The stress-activated c-Jun N-terminal protein kinase (JNK) and p38 mitogen-activated protein (MAP) kinase (p38) regulate apoptosis induced by several forms of cellular insults. Potential targets for these kinases include members of the Bcl-2 family proteins, which mediate apoptosis generated through the mitochondria-initiated, intrinsic cell death pathway. Indeed, the activities of several Bcl-2 family proteins, both pro- and anti-apoptotic, are controlled by JNK phosphorylation. For example, the pro-apoptotic activity of BimEL, a member of the Bcl-2 family, is stimulated by JNK phosphorylation at Ser-65. In contrast, there is no reported evidence that p38-induced apoptosis is due to direct phosphorylation of Bcl-2 family proteins. Here we report evidence that sodium arsenite-induced apoptosis in PC12 cells may be due to direct phosphorylation of BimEL at Ser-65 by p38. This conclusion is supported by data showing that ectopic expression of a wild type, but not a non-phosphorylatable S65A mutant of BimEL, potentiates sodium arsenite-induced apoptosis and by experiments showing direct phosphorylation of BimEL at Ser-65 by p38 in vitro. Furthermore, sodium arsenite induced BimEL phosphorylation at Ser-65, which was blocked by p38 inhibition. This study provides the first example whereby p38 induces apoptosis by phosphorylating a member of the Bcl-2 family and illustrates that phosphorylation of BimEL on Ser-65 may be a common regulatory point for cell death induced by both JNK and p38 pathways.

Apoptosis plays a critical role in the proper development of the nervous system and the maintenance of homeostasis in the adult brain. Inappropriate apoptosis may contribute to various neurodegenerative conditions including stroke, epilepsy, and Parkinson disease. Furthermore, many environmental toxicants exert neurotoxicity by inducing apoptosis. For example, heavy metals, including arsenic, lead, mercury, and lithium, all induce neuronal apoptosis (1–5). Hence, elucidation of mechanisms that regulate neuronal apoptosis may provide new insights concerning strategies to counteract apoptosis associated with neurodegenerative diseases or those induced by neural toxicants.

JNK and p38 are stress-activated MAP kinases that are preferentially activated by cell stress-inducing signals, including oxidative stress, environmental stress, and toxic chemical insults (6–8). Sustained activation of JNK or p38 is implicated in the induction of many forms of neuronal apoptosis in response to a variety of cellular injuries (1, 8–15). Apoptosis induced by cellular stress is often mediated through the mitochondria-initiated cell death pathway (16). The Bcl-2 family proteins regulate this process by modulating the membrane potential and function of mitochondria. There has been intense interest in understanding how both pro- and anti-apoptotic kinase-signaling pathways regulate the function of Bcl-2-related proteins. For example, the activities of many Bcl-2 family proteins, including BAD, Bcl-2, and Bcl-xL, are regulated by protein phosphorylation (12, 17–26).

Bim is a BH3 domain-only pro-apoptotic protein and a member of the Bcl-2 family with three major forms generated by alternative splicing: BimEL, Bimε, and BimS (27, 28). BimEL is the most abundant isoform in neurons (29, 30). Recent studies suggest that JNK induces apoptosis by directly phosphorylating BAD, BimEL, and BimS (31–36). In addition, JNK also phosphorylates and inactivates the anti-apoptotic Bcl-2 and Bcl-xL (12, 20, 37, 38). In contrast to extensive studies concerning the regulation of Bcl-2 family members by JNK, there is no evidence that p38 regulates apoptosis through direct phosphorylation of Bcl-2 family proteins.

The objective of this study was to determine whether p38-induced apoptosis is dependent on p38-catalyzed phosphorylation of BimEL using arsenite-induced apoptosis in PC12 cells as an in vitro model. Our data suggest that sodium arsenite induces apoptosis by a mechanism that depends on p38 activity and BimEL function. Significantly, p38 was both necessary and sufficient to induce BimEL phosphorylation at Ser-65, a known JNK phosphorylation site that plays a key role in JNK-induced apoptosis (33, 35). These results define a novel mechanism for p38-mediated apoptosis and identify Bim phosphorylation at Ser-65 as a common mechanism underlying apoptosis induced through the JNK and p38-signaling pathways.

