A Bex-cycle built for two

Neurotrophins promote neuronal survival, but it has been recognized that they also stimulate differentiation. Indeed, in early studies by Hamburger, Levi-Montalcini and Cohen, nerve growth factor (NGF) not only promoted survival, but also caused marked neurite outgrowth when added to explants of sensory ganglia (Cohen *et al*, 1954). The ability of NGF to promote differentiation was particularly well illustrated when Greene & Tischler (1976) showed that a pheochromocytoma cell line (PC12) treated with NGF exited the cell cycle and differentiated into a sympathetic neuron-like phenotype. Since then, it has been established that the survival and differentiation effects of the neurotrophins are primarily mediated by binding to members of the Trk family of tyrosine kinase receptors (Huang & Reichardt, 2003); however, the first receptor identified for NGF was a 75-kDa protein referred to as p75 (Chao *et al*, 1986; Radeke *et al*, 1987). In the past few years, the p75 receptor has received a lot of attention because of its multifunctional signalling ability. This receptor can promote cell survival or induce apoptosis, regulate neurite outgrowth and promote peripheral myelination, depending on the specific cellular context, the expression of co-receptors and the activating ligand (Barker, 2004).

The role of p75 in promoting differentiation and cell-cycle arrest has been largely overlooked in recent years, even though this was one of the first cellular responses to be shown to result from the binding of NGF to the receptor. Long considered to be simply a co-receptor for the Trks, p75 came to be recognized as a *bona fide* signalling molecule when it was shown to activate sphingomyelinase. This resulted in the production of ceramide, which led to the differentiation of T9 glioma cells as indicated by their cell-cycle arrest and process outgrowth (Dobrowsky *et al*, 1994). Although the focus of most subsequent research was on the role of p75 in mediating apoptosis and inhibition of neurite extension, several studies suggested a link between p75 and differentiation and/or cell cycle arrest. For example, prostate tumour cells accumulate in G0/G1 after p75 expression (Khwaja *et al*, 2006), and when mutant PC12 cells—expressing very low levels of p75, but wild-type amounts of TrkA—were treated with NGF, they extended processes but failed to stop dividing (Ito *et al*, 2002). Several of the proteins identified through their ability to bind to the intracellular domain of p75 can also induce cell-cycle arrest when they are over-expressed in dividing cells. These include neurotrophin-receptor-interacting factor (NRIF; Benzel *et al*, 2001), Schwann cell factor 1 (SC1; Chittka & Chao, 1999) and the melanoma-associated antigens (MAGE) family proteins known as neurotrophin-receptor-interacting MAGE homologue (NRAGE; Salehi *et al*, 2000) and necdin (Tcherpakov *et al*, 2002). However,

no direct role for these proteins in mediating neurotrophin-induced cell-cycle arrest or differentiation has been established.

In the 22 March 2006 issue of *The EMBO Journal*, Ibanez and colleagues provide an important molecular link between the p75 receptor and the regulation of the cell cycle (Vilar *et al*, 2006). Using phage display to screen for peptides that bind to the intracellular domain of p75, they isolated a fragment of brain-expressed X-linked 1 (Bex1, also referred to as Rex3). Bex1 is a member of a protein family first identified in a screen for genes whose expression was reduced after retinoic-acid-mediated differentiation of F9 teratocarcinoma cells (Faria *et al*, 1998). Little is known about these small, 115–130 amino-acid proteins and no clues are provided by their sequence. Six family members have been identified in mammals, although Bex5 has only been found in humans, and all are expressed at high levels in the brain (Alvarez *et al*, 2005). Interestingly, Bex3, also referred to as p75NTR-associated cell death executor (NADE), has been shown to interact with the intracellular domain of p75 and to mediate an apoptotic signal from the receptor after ligand binding (Mukai *et al*, 2000). However, this finding remains controversial, as other researchers have not been able to reproduce this result using rat or human Bex3 (Alvarez *et al*, 2005; Tong *et al*, 2003).

