Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 3145–3149, April 1991
Developmental Biology

Negative regulation of the major histocompatibility complex class I promoter in embryonal carcinoma cells

(DNA-binding protein)

JAMES R. FLANAGAN*†, MASAHIRO MURATA‡§, PETER A. BURKE*¶, YASUAKI SHIRAYOSHI*‖, ETTORE APPELLA**, PHILLIP A. SHARP‡, AND KEIKO OZATO*††

*Laboratory of Developmental and Molecular Immunity, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892; ‡Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139; and **Laboratory of Cell Biology, National Cancer Institute, Bethesda, MD 20892

Contributed by Phillip A. Sharp, January 10, 1991

ABSTRACT **Transcription of major histocompatibility complex (MHC) class I genes is negatively regulated in undifferentiated F9 mouse embryonal carcinoma cells via the conserved upstream regulatory region. This region contains constitutive enhancers and an inducible enhancer, the interferon consensus sequence (ICS), that is responsible for interferon-induced transcription. A series of mutations in the ICS, but not in the enhancer elements, resulted in an increase in expression of the MHC class I promoter in F9 cells. However, these ICS mutants did not increase promoter activity in F9 cells differentiated after retinoic acid treatment. Results of mobility-shift DNA-binding assays and methylation interference experiments showed that undifferentiated F9 cells contained a factor(s) that bound to a sequence within the 5′ and central part of the ICS. This binding site, termed the MHC negative regulatory element (NRE), coincided with the site of mutations that increased promoter activity in F9 cells and was distinct from the element to which interferon-response factors bind. The factor(s) that binds to the MHC NRE was not detected in differentiated F9 cells treated with retinoic acid or in other cells expressing MHC class I genes. Finally, introduction of concatenated, double-stranded NRE oligomers, but not oligomers of unrelated sequences, into F9 cells abolished negative regulation of the MHC class I promoter activity, providing evidence that the NRE binding factor is responsible for repression of the MHC class I genes in F9 cells.**

Embryonal carcinoma (EC) cells resemble early mammalian embryos in many aspects (1). In the undifferentiated state, these cells express high levels of c-*myc* gene product, heat shock proteins, and a surface antigen that is embryo-specific. One of the most distinctive characteristics of undifferentiated EC cells is their capacity to repress transcription of various genes. A number of viral genes including those of polyoma virus, simian virus 40, and murine leukemia virus have been shown to be transcriptionally repressed (2–4) due to negative regulatory mechanisms (5–8). The sequences specifying such repression have been extensively studied (8–11). The presence of a nuclear factor in EC cells that may be involved in negative regulation has been reported for murine leukemia virus (11, 12). In addition, the interferon β (IFN-β) gene is reported to be under negative control in F9 mouse EC cells, and this correlates with the presence of a factor that binds to a sequence upstream of the gene (13). Lenardo *et al.* (14) showed that F9 cells contain a factor, called NFA-3, that binds to the immunoglobulin (Ig) octamer sequence, which may negatively regulate the activity of the Ig κ enhancer. These reports suggest that there are multiple factors and mechanisms that govern repression of various cellular genes in EC cells. The repression seen in EC cells may reflect normal developmental processes, as early embryos are refractory to viral infection (15) and fail to express many cellular genes (1, 16, 17). When embryos develop, and when EC cells differentiate following retinoic acid treatment, negative regulation ceases. Concomitantly, a number of cellular genes are induced, including the Endo A and B (16), collagen type IV, plasminogen activator (17), IFN-β (13), and major histocompatibility complex (MHC) class I genes (18). Transcription of MHC class I genes is repressed in F9 cells via the conserved regulatory region (nucleotides −203 to −139 relative to the RNA initiation site) that contains the MHC class I enhancers and the IFN consensus sequence (ICS) (ref. 19 and Fig. 1). This repression ceases following differentiation, which parallels induction at low levels of endogenous MHC class I genes. A similar progression of events occurs in mouse embryos: at very early stages MHC class I gene expression is negligible, followed by expression at low levels at somite stage (20). In the present study the site of negative regulation is mapped to a sequence that resides within the ICS. Evidence for expression of a nuclear factor(s) in undifferentiated, but not in differentiated, F9 cells that binds specifically to the negative regulatory element (NRE) is presented, which indicates that transcriptional repression of MHC class I genes is mediated by binding of this factor to the NRE.

