Bcl-2 Activates the Transcription Factor NFκB through the Degradation of the Cytoplasmic Inhibitor IκBa*

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Nuclear factor κB (NFκB) is a ubiquitously expressed transcription factor that is regulated by the cytoplasmic inhibitor protein IκBα. Biological agents such as tumor necrosis factor α (TNFα), which activate NFκB, result in the rapid degradation of IκBα. Adenoviral-mediated gene transfer of Bcl-2 prevents apoptosis of neonatal ventricular myocytes induced by TNFα. In view of the growing evidence that NFκB may play an important role in regulating apoptosis, we determined whether TNFα and Bcl-2 could modulate the activity of NFκB in ventricular myocytes. Stimulation of myocytes with TNFα resulted in a 2.1-fold increase in NFκB-depending gene transcription and nuclear DNA binding. Similarly, a 1.9-fold increase in NFκB-dependent gene transcription was observed in myocytes expressing Bcl-2. Nuclear DNA binding activity of NFκB was significantly increased in myocytes expressing Bcl-2, with a concomitant reduction in IκBα protein level. The Bcl-2-mediated loss of IκBα could be prevented by the proteasome inhibitor lactacystin, consistent with the notion that the targeted degradation of IκBα consequence to overexpression of Bcl-2 utilizes the ubiquitin-proteasome pathway. This was further tested in human 293 cells in which the N-terminal region of IκBα was identified to be an important regulatory site for Bcl-2. Deletion of this region or a serine to alanine substitution mutant at amino acids 32 and 36, which are defective for both phosphorylation and degradation, were more resistant than wild type IκBα to the inhibitory effects of Bcl-2. To our knowledge, this provides the first evidence for the regulation of IκBα by Bcl-2 and suggests a link between Bcl-2 and the NFκB signaling pathway in the suppression of apoptosis.

The nuclear factor κB (NFκB) was first identified as a key regulatory molecule necessary for the activation of B lymphocytes gene transcription (1, 2). Because of these initial observations, it is now widely appreciated that NFκB is a ubiquitously expressed transcription factor involved in the activation of genes associated with inflammation, cell adhesion, and viral gene transcription (reviewed in Refs. 3 and 4). NFκB belongs to a family of transcription factors with Rel homology and include Rel-A, c-Rel, RelB, and Drosofphila dorsal proteins (5–7). The predominant form of NFκB exists in mammalian cells as a heterodimeric complex of 50-kDa and 65-kDa/Rel protein subunits (8–10). NFκB activity can be induced in a number of cell types by a variety of agents, including ionizing radiation, phorbol esters, and proinflammatory cytokines such as interleukin–1 and tumor necrosis factor alpha (TNFα) (11, 12).

In contrast to other transcription factors that are typically located within the nucleus of the cell, NFκB is sequestered in the cytoplasm by the inhibitor protein IκBα (5, 13–15). IκBα prevents the nuclear targeting of NFκB by interaction via its conserved ankyrin repeats (7, 16, 17).

The mechanism by which biological signals activate NFκB in vivo remains elusive; however, recent studies suggest that NFκB activation requires the phosphorylation and degradation of IκBα (18, 19). Presumably, the inducible degradation of IκBα permits NFκB to translocate to the nucleus and affect gene transcription (11, 20). In this regard, the N-terminal domain of IκBα represents an important site of regulation, since N-terminal deletion mutations or substitution of the conserved serine residues 32 and 36 with alamines render the IκBα molecule constitutively active and resistant to biological signals that would otherwise trigger its phosphorylation and degradation (18, 21). Thus, the coordinated regulation of NFκB by IκBα underscores the biological importance of NFκB as a multifunctional transcription factor.

An anti-apoptotic function for NFκB has recently been described (22–24). This is largely substantiated by studies in which fibroblast derived from RelA−/− mice were found to be more sensitive to the cytotoxic effects of TNFα than RelA+/+ wild type controls (25, 26). Replacement of p65/NFκB into RelA-deficient cells restored resistance to TNFα-mediated apoptosis, indicating a potentially important role for NFκB in regulating apoptosis. These observations are consistent with the enhanced susceptibility of certain cells to TNFα-mediated apoptosis in the presence of the protein synthesis inhibitor cycloheximide (23).

