Bim is a proapoptotic, BH3-domain-only member of the Bcl-2 family that plays a role in death of trophic factor-deprived sympathetic neurons as well as in other paradigms of apoptotic death. We report here that nerve growth factor (NGF) leads to both a slow down-regulation of Bim expression in neuronal PC12 cells and rapid Bim phosphorylation. Both effects appear to be mediated by the MEK/ERK pathway. An assay for Bim-mediated death revealed that NGF-promoted phosphorylation suppresses the proapoptotic activity of Bim. The phosphorylation sites responsible for this effect in the extra long form of Bim were identified as Ser-109 and Thr-110. Thus, NGF protects neurons from the proapoptotic effects of Bim both by acute phosphorylation and the longer term repression of expression.

Bcl-2 family members are major components of the apoptotic mechanism and are characterized by one or more of the four conserved Bcl-2 homology (BH) domains present in Bcl-2 (reviewed in Ref. 1). Some members such as Bcl-2 and Bcl-XL promote cell survival, whereas others such as Bax, Bak, Bad, Bik, and Bim are proapoptotic. Among the proapoptotic species, a number (e.g. Bik, Bad and Bik) contain a single BH3 domain. These BH3-only proteins appear to play critical roles in cell death by both neutralizing prosurvival Bcl-2 family members and activating proapoptotic members of the family (2).

One important mechanism by which the activity of proapoptotic BH3-only proteins is regulated is posttranslational modification. A well established example is phosphorylation of Bad by the PI3 kinase/AKT signaling pathway. Phosphorylated Bad is sequestered by binding to the 14-3-3 protein and is thereby unable to heterodimerize with Bcl-2 or Bcl-XL (3, 4). In contrast, phosphorylation of Bik is required for its efficient apoptotic activity (5). Another means by which BH3-only proteins are regulated is via their expression. Bik, Hrk, and PUMA are examples of BH3-only proteins that are produced in apoptotic paradigms (6–8).

The present study concerns regulation of the BH3-only protein Bim. Bim was isolated by an expression screen for Bcl-2-binding proteins (9). There are three major isoforms, designated BimEL, BimL, and BimS, which are probably generated by alternative splicing. All isoforms promote apoptosis, with the shortest (BimS) being the most potent (9). Bim is expressed in hematopoietic, epithelial, neuronal, and germ cells (10) and is required for hematopoietic homeostasis and apoptosis of autoreactive thymocytes (2). In the nervous system, Bim appears to influence neuronal apoptosis. Both induced and developmental deaths are delayed in brain and peripheral neurons of Bim null mice (11). Moreover, BimEL is rapidly induced in NGF-deprived sympathetic neurons (11, 12), and Bim deletion delays death in this model (11). It was reported that Bim induction induced by NGF deprivation is partially mediated by the JNK pathway (12, 13). However, the NGF-dependent signaling pathway that mediates the repression of Bim expression is unknown. With respect to posttranslational modification, Bim is phosphorylated in response to IL-3 in hematopoietic cells (14), but the consequences of this have not been described.

Neurotrophic factors such as NGF play a major role in supporting the survival of neurons, but the mechanisms by which this occurs are only partially understood. Because Bim plays an important role in mediating the death of neurons and other cell types, we investigated how NGF regulates Bim so as to promote survival. We show that NGF actively down-regulates Bim expression via a MEK/ERK-dependent pathway, thereby repressing the proapoptotic influence of Bim. NGF also promotes rapid phosphorylation of Bim by a MEK/ERK-dependent pathway; such phosphorylation compromises the apoptotic activity of Bim and thereby represents an additional mechanism by which NGF provides protection from Bim-mediated death. Thus, NGF affects Bim activity on two distinct time scales, i.e. a fast phosphorylation followed by a slow down-regulation.

**EXPERIMENTAL PROCEDURES**

**Materials**—Cell growth medium RPMI 1640, Platinum Taq DNA polymerase, and LipofectAMINE 2000 were from Invitrogen. t-butyloxycarbonyl-aspartyl(OMe)-fluoromethyl ketone (Baf) was from Enzyme Systems Products, Hoechst dye 33342 and anti-human NGF antiserum were from Sigma, and the Bim antibody was from StressGen. A protein phosphatase was from New England Biolabs, and LY294002 and U0126 were from Calbiochem. Human recombinant NGF was a kind gift from Genentech, and CEP-1347 was a kind gift from Cephalon.

