*Proc. Natl. Acad. Sci. USA*
Vol. 84, pp. 2653–2657, May 1987
Biochemistry

# A common positive trans-acting factor binds to enhancer sequences in the promoters of mouse *H-2* and $\beta_2$-microglobulin genes

**(enhancer–interferon response sequence/DNA binding protein/"footprint"/*in vivo* competition)**

ALAIN ISRAËL, AKINORI KIMURA, MARK KIERAN, OSAMU YANO, JEAN KANELLOPOULOS, ODILE LE BAIL, AND PHILIPPE KOURILSKY

Unité de Biologie Moléculaire du Gène, Unité 277, Institut National de la Santé et de la Recherche Médicale, Unité Associée 535, Centre National de la Recherche Scientifique, Institut Pasteur, 25 rue du Dr. Roux, 75724 Paris Cédex 15, France

*Communicated by Pierre Chambon, December 29, 1986*

**ABSTRACT** **Using gel retardation and *in vitro* "footprinting", we have analyzed the interactions between nuclear proteins derived from various mouse cells and the enhancer and interferon response sequences of the *H-2K*$^b$ gene. We have found that a protein factor binds a site in the enhancer sequence that partially overlaps the interferon response sequence. This factor also binds to a similar sequence lying in the opposite orientation in the promoter of the mouse $\beta_2$-microglobulin gene, suggesting a common regulatory mechanism. Transfection competition experiments indicate that this factor acts as a positive element in the expression of *H-2* and $\beta_2$-microglobulin genes.**

The class I *H-2* genes encoding the major histocompatibility antigens of the mouse are expressed on most somatic cells of the adult organism but not on the surface of the nonfertilized egg and in undifferentiated embryonal carcinoma cells (1–3). Their expression is stimulated by $\alpha$-, $\beta$, and $\gamma$-interferons, at least in part at the transcriptional level (4–8). Similarly, $\beta_2$-microglobulin ($\beta_2$m), the nonpolymorphic light chain that is noncovalently associated with H-2 antigens, is not expressed in embryonal carcinoma cells and is positively regulated by interferons. However, its synthesis follows a different time course during embryonic development (9, 10), so that it is not clear whether common regulatory mechanisms control the expression of the $\beta_2$m and *H-2* genes.

We have earlier analyzed in detail the promoter of the *H-2K*$^b$ gene and shown that it contains two enhancer-like sequences, one of which overlaps with a consensus interferon response sequence (IRS) (11). This IRS is necessary for stimulation by interferon but requires the overlapping enhancer to be functional: it acts as a modulator of the enhancer and the two sequences together behave like an inducible enhancer (12).

Since these sequences are likely to be the site of interaction of trans-acting molecules that regulate class I gene expression, we have prepared nuclear extracts derived from mouse cells and assayed for specific DNA–protein interactions by a gel retardation assay, followed by DNase I or methidium-propyl-EDTA·Fe(II) protection experiments.

## MATERIALS AND METHODS

**Preparation of Nuclear Extracts.** Nuclear extracts derived from 3T6 mouse cells were prepared according to ref. 13. Extracts were aliquotted and kept at −70°C. The protein concentration was determined with the Bio-Rad assay.

**Gel Retardation Assay.** DNA probes were end-labeled using the large fragment of DNA polymerase I or polynucleotide kinase (Boehringer Mannheim). For binding reactions, 2–5 $\mu$g of nuclear extract was incubated in 20 $\mu$l for 10 min at 20°C with 2 $\mu$g of poly(dI-dC) and 1 $\mu$g of the competitor plasmid in 50 mM NaCl/20 mM phosphate buffer, pH 6/5 mM $MgCl_2$/10% (vol/vol) glycerol/1 mM dithiothreitol. Labeled fragment (2000–5000 dpm) was added, incubation was continued for 15 min, and the mixture was loaded on a 5% acrylamide gel in 0.5× Tris borate/EDTA buffer, which was run for 150 min at 12 V/cm. The gel was dried and autoradiographed overnight.

**"Footprinting" Experiments.** DNase I footprinting analysis was performed as described (14) with the binding reaction being scaled up 4 times. For methidium-propyl-EDTA·Fe(II) footprinting, $MgCl_2$ was omitted from the binding reaction mixture. Partial cleavage was carried out by addition of 10 $\mu$g of methidium-propyl-EDTA·Fe(II) per ml, 10 $\mu$M ferrous ammonium sulfate, and 5 mM dithiothreitol. After 5 min at 30°C, the reaction mixture was loaded on a polyacrylamide gel, which was then processed as for DNase I footprinting (14).

