Review

Control of the cell cycle by neurotrophins: lessons from the p75 neurotrophin receptor

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Summary. Although traditionally little attention has been paid to the interplay between neurotrophins and the cell cycle, a number of recent findings suggest an important role for these growth factors in the regulation of this aspect of the cellular physiology.

In this article, we review the evidence from a number of studies that neurotrophins can influence cell cycle progression or mitotic cycle arrest both in the nervous system as well as in other cell types. The contrary response of different cells to neurotrophins in terms of cell cycle regulation derives in part from the fact that these factors use two different receptor types to transmit their signals: members of the Trk family and the p75 neurotrophin receptor ($p75^{NTR}$). With this in mind, we outline the current state of our knowledge regarding the molecular basis underlying the control of cell cycle progression by neurotrophins. We focus our interest on the receptors that transduce these signals and, in particular, the striking finding that $p75^{NTR}$ interacts with proteins that can promote mitotic cycle arrest. Finally, we discuss the mechanisms of cell death mediated by $p75^{NTR}$ in the context of cell cycle regulation.

Key words: Trk, differentiation, cell cycle, apoptosis, cancer

Introduction

A prominent feature of the mature vertebrate nervous system is that the main cellular components, the neurons, are postmitotic. This implies that precursors that proliferate vigorously during the initial stages of development, must withdraw from the cell cycle in order to differentiate. Both proliferation and cell cycle arrest are tightly regulated by a number of intrinsic and extrinsic signals that include proneural and neurogenic gene products as well as other extracellular factors. Although traditionally, little attention has been paid to

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the interplay between neurotrophins and cell cycle regulation, recent advances suggest that these growth factors fulfill critical roles in the control of the mitotic cycle, both in the nervous system and also in other cell types.

The neurotrophin family is made up of non-covalently-associated, homodimeric proteins: namely, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin3 (NT3), and neurotrophin4/5 (NT4/5). For many years, these molecules have been known to be key players during the development of the nervous system. Thus, they are involved in regulating survival and death as well as controlling synaptic plasticity and the maintenance of neural networks (for recent reviews, see Bibel and Barde, 2000; Huang and Reichardt, 2001). However, the molecular basis underlying these functions is still not fully understood. Indeed, understanding the behavior of neurotrophins is complicated by the fact that their signals are mediated through two different types of receptors: the members of the tropomyosin receptor kinase (Trk) family, and the p75 neurotrophin receptor ($p75^{NTR}$).

The Trk family of receptor tyrosine kinases (RTKs) is made up of three different neurotrophin receptors, each with a different specificity: TrkA binds to NGF, TrkB to BDNF and NT4/5, and TrkC binds to NT3 (reviewed by Bothwell, 1995). These receptors are monomeric proteins with two immunoglobulin (Ig) domains in the extracellular region, and a tyrosine kinase domain located in the cytoplasmic tail (Fig. 1). The binding of neurotrophins to the Ig domains triggers dimerization of the receptor and activation of the kinase, thus initiating signal transduction (see below). Importantly, both the TrkB and TrkC genes undergo alternative splicing and can produce different isoforms with truncations or insertions in the tyrosine kinase domain (Klein et al., 1990; Lamballe et al., 1993). These alterations confer distinct biological properties to the receptors.

$p75^{NTR}$ is a member of the tumor necrosis factor receptor family and it is expressed by a variety of neuronal and non-neuronal cell types. Structurally,

p75^NTR is a single transmembrane receptor with an intracellular tail that contains a type II death domain (Fig. 2). Initially, p75^NTR was identified as a receptor for NGF (Johnson et al., 1986; Radeke et al., 1987) but as other members of the neurotrophin family were described, it became clear that it was able to bind all the neurotrophins (Rodríguez-Tébar et al., 1990, 1992). The transcendence of the identification of p75^NTR as a universal neurotrophin receptor was devalued by the lack of any obvious catalytic motif within its cytoplasmic domain that could mediate its cellular function. Indeed, when the members of the Trk family were shown to specifically bind and transmit the trophic signals initiated by the neurotrophins (reviewed by Barbacid, 1994), p75^NTR was considered as a secondary player in studies of the neurotrophin family. It was not until the mid-nineties that it became clear that p75^NTR itself can transmit signals and that it fulfilled specific functions during both development as well as in adulthood. Indeed, it has been associated with the processes of axonal outgrowth (Yamashita et al., 1999; Bentley and Lee, 2000), Schwann cell migration (Anton et al., 1994; Bentley and Lee, 2000), and the most studied effect, the induction of cell death (for a recent review, see Miller and Kaplan, 2001).

