KBF1 (p50 NF-κB Homodimer) Acts as a Repressor of H-2Kb Gene Expression in Metastatic Tumor Cells

By Daniel Plaksin,* Patrick A. Baueurle,‡ and Lea Eisenbach*

From the *Department of Cell Biology, The Weizmann Institute of Science, Rehovot 76100, Israel; and the ‡Laboratorium für Molekulare Biologie der Ludwig-Maximilians-Universität München, D-8033 Martinsried, Germany

Summary

Downregulation of major histocompatibility complex class I expression is causally related to high malignancy and low immunogenicity of certain murine tumors. In this study, we have analyzed the roles of the nuclear factors KBF1/p50 and p65 in regulation of class I expression in high and low metastatic tumor cells. Low class I-expressing cells show higher levels of KBF1/p50 and NF-κB (p50/p65) binding activity than high class I-expressing cells. However, an excess of KBF1 over NF-κB is observed in low expressing cells, while an excess of NF-κB over KBF1 is observed in high expressing cells. Stable transfection of a p65 expression vector into low class I-expressing cells activated H-2 transcription and cell surface expression, while stable transfection of p50 expression vector into high expressing cells suppressed H-2Kb transcription and cell surface expression. Our studies suggest that KBF1 has the potential of downregulating class I gene expression, whereas dimers containing the p65 subunit are activators of class I gene expression.

Class I molecules of the MHC present peptide fragments from intracellularly processed antigens to CTL (1, 2). Reduced levels of expression of H-2 class I proteins were observed in spontaneous, chemically induced, and virally transformed murine tumors (3–8), human small cell carcinomas (9), neuroblastomas (10), eccrine protocarcinomas (11), cervical carcinomas (12), and Burkitt's lymphoma lines (13). Moreover, examination of 44 primary tumors and autologous metastases showed significant downregulation of class I in metastases (14). In many of the experimental tumor systems, the lack of H-2 expression determined reduced immunogenicity and high tumorigenicity.

The Lewis lung carcinoma (3LL) tumor that originated spontaneously in a C57Bl/H-2b mouse almost lacks cell surface expression of H-2Kb antigen (3). Screening a large number of newly generated single cell clones of 3LL showed that the higher the metastatic phenotype of a clone, the lower the expression of H-2Kb glycoprotein; H-2Db, on the other hand, is expressed to some extent on most clones (15). The low metastatic H-2Kb-positive clones were shown to be significantly more immunogenic than syngeneic and in allogeneic hosts, compared with high metastatic H-2Kb-negative cells (15). It therefore appeared that putative peptides derived from tumor antigens could be recognized by CTL only in association with H-2Kb gene products, while H-2Db molecules do not serve as a restriction element for antitumor CTL (16). In vitro treatment by IFN-γ transiently activated the expression of H-2Kb gene in the metastatic clones (17). When cells treated for 5 d with IFN-γ were injected intravenously into mice, a significant decrease in their metastatic competence was observed (17–19). Since IFN-γ is known to affect many different functions of the cells, we tested whether the reduction of metastatic competence by IFN-γ can be attributed directly to the induction of H-2Kb expression, by introducing H-2Kb gene into cells of a highly metastatic, Kb-negative clone, called D122. We demonstrated that cells of a highly metastatic clone, transfected with H-2Kb gene, reverted to low or nonmetastatic phenotypes. This was correlated with the acquisition of H-2K-restricted immunogenic properties (20, 21). Low levels of class I cell surface expression in most tested tumors, of human or murine origin, are the result of low mRNA levels, and only rarely are defects in protein transport, peptide processing, or MHC–peptide assembly described (22, 23). The unique model of the 3LL carcinoma enables a direct analysis of the transcriptional mechanism responsible for the differential class I expression in high and low expressing clones.

