Neurotrophin signal transduction in the nervous system

David R Kaplan* and Freda D Miller†

Neurotrophins use two types of receptors, the Trk tyrosine kinase receptors and the p75 neurotrophin receptor (p75NTR), to regulate the growth, development, survival and repair of the nervous system. These receptors can either collaborate with or inhibit each other's actions to mediate neurotrophin effects. The development and survival of neurons is thus based upon the functional interplay of the signals generated by Trk and p75NTR. In the past two years, the signaling pathways used by these receptors, including Akt and MAPK-induced signaling via Trk, and JNK, p53, and NF-κB signaling via p75NTR, have been identified. In addition, a number of novel p75NTR-interacting proteins have been identified that transmit growth, survival, and apoptotic signals.

Addresses
*Brain Tumor Research Center, Montreal Neurological Institute, 3801 University Street, Montreal, PQ, Canada, H3A 2B4; e-mail: mcdv@musica.mcgill.ca
†Center for Neuronal Survival, Montreal Neurological Institute, 3801 University Street, Montreal, PQ, Canada, H3A 2B4; e-mail: mdfm@musica.mcgill.ca

Current Opinion in Neurobiology 2000, **10**:381–391

0959-4388/00/$ – see front matter

Abbreviations

FKHRL1	Forkhead 1
JNK	Jun amino-terminal kinase
MAPK	mitogen activated protein kinase
MEK	MAPK kinase
NGF	nerve growth factor
NRIF	neurotrophin receptor interacting factor
PI-3K	phosphoinositide 3-kinase
P75NTR	p75 neurotrophin receptor
TNFR1	tumor necrosis factor receptor 1

Introduction

Neurotrophins mediate the survival, differentiation, growth, and apoptosis of neurons by binding to two types of cell surface receptors, the Trk tyrosine kinases and the p75 neurotrophin receptor (p75NTR). These receptors, often present on the same cell, coordinate and modulate the responses of neurons to neurotrophins. The functions of the neurotrophin receptors vary markedly, from the sculpting of the developing nervous system to the regulation of the survival and regeneration of injured neurons. Strikingly, while Trk receptors transmit positive signals such as enhanced survival and growth, p75NTR transmits both positive and negative signals. The signals generated by the two neurotrophin receptors can either augment or oppose each other. Trk and p75NTR thus exist in a paradoxical relationship, each acting to suppress or enhance the other's actions. How the two neurotrophin receptors act together to regulate the responses of cells to neurotrophins, and the nature of the intracellular signals used by these receptors to exert their effects, are the key questions in neurotrophin signal transduction. In the past two years, several of the intracellular signaling proteins and signal transduction pathways used by these receptors to promote neurotrophin actions have been identified. In this review, we discuss the latest findings in neurotrophin signaling, emphasizing the mechanisms used by the neurotrophin receptors to promote neuronal survival and apoptosis in primary neurons and *in vivo* systems.

Trk-mediated survival often emanates from Ras

A potent activity of neurotrophins, particularly in sympathetic and sensory neurons, is neuronal survival. Both during development and in culture, the survival of these neurons is absolutely dependent upon a constant exposure to optimal amounts of neurotrophins. The first neurotrophin-activated signaling protein shown to mediate survival of these neurons was the small GTP-binding protein Ras. Inhibition of Ras activity decreased survival of most, but not all, populations of sympathetic neurons [1,2], whereas increasing Ras activity as a result of deletion of neurofibromatosis-1 (NF-1), a Ras regulatory inhibitor, allowed peripheral neurons to survive in culture in the absence of neurotrophins [3]. Ras, which in most cases is responsible for 40–60% of neurotrophin-dependent survival, does not act directly to promote survival. Rather, it functions by translating and directing neurotrophin-initiated signals into multiple signaling pathways. Recent data indicate that two of these signaling pathways, PI-3K/Akt and MEK/MAPK, are the major effectors of neurotrophin and Ras-activated survival (Figure 1). In the first part of this review, we discuss recent data supporting this conclusion.

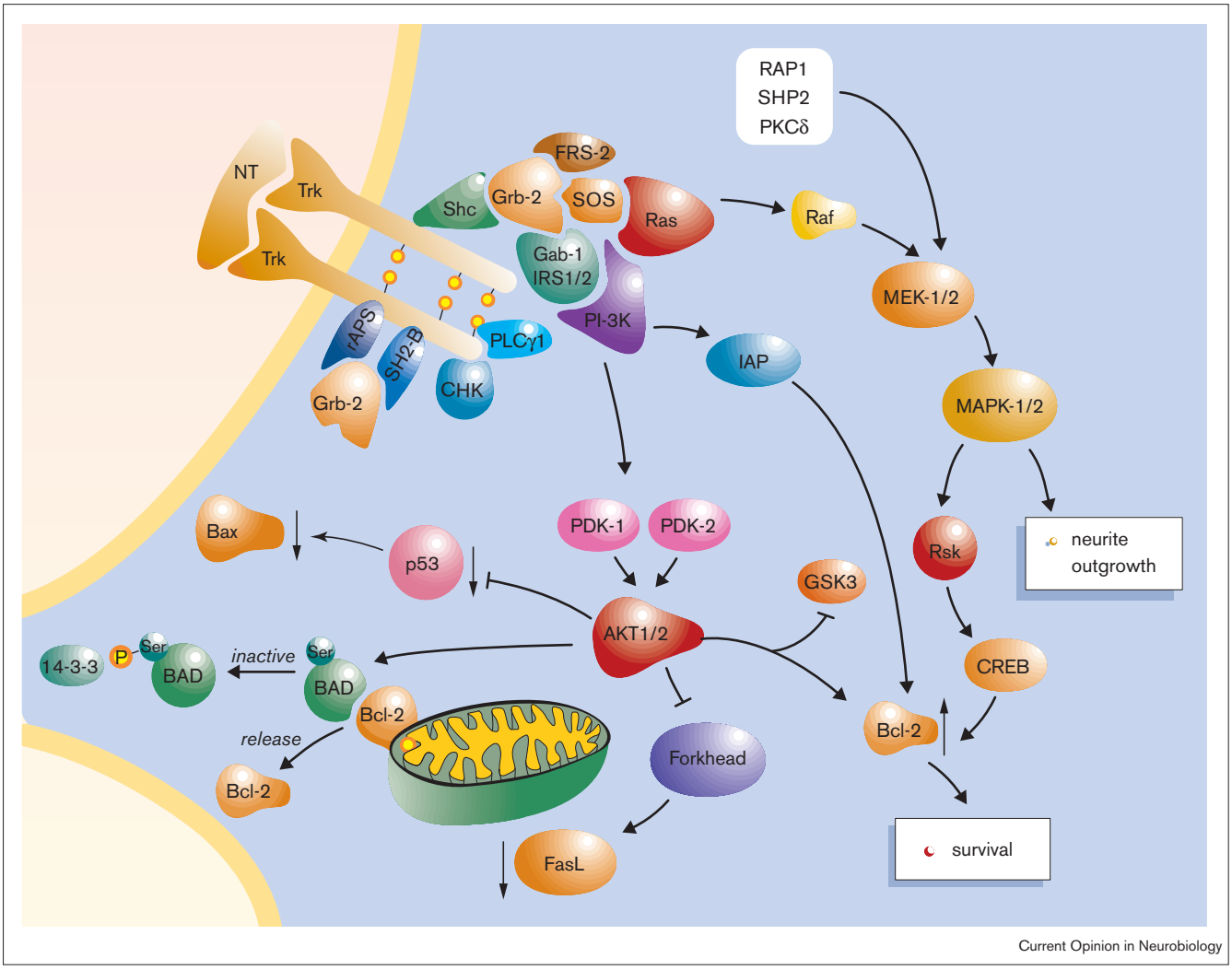
The PI-3K/Akt survival pathway

PI-3K was first identified as a regulator of neurotrophin-mediated survival responses by Cooper and colleagues in nerve growth factor (NGF)-dependent PC12 cells [4]. Subsequently, many groups showed that in cerebellar, sympathetic, sensory, cortical and motor neurons, PI-3 kinase activity was responsible for as much as 80% of neurotrophin-regulated cell survival [5–8,9•,10••,11,12,13••,14,15], indicating that PI-3K is the major survival-promoting protein for neurons. Not all studies, however, supported this conclusion. In particular, one group reported that inhibition of PI-3K activity did not decrease NGF-mediated survival of rat sympathetic neurons [16], while three other groups reported that it did [5,9•,10••,12]. Whether these contrasting data are due to differences in culture conditions or inhibitor concentrations is not known, but it highlights the pitfalls of relying upon dominant-inhibitory mutants or selective inhibitors without biochemical confirmation that these reagents specifically block the activity of PI-3K.

