NF-κB1 (p50) Homodimers Contribute to Transcription of the bcl-2 Oncogene*

John F. Kurland‡, Reinhard Kodym‡§, Michael D. Story‡, Kevin B. Spurgers‡, Timothy J. McDonnell, and Raymond E. Meyn‡¶

From the Departments of ‡Experimental Radiation Oncology and ¶Molecular Pathology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

The bcl-2 proto-oncogene is frequently expressed in human cancer. Although bcl-2 was first cloned as the t(14;18) translocation breakpoint from human follicular B-cell lymphoma, it has become apparent that many cell types express bcl-2 because of transcriptional regulation. As such, several transcription factors have been demonstrated to activate expression of bcl-2, including NF-κB. We investigated the role of NF-κB1 (p50) homodimers in the expression of Bcl-2 in two murine B-cell lymphoma cell lines: LY-as, an apoptosis-proficient line with low Bcl-2 protein expression and no nuclear NF-κB activity, and LY-ar, a nonapoptotic line with constitutive p50 homodimer activity and 30 times more Bcl-2 protein expression than LY-as. We found that nuclear p50 homodimer activity correlated with Bcl-2 expression in these cell types and identified several sites within the bcl-2 5′-flanking region that p50 was capable of binding. In vitro transcription revealed that recombinant p50 enhanced the production of run-off transcripts from the bcl-2 P1 promoter. Additional in vitro transcription experiments suggested the sites by which p50 afforded this effect. We conclude that the p50 homodimer is capable of transcriptional activation of the bcl-2 gene and suggest that its nuclear activity contributes to the expression of bcl-2 in LY-ar cells.

The proto-oncogene bcl-2 was first cloned as the t(14;18) translocation breakpoint from human follicular center B-cell lymphoma. This translocation moves the bcl-2 open reading frame from its normal promoter and regulatory sequences on chromosome 18 and places it under the control of the immunoglobulin heavy chain enhancer (E<sub>H</sub>) on chromosome 14, leading to aberrant expression (1). Since its identification, Bcl-2 has become the archetypal member of a related family of proteins involved in the regulation of apoptotic cell death, with its overexpression leading to prolonged survival of lymphocyte and promyeloid cells that ordinarily respond to a variety of apoptotic signals (1, 2).

However, not all cell types displaying Bcl-2 overexpression harbor the t(14;18) translocation. Some cases of chronic lymphocytic leukemia are characterized by variant translocations of the bcl-2 gene including t(2;18) and t(22;18), which place bcl-2 into the gene loci of the immunoglobulin κ or λ light chains, respectively (1). Furthermore, a number of non-Hodgkin’s lymphomas have been described that express bcl-2 despite any evidence for gene rearrangements (56), suggesting that expression is controlled through transcription initiating at the bcl-2 promoter. Other cancer types may also express bcl-2 by traditional transcription mechanisms. For example, Wilms’ tumors have been reported to avoid apoptosis by up-regulation of bcl-2 through WT1 (3). In normal cell types, bcl-2 expression has been attributed to transcription factors such as Aiolos and/or c-Myb in T-cells (4, 5), CAMP-responsive element-binding protein in B-cells (6, 7), and the Brn-3a Pou family in neuronal cells (8). Other transcription factors may also be important in the expression of bcl-2 including the NF-κB family, which has been implicated in the expression of both bcl-2 and bcl-X<sub>L</sub> in primary hippocampal neurons (9) and also in the maturation of B-cells, where peripheral B-cells are prevented from undergoing apoptosis by NF-κB-mediated Bcl-2 up-regulation (55). Thus, depending on the cell type and possibly the stage of differentiation, various transcription factors may regulate expression of the bcl-2 gene.