Recently, programmed cell death has been documented in cardiac tissues in a number of disease conditions (27–29). Since ventricular myocytes are terminally differentiated and have exited the cell cycle, the loss of potentially viable cardiac cells after myocardial injury has profound clinical implications with respect to cardiac structure and function, given the lack of de novo myocyte regeneration and the meager ability of the heart to repair itself.

Although the mechanisms that govern apoptosis in cardiac cells remain poorly defined, there is evidence that the bcl-2 gene product may play a critical role in this process. We have...
recently demonstrated that adenovirus-mediated gene transfer of bcl-2 to ventricular myocytes was sufficient to prevent apoptosis provoked by either p53 or deregulated expression of E2F-1 (29, 30). Given that Bcl-2 can delay or prevent apoptosis by a diverse number of death-promoting signals, it likely impinges on one or more signaling factors that lead to cell death. Precedence for the modulation of gene transcription by Bcl-2 has been documented (31, 32). In this regard, Bcl-2 has been shown to block interleukin 2-dependent gene transcription and nuclear import of the transcription factor NF-AT4 in T-lymphocytes. Here, the BH4 domain of Bcl-2 has reportedly been suggested to bind to and sequester the calcium-activated phosphatase calcineurin, whose activity is crucial for the signal-induced dephosphorylation and nuclear import of NF-AT4 (31).

Since NFkB has been suggested to play a beneficial role in preventing apoptosis provoked under certain conditions, we ascertained whether Bcl-2 modulates the activity of NFkB in neonatal ventricular myocytes.

**MATERIALS AND METHODS**

**Cell Culture and Transfection**—Neonatal ventricular myocytes were isolated from Sprague-Dawley rat hearts and maintained as primary cultures as described previously (33). After overnight incubation in Dulbecco’s modified Eagle’s medium (DMEM/Ham’s nutrient mixture F-12 1:1, 17 mM HEPES, 3 mM NaHCO3, 2 mM l-glutamine, 50 μg/ml gentamicin, and 10% fetal bovine serum, cells were transferred to serum-free medium. Myocyte cultures were infected with 20 plaque-forming units of recombinant adenovirus per cell, which encode the bcl-2 gene product, and incubated for 4 h. This titration of virus achieves gene delivery to ≥95% of neonatal ventricular cells under these conditions (29, 34). Myocytes were transfected immediately after removal of viral stocks with DMEM containing 2.5% calf serum, 5 μg of luciferase reporter plasmid, 2.5 μg of CMV-δ-gal, and varying concentrations of IxBα expression plasmids described below. Constructs containing NFkB response elements and the herpes simplex virus thymidine kinase promoter were previously described (33, 35). Myocytes were maintained in serum-free medium and harvested 24 h after transfection. To control for potential differences in transfection efficiency among different myocyte cultures, luciferase activity was normalized to β-galactosidase activity and expressed as relative light units. Myocytes were stimulated with 10 nM/ml human recombinant TNFα for 24 h (R & D systems).

Human embryonic kidney 293 cells (American Type Culture Collection) were maintained in DMEM containing 10% fetal bovine serum (Life Technologies, Inc.) as previously reported (29, 34). For transfection experiments in 293 cells, cells were incubated for 3 h in DMEM containing Superfect (Qiagen), 5 μg of CMV-driven eukaryotic expression vectors of Bcl-2 (CMV Bcl-2) (36), and epitope-FLAG-tagged derivatives of each of the wild type IκBα, N-terminal deletion mutant encoding amino acids 37–317, (ΔNικBα), or serine to alanine substitution mutant amino acids 32 and 36, respectively (SA32/SA36IκBα) (18) (kindly provided by D. Ballard). Cells were transfected with the CMV-driven eukaryotic expression vector without the cDNA insert for all transfection controls. After transfection, cells were washed and maintained in 10% fetal bovine serum, DMEM for 24 h. Data were obtained from at least n = 4 independent cultures, with replicates of 3 for each condition. Results were compared by Student t test, using a significance level of p ≤ 0.05. Recombinant Adenoviruses—Adenoviruses were propagated, harvested, titered, and purified as previously reported (29, 34). AdCMV-Bcl-2 denotes the full-length human Bcl-2 cDNA driven by the human CMV enhancer-promoter as described previously (29, 37). The adenovirus AdDi312 designated AdNCTL was used for control for viral infection (kindly provided by T. Shenk) (33).