PI-3K is a target of Ras

An intimate connection between Ras and PI-3K activity in PC12 cells was first reported by Downward and

Figure 1



Trk signaling pathways regulating survival and neurite growth in neuronal cells. Neurotrophin (NT) binding to Trk stimulates receptor transphosphorylation, resulting in the recruitment of a series of signaling proteins to docking sites on the receptor. These proteins include Shc, which activates Ras through Grb-2 and SOS [108], FRS-2 [109,110], rAPS [110], SH2-B [111••], and CHK [112], which participate in activating MAPK, and PLC-γ1. Shc and FRS-2 bind to phosphorylated Tyr490 of TrkA [108], while PLC-γ1 and CHK bind to phosphorylated Tyr 785 [108,112]. MAPK activity is also regulated through Raf [39•], Rap1 [39•], SHP-2 [113], and PKCδ [114]. The pathways used by MEK and MAPK to regulate neurite growth and survival are discussed in the text. Trk activates PI-3K through the Ras and the Gab-1/IRS-1/IRS-2 family of adapter proteins [18,19,115]. PI-3K activity stimulates the activities of PDK1 and PDK2, which in turn activate Akt [26,27]. The targets of PI-3K/Akt anti-apoptotic activity, including BAD, Forkhead, GSK-3, Bcl-2, IAP, and the p53 cell death pathway, are discussed in the text.

colleagues [17], who demonstrated that Ras directly interacted with PI-3K and that inhibition of Ras suppressed NGF-mediated PI-3K activity. More recently, Ras was shown to activate PI-3K survival promoting pathways in peripheral neurons using two approaches. First, Ras effector mutants that were selective for activating PI-3K, but not those selective for activating MEK/MAPK or RalGDS (Ras-related guanine-nucleotide dissociation stimulator), induced survival [9•], while Ras-mediated survival was blocked by the PI-3K inhibitor LY294002 [9•,11]. Ras is not, however, the only means by which Trk activates PI-3K. In the Trk system, PI-3K activation is probably due to the combined actions of Ras and Gab-1, an adapter protein that binds and stimulates PI-3K [18] and which, when overexpressed, potently stimulated NGF-independent survival [19].

Akt is a crucial mediator of PI-3K-induced survival activity

PI-3K, like Ras, stimulates the activities of many signaling proteins. Among these is the serine/threonine kinase Akt (or protein kinase B), a target of NGF-induced PI-3K activity [20,21]. Greenberg and colleagues [6] first reported a role for Akt in neuronal survival, showing that cerebellar neurons required Akt for 20% of IGF-1-induced survival.

Subsequently, Akt activity was shown, using dominant-inhibitory Akt, to be necessary for approximately 80% of NGF-induced survival of sympathetic neurons [5,10••,12]. Akt not only mediates growth factor-regulated cell survival, but also neuronal survival promoted by depolarization [10••,*22*•,23]. In cerebellar neurons, Akt induced survival by stimulating Ca^{2+} influx through L-type calcium channels [*22*•], while in sympathetic neurons, it promoted survival by acting downstream of L-type channels and Ras/PI-3K [10••]. In the latter case, suboptimal levels of NGF and KCl synergistically stimulated maximal Akt activity and neuronal survival, indicating that Akt is a convergence point for diverse survival signals. During development, neurons exposed to suboptimal levels of neurotrophins but which are active may have a competitive advantage over those that are not active, due to increased amounts of Akt activity.

Akt is most probably not the only target of PI-3K-induced survival activity. Inhibition of PI-3K is often more effective than inhibition of Akt at suppressing survival responses. Another potential target of PI-3K is the IAP (inhibitor of apoptosis) family of caspase inhibitors, which includes X-linked inhibitor of apoptosis protein (XIAP), neuronal apoptosis-inhibitory protein (NAIP), and human inhibitor of apoptosis protein (HIAP, also known as inhibitor of T-cell apoptosis [ITA]; see [24]). HIAP/ITA levels were induced by NGF in chick sensory and sympathetic neurons in a manner dependent upon PI-3K activity, and suppression of XIAP levels decreased NGF-induced survival [25••]. Whether IAP levels or activity are also controlled by Akt, either at the transcriptional level or via phosphorylation, is not yet known.

The targets of Akt

In neurons, Akt has only been shown to regulate survival, and not any other response such as neurite outgrowth or differentation. Thus, all of the proposed direct targets of Akt activity identified in the past year have been proteins that regulate cell survival in many cell systems: these include Bad, an inhibitor of the Bcl-*2* anti-apoptotic protein; pro-caspase-9, which is cleaved into the pro-apoptotic caspase-9; and Forkhead, a transcription factor that induces apoptosis by increasing levels of Fas ligand (FasL) (Figure 1). In each case, Akt suppresses apoptosis by phosphorylating the apoptotic protein in the Akt consensus phosphorylation site RXRXXS/T (single-letter amino acid code). How Akt regulates the activity of its many targets, as well as the mechanisms whereby PI-3K regulates Akt activity, have been extensively discussed in three recent reviews [26,27,28•]. Therefore, we will discuss whether Akt phosphorylation of one or more of these targets is responsible for neurotrophin-induced neuronal survival.

The first reported target of Akt-mediated survival activity was Bad. Phosphorylation by Akt at Ser136 in Bad induced its association with 14-3-3, and prevented it from associating with and inactivating the anti-apoptotic Bcl-*2* and Bcl-XL proteins [29,30]. Evidence for the importance of Akt-induced Bad phosphorylation derives from overexpression experiments in cerebellar neurons, whereby insulin-like growth factor 1 (IGF-1) or constitutively active Akt suppressed the apoptotic activity of wild-type Bad, but not of Bad mutated at Ser136 [29]. These experiments provide compelling evidence for Akt regulation of ectopically expressed Bad, but three other lines of evidence indicate that endogenous Bad phosphorylation might not be important for growth factor-mediated neuronal survival. First, endogenous Bad has not been reported to be phosphorylated in neurotrophic factor-treated neurons, except for increases in brain-derived neurotrophic factor (BDNF)-treated cerebellar neurons [31•]. Second, analysis of the Bax knockout mice indicate that Bax is the apoptotic Bcl-*2* family member that is required for cerebellar neuron cell death [32]. Third, neurons from the Bad knockout mouse do not show alterations in apoptosis [33].

The proteolytic cleavage and activation of pro-caspase-9 was also shown to be effectively inhibited by Akt *in vitro* and in overexpression experiments. However, the lack of conservation of the Ser196 Akt phosphorylation site in nonhuman procaspase-9 [34], as well as the dearth of reports of Akt-induced phosphorylation of endogenous procaspase-9 in neurons, does not support a role for this phosphorylation event in neurotrophic factor mediated neuronal survival. The third and best candidate for a direct Akt target in neurons is Forkhead 1 (FKHRL1). Genetic studies in *C. elegans* first indicated that the activity of a Forkhead family member, DAF16 (Dauer-formation-16), which contains an Akt consensus phosphorylation site, was suppressed by Akt [35]. Greenberg's group then showed that ectopic expression of FKHRL1 mutated at the Thr32 and Ser315 Akt phosphorylation sites increased apoptosis of cerebellar neurons cultured in IGF-1 by 20% [36••]. Apoptosis induced by FKHRL1 was reduced by inhibition of FasL binding to its receptor, indicating that FKHRL1 stimulates apoptosis, in part, by inducing the transcription of cell death ligands such as Fas. While endogenous Forkhead has not been shown to be phosphorylated by Akt in neurons, the strong genetic evidence for this protein as an Akt target, coupled with the compelling *in vitro* and non-neuronal cell data showing regulation of Forkhead by Akt phosphorylation [36••], makes Forkhead an attractive target for Akt in mammalian neurons.

These results suggest that a signaling pathway consisting of Ras/PI-3K/Akt is the major regulator of neuronal survival. Akt may suppress apoptosis directly by inhibiting the activities of Forkhead or Bad, indirectly by suppressing GSK-3 apoptotic activities [37,38•], increasing IAP, Bcl-*2*, or Bcl-XL levels, or, as will be discussed below, by blocking the function of the primary neuronal apoptotic pathway in neurons, JNK-p53-Bax (Figure 1). Akt probably mediates cell survival at a number of levels, depending upon the cell type, target availability, and the requirement for transcriptional or post-transcriptional events to suppress apoptosis.

MEK/MAP kinase – a second survival pathway used by neurotrophins?

A second survival-promoting pathway used by neurotrophins consists of the Ras-MEK-MAPK pathway (Figure 1). This pathway has many roles in neurons, including synaptic plasticity, long-term potentiation, and survival (reviewed in [39•]). The evidence for the contribution of this pathway to neuronal survival is, however, conflicting. While NGF induces a strong and sustained activation of MAPK in sympathetic neurons and PC12 cells, most studies have found that inhibition of MEK has minimal effects on NGF-dependent neuronal survival [9•,40–42]. Thus, although the selective activation of MEK/MAPK can promote neuronal survival [9•,19,42,43], the lack of dramatic effects of MEK inhibitors (<20% decreases in cell survival) in these same cell types indicates that MEK activity is sufficient, but not necessary, for most of neurotrophin-mediated survival. The only exception to this generalization is in P6 rat cerebellar neurons, where the data are conflicting, with one report showing no role for MEK/MAPK in BDNF or insulin-dependent survival [44], and another demonstrating that MEK activity was required for survival promoted by these factors [31•]. However, in the latter study, BDNF-mediated survival was suppressed by only 20–30% when MEK was inhibited, indicating that MEK-MAPK is only one contributor to neurotrophin-mediated cerebellar neuron survival.

The major role for MEK-induced survival pathways may be to protect neurons from death due to injury or toxicity, rather than from trophic factor withdrawal. In cortical neurons, constitutively active MEK protected, while inhibition of MEK blocked BDNF-regulated neuroprotection from camptothecin-induced apoptosis [13••]. In this study, MEK played no role in serum or BDNF-induced survival under basal conditions. Rather, PI-3K mediated this effect. Similarly, MEK/MAPK protected sympathetic neurons against apoptosis due to cytosine arabinoside [45•], cerebellar neurons from apoptosis caused by oxidative stress [46], and retinal ganglion cells from death following axotomy [47••]. MEK may also play a more prominent role in TrkB-induced survival. We have shown that TrkB, unlike TrkA, uses both MEK and PI-3K to promote the survival of sympathetic neurons (J Atwal, F Miller, D Kaplan, unpublished data).