**References**

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3. The abbreviations used are: JNK, c-Jun N-terminal kinase; sh, short hairpin; MAP, mitogen-activated protein; GFP, green fluorescent protein; eGFP, enhanced GFP; PDE2, phosphodiesterase 2; Z, benzylxycarbonyl; fmk, fluoromethyl ketone; NAC, N-acetyl-cysteine; GSH, glutathione; HEK, human embryonic kidney; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; polII, polymerase II.
**EXPERIMENTAL PROCEDURES**

**Plasmids**—The following plasmids have been described previously: constitutively active pRC/RSV-FLAG-MKK3 (Glu), dominant negative pRC/RSV-FLAG-MKK3 (Ala), wild-type pCMV-FLAG-p38 (9), pEFBsos-FLAG-BimEL (27), the human U6-RNA polIII promoter-driven shRNA constructs of Bim and vector control (35), the human U6-RNA polIII promoter-driv en shRNA construct of green fluorescent protein (GFP) (39), and pEFpGK-EE-BimEL S65A (33). The pIRE2-DsRed2 vector was purchased from Clontech. The luciferase-shRNA and the shRNA of a scrambled phosphodiesterase 2 (PDE2) were cloned into a pBS-SKII vector containing a human U6-RNA polIII promoter (pBS-hU6) (40). Because the addition of a 27-nucleotide U6 leader sequence enhances shRNA efficiency (41), we added this leader sequence by PCR to the pBS-hU6 vector before cloning in the luciferase shRNA. The DNA sequence used to construct the luciferase shRNA has been described previously (42). The DNA sequence used to construct the scrambled PDE2 shRNA was 5′-GATCAGACCTTGAGAGA-3′.

**Reagents**—Sodium arsenite (Sigma) was dissolved in Dulbecco’s modified Eagle’s medium (Mediatech, Inc., Herndon, WA) at 1000 × stock. Z-VAD-fmk (R & D Systems, Inc., Minneapolis, MN), SP600125 (EMD Biosciences, San Diego, CA), SB203580, and SB202190 (Sigma) were dissolved in dimethyl sulfoxide (Me2SO) as 1000× stock. An equal volume of Me2SO was used as a vehicle control, which did not cause any measurable toxicity. N-Acetyl-cysteine (NAC) and glutathione (GSH, Sigma) were dissolved in water. Recombinant BimEL or BimEL 3A proteins were purchased from Upstate Biotechnology, Inc. (Lake Placid, NY). The following antibodies were purchased commercially: anti-phospho-p38 (Cell Signaling Technology), anti-phospho-c-Jun and anti-c-Jun (Cell Signaling Technology, Inc.), anti-total Bim (Stressgen Biotechnologies, Inc., San Diego, CA), and anti-EE epitope (Covance Research Products, Inc., Berkeley, CA). The anti-DsRed was from BD Biosciences, and anti-p-Ser-65 Bim was either from Upstate Biotechnology, Inc. (Ann Arbor, MI) or from Upstate Biotechnology, Inc. (Lake Placid, NY). The following antibodies were purchased commercially: anti-phospho-p38 (Cell Signaling Technology), anti-phospho-c-Jun and anti-c-Jun (Cell Signaling Technology, Inc.), anti-total Bim (Stressgen Biotechnologies, Inc., San Diego, CA), and anti-EE epitope (Covance Research Products, Inc., Berkeley, CA). The anti-DsRed was from BD Biosciences, and anti-p-Ser-65 Bim was either from Upstate Biotechnology, Inc. (Lake Placid, NY), or generated as described previously (35).