In the following pages, we will discuss recent findings that highlight the importance of neurotrophins and in particular p75^NTR-mediated signals in the regulation of the mitotic cycle both in the nervous system as well as in other cell types. We will also discuss the mechanisms controlling neurotrophin and p75^NTR-mediated cell death in the context of cell cycle regulation.
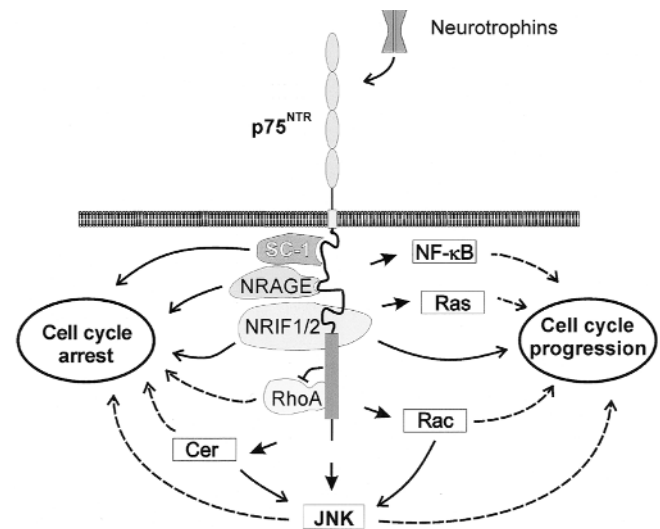
Neurotrophins and cell cycle regulation

Neurotrophin-dependent regulation of the mitotic cycle in non-neural cells

During recent years, many reports have been published describing the mitogenic effects of neurotrophins in tumor cell lines and primary cultures of non-neural origin. While in some cases there is no indication as to which neurotrophin receptor might be mediating this effect (e.g. NGF-dependent changes in DNA synthesis in rat splenic mononuclear cells; Thorpe
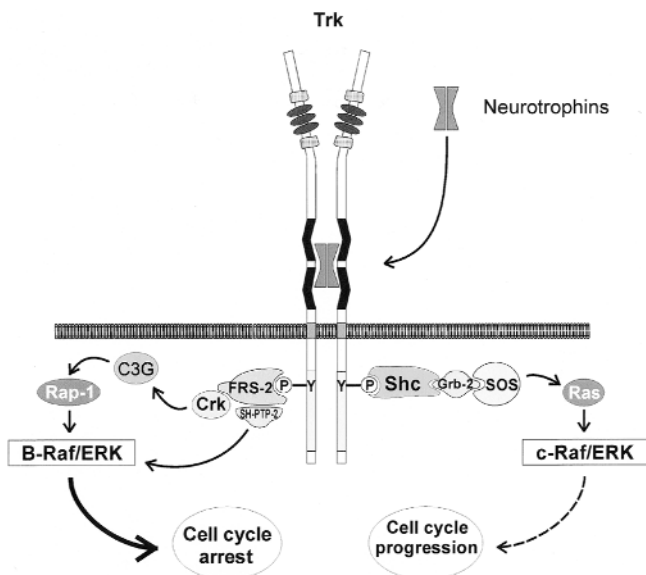
Fig. 1. Structure of Trk receptors and their signal transduction pathways regulating cell cycle progression. Trk receptors are transmembrane proteins containing three characteristic tandem leucine-rich motifs (dark grey ovals) flanked by two cysteine clusters (light grey rectangles) and two immunoglobulin domains (black segments) in the extracellular region. They have one single transmembrane motif (dark grey segment) and one tyrosine kinase domain (light grey segment) located in the cytoplasmic tail. Upon binding to their neurotrophin ligands, Trk receptors dimerize and activate their tyrosine kinase domain, thus becoming autophosphorylated on specific tyrosine residues. Phosphorylated Y490 (Y; in the human sequence) plays an important role in the initiation of signaling cascades that end up regulating the cell cycle. Depending on the adaptor proteins that bind to Y490, Trk receptors may transduce cell cycle promotion or cell cycle arrest (see text). Thick arrow: prolonged activity, dashed arrow: transient activity.

Fig. 2. Structure of p75^NTR and signal pathways initiated upon its activation by neurotrophins which may end up in cell cycle regulation. p75^NTR is a transmembrane receptor characterized by an extracellular domain containing four cysteine repeats (ovals), a single transmembrane domain (small rectangle), and an intracellular tail containing a highly conserved juxtamembrane motif (black line), and a class II death domain (large rectangle). Binding of neurotrophins to p75^NTR stimulates a number of pathways depending on the cellular context. A number of proteins have been described that interact with the intracellular domain of p75^NTR and that are involved in the regulation of the cell cycle (SC-1, NRAGE, NRIF1/2). RhoA can also interact with the intracellular domain of p75^NTR and might indirectly regulate the cell cycle (dashed arrow). Additional pathways initiated by p75^NTR, and indicated with short arrows, may indirectly end up in cell cycle regulation (dashed arrows). These pathways include the activation of ceramide (Cer), c-Jun N-terminal kinase (JNK), Rac, NF-kB, and Ras (see text).

and Perez-Polo, 1987), in the majority of cases, neurotrophin-dependent mitogenic activity is related to the expression of Trk receptors. This fact is not surprising since the first member of this family, TrkA, was originally isolated as an oncogene from a colon carcinoma. The transformation of TrkA involves the fusion of the extracellular portion of the TrkA protein with that of tropomyosin, leading to the constitutive activation of the protein kinase (Martín-Zanca et al., 1986). As we will discuss below, the molecular basis for the mitogenic capacity of the Trk receptors probably derives from their interactions with the many intracellular signaling pathways that are regulated by mitogens.

