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A Poly(dA-dT) Upstream Activating Sequence Binds High-Mobility Group I Protein and Contributes to Lymphotoxin (Tumor Necrosis Factor-β) Gene Regulation

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Lymphotoxin (LT; also known as tumor necrosis factor-β) is a pleiotropic cytokine whose expression is tightly regulated in most cells and is repressed prior to activation signals. In some early B cells and Abelson murine leukemia virus-transformed pre-B-cell lines, LT mRNA is constitutively expressed. To examine the molecular regulation of the LT gene in a constitutively expressing cell line, we studied the Abelson murine leukemia virus-transformed lines PD and PD31. As demonstrated by primer extension analysis, constitutively expressed pre-B-cell-derived and inducibly expressed T-cell-derived LT mRNA were initiated at the same cap sites and predominant cap site utilization was conserved. Furthermore, we delineated an upstream activating sequence that was an important functional component of lymphotoxin transcriptional activation in PD and PD31 cells. The upstream activating sequence was localized to an essentially homopolymeric A+T-rich region (LT−612/−580), which was bound specifically by recombinant human high-mobility group I protein (HMG-I) and a PD/PD31 nuclear extract HMG-I (HMG-I-like) protein. The nuclear extract-derived HMG-I-like protein was recognized by anti-HMG-I antibody and bound to LT DNA to effect an electrophoretic mobility shift identical to that of bound recombinant human HMG-I. These findings implicate HMG-I in the regulation of constitutive lymphotoxin gene expression in PD and PD31 cells. HMG-I and HMG-I-like proteins could facilitate the formation of active initiation complexes by altering chromatin structure and/or by creating recognition sites for other activator DNA-binding proteins, some of which may be unique to or uniquely modified in these constitutive LT mRNA producers.

Lymphotoxin (LT; also known as tumor necrosis factor-β), the product of a gene encoded within the major histocompatibility complex (34, 35) is a pleiotropic cytokine whose expression is tightly regulated in most cells. LT transcription is induced in T cells by activation with specific antigen in the context of syngeneic major histocompatibility complex, mitogens, or phorbol esters (reviewed in reference 39). Lipopolysaccharide, phorbol esters, *Staphylococcus aureus* Cowan 1, B-cell growth factor, and anti-μ antibodies, alone and in combination, can also induce its production by some B cells (37; reviewed in reference 59). LT is regulated differently in some early B-cell lines and Abelson murine leukemia virus (A-MuLV)-transformed murine pre-B cell lines in that these cells constitutively produce high levels of LT mRNA (15, 29).

The mechanism(s) of constitutive LT mRNA accumulation in the A-MuLV-transformed pre-B-cell lines PD and PD31 may operate on several levels. One possible explanation for LT mRNA accumulation is that the promoter, which is repressed in normal T and B cells prior to cellular activation (reviewed in references 16 and 39), functions constitutively in these pre-B-cell lines. Constitutive LT promoter activity could be associated with the function of upstream positive regulatory elements that transcriptionally activate the promoter. In this case, the positive regulatory elements would probably be recognized by DNA-binding proteins that are present and/or modified in a cell-type-specific manner in constitutive LT mRNA expressors, thereby activating element function. Posttranscriptional regulation of mRNA stability may also contribute to the regulation of LT in these cells (6, 15, 45).

In previous studies, we identified the sites of LT transcriptional initiation in activated T cells and a minimal LT promoter unit, which functions in T cells but not mature B cells (16, 38). Furthermore, the activity of the minimal promoter unit in T cells requires a functional NF-κB site (38). Here, we continue the analysis in early B-cell lines that express LT mRNA constitutively by examining the contribution of 5′ DNA sequences to LT regulation. We implicate a poly(dA-dT)-rich upstream activator sequence (UAS) as a component of the regulatory machinery controlling constitutive LT gene expression in PD and PD31 cells. Furthermore, the LT UAS was recognized specifically and bound with high affinity by both recombinant human high-mobility group I protein (rhuHMG-I) and an HMG-I (HMG-I-like) protein found in nuclear extracts of PD and PD31 cells. HMG-I is a well-characterized nonhistone chromosomal protein (distinct from HMG-1) which specifically recognizes the minor groove of double-stranded poly(dA-dT) DNA (11, 53) and has been implicated as an important component of active chromatin structures. Here we demonstrate that HMG-I-like proteins bind to the poly(dA-dT)-rich UAS and implicate HMG-I in LT gene regulation.

MATERIALS AND METHODS

Cell lines. The pre-B-cell line PD (47) was derived by in vitro A-MuLV transformation of bone marrow cells from an

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adult NIH Swiss mouse. The PD31 cell line was originally derived from PD and is distinct from the parental cell line with reference to kappa chain gene rearrangement (2), heavy- and light-chain gene expression (31), LT protein expression, and growth properties in culture (15). The PD cell line, obtained from R. Laskov, Hebrew University, Israel, was maintained in Dulbecco modified Eagle medium (GIBCO, Grand Island, N.Y.) supplemented with 10% fetal calf serum (GIBCO) and 50 μM β-mercaptoethanol. The PD31 cell line, obtained from J. Hesse, National Institutes of Health, Bethesda, Md., was grown in RPMI 1640 (GIBCO) plus 10% fetal calf serum and 50 μM β-mercaptoethanol. Both cell lines were maintained at 37°C and 5% CO_2. The murine T-cell clone F128, which is specific for myelin basic protein, was maintained in medium (Click's plus 10% Hyclone and 2% T-cell growth factor) with antigen and syngeneic spleen cells (52, 63). It was grown in the absence of feeder cells for 9 days prior to stimulation through T-cell receptor cross-linking with anti-CD3 antibodies (41).

Primer extension analysis. A synthetic oligonucleotide with the sequence 5'-GAAAAGGCTGTGGCTCAAGAAAG GAGGTAGGATCC-3' was labeled with T4 polynucleotide kinase in the presence of [γ-^{32}P]ATP and used for primer extension analysis as described previously (16, 24). This oligonucleotide is complementary to murine lymphotoxin mRNA 73 to 107 bp downstream of the putative proximal cap site of the promoter; use of this oligonucleotide is described elsewhere (16) and was previously referred to by the protected coding strand sequences, rather than the noncoding strand sequences which are shown here.

Plasmids. Plasmids pLT−293CAT and pLT−662CAT, which consist of LT 5' sequences linked to the chloramphenicol acetyltransferase (CAT) gene, have been previously described as LTCAT1+ and LTCAT2+ (16). Additional fragments were isolated from LT−662 by using restriction enzymes (indicated in parentheses) to define their 5' termini and were designated as follows: LT−612 (*Taq*I), LT−580 (*Bsr*I), LT−508 (*Stu*I), and LT−445 (*Ban*I). LT−612 and LT−580 were generated by partial digestion of the LT−662 fragment with the indicated enzyme. Isolated LT upstream sequences were blunt ended, ligated to *Hin*dIII linkers, and inserted into the unique *Hin*dIII site upstream of the CAT gene in pSVOCAT (20). The 5' deletion mutants share a common 3' end (LT+77) (see Fig. 2A). Plasmids were twice banded on CsCl gradients (52) and then extensively dialyzed prior to use in transfections.

Transfection and CAT assays. PD and PD31 cells were isolated from early-logarithmic-growth-phase cultures and transiently transfected by DEAE-dextran-mediated DNA transfer as previously described (16). To optimize transfection efficiency, the PD31 cells were incubated in the DEAE-dextran-DNA cocktail for 60 min rather than 30 min. Cells were harvested 48 h posttransfection and lysed in a detergent solution (0.01 M Tris · Cl [pH 7.8], 0.15 M NaCl, 1.5 M $MgCl_2$, 0.65% Nonidet P-40). Cell extracts were heat treated (16), and 100 μg of total protein (4) was assayed for CAT enzyme activity as described by Gorman et al. (20) with 2.4 mM acetyl coenzyme A (4-h incubation). Acetylated forms of [^{14}C]chloramphenicol were visualized by autoradiography, and CAT activity was quantitated by densitometric analysis (GS-300 Transmittance/Reflective Scanning Densitometer and GS-300 Data System; Hoefer Scientific Instruments). The data were standardized within each transfection by first subtracting pSVOCAT-driven background activity from the activity of each construct and then presenting the resultant numbers relative to pLT−662CAT activity (designated 100%).

