

## Nuclear Factor- $\kappa$ B to the Rescue of Cytokine-induced Neuronal Survival

Patrik Ernfors

Laboratory of Molecular Neurobiology, Department of Medical Biochemistry and Biophysics, Karolinska Institute, S-171 77 Stockholm, Sweden

During the development of the vertebrate nervous system, a balance is maintained between the formation of neurons and their synapses and neuronal death and synaptic remodeling. Neurotrophic cytokines are known to promote the survival of certain classes of neurons during development. The signaling pathways activated by these cytokines, however, have not been defined. In this issue, Middleton et al. (2000) provides evidence that neurotrophic cytokines activate the transcription factor nuclear factor kappa B (NF $\kappa$ B), which is crucial for the survival of cytokine dependent neurons.

In many populations of developing neurons, more than half of the neurons generated die by apoptosis. This is believed to ensure that the correct number and type of neurons innervate target cells. The survival or death decision of neurons appears not to be predetermined, but is instead the consequence of the integration of multiple intracellular signaling pathways activated by external stimuli. Such external stimuli include neurotrophic factors, which are present in limiting quantities. Neurons that obtain an adequate supply of the required neurotrophic factor survive, whereas neurons that are unsuccessful in the competition die.

Neurotrophic cytokines are a family of neurotrophic factors that play an important role in regulating neuronal survival in the developing nervous system. Middleton et al. (2000) describes new results showing that neurotrophic cytokines (including ciliary neurotrophic factor [CNTF]; leukemia inhibitory factor [LIF]; cardiotrophin-1 [CT-1]; and interleukin-6 [IL-6]) activate NF $\kappa$ B and that this pathway is essential for the survival of developing sensory neurons. When the authors introduce a NF $\kappa$ B repressor (super-repressor I $\kappa$ B) into embryonic sensory neurons or culture cells lacking the NF $\kappa$ B subunit p65, the neurons show impaired survival response to cytokines. Moreover, Middleton et al. (2000) finds that p65 null mutant mice display an increased apoptosis of cytokine-dependent neurons during development *in vivo*. They therefore conclude that NF $\kappa$ B plays a key role in mediating the survival response of developing sensory neurons to cytokines.

### NF $\kappa$ B

NF $\kappa$ B is activated by numerous, diverse signals through a few common intracellular mediators. When cytokines bind to their receptors, the receptor associates with TNF recep-

tor-associated factors (TRAF) 2 or 6, which in turn activates the NF $\kappa$ B-inducing kinase (NIK) via activation of the TAT-associated kinase-1 (TAK1). NIK phosphorylates and activates I $\kappa$ B kinase (IKK) which phosphorylates the inhibitory NF $\kappa$ B binding protein I $\kappa$ B, leading to its degradation and the release and translocation of NF $\kappa$ B to the nucleus (Ninomiya-Tsuji et al., 1999, and references therein; Fig. 1). TRAF6 also mediates NF $\kappa$ B activation following the binding of NGF to the p75 neurotrophin receptor (Khursigara et al., 1999; Fig. 1).