Of the transcription factors mentioned, NF-κB in particular has become especially interesting because it has been implicated in the control of several Bcl-2 family members including A1, Bcl-X<sub>L</sub>, and Bcl-2 itself (9, 10–12, 55). NF-κB was first described by Sen and Baltimore (13) as a B-cell factor that binds to a site in the enhancer region of the gene encoding the immunoglobulin κ light chain. NF-κB has since been shown to activate transcription of numerous genes involved in inflammation and immunity such as granulocyte-macrophage colony-stimulating factor, IL-2, IL-6, and IL-8 (14). The NF-κB family of transcription factors comprises a set of proteins that share what is referred to as a Rel homology domain, including mammalian p50 (NF-κB1), p65 (RelA), p52, c-Rel, and Rel-B. NF-κB is usually described as a dimer composed of the p50 and p65 subunits and is normally sequestered in the cytoplasm in complex with the specific inhibitors IκBα or IκBβ. Upon stimulation by certain cytokines such as TNFα and IL-1 or by stress factors including double-stranded RNA, bacterial lipopolysaccharide, phorbol esters, UV light, γ-radiation, protein synthesis inhibitors, and/or viruses, NF-κB is translocated to the nucleus where it then associates with the DNA-binding motif 5′-GG-GACTTTC-3′ (consensus 5′-GGGNNYYCC-3′) (15). It is thought that nuclear localization is exclusively achieved by first releasing NF-κB from its cytoplasmic complex with IκB
NF-κB1 (p50) Homodimers and Bcl-2 Expression

The release, initiated by the phosphorylation of IκBα on serines 32 and 36, is followed by degradation of the inhibitor via the ubiquitin-proteasome pathway (16, 19). This overall process is controlled by an auto-feedback loop in which NF-κB regulates the expression of IκBα (17).

A nuclear form of NF-κB is constitutive in various cell types, including B-cell lymphomas, and this constitutive activation may arise via a mechanism distinct from that of the classical transient pathway described above. In one such mechanism, hypophosphorylated IκBβ acts as a chaperone, protecting NF-κB from being bound and inhibited by IκBα, and draws NF-κB into the nucleus (20–22). Interestingly, constitutive activation of NF-κB through an IκBα chaperone may not be unique to IκBβ. Bcl-3, a homologue of the IκB proteins, has been shown to react with the cytoplasmic pool of p50 complexed with its precursor, p105, thereby forming p50 homodimers that translocate to the nucleus in a p50/p50/Bcl-3 complex (23). It is evident that different routes to NF-κB activation, as well as different dimer forms of NF-κB, may provide distinct mechanisms for differential control of various genes containing κB binding sites within their promoter regions, including anti-apoptotic proteins such as inhibitor of apoptosis proteins (24) and members of the Bcl-2 family.

We have investigated the role of a constitutively nuclear form of NF-κB that arises during the progression of an apoptosis-sensitive murine B-cell lymphoma cell line displaying little Bcl-2 protein expression (LY-as) to an apoptosis-resistant line with a 30-fold increase in Bcl-2 (LY-ar) (27, 28). By use of electrophoretic mobility shift assays (EMSA) and NF-κB family member-specific antibodies, we provide evidence that p50 homodimers make up the activated nuclear NF-κB complex in LY-ar cells. We demonstrate that reversing the activity of this NF-κB dimer form leads to a decrease in Bcl-2 transcripts in the LY-ar cell type. Additionally, EMSA using oligonucleotides representing putative NF-κB sites revealed that several sites within the Bcl-2 P1 promoter/5′-flanking region are capable of interacting with p50. Lastly we demonstrate, through in vitro transcription, that p50 is capable of enhancing transcription from the Bcl-2 P1 promoter. We conclude that p50 homodimers are capable of enhancing transcription and that Bcl-2 is likely a target gene for this NF-κB dimer form.

EXPERIMENTAL PROCEDURES

Cell Culture and Treatments—LY-ar and LY-as murine B-cell lymphoma cell lines were maintained in RPMI 1640 culture medium supplemented with 10% fetal bovine serum, 1% (v/v) 200 mM L-glutamine, and 1% (v/v) 10,000 units/ml penicillin/streptomycin in an atmosphere of 5% CO2 at 37 °C. Bcl-2 levels were examined in the presence of 30 ng/ml recombinant murine TNF-α (Pharmingen). The gels were visualized by phosphorimaging.

