The Cytoplasmic and Transmembrane Domains of the p75 and Trk A Receptors Regulate High Affinity Binding to Nerve Growth Factor*

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Ligand-induced receptor oligomerization is an established mechanism for receptor-tyrosine kinase activation. However, numerous receptor-tyrosine kinases are expressed in multicomponent complexes with other receptors that may signal independently or alter the binding characteristics of the receptor-tyrosine kinase. Nerve growth factor (NGF) interacts with two structurally unrelated receptors, the Trk A receptor-tyrosine kinase and p75, a tumor necrosis factor receptor family member. Each receptor binds independently to NGF with predominantly low affinity (K_d = 10^{-9} M), but they produce high affinity binding sites (K_d = 10^{-11} M) upon receptor co-expression. Here we provide evidence that the number of high affinity sites is regulated by the ratio of the two receptors and by specific domains of Trk A and p75. Co-expression of Trk A containing mutant transmembrane or cytoplasmic domains with p75 yielded reduced numbers of high affinity binding sites. Similarly, co-expression of mutant p75 containing altered transmembrane and cytoplasmic domains with Trk A also resulted in predominantly low affinity binding sites. Surprisingly, extracellular domain mutations of p75 that abolished NGF binding still generated high affinity binding with Trk A. These results indicate that the transmembrane and cytoplasmic domains of Trk A and p75 are responsible for high affinity site formation and suggest that p75 alters the conformation of Trk A to generate high affinity NGF binding.

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Growth factor receptor-tyrosine kinases, such as the epidermal growth factor receptor (EGFR), undergo ligand-induced oligomerization, leading to receptor activation. Although homodimerization of receptor-tyrosine kinases is sufficient to initiate transmembrane signaling, an increasing number of receptor-tyrosine kinases have been found to be co-expressed with heterologous co-receptors lacking intrinsic kinase activity. Examples of such heteromeric receptors include the receptors for vascular endothelial growth factor, the flt-1 receptor-tyrosine kinase and neuropilin-1 (1); the receptors for the glial-derived neurotrophic growth factor (GDNF), the ret receptor-tyrosine kinase and GFRα (2); and the receptors for nerve growth factor (NGF), the Trk A receptor-tyrosine kinase and the p75 neurotrophin receptor (3). With the vascular endothelial growth factor and NGF ligands, co-expression of their receptor-tyrosine kinase and heterologous co-receptor generates receptor complexes that exhibit higher affinity binding constants than those exhibited by homodimeric receptor-tyrosine kinase complexes. For GDNF receptor activation, expression of both the ret receptor-tyrosine kinase and GFRα subunit is required for ligand binding because GDNF is unable to activate homodimeric ret receptor complexes (4).

Several models have been proposed to accommodate co-operative roles for these dual receptor systems. A model in which the nonreceptor-tyrosine kinase first binds the ligand, alters the local concentration, and then passes the ligand to the receptor-tyrosine kinase has been proposed. A second, conformational model predicts that co-expression of both receptor-tyrosine kinase and co-receptor alters the conformation of the receptor-tyrosine kinase through allosteric interactions, generating a higher affinity binding site by altering the association or dissociation constants of the ligand with its receptor-tyrosine kinase.

We have utilized the NGF receptors as a model system to clarify a mechanism that generates high affinity site formation. NGF belongs to the neurotrophin family of survival and differentiation factors, which also includes BDNF, NT-3, and NT-4/5. NGF-responsive neurons exhibit two classes of binding sites based upon equilibrium binding reactions (5). The transmembrane proteins responsible for high affinity NGF binding (K_d = 10^{-11} M) are the Trk A receptor and the p75 neurotrophin receptor (which binds all neurotrophins (6)). Kinetic analysis of NGF binding indicates that each receptor binds NGF with a relatively low affinity K_d between 10^{-9} and 10^{-10} M (7). Although p75 displays very rapid rates of association and dissociation with NGF, the Trk A receptor has much slower on- and off-rates. When Trk A receptors are co-expressed with p75, the rate of association is accelerated 25-fold, generating a new kinetic site exhibiting high affinity binding properties (7). Thus, one function of p75 is to increase the binding affinity of NGF. How this is accomplished has not been determined.

