Cleavage of p75 Neurotrophin Receptor by α-Secretase and γ-Secretase Requires Specific Receptor Domains*§

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The p75 neurotrophin receptor (p75NTR), a member of the tumor necrosis factor superfamily of receptors, undergoes multiple proteolytic cleavage events. These events are initiated by an α-secretase-mediated release of the extracellular domain followed by a γ-secretase-mediated intramembrane cleavage. However, the specific determinants of p75NTR cleavage events are unknown. Many other substrates of γ-secretase cleavage have been identified, including Notch, amyloid precursor protein, and ErbB4, indicating there is broad substrate recognition by γ-secretase. Using a series of deletion mutants and chimeric receptors of p75NTR and the related Fas receptor, we have identified domains that are essential for p75NTR proteolysis. The initial α-secretase cleavage was extracellular to the transmembrane domain. Unfortunately, deletion mutants were not capable of defining the requirements of ectodomain shedding. Although this cleavage is promiscuous with respect to amino acid sequence, its position with respect to the transmembrane domain is invariant. The generation of chimeric receptors exchanging different domains of noncleavable Fas receptor with p75NTR, however, revealed that a discrete domain above the membrane is sufficient for efficient cleavage of p75NTR. Mass spectrometric analysis confirmed the cleavage can occur with a truncated p75NTR displaying only 15 extracellular amino acids in the stalk region.

The p75 neurotrophin receptor, p75NTR, is a member of the TNF receptor superfamily that includes the Fas (CD95) antigen, CD30, and CD40 (1). This family of receptors is distinguished with multiple cysteine-rich domains for ligand binding, a single transmembrane sequence, and a noncatalytic cytoplasmic domain (2). X-ray crystallographic analyses show a great deal of similarity between the cysteine-rich repeats of p75NTR and the p55 TNF receptor (3, 4). The intracellular region of several receptors, including the p55 TNF receptor, Fas receptor (FasR), and p75NTR, contains a death domain sequence (5). The death domain serves as a protein-protein docking site and is required for initiating TNF- and Fas-dependent cytotoxicity. Activation of TNF receptor members leads to the recruitment of proteins including TNF-associated factors, death domain-containing proteins TRADD (6), FADD (7), and RIP (8).

p75NTR is recognized by all the neurotrophins (nerve growth factor, brain-derived nerve factor (BDNF), NT-3, and NT-4) that promote differentiation, growth, and survival of diverse cell types in the nervous system (9, 10). In addition, neurotrophins also initiate signaling through tropomyosin-related kinase tyrosine kinase receptors, which are capable of forming high affinity binding sites with p75NTR that potentiate responses to low concentrations of neurotrophins (11). In the absence of tropomyosin-related kinase receptors, p75NTR is capable of independent signaling that activates NF-κB or c-Jun N-terminal kinase activity (12, 13). In selected cell types, p75NTR can initiate cell death (14–17). Alternatively, p75NTR can serve as a co-receptor with Nogo receptor in blocking regeneration in the central nervous system (18–20).

Intramembrane cleavage events have been described recently for p75NTR (21, 22). Proteolysis through regulated intramembrane proteolysis has emerged as a highly conserved mechanism in receptor signaling (23–25). Presenilin-dependent γ-secretase activity is responsible for the intramembrane proteolysis of an increasing number of membrane proteins, including Notch, ErbB4 tyrosine kinase receptors, CD44, low density lipoprotein, and β-amyloid precursor protein (24). γ-Secretase is a large protein complex with an unusual aspartyl protease activity that cleaves substrates within the transmembrane domain and requires presenilins and other components, such as nicastrin, Pen-2, and Aph-1 (26).

Prior to γ-secretase cleavage, p75NTR undergoes extracellular cleavage events that release the ectodomain of the receptor. Previous studies indicated that the γ-secretase cleavage occurs in the middle of the transmembrane domains (22). However, the requirements for the initial α-secretase events remain unknown. In this study we defined the specific domains in p75NTR that are susceptible to α-secretase and subsequent γ-secretase cleavage. Analyses of a series of chimeric receptors identified a 15-amino acid region of p75NTR that is sufficient for inducing α-secretase cleavage. We also found that the initial α-secretase cleavage is required for the subsequent γ-secretase cleavage. Therefore, susceptibility to proteolytic cleavage is dependent upon specific regions and structural features of p75NTR.