Bcl-2 Promoter Construct Design—A three-step cloning strategy based on previous studies was used to create the Bcl-2 promoter constructs (50). Briefly, a 308-bp probe was polymerase chain reaction-amplified with primer-generated XhoI and HindIII sites from a 7.8-kilobase genomic HindIII fragment of the Bcl-2 gene. This 308-bp probe covers a region just 5′ to the AccI site to just 5′ of the initiation codon. This probe was cloned into the XhoI and HindIII sites of the pGL3 basic luciferase vector (Promega), producing pGL3-308 Bcl-2. Next, a 486-bp XhoI/AccI restriction fragment was cloned into pGL3-308 Bcl-2, producing pGL3-748 Bcl-2. Finally, a 2100-bp XhoI fragment was cloned into pGL3-748 Bcl-2, producing pGL3-2.8 Bcl-2. The sequence contained within the construct is available as GenBank™ accession numbers X51898 and M13994.

Additionally, truncated bcl-2 promoter constructs were generated from pGL3-2.8 Bcl-2 and cloned into the G-less cassette-containing construct, pMLC ATG150, generously provided by Dr. Michele Sawadogo. Three constructs containing bcl-2 promoter elements were made by restriction digestions: Bcl-2 promoter region from -2.8 to -2.2 Bcl-2 to generate fragments that were then blunt-ended (by fill-in reaction with T4 DNA polymerase) and cloned into a blunt-ended BamHI site within the pMLC ATG150 cloning region. Fragments cloned included a 1322-bp fragment generated by digestion with XhoI and AgeI, an 800-bp fragment generated by digestion with AvaII and AgeI, and a 727-bp fragment generated by digestion with HphI and AgeI. All constructs were checked for proper orientation upstream of the G-less cassette.

In Vitro Transcription—In vitro transcription reactions were performed similarly to the one described by Dignam et al. (26) and the in vitro transcription system protocol provided by the manufacturer (Promega). Briefly, 25-μl reaction mixtures were prepared in a buffer containing HeLaScribe® nuclear extract (58.2 μg/reaction) with ATP, UTP, CTP (provided at 0.6 mM), and GTP (provided at 0.024 mM) in a buffer solution containing 6 mM MgCl2, [32P]GTP (3,000 Ci/mmol, 10 μCi/ml) was used to label run-off transcripts. The reactions were performed in the presence of 115 ng of recombinant human p50 (Promega) or BSA, and 500 ng of linearized human bcl-2 P1 promoter/5′-flanking region was used as a template. The reactions were initiated by addition of HeLa extract and performed at 27 °C for 60 min. The reactions were terminated by the addition of 175 μl of stop solution containing 0.3 μl Tris-HCl, pH 7.40, 0.3 mM sodium acetate, 0.5% SDS, 2 μl EDTA, and 3 μg/ml yeast tRNA. RNA was precipitated with RNAeasy, isopropanol, and ethanol, pelleted, dried, and resuspended in nuclease-free water. The samples were heated to 90 °C prior to loading onto gels. RNA was resolved on a 6% acrylamide, 7 μM urea denaturing gel run at 300 V. Gels were dried, and run-off transcripts were visualized by phosphorimaging and quantified with Imagequant™ software.

In vitro transcription reactions using G-less cassette, whole-plasmid constructs as a template were performed in a similar manner. Changes to the protocol included the following: 1) 43.7 μg of HeLaScribe® nuclear extract was used in each reaction; 2) GTP was omitted from the reaction, and CTP was provided at 0.024 mM; 3) MglCl was provided at 7 μM. 4) [α-32P]CTP (3,000 Ci/mmol, 10 μCi/ml) was used to label G-less cassette transcripts; 4) 0.1 μl 3′-O-methyl GTP and 25 units of RNase T1 were included; and 5) the reactions were performed at 30 °C. In one set of reactions, 4 μg of antibodies to p50 or RelB were also included in the reaction (Santa Cruz).
RESULTS

Nuclear NF-κB Activity and Bcl-2 Expression in LY-as and LY-ar Cells: Overexpression of Bcl-2 in LY-ar Cells Correlates with Constitutively Active p50 Homodimers—Two murine B-cell lymphoma cell lines, isolated from a mouse lymphoma (LY-TH) and grown in vitro, were used in these studies. The line designated LY-as is sensitive to radiation-induced apoptosis, and the line designated LY-ar was derived from a population of the sensitive cells after maintenance in culture on the basis of its resistance to apoptosis (27). The resistant cell line was previously shown to have 30 times the amount of Bcl-2 protein expression as the sensitive cell line (28), which apparently accounts for the difference in propensity for apoptotic cell death. A representative Bcl-2 Western blot is provided (Fig. 1A).

