Biochemical Pharmacology, Vol. 60, pp. 1015–1021, 2000.

ISSN 0006-2952/00/$–see front matter
PII S0006-2952(00)00372-5

c-Jun and the Transcriptional Control of Neuronal Apoptosis

Jonathan Ham,† Andreas Eilers,‡ Jonathan Whitfield,§ Stephen J. Neame§ and Bina Shah§*

*Cancer Biology and Molecular Haematology Unit, Camelia Botnar Laboratories, Institute of Child Health, University College London, London WC1N 1EH, U.K.; ‡Max Delbrück Centrum, 13122 Berlin, Germany; and §Eisai London Research Laboratories, Bernard Katz Building, University College London, London WC1E 6BT, U.K.

ABSTRACT. There has been considerable interest in the molecular mechanisms of apoptosis in mammalian neurons because this form of neuronal cell death is important for the normal development of the nervous system and because inappropriate neuronal apoptosis may contribute to the pathology of human neurodegenerative diseases. The aim of recent research has been to identify the key components of the cell death machinery in neurons and understand how the cell death programme is regulated by intracellular signalling pathways activated by the binding of neurotrophins or death factors to specific cell surface receptors. The aim of this commentary was to review research that has investigated the role of the Jun N-terminal kinase (JNK)/c-Jun signalling pathway in neuronal apoptosis, focusing in particular on work carried out with developing sympathetic neurons. Experiments with sympathetic neurons cultured *in vitro*, as well as with cerebellar granule neurons and differentiated PC12 cells, have demonstrated that JNK/c-Jun signalling can promote apoptosis following survival factor withdrawal. In addition, experiments with *Jnk*(−/−) knockout mice have provided evidence that *Jnk3* may be required for apoptosis in the hippocampus *in vivo* following injection of kainic acid, an excitotoxin, and that *Jnk1* and *Jnk2* are required for apoptosis in the developing embryonic neural tube. However, in the embryonic forebrain, *Jnk1* and *Jnk2* have the opposite function and are necessary for the survival of developing cortical neurons. These results suggest that JNKs and c-Jun are important regulators of the cell death programme in the mammalian nervous system, but that their biological effects depend on the neuronal type and stage of development. BIOCHEM PHARMACOL **60**;8:1015–1021, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. apoptosis; sympathetic neurons; nerve growth factor; development; c-Jun; Jun N-terminal kinase; cytochrome *c*; caspase

NEURONAL CELL DEATH

Apoptosis is one of the major forms of cell death in the mammalian nervous system. For example, during embryonic and early postnatal development, approximately 50% of the neurons formed by neurogenesis die by apoptosis. This death is required for the formation of appropriate connections between neurons and their targets and for morphogenetic processes, such as neural tube closure [1, 2]. In addition, there is increasing evidence that cell death by apoptosis may contribute to the pathology of human neurodegenerative disease [3].

Sympathetic neurons have proved to be a particularly useful model system for *in vitro* studies of the molecular mechanisms of developmental neuronal cell death (Fig. 1). These cells normally die by apoptosis between days 3 and 7 of postnatal development in the rat and require NGF‖ for survival at this time [4]. When deprived of NGF in culture, sympathetic neurons undergo a form of cell death that has the classic features of apoptosis: rates of RNA and protein synthesis fall, the cytoplasm and nucleus shrink, mitochondria release cytochrome *c* into the cytoplasm, caspases are activated and required for cell death, chromatin condenses and the DNA is degraded into oligonucleosomal fragments, phosphatidylserine redistributes from the inner to the outer surface of the plasma membrane, neurites fragment, and the cells ultimately detach from their substrate [5–13]. Interestingly, NGF withdrawal-induced death is blocked by inhibition of transcription or translation, suggesting that new gene expression and protein synthesis are required for cell death to occur [5]. The goal of our research has been: 1) to identify pro-apoptotic genes that are induced in sympathetic neurons following NGF withdrawal; 2) to understand how their protein products promote apoptosis; and 3) to

† Corresponding author: Dr. Jonathan Ham, Cancer Biology & Molecular Haematology Unit, Institute of Child Health, University College London, 30 Guilford Street, London WC1N 1EH, U.K. Tel. +44-020-7905.2294; FAX +44-020-7813.8100; E-mail: J.Ham@ich.ucl.ac.uk

‖ *Abbreviations:* NGF, nerve growth factor; AP-1, activator protein-1; JNK, Jun N-terminal kinase; CAT, chloramphenicol acetyltransferase; ATF-2; activating transcription factor-2; MEKK1, MEK kinase 1; SEK1, stress-activated protein kinase/extracellular signal-regulated kinase; JIP-1, JNK-interacting protein 1; JBD, JNK-binding domain; $p75^{NTR}$, p75 neurotrophin receptor; and dn-Jun, dominant negative c-Jun.