EXPERIMENTAL PROCEDURES

Materials—Phorbol-12 myristate 13-acetate (PMA) was purchased from Sigma. TAPI-1, GM 6001, clasto-lactacystin β-lactone (27), and compound E (γ-secretase inhibitor XVIII) were purchased from Calbiochem.
Plasmids—Deletion constructs Δ204–209, Δ210–215, and Δ216–221 in p75NTR were produced by site-directed mutagenesis using rat pCDNA3-p75NTR as a template. Deletion constructs of human pRES-p75NTR (P2 chimeric receptors of p75NTR and the Fas receptor called B9 and A7) were from A. Le Bivic (Faculte des Sciences de Luminy, Marseille, France). The construction of p75NTR, Δ14 was described previously (22). The deletion construct HA p75NTR, Δ15 lacking the entire extracellular domain except 15 amino acids proximal to the membrane was generated using a PCR strategy. Rat pCDNA3-p75NTR was used as a template, and the insert was cloned into pCDNA3.1 using XbaI and KpnI sites. The construction of the P1 and P2 chimeric receptors of p75NTR and the Fas receptor (called B9 and A7) was described previously (28). Additional chimeras in this study were produced using a similar hybrid PCR-based strategy and appropriate pairs of primers. All of the constructs were verified by automated DNA sequencing.

Cell Culture Treatments and Transfection—PC12 cells were maintained in Dulbecco’s modified Eagle’s medium containing 5% fetal bovine serum and 10% horse serum supplemented with 2 μg/ml gentamicin. HEK293 cells were maintained in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum. For transient transfection, Lipofectamine 2000 (Invitrogen) reagents were used according to the manufacturer’s protocol. In cleavage experiments cells were treated for 45 min with 100 ng/ml PMA and overnight with 1 μM compound E, 20 μM TAPI-1, 10 μM GM 6001, and 5 μM clasto-lactacystin-β-lactone. Cells were harvested 48 h after transfection, washed once with cold phosphate-buffered saline on ice, and lysed in radioimmune precipitation assay III buffer (10 mM Tris, pH 8, 1 mM EDTA, 150 mM NaCl, 1% Nonidet P-40, 0.1% SDS, 0.1% deoxycholate, 2 μg/ml aprotinin, 1 μg/ml leupeptin, and 25 μg/ml phenylmethylsulfonyl fluoride). Protein concentrations were determined by Bradford assay.

Schwann Cell Culture—Schwann cells were isolated from postnatal day 3 Sprague-Dawley rat pups (29). Cells were maintained in the logarithmic phase of growth in Dulbecco’s modified Eagle’s medium supplemented with 10% heat-inactivated fetal calf serum (Invitrogen), 2% glutamine, and 0.1% penicillin-streptomycin, and 2 μM forskolin (Sigma).

Dorsal Root Ganglion Neuronal Cultures—Dorsal root ganglion neuron cultures were prepared as described previously (30). Neurons were cultured on ammonium-collagen-coated 60-mm tissue culture dishes, using 30–40 ganglia per dish, in the presence of “C” medium (Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum, 50 ng/ml nerve growth factor (Harlan Bioproducts for Science), 2 mM glutamine, and 0.1% non-essential amino acids). Neuronal cultures were maintained in primary rat Schwann cells using pharmacological inhibitors and activators. Primary Schwann cells were treated overnight with compound E (Cpd.E) (1 μM) or clasto-lactacystin-β-lactone (5 μM) or TAPI-1 (20 μM) and 45 min with PMA (100 ng/ml), lysed, and analyzed by Western blot using 9992 antisera against the ICD of p75. B, analysis of p75NTR proteolytic processing in freshly dissociated dorsal root ganglion (DRG) neuron cultures and PC12 cells. Cells were treated for 45 min with PMA (100 ng/ml) or overnight with compound E (1 μM), lysed, and analyzed by Western blot using 9992 antisera against the ICD of p75NTR.

