Insulin-like Growth Factor-I Induces bcl-2 Promoter through the Transcription Factor cAMP-Response Element-binding Protein*

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Insulin-like growth factor-I (IGF-I) is known to prevent apoptosis induced by diverse stimuli. The present study examined the effect of IGF-I on the promoter activity of bcl-2, a gene with antiapoptotic function. A luciferase reporter driven by the promoter region of bcl-2 from −1640 to −1287 base pairs upstream of the translation start site containing a cAMP-response element was used in transient transfection assays. Treatment of PC12 cells with IGF-I enhanced the bcl-2 promoter activity by 2.3-fold, which was inhibited significantly (p < 0.01) by SB203580, an inhibitor of p38 mitogen-activated protein kinase (MAPK). Cotransfection of the bcl-2 promoter with MAPK kinase 6 and the β isoform of p38 MAPK resulted in 2–3-fold increase in the reporter activity. The dominant negative form of MAPKAP-K3, a downstream kinase activated by p38 MAPK, and the dominant negative form of cAMP-response element-binding protein, inhibited the reporter gene activation by IGF-I and p38β MAPK significantly (p < 0.01). IGF-I increased the activity of p38β MAPK introduced into the cells by adenoviral infection. Thus, we have characterized a novel signaling pathway (MAPK kinase 6/p38β MAPK/MAPKAP-K3) that defines a transcriptional mechanism for the induction of the antiapoptotic protein Bcl-2 by IGF-I through the nuclear transcription factor cAMP-response element-binding protein in PC12 cells.

The products of the bcl-2 gene belong to a growing family of proteins that are involved in the regulation of mammalian apoptosis. They include proapoptotic (Bax, Bad, Bid, and Bik) and antiapoptotic (Bcl-2, Bcl-xL, and Brag-1) proteins (1). Complex interplay between these two groups of proteins seems to decide the fate of cells when exposed to apoptotic stimuli. In transgenic mice overexpressing the bcl-2 gene, the loss of neuronal cells by natural cell death as well as experimental ischemia is significantly reduced (2). In bcl-2 gene-ablated mice, loss of neurons and apoptosis in thymus and spleen has been observed (3). The expression pattern of Bcl-2 during murine embryogenesis by immunohistological analysis shows that this protein is restricted to zones of survival (4). Hence, expression of Bcl-2 appears to be a key regulatory step in promoting cell survival.

Insulin-like growth factor-I (IGF-I) is known to exert antiapoptotic action in several cell types. One of the mechanisms by which IGF-I promotes cell survival is through down-regulation of the proapoptotic protein Bad. IGF-I stimulates the phosphorylation of Bad by activating phosphatidylinositol 3-kinase and Akt, leading to the sequestration of phospho-Bad in cytosol by the protein 14-3-3 (5). In addition to this cytosolic covalent modification, IGF-I-mediated expression of the antiapoptotic protein Bcl-xL could play a role in the promotion of cell survival (6). This growth factor has been shown to inhibit the down-regulation of Bcl-2 protein induced by hypoxia in cultured rat cortical neurons and by interleukin-3 deprivation in murine myeloid progenitor cells (7, 8). The mechanism by which IGF-I sustains the expression of bcl-2 has not been studied. IGF-I is likely to increase the bcl-2 expression at the transcriptional level, since bcl-2 promoter is positively regulated by the nuclear transcription factor CREB, and IGF-I can activate this transcription factor (9). The bcl-2 gene consists of three exons with an untranslated first exon. It has a TATA-less GC-rich promoter with positive and negative regulatory elements (10–12). The presence of a CRE site in a region between −1526 and −1552 upstream of the translation start site has been reported (12). Phosphorylation of CREB by PKC in B lymphocytes leads to induction of the bcl-2 gene in a CRE-dependent fashion and protection from apoptosis (12). In a recent study, we demonstrated that insulin-like growth factor-I-induced CREB activation involves p38 MAPK-mediated signaling pathway in PC12 cells (9). Hence, activation of p38 MAPK could stimulate the bcl-2 promoter activity through CREB in these cells.