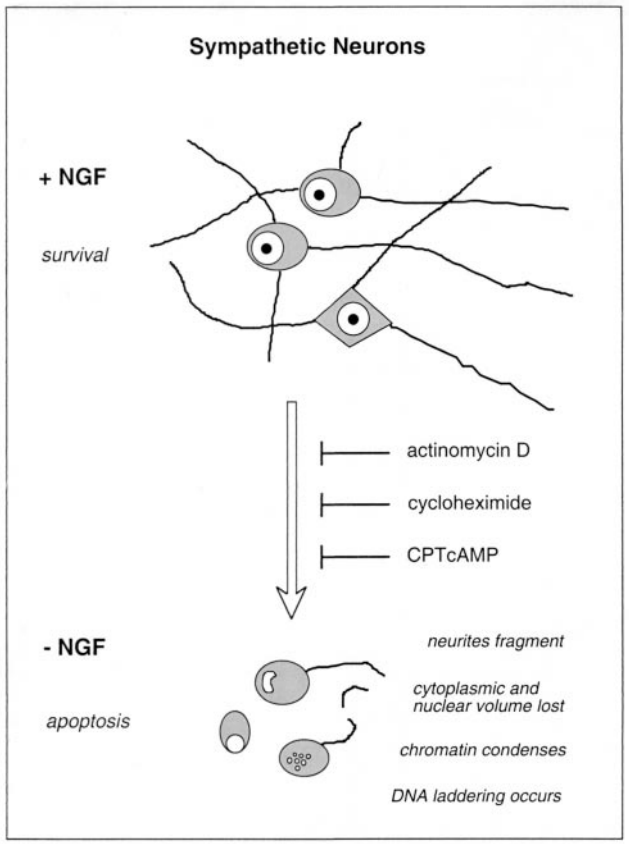

FIG. 1. The sympathetic neuron cell death model. Sympathetic neurons are isolated from 1-day-old rats and cultured in Dulbecco's modified Eagle's medium containing glutamine, penicillin/streptomycin, 10% foetal calf serum, and NGF at 50 ng/mL on tissue culture plastic or glass coverslips coated with poly-L-lysine and laminin [18]. Fluorodeoxyuridine and uridine are added to 20 μM to inhibit the proliferation of any non-neuronal cells that are present. After 7 days *in vitro*, the neurons have well-defined nuclei and nucleoli and have formed an extensive network of neurites. If the neurons are then deprived of NGF, they die by apoptosis, with most of the cells dead at 72 hr after NGF withdrawal. NGF withdrawal-induced death is effectively blocked by inhibitors of transcription and protein synthesis or membrane-permeant cAMP analogues, such as 8-(4-chlorophenylthio)adenosine 3′,5′-cyclic monophosphate (CPTcAMP).

identify the transcription factors and intracellular signalling pathways that regulate the expression of the cell death genes.

C-JUN AND NEURONAL APOPTOSIS

We hypothesized that if NGF withdrawal activates a programme of gene expression that promotes apoptosis in sympathetic neurons, then immediate early response genes, such as members of the Jun and Fos (AP-1) family, might be amongst the first genes to be activated [14]. The AP-1 family [15] consists of the basic/leucine zipper transcription factors c-Fos, Fos B, Fra-1, Fra-2, c-Jun, Jun B, and Jun D, which form homodimers or heterodimers with each other and which bind to a specific DNA sequence, the AP-1 site (5′-TGACTCA-3′). To investigate the pattern of expression of these proteins before and after NGF withdrawal, we carried out immunoblotting and immunocytochemistry experiments with affinity-purified antibodies specific for each family member [14]. We found that sympathetic neurons express c-Jun, Jun B, Jun D, and Fra-1, but not c-Fos or Fos B. After NGF withdrawal, c-Jun protein levels increased substantially and this occurred before the cell death commitment point, whereas the levels of other members of the family did not increase. In addition, the N-terminal c-Jun transcriptional activation domain became more phosphorylated, which increases the ability of c-Jun to activate the transcription of target genes. We also found that microinjection of an expression vector for a c-Jun dominant negative mutant, which inhibited AP-1 activity, protected sympathetic neurons against NGF withdrawal-induced death, whereas overexpression of wild-type c-Jun was sufficient to induce apoptosis in the presence of NGF [14]. Furthermore, Estus *et al.* [16] showed that microinjection of an antibody specific for c-Jun inhibited cell death after NGF deprivation, whereas antibodies against Jun B or Jun D had no effect. Taken together, these results indicate that AP-1 activity is required for apoptosis in NGF-deprived sympathetic neurons and suggest that c-Jun plays a key role.

REGULATION OF C-JUN EXPRESSION AND ACTIVITY IN SYMPATHETIC NEURONS: THE ROLE OF JUN N-TERMINAL KINASE

To understand how NGF withdrawal causes increased expression of c-Jun protein and increased Jun N-terminal phosphorylation, we studied the c-*jun* promoter and the role of stress-activated protein kinases, such as JNKs and p38 kinase, which are known to regulate c-*jun* gene expression and c-Jun phosphorylation [15, 17]. In microinjection experiments with a c-*jun* promoter/CAT reporter gene, we found that NGF withdrawal caused a substantial increase in the level of CAT expression, indicating that the c-*jun* promoter had been activated [18]. This activation was abolished by mutating the jun1 and jun2 TPA responsive elements (TREs), which were previously described as binding sites for c-Jun/ATF-2 heterodimers in non-neuronal cells [19]. Multiple copies of the jun2 TRE functioned as an enhancer activated by NGF withdrawal, when cloned upstream of the heterologous simian virus 40 (SV40) early promoter [18]. Finally, expression of dominant negative c-Jun in sympathetic neurons prevented the increase in c-Jun protein levels that normally occurs after NGF deprivation [18]. These observations suggest that c-Jun, possibly as a heterodimer with ATF-2, is required for induction of c-*jun* gene expression following NGF withdrawal.