Purification of HA-tagged p75 Extracellular Domain—HEK293 cells were transiently transfected with HA-p75NTR-Δ15 using Lipofectamine 2000 (Invitrogen) and incubated in serum-free Dulbecco’s modified Eagle’s medium. On day 2, the medium was harvested, clarified by centrifugation, and incubated overnight at 4 °C with 1 ml of 1% triton (settled resin volume) of anti-HA affinity matrix (Roche Applied Science). The anti-HA affinity matrix was collected by brief centrifugation and washed with 20 ml of washing buffer (20 mM Tris, pH 7.5, 0.1 mM NaCl, 0.1 mM EDTA, and 0.05% Tween 20). The bound protein was eluted with 0.1 M glycine, pH 2.0. The affinity purified proteins were analyzed by the dot-blot method using monoclonal HA.11 (Covance). The fraction positive for HA immunoactivity was analyzed by MALDI Q-TOF mass spectrometry.

MALDI Q-TOF Mass Spectrometry—The concentrated affinity-puriﬁed supernatant fraction (20 μl) was dried under vacuum and redissolved in 5 μl of 0.1% trifluoroacetic acid in 2% acetonitrile. The reconstituted solution was desalted using a U-C18 reverse-phase ZipTip column (Millipore). The eluate (50% acetonitrile in 0.1% trifluoroacetic acid) was dried and redissolved in 2.5 μl of 2,6-dihydroxyacetophenone MALDI matrix solution. The 2,6-dihydroxyacetophenone matrix solution (50 μM) was prepared by dissolving 15.2 mg of 2,6-dihydroxyacetophenone in 1 ml of water/methanol (10:90, v/v) followed by the addition of 100 mM dimannoside citrate in water at a ratio of 1:1 (v/v). The sample matrix mixture (1.2 μl) was spotted onto the MALDI sample stage and air-dried. Positive ion MALDI Q-TOF mass spectra were acquired with a Micromass Q-TOF Ultima MALDI mass spectrometer (Waters). The instrument was operated in V mode with a mass resolution of ~10,000, which enabled the mass accuracy of 5~10 ppm after internal calibration. Mass spectra were acquired and processed using Masslynx 4.0 software (Micromass) with standard instrument settings. A total of 260 laser shots were averaged per mass spectrum, the background subtracted, and the spectrum smoothed using mass windows appropriate for the significant peak widths.

RESULTS

It has been known for many years that p75NTR undergoes ectodomain shedding (31, 32). Ectodomain cleavage of full-length p75NTR releases the extracellular domain from an ~28-kDa membrane-bound C-terminal fragment (CTF). The CTF is subsequently cleaved by γ-secretase to give rise to the soluble p75NTR intracellular domain, p75-ICD (21, 22).

The cleavage of p75NTR has been followed primarily in trans-
To examine whether p75NTR is cleaved endogenously, we tested primary Schwann cells isolated from rat sciatic nerve. The mature 75-kDa receptor is abundantly expressed in Schwann cells (33, 34). Western blotting with an antibody directed against the cytoplasmic domain of p75NTR revealed an immature underglycosylated form at 45 kDa and fragments at 24 kDa consistent with the p75NTR CTF. A protein at 19 kDa consistent with a γ-secretase cleavage event (p75-ICD) was also observed (Fig. 1A).

We examined whether these fragments were sensitive to inhibitors of γ-secretase. Synthetic γ-secretase inhibitors can block the intramembrane cleavage and cause an accumulation of the precursor domain (CTF). In contrast, the mature domain (ICD) is not detected in the absence of γ-secretase activity (35–37).