**Western Blot Analysis**—For immunodetection of p65/NFkB and IxBα/MAD-3 proteins, cardiac myocytes and 293 cells were harvested in 1.0% Nonidet P-40 buffer, 0.5% sodium dodecyl sulfate, 150 mM NaCl, 50 mM Tris-HCl, pH 7.4. Cell lysates (100 μl) were resolved on a 10% sodium dodecyl sulfate-polyacrylamide gel at 140 V for 4 h and electrophoretically transferred to polyvinylidene difluoride membrane (Boehringer Mannheim). For detection of p65/NFkB, the polyvinylidene difluoride membrane was incubated for 3 h at 4 °C with rabbit antibody directed toward murine p65 subunit of NFkB clone C20 (1 μg/ml Santa Cruz Biotechnology) in 150 mM NaCl, 50 mM Tris-HCl, pH 7.4, 0.3% Tween 20, 0.1% bovine serum albumin (TBS-Tween). For detection of IxBα/MAD-3 protein, the polyvinylidene difluoride filter was incubated overnight with a rabbit antibody directed toward IxBα/MAD-3 protein clone C21 (1 μg/ml Santa Cruz Biotechnology) in TBS-Tween. Expression of Bcl-2 was detected with a murine antibody directed toward Bcl-2 (kindly provided by S. Korsmeyer). For detection of transfected IxBα-FLAG-tagged proteins, cell lysates were incubated with 100 μg/ml mouse anti-FLAG M2 antibody (Eastman Kodak Co.) and immunoprecipitated with mouse anti-FLAG protein G-agarose beads (Amersham Pharmacia Biotech), and stored at –80 °C. Proteins were detected by chemiluminescence reaction with horseradish-peroxidase-conjugated sheep antibody against mouse or rabbit IgG using ECL reagents (Amersham Pharmacia Biotech).

**Electromobility Gel Shift Assay**—Nuclear extracts of cardiac myocytes were prepared as described previously by McKinsey et al. (19) with certain modifications. Briefly, 3 × 105 cells were pelleted and resuspended in 200 μl of buffer A (10 mM HEPES, pH 7.9, 60 mM KCl, 1.0 mM EDTA, 1.0 mM dithiothreitol, protease inhibitors, 0.3% Nonidet P-40). Cells were allowed to swell on ice for 15 min and centrifuged at 1,000 × g at 4 °C. The supernatant was extracted and stored at –80 °C. The remaining cell pellet was resuspended in 50 μl of buffer C (200 mM HEPES, pH 7.9, 0.4 mM NaCl, 1.0 mM EDTA, 1.0 mM EGTA, 1.0 mM dithiothreitol, 1.0 mM phenylmethylsulfonyl fluoride) and rocked vigorously at 4 °C for 15 min. The nuclear extract was centrifuged for 5 min at 10,000 × g and stored at –80 °C. Analysis of DNA binding activities by electromobility shift analysis was carried out as described previously (19) using a 32P-labeled double oligonucleotide probe containing NFkB consens binding sites 5’-AGTTGAGGGAGTCCGAGGC3’- (18). DNA binding reactions (20 μl) were carried out on ice and contained 2 μg of nuclear extract, 2 μg of double-stranded probe, poly(dI-dC) (Amersham Pharmacia Biotech), and 10 μg of bovine serum albumin in 20 mM HEPES, pH 7.9, 5% glycerol, 1 mM EDTA, 5 mM dithiothreitol. Nuclear-protein complexes were resolved on a native 5% polyacrylamide gel in 1× Tris-buffere d EDTA, pH 8.0, and detected by autoradiography (38).

