Binding of A/T-rich DNA by Three High Mobility Group-like Domains in c-Abl Tyrosine Kinase*

Yong-Jie Miao and Jean Y. J. Wang‡

From the Department of Biology and Center for Molecular Genetics, University of California, San Diego, La Jolla, California 92039-0347

The c-Abl tyrosine kinase has been shown previously to bind DNA. Using polymerase chain reaction-based binding site-selection methods, no consensus high affinity binding site for c-Abl was found. Instead, oligonucleotides with runs of A/T sequences were isolated, and purified c-Abl was shown to bind A/T-containing oligonucleotides better than those without A/T sequences. DNA binding of c-Abl was dependent on three high mobility group 1-like boxes (HLBs), which bound cooperatively to the A/T-rich oligonucleotides. To distinguish binding to A/T sequences per se from binding to nonspecific DNA with a bend at the A/T-rich region, two oligonucleotides were compared for binding to c-Abl. Both oligonucleotides contained A/T sequences. In one, the A/T motif was part of an 80-mer duplex DNA. In another, the A/T motif was in the duplex arm of an 80-mer “bend DNA” containing an internal unpaired 20-mer region to provide a flexible hinge. Interestingly, the HLBs of c-Abl bound better to the oligonucleotide containing the bubble, suggesting a higher affinity for bent DNA rather than A/T sequences per se. Taken together, these observations define a new class of DNA binding domains, the HLBs, which do not bind DNA with a high degree of sequence specificity, but may selectively bind to bent DNA or to sequences that are easier to distort.

Protein-tyrosine kinases comprise a large family of enzymes with more than 100 members found in higher eukaryotes. The majority of protein-tyrosine kinases are localized at the plasma membranes, where they are involved in the transduction of extracellular signals (1). However, not all of the protein-tyrosine kinases are localized at the cell periphery (2). The tyrosine kinase domain of c-Abl and inhibits the enzyme activity. With the activation of the G1 cyclin-dependent kinases and the phosphorylation of RB, c-Abl is released and activated (4, 5). Thus, c-Abl tyrosine kinase is a downstream target of regulation by the cyclin-dependent kinases at G1/S transition.

An unique function found with c-Abl but not other tyrosine kinases is the ability of c-Abl to bind DNA. The DNA binding function of c-Abl is also regulated in the cell cycle. Previously, the DNA binding function of c-Abl was mapped to a 99-amino acid domain in the C-terminal region of the protein (6). As cells enter mitosis, this region of c-Abl becomes hyperphosphorylated on Ser/Thr residues (7). This hyperphosphorylation is correlated with the inactivation of DNA binding (6). Several other DNA-binding proteins, such as Myc and Myb, have also been shown to become hyperphosphorylated and inactivated during mitosis (8). Taken together, the cell cycle-dependent regulation of c-Abl tyrosine kinase and DNA binding functions suggest that this nuclear tyrosine kinase may play a role in cell cycle progression.

The C-terminal repeated domain (CTD) of RNA polymerase II has been identified as a nuclear substrate of c-Abl. Our laboratory has shown that the large subunit of RNA polymerase II is tyrosine-phosphorylated in vivo, and that c-Abl can phosphorylate the CTD to a high stoichiometry in vitro (6). Tyrosine phosphorylation of the CTD requires not only the tyrosine kinase activity of c-Abl, but also other CTD-interacting domains (CTD-IDs) found in c-Abl. Mutation of the CTD-IDs in c-Abl can abolish its CTD kinase activity, but does not affect its autokinase activity or its ability to phosphorylate enolase, a nonspecific substrate (10, 11). One CTD-ID is the Abl Src homology 2 domain, which binds to the tyrosine-phosphorylated CTD (10). Another CTD-ID is found at the C terminus of c-Abl, and this domain binds to the unphosphorylated CTD (11). The CTD-IDs are also required for c-Abl to phosphorylate the CTD in vivo and to form a complex with RNA polymerase II (11). These findings suggest that c-Abl is likely to be a physiologically relevant CTD kinase, and it may participate in the regulation of transcription during cell cycle progression.

