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Signaling of Neuronal Cell Death by the p75NTR Neurotrophin Receptor

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Abstract

The neurotrophin receptor (p75NTR) is best known for mediating tropic support by participating in the formation of high-affinity nerve growth factor (NGF) receptor complexes with *trk*A, however, p75NTR more recently has been shown to act as a bona fide death-signaling receptor, which can signal independently of *trk*A. This article discusses the evidence for an active role of p75NTR in neuronal cell death and the mechanisms controlling this process, including roles for Bcl-2 family members, the c-*jun* stress kinase JNK, the transcription factor nuclear factor kappa B (NFκB), and caspases.

Index Entries: p75NTR; apoptosis; cell death; signaling; Bc1-2 family; nerve growth factor; neurotrophin; neuronal survival; knockout; caspase.

Dual Role for p75NTR

The trophic actions of nerve growth factor (NGF) in developing neurons have been extensively studied and essentially involve NGF dimers binding to a high-affinity receptor complex able to generate a signal-transduction phosphorylation cascade. The cytoplasmic domain of *trk* can autophosphorylate, initiating a signal cascade, resulting in cell survival, growth-promoting responses, and neuronal plasticity *(1–4)*. This process involves p75 neurotrophin receptors (p75NTR) that colocalize and heterodimerize with *trk*A receptors via the extracellular domains *(3,5)*. Formation of these heterodimer complexes produces high-affinity receptors for NGF, whereas both *trk*A and p75NTR independently ligate NGF at a lower affinity *(6)*. Because the affinity of p75NTR for each of the neurotrophins is equivalent, the specificity of the high-affinity receptor is determined by the *trk*-component *(7)*.

Although the requirement for p75NTR in the formation of high-affinity neurotrophin receptor complex survival signaling is well characterized, its importance in vivo in promoting

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survival is ambiguous given the increasing evidence that p75NTR is involved in neuronal cell death. This ambiguity is born out in the phenotype of mice deficient for functional p75NTR *(see* Table 1*)*.

Apoptotic Transducer—p75NTR

The suggestion that p75NTR acts to promote cell death arose from two observations: the in vitro overexpression of p75NTR led to increased cell mortality *(8)* and decreased expression of p75NTR (achieved with the use of antisense oligonucleotides) resulted in increased survival of early-postnatal neurons in vitro after withdrawal of NGF *(9)*. Although these experiments suggested a role for p75NTR in promoting neuronal death, the evidence that p75NTR may function in a similar way in vivo has only recently been obtained.

The overexpression of the transmembrane and cytoplasmic domain of p75NTR in neurons in transgenic mice caused a significant increase in neuronal cell death during early postnatal life both in those neurons that normally express p75NTR and in those that do not *(10)*. Mice depleted for functional p75NTR by gene "knockout" display a phenotype that reflects a function for p75NTR both in trophic support and neuronal death. These mice not only have reduced numbers of dorsal root ganglia (DRG) neurons owing to inability to form high-affinity NGF receptors *(11,12)*, but also increased numbers of sympathetic and basal forebrain neurons, explained by absent or delayed naturally occurring cell death, as shown in vitro, during early postnatal development *(13–15)*. Similarly, we find that cultured postnatal DRG sensory neurons are more resistant to death after NGF withdrawal compared to DRG neurons from control mice *(16)*.

Axotomy of sensory and motor neurons results in upregulation of p75NTR, loss of acetylcholine esterase immunoreactivity, and apoptosis *(17,18)*. This cell death can be extensively halted by the application of p75NTR antisense oligonucleotides to the axotomized neurons *(19)*. In mice depleted for functional p75NTR, facial motor neurons of adult mice were less susceptible to cell death after axotomy *(20)*, but this does not appear so in the neonatal DRG *(21)*. Transgenic overexpression of p75NTR also results in an increase in the number of apoptotic neurons (above that normally observed) after axotomy *(10)*. Together these in vivo data argue for the extent of neuronal death to be dependent on the level of p75NTR expression.