We were unable to demonstrate by Southern blot evidence for a translocation event underlying the overexpression of Bcl-2 in the LY-ar cell population (data not shown). This, along with the finding that resistant cells emerge from the sensitive cell population in a reproducible manner with high frequency, suggests that Bcl-2 expression is altered at the level of transcription. Because of previous studies implicating NF-κB as a factor involved in B-cell lymphoma development and Bcl-2 family member expression, we compared NF-κB activity in nuclear extracts from LY-as and LY-ar cells. EMSA revealed that LY-ar cells have a constitutive form of NF-κB, whereas LY-as cells do not (Fig. 1B). To determine what members of the NF-κB family make up the constitutive form of NF-κB in LY-ar cells, EMSA supershift experiments were performed in which antibodies to known NF-κB family members were added to the DNA binding reaction. The only reactive antibody was anti-p50, which efficiently shifted the DNA-binding complex, indicating that the constitutive form of NF-κB found in LY-ar cells is most likely composed of p50 homodimers (Fig. 1C). Thus, the increase in Bcl-2 expression found between LY-as and LY-ar cells correlated with constitutive activation of p50 homodimers.

The Protease Inhibitor Calpain Inhibitor I Reverses Nuclear p50 Homodimer Activity and Decreases bcl-2 Transcripts in LY-ar Cells—To attempt to modulate p50 homodimer activity, we incubated LY-ar cells in the presence of calpain inhibitor I, a proteasome inhibitor shown to reverse NF-κB activity (18, 47). EMSA revealed that treatment with 5 μM calpain inhibitor I for 12 h led to reversal of p50 homodimer DNA binding in LY-ar nuclear extracts (Fig. 2A). To determine whether reversing nuclear p50 homodimer activity had an effect on the amount of bcl-2 transcript, RNase protection assay was performed on calpain inhibitor I-treated LY-ar cells. Detectable bcl-2 transcripts were diminished in LY-ar cells following treatment with calpain inhibitor I (Fig. 2B). The correlation of loss of nuclear p50 activity with a decrease in bcl-2 expression is consistent with a role for p50 homodimers in the regulation of bcl-2 transcription.

Inducers of Various Forms of NF-κB Lead to Variable Expression of Bcl-2 Protein in LY-as Cells—Because TPA and TNFα activate NF-κB (15), we treated LY-as cells with these agents, and nuclear NF-κB activity and Bcl-2 protein expres-
NF-κB homodimers and Bcl-2 expression

Recombinant Human p50 Is Capable of Enhancing in Vitro Transcription Assay Run-off Transcripts from the bcl-2 P1 Promoter—To assess whether p50 was capable of mediating transcription of the bcl-2 gene, an in vitro transcription assay using the human bcl-2 P1 promoter was performed. Representations of the bcl-2 promoter construct and linearized construct used as a template in the transcription assay are provided (Fig. 4A). The addition of recombinant human p50 to HeLa nuclear extracts led to a roughly 6-fold increase in run-off transcripts corresponding in size to that predicted based on initiation sites (4B) for the bcl-2 gene (Fig. 4C). Because the addition of p50 alone led to enhanced transcription, these data indicate that p50 is indeed capable of acting as a transcriptional activator and suggest that bcl-2 is a target gene for its activity.