Both c-Jun and ATF-2 are substrates for JNKs, which phosphorylate their transcriptional activation domains at specific serine and threonine residues and thereby potentiate their transcriptional activity [15, 17]. In the case of c-Jun, serines 63 and 73 are the major phosphorylation sites. In addition, p38 kinase can phosphorylate and activate ATF-2, but not c-Jun [17]. We therefore investigated whether JNK or p38 was activated by NGF withdrawal in

sympathetic neurons. By carrying out immune complex kinase assays using antibodies specific for JNK or p38 to immunoprecipitate the kinases from cell extracts and the appropriate substrates, glutathione *S*-transferase (GST) c-Jun or GST ATF-2, we were able to show that JNK, but not p38, activity increased after NGF withdrawal and that this increase was prevented by the addition of neuroprotective agents, such as 8-(4-chlorophenylthio)adenosine 3′,5′-cyclic monophosphate (CPTcAMP) and *N*-acetylcysteine [18]. The increase in JNK activity preceded the increase in c-Jun N-terminal phosphorylation that was observed as a c-Jun mobility shift in immunoblots [14]. Consistent with the increase in JNK activity, we were able to show by immunocytochemistry using an antibody specific for c-Jun phosphorylated at serine 63 [20] that c-Jun became more phosphorylated at this site after NGF withdrawal [18]. Similar observations were reported by Virdee *et al.* [21], using a slightly different sympathetic neuron culture system.

To determine whether the JNK pathway was necessary for c-Jun phosphorylation, c-*jun* promoter activation, or NGF withdrawal-induced death, we performed microinjection experiments using expression vectors for an activated form of MEKK1, a dominant negative mutant of SEK1 (SEK*AL*), or the JNK-binding domain of JIP-1 [18 and *]. MEKK1 is a JNK kinase kinase that potently activates the JNK, but not p38 or MAPK, pathway, and SEK1 is a JNK kinase that is a direct target of MEKK1 and which, in turn, activates JNK [15, 17]. SEK*AL* has been mutated so that it can no longer be activated by MEKK1 [22]. JIP-1 is a cytoplasmic scaffold protein that binds JNK and its upstream activating kinases and facilitates JNK activation [23]. When separated from the rest of JIP-1, the JNK-binding domain acts as a decoy substrate and potently and selectively inhibits JNK, but not p38 or mitogen-activated protein kinase (MAPK) [24].

We found that overexpression of MEKK1 increased c-Jun phosphorylation and induced apoptosis in the presence of NGF [18]. These effects were blocked by co-expression of SEK*AL*, suggesting that activated MEKK1 was acting via a SEK1-dependent pathway (Fig. 2). Interestingly, overexpression of SEK*AL* alone did not prevent NGF withdrawal-induced death or the increase in c-Jun protein levels and c-Jun phosphorylation that occur after NGF deprivation [18]. These observations indicate that activation of the JNK pathway by an upstream activator such as MEKK1 is sufficient to increase expression of phosphorylated c-Jun and induce apoptosis in the presence of NGF; similar results were obtained with differentiated PC12 cells deprived of NGF [25]. However, in sympathetic neurons, the physiological signalling pathway by which NGF withdrawal induces c-Jun expression, increased c-Jun phosphorylation, and apoptosis involves a kinase cascade that cannot be inhibited by SEK*AL* (Fig. 2).

Finally, to determine whether JNK activity was necessary for c-Jun phosphorylation and cell death in the sympathetic neuron model, we investigated the effect of overexpressing the JNK-binding domain of JIP-1. We found that the JIP-1 JBD efficiently inhibited c-Jun phosphorylation in sympathetic neurons and prevented c-*jun* CAT activation and