In the light of this, it is therefore not surprising that one of the first biological responses attributed to the Trk receptors was their mitogenic effects. In fibroblasts, cells that do not normally express either p75^{NTR} or Trk receptors, exposure to neurotrophins elicits a growth response if expression of a Trk receptor is induced (Cordon-Cardo et al., 1991; Glass et al., 1991; Klein et al., 1991; Lamballe et al., 1991; Soppet et al., 1991). This demonstrates the potential of these molecules to promote proliferative signals and subsequently, many other examples of the mitogenic effects of Trk activation have been reported.

The mitogenic effects of neurotrophins can also be seen when NGF induces proliferation mediated by TrkA, in human breast cancer cells, primary human myoblasts, and thecal cells from antral ovarian follicles (Dissen et al., 2000; Rende et al., 2000; Chiarenza et al., 2001; Descamps et al., 2001). In addition, concentrations of NGF that activate only high-affinity receptors have been shown to promote proliferation in human B lymphocytes and lymphoblastoid B-cell lines cultured in vitro (Otten et al., 1989; Kimata et al., 1991). NGF is also capable of triggering proliferation in corneal cells (You et al., 2000). In vivo, overexpression of truncated forms of TrkC lacking its tyrosine kinase domain prevents proliferation of cardiac myocytes, suggesting that TrkC might promote mitogenic effects during early heart development (Lin et al., 2000). In addition, BDNF, NT3 and NT4/5 all promote proliferation in murine skin (Botchkarev et al., 1999). However, whether or not Trk receptors are mediators of this latter effect remains uncertain.

In contrast, the presence of p75^{NTR} seems to trigger withdrawal from the cell cycle in some tumor cell lines that show a mitogenic response to neurotrophins. This indicates that, as previously described for pro-apoptotic signalling (Yoon et al., 1998; Davey and Davies, 1998) and in neurite outgrowth (Kohn et al., 1999), p75^{NTR} produces an opposite effect to that of the Trk receptors in terms of cell cycle progression. This has been demonstrated in some pancreatic cancer cell lines whose response to NGF, either mitogenic or anti-mitogenic, depends on the balance of TrkA and p75^{NTR} expression (Zhu et al., 2001). In these cells, NGF-induced mitogenesis is associated with TrkA phosphorylation,

whereas those cell lines with low TrkA levels and high or moderate p75^{NTR} expression respond to NGF by reducing their growth. A similar effect was also observed in a human prostate epithelial tumor cell line, which expresses TrkA and responds to NGF by promoting mitogenesis. This NGF-dependent growth response can be prevented in this cell line by the stable expression of p75^{NTR} (Pflug and Djakiew, 1998).

Neurotrophin-dependent regulation of the mitotic cycle in cells of neural origin

As well as in non-neural cells, there is increasing evidence that neurotrophins can provide either positive or negative mitogenic signals in neural cells. In vitro, NGF induces mitogenesis in chromaffin cells from young rats (Lillien and Claude, 1985), cochleovestibular ganglia isolated from chick embryos (Represa and Bernd, 1989), precursor cells from the rat striatum (Cattaneo and McKay, 1990), and cerebellar neuroblasts (Confort et al., 1991). Additionally, NT3 is mitogenic in cultures of quail neural crest cells (Kalcheim et al., 1992), and addition of exogenous NT4/5 to purified cultures of oligodendrocyte/type-2 astrocyte progenitors maintained in an undifferentiated state results in an increase in proliferation (Scarisbrick et al., 2000). In vivo, further examples of mitogenic signals initiated by neurotrophins can be found, although in these cases indirect mechanisms cannot be ruled out. Hence, both NGF and NT3 have been shown to stimulate cell proliferation in premigratory precursors of cerebellar granule neurons (Muller et al., 1994; Katoh-Semba et al., 2000). Moreover, the presence of fibroblasts producing BDNF or NT3 augmented the number of BrdU-positive oligodendrocytes following spinal cord injury (McTigue et al., 1998).

Anti-mitogenic signals that induce cell cycle withdrawal can also be observed upon treatment with neurotrophins in cells of neural origin. Perhaps the best documented example of cell cycle withdrawal induced by a neurotrophin is the classical differentiation of PC12 cells in response to NGF (Greene and Tischler, 1976). When these cells are exposed to NGF, cell division is inhibited, resulting in the cells becoming large, rounded, and developing abundant neurite networks. Additionally, in cultures of rat cortical precursor cells, exogenous NT3 appears to suppress proliferation and promote neuronal differentiation (Ghosh and Greenberg, 1995). In the chick retina, NT3 is involved in the withdrawal from the cell cycle of precursors that differentiate as G4-positive neurons (de la Rosa et al., 1994). Furthermore, inhibition of NT3 activity in the embryonic chick retina in vivo results in a reduction in neuronal differentiation and the maintenance of mitotic precursors (Bovolenta et al., 1996). In vivo, application of NT3 early in development leads to a marked decrease in the number of proliferating neuroblasts in sensory ganglia (Ockel et al., 1996).