**Cell Culture**—PC12 cells were cultured as described previously in collagen-coated dishes with RPMI 1640 medium supplemented with 10% heat-inactivated horse serum and 5% fetal bovine serum (15). Neuronal differentiation was induced with NGF (100 ng/ml) in medium with 1% horse serum. For NGF deprivation, on day 7 of treatment the cultures were washed with NGF-free medium twice, and anti-NGF antibody (1:100) was added. Control cells were washed with serum-free
medium and maintained in medium supplied with NGF without serum.  

**Bim Expression Plasmid Constructs**—Polyadenylated RNA from PC12 cells was reverse-transcribed and cDNA encoding BimEL was amplified by PCR using Platinum Taq (Invitrogen) according to the manufacturer's protocol. The primers for the amplification were 5'-GAATTCGAACCATGGCCAAGCAACCTTCTGA-3' and 5'-GTCGACT-CAATGCCTTCTCCATACCA-3' (EcoRI and SalI linker sequences are underlined). The amplified products were digested with EcoRI and SalI, gel purified, and subcloned into PCMS-EGFP (Clontech).  

**Site-directed Mutagenesis**—Point mutations were introduced into BimEL by PCR-based site-directed mutagenesis using Phusion DNA polymerase (Stratagene) according to the manufacturer's protocol and were verified by sequencing. Similarly, BimS and BimELA(292–381) were generated by deletion of desired sequences of BimEL using site-directed mutagenesis and were verified by sequencing.  

**Transfections**—DNA was prepared with a Plasmid Maxi kit (Qiagen). PC12 cells were transfected with 3 μg of plasmid in 6-well dishes and 0.5 μg of plasmid in 24-well dishes overnight after plating using LipofectAMINE 2000. Six h later, medium with LipofectAMINE 2000 was refreshed with complete medium with or without NGF or BAF as indicated.  

**Assessment of Cell Survival**—The number of intact, enhanced green fluorescent protein (EGFP)-positive cells per well and the proportions of the apoptotic nuclei were assessed as described previously (16). All experiments were performed at least in triplicate, and results are reported as means ± S.E.

**Western Immunoblotting**—PC12 cells were lysed, and protein was analyzed by Western immunoblotting as described previously (16), except that samples were incubated at 70 °C for 10 min in Nupage (Invitrogen) sample buffer and separated by Nupage 4–12% or 12% Bis-Tris polyacrylamide gel electrophoresis prior to blotting on Hybond. Detection was carried out using enhanced chemiluminescence according to the instructions of the manufacturer (Amersham Biosciences) except in the blots shown in Figs. 1B and 2B, which employed SuperSignal West Dura extended duration substrate (Pierce).  

**RESULTS**

**NGF Reversibly Promotes Bim Phosphorylation and Depresses Bim Expression**—To examine the regulation of Bim by NGF, we employed PC12 cells. NGF promotes PC12 cell neuronal differentiation (15), whereas NGF withdrawal in the absence of serum results in their apoptotic death (16, 17). The mechanisms by which NGF promotes survival appear to be similar in PC12 cells and sympathetic neurons (16–19), and PC12 cells provide the advantage that they may be compared before and after NGF exposure. As shown in Fig. 1, Western immunoblotting revealed the expression of Bim in both naive (NGF-ununtreated) and neurally differentiated PC12 cells. The major form was BimEL, with lesser amounts of BimL and BimS. Brief exposure (10 min) of naive cells to NGF induced a mobility shift of BimEL so that a portion migrated more slowly (Fig. 1A). A time course revealed that the shift was present within 5 min, maximal by 30–60 min, and then slowly declined but was still apparent by 24 h (Fig. 1B). A shift of BimL was also detectable by 30 min of treatment. In contrast, there was no apparent shift for BimS.  

When cells were subjected to long term (7 days) treatment with NGF to induce neuronal differentiation, there was a substantial drop in both the levels of the Bim protein and the proportion that migrated more slowly (Fig. 1, A and C). NGF deprivation for 8 h (a time before cell death is apparent) significantly induced the expression of BimEL as it does in sympathetic neurons (11, 12). When the deprived cells were re-exposed to NGF for 10 min, the more slowly migrating form of BimEL was once again prominent (Fig. 1C).  