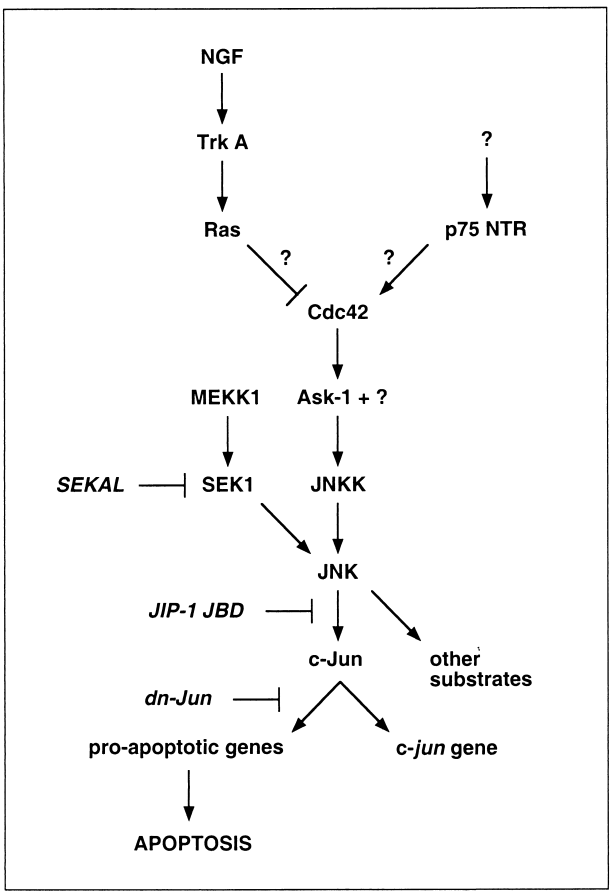

FIG. 2. Relationship between the JNK pathway and c-Jun expression and apoptosis in sympathetic neurons. After NGF withdrawal, JNK activity, c-Jun levels, and c-Jun phosphorylation increase. Expression of c-Jun protein and apoptosis are blocked by dominant negative Jun (dn-Jun), which inhibits AP-1 activity. NGF withdrawal-induced death is also blocked by injection of antibodies against c-Jun, but not Jun B or Jun D [16]. These results suggest that c-Jun activates target genes that promote apoptosis, as well as the c-*jun* gene itself. In some systems, JNKs are activated by SEK1, which in turn is activated by MEKK1. Overexpression of MEKK1 in sympathetic neurons increases the level of c-Jun protein and induces apoptotic cell death, suggesting that activation of the JNK pathway is sufficient to trigger apoptosis. The induction of c-Jun expression and apoptosis by MEKK1 is blocked by co-expression of the SEK1 dominant negative mutant SEK*AL*. However, expression of SEK*AL* does not block the expression of c-Jun induced by NGF withdrawal or NGF withdrawal-induced death [18]. Cdc42 and ASK1 are upstream activators of the JNK pathway [26, 27]. JNK activity is necessary for c-Jun phosphorylation, c-*jun* promoter activation, and NGF withdrawal-induced death, because these events are blocked when the JNK-binding domain of JIP-1, a selective JNK inhibitor, is expressed in sympathetic neurons. The JNK pathway is antagonised by Ras signalling [29] and may be activated by signalling from $p75^{NTR}$ [28].

* Eilers A, Whitfield J, Shah B and Ham J, manuscript submitted for publication.

cell death after NGF withdrawal.* These results support the model shown in Fig. 2, in which NGF withdrawal triggers JNK activation by a pathway that cannot be inhibited by SEK*AL*, activated JNK then phosphorylates c-Jun and possibly other JNK substrates, such as ATF-2 and p53, and phosphorylated c-Jun then activates the transcription of the c-*jun* gene itself and other target genes, some of which encode proteins that promote caspase activation and apoptosis.

UPSTREAM ACTIVATORS OF THE JNK/C-JUN PATHWAY IN SYMPATHETIC NEURONS

Microinjection experiments with activated or dominant negative mutants of JNK kinase kinases, such as apoptosis signal-regulating kinase (ASK) 1, and of Rho family GTPases, such as Cdc42, indicate that these or closely related proteins are likely to be crucial components of the upstream signalling pathway that triggers JNK activation in sympathetic neurons (Fig. 2) [26, 27]. However, whether activation of the JNK pathway following NGF withdrawal is the result of inactivation of a Trk A-dependent signalling pathway that antagonises the JNK pathway or the result of a pro-apoptotic signal from the $p75^{NTR}$ is not yet clear (Fig. 2). There is evidence that both processes could occur. For example, experiments with sympathetic neurons from $p75^{NTR}$ (−/−) knockout mice suggest that p75 may be required for NGF withdrawal-induced death, and binding of brain-derived neurotrophic factor (BDNF) to $p75^{NTR}$ in sympathetic neurons has been shown to increase c-Jun phosphorylation and induce apoptosis [28]. The same authors have suggested that sympathetic neurons synthesize BDNF, which may act in an autocrine manner [28]. On the other hand, the Trk A antagonism model is supported by the observation that activated forms of the Ras GTPase, which is normally activated following the binding of NGF to Trk A, have been shown to suppress the JNK pathway [29]. The molecular mechanism by which this may occur is not yet known.

