CREB Proteins Function as Positive Regulators of the Translocated \textit{bcl-2} Allele in t(14;18) Lymphomas*

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The translocated and normal \textit{bcl-2} alleles in the DHL-4 cell line with the t(14;18) translocation were separated by pulsed field electrophoresis. An \textit{in vivo} footprint over a cAMP response element (CRE) in the \textit{bcl-2} 5′-flanking sequence was identified on the translocated allele. Electrophoretic mobility shift assays with the \textit{bcl-2} CRE demonstrated complexes with mobilities identical to those with a consensus CRE. UV cross-linking experiments revealed that proteins with molecular masses of 34, 43, and 67 kDa bound to the \textit{bcl-2} CRE site. Electrophoretic mobility shift assay with an antibody specific to the phosphorylated cAMP response-binding protein (CREB) demonstrated that phosphorylated CREB was present in DHL-4 cells. Treatment with phorbol 12-myristate 13-acetate (PMA) led to an increase in both the amount of phosphorylated CREB and the \textit{bcl-2} promoter activity. The response to PMA was dependent on an intact CRE site. The activity of the \textit{bcl-2} promoter was increased 20-fold in a construct with the immunoglobulin heavy chain enhancers, and mutation of the CRE site abolished most of the induction. The addition of PMA increased the activity of the \textit{bcl-2}-immunoglobulin enhancer construct by 3.5-fold. Access to the CRE site is blocked in the silent normal \textit{bcl-2} allele, while CREB proteins bind to the site on the translocated allele. We conclude that the CRE site functions as a positive regulatory site for the translocated \textit{bcl-2} allele in t(14;18) lymphomas.

The \textit{bcl-2} gene was originally identified by its involvement in the t(14;18) translocation that is associated with human follicular lymphoma (1). The translocation of \textit{bcl-2} to the immunoglobulin heavy chain (IgH) locus leads to deregulated expression of \textit{bcl-2}, and high levels of \textit{bcl-2} mRNA are detected in cells with the t(14;18) translocation (2, 3). Although the mechanism of the deregulation of \textit{bcl-2} is unknown, regulatory elements of the immunoglobulin locus may play a role. The deregulated \textit{bcl-2} gene is believed to play a role in the pathogenesis of follicular lymphoma. Transgenic mice containing a \textit{bcl-2}-Igminigene show a polyclonal expansion of B cells with prolonged cell survival but no increase in cell cycling. Progression to high grade lymphomas is seen in these mice (4).

The major transcriptional promoter for \textit{bcl-2}, P1, is located 1386–1423 bp upstream of the translation start site (5). This is a TATA-less, GC-rich promoter that displays multiple start sites. A minor promoter, P2, utilized in some cell types, is located 1.3 kilobases downstream from the first one (5). Little information is available concerning the transcriptional control of the \textit{bcl-2} gene. A negative regulatory element upstream of the P2 promoter has been described (6). The proteins that bind to this element have not been identified, although p53 was shown to mediate down-regulation of \textit{bcl-2} either directly or indirectly through a 195-base pair segment of this region (7). We have previously described three \(\pi1\) binding sites that are negative regulators of \textit{bcl-2} expression in pre-B cells (8). Normal pre-B cells express very little \textit{bcl-2}, and extensive cell death by apoptosis occurs at this developmental stage. Levels of \textit{Bcl}-2 protein are increased in mature B cells. We have found that the three \(\pi1\) sites are not functional in mature B cells (8). We have recently characterized the regulatory regions, including a cAMP-responsive element (CRE), that are responsible for the positive regulation of \textit{bcl-2} expression during B-cell activation in mature B cells and during rescue from calcium-dependent apoptosis of immature B cells. We have found that a CRE element mediates the increase in \textit{bcl-2} expression following surface immunoglobulin cross-linking in mature B cells or treatment with phorbol esters in both mature and immature B cells.