The NGF-dependent Bim mobility shift and the report that BimEL and BimL are phosphorylated in response to IL-3 in Baf-3 cells (14) prompted us to test whether the slowly migrating form of BimEL in NGF-treated cells is generated by phosphorylation. Accordingly, we exposed lysates of cells exposed to NGF for 30 min to either buffer alone or to α protein phosphatase. Phosphatase treatment completely eradicated the NGF-promoted mobility shift (Fig. 2A). Taken together, our results thus show that NGF promotes both rapid phosphorylation of BimEL and BimL and long term down-regulation of Bim protein expression. The withdrawal of NGF from neuronally differentiated cells re-induces Bim expression, whereas NGF re-addition promotes rapid Bim re-phosphorylation.

**NGF Regulates Both Bim Phosphorylation and Expression via the MEK/MAPK Pathway**—To explore the signaling pathways underlying NGF-promoted BimEL phosphorylation, we pretreated cells with LY294002 (50 μM), a specific PI3 kinase inhibitor (20), and U0126 (50 μM), a specific MEK1/2 inhibitor (21), and then exposed them to NGF for 15 min. Both compounds successfully distinguished between actions promoted by the PI3K and Ras/MEK/ERK pathways in PC12 cells and other cell types (22, 23). Western immunoblotting revealed that U0126 completely blocked BimEL phosphorylation but that, in contrast, LY294002 had no effect (Fig. 2A). Similar results were achieved with BimL after a 30 min exposure to NGF (Fig. 2B).  

We next probed the signaling pathway by which NGF regulates Bim expression by treating neuronally differentiated cells with 50 μM LY294002 or U0126 in the presence of NGF. Inhibition of the PI3K had little or no effect on Bim expression,
Fig. 2. NGF phosphorylates and down-regulates Bim via the MEK/MAPK pathway. A, the NGF-induced shift in BimEL electrophoretic migration is due to phosphorylation and is blocked by an inhibitor of MEK but not of PI3K. PC12 cells were treated with NGF for 15 min, and cell extracts were then exposed to either buffer alone (Control) or a protein phosphatase for 30 min at 30 °C as described under “Experimental Procedures.” To test whether phosphorylation was through PI3K or MEK, cells were treated with NGF for 15 min in the presence of 50 μM LY294002 or 50 μM U0126, respectively. Western immunoblotting was as described in the Fig. 1A legend. B, NGF-promoted phosphorylation of BimL is also mediated by the MEK/MAPK pathway. Cells were treated with NGF for 30 min in the presence of 50 μM LY294002 or 50 μM U0126. Western immunoblotting was as described in the Fig. 1B legend. C, the repression of Bim expression by NGF is at least in part mediated by the MEK/MAPK pathway. PC12 cells were treated with NGF for 7 days and then deprived of NGF for 8 h or treated with 50 μM LY294002 or 50 μM U0126 for 8 h in presence of NGF. Western immunoblotting was as described in the Fig. 1C legend. D, induction of the Bim protein after NGF deprivation is partially blocked by inhibition of JNK signaling. PC12 cells were treated with NGF for 7 days and then deprived of NGF for 24 h in presence or absence of 200 nM CEP-1347. Western immunoblotting was performed as described in the Fig. 1C legend.

whereas, in contrast, the inhibition of MEK led to pronounced BimEL induction (Fig. 2C). Our results thus suggest that NGF-promoted repression of Bim expression as well as rapidly elevated Bim phosphorylation are mediated at least in part via the MEK/ERK pathway, and not by the PI3K pathway.

If the MEK/ERK pathway mediates the repression of Bim expression, then what is the mechanism by which Bim is induced following NGF deprivation? Previous results with NGF-deprived cultured sympathetic neurons have indicated that the JNK signaling pathway plays a partial role in Bim induction (12, 13). As shown in Fig. 2D, this also appears to be the case for PC12 cells. When neurally differentiated PC12 cells were deprived of NGF for 24 h and simultaneously exposed to CEP-1347, a specific inhibitor of apoptosis and of the JNK pathway in PC12 cells (16), there was a partial blockade of the induction of BimEL.