Ibanez and colleagues show that the regulation of Bex1 stability and subcellular localization is important for the cell-cycle arrest associated with differentiation. The amount of Bex1 is dynamically regulated in PC12 cells—it is stabilized through phosphorylation by Akt in response to serum or NGF, but undergoes degradation in serum-free media. In the presence of serum, these cells remain in a proliferative state, whereas low serum or NGF treatment results in cell-cycle arrest. Thus, the effects of NGF seem puzzling at first as it stabilizes Bex1, similar to serum, but causes mitotic arrest, similar to serum withdrawal; however, this is where p75 comes into play. NGF, acting through TrkA, stimulates Akt, which phosphorylates and thereby stabilizes Bex1. When Bex1 accumulates, or is overexpressed, a large portion of the protein is localized in the nucleus, and overexpression of Bex1 prevents cell-cycle arrest. However, NGF also binds p75 and this results in nuclear export of Bex1. Therefore, the two neurotrophin receptors work together—TrkA stabilizes Bex1 and p75 translocates it out of the nucleus (Fig 1). It is interesting to speculate that Bex1 acts in the nucleus to influence the transcription of cell-cycle regulators, as its movement in and out of this organelle seems to be crucial for its effects on proliferation and arrest. Perhaps Bex1 prevents the transcription of genes such as the cell-cycle inhibitor p21, which is upregulated by TrkA (Yan & Ziff, 1995), but through p75-mediated Bex1 export, the TrkA road to mitotic arrest is cleared.

The mechanism by which p75 promotes Bex1 translocation to the cytoplasm remains unknown; however, the intracellular domain of the receptor has been detected in the nucleus (Kanning *et al*, 2003; Frade, 2005), indicating that the interaction of Bex1 with p75 might directly affect the localization of Bex1. The receptor is cleaved in the membrane by the γ -secretase complex, which releases the soluble intracellular domain. Therefore, it is plausible

Fig 1 | Neurotrophin regulation of Bex1 stability and localization. Neurotrophin binding to TrkA stabilizes Bex1 through Akt-dependent phosphorylation. At the same time, binding to p75 stimulates nuclear export of Bex1, which blocks the activation of NF-κB and allows for cell-cycle arrest. Bex1, brain-expressed X-linked 1; RIP2, receptor-interacting protein 2.

that this fragment could enter the nucleus, bind Bex1 and somehow facilitate its export.

When Bex1 is out of the nucleus, Ibanez and colleagues show that its association with p75 prevents the receptor from activating the NF-κB pathway. NF-κB is a transcription factor with a range of targets and has many cellular effects, including the stimulation of proliferation (Aggarwal, 2004). When Bex1 binds to p75, this displaces receptor-interacting protein 2 (RIP2)—an adaptor protein necessary for p75 to activate NF-κB—and results in the attenuation of this pathway (Fig 1). The authors also show that overexpression of Bex1 in PC12 cells prevents NGF activation of NF-κB, whereas knocking down Bex1 with small interfering RNA enhances this activation. Although this ability of Bex1 to shut down NF-κB is consistent with NGF-induced cell-cycle arrest, it raises some questions about the role of NF-κB in survival and differentiation. Several previous studies have shown that neurotrophin activation of NF-κB promotes survival (Foehr *et al*, 2000; Gentry *et al*, 2000; Hamanoue *et al*, 1999; Maggirwar *et al*, 1998) and neurite outgrowth (Foehr *et al*, 2000; Gutierrez *et al*, 2005), which indicates that NGF would elicit a signal that inhibits the transcription factor. This paradox remains to be resolved, but there might be a key temporal aspect to NF-κB activation during neuronal differentiation. It is possible that activation is initially decreased by Bex1 in progenitor cells, allowing for cell-cycle arrest, whereas later, in mature neurons, NF-κB is activated, thereby promoting survival and neurite outgrowth.

Perhaps the most exciting aspect of the Ibanez study is the effects of Bex1 on neural stem cells and the implication that p75 might regulate their differentiation. The authors show that Bex1 messenger RNA is expressed in proliferating neural stem cells derived from the

subventricular zone of postnatal rats, but is downregulated in the differentiating cells. Furthermore, ectopic overexpression of Bex1 in these stem cells inhibited their differentiation into neurons. These results are particularly interesting in relation to recent studies that suggest a role for p75 in the differentiation of neural stem cells. The receptor is expressed by dividing cells in the subventricular zone of adult rodents, a region where neural stem cells originate (Giuliani *et al*, 2004), and in neurosphere cultures, similar to those used by Ibanez and colleagues (Hosomi *et al*, 2003). In addition, the ability of the neurotrophin brain-derived neurotrophic factor to induce neuronal differentiation was abrogated in neural stem cells derived from p75^{-/-} mice (Hosomi *et al*, 2003). Although it is not known how p75 induces the differentiation of these cells, these new data indicate that the receptor uses its ability to pull Bex1 out of the nucleus as a mechanism. It is important to understand the molecular signals that influence neuronal differentiation of neural stem cells because these cells have been highly touted for their potential therapeutic use. This new study provides some insight into this process, suggesting that neurotrophins might use Bex1 to regulate cell-cycle arrest.

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