Efforts have been made to identify which neurotrophin receptors are responsible for either the

positive or negative effects on cell cycle progression. This ongoing analysis seems to indicate that cell cycle regulation by both Trk and p75^{NTR} in neural cells is much more flexible than in those cells of non-neural origin. Thus, Trk activity can either prevent or promote mitogenesis, and the same can be seen for p75^{NTR}.

Several examples can be found of proliferation induced by neurotrophins and mediated by Trk receptors. Exposure of cultured human Müller glial cells to NGF in serum-free conditions provokes a concentration-dependent increase in cell number and BrdU incorporation. This effect is inhibited by K252α, a specific antagonist of the family of neurotrophin-linked RTKs, suggesting that TrkA is involved (Ikeda and Puro, 1994). Proliferation is also potentiated by NGF in glioblastoma multiforme cell lines, where TrkA and p75^{NTR} are both expressed, and this effect is also blocked by K252α (Singer et al., 1999). However, the high dose of NGF that was used in this study might undermine the relevance of these data. All neurotrophins are capable of inducing proliferation in Y-79 neuroblastoma cells through the activation of Trk receptors (Wagner et al., 2000), and oligodendrocyte precursors can respond to NT3 through TrkC by increasing the expression of proteins involved in S-phase entry (Kumar et al., 1998). Furthermore, the mitogenic effect of platelet-derived growth factor is consistently potentiated by NT3 in optic nerve oligodendrocyte precursors, but not in spinal cord oligodendrocyte precursors that do not express TrkC (Barres et al., 1994; Robinson and Miller, 1996). These responses to NT3 were sensitive to the presence of K252α (Kumar et al., 1998).

Examples where different Trk receptors expressed by a particular cell type can trigger distinct mitogenic effects can also be found in the literature. Thus, TrkB induces the growth of SH-SY5Y neuroblastoma cells when activated by BDNF, while NGF inhibits growth when these same cells are transfected with its receptor TrkA (Eggert et al., 2000). Similarly, Trk receptors seem to initiate antimitotic responses in other cells of neural origin. Thus, TrkA is involved in NGF-induced growth arrest of PC12 cells since the NGFΔ9/13 mutant protein which binds poorly to TrkA and therefore, does not stimulate kinase activity, does not cause the cell cycle arrest that is seen with native NGF (Hughes et al., 2001). In addition, non-catalytic TrkC isoforms generated by alternative splicing have been implicated in cell cycle withdrawal and differentiation. This is consistent with the expression pattern reported for the mouse TrkC NC2 isoform in the developing nervous system, which is associated with regions undergoing abundant neuronal differentiation. TrkC NC2 expression coincides with the exit of neuronal progenitors from the cell cycle and it is maintained in differentiated cerebellar neurons in conjunction with p75^{NTR} expression (Menn et al., 1998, 2000). In addition, a truncated form of TrkC is also involved in neural crest differentiation in collaboration with p75^{NTR} (Hapner et al., 1998). These studies suggest

that p75^{NTR}, in collaboration with truncated TrkC, might be involved in providing the signals for cell cycle withdrawal that are associated with neuronal differentiation. Interestingly, infection of early chick retinas with retroviral vectors that over-express a truncated isoform of TrkC produces clones with a significant reduction in their size and mainly composed of neural phenotypes that correspond to those born at the early stages of retina development (Prada et al., 1991; Das et al., 2000). This suggests that the presence of exogenous truncated TrkC triggers cell cycle withdrawal and differentiation in this tissue. Similar truncated isoforms of TrkC are observed in the vertebrate retina (Valenzuela et al., 1993), suggesting that endogenously-expressed truncated TrkC might be involved in cell cycle arrest and differentiation, maybe through its interaction with NT3, a neurotrophin which promotes neurogenesis in the chick retina both in vivo and in vitro (de la Rosa et al., 1994; Bovolenta et al., 1997). In addition, p75^{NTR} might cooperate in provoking the cell cycle arrest and differentiation promoted by NT3 since this receptor is expressed by the first phenotypes produced in the chick retina (von Bartheld et al., 1991). The possibility that over-expression of truncated TrkC competes with endogenous full length TrkC, inhibiting a putative proliferative effect of this receptor cannot be ruled out, although it seems unlikely (Das et al., 2000). At concentrations at which NT3 binds to its high affinity receptor, proliferation is not induced in cultured retinal precursors (de la Rosa et al., 1994).