**Cell Culture and Transient Transfection**—Rat pheochromocytoma PC12 cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% horse serum, 5% fetal bovine serum, 1% glutamine, and 0.5% penicillin-streptomycin. The cells were maintained in plates coated with rat tail collagen (Biomedical Technologies, Inc., Stoughton, MA) at 37 °C with 7.5% CO2. Transient transfection was performed using Lipofectamine 2000 (Invitrogen) in regular growth medium. HEK293 cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum and 0.5% penicillin-streptomycin. Cells were maintained at 37 °C with 5% CO2. Transient transfection was performed using FuGENE 6 (Roche Applied Science) according to the manufacturer’s instructions. For transient transfections, both PC12 and HEK293 cells were plated a day before transfection onto plates coated with poly-d-lysine (Sigma).

**Western Analysis and Protein Kinase Assay**—Cell lysates were prepared as described previously (43). Thirty micrograms of protein were used for Western analysis. For the in vitro kinase assay measuring p38 phosphorylation of Bim, 250 ng of recombinant, active p38α protein and 1 μg of recombinant BimEL protein or BimEL 3A protein were incubated with 100 μM ATP in kinase assay buffer as described by the protocol from Upstate Biotechnology, Inc. The recombinant BimEL 3A and BimEL 3A and p38α were from Upstate Biotechnology, Inc. The kinase reaction was carried out in 50 μl of total volume and at 37 °C for 30 min. The kinase reaction was stopped by adding SDS loading buffer and analyzed for Bim phosphorylation at Ser-65 by Western blotting using the phosho-Ser-65 Bim antibody (Upstate Biotechnology, Inc.).

**Cell Viability and Apoptosis Assays**—Cell viability was measured by MTT metabolism (44). Apoptosis was determined by nuclear condensation and/or fragmentation after staining with the DNA dye Hoechst 33258 (bis-benzimide) (44). The transfection efficiency of PC12 cells was ~30–40%. To facilitate quantification of apoptosis in transfected cells, cells were co-transfected with eGFP or DsRed2 as a marker for transfection. At least 2,000 non-transfected or 1,000 transfected (eGFP + or Red2 + immunostaining) cells were counted for each data point. To obtain unbiased counting, slides were coded and the cells were scored blindly without prior knowledge of treatment.

**Calf Intestinal Alkaline Phosphatase Treatment**—Cell lysates to be treated with phosphatases were prepared in lysis buffers lacking phosphatase inhibitors (45). Ten units of calf intestinal alkaline phosphatase (Fermenta, Inc.) and 10 mM MgCl2 were added to 150 μg of protein lysates, and the mixture was incubated at 37 °C for 60 min. The reaction was stopped by adding SDS loading buffer.

**Data Analysis**—Data were from at least three independent experiments. Statistical analysis of data was performed using one-way analysis of variance (Figs. 2–4 and 7; error bars represent S.E., ***, p < 0.001).**

**RESULTS**

**Sodium Arsenite Induces Apoptosis in PC12 Cells**—PC12 cells were treated with 0–50 μM sodium arsenite, and cell viability was assayed at 0, 8, 24, and 48 h using the MTT metabolism assay (Fig. 1A). Sodium arsenite reduced cell viability in a dose- and time-dependent manner. To determine whether the loss of cell viability was due to apoptosis, PC12 cells were treated with 0 or 15 μM sodium arsenite for 24 h and stained with the DNA dye Hoechst to visualize nuclear morphology. Sodium arsenite caused morphological changes characteristic of apoptosis, including nuclear condensation and fragmentation (Fig. 1B). Induction of the apoptotic phenotype by sodium arsenite was dependent on the time of incubation with increasing apoptosis over time (Fig. 1C).