**Fig. 2. Ectodomain shedding is a necessary step for the γ-secretase cleavage of p75NTR.** A, the p75NTR CTF and ICD are the precursor and product of a proteolytic reaction catalyzed by a γ-secretase-like activity. HEK293 cells were transfected with N-terminal HA-tagged p75NTR expression vector. After transfection, the cells were incubated overnight with compound E (Cpd.E) or 45 min with PMA (100 ng/ml). Lysates were analyzed by Western blot using 9992 antisera against the ICD of p75NTR. B, the CTFs generated by proteolysis are specifically detected with the antibody directed against the ICD of p75NTR but not with an N-terminal HA-directed antibody. HEK293 cells were transfected with an N-terminal HA-tagged p75NTR expression vector and analyzed by Western blot using 9992 antisera (WB: 9992) against the ICD of p75NTR or with anti-HA antibody (WB: HA). C, effect of matrix metalloproteinases blockade on γ-secretase cleavage event. HEK293 cells were transfected with p75NTR expression vector. After transfection, the cells were incubated 45 min with PMA (100 ng/ml) or overnight with GM 6001 (10 μM), or pretreated overnight with GM 6001 (10 μM) and then stimulated 45 min with PMA (100 ng/ml). Lysates were analyzed by Western blot using 9992 antisera against the ICD of p75NTR.

**Fig. 3. Deletion analysis of the stalk domain of p75NTR.** A, small deletions of the stalk domain in the proximity of the transmembrane domain do not abolish ectodomain shedding. HEK293 cells were transfected with nonoverlapping deletions of three sets of six amino acids from the stalk domain of p75NTR (Δ204–209, Δ210–215, and Δ216–221). Transfected cells were incubated with compound E (Cpd.E) (1 μM) overnight, and lysates were analyzed by Western blot using 9992 antisera against the ICD of p75NTR. B, large deletions of the stalk domain do not abolish ectodomain shedding. HEK293 cells were transfected with two broad deletions of the p75NTR stalk domain (Δ168–218 and Δ187–218). After transfection the cells were incubated overnight with compound E (1 μM). Lysates were analyzed by Western blot using 9992 antisera against the ICD of p75NTR. C, stalk and transmembrane domains protein sequence alignment of p75NTR deletion constructs.
p75 Receptor Cleavage

To test the generality of this cleavage event, PC12 cells and dorsal root ganglion neurons were tested for proteolytic processing of p75NTR. A similar set of p75NTR fragments was detected in primary dorsal root ganglion neurons and in PC12 cells (Fig. 1B). Treatment with PMA and compound E yielded the same pattern of production of the CTF and ICD fragments (Fig. 1), indicating that cleavage of p75NTR occurs in both glial and neuronal cell backgrounds.

Defining the α-Secretase Cleavage Event—To define and map the cleavage sites further, we expressed an N-terminal HA-tagged p75NTR cDNA in HEK293 cells. After treatment with PMA and compound E, p75NTR proteolytic products were observed at the expected sizes (Fig. 2A). As expected, the fragments were specifically detected with the antibody directed against the C terminus of the receptor (Fig. 2B, 9992) but not with the N-terminal HA tag (Fig. 2B). The increase in CTFs and ICD fragments obtained in the presence of PMA could be blocked by pretreatment with GM 6001, a broad range matrix metalloproteinase inhibitor (38), confirming that the removal of the extracellular domain is important for intramembrane proteolysis by α-secretase (Fig. 2C).

To identify the α-secretase cleavage site, we produced a series of deletions of the stalk domain of p75NTR that extended from the transmembrane domain to the cysteine-rich repeats of the extracellular domain. This region consists of 62 amino acids and is particularly rich in serine and threonine residues. Glycosylation of p75NTR on O-linked sugars occurs in this domain (39). Together with N-linked glycosylation at the N-terminal region, these posttranslational modifications account for almost half of the apparent molecular weight of the receptor. We focused on the stalk domain for a number of reasons. First, according to the apparent mass of the CTF, the cleavage is predicted to occur in the proximity of the transmembrane domain. Second, the stalk domain proximal to the transmembrane domain is very highly conserved (>89%). Also, proteolytic cleavage events by metalloproteinases have been identified in the stalk domain of other receptors. We produced three nonoverlapping deletions of six amino acids (∆204–209, ∆210–215, and ∆216–221) that spanned this conserved region. We also tested two larger deletions (∆168–218 and ∆187–218). All the mutants expressed in HEK293 cells produced the CTFs, as assessed by a specific antibody against p75NTR (Fig. 3).