As is the case for non-neural cell types, p75^{NTR} can sometimes also transmit proliferation signals in neural cells. Thus, in vivo, NGF augments proliferation in primary sympathetic ganglia of chick embryos, presumably by acting through p75^{NTR} since TrkA has not been detected in the avian sympathetic ganglia until later on in development (Goldstein et al., 1997). Although the presence of NGF also reduced the low level of cell death that is observed in sympathetic ganglia in vivo, the effect of NGF on proliferation was apparent owing to the twofold increase in the proportion of cells in S-phase.

One explanation of the pro-mitogenic vs. anti-mitogenic effects of p75^{NTR} and/or Trk receptors might lie in the cell cycle-dependent expression of these receptors. Thus, p75^{NTR} protein has been reported to be increased on the surface of neural crest-derived cells during mitosis in vivo (Young, 2000). In contrast, PC12 cells present higher superficial levels of p75^{NTR} in their surface at late G1, S and G2, whereas TrkA is more highly expressed in M-phase and early G1 (Urdiales et al., 1998). In PC12 cells, NGF triggers differentiation during the G1/G0 phase, confirming that TrkA plays a crucial role in cell cycle withdrawal (see above), whereas at other phases of the cell cycle when p75^{NTR} is predominantly expressed at the cell surface, NGF provokes survival (Urdiales et al., 1998). It is thus conceivable that, in a particular cell type, the decision to progress or withdraw from the mitotic cycle could be

mediated by the neurotrophin receptor, Trk or p75^{NTR}, which is being expressed at the time when the decision takes place, normally at G1/G0.

Molecular basis for cell cycle regulation dependent on neurotrophins

Trk-dependent signals regulating the cell cycle

Neurotrophin binding to Trk receptors results in dimerization and activation of their tyrosine kinase domain, resulting in autophosphorylation of conserved tyrosines in the cytoplasmic tail. Seven of these phospho-tyrosines create docking sites for adaptor proteins that couple these receptors to intracellular signaling cascades, including the extracellular signal-regulated kinase (ERK) pathway, the phosphatidyl-inositol-3-OH kinase/Akt kinase pathway, and the phopholipase C-γ pathway (reviewed by Kaplan and Miller, 2000; Patapoutian and Reichardt, 2001). These signaling cascades are responsible for the multiplicity of cellular events controlled by the Trk receptors.

In a similar manner to other RTKs, Trk receptors can provide signals to stimulate proliferation, or cell cycle withdrawal and differentiation, depending on the cellular context in which the transduction pathway is activated (Marshall, 1995; Grewal et al., 1999; Meakin et al., 1999). The molecular basis that determines whether exposure to neurotrophins results in proliferation or cell cycle arrest seems to depend on whether there is a transient or prolonged activation of the ERK pathway (Marshall, 1995; Patapoutian and Reichardt, 2001). In each case, the recruitment of different adaptor proteins to the phosphotyrosine Y490 in the human sequence initiates defined signal transduction cascades (Fig. 1).

Signals triggering cell cycle progression upon Trk activation seem to depend on Shc recruitment to Y490. This interaction is subsequently translated into the recruitment of the Grb-2 and Ras exchange factor Son of sevenless (SOS), which in turn promotes transient activation of Ras (Borrello et al., 1994; York et al., 1998), thereby triggering the c-Raf/ERK pathway and proliferation (York et al., 1998). Alternatively, the prolonged activation of ERK can be initiated by recruitment of the adaptor protein fibroblast growth factor receptor substrate (FRS)-2 to Y490 (Meakin et al., 1999). FRS-2 is then phosphorylated, thereby creating docking sites for adaptor proteins such as Crk and Src-homology protein kinase phosphatase (SH-PTP)-2 (Meakin et al., 1999). Once bound to FRS-2, Crk binds and activates the guanine nucleotide exchange factor C3G (Nosaka et al., 1999), which in turn activates the small G protein Rap-1. Rap-1 activates B-raf and ERK signaling in a prolonged manner (York et al., 1998; 2000), thus promoting cell cycle withdrawal and differentiation. Prolonged activation of ERK seems also to be facilitated by other proteins like SH-PTP-2 (Wright et al., 1997).

A further degree of complexity to be added to the control of cell cycle via Trk receptors relates to the integrity of their tyrosine kinase homology region. Thus, splice variants of TrkC with amino acid insertions in the kinase domain do not mediate the mitogenic activity of NT3 in fibroblasts (Valenzuela et al., 1993; Guiton et al., 1995). The presence of these insertions is manifested by the lack of high affinity binding of Shc and phospholipase C-γ to the TrkC variants, and this interferes with the biological response to NT3 in fibroblasts (Guiton et al., 1995). Additionally, the signaling triggered by Trk receptors in terms of cell cycle regulation is also dependent on functional cross-talk between Trk and p75^{NTR} (see below).

p75^{NTR}-dependent signals regulating the cell cycle

We have seen that p75^{NTR} can regulate the cell cycle in both cells of neural and non-neural origin. Possible mechanisms that might underlie this behavior have been highlighted recently. Proteins that interact with the intracellular domain of p75^{NTR} and second messengers that are activated by this receptor and that are capable of regulating the cell cycle have been described.