***In Vivo* Competition.** 3T6 cells were transfected as described (11) with a total of 22 $\mu$g of DNA including 2.5 $\mu$g of the chloramphenicol acetyltransferase (CAT) construct (pdl393 or $\beta_2$m DdeconaCAT), various amounts (see Fig. 4) of a pUC18 derivative containing two copies of the −195 to −157 region from the K$^b$ promoter, pUC18 DNA to a total of 20 $\mu$g, and 2 $\mu$g of the thymidine kinase-$\beta$-galactosidase (TK-$\beta$gal) construct (11) as an internal control. Forty hours after transfection, activity was measured and normalized with respect to $\beta$-galactosidase activity (11).

**Methylation Interference.** End-labeled DNA fragments were partially methylated at the guanine residues with dimethyl sulfate as described (15). Methylated DNA was precipitated twice, rinsed with 70% ethanol, dried, and redissolved in 10 $\mu$l of 10 mM Tris·HCl/1 mM EDTA, pH 8. Binding and electrophoresis were carried out as for footprinting experiments. The retarded and unretarded bands were electroeluted, ethanol-precipitated, and treated with piperidine as described in ref. 15, before fractionation in a thin 6% polyacrylamide gel.

## RESULTS

**Specific Interaction of Nuclear Proteins with the Enhancer–IRS Region of the *H-2K*$^b$ Gene.** Deletion analysis of the *H-2K*$^b$ promoter had defined several regions that contribute to its activity (11). These include an enhancer-like sequence (enhancer B: −120 to −61), and another enhancer sequence (enhancer A: −193 to −159), which overlaps an IRS (−165 to −137). Trans-acting proteins that bind purified restriction

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.

Abbreviations: $\beta_2$m, $\beta_2$-microglobulin; IRS, interferon response sequence; CAT, chloramphenicol acetyltransferase; TK, thymidine kinase; SV40, simian virus 40; $\beta$gal, $\beta$-galactosidase.

fragments carrying these various sequences have been searched by a gel retardation assay (16, 17).

Using a nuclear extract derived from 3T6 mouse cells, the only specific interaction we could detect in the promoter was with a *Dde* I/*Ava* II fragment (−215 to −120) that includes the overlapping enhancer–IRS sequences (Fig. 1*C*). Two retarded bands (R-I and R-II) were observed. Treatment of the extract with proteinase K destroyed the binding activity. The results of the retardation assay were judged specific when the retarded bands were competed away specifically by an excess of cold homologous DNA (see below).

Since two sequences that functionally cooperate (12) are included in the *Dde* I/*Ava* II fragment, we tried to characterize this interaction by competition with various subfragments (Fig. 1*A*). Binding of the *Dde* I/*Ava* II fragment could be efficiently competed away by plasmids carrying the homologous sequence (Dd-cIIconaCAT, lane 9) but not by conaCAT or TKCAT (containing, respectively, the conalbumin promoter and the TK promoter: lanes 12 and 14). A weak competition was seen with plasmids carrying the SV40 enhancer (SVEconaCAT in lane 5 and pSV2CAT in lane 15). A plasmid carrying only the enhancer A sequence with a cytosine to adenine transversion at −160 and a thymine to guanine at −158 (Dd-HfconaCAT) (lane 10, and line 10 in Fig. 1*B*) could compete only partially with the *Dde* I/*Ava* II fragment (this sequence also displays a reduced enhancer activity; data not shown). A plasmid containing the intact enhancer A sequence (from −195 to −157) (Sau-SauconaCAT, lane 8 and line 8) is as efficient a competitor as Dd-cIIconaCAT. A plasmid carrying the enhancer A sequence (Sau-HfconaCAT, lane 11 and line 11) with two cytosine to guanine transversions at positions −160 and −161, which has lost enhancer activity (figure 6 in ref. 11), did not compete with the *Dde* I/*Ava* II fragment. The enhancer B sequence (Av-SauconaCAT, lane 6) or the IRS (Hf-SauconaCAT, lane 7 and line 7) did not compete, nor did two fragments derived from the homologous region in the promoter of a gene from the *Qa* region expressed only in the liver, *Q10* (19, 20), and carried in two plasmids [Hp-Ha(Q10)conaCAT and Hp-Hf(Q10)conaCAT] (lanes 3 and 4 and lines 3 and 4), which show a very weak enhancer activity in mouse fibroblasts (figure 6 in ref. 11).

The slowest migrating R-I band is also competed, although partially, by SVEconaCAT and pSV2CAT (lanes 5 and 15). A fragment that contains only the enhancer A sequence derived from the Dd-HfconaCAT plasmid (−217 to −154, including the cytosine to adenine transversion at −160 and the thymine to guanine transversion at −158; see Fig. 1*B*, line 10) shows a weak retarded band that can be competed away by the Dde-cIIconaCAT plasmid and, interestingly, by a plasmid containing the SV40 72-base-pair (bp) repeats (data not shown).