To understand the role of c-Abl in transcription regulation, it is necessary to know whether the DNA binding domain of c-Abl can select specific DNA sequences. A previous report has suggested that c-Abl can bind to a palindromic sequence, EP, found in the hepatitis B enhancer (12). Experiments described in that report were consistent with the presence of c-Abl in the EP-binding complex. However, those results did not demonstrate a direct interaction between c-Abl and the palindromic sequence of EP. Using a PCR-based method to select and amplify oligonucleotide sequences, we found that c-Abl did not select the palindromic enhancer sequence of EP. Instead, oligonucleotides with a high A/T content were obtained. The structural basis for the binding of c-Abl to A/T-rich sequences is the presence of three sets of amino acid sequences that are distantly related to the high mobility group 1 (HMG1) domain found in a large number of DNA-binding proteins (13, 14). The

* This work was supported by Grant CA 43054 (to J. Y. J. W.) from the National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Dept. of Biology, 0347, University of California, San Diego, 9500 Gilman Dr., La Jolla, CA 92039-0347. Tel.: 619-534-6523; Fax: 619-534-2821; E-mail: jywang@uscd.edu.

1 The abbreviations used are: CTD, C-terminal repeated domain; HMG, high mobility group; HLB, HMG-like box; CTD-ID, CTD-interacting domain; EMSA, electrophoretic mobility shift assay; PCR, polymerase chain reaction; GST, glutathione S-transferase.
three HMG-like domains (HLBs) were shown to bind DNA cooperatively and were likely to bind better to bent DNA.

**MATERIALS AND METHODS**

**Cell Culture and Nuclear Extracts—** NIH3T3 cells and 3T3 Abl knockout (Abl−/−) cells (a gift from Dr. D. Baltimore, Massachusetts Institute of Technology, Cambridge, MA) were cultured at 37°C in Dulbecco’s modified Eagle’s medium supplemented with 10% calf serum. Sf9 cells were cultured at 27°C in Grace’s insect media (Life Technologies, Inc., supplemented with 0.3% yeastolate (Difco), 0.3% lactalbumin hydrolysate (Difco), and 10% heat-inactivated fetal bovine serum (HyClone). Nuclear and whole cell extracts from NIH3T3 cells (Abl+/+ and Abl−/−) knockout cells (Abl−/−) were prepared as described previously (15).

**Reconstitution of Abl−/− Cell Clones—** Three Abl knockout cells (Abl−/−) were co-transfected with pBMCV-Abl-Tag and a pRSV-based vector containing the hygromycin resistance gene at a ratio of 5:1 using the calcium phosphate method. After 24 h of transfection, cells were split 1:10 and were incubated for another 24 h. Stable transfectants were selected for 14 days in media containing 0.1% G418. Individual colonies were isolated and checked for expression of c-Abl-Tag by Western blot using 8E9, a c-Abl-specific antibody. Two stable clones (clones 9 and 10) expressing c-Abl were isolated. Nuclear extracts from each clone were made and used for electrophoretic mobility shift assay (EMSA).