Targets of MEK/MAPK survival activity

Akt induces survival by inhibiting the activity of apoptotic proteins. In contrast, MEK/MAPK induces survival by stimulating the activity or expression of anti-apoptotic proteins, including Bcl-2 and the transcription factor CREB (cAMP response element binding protein). NGF potently increased Bcl-2 levels in sympathetic neurons [48••], which in turn protected these and other neurons from apoptotic cell death [49]. Inhibition of MEK/MAPK activity in PC12 cells completely blocked NGF's ability to increase Bcl-2 levels [50], suggesting that Bcl-2 is a transcriptional target of the MEK/MAPK pathway. CREB activity is also required for Bcl-2 expression and survival induced by NGF in sympathetic neurons [51••], suggesting a MEK/MAPK–CREB–Bcl-2 survival pathway. CREB, like Bcl-2, is clearly a key mediator of neuronal survival, as dominant-inhibitory forms of CREB induced apoptosis of virtually all sympathetic neurons grown in NGF [51••], and 25% of cerebellar neurons grown in BDNF [31•]. The activation of CREB by survival factors is likely to be due to phosphorylation at Ser133 by multiple kinases, including MAPK activated-Rsk [31•], p38MAPK [52], and Akt [53]. p38MAPK is, however, an unlikely candidate for this role, since it induces apoptosis [43] or neurite outgrowth [54] in PC12 cells, but not survival. MEK/MAPK is also an unlikely CREB activator in sympathetic neurons, as MEK activity is not required for NGF-mediated survival. Perhaps CREB is activated by Akt in sympathetic neurons, as occurs in 293T cells [53], while it may be activated by both Akt and MAPK in cerebellar neurons.

p75NTR as a signaling receptor

Although p75NTR was the first-isolated neurotrophin receptor, as well as the first-reported member of the p75NTR/Fas/TNFR1 (tumor necrosis factor receptor 1) family (reviewed in [55]), our understanding of its physiological role and the underlying signaling mechanisms has lagged considerably behind our understanding of the Trk neurotrophin receptors. In particular, studies on p75NTR have been complicated by the fact that it can interact directly with Trk [56], and by the finding that its signaling capacity is modified by the coincident activation of Trk receptors. Nonetheless, the past year has seen the emergence of a consensus regarding the signaling pathways activated by p75NTR and of its potential biological functions, and has led to the elucidation of a number of p75NTR-interacting proteins (Figure 2).

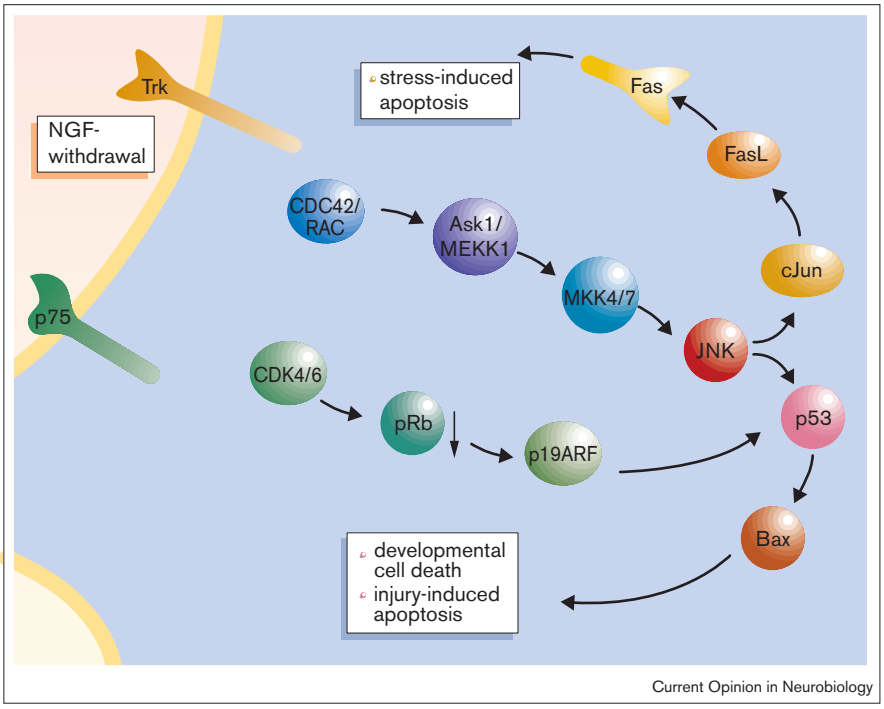
p75NTR as an apoptotic receptor independent of Trk

The original finding that p75NTR could mediate neuronal apoptosis in a neural cell line [57] has, over the past several years, been extended to a large number of primary neural cells, both in culture and *in vivo*. In particular, ligand-dependent activation of p75NTR has been shown to cause the apoptosis of cultured neonatal sympathetic neurons [58••], motor neurons [59,60], sensory neurons [61,62•], oligodendrocytes [63] and Schwann cells [64•]. Still controversial, however, is the role of p75NTR in regulating survival of basal forebrain cholinergic neurons; while Yeo *et al.* [65] reported increased numbers of cholinergic neurons in *p75NTR*$^{-/-}$ animals, Van der Zee *et al.* [66] have withdrawn their earlier report describing similar findings, and Peterson *et al.* [67] recently published a report indicating that neuronal number is actually decreased in *p75NTR*$^{-/-}$ animals. Whether this discrepancy is due to differing genetic backgrounds and/or other potential confounds associated with the *p75NTR*$^{-/-}$ animals (see discussion below) remains to be clarified.

In addition to confirming that p75NTR plays an important role in regulating neural apoptosis, a number of major

Figure 2

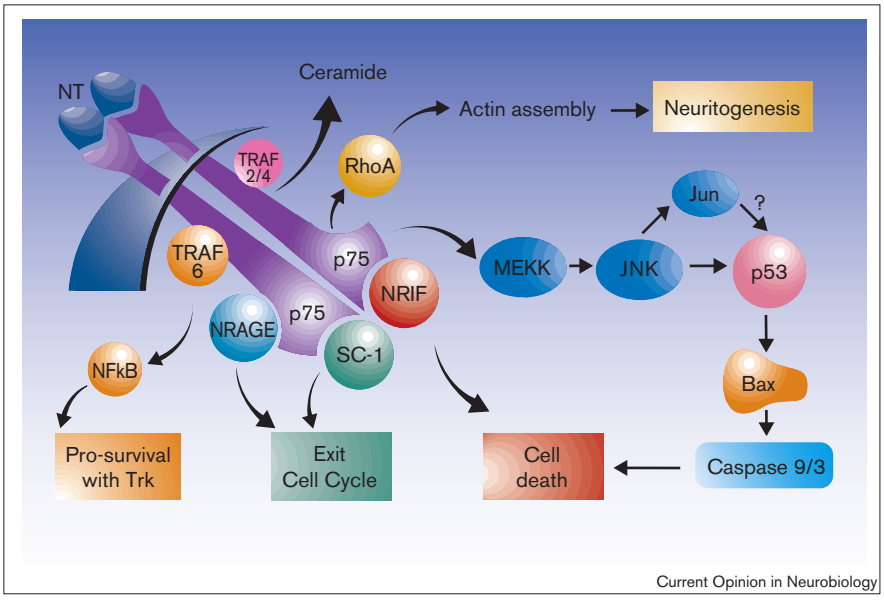
Cell death pathways induced by NGF withdrawal from neurons. Two pathways are activated by withdrawal of NGF from sympathetic neurons. The first consists of cdc42/Rac [116], Ask1 [117•] and possibly MEKK1, MKK4/7, JNK [48••], and p53 [48••]. JNK isoforms induce cell death through c-Jun [118] and increase in FasL [119••], or by increases in p53 and Bax levels or activity [48••]. A second pathway involves the activation of cell cycle regulatory molecules such as CDK4/6 [120], which results in increased pRb phosphorylation, and possibly the subsequent activation of p53 through p19ARF [121••]. We hypothesize that each pathway converges upon and activates p53 to cause cell death. JNK3 is involved in stress-induced cell death [119••], while JNK1 and 2 are involved in developmental cell death [122].



conclusions can be derived from these studies. First, in all of these cells, the apoptotic actions of p75NTR were ligand-mediated, indicating that ligand binding to p75NTR does not abolish its ability to mediate apoptosis, as previously suggested [68]. Second, these studies indicate that p75NTR signals apoptosis in a Trk-independent fashion. For example, p75NTR activation caused apoptosis when sympathetic neurons were maintained in KCl [10••,48••], when sensory neurons were maintained in ciliary neurotrophic factor (CNTF) [62•], and when Schwann cells were maintained in IGF plus neuregulin [64•] — all Trk-independent survival signals. Third, in all of these studies, p75NTR only mediated apoptosis when Trk was inactive or suboptimally activated, leading to the conclusion that Trk activation silences p75NTR apoptotic signaling. For example, robust Trk

Figure 3

p75NTR signaling pathways. p75NTR binds a number of interacting proteins, including TRAF2, 4, and 6, NRAGE, SC-1, and RhoA, which play roles in cell survival, cell cycle regulation, and neurite outgrowth. p75NTR also increases ceramide levels and activates the JNK-p53-Bax cell death pathway. See text for a discussion of these proteins and pathways.



activation blocked p75NTR-mediated death of sympathetic [58••] and trigeminal mesencephalic sensory neurons [62•], and expression of exogenous TrkA in oligodendrocytes inhibited NGF-induced apoptosis [69••]. Thus, the outcome of neurotrophin-mediated p75NTR signaling depends on the expression of Trk receptors; NGF has the potential to be pro-apoptotic for cells that do not express TrkA (such as oligodendrocytes [69••]), while BDNF would be pro-apoptotic for those cells that do not express TrkB (such as sympathetic neurons [58••]).