During development, p75NTR is widely expressed in the central and peripheral nervous systems, including in spatial and temporal association with cells undergoing apoptosis. In the rodent, p75NTR is highly expressed in early embryonic central and peripheral nervous system during neuronal development at a time when trophic factors are required for survival *(22,23)*, and subsequently while neurons are extending neuritic processes and as a part of the fine-tuning of the neural network *(14,24)*. During postnatal development, p75NTR expression is down regulated in most parts of the central nervous system, but is rapidly induced following trauma, such as ischemia and nerve lesion *(17,25,26)*. Intriguingly, in the aged rat brain and Alzheimer's patients, high levels of p75NTR are observed in basal forebrain and hippocampus, where there is extensive death of neurons involved in learning and memory *(24, 27)*. Furthermore, these cells are larger and have higher choline acetyltransferase activity in mice depleted for p75NTR *(15)*. These data further implicate p75NTR in, not only naturally occurring cell death, but also cell death resulting from damage and disease.

The best in vivo demonstration of p75NTR-mediated neuronal death during development comes from the embryonic E4 chick retina where ganglion cells express p75NTR, but not *trk*A. Application of antibodies that block NGF binding to p75NTR inhibited death of retinal ganglia cells *(28)*, and separation of the retinal cells from microglia (a source of endogenous NGF) in explant culture also reduces cell death *(29)*, indicating that activation of p75NTR by

Table 1
Phenotypes of Mice Deficient for the Bcl-2 Family and p75NTR

	Bcl-2$^{-/-}$	Bcl-x$^{-/-}$	Bax$^{-/-}$	p75$^{-/-}$
Life-span	2–24 wk Most die at 7–8 wk	E13	Normal	Normal
Major phenotype	Poly cystic kidney Hypopigmented hair Lymphoid apoptosis	CNS apoptosis	Hyperplasia of non-neural lineages Lymphocytes, ova/testes	Loss of innovation of sensory and nociceptive neurons
Number of CNS neurons	Facial: P9 71% P44 67%	Reduced	51% Increase 13% in Heterozygotes	Increase in basal forebrain neurons
Number of DRG neurons	P9 90% P44 56%	Reduced		Lower—but those that survive are more resistant to death in vitro
Number of SCG neurons	P10 60% P44 58%	Reduced	100% Increase	24% Increase between PO-P23 more resistant to death in vitro
Axotomy effects Facial MN	No difference to wt (10% survival)		100% Survival	Increased survival
Other	Normal number of neurons PO, which respond normally to growth factors	Apoptosis of liver. Reduced T- and B-cells spleen, and so forth	Survive GF w/d in vitro cells do not die, but appear atrophic —recover with NGF treatment	Required in early embryonic development to form high-affinity NGF receptor with *trk*A, therefore, loss of DRG around E15
Normal expression pattern	Expressed in most CNS and PNS from E13 to birth, down-regulated by adult except in PNS, present in glial cells	Adult: sympa thetic ganglia, DRG, and large neurons of the brain-stem and motor cortex	Adult: sympathetic ganglia, DRG, and large neurons of the brainstem and motor cortex, absent from glial cells	Expressed in most neurons through development, but is downregulated after birth in CNS, remains high in DRG
Overexpression (transgenic)	Prevents death induced by growth factor withdrawal or axotomy, high levels result in increased cell death	Prevents majority of cell death after facial axotomy 65% compared to 15% survival in wild-type	No increase in death after axotomy or growth factor withdrawal, no change in numbers of neurons	Increased death after axotomy

NGF was required to initiate cell death. Others have also shown that activation of p75NTR signaling pathway requires ligand binding. Oligodendrocytes, which are cultured to express high levels of p75NTR, but not *trk*A, can be killed by the addition of high levels of NGF *(30)*. Also, brain-derived neurotrophic factor (BDNF) ligation to p75NTR in sympathetic neurons (not expressing *trk*B) also results in cell death *(13)*. In addition, NGF (but not BDNF) can induce apoptosis of embryonic trigeminal mesencephalic neurons cultured in ciliary neurotrophic factor (CNTF) *(31)*. Ligands for p75NTR do not, however, consistently induce apoptosis. Application of soluble exogenous NGF, BDNF, or NT-3 to the retina appears to be unable to induce increased retinal death and instead activates survival signaling pathways given expression of the appropriate *trk* receptor *(28,30,33)*. Similarly, others have been unable to reproduce the effects of NGF on oligodendrocytes, probably owing to the presence of *trk*A in their culture conditions *(34–36)*.