Analysis of the mouse MHC class I promoter has revealed several regions important for the expression and regulation of these genes through specific binding of proteins (24–34). The promoter-enhancer region of class I genes includes two main enhancer sequences, A and B. Enhancer A overlaps with the IFN-responsive sequence (IRS) (−163, −137). Both enhancers are conserved within the promoter of several genes coding for classical transplantation antigens. In most cells or tissues of human, rat, or mouse origin, a nuclear factor called

1651 J. Exp. Med. © The Rockefeller University Press • 0022-1007/93/06/1651/12 $2.00
Volume 177 June 1993 1651–1662
KBF1 interacts specifically with two cis-acting GC-rich palindromes, within the \( \lambda \) enhancer (25, 35, 36). This constitutive nuclear factor also binds to a similar sequence, within the promoter of the mouse \( \beta_2 \)-m gene (35). KBF1 was thus expected to play a role in the coordinate up regulation of class I and \( \beta_2 \)-m gene expression (35).

Another factor binding to the palindrome sites in class I gene enhancers and the \( \beta_2 \)-m promoter is NF-\( \kappa \)B (28, 37). This inducible factor is thought to displace the constitutive KBF1 upon treatment of cells with TNF and phorbol ester, thereby replacing a weakly trans-activating factor by a strong activator (36). NF-\( \kappa \)B is composed of two DNA binding subunits called p50 and p65 (38-40). A third subunit, called IxB, serves to inhibit the DNA binding and nuclear translocation of the p50-p65 heterodimer (41). The release of IxB in response to a multitude of external stimuli provides the molecular basis for the inducible activation of NF-\( \kappa \)B (reviewed in reference 42). p50 and p65 share sequence homology and are members of a novel class of transcription factors also including the Rel protooncogene (for review, see reference 43).

KBF1, by all criteria tested, is identical to a homodimer of the p50 subunit of NF-\( \kappa \)B (44-46). p50 homodimers escape inducible control because they cannot detectably interact with purified IxB proteins, unlike dimers containing p65. A unique feature of p50/KBF1 is its synthesis of a large non-DNA binding precursor molecule, p105, with endogenous IxB activity in its COOH-terminal portion (47-49).

KBF1 (p50 NF-\( \kappa \)B homodimer) is present in most differentiated cells where MHC class I antigens are expressed. However, its binding activity is absent in undifferentiated embryonal carcinoma (EC) cells, where class I and \( \beta_2 \)-m genes are silent. It is induced when the cells are triggered to differentiate (28), and the appearance of KBF1 binding activity in EC cells parallels the onset of H-2 class I and \( \beta_2 \)-m gene expression (mRNA and proteins). KBF1-like binding activity is undetectable in brain nuclear extracts, which correlates with the lack of H-2 class I antigens in this organ (50). Its absence from fetal mouse liver nuclear extracts is however in contrast to class I expression from mid somite stage of gestation onwards (50). In stage-specific T cell lines, the level of class I transcription has been shown to correlate with the amount of KBF1-like binding activity (51). Another factor, H-2 transcription factor (H2TF1), binds also to enhancer A at exactly the same nucleotide residues as KBF1 (52), but its relationship to KBF1 is unclear at present. Recent reports show that homodimers of p50 (KBF1) lack significant trans-activating potential in transient transfection assays but rather have the potential of downregulating \( \kappa \)-specific gene expression (53-55). In contrast, in vitro transcription studies showed that p50 dimers act as activators of gene transcription (56, 57).

These conflicting observations prompted us to test whether stable expression of KBF1 in 3LL carcinoma cell lines positively or negatively influences class I gene expression. Our data suggest that KBF1 is a negative regulator of class I expression, whereas the p65 subunit of NF-\( \kappa \)B is a positive regulator. These results suggest that the balance between the two NF-\( \kappa \)B subunits is crucial for the level of class I expression.

Materials and Methods

Tumor Cells. Tumor cells were maintained in DME, 10% FCS, and supplements described elsewhere (4). The Lewis lung carcinoma (3LL) is a malignant tumor that originated spontaneously in C57BL/6 (H-2\( ^{b} \)) mice (4). A9 and D122 are low and high metastatic clones, respectively, cloned from the 3LL carcinoma cells by limiting dilution (4).