Elevation of intracellular cAMP levels can result in either stimulation or repression of specific gene expression, and most of these genes contain one or more CREs. cAMP binds to the regulatory subunit of protein kinase A and releases the active catalytic subunit. This subunit phosphorylates the transactivation domain of CRE-binding protein (CREB), which then induces the expression of genes containing CREs. In addition, it has been demonstrated that CREB can be phosphorylated and transcriptionally activated by Ca\(^2+\)/calmodulin-dependent protein kinases I and II and protein kinase C (10–12). A number of CREB proteins have been described including CREB, CRE modulator, and several activating transcription factors (ATFs). The CREB proteins are basic leucine zipper transcription factors and are active as either homo- or heterodimers. Some of the ATFs heterodimerize with members of the Jun/Fos family of proteins (for reviews, see Refs. 13–15).

We are studying \textit{in vivo} protein binding to both the normal and translocated \textit{bcl-2} alleles in follicular lymphoma cells with a t(14;18) translocation. We identified an \textit{in vivo} footprint at a CRE site in the 5′-flanking sequence of the translocated \textit{bcl-2}...
gene. We demonstrated that CREB family proteins bound to this site in vitro and that the maximal increase in bcl-2 promoter expression mediated by the IgH enhancers in transient transfection experiments was dependent on the CRE site. Our results suggest that the CRE site functions as a positive regulatory element for the translocated bcl-2 allele in follicular lymphoma with the t(14;18) translocation.

EXPERIMENTAL PROCEDURES

Plasmid Constructs—A DNA fragment from BamHI (~3934) to SacI (~1287) of the human bcl-2 gene (a generous gift from Michael Cleary, Stanford) was inserted into a luciferase reporter vector with PsiI linkers. Numbering of the bcl-2 sequence is relative to the translation start site. Portions of the bcl-2 5'-flanking sequence were made by digestion with restriction enzymes SacII (~1640) and BsrFI (~1526) or by polymerase chain reaction (PCR) subfragment cloning. A construct with a mutated CRE site was generated from the ~1640 construct by replacement of the CRE sequence at ~1545 with GTACTAGTG (the bases that differ from the wild-type sequence are underlined). The murine immunoglobulin heavy chain gene enhancer sequences, HS12 (16), HS13, and HS14 (17), were cloned into the luciferase gene in the construct of the bcl-2 promoter constructs (the HS12 site is also called the immunoglobulin heavy chain gene 3' enhancer). All plasmid sequences were confirmed by the dideoxynucleotide method (Sequenase, U. S. Biochemical Corp.).

Cell Lines and Transient Transfection Assays—DHL-4 cells were cultured in RPMI medium supplemented with 10% fetal calf serum, 100 units/ml penicillin, 100 mM streptomycin, and 2 mM l-glutamine. This cell line has a t(14;18) translocation.

DNA transfections were performed with cells in log phase (5 × 10⁶ cells/ml). The cells were washed with RPMI and resuspended at 3 × 10⁶ cells/ml in RPMI medium containing 25 mM of DEAE-dextran (18). A total of 10–20 μg of plasmid DNA was added, and electroporation was performed with a Bio-Rad gene pulser at 960 volts (5.5 V/cm) at room temperature with a pulse time of 55 s. The transfected cells were cultured in 25 ml of supplemented RPMI medium for 48 h. Reporter gene activity was determined by the luciferase assay performed with a Bio-Rad gene pulser at 960 volts (5.5 V/cm) at room temperature with a pulse time of 55 s. The transfected cells were cultured in 25 ml of supplemented RPMI medium for 48 h. Reporter gene activity was determined by the luciferase assay performed with a Bio-Rad gene pulser at 960 volts (5.5 V/cm) at room temperature with a pulse time of 55 s. The transfected cells were cultured in 25 ml of supplemented RPMI medium for 48 h. Reporter gene activity was determined by the luciferase assay performed with a Bio-Rad gene pulser at 960 volts (5.5 V/cm) at room temperature with a pulse time of 55 s. The translocated bcl-2 gene yielded a 520-kilobase band.

In Vivo Dimethyl Sulfate Treatment and DNA Isolation—Treatment of cells with dimethyl sulfate was performed as described previously (20). After electrophoresis of the DNA, one lane of the gel was transferred to a filter; a probe for bcl-2, pFL1 (22), and a probe for JH (23) were used sequentially to locate the two bcl-2 alleles. The DNA in these two regions was eluted from the gel. Cleavage with piperidine was performed according to the Maxam-Gilbert procedure (24).

