Neuron, Vol. 29, 563–566, March, 2001, Copyright ©2001 by Cell Press

A Convoluted Way to Die

Minireview

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The neurons that fail to establish proper connections with their respective postsynaptic targets die naturally during development due to the lack of sufficient trophic support. The first hint that such naturally occurring neuronal cell death requires the active participation of neurons came from the seminal work by Eugene Johnson and colleagues who showed that sympathetic neuronal cell death induced by NGF deprivation can be inhibited by macromolecular synthesis inhibitors such as cycloheximide and actinomycin D (reviewed in Yuan and Yankner, 2000). These findings suggested that certain death-inducing molecules must be synthesized in order for neurons to die. The identity of such molecules and how they function to induce neuronal death have been the subject of many studies and remained largely a mystery until recently. It turns out that one of these molecules is none other than BIM (the *B*CL-2 *i*nteracting *m*ediator of cell death), a BH3-only member of the BCL-2 family. Previous works from Andrew Strasser's lab have shown that there are three BIM isoforms; BIM_S, BIM_L, and BIM_{EL}, all of which are proapoptotic to different extents (O'Connor et al., 1998). In this issue of *Neuron*, two papers from the Eugene Johnson and Jonathan Ham laboratories, respectively (Whitfield et al., 2001; Putcha et al., 2001), show that BIM_{EL} is induced in a variety of neuronal cell death paradigms including sympathetic neurons upon removal of NGF, neonatal sciatic nerve axotomy, and K^+-deprived cerebellar granule neurons. Furthermore, they show that inhibition of BIM by antisense and genetic knockout approaches significantly reduced sympathetic neuron death induced by NGF deprivation. Thus, BIM clearly fits the criteria of a death-inducing molecule that is synthesized in order for neurons to die.

Upregulation of BIM expression during apoptosis is not limited to neurons: BIM is also induced in cytokine-deprived murine IL3-dependent cells (Shinjyo et al., 2001). In addition, BIM is essential for the execution of trophic factor deprivation–induced apoptosis in the lymphoid cell lineages (Bouillet et al., 1999). Thus, all trophic factor deprivation–induced apoptosis in different mammalian cell types may be regulated by BIM or BIM-like BH3-only members of the BCL-2 family. Since BIM most likely acts by inhibiting proapoptotic members of the BCL-2 family (O'Connor et al., 1998), this demonstrates again the beautiful evolutionary convergence on the mechanism of apoptosis from *C. elegans* to mammals, as programmed cell death during *C. elegans* development is initiated by transcriptional upregulation of Egl-1, another BH3-only member of the BCL-2 family that acts by inhibiting Ced-9, an antiapoptotic member of the BCL-2 family (Conradt and Horvitz, 1998).

BIM may not be the only BH3-only member that is induced by trophic factor deprivation. Previously, DP5/Hrk, another BH3-only member of the BCL-2 family, was shown to be induced in rat sympathetic neurons upon NGF deprivation and in cortical neurons stimulated by amyloid β protein (reviewed in Yuan and Yankner, 2000). Although it is not clear if inhibition of DP5/Hrk may reduce neuronal cell death, coinduction of DP5/Hrk and BIM suggests that multiple BH3-only members of the BCL-2 family may be induced in order for neurons to die.

The BH3-Only Members of BCL-2 Family: Critical Intracellular Mediators of Death Signaling

The BH3-only members of BCL-2 family include Egl-1, BIM, Hrk/DP5, BIK, BID BAD, and NOXA, and the list is still expanding (Huang and Strasser, 2000). Although what identifies these killer proteins as a group is only a limited homology within a stretch of nine–amino acid sequence, termed the "BH3 domain," previous structural studies of BID suggest that they may share a surprising high level of homology with other members of the BCL-2 family in their tertiary structures, consisting of four hydrophobic α helixes surrounding two central hydrophobic helixes, which are the business center of the molecule (Chou et al., 1999; McDonnell et al., 1999). Their overall structural similarities indicate that they may act in similar fashion to regulate cell death.