Mechanisms of Signaling

The nature of the signaling mechanism by which p75NTR acts is particularly hotly debated and it remains unclear how p75NTR signals death in the presence of *trk*, since, as we have seen above, the activation of *trk* usually overrides the effect of p75NTR signaling *(9,10,19,37)*, and antibody-induced *trk*A activation alone is sufficient to mediate a NGF-like survival signal after axotomy *(37)*. Thus, in the presence of *trk*A, NGF ligation to the *trk*A/p75NTR complex results in survival and not p75NTR-mediated death (Fig. 1).

*trk*A may inhibit p75NTR death signaling by at least two mechanisms: First, in the presence of *trk*A, p75NTR death signaling pathway is not activated; p75NTR forms high-affinity receptors for growth factors and, in the ligated state, acts to transduce *trk* phosphorylation cascades *(38)*, and may also signal via a p75NTR-associated kinase *(39)* (Fig. 1). Second,

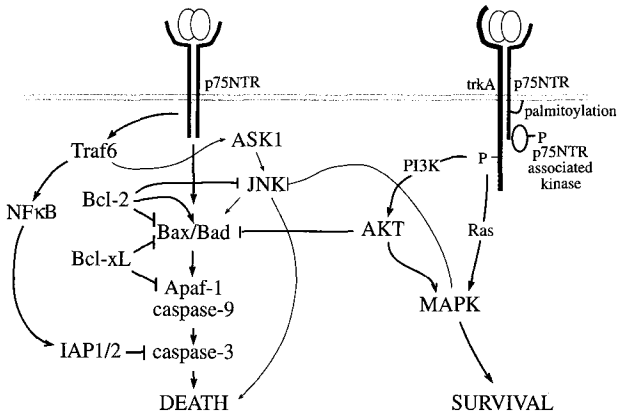

Fig. 1. Putative p75NTR death signaling pathways. Binding of neurotrophin to the trkA-p75NTR receptor complex initiates survival signaling through the PI3K/Ras-MAPK pathways. Survival signaling inhibits (T) p75NTR death signaling by a number of possible mechanisms: physically by palmitoylation or binding by survival signaling molecules; or through AKT and MAPK inhibition of death signaling pathways. In addition, in the absence of trk, ligand-activated p75NTR can bind Traf6, which through NFκB can inhibit cell death via IAPs. Traf6 activation may also influence cell death through the JNK death pathway. The Bcl-2 family is heavily implicated in p75NTR death signaling: Bcl-2 capable of both inhibiting and promoting death; and Bcl-xL inhibiting death, possibly through interaction with Apaf-1, caspase-9, and caspase-3. Bold lines indicate pathways that have been shown to influence neuronal death, whereas pathways that have been implicated in nonneural cell death are shown in thin lines.

NGF binding to p75NTR may lead to p75NTR death signaling, but activation of *trk* inhibits the cascade at some point down stream.

There are a number of ways by which *trk* might specifically inhibit the initiation of p75NTR signaling. The presence or association of p75NTR with *trk*A may compete with a p75NTR-associated death-transducing protein, thus physically blocking the death pathway. This is observed in the tumor necrosis factor receptor (TNFR) death pathway where a Suppressor of Death Domain (SODD) protein suppresses constitutive death signaling *(40)*. Similarly, *trk* palmitoylation of p75NTR *(38)*

may physically restrict availability of p75NTR to promote death signals by regulating protein–protein or protein–membrane interactions, or by restricting the cellular localization of p75NTR.

Inhibition of the activated p75NTR death pathway may be owing to competition between downstream signaling cascades (Fig. 1). *trk* signaling activates phosphotidylinositol 3-phosphate kinase (P13K), as well as *ras* and mitogen-activated protein (MAP) kinases *(41,42)*, and inhibits death-associated c-*jun* activating kinase (JNK) activity in oligodendrocytes *(35)*. MAPK, in turn, can phosphorylate and activate anti apoptotic proteins Bcl-2 or Bcl-x_L *(43,44)*. In addition, PI3K activated Akt has been shown to phosphorylate and inactivate the death-promoting Bax and Bad proteins as well as the Forkhead transcription factor, FKHRL, which controls expression of cell death genes *(41,45–47)*. In contrast, *trk* signaling does not appear to suppress the p75NTR-mediated activation of NFκB *(35)*, a transcription factor that induces expression of survival proteins, such as Inhibitors of Apoptosis (IAPs), which in turn suppress the killing pathway *(48)*.