Ligation-mediated PCR—Chemically modified and cleaved DNA was then subjected to amplification by ligation-mediated PCR essentially as described by Mueller and Wold (25), Pfiefer et al. (26), and Garrity and Wold (27). Sequenase was used for first strand synthesis, and Tsq DNA polymerase was used for PCR. Conditions used for amplification were 95°C for 2 min, 61°C for 2 min, and 76°C for 3 min. After 20–22 cycles of PCR, samples were hybridized with end-labeled primers (primer 3 or primer set) and amplified by one more cycle of PCR. The reaction mix was resolved in a 6% polyacrylamide denaturing gel. Footprinting on each strand was repeated at least four times with genomic DNA samples prepared from at least three separate batches of dimethyl sulfate-treated cells. The primers used for PCR were synthesized in an Applied Biosystems 393 DNA synthesizer and purified by gel filtration on an Applied Biosystems oligonucleotide purification cartridge. The common linkers used were GCGGTAGCAGCCGGAGATCCTGAAATC and GAATTCGACATC. The primers for the coding strand were GGCGCCGCCGAGAAG, GGAGAAAGGGGGCCCGAG, and GGGCGCGGACGGCCGTCG. The noncoding strand primers were GGCGGTAGCGGCTGCTC, TGCGCTAGGAGGCCGCTTTC, and TGCGCTAGGAGGCCGCTTTC.
CREB Regulates the Translocated bcl-2 Gene

mediated PCR was performed on each one. With primer sets that cover the region surrounding the CRE site in the 5’ region, we found a footprint over this site on the translocated bcl-2 allele that was not present on the normal silent bcl-2 allele (Fig. 2). Three guanine residues were protected on the coding strand and one guanine residue demonstrated protection on the noncoding strand.

CREB Proteins in DHL-4 Cells Bind to the bcl-2 CRE Site in Vitro—The bcl-2 CRE site differs from the CRE consensus sequence by one base. We wished to determine which CREB family members present in DHL-4 cells bind to the bcl-2 CRE site in vitro. Nuclear extracts were prepared from DHL-4 cells, and EMSA was performed with the bcl-2 CRE site and a consensus CRE site. Four complexes were formed with both the bcl-2 CRE and the consensus CRE oligonucleotides (Fig. 3, lanes 1 and 4). Competition with excess cold oligonucleotides demonstrated that the consensus CRE element competed against the bcl-2 CRE site (Fig. 3, lane 3) and that the bcl-2 CRE oligonucleotide competed against the consensus CRE site (Fig. 3, lane 5).

Antibodies against different CREB family members were used in EMSA to determine which proteins were present in the gel shift complexes. Complexes 1–3 formed with the bcl-2 CRE site were supershifted with an antibody that recognizes all CREB/ATF family members (Fig. 4, lane 2). Complex 2 was supershifted with an antibody against CREB (Fig. 4, lane 3), and complex 1 was supershifted with an antibody against ATF-2 (Fig. 4, lane 4).

To further characterize the proteins that bind to the bcl-2 CRE site, UV cross-linking followed by denaturing polyacrylamide gel electrophoresis was performed with each of the EMSA complexes 1–3 (Fig. 5A). UV cross-linking of EMSA complex 1 yielded a protein of 67 kDa after correction for bound oligonucleotide (Fig. 5A, lane 1). A faintly labeled protein of 140 kDa was also observed, which may represent a homodimeric complex of the 67-kDa protein. UV cross-linking of EMSA complex 2 revealed a protein of 43 kDa after correction for the bound oligonucleotide (Fig. 5A, lane 2). A protein of 90 kDa was also observed. It is possible that this complex is a dimer of the 43-kDa protein. EMSA complex 3 yielded two proteins of corrected molecular masses of 34 and 43 kDa (Fig. 5A, lane 3). Proteins of 70–90 kDa were also observed, which may represent dimeric forms of the 34- and 43-kDa proteins. The EMSA complexes and the proteins in each complex are similar to those found in a mature B cell line that lacks a translocation of bcl-2 (DHL-9), but they are not identical. ²