**Assays of Apoptosis**—Cardiac myocytes were identified by indirect immunocytochemistry using MF20 hybridoma supernatant (generously provided by D. Bader, 1:5 dilution) against sarcomeric myosin heavy chain and 10 μg/ml rhodamine-conjugated sheep F(ab)2 anti-mouse IgG (Boehringer Mannheim). Nuclear morphology and nucleosomal DNA fragmentation of cardiac nuclei was determined by counter staining with Hoechst 33258 dye for nuclear DNA as described previously (29, 33, 39). Myocytes stained positive for both myosin heavy chain and Hoechst dye 33258 and displayed characteristic nuclear features of apoptosis were counted and scored as apoptotic as described previously (29, 33, 39). Duplicate cultures using ≥200 cells for each condition were calculated. Genomic DNA was isolated from ventricular myocytes for nucleosomal DNA fragmentation by gel electrophoresis as described previously (29, 33).
RESULTS AND DISCUSSION

To monitor signals that lead to the downstream activation of NFκB, ventricular myocytes were transfected with a luciferase reporter gene that contains putative binding sites for NFκB (18, 35). A 2.1-fold increase (p < 0.001) in luciferase reporter gene activity was observed in the presence of TNFα compared with unstimulated control cells or those cells transfected with the constitutively active herpes simplex virus thymidine kinase promoter, which lacks NFκB binding sites. Similarly, expression of Bcl-2 in ventricular myocytes resulted in a 1.9-fold increase (p < 0.0002) in NFκB-dependent transcription compared with vector-transfected control cells (Fig. 1). Furthermore, gel shift experiments indicated that NFκB binding activity was increased by TNFα in myocytes compared with vehicle-treated control cells (Fig. 3, lane 2 versus lane 3). Together, these findings confirm that ventricular myocytes are functionally coupled to biological signals that activate nuclear NFκB DNA binding activity and direct NFκB-dependent gene transcription.

Moreover, our observations indicated that stimulation of neonatal ventricular myocytes with TNFα did not provoke apoptosis as indicated by Hoechst 33258 staining (percent apoptosis; Fig. 2, control (CNTL) versus TNF, 4.7 ± 0.62% versus 4.9 ± 1.73%, p = 0.471), similar to that reported for other cell types (23, 40). However, the combination of TNFα plus the protein synthesis inhibitor cycloheximide (CHX) resulted in a significant increase in myocyte death as illustrated by increased chromatin condensation by Hoechst 33258 staining and nucleosomal DNA laddering compared with control cells or those stimulated with TNFα (percent apoptosis; Fig. 2, control (CNTL) versus TNF + CHX, 4.7 ± 0.62% versus 38 ± 8.8%, p < 0.0002; TNFα versus TNFα + CHX; 4.9 ± 1.73% versus 38 ±
Fig. 3. Electromobility gel shift analysis of ventricular myocytes. Equivalent amounts of nuclear extract (5 μg) from ventricular myocytes were prepared after treatment and analyzed for NFκB binding activity with a 32P-labeled oligonucleotide probe containing NFκB binding sites. Lane 1, free probe; lanes 2 and 11, vehicle-treated myocytes; lane 3, TNFα (10 ng/ml)-stimulated myocytes; lane 4, Bcl-2-expressing myocytes. Lane 12 represents nuclear extract from myocytes infected with control virus. Competition binding analysis of nuclear extract with 100-fold excess cold probe is shown for the above samples; lane 5, vehicle-treated controls; lane 6, TNFα (10 ng/ml)-treated myocytes; and lane 7, Bcl-2-expressing myocytes. Supershift analysis is shown in lanes 8–9, respectively, myocyte nuclear extract was incubated with a rabbit antibody directed toward the p65 subunit of NFκB (see “Materials and Methods” for details). CNTL, uninfected control; AdCNTL, adenovirus control.

8.8%, p < 0.001, Fig. 2). Interestingly, expression of Bcl-2 in ventricular myocytes reduced the incidence of cell death triggered by the combination of TNFα and cycloheximide (Fig. 2, percent apoptosis; TNF + CHX versus TNF + CHX + Bcl-2, 38 ± 8.8% versus 19.3 ± 5.81%, p < 0.01). This observation is concordant with a recent report documenting the ability of the adenovirus E1B 19-kDa protein to prevent apoptosis provoked by TNFα and cycloheximide in BRK cells (40).

The unmasking of the cytotoxic effects of TNFα by cycloheximide suggests that activation of downstream genes may play a crucial role in preventing the TNFα-mediated cytotoxicity. In this regard, it has recently been suggested that the transcription factor NFκB may be important in preventing the cytotoxic effects mediated by TNFα (23–25).