How p75NTR in the presence of *trk* is activated to signal death remains speculative. Ligand activation of p75NTR by neurotrophins has only been observed in cells not expressing the appropriate *trk* for the ligand, namely E4 chick retinal ganglia *(29)*, oligodendrocytes *(42)*, trigeminal neurons *(31)*, and sympathetic neurons *(13)*. Consistent with this, a ligand-acting p75NTR-specific antibody is sufficient to cause apoptosis in the absence of *trk*A *(49)*. In contrast, p75NTR appears to be constitutively active in cells expressing *trk*, after neurotrophin withdrawal or axotomy *(9, 19)*. However, an alternative interpretation is that a growth factor from an alternative source can activate p75NTR in the absence of the neurotrophin on which the cell is dependent *(13,28,32,49)*.

Rather than debating whether p75NTR is ligand or constitutively activated, it may be more accurate to determine whether activated p75NTR is in a monomeric or multimeric state. It is generally believed that ligand activation causes multimerization *(50)*, and the activation of p75NTR by dimeric neurotrophins supports this concept. However, Bredesen and colleagues argue that a number of receptors, including p75NTR, are inhibited from signaling while multimeric *(51)*. Thus, addition of "inappropriate" growth factors may disengage p75NTR from the *trk* complex, allowing it to signal in the monomeric state. In fact, the high concentrations *(13,30)* or application bound to glass beads (forming highly concentrated pockets *[29]*) of neurotrophins needed to ligand-activate p75NTR in the absence of *trk* support this hypothesis. Alternatively, the high concentrations of growth factor used in these experiments may have initiated nonbiological effects, or the presentation of growth factor may be particularly important *(29)*.

Neuronal and Nonneuronal Apoptotic Signaling Pathways

Apoptosis has been most extensively studied in the hemopoetic system, and many protein components of the death signaling pathways have been isolated. Thus, these death pathways are fundamentally well understood. In contrast, neuronal apoptosis is less well characterized, although at least some components of the apoptosis pathways appear to be conserved in all cell types. Taking together the known components of apoptotic pathways, there appears to be a minimum of a four-step sequence *(52)*. First is an apoptotic stimulus, such as growth factor or serum deprivation, ionizing radiation, or Fas/tumor necrosis factor (TNF) ligand-receptor docking. This in turn activates modulators of apoptosis, such as the Bcl-2 family members, death domain and death effector molecules, kinases, phosphatases, and ceremide. Successful signal transduction by the death modulators leads to caspase activation. The result of caspase activation is protein cleavage of caspase-substrate proteins, which in turn results in the morpho-

logical changes, such as membrane "blebbing" and DNA fragmentation, characteristic of the apoptotic process *(53)*. In particular, caspases (proteases that cleave after aspartate residues) appear to be centrally involved in apoptosis of all cell types and stimuli, and activation of a caspase inevitably results in subsequent dismantling of the cell *(54)*. However, the precise signaling pathways resulting in caspase activation for each apoptotic stimulus are not nearly as well resolved.

The best example of mammalian apoptotic signaling is the Fas/TNFR pathway, which is active in the immune system (for comprehensive review, see *55*). Briefly, the induction of apoptotic signaling begins with ligand binding and the formation of dimer or trimer receptor complexes. Cytoplasmic signaling follows the recruitment of a number of death domain-containing proteins, which bind to the cytoplasmic death domain of the receptor and, stepwise, to each other, initiating signal transduction cascades or resulting directly in caspase activation. The simplest death cascade involves three death domain and/or death effector domain-containing adapter proteins (FADD, TRADD, and caspase-8) linking the initiating and effector parts of the apoptotic cascade. However, even Fas/TNF apoptosis signaling is not necessarily quite so direct and can involve many different interacting proteins (including RIP, RAIDD, and TRAF proteins) before receptor trimerization results in a phosphorylation cascade and/or caspase activation *(55)*.