MATERIALS AND METHODS

Cells. F9, F9Tk⁻, P19, and PCC3 EC cells (21) were cultured in appropriate media (19, 21). To induce differentiation, cells were treated with 0.5 μM all-*trans*-retinoic acid (Eastman Kodak) for 3–7 days.

***H-2L^d* Constructs and Transfection.** Plasmids pL^{d}CAT1.4K and pL^{d}CAT123 (19) contain CAT coding sequences under control of the *H-2L^d* promoter (see Fig. 1). Mutant CAT constructs containing 2-base-pair (bp) substitutions in the enhancer elements and in the ICS (Fig. 1) were prepared by site-directed mutagenesis (22, 23). Transfection was per-

Abbreviations: CAT, chloramphenicol acetyltransferase; EC, embryonal carcinoma; IFN, interferon; ICS, IFN consensus sequence; IRE, IFN response element; MHC, major histocompatibility complex; NRE, negative regulatory element.

†Present address: Department of Internal Medicine, University of Iowa, School of Medicine, Iowa City, IA 52242.
§Present address: Central Research Laboratories, Ajinomoto Co., Inc., Kawasaki-ku, Kawasaki 210, Japan.
¶Present address: Department of Surgery, New England Deaconess Hospital, Deaconess-Harvard Surgical Service, Boston, MA 02115.
‖Present address: Meiji Institute of Health Science, 540 Naruda, Odawara 250, Japan.
††To whom reprint requests should be addressed at: Building 6, Room 2A01, Laboratory of Developmental and Molecular Immunity, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

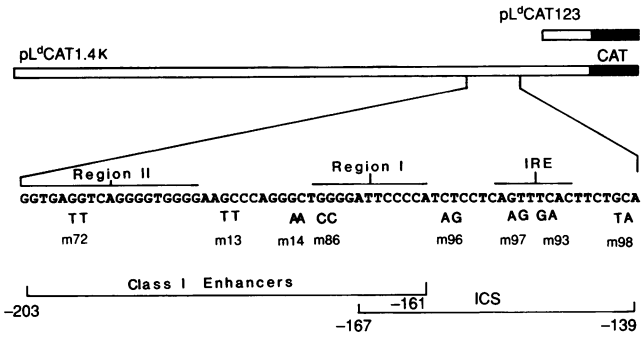

FIG. 1. Schematic representation of plasmids pL^{d}CAT1.4K and pL^{d}CAT123. The reporter gene is the bacterial chloramphenicol acetyltransferase (CAT) gene. The two conserved regulatory elements, MHC class I enhancers and ICS, are shown. The enhancer region has two elements, region I and region II, while the ICS contains the IFN response element (IRE). Nucleotide substitutions of mutants (m) produced from pL^{d}CAT1.4K are indicated.

formed by the calcium phosphate precipitation method with *N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (Bes) buffer (19). About 5×10^5 F9Tk$^-$ cells were incubated in a 100-mm plate with a mixture of DNA [a total of 20 μg containing CAT constructs, pCH110 (24), and, as carrier DNA, pUC] overnight, then washed and further incubated 24 hr before harvest for CAT assay (19). In some experiments carrier DNA was not used. At least three different preparations of plasmid DNA were tested for each CAT construct, and in some experiments the expression of β-galactosidase activity was used to normalize transfection efficiency. Within a single series of experiments, variability of transfection efficiency was <20%. For competition *in vivo*, double-stranded oligodeoxynucleotides were phosphorylated and concatenated by incubation with T4 kinase and ligase, respectively. This procedure yielded DNA of ≈1 kilobase (kb) (20- to 25-mers) (25). Concatenated oligomers were purified by phenol/chloroform extraction and ethanol precipitation before use in transfection.