Nuclear extracts. Cells were harvested from early-logarithmic-growth-phase cultures and washed in cold phosphate-buffered saline, and the cell pellet was kept at −70°C until use. Nuclear extracts were prepared as outlined by Dignam et al. (8) and Lenardo et al. (30). Buffers A, C, and D, which contained 0.5 mM phenylmethylsulfonyl fluoride and 0.5 mM dithiothreitol to minimize proteolysis, were those described by Dignam et al. (8). Frozen pellets, containing 5×10^6 to 22×10^6 cells, were thawed in the presence of an equal volume of buffer A. Cell membranes were disrupted by 15 strokes of a Dounce homogenizer (pestle B), and the intact nuclei were pelleted for 20 min in a microcentrifuge (4°C). The nuclei were then extracted with 2 volumes of buffer C for 20 min on ice. The resultant nuclear extract was clarified by centrifugation (as above), dialyzed against buffer D overnight, and reclarified, and the total protein concentration was determined (4). Aliquots of nuclear extract were used immediately or stored at −70°C prior to use.

Purification of rhuHMG-I. Bacterially derived rhuHMG-I (21, 36) was expressed and purified by reverse-phase high-pressure liquid chromatography (HPLC) as previously described (12–14).

EMSAs. Electrophoretic mobility shift assays (EMSAs) were carried out essentially as described by Ausubel et al. (3), with modifications. The double-stranded 154-bp LT−662/−508 fragment was generated by *Acc*I and *Stu*I enzymatic digestion of LT 5' DNA and isolated by standard techniques (51). This fragment was Klenow end labeled by using [^{32}P]dATP and/or [^{32}P]dGTP (3,000 Ci/mmol; Amersham) and purified over a Sephadex G-50 column. For the "stairway" EMSA, aliquots of column purified ^{32}P-labeled LT−662/−508 were further digested with one of the following restriction enzymes: *Taq*I, *Bsr*I, or *Mae*III. The resultant 50-bp (LT−662/−612), 82-bp (LT−662/−580), and 118-bp (LT−662/−544) ^{32}P-labeled fragments (see Fig. 6) were gel purified on 12% nondenaturing polyacrylamide gels (51).

Two complementary oligonucleotides, a 34-mer (5'-TCGAA AAAAGCAAAAAAAAAAAAAAAAACTGGC) and a 31-mer (5'-GCCAGTTTTTTTTTTTTTTTTTGCTTTTTT), that corresponded to the coding and noncoding strands, respectively, of the LT poly(dA-dT)-rich UAS were synthesized (Yale Medical School Protein and Nucleic Acid Chemistry Facility). The oligonucleotides were mixed in an equimolar ratio and annealed in the presence of 150 mM NaCl. The resultant double-stranded synthetic oligonucleotide (LT−612/−580) was Klenow end labeled with [^{32}P]dCTP (3,000 Ci/mmol; Amersham) and gel purified as described above.

Binding assays with nuclear extract were carried out in buffer D that contained about 5 ng of ^{32}P-labeled DNA fragment (10^4 cpm), 2 μg of poly(dG-dC) (Boehringer Mannheim Biochemicals), and 5 to 10 μg of nuclear extract in a total volume of 25 μl. Binding assays involving HPLC-purified rhuHMG-I consisted of about 15 ng of purified protein, 0.5 μg of poly(dG-dC), and 1.25 μg of acetylated bovine serum albumin (Promega). The extract and probe were incubated for 15 min at room temperature and then analyzed on 4% polyacrylamide nondenaturing 0.5× TBE gels (acrylamide-to-bisacrylamide ratio, 37.5:1). Gels were dried and exposed to Kodak XAR-2 film (−70°C) without intensifying screens unless otherwise noted. Autoradiographs of the EMSAs were analyzed densitometrically (as above).

For DNA competition studies, 5 to 30 ng of the indicated

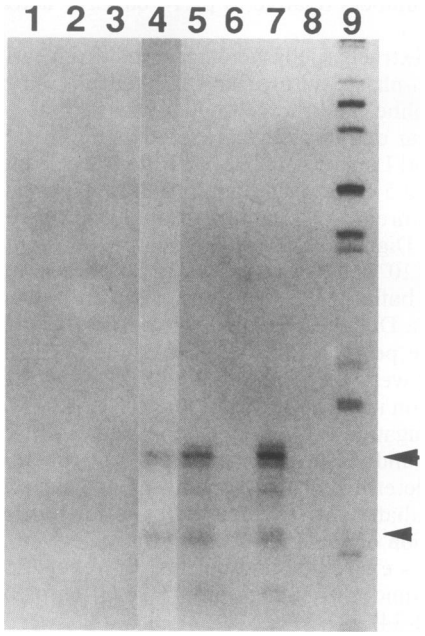

FIG. 1. Quantitative primer extension analysis of murine LT RNA from PD and PD31 pre-B cells and an activated T-cell clone demonstrates identical transcription initiation site utilization. Extension products from an LT-specific ^{32}P-labeled oligonucleotide primer with total cellular RNA templates were resolved on a 6% polyacrylamide–8 M urea gel and visualized by autoradiography. Lanes: 1, LT oligonucleotide alone; 2, tRNA (40 μg); 3, WEHI 164 RNA (40 μg); 4, PD RNA (50 μg); 5, PD31 RNA (40 μg); 6, unstimulated F128 T-cell clone RNA (10 μg); 7, anti-CD3-stimulated F128 T-cell clone RNA (10 μg); 8, blank; 9, pBR322-*Msp*I marker (3 × 10^2 cpm). All samples were run on the same gel except lane 4. The positions of the 107-bp (large arrow) and 92-bp (small arrow) products are indicated and correspond to transcripts initiated at the promoter-proximal and -distal cap sites, respectively.

unlabeled competitor DNA was added to binding reactions in addition to labeled probe. For antibody competition experiments, a rabbit-derived immunoglobulin G (IgG) polyclonal antibody generated against native murine HMG-I was used (9). Nuclear extract was preincubated with anti-HMG-I antibody (1:10 dilution) for 15 min at room temperature (with gentle shaking) prior to addition of labeled probe. Affinity-purified rabbit IgG specific for murine IgG (Jackson Immunoresearch Laboratories, Inc., Westgrove, Pa.) was used as an antibody control under identical conditions.

RESULTS

Characterization of LT gene transcription initiation sites by primer extension. RNAs derived from the murine pre-B-cell lines PD and PD31 were analyzed by primer extension to determine whether transcription initiation site usage in constitutive LT mRNA-expressing cells was the same as that previously identified in murine T cells activated by T-cell receptor cross-linking by anti-CD3 antibody (16). Primer extension was performed by using a ^{32}P-labeled oligonucleotide complementary to murine LT mRNA 73 to 107 bp downstream from the TATAAA proximal cap site. Two primer extension products were seen with RNA from PD and PD31 pre-B cells and an activated T-cell clone (Fig. 1, lanes 4, 5, and 7). A predominant product of 107 bp (Fig. 1, large arrow), corresponding to the size predicted for RNA transcripts initiated at the most proximal cap site, was noted in these cell lines. A less abundant product of 92 bp (Fig. 1, small arrow) indicated that a distal cap site (located 15 bp downstream of the proximal cap site) was also utilized, although to a lesser extent. Our data demonstrate a strong preference for the same TATAAA proximal cap site usage in these constitutive LT producers as had already been demonstrated in LT-inducible T-cell clones (16). Note that RNA derived from WEHI 164 cells (Fig. 1, lane 3) and nonactivated F128 T cell clones (lane 6) were negative for LT mRNA; these findings were expected because WEHI 164 cells do not express LT mRNA, as determined by RNase protection and Northern (RNA) blot analyses (data not shown), and F128 T cell clones require activation signals for LT expression.

Delineation of functional UAS in PD and PD31 cells. The A-MuLV-transformed pre-B-cell lines PD and PD31 constitutively accumulate high levels of LT mRNA (29) and, as shown in Fig. 1, utilize the same transcription initiation sites as do T cells (15). To assess the role of upstream sequences in the regulation of the LT gene, a battery of LTCAT constructs (LTCATs) containing various fragments of LT gene 5′ DNA linked to the bacterial gene CAT (Fig. 2A) were transfected into PD and PD31 cells. Previous studies (16, 38) identified a promoter unit (LT−293), which functions in transiently transfected murine and human T cells but is inactive in mature B cells and plasmacytomas. The current data confirmed and extended our initial results, demonstrating that the LT promoter unit (LT−293) did not function in pre-B cells. In contrast to the results obtained with T cells and more mature B cells, however, some of the larger LTCATs were transcriptionally active in these pre-B cells.