**CASTing Procedure—** Cyclic amplification and selection of targets (CASTing) was performed as described previously (16). Briefly, an 81-mer pair oligonucleotide containing a random stretch of 35 nucleotides (5′-CTCCGTACCTCGAGTACCTTAAGGCTCAAGAGCTGATCGGATCTGAAC-3′) and a 5′-end PCR primer (5′-CTCCGTACCTCGAGTACCTTAAGGCTCAAGAGCTGATCGGATCTGAAC-3′) were synthesized. Five micrograms of the oligonucleotide was converted to double-stranded DNA by extension of excess 3′ end PCR primer for 30 min at 72°C with Taq DNA polymerase. Ten micrograms of this double-stranded DNA was mixed with 4 μl of nuclear extract (see below) in 20 μl of binding buffer (0.1 M NaCl, 1.8 mM MgCl2, 10 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml each of aprotinin, leupeptin, and phenanthroline) at 4°C. The mixture was incubated at room temperature on a rotator for 1 h. The magnetic beads were retrieved from solution with a magnet and were washed three times with binding buffer plus 0.1% bovine serum albumin, and then once with a 50-μl wash with PCR buffer. The beads were resuspended in 100 μl of PCR mixture and 0.5 μg of each of the two PCR primers, 2 μM MgCl2, and 125 μM dNTP. After heating the PCR mixture at 95°C for 5 min, Taq DNA polymerase (2 units) was added. PCR was performed for 10—20 cycles (94°C, 1 min; 65°C, 1 min; 72°C, 1 min) after which the primer concentration was halved to 0.25 μM. The supercoiled 2.0 Mb NifII′ column (Probond™, Invitrogen) equilibrated in buffer A (20 mM HEPES, pH 7.8, 500 mM KCl, 0.2 mM EDTA, 1 mM DTT). After washing the column with washing buffer B (20 mM HEPES, pH 6.3, 500 mM KCl, 0.2 mM EDTA, 1 mM DTT), elution was carried out with buffer B containing 300 mM imidazole. The eluate was dialyzed against buffer C (20 mM HEPES, pH 7.5, 50 mM KCl, 1.5 mM MgCl2, 1 mM DTT, 0.05% Nonidet P-40), and was applied to a heparin-Sepharose column. This column was eluted with a 20-ml linear gradient of 0.05—1 M KCl in buffer C. Abl fractions at 400 mM KCl were pooled and concentrated by using a Centricon 10 unit (Amicon). In some preparations, Ni2+ column fractions containing c-Abl were subsequently fractionated on native-DNA cellulose and c-Abl was detected in fractions eluted with 400 mM KCl.

**Oligonucleotides—** 18/26, 5′-CCAGTATGCTCTTAATAGGTTTTGAGGTAGATGTTGGAGCTGATC-3′; 18/26, 5′-AGAGCGGTCTATCGAGCTTAATTGGAATTCGGGAACGGCCCTACTGAACTGAGA-CCTGG-3′; and 18/26, 5′-CTCCACACATCCTACCTGACATCTAAATGACGGATCCTATGGTTGTTG-3′ were used for EMSA. The 80-mer bubble was synthesized using a 5′-end biotin-labeled 80-mer top strand 5′-biotin-CGAATTCCATCAATGATCTGACATCCATCTCTCCATGGTTGTTG-3′.

**EMSA—** The gel shift reaction mixture (20 μl) contained 2 ng of 5′-labeled clone 18/26 or clone 18, 20 mM HEPES, pH 7.5, 50 mM KCl, 1.5 mM MgCl2, 10 mM DTT, 0.1% Triton, 3% glycerol, and 0.5 mg/ml bovine serum albumin. The reactions were initiated by adding purified c-Abl or GST-fusion proteins to the concentrations indicated, as indicated in the figure legends, and then incubated at room temperature for 15 min. The samples were then loaded onto a 5% polyacrylamide gel (55:1 acrylamide/bisacrylamide) gel that had been prerun for 15 min. The gel was run at 4°C at 300 V for 3 h. Antibodies were preincubated with c-Abl for 15 min at room temperature prior to adding to the mixture. For EMSA done with whole cell extracts, 2 μg of extract was used in each reaction in the presence of 1 μg of sonicated salmon sperm DNA. The gel was subsequently dried and subjected to autoradiography. The data for the DNA binding curve were quantitated using the Molecular Dynamics PhosphorImager.