Direct interactions between the p75 and Trk A receptors...
have been difficult to document biochemically, although ligand-induced receptor homodimers can be readily detected in affinity cross-linking reactions (8). However, immunoprecipitation experiments using 125I-labeled NGF as a ligand (12). Photobleaching recovery experiments using fluorescently tagged p75 receptors or using monovalent antibody detection of Trk A and p75 have also revealed physical clustering of p75 with Trk A mediated by both intracellular and extracellular domains of p75 and Trk A and augmented by Trk A kinase activity (13). These studies have recently been extended to other Trk family members with the demonstration of a Trk B and p75 interaction by co-immunoprecipitation mediated by intracellular and extracellular domains (14). Furthermore, co-expression of p75 with Trk B was found to modulate the ligand specificity of Trk B (14).

In addition to altering ligand binding, co-expression of p75 with Trk A can also influence Trk A signaling (15–18). The binding of NGF to Trk A receptors activates its cytoplasmic kinase, resulting in the phosphorylation of cytoplasmic tyrosine residues followed by the binding and activation of multiple downstream targets, including Shc, PLCγ, FRS-2, and SH2B (19–21), which mediate effects such as survival and differentiation. The p75 receptor is a member of the tumor necrosis factor family of receptors and contains a putative cytoplasmic death domain (22). Although the p75 receptor can regulate Trk activity, and alter the specificity of Trk receptors for neurotrophin ligands (14, 16, 23), the p75 receptor can also mediate cell death when expressed alone or in combination with other Trk receptors (24–26). Neurotrophin binding to p75 can result in sphingomyelinase activation (27) and recruitment of TRAF-6, a mediator of tumor necrosis factor receptor activation (28), RhoA (29), and NRIF (30). Thus, receptor-mediated signal transduction by the neurotrophins is unique among polypeptide growth factors because two different transmembrane signaling receptors can be activated by a neurotrophin ligand with distinctive biological outcomes. In addition, the ability of each receptor subunit to modulate the signaling cascades initiated by the co-receptor suggests that the receptors directly interact.

One potential mechanism for the p75 receptor’s ability to influence high affinity binding may be to increase the effective concentration of neurotrophin at the cell surface and thus enhance NGF binding to Trk A (16). A similar mechanism involving heparin sulfate facilitation of fibroblast growth factor binding to FGFR2 has been postulated (31), but a more complex role in inducing conformational changes in the FGFR2 complex has become apparent with crystallographic analysis (32). Another mechanism for p75 regulation of high affinity site formation is that the conformation of Trk A may be altered in the presence of p75, and this allosteric regulation facilitates Trk A-ligand binding and subsequent signaling functions (7, 10). To test these hypotheses, we undertook structure/function analysis of the p75 and Trk A receptors to identify the domains of each receptor required for high affinity ligand binding.

MATERIALS AND METHODS

Baculovirus Vectors—The generation of the baculovirus constructs containing cDNAs for wild type and mutant p75 and Trk A have been described (33), as has the generation of chimeric cDNAs encoding Trk A-torso constructs (34). The cDNAs encoding the epidermal growth factor receptor-p75 chimeras (constructs EN10 and EN31) (35) were released by EcoRI digestion from the Bluescript vector and ligated into the Nco to EcoRI site of the baculovirus expression vector using polymerase chain reaction primers.

Mammalian Expression Vectors—The cDNA for the mutant p75 construct (p75–105) (36) was excised from Bluescript using EcoRI digestion and subcloned into the pCMV expression vector. The construction of pCMV5 plasmids encoding the native Trk A and p75 and a Trk A-Trk B chimeric receptor (3.2B) has been described (37). This 3.2B Trk A-Trk B chimera contains the Trk A sequence in the IgG1C1 and C2 domains with the leucine-rich repeats, transmembrane, and intracellular domains of Trk B origin. A second Trk A-Trk B chimeric receptor containing the Trk A IgG1C1 and C2 domains and the Trk A transmembrane domain with Trk B leucine-rich repeats and intracellular domain was constructed by domain swapping using the polymerase chain reaction primers and techniques described in detail (37). The nucleotide sequence of each cloned fragment from Trk A and Trk B was determined using the dideoxynucleotide chain termination method.