These results suggest that specific binding takes place between −195 and −157 and that nucleotides that have been shown to be critical for enhancer activity (11) are also important for binding the protein.

**Binding of a Similar Factor to the $\beta_2$m Enhancer.** We then asked whether the $\beta_2$m and the $K^b$ gene might be coregulated. Using extracts from 3T6 cells, we performed competition experiments between the labeled $K^b$ *Dde* I/*Ava* II fragment described above and various subfragments derived from the $\beta_2$m promoter. No obvious homology had been

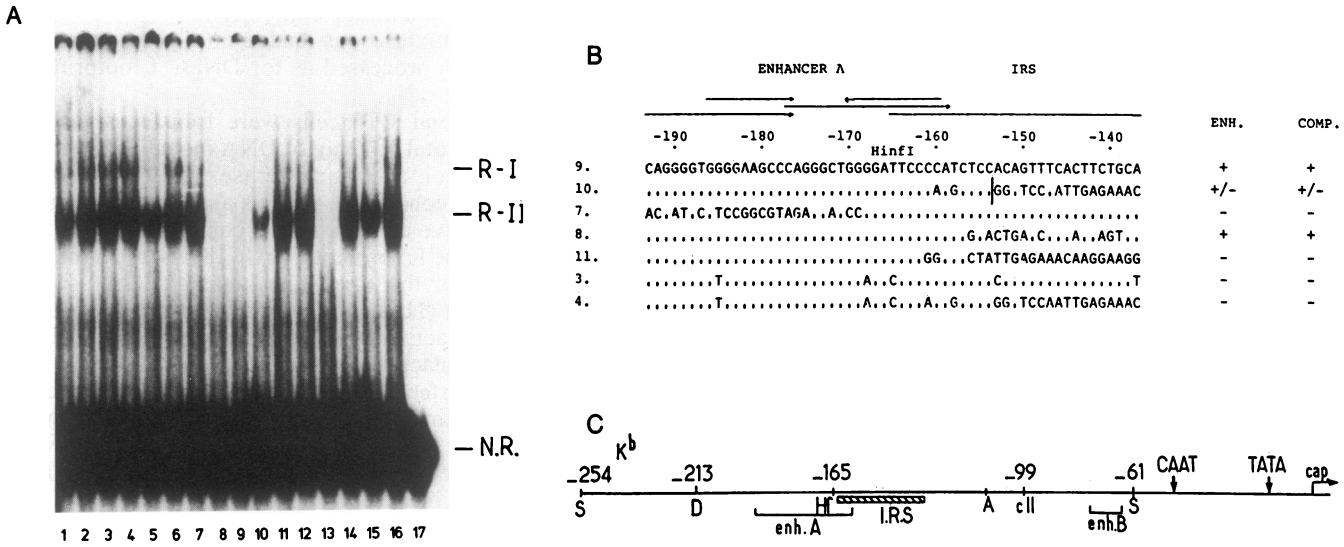


FIG. 1. Mobility shift competition experiments. (*A*) The *Dde* I/*Ava* II fragment (−215 to −120) was labeled with [$\gamma^{32}$P]ATP at the *Dde* I extremity. Binding and electrophoresis were performed as described. Competition plasmids were as follows: lane 1, pHf-cII contains the *Hin*fI/*Hin*cII fragment (−166 to −99) cloned in pBR322; lane 2, pBP is a derivative of pBR322 deleted between *Bam*HI and *Pvu* II; lane 3, Hp-Ha(Q10)conaCAT contains the *Hpa* II/*Hae* III fragment (−276 to −125) derived from the Q10 promoter cloned in pconaCAT (11); lane 4, Hp-Hf(Q10)conaCAT contains the *Hpa* II/*Hin*fI fragment (−276 to −166) derived from the Q10 promoter; lane 5, SVEconaCAT contains the two 72-bp repeats of simian virus 40 (SV40); lane 6, Av-SauconaCAT contains the *Ava* II/*Sau*3A fragment (−121 to −61) derived from the $K^b$ promoter [this fragment includes enhancer B (11)]; lane 7, Hf-SauconaCAT contains the *Hin*fI/*Sau*3A fragment (−165 to −61) (the relevant features of this construct are shown in *B*); lane 8, Sau-SauconaCAT contains the enhancer A sequence (−195 to −157); lane 9, Dd-cIIconaCAT contains the *Dde* I/*Hin*cII fragment (−213 to −99); lane 10, Dd-HfconaCAT contains the *Dde* I/*Hin*fI fragment (−213 to −161, see *B*); lane 11, Sau-HfconaCAT contains the *Sau*3A/*Hin*fI fragment (−254 to −162, see *B*); lane 12, pconaCAT is the plasmid in which fragments have been cloned to assay enhancer activity (11); lane 13, $\beta_2$m DdeconaCAT contains a sequence derived from the $\beta_2$m promoter (−287 to −60) cloned in pconaCAT. This fragment had been shown to exhibit enhancer activity (11); lane 14, pTKCAT contains the TK promoter linked to the CAT gene; lane 15, pSV2CAT contains the SV40 enhancer and early promoter linked to the CAT gene (18); lane 16, pCATdel contains the CAT gene with no promoter in front; lane 17, control with no extract added. (*B*) Sequence of the enhancer A–IRS region in the various constructs used for competition as shown in *A*. Numbers in front of each line correspond to lane numbers in *A*. The nucleotides differing from the $K^b$ region are indicated. The vertical bar in the Dd-HfconaCAT sequence (line 10) indicates the 3′ limit of the labeled fragment used for retardation (see text). Enhancer activity (ENH.) was determined in ref. 11; +/− indicates an enhancer activity lower than wild type. Competition (COMP.) relates to the results shown in *A*; +/− indicates partial competition in the conditions described above. (*C*) Map of the H-2$K^b$ promoter with relevant restriction sites and regulatory regions. S, *Sau*3A; D, *Dde* I; Hf, *Hin*fI; cII, *Hin*cII; A, *Ava* II.