Mobility-Shift DNA-Binding Assay and Methylation Interference Experiments. Nuclear extracts were prepared by the method of Dignam *et al.* (26) except that the protease inhibitors aprotinin and leupeptin were added at 1 μg/ml throughout. Electrophoresis of DNA–protein complexes was performed as described (27), using three probes: the NRE of the *H-2L^d* gene, a shorter probe corresponding to the NRE of the *H-2K^b* gene (Table 1), and a 90-bp probe containing both the enhancer elements and the ICS of the *H-2K^b* gene. These probes were prepared from plasmids harboring the relevant sequences. The NREs of the *L^d* and *K^b* genes differ at a single site, position −155 (Table 1). Extract protein (10 μg) was mixed with ^{32}P-labeled probes (≈10^4 cpm/ng) and poly(dI-dC) (2 μg; Pharmacia) in 10 μl of binding buffer [50 mM NaCl/10 mM Tris·HCl, pH 7.8/1 mM dithiothreitol/0.5 mM EDTA/10% (vol/vol) glycerol]. After incubation at room temperature for 20 min, this mixture was electrophoresed through a 4% polyacrylamide gel in Tris/borate buffer (pH 7.9). Mobility-shift assays were also performed using a different binding buffer, 10 mM Hepes, pH 7.4/1 mM $MgCl_2$/40 mM KCl (22, 23). Methylation interference experiments were performed using nuclear extracts from undifferentiated F9 cells (27).

RESULTS

Mapping of the NRE by Site-Directed Mutagenesis. A series of eight mutants containing 2-bp substitutions in the MHC enhancer and ICS was constructed by site-directed mutagenesis (Fig. 1; refs. 22 and 23). These mutants were generated from plasmid pL^{d}CAT1.4K, which has a 1.4-kb upstream region of *H-2L^d* positioned upstream of the CAT gene (19). Mutants m72, m13, m14, and m86 had substitutions in the enhancer elements (region I and region II) and their vicinity, while m96, m97, m93, and m98 had substitutions in the ICS (Fig. 1). The ICS region contains an IRE (Fig. 1) recognized by IFN response factors (22, 28–31). Mutant constructs were tested for activity by transfection into undifferentiated F9 cells. Two controls were tested in parallel, the parental plasmid pL^{d}CAT1.4K and pL^{d}CAT123, from which the enhancers and the ICS had been deleted (Fig. 1). Results are summarized in Table 2. In undifferentiated F9 cells the levels of CAT activity generated by pL^{d}CAT1.4K were lower than those generated by pL^{d}CAT123, as expected: the region from −203 to −139, encompassing the MHC enhancers and ICS, was previously found to mediate negative regulation of the MHC class I promoter in F9 cells (19). The opposite relationship prevails in fibroblasts, in which region I and region II act as functional enhancers (23, 32, 33). All four mutants in the enhancer region (m72, m13, m14, and m86) generated

Table 1. NRE oligonucleotides and summary of factor binding

Name	Sequence	Competition*
	−175 • −137	
NRE (*L^d*)	GCTGGGGATTCCCCATCTCCTCAGTTTCACTTCTGCACC	+
	CGACCCCTAAGGGGTAGAGGAGTCAAAGTGAAGACGTGG	
	• • • •	
	−169 −137	
ICS m232	GATTCATAATCTCCTCAGTTTCACTTCTGCACC	+
ICS m234	GATTCCCCGGATCCTCAGTTTCACTTCTGCACC	−
ICS m238	GATTCCCCATCTCCTCCTGTTCACTTCTGCACC	−
ICS m228	GATTCCCCATCTCCTCAGTTTTGATTCTGCACC	−
ICS m242	GATTCCCCATCTCCTCAGTTTCACGCATGCACC	+
	−161 −147	
Short NRE (*K^b*)	ATCTCCACAGTTTCA	+
Mutant short NRE	ATCTACAGAGTTTAA	−
	−176 −158	
Region I	GGCTGGGGATTCCCCAGGCTGGGGATTCCCCATCT	−
lac operator	GGTGGAATTGTGAGCGGATAACAATTCCATGCATG	−

Only the upper strands (except for the NRE) are shown. The NRE used for competition *in vivo* had linkers at the 5′ and 3′ ends (GATCT and GCATG). Bases different from the parental type are underlined. The guanine residues showing interference (Fig. 4) are marked (•). The proposed NRE factor binding site is boxed.

*Summary of results in Fig. 3*A*.