The p38 MAPK belongs to the MAPK superfamily, the other members being extracellular signal-regulated kinase 1/2 and stress-activated protein kinase/N-terminal Jun kinase. Activation of p38 MAPK has been observed during apoptosis mediated by diverse stimuli such as growth factor withdrawal and exposure to UV irradiation (13). p38 MAPK is important for programmed cell death, since its specific inhibitor SB203580 can prevent apoptosis (14). However, studies have demonstrated that p38 MAPK can be activated by growth factors, leading to induction of growth-promoting genes (9, 15). Differentiation of PC12 cells into a neuronal cell type and adipogenesis have been shown to require p38 MAPK (16, 17).
The apparent discrepancy between these observations can probably be explained by the existence of several p38 MAPK isoforms with distinct functions. So far, four isoforms, α, β, γ, and δ, have been identified with several splice variants (18–21). In cardiomyocytes, β isozyme was shown to exert hypertrophic action, whereas p38α induces apoptosis (22). Identification of isoform specific regulation by trophic versus toxic factors should clarify this confusing scenario.

The objectives of the present investigation were (a) to examine the IGF-I mediated activation of bcl-2 promoter in PC12 cells and characterize the signaling pathway involved in this activation and (b) to examine the isozyme specific role of p38 MAPK in the activation of bcl-2 promoter. We demonstrate that IGF-I induced bcl-2 promoter activity proceeds in part through a novel signaling pathway involving MAPK kinase 6/p38 MAPK and requires CREB.

**EXPERIMENTAL PROCEDURES**

**Materials**—Cell culture media and supplies were from Life Technologies, Inc. (Beverly, MA) and Gemini Bio Products, Inc. (Calabasas, CA). SB 203580 was obtained from Calbiochem. PD98059 was purchased from Beecham, King of Prussia, PA). The dominant negative CREB (pRS-VKCREB) was provided by Dr. Richard Goodman (Oregon Health Sciences University, Portland, OR). The luciferase assay kit was purchased from Analytical Luminescence Laboratory (San Diego, CA). Antibodies specific for CREB, phospho-CREB (Ser-133), p38 MAPK, CII, PKC, ERK1, and ATF-2, and the ATF-2 fusion protein was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-phospho-CREB antibody and other fine chemicals were purchased from Sigma.

**Preparation of Recombinant Adenovirus**—cDNA encoding full-length FLAG epitope-tagged p38β or MAPK kinase 6 (WT) were subcloned into HindIII and XbaI sites in the plasmid pACCMVpLPα, which includes the left end of the adenovirus chromosome with the E1A gene and the 5′- half of the E1B gene replaced by the cytomegalovirus major immediate early promoter, a multiple cloning site, and intron and polyadenylation sequences from SV40 (23). Recombinant adenovirus containing these genes was subsequently amplified by serial passage in HEK-293 cells (24). Plasmids containing the appropriate constructs in pACCMVpLPα were cotransfected into 293 cells with the SV40 promoter was included to normalize the transfection efficiency. Statistical analysis was carried out by Student’s t test. The PC12 cells were infected with adenovirus p38β linked to FLAG epitope and MAPK kinase 6 (WT) and exposed to IGF-I as described in the legend to Fig. 7. After washing the cells with PBS, 200 μl of ice-cold cell lysis buffer (20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM sodium orthovanadate, 10 μg/ml leupeptin, 500 μM o-phenanthroline, and 1 mM phenylmethylsulfonyl fluoride) was added. The cells were scraped, lysed by sonication, and centrifuged for 20 min. The supernatant (300 μg of protein) was mixed with 15 μg of FLAG antibody overnight at 4 °C. Protein A-Sepharose (20 μl) was added and gently rocked for 3 h at 4 °C. After centrifugation, the pellet was washed twice with cell lysis buffer and twice with kinase assay buffer (25 mM Tris (pH 7.5), 5 mM β-glycerophosphate, 2 mM dithiothreitol, 0.1 mM sodium orthovanadate, 10 mM MgCl2). The pellet was suspended in 30 μl of kinase buffer with 200 μM ATP and 2 μg of ATP-2 fusion protein and incubated for 30 min at 30 °C. The reaction was terminated by the addition of 10 μl of 4× Laemmli sample buffer. These samples were electrophoresed and immunoblotted with antibody to phospho-ATF-2. The intensities of the bands were quantitated by scanning.