Expression in Mammalian Cells—293 cells were cotransfected with the pCMV p75 or pCMV mutant p75 (p75–105) construct and the pMEX construct, which encodes a neomycin resistance gene, using the calcium phosphate precipitation method (37). Following selection in media containing 250 μg/mL of G418, colonies were subcloned and expanded. The level of expression of native or mutant p75 was determined using whole cell lysates in Western blot analysis with the 9992 antisera specific for Trk A and p75 (36). Control cell lines expressing mutant p75 were subsequently transiently transfected with pCMV5 encoding Trk A using the calcium phosphate method and incubation of the cells with DNA for 4 h followed by replacement with fresh media. Twenty eight h after the addition of DNA, cells were harvested in phosphate-buffered saline containing 1 mM EDTA, and snap frozen in liquid nitrogen.

Expression in Sf9 Cells—Sf9 insect cells were maintained in TMN-FH medium from JRH Biosciences supplemented with 9% heat-inactivated fetal bovine serum and 50 μg/mL gentamicin at 28 °C. PC12 nnn5 cells expressing mutant Trk A receptors were cultured as described (33). Human embryonic kidney cell line 293 was maintained as described (35).

Baculovirus Expression in Sf9 Cells—To express a single NGF receptor, recombinant baculovirus was added to Sf9 cells (2–10 6 cells in a 25 cm 2 flask) at a multiplicity of infection of 1 for the p75 virus or 4 for the Trk A virus. For co-expression experiments, baculovirus encoding Trk A and p75 were added to Sf9 cells at a m.o.i. of 16 followed immediately by the addition of virus encoding p75 at a m.o.i. of 1. To vary the ratio of infection, the m.o.i. of virus encoding Trk A was varied from 2 to 40, and the m.o.i. of virus encoding p75 was varied from 1 to 10. After 60 h of infection, cells were pelleted by centrifugation, washed in phosphate-buffered saline, and snap frozen in liquid nitrogen.

Equilibrium Binding Assay Using Radiiodinated NGF—Mouse NGF (Bioproducts for Science, renin free) was radiiodinated using lactoperoxidase and hydrogen peroxide as described (38). The specific activity averaged 3000 dpm/fmol and was used within 2 weeks of radiiodination. Crude membrane preparations from snap frozen cells were prepared as described (7). The equilibrium binding assay conditions utilizing membrane preparations have been described in detail (7, 38), utilizing 125I-NGF concentrations from 0.0005 to 4 nM, and each condition was assayed in triplicate in the presence or absence of excess unlabeled NGF (0.8 μM) (39). The specific counts and background averaged 60–85% of the total counts. In indicated experiments, equilibrium binding studies were performed using the whole cells binding assay (37). Cells were resuspended at 0.75 – 10 6/mL final concentration, and binding to radiiodinated NGF (0.0005 to 4 nM) in the presence or absence of excess unlabeled NGF (0.8 μM) proceeded for 2 h at 0.4 °C. Cell-bound NGF was separated from free NGF by pelleting through calf serum. The Scatchard plot analysis was performed using the LIGAND program as described (7) and additionally analyzed using the PRISM program to perform nonlinear regression and directly compare curve fits to determine whether they were similar with 95% confidence limits.