**p38 Activation Is Required for Sodium Arsenite-induced Apoptosis in PC12 Cells**—Activation of p38 is required for sodium arsenite-induced apoptosis in primary cultured cortical, cerebellar neurons and in non-neuronal cells (1, 2, 46). To evaluate the contribution of p38 MAP kinase for arsenite-induced apoptosis in PC12 cells, p38 activity was monitored by Western analysis using an anti-phospho-p38 antibody that specifically recognizes phosphorylated and activated p38 (Fig. 2, A and B). Treatment with sodium arsenite at 15 μM, or higher concentrations, induced p38 phosphorylation (Fig. 2A), indic-
ative of p38 activation. Activation of p38 was detectable 1 h after treatment, reached a maximum at 8 h, and persisted for at least 24 h (Fig. 2B).

To determine whether p38 activation contributes to sodium arsenite-induced apoptosis, PC12 cells were transiently transfected with a dominant interfering mutant of MAP kinase kinase 3 (dnMKK3) to selectively block p38 activation. MKK3 is an upstream kinase that activates and phosphorylates p38 (47). Expression of dnMKK3 significantly inhibited apoptosis induced by 15 μM sodium arsenite (Fig. 2C; *** p < 0.001). In addition, treatment with 10 μM SB202190 or SB203580, inhibitors for p38, also protected PC12 cells from sodium arsenite toxicity (Fig. 2D; *** p < 0.001 (and data not shown)). These data suggest that p38 is required for apoptosis in PC12 cells caused by sodium arsenite treatment.

Sodium Arsenite-induced Apoptosis and p38 Activation Are Mediated through Oxidative Stress—Because sodium arsenite can induce oxidative stress (48–50) and p38 is activated by oxidative stress in some systems (6–8), we considered the possibility that arsenite stimulation of p38 activity and subsequent apoptosis in PC12 cells may be due to oxidative stress. Indeed, sodium arsenite-induced p38 activation was attenuated by treatment with either of two antioxidants, GSH or NAC (Fig. 3A). Furthermore, GSH and NAC protected PC12 cells from sodium arsenite-induced cell apoptosis (Fig. 3B and C). These data suggest that sodium arsenite causes oxidative stress in PC12 cells, which leads to p38 activation and apoptosis.

BimEL Protein Is Required for Sodium Arsenite-induced Apoptosis—Because Bcl-2 family members, including BimEL, are implicated in mitochondria-initiated cell death, we examined the effect of reducing BimEL expression on arsenite-medi-
ated apoptosis. This was accomplished using a DNA template-based RNA interference method to specifically lower the expression of endogenous BimEL (35). Transient transfection of PC12 cells with a plasmid DNA encoding a BimEL hairpin RNA (35) almost completely knocked down BimEL protein expression 2 days after transfection (Fig. 4A). This decrease in Bim protein significantly protected cells from apoptosis induced by constitutive p38 activation. PC12 cells were pretreated with 5 mM NAC (B) or 1 and 10 mM GSH (C) for 2 h and then stimulated with 0 or 15 μM sodium arsenite for 24 h. NT, no treatment. At least 2000 cells were counted for each data point. ***,

p < 0.001.

Sodium Arsenite Stimulation of BimEL Phosphorylation Depends on p38 but Not JNK Activity—To investigate the relationship between BimEL and p38 in sodium arsenite-induced apoptosis, we determined whether p38 phosphorylates BimEL, thereby regulating its pro-apoptotic activity. HEK293 cells were transiently transfected with a BimEL expression plasmid, and p38 was activated by co-transfecting the cells with constitutively active MKK3 (caMKK3) and wild-type p38α. Apoptosis in transfected cells (eGFP) was scored 2 days post-transfection. C, expression of shBimEL inhibits sodium arsenite-induced apoptosis. PC12 cells were transfected with plasmid DNA encoding an empty vector control, shBimEL, shGFP, shLuciferase (shLucif), or a scrambled shPDE2. Apoptosis in transfected cells (Red2–) was scored 2 days post-transfection. At least 1000 transfected cells were counted for each data point. ***, p < 0.001.