detected between the $K^b$ and $\beta_2$m promoter sequences (11), but a plasmid containing a 227-bp *Dde* I fragment (−287 to −60) could compete away the $K^b$ *Dde* I/*Ava* II fragment (Fig. 1*A*, lane 13). This fragment, previously shown to exhibit enhancer activity (11), was labeled at both ends and cut with *Ava* II, yielding a 126-bp fragment (−186 to −60). In the retardation assay, the latter displays a retarded band that comigrates with the R-II band shown in Fig. 1. Competition experiments gave the same results as those shown in Fig. 1, suggesting that a similar factor could bind both promoters (data not shown). Competition experiments with increasing amounts of both the homologous or the heterologous competitor show that the affinity of the binding factor for the two sequences is roughly similar (data not shown).

**Footprinting Analysis of Binding on Both Enhancers.** To map more precisely the region(s) responsible for binding on the two promoters, we performed *in vitro* footprinting experiments using either DNase I or methidium-propyl-EDTA·Fe(II) as the cleaving agent.

We first analyzed the DNase I protection pattern (21) of bands R-I and R-II obtained with the $K^b$ *Dde* I/*Ava* II fragment. With the noncoding strand labeled at the *Dde* I extremity, the fastest migrating band R-II exhibits a pattern of protection that extends from the 3′ extremity to the middle of enhancer A (−158 to −175) (Fig. 2*A*). R-I is characterized by a protection pattern starting also at −158 but extending further beyond the limit of enhancer A (−199: compare lane R-I with lane NR on its left). The area protected in R-II corresponds to one of the direct repeats shown on the left (arrows), while in R-I the two repeats are protected.

These protection areas are bordered on their common 3′ end by DNase I hypersensitive sites. The IRS occasionally showed protection in both R-I and R-II, but this result was irreproducible (the observation was made twice in 10 experiments) and we cannot conclude firmly that a protein binds the IRS. The results obtained with extracts derived from untreated or from $\alpha$- and $\beta$-interferon-treated 3T6 cells were similar. Progressive deletion with BAL-31 starting from the *Ava* II site indicated that the 3′ limit of the region required for binding is the thymine at position −158 (data not shown).

To localize more precisely the protected area, we also used methidium-propyl-EDTA·Fe(II), a chemical cleaving agent that shows no cutting specificity and yields smaller and more accurate protected areas (22, 23). Fig. 2 *B* and *C* shows the protection patterns obtained with the *Dde* I/*Ava* II fragment from $K^b$ and the *Ava* II/*Dde* I fragment from $\beta_2$m, respectively (only band R-II is shown for $K^b$): the protected area is now restricted to 13 bp, which correspond in the $K^b$ promoter to the overlapping part between enhancer A and the IRS. The protected area on the noncoding strand is shifted 2 bp on the 3′ side as compared to the coding strand (Fig. 2*B*, compare lanes 3 and 11). Methylation interference experiments were