The pLT−662CAT exhibited high levels of LT promoter activity in PD and PD31 transfectants (Fig. 2B). In fact, LT−662-driven CAT levels in many of the transfection experiments were comparable to those of RSVCAT (data not shown), which is driven by the powerful Rous sarcoma virus enhancer and promoter (19). To allow for comparison among transfection experiments, CAT activity driven by the various constructs was standardized against pLT−622CAT activity levels (designated 100%). The histograms shown in Fig. 2B represent the average of the relative CAT activity levels for each construct from eight separate transfections into each cell line. To delineate LT promoter-activating elements contained within LT−662, the ability of pLT−293CAT, pLT−445CAT, pLT−508CAT, pLT−580CAT, and pLT−612CAT (Fig. 2A) to drive CAT expression in PD and PD31 transfectants was assessed. As shown in Fig. 2B, considerable recovery (about 80%) of pLT−662CAT transcriptional activation levels was achieved with pLT−612CAT in both PD and PD31 transfectants. Constructs containing LT 5′ fragments smaller than LT−612 were minimally active in comparison with either pLT−612CAT or pLT−662CAT. Missense constructs, which contained inverted LT 5′ fragments, were also inactive (data not shown). We were therefore able to localize an important component of LT promoter transcriptional activation to the 32-bp region between −612 and −580.

We next concentrated our efforts on further characterizing the UAS localized between −612 and −580. This sequence was noteworthy because it was composed almost entirely of a homopolymeric tract of dA-dT sequences (Fig. 3A). Tracts of poly(dA-dT) DNA deviate from standard B-DNA in that they have a shorter helix repeat of 10.0 bp (40, 46), contain a distinctly narrow minor groove (1), and are associated with kinks or bends in the DNA (26). Moreover, poly(dA-dT)

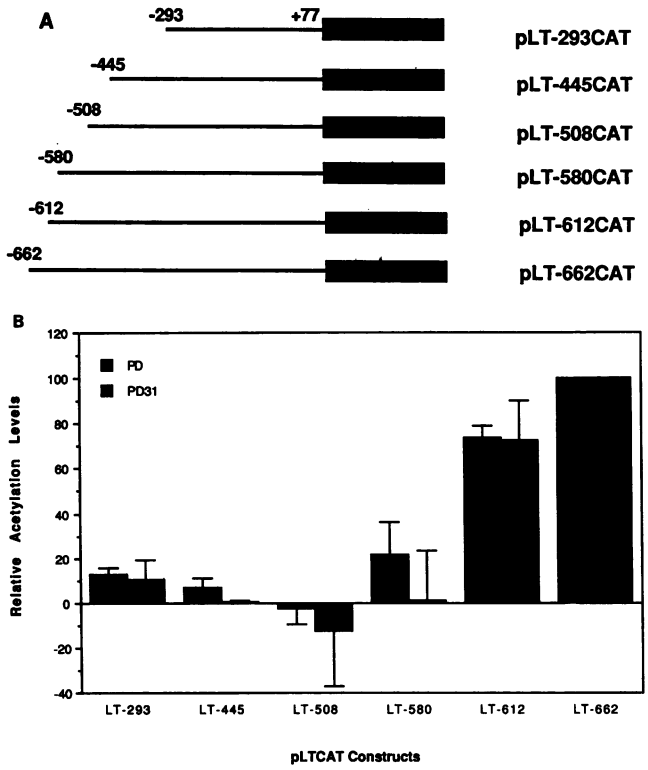

FIG. 2. A 32-bp region between −612 and −580 contains an important LT UAS. Schematic diagram of LTCAT constructs (A) depicting LT 5′ DNA and linker sequences (lines) and CAT gene (black box). All LTCATs had a common 3′ LT sequence terminus (+77) and the indicated 5′ termini. Control missense plasmids contained inverted LT 5′ sequences (not shown). (B) PD and PD31 cells (1.5×10^7) were transfected with 20 μg of the indicated LTCATs. Histograms were plotted as percent CAT activity relative to pLT−662CAT levels as determined by densitometric scanning (for details, see Materials and Methods). Note that LT promoter usage was identical in transfected PD and PD31 cells. The majority of pLT−662CAT-driven promoter activity was recovered with pLT−612/+77, which included a 32-bp UAS; LTCATs that lacked this region were minimally active. Histograms represent the average of eight separate transfections and CAT assays for each cell line; error bars equal one standard deviation.

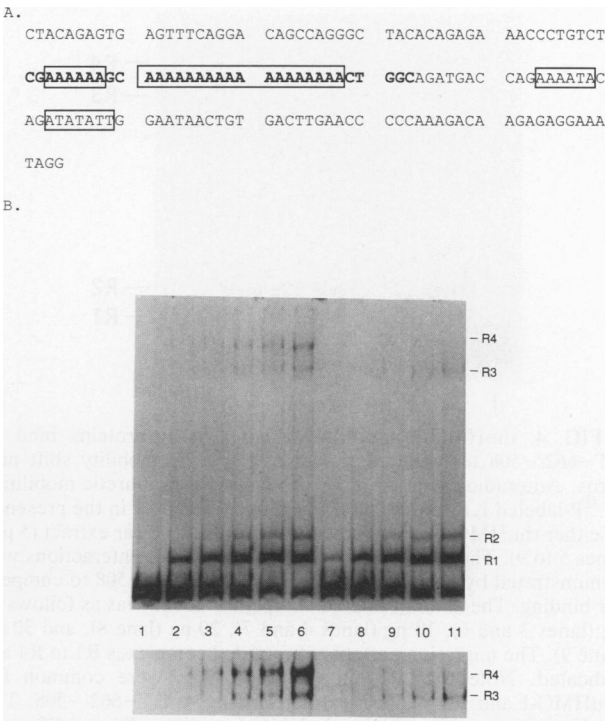

FIG. 3. PD and PD31 nuclear extracts display near-identical shift patterns when bound to LT−662/−508. Nucleotide sequence of LT−662/−508 coding strand (A) indicating the poly(dA-dT)-rich UAS localized between −612 and −580 (bold type), potential HMG-I-binding sites (boxed), and flanking sequences. (B) Autoradiogram of EMSA showing ^{32}P-labeled LT−662/−508 incubated in the presence of PD (lanes 2 to 6) or PD31 (lanes 7 to 11) nuclear extracts. Nuclear extract protein amounts were as follows: 0 μg (lane 1), 1 μg (lanes 2 and 7), 2 μg (lanes 3 and 8), 3 μg (lanes 4 and 9), 4 μg (lanes 5 and 10), and 5 μg (lanes 6 and 11). The migration patterns of retarded complexes R1 through R4 are indicated. The top autoradiograph was exposed for 18 h. To facilitate visualization of the R3 and R4 complexes, a longer exposure (3 days, with one intensifying screen) of a portion of the same gel is shown in the bottom panel.

UASs are required for the constitutive expression of several genetic loci in yeasts (for a review, see reference 58). Interestingly, it has been observed that the longer the poly(dA-dT) stretch, the more active its transcriptional stimulation (48, 56), and the 32-bp LT UAS is fairly large. Additionally, the UAS identified here includes the 3′ end of a previously identified murine (*Alu*-like) B1 repetitive element (16); repetitive elements have been previously implicated in the regulation of gene expression (28, 50, 64). Also of note was the presence of several potential HMG-I-binding sites, characterized by stretches of at least 6 bp of dA-dT sequences (57), in the LT−662/−508 region. The A+T-rich UAS clearly included potential recognition sites for HMG-I (Fig. 3A). To examine further the role of the UAS, we investigated patterns of DNA-binding protein interactions in this region.