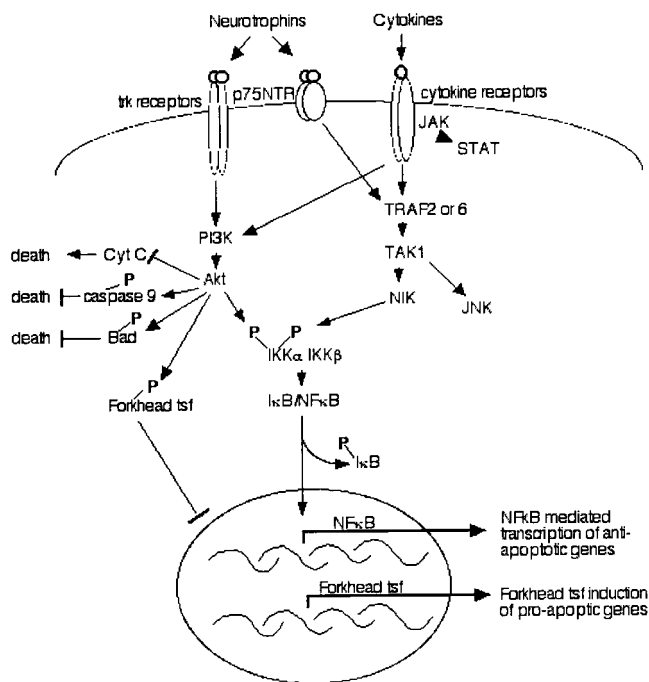
NF $\kappa$ B is activated *in vivo* in a number of different animal model systems as well as human neurodegenerative diseases. Whether the elevated NF $\kappa$ B activity contributes to cell survival or cell death has been a controversial issue. Whereas some studies report that elevated activity of NF $\kappa$ B in cerebral ischemia, oxidative stress, and excitotoxicity promote cell death of central neurons (Post et al., 1998; Schneider et al., 1999), other findings suggest it is protective against oxidative stress (Lezoualc'h et al., 1998; Yu et al., 1999) and exposure to b-amyloid (Barger et al., 1995; Kaltschmidt et al., 1999). In contrast to the conflicting results obtained in studies of pathological conditions affecting adult neurons, results on the role of NF $\kappa$ B in the developing nervous system are more consistent. NF $\kappa$ B activity protects sympathetic neurons against oxidative cell death (Lezoualc'h et al., 1998) and sensory and sympathetic neurons against trophic factor deprivation (Maggirwar et al., 1998; Hamanoue et al., 1999; Middleton et al., 2000).

### Neurotrophic Factors and Receptor Signaling

In addition to neurotrophic cytokines, the neurotrophic factors of the neurotrophin family are essential for the survival of many kinds of neurons during development and the intracellular signaling pathways mediating their effect are beginning to be understood. The neurotrophin family members, including NGF, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT3), and neurotrophin-4 (NT4), mediate their effects through the trk tyrosine kinase receptors which activate Ras/MAPK, PI3K/Akt, and PLC $\gamma$  signaling pathways (Barbacid, 1995).

An important substrate for the survival effects of the neurotrophin receptors is PI3K, which activates the serine-threonine kinase Akt. Akt has been shown to be necessary and sufficient for neurotrophin-mediated neuronal survival of sympathetic neurons (Crowder and Freeman, 1998; Vaillant et al., 1999). Activated Akt prevents apoptosis by inactivating the intracellular apoptosis-promoting protein Bad (Datta et al., 1997), by inhibiting mitochon-

Address correspondence to Patrik Ernfors, Lab. Mol. Neurobiol., MBB, Karolinska Institutet, Brezeliusväg 3, plan 3, 171 77 Stockholm, Sweden. Tel.: 46 8 728 7659. Fax: 46 8 728 76 56. E-mail: patrik@cajal.mbb.ki.se



**Figure 1.** Convergence between cytokine and neurotrophin survival signaling pathways. Neurotrophins bind to their corresponding trk tyrosine kinase receptors leading to the activation of PI3K and Akt. Akt then (a) prevents mitochondrial cytochrome C release; (b) inhibits caspase-9; (c) inactivates bad; (d) phosphorylates forkhead transcription factors thus preventing transcriptional activation of cell death-promoting genes; (e) activates IKK $\alpha$  leading to phosphorylation of I $\kappa$ B, release and nuclear translocation of NF $\kappa$ B, and transcriptional activation of anti-apoptotic genes. Cytokine receptors (a) activate NIK via TAK1 association with TRAF2 or 6 leading to activation of IKK $\alpha$ ; (b) activate PI3K and Akt resulting in the activation of IKK $\alpha$ . PI3K and NIK phosphorylate IKK $\alpha$  at distinct sites and data suggest that phosphorylation of both is required for activation of IKK $\alpha$  in some cells. Cytokine receptors can also activate the JAK/STAT and jun-NH<sub>2</sub>-terminal kinase (JNK) pathways. p75NTR activates NF $\kappa$ B by interacting with TRAF6.

drial cytochrome C release, which is required for activation of the intracellular suicidal program, and by phosphorylating and inactivating caspase-9 (Crowder and Freeman, 1998; Ashcroft et al., 1999; Vaillant et al., 1999). In addition, Akt also prevents apoptosis by phosphorylation and inactivation of a forkhead transcription factor (Brunet et al., 1999).