A fourth and somewhat surprising conclusion is that p75NTR is, for some cells, essential for apoptosis following growth factor withdrawal. Barrett and Bartlett [61] first showed that sensory neuron survival following neurotrophin withdrawal was enhanced when p75NTR levels were decreased. More recent work extended this finding to other primary cells; apoptosis of *p75NTR*$^{-/-}$ sympathetic neurons was greatly delayed following NGF withdrawal [58••], and *p75NTR*$^{-/-}$ Schwann cells showed enhanced survival in the absence of survival factors [64]. Interestingly, as no exogenous p75NTR ligand is present following growth factor withdrawal, these data may suggest that p75NTR can signal apoptosis in a ligand-independent fashion [68]. However, as both sympathetic neurons and Schwann cells make endogenous p75NTR ligands, these data raise the equally interesting possibility of an autocrine p75NTR-driven apoptosis loop that is suppressed by survival factors.

p75NTR mediates apoptosis following injury and during development

The past year has also seen a number of studies indicating that the apoptotic function of p75NTR is important following neural injury and during development. The first suggestion that p75NTR might be involved in injury-induced apoptosis originated with studies showing that neuron-specific expression of the p75NTR intracellular domain caused the death of injured facial motor neurons in transgenic mice [70]. More recently, endogenous p75NTR was shown to play a role in the death of injured neonatal facial motor neurons [59,71], and, following seizure in adult animals, neuronal apoptosis was accompanied by induction of p75NTR in the dying neurons [72•]. In this regard, exogenous BDNF exacerbated the death of CA3 pyramidal neurons following kainic acid treatment [73], suggesting that both endogenous and exogenous neurotrophins might act through p75NTR to cause apoptosis in the damaged nervous system. Recent evidence also indicates that p75NTR is essential for rapid and appropriate apoptosis during developmental cell death (reviewed in [74]). In particular, apoptosis is significantly reduced in embryonic retinae of *NGF*$^{-/-}$ and *p75NTR*$^{-/-}$ mice [75,76], and the period of naturally-occurring sympathetic neuron death is greatly delayed in the *p75NTR*$^{-/-}$ mice [58••]. Moreover, 75NTR is essential for maintaining the specificity of neuronal survival responses to different neurotrophins; sympathetic neurons of *p75NTR*$^{-/-}$ but not wild-type mice utilized NT3 as a survival ligand both *in vivo* [77••] and in culture [78]. Previous work demonstrating that NT3 activates TrkA on sympathetic neurons but does not maintain survival [79], and that p75NTR activation has no effect on sympathetic neuron TrkA activation [48••,58••], suggests that p75NTR 'selects' survival ligands not by regulating TrkA activation, as is seen in some cell lines (reviewed in [80]), but by antagonistically signalling neuronal apoptosis.

p75NTR apoptotic signal transduction

How does p75NTR signal apoptosis? One recently elucidated pathway involves JNK (Jun amino-terminal kinase)-p53-Bax, which is activated by p75NTR activation and following NGF withdrawal ([48••], Figures 2 and 3). p53 appears to be a key death sensor in this pathway, with the levels of this protein determining whether neurons undergo apoptosis *in vivo* and in culture [48••]. MEKK and JNK function upstream of p53 in p75NTR-mediated apoptosis, while cdc42/Rac1, Ask1, MKK (mitogen-activated protein kinase kinase), JNK, c-jun, and p53 have been shown to act in a signaling pathway regulating NGF-withdrawal-induced apoptosis (Figures 2 and 3). The presence of apoptotic proteins common to both p75NTR and NGF-withdrawal-induced cell death pathways, and the observation that *p75NTR*$^{-/-}$ sympathetic neurons are greatly delayed in their death following NGF withdrawal, suggests that a major component of NGF withdrawal-induced apoptosis involves p75NTR-driven activation of the JNK-p53-Bax pathway. Although it is not yet known whether this pathway is important for apoptosis in other cells, it is intriguing that p75NTR is induced in dying cells following seizure [72•], and that seizure-induced apoptosis requires JNK3 [81] and p53 [82]. Also intriguing is the finding that p75NTR-mediated apoptosis of oligodendrocytes involves the same pattern of caspase activation as did radiation-induced oligodendrocyte apoptosis [83], which is known to require p53 [84]. Thus, although it is as yet unclear how p75NTR activates the JNK-p53-Bax cell death pathway, this pathway may well play a key role in a variety of p75NTR-driven apoptotic events. Interestingly, in sympathetic neurons TrkA activation, which inhibits p75NTR-mediated apoptosis, silences this JNK-p53 death pathway via Ras and perhaps PI-3K/Akt [9•], while in oligodendrocytes, TrkA activation selectively silences JNK activation coincident with its repression of p75NTR-mediated apoptosis [69••].

A second potential p75NTR-dependent apoptotic pathway involves the recently-reported neurotrophin receptor interacting factor (NRIF) [85••]. NRIF is a ubiquitously-expressed zinc finger protein that interacts with p75NTR in GST pulldown assays. Analysis of the *NRIF*$^{-/-}$ mice revealed a deficit in apoptosis in the embryonic retina that was similar to that seen in the *NGF*$^{-/-}$ and *p75NTR*$^{-/-}$ mice [76,85••], raising the possibility that p75NTR might signal apoptosis in some cells via NRIF.

Finally, the past year has seen the description of a number of additional p75-interacting proteins that may regulate cell survival, including members of the TRAF (tumor

necrosis factor receptor-associated factor) family [86•] (see the discussion below), and two novel proteins, the zinc finger motif protein SC-1 [87•] and NRAGE (neurotrophin receptor-interacting MAGE [melanoma antigen gene] homologue) (A Salehi, P Barker, personal communication). One of these latter two proteins, SC-1, is a zinc finger protein that, like NRIF, associates with p75NTR in GST pulldown assays. Interestingly, NGF-mediated activation of p75NTR led to translocation of SC-1 from the cytoplasm to the nucleus and inhibited cellular proliferation [87•] suggesting that it, like other members of the same family, may play a role in growth arrest. The second protein, NRAGE, is a member of the MAGE family, and can be coimmunoprecipitated with p75NTR from PC12 nnr5 cells (A Salehi, P Barker, personal communication). Like SC-1, NRAGE appears to play a role in growth arrest. It is unclear whether these two proteins play any role in apoptosis but it is intriguing that cell cycle deregulation is thought to be involved in many types of neuronal apoptosis (Figure 3).

p75NTR as a signaling receptor in the presence of Trk activation

One of the major conclusions that can be derived from recent studies on the neurotrophin receptors is that the signaling capacity and biological role of p75NTR is a function of cellular Trk activation status. In particular, as discussed above, Trk signaling silences p75NTR-mediated apoptotic pathways such as the JNK-p53 pathway, while leaving other p75NTR pathways 'intact'. Moreover, emerging evidence indicates that *crosstalk* between these two receptors is bidirectional, with p75NTR modulating certain Trk signaling pathways. We will discuss these issues by focusing on p75NTR-mediated NF-κB signaling, and by examining how p75NTR modulates the morphological growth of neurons.

The finding that p75NTR caused activation of the transcription factor NF-κB in Schwann cells [88] has recently been extended to oligodendrocytes [89] and sensory neurons [90]. Unlike the JNK-p53 pathway, p75NTR-mediated activation of the NF-κB pathway is not silenced by coincident TrkA activation [69••]. Two recent publications suggest that NF-κB activation represents a p75NTR-mediated prosurvival pathway that collaborates with Trk. Specifically, Maggirwar *et al.* [91•] demonstrated that NGF treatment of sympathetic neurons led to NF-κB activation, and that this activation was important for NGF-mediated survival. Although this study did not examine the relative roles of TrkA versus p75NTR, Hamanoue *et al.* [92•] demonstrated that NGF-induced NF-κB activation in sensory neurons required p75NTR and that this pathway was important for survival. How does p75NTR activate NF-κB? Recent work demonstrated that p75NTR interacts with TRAF6, and that dominant-negative TRAF6 blocked p75NTR-mediated NF-κB activation in Schwann cells [86•]. In non-neuronal cells, TRAF6 also forms a signaling complex with TRANCE (tumor necrosis factor-related activation-induced cytokine) to activate Akt [93]. Thus, p75NTR and Trk may synergistically activate Akt's survival-promoting activity in cells where p75NTR can associate with TRAF6 or other TRAF family members. In this regard, a related publication demonstrates that other TRAF family members interact with p75NTR, at least as assayed by GST pulldowns [94].

p75NTR and the regulation of neuronal growth

A second biological function of p75NTR in the presence of Trk activation is the modulation of neuronal growth. Such a role for p75NTR was first suggested by the finding that sympathetic innervation patterns were perturbed in *p75NTR*$^{-/-}$ mice [95]. More recent studies led to the conclusion that ligand-mediated p75NTR activation inhibits TrkA-mediated growth and target innervation of sympathetic neurons [96•]. Remarkably, elimination of p75NTR even led to robust sprouting of adult sympathetic nerve fibers on CNS myelin [97••]. The finding that p75NTR inhibits neuronal growth is not limited to sympathetic neurons; BDNF-mediated activation of NGF-dependent sensory neurons also inhibits their growth [90], and there is cholinergic hyperinnervation of the hippocampus and hypertrophy of basal forebrain cholinergic neurons in *p75NTR*$^{-/-}$ animals [65]. Together these studies raise the possibility that neurotrophins act as growth inhibitors via p75NTR, providing a mechanism for regulating the specificity or density of axonal growth and target innervation and/or for axon collateral elimination.