Importantly, the TNF/Fas signaling pathway involves proteins, which interact with each other through their death or death effector domains. Overexpression of these proteins, and in some cases the domain alone, is sufficient to induce apoptosis *(52)*. In contrast, apoptosis resulting from stress stimuli, such as radiation, drug treatment, or growth factor withdrawal, appears to involve alternative pathways to death domain protein transduction, although these signals also proceed to caspase activation.

When p75NTR was earning credibility as a death inducer, it was recognized that the cytoplasmic domain of p75NTR shared homology with the death domain proteins involved in the Fas/TNF death pathway *(56)*, thus cementing p75NTR in the death-receptor family. The death domain motif contains large numbers of polar residues and is believed to be primarily involved in protein–protein interactions via both hetero- and homodimerization *(50,57)*. Death domain-containing proteins are highly variable in overall structure and include transmembrane receptors, kinases, death effector domain proteins, and proteins containing ankyrin repeat motifs, which suggests variable functions for this family of proteins *(56)*. Indeed, it has recently been suggested that *Drosophila* death domain-containing proteins are not involved with induction of apoptosis at all and that the domain merely functions in protein–protein interactions *(57)*. Even so, on the premise that many death domain-containing proteins are involved in transducing death signals and interact with each other via that domain, it would be predicted that p75NTR would transduce signals in a parallel pathway to Fas/TNF. We and others have used the yeast two-hybrid system (used to identify many of the death domain proteins) to investigate interactions between the death domain of p75NTR and other death domain homologous proteins, and found no specific interaction to support this prediction *(58–60)*. In support of this supposition, it has been found that the death domain of p75NTR does not spontaneously form dimers in experimental conditions found to dimerize the TNF receptor death domain, and has a different tertiary structure *(61)*. This indicates that p75NTR encodes a homologous death domain sequence without functional protein binding capacity. Accordingly, we hypothesize that the p75NTR death signaling cascade is unlikely to be a parallel cascade to TNF signaling and have shown that the juxtamembrane region of the p75NTR cytoplasmic domain and not the "death domain" is both required and sufficient to propagate the death signal *(58)*. To support this hypothesis further, TRAF6 (which activates stress pathways rather than "death domain" proteins) has been shown to interact with p75NTR in the

juxtamembrane region and not the "death domain" *(59)*. Therefore, by what pathways might p75NTR transduce apoptotic signals?

The Bcl-2 Family of Apoptotic Mediators

Modulators of death signaling, such as the Bcl-2 family and cell-cycle regulators that are important in stress-induced apoptosis, might also be central to p75NTR-induced apoptosis. It is unlikely that apoptosis resulting from a "clash" of mitogenic signaling and growth arrest via c-*myc* and cyclin D regulatory gene classes occurs in post mitotic neurons, although these stimuli may induce apoptosis regardless of their role in cell-cycle effects *(52)*. The Bcl-2 family, in contrast, is involved in both protection from, and induction of, neuronal apoptosis *(62)*. Bcl-2 is a generic apoptotic inhibitor protein, and frenzied research continues for the precise method of protection *(63–66)*. In cell-culture conditions, Bcl-2 is a potent inhibitor of stress, radiation, serum, or growth factor-induced apoptosis, with apoptotic family members (Bax, Bad, Bim) involved in inducing apoptosis. However, Bcl-2 and Bcl-x_L are poor inhibitors of TNF-induced apoptosis *(67,68)*.

Bcl-2 family members are functional in neuronal apoptosis after a variety of stimuli, including NGF deprivation in the neuronal-like PC12 cell line. Since PC12 cells are often still dividing even under neuronally differentiated conditions, it is difficult to interpret whether the death is stress-induced or triggered by p75NTR signaling or a combination of both *(69)*. However, exogenous Bcl-2 and Bcl-x_L are able to protect PC12 cells from most apoptotic stimuli *(44,70)*. Similarly, in vivo evidence supports the hypothesis that Bcl-2 family members function in neurons as in other cell types (*see* Table 1).