Nuclear extracts from PD and PD31 cells bind to the UAS containing LT−662/−508 region. EMSAs were performed to assess binding patterns of nuclear proteins to the LT−662/−508 fragment. The LT−662/−508 fragment was used because it includes the UAS identified functionally in transfection studies in PD and PD31 cells. Figure 3B depicts an EMSA in which nuclear extracts from PD and PD31 cells were evaluated for their ability to bind to LT−662/−508. An identical pattern of retarded species was observed with extracts from the two cell lines. The electrophoretic mobilities of the predominant retarded complexes are indicated by bold dashes and enumerated R1, R2, R3, and R4 (Fig. 3B). Identical PD and PD31 extract-mediated EMSA patterns supported the transfection results, which showed that the LTCATs were regulated similarly in the two cell lines. Because our findings with PD and PD31 nuclear extracts were consistently identical, we interpreted the EMSA data to reflect the typical protein-DNA interactions of LT−662/−508 in these cells. (For the purposes of brevity, we show no additional duplicate data for PD- and PD31-derived nuclear extracts.)

Titrations of both nuclear extracts (1 to 5 μg) suggested that the multiple retarded complexes were due to protein-DNA interactions of various affinities (Fig. 3B). Retarded complex R1 clearly represented a protein-DNA interaction of high affinity, since it was apparent with the addition of only 1 μg of either nuclear extract. The retarded complexes

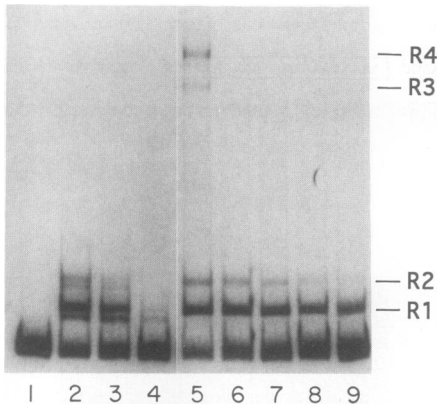

FIG. 4. rhuHMG-I and PD nuclear extract proteins bind to LT−662/−508 to facilitate similar and specific mobility shift patterns. Autoradiogram of EMSA showing electrophoretic mobilities of ^{32}P-labeled LT−662/−508 probe alone (lane 1) or in the presence of either rhuHMG-I (15 ng; lanes 2 to 4) or PD nuclear extract (5 μg; lanes 5 to 9). The specificity of these protein-DNA interactions was demonstrated by the ability of unlabeled LT−662/−508 to compete for binding. The amount of cold competitor added was as follows: 5 ng (lanes 3 and 6), 10 ng (lanes 4 and 7), 20 ng (lane 8), and 30 ng (lane 9). The migration patterns of retarded complexes R1 to R4 are indicated. Note that R1 and R2 complexes were common for rhuHMG-I and PD nuclear extract binding to LT−662/−508. The doublet pattern observed for rhuHMG-I-mediated R1 and R2 complexes was due to HPLC copurification of a truncated rhuHMG-I that retained full binding activity but lacked the carboxyl terminus of the full-length rhuHMG-I (see Results).

R2, R3, and R4 required higher concentrations (2 to 3 μg) of nuclear extract, indicating lower-affinity interactions. The degree of mobility shift observed for the nuclear extract-mediated R3 and R4 complexes indicated that they were due to high-molecular-weight DNA-binding protein interactions. It should be stressed that the gel conditions used here favor the visualization of only high-affinity protein-DNA interactions; many protein-DNA interactions cannot survive such high-stringency gels (3). Complexes R1, R2, R3, and R4 therefore all represented high-affinity interactions, but of various degrees.

Comigration of complexes R1 and R2 mediated by nuclear extracts and rhuHMG-I. HMG-I is a DNA-binding protein which specifically recognizes the minor groove of double-stranded poly(dA-dT) DNA (11, 53). As noted above, there were several potential HMG-I-binding sites, including the A+T-rich UAS, located within the LT−662/−508 DNA fragment (Fig. 3A). As previously indicated (10, 43, 49), however, not all dA-dT tracts equal to or greater than 6 bp bind HMG-I with equal efficiency. Flanking sequences have been postulated to account for the differential affinity of HMG-I for various potential sites.

To determine whether bona fide HMG-I would recognize these potential binding sites, we evaluated the ability of HPLC-purified rhuHMG-I to bind to LT−662/−508. Clearly, rhuHMG-I bound to the LT−662/−508 fragment, as demonstrated by a pattern of retarded complexes (Fig. 4, lane 2). The presence of two doublet shifts for rhuHMG-I in this figure was due to the HPLC copurification of a truncated rhuHMG-I species along with full-length rhuHMG-I. This truncated rhuHMG-I lacked the carboxyl terminus of the full-length protein but retained full binding activity. Full-length rhuHMG-I bound to LT−662/−508 to produce two retarded complexes (R1 and R2) with mobility shifts identical to those of the PD and PD31 nuclear extract-mediated R1 and R2 complexes (Fig. 3, see also Fig. 5 and 6). Judging from the mobility shifts of the retarded complexes, R1 was composed of a single rhuHMG-I molecule bound to one of the high-affinity binding sites within LT−662/−508, and the pattern of the R2 complex was consistent with binding by two HMG-I molecules. The identical pattern of R1 and R2 bands (compare Fig. 4, lanes 2 and 5; see also Fig. 5 and 6) seen in the presence of either nuclear extract or purified full-length rhuHMG-I suggested that PD nuclear extract contained HMG-I or an HMG-I-like protein.

The specificity of retarded complexes was demonstrated by the ability of unlabeled LT−662/−508 to compete for binding of purified rhuHMG-I and nuclear extract to labeled LT−662/−508. As determined densitometrically, approximately a 10:1 molar ratio of unlabeled to labeled probe was required to effect a 50% reduction in HMG-I-specific retarded complex R2 (Fig. 4, compare lane 3 with lane 2), whereas about a 20:1 ratio was necessary to reduce R1 complex formation by 50% (compare lane 4 with lane 2). For PD nuclear extract-mediated R2 complex, a 20:1 to 40:1 molar ratio of unlabeled to labeled probe was required to effect a 50% reduction (compare lane 7 with lane 5). The PD nuclear extract-mediated complex R1 required a 60:1 ratio to reduce binding by 31% (compare lane 9 to 5), implying a higher-affinity association than those of the other complexes. The R3 and R4 retarded complexes, observed only with nuclear extracts, were abolished at a molar ratio of only 10:1 (unlabeled to labeled), indicating lower binding affinity interactions than those of the R1 and R2 complexes.

Anti-HMG-I specific antibodies altered the pattern of PD nuclear extract-associated retarded complexes in EMSAs. To determine whether the protein-DNA complexes R1 and R2 observed in the presence of PD nuclear extract were due to HMG-I or an HMG-I-like protein, we examined the effect of a rabbit polyclonal antibody generated against purified native murine HMG-I (9) in EMSAs. The addition of specific antibody to binding reactions (27) can cause a variety of changes in protein-DNA complex formation. If the antibody recognizes a protein component of a protein-DNA complex, antibody can block complex formation and/or form an antibody-protein-DNA ternary complex, thereby resulting in a supershifted complex.

The IgG-purified anti-HMG-I effected changes in several of the protein-DNA complexes normally observed with PD nuclear extract or rhuHMG-I and LT−662/−508 (Fig. 5). The presence of anti-HMG-I antibody in binding reactions resulted in a reduction in the amounts of PD nuclear extract and rhuHMG-I-mediated R1 and R2 retarded complexes (Fig. 5, compare lane 3 with lane 4 and lane 6 with lane 7). For example, as determined densitometrically, levels of PD nuclear extract-mediated R1 and R2 complexes were reduced by 23 and 67%, respectively. The data demonstrated that PD nuclear extract contained HMG-I or an HMG-I-like protein, with electrophoretic mobility similar to that of rhuHMG-I, that was an integral component of the R1 and R2 complexes. Until sequence analysis of the nuclear protein confirms that it is HMG-I, it will be referred to as PD HMG-I protein. The control antibody, affinity-purified rabbit anti-IgG, did not alter EMSA binding patterns seen for free probe alone, nuclear extract, or rhuHMG-I (Fig. 5, compare lanes 1 and 2, lanes 3 and 5, and lanes 6 and 8).