Table 2. Activity of mutant pL^{d}CAT1.4K in F9 cells

CAT construct	Relative activity (*P* value)		
	− RA	+ RA	*n**
pL^{d}CAT1.4K	1.0	1.0	
pL^{d}CAT123	2.5 (<0.03)	1.2 (<0.05)	5
m72	1.5 (NS)		4
m13	1.5 (NS)		4
m14	1.4 (NS)		4
m86	1.2 (NS)		4
m96	4.4 (<0.05)	1.1 (<0.05)	4
m97	2.5 (<0.03)	1.1 (<0.06)	4
m93	6.7 (<0.06)	0.95 (<0.06)	4
m98	2.8 (<0.06)	0.1 (<0.06)	4

F9 cells before (−RA) and after (+RA) retinoic acid treatment (0.5 μM, 7 days) were transfected with pL^{d}CAT constructs. CAT activity was normalized by β-galactosidase activity and is expressed relative to the pL^{d}CAT1.4K value. Significance (*P*) was assessed by a nonparametric statistics signs test. NS, not significantly different from pL^{d}CAT1.4K.
*Number of experiments.

low CAT activity, comparable to the activity from pL^{d}CAT1.4K. In contrast, CAT activity generated by the ICS mutants was markedly higher than that from pL^{d}CAT1.4K (Table 2). The highest levels of activity were produced by m96 and m93, which exceeded those produced by pL^{d}CAT123. CAT activity produced by m97 and m98 was lower than that produced by m96 and m93, even though the m97 mutation is in the region immediately flanking the mutations in m96 and m93. These data suggest that MHC class I genes are negatively regulated in F9 cells by a sequence within the ICS. In fibroblasts, however, the levels of CAT activity from these ICS mutants are comparable to the levels generated by pL^{d}CAT1.4K (22, 25). These mutants were also tested by transfection into differentiated F9 cells after treatment with retinoic acid (Table 2). In these cells the parental plasmid pL^{d}CAT1.4K and the deletion mutant pL^{d}CAT123 gave similar levels of activity, indicating that negative regulation as mediated by this region is not functional after differentiation (19). Consistent with this result, all ICS mutants generated CAT activity comparable to that of pL^{d}CAT1.4K in retinoic acid-treated F9 cells. Thus, mutations in the ICS region increase CAT activity only in undifferentiated F9 cells.

Induction of MHC Class I Promoter Activity by Competition *in Vivo*. The first indication that the negative factor might be present at a limiting concentration was that the relative CAT activity of pL^{d}CAT1.4K and pL^{d}CAT123 depended on the amounts of DNA transfected into F9 cells. When F9 cells were transfected with <10 μg of plasmid, the activity produced by pL^{d}CAT123 was higher than that from pL^{d}CAT1.4K. However, when 15 μg of plasmid was transfected, the levels of CAT activity from pL^{d}CAT1.4K became slightly higher than that from pL^{d}CAT123 (data not shown). A similar effect has been reported with the polyoma virus enhancer in F9 cells (6). Oligomers of regulatory sequences have been shown to compete efficiently for the activity of a DNA-binding factor *in vivo* (25, 34). The oligomer corresponding to region I and the ICS, termed the NRE (Table 1) was concatenated, and transfected together with pL^{d}CAT1.4K into undifferentiated F9 cells. A clear increase in CAT activity was observed when increasing amounts of the NRE oligomer were transfected with pL^{d}CAT1.4K (Fig. 2). The levels of CAT activity generated by pL^{d}CAT1.4K were comparable to those generated by pL^{d}CAT123 when the amount of the NRE oligomer was increased to 1–2 μg. In contrast, oligomers for region I or the *lac* operator sequence (35) did not increase pL^{d}CAT1.4K activity (Fig. 2). The NRE oligomer had no effect on CAT activity when cointroduced

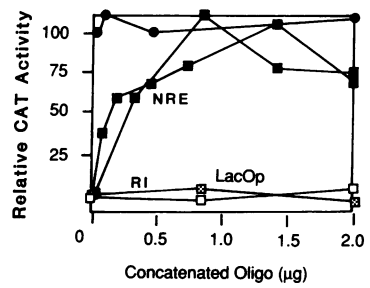

FIG. 2. Induction of expression of pL^{d}CAT1.4K by competition with the NRE oligomer. Concatenated double-stranded oligomer for the NRE (■), *lac* operator (Lac Op, □), or region I (RI, ⊠) was cotransfected with pL^{d}CAT1.4K (5 μg) into F9 cells. As a control, plasmid pL^{d}CAT123 and the NRE oligomer were cotransfected in parallel (●). CAT activity was normalized relative to the activity of 5 μg of pL^{d}CAT123 (100) and pL^{d}CAT1.4K (0) without oligomers. Percent chloramphenicol conversions for pL^{d}CAT123 were 0.3–0.6%, and for pL^{d}CAT1.4K 0.05–0.1%.

with pL^{d}CAT123. These results agree with the site-directed mutagenesis data and confirm that expression of the MHC class I promoter is negatively regulated by a titratable factor that binds the NRE. That the region I oligomer did not increase the levels of CAT activity from pL^{d}CAT1.4K indicates that the primary site of negative regulation resides within the ICS.