**RESULTS**

**Selection of Sequences Preferred by c-Abl—** To determine if c-Abl can select specific sequences, we used the CASTing method (16). Nuclear extract prepared from NIH3T3 cells was incubated with an excess amount of oligonucleotides, each containing a 35-nucleotide core of random sequences flanked by two PCR primers (see “Materials and Methods”). Those oligonucleotides were selected with Abl−/− and eluted onto magnetic beads coated with the anti-Abl monoclonal antibody 8E9. After washing, the bound oligonucleotides were released, amplified by PCR, and then subjected to another
round of selection. After 10 rounds, the PCR products were cloned and sequenced. No consensus sequence was found among the selected oligonucleotides. However, 24 out of 31 clones sequenced (77%) contained runs of A/T sequences that were between 5 and 9 nucleotides in length (Fig. 1A). Moreover, most clones contained more than one run of A/T. These clustered runs of A/T are boxed in the sequence and are referred to as the “A/T-rich motif” (Fig. 1A).

To determine if the selection of the A/T-rich motif was dependent on c-Abl, a control CASTing experiment using nuclear extracts from Abl-null 3T3 cells was performed. The 8E9-coated magnetic beads brought down the c-Abl protein from the NIH3T3 cell extracts (Fig. 1C, lower panel, lane 1) but did not bring down any detectable Abl protein from the Abl-null 3T3 cell extracts (lane 2) when an equal amount of total protein was used (Fig. 1C, upper panel). Seventeen clones were sequenced from CASTing selection performed with the Abl-null 3T3 extracts. Among them, six (clones 3, 8, 9, 13, 14, and 15) contained A/T runs of 5 and 6 nucleotides in length (Fig. 1B). Only one clone (clone 2) contained the type of A/T-rich motif found among the c-Abl-selected clones. Thus, the selection of A/T-rich motifs was more efficient when the c-Abl protein was present in the nuclear extracts.

To further examine the protein-DNA complexes formed with the A/T-rich motif, an oligonucleotide was synthesized based on the sequence of two selected clones, 18 and 26 (Fig. 1A). These two clones differed by only 1 nucleotide and contained a 34-nucleotide identical sequence oriented in opposite directions relative to the PCR primer arms. Whole cell extracts from NIH3T3 cells, Abl-null 3T3 cells, and NIH3T3 cells stably transfected with a pBK-based Abl-TAG expression plasmid were made and used in electrophoretic mobility shift assays (EMSAs). Many protein-DNA complexes were formed with the 18/26 probe (Fig. 2). The A-complex, migrating at the top of the lane, could be competed away with the inclusion of unlabeled 18/26 probe (Fig. 2A, compare lanes 2 and 3 or lanes 6 and 7). The amount of this complex could be correlated with the amount of c-Abl. A decreased amount of this complex was found in the Abl-null nuclear extract (lane 4), whereas an increased amount of the complex was observed in the transfected cell extract (lane 6). To further document the correlation between the specific gel shift complex and the amount of c-Abl, a pBK-based vector expressing c-Abl was introduced into the Abl-null cells. Two stably transfected clones expressing c-Abl were isolated. Again, the amount of this gel shift complex was found to parallel the level of c-Abl in the extracts (Fig. 2C, lanes 4 and 5). These observations suggested that c-Abl might be present in the A-complex. Two antibodies, the 8E9 antibody that reacts with...
an arrow by the were loaded in each lane.

His6-tagged c-Abl was purified in a two-step process, beginning with the recombinant baculovirus expression system. The Abl-Tag in the presence (with 6 histidines (His6-tag) and overproduced in insect Sf9 cells murine c-Abl type IV protein was tagged at the N terminus used purified c-Abl protein in DNA binding reactions. The to determine if c-Abl indeed prefers A/T-rich motifs, we DNA—A-complex. due to some indirect effect of c-Abl on the formation of the present in the A-complex, and the observed correlation could be of the c-Abl in the A-complex. Alternatively, c-Abl might not be present in the A-complex, and the observed correlation could be due to some indirect effect of c-Abl on the formation of the A-complex.