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The Ratio of Expression of p75 and Trk A Regulates the Percentage of High Affinity Binding Sites—The level of expression of the p75 and Trk A receptors are highly regulated during neuronal development and in response to injury. To determine the optimal levels of expression of each receptor that confers the maximal percentage of high affinity binding sites, binding studies were performed using cells that expressed varying ratios of Trk A:p75. In prior studies using PC12 cells, we had noted that expression of Trk A:p75 at ratios of 1:0.8 yielded a higher percentage of high affinity sites as compared with PC12 cells with a 1:20 ratio of Trk A:p75 (42). Transient expression of Trk A and p75 in Sf9 cells using baculovirus expression vectors allowed the two receptors to be expressed at defined ratios by altering the ratio of infectious virus and generated Sf9 cells infected with virus, cultured for 24 h, and harvested as described under “Materials and Methods.” Cell lysates containing equivalent concentrations of protein were separated by SDS-PAGE, and Western blot analysis was performed using antisera to the intracellular domain of p75 or the C-terminal tail of Trk A as indicated. The positions of the molecular size markers are indicated in kDa. Lanes B–E, cell lysates (10 μg) from Sf9 cells infected with baculoviral vectors encoding Trk A and p75, which were utilized in the corresponding equilibrium binding experiment (lanes B–E) and from Trk A-PC12 cells (50 μg) (lane F) (42) were probed in Western blot analysis using the indicated antisera. b–e, equilibrium binding of 125I-NGF to cell membranes. Membrane preparations were incubated with increasing concentrations of labeled NGF (0.0005–4 nM) in the presence or absence of excess unlabeled ligand. Values represent means of triplicate determinations of specific binding, which averaged 60–85%. Bound counts are represented as femtomoles. The protein content of the membranes added to each condition was 1.0 μg (20:1 and 1:15 ratio) and 1.5 μg (1:5 and 1:0.8 ratio).

Indeed, the percentage of high affinity sites and the $K_d$ of the high affinity site observed in Sf9 cells expressing Trk A:p75 at a 1:0.8 ratio is comparable with that observed using Trk A-overexpressing PC12 cells at a 0.8:1 ratio (7, 42), demonstrating that binding results obtained with whole cells and membrane preparations are consistent and that receptor expression in different cell types yields binding sites with similar affinity (3, 7, 42). Thus, the ratio of expression of Trk A to p75 can significantly alter the number of high affinity sites with a higher percentage of the total receptors exhibiting high affinity binding when the receptors are expressed in near equimolar ratios.

The Transmembrane and Cytoplasmic Domains of Trk A Affect High Affinity Site Formation—The region of the extracellular domain of Trk A that mediates binding to NGF has been mapped to the juxtamembrane IgG C2 domains (40, 41). However, the effects of alterations within the transmembrane and cytoplasmic domains upon NGF binding are unknown. To assess the role of each of these domains, the transmembrane and cytoplasmic domains of the torso receptor kinase were exchanged with the Trk A receptor. Torso is a distantly related receptor-tyrosine kinase to Trk A, and a series of chimeric Trk A-torso receptors were generated (34) to test the contribution of each domain within the context of the full-length receptor-tyrosine kinase (Fig. 2a). These constructs were co-expressed with p75 at a Trk:p75 ratio of 1:1 (Fig. 2b). This ratio of expression results in a 13% high affinity sites formation ($K_d = 1.7 \times 10^{-11}$ M and Tables I and II), expressed as a percentage of total binding, upon co-expression of native Trk and p75 (Fig. 2c). Both the transmembrane and cytoplasmic domains of Trk A appear to contribute to high affinity sites formation when co-expressed with p75 as chimeric proteins with torso sequences substituted for either of these two Trk A domains displayed reduced percentages of high affinity sites to $\sim 4$–6% of the total binding sites (305:p75, 4% binding with $K_d = 1.8 \times$...
10^{-11} \text{ M}; 303:p75, 6\% binding with K_d = 7 \times 10^{-11} \text{ M}(Fig. 2, D and E, and Tables I and II). Chimeric receptors, encoding the Trk A extracellular sequence and the torso transmembrane and cytoplasmic domains similarly yield a reduced percentage of high affinity sites upon co-expression with p75 (325:p75, 4\% binding with K_d = 4 \times 10^{-11} \text{ M}) (Fig. 2 and Tables I and II). Although the generation of chimeric receptors from structurally distinct proteins can alter protein conformation, these results suggest that the cytoplasmic and transmembrane domains of Trk A can influence the ability of the extracellular domain to bind NGF with high affinity at equilibrium.

