Identification of a *cis*-Acting Element in the Class I Major Histocompatibility Complex Gene Promoter Responsive to Activation by Retroviral Sequences

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The infection of cells with Moloney murine leukemia virus (M-MuLV) causes an increase in specific cellular gene products, including the major histocompatibility complex (MHC) class I antigens. This upregulation occurs through a transactivation process mediated by the long terminal repeat (LTR) of M-MuLV, and we show here that the gene activation response to the LTR requires at least one specific *cis* element within the MHC proximal promoter region. Nested deletions of MHC class I *H-2K^b* gene promoter sequence were subcloned into a chloramphenicol acetyltransferase (CAT) reporter vector and then transiently introduced into BALB/c-3T3 cells expressing M-MuLV or cotransfected into BALB/c-3T3 cells with a vector containing subgenomic portions of the virus, including the LTR. CAT activity assays demonstrated that a minimal *H-2K^b* gene promoter (–64 to +12) contained elements sufficient for this transactivation. DNase I footprinting assays located a protein-binding site in the region of –64 to –34 bp from the transcriptional start site, and point mutation analysis confirmed the location of this *cis*-acting element, designated the *let* response element (LRE), and defined a binding motif. This LRE is distinct from binding sites for currently known transcription factors in the class I MHC gene promoter and is conserved in the promoters of human and murine MHC class I genes. Mutation of the LRE resulted in dramatic reduction in both DNA-protein binding activity in electrophoretic mobility shift assay and in the ability of the mutated promoter to respond to retroviral transactivation. Addition of the LRE to a heterologous promoter conferred the ability to respond to retroviral transactivation.

Induction of T-lymphocyte leukemias by retroviruses involves the regulated expression of both viral and cellular genes. Activation of cellular genes by infection with retroviruses has been most fully characterized for human T-cell lymphotropic virus types 1 and 2, human immunodeficiency viruses, and bovine leukemia virus (12, 36, 45). In these cases, transactivation of the viral and cellular genes is mediated by a viral gene product designated Tax or Tat (25). Murine leukemia viruses (MuLVs) are not known to possess any structural genes similar to *tax* or *tat*. The induction of certain cellular gene products after infection with Moloney MuLV (M-MuLV) results from the long terminal repeat (LTR)-encoded transactivator (*let*) activity (8). We have defined a novel transcript arising within the LTR sequence, and its presence correlates with the transactivational activity (7). The U3 region of the LTR contains elements essential for *let* activity and is known to be a determinant of latency and disease specificity of the virus (4, 27). We have previously reported that M-MuLV is capable of up-regulating the transcription of class I major histocompatibility antigen complex (MHC) genes and β_2 -microglobulin genes in murine fibroblasts (14, 44) and other members of the immunoglobulin gene superfamily in human T lymphocytes (24). These proteins, including the MHC class I antigens, have been reported to function as T-cell activation molecules (5, 20, 24). Both endogenous and transiently transfected MHC genes are upregulated in both human or mouse cells expressing murine

retroviruses, indicating that the transactivation occurs regardless of the location of responsive genes.

The expression and modulation of MHC class I gene expression in cells plays a crucial role in the ability of these proteins to present antigen or otherwise interact with the cellular immune system. The regulation of MHC class I gene expression results from the specific binding of factors within *cis*-acting DNA sequences 5' to the coding region, and this transcriptional regulation may be the most important determinant of the level of cell surface MHC antigen expression (2, 22, 29, 40). Determination of the retrovirus-responsive *cis*-acting MHC promoter elements and their interactions with DNA-binding proteins is an essential first step toward understanding the mechanisms whereby retroviruses induce transcriptional up-regulation of the MHC genes. In this study, we have dissected the *H-2K^b* promoter and identified a *cis*-acting element that is involved in mediating transactivation by the virus. This retrovirus-responsive *cis*-acting element appears to be a novel regulatory site and can be distinguished from other highly conserved sequences in the class I MHC promoter, termed enhancer A, enhancer B, and interferon-responsive sequences (IRS) (29). Alteration of this *cis*-acting element by point mutations abrogated transactivation in transient assays, coincident with a loss of protein-DNA binding activity to this element in electrophoretic mobility shift analyses (EMSAs). This novel *cis*-acting *H-2K^b* gene sequence element conferred responsiveness to the MuLV LTR when attached to a heterologous promoter. Transient transfection of such chimeric genes into mouse fibroblasts or human T cells expressing murine retrovirus stimulated transcription of the reporter gene. MuLV-responsive gene regulatory elements like the one described herein may relate to the ability of these retroviruses to cause