Figure 3: Sodium arsenite-induced p38 activation and apoptosis are mediated by oxidative stress. A, treatment with anti-oxidant glutathione (GSH) or N-acetyl-cysteine (NAC) inhibits sodium arsenite-induced p38 activation. PC12 cells were preincubated for 2 h with GSH or NAC before sodium arsenite treatment. Total p38 was used as a loading control. Anti-oxidants inhibit sodium arsenite-induced apoptosis. PC12 cells were pretreated with 5 mM NAC (B) or 1 and 10 mM GSH (C) for 2 h and then stimulated with 0 or 15 μM sodium arsenite for 24 h. NT, no treatment. At least 2000 cells were counted for each data point. ***,

p < 0.001.

Figure 4: Sodium arsenite-induced PC12 cell apoptosis requires BimEL. A, expression of shBimEL reduces BimEL protein levels. PC12 cells were transfected with a BimEL RNA interfering plasmid (shBimEL) or an empty vector control. Twenty-four hours later, the cells were treated with 15 μM sodium arsenite for another 24 h. Cell lysates were analyzed by Western blotting using an anti-BimEL antibody. β-Actin was used as a loading control. To prevent protein degradation due to caspase activation, the cells were pretreated with 10 μM Z-VAD-fmk, a pan-caspase inhibitor, for 1 h before the addition of sodium arsenite. B, expression of shBimEL inhibits apoptosis induced by constitutive p38 activation. PC12 cells were transfected with 0–1.5 μg of shBimEL plasmid DNA ± co-transfection of constitutively active (ca) MKK3 and wild-type p38α. Apoptosis in transfected cells (eGFP) was scored 2 days post-transfection. C, expression of shBimEL inhibits sodium arsenite-induced apoptosis. PC12 cells were transfected with plasmid DNA encoding an empty vector control, shBimEL, shGFP, shLuciferase (shLucif), or a scrambled shPDE2. Apoptosis in transfected cells (Red2–) was scored 2 days post-transfection. At least 1000 transfected cells were counted for each data point. ***, p < 0.001.

Sodium Arsenite Stimulation of BimEL Phosphorylation Depends on p38 but Not JNK Activity—To investigate the relationship between BimEL and p38 in sodium arsenite-induced apoptosis, we determined whether p38 phosphorylates BimEL, thereby regulating its pro-apoptotic activity. HEK293 cells were transiently transfected with a BimEL expression plasmid, and p38 was activated by co-transfecting the cells with constitutively active MKK3 (caMKK3) and wild-type p38α. Apoptosis in transfected cells (eGFP) was scored 2 days post-transfection. C, expression of shBimEL inhibits sodium arsenite-induced apoptosis. PC12 cells were transfected with plasmid DNA encoding an empty vector control, shBimEL, shGFP, shLuciferase (shLucif), or a scrambled shPDE2. Apoptosis in transfected cells (Red2–) was scored 2 days post-transfection. At least 1000 transfected cells were counted for each data point. ***, p < 0.001.
shift was eliminated when cell lysates were pretreated with a calf intestine alkaline phosphatase, demonstrating that the gel shift of Bim\textsubscript{EL} is due to its phosphorylation. These data indicate that p38 activation is sufficient to induce Bim\textsubscript{EL} phosphorylation in transfected cells.

To determine whether the endogenous Bim\textsubscript{EL} protein is phosphorylated by p38 after sodium arsenite treatment, PC12 cells were treated with 15 \(\mu\text{M}\) sodium arsenite for 8 h, and cell lysates were analyzed by Western blotting using an anti-Bim antibody. Sodium arsenite caused a gel shift of the endogenous Bim\textsubscript{EL} (Fig. 5B). This shift was abolished by treatment with SB203580, a specific inhibitor for p38 (51, 52).