A ROLE FOR C-JUN IN OTHER MODELS OF NEURONAL CELL DEATH

To determine whether c-Jun also promotes apoptosis in CNS neurons, we studied its role in cerebellar granule neurons [30]. Highly purified cultures of cerebellar granule neurons can be isolated in quantity from 8-day-old rats and maintained in medium containing 10% serum and 25 mM KCl [31]. The KCl causes membrane depolarisation and calcium influx and provides a survival signal. Removal of the serum and KCl triggers cell death by apoptosis in a transcription-dependent manner. We found that after KCl/serum deprivation, c-Jun protein levels increased, whereas the levels of other AP-1 family members did not [30]. Transient transfection of the granule neurons with expression vectors for Bcl-2 or dn-Jun protected them against apoptosis induced by survival signal withdrawal. Furthermore, c-Jun became phosphorylated on serine 63 within 60 min of KCl/serum deprivation and this phosphorylation persisted for several hours. To demonstrate the importance of c-Jun phosphorylation, we made use of c-Jun phosphorylation site mutants. c-Jun(asp) is an activated form of c-Jun in which the known and potential serine and threonine phosphorylation sites in the transactivation domain have been mutated to aspartate, which mimics phosphorylation [32]. In c-Jun(ala), the same sites have been changed to alanine. This protein cannot be phosphorylated and has a greatly reduced ability to activate transcription. When expressed in granule neurons, c-Jun(asp) was a potent inducer of apoptosis, wild-type c-Jun was weaker, and c-Jun(ala) did not induce apoptosis. Furthermore, after the removal of serum and KCl, c-Jun(ala) acted as an inhibitor of apoptosis, suggesting that phosphorylation of c-Jun is necessary for the induction of cell death after survival signal withdrawal [30]. In addition to a pro-apoptotic role in sympathetic neurons and cerebellar granule neurons deprived of survival factors, the c-Jun pathway has also been shown to promote apoptosis in differentiated PC12 cells deprived of NGF [25], in striatal neurons treated with neurotoxic concentrations of dopamine [33], or in a hippocampal neuron cell line transfected with an expression vector for polyglutamine-expanded Huntingtin [34]. Deregulated expression of c-Jun has also been reported to induce apoptosis in NIH3T3 fibroblasts [35].

Initially, it was difficult to study the role of c-Jun in the nervous system *in vivo*, because c-*jun* (−/−) knockout mice died during embryonic development at E12.5 due to hepatic failure [36]. However, knockouts of the three mammalian JNK genes, *Jnk1*, *Jnk2*, and *Jnk3*, have been informative. Expression of JNK3 is largely restricted to the nervous system [37], whereas JNK1 and JNK2 are expressed in many tissues [17]. *Jnk3* (−/−) knockout mice survive development, are viable, and have a normal brain structure [38]. Interestingly, compared to wild-type mice, *Jnk3* (−/−) mice are much less sensitive to the effects of kainic acid injection, which normally induces epileptic seizures and the apoptotic death of hippocampal CA1 and CA3 neurons. In contrast, inactivation of *Jnk1* or *Jnk2* had no effect on kainic acid-induced seizures or apoptosis [38]. This suggests that JNK3 might be required for stress-induced neuronal apoptosis in the adult hippocampus. However, because kainic acid-induced seizures were blocked by inactivation of *Jnk3*, it is also possible that the inhibition of apoptosis could be secondary to the effect on seizure activity. A c-*jun* "knock-in" mouse has also been described, in which the c-*jun* gene was replaced by a mutant c-*jun* gene (*Jun*AA) where the codons for serines 63 and 73 were changed to alanine [39]. Like the *Jnk3* (−/−) mouse, the *Jun*AA mouse survives development, and neuronal death in the CA1 and CA3 fields of the hippocampus after kainic acid injection is greatly reduced, suggesting that c-Jun is the key substrate for JNK3 in the kainic acid model [39]. Finally, disruption

* Eilers A, Whitfield J, Shah B and Ham J, manuscript submitted for publication.

of *Jnk1* or *Jnk2* alone has no effect on the nervous system, but a *Jnk1* (−/−) *Jnk2* (−/−) double knockout mouse dies during embryonic development with a pronounced neural phenotype [40, 41]. In the hindbrain, the neural tube fails to close due to a deficiency in apoptosis. However, the opposite effect is seen in the developing cerebral cortex, where apoptosis is increased [40, 41]. This result indicates that JNK1 and JNK2 are required for developmental cell death in the neural tube, but promote neuronal survival in the developing cortex. Thus, although JNKs and c-Jun may be pro-apoptotic in many neuronal cell types, they can have other roles. For example, it has been known for some time that increased expression of c-Jun is associated with axonal regeneration in adult peripheral neurons that have been axotomised, suggesting that c-Jun may be required for the expression of genes necessary for regeneration [42].