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leukemias and lymphomas and possibly to confer virulence in infected host cells.

MATERIALS AND METHODS

Cell and cultures. BALB/c-3T3 and NIH 3T3 fibroblasts were obtained through the American Type Culture Collection (Rockville, Md.). Mo-BALB, a chronically Mo-MuLV-infected cell line, was described previously (44). All cell lines were maintained in Dulbecco's modified Eagle medium supplemented with 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, and antibiotics (GIBCO Laboratories, Grand Island, N.Y.).

Retroviral vectors and *H-2K^b* promoter mutant constructions. The proviral clone pMoV-9 was described previously (9, 11). The pZIP-NEO-SV(X) vector was provided by C. Cepko (6). The vectors pML4 and pMNC-stuffer, containing the 3' LTR alone and both 5' and 3' Mo-MuLV LTRs, respectively, have been described elsewhere (7). The construction of *H-2K^b* promoter deletions has been previously described (24). The 5' deletion mutant plasmids pK^bEN-CAT, pK^bHN-CAT, pK^bPvN-CAT, pK^bPsN-CAT, and pK^bCore contain 4.2 kb, 2.1 kb, 1.2 kb, 650 bp, and 64 bp, respectively, of promoter sequences in a promoterless chloramphenicol acetyltransferase (CAT) expression vector. pK^bCore derivatives with base substitutions were constructed by replacing the *Bam*HI-*Sac*II fragment (−64 to −35) of the *H-2K^b* promoter sequence with double-stranded oligonucleotides containing the desired point mutations. For heterologous promoter analysis of *cis*-acting regulatory elements 5' to the TATA box of the *H-2K^b* gene, point mutations were introduced into the sequences at −64 to −35 of the *H-2K^b* promoter. *Xba*I linkers were attached, and the mutated promoters were subcloned into the *Xba*I site of plasmid pJE-38 CAT (42). All mutations generated were verified by restriction enzyme digestion and DNA sequencing.

Transfection and CAT assay. Transient transfections were accomplished by using either the calcium phosphate method (44) or the DEAE-dextran procedure (30), with a modification. In all of the cotransfection experiments, 5 µg each of CAT reporter plasmid and retroviral vector was used for a transfection in a 100-mm-diameter dish. The cell extracts were harvested 40 to 48 h after transfection. The CAT activities were measured as described previously (19). To control for transfection efficiency, the plasmid pRSVβ-Gal was included in transient transfections. Extracts from cells cotransfected with pRSVβ-Gal construct (0.5 µg per dish) were prepared as described previously (41) and assayed for β-galactosidase activity on a cytofluorometer.