Binding of an F9-Specific Nuclear Factor(s) to the MHC NRE. The potential existence of a nuclear factor(s) that binds to the NRE in F9 cells was examined by mobility-shift assays using three probes: (*i*) the NRE sequence tested for competition *in vivo*, (*ii*) a short NRE sequence corresponding to the core region of the NRE derived from *H-2K^b* (Table 1), and (*iii*) a 90-bp fragment derived from the upstream region of *H-2K^b* and containing the enhancers and the ICS. The DNA sequences of *H-2K^b* and *H-2L^d* differ at a single site, position −155 (A vs. T) in the conserved regulatory region −203 to −139. With nuclear extracts from undifferentiated F9 cells, all three probes produced two prominent bands that migrated very slowly, marked N in Fig. 3*A*. In each case, formation of the bands N was efficiently inhibited by excess unlabeled NRE (data not shown) or short NRE (Fig. 3*A*) competitor. A mutant short NRE oligomer (Table 1) that contained three nucleotide substitutions failed to compete for bands N. The sequence specificity required for generating bands N was further evaluated by testing the ability of additional mutant NRE oligomers to compete (Table 1, Fig. 3*A*). Mutant oligomers m234, m238, and m228, which had three base changes in the central part of the NRE, failed to compete for bands N. On the other hand, oligomers m232 and m242, which had base changes in the 5′ or the 3′ end of the NRE, competed for bands N. Bands N were detected in extracts from both F9 and F9Tk$^-$ cells, as well as by using a different binding buffer for the mobility-shift assay (*Materials and Methods*) (data not shown). These results indicate that undifferentiated F9 cells contain a factor(s) that binds to the NRE and that the nucleotide difference at position −155 in *H-2L^d* and *H-2K^b* does not affect binding of the factor to the NRE. This binding site coincides with the sites of mutations in pL^{d}CAT1.4K, which generated increased levels of CAT activity in F9 cells (Table 2, Fig. 1). Bands N were not detected in extracts of differentiated F9 cells (Fig. 3*B*). Furthermore, extracts from two other EC cell lines, P19 and PCC3, generated a slowly migrating band equivalent to one of the N bands (Fig. 3*B*); the NRE oligomer competed for this band, but the mutant NRE m238 did not (data not shown). In contrast, extracts from mouse NIH 3T3 cells and mouse liver did not produce bands N.