Mice lacking functional genes for the apoptotic-enhancer, Bax, show increased survival of sympathetic and motor neurons during development, resulting in supernumerary cell numbers. Furthermore, NGF deprivation does not cause apoptosis in neurons lacking Bax, despite the appearance of cell atrophy, and this morphology is fully reversible by subsequent NGF application *(71)*. This suggests a requirement for Bax in p75NTR-mediated apoptosis. Consistent with Bax functioning in neurons, mice deficient for Bcl-x_L (which is able to prevent Bax-induced apoptosis *(62,64)*, have dramatic increases in apoptosis in the central nervous system and sensory neurons. These mice have no other gross morphological changes, but the substantial neuronal apoptosis results in death of the animal around embryonic d 13 *(72)*. Furthermore, Bax deficiency can prevent the apoptosis that is occurring in Bcl-x_L-deficient neurons *(73)*. In contrast, Bcl-2-depleted mice show a major compromise of the lymphoid system *(74,75)*, but have a dramatically less obvious neuronal phenotype despite being highly expressed in the developing nervous system *(76)*. Endogenous Bcl-2 regulates the transient survival of a subset of sensory neurons during embryogenisis *(77)*. However, this was not observed by others who reported the Bcl-2-deficient mice had a postnatal loss of a wide variety of neuronal populations corresponding to the onset of kidney degeneration owing to polycystitis and not to any known periods of naturally occurring cell death (Table 1). The phenotype of Bcl-2-deficient mice thus indicates that Bcl-2 itself may play a subsidiary or alternative role to Bcl-x_L in modulating neuronal apoptosis. Experiments utilizing transgenic technology have demonstrated that Bcl-2 is able to rescue NGF dependent neurons from a wide variety of cell death stimuli including axotomy *(78–82)*. However, there are a number of neuronal populations and experimental paradigms where Bcl-2 is ineffective at rescuing neuronal apoptosis *(78,82,83)*. Given the somewhat artificial expression of Bcl-2 in transgenic studies, it is possible that Bcl-2 is not necessarily involved in inhibiting p75NTR-mediated apoptosis *per se*, but may act, in these experiments, in a redundant manner mimicking Bcl-x_L action *(7)*. In support of this hypothesis, we recently elucidated the roles of Bcl-2 and Bcl-x_L

in mediating p75NTR death signaling in sensory neurons from newborn mice. Bcl-x_L was a potent inhibitor of p75NTR-mediated neuronal death, whereas in contrast, Bcl-2 actually promoted p75NTR killing, despite being able to protect the same cells against growth factor withdrawal *(58)*. Others have also reported the ability of Bcl-2 to kill *(80,84)*. This suggests that although Bcl-x_L is a potent antiapoptotic protein, Bcl-2 has two separable functions dependent on the nature of the death stimulus.

From the above experiments, it is obvious that the Bcl-2 family is involved in modulating neuronal apoptosis, including that initiated by p75NTR signaling (Fig. 1). p75NTR mediated apoptosis appears to be extensively regulated specifically by the Bax and Bcl-x_L members of the family, whereas Bcl-2 itself, although having the ability to prevent neuronal death, may play a very different role in the p75NTR signaling pathway.

Stress-Induced Signaling Pathways

Other components of stress-induced apoptosis signaling, such as JNK activation, may also be involved in p75NTR apoptosis transduction (Fig. 1). Traditionally, apoptosis mediated through the JNK pathway can be blocked by Bcl-2, indicating this is a stress response *(70)*. Overexpression of components of the JNK signalling pathway (which involves MEKK, SEK, JNKK, JNK, and c-*jun*) can lead to apoptosis *(85)*, although JNK activation is not always an apoptotic signaling cascade *(86)*. Apoptosis following JNK activation is mediated by the TNF receptor, signaling via TRAFs through ASK1, Daxx, RIP, but not TRADD *(87–91)*. JNK signaling is necessary for apoptosis in motor neurons and PC12 cells expressing p75NTR after growth factor deprivation *(92,93)*, and is recorded in sympathetic neurons in response to BDNF *(13)*, and in oligodendrocytes in response to NGF *(35)*. The observed increases in JNK activity may be owing to changes in strength of either *trk* or p75NTR signals, and may be accompanied by decreased MAPK activation *(35,94)*. Blocking the JNK pathway hinders apoptosis induced by p75NTR in the absence of *trk* expression, indicating that the JNK signaling pathway may be required for p75NTR death signaling *(35)*. However, JNK activation is not always observed during p75NTR-induced apoptosis *(10,36)*, suggesting that there may be other p75NTR death-signaling cascades.