**Phosphorylation Affects the Proapoptotic Activity of Bim**—To assess the effect of phosphorylation on the proapoptotic activity of Bim, we devised an over-expression system in which the coding region of rBimEL was inserted into the pCMS-EGFP vector and transiently transfected into PC12 cells. By comparison of endogenous and exogenous proteins on Western blots and with a transfection efficiency of about 50%, we estimate that BimEL was over-expressed by 20–40-fold in the presence of the pan-caspase inhibitor BAF (data not shown). At 24 h, the counts of surviving transfected cells (monitored by EGFP expression) revealed a large (95%) decrease for those transfected with BimEL as compared with those transfected with an empty vector (Fig. 3A) or a vector containing a truncated cyclin B2 insert of similar size to BimEL (data not shown). In contrast, there was no difference for cells transfected with BimS in the presence of BAF, thus ruling out a difference in initial transfection efficiency. The promotion of death by BimEL was also verified by staining nuclei with Hoechst 33342. Approximately 60% of nuclei in cells transfected with BimEL showed apoptotic morphology compared with ~5% in control cultures (Fig. 3D). These findings are consistent with reports that Bim over-expression causes apoptosis of 293T human embryonic kidney cells (9) and sympathetic neurons (12). We next examined whether NGF rescues PC12 cells from death induced by BimEL. The addition of NGF within 6 h after transfection rescued 25–40% of the cells from death promoted by BimEL at 24 h (Fig. 3A). This represents a 5–10-fold increase in survival promoted by NGF. Consistent with this, NGF promoted a nearly 60% drop in the percentage of apoptotic nuclei in BimEL-transfected cultures (Fig. 3D). Next we addressed whether rescue by NGF is due at least in part to stimulation of Bim phosphorylation. We reasoned that because BimS is the most potent death-inducing Bim isoform (9) and does not appear to undergo NGF-dependent phosphorylation, the critical phosphorylation sites for putative regulation of BimEL should be absent in BimS. If this is so, then NGF should be less effective in rescuing cells from death promoted by BimS than by BimEL. As shown in Fig. 3C, NGF rescued about half as many cells from death evoked by BimS as compared with BimEL. Conversely, the proportion of apoptotic nuclei was nearly twice as high in NGF-treated BimS-overexpressing cells than that in cells with over-expressed BimEL (Fig. 3D). Nevertheless, despite its higher apoptotic activity in presence of NGF, the transfected BimS protein was consis-
tently present at lower levels than the BimEL protein (Fig. 3E). These findings indicate that NGF-promoted phosphorylation regulates BimEL proapoptotic activity.

To narrow the region of BimEL responsible for NGF regulation, we evaluated a construct, BimELΔ-(292–381), with a deletion corresponding to the sequence of BimL that is absent in BimS (see Fig. 3B). As for BimS, NGF was significantly less effective in suppressing death promoted by BimELΔ-(292–381) than by BimEL (Fig. 3, C and D). This suggests that the site for NGF regulation lies within the BimEL sequence encoded by bases 292–381 (amino acids 98–127). To identify specific sites required for rescue by NGF, we generated BimEL constructs with point mutations at combinations of Ser and Thr residues within the region of amino acids 98–127 and tested them in our apoptosis assay. BimEL(S109G/T110A), BimEL(S109G), and BimEL(T110A) showed a resistance to rescue by NGF indistinguishable from that of BimS and BimELΔ-(292–381) than with BimEL. PC12 cells were transfected with equal amounts of pCMS-EGFP vector encoding BimEL, BimS, or BimELΔ-(292–381) in the presence or absence of NGF. 24 h after transfection, surviving (green fluorescent) cells were counted. Values are the means of counts from four independent experiments ± S.E. Symbols denote statistically significant differences (single asterisk indicates that BimS differed from BimEL in presence of NGF, p < 0.03; double asterisks indicate that BimELΔ-(292–381) differed from BimEL in the presence of NGF, p < 0.006). D. NGF suppresses the proapoptotic activity of BimEL by phosphorylation of Ser-109 or Thr-110. PC12 cells were transfected with empty vector (pCMS-EGFP) (lane 1) or with DNA encoding full-length BimEL (lane 2), BimS (lane 3), or mutants encoding BimELΔ-(292–381) (lane 4), BimEL(S109G/T110A) (lane 5), BimEL(S109G) (lane 6), BimEL(T110A) (lane 7), BimEL(S100G/S105G/T112A) (lane 8), or BimEL(S100G/T112A) (lane 9) in the presence and absence of NGF. 24 h later, the percentages of apoptotic nuclei were determined by scoring per condition at least 100 Hoechst 33242-stained nuclei of cells expressing EGFP. Values are the means of 4–5 independent experiments ± S.E. (single asterisks indicate that BimEL(S109G/T110A) and BimEL(T110A) differed from BimEL in the presence of NGF, p < 0.005; double asterisks indicate that BimS, BimELΔ-(292–381), and BimEL(S109G) differed from BimEL in the presence of NGF, p < 0.0005). E, expression of various constructs in PC12 cells in the presence of BAF. PC12 cells were transfected with equal amounts of expression vector encoding BimEL, BimS, or BimELΔ-(292–381) in the presence of 50 µM BAF for 24 h, and during the last 30 min they were treated with or without NGF. Cells were lysed and analyzed by Western immunoblotting as in described in the Fig. 1C legend.
by promoting phosphorylation of either Ser-109 or Thr-110.