### Convergence in Neuronal Survival/Death Pathways by Cytokines and Neurotrophins

Recent results provide a direct link from cytokine receptors to the PI3K/Akt pathway and from Akt to NF $\kappa$ B activity via IKK $\alpha$ -IKK $\beta$  activation, I $\kappa$ B degradation, and subsequent NF $\kappa$ B nuclear translocation (Chen et al., 1999; Kane et al., 1999; Ozes et al., 1999; Romashkova and Makarov, 1999). This raises the question whether neurotrophins and cytokines accomplish their survival promoting effects largely through the same intracellular signaling pathways (Fig. 1). NF $\kappa$ B has been shown to

participate in NGF-elicited, p75 neurotrophin receptor-mediated neuronal survival, but its relative contribution is not as important as it is in mediating the survival response of developing sensory neurons to cytokines (Maggirwar et al., 1998; Hamanoue et al., 1999). Furthermore, in contrast to NGF, other members of the neurotrophin family, including BDNF and NT3, do not activate NF $\kappa$ B (Middleton et al., 2000).

How is specificity generated? An essential intracellular mechanism for regulating speed and specificity of signal transduction is the restriction of the subcellular localization of signaling components. This is achieved through anchor proteins bound to specific subcellular structures (proteins or lipids) and scaffold proteins which assemble various signaling components. Recently, two scaffold proteins which could provide a platform for the assembly of NF $\kappa$ B signaling components were reported (Scheidereit, 1998). Such higher order control of signaling and interactions between signaling pathways highlights the importance of functional studies on real primary cells, tissues and animals.

The possible participation of the PI3K/Akt pathway in survival signaling by neurotrophic cytokines has yet to be directly examined. Recent results on Akt signaling confirms the importance of context since it can act in different ways in different cell types and following activation by different ligands. For example, Akt is necessary for tumor necrosis factor-mediated NF $\kappa$ B activation in epithelial but not in fibroblast cells (Ozes et al., 1999; Romashkova and Makarov, 1999). Furthermore, whereas both BDNF and platelet-derived growth factor (PDGF) leads to phosphorylation of Akt, NF $\kappa$ B is activated only after PDGF treatment (Middleton et al., 2000; Romashkova and Makarov, 1999).

In light of the new results implying an important role for cytokine-induced NF $\kappa$ B activation in survival/death signaling during development of the peripheral nervous system, the next issues to be addressed will almost certainly be whether Akt participates in neuronal survival by cytokines, and whether cytokine activation of NF $\kappa$ B involves NIK and/or Akt. A bigger challenge, however, will be to determine when and how cytokine and neurotrophin signaling pathways converge and their consequences for physiological as well as disease processes in the nervous system.

Submitted: 15 December 1999

Accepted: 5 January 2000

### References

- Ashcroft, M., R.M. Stephens, B. Hallberg, J. Downward, and D.R. Kaplan. 1999. The selective and inducible activation of endogenous PI 3-kinase in PC12 cells results in efficient NGF-mediated survival but defective neurite outgrowth. *Oncogene*. 18:4586-4597.
- Barbacid, M. 1995. Neurotrophic factors and their receptors. *Curr. Opin. Cell Biol.* 7:148-155.
- Barger, S.W., D. Horster, K. Furukawa, Y. Goodman, J. Kriegstein, and M.P. Mattson. 1995. Tumor necrosis factors alpha and beta protect neurons against amyloid beta-peptide toxicity: evidence for involvement of a kappa B-binding factor and attenuation of peroxide and Ca<sup>2+</sup> accumulation. *Proc. Natl. Acad. Sci. USA*. 92:9328-9332.
- Brunet, A., A. Bonni, M.J. Zigmond, M.Z. Lin, P. Juo, L.S. Hu, M.J. Anderson, K.C. Arden, J. Blenis, and M.E. Greenberg. 1999. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. *Cell*. 96: 857-868.
- Chen, R.H., M.C. Chang, Y.H. Su, Y.T. Tsai, and M.L. Kuo. 1999. Interleukin-6