Given that Bcl-2 has been shown to prevent apoptotic cell death in a variety of cell types including ventricular myocytes (29), we ascertained whether Bcl-2 might enhance the activation of NFκB. For these experiments, we utilized recombinant adenovirus to deliver Bcl-2 to ventricular myocytes with uniformity and high efficiency (33, 34, 39). Electromobility shift analysis of nuclear extract prepared from ventricular myocytes expressing Bcl-2 displayed a significant increase in nuclear DNA binding activity of NFκB compared with uninfected control cells or those infected with a control virus (Fig. 3, lane 4 versus lane 2 and lane 12). Moreover, competition binding assays with 100-fold excess probe (lanes 5–7) as well as supershift experiments with antibodies directed toward the p65 subunit of NFκB (lanes 8–10) confirmed the migrating complex to contain p65/NFκB.

Since NFκB activity is largely governed by IκBα, which sequesters NFκB in the cytoplasm, we determined whether the observed increase in nuclear NFκB binding activity is related to decreased IκBα protein content. Protein extracts of ventricular myocytes were subjected to Western blot analysis and probed with a rabbit antibody directed toward IκBα/MAD-3. As shown in Fig. 4A, IκBα levels were profoundly suppressed in ventricular myocytes expressing Bcl-2 compared with control cells. These observations suggested that the increased nuclear NFκB DNA binding activity in myocytes expressing Bcl-2 may be a consequence of the enhanced degradation of IκBα. To test this possibility, we used lactacystin, an inhibitor of the threonine protease of the proteasome, to determine whether inhibition of proteasome-mediated degradation can prevent the Bcl-2 suppression of IκBα. By Western blot analysis, our experiments demonstrate comparable levels of IκBα in untreated...
control myocytes and those treated with lactacystin either in the presence or absence of Bcl-2 (Fig. 4). These findings support the hypothesis that Bcl-2 may target the degradation of IκBα through a proteasome-dependent mechanism. Furthermore, lactacystin prevented the nuclear localization of NFκB mediated by either Bcl-2 or TNFα in ventricular myocytes as indicated by immunocytochemistry.2

Given that the N terminus of IκBα is necessary for signal-induced phosphorylation and degradation by agents that activate NFκB (18), it might also serve as a potential target site for the actions of Bcl-2. To test this possibility, we used 293 cells for these experiments, since the transfection efficiency of neonatal ventricular myocytes by conventional methodologies for plasmid DNA is too low for global changes in gene expression to be determined2 (34). We transfected 293 cells with IκBα eukaryotic expression plasmids of either wild type or N-terminal mutants of IκBα (ΔN-IκBα) and (SA32/SA36) IκBα, which are defective for phosphorylation and degradation (18), in the presence and absence of Bcl-2. Cell extracts were prepared and immunoprecipitated with a murine antibody directed toward FLAG sequences followed by Western blot analysis for IκBα. As shown in Fig. 5A, Bcl-2 resulted in a significant reduction in the level of the wild type IκBα protein compared with vector-transfected control cells. In contrast, no apparent change in the levels of either the N-terminal mutant or SA32/SA36 mutant of IκBα was observed in the presence of Bcl-2 compared with their respective controls. Comparable levels of Bcl-2 protein were detected among the different groups, indicating that the observed effects were not a result of differences in Bcl-2 expression, (Fig. 5B). These findings suggest that the N terminus of IκBα may be the site by which Bcl-2 targets the degradation of IκBα. No change in the expression of either the wild type or mutant forms of IκBα was observed in cells transfected with the eukaryotic expression vector lacking the Bcl-2 cDNA (Fig. 5C), suggesting that observed effects were related to Bcl-2 expression alone and were not due to anomalies in cell transfection or promoter competition.

We extended these observations by testing whether Bcl-2 abrogates the inhibitory effects of IκBα on NFκB-dependent gene transcription in ventricular myocytes. The wild type and mutant forms of IκBα inhibited NFκB-dependent gene transcription equivalently. However, the inhibitory effects imposed by the wild type but not the N-terminal deletion mutant or serine-alanine 32/36 substitution mutant could be abrogated by Bcl-2.2 These findings support a model in which the N-terminal domain of IκBα is an important site for Bcl-2 regulation.