Activation of the transcription factor NFκB is similarly stimulated by TRAF proteins in response to TNF receptor trimerization, in a parallel pathway to JNK signal transduction *(86,88)*. In contrast to JNK activation, protein expression resulting from NFκB activation acts to prevent apoptosis in most circumstances, including in PC12 cells *(48,95,96)*. Schwann cells and oligodendrocytes (cells lacking *trk*A) are capable of activating NFκB through p75NTR and TRAF6 in the presence of NGF (Fig. 1; *30,35,36,59,97*). The result of NFκB-regulated transcription in this circumstance is likely to be mediation of NGF effects, such as cell migration or survival, rather than apoptosis *(98)*, with NFκB activation leading to inhibitor of apoptosis (IAP) expression and caspase inhibition (48). In support of the proposal that NFκB is not involved in the p75NTR death pathway, Majadan and colleagues *(10)* found no increase in NFκB activation in p75NTR overexpressing mouse brain undergoing apoptosis compared with normal brain, nor was the level of p75NTR-mediated NFκB activation altered when *trk* signaling was initiated *(35)*.

Ceremide has also been associated with apoptosis, particularly with respect to JNK and NFκB signaling pathways *(99–101)*, as well as being implicated in p75NTR death signaling *(102,103)*. However, although NGF may activate the sphingomyelin cycle, changes in ceremide production appear to be downstream of caspase activation and may be the result of apoptotic processes rather than a mediation event *(see 55)*.

In summary, signaling pathways resulting from apoptotic stimuli can be broadly divided

into two groups, that which is induced by stress on the cells, such as radiation, UV, or drug treatment, and that which is "active", such as Fas/TNF-induced apoptosis. It is into this second class that we believe p75NTR-mediated apoptosis falls. However, unlike Fas/TNF signaling, the evidence suggests that p75NTR-mediated apoptosis does not involve "death domain"-containing proteins and is mediated by stress-activated JNK and Bcl-2 family members.

Caspases in Neuronal Death

The "point of no return" of an apoptotic cell is arguably once the death-effector caspases are activated. There are currently over 10 identified caspase proteins, and they have been divided into three classes *(54)*. The first group includes interleukin-converting enzyme (ICE), and the caspases in this group are probably not involved in apoptosis *per se*, but regulate inflammation and cytokine function. The second and third groups are involved with cell death with group three (upstream) functioning in the cleavage and activation of the group two (downstream) caspases, which then cleave other proteins resulting in morphological changes characteristic of apoptosis and dismantling of the cell *(53,54)*.

Despite the lack of knowledge surrounding the signaling components of p75NTR-induced apoptosis, p75NTR transduction almost certainly leads to caspase activation and apoptosis, since broad specificity caspase inhibitors have been shown to inhibit death resulting from NGF withdrawal *(8,70,104,105)*. Caspase-3 cleavage and activation occur in dying neurons in vitro, and the apoptotic process can be inhibited by caspase-3 peptide blockers *(106,107)*, supporting the idea that caspase-3 is involved in neuronal death. Caspase-2 has also been implicated in neuronal death resulting from growth factor withdrawal and axotomy *(93,108)*. However, these data are not supported in vivo by the caspase-2-deficient mice, which have no impaired neuronal programmed cell death *(109)*.

In contrast, in vivo evidence strongly implicates caspase-9 and caspase-3 as important neural caspases. Mice lacking either the caspase-9 or caspase-3 genes have dramatic neural phenotype, resulting in supernumerary neurons, oversized brains, and death early in postnatal life owing to the dearth of apoptosis during development of the nervous system (Table 2) *(110–112)*. Furthermore, mice deficient for the CED-4 homolog Apaf-1 also have a dramatic neuronal phenotype *(113)*, reflecting the phenotypes of caspase-9- and 3-deficient mice, and in stark contrast to the Bcl-x_L-deficient mice *(64)*. Surprisingly, the phenotype of each mutant mouse was generally confined to neural tissues, suggestive of a common pathway. Supporting this idea is evidence that Bcl-x_L, Apaf-1, and caspase-9 form a complex, with Bcl-x_L inhibiting caspase activation *(114)*, and both caspase-9- and Apaf-1-deficient mice have impaired caspase-3 cleavage, suggesting that caspase-9 is a direct upstream activator of caspase-3 *(112)*.