**Transfection Procedure**—Transient transfection was carried out using LipofectAMINE Plus reagent (Life Technologies, Inc.). PC12 cells were cultured to 60–80% confluence for transfection experiments in 6 × 35-mm plates. For each well, 1 μg of plasmids, 3 μl of Plus reagent, and 10 μg of LipofectAMINE reagent were used as per the manufacturer’s instructions. The plasmid containing the β-galactosidase gene driven by the CMV promoter was included to normalize the transfection efficiency. DNA and LipofectAMINE reagent were diluted in 300 μl of serum-free medium without antibiotics, mixed together, and incubated at room temperature for 30 min. The culture plates were washed with PBS, and 800 μl of serum- and antibiotic-free medium was added. The 200 μl of the plasmid LipofectAMINE mixture was then added to each well, and the plates were incubated at 37 °C for 5 h. Then 1 ml of high serum medium (20% fetal bovine serum and 10% heat-inactivated horse serum) was added, and the cells were incubated for approx. 24 h with growth factors for luciferase assay. The cells were washed in PBS and lysed with 100 μl of reporter lysis buffer. The cells were lysed by freezing and thawing, and lysate was centrifuged at 14,000 RPM for 30 min. The supernatant was used for the assay of luciferase and β-galactosidase. Luciferase assays were carried out using the enhanced luciferase assay kit (Analytical Luminescence Laboratory, San Diego, CA) on a Monolight 2010 luminometer. The β-galactosidase assay was performed according to the method of Wadzinski et al. (29).

Statistical analysis was carried out by Student’s t test.
RESULTS

Basal bcl-2 Promoter Activity Is Positively Regulated by CREB—Previous studies have shown the regulation of bcl-2 promoter activity by positive and negative regulatory elements in the 5′ upstream regions (10–12). To explore the importance of CRE in bcl-2 expression, we first characterized these regulatory regions of bcl-2 promoter in PC12 cells. The sequence from −3934 to −1287 was able to drive the expression of a luciferase gene from a promoterless reporter construct (Fig. 1). Truncation of the 5′-end from −3934 to −1640 led to a 2.5-fold increase in the promoter activity (Fig. 1). This increase seems to be due to the loss of negative regulatory regions identified by previous studies (10, 11). This truncated promoter region contains a CRE site between −1611 and −1526. Mutation of the CRE site decreased the luciferase activity by 50%. Additionally, cotransfection of the CRE-containing reporter construct with dominant negative CREB (KCREB) significantly (p < 0.001) decreased the luciferase induction. Progressive deletion from the 5′-end of the CRE site-containing region resulted in a 68% decrease of reporter activity. These experiments clearly demonstrate the positive regulation of basal bcl-2 promoter activity by the nuclear transcription factor CREB.

IGF-I Stimulates the Expression of Bcl-2 by Inducing Its Promoter—IGF-I is known to up-regulate the expression of the pro-cell survival protein Bcl-xL (30). We wanted to examine if this growth factor can increase the expression of Bcl-2. When PC12 cells were treated with 50 and 100 ng/ml concentrations of IGF-I, there was a significant (p < 0.001) increase in the expression of Bcl-2 as shown by the immunoblot (Fig. 2A). To understand the mechanism by which IGF-I stimulates the expression of Bcl-2, we examined the effect of this growth factor on the promoter activity of bcl-2 gene. When PC12 cells were transiently transfected with a luciferase reporter driven by the CRE site containing truncated bcl-2 promoter, IGF-I (100 ng/ml) was also able to increase its activity in a time-dependent manner (Fig. 2B). Treatment of the PC12 cells with this growth factor for 15 h led to a 2.3-fold increase in the bcl-2 promoter activity over the untreated cells. When the promoter was cotransfected with dominant negative CREB, IGF-I stimulated reporter activity was decreased by 46% (Table I), indicating that this growth factor induces bcl-2 promoter through activation of CREB.