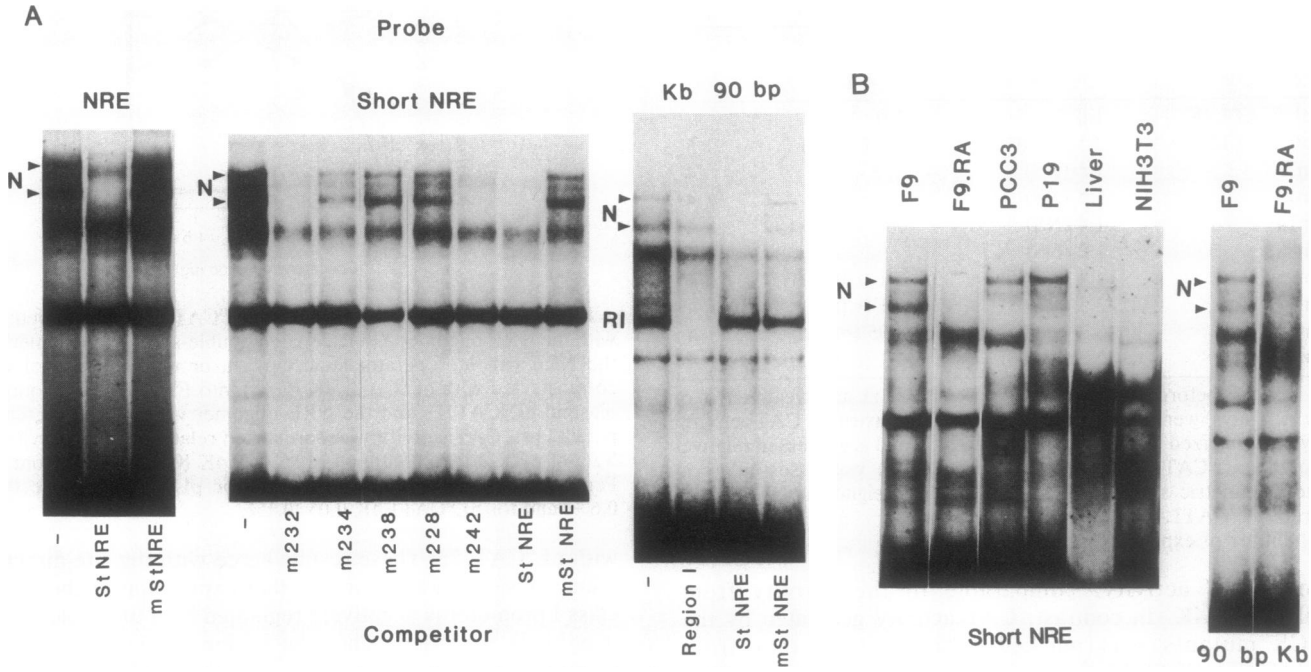

FIG. 3. A nuclear factor(s) expressed in F9 cells binds to the NRE. (*A*) Competition experiments. Nuclear extract proteins (10 μg) from undifferentiated F9 cells were tested by mobility-shift DNA-binding assay using ^{32}P-labeled NRE, the short NRE, or a 90-bp-long upstream fragment of the K^b gene as probes. Competitor oligomers (Table 1) were added at 100 times molar excess. −, No competitor; St NRE, short NRE; mSt NRE, mutant short NRE. (*B*) Cell-type specificity. Extract proteins from undifferentiated F9 cells, differentiated F9 cells (induced with retinoic acid, F9.RA), undifferentiated P19 and PCC3 cells, NIH 3T3 cells, and mouse liver were tested with the short NRE or the 90-bp K^b fragment as probes.

Methylation Interference Experiments. Methylation interference was used to determine the critical guanine residues involved in contacting the protein in bands N (Fig. 4). In the coding strand only the guanine at position −152 exhibited interference (see Fig. 4, Table 1). In the noncoding strand, modification of any of six guanines, at position −148, −154, −156, −157, −158, or −160, showed strong interference. These results agree with the results of competition in Fig. 3*A* and map the site involved in forming bands N approximately from −160 to −148. These results confirm that the protein responsible for forming bands N is distinct from previously identified factors that bind to the IRE, which show methylation interference only from −152 to −142 (22, 28–31).

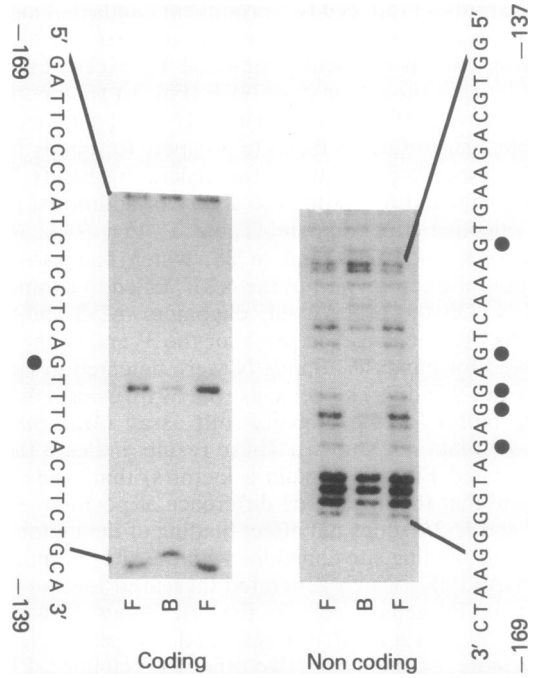

FIG. 4. Methylation interference. These experiments were performed as described (27) by using nuclear extracts from undifferentiated F9 cells and the NRE probe. The guanine (G) residues exhibiting interference are marked (●). B and F stand for bound and free probe, respectively.