To more precisely map the domains of Trk A that are necessary to confer high affinity binding when co-expressed with p75, a series of chimeric Trk A-Trk B receptors were utilized. These highly related receptors, which share 70–85\% identity across all domains, nonetheless discriminate between the related neurotrophins NGF and BDNF. Although the IgG C2 domain of Trk A is sufficient to confer low affinity NGF binding, co-expression of a Trk chimera of Trk A IgGC plus Trk A transmembrane domains with native p75 yields high affinity sites (14\% binding with K_d = 2.6 \times 10^{-11} \text{ M}) (Fig. 3c and Tables I and II). These results are comparable with those obtained upon co-expression of Trk A and p75 in 293 cells (Fig. 3e, 12\% binding with K_d = 2.3 \times 10^{-11} \text{ M}). These results suggest that the highly related intracellular domain of Trk B (87\% identical to Trk A) can effectively substitute for the intracellular domain of Trk A in terms of high affinity site formation as the K_d of the high affinity site is similar to that obtained with native Trk A co-expression with p75 (compare with Figs. 1d and 2c). In contrast, co-expression of a Trk chimera of Trk A IgGC plus Trk B transmembrane domain with native p75 yields only intermediate and low affinity binding sites (K_d = 1.8 \times 10^{-10} \text{ M and K_d = 0.9 \times 10^{-9} M, respectively}) (Fig. 3d and Tables I and II). Thus, both the IgGC2 and transmembrane domains of Trk A are required for the generation of high affinity binding sites (Fig. 3, C and D, and Tables I and II).

To further assess the contributions of the cytoplasmic do-

### Table I

Schematic representation of the structural domains of the p75 and Trk A receptor required for high affinity NGF binding

Panel A, the cytoplasmic and transmembrane domains of Trk A contribute to high affinity site generation upon co-expression with native p75. White areas, native Trk A; hatched areas, torso; black areas, Trk B. Panel B, the transmembrane domain but not the extracellular domain of p75 is required for high affinity site generation upon co-expression with Trk A. White areas, p75; hatched areas, EGFR; line, extracellular mutation in p75 that impairs NGF binding. The mutant p75 ‘lack tail’ refers to results obtained with a p75 construct encoding only the membrane proximal eight amino acids of the cytoplasmic tail (38).
main of Trk A, a point mutation, K538A, which renders Trk A kinase inactive (33), was expressed by transfection of PC12 nnr5 cells expressing endogenous p75. In the PC12 nnr5 cells, the ratio of K538A:Trk A:p75 was 1:0.7 (Fig. 4a); however, high affinity binding sites (Kd < 1 x 10^-10 M) were not generated (Fig. 4b and Table I). Thus, not only is the cytoplasmic domain of Trk A required for high affinity site generation, but kinase activation contributes to the formation of this site (Tables I and II).

**Domains of p75 That Contribute to High Affinity Site Formation**

- The extracellular domain of p75 interacts with NGF through the four cysteine-rich sequences, although the most
N-terminal sequence appears dispensable for low affinity binding (36, 43). In prior studies, we have demonstrated that the cytoplasmic domain of p75 is required for high affinity site formation because expression of a truncated p75 encoding only eight amino acids within the cytoplasmic domain in cells that express Trk A results in only low affinity binding sites (38).

To assess whether p75 extracellular sequences contribute to high affinity binding, chimeric p75 receptors were generated, exchanging the extracellular domain of the EGF receptor for the extracellular domains of p75, EGFR-p75(10) (Fig. 5a) (35). This chimeric receptor, which lacks the extracellular domain of p75, does not bind NGF (35). Co-expression of this chimERIC EGFR-p75 with native Trk A in SF9 cells at a ratio of 1:1 (TrkA: chimeric EGFR-p75(10), Fig. 5b) resulted in both high and low affinity sites. Approximately 14% of the sites exhibited a $K_d = 1.9 \times 10^{-11} \text{ M}$ with the majority as low affinity ($K_d = 1.3 \times 10^{-9} \text{ M}$) (Fig. 5c and Tables I and II). These results suggest that the binding of NGF to p75 is dispensable for high affinity site formation. However, both the intracellular and transmembrane domains of p75 promote high affinity site formation. To assess whether the transmembrane domain of p75 contributes to high affinity site formation, a second chimeric EGF receptor-p75 receptor was utilized that contained both extracellular and transmembrane sequences from the EGF receptor and the cytoplasmic domain of p75 (31 construct, Fig. 5a). When this construct EGFR-p75(31) was co-expressed with Trk A at a ratio of 1:1 (TrkA: chimeric EGFR/p75(31), Fig. 5b), no high affinity sites were detectable ($K_d = 1.6 \times 10^{-9} \text{ M}$ and Tables I and II)(Fig. 5d). These studies suggest that upon co-expression with Trk A, the extracellular domain of p75 is dispensable for high affinity site formation, whereas the transmembrane domain of p75 is required.