Western analysis of the noncross-linked EMSA proteins was performed to obtain a better estimation of their molecular masses and to confirm the cross-reactivity with anti-CREB antibodies. The EMSA complexes were isolated as above but were not exposed to UV light prior to denaturing SDS-polyacrylamide gel electrophoresis and Western blotting. As shown in Fig. 5B, the major proteins present in each of the EMSA

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**Fig. 2.** In vivo footprint analysis by ligation-mediated PCR of the bcl-2 CRE site in DHL-4 cells. The region illustrated is labeled by nucleotide number relative to the bcl-2 translation start site. N denotes the normal bcl-2 allele, V denotes in vitro methylated DNA, and T denotes the translocated bcl-2 allele. The protected guanines are marked by closed circles. Protection of guanine is 88% at position −1545, 82% at position −1543, 77% at −1540, and 54% at position −1541 on the noncoding strand.

**Fig. 3.** EMSA of the bcl-2 CRE (bCRE) site (lanes 1–3) and the consensus CRE (cCRE) site (lanes 4–6) with DHL-4 nuclear extracts. Lanes 1 and 4 contain no competitor oligonucleotide, lanes 2 and 5 contain a 50-fold molar excess of cold bcl-2 CRE site oligonucleotide (b), and lanes 3 and 6 contain a 50-fold molar excess of cold consensus CRE site oligonucleotide (c). The specific complexes are labeled 1–4.

**Fig. 4.** EMSA with CREB protein antibodies. The bcl-2 CRE site and DHL-4 nuclear extracts were used. Lane 1, preimmune serum (PI); lane 2, an antibody that recognizes all CREB/ATF family members (ATF-1/CREB); lane 3, an antibody specific for CREB (CREB); lane 4, an antibody specific for ATF-2 (ATF-2). The complexes are labeled 1–4, and the supershifted complexes 1 and 2,3 are indicated.

**Fig. 5.** Denaturing SDS-polyacrylamide gel and Western analysis of the EMSA complexes formed with DHL-4 nuclear extracts and the bcl-2 CRE site. A, denaturing SDS-polyacrylamide gel analysis of the UV cross-linked EMSA complexes. The lanes contain proteins from the corresponding EMSA complexes 1–3 (Fig. 3). The migration of the molecular mass markers is shown on the left. B, Western blot analysis of the noncross-linked EMSA complexes using the ATF-1/CREB antibody. The EMSA complexes were isolated and subjected to denaturing SDS-polyacrylamide gel analysis followed by Western blotting. The lanes are labeled as described above for panel A. The molecular mass of the protein in lane 1 is 67 kDa, 43 kDa in lane 2, and 43 and 34 kDa in lane 3.
complexes reacted with the ATF-1/CREB antibody. The molecular mass of the protein in EMSA complex 1 was 67 kDa (Fig. 5B, lane 1). The protein in EMSA complex 2 had a molecular mass of 43 kDa, and the proteins in EMSA complex 3 had molecular masses of 43 and 34 kDa (Fig. 5B, lanes 2 and 3).

We used an antibody that is specific for the phosphorylated form of CREB to determine whether phosphorylated CREB protein was present in DHL-4 cells and whether activation of protein kinase C or protein kinase A resulted in phosphorylation of CREB. As shown in Fig. 6, lanes 3, phosphorylated CREB protein was present in untreated DHL-4 cells. Treatment of DHL-4 cells with PMA for 30 min resulted in an increase in the amount of phosphorylated CREB protein (Fig. 6, lane 4). Treatment with forskolin alone or with both PMA and forskolin for 30 min also resulted in the phosphorylation of CREB (Fig. 6, lanes 5 and 6).

The CRE Site Demonstrates Functional Activity in the Presence of the Immunoglobulin Enhancers—To determine whether the CRE site in the bcl-2 5' region had any functional activity in the absence and the presence of immunoglobulin regulatory elements in DHL-4 cells, transient transfection experiments were performed. The constructs for the transient transfection experiments are illustrated in Fig. 7A. We demonstrated that the mutated CRE site did not bind CREB proteins.3 As shown in Fig. 7B, mutation of the bcl-2 CRE site resulted in a decrease in activity of approximately 4.5-fold. We used a minigenne model of the bcl-2-IgH translocation to examine the influence of several immunoglobulin enhancers. An increase of 20-fold over the activity of the bcl-2 promoter alone was obtained with a combination of the 4 DNase I hypersensitive sites located 3' of the murine immunoglobulin heavy chain gene. The maximal activity was dependent on the CRE site; mutation of this site abolished most of the induction (Fig. 7C).