IGF-I-mediated Induction of bcl-2 in PC12 Cells Involves p38 MAPK—Having shown the role of CREB in driving the bcl-2 promoter and its induction by IGF-I, we then proceeded to examine the signaling pathways known to be stimulated by IGF-I that are involved in phosphorylation of CREB on serine 133. CREB has been shown to be phosphorylated and activated by signaling cascades mediated by protein kinase A, protein kinase C, Ca2+, Ras, and phosphatidylinositol-3 kinase. In a recent study, we demonstrated that IGF-I-stimulated induction of chromogranin A, a neuroendocrine-specific gene responsive to CREB activation, involves the signaling mediated by MAPK kinase 6 and p38 MAPK (9). Hence, we examined the stimulation of bcl-2 promoter activity by IGF-I in the presence of IGF-I (50 and 100 ng/ml) for 24 h. After washing the cells in ice-cold PBS, 100 μl of Laemmli sample buffer was added, and the lysate was prepared by sonication. Protein samples were separated by SDS-PAGE and immunoblotted for Bcl-2. B, PC12 cells were transfected with the bcl-2 promoter and pRSV β-galactosidase. After 24 h of transfection, the cells were exposed to IGF-I (100 ng/ml) for the indicated periods of time in the absence and presence of IGF-I (100 ng/ml). At the end of incubation period, cell lysates were prepared for the assay of luciferase and β-galactosidase. The values represent means ± S.E. of three observations, each being the average of duplicate measurements.
TABLE I

Effect of KCREB on IGF- and p38 MAPK-mediated activation of bcl-2 promoter

| Cotransfection and treatment | Cotransfection of reporter with KCREB | Without KCREB | With KCREB |
|-----------------------------|---------------------------------------|---------------|------------|
| Control                     | 100                                   | 510 ± 35.5    | 1243 ± 11.9 |
| IGF-I (100 ng/ml)           | 2317 ± 25.3                           | 1010 ± 12.3   | 1187 ± 16.8 |
| MKK6 (Glu)                  | 2060 ± 13.6                           | 1820 ± 30.1   | 1182 ± 40.8 |
| p38 MAPK                    | 2247 ± 18.0                           | 1820 ± 30.1   |
| p38 MAPK + IGF-I            | 3733 ± 52.5                           | 1820 ± 30.1   |

*p < 0.001.

FIG. 3. IGF-I activates the bcl-2 promoter through the p38 MAPK-mediated signaling pathway. PC12 cells cultured in 6 x 35-mm wells were transfected with CRE-containing bcl-2 promoter construct along with pRSV β-galactosidase for 5 h by the LipofectAMINE Plus method. After 24 h of transfection, the cells were preincubated with PD98059 (40 μM) and SB203580 (10 μM) for 30 min and later exposed to IGF-I (100 ng/ml) for 24 h. The cells were lysed and assayed for luciferase and β-galactosidase activity. The values are means ± S.E. of three observations, each carried out in duplicate.

FIG. 4. Activation of bcl-2 promoter activity by MAPK kinase 6. PC12 cells cultured to 60–75% confluence in 6 x 35-mm wells were transfected with CRE-containing bcl-2 promoter construct and wild type and constitutively active forms (Glu) of MAPK kinase 6 in serum- and antibiotic-free medium. For each well, 1 μg of total plasmids, 3 μl of Plus reagent, and 10 μg LipofectAMINE reagent were used. After 24 h of transfection, the cells were incubated in the absence and presence of IGF-I for another 24 h, lysed, and assayed for luciferase and β-galactosidase. The values represent means ± S.E. of three observations, each carried out in duplicate.