Two such proteins are the neurotrophin receptor interacting factor (NRIF)1 and NRIF2, zinc finger-containing proteins that interact with the intracellular domain of p75^{NTR} and that can influence the mitotic cycle (Casademunt et al., 1999; Benzel et al., 2001). In the C57/BL6 mouse strain, the functional null mutant of the *nrif1* gene results in significantly smaller embryos which do not survive beyond E12, a phenotype that is consistent with cell cycle arrest (Benzel et al., 2001). Strikingly, when either NRIF1 or NRIF2 are transiently expressed in human embryonic kidney 293T cells, the rate of proliferation is also markedly decreased (Benzel et al., 2001) suggesting that quantitative changes in the levels of both NRIF molecules can interfere with the progression of the mitotic cycle.

Another zinc finger protein that interacts with the intracellular tail of p75^{NTR} is SC-1. This protein contains six zinc finger motifs and one positive regulatory (PR) domain, previously identified as a common domain in several transcription factors including the tumor suppressor, retinoblastoma-interacting zinc finger (RIZ) protein. SC-1 interacts with p75^{NTR} and in response to the specific activation of this receptor by NGF, it translocates to the nucleus in COS cells, an effect that is blocked by the expression of TrkA (Chittka and Chao, 1999). The presence of SC-1 in the nucleus correlates with the loss of BrdU incorporation, indicating that this protein is a clear candidate for mediating the anti-proliferative effects triggered by the activation of p75^{NTR}. Disrupting the translocation of SC-1 to the nucleus may represent a mechanism to prevent growth arrest in cells co-expressing both TrkA and p75^{NTR} and as such provide a means by which TrkA can promote mitogenesis (see above).

A further p75^{NTR} interacting protein that has recently been implicated in cell cycle regulation is the

neurotrophin-receptor-interacting MAGE homolog, NRAGE (Salehi et al., 2000). This protein is a member of the melanoma antigen (MAGE) family of proteins, initially characterized as precursors of a number of cell-surface antigens expressed by tumor cells. NRAGE induces cell cycle arrest when over-expressed in human embryonic kidney 293 cells. Within the developing CNS, NRAGE colocalizes with p75^{NTR} in the mantle zone, a region where neurons are born, suggesting that NRAGE might participate in the mechanisms that control the arrest of growth that takes place during neurogenesis. Interestingly, TrkA seems to compete with NRAGE for the same binding site in p75^{NTR}. Thus, over-expression of NRAGE blocks the physical association of p75^{NTR} with TrkA (Salehi et al., 2000), again emphasising the opposing interactions between these two receptors.

The small GTPase RhoA interacts with, and is activated by the cytoplasmatic domain of p75^{NTR}, in the absence of neurotrophin binding. Upon interacting with neurotrophins, RhoA is inactivated, thus promoting axonal outgrowth (Yamashita et al., 1999). Interestingly, RhoA is known to be involved in controlling the formation of the cytoskeletal ring during cytokinesis, presumably by activation of citron kinase, Rho kinase I/II, formin-homology proteins, and a regulatory subunit of a myosin phosphatase (for a review, see Glotzer, 2001). In addition, RhoA is required for the expression of cyclin D1 in mid-G1, through a mechanism involving sustained ERK activity (Welsh et al., 2001), a finding that suggests a new link between the cell cycle and signals derived from Trk and p75^{NTR} activation. The inhibition of RhoA upon interaction of neurotrophins with p75^{NTR}, might therefore be part of the complex molecular process involved in cell cycle arrest and the morphological differentiation observed in different cells upon activation of this receptor.

Some second messengers such as ceramide, which are generated upon p75^{NTR} activation might also influence cell cycle progression (Dobrowsky et al., 1994; Dobrowsky and Carter, 1998). Numerous studies have demonstrated that ceramide is able to trigger G1/G0 arrest by inducing hypophosphorylation of Rb (Dbaibo et al., 1995; Venable et al., 1995). This may result from cyclin-dependent kinase (CDK) 2 inhibition by the ceramide-activated protein phosphatase (CAPP; Dobrowsky et al., 1993; Galadari et al., 1998; Lee et al., 2000), and the expression of the CDK inhibitor p21cip1/waf1 (Alesse et al., 1998; Kim et al., 2000; Lee et al., 2000). Another target of ceramide is the c-jun N-terminal kinase (JNK), which has been shown to participate in ligand-dependent apoptosis induced by p75^{NTR} (see Kaplan and Miller, 2000). Interestingly, there is increasing evidence that stress kinases like JNK are involved in either promoting or preventing cell-cycle progression (for a recent review, see Pearce and Humphrey, 2001), thus suggesting another potential link between p75^{NTR} signalling and the regulation of the cell cycle.