Recombinant Human p50 Likely Enhances Transcription through Interaction with NF-κB Sites 4 and 7—We sought to delineate the regions within the bcl-2 promoter through which p50 enhanced transcription. To do this, we cloned portions of the bcl-2 promoter upstream of the P1 start site into the G-less cassette containing construct, pMLCαAT1GΔ50, which also contains the last 50 nucleotides of the adenovirus major late promoter to provide an initiation site for transcription. Truncated bcl-2 5′-flanking regions were cloned into the minimal promoter construct, and in vitro transcription reactions were performed. Representations of the truncated constructs used are provided (Fig. 5A). Addition of recombinant human p50 to HeLa nuclear extracts led to enhanced transcription of the G-less cassette for both the XhoI/AgeI and AvrII/AgeI constructs, with roughly 8- and 3-fold increases, respectively. However, addition of p50 led to little or no increase in transcription from the HphI/AgeI construct. To demonstrate that the effect was specific to p50, antibodies were added to the transcription reaction. Upon addition of anti-p50 antibody, but not antibody to RelB, transcription of the G-less cassette was diminished (Fig. 5B). These data demonstrate specific enhancement of transcription by p50 using regions of the bcl-2 promoter known to have sequences with which p50 can interact, primarily im-
In the present study we investigated the activation of p50/p50 homodimers in a murine B-cell lymphoma. We provided correlative evidence that p50/p50 is important to Bcl-2 expression in the LY-ar cell type and provided additional evidence that p50 can bind regions within the bcl-2 promoter/5′-flanking region. Lastly, we demonstrated that recombinant p50 is capable of enhancing transcription from the bcl-2 P1 promoter and showed which sites likely contribute to transcription. As such, our data implicate bcl-2 as a target gene for p50/p50-mediated regulation and suggest that constitutive nuclear activation of p50 homodimers in LY-ar cells contributes to the overexpression of Bcl-2 observed for that cell type.

Although our data strongly imply that p50 homodimers function to enhance transcription, there have been conflicting reports concerning the role p50/p50 plays in transcription. Some reports support the ability of p50 to transactivate, particularly in the presence of Bcl-3 (39–41, 49), whereas others maintain p50 acts as a repressor, binding NF-κB sites normally activated by p50/p65 (34, 42, 43, 51).

In an example of the latter case, Udalova et al. (51) have recently provided evidence that the TNF promoter contains an NF-κB binding site that typically binds both p50/p50 and p50/p65, but a single base mutation within the site specifically inhibits p50/p50 binding. Using an adenoviral reporter assay, the group demonstrated that the ability to bind p50/p50 reduces the enhancer effect of the site, with a reduction in lipopolysaccharide-inducible gene expression. Similarly, Richard et al. (43) showed that IL-9 could produce a prolonged up-regulation of p50 homodimers within the nucleus, but the authors were unable to measure an increase in luciferase expression from a promoter construct containing two palindromic NF-κB sites. However, p50 homodimer activation could prevent luciferase expression when the cells were subsequently activated by...
NF-κB1 (p50) Homodimers and Bcl-2 Expression

TNFα, suggesting that p50 homodimers are repressors of transcription rather than activators. Additional evidence of p50 as a repressor comes from Mori et al. (36), who presented evidence that p50 homodimers, which were constitutively activated in nontransformed adult T-cells, could not activate transcription of constructs containing five repeats of the κB motif from the IL-2Rα gene. However, they showed that p50/p65, which was activated in leukemic cells, could activate transcription of those constructs.

From the reports described above, p50 appears to generally act as a repressor of constructs containing tandem repeats of κB motifs. In two reports, however, in which recombinant p50 was added to HeLa nuclear extracts, three tandem repeats of the H-2KκB motif acted as a potent enhancer of transcription (39, 52). Interestingly, p50 homodimers are capable of binding all κB motifs with similar efficiency, but only a subset of those, including the H-2KκB motif, can be transcriptionally activated by p50 (39). It is possible that constructs containing κB tandem repeats other than the H-2KκB motif may provide a sequence with which p50 forms a repressing rather than an activating complex. Alternatively, transcriptional activity of p50 may be cell type-dependent or dependent on undefined cofactors. Our data suggest that κB sequences within the bcl-2 promoter can interact with p50 homodimers to enhance transcription. It is entirely possible that there are many κB sequences like the H-2KκB motif that bind p50/p50 to enhance or activate transcription.