Preparation of nuclear extracts and EMSAs. Nuclear extracts of BALB/c and Mo-BALB cells were prepared essentially as described previously (1). The protein concentration of nuclear extracts was determined by the Bio-Rad protein assay. All nuclear extracts recovered were quickly frozen in aliquots and stored at −70°C. In EMSAs, the 30-bp (*Bam*HI-*Sac*II) and 113-bp (*Hind*II-*Hind*III) fragments were isolated from the pK^bCore or mutant constructions and end labeled either with [α -³²P]dATP and the Klenow fragment of DNA polymerase I or with [γ -³²P]ATP and T4 polynucleotide kinase. Binding reaction mixtures were preincubated for 10 min on ice in a total volume of 15 µl containing 10 mM HEPES (pH 7.8), 50 mM potassium glutamate, 5 mM MgCl₂, 1 mM dithiothreitol, 5% glycerol, 0.5 µg of poly(dI-dC), and 3 to 6 µg of nuclear extracts. After addition of radiolabeled DNA (5 × 10³ cpm), the reaction mixtures were incubated for an additional 30 min on ice, then loaded onto 5% polyacrylamide nondenaturing gel, and subjected to electrophoresis at 200 V in 0.5× Tris borate-EDTA buffer at 4°C. For the competition studies, unlabeled competitor DNAs were preincubated together with the nuclear extract in the binding reactions prior to the addition of labeled DNA probes. Double-stranded synthetic oligonucleotides containing three copies of AP-1-binding consensus sequences or DNA fragments containing *H-2K^b* promoter were used as specific competitors. The oligonucleotide (CFM1) used as a nonspecific competitor was 5'-CTTGA CAAGGCAACTTGACCAAGAGTCTG-3'. For quantitation, the shifted bands and the free probe were cut from the gel, and the radioactivity was quantitated by liquid scintillation counting.

DNase I footprinting assay. The binding reaction mixtures for DNase I footprint analysis were incubated for 25 min on ice in 50 µl of buffer containing 10 mM HEPES (pH 7.8), 50 mM potassium glutamate, 5 mM MgCl₂, 1 mM dithiothreitol, 5% glycerol, 2% polyvinyl alcohol, 2.5 µg of poly(dI-dC), 2.5 × 10⁴ cpm of DNA probe, and 50 µg of nuclear extract. After addition of 5 mM MgCl₂ and 5 mM CaCl₂, the reactions were treated with DNase I to a final concentration of 0.1 mg/ml for 1 min on ice and then terminated with the addition of 20 mM EDTA, 1% sodium dodecyl sulfate, and 250 µg of RNA per ml. Reaction mixtures were extracted with equal volumes of phenol-chloroform and precipitated with ethanol. Reaction products were separated on a standard sequencing gel.

RESULTS

Transcriptional activation of the *H-2K^b* gene by Mo-MuLV sequences. To determine whether the upregulation of class I MHC antigen expression and transcript expression as a result of Mo-MuLV infection was the result of a transactivation process, the effects of the virus or subgenomic fragments of the

FIG. 1. Transient expression of MHC promoter-CAT chimeric genes after cotransfection with Mo-MuLV vectors. BALB cells were cotransfected with the LTR-expressing vector pML4 (+) or a control plasmid (−) plus individual MHC-CAT reporter vectors, including pK^bEN-CAT (lanes 1 and 2), pK^bHN-CAT (lanes 3 and 4), pK^bPsN-CAT (lanes 5 and 6), and pK^bCore (lanes 7 and 8), containing 4.2 kb, 2.1 kb, 650 bp, and 64 bp, respectively, of the *H-2K^b* gene promoter. Each plate received 5 µg of CAT reporter plasmid and 5 µg of pML4 (lanes 2, 4, 6, and 8) or pBR322 (lanes 1, 3, 5, and 7). At 48 h posttransfection, cells were harvested and prepared for CAT activity assay as described in Materials and Methods. An autoradiogram of the separated reaction products is shown. Transfection efficiencies, as assessed by quantitation of the product of the cotransfected pRSVβ-Gal, were similar for all samples.