We have shown previously that the bcl-2 CRE site is responsive to PMA in a mature B-cell line that lacks a translocation of bcl-2 and also in a more immature B-cell line.2 The bcl-2 promoter linked to the immunoglobulin enhancers also responded to PMA with an increase in activity of approximately 3.5-fold (Fig. 7C).

DISCUSSION

We have used in vivo footprinting to examine the CRE site in the bcl-2 5' region, and we have demonstrated that it is occupied on the translocated bcl-2 gene. The normal allele, which is transcriptionally silent, did not show any protection at this site. Although the bcl-2 CRE site differs from the consensus CRE site by one base, we have shown that CREB proteins in DHL-4 cells bind to this site. Four complexes were observed with EMSA with the bcl-2 CRE site. Complex 4 did not show reactivity with any of the CREB family antibodies, and we have shown previously that the protein in this complex binds 5' of the CRE site. In a mature B cell line, this site has very little transcriptional activity.2 This complex has not been further characterized. Complexes 1, 2, and 3 were supershifted with an antibody that is reactive against all CREB/ATF family members. In addition, complex 1 was supershifted by an antibody against ATF-2. An antibody against CREB supershifted complex 2. From the molecular masses and the antibody studies, we believe that EMSA complex 1 is composed of ATF-2 (67 kDa), and EMSA complex 2 contains CREB (43 kDa). EMSA

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3 E. Mochon and L. M. Boxer, unpublished data.
complex 3 most likely contains CREB and ATF-1 (34 kDa). Similar results were obtained with this site in the mature B-cell line DHL-9, but proteins of molecular masses 63 and 67 kDa were present in EMSA complex 1.

We have demonstrated that the CRE site has functional activity in DHL-4 cells. Mutation of this site led to a 4.5-fold decrease in promoter activity. The addition of 4 DNase I hypersensitive sites with enhancer activity from the IgH locus to the bcl-2 promoter construct resulted in an increase in promoter activity of greater than 20-fold. These regulatory elements have been shown to result in increased c-myc promoter activity and to lead to the shift in promoter usage from P2 to P1, which is characteristic of the translocated c-myc allele in Burkitt’s lymphoma (17). The IgH intron enhancer has a weak effect on the bcl-2 promoter (approximately 2-fold increase), and in the presence of the four IgH hypersensitive sites, it adds very little to the increase in bcl-2 promoter activity.

Although the minigene construct that we used does not reproduce the large distance between the immunoglobulin locus and the 5′ region of the translocated bcl-2 gene, it is quite likely that similar interactions between regulatory proteins occur despite their separation by approximately 250 kilobases. We have also shown that phosphorylation of CREB in response to PMA resulted in higher bcl-2 promoter activity even in the presence of the immunoglobulin regulatory elements.

Two different transactivation domains have been described in the CREB protein. The kinase-inducible domain requires phosphorylation at serine 133 for activity, while the constitutive transactivation domain mediates basal expression (29, 30). Phosphorylation at serine 133 for activity, while the constitutive transactivation domain mediates basal expression (29, 30).

Because the CRE site functions as a positive regulatory element in transfection studies, we speculate that it is involved in the expression of the translocated bcl-2 allele in t(14;18) lymphomas. This site is unoccupied in the normal bcl-2 allele, as indicated by in vivo footprinting. How access to this site is restricted is not clear. We are investigating whether the normal allele is methylated as a potential explanation. A previous study demonstrated that one bcl-2 allele was hypomethylated relative to the other bcl-2 allele in t(14;18) lymphomas (9).

It is likely that the deregulation of the translocated bcl-2 allele is a consequence of interactions between the bcl-2 promoter region and regulatory elements of the immunoglobulin locus. We have demonstrated that several of the immunoglobulin heavy chain enhancers increase bcl-2 promoter activity and that the maximal increase is dependent on an intact bcl-2 CRE site. We are currently investigating the interactions between the transcription factors that bind these regulatory elements and the transcription factors that bind to the translocated bcl-2 promoter in t(14;18) lymphoma cells.