The mechanism by which Bcl-2 leads to the nuclear activation of NFκB is unknown but may be related to inactivation of cytoplasmic inhibitor protein IκBα. Precedence for factors other than NFκB that are regulated by Bcl-2 have recently been reported (32). This is exemplified by the observation that NF-AT4, which is necessary for interleukin 2-dependent gene transcription and activation-induced apoptosis in T-lymphocytes, can be modulated by Bcl-2 (31, 42). The BH4 domain of Bcl-2 binds to and sequesters the calcium-activated phosphatase calcineurin, which is crucial for the signal-induced dephosphorylation and nuclear targeting of NF-AT4. Moreover, Bcl-2 can also interact with a variety of cellular proteins including Raf-1, Bag-1, Bax, and others (43–46). Thus, it is tempting to speculate that Bcl-2 may modulate IκBα by interacting with one or more cellular proteins that either directly or indirectly activate NFκB. Alternatively, Bcl-2 may influence NFκB by altering the activity of one or the recently identified IκB kinases (47).

To our knowledge, the data presented under the conditions tested provide the first evidence for the regulation of IκBα by Bcl-2. Although a direct requirement for activation of NFκB by suppression of apoptosis by Bcl-2 was not proven, our data nevertheless suggest a tentative link between Bcl-2 and the NFκB signaling pathway for rescue from apoptosis. It should be mentioned, however, that protection from apoptosis may not be a universal feature of NFκB activation, since NFκB has also been suggested to be a critical requirement for induction of apoptosis under certain conditions (41). Thus, whether NFκB operates as a pro- or anti-apoptotic factor may depend on the context of the cell type and ensuing stimulus for apoptosis. Future studies are directed toward determining the physiological significance of these observations and the mode by which Bcl-2 modulates NFκB activity in response to apoptotic signals.

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2 D. de Moissac and L. A. Kirshenbaum, unpublished data.
REFERENCES