Purified c-Abl Binds with Higher Affinity to A/T-rich DNA—To determine if c-Abl indeed prefers A/T-rich motifs, we used purified c-Abl protein in DNA binding reactions. The murine c-Abl type IV protein was tagged at the N terminus with 6 histidines (His6-tag) and overproduced in insect Sf9 cells using the recombinant baculovirus expression system. The His6-tagged c-Abl was purified in a two-step process, beginning with a Ni2+ column followed by a heparin-Sepharose column (see “Materials and Methods”). Approximately 100-fold increase in specific activity was achieved. When analyzed by SDS-polyacrylamide gel electrophoresis, c-Abl represented greater than 90% of the protein in the purified fraction (Fig. 3B, lane 3). The purified c-Abl bound to the 18/26 probe and formed a stable complex that was detectable by EMSA (Fig. 3A, lane 2). The 8E9 antibody did not react with the 18/26 probe (lane 4), but it was able to supershift this complex (lane 3). As a control, an antibody against Cdc2 had no effect on this protein-DNA complex (lane 5). These results showed that c-Abl protein was present in the protein-DNA complex. Binding preference of c-Abl to an A/T-rich motif was tested by competition. Three unlabeled oligonucleotides were used: 1) 18, which contained the identical sequence as the labeled probe, 2) 30, which contained an A/T-rich motif, and 3) 10, which contained no A/T-rich motif (Fig. 1A). Each oligonucleotide competed for binding to c-Abl, but with different efficiencies (lanes 6–30). The binding data were quantitated using PhosphorImager (Fig. 3C).

When compared to the competition with oligonucleotide 18, oligonucleotide 30 was about 8-fold less efficient. However, oligonucleotide 10, which lacked the A/T-rich motif, required more than 60-fold the amount to achieve the same degree of competition. These observations supported the interpretation of the CASTing results and suggested that c-Abl preferentially bound to A/T-rich DNA.

The c-Abl DNA Binding Domain Has Three HMG1-like Boxes—Previously, the DNA binding domain of c-Abl was determined to be the 99-amino acid region between the StuI and SalI sites of the murine c-abl sequence (6). Several searches of the protein sequence data bases were conducted, but no apparent similarity with any of the known DNA binding domains was detected. After finding that c-Abl bound better to A/T-rich DNA, we repeated the search by focusing on DNA binding domains that are known to select A/T-rich sequences, i.e. the HMG domains. Such a focused search revealed that the 99-amino acid region and two other regions of c-Abl were distinctly related to the HMG box (Fig. 4A). These were designated as HMG-like boxes (HLBs) 1–3. HLB-1 lies between the NarI and XhoI restriction sites of murine c-abl, HLB-2 lies between XhoI and SalI, and HLB-3, between SalI and StuI, which corresponds to the previously assigned 99-amino acid DNA binding domain (6).

A multiple sequence alignment of several HMG box sequences is shown in Fig. 4A. These sequences were divided into three groups. The top group consists of HMG boxes that are found in sequence-specific DNA-binding proteins. The middle group consists of HMG boxes that do not exhibit clear sequence specificity. The bottom group contain the three c-Abl HLBs that are quite divergent from the HMG boxes. HLB-1, -2, and -3 of c-Abl share 34%, 30%, and 35% similarity to the human SRY sequence, respectively.

Cooperative Binding to DNA among the Three HLBs—To
determine the roles that the three HLBs have in DNA binding, a series of GST fusion proteins containing different combinations of the HLBs (Fig. 5B) were tested for binding to the 18/26 probe (Fig. 5A). The fusion protein containing HLB3 could bind 18/26 by itself, which is consistent with the previous assignment of this region as the DNA binding domain of c-Abl (6). The fusion protein containing HLB2 did not form a stable complex with 18/26. This was also consistent with the results of previous experiments in which DNA binding was measured by Southwestern blotting and binding to DNA cellulose (6). Interestingly, the fusion protein containing both HLB 2 and 3 bound to DNA with an affinity higher than HLB3 alone. Addition of HLB1 in the fusion protein GNS caused an additional, albeit less dramatic, increase in the binding affinity. In fact, GNS bound the 18/26 probe as well as the full-length c-Abl protein (Fig. 5A). These results suggested that all three HLBs of c-Abl contributed to the binding of the A/T-rich sequence.