of pharmacological inhibitors specific for different MAP kinases. The transfected cells were preincubated with PD98059 (40 μM), an inhibitor of MAPK kinase 1/2 (and therefore extracellular signal-regulated kinase 1/2) and SB203580 (10 μM), a specific inhibitor of p38 MAPK (Fig. 3). IGF-I-stimulated reporter activity was decreased (40%; p < 0.01) by SB203580 (Fig. 3). The MAPK kinase inhibitor (PD98059), on the other hand, increased the bcl-2 promoter activity by 80% (p < 0.001) and 52% (p < 0.01) in the absence and presence of IGF-I (Fig. 3). The bcl-2 gene has been shown to contain negative regulatory elements that respond to the Ets family of proteins, which are activated through the MAPK kinase/extracellular signal-regulated kinase pathway (11). Further studies are needed to examine this pathway. The results of the experiments with SB203580 demonstrate the involvement of p38 MAPK-mediated signaling pathway in the induction of bcl-2 by IGF-I. We next examined the role of this pathway in the regulation of bcl-2 expression in more detail.

MAPK Kinase 6-Mediated Activation of the bcl-2 Promoter—In the next series of experiments in PC12 cells, the bcl-2 reporter construct was cotransfected with wild type and dominant active forms of MAPK kinase 6, the upstream kinase known to activate p38 MAPK. The results of these studies indicated that MAPK kinase 6 is an activator of bcl-2 promoter (Fig. 4). The wild type and constitutively active (Glu) forms of MAPK kinase 6 stimulated the luciferase activity by 2–3-fold (p < 0.001). MAPK kinase 6 (Glu)-mediated bcl-2 promoter activity was decreased by 51% when the cells were cotransfected with KCREB (Table I). There were relatively smaller increases of 40 and 78% in promoter activity when cotransfected with wild type and constitutively active forms of MAPK kinase 3, respectively. This is likely to be due to differences observed in the activation pattern of p38 MAP kinases by IGF-I (Fig. 3). The MAPK kinase 6 increased the activity by 2.1–2.8-fold. Although other isoforms of p38 MAPK, α, γ, and δ, have been shown to be activated by both MAPK kinase 3 and MAPK kinase 6, whereas the β isoform is preferentially activated by MAPK kinase 6 (18, 20, 31).

p38 MAPK Activates the bcl-2 Promoter—In order to specifically understand the role of p38 MAP kinase isoforms in regulating the bcl-2 promoter activity, we overexpressed them individually in PC12 cells. Among the various isoforms of p38 MAPK, α, γ, and δ, have been shown to be activated by both MAPK kinase 3 and MAPK kinase 6, whereas the β isoform is preferentially activated by MAPK kinase 6 (18, 20, 31).
Induction of bcl-2 Promoter by IGF-I

Fig. 5. Activation of bcl-2 promoter activity by p38β MAPK. PC12 cells cultured in 6 × 35-mm wells were transfected with increasing amounts of various isoforms of p38 MAPK (A) or p38β and MAPK kinase 6 (Glu) (B). The transfection was carried out using LipofectAMINE Plus reagent in serum- and antibiotic-free medium (1 μg of total plasmids, 3 μl of Plus reagent, and 10 μg of LipofectAMINE reagent/well). After 24 h of transfection, the indicated sets of cells were treated with a 10 μM concentration of SB203580 for another 24 h (B). Luciferase and β-galactosidase were assayed in the cell lysates. The values represent means ± S.E. of three observations.

Fig. 6. Inhibition of p38β MAPK-mediated activation of bcl-2 by a dominant negative form of MAPKAP-K3. PC12 cells cultured to 60–75% confluence were transfected with the CRE-site containing bcl-2 promoter linked to the luciferase reporter and the indicated plasmids in serum- and antibiotic-free medium by the LipofectAMINE Plus method. After 24 h of transfection, the cells were treated in the absence and presence of IGF-I (100 ng/ml) for another 24 h. Luciferase and β-galactosidase were assayed in the cell lysates. Results are means ± S.E. of four independent experiments.