- inhibits transforming growth factor-beta-induced apoptosis through the phosphatidylinositol 3-kinase/Akt and signal transducers and activators of transcription 3 pathways. *J. Biol. Chem.* 274:23013–23019.
- Crowder, R.J., and R.S. Freeman. 1998. Phosphatidylinositol 3-kinase and Akt protein kinase are necessary and sufficient for the survival of nerve growth factor-dependent sympathetic neurons. *J. Neurosci.* 18:2933–2943.
- Datta, S.R., H. Dudek, X. Tao, S. Masters, H. Fu, Y. Gotoh, and M.E. Greenberg. 1997. Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. *Cell.* 91:231–241.
- Hamanoue, M., G. Middleton, S. Wyatt, E. Jaffray, R.T. Hay, and A.M. Davies. 1999. p75-mediated NF-kappaB activation enhances the survival response of developing sensory neurons to nerve growth factor. *Mol. Cell Neurosci.* 14: 28–40.
- Kaltschmidt, B., M. Uherek, H. Wellmann, B. Volk, and C. Kaltschmidt. 1999. Inhibition of NF-kappaB potentiates amyloid beta-mediated neuronal apoptosis. *Proc. Natl. Acad. Sci. USA.* 96:9409–9414.
- Kane, L.P., V.S. Shapiro, D. Stokoe, and A. Weiss. 1999. Induction of NF-kappa B by the Akt PKB kinase. *Curr. Biol.* 9:601–604.
- Khursigara, G., J.R. Orlinick, and M.V. Chao. 1999. Association of the p75 neurotrophin receptor with TRAF6. *J. Biol. Chem.* 274:2597–2600.
- Lezoualc'h, F., Y. Sagara, F. Holsboer, and C. Behl. 1998. High constitutive NF-kappaB activity mediates resistance to oxidative stress in neuronal cells. *J. Neurosci.* 18:3224–3232.
- Maggirwar, S.B., P.D. Sarmiere, S. Dewhurst, and R.S. Freeman. 1998. Nerve growth factor-dependent activation of NF-kappaB contributes to survival of sympathetic neurons. *J. Neurosci.* 18:10356–10365.
- Middleton, G., M. Hamanoue, Y. Enokido, S. Wyatt, D. Pennica, E. Jaffray, R.T. Hay, and A.M. Davies. 2000. Cytokine-induced nuclear factor- $\kappa$ B activation promotes the survival of developing neurons. *J. Cell Biol.* 148:325–332.
- Ninomiya-Tsuji, J., K. Kishimoto, A. Hiyama, J. Inoue, Z. Cao, and K. Matsumoto. 1999. The kinase TAK1 can activate the NIK-I kappaB as well as the MAP kinase cascade in the IL-1 signalling pathway. *Nature.* 398:252–256.
- Ozes, O.N., L.D. Mayo, J.A. Gustin, S.R. Pfeffer, L.M. Pfeffer, and D.B. Donner. 1999. NF-kappaB activation by tumour necrosis factor requires the Akt serine-threonine kinase. *Nature.* 401:82–85.
- Post, A., F. Holsboer, and C. Behl. 1998. Induction of NF-kappaB activity during haloperidol-induced oxidative toxicity in clonal hippocampal cells: suppression of NF-kappaB and neuroprotection by antioxidants. *J. Neurosci.* 18: 8236–8246.
- Romashkova, J.A., and S.S. Makarov. 1999. NF-kappaB is a target of AKT in anti-apoptotic PDGF signalling. *Nature.* 401:86–90.
- Scheidereit, C. 1998. Signal transduction. Docking IkappaB kinases. *Nature.* 395:225–256.
- Schneider, A. A. Martin-Villalba, F. Weih, J. Vogel, T. Wirth, and M. Schwaninger. 1999. NF-kappaB is activated and promotes cell death in focal cerebral ischemia. *Nat. Med.* 5:554–559.
- Vaillant, A.R., I. Mazzoni, C. Tudan, M. Boudreau, D.R. Kaplan, and F.D. Miller. 1999. Depolarization and neurotrophins converge on the phosphatidylinositol 3-kinase-Akt pathway to synergistically regulate neuronal survival. *J. Cell Biol.* 146:955–966.
- Yu, Z., D. Zhou, A.J. Bruce-Keller, M.S. Kindy, and M.P. Mattson. 1999. Lack of the p50 subunit of nuclear factor-kappaB increases the vulnerability of hippocampal neurons to excitotoxic injury. *J. Neurosci.* 19:8856–8865.