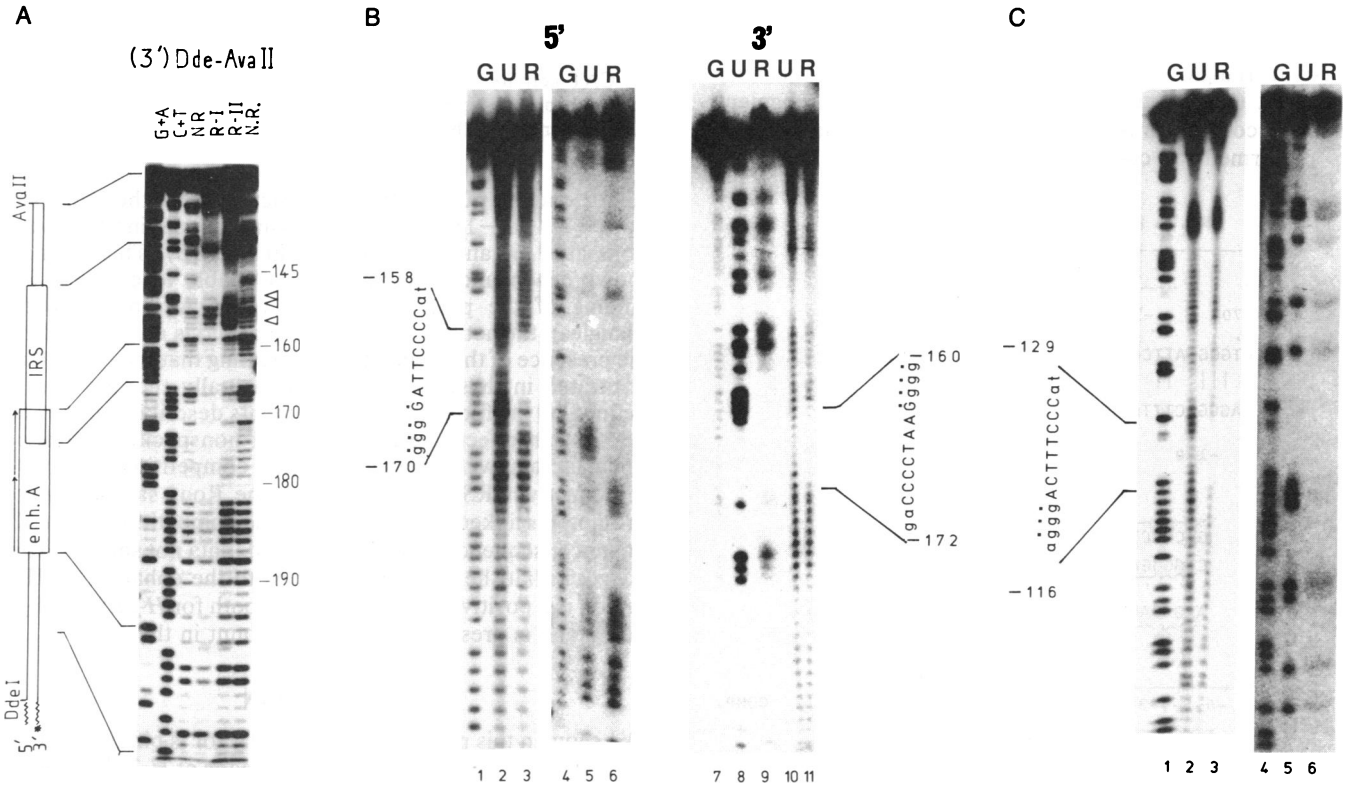


FIG. 2. (*A*) DNase I footprint analysis of bands R-I and R-II shown in Fig. 1. The *Dde* I/*Ava* II fragment was labeled with the Klenow enzyme at the *Dde* I extremity. The binding reaction, as described in Fig. 1, was scaled up 4 times. After binding, samples were treated with DNase I (2 $\mu$g/ml) for 1 min at 20°C. Digestion was stopped by the addition of 20 mM EDTA and electrophoresis of complexes was performed as usual. Appropriate bands were excised from the gel after autoradiography and the DNA was electroeluted. After ethanol precipitation, DNA was analyzed on a 6% polyacrylamide/urea sequencing gel. G+A and C+T are the purine and pyrimidine specific sequence ladders (15). NR, unretarded band; R-I and R-II, retarded bands shown in Fig. 1. R-I should be compared to NR on its left and R-II to N.R. on its right. Open arrows indicate hypersensitive sites. (*B*) Methidium-propyl-EDTA·Fe(II) footprinting and dimethyl sulfate interference on both strands of the $K^b$-derived *Dde* I/*Ava* II fragment. Methidium-propyl-EDTA·Fe(II) cleavage and dimethyl sulfate interference were carried out as described. 5′, coding strand; 3′, noncoding strand; G, purine-specific sequencing ladder; U, unretarded band; R, retarded band. Lanes 2, 3, 10, and 11, methidium-propyl-EDTA·Fe(II) protection; lanes 5, 6, 8, and 9, dimethyl sulfate interference. Protected sequences are shown on the side: lowercase letters correspond to partially protected nucleotides. Dots over Gs indicate the residues whose methylation prevents binding. (*C*) Methidium-propyl-EDTA·Fe(II) footprinting and dimethyl sulfate interference on the noncoding strand of the $\beta_2$m-derived *Ava* II/*Dde* I fragment labeled at the *Dde* I end. Symbols are the same as in *B*. Lanes 2 and 3, methidium-propyl-EDTA·Fe(II) protection; lanes 5 and 6, dimethyl sulfate interference.