Previous works from Johnson's lab have shown that the protein synthesis step in trophic factor–deprived sympathetic neurons is required to induce the translocation of BAX, a proapoptotic member of the BCL-2 family, from cytosol to mitochondria, that is critical for neurons to die (Putcha et al., 1999). $Bax^{-/-}$ sympathetic neurons are highly resistant to trophic factor deprivation–induced cell death (Miller et al., 1997). Therefore, one possibility is that transcriptionally upregulated BIM or BIM-like molecule(s) are responsible for mitochondrial translocation of BAX. Although it has not been examined whether BAX remains cytosolic in *Bim* knockout neurons, Johnson and colleagues (Putcha et al., 2001) found that induced BIM resides in the mitochondria as an integral membrane protein, which makes it unlikely that BIM is responsible for the translocation of BAX from the cytosol to mitochondria. Since BIM has been shown to interact with BCL-2 and BCL-W, but not BAX, BIM may function to inactivate BCL-2-related antiapoptotic proteins and contribute indirectly to the multimerization and integration of BAX into the mitochondrial outer membrane.

If BIM does not act by inducing BAX translocation, then there must be another factor that needs to be synthesized in trophic factor–deprived sympathetic neurons to induce BAX translocation. Thus, the protein synthesis step in trophic factor–deprived sympathetic neurons may have produced several death-inducing activities that act to coordinate the death process. Interestingly, BID, another BH3-only member of the BCL-2 family that is normally cytosolic in living cells, has been

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shown to induce the conformational change and mitochondrial translocation of BAX (Eskes et al., 2000). Although it has not yet been shown, it is interesting to speculate that perhaps a BID-like cytosolic BH3-only protein(s) may be involved in regulating BAX translocation in neurons. BID itself, however, is unlikely to be involved, as no cleavage of BID was detected during sympathetic neuronal death (Whitfield et al., 2001).

Multiple Controls of the BH3-Only Killer Activities

Regulation of the BH3-only member of the BCL-2 family can be achieved at both transcriptional and posttranscriptional levels. While Egl-1, BIM, and DP5/Hrk are regulated at the transcriptional level, BID has been shown to be regulated by proteolytic cleavage (Li et al., 1998; Luo et al., 1998). Cleavage of BID by caspase-8 in the Fas/TNF signaling pathway induces the mitochondrial translocation of truncated BID and thereby transmits the death signal from cytoplasmic membrane to mitochondria. BAD, on the other hand, has been shown to be regulated by protein phosphorylation, which allows its sequestration by chaperone protein 14-3-3 and inhibition of its proapoptotic activity (Zha et al., 1996).

BIM is not only regulated at the transcriptional level but also at the postranscriptional level as well. BIM is normally expressed in hematopoietic, epithelial, neuronal, and germ cells (O'Reilly et al., 2000). In the nervous system, BIM expression was detected primarily in neurons of the gray matter but not in glial cells, astrocytes, or oligodendrocytes. At the protein level, BIM_{EL} is the predominant form expressed in neurons. Immunostaining of BIM revealed a punctate pattern, suggesting its association with cytoplasmic structures. Since expression of BIM is highly toxic to cells, BIM must be inhibited in healthy cells. At least one of the known inhibitory mechanisms is through sequestration to the microtubule-associated dynein motor complex by direct interaction of BIM with LC8 cytoplasmic dynein light chain (Puthalakath et al., 1999). In addition, BIM_{EL} and BIM_{L} are phosphorylated in IL3-dependent hematopoietic cells upon addition of IL3 by a Ras/Raf/MAPK or Ras/PI3 kinase–dependent mechanism (Shinjyo et al., 2001). Since the survival of sympathetic neurons in the presence of NGF is at least partly dependent upon the PI3 kinase, it is highly likely that constitutively expressed BIM in sympathetic neurons, albeit at low levels, may be suppressed by phosphorylation as well.

