights into $p75^{NTR}$ action.

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