virus on the levels of exogenous or transiently introduced (exogenous) *H-2K^b* gene expression have been monitored. A previous report demonstrated that expression of chimeric genes consisting of different lengths of the *H-2K^b* gene promoter sequence linked to the reporter gene CAT was stimulated when they were transiently transfected into human T cells infected with Mo-MuLV (24). Plasmid pK^bEN-CAT, which included 4.2 kb of upstream promoter sequences, contained the sequences essential for effective promoter activity. Nested deletions of this chimeric CAT expression vector were performed to localize the essential element responsible for transactivation. Transient expression of these CAT vectors in cells containing the infectious Mo-MuLV revealed that the proximal 64 bp of the promoter retained the ability to respond to Mo-MuLV transactivation. More recently, the LTR of Mo-MuLV has been shown to encode a transactivational activity (8). The ability of the LTR alone to transactivate MHC-CAT expression vectors was examined. The vector pML4, which contains an Mo-MuLV LTR in a pBR322 plasmid backbone (8), was cotransfected into BALB/c-3T3 cells with serially truncated *H-2K^b* promoter constructs, and cytoplasmic CAT activity was assayed 40 to 48 h after transfection. A consistent 20- to 50-fold increase in CAT enzyme activity was observed in the cells cotransfected with the pML4 plasmid in comparison with those cells receiving plasmid pBR322 as a control (Fig. 1). As reported previously for assays using different reporter genes (8, 43), the same relative magnitude of transactivation was found in cotransfections with constructs containing the entire Mo-MuLV genome (pMoV9) or a deleted retroviral construction containing two LTRs [pZip-NEO-SV(X) or pMNC-stuffer] (data not shown). These results suggested that the MuLV LTR sequence alone was sufficient to influence the transcription of the *H-2K^b* gene and that the responsive sequences of the MHC gene lie within the 64 bp upstream of the transcriptional start site of the *H-2K^b* gene.

Identification of nuclear protein binding sites in the *H-2K^b* promoter. The reporter gene assays localized an LTR-responsive *cis*-acting sequence to within 64 bp of the start site of the gene. An analysis of this sequence by using a regulatory transcription factor database (16, 17, 35) revealed no known transcription factor-binding motifs within this region, with the exception of the TATA- and CAAT-factor-binding (CBF)

FIG. 2. EMSAs using the fragment containing the sequence from -64 to -35 bp of the *H-2K^b* promoter. Radiolabeled DNA was incubated with extracts from BALB (B; lanes 1 and 2) and Mo-BALB (M; lanes 3 to 6) cells. Three micrograms of nuclear extract was used in lanes 1 and 3, and 6 μg of nuclear extract was used in the other lanes. A 100-fold molar excess of the same unlabeled promoter fragment as used for the probe was added in lane 5. The unlabeled nonspecific oligonucleotide CFM1 was used at a 100-fold molar excess in lane 6. The figure is an autoradiogram.

elements. To investigate nuclear factor binding to this region, nuclear protein extracts were prepared from infected and non-infected cells and used in EMSAs. A 30-bp *Bam*HI-*Sac*II DNA fragment including nucleotides -64 to -35 was isolated from pK^bCore and used as a probe. Figure 2 shows the results of EMSA using this probe with nuclear extracts from uninfected BALB cells or Mo-BALB cells. Nuclear extracts from both infected and uninfected cells contained binding activity, but the magnitude of the binding was substantially greater in extracts from infected cells (an average from multiple experiments of 8.6-fold-greater binding). Competition experiments demonstrated complete competition of this binding activity from infected cells when a molar excess of the unlabeled 30-bp fragment was used, but a similar molar excess of the nonspecific fragment CFM1 (see Materials and Methods) did not compete the labeled complex. EMSAs also demonstrated quantitative increases in nuclear protein binding to this fragment when extracts from BALB cells stably expressing an LTR alone (7) were used as a source of nuclear extract, in comparison to control-transfected cells (data not shown).