DisCUSSION

IGF-I has been shown to exert antiapoptotic action in several cell types. It stimulates the phosphorylation of the proapoptotic protein Bad though phosphatidylinositol 3-kinase and its downstream kinase Akt leading to its sequestration in cytosol by the protein 14-3-3 (5). Previous reports have demonstrated the ability of IGF-I to inhibit the down-regulation of Bcl-2 protein induced by hypoxia in cultured rat cortical neurons and by interleukin-3 deprivation in murine myeloid progenitor cells (7, 8). We now demonstrate that IGF-I induces promoter activity of the bcl-2 gene through activation of CREB via a novel signaling pathway mediated by MAPK kinase 6/p38β MAPK/MAPKAP-K3 (Fig. 8).

CREB is phosphorylated on serine 133 by protein kinases belonging to several different families in addition to protein kinase A. Some of the kinases involved are RSK2, calmodulin-dependent protein kinase IV, and Akt (34–36). A number of growth factors activate CREB through a p38 MAPK-mediated signaling pathway (9, 15, 37). Downstream of p38 MAPK, many kinases capable of phosphorylating CREB have been identified. Fibroblast growth factor- and UV irradiation-mediated activation of p38 MAPK leads to the activation of CREB through MAPKAP-K2 (37, 38). A recently identified mitogen and stress-activated protein kinase, MSK1, phosphorylates CREB in response to the activation of extracellular signal-regulated kinase and p38 MAPK (39). Another kinase identified as MAPKAP-K3 and 3pk, capable of phosphorylating CREB, was examined in this study using a triple mutant dominant negative form (33, 40). When cotransfected with this mutant form of MAPKAP-K3, the induction of bcl-2 by p38β MAPK is significantly impaired. This suggests that at least one of the targets of p38β MAPK important for CREB and bcl-2 regulation is MAPKAP-K3. This may not be the only critical CREB kinase

by MAPK kinase 6 and p38β MAPK, we further examined the mechanism by which this pathway can activate the transcription factor CREB through phosphorylation. A recently identified kinase, MAPKAP-K3, also known as 3pk, has been shown to be activated by p38 MAPK and extracellular signal-regulated kinase (33). When we cotransfected the reporter construct to be activated by p38 MAPK and extracellular signal-regulated kinase, MAPKAP-K3, also known as 3pk, has been shown to be activated by p38 MAPK and extracellular signal-regulated kinase (33). When cotransfected with this mutant form of MAPKAP-K3, 50 and 44% decreases (p < 0.01) in the p38β MAPK induced luciferase activity were observed in the absence and presence of IGF-I, respectively (Fig. 6). Further, when a dominant negative CREB (KCREB) was included in the transfection assay, induction by p38β MAPK decreased by 47–51% in the absence and presence of IGF-I (Table I). These experiments suggest the presence of a signaling pathway mediated by MAPK kinase 6/p38β MAPK/MAPKAP-K3, leading to the phosphorylation of CREB and subsequent induction of the CRE-containing bcl-2 promoter.

Increased Expression of Bcl-2 Protein by p38β MAPK and MAPK Kinase 6—The experiments described so far clearly demonstrate the induction of bcl-2 promoter by p38 MAPK. Next, we wanted to examine the effect of this signaling pathway on the expression of Bcl-2. Optimal transfection efficiency in PC12 cells is 10–15% with our current transfection strategy. For reporter assays, therefore, we introduced MAPK kinase 6 (WT) and p38β by adenoviral infection. We chose the wild type of MAPK kinase 6, since its effect can be further enhanced by IGF-I. Infection of the cells with increasing concentrations of adenoviral p38β and MAPK kinase 6 (WT) resulted in increased levels of phospho-p38, phospho-CREB, and Bcl-2 as shown by the immunoblots, the maximum increases being 2.2–3.4-fold (Fig. 7A). When PC12 cells infected with adenoviral p38β and MAPK kinase 6 (WT) were treated with IGF-I (100 ng/ml) for 10 min, p38 MAPK was activated by the growth factor as shown by a 64% increase in phosphorylation of p38 (Fig. 7B). We further confirmed IGF-I-mediated stimulation of p38 MAPK activity by immunoprecipitation kinase assay using ATF-2 as the substrate. As shown by the immunoblot for phospho-ATF-2, IGF-I was able to stimulate p38 MAPK activity significantly (47%; p < 0.01; Fig. 7B).