Preincubation of either PD nuclear extract or rhuHMG-I with antibody to HMG-I also resulted in the appearance of a novel species of retarded complex (Fig. 5, lanes 4 and 7,

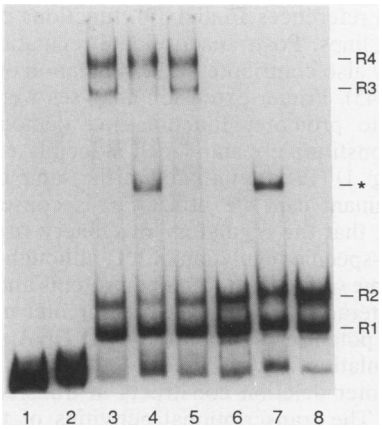

FIG. 5. Anti-HMG-I antibody specifically alters EMSA binding patterns of PD nuclear extract and rhuHMG-I to LT−662/−508. Autoradiogram of EMSA showing electrophoretic mobility of ^{32}P-labeled LT−662/−508 probe incubated in the presence of PD nuclear extract (10 μg; lanes 3 to 5) or rhuHMG-I (15 ng; lanes 6 to 8). Parallel binding assays were performed without antibody (lanes 3 and 6) or in the presence of either anti-HMG-I IgG (lanes 2, 4, and 7) or control IgG (lanes 5 and 8). The electrophoretic mobility of free probe (lane 1) was unchanged when incubated with anti-HMG-I IgG (lane 2).

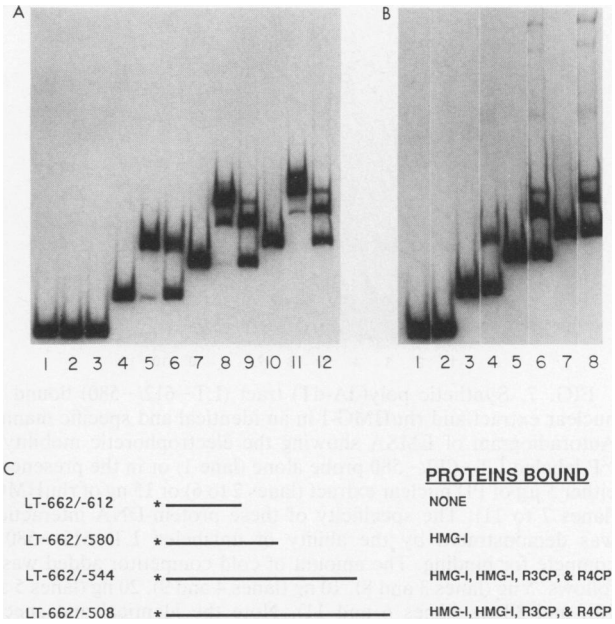

FIG. 6. Delineation of binding sites for two HMG-I molecules and the R3 and R4 complex protein(s) within the LT−662/−508 region. (A) Autoradiography of stairway EMSA showing electrophoretic mobilities of four different ^{32}P-labeled probes: LT−662/−612 (lanes 1 to 3), LT−662/−580 (lanes 4 to 6), LT−662/−544 (lanes 7 to 9), and LT−662/−508 (lanes 10 to 12) incubated alone (lanes 1, 4, 7, and 10) or in the presence of either 15 ng of rhuHMG-I (lanes 2, 5, 8, and 11) or 5 μg of PD nuclear extract (lanes 3, 6, 9, and 12). (B) Autoradiography of similar stairway EMSA with twice the amount of PD nuclear extract to facilitate visualization of high-mobility-shifted complexes R3 and R4. The four ^{32}P-labeled probes are as in panel A: LT−662/−612 (lanes 1 and 2), LT−662/−580 (lanes 3 and 4), LT−662/−544 (lanes 5 and 6), and LT−662/−508 (lanes 7 and 8) incubated alone (lanes 1, 3, 5, and 7) or in the presence of 10 μg of PD nuclear extract (lanes 2, 4, 6, and 8). Note that the smallest fragment (LT−662/−612) contained no binding sites, whereas the larger fragments (LT−662/−580 and LT−662/−544) contained one and two HMG-I-binding sites, respectively. The high-mobility-shifted bands (R3 and R4) were visible with LT−662/−544 and LT−662/−508 probes and best visualized at the higher PD nuclear extract concentration (panel B). (C) Schematic diagram of the four probes used above, indicating the ^{32}P end label (*) and the proteins that bind to these DNA fragments: PD HMG-I and rhuHMG-I (HMG-I), PD nuclear extract R3 complex proteins (R3CP), and R4 complex proteins (R4CP).

asterisk). The novel, intermediately migrating species was best explained as a supershifted ternary complex composed of HMG-I, LT DNA, and anti-HMG-I antibody. The formation of ternary complexes was anti-HMG-I antibody specific in that control antibody did not facilitate the assembly of PD HMG-I or rhuHMG-I ternary complexes (Fig. 5, lanes 5 and 8).

The reduction of nuclear extract-specific retarded complex R3 by 83% (as determined densitometrically) in the presence of anti-HMG-I antibody was particularly interesting because such a slowly migrating complex could not be accounted for solely by PD HMG-I binding and, furthermore, was not observed with rhuHMG-I. The ability of anti-HMG-I antibody to abrogate R3 complex formation indicated that antibody binding to either PD HMG-I or a DNA-binding protein (R3 complex component), which was antigenically similar to HMG-I, sterically hindered R3 complex formation. The potential role of HMG-I in facilitating the binding of other proteins to DNA has been previously proposed (10). The implications of such facilitated binding are discussed below.

Delineation of binding sites within LT−662/−508 by stairway EMSA. A stairway EMSA was performed to assess binding contributions of different regions of the LT−662/−508 fragment. To facilitate this analysis, LT−662/−508 was end labeled with [^{32}P]dATP and aliquots of labeled LT−662/−508 were digested with *Taq*I (to generate LT−662/−612), *Bsr*I (to generate LT−662/−580), or *Mae*III (to generate LT−662/−544). Each of the above 5'-end-labeled LT−662/−508 derivatives (Fig. 6) was then gel purified and examined for its ability to bind rhuHMG-I and PD nuclear extract. As shown in Fig. 6, LT−662/−612 bound neither rhuHMG-I nor PD nuclear extract (Fig. 6A, compare lanes 2 and 3 with lane 1; Fig. 6B, compare lane 2 with lane 1). LT−662/−580, which included the 32-bp poly(dA-dT) UAS, bound both rhuHMG-I and PD nuclear extract (Fig. 6A, compare lanes 5 and 6 with lane 4; Fig. 6B, compare lane 4 with lane 3). As suggested by the single species of retarded complex, this UAS included one binding site recognized by rhuHMG-I and PD HMG-I. LT−662/−544 included all of the potential HMG-I-binding sites, as determined by sequence analysis (Fig. 3A), found within the full-length LT−662/−508. The region between −580 and −544 conferred an additional site for HMG-I-specific binding, which restored the R1/R2 banding pattern seen with LT−662/−508 (Fig. 6A, compare lanes 8 and 11 and lanes 9 and 12; Fig. 6B, compare lanes 6 and 8). In addition, this region contained the DNA sites required for the interactions with the high-molecular-weight DNA-binding proteins that formed the nuclear extract-mediated complexes R3 and R4 (Fig. 6B, compare lanes 6 and 8). Taken together, these data demonstrate that the binding sites required for the formation of retarded complexes R1 to R4 were included within the region from −612 to −544.

A synthetic poly(dA-dT) UAS bound rhuHMG-I and PD HMG-I. The binding properties of a minimal poly(dA-dT)

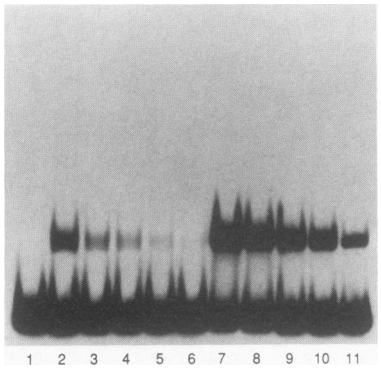

FIG. 7. Synthetic poly(dA-dT) tract (LT−612/−580) bound PD nuclear extract and rhuHMG-I in an identical and specific manner. Autoradiogram of EMSA showing the electrophoretic mobility of ^{32}P-labeled LT−612/−580 probe alone (lane 1) or in the presence of either 5 μg of PD nuclear extract (lanes 2 to 6) or 15 ng of rhuHMG-I (lanes 7 to 11). The specificity of these protein-DNA interactions was demonstrated by the ability of unlabeled LT−612/−580 to compete for binding. The amount of cold competitor added was as follows: 5 ng (lanes 3 and 8), 10 ng (lanes 4 and 9), 20 ng (lanes 5 and 10), and 30 ng (lanes 6 and 11). Note the identical and specific electrophoretic mobility of retarded complexes in the presence of either PD nuclear extract or rhuHMG-I.