Despite the absence of Ser-109 and Thr-110, BimELA-(292–381) retained a mobility shift in response to NGF treatment (Fig. 3E). This shift indicates that NGF may mediate phosphorylation at additional sites of BimEL outside of amino acids 98–127. However, it is unclear whether such sites might contribute to NGF-mediated protection from death.

**DISCUSSION**

**NGF Represses Bim Expression**—Our aim was to study the mechanisms by which NGF protects neurons from death promoted by the BH3-only protein Bim. Recent studies established that NGF withdrawal from sympathetic neurons induces Bim, which then contributes to death (11, 12). Such induction could arise through the loss of NGF-dependent suppression of Bim expression and/or because the death mechanisms triggered by NGF deprivation lead to Bim induction. Sympathetic neurons cannot be easily maintained without NGF, and thus it is problematic to use them to distinguish between these possibilities. PC12 cells, in contrast, survive without NGF in the presence of serum, and with them we found that NGF reversibly downregulates Bim. Thus, NGF actively represses Bim expression, and this contributes to the survival-promoting actions of NGF.

Our findings indicate that a blockade of MEK, but not of PI3K, up-regulates Bim in neurally differentiated PC12 cells in the presence of NGF. This indicates that NGF suppresses Bim expression at least in part through the well described Ras-dependent MEK/MAPK signaling cascade but not through the PI3K/AKT pathway. Consistent with this, each of these pathways independently regulates the survival of PC12 cells and sympathetic neurons (24, 25). Such a situation may be cell- and/or signal-specific in that Shinjyo et al. (14) reported that IL-3 down-regulates Bim in hematopoietic cells via both PI3K- and MAPK-dependent pathways. At least for the PI3K-dependent induction of Bim by IL-3, withdrawal required activation of the transcription factor FKHR-L1 (26).

Taken together, past and the present findings indicate that NGF represses Bim expression in neuronal cells by two mechanisms. As shown in past work, NGF suppresses apoptotic pathways that otherwise lead to induction of proapoptotic proteins such as Bim (11, 12). For instance, NGF partly inhibits Bim induction by suppressing the proapoptotic activation of the JNK pathway (Refs. 12 and 13, and Fig. 2D). Additionally, as shown here, NGF actively suppresses Bim expression through the activation of MEK. These two mechanisms combine to assure that Bim expression remains at sub-apoptotic levels.

**NGF Deprivation and Induction of Bim Expression**—As with sympathetic neurons, we found here that NGF withdrawal induces Bim expression in PC12 cells. Based on the above considerations, this is likely to result from several distinct mechanisms. One is via activation of the JNK/cJun apoptotic pathway. Interference with this pathway partly blocks Bim induction in sympathetic neurons (12, 13), and we have found that this is also the case for NGF-deprived PC12 cells (Fig. 2D). A second event is cessation of MEK signaling and the consequent reversal of Bim suppression. Neither mechanism alone appears to fully account for Bim induction after NGF withdrawal; interference with the JNK/cJun pathway only partially blocks Bim induction (Refs. 12 and 13, and Fig. 2D), and the blockade of MEK activity with U0126 is less effective in inducing Bim than is the removal of NGF (Fig. 2C). Thus NGF withdrawal triggers multiple mechanisms that appear to contribute to the elevation of Bim to levels that promote apoptosis.