It appears that at least one such κB motif has already been identified. The p-selectin promoter was shown to contain a sequence that preferentially bound p50 or p52 homodimers as opposed to p65-containing complexes, and p50 homodimers were shown to be transcriptionally active in luciferase assays performed using the p-selectin promoter region. Interestingly, the authors demonstrated that p50 homodimers alone could repress luciferase expression, but when Bcl-3 was co-expressed, repression was reversed (40). This not only provided evidence that p-selectin is a possible p50/p50 target gene but also implied that p50/p50 as an activator may be dependent upon other cofactors. Indeed, other Bcl-3-interacting proteins have been identified, and Tip60, a histone acetylase, has been shown to bind Bcl-3 and lead to superactivation of luciferase constructs driven by the p-selectin promoter when co-expressed with p50 and Bcl-3 in Drosophila SL2 cells (49). It is possible that certain genes contain κB sequences that preferentially bind p50/p50 in complex with transcriptionally activating cofactors such as Bcl-3 and Tip60, whereas other genes are less likely to promote transactivation and may even be repressed when bound by p50/p50 homodimers.

The control of bcl-2 expression by various transcription factors has been described in several reports (3–9, 55). We describe a cell system in which constitutive activation of the p50 homodimer form of NF-κB appears to contribute to the expression of the bcl-2 oncogene, creating an apoptosis-resistant phenotype. The path to apoptosis resistance may involve a selective process where some cells undergo a stress response that activates p50 homodimers. The subsequent Bcl-2 expression would provide a survival advantage to those cells, and they would dominate the population. It is interesting and perhaps unique that the constitutive form of NF-κB present in LY-ar cells is composed exclusively of p50 homodimers. Indeed, most investigations into transcriptional regulation by NF-κB have focused on the classical p50/p65 form, and previous reports on constitutively active NF-κB in transformed cells have described forms composed of p50/p65, p50/RelB, c-Rel/p50, or p65/e-Rel (21, 30–33). Previously, only primary cell types such as unstimulated T-cells and macrophages have been described as containing primarily the p50/p50 form (34–36), and to our knowledge, there is only a single recent report suggesting that p50 activation has an important role in tumor cell development (53). However, it is often the case that NF-κB activation in transformed cell types by cytokines or phorbol esters involves multiple dimer forms including the p50 homodimer, e.g. HeLa cells activated by TNFα (37), and it is possible the p50 homodimer plays an important role in such contexts. An interesting question for future studies is, therefore, what causes the sole activation of p50 in LY-ar cells? It has been previously reported that Bcl-3-p50 complexes are typically more abundant in transformed or primary B-cells than in non-B-cells (23, 38). Moreover, it has been demonstrated that constitutively active p50 homodimers are formed through an interaction between Bcl-3 and the cytoplasmic p50/p105 pool, leading to dimer rearrangement in which the p50/p50 complex is formed, which in turn migrates to the nucleus with Bcl-3 (23). In such a situation, the relative amounts of the various NF-κB and IκB members may critically influence whether there will be sufficient quantity of p50 to complex with p105. LY-ar cells may favor this type of activation because p105 expression is greater in the LY-ar line than the LY-as line (data not shown). It has also been reported that p105 is a target for IκBα kinases, leading to p105 processing and rapid formation of Bcl-3-p50/p50 complexes when NF-κB is induced by TNF, IL-1β, or TPA in HeLa cells (37). This too may be important to the activation of p50/p50 in the LY-ar cell type. Indeed, there may be more than one pathway to p50 homodimer activation, and in light of the data presented here and elsewhere, it appears that this can lead to activation of a certain subset of genes. It is intriguing to speculate that like p50/p65, p50/p50 affords transcriptional regulation of a class of genes whose members still remain to be identified.