Transcriptional Regulation of the BH3-Only Killers

Transcription is not required for apoptosis induced by activation of death receptors, such as Fas and TNFR, or by disturbance in cell homeostasis, such as DNA damage or disruption of cytoskeleton in nonneuronal cells. For these types of apoptotic stimuli, the preassembled apoptotic apparatus in cells is fully equipped to handle the signal transduction and execution of the death. Developmental cell death, which may be largely due to the limiting amount of trophic factors available, and a number of other cell death paradigms, are unique since they require de novo synthesis of death-inducing molecules to carry out the execution order. The transcriptional upregulation of *egl-1* is critical for the onset of programmed cell death during *C. elegans* development (Conradt and Horvitz, 1998). In *C. elegans* hermaphrodites, the expression of *egl-1* is suppressed in a pair of hermaphrodite specific neurons, HSN, by Tra-1, a zinc finger protein that is the terminal global regulator of somatic sexual fate in *C. elegans* (Conradt and Horvitz, 1999). It is not clear, however, which transcriptional factor positively regulates *egl-1* expression in dying cells in *C. elegans*.

The expression of BIM, on the other hand, may be regulated in lymphocytes by FKHR-L1 (Dijkers et al., 2000), a transcriptional factor whose activity is suppressed by the PI3 kinase/Akt pathway. In Ba/F3 cells, whose survival in culture is IL3 dependent, BIM expression is induced upon IL3 deprivation and also by treatment with PI3 kinase inhibitor. FKHR-L1 has been shown to be a potent inducer of neuronal apoptosis. FKHR-L1 activity is inhibited by Akt-mediated phosphorylation on three sites, and IL3 withdrawal resulted in dephosphorylation of FKHR-L1. Overexpression of triple mutant of FKHR-L1, which is unable to be phosphorylated by Akt, causes cell death in cerebellar granule neurons (Brunet et al., 1999) and induces BIM expression in IL3-maintained Ba/F3 cells (Dijkers et al., 2000). Therefore, it will be very interesting to examine whether FKHR-L1 is involved in regulating BIM expression in neurons.

The Signal Transduction Pathway to BIM Induction

Jonathan Ham's paper (Whitfield et al., 2001) examined the role of c-Jun in regulating BIM expression in neurons. Activation of c-Jun N-terminal kinase (JNK) and the phosphorylation of c-Jun are critical events in sympathetic neuronal cell death (reviewed in Yuan and Yankner, 2000). Ham and colleagues showed that expression of a dominant-negative c-Jun resulted in about 40% reduction in the levels of BIM expressed in NGF-deprived sympathetic neurons. The authors concluded that the activation of transcriptional factor c-Jun contributes to the induction of BIM expression.

c-Jun is activated by phosphorylation with JNK. Using CEP-1347, a chemical inhibitor of the JNK pathway, but not JNK itself, Johnson and colleagues showed, judging from their Western blot, that inhibition of JNK pathway resulted in about similar levels of reduction in BIM expression in NGF-deprived sympathetic neurons as using the dominant-negative c-Jun mutant as done by the Ham group. Thus, both groups are in agreement that JNK and c-Jun most likely play a role in upregulating BIM expression, but the activation of JNK pathway per se is not sufficient to induce BIM expression (Whitfield et al., 2001; Putcha et al., 2001).

Johnson and colleagues proposed that trophic factor deprivation–induced sympathetic neuronal cell death requires the activation of parallel pathways, each of them is necessary but not sufficient to induce neuronal cell death. One of the pathways is the induction of BH3-only proteins like BIM and DP5/Hrk, which may be necessary for inactivation of BCL-2-like antiapoptotic proteins and facilitates the mitochondrial translocation of BAX. The second pathway is the induction of c-Jun and activation of JNK, which may be necessary for inactivating a separate antiapoptotic mechanism in neurons, termed "competence-to-die" by Johnson (Deshmukh and Johnson, 1998). Now we know that the JNK/c-Jun pathway also plays a role in the upregulation of BIM. Thus, the neuronal cell death pathway intertwines at more than one level to coordinate the death process (Figure 1).