How does p75NTR inhibit TrkA-mediated growth? Recent studies indicate that p75NTR activation causes a selective downregulation of the TrkA-dependent Raf–MEK–MAPK pathway, which is a major growth pathway for sympathetic neurons (R Aloyz, FD Miller, DR Kaplan, unpublished data). Although the p75NTR-derived signals that are responsible for negatively regulating this pathway are currently unknown, it is intriguing that elevation of ceramide, a known downstream p75NTR effector, inhibits NGF-dependent growth of distal sympathetic neurites [98] and the activation of Raf-1 and Akt in non-neuronal cells [99,100]. In this regard, p75NTR-mediated ceramide increases are thought to modulate the growth of cultured hippocampal neurons [101].

p75NTR probably also regulates axonal growth via a recently-described modulation of the growth regulatory protein Rho. Yamashita *et al.* [102••] demonstrated that, in 293 cells, transfection of p75NTR led to a robust activation of Rho, and that neurotrophin binding to p75NTR largely suppressed this p75NTR-dependent Rho activation. As Rho activation has been shown to inhibit neuronal growth [103•], this work raises the possibility that p75NTR might regulate axonal growth either positively or negatively, depending on the proportion of unliganded to liganded p75NTR present in the local microenvironment.

At this point, it is worth mentioning a number of limitations to some of the current approaches in studying p75NTR function. The first two caveats involve the *p75NTR*$^{-/-}$ mice. The homologous recombination strategy used to generate these mice left intact a p75NTR splice variant that lacks

exon III, and which generates a protein that is incapable of binding neurotrophins [104], making these animals hypomorphs rather than true knockouts. A second caveat with these mice involves the fact that p75NTR is now known to regulate both the migration [105] and survival [64•,106] of Schwann cells, meaning that any perturbations in growth or innervation pattern observed in the PNS of these animals might be attributable to a Schwann cell phenotype rather than to a neuronal phenotype. The final issue involves the use of p75NTR antibodies to assess the biological role of this receptor and in particular the use of MC192 [107]. Although many studies have used MC192 to 'block' neurotrophin binding to p75NTR, this antibody has now been shown to cause NF-κB translocation in sensory neurons [90], presumably by functioning as an 'activating' antibody. This finding obviously changes the interpretation of many experiments that have been published previously, and makes it a poor choice as a blocking antibody for future experiments.

Conclusions

Neurotrophins regulate neuronal survival and apoptosis at several levels. Trk uses at least two mechanisms, Ras/PI-3K/Akt-induced suppression of apoptotic proteins and pathways, and MEK/MAPK activation of anti-apoptotic proteins, to stimulate survival. p75NTR can potentiate Trk activity through the activation of NF-κB. In most cases, however, p75NTR functions as a ligand-stimulated apoptotic receptor, inducing the activity of the JNK–p53–Bax apoptosis pathway, and of other proteins that regulate cell death such as NRIF. Trk, through Ras and probably PI-3K/Akt, can interfere with p75NTR-induced apoptosis by suppressing the JNK–p53–Bax pathway upstream of JNK, or by inhibiting the activities of cell death proteins such as Forkhead. p75NTR, in turn, can suppress Trk-induced cell survival and growth pathways, possibly through ceramide-mediated inhibition of Akt and Raf activities. This functional crosstalk between Trk and p75NTR signaling pathways appears to be a key process in determining how the nervous system develops and is repaired following injury. In the next year, the characterization of both the newly identified and novel targets of Trk and p75NTR will allow us to identify the mechanisms used by these receptors to develop, maintain, and repair the nervous system.

Acknowledgements

We thank Ute Zirrgiebel and Frank Zuhl for generating the figures for this review.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Borasio GD, Markus A, Wittinghofer A, Barde Y-A, Heumann R: **Involvement of ras p21 in neurotrophin-induced response of sensory but not sympathetic neurons.** *J Cell Biol* 1993, **121**:665-672.
2. Nobes CD, Tolkovsky AM: **Neutralizing anti-p21ras Fabs suppress rat sympathetic neuron survival induced by NGF, LIF, CNTF and cAMP.** *Eur J Neurosci* 1995, **7**:344-350.
3. Vogel KS, Brannan CI, Jenkins NA, Copeland NG, Parada LF: **Loss of neurofibromin results in neurotrophin-independent survival of embryonic sensory and sympathetic neurons.** *Cell* 1995, **82**:733-742.
4. Yao R, Cooper GM: **Requirement for phosphatidylinositol-3 kinase in the prevention of apoptosis by nerve growth factor.** *Science* 1995, **267**:2003-2006.
5. Crowder RJ, Freeman RS: **Phosphatidylinositol 3-kinase and Akt protein kinase are necessary and sufficient for the survival of nerve growth factor-dependent sympathetic neurons.** *J Neurosci* 1998, **18**:2933-2943.
6. Dudek H, Datta SR, Franke TF, Birnbaum MJ, Yao R, Cooper GM, Segal RA, Kaplan DR, Greenberg ME: **Regulation of neuronal survival by the serine-threonine protein kinase Akt.** *Science* 1997, **275**:661-664.
7. D'Mello SR, Borodezt K, Soltoff SP: **Insulin-like growth factor and potassium depolarization maintain neuronal survival by distinct pathways: possible involvement of PI 3-kinase in IGF-1 signaling.** *J Neurosci* 1997, **17**:1548-1560.
8. Bartlett SE, Reynolds AJ, Weible M, Heydon K, Hendry IA: **In sympathetic but not sensory neurons, phosphoinositide-3 kinase is important for NGF-dependent survival and the retrograde transport of 125I-βNGF.** *Brain Res* 1997, **761**:257-262.
9. • Mazzoni IE, Said FA, Aloyz R, Miller FD, Kaplan D: **Ras regulates sympathetic neuron survival by suppressing the p53-mediated cell death pathway.** *J Neurosci* 1999, **19**:9716-9727.

Demonstrates that one of the major mechanisms that Ras uses to support neurotrophin-induced neuronal survival is via suppression of a JNK-p53-Bax cell death pathway.

10. •• Vaillant AR, Mazzoni I, Tudan C, Boudreau M, Kaplan DR, Miller FD: **Depolarization and neurotrophins converge on the phosphatidylinositol 3-kinase-Akt pathway to synergistically regulate neuronal survival.** *J Cell Biol* 1999, **146**:955-966.

First report that neuronal depolarization mediates survival via activation of a Ras-PI-3K-Akt pathway, and that neurotrophins and depolarization synergistically support survival via convergence on this pathway.

11. Klesse LJ, Parada LF: **p21 Ras and phosphatidylinositol-3 kinase are required for survival of wild-type and NF1 mutant sensory neurons.** *J Neurosci* 1998, **18**:10420-10428.
12. Virdee K, Xue L, Hemmings BA, Goemans C, Heumann R, Tolkovsky AM: **Nerve growth factor-induced PKB/Akt activity is sustained by phosphoinositide 3-kinase dependent and independent signals in sympathetic neurons.** *Brain Res* 1999, **837**:127-142.
13. •• Hetman M, Kanning K, Cavanaugh JE, Xia Z: **Neuroprotection by brain-derived neurotrophic factor is mediated by extracellular signal-regulated kinase and phosphatidylinositol 3-kinase.** *J Biol Chem* 1999, **274**:22569-22580.

Demonstrates that the ERK pathway plays a major role in neuronal survival following cellular insult, while PI-3K is the dominant pathway for neurotrophin-mediated basal survival.

14. Dolcet X, Egea J, Soler RM, Martin-Zanca D, Comella JX: **Activation of phosphatidylinositol 3-kinase, but not extracellular-regulated kinases, is necessary to mediate brain-derived neurotrophic factor-induced motoneuron survival.** *J Neurochem* 1999, **73**:521-531.
15. Williams EJ, Doherty P: **Evidence for and against a pivotal role of PI 3-kinase in a neuronal cell survival pathway.** *Mol Cell Neurosci* 1999, **13**:272-280.
16. Philpott KL, McCarthy MJ, Klippel A, Rubin LL: **Activated phosphatidylinositol 3-kinase and Akt kinase promote survival of superior cervical neurons.** *J Cell Biol* 1997, **139**:809-815.
17. Rodriguez-Viciana P, Warne PH, Dhand R, Vanhaesebroeck B, Gout I, Fry MJ, Waterfield MD, Downward J: **Phosphatidylinositol-3-OH kinase as a direct target of Ras.** *Nature* 1994, **370**:527-532.
18. Holgado-Madruga M, Moscatello DK, Emlet DR, Dieterich R, Wong AJ: **Grb2-associated binder-1 mediates phosphatidylinositol 3-kinase activation and the promotion of cell survival by nerve growth factor.** *Proc Natl Acad Sci USA* 1997, **94**:12419-12424.
19. Korhonen JM, Said FA, Wong AJ, Kaplan DR: **Gab1 mediates neurite outgrowth, DNA synthesis, and survival in PC12 cells.** *J Biol Chem* 1999, **274**:37307-37314.
20. Andjelkovic M, Suidan HS, Meier R, Frech M, Alessi DR, Hemmings BA: **Nerve growth factor promotes activation of the alpha, beta and gamma isoforms of protein kinase B in PC12 pheochromocytoma cells.** *Eur J Biochem* 1998, **251**:195-200.