Because JNK also phosphorylates Bim\textsubscript{EL}, we performed additional experiments to exclude the possibility that the effect of SB203580 on Bim\textsubscript{EL} phosphorylation is due to inhibition of JNK. SB203580 did not block sodium arsenite-induced c-Jun phosphorylation (Fig. 5C), indicating that SB203580 does not interfere with JNK signaling under these conditions. Furthermore, although the JNK inhibitor SP600125 attenuated sodium arsenite-induced c-Jun phosphorylation (Fig. 5D), it did not inhibit Bim\textsubscript{EL} phosphorylation (Fig. 5E). This JNK inhibitor also did not protect PC12 cells from sodium arsenite-induced apoptosis (data not shown). Based on these observations, we conclude that p38 activation is sufficient to induce Bim\textsubscript{EL} phosphorylation and that p38 is required for Bim\textsubscript{EL} phosphorylation after sodium arsenite treatment.
p38 Phosphorylation of BimEL

FIGURE 6. p38 MAP kinase phosphorylates BimEL at Ser-65. A, activation of p38 signaling induces BimEL phosphorylation at Ser-65. HEK293 cells were co-transfected with plasmid DNA encoding BimEL and a vector control (V) or caMKK3+p38CA. Twenty-four hours after transfection, the cells were treated with 10 μM SB203580 or vehicle control for another 24 h. B, sodium arsenite induces BimEL phosphorylation at Ser-65 in a p38-dependent manner. PC12 cells were preincubated with 10 μM SB203580 or vehicle control for 1 h and then treated with 0–30 μM sodium arsenite for 8 h. C, p38 is sufficient to phosphorylate BimEL at Ser-65 in vitro. A recombinant wild-type BimEL protein or a BimEL 3A mutant protein (S55A/S65A/S73A) was incubated with a purified, active p38α together with ATP in an in vitro kinase assay. The cell lysates from A and B or the in vitro kinase reaction product from C were analyzed for BimEL phosphorylation at Ser-65 by Western blotting using an antibody specific to BimEL phosphorylated at Ser-65. Total BimEL was used as a loading control.

65 residue is replaced by a non-phosphorylatable alanine. One day after transfection, the cells were treated with 15 μM sodium arsenite or vehicle control for 24 h. In untreated cells, the wild type (but not the S65A mutant BimEL) induced apoptosis (Fig. 7A), consistent with previous reports using cultured cerebellar granule neurons (33, 35). Furthermore, the wild type (but not the S65A mutant BimEL) potentiated PC12 cell apoptosis after sodium arsenite treatment. This is also consistent with the report that overexpression of BimEL potentiates insulin withdrawal-induced apoptosis in cerebellar granule neurons (35). These data suggest that Ser-65 phosphorylation is important for BimEL to mediate sodium arsenite-induced apoptosis.

DISCUSSION

The objective of this study was to determine whether p38 MAP kinase induces apoptosis by directly phosphorylating and modulating the activity of the Bcl-2 family protein BimEL. Using sodium arsenite-induced apoptosis in PC12 cells as a model, we have discovered that p38 activation is sufficient to induce BimEL phosphorylation at Ser-65 and is required for Ser-65 phosphorylation of the endogenous BimEL after sodium arsenite treatment. Furthermore, we have found that p38 directly phosphorylates BimEL at Ser-65 in vitro. Expression of the wild type (but not S65A mutant of BimEL) potentiated sodium arsenite-induced apoptosis. Taken with existing evidence that a phospho-mimic mutant of BimEL at Ser-65 (S65E BimEL) is a more potent inducer of apoptosis than the wild-type BimEL (33, 35), our data define a novel mechanism for p38 induction of apoptosis that is mediated through phosphorylation of BimEL at Ser-65 and BimEL activation.