Surprisingly, even a deletion that removes most of the stalk domain except for 12 amino acids (∆168–218) was processed. It is important to note that the CTFs generated by the deletion...
constructs all migrated with the same mobility, suggesting that the α-cleavage site is located below the most C-terminal deletion (Δ216–221). Another study found that the initial sequence of the transmembrane domain (NLIPV) is also not needed for α-secretase cleavage (40). Alternatively, the cleavage site or the recognition site for α-secretase may not entirely be determined by sequence but by a location relative to a distance from the membrane. The enzyme-substrate recognition site may be located distal to the cleavage site. This mechanism is plausible, because it has been suggested for several transmembrane proteins undergoing extracellular domain shedding by matrix metalloproteinases, such as amyloid precursor protein and L-selectin (41, 42).

**Mass Spectrometry Analysis**—To identify the α-cleavage site by an independent approach, we purified the truncated fragment released in the cell medium. Because of extensive glycosylation of p75NTR in the extracellular domain, it was not feasible to use mass spectrometry to identify the glycosylated product of the full-length receptor released in the medium. We therefore generated a shortened p75NTR construct (Δ15) that lacked all four cysteine-rich repeats and a large segment of the juxtamembrane stalk. A HA epitope tag was placed at the N terminus of Δ15. Expression of the Δ15 p75NTR construct in HEK293 cells produced a full-length fragment and smaller fragments consistent with the size of the CTF and ICD, as assessed by anti-p75 antibody (Fig. 4A).

Conditioned media from cells transiently expressing Δ15 were collected and purified using an HA affinity resin. The fractions were analyzed by dot blot and by MALDI Q-TOF mass spectrometry. The MALDI Q-TOF mass spectrometry analysis detected two different peptide ions with monoisotopic masses (M + H⁺) of 1988.9 and 1689.7 corresponding to the peptide sequences of HA-MGSSQPVVT and HA-MGSSQP, respectively (Fig. 4B). The inset to Fig. 4B shows the sequence of peptide g m/z 1988.9. The peptides were also sequenced with collision-induced dissociation (tandem mass spectrometry), which confirmed the results obtained by MALDI Q-TOF mass spectrometry.

Proteolytic processing was previously observed in two deletion mutants (Δ210–215 and Δ216–221) that lacked sequences in the stalk domain (Fig. 3A). The cleavage sites that were utilized in the two deletions possess sequences that are different from the Δ15 cleavage site determined by mass spectrometry. A recent investigation of the matrix metalloproteinase TNF-α-converting enzyme cleavage of p75NTR implied that one potential cleavage occurred after the amino acid sequence MGSSQP (40), which is consistent with our mass spectrometry analysis. An alternative explanation is that the cleavage site is not determined by a specific sequence motif but occurs at an invariant location relative to the membrane. In this case, specificity of cleavage may be determined by sequences located at some distance away from the cleavage site.

**Chimeric Receptor Analysis**—To assess which domains are most responsible for the initial cleavage of p75NTR, we generated a series of chimera receptors between p75NTR and the FasR, a related member of the TNF receptor superfamily. The FasR is also a type I transmembrane receptor containing canonical cysteine-rich domains followed by a short stalk region and a single membrane-spanning domain. In addition, the FasR contains a death domain in the cytoplasmic region.

We first tested whether FasR is proteolytically processed in a fashion similar to p75NTR processing. The FasR was expressed in HEK293 cells and lysates probed with an antibody
directed against the intracellular domain of FasR (Fig. 5A). The full-length Fas receptor (45 kDa) could be detected; however, no other fragments were detected after PMA or compound E treatment. These results imply that FasR is not an efficient substrate for \( \alpha \)/H9251-secretase and \( \gamma \)/H9253-secretase proteolytic processing.