MOLECULAR ORDERING OF THE NEURONAL CELL DEATH PATHWAY

A number of landmark events have been identified in the cell death pathway activated by NGF withdrawal in sympathetic neurons. These include c-Jun induction, cytochrome *c* relocalisation from mitochondria to the cytosol, caspase activation, and DNA fragmentation (Fig. 3). In purified, cell-free systems, it has been shown that caspase-3 activation requires cytochrome *c*, caspase-9, apoptotic protease activating factor 1 (Apaf-1), and dATP [43], and it has been shown in microinjection experiments using a blocking antibody that cytochrome *c* is required for NGF withdrawal-induced death in sympathetic neurons [12]. Anti-apoptotic members of the Bcl-2 family, such as Bcl-2 and Bcl-xL, prevent the release of mitochondrial cytochrome *c*, whereas pro-apoptotic family members, such as Bax, have the opposite effect [44]. It seems likely that c-Jun functions at a step that is upstream of cytochrome *c* release. To determine whether this is the case, we investigated whether the redistribution of cytochrome *c* that occurs in NGF-deprived sympathetic neurons could be blocked by expressing dominant negative c-Jun. To carry out these experiments, we used recombinant adenoviruses as a gene delivery system. Recombinant adenoviruses efficiently infect postmitotic neurons, and a given protein can be expressed in the majority of neurons in a tissue culture dish [45]. We found that recombinant adenoviruses expressing dn-Jun or Bcl-2 inhibited NGF withdrawal-induced death of sympathetic neurons, whereas viruses expressing β-galactosidase or MEKK1 did not.* Furthermore, expression of dn-Jun or Bcl-2, but not β-galactosidase, also inhibited the release of cytochrome *c* from mitochondria, visualised by carrying out immunocytochemistry with an anti-cytochrome *c* antibody.† These results suggest that c-Jun promotes neuronal cell death by regulating the expression of proteins that directly or indirectly control cytochrome *c* release (Fig. 3). One possible candidate is the Fas ligand gene. The Fas ligand promoter contains an AP-1 site that enables the promoter to be activated by the JNK pathway [46]. There is evidence that Fas ligand/Fas signalling contributes to the survival signal withdrawal-induced death of differentiated PC12 cells, cerebellar granule neurons, and motoneurons [47, 48], but it is not known yet whether this also occurs in sympathetic neurons.

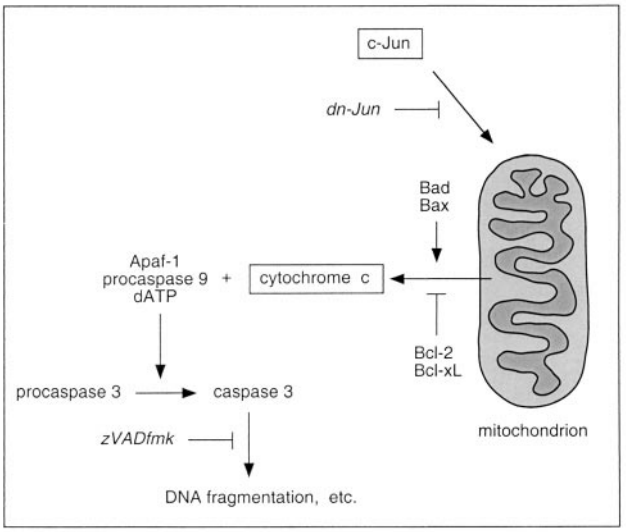

FIG. 3. The role of mitochondria in sympathetic neuron apoptosis. NGF withdrawal causes cytochrome *c* to be released from the mitochondria into the cytosol [12, 13], and cytochrome *c* is required for NGF withdrawal-induced death [12]. Cytosolic cytochrome *c* forms a complex with procaspase-9 and Apaf-1, and in the presence of dATP, procaspase-9 is activated and cleaves procaspase-3 to form activated caspase-3 [43]. Caspase-3 in turn triggers the biochemical and morphological events characteristic of apoptosis by cleaving various protein substrates. Peptide caspase inhibitors, such as benzyloxycarbonyl-Val-Ala-Asp [Ome] fluoromethylketone (zVADfmk), inhibit caspase-3 and other caspases and protect sympathetic neurons against NGF withdrawal-induced death. The release of cytochrome *c* from mitochondria is inhibited by the anti-apoptotic Bcl-2 and Bcl-xL proteins and stimulated by pro-apoptotic Bcl-2 family members, such as Bad and Bax [44]. c-Jun acts upstream of cytochrome *c* release because expression of dominant negative Jun in sympathetic neurons prevents the redistribution of cytochrome *c* that normally occurs after NGF withdrawal and blocks downstream events, such as chromatin condensation and nuclear shrinkage. c-Jun may regulate the expression of proteins that promote cytochrome *c* release.

CONCLUSIONS

The work reviewed in this commentary suggests that one function of the JNK/c-Jun pathway is to promote apoptosis in postmitotic neurons following survival factor withdrawal or exposure to excitotoxins, such as kainic acid. However, increased expression of c-Jun is also associated with regeneration of axons in the adult peripheral nervous system [42], and c-Jun is required for proliferation of fibroblasts [39]. An important challenge will be to work out which factors

* Whitfield J, Neame SJ and Ham J, unpublished observations.
† Whitfield J, Neame SJ and Ham J, unpublished observations.

determine the biological role of c-Jun in a particular cell type in a given physiological situation. The identification of c-Jun target genes that encode proteins important for apoptosis, neuronal regeneration, or cell proliferation will be essential for answering such questions. In addition, *Jnk* knockout mice or knock-in or transgenic mice expressing specific c-*jun* mutants will be useful for learning more about the normal physiological role of c-Jun in the nervous system *in vivo*. Such mice might also be useful for investigating whether the c-Jun pathway promotes neuronal death in animal models of human neurodegenerative diseases, such as Alzheimer's disease, the motoneuron diseases amyotrophic lateral sclerosis and spinal muscular atrophy, or Huntington's disease, for which transgenic mouse models have been developed [3].