UAS were assessed to eliminate any contributions arising from flanking sequences, which could have affected the results of the stairway EMSA. Complementary oligonucleotides which spanned the LT−612/−580 region were synthesized and annealed to form the double-stranded LT−612/−580 fragment. LT−612/−580 was ^{32}P labeled and examined for its ability to bind rhuHMG-I and PD HMG-I (Fig. 7). The synthetic UAS clearly bound rhuHMG-I with high affinity to produce a retarded complex; the specificity of this interaction was demonstrated by the ability of unlabeled LT−612/−580 to compete with labeled probe for binding to rhuHMG-I. Furthermore, the inability of other control double-stranded synthetic oligonucleotides to bind PD HMG-I or rhuHMG-I demonstrated that binding to LT−612/−580 was sequence specific (data not shown). As determined by densitometric scanning, a 20:1 to 40:1 molar ratio of unlabeled to labeled LT−612/−580 resulted in a 50% reduction in rhuHMG-I–labeled DNA complex formation (Fig. 7, compare lanes 7 and 10). As expected, the UAS also bound PD HMG-I to produce a retarded complex that comigrated with the rhuHMG-I retarded complex. The amount of the PD HMG-I retarded complex was reduced by 50% in the presence of an excess molar ratio of 10:1 for unlabeled versus labeled probe (Fig. 7, compare lanes 2 and 3). Preincubation with anti-HMG-I antibody (as above) prior to the addition of labeled LT−612/−580 resulted in a specific decrease in retarded complex formation of 46% for PD and 64% for PD31 nuclear extracts (data not shown). Taken together, these data demonstrated unequivocally that the UAS localized within LT−612/−580 contained a high-affinity binding site for purified rhuHMG-I and a PD HMG-I protein of similar electrophoretic mobility.

DISCUSSION

This study focuses on the contributions of upstream flanking sequences to LT regulation in the A-MuLV-transformed pre-B-cell lines PD and PD31. One possible explanation for LT mRNA accumulation is that the promoter, which is normally repressed prior to cellular activation (reviewed in references 16 and 39), functions constitutively in these cell lines. Posttranscriptional regulation of mRNA stability may also contribute to the regulation of LT in these cells (6, 15, 45). Primer extension analyses were performed to investigate promoter function and demonstrated that pre-B-cell constitutively and T-cell inducibly expressed LT mRNAs (Fig. 1) (16) are initiated at the same cap sites and that predominant cap site utilization is conserved. These data suggest that the regulatory machinery responsible for the cell type-specific regulation of LT, although distinct with regard to some of the DNA-binding proteins and recognition elements, interacts with a common promoter unit.

Here, the potential contribution of 5′ DNA sequences to LT gene regulation was assessed by examining the behavior of LT promoter deletion constructs in transfected PD and PD31 cells. The transcriptional activities of the LTCATs were fundamentally different in these constitutive LT mRNA producers, when compared with those observed in other lymphocyte transfectants. Larger LT 5′ fragments, which are silent in murine T-cell lymphomas and mature B cells, were transcriptionally active in PD and PD31 transfectants. We delineated the PD-PD31-responsive UAS that was necessary and sufficient for transcriptional activation of the LT gene promoter to a 32-bp poly(dA-dT)-rich tract (−612 to −580). A minimal deletion of this region (pLT−580CAT) resulted in a transcriptionally inactive construct in transfection assays.

This demonstration of poly(dA-dT) UAS function in LT gene regulation is reminiscent of the constitutively expressed *pet56*, *his3*, and *ded1* regulatory loci of yeast, which are controlled by upstream poly(dA-dT) elements. Both in vivo and in vitro experiments have demonstrated unequivocally that the poly(dA-dT) tracts are necessary and sufficient for constitutive expression (7, 32, 56, 57). The mechanism(s) through which poly(dA-dT) elements activate transcription is not yet resolved (reviewed in reference 58). The unusual conformation of double-stranded poly(dA-dT) DNA may create a nucleosome-free chromatin structure that confers accessibility to DNA-binding proteins (56), thereby functioning as a nonspecific entry point. Alternatively, DNA-binding proteins may specifically bind poly(dA-dT) tracts and modulate transcriptional activity (32). One likely candidate for this latter mechanism is the yeast protein datin, which specifically binds A+T-rich DNA (62). It has been suggested that, depending upon the circumstances, datin can function as either an activator or a repressor of yeast transcription.

We characterized the protein-binding properties of the functional UAS by performing EMSAs with a 5′ fragment of LT gene DNA (LT−662/−508), which included the poly(dA-dT) tract and flanking sequences. We found that rhuHMG-I and an abundant HMG-I-like protein detected in PD and PD31 nuclear extracts (designated PD HMG-I) bound to LT−662/−508. PD HMG-I had an electrophoretic mobility similar to that of rhuHMG-I and reacted with anti-HMG-I antibody (Fig. 4 and 5). Use of a synthetic UAS (LT−612/−580) and LT−662/−508 subfragments as probes in EMSAs demonstrated that the poly(dA-dT)-rich LT UAS bound rhuHMG-I and PD HMG-I protein with high affinity (Fig. 6 and 7). These studies demonstrate that HMG-I-like proteins bind to the poly(dA-dT)-rich UAS and presumably contribute to LT gene transcriptional regulation.

In contrast, LT−580, which contained one high-affinity HMG-I-binding site between −580 and −544 (as determined by EMSAs), did not activate the LT promoter to express CAT. These data demonstrated that the binding of one HMG-I molecule to a high-affinity site was not sufficient for

transcriptional activation. On the other hand, LT−612 and LT−662, which both contained an additional high-affinity HMG-I-binding site (localized within −612/−580), drove high levels of CAT expression. These data emphasize that it is not sufficient to demonstrate binding and therefore assume function but, rather, that it is essential to demonstrate both element function and binding.

HMG-I, a well-characterized nonhistone chromosomal protein, is an important component of active chromatin structures. It has been implicated in metaphase chromatin condensation (33), heterochromatin nucleosome phasing (53), nuclear matrix-DNA interactions (11), 3'-end processing of genes (42, 49), and the formation of active initiation complexes (61, 65). HMG-I is preferentially expressed at high levels in undifferentiated, neoplastically transformed, and rapidly proliferating cells (5, 14, 17, 18, 22, 23, 33, 60); it is predicted to undergo a wide variety of posttranslational modifications (14), some of which are expected to alter its affinity for DNA. Such modifications may contribute to HMG-I-modulated cell cycle progression and tissue-specific gene expression; for example, cdc2 kinase phosphorylation of the principal binding domain of murine HMG-I significantly weakens its association with DNA (44).

HMG-I binding to the LT UAS could induce conformational changes in the secondary structure of the DNA and/or chromatin that create a transcriptionally active locus. Such conformational changes are applicable to both the endogenous LT gene and the LTCAT constructs, because it is known that mammalian cells are capable of rapidly assembling DEAE-dextran-transfected plasmids into nucleosome-containing minichromosomes (43). As suggested previously, HMG-I–DNA interactions may act through the nonspecific mechanism of increased accessibility for DNA-binding proteins (23). Conformation-dependent transcriptional activation could also be mediated via HMG-I–DNA complexes serving as targets, which are specifically recognized by other DNA-binding proteins. For example, there may be unique or uniquely modified DNA-binding proteins in LT constitutive expressors that recognize these HMG-I–DNA complexes.

Similarly, anti-HMG-I antibody abrogated formation of the protein-DNA complex designated R3 in addition to mediating the appearance of supershifted complexes and reducing levels of HMG-I containing complexes R1 and R2 (Fig. 5). A DNA-binding protein, or complex of proteins, of high molecular weight undoubtedly contributed the relatively large mobility shift observed for the R3 complex. The ability of anti-HMG-I antibody to abrogate R3 complex formation indicated that antibody binding to either PD HMG-I or a DNA-binding protein (R3 complex component), which was antigenically similar to HMG-I, sterically hindered R3 complex formation. The former implies that HMG-I can facilitate the association of additional DNA-binding proteins to their substrates, acting as a seed for transcriptional modifier complex formation. Although a role for HMG-I protein in cooperative binding has been suggested previously (10), our findings represent the first supportive evidence for such interactions.