As mentioned above, various types of cells are characterized by p50/p50 activation as well as by p50/p65 activation. Several strategies have been undertaken in an attempt to reverse NF-κB activation in these cells, thereby shutting down cell survival signals and potentially sensitizing the tumor cells to cytotoxic treatments. One method is to use dominant negative IκBα, which acts as a constitutive repressor of NF-κB because it contains serine-to-alanine mutations at serines 32 and 36, thereby preventing the phosphorylation and degradation of IκBα (44, 45). However, IκBα is known to primarily or preferentially interact with the p65-containing forms of NF-κB (15). With evidence that p50 homodimer activation can also lead to up-regulation of cell survival proteins, strategies that target p50 homodimers in addition to the other forms of NF-κB may be useful, particularly in cells containing both the p50 homodimer and the classical NF-κB heterodimer. Another IκB molecule, IκBγ, is known to preferentially interact with p50 homodimers (46), and one can envision that its overexpression, much like the IκBα dominant negative overexpression in the case of classical NF-κB, could lead to a reversal of p50 homodimer activity in the nucleus. Alternatively, proteasome inhibitors have been explored and shown to prevent NF-κB activation (18, 47). We demonstrated that calpain inhibitor I reversed constitutive p50/p50 activity in EMSA (Fig. 2A). It may therefore be predicted that the use of various proteasome inhibitors could lead to the inhibition of p50 activity as well.

In conclusion, p50 homodimers have been a less investigated form of the NF-κB family. However, recent interest in its binding partner Bcl-3, as well as the realization that p50 dimers may play key roles in cell survival, inflammation, and immunity in a manner similar to the other forms of NF-κB, will undoubtedly shed additional light on its mechanisms. Identification of genes that are transactivated or repressed by p50/p50
activity will be an interesting subject for future studies as well as strategies for reversing its influences.

Acknowledgments—We thank Michele Sawadogo for generously providing the G-less cassette construct pMLC2AT1G50, Marvette Hobbs for technical assistance, and Walter J. Pagel for assisting in the preparation of this manuscript.