Chimeric receptors were therefore generated between the extracellular portion of the FasR and the intracellular portion of p75NTR. The initial FasR chimeric receptors contained the p75NTR transmembrane and cytoplasmic domains (designated P1) or only the cytoplasmic domain of p75NTR (P2). These two chimeric receptors were transiently transfected in HEK293 cells along with wild-type p75NTR. Both chimeric receptors were expressed appropriately but did not undergo processing as wild-type p75NTR (Fig. 5B). The lack of ICD production by these chimeras (P1 and P2) was not due to enhanced degradation, because treatment with lactacystin did not result in the appearance of detectable fragments (data not shown).

To determine whether the extracellular and transmembrane domains of p75NTR were directly involved in \( \alpha \)/H9251-secretase cleavage, each domain was introduced into the corresponding location in the FasR. Expression of a Fas chimera containing the p75NTR extracellular domain (F1) yielded a proteolytic product of \( \sim 20 \) kDa, the expected size of a CTF produced from FasR (Fig. 6A). The FasR\( _{TM-ICD} \) (where TM = transmembrane) calculated molecular mass is 18.5 kDa. When a Fas chimeric receptor carrying both the extracellular and transmembrane domains of p75NTR (F2) was expressed, a second product corresponding to \( \gamma \)-secretase cleavage was also detected, and its production was specifically inhibited by compound E (Fig. 6B). These data strongly suggest that the extracellular and the transmembrane domains of p75NTR are necessary and sufficient for promoting \( \alpha \)- and \( \gamma \)-secretase processing of the receptor.

To define further the domain of p75NTR that is susceptible to \( \alpha \)- and \( \gamma \)-secretase cleavages, we generated a chimeric receptor in which the transmembrane domain of the FasR was introduced into p75NTR (P3). We observed a constitutively high level of CTFs produced from the P3 receptor (Fig. 7A). The level of CTFs was increased by PMA treatment but not by compound E. The absence of an ICD fragment from the P3 chimeric receptor is consistent with the role of the p75NTR transmembrane domain in processing the CTFs by \( \gamma \)-secretase. A greater accumulation of CTFs was therefore observed. A converse construct in which the p75NTR transmembrane domain was introduced into FasR (F3) produced no cleavage products, indicating that the p75 transmembrane sequence was not sufficient to trigger \( \alpha \)-secretase cleavage (Fig. 7B).

Finally, we exchanged the stalk domains between the two receptors. p75NTR carrying the stalk domain of FasR was no longer processed and displayed insensitivity to PMA and compound E treatments (Fig. 8A). On the other hand, when the stalk domain of p75NTR was tested in the FasR (F4), the CTF was produced. The CTF was also observed in other chimeras expressing the p75NTR extracellular domain (Fig. 6). To narrow down the sequence required for cleavage, we introduced the 15 amino acids of p75NTR proximal to the transmembrane region.
to the corresponding region of the FasR (F5). The CTF was observed in the F5 chimeric receptor (Fig. 8C). The production of the p75NTR CTF was inhibited by GM 6001 (Fig. 8C), indicating that a metalloproteinase-like activity catalyzed this reaction.

Taken together, the analysis of the chimeric receptors together with the mass spectrometric analysis strongly suggests that the 15 amino acids of the stalk domain of p75NTR are necessary and sufficient for inducing the extracellular shedding of the receptor. Furthermore, this event is required for further processing of p75NTR by γ-secretase.

**DISCUSSION**

Regulated intramembrane proteolysis is a conserved mechanism that has been proposed to regulate intracellular signaling events. As numerous transmembrane protein substrates for γ-secretase cleavage have been identified, an understanding of the mechanisms by which intramembrane proteases selectively recognize and cleave proteins is increasingly important. Previous studies indicate that ectodomain shedding is responsible for presenilin-dependent transmembrane cleavage (43). However, the substrate requirements for cleaving diverse membrane spanning proteins are not completely defined.