To confirm that binding of NGF to p75 is not required for high affinity site formation, a mutant p75 receptor that is incapable of binding to NGF because of a five amino acid deletion in the third cysteine repeat was utilized in co-expression studies with Trk A (Fig. 6a) (36). The inability of this mutant p75 to bind NGF was confirmed in cross-linking experi-
iments utilizing radioiodinated NGF. Although a 90-kDa cross-linked product is detectable in PC12 cells bearing native p75 receptors (13 kDa for NGF and 75 kDa for p75), no product is detectable in cells expressing the mutant p75 (50) (Fig. 6c), consistent with earlier studies (36). However, upon co-expression of the mutant p75 (50) with Trk A at ratios comparable with those in Trk-PC-12 cells (or -1:1, Trk:p75, Fig. 6b), both high and low affinity sites for NGF were generated (8% of sites with \( K_d = 2.8 \times 10^{-11} \) M, 92% of sites with \( K_d = 1.4 \times 10^{-9} \) M) (Fig. 6d and Tables I and II). In contrast, cells expressing Trk A alone yielded only low affinity NGF binding sites (\( K_d = 1.5 \times 10^{-9} \) M) (Fig. 6d).

Collectively, these results suggest that the extracellular domain of p75 is not required for high affinity site formation.
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(Tables I and II). Strikingly, these chimeric receptor data support a model in which both the transmembrane and cytoplasmic domains of p75 are required for high affinity binding, most probably through a conformational change in the Trk A receptor.

**DISCUSSION**

NGF-responsive neurons of the peripheral nervous system require a continuous supply of subpicomolar concentrations of this neurotrophin for survival and maintenance, yet during development, they exhibit critical periods in which the sensitivity and requirements for NGF are distinctive from those exhibited by postnatal animals (44). These observations suggest that the ability of neurons to respond to neurotrophins can be exquisitely modulated in part by regulating downstream signaling events but also by the regulation of receptors on their cell surface. Our findings that the number of high affinity sites can be altered simply by changing the ratio of expression of the two NGF receptor subunits indicates that this receptor system imparts a cell with the ability to rapidly modulate cell responsiveness to limiting concentrations of ligand. In this regard, these studies are in agreement with those examining the survival of sensory neurons in animals that are haploinsufficient for p75 (45) in which lower levels of expression of the p75 receptor result in a survival disadvantage in a gene dose-dependent manner. These observations also imply that the massive up-regulation of both Trk A and p75 noted following injury may function not only to increase the total number of binding sites but may also regulate the generation of binding sites with higher affinity, thus augmenting Trk A signaling and NGF internalization in response to stress.

Two hypothetical models of potential p75-Trk A interactions have been proposed to accommodate the cooperative roles of the two receptors (46). The presentation or sequestration model postulates that NGF first binds rapidly to p75, increasing the local concentration of NGF for Trk A. This model does not require a direct interaction of p75 with Trk A receptors and is supported by two classes of experiments: ligand mutagenesis studies in which neurotrophins deficient in p75 binding exhibit Trk activation that is modestly impaired (47) and studies in which ligand blocking antibodies to p75 reduce Trk activation (16). A conformational model invokes an interaction of Trk A with p75 in the absence of ligand. This conformational model is supported by photobleaching recovery experiments, demonstrating preformed complexes of Trk A and p75 that can be detected in naive cells but that will undergo further aggregation in the presence of exogenous ligand. Evidence for this interactive model has also been provided in co-immunoprecipitation studies, documenting p75-Trk A complexes (9, 10) and p75-TrkB complexes (14). In the conformational model, co-expression of both receptors could result in a conformational change in Trk A, leading to an accelerated rate of association and thus, a high affinity binding site. This latter model is consistent with the data presented here. It should be emphasized, however, that neither of these models accurately depicts the exact stoichiometry of p75-Trk A, but both models are consistent with the dimerization of Trk following binding of ligand.