To more precisely delineate the DNA-protein interactions, DNase I footprinting analysis was used (Fig. 3). A 113-bp *H-2K^b* DNA fragment (-100 to +12 bp, with respect to the start site), uniquely labeled at the 5' end, was used. No protection of the DNA sequence was observed in the absence of nuclear protein (lane 1). Nuclear proteins from BALB and Mo-BALB cells bound to, and partially protected, the upstream promoter region adjacent to the CAAT box on the antisense strand. Proteins in extracts from Mo-BALB cells interacted more strongly with 13 nucleotides centered around position -54 (5'-CACCAAGAACCAA-3'), which has a symmetric structure. This protected site appears to be a novel DNA protein-binding motif, not previously identified as a binding site for nuclear proteins. Within the region from -64 to -35 bp, the CAAT box element represented the only previously recognized protein-DNA binding site. Nuclear extracts from Mo-BALB cells protected the CBF binding site (CCAAT), and its flanking regions, slightly more effectively than extracts from uninfected BALB cells, but neither type of nuclear extract protected completely. The bracketed region in

FIG. 3. DNase I footprint analysis of the proximal *H-2K^b* promoter region. The analysis of the noncoding strand is shown. The end-labeled 112-bp DNA promoter fragment was incubated without extract (lane N), with 50 μg of extracts from BALB cells (lanes B), or with 50 μg of extracts from Mo-BALB cells (lanes Mo). Lane G contains a G-specific sequencing reaction. The figure is an autoradiogram of a sequencing gel. A footprint over the distal promoter element is evident in the lanes containing extracts from Mo-MuLV-infected cells. The protected sequences would be read as 5'-CACCAAGAACCAATC-3' on the sense strand and contain the CATAT box and an adjacent direct repeat structure.

Fig. 3 was invariably protected relative to the control in repeated experiments using independently generated nuclear extracts. Another promoter region, covering the area from -73 to -84 bp, was also protected by extracts from virus- or LTR-expressing cells. Because this protected region lies upstream of the core MHC promoter sequence that we have shown to be required and sufficient for response to the LTR (-64 bp), this distal region was not studied further.

Site-directed mutagenesis of the nuclear protein-binding motif abolishes DNA-protein binding and transactivation. Point mutations were introduced in the putative *cis*-acting regulatory elements of the *H-2K^b* promoter (-58 to -47) to assess their contributions to transactivation activity by Mo-MuLV. These mutated promoter elements, driving a CAT reporter gene, were transiently transfected into BALB cells together with the LTR-expressing plasmid pML4 or the control plasmid pBR322, and the cell lysates were analyzed for CAT activity. A mutation in the CAAT box (pK^bCoCM5) diminished basal promoter activity somewhat in comparison to the wild-type promoter vector (pK^bCore), but the relative inducibility of the promoter by the LTR was unchanged (Fig. 4A). This 1-bp substitution was sufficient to interfere with binding of the CBF (data not shown). A 2-bp substitution further upstream in the CAAT box (pK^bCoACM) reduced the magnitude of the transcriptional induction by Mo-MuLV, but transactivation could still be demonstrated. Thus, the CAAT

A		
Plasmid	Sequence	Fold induction
	-60 -45	
pK ^b Core	5' ATCACCAAGAACCAATCAGT 3'	23 ± 3.1
pK ^b CoSM578	AG.....	22 ± 3.5
pK ^b CoSM556	TC.....	14 ± 2.4
pK ^b CoSM545	CA.....	8.6 ± 2.2
pK ^b CoAM	AC.....	1.8 ± 0.5
pK ^b CoACM	GC.....	17 ± 2.5
pK ^b CoCM5	G.....	20 ± 4.3

FIG. 4. Functional analysis of the pK^bCore promoter and mutated derivatives. (A) Summation of transactivational activity. The nucleotide sequences of the wild-type -62 to -43 bp promoter region of pK^bCore and mutant promoters are shown. All sequences shown are on the coding strand. The wild-type and various promoter mutant reporter genes indicated with the replaced nucleotides were cotransfected with plasmid pML4 or pBR322 and with pRSVβ-Gal, and the activities of CAT and β-galactosidase were determined and quantitated as described in Materials and Methods. The specific CAT activity data shown are the means ± standard errors of the means of at least three independent experiments and are expressed as levels of CAT activity enhanced by the cotransfected LTR relative to that induced by the cotransfected (control) plasmid pBR322. (B) Disruption of the retrovirus-responsive *cis*-acting promoter elements. Equal amounts of pK^bCore (lanes 1 and 2) or pK^bCoAM (lanes 3 and 4) were used as CAT reporter vector. Cotransfections with pML4 or pBR322 (as a control) and CAT activity assays were performed as described in Materials and Methods. An autoradiograph of the thin-layer chromatography plate is shown. Upper spots indicate the 3-acetyl chloramphenicol reaction product. For quantitation, the spots were cut from the plate and counted by liquid scintillation counting to generate the data presented in panel A.