DISCUSSION

Negative regulation of MHC class I promoter expression in F9 cells mediated by the conserved upstream regulatory region has been investigated. This negative regulation was relieved by competition *in vivo* (Fig. 2), which correlated with increased promoter activity observed by mutations placed in the NRE in pL^{d}CAT1.4K (Table 1). This confirms that negative regulation is caused by a titratable negative factor(s) rather than by interference with the activities of positive factor(s). In accordance, a factor(s) that specifically binds to the NRE, bands N in Fig. 3, was detected in extracts of F9 and other EC cells. Further, negative regulation was observed only in undifferentiated cells: it ceased following differentiation of F9 cells by retinoic acid treatment and concomitantly, binding activity to the NRE became undetectable (Table 1, Fig. 3*B*). These data provide strong evidence that the factor that binds to the NRE represents an essential component of the negative regulation of MHC class I genes. Results of functional studies as well as mobility-shift and methylation interference experiments locate the primary site of negative regulation in the 5′ and central part of the ICS, the NRE. The NRE maps between −160 and −148 and is distinct from the IRE, which maps to an overlapping sequence (22, 28–31). The present study indicates that a sequence in the ICS has a more important role than the MHC enhancer elements (between −203 and −161), although previous work indicated involvement of the enhancer region (19). Negative regulation mediated by the NRE may occur in

other cells as well—e.g., in cells of the central nervous system, where MHC class I genes are not expressed (P. T. Massa, S. Hirschfeld, B.-Z Levi, L. A. Quigley, K.O., and D. E. McFarlin, unpublished work). Further, Baniahmad *et al.* (36) noted that the NRE described here is similar in sequence to the silencer elements of the lysozyme gene and other genes. However, down-regulation of MHC class I genes probably results from more than one mechanism: a sequence further upstream and distinct from the NRE has been shown to control repression of MHC class I gene expression in cells transformed by the oncogenic adenovirus 12 (37).

It is not known how the NRE elicits its function. It is not likely that binding of a negative factor to the NRE physically excludes factors from interacting with the enhancers (38), as the MHC class I enhancers map to regions distinct from the NRE, and the enhancer activity is low in F9 cells (ref. 19 and Fig. 2). Since the negative regulation studied in this work is likely to be mediated by direct binding of a factor to the NRE, indirect mechanisms due to protein–protein interaction, such as "squelching" (38, 39), are also unlikely. The negative factor in F9 cells may directly act on the basal transcription complex and reduce the rate of transcription mediated by the TATA element and initiation sequences. Negative regulation has been reported to occur in F9 cells for a number of other genes, including genes in polyoma virus and simian virus 40, in which the repression appears to be mediated by the enhancer region. More than one negative regulatory factor has been implicated for murine leukemia virus (11, 12). Additionally, factors proposed to be responsible for negative regulation, such as the octamer-binding protein NFA-3 (14) and a factor that binds to the regulatory region of the IFN-β gene (13), are expressed in F9 cells. This suggests that negative regulation is a common mechanism of gene regulation in EC cells. It is not clear whether these factors are unrelated to each other or whether they have a common component. Analysis of a recently cloned protein that recognizes the NRE as well as the CAAT sequence (M.M., T. Nagata, K.O., and P.A.S., unpublished work) may provide clues to the mechanism of negative regulation of MHC class I and other genes in F9 cells.

We thank Drs. A. S. Baldwin, Jr., P. H. Driggers, and W. Anderson for K^b probes, for oligonucleotides, and for a gift of P19 cells. We thank Ms. W.-H. Mak for technical assistance. This work was supported by Grant PO1-CA42063 and Cancer Center Support Grant (core) P30-CA14051 from the National Cancer Institute, by a cooperative agreement (CDR-8803014) with the National Science Foundation, and by a grant from Ajinomoto Co., Inc., Japan, to P.A.S.