21. Aschroft M, Stephens RM, Hallberg B, Downward J, Kaplan DR: **The selective and inducible activation of endogenous PI 3-kinase in PC12 cells results in efficient NGF-mediated survival but defective neurite outgrowth.** *Oncogene* 1999, **18**:4586-4597.

22. • Blair LA, Bence-Hanulec KK, Mehta S, Franke T, Kaplan D, Marshall J: **Akt-dependent potentiation of L channels by insulin-like growth factor-1 is required for neuronal survival.** *J Neurosci* 1999, **19**:1940-1951.
This report shows that one way Akt can support neuronal survival is by directly regulating the activity of calcium channels.

23. Crowder RJ, Freeman RS: **The survival of sympathetic neurons promoted by potassium depolarization, but not by cyclic AMP, requires phosphatidylinositol 3-kinase and Akt.** *J Neurochem* 1999, **73**:466-475.

24. Le Casse EC, Daird S, Korneluk RC, MacKenzie AE: **The inhibitors of apoptosis (IAPs) and their emerging role in cancer.** *Oncogene* 1998, **17**:3247-3259.

25. •• Wiese S, Digby MR, Gunnersen JM, Gotz R, Pei G, Holtmann B, Lowenthal J, Sendtner M: **The anti-apoptotic protein ITA is essential for NGF-mediated survival of embryonic chick neurons.** *Nat Neurosci* 1999, **2**:978-983.
First demonstration that an IAP family member is part of a neurotrophin-mediated neuronal survival pathway.

26. Datta SR, Brunet A, Greenberg ME: **Cellular survival: a play in three Akts.** *Genes Dev* 1999, **13**:2905-2927.

27. Chan TO, Rittenhouse SE, Tsichlis PN: **AKT/PKB and other D3 phosphoinositide-regulated kinase: kinase activation by phosphoinositide-dependent phosphorylation.** *Annu Rev Biochem* 1999, **68**:965-1014.

28. • Vanhaesebroeck B, Alessi DR: **The PI3K–PDK1 connection: more than just a road to PKB.** *Biochem J* 2000, **346**:561-576.
A critical, excellent review on the regulation of Akt and its substrates.

29. Datta SR, Dudek H, Tao X, Masters S, Fu H, Gotoh Y, Greenberg ME: **Akt phosphorylation of Bad couples survival signals to the cell-intrinsic death machinery.** *Cell* 1997, **91**:231-241.

30. Del Peso L, Gonzalez-Garcia M, Page C, Herrera R, Nunez G: **Interleukin-3-induced phosphorylation of BAD through the protein kinase Akt.** *Science* 1997, **278**:687-689.

31. • Bonni A, Brunet A, West AE, Datta SR, Takasu MA, Greenberg MA: **Cell survival promoted by the Ras-MAPK signaling pathway by transcription-dependent and -independent mechanisms.** *Science* 1999, **286**:1358-1362.
This report demonstrates that the MAP kinases and Rsks regulate a portion of cerebellar neuron survival by inhibiting the activity of BAD and stimulating the activity of CREB.

32. Miller TM, Mouler KL, Knudson CM, Creedon DJ, Deshmukh M, Korsmeyer SJ, Johnson EM Jr: **Bax deletion further orders the cell death pathway in cerebellar granule cells and suggests a caspase-independent pathway to cell death.** *J Cell Biol* 1997, **139**:205-217.

33. Shindler KS, Yunker AM, Cahn R, Zha J, Korsmeyer SJ, Roth KA: **Trophic support promotes survival of bcl-x-deficient telencephalic cells *in vitro.*** *Cell Death Differ* 1998, **5**:901-910.

34. Fujita E, Jinbo A, Matuzaki H, Konishi H, Kikkawa U, Momoi T: **Akt phosphorylation site found in human caspase-9 is absent in mouse caspase-9.** *Biochem Biophys Res Commun* 1999, **264**:550-555.

35. Paradis S, Ruvkun G: ***Caenorhabditis elegans* Akt/PKB transduces insulin receptor-like signals from AGE-1 PI3 kinase to the DAF-16 transcription factor.** *Genes Dev* 1998, **12**:2488-2498.

36. •• Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS, Anderson MJ, Arden KC, Blenis J, Greenberg ME: **Akt promotes cell survival by phosphorylating and inhibiting a forkhead transcription factor.** *Cell* 1999, **96**:857-868.
First report that a member of the forkhead family of apoptosis-inducing transcription factors is a direct target of Akt, and that a portion of Akt's prosurvival activity depends upon this mechanism.

37. Pap M, Cooper GM: **Role of glycogen synthase kinase-3 in the phosphatidylinositol 3-kinase/Akt cell survival pathway.** *J Biol Chem* 1998, **273**:19929-19932.

38. • Hetman M, Cananaugh JE, Kimelman D, Xia Z: **Role of glycogen synthase kinase-3beta in neuronal apoptosis induced by trophic withdrawal.** *J Neurosci* 2000, **20**:2567-2574.
Reports that inhibition of GSK-3 activity, a result of PI-3 kinase activity, protects cortical neurons from cell death.

39. • Grewal SS, York RD, Stork PJS: **Extracellular-signal-regulated kinase signalling in neurons.** *Curr Opin Neurobiol* 1999, **9**:544-553.
A recent review on MAP kinase signalling in neurons.

40. Creedon DJ, Johnson EM Jr, Lawrence JC Jr: **Mitogen-activated protein kinase-independent pathways mediate the effects of nerve growth factor and cAMP on neuronal survival.** *J Biol Chem* 1996, **271**:20713-20718.

41. Virdee K, Tolkovsky AM: **Inhibition of p42 and p44 mitogen-activated protein kinase activity by PD98059 does not suppress nerve growth factor-induced survival of sympathetic neurons.** *J Neurochem* 1996, **67**:1801-1805.

42. Klesse LJ, Meyers KA, Marshall CJ, Parada LF: **Nerve growth factor induces survival and differentiation through two distinct signaling cascades in PC12 cells.** *Oncogene* 1999, **18**:2055-2068.

43. Xia Z, Dickens M, Raingeaud J, Davis RJ, Greenberg ME: **Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis.** *Science* 1995, **270**:1326-1331.

44. Gunn-Moore FJ, Williams AG, Toms NJ, Tavare JM: **Activation of mitogen-activated protein kinase and p70S6 kinase is not correlated with cerebellar granule cell survival.** *Biochem J* 1997, **324**:365-369.

45. • Anderson CNG, Tolkovsky AM: **A role for MAPK/ERK in sympathetic neuron survival: protection against a p53-dependent, JNK-independent induction of apoptosis by cytosine arabinoside.** *J Neurosci* 1999, **19**:664-673.
Demonstrates, like [13••], that a major role for MEK/MAP kinase is maintenance of neuronal survival in response to insults, in this case a DNA-damaging agent.

46. Skaper SD, Floreani M, Negro A, Facci L, Giusti P: **Neurotrophins rescue cerebellar granule neurons from oxidative stress-mediated apoptotic death: selective involvement of phosphatidylinositol 3-kinase and the mitogen-activated protein kinase pathway.** *J Neurochem* 1998, **70**:1859-1868.

47. •• Shen S, Wiemelt AP, McMorris FA, Barres BA: **Retinal ganglion cells lose trophic responsiveness after axotomy.** *Neuron* 1999, **23**:285-295.
An interesting paper demonstrating that the inability of neurotrophins to support the survival of injured CNS neurons is at least partially due to a decrease in trophic factor responsiveness.

48. •• Aloyz RS, Bamji SX, Pozniak CD, Toma JG, Atwal J, Kaplan DR, Miller FD: **p53 is essential for developmental neuron death as regulated by the TrkA and p75 neurotrophin receptors.** *J Cell Biol* 1998, **143**:1691-1703.
Identifies p53 as a key cell death sensor for both p75NTR-mediated apoptosis and developmental neuron death *in vivo*. This report also demonstrates an increase in Bcl2 and Bcl-xl levels and a decrease in Bax levels in sympathetic neurons with NGF treatment.

49. Michaelidis TM, Sendtner M, Cooper JD, Airaksinen MS, Holtmann B, Meyer M, Thoenen H: **Inactivation of bcl-2 results in progressive degeneration of motoneurons, sympathetic and sensory neurons during early postnatal development.** *Neuron* 1996, **17**:75-89.

50. Liu Y-Z, Boxer LM, Latchman DS: **Activation of the Bcl-2 promoter by nerve growth factor is mediated by the p42/p44 MAPK cascade.** *Nucleic Acids Res* 1999, **27**:2086-2090.

51. •• Riccio A, Ahn S, Davenport CM, Blendy JA, Ginty DD: **Mediation by a CREB family transcription factor of NGF-dependent survival of sympathetic neurons.** *Science* 1999, **286**:2358-2361.
This report demonstrates that CREB is both necessary and sufficient for sympathetic neuron survival in response to NGF, and that one of the targets of CREB-dependent transcriptional activity is Bcl-2.

52. Xing J, Korhnhauser JM, Xia Z, Thiele EA, Greenberg ME: **Nerve growth factor activates extracellular signal-regulated kinase and p38 mitogen-activated protein kinase pathways to stimulate CREB serine 133 phosphorylation.** *Mol Cell Biol* 1998, **18**:1946-1955.

53. Du K, Montminy M: **CREB is a regulatory target for the protein kinase Akt/PKB.** *J Biol Chem* 1998, **273**:32377-32379.

54. Morooka T, Nishida E: **Requirement of p38 mitogen-activated protein kinase for neuronal differentiation in PC12 cells.** *J Biol Chem* 1998, **38**:24285-24288.