HMG-I, which recognizes the minor groove of double-stranded dA-dT sequences (11, 25, 53, 55), could interact with major groove-binding activator and repressor proteins via its acidic carboxy-terminal domain. The acidic carboxyl domain of HMG-I may contribute to intermolecular protein-protein complex formation and/or protein-DNA complex formation. Additionally, some major groove-binding proteins make contacts in the minor groove (10, 54); HMG-I binding, under these circumstances, could clearly influence these interactions. HMG-I may also stabilize protein-DNA complexes. Clearly, HMG-I and HMG-I-like molecules could function, via the suggested mechanisms, to enhance or repress transcription, depending upon the circumstances.

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REFERENCES

1. **Alexeev, D. G., A. A. Lipanov, and I. Y. Skuratovskii.** 1987. Poly (dA) · poly(dT) is a B-type double helix with a distinctively narrow minor groove. Nature (London) **325**:821–823.
2. **Alt, F., N. Rosenberg, S. Lewis, E. Thomas, and D. Baltimore.** 1981. Organization and reorganization of immunoglobulin genes in A-MuLV-transformed cells: rearrangement of heavy but not light chain genes. Cell **27**:381–390.
3. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl.** 1987. Current protocols in molecular biology, vol. 2. Greene Publishing Associates and Wiley-Interscience, New York.
4. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
5. **Bussemakers, M. J. G., W. J. M. van de Ven, F. M. J. Debruyne, and J. A. Schalken.** 1991. Identification of high mobility group protein I (Y) as potential progression marker for prostate cancer by differential hybridization analysis. Cancer Res. **51**:606–611.
6. **Caput, D., B. Beutler, K. Hartog, R. Thayer, S. Brown-Shimer, and A. Cerami.** 1986. Identification of a common nucleotide sequence in the 3'-untranslated region of mRNA molecules specifying inflammatory mediators. Proc. Natl. Acad. Sci. USA **83**:1670–1674.
7. **Chen, W., S. Tabor, and K. Struhl.** 1987. Distinguishing between mechanisms of eukaryotic transcriptional activation with bacteriophage T7 RNA polymerase. Cell **50**:1047–1055.
8. **Dignam, J. D., R. M. Lebovitz, and R. G. Roeder.** 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. Nucleic Acids Res. **11**:1475–1489.
9. **Disney, J. E., K. R. Johnson, N. S. Magnuson, S. R. Sylvester, and R. Reeves.** 1989. High-mobility group protein HMG-I localizes to G/Q- and C-bands of human and mouse chromosomes. J. Cell Biol. **109**:1975–1982.
10. **Eckner, R., and M. L. Birnstiel.** 1989. Cloning of cDNAs coding for human HMG I and HMG Y proteins: both are capable of binding to the octamer sequence motif. Nucleic Acids Res. **17**:5947–5959.
11. **Elton, T. S., M. S. Nissen, and R. Reeves.** 1987. Specific A · T DNA sequence binding of RP-HPLC purified HMG-I. Biochem. Biophys. Res. Commun. **143**:260–265.
12. **Elton, T. S., and R. Reeves.** 1985. Microheterogeneity of the mammalian high mobility group (HMG) proteins 1 and 2 investigated by reverse-phase high performance liquid chromatography. Anal. Biochem. **144**:403–416.
13. **Elton, T. S., and R. Reeves.** 1985. Microheterogeneity of the mammalian high-mobility group proteins 14 and 17 investigated by reverse-phase high-performance liquid chromatography. Anal. Biochem. **146**:448–460.