1. Sen, R., and Baltimore, D. (1986) Cell 46, 705–716
2. Lenardo, M. J., and Baltimore, D. (1989) Cell 58, 227–229
3. Grilli, M., Chiu, J. J., and Lenardo, M. J. (1993) Int. Rev. Cytol. 143, 1–62
4. Riviere, Y., Blank, V., Kourilsky, P., and Israel, A. (1991) Nature 350, 625–626
5. Baeuerle, P. A., and Baltimore, D. (1989) Genes Dev. 3, 1689–1698
6. Nolan, G. P., Ghosh, S., Liou, H. C., Tempst, P., and Baltimore, D. (1991) Cell 64, 961–969
7. Blank, V., Kourilsky, P., and Israel, A. (1992) Trends Biochem. Sci. 17, 135–140
8. Hansen, S. K., Nerlov, C., Zabel, U., Verde, P., Johnsen, M., Baeuerle, P. A., and Blasi, F. (1992) EMBO J. 11, 193–213
9. Urban, M. B., and Baeuerle, P. A. (1991) New Biol. 3, 279–288
10. Ghosh, S., Gifford, A. M., Riviere, L. R., Tempst, P., Nolan, G. P., and Baltimore, D. (1993) Cell 73, 1019–1029
11. Beg, A. A., Finco, T. S., Nantermet, P. V., and Baldwin, A. S., Jr. (1993) Mol. Cell. Biol. 13, 3301–3310
12. Krasnow, S. W., Zhang, L. Q., Leung, K. Y., Osborn, L., Kunkel, S., and Nabel, G. J. (1991) Cytokine 3, 372–379
13. Baeuerle, P. A., and Baltimore, D. (1988) Science 242, 540–546
14. Williams, R. S. (1993) Am. J. Med. Sci. 306, 129–136
15. Haskell, S., Beg, A. A., Tempkin, S. M., Morris, J. S., Yurochko, A. D., Sampson-Johannes, A. Mondal, K., Ralph, P., and Baldwin, A. S., Jr. (1991) Cell 65, 1281–1289
16. Beg, A. A., Ruben, S. M., Scheinman, R. I., Haskell, S., Rosen, C. A., and Baldwin, A. S., Jr. (1992) Genes Dev. 6, 1899–1913
17. Inoue, J., Kerr, L. D., Rashid, D., Davis, N., Bose, H. R., Jr., and Verma, I. M. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 4333–4337
18. Brockman, J. A., Scherer, D. C., McKinsey, T. A., Hall, S. M., Qi, X., Lee, W. Y., and Ballard, D. W. (1995) Mol. Cell. Biol. 15, 2809–2818
19. McKinsey, T. A., Brockman, J. A., Scherer, D. C., Al Murrani, S. W., Green, P. L., and Ballard, D. W. (1996) Mol. Cell. Biol. 16, 2063–2068
20. Grimm, S., and Baeuerle, P. A. (1993) Biochem. J. 290, 297–308
21. Brown, K., Gerstberger, S., Carlson, L., Franzoso, G., and Siebenlist, U. (1995) Science 267, 1485–1488
22. Wu, M., Lee, H., Bellas, R. F., Schauer, S. L., Arsura, M., Katz, D., FitzGerald, M. J., Rothstein, T. L., Sherr, D. H., and Sonenshein, G. E. (1996) EMBO J. 15, 4682–4690
23. Wang, N. M., M. W., and Baldwin, A. S., Jr. (1996) Science 274, 784–787
24. Van Antwerp, D. J., Martin, S. J., Kafri, T., Green, D. R., and Verma, I. M. (1996) Science 274, 787–789
25. Beg, A. A., and Baltimore, D. (1996) Science 274, 782–784
26. Bellas, R. F., FitzGerald, M. J., Fausto, N., and Sonenshein, G. E. (1997) Am. J. Pathol. 151, 891–896
27. Tanaka, M., Ita, H., Adachi, S., Akimoto, H., Nishikawa, T., Kasajima, T., Marumo, F., and Hiroe, M. (1994) Circ. Res. 75, 426–433
28. Isner, J. M., Kearney, K., Bortman, S., and Passeri, J. (1995) Circulation 91, 2703–2711
29. Kirshenbaum, L. A., and de Moissac, D. (1997) Circulation 96, 1580–1585
30. Agah, R., Kirshenbaum, L. A., Abdellatif, M., Truong, L. D., Chakraborty, S., Michael, L. H., and Schneider, M. D. (1997) J. Clin. Invest. 100, 2722–2728
31. Shibasaki, F., Kondo, E., Nakagi, T., and McKeon, F. (1997) Nature 386, 728–731
32. Ivanov, V. N., Deng, G., Podack, E. R., and Malek, T. R. (1995) Int. Immunol. 7, 1709–1720
33. Kirshenbaum, L. A., and Schneider, M. D. (1995) J. Biol. Chem. 270, 7791–7794
34. Kirshenbaum, L. A., Macellan, W. R., Mazur, W., French, B. A., and Schneider, M. D. (1993) J. Clin. Invest. 92, 381–387
35. Wahl, C., Liptay, S., Adler, G., and Schmidt, R. M. (1998) J. Clin. Invest. 101, 1163–1174
36. Yin, X. M., Olival, Z. N., and Korsmeyer, S. J. (1994) Nature 369, 321–323
37. Subramanian, T., Tarodi, B., and Chennadurai, G. (1995) Cell Growth Differ. 6, 131–137
38. Ballard, D. W., Lynn, S. P., Gardner, J. F., and Voss, E. W., Jr. (1984) J. Biol. Chem. 259, 3492–3498
39. Kirshenbaum, L. A., Abdellatif, M., Chakraborty, S., and Schneider, M. D. (1996) Dev. Biol. 179, 402–411
40. White, E., Sambhivali, P., Debbas, M., Wold, W. S., Kuscher, D. I., and Gooding, L. R. (1992) Mol. Cell. Biol. 12, 2570–2580
41. Lin, K.-I., Lee, S.-H., Narayanan, R., Baraban, J. M., and Hardwick, J. M. (1995) J Cell Biol. 131, 1149–1161
42. Linette, G. G., Li, Y., Roth, K., and Korsmeyer, S. J. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 9545–9552
43. Wang, H. G., Rapp, U. R., and Reed, J. C. (1996) Cell 87, 629–638
44. Wang, H. G., Takayama, S., Rapp, U. R., and Reed, J. C. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 7063–7068
45. Oltvi, Z. N., Milliman, C. L., and Korsmeyer, S. J. (1997) Cell 73, 609–619
46. Takayama, S., Sato, T., Krajewski, S., Kochel, K., Irie, S., Millan, J. A., and Reed, J. C. (1995) Cell 80, 279–284
47. Worch, J. D., Gao, X., Cao, Z., Rothe, M., and Goeddel, D. V. (1997) Science 287, 866–869