Several studies have highlighted the association between cell survival and the activation and nuclear translocation of NF-κB as the result of NGF activating p75^{NTR} (Carter et al., 1996; Bhakar et al., 1999; Cosgaya and Shooter, 2001). Members of the NF-kB family are known to potentiate the proliferative response to mitogenic factors, and the best explored link between NF-kB activation and cell cycle progression involves transcriptional activation of the *cyclin D1* gene (for a review see Joyce et al., 2001). Therefore, NF-κB might participate in the positive modulation of the cell cycle observed on activation of p75^{NTR}. Thus, depending on the prevalence of the NF-κB or ceramide/JNK pathways, the response to p75^{NTR} activation might involve either proliferation or cell cycle arrest, respectively. This situation is reminiscent of the cell death/survival signals regulated by p75^{NTR}, which have also been shown to depend on the activation of either of these second messengers (see Kaplan and Miller, 2000).

Another small GTPase that has been shown to mediate responses to p75^{NTR} is the protooncogene Rac. Rac is activated by the interaction of NGF with p75^{NTR} and, by activating JNK, promotes cell death in cortical oligodendrocytes (Harrington et al., 2002). Interestingly, Rac has been implicated in cell cycle regulation, mainly as a result of integrin-mediated cell adhesion inducing cyclin D1 or as a downstream effector of Ras (reviewed by Ridley, 2001). Therefore, Rac might be another potential candidate for the mitogenic effects promoted by p75^{NTR}.

Finally, some evidence exists that p75^{NTR} and Trk receptors can share signaling pathways that regulate the cell cycle. Thus, the stimulation of p75^{NTR} with NGF has been shown to activate Ras and induce a fast and transient phosphorylation of p44 MAPK and p42 MAPK (ERK1 and ERK2, respectively) in P2 rat cerebellar cultures and PCNA cells (Susen et al., 1999). In this case, the NGF-mediated signals were independent of TrkA which is not expressed in these cells. Thus, it can be inferred from these findings that a signal transduction pathway based on the transient activation of ERK, known to result in a proliferative response when initiated by Trk receptors (see above), could also represent an alternative mechanism by which p75^{NTR} promotes cell cycle progression.

Cell cycle regulation and apoptosis mediated by p75^{NTR}

The recent findings demonstrating that p75^{NTR} can interact with molecules involved in cell cycle regulation raises the intriguing possibility that in some cases the known apoptotic effect of p75^{NTR} may be secondary to conflicting signals for cell division and growth arrest (O'Connor et al., 2000). This possibility may be particularly important in cases where postmitotic neurones either lack antiproliferative signals or receive proliferative inputs.

Cumulative evidence indicates that once a neuronal

precursor has withdrawn from the mitotic cycle, re-entry is not allowed and, that if the control of this process breaks down, the cell dies (Liu and Greene, 2001). Recent data also suggest that the mechanisms to prevent re-entry into the cell cycle of newly-born neurons depend, at least in part, on the continuous presence of neurotrophins at critical stages. Thus, when the SH-SY5Y neuroblastoma cell line is sequentially exposed to retinoic acid and BDNF in serum-free medium, cells withdraw from the cell cycle and generate a homogeneous population of cells with a neuronal morphology. Cell survival is therefore dependent on the continuous presence of BDNF, and removal of this neurotrophin induces cell death which appears to result form the attempt to re-enter the cell cycle (Encinas et al., 2000). A similar situation is observed in vivo when dorsal root ganglion neurons, in the absence of NT3, fail to detain the cell cycle, override the G1 restriction point, and die in S phase after having upregulated several proteins required for entry into the cell cycle (ElShamy et al., 1998). Other studies have reached similar conclusions. Thus, survival of sympathetic neurons in culture is dependent on a permanent supply of NGF, which mediates its effects through the activation of TrkA, a receptor highly expressed by this neuronal population. In the absence of NGF, these neurons die concomitant with the upregulation of cyclin D1 (Freeman et al., 1994). Inhibitors of cyclin-dependent kinases as well as dominant negative forms of CDK4/6 are able to prevent their death (Park et al., 1996, 1997), suggesting that the apoptotic process is a consequence of an attempt to re-enter the cell cycle. Sympathetic neurons can be maintained alive in the absence of NGF by treatment with KCl and, under these circumstances, BDNF can trigger $p75^{NTR}$-mediated apoptosis in these neurons (Bamji et al., 1998). Whether or not this effect is the result of sympathetic neurons attempting to re-enter into the cell cycle, as occurs after NGF deprivation in the absence of KCl, is unknown. Nevertheless, this might well be the case, as the signals provoked by BDNF treatment are similar to those observed after NGF-deprivation. In both cases the activation of the JNK-p53-Bax pathway has been shown to be necessary for the induction of cell death (Aloyz et al., 1998).