The in vivo data also support the idea of neuronal programmed cell death being a different pathway to the "death domain" pathway activated by the TNFR family. Thymocytes isolated from the caspase-9 and Apaf-1 mutant mice displayed a normal susceptibility to Fas-induced cell death, but resistance to stress or chemical apoptotic stimuli, separating these two death pathways *(112,113)*. Furthermore, lymphoblasts from caspase-2-deficient mice were resistant to Fas-, but not perferin-mediated death consistent with a role for this protein in mediating death domain/caspase-8, but not "stress-type" neuronal death *(109)*.

p75NTR Signaling Pathways

There is still much to resolve in defining the signaling pathway resulting from p75NTR transduction. The question of whether p75NTR is ligand-activated and what the normal ligand is also remains unresolved, despite the knowledge that p75NTR is able to initiate signal transduction on neurotrophin ligation in

Table 2
Phenotypes of Mice Deficient for Caspases and Apaf-1

	Caspase-3$^{-/-}$	Caspase-9$^{-/-}$	Apaf-1$^{-/-}$	Caspase-2$^{-/-}$
Life-span	Death from E13 to 1–3 wk	Death by P3	Lethal before birth	Appears normal
Major problems	E12 brain development affected hyperplasia supernumerary cells	Perturbation in cortical morphology present by E12 exencephaly of entire cranial tissue	Ectopic brain masses morphology present by E12 exencephaly of entire cranial tissue	Excess numbers of germ cells apoptosis-resistant oocytes
	Brain has ectopic cell mass with cortex characteristics	Hindbrain neural tube remains open		Newborn has 73% facial motor neurons compared to WT
	Particular overproduction of neurons of the ventricular zone	Particular overproduction of neurons of the ventricular zone	Particular overproduction of neurons of the ventricular zone	Sympathetic neurons not resistant to NGF withdrawal or axotomy or ischemia
		Impaired caspase-3 processing	Cytochrome-c release is not impaired, impaired processing of caspase-3, 8, and 2 after stress/chemical-induced death	
Other	Reduced numbers of thymocytes, but equally sensitive to all apoptotic stimuli	Fewer thymocytes, but equally sen sitive to Fas, but not stress-induced apoptosis	Fewer thymocytes, but equally sensitive to Fas, but not stress-induced apoptosis	Lymphobasts are resistant to perferin, but not Fas-induced apoptosis
Normal expression pattern	Ubiquitous in all tissues from at least E7	Appears ubiquitous	Appears ubiquitous	High levels in embryo, including brain E9–E16, low-level expression in heart, lung, and brain

both neurons and glial cells. In *trk*A free cells, it appears that NGF ligation to p75NTR initiates apoptotic pathways, which contrasts with when *trk*A is present and NGF stimulated pathways lead to survival. How p75NTR-mediated death is initiated in cells expressing *trk*A remains an enigma. The proteins involved with transducing the death signal from p75NTR are also in question, and we have proposed a possible interaction of pathways and known death-mediating protein (Fig. 1).

Unlike TNF-initiated apoptosis, p75NTR pathways probably do not involve death domain-containing proteins, and Bcl-2 itself is

not centrally involved in preventing p75NTR-regulated apoptosis, although it may play a secondary role. Although p75NTR has been shown to signal through JNK and NFκB, possibly via TRAF6, the pathway by which JNK activates cell death, and whether this is the primary p75NTR death pathway remain uncertain. Evidence that inhibition of JNK arrests p75NTR-mediated apoptosis places JNK as a strong candidate in the central p75NTR death pathway. Furthermore, there is evidence to suggest that JNK might regulate the activity of Bcl-2 family members *(115,116).*

The other proteins implicated in p75NTR-mediated apoptosis are antiapoptotic protein Bcl-x_L, apoptosis inducer Bax, and caspases-9 and 3. With the activation of caspases, the apoptotic pathway is complete. However, the pathways connecting pro-apoptotic Bcl-2 proteins with caspase activation are not yet resolved despite the knowledge of interactions among Bcl-x_L, Apaf-1, and caspase-9 *(114,117).* It is the "missing link(s)" among the p75NTR cytoplasmic domain, JNK, and Bcl-2 family members, which is going to be most interesting to understand, particularly in terms of piecing together the dual role of p75NTR as an apoptotic receptor and as a helper in *trk* survival signaling.

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