The structural-functional relationship of the domains of Trk A and p75, which are required for high affinity binding, suggest that interactions between the two receptors occur via transmembrane and cytoplasmic domains. These observations are consistent in part with those of Ross and colleagues (34), who have determined that deletions in the cytoplasmic domain of p75 or alterations in the transmembrane or cytoplasmic domain of Trk A result in decreases in the degree of copatching of the receptors when they are co-expressed in SF9 cells. These studies also suggest that Trk A kinase activity promoted Trk A-p75 interactions, which is similar to the requirement for kinase activation in high affinity site formation described here (Fig. 4). However, these investigators have also detected potential interactions of the extracellular domains of the receptors by the copatching technique. In studies examining interactions between Trk B and p75 using co-immunoprecipitation analysis (14), both the intracellular domains and extracellular domains contributed to receptor complex formation. These modest contradictions with the current study may reflect, in part, the differences in techniques used to assess potential receptor interactions. The copatching and photobleaching recovery techniques are extremely sensitive in detecting biophysical interactions in living cells, yet they do not yield any information as to whether these interactions could augment or inhibit ligand recognition and the affinity of ligand binding. Indeed, a small conformational change induced by the interaction of distant structural domains could induce a large effect on the ability of a protein to bind a substrate (in the case of an allosteric enzyme) or a ligand (in the case of neurotrophin receptor). Thus, discrete mutations in the Trk A intracellular domains could result in slight rotational changes of the extracellular domains, particularly in the juxtamembrane extracellular sequences required for NGF binding. The abilities of structural mutations to act at a distance has been well documented in the biology of transmembrane receptors with the cytoplasmic domains of the GPIIIb/IIIA complex exhibiting “inside-out” modulation of ligand binding (50) and the transmembrane domain of the EGF receptor altering the affinity of interaction with EGF (48). It should also be noted that although the extracellular domain of p75 is not required for high affinity site formation, this finding does not exclude potential interactions between the extracellular domains of Trk receptors and p75, which were noted using different assessments of the multimeric receptor complexes (14, 34).

The ability of p75 to distinguish between the transmembrane domains of Trk A and Trk B with regard to high affinity NGF binding site formation suggests that even between highly related receptors, transmembrane domain interactions may be restrictive. In this regard, it is notable that the transmembrane domains of Trk A and Trk B are more divergent (50% similarity) than are the cytoplasmic domains (87% similarity). Indeed, the cytoplasmic domain of Trk B is capable of substituting for the Trk A cytoplasmic domain in high affinity site formation (Fig. 3, C and E). This ability of p75 to interact distinctively with Trk A and Trk B transmembrane domains is supported by the recent study of Vesa et al. (49) in which p75 expression with Trk A augmented Trk A signaling, whereas co-expression of p75 and Trk B had a negative effect on Trk B signaling in response to its cognate ligand BDNF. Furthermore, co-expression of p75 with Trk B restricted Trk B activation to BDNF with reductions in activation noted to NT-3 and NT-4 (14). In contrast, co-expression of p75 with Trk C resulted in a relaxation in the absolute specificity for NT-3 (49). Collectively, these studies suggest that p75, potentially via interactions with Trk transmembrane domains, can induce distinct alterations in Trk receptor conformation. In the case of Trk A, NGF binding affinity is enhanced; with Trk B, binding is more restrictive for BDNF, whereas with Trk C, the specificity of binding solely to NT-3 is relaxed. These results suggest that during pathological conditions that up-regulate p75 in neurons that co-express multiple Trk receptors, increased p75 expression could enhance NGF-dependent functions, inhibit BDNF responsivity, and broaden the ligand specificity of Trk C.

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