also performed to pinpoint the guanine residues that are in close contact with the binding factor (Fig. 2*B*): methylation of the four guanines (−160 to −163) on the noncoding strand prevents binding of the protein, while methylation of guanine at −172 has only a partial effect (lanes 8 and 9). On the coding strand, methylation of the four guanines (−167 to −170) prevents binding (lanes 5 and 6). Methidium-propyl-EDTA·Fe(II) footprinting on the noncoding strand of the $\beta_2$m 126-bp *Ava* II/*Dde* I fragment is shown in Fig. 2*C*: protection is visible on a 14-bp fragment (−116 to −129: lanes 2 and 3) and methylation of the three guanines (−117 to −119) prevents binding. These results are summarized in Fig. 3*B*.

These data prompted us to analyze in more detail the sequences of the $K^b$ and $\beta_2$m promoters. We found that the protected area detected in the $\beta_2$m promoter belongs to a 33-bp fragment that can be aligned with the enhancer A–IRS region of $K^b$, but in the opposite orientation (Fig. 3*A*). Interestingly, this alignment extends in the IRS sequence, suggesting a potential role for this sequence in the previously described stimulation of $\beta_2$m expression by interferon (6, 24).

***In Vivo* Competition Experiments.** To try and define whether the protein(s) binding to this sequence acts positively or negatively, we performed *in vivo* competition experiments. We cloned the enhancer A sequence (−195 to −157), including the binding site, into pUC18. We then transfected 3T6 cells with a constant amount of the dl393 plasmid (which consists of 393 bp of the $K^b$ promoter linked to the CAT gene) together with increasing amounts of the pUC18 construct and we found that the activity of the $K^b$ promoter goes down (Fig. 4) (solid triangles). This also holds true for a $\beta_2$m enhancer construct (solid squares). Since the enhancer A fragment used as a competitor is somewhat larger than the binding site, we performed the competition with the *Sau*3A/*Hin*fI fragment cloned in pUC18: this construct includes the sequence from −254 to −157 with two cytosine to guanine transversions at −160 and −161 (line 11 in Fig. 1*B*). This plasmid shows no enhancer activity and is unable to compete for binding of the protein factor *in vitro*. As shown (open triangles), the activity of the dl393 plasmid is not affected by the presence of this competitor, indicating that the factor that is titrated in these experiments is actually the one that is defined by the footprinting experiments described above. To rule out an effect due to titration of nonspecific transcriptional factors, we performed the same competition assay with the construct RSVCAT, in which the Rous sarcoma virus promoter is linked to the CAT gene: no competition by the $K^b$ enhancer sequence could be observed (data not shown). This suggests that the protein that binds to the enhancer A–IRS region is a positive regulatory factor both for *H-2K*$^b$ and $\beta_2$m genes and is present in a limited amount in the cell.

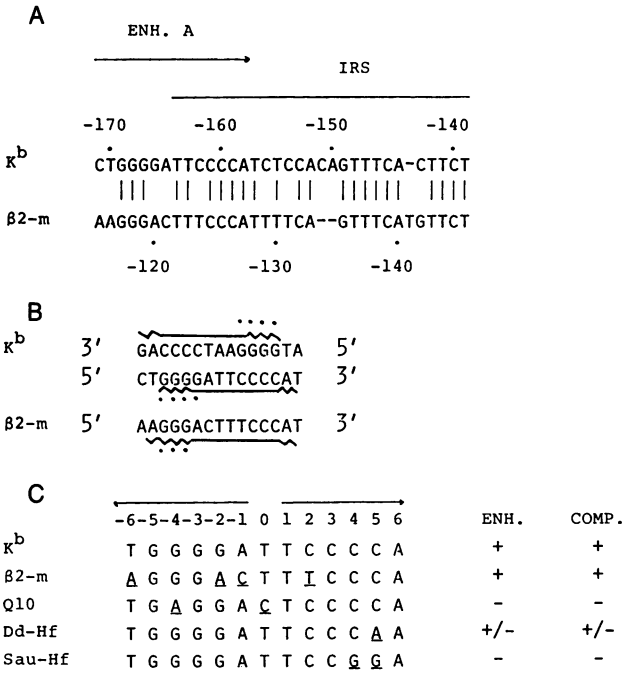


FIG. 3. (*A*) Alignment of the enhancer A–IRS region of $K^b$ with the corresponding region of $\beta_2$m. The matched nucleotides are indicated by vertical lines. (*B*) Summary of the results of methidium-propyl-EDTA·Fe(II) protection and methylation interference: both strands are shown for the $K^b$ promoter, and the noncoding strand is shown for $\beta_2$m. Wavy lines, partial methidium-propyl-EDTA·Fe(II) protection; straight lines, full protection. Dots over the G residues show the results of methylation interference. (*C*) The palindromic sequences that include the binding site in the $K^b$ promoter and the corresponding sequences in various genes and constructs. Enh. and comp. are as described in legend to Fig. 1*B*.