The HLBs of c-Abl Bind Bubble DNA—An interesting feature of the HMG domain is its ability to induce DNA bending upon binding (14). The preference for A/T sequences by HMG domains is most likely due to the fact that A/T sequences are more flexible in adopting a bent conformation (14). Our observations that c-Abl preferred A/T-rich oligonucleotides and that c-Abl contained three HMG-like domains prompted us to test if c-Abl bound to A/T sequences per se or preferred A/T-rich sequences because they were easier to bend. Two 80-mer oligonucleotides were prepared based on the sequence of probe 18/26 (see “Materials and Methods”). Both oligonucleotides contained the A/T sequence of 18/26 in the left 30-mer region. The duplex oligonucleotide contained a central 20-mer of T-A homoduplex, whereas the bubble oligonucleotide contained a central 20-mer of unpaired T/C. The T-A duplex or the unpaired T/C are flanked by identical 30-mer right and left arms (Fig. 6B). Binding of the GNS protein (Fig. 5B) to these two oligonucleotides...
and to a single-stranded 80-mer containing only the T-strand was determined as a function of protein concentration (Fig. 6A). Consistent with previous results (6), GNS bound duplex DNA better than single-stranded DNA. Interestingly, GNS was found to bind the bubble DNA better than the A/T-rich duplex. These results suggested that the HLBs of c-Abl did not bind to A/T sequences per se. The single-stranded region in the bubble DNA might function as a flexible hinge for bending, and thus allowing the HLBs to bind cooperatively to the two arms of a bent DNA.

**DISCUSSION**

The DNA binding function of c-Abl is shown here to be mediated by three HMG-like boxes, which do not select DNA sequences with a high degree of specificity. The HLBs of c-Abl are quite divergent from the HMG domains and cannot be classified as a member of the HMG family (14). However, the preference for binding to A/T-rich oligonucleotides and to bubble DNA indicates that the HLBs may be functionally similar to the HMG box. The HMG box is a DNA binding domain of about 80 amino acids found in a number of proteins that bind to DNA.
with different degrees of sequence specificity. Those that bind DNA with little sequence specificity, including HMG-1, HMG-2, and UBF, tend to contain more than one HMG box. UBF, a transcription factor for RNA polymerase I, contains four HMG boxes (18). The cooperative binding of the three HLBs in c-Abl is similar to the cooperative binding of the four HMG boxes in UBF (19). HMG family members that can discriminate between DNA sequences include the T-cell transcription factors, TCF-1/LEF-1, and the testis determination factor, SRY (19–23). Although these HMG proteins show preference for some specific sequences, their sequence recognition capabilities, as argued by Grosschedl, are quite modest (14). Moreover, the consensus binding sequence of TCF-1/LEF-1, 5′-(A/T)(A/T)CAAAAG-3′, and the preferred binding sequence for SRY, 5′-(A/T)AAACAAT/A)-3′, are A/T-rich (20–22, 24, 25). Selection of the A/T-rich sequence by the HMG box is likely due to the fact that binding by an HMG domain requires a large distortion in the DNA backbone. This has been shown by an NMR study of the SRY-DNA complex (26). The binding to bent DNA can explain the cooperative binding of the three HLBs in c-Abl. It will be of interest to determine the structure of the c-Abl HLBs and the way they bind DNA.