References

1. Oppenheim RW, Cell death during the development of the nervous system. *Annu Rev Neurosci* **14:** 453–501, 1991.
2. Jacobson MD, Weil M and Raff MC, Programmed cell death in animal development. *Cell* **88:** 347–354, 1997.
3. Pettmann B and Henderson CE, Neuronal cell death. *Neuron* **20:** 633–647, 1998.
4. Wright LL, Cunningham TJ and Smolen AJ, Developmental neuron death in the rat superior cervical sympathetic ganglion: Cell counts and ultrastructure. *J Neurocytol* **12:** 727–738, 1983.
5. Martin DP, Schmidt RE, DiStefano PS, Lowry OH, Carter JG and Johnson EM, Inhibitors of protein synthesis and RNA synthesis prevent neuronal death caused by nerve growth factor deprivation. *J Cell Biol* **106:** 829–844, 1988.
6. Deckwerth TL and Johnson EM, Temporal analysis of events associated with programmed cell death (apoptosis) of sympathetic neurons deprived of nerve growth factor. *J Cell Biol* **123:** 1207–1222, 1993.
7. Edwards SN and Tolkovsky AM, Characterization of apoptosis in cultured rat sympathetic neurons after nerve growth factor withdrawal. *J Cell Biol* **124:** 537–546, 1994.
8. Rimon G, Bazenet CE, Philpott KL and Rubin LL, Increased surface phosphatidylserine is an early marker of neuronal apoptosis. *J Neurosci Res* **48:** 563–570, 1997.
9. Martinou I, Fernandez P-A, Missotten M, White E, Allet B, Sadoul R and Martinou J-C, Viral proteins E1B19K and p35 protect sympathetic neurons from cell death induced by NGF deprivation. *J Cell Biol* **128:** 201–208, 1995.
10. Deshmukh M, Vasilakos J, Deckwerth TL, Lampe PA, Shivers BD and Johnson EM, Genetic and metabolic status of NGF-deprived sympathetic neurons saved by an inhibitor of ICE family proteases. *J Cell Biol* **135:** 1341–1354, 1996.
11. McCarthy MJ, Rubin LL and Philpott KL, Involvement of caspases in sympathetic neuron apoptosis. *J Cell Sci* **110:** 2165–2173, 1997.
12. Neame SJ, Rubin LL and Philpott KL, Blocking cytochrome *c* activity within intact neurons inhibits apoptosis. *J Cell Biol* **142:** 1583–1593, 1998.
13. Deshmukh M and Johnson EM, Evidence of a novel event during neuronal death: Development of competence-to-die in response to cytoplasmic cytochrome *c*. *Neuron* **21:** 695–705, 1998.
14. Ham J, Babij C, Whitfield J, Pfarr CM, Lallemand D, Yaniv M and Rubin LL, A c-Jun dominant negative mutant protects sympathetic neurons against programmed cell death. *Neuron* **14:** 927–939, 1995.
15. Karin M, Liu Z-G and Zandi E, AP-1 function and regulation. *Curr Opin Cell Biol* **9:** 240–246, 1997.
16. Estus S, Zaks WJ, Freeman RS, Gruda M, Bravo R and Johnson EM, Altered gene expression in neurons during programmed cell death: Identification of c-*jun* as necessary for neuronal apoptosis. *J Cell Biol* **127:** 1717–1727, 1994.
17. Ip YT and Davis RJ, Signal transduction by the c-Jun N-terminal kinase (JNK)—from inflammation to development. *Curr Opin Cell Biol* **10:** 205–219, 1998.
18. Eilers A, Whitfield J, Babij C, Rubin LL and Ham J, Role of the Jun kinase pathway in the regulation of c-Jun expression and apoptosis in sympathetic neurons. *J Neurosci* **18:** 1713–1724, 1998.
19. Herr I, van Dam H and Angel P, Binding of promoter-associated AP-1 is not altered during induction and subsequent repression of the c-*jun* promoter by TPA and UV irradiation. *Carcinogenesis* **15:** 1105–1113, 1994.
20. Lallemand D, Ham J, Garbay S, Bakiri L, Traincard F, Jeannequin O, Pfarr CM and Yaniv M, Stress-activated protein kinases are negatively regulated by cell density. *EMBO J* **17:** 5615–5626, 1998.
21. Virdee K, Bannister AJ, Hunt SP and Tolkovsky AM, Comparison between the timing of JNK activation, c-Jun phosphorylation and onset of death commitment in sympathetic neurons. *J Neurochem* **69:** 550–561, 1997.
22. Yan M, Dai T, Deak JC, Kyriakis JM, Zon LI, Woodgett JR and Templeton DJ, Activation of stress-activated protein kinase by MEKK1 phosphorylation of its activator SEK1. *Nature* **372:** 798–800, 1994.
23. Whitmarsh AJ, Cavanagh J, Tournier C, Yasuda J and Davis RJ, A mammalian scaffold complex that selectively mediates MAP kinase activation. *Science* **281:** 1671–1674, 1998.
24. Dickens M, Rogers JS, Cavanagh J, Raitano A, Xia Z, Halpern JR, Greenberg ME, Sawyers CL and Davis RJ, A cytoplasmic inhibitor of the JNK signal transduction pathway. *Science* **277:** 693–696, 1997.
25. Xia Z, Dickens M, Raingeaud J, Davis RJ and Greenberg ME, Opposing effects of ERK and JNK–p38 MAP kinases on apoptosis. *Science* **270:** 1326–1331, 1995.
26. Bazenet CE, Mota MA and Rubin LL, The small GTP-binding protein Cdc42 is required for nerve growth factor withdrawal-induced neuronal death. *Proc Natl Acad Sci USA* **95:** 3984–3989, 1998.
27. Kanamoto T, Mota MA, Takeda K, Rubin LL, Miyazono K, Ichijo H and 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* **20:** 196–204, 2000.
28. Bamji SX, Majdan M, Pozniak CD, Belliveau DJ, Aloyz R, Kohn J, Causing CG and Miller FD, The p75 neurotrophin receptor mediates neuronal apoptosis and is essential for naturally occurring sympathetic neuron death. *J Cell Biol* **140:** 911–923, 1998.
29. Mazzoni IE, Said FA, Aloyz R, Miller FD and Kaplan D, Ras regulates sympathetic neuron survival by suppressing the p53-mediated cell death pathway. *J Neurosci* **19:** 9716–9727, 1999.
30. Watson A, Eilers A, Lallemand D, Kyriakis J, Rubin LL and Ham J, Phosphorylation of c-Jun is necessary for apoptosis induced by survival signal withdrawal in cerebellar granule neurons. *J Neurosci* **18:** 751–762, 1998.
31. D'Mello SR, Galli C, Ciotti T and Calissano P, Induction of apoptosis in cerebellar granule neurons by low potassium: Inhibition of death by insulin-like growth factor 1 and cAMP. *Proc Natl Acad Sci USA* **90:** 10989–10993, 1993.
32. Papavassiliou AG, Treier M and Bohmann D, Intramolecular signal transduction in c-Jun. *EMBO J* **14:** 2014–2019, 1995.
33. Luo Y, Umegaki H, Wang X, Abe R and Roth GS, Dopamine