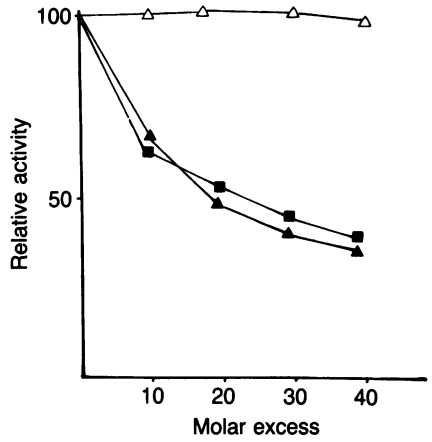


FIG. 4. *In vivo* competition between pdl393 (▲) containing 393 bp from the $K^b$ promoter linked to the CAT gene, or $\beta_2$m Dde conaCAT (■) carrying the *Dde* I fragment (−287 to −60) cloned in pconaCAT, with a pUC18 derivative containing two copies of the −195 to −157 region from the $K^b$ promoter. The line marked with open triangles represents competition between pdl393 and a pUC18 derivative containing the −254 to −157 *Sau*3A/*Hin*fI fragment that shows no enhancer activity due to two cytosine to guanine transversions at −160 and −161 (see Fig. 1, lane 11 and line 11). A total of 22 μg of DNA containing 2.5 μg of the CAT construct, increasing amounts of the pUC construct, pUC18 DNA to a total of 20 μg, and 2 μg of TK-βgal (11) was transfected into 3T6 cells as described (11). Forty hours after transfection, CAT activity was measured and normalized with respect to βgal activity (11). On the vertical axis, 100 represents the CAT activity when no competitor plasmid is added. Relative activity is plotted against molar excess of the enhancer region in the pUC construct vs. the CAT construct.

## DISCUSSION

We show in this paper that a protein factor is binding to a 13- to 14-bp sequence in the enhancer regions of *H-2K*$^b$ and $\beta_2$m. The *H-2K*$^b$ sequence is perfectly conserved in the $K^k$, $K^d$, and $L^d$ promoters (11). Inside a 33-bp fragment that can be aligned between the $K^b$ and $\beta_2$m promoters, the protected areas occupy homologous positions. Preliminary results indicate that the binding activities specific for the two promoters copurify through four chromatographic purification steps, including an affinity column made with the $K^b$ enhancer A–IRS region (O.Y., J.K., M.K., A.I., and P.K., unpublished data). Together with the fact that a 13-bp sequence probably accommodates only one protein, we conclude that a common or closely related factor is binding to both enhancer sequences. The alignment shown in Fig. 3*A* indicates a similarity between the two promoters that extends

from the proximal part of enhancer A to the IRS region. Outside of these sequences, we found no obvious homology.

The enhancer A of $K^b$ is present as two imperfect direct repeats (11), and DNase I footprinting (Fig. 2*A*) indicates that the upstream repeat can be protected as well: the significance of this sequence is unclear at the moment, since it is not present in the $\beta_2$m promoter, and a plasmid that contains it is unable to compete with the $K^b$ promoter *in vivo* (Fig. 4, open triangles). The binding activity described here recognizes the overlapping part between the enhancer and IRS sequences. *In vivo* competition experiments described above and analysis of several mutant sequences (see below) indicate that it is necessary for enhancer function and for basal level of promoter expression. We have shown previously that enhancer activity is necessary for interferon induction (12), but the mechanism by which interferon stimulates transcription remains unclear: we have not detected any reproducible protection on the conserved IRS sequence, and the pattern of protection we observed was unchanged whether we used extracts derived from untreated or from interferon-treated cells.

It is interesting to note that the region of binding in the $K^b$ promoter forms a perfect palindrome (Fig. 3*C*). Palindromes are often recognition sequences both for prokaryotic (reviewed in ref. 25) and eukaryotic (26) regulatory proteins. The protection pattern in Fig. 3*B* indicates a rotational binding symmetry in relation with the palindromic sequence: this suggests that the protein binds as a dimer, as several prokaryotic regulatory proteins do (25). An extensive mutational analysis will be necessary to define the functionally important residues in the enhancer sequences. However, some of them are already obvious from the available data: Fig. 3*C* shows the corresponding sequence in the constructs Dd-HfconaCAT and Sau-HfconaCAT (described in Fig. 1*B*) and in the promoters of the *Q10* and $\beta_2$m genes. Clearly, there is a good correlation between the protected area as determined by methidium-propyl-EDTA·Fe(II) footprinting and the nucleotides that have been shown to be important for enhancer activity.