REFERENCES

1. Yunis, J. J. (1983) Science 221, 227–236
2. Cleary, M. L., Smith, S. D., and Sklar, J. (1986) Cell 47, 19–28
3. Graninger, W. B., Seto, M., Boutain, B., Goldman, P., and Korsmeyer, S. J. (1987) J. Clin. Invest. 80, 1512–1515
4. McDonnell, T. J., and Korsmeyer, S. J. (1991) Nature 349, 254–256
5. Seto, M., Jaeger, U., Hackett, R. D., Graninger, W., Bennett, S., Goldman, P., and Korsmeyer, S. J. (1988) EMBO J. 7, 123–131
6. Young, R. L., and Korsmeyer, S. J. (1995) Mol. Cell. Biol. 15, 1038–1039
7. Miyashita, T., Harigai, M., Hanada, M., and Reed, J. C. (1994) Cancer Res. 54, 3131–3135
8. Chen, H. M., and Boxer, L. M. (1995) Mol. Cell. Biol. 15, 3840–3847
9. Hanada, M., Delia, D., Aiello, A., Stadtmauer, E., and Reed, J. C. (1993) Blood 82, 1820–1828
10. Dash, P. K., Karl, K. A., Colicos, M. A., Pwyres, R., and Kandel, E. R. (1991) Proc. Natl. Acad. Sci. U.S.A. 88, 5061–5065
11. Sheng, M., Thompson, M. A., and Greenberg, M. E. (1991) Science 252, 1427–1430
12. Yamamoto, K. K., Gonzalez, G. A., Biggs, W. H. I., and Montminy, M. R. (1991) Nature 344, 494–498
13. Lalli, E., and Sassone-Corsi, P. (1994) J. Biol. Chem. 269, 17359–17362
14. Lee, K. A. W., and Mason, N. (1995) Biochem. Biophys. Acta 1174, 221–233
15. Meyer, T. E., and Habener, J. F. (1993) Endocrinology 124, 269–290
16. Darivach, F., Williams, G. T., Campbell, K., Pettersson, S., and Neuberger, M. S. (1991) Eur. J. Immunol. 21, 1499–1504
17. Madisen, L., and Grouincke, M. (1994) Genes & Dev. 8, 2212–2226
18. Gauss, G. H., and Lieber, M. R. (1992) Nucleic Acids Res. 20, 6739–6740
19. Zelenetz, A., Chu, G., Galli, N., Baggi, C., Horning, S. J., Dayton, T. A., Cleary, M. L., and Levy, R. (1991) Blood 78, 1552–1560
20. Arcinas, M., and Boxer, L. M. (1994) Oncogene 9, 2699–2706
21. Ji, L., Arcinas, M., and Boxer, L. M. (1994) Mol. Cell. Biol. 14, 7967–7974
22. Cleary, M. L., and Sklar, J. (1985) Proc. Natl. Acad. Sci. U.S.A. 82, 7439–7443
23. Ravetch, J. V., Siebenlist, U., Korsmeyer, S., Waldmann, T., and Leder, P. (1981) Cell 27, 583–591
24. Maxam, A., and Gilbert, W. (1980) Methods Enzymol. 65, 499–560
25. Mueller, P., and Bold, W. (1989) Science 246, 780–786
26. Pfeifer, G., Steigerwald, S. D., Mueller, P. R., Bold, W., and Riggs, A. D. (1989) Science 246, 810–815
27. Garrity, P., and Bold, W. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 1021–1025
28. Chodosh, L. A. (1988) in Current Protocols in Molecular Biology (Ausubel, F. M., ed) pp. 12.5.1–12.5.6, Greene Publishing and Wiley-Interscience, New York
29. Quinn, P. G. (1993) J. Biol. Chem. 268, 10999–10009
30. Xing, L., and Quinn, P. G. (1994) J. Biol. Chem. 269, 28732–28736
31. Frankfurt, O. S., Byrne, J. J., Seekinger, D., and Sugarbaker, E. V. (1993) Oncology Res. 5, 37–42

*B. E. Wilson, L. Ji, and L. M. Boxer, unpublished data.
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