Our results do not support the previous report that c-Abl binds to the palindromic sequence in the hepatitis B viral enhancer, EP (12). We have performed selection not only with the CASTing method, but also with the SAAB method (27), which uses EMSA to select and amplify oligonucleotides bound to c-Abl. In no case did we observe the selection of the EP sequence. Proteins that bind DNA with low specificity are not necessarily randomly distributed on cellular DNA (28). In fact, HMG proteins have been shown to be recruited to specific promoter complexes, such as the NF-κB/DSP1 complex in the interferon-responsive element (28, 29). The c-Abl tyrosine kinase may well be present in the EP-enhancer complex. However, our results would suggest that the EP complex must also contain a DNA-binding protein that recognizes the EP sequence. Binding of DNA by HMG proteins can promote the binding of other nuclear proteins, such as the progesterone receptor, to the bent site (30). Perhaps c-Abl, through DNA binding, can promote the formation of the EP complex.

The association of c-Abl with a specific transcription factor has been reported. Our laboratory has shown that RB can simultaneously bind to c-Abl and the transcription factor E2F. In fact, c-Abl and E2F can be co-immunoprecipitated from the nuclear extracts prepared from RB-positive cells (31). E2F is a family of transcription factors composed of E2F/DP heterodimers. The E2F-binding site is found in several cell cycle-regulated promoters, including cyclin A, cyclin E, and Cdc2 (32). The assembly of an E2F-c-Abl complex through RB suggests that c-Abl may participate in the regulation of promoters that contain E2F sites. Recently, the v-Abl tyrosine kinase has been shown to form a complex with another transcription factor, CREB, that binds to the cyclic AMP-responsive element. In this instance, v-Abl is shown to enhance transcription from the cyclic AMP-responsive element (33).

The c-Abl tyrosine kinase has been shown to be activated in transient co-transfection assays. Welch and Wang showed that c-Abl can superactivate a 5×Gal4-Fos-CAT reporter in conjunction with Gal4-VP16 (4). This superactivation function requires both the tyrosine kinase activity and the DNA binding function. Deletion of the HLBs 2 and 3, as
in the mutant c-Abl ΔXS, abolished the supertransactivation function of c-Abl (4). Recently, Baskaran et al. have extended this observation to show that c-Abl can also stimulate a fos-CAT reporter containing the basal fos promoter (11). Again, both the tyrosine kinase and the DNA binding function are required. Identification of the three HLBs and their binding to bent DNA form the basis for future studies on the mechanism by which c-Abl tyrosine kinase modulates transcription.

Acknowledgments—We thank Dr. Jeff R. Huth (NIDDK, National Institutes of Health) for providing sequence alignment in Fig. 4A, Dr. David Baltimore (MIT) for 3T3 Abl null cells, Dr. Michael Dahmus for His-tagged c-Abl baculovirus, and Dr. Edward T. Kipreos for GXS, GXU, and GUS constructs. We also thank Erik Knudsen and Dr. Laura Lee Whitaker for critical comments concerning the manuscript.