induces apoptosis through an oxidation-involved SAPK/JNK activation pathway. *J Biol Chem* **273:** 3756–3764, 1998.
34. Liu YF, Expression of polyglutamine-expanded Huntingtin activates the SEK1–JNK pathway and induces apoptosis in a hippocampal neuronal cell line. *J Biol Chem* **273:** 28873–28877, 1998.
35. Bossy-Wetzel E, Bakiri L and Yaniv M, Induction of apoptosis by the transcription factor c-Jun. *EMBO J* **16:** 1695–1709, 1997.
36. Hilberg F, Aguzzi A, Howells N and Wagner EF, c-Jun is essential for normal mouse development and hepatogenesis. *Nature* **365:** 179–181, 1993.
37. Mohit AA, Martin JH and Miller CA, $p49^{3F12}$ kinase: A novel MAP kinase expressed in a subset of neurons in the human nervous system. *Neuron* **14:** 67–78, 1995.
38. Yang DD, Kuan C-Y, Whitmarsh AJ, Rincon M, Zheng TS, Davis RJ, Rakic P and Flavell RA, Absence of excitotoxicity-induced apoptosis in the hippocampus of mice lacking the *Jnk3* gene. *Nature* **389:** 865–870, 1997.
39. Behrens A, Sibilia M and Wagner EF, Amino-terminal phosphorylation of c-Jun regulates stress-induced apoptosis and cellular proliferation. *Nat Genet* **21:** 326–329, 1999.
40. Kuan C-Y, Yang DD, Samanta Roy DR, Davis RJ, Rakic P and Flavell RA, The Jnk1 and Jnk2 protein kinases are required for regional specific apoptosis during early brain development. *Neuron* **22:** 667–676, 1999.
41. Sabapathy K, Jochum W, Hochedlinger K, Chang L, Karin M and Wagner EF, Defective neural tube morphogenesis and altered apoptosis in the absence of both JNK1 and JNK2. *Mech Dev* **89:** 115–124, 1999.
42. Herdegen T, Skene P and Bahr M, The c-Jun transcription factor—bipotential mediator of neuronal death, survival and regeneration. *Trends Neurosci* **20:** 227–231, 1997.
43. Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES and Wang X, Cytochrome *c* and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell* **91:** 479–489, 1997.
44. Martinou J-C, Key to the mitochondrial gate. *Nature* **399:** 411–412, 1999.
45. Slack RS and Miller FD, Viral vectors for modulating gene expression in neurons. *Curr Opin Neurobiol* **6:** 576–583, 1996.
46. Kasibhatla S, Brunner T, Genestier L, Echeverri F, Mahboubi A and Green DR, DNA damaging agents induce expression of Fas ligand and subsequent apoptosis in T lymphocytes via the activation of NF-kB and AP-1. *Mol Cell* **1:** 543–551, 1998.
47. Le-Niculescu H, Bonfoco E, Kasuya Y, Claret F-X, Green DR and 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* **19:** 751–763, 1999.
48. Raoul C, Henderson CE and Pettmann B, Programmed cell death of embryonic motoneurons triggered through the Fas death receptor. *J Cell Biol* **147:** 1049–1061, 1999.