box appeared to govern the level of transcription but was not sufficient or required for MHC gene transactivation. However, the substitution of two nucleotides immediately upstream of the CAAT box (pK^bCoAM) abolished almost all transactivation by the LTR (Fig. 4). Although the basal activity of the mutant promoter was about one-half of that of wild-type promoter pK^bCore, the relative loss of responsiveness to transactivation by the LTR was significantly more severe (at least 10-fold). One of the nucleotides mutated in pK^bCoAM, nucleotide -54, lies in the center of the direct repeat sequence extending from -59 to -49 (5'-ACCAAGAACCA-3'). Mutation of nucleotides adjacent to nucleotide -54 (pK^bCoSM545 and pK^bCoSM556) led to consistent and statistically significant 1.8- to 3.5-fold decreases in the magnitude of transactivation by the LTR. When the sequence was mutated further upstream (pK^bCoSM578), however, no effect on transactivation of the promoter was observed. Thus, the more proximal half of the palindromic sequence (adjacent to the CAAT box) appeared to be less sensitive to effects from nucleotide mutations or substitutions than did the more distal half. These results suggested that *H-2K^b* promoter transactivational activity in re-

FIG. 5. Effects of point mutations in the *cis*-acting promoter element on nuclear protein binding. Oligonucleotides of wild-type (W) promoter sequence from -64 to -35 bp of pK^bCore (lanes 1 to 7) or mutant (A) promoter sequence of pK^bCoAM (lanes 8 and 9) were labeled with [³²P]ATP and incubated with 6 μg of nuclear extracts from BALB (B) or Mo-BALB (M) cells as indicated. As competitors, 100-fold molar excesses of either unlabeled wild-type or mutant oligonucleotide were incubated for 20 min with the nuclear extracts before the addition of the labeled probe.

sponse to Mo-MuLV requires a *cis*-acting element centered at approximately -54 bp.

To determine if the loss of ability of the mutated promoter elements to mediate transactivation correlated with changes in DNA-binding protein activity, EMSAs were performed with oligonucleotides containing the mutated sequences, either as labeled probes or as competitors. Labeled double-stranded oligonucleotides containing sequences from -64 to -35 bp of the wild-type pK^bCore (called pK^bCo) showed prominent nuclear protein-binding complex formation in EMSA using extracts from Mo-MuLV-infected cells (approximately 45% of the probe was complexed when 8 μg of nuclear extract was used), and some complexes were also generated by extracts from uninfected cells (approximately 30% of the probe was complexed with 8 μg of nuclear extract) (Fig. 5, lanes 2 and 3). A two-base substitution in the middle of the direct repeat sequence (pK^bCoAM) sharply decreased the formation of nuclear protein complexes (lanes 8 and 9), with an average of only 7 and 15% of the mutant probe complexed by extracts from uninfected and infected cells, respectively. These decreases in DNA-protein binding thus correlated well with the functional effects observed with the mutant promoter sequence. Competition experiments also generated consistent results. Whereas a molar excess of unlabeled wild-type oligonucleotide effectively competed complex formation with a labeled wild-type probe (lanes 4 and 5), the unlabeled mutated oligonucleotide did not effectively compete (less than 50% competition) (lanes 6 and 7). EMSAs carried out with promoter fragments of the other promoter mutants, such as pK^bCoSM578 and pCoACM, which did not interfere with transactivation by the LTR, showed similar patterns and magnitude of DNA-nuclear protein complex formation as the wild-type promoter fragment (data not shown).