55. Casaccia-Bonnefil P, Kong H, Chao MV: **Neurotrophins: the biological paradox of survival factors eliciting apoptosis.** *Cell Death Differ* 1998, **5**:357-364.

56. Bibel M, Hoppe E, Barde Y-A: **Biochemical and functional interactions between the neurotrophin receptors trk and p75NTR.** *EMBO J* 1999, **18**:616-622.

57. Rabizadeh S, Oh J, Zhong LT, Yang J, Bitler CM, Butcher LL, Bredesen DE: **Induction of apoptosis by the low-affinity NGF receptor.** *Science* 1993, **261**:345-348.

58. •• Bamji SX, Majdan M, Pozniak CD, Belliveau DJ, Aloyz R, Kohn J, Causing CG, Miller FD: **The p75 neurotrophin receptor mediates neuronal apoptosis and is essential for naturally occurring sympathetic neuron death.** *J Cell Biol* 1998, **140**:911-923.
The first report that endogenous p75NTR signaling leads to death of post-mitotic neurons, and demonstration of an essential role for p75NTR in naturally-occurring sympathetic neuron death *in vivo*.

59. Wiese S, Metzger F, Holtmann B, Sendtner M: **The role of p75NTR in modulating neurotrophin survival effects in developing motoneurons.** *Eur J Neurosci* 1999, **11**:1668-1676.

60. Sedel F, Bechade C, Triller A: **Nerve growth factor (NGF) induces motoneuron apoptosis in rat embryonic spinal cord.** *Eur J Neurosci* 1999, **11**:3904-3912.

61. Barrett GL, Bartlett PF: **The p75 nerve growth factor receptor mediates survival or death depending on the stage of sensory neuron development.** *Proc Natl Acad Sci USA* 1993, **91**:6501-6505.

62. • Davey F, Davies AM: **TrkB signalling inhibits p75-mediated apoptosis induced by nerve growth factor in embryonic proprioceptive neurons.** *Curr Biol* 1998, **8**:915-918.
Another report supporting the concept that p75NTR mediates neuronal apoptosis only when Trk signalling is suboptimal or absent.

63. Casaccia-Bonnefil P, Carter BD, Dobrowsky RT, Chao MV: **Death of oligodendrocytes mediated by the interaction of nerve growth factor with its receptor p75.** *Nature* 1996, **383**:716-719.

64. • Soilu-Hanninen M, Ekert P, Bucci T, Syroid D, Bartlett PF, Kilpatrick TJ: **Nerve growth factor signaling through p75 induces apoptosis in Schwann cells via a Bcl2-independent pathway.** *J Neurosci* 1999, **19**:4828-4838.
First report that p75NTR regulates Schwann cell survival, a finding that has implications for our understanding of the p75NTR$^{-/-}$ mouse phenotype.

65. Yeo TT, Chua-Couzens J, Butcher LL, Bredesen DE, Cooper JD, Valletta JS, Mobley WC, Longo FM: **Absence of p75NTR causes increased basal forebrain cholinergic neuron size, choline acetyltransferase activity, and target innervation.** *J Neurosci* 1997, **17**:7594-7605.

66. Hagg T: **Neuronal cell death: retraction.** *Science* 1999, **285**:340.

67. Peterson DA, Dickinson-Anson HA, Leppert JT, Lee KF, Gage FH: **Central neuronal loss and behavioural impairment in mice lacking neurotrophin receptor p75.** *J Comp Neurol* 1999, **404**:1-20.

68. Bredesen DE, Ye X, Tasinato A, Sperandio S, Wang JJ, Assa-Munt N, Rabizadeh S: **p75NTR and the concept of cellular dependence: seeing how the other half die.** *Cell Death Differ* 1998, **5**:365-371.

69. •• Yoon SO, Carter BD, Casaccia-Bonnefil P, Chao MV: **Competitive signaling between TrkA and p75 nerve growth factor receptors determines cell survival.** *J Neurosci* 1998 **18**:3273-3281.
First demonstration that the apoptotic potential of p75NTR in response to a given neurotrophin is determined by the Trk receptor repertoire on the cell. Also the first demonstration that Trk signalling selectively silences some p75NTR pathways and not others.

70. Majdan M, Lachance C, Gloster A, Aloyz R, Zeindler C, Bamji S, Bhakar A, Belliveau D, Fawcett J, Miller FD, Barker PA: **Transgenic mice expressing the intracellular domain of the p75 neurotrophin receptor undergo neuronal apoptosis.** *J Neurosci* 1997, **17**:6988-6998.

71. Ferri CC, Moore FA, Bisby MA: **Effects of facial nerve injury on mouse motoneurons lacking the p75 low-affinity neurotrophin receptor.** *J Neurobiol* 1998, **34**:1-9.

72. • Roux PP, Colicos MA, Barker PA, Kennedy TE: **p75 neurotrophin receptor expression is induced in apoptotic neurons after seizure.** *J Neurosci* 1999, **19**:6887-6896.
Intriguing demonstration that p75NTR is upregulated in virtually all apoptotic CNS neurons at longer timepoints following seizure.

73. Rudge JS, Mather PE, Pasnikowski EM, Cai N, Corcoran T, Acheson A, Anderson K, Lindsay RM, Wiegand SJ: **Endogenous BDNF protein is increased in adult rat hippocampus after a kainic acid induced excitotoxic insult but exogenous BDNF is not neuroprotective.** *Exp Neurol* 1998, **149**:398-410.

74. Majdan M, Miller FD: **Neuronal life and death decisions: functional antagonism between the Trk and p75 neurotrophin receptors.** *Int J Devl Neurosci* 1999, **17**:153-161.

75. Frade JM, Rodriguez-Tebar A, Barde Y-A: **Induction of cell death by endogenous nerve growth factor through its p75 receptor.** *Nature* 1996, **383**:166-168.

76. Frade JM, Barde Y-A: **Genetic evidence for cell death mediated by nerve growth factor and the neurotrophin receptor p75 in the developing mouse retina and spinal cord.** *Development* 1999, **126**:683-690.

77. •• Brennan C, Rivas-Plata K, Landis SC: **The p75 neurotrophin receptor influences NT-3 responsiveness of sympathetic neurons *in vivo*.** *Nat Neurosci* 1999, **2**:699-705.
Demonstrates that p75NTR plays a role in determining which neurotrophins can signal survival through TrkA *in vivo* by showing that NT-3 becomes a survival factor for sympathetic neurons in p75NTR$^{-/-}$ mice.

78. Lee KF, Davies AM, Jaenisch R: **p75-deficient embryonic dorsal root sensory and neonatal sympathetic neurons display a decreased sensitivity to NGF.** *Development* 1994, **12**:1027-1033.

79. Belliveau DJ, Krivko I, Kohn J, Lachance C, Pozniak C, Rusakov D, Kaplan D, Miller FD: **NGF and NT-3 both activate TrkA on sympathetic neurons but differentially regulate survival and neuritogenesis.** *J Cell Biol* 1997, **136**:374-388.

80. Barker PA: **p75NTR: a study in contrasts.** *Cell Death Differ* 1998, **5**:346-356.

81. Yang DD, Kuan C, Whitmarsh AJ, Rincon M, Zheng TS, Davis RJ, Rakic P, Flavell RA: **Absence of excitotoxicity-induced apoptosis in the hippocampus of mice lacking the Jnk3 gene.** *Nature* 1997, **389**:865-870.

82. Morrison RS, Wenzel HJ, Kinoshita Y, Robbins CA, Donehower LA, Schwartzkroin PA: **Loss of the p53 tumor suppressor gene protects neurons from kainate-induced cell death.** *J Neurosci* 1996, **16**:1337-1345.

83. Gu C, Casaccia-Bonnefil P, Srinivasan A, Chao MV: **Oligodendrocyte apoptosis mediated by caspase activation.** *J Neurosci* 1999, **19**:3043-3049.

84. Schwartz D, Rotter V: **p53-dependent cell cycle control: response to genotoxic stress.** *Semin Cancer Biol* 1998, **8**:325-336.

85. •• Casademunt E, Carter BD, Benzel I, Frade JM, Dechant G, Barde Y-A: **The zinc finger protein NRIF interacts with the neurotrophin receptor p75NTR and participates in programmed cell death.** *EMBO J* 1999, **18**:6050-6061.
First identification of NRIF, a protein that interacts with p75NTR and that plays an essential role during naturally-occurring cell death in the nervous system *in vivo*.

86. • Khursigara G, Orlinick JR, Chao MV: **Association of the p75 neurotrophin receptor with TRAF6.** *J Biol Chem* 1999, **274**:2597-2600.
First demonstration that members of the TRAF family interact with p75NTR. Also shows that TRAF6 is essential for p75NTR-regulated NF-κB activation.

87. • Chittka A, Chao MV: **Identification of a zinc finger protein whose subcellular distribution is regulated by serum and nerve growth factor.** *Proc Natl Acad Sci USA* 1999, **96**:10705-10710.
Reports a novel p75NTR interactor that may play a role in cell cycle regulation.

88. Carter BD, Kaltschmidt C, Kaltschmidt B, Offenhauser N, Bohm-Matthaei R, Bauerle PA, Barde Y-A: **Selective activation of NF-κB by nerve growth factor through the neurotrophin receptor p75.** *Science* 1996, **272**:542-545.

89. Ladiwala U, Lachance C, Simoneau SJJ, Bhakar A, Barker PA, Antel JP: **p75 neurotrophin receptor expression on adult human oligodendrocytes: signaling without cell death in response to NGF.** *J Neurosci* 1998, **18**:1297-1304.