The stress-activated JNK and p38 MAP kinases have been implicated in the induction of apoptosis in response to many forms of apoptotic signals. There is considerable interest in understanding how the pro-apoptotic kinase-signaling pathways regulate the activity of Bcl-2 family proteins, which are key components of the cell death machinery. JNK induces apoptosis by directly phosphorylating and activating pro-apoptotic BAX, BimEL, and BimL (31–36). In contrast, relatively little is known regarding p38 phosphorylates and inactivates anti-apoptotic Bcl-2 and Bcl-xL (12, 20, 37, 38). In contrast, relatively little is known regarding p38-induced phosphorylation and regulation of the Bcl-2 family proteins. Although p38 may be involved in the phosphorylation of Bcl-xL, BAX, and BimEL, the evidence has been limited and indirect. For example, activation of the p38-signaling pathway mediates tumor necrosis factor-induced Bcl-xL phosphorylation; however, the nature of the kinase responsible for this phosphorylation, the site, and the functional consequence of this phosphorylation are unknown (54). p38 has also been implicated in increasing or decreasing BAX phosphorylation at Ser-112, but these events are indirect via p38 regulation of other intermediate kinases or phosphatases (55, 56). Although it was stated that p38 can phosphorylate BimEL at Ser-65 in vitro (53),
no actual data were shown. Data reported in our study suggest that p38 MAP kinase induces apoptosis by directly phosphorylating BimEL at Ser-65. To our knowledge, this is the first evidence that p38 MAP kinase regulates apoptosis by directly phosphorylating and regulating the activities of a Bcl-2 family protein.

Recent studies suggest that BimEL is a substrate for ERK1/2 MAP kinase (36, 53, 57–60). ERK1/2 phosphorylation of BimEL may inhibit its association with BAX and its pro-apoptotic activity (57, 60), as well as causing proteasome-mediated Bim degradation (53). Mutation of Ser-65 to alanine blocks ERK1/2 phosphorylation at Ser-65 induced by the survival growth factor interleukin-3 is blocked by inhibition of ERK1/2 (60). It is intriguing that BimEL phosphorylation at Ser-65 by JNK and p38 enhances its pro-apoptotic activity (33, 35), whereas ERK1/2 phosphorylation at this same site antagonizes its activity (53, 57, 60). It is possible that ERK1/2, JNK, and p38 phosphorylate BimEL at additional sites, leading to different patterns of BimEL phosphorylation that specify whether phosphorylated BimEL potentiates or antagonizes apoptosis.

Although JNK and p38 are both proline-directed MAP kinases and have been implicated in many different forms of apoptosis, downstream targets that mediate their apoptotic activity have not been completely elucidated. JNK-induced neuronal apoptosis requires c-Jun (1, 9, 12, 14, 15, 61, 62), which is not a substrate of p38. Bcl-2 is phosphorylated and inactivated by JNK (12, 20, 23, 63, 64); however, it does not seem to be directly phosphorylated by p38. Here we suggest BimEL phosphorylation at Ser-65 as a convergent regulatory point for regulation of apoptosis by JNK and p38.

Our data using RNA interference technology suggest a critical role for BimEL in sodium arsenite-induced apoptosis in PC12 cells. A recent report (65) suggests that sodium arsenite-induced apoptosis is largely unaffected in cortical neurons prepared from Bim knock-out mice. Although the reason for this apparent discrepancy is currently unclear, it is possible that sodium arsenite may induce apoptosis by different mechanisms in post-mitotic cortical neurons than in proliferating PC12 cells. Alternatively, it is also possible that there is developmental compensation by other BH3-only Bcl-2 families of proteins in the Bim knock-out mice. The fact that sodium arsenite induces expression of Bim proteins in cortical neurons (65) is consistent with the notion that BimEL may play an important role in sodium arsenite-induced apoptosis in cortical neurons from the normal brain.

In conclusion, our data demonstrate that sodium arsenite treatment induces oxidative stress in PC12 cells and causes sequential activation of p38 MAP kinase, phosphorylation of BimEL on Ser-65, and apoptosis (Fig. 7B). These data identify a novel mechanism by which p38 activation induces phosphorylation of the BH3-only pro-apoptotic BimEL protein at Ser-65, thereby leading to the induction of apoptosis.

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