Gene Expression. RNAs were prepared from 1-3 x 10\(^6\) cells, propagated in tissue culture, by the method of Chirgwin et al. (58). Northern blots were prepared from formaldehyde-containing agarose gels loaded with 40 \( \mu \)g total RNA per lane as described (21) and assayed by hybridization to \( ^{32}P \)-labeled probes. The following probes were used: for the H-2K\( ^{b} \) probe, the H-2K\( ^{b} \)-specific 30-mer oligonucleotide, which codes for amino acids 290-299 of the H-2K\( ^{b} \) protein; for the H-2D\( ^{b} \) probe, the H-2D\( ^{b} \)-specific 30-mer oligonucleotide, which codes for amino acids 293-302 of the H-2D\( ^{b} \) protein; for the p50 probe, a 1.5-kbp insert of pBL50 (a gift of S. Ruben, Roche Institute, NJ) (59); for the p65 probe, a 2.0-kbp insert of pBL69 (a gift of S. Ruben) (59); for the \( \beta \)-actin probe, a 4.3-kbp insert of pAC18.1 (a gift of U. Nudel; Weizmann Institute, Rehovot, Israel) (60). Hybridizations were performed in 50% formamide at 42°C. Blots were washed in 0.1 X SSC, 0.1% SDS at 60°C. Blots hybridized to oligonucleotide probes were washed in 0.5 X SSC, 0.1% SDS at 50°C.

Plasmids, Constructs, Antibodies, and Proteins. Plasmids RcCMV-p50\(^{50-100} \), RcCMV-p65\(^{50-100} \) were described previously (53). PUC-365-CAT was prepared by cloning of a BamHI-XbaI restriction fragment from pH-2-CAT (a gift of A. Israel, Pasteur Institute, Paris) (61) into a Puc19 vector. PUC-142-CAT and PUC-190-CAT were subcloned from p138H-2K CAT and p190H-2K CAT (a gift from A. Baldwin, Massachusetts Institute of Technology, Cambridge, MA) (52), respectively, into a Puc19 vector (52). p38-H-2K-CAT (a gift from A. Baldwin) and the \( \beta \) galactosidase-containing expression vector pCH110 (Pharmacia Fine Chemicals, Piscataway, NJ). Recombinant p50 and p65 were prepared by the MAXIMAC baculovirus expression system (a gift of D. Baltimore, The Rockefeller University, New York) (56), and antibodies to KBF1 \( \alpha \)2 and \( \alpha \)3 were a gift of A. Israel (44). IxB recombinant MAD-3 was expressed in bacteria and purified as described (61a). Antibodies to c-rel (a gift of L. Verma, The Salk Institute, San Diego, CA), and antibodies to \( c - \) rel (a gift of M. Hannink, University of Missouri, Columbia, MO) were used.

Preparation of Stable Transfectants. Transfections of H-2K\( ^{b} \), 2.9-K\( ^{R} \), 4.1-K\( ^{R} \), and RcCMV-p65 expression vectors into the high metastatic clone D122 (3LL) and transfection of RcCMV-p50 into the low metastatic clone A9 (3LL) were performed by the calcium phosphate technique, in cotransfection with a 1:9 ratio of PSV\( \kappa \)-neomycin resistance gene (PSV\( \kappa \)-neomycin) (21). Control transfection was done with PSV\( \kappa \)-neo alone. To increase the efficiency of transfection, a 3-min treatment with DME, 15% glycerol was preformed after incubation with DNA precipitates. Colonies growing in 400 \( \mu \)g/ml (D122 cells) and 800 \( \mu \)g/ml (A9 cells) G418 (GIBCO BRL, Gaithersburg, MD) 4 wk after transfection were expanded and analyzed for expression of the inserted genes. Control transfectants carrying PSV\( \kappa \)-neomycin show hybridization patterns, and MHC class I expression equal to parental cells. Positive clones were checked for plasmid DNA integration by Southern blots.