REFERENCES
1. van der Geer, P., Hunter, T., and Lindberg, R. A. (1994) Annu. Rev. Cell Biol. 10, 251–337
2. Wang, J. Y. J. (1994) Trends Biochem. Sci. 19, 373–376
3. Wang, J. Y. J. (1993) Curr. Opin. Genet. Dev. 3, 35–43
4. Welch, P. J., and Wang, J. Y. J. (1995) Cell 75, 779–790
5. Welch, P. J., and Wang, J. Y. J. (1995) Mol. Cell Biol. 15, 5542–5551
6. Kipreos, E. T., and Wang, J. Y. J. (1992) Science 256, 382–385
7. Kipreos, E. T., and Wang, J. Y. J. (1994) Science 264, 217–220
8. Luscher, B., and Eisenman, R. N. (1992) J. Cell Biol. 118, 775–784
9. Baskaran, R., Dahmus, M. E., and Wang, J. Y. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 11167–11171
10. Duyvetter, J., Baskaran, R., and Wang, J. Y. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 1555–1559
11. Baskaran, R., Chiang, G. G., and Wang, J. Y. (1996) Mol. Cell Biol. 16, 3561–3569
12. Dikstein, R., Heffetz, D., Ben-Neriah, Y., and Shaul, Y. (1992) Cell 69, 751–757
13. Landsman, D., and Bustin, M. (1993) Bioessays 15, 539–546
14. Grosschedl, R., Giese, K., and Pagel, J. (1994) Trends Genet. 10, 94–100
15. Lassar, A. B., Davis, R. L., Wright, W. E., Kedesch, T., Murre, C., Voronova, A., Baltimore, D., and Weintraub, H. (1991) Cell 66, 305–315
16. Wright, W. E., Binder, M., and Funk, W. (1991) Mol. Cell. Biol. 11, 4104–4110
17. Guan, K., and Dixon, J. E. (1994) Anal. Biochem. 192, 262–267
18. Jantzen, H. M., Admon, A., Bell, S. P., and Tjian, R. (1990) Nature 344, 830–836
19. Jantzen, H. M., Chow, A. M., King, D. S., and Tjian, R. (1992) Genes Dev. 6, 1950–1963
20. Travis, A., Amsterdam, A., Belanger, C., and Grosschedl, R. (1991) Genes Dev. 5, 880–894
21. van de Wetering, M., Oosterwegel, M., Dooyies, D., and Clevers, H. (1991) EMBO J. 10, 123–132
22. Waterman, M. L., Fischer, W. H., and Jones, K. A. (1991) Genes Dev. 5, 656–669
23. Sinclair, A. H., Berta, P., Palmer, M. S., Hawkins, J. R., Griffiths, B. L., Smith, M. J., Foster, J. W., Frischauf, A. M., Lovell-Badge, R., and Goodfellow, P. N. (1990) Nature 346, 240–244
24. Gabby, J., Collignon, J., Koopman, P., Capell, B., Economou, A., Munsterberg, A., Vivian, N., Goodfellow, P., and Lovell-Badge, R. (1990) Nature 346, 245–250
25. Harley, V. R., Lovell-Badge, R., and Goodfellow, P. N. (1994) Nucleic Acids Res. 22, 1500–1501
26. Werner, M. H., Huth, J. R., Gronenborn, A. M., and Cleare, G. M. (1995) Cell 81, 705–714
27. Blackwell, T. K., and Weintraub, H. (1990) Science 250, 1104–1110
28. Tjian, R., and Maniatis, T. (1994) Cell 77, 5–8
29. Lehming, N., Thanos, D., Brickman, J. M., Ma, J., Maniatis, T., and Ptasnke, M. (1994) Nature 371, 175–179
30. Onate, S. A., Prendergast, P., Wagner, J. P., Nissen, M., Reeves, R., Pettijohn, D. E., and Edwards, D. P. (1994) Mol. Cell. Biol. 14, 3376–3391
31. Welch, P. J., and Wang, J. Y. J. (1995) Genes & Dev. 9, 31–46
32. DeGregori, J., Kowalik, T., and Nevins, J. R. (1995) Mol. Cell. Biol. 15, 4215–4224
33. Birchell-Roberts, M. C., Ruscetti, F. W., Kasper, J. J., Bertolette, D. C., III, Yao, Y. D., Bang, O. S., Roberts, M. S., Turley, J. M., Perris, D. K., and Kim, S.-J. (1995) Mol. Cell. Biol. 15, 6088–6099
34. Feller, S. M., Knudsen, B., and Hanafusa, H. (1994) EMBO J. 13, 2341–2351
Binding of A/T-rich DNA by Three High Mobility Group-like Domains in c-Abl Tyrosine Kinase
Yong-Jie Miao and Jean Y. J. Wang

J. Biol. Chem. 1996, 271:22823-22830.
doi: 10.1074/jbc.271.37.22823

Access the most updated version of this article at http://www.jbc.org/content/271/37/22823

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 34 references, 16 of which can be accessed free at http://www.jbc.org/content/271/37/22823.full.html#ref-list-1