1. Martin, G. R. (1980) *Science* **209**, 768–776.
2. Kelly, F. & Condamine, H. (1982) *Biochim. Biophys. Acta* **651**, 105–141.
3. Linney, E., Davis, B., Overhauser, J., Chao, E. & Fan, H. (1984) *Nature (London)* **308**, 470–472.
4. Gautsch, J. W. & Wilson, M. (1983) *Nature (London)* **301**, 32–37.
5. Gorman, C. M., Rigby, P. W. J. & Lane, D. P. (1985) *Cell* **42**, 519–526.
6. Sasson-Corsi, P., Fromental, C. & Chambon, P. (1987) *Oncogene Res.* **1**, 113–119.
7. Cremisi, C. & Babinet, C. (1986) *J. Virol.* **59**, 761–763.
8. Furukawa, K., Yamaguchi, Y., Ogawa, E., Shigesada, K., Satake, M. & Ito, Y. (1990) *Cell Growth Differ.* **1**, 135–147.
9. Sleigh, M. J., Lockett, T. J., Kelly, J. & Lewy, D. (1987) *Nucleic Acids Res.* **15**, 4307–4324.
10. Wasylyk, B., Imler, J. L., Chatton, B., Schatz, C. & Wasylyk, C. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7952–7956.
11. Flamant, F., Gurin, C. C. & Sorge, J. A. (1987) *Mol. Cell. Biol.* **7**, 3548–3553.
12. Tsukiyama, T., Niwa, O. & Yokoro, K. (1989) *Mol. Cell. Biol.* **9**, 4670–4676.
13. Haggarty, A., Ponton, A., Paterno, G. D., Daigneault, L. & Skup, D. (1988) *Nucleic Acids Res.* **16**, 10575–10592.
14. Lenardo, M. J., Staudt, L., Robbins, P., Kuang, A., Mulligan, R. C. & Baltimore, D. (1989) *Science* **243**, 544–546.
15. Barklis, E., Mulligan, R. & Jaenish, R. (1986) *Cell* **47**, 391–399.
16. Oshima, R. G. (1982) *J. Biol. Chem.* **257**, 3414–3421.
17. Strickland, S., Smith, K. K. & Marotti, K. R. (1980) *Cell* **21**, 347–355.
18. Artzt, K. & Jacob, F. (1974) *Transplantation* **17**, 632–634.
19. Miyazaki, J., Appella, E. & Ozato, K. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 9537–9541.
20. Ozato, K., Wan, Y.-J. & Orrison, B. M. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 2427–2431.
21. McBurney, M. W. & Rogers, B. J. (1982) *Dev. Biol.* **89**, 503–508.
22. Shirayoshi, Y., Burke, P. A., Appella, E. & Ozato, K. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 5884–5888.
23. Burke, P. A., Hirschfeld, S., Shirayoshi, Y., Kasik, J., Hamada, K., Appella, E. & Ozato, K. (1989) *J. Exp. Med.* **169**, 1309–1321.
24. Herbomel, P., Bourachot, B. & Yaniv, M. (1984) *Cell* **39**, 653–662.
25. Flanagan, J. R., Kreig, A. M., Max, E. E. & Khan, A. S. (1989) *Mol. Cell. Biol.* **9**, 739–746.
26. Dignam, J. D., Martin, P. L., Shasty, B. S. & Roeder, R. G. (1983) *Methods Enzymol.* **101**, 582–599.
27. Baldwin, A. S., Jr. (1988) *Curr. Protocol Mol. Biol.* **3**, 1231–1236.
28. Levy, D. E., Kessler, D. S., Pine, R., Reich, N. & Darnell, J. E., Jr. (1988) *Genes Dev.* **2**, 383–393.
29. Blanar, M. A., Baldwin, A. S., Jr., Flavell, R. A. & Sharp, P. A. (1989) *EMBO J.* **8**, 1139–1144.
30. Harada, H., Fujita, T., Miyamoto, M., Kimura, Y., Maruyama, M., Furia, A., Miyata, T. & Taniguchi, T. (1989) *Cell* **58**, 729–739.
31. Driggers, P. H., Ennist, D. L., Gleason, S. L., Mak, W.-H., Marks, M. S., Levi, B.-Z., Flanagan, J. R., Appella, E. & Ozato, K. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 3743–3747.
32. Israel, A., Kimura, A., Kieran, M., Yano, O., Kanellopoulos, P., LeBail, O. & Kourilsky, P. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 2653–2657.
33. Baldwin, A. S., Jr., & Sharp, P. A. (1987) *Mol. Cell. Biol.* **7**, 305–313.
34. Xiao, H. & Lis, J. T. (1986) *Mol. Cell. Biol.* **7**, 3200–3206.
35. Gilbert, W. & Maxam, A. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 3581–3584.
36. Baniahmad, A., Muller, M., Steiner, C. H. & Renkawitz, R. (1987) *EMBO J.* **6**, 2297–2303.
37. Katoh, S., Ozawa, K., Kondoh, S., Soeda, E., Israel, A., Shiroki, K., Fujinaga, K., Itakura, K., Gachelin, G. & Yokoyama, K. (1990) *EMBO J.* **9**, 127–135.
38. Levine, M. S. & Manley, J. L. (1989) *Cell* **59**, 405–408.
39. Adler, S., Waterman, M. L., He, X. & Rosenfeld, M. (1988) *Cell* **52**, 685–695.