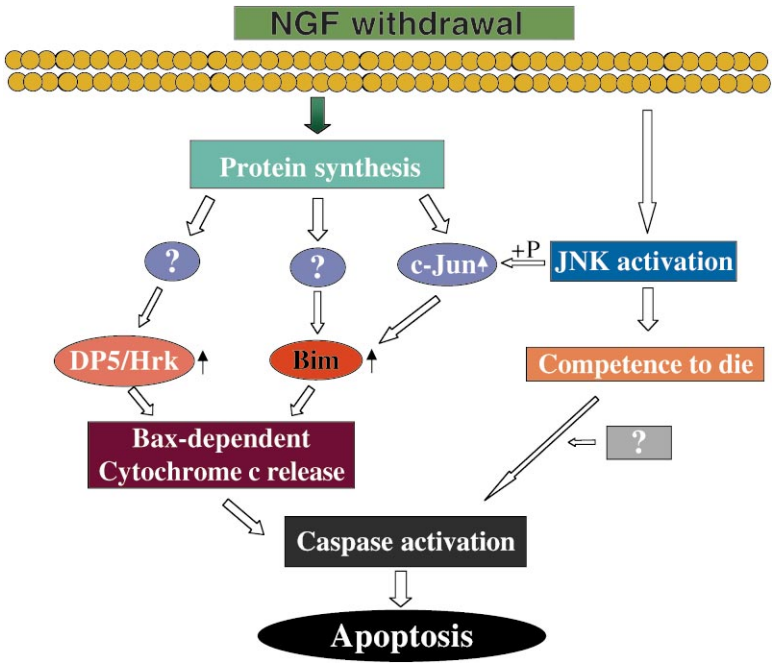

Figure 1. The Neuronal Cell Death Pathway in Sympathetic Neurons

The Overall Picture and Final Thoughts

The current picture of sympathetic neuronal cell death pathway is as follows (Figure 1). Upon removal of NGF, neurons induce at least four proteins that may play critical roles in apoptotic signal transduction: BIM, DP5/Hrk, and two transcription factors of the AP-1 family—c-Jun and c-Fos. Because c-Jun is necessary but maybe not sufficient to induce BIM expression, c-Jun and another factor(s) may act together to regulate the expression of BIM, DP5/Hrk, or other BH3-like proteins. c-Fos is unlikely to be involved as its induction is a very late event. The mechanism by which neurons activate c-Jun is at least partially through JNK. Activation of JNK is critical for the development of competence-to-die, which may act by releasing the cytoplasmic "brake" for caspase activation. Upregulated BIM, DP5/Hrk, etc., may act to neutralize the neuronal antiapoptotic arsenals such as BCL-2 and BCL-xL, thereby facilitating BAX aggregation and mitochondrial insertion. Thus, the neuronal apoptotic pathway is built in such a way to assure that only when both proapoptotic signaling pathways are activated is the green light to caspase activation turned on to be swiftly followed by neuronal death.

The existing information showed that certain apoptotic pathways, such as death receptor–induced cell death, are incredibly direct: there are very few signal transduction steps that go from an extracellular death signal to the activation of caspases, which results in the quick final demise of a cell. In contrast, neurons seem to have evolved an intricate and convoluted way to die. Why? One reasonable answer is that most cell types in our body are programmed to live for a short time and are turned over rather quickly. Therefore, they are highly disposable—from the organism point of view, it is much better to eliminate a compromised cell than try to mend it. Neurons, on the other hand, are much more precious—throughout our lives, we maintain roughly the same set of neurons as when we are born, if we are fortunate enough to live free of neurodegenerative diseases. Furthermore, mature neurons are nonproliferative and, therefore, it is unlikely that a defective neuron may overproliferate and turn cancerous. Thus, the organism seems to have adapted an entirely different policy for neurons: to keep them alive any way it can.