14. **Elton, T. S., and R. Reeves.** 1986. Purification and postsynthetic modifications of Friend erythroleukemic cell high mobility group protein HMG-I. Anal. Biochem. **157**:53–62.
15. **Fashena, S. J.** 1992. Ph.D. thesis. Yale University, New Haven, Conn.
16. **Fashena, S. J., W.-L. Tang, T. Sarr, and N. H. Ruddle.** 1990. The murine lymphotoxin gene promoter: characterization and negative regulation. J. Immunol. **145**:177–183.
17. **Giancotti, V., E. Buratti, L. Perissin, S. Zorzet, A. Balmain, G. Portella, A. Fusco, and G. Goodwin.** 1989. Analysis of the HMG-I nuclear proteins in mouse neoplastic cells induced by different procedures. Exp. Cell Res. **184**:538–545.
18. **Giancotti, V., B. Pani, P. D'Andrea, M. T. Berlingieri, P. P. DiFiore, A. Fusco, G. Veccio, R. Philp, C. Crane-Robinson, R. H. Nicolas, C. A. Wright, and G. H. Goodwin.** 1987. Elevated levels of a specific class of nuclear phosphoproteins in cells transformed with v-*ras* and v-*mos* oncogenes and by co-transfection with c-*myc* and polyoma middle T genes. EMBO J. **6**:1981–1987.
19. **Gorman, C. M., G. T. Merlino, M. C. Willingham, I. Pastan, and B. H. Howard.** 1982. The Rous sarcoma virus long terminal repeat is a strong promoter when introduced into a variety of eukaryotic cells by DNA-mediated transfection. Proc. Natl. Acad. Sci. USA **79**:6777–6781.
20. **Gorman, C. M., L. F. Moffat, and B. F. Howard.** 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol. Cell. Biol. **2**:1044–1051.
21. **Johnson, K. R., J. E. Disney, C. R. Wyatt, and R. Reeves.** 1990. Expression of mRNAs encoding mammalian chromosomal proteins HMG-I and HMG-Y during cellular proliferation. Exp. Cell Res. **187**:69–76.
22. **Johnson, K. R., D. A. Lehn, T. S. Elton, P. J. Barr, and R. Reeves.** 1988. Complete murine cDNA sequence, genomic structure, and tissue expression of the high mobility group protein HMG-I(Y). J. Biol. Chem. **263**:18338–18342.
23. **Johnson, K. R., D. A. Lehn, and R. Reeves.** 1989. Alternative processing of mRNAs encoding mammalian chromosomal high-mobility-group proteins HMG-I and HMG-Y. Mol. Cell. Biol. **9**:2114–2123.
24. **Jones, K. A., K. R. Yamamoto, and R. Tjian.** 1985. Two distinct transcription factors bind to the HSV thymidine kinase promoter in vitro. Cell **42**:559–572.
25. **Karlson, J. R., E. Mork, J. Holtland, S. G. Laland, and T. Lund.** 1989. The amino acid sequence of the chromosomal protein HMG-Y, its relation to HMG-I and possible domains for the preferential binding of the proteins to stretches of A-T base pairs. Biochem. Biophys. Res. Commun. **158**:646–651.
26. **Koo, H.-S., H.-M. Wu, and D. M. Crothers.** 1986. DNA bending at adenine-thymine tracts. Nature (London) **320**:501–506.
27. **Kristie, T. M., and B. Roizman.** 1986. α4, the major regulatory protein of herpes simplex virus type 1, is stably and specifically associated with promoter-regulatory domains of α genes and of selected other viral genes. Proc. Natl. Acad. Sci. USA **83**:3218–3222.
28. **Laimins, L., M. Holmgren-Konig, and G. Khoury.** 1986. Transcriptional "silencer" element in rat repetitive sequences associated with the rat insulin 1 gene locus. Proc. Natl. Acad. Sci. USA **83**:3151–3155.
29. **Laskov, R., G. Lancz, N. H. Ruddle, K. M. McGrath, S. Specter, T. Klein, J. Y. Djeu, and H. Friedman.** 1990. Production of tumor necrosis factor (TNF-α) and lymphotoxin (TNF-β) by murine pre-B and B cell lymphomas. J. Immunol. **144**:3424–3430.
30. **Lenardo, M. J., C.-M. Fan, T. Maniatis, and D. Baltimore.** 1989. The involvement of NF-κB in β-interferon gene regulation reveals its role as widely inducible mediator of signal transduction. Cell **57**:287–294.
31. **Lewis, S., N. Rosenberg, F. Alt, and D. Baltimore.** 1982. Continuing kappa-gene rearrangement in a cell line transformed by Abelson murine leukemia virus. Cell **30**:807–816.
32. **Lue, N. F., A. R. Buchman, and R. D. Kornberg.** 1989. Activation of yeast RNA polymerase II transcription by a thymidine-rich upstream element *in vitro*. Proc. Natl. Acad. Sci. USA **86**:486–490.
33. **Lund, T., J. Holtlund, M. Fredriksen, and S. G. Laland.** 1983. On the presence of two new high mobility group-like proteins in HeLa S3 cells. FEBS Lett. **152**:163–167.
34. **Muller, U., C. V. Jongeneel, S. A. Nedospasov, K. F. Lindahl, and M. Steinmetz.** 1987. Tumour necrosis factor and lymphotoxin genes map close to H-2D in the mouse major histocompatibility complex. Nature (London) **325**:265–267.
35. **Nedospasov, S. A., B. Hirt, A. N. Shakov, V. N. Dobrynin, E. Kawashima, R. S. Accolla, and C. V. Nedospasov.** 1986. The genes for tumor necrosis factor (TNF-alpha) and lymphotoxin (TNF-beta) are tandemly arranged on chromosome 17 of the mouse. Nucleic Acids Res. **14**:7713–7725.
36. **Nissen, M. S., T. A. Langan, and R. Reeves.** 1991. Phosphorylation by cdc2 kinase modulates DNA binding activity of high mobility group I nonhistone chromatin protein. J. Biol. Chem. **266**:19945–19952.
37. **O'Garra, A., G. Stapleton, V. Dhar, M. Pearce, J. Schumacher, H. Rugo, D. Barbis, A. Stall, J. Cupp, K. Moore, P. Vieira, T. Mosmann, A. Whitmore, L. Arnold, G. Haughton, and M. Howard.** 1990. Production of cytokines by mouse B cells: B lymphomas and normal B cells produce interleukin 10. Int. Immunol. **2**:821–832.
38. **Paul, N. L., M. J. Lenardo, K. D. Novak, T. Sarr, W.-L. Tang, and N. H. Ruddle.** 1990. Lymphotoxin activation by human T-cell leukemia virus type I-infected cell lines: role for NF-κB. J. Virol. **64**:5412–5419.
39. **Paul, N. L., and N. H. Ruddle.** 1988. Lymphotoxin. Annu. Rev. Immunol. **6**:407–438.
40. **Peck, L. J., and J. C. Wang.** 1981. Sequence dependence of the helical repeat of DNA in solution. Nature (London) **292**:375–378.
41. **Portoles, P., J. Rojo, A. Golby, M. Bonneville, S. Gromkowski, L. Greenbaum, C. A. Janeway, D. B. Murphy, and K. Bottomly.** 1989. Monoclonal antibodies to murine CD3ε define distinct epitopes, one of which may interact with CD4 during T cell activation. J. Immunol. **142**:4169–4175.
42. **Reeves, R., T. S. Elton, M. S. Nissen, D. Lehn, and K. R. Johnson.** 1987. Posttranscriptional gene regulation and specific binding of the nonhistone protein HMG-I by the 3′ untranslated region of bovine interleukin 2 cDNA. Proc. Natl. Acad. Sci. USA **84**:6531–6535.
43. **Reeves, R., C. M. Gorman, and B. Howard.** 1985. Minichromosome assembly of non-integrated plasmid DNA transfected into mammalian cells. Nucleic Acids Res. **13**:3599–3615.
44. **Reeves, R., T. A. Langan, and M. S. Nissen.** 1991. Phosphorylation of the DNA-binding domain of nonhistone high-mobility group I protein by cdc2 kinase: reduction of binding affinity. Proc. Natl. Acad. Sci. USA **88**:1671–1675.
45. **Reeves, R., and N. S. Magnuson.** 1990. Mechanisms regulating transient expression of mammalian cytokine genes and cellular oncogenes. Prog. Nucleic Acid Res. Mol. Biol. **38**:241–282.
46. **Rhodes, D., and A. Klug.** 1981. Sequence-dependent helical periodicity of DNA. Nature (London) **292**:378–380.
47. **Rosenberg, N., and D. Baltimore.** 1976. A quantitative assay for transformation of bone marrow cells by Abelson murine leukemia virus. J. Exp. Med. **143**:1453–1463.
48. **Russell, D. W., M. Smith, D. Cox, V. M. Williamson, and E. T. Young.** 1983. DNA sequences of two yeast promoter-up mutants. Nature (London) **304**:652–654.
49. **Russnak, R. H., E. P. M. Candido, and C. R. Astell.** 1988. Interaction of the mouse chromosomal protein HMG-I with the 3′ ends of genes *in vitro*. J. Biol. Chem. **263**:6392–6399.
50. **Saffer, J. D., and S. J. Thurston.** 1989. A negative regulatory element with properties similar to those of enhancers is contained within an *Alu* sequence. Mol. Cell. Biol. **9**:355–364.
51. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
52. **Sheehan, K. C. F., N. H. Ruddle, and R. D. Schreiber.** 1989. Generation and characterization of hamster monoclonal antibodies that neutralize murine tumor necrosis factors. J. Immunol. **142**:3884–3893.

53. **Solomon, M. J., F. Strauss, and A. Varshavsky.** 1986. A mammalian high mobility group protein recognizes any stretch of six A · T base pairs in duplex DNA. Proc. Natl. Acad. Sci. USA **83:**1276–1280.
54. **Staudt, L. M., H. Singh, R. Sen, T. Wirth, P. A. Sharp, and D. Baltimore.** 1986. A lymphoid-specific protein binding to the octamer motif of immunoglobulin genes. Nature (London) **323:** 640–643.
55. **Strauss, F., and A. Varshavsky.** 1984. A protein binds to a satellite DNA repeat at three specific sites that would be brought into mutual proximity by DNA folding in the nucleosome. Cell **37:**889–901.
56. **Struhl, K.** 1985. Naturally occurring poly(dA-dT) sequences are upstream promoter elements for constitutive transcription in yeast. Proc. Natl. Acad. Sci. USA **82:**8419–8423.
57. **Struhl, K.** 1986. Constitutive and inducible *Saccharomyces cerevisiae* promoters: evidence for two distinct molecular mechanisms. Mol. Cell. Biol. **6:**3847–3853.
58. **Struhl, K.** 1989. Molecular mechanisms of transcriptional regulation in yeast. Annu. Rev. Biochem. **58:**1051–1077.
59. **Turetskaya, R. L., S. J. Fashena, N. L. Paul, and N. H. Ruddle.** Genomic structure, induction, and production of TNF-β. *In* B. Aggarwal and J. Vilcek (ed.), Tumor necrosis factors: structure, function, and mechanism of action, in press. Marcel Dekker, Inc.
60. **Vartiainen, E., J. Palvimo, A. Mahonen, A. Linnala-Kankkunen, and P. H. Maenpaa.** 1988. Selective decrease in low-M_r HMG proteins HMG-I and HMG-Y during differentiation of mouse teratocarcinoma cells. FEBS Lett. **228:**45–48.
61. **Wegner, M., G. Zastrow, A. Klavinius, S. Schwender, F. Muller, H. Luksza, J. Hoppe, J. Wienberg, and F. Grummt.** 1989. Cis-acting sequences from mouse rDNA promote plasmid DNA amplification and persistence in mouse cells: implication of HMG-I in their function. Nucleic Acids Res. **17:**9909–9932.
62. **Winter, E., and A. Varshavsky.** 1989. A DNA binding protein that recognizes oligo(dA) · oligo(dT) tracts. EMBO J. **8:**1867–1877.
63. **Wong, R. L., N. H. Ruddle, S. G. Padula, E. G. Lingenheld, C. M. Bergman, R. V. Rugen, D. I. Epstein, and R. B. Clark.** 1988. Subtypes of helper cells: noninflammatory type 1 helper T cells. J. Immunol. **141:**3329.
64. **Wu, J., G. J. Grindlay, P. Bushel, L. Mendelsohn, and M. Allan.** 1990. Negative regulation of the human ε-globin gene by transcriptional interference: role of an Alu repetitive element. Mol. Cell. Biol. **10:**1209–1216.
65. **Yang-Yen, H.-F., and L. I. Rothblum.** 1988. Purification and characterization of a high-mobility-group-like DNA-binding protein that stimulates rRNA synthesis in vitro. Mol. Cell. Biol. **8:**3406–3414.