This MHC gene promoter element is sufficient to confer transactivational responsiveness. The wild-type or mutated *H-2K^b* gene regulatory sequences from -64 to -35 bp were used to generate chimeric gene constructs using heterologous

TABLE 1. *H-2K^b* promoter sequences transferred to a heterologous promoter confer the ability to respond to retroviral transactivation

Plasmid	Promoter-CAT vector ^a		Fold induction ^b
	Minimal promoter	Inserted sequence	
pJE-38 CAT	JE -38/+10		1.0 ± 0.3
pK ^b Co/JE CAT	JE -38/+10	<i>H-2K^b</i> -65/+35	7.2 ± 1.5
pK ^b CoA/JE CAT	JE -38/+10	<i>H-2K^b</i> -65/+35 mutant	1.7 ± 0.4

^a For the construction of the vectors, complementary oligonucleotides containing the sequences from -60 to -35 bp of pK^bCore and pK^bCoAM were annealed and inserted into the *Xba*I site of pJE-38 CAT, upstream of position -38, yielding the fusion gene vectors pK^bCo/JE and pK^bCoA/JE CAT.

^b To measure the inducibility of the fusion gene promoters, each vector was cotransfected together with pML4 into BALB/c-3T3 cells as described in Materials and Methods. The relative (fold) increases in CAT activity were determined as described in the legend to Fig. 4.

promoters. The minimal promoter utilized was derived from the rat JE/MCP-1 gene and comprised 38 bp of sequence adjacent to the transcriptional start site of the gene (called JE-CAT). The wild-type and mutant oligonucleotides used in the EMSA studies (pK^bCo and pK^bCoAM, respectively) were ligated upstream of this promoter to generate pK^bCo/JE and pK^bCoA/JE. These plasmids were cotransfected with an LTR (pML4), or with pBR322 as a control, into BALB cells, and the resultant CAT activity was measured (Table 1). The pJE-38 CAT construct did not demonstrate any transactivation by the LTR (consistent with our previous reports (43)). However, the presence of the wild-type -64 to -35 *H-2K^b* promoter sequences in pK^bCo/JE conferred the ability to respond to the LTR transactivational activity. Mutation of the DNA-protein binding motif in vector pK^bCoA/JE abrogated the ability of the chimeric promoter to respond to the LTR. Thus, the -64 to -35 sequence of the MHC gene promoter is both necessary and sufficient for the transactivational response to MuLVs.

DISCUSSION

Regulation of class I MHC protein or gene expression is a well-recognized strategy used by oncogenic viruses or oncogenes in their complex interactions with the host cellular immune system (31, 33, 34, 38, 39). Abnormal regulation (induction) of MHC genes has been observed in tumors generated in diverse ways, including chemically transformed cells, radiation-induced tumor cells, and spontaneously arising leukemias in high-incidence mouse strains (18; for a review, see reference 10). Attempts have been made to correlate changes in MHC expression with the metastatic or tumorigenic potential of the transformed cell (13). We have shown here, and in previous studies (15, 44), that cells increase their expression of class I MHC proteins and genes after infection with Mo-MuLV, and this upregulation occurs by transactivation of the responsive genes rather than by sequestration of putative transcriptional repressors by sequences within the viral genome (7, 8, 43). Previous studies have shown that a 639-bp sequence of the MHC gene promoter contains enhancer sequences responsive to Mo-MuLV activation and that shortening the sequence to 64 bp decreases somewhat the level of basal gene expression but does not alter the magnitude of the relative induction by Mo-MuLV or the viral LTR alone (24). In this study, we have mapped and characterized a *cis*-regulatory element which is essential for the transactivational response to the retrovirus, and which we designate the *let* response element (LRE), to a 12-bp region spanning -58 to -47 of the *H-2K^b* gene promoter. It is likely from deletional analysis that the region up-

stream of this element to -650 bp must also contain at least one positive regulatory element which affects the absolute level of basal and inducible expression.