However, in the $\beta_2$m sequence, there are two mismatches in the palindrome (thymine to adenine and adenine to cytosine at positions $-6$ and $-1$ of the palindrome, respectively). Guanine to adenine at $-2$ and cytosine to thymine at $+2$ are silent substitutions as far as palindromic character is concerned. This suggests that the recognition site is degenerate.

Using a nucleotide data bank* and the program described in ref. 27, we did not find any other eukaryotic promoter with a sequence similar to the $K^b$ and $\beta_2$m binding sites. The binding activity described here could thus have some specificity for the *H-2* and $\beta_2$m genes.

*National Institutes of Health (1986) Genetic Sequence Databank: GenBank (Research Systems Div., Bolt, Beranek, and Newman, Cambridge, MA), Tape Release 44.0.

We thank P. Dervan for kindly providing methidium-propyl-EDTA·Fe(II); B. Arcangioli and researchers in the laboratory of M. Yaniv for help and advice in the retardation and footprinting assays; and J. M. Panaud, J. Lobrot, and B. de Cougny from the photographic service of the Institut Pasteur for their skillful assistance. We also thank Dr. J. M. Claverie for his help in searching sequence homologies and V. Caput for typing the manuscript. M.K. was recipient of an Institut National de la Santé et de la Recherche Médicale fellowship.

1. Croce, C. M., Linnenbach, A., Huebner, K., Parnes, J. R., Margulies, D. H., Appella, E. & Seidman, J. G. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 5754–5758.
2. Morello, D., Daniel, F., Baldacci, P., Cayre, Y., Gachelin, G. & Kourilsky, P. (1982) *Nature (London)* **296,** 260–262.
3. Morello, D., Gachelin, G., Dubois, P., Tonigaki, N., Pressman, D. & Jacob, F. (1978) *Transplantation* **26,** 119–125.
4. Lindahl, P., Leary, P. & Gresser, I. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 2785–2789.
5. Burrone, O. R. & Milstein, C. (1982) *EMBO J.* **1,** 345–349.
6. Wallach, D., Fellous, M. & Revel, M. (1982) *Nature (London)* **299,** 833–836.
7. Fellous, M., Nir, U., Wallach, D., Merlin, G., Rubinstein, M. & Revel, M. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 3082–3086.
8. Rosa, F., Berissi, H., Weissenbach, J., Maroteaux, L., Fellous, M. & Revel, M. (1983) *EMBO J.* **2,** 239–243.
9. Morello, D., Duprey, P., Israël, A. & Babinet, C. (1985) *Immunogenetics* **22,** 441–452.
10. Ozato, K., Wan, Y. J. & Orrison, B. M. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 2427–2431.
11. Kimura, A., Israël, A., Le Bail, O. & Kourilsky, P. (1986) *Cell* **44,** 261–272.
12. Israël, A., Kimura, A., Fournier, A., Fellous, M. & Kourilsky, P. (1986) *Nature (London)* **322,** 743–746.
13. Piette, J., Kryszke, M. H. & Yaniv, M. (1985) *EMBO J.* **4,** 2675–2685.
14. Arcangioli, B. & Lescure, B. (1985) *EMBO J.* **4,** 2627–2633.
15. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65,** 499–560.
16. Garner, M. M. & Revzin, A. (1981) *Nucleic Acids Res.* **9,** 3047–3060.
17. Fried, M. & Crothers, D. M. (1981) *Nucleic Acids Res.* **9,** 6505–6525.
18. Gorman, C. L., Moffat, L. T. & Howard, B. (1982) *Mol. Cell. Biol.* **2,** 1044–1051.
19. Devlin, J. J., Lew, A. M., Flavell, R. A. & Coligan, J. E. (1985) *EMBO J.* **4,** 369–374.
20. Kress, M., Cosman, D., Khoury, G. & Jay, G. (1983) *Cell* **34,** 189–196.
21. Galas, B. J. & Schmitz, A. (1978) *Nucleic Acids Res.* **5,** 3157–3170.
22. Van Dyke, M. W. & Dervan, P. B. (1983) *Nucleic Acids Res.* **11,** 5555–5567.
23. Sawadogo, M. & Roeder, R. G. (1985) *Cell* **43,** 165–175.
24. Heron, I., Hokland, M. & Berg, K. (1978) *Proc. Natl. Acad. Sci. USA* **77,** 6215–6219.
25. Pabo, C. O. & Sauer, R. T. (1984) *Annu. Rev. Biochem.* **53,** 293–321.
26. Giniger, E., Vornum, S. M. & Ptashne, M. (1985) *Cell* **40,** 767–774.
27. Claverie, J. M. & Sauvaget, I. (1985) *Cabios* **1,** 95–104.