REFERENCES

1. Strasser, A., Wang, D. C. S., and Vaux, D. L. (1997) Nature 389, 231–237
2. Chao, D. T., and Korsemeyer, S. J. (1998) Annu. Rev. Immunol. 16, 913–941
3. Palombella, V. J., Rando, O. J., Goldberg, A. L., and Maniatis, T. (1994) Cell 78, 773–785
4. Bauerle, P. A., and Baltimore, D. (1996) Cell 87, 13–20
5. Qin, B., and Baltimore, D. (1998) Science 279, 1068–1072
6. McDonald, W. F., and Baltimore, D. (1991) Science 252, 1021–1024
7. Paguzhenthi, S., Miller, E., Sable, C., Young, P., Heidenreich, K. A., Boxer, L. M., and Wingender, E. (1999) Nucleic Acids Res. 27, 1333–1337
8. Takahashi, K., and Yamamoto, K. R. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 12685–12688
9. Yamada, K., and Takahashi, K. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 12688–12693
10. Wang, C. Y., Guttridge, D. C., Mayo, M. W., and Baldwin, A. S., Jr. (1999) Biochim. Biophys. Acta 1433, 151–178
11. Lee, H. H., Dadgostar, H., Cheng, Q., Shu, J., and Cheng, G. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 1333–1337
12. Wang, C. Y., Guttridge, D. C., Mayo, M. W., and Baldwin, A. S., Jr. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 1333–1337
13. Palombella, V. J., Rando, O. J., Goldberg, A. L., and Maniatis, T. (1994) Cell 78, 773–785
14. Bauerle, P. A., and Baltimore, D. (1998) Science 279, 1068–1072
15. Qin, B., and Baltimore, D. (1996) Cell 87, 13–20
16. McDonald, W. F., and Baltimore, D. (1991) Science 252, 1021–1024
17. Takahashi, K., and Yamamoto, K. R. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 12685–12688
18. Yamada, K., and Takahashi, K. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 12688–12693
19. Wang, C. Y., Guttridge, D. C., Mayo, M. W., and Baldwin, A. S., Jr. (1999) Biochim. Biophys. Acta 1433, 151–178
20. Wang, C. Y., Guttridge, D. C., Mayo, M. W., and Baldwin, A. S., Jr. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 1333–1337
21. Palombella, V. J., Rando, O. J., Goldberg, A. L., and Maniatis, T. (1994) Cell 78, 773–785
22. Bauerle, P. A., and Baltimore, D. (1996) Cell 87, 13–20
23. Qin, B., and Baltimore, D. (1998) Science 279, 1068–1072
24. McDonald, W. F., and Baltimore, D. (1991) Science 252, 1021–1024
25. Takahashi, K., and Yamamoto, K. R. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 12685–12688
26. Yamada, K., and Takahashi, K. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 12688–12693
27. Wang, C. Y., Guttridge, D. C., Mayo, M. W., and Baldwin, A. S., Jr. (1999) Biochim. Biophys. Acta 1433, 151–178
28. Wang, C. Y., Guttridge, D. C., Mayo, M. W., and Baldwin, A. S., Jr. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 1333–1337
29. Palombella, V. J., Rando, O. J., Goldberg, A. L., and Maniatis, T. (1994) Cell 78, 773–785
30. Bauerle, P. A., and Baltimore, D. (1996) Cell 87, 13–20
31. Qin, B., and Baltimore, D. (1998) Science 279, 1068–1072
32. McDonald, W. F., and Baltimore, D. (1991) Science 252, 1021–1024
33. Takahashi, K., and Yamamoto, K. R. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 12685–12688
34. Yamada, K., and Takahashi, K. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 12688–12693
35. Wang, C. Y., Guttridge, D. C., Mayo, M. W., and Baldwin, A. S., Jr. (1999) Biochim. Biophys. Acta 1433, 151–178
36. Wang, C. Y., Guttridge, D. C., Mayo, M. W., and Baldwin, A. S., Jr. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 1333–1337
37. Palombella, V. J., Rando, O. J., Goldberg, A. L., and Maniatis, T. (1994) Cell 78, 773–785
38. Bauerle, P. A., and Baltimore, D. (1996) Cell 87, 13–20
39. Qin, B., and Baltimore, D. (1998) Science 279, 1068–1072
40. McDonald, W. F., and Baltimore, D. (1991) Science 252, 1021–1024
41. Takahashi, K., and Yamamoto, K. R. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 12685–12688
42. Yamada, K., and Takahashi, K. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 12688–12693
43. Wang, C. Y., Guttridge, D. C., Mayo, M. W., and Baldwin, A. S., Jr. (1999) Biochim. Biophys. Acta 1433, 151–178
44. Wang, C. Y., Guttridge, D. C., Mayo, M. W., and Baldwin, A. S., Jr. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 1333–1337
45. Palombella, V. J., Rando, O. J., Goldberg, A. L., and Maniatis, T. (1994) Cell 78, 773–785
46. Bauerle, P. A., and Baltimore, D. (1996) Cell 87, 13–20
47. Qin, B., and Baltimore, D. (1998) Science 279, 1068–1072
48. McDonald, W. F., and Baltimore, D. (1991) Science 252, 1021–1024
49. Takahashi, K., and Yamamoto, K. R. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 12685–12688
50. Yamada, K., and Takahashi, K. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 12688–12693
51. Wang, C. Y., Guttridge, D. C., Mayo, M. W., and Baldwin, A. S., Jr. (1999) Biochim. Biophys. Acta 1433, 151–178
52. Wang, C. Y., Guttridge, D. C., Mayo, M. W., and Baldwin, A. S., Jr. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 1333–1337
53. Palombella, V. J., Rando, O. J., Goldberg, A. L., and Maniatis, T. (1994) Cell 78, 773–785
54. Bauerle, P. A., and Baltimore, D. (1996) Cell 87, 13–20
55. Qin, B., and Baltimore, D. (1998) Science 279, 1068–1072
56. McDonald, W. F., and Baltimore, D. (1991) Science 252, 1021–1024
NF-κB1 (p50) Homodimers Contribute to Transcription of the bcl-2 Oncogene
John F. Kurland, Reinhard Kodym, Michael D. Story, Kevin B. Spurgers, Timothy J. McDonnell and Raymond E. Meyn

J. Biol. Chem. 2001, 276:45380-45386.
doi: 10.1074/jbc.M108294200 originally published online September 20, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M108294200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 57 references, 34 of which can be accessed free at
http://www.jbc.org/content/276/48/45380.full.html#ref-list-1