**NGF Regulates Bim Phosphorylation and Apoptotic Activity**—In addition to relatively slow effects on Bim expression, NGF rapidly promoted phosphorylation of BimEL and BimL but not of BimS. Our results indicated that this phosphorylation suppresses the proapoptotic activity of Bim. NGF reduced death promoted by exogenous BimEL and was significantly less effective in blocking death promoted by exogenous BimS. This was also the case for a mutant form of BimEL with the deletion of a domain present in BimEL and BimL but not in BimS. NGF also less effectively suppressed death evoked by BimEL constructs with point mutations at either of two potential sites of phosphorylation within the domain not present in BimS.

Although our data support a model in which NGF-promoted phosphorylation compromises the proapoptotic activity of Bim, there are several issues that must be addressed. For instance, if this model is correct, why does NGF only rescue a proportion of cells after Bim transfection? A likely possibility is that we utilized an over-expression system such that, if even a fraction of Bim was in a non-phosphorylated state, it was sufficient to induce death. In support of this, immunoblotting indicated that a detectable fraction of endogenous and exogenous Bim remained non-phosphorylated in the presence of NGF. An alternative is that phosphorylated Bim, although less efficient in promoting death, remains at least some proapoptotic activity. A second question pertains to the effects of NGF on death induced by BimS and several Bim mutant forms. In these cases, although NGF was significantly less effective in blocking death than in the case of wild-type BimEL, why does it nevertheless provide some degree of protection? An attractive explanation is that NGF rescues cells by multiple means, including actions downstream of mitochondria (27), and that such mechanisms provide partial protection even from over-expressed forms of Bim that are not subject to regulation by phosphorylation.

Our findings indicate that NGF-promoted Bim phosphorylation is mediated by the MEK/ERK pathway. This contrasts with the BH3-only protein Bad that is targeted for phosphorylation and the consequent suppression of proapoptotic activity by the PI3K/AKT pathway (4). In the latter case, phosphorylation promotes interaction with the 14-3-3 protein and sequestration away from mitochondria. We do not presently know how NGF-promoted Bim phosphorylation modifies its proapoptotic activity. It is intriguing that the putative phosphorylation sites at Ser-109 and Thr-110, which are important for rescue by NGF, lie within the DKST region that has been identified for binding of Bim to the dynein light chain LC8 (28). It has been reported that Bim is sequestered to the microtubule-associated dynein motor complex of healthy cells and released by apoptotic stimuli (28). Thus, it may be that phosphorylation promotes such sequestration of Bim. On the other hand, the Bim-LC8 interaction was discovered in a yeast two-hybrid system and occurs in mammalian co-expression systems, and in neither case was there evidence that phosphorylation occurred or was required (28). In addition, we have been unable to find evidence in PC12 cells that NGF-promoted phosphorylation of Bim alters its association with the microtubule cytoskeleton (data not shown). Thus, phosphorylation of Ser-109/Thr-110 may regulate interaction of Bim with yet an additional partner.

**Bim and the Mechanism of NGF-promoted Neuronal Survival**—Our and other findings indicate that NGF regulates Bim at several levels. NGF depresses Bim expression and suppresses apoptotic pathways that contribute to its induction. NGF also acutely promotes Bim phosphorylation, which in turn inhibits its proapoptotic activity. This multi-level mechanism may have evolved not only to provide multiple means to protect healthy cells from a potentially lethal molecule like Bim, but also to do so over different temporal scales. Repression and induction of Bim by NGF addition and withdrawal, respectively, occur over a time course of hours to tens of hours. This assures that death occurs only after a prolonged time of NGF withdrawal and not...
during a temporary loss of trophic support. Moreover, the acute survival-promoting phosphorylation of Bim provides neurons with a rapid protection mechanism upon the reversal of prolonged trophic factor deprivation. In this regard, it is significant that acute Bim phosphorylation occurred not only for "naive" PC12 cells, but also for neuronally differentiated cells in which long term NGF withdrawal was followed by NGF restoration. Although Bim phosphorylation was maintained during continuous exposure to NGF, the proportion of the total Bim protein that was phosphorylated was diminished at 24 h and substantially lowered by 1 week of NGF treatment. This presumably reflects the slow fall in ERK signaling that occurs during continuous NGF exposure (29). However, this loss of phosphorylation is offset by the repression of Bim expression that occurs over this time. Finally, the regulation of Bim expression and phosphorylation by IL-3 in addition to NGF suggests that the present findings are relevant to additional trophic agents and cell types.

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