What does this complicated neuronal apoptosis pathway tell us about the therapeutic opportunity of neurodegenerative diseases? Many studies have demonstrated that apoptotic mechanisms contribute to both acute and chronic neurodegenerative diseases (Yuan and Yankner, 2000). Inhibition of caspase activation has shown beneficial effects in a number of animal models of neurodegenerative models. Convoluted neuronal cell death pathways may be good news to us: as multiple controlling points in the neuronal death pathways may provide additional flexibility in choosing the best therapeutic points. One word of caution, however, is that it remains to be seen whether activation of a single neuronal cell death pathway, which is not sufficient for neurons to die in short-term culture, can be ignored in the long term and whether a "half-dead" neuron can be fully functional. Nevertheless, we have made tremendous progress in understanding the mechanisms of neuronal cell death in the last decade, and this growing body of information will very likely help us to develop novel treatments of neurodegenerative diseases in the next decade.

Selected Reading

Bouillet, P., Metcalf, D., Huang, D.C., Tarlinton, D.M., Kay, T.W., Kontgen, F., Adams, J.M., and Strasser, A. (1999). Science *286*, 1735–1738.

Brunet, A., Bonni, A., Zigmond, M.J., Lin, M.Z., Juo, P., Hu, L.S., Anderson, M.J., Arden, K.C., Blenis, J., and Greenberg, M.E. (1999). Cell *96*, 857–868.

Chou, J.J., Li, H., Salvesen, G.S., Yuan, J., and Wagner, G. (1999). Cell. *96*, 615–624.

Conradt, B., and Horvitz, H.R. (1998). Cell *93*, 519–529.

Conradt, B., and Horvitz, H.R. (1999). Cell *98*, 317–327.

Deshmukh, M., and Johnson, E.M. (1998). Neuron *21*, 695–705.

Dijkers, P.F., Medemadagger, R.H., Lammers, J.J., Koenderman, L., and Coffer, P.J. (2000). Curr. Biol. *10*, 1201–1204.

Eskes, R., Desagher, S., Antonsson, B., and Martinou, J.C. (2000). Mol. Cell. Biol. *20*, 929–935.

Huang, D.C., and Strasser, A. (2000). Cell *103*, 839–842.

Li, H., Zhu, H., Xu, C.J., and Yuan, J. (1998). Cell *94*, 491–501.

Luo, X., Budihardjo, I., Zou, H., Slaughter, C., and Wang, X. (1998). Cell. *94*, 481–490.

McDonnell, J.M., Fushman, D., Milliman, C.L., Korsmeyer, S.J., and Cowburn, D. (1999). Cell *96*, 625–634.

Miller, T.M., Moulder, K.L., Knudson, C.M., Creedon, D.J., Deshmukh, M., Korsmeyer, S.J., and Johnson, E.M. (1997). J. Cell Biol. *139*, 205–217.

O'Connor, L., Strasser, A., O'Reilly, L.A., Hausmann, G., Adams, J.M., Cory, S., and Huang, D.C. (1998). EMBO J. *17*, 384–395.

O'Reilly, L.A., Cullen, L., Visvader, J., Lindeman, G.J., Print, C., Bath, M.L., Huang, D.C., and Strasser, A. (2000). Am. J. Pathol. *157*, 449–461.

Putcha, G.V., Deshmukh, M., and Johnson, E.M., Jr. (1999). J. Neurosci. *19*, 7476–7485.

Putcha, G.V., Moulder, K.L., Golden, J.P., Bouillet, P., Adams, J.A., Strasser, A., and Johnson, E.M., Jr. (2001). Neuron *29*, this issue, 615–628.

Puthalakath, H., Huang, D.C., O'Reilly, L.A., King, S.M., and Strasser, A. (1999). Mol. Cell *3*, 287–296.

Shinjyo, T., Kuribara, R., Inukai, T., Hosoi, H., Kinoshita, T., Miyajima, A., Houghton, P.J., Look, A.T., Ozawa, K., and Inaba, T. (2001). Mol. Cell. Biol. *21*, 854–864.

Whitfield, J., Neame, S.J., Paquet, L., Bernard, O., and Ham, J. (2001). Neuron *29*, this issue, 629–643.

Yuan, J., and Yankner, B.A. (2000). Nature *407*, 802–809.

Zha, J., Harada, H., Yang, E., Jockel, J., and Korsmeyer, S.J. (1996). Cell *87*, 619–628.