The results from this study indicate that p75NTR molecules lacking the bulk of the extracellular domain serve as efficient substrates for γ-secretase action. It is worth noting that NRH2, a p75 homolog that lacks the cysteine-rich domains, is also efficiently cleaved by α-secretase activity (21). The extracellular domain of NRH2 is short and does not have homology with p75NTR. Yet, NRH2 is a target of not only α-secretase but also γ-secretase activities. It is striking that the action of α-secretase does not appear to depend strictly upon a specific amino acid motif in these molecules. Deletion mutants in p75NTR indicate that α-secretase is effective in cleaving the receptor when large portions of the stalk domain are missing (40). We have confirmed these results here with deletions in sequences above the transmembrane domain. Remarkably, we found that the sizes of the CTFs and the ICD fragments produced by each deletion mutant are nearly identical, indicating that the α-secretase is recognizing a structural feature of the receptor for its proteolytic activity.

However, the structural requirements for α-secretase and γ-secretase cleavages display some degree of sequence specificity. Other related TNF receptor members, such as the Fas receptor, do not undergo cleavage like p75NTR, despite the overall similarity in structural features between the two receptors. Analysis of FasR/p75NTR chimeric receptors clearly indicated that the stalk and transmembrane domains of the two receptors behave very differently in α-secretase and γ-secretase cleavage. A 15-amino acid sequence in the stalk domain of p75NTR was sufficient to confer ectodomain shedding of the FasR. Likewise, the transmembrane sequence of p75NTR behaved as a preferred substrate for γ-secretase action over the transmembrane of FasR. Conversely, the FasR stalk and transmembrane sequences make p75NTR resistant to cleavage. Treatment of cleavage-resistant chimeric receptors with lactacystin indicated that degradation did not account for the lack of cleaved fragments (supplemental data). Because p75NTR and FasR share a similar overall structure, these chimeric receptor
experiments indicate there are also sequence requirements for efficient cleavage.

Cleavage of p75NTR by regulated intramembrane proteolysis is of particular importance for a number of reasons. First, each product of the cleavage may serve multiple functions. The release of the extracellular domain generates a binding protein for many potential ligands, including neurotrophins, pro-neurotrophin precursors, β-amyloid, and the rabies virus glycoprotein (13, 44). Second, the ICD domain of p75NTR has the potential of binding many intracellular proteins, including TNF-associated factor 6, SC-1, NADE, NRAGE, and RhoA (12, 45). The p75NTR cytoplasmic domain may bring these proteins to function in different cellular compartments. The cleavage of the CTF also gives rise to a small peptide the significance of which is unknown but that is analogous to the Aβ peptides generated from amyloid precursor protein. Another compelling reason that p75NTR cleavage is important is that many cell types up-regulate p75 receptors under pathological or inflammatory conditions. For example, after seizures, there is prominent expression of p75NTR in cortical neurons (46). Induction of p75NTR has been observed in many cell types, including oligodendrocytes, Schwann cells, microglia, macrophages, and smooth muscle cells (10, 45). The early induction and cleavage of p75NTR in selective neurons that are destined to undergo intramembraneous γ-secretase cleavage of amyloid precursor protein (24) suggests that p75NTR cleavage may be linked to other events occurring during neurodegeneration.

There is currently little evidence that neurotrophin ligands of p75NTR have a major impact upon the cleavage or upon the regulation of γ-secretase activities. In addition, the precise proteases responsible for the α-secretase-mediated cleavage of p75NTR have not been firmly established, although members of the ADAM (a disintegrin and metalloprotease) family are likely candidates (40, 47). The growing number of substrates undergoing intramembraneous cleavage raises the issue of how particular target sequences are recognized by γ-secretase. Blocking the shedding and the intramembraneous cleavage events of p75NTR using receptor sequences resistant to proteolysis will represent an important approach to defining the physiological function of these processing events.

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2 T. W. Kim, personal communication.

3 N. Zampieri, unpublished results.