90. Kimpinski K, Jelinski S, Mearow K: **The anti-p75 antibody, MC192, and brain-derived neurotrophic factor inhibit nerve growth factor-dependent neurite growth from adult sensory neurons.** *Neurosci* 1999, **93**:253-263.

91. • Maggirwar SB, Sarmiere PD, Dewhurst S, Freeman RS: **Nerve growth factor-dependent activation of NF-κB contributes to survival of sympathetic neurons.** *J Neurosci* 1998, **18**:10356-10365.
First demonstration that NF-κB is part of a neurotrophin-regulated survival pathway.

92. • Hamanoue M, Middleton G, Wyatt S, Jaffray E, Hay RT, Davies AM: **p75-mediated NF-κB activation enhances the survival response of developing sensory neurons to nerve growth factor.** *Mol Cell Neurosci* 1999, **14**:28-40.
Demonstrates that p75NTR can potentiate Trk-induced survival activity via NF-κB.

93. Wong BR, Besser D, Kim N, Arron JR, Vologodskaia M, Hanafusa H, Choi Y: **TRANCE, a TNF family member, activates Akt/PKB through a signaling complex involving TRAF6 and c-Src.** *Mol Cell* 1999, **4**:1041-1049.

94. Ye X, Mehlen P, Rabizadeh S, VanArsdale T, Zhang H, Shin H, Wang JJL, Leo E, Zapata J, Hauser CA *et al.*: **TRAF family proteins interact with the common neurotrophin receptor and modulate apoptosis induction.** *J Biol Chem* 1999, **274**:30202-30208.

95. Lee KF, Bachman K, Landis S, Jaenisch R: **Dependence on p75 for innervation of some sympathetic targets.** *Science* 1994, **263**:1447-1449.

96. • Kohn J, Aloyz RS, Toma JG, Haak-Frendscho M, Miller FD: **Functionally antagonistic interactions between the TrkA and p75 neurotrophin receptors regulate sympathetic neuron growth and target innervation.** *J Neurosci* 1999, **19**:5393-5408.
Reports that, when Trk signalling is robust, BDNF-mediated p75NTR activation leads to decreased sympathetic neuron growth both in culture and *in vivo*.

97. •• Walsh GS, Krol KM, Crutcher KA, Kawaja MD: **Enhanced neurotrophin-induced axon growth in myelinated portions of the CNS in mice lacking the p75 neurotrophin receptor.** *J Neurosci* 1999, **19**:4155-4168.
The first report demonstrating that p75NTR plays a growth-inhibitory role by showing that in p75NTR$^{-/-}$ mice, adult sympathetic neurons will grow robustly on CNS myelin when exposed to increased NGF.

98. Posse de Chaves EI, Bussiere M, Vance DE, Campenot RB, Vance JE: **Elevation of ceramide within distal neurites inhibits neurite growth in cultured rat sympathetic neurons.** *J Biol Chem* 1997, **272**:3028-3035.

99. Muller G, Storz P, Bourtelle S, Doppler H, Pfizenmaier K, Mischak H, Philipp A, Kaiser C, Kolch W: **Regulation of Raf-1 kinase by TNF via its second messenger ceramide and *crosstalk* with mitogenic signalling.** *EMBO J* 1998, **17**:732-742.

100. Zhou H, Summers SA, Birnbaum MJ, Pittman RN: **Inhibition of Akt kinase by cell-permeable ceramide and its implications for ceramide-induced apoptosis.** *J Biol Chem* 1998, **273**:16568-16575.

101. Brann AB, Scott R, Neuberger Y, Abulafia D, Boldin S, Fainzilber M, Futerman AH: **Ceramide signaling downstream of the p75 neurotrophin receptor mediates the effects of nerve growth factor on outgrowth of cultured hippocampal neurons.** *J Neurosci* 1999, **19**:8199-8206.

102. •• Yamashita T, Tucker KL, Barde Y-A: **Neurotrophin binding to the p75 receptor modulates Rho activity and axonal outgrowth.** *Neuron* 1999, **24**:585-593.
An intriguing report indicating that p75NTR regulates Rho activity, thereby providing a mechanism whereby p75NTR could directly modulate growth.

103. • Lehmann M, Fournier A, Selles-Navarro I, Dergham P, Sebok A, Leclerc N, Tigyi G, McKerracher L: **Inactivation of Rho signaling pathway promotes CNS axon regeneration.** *J Neurosci* 1999, **19**:7537-7547.
An interesting publication, particularly in light of [102••], demonstrating that decreased Rho activity leads to increased axonal growth.

104. Dechant G, Barde YA: **Signalling through the neurotrophin receptor p75NTR.** *Curr Opin Neurobiol* 1997, **7**:413-418.

105. Anton ES, Weskamp G, Reichardt LF, Matthew WD: **Nerve growth factor and its low affinity receptor promote Schwann cell migration.** *Proc Natl Acad Sci USA* 1994, **91**:2795-2799.

106. Ferri CC, Bisby MA: **Improved survival of injured sciatic nerve Schwann cells in mice lacking the p75 receptor.** *Neurosci Lett* 1999, **272**:191-194.

107. Chandler CE, Parsons LM, Hosang M, Shooter EM: **A monoclonal antibody modulates the interaction of nerve growth factor with PC12 cells.** *J Biol Chem* 1985, **259**:6882-6889.

108. Kaplan DR, Miller FD: **Signal transduction by the neurotrophin receptors.** *Curr Opin Cell Biol* 1997, **9**:213-221.

109. Kouhara H, Hadari YR, Spivak-Kroizman T, Schilling J, Bar-Sagi D, Schlessinger J: **A lipid-anchored Grb2-binding protein that links FGF-receptor activation to the Ras/MAPK signaling pathway.** *Cell* 1997, **89**:693-702.

110. Meakin SO, MacDonald JIS, Gryz EA, Kubu CJ, Verdi JM: **The signaling adapter FRS-2 competes with Shc for binding to the nerve growth factor receptor TrkA.** *J Biol Chem* 1999, **274**:9861-9870.

111. •• Qian X, Riccio A, Zhang Y, Ginty DD: **Identification and characterization of novel substrates of Trk receptors in developing neurons.** *Neuron* 1998, **21**:1017-1029.
Identification of rAPS and SH2-B as Trk interacting proteins involved in modulating NGF-dependent survival signalling.

112. Yamashita H, Avraham S, Jiang S, Dikic I, Avraham H: **The Csk homologous kinase associates with TrkA receptors and is involved in neurite outgrowth of PC12 cells.** *J Biol Chem* 1999, **274**:15059-15065.

113. Wright JH, Drueckes P, Bartoe J, Zhao Z, Shen S-H, Krebs EG: **A role for the SHP-2 tyrosine phosphatase in nerve growth factor-induced PC12 cell differentiation.** *Mol Biol Cell* 1997, **8**:1575-1585.

114. Corbic KC, Foster DA, Rosner MR: **Protein kinase Cδ mediates neurogenic but not mitogenic activation of mitogen-activated protein kinase in neuronal cells.** *Mol Cell Biol* 1999, **19**:4209-4218.

115. Yamada M, Ohnishi H, Sano S, Nakatani A, Ikeuchi T, Hatanaka H: **Insulin receptor substrate (IRS)-1 and IRS-2 are tyrosine phosphorylated and associated with phosphatidylinositol 3-kinase in response to brain-derived neurotrophic factor in cultured cerebral cortical neurons.** *J Biol Chem* 1997, **272**:30334-30339.

116. Bazenet CE, Mota MA, Rubin LL: **The small GTP-binding protein Cdc42 is required for nerve growth factor withdrawal-induced neuronal death.** *Proc Natl Acad Sci USA* 1998, **95**:3984-3989.

117. • Kanamoto T, Mota M, Takeda K, Rubin LL, Miyazono K, Ichijo H, Bazenet CE: **Role of apoptosis signal-regulating kinase in regulation of the c-Jun N-terminal kinase pathway and apoptosis in sympathetic neurons.** *Mol Cell Biol* 2000, **20**:196-204.
Demonstrates that Ask1 is a crucial element of NGF withdrawal-induced death of sympathetic neurons and PC12 cells.

118. Eilers A, Whitfield J, Babij C, Rubin LL, Ham J: **Role of the jun kinase pathway in the regulation of c-Jun expression and apoptosis in sympathetic neurons.** *J Neurosci* 1998, **18**:1713-1724.

119. •• Le-Niculescu H, Bonfoco E, Kasuya Y, Claret F-X, Green DR, Karin M: **Withdrawal of survival factors results in activation of the JNK pathway in neuronal cells leading to Fas ligand induction and cell death.** *Mol Cell Biol* 1999, **19**:751-763.
Definitively demonstrates that a JNK-c-jun-Fas ligand pathway is an important mediator of stress-induced neuronal apoptosis in PC12 cells and cerebellar neurons.

120. Park DS, Levine B, Ferrari G, Greene LA: **Cyclin dependent kinase inhibitors and dominant negative cyclin dependent kinase 4 and 6 promote survival of NGF-deprived sympathetic neurons.** *J Neurosci* 1997, **17**:8975-8983.

121. Sherr CJ: **Tumor surveillance via the ARF-p53 pathway.** *Genes Dev* 1998, **12**:2984-2991.

122. •• Kuan C-Y, Yang DD, Roy DRS, Davis RJ, Rakic P, Flavell RA: **The Jnk1 and Jnk2 protein kinases are required for regional specific apoptosis during early brain development.** *Neuron* 1999, **22**:667-676.
Shows that mice lacking Jnk1 and Jnk2 exhibit dysregulated neuronal apoptosis in various developing neural structures.