Interferons are capable of inducing class I MHC expression on mesenchymal cells (26), and endogenous cellular beta interferon can be elaborated by mesenchymal cells in response to infection by certain viruses. Interferon has been ruled out as a mediator of the MHC response to MuLVs, as MuLV-infected cells do not produce detectable amounts of beta interferon and do not exhibit an interferon-induced antiviral state (14, 44). Furthermore, the IRS in the class I gene promoter lies approximately -165 to -137 bp relative to the cap site in *H-2K^b* (23), and a minimal MHC promoter ending at -64 bp, deleting the IRS, remained responsive to the retroviral LTR. MuLV has been shown to chronically induce NF-κB DNA-binding activity in infected cells (32). The MHC promoter is known to have an NF-κB-binding site and to be responsive to stimuli that increase NF-κB-binding activity (2, 3, 21, 28, 37). This NF-κB-binding site lies between 190 and 138 bp upstream of the cap site and so can also be eliminated as the element responsible for transactivation of MHC gene by MuLV. We have previously reported an induction of AP-1 complex DNA-binding activity consisting of c-Jun and c-Fos in cells expressing an MuLV genome or isolated LTR or a U3/R LTR fragment, with coincident induction of certain AP-1-inducible genes and their encoded proteins, including *c-jun* and the *collagenase IV* gene (10a, 43). The *H-2K^b* MHC promoter region studied herein contains no recognizable AP-1-binding motif. Furthermore, treatment of fibroblast cells with phorbol esters does not induce transcription of endogenous or transfected class I MHC genes (6a), making AP-1 unlikely to mediate any of the virus-induced transactivation we observe.

Using DNA fragments containing the *cis*-acting LRE identified in this report, we could detect at least one nuclear protein in EMSA using cell extracts from both uninfected and infected cells. DNase I footprinting analysis demonstrated that nuclear proteins from Mo-MuLV-infected cells protected the 12-bp LRE, and more particularly the internal AAGAA core motif, more efficiently than did nuclear proteins from uninfected cells. The well-characterized basal promoter element, the CAAT box, is the known *cis*-acting element lying closest to the LRE (28, 37), and we observed protein binding to this element in footprinting assays and also in EMSA (unpublished data). Functional mutational analysis of the role of the CAAT box in the retroviral transactivational process showed that mutations in the CAAT box element which abolished CBF binding to the promoter did not significantly abrogate the ability of the promoter to respond to the retrovirus. Thus, the CBF is unlikely to mediate or modulate the transactivation process.

It may be noteworthy that there is some conservation of the LRE site between human and murine genes, with both HLA-A2 and HLA-B7 containing the sequence ...AGAA(G)CCA at ... at homologous regions in their proximal promoters. Chimeric reporter genes driven by the HLA-B7 promoter are transactivated by murine and human retroviral LTRs (8a). The fact that the LRE DNA-binding activity was present in both infected and uninfected cells, both of which express class I MHC antigens and genes, albeit at different levels, suggests that this factor may play a role in basal expression of MHC genes as well as in virus-stimulated expression. The decrease in basal expression that we observe during transient expression assays when this element is mutated is consistent with such a speculation.

In summary, the LTR transactivator *let* appears to require certain conserved *cis*-acting elements for the specific induction of cellular genes, including the LRE-binding motif identified

here or the tetradecanoylphorbol ester-response element. The precise molecular mechanism(s) by which the *let* gene and its novel transcript can regulate gene expression through these promoter elements and their cognate DNA-binding proteins remains to be determined. The *let* gene is unlikely to encode a functional protein product and may operate through RNA-protein complexes (8). We have found that the LTRs of the human retroviruses human immunodeficiency virus and human T-cell lymphotropic virus encode transcripts and transactivational activities similar to those of the murine retroviruses, suggesting that this mechanism of gene regulation by viruses may be generalizable (8b).

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