An additional example of cell death regulated by neurotrophins in the context of cell cycle regulation comes from studies in the developing chick retina. In this tissue, the presence of BDNF can prevent apoptosis in newly-born neurons that die during early stages of development as a consequence of the interaction of NGF with $p75^{NTR}$ (Frade et al., 1996, 1997). The antiapoptotic effect of BDNF is likely to be triggered by TrkB as this receptor is expressed early in retinal development (Frade et al., 1997). When retinal precursor cells are cultured without BDNF in conditions that favor differentiation, a percentage of these cells express the neuronal marker G4 and die in the presence of NGF as a consequence of $p75^{NTR}$ activation. This induction of apoptosis is prevented by BDNF, and occurs after a rise of cyclin B2 levels and in the presence of mitotic figures amongst the G4-positive neurons (Frade, 2000). Interestingly, BDNF prevents both the ectopic expression of cyclin B2 and cell cycle re-entry in post-mitotic, NGF-treated neurons expressing G4 (Frade, 2000), and the effect of BDNF can be mimicked by drugs that prevent cell cycle progression.

A possible molecular link between the $p75^{NTR}$-dependent upregulation of cyclin B2 in retinal neurons and cell death might reside in the activation of the tumor suppressor gene p53, as described for sympathetic neurons. Indeed, the inappropriate activation of cell cycle regulatory molecules is known to induce the expression of p19ARF, which subsequently activates p53 and leads to apoptosis via a pathway independent of DNA damage (Sherr, 1998). Alternatively, p53 might be directly activated by JNK, a second messenger involved in $p75^{NTR}$-induced cell death which has been clearly linked to the activation of p53 in the sympathetic neuron paradigm (Aloyz et al., 1998).

Another protein that seems to participate in the induction of cell death in retinal neurons is NRIF1 (Casademunt et al., 1999; Benzel et al., 2001). The role of NRIF1 in mediating apoptosis in the mouse embryonic retina was highlighted by the significant reduction in the levels of cell death in retinas from mutant mice in which the NRIF1-binding site for $p75^{NTR}$ is absent (Casademunt et al., 1999). This effect is indistinguishable from that observed in the retina of $p75^{NTR-/-}$ and $ngf^{+/-}$ mutants (Frade and Barde, 1999).

Mice mutant for *nrif1* in the C57/BL6 genetic background are embryonically lethal due to a general failure in the progression of the cell cycle at early stages of development (Benzel et al., 2001). This fact suggests an important role for NRIF1 in mitotic cycle progression. Strikingly, *nrif1* mutants are viable in the Sv129 background, and no effects in terms of cell cycle arrest are observed in these mice. This apparent contradiction is explained by the upregulation of NRIF2 in the Sv129 background, which probably compensates for most NRIF1 functions. Nevertheless, in these mice, NRIF2 does not seem to be able to replace NRIF1 in its ability to regulate cell death since the functional depletion of NRIF1 results in a lower apoptosis in the mouse retina. A role for NRIF1 in triggering $p75^{NTR}$-dependent apoptosis in the developing mouse retina would be consistent with NRIF1 being capable of promoting $p75^{NTR}$-dependent re-entry into the cell cycle in postmitotic cells, similar to that observed in the chick retina. Nevertheless, an alternative explanation based on the pro-apoptotic effects of NRIF1, independent of its role in cell cycle regulation, cannot be ruled out.

Another link between cell death and $p75^{NTR}$-dependent regulation of the mitotic cycle is NRAGE, a molecule capable of preventing cell cycle progression. Interestingly, this molecule has been directly associated with $p75^{NTR}$-dependent apoptosis in the MAH sympathoadrenal cell line, which lacks $p75^{NTR}$ and TrkA receptors. These cells can be induced to die in the

presence of NGF only when NRAGE and p75^{NTR} are co-expressed (Salehi et al., 2000). The interaction of NGF with p75^{NTR} in PC12 cells has been shown to trigger the translocation of NRAGE from the cytoplasm to the cell membrane (Salehi et al., 2000). Therefore, it is conceivable that the diminution of the cytoplasmic pool of NRAGE induced by the interaction of NGF with p75^{NTR} might favour cell cycle progression, and this might account for cell death in cells already committed to withdraw from the mitotic cycle.

Conclusion

All the findings described above highlight the important role that the neurotrophins have in the regulation of the cell cycle, a particular aspect of the neurotrophin physiology that has not been sufficiently acknowledged in the past. The role of neurotrophins in cell cycle regulation has critical consequences for our understanding of physiological processes such as precursor cell expansion, tissue homeostasis, apoptosis, and differentiation of cells that, like neurons, are characterized by their post-mitotic status. In addition, a role of neurotrophins in regulating the cell cycle in pathogical situations like cancer and degenerative diseases can also be envisaged. Therefore, it is foreseeable that in the near future, stronger efforts will be made to deepen our understanding of the molecular events that underlie cell cycle regulation by neurotrophins. This will also help us to determine to what extent this aspect of neurotrophin physiology influences normal and pathological cellular functions.

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