DISCUSSION

IGF-I has been shown to exert antiapoptotic action in several cell types. It stimulates the phosphorylation of the proapoptotic protein Bad though phosphatidylinositol 3-kinase and its downstream kinase Akt leading to its sequestration in cytosol by the protein 14-3-3 (5). Previous reports have demonstrated the ability of IGF-I to inhibit the down-regulation of Bcl-2 protein induced by hypoxia in cultured rat cortical neurons and by interleukin-3 deprivation in murine myeloid progenitor cells (7, 8). We now demonstrate that IGF-I induces promoter activity of the bcl-2 gene through activation of CREB via a novel signaling pathway mediated by MAPK kinase 6/p38β MAPK/MAPKAP-K3 (Fig. 8).

CREB is phosphorylated on serine 133 by protein kinases belonging to several different families in addition to protein kinase A. Some of the kinases involved are RSK2, calmodulin-dependent protein kinase IV, and Akt (34–36). A number of growth factors activate CREB through a p38 MAPK-mediated signaling pathway (9, 15, 37). Downstream of p38 MAPK, many kinases capable of phosphorylating CREB have been identified. Fibroblast growth factor- and UV irradiation-mediated activation of p38 MAPK leads to the activation of CREB through MAPKAP-K2 (37, 38). A recently identified mitogen and stress-activated protein kinase, MSK1, phosphorylates CREB in response to the activation of extracellular signal-regulated kinase and p38 MAPK (39). Another kinase identified as MAPKAP-K3 and 3pk, capable of phosphorylating CREB, was examined in this study using a triple mutant dominant negative form (33, 40). When cotransfected with this mutant form of MAPKAP-K3, the induction of bcl-2 by p38β MAPK is significantly impaired. This suggests that at least one of the targets of p38β MAPK important for CREB and bcl-2 regulation is MAPKAP-K3. This may not be the only critical CREB kinase
shown to exert distinct isozyme specific effects (22). The p38 MAPK has been shown to be involved in a p38 MAPK-dependent manner (42). The apparent discord in the results is probably due to the presence of multiple p38 MAPK isoforms with distinct functions. Isozyme-specific functional responses to p38 MAPK family members have been identified. More recently, several growth factors have been shown to activate p38 MAPK (9, 15, 41). The antiproliferative protective response. In HeLa cells, apoptosis in-duced by Fas ligation and UV irradiation are blocked by p38 MAPK play distinct roles. This does not appear to be an artifact of transient transfection strategies, as infection of PC12 cells with adenoviral p38 MAPK and MAPK kinase 6 leads to increased phosphorylation of CREB and elevated expression of Bcl-2.

CRED has been shown to promote cell survival and prevent apoptosis against diverse stimuli. Expression of the dominant negative form of CREB in human melanoma cells leads to increased susceptibility to apoptosis (44, 45). Tissue-specific expression of the dominant negative form of CREB in transgenic mice leads to progressive cardiac dysfunction, defective interleukin-2 production in thymocytes, and dwarfism resulting from somatotroph hypoplasia (46–48). Induction of the antia apoptotic protein Bcl-2 as shown by us in PC12 cells and by Wilson et al. (12) in B lymphocytes in a CREB-dependent manner provides a transcriptional mechanism by which this factor can promote cell survival. CREB is activated in the nervous system by numerous neurotrophic factors that promote survival and differentiation. These in vivo and in vitro studies clearly demonstrate the importance of CREB in cell survival. Our work on bcl-2 provides insight as to the mechanism of the cytoprotection by CREB.

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Fig. 8. Proposed signaling pathway mediated by IGF-I leading to bcl-2 induction. IGF-I activates CREB through the signaling pathway involving MAPK kinase 6, p38 β MAPK, and MAPKAP-K3, resulting in the induction of bcl-2 promoter containing the cAMP-response element binding site.

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Induction of bcl-2 Promoter by IGF-I

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