Cell Surface and Cellular H-2 Proteins. To determine cell surface H-2 expression, protein A-purified mAbs 20-8-4 (c\( \kappa \)K\( ^{b} \)) and 28-14-8 (c\( \kappa \)D\( ^{b} \)) (62) were iodinated by chloramine T. 500 ng of labeled antibody was mixed with 5 x 10\(^6\) freshly trypsinized cells in 0.1 ml PBS in BSA-coated tubes. Triplicate samples were incubated...
 DNAs (20/~g of the CAT derivative plus 4/~g of pCH110). Then, counter. Immunoprecipitation of class I proteins from [35S]methio-

Nielson et al. (64). Experiments were repeated at least three times. Schiile et al.

incubated for 12 h with calcium phosphate-precipitated plasmid

processed for enzymatic assays. CAT activity, using 30/zg of total

cell extract protein, was determined as described by Schreiber

stranded 52-bp oligodeoxynucleotide containing the entire enhancer

used in the binding assay were: AP1 (-203 to -190), APIAX

was used as probes. The nonlabeled competitor oligonucleotides

be labeled oligomer. Rabbit anti-pS0 antiserum was added to the nu-

DNAs were added to the binding reactions 2 rain before the la-

When a control antisera was added to the extract. DNA-protein

DNA-protein binding was conducted in 20-/zl volumes. Nu-

No disruption of the nucleoprotein complex binding was observed

DNA-protein complexes were resolved on 4% polyacrylamide gel (acrylamide/bis-

were added to the preincubated nuclear extracts. Unlabeled competitor

No gross mutations, deletions, or rearrangements occurred in the K b genomic clones. Two genomic clones, 2.9-K b and

Restriction analysis with a variety of restriction enzymes indicated that no gross mutations, deletions, or rearrangements occurred in the K b genomic clones. Two genomic clones, 2.9-K b and 4.1-K b, 10.5 kbp in length, which include the entire K b promoter, were subcloned into Bluescript and were used for transfection

DNAs were ligated to predigested X

metabotropic cell surface expression is readily detectable on D122 cells and it is approximately threefold lower on D122 cells as compared with A9 cells (see Figs. 6 D and 7 C) (21). We have shown before that transfection of D122 cells with a plasmid containing the K b gene cloned from a normal genomic library and driven by an autologous promoter yielded K b-

positive clones, provided that a high copy number of the gene was inserted (21). To test whether the D122 endogenous K b gene can be expressed under similar conditions, we cloned the gene from a D122 genomic library (λdash). Restriction analysis with a variety of restriction enzymes indicated that no gross mutations, deletions, or rearrangements occurred in the K b genomic clones. Two genomic clones, 2.9-K b and 4.1-K b, 10.5 kbp in length, which include the entire K b

A

B

Figure 1. H-2K b expression on A9 and D122 cells. (A) Cell surface expression was determined by RIA. (B) RNA and cytoplasmic K b heavy chain in A9 (lanes 1) and D122 (lanes 2) cells. Northern blot and hybridization were performed as described in Materials and Methods. Immunoprecipitation was performed with antibody 20-8-4 as described (4).
and able to be functionally expressed, even in the original D122 cells.

**Differential Activity of Enhancer A in A9 and D122 Cells.** Since the endogenous K\(^b\) promoter can be activated in D122 cells upon transfection of multiple copies of the gene, it seems reasonable to assume that trans-acting silencer factors exist in D122 cells and these are diluted by excess promoter sequences. To test the activity of enhancer elements of the K\(^b\) gene in A9 and D122 cells, deletions of the K\(^b\) promoter were fused to the bacterial CAT gene (Fig. 3). These recombinants were transiently cotransfected into A9 and D122 cells with pCH110, which express the bacterial \(\beta\)-galactosidase under the regulation of the SV40 promoter, and served as an internal control. CAT activity was normalized to \(\beta\)-galactosidase activity for each transfection. Plasmids containing enhancer B, with endpoints at \(-117\) and \(-142\), showed low and similar activities upon transfection into the two cell types (Fig. 3 B). In contrast, the plasmid containing the endpoint at \(-190\) was at least twofold more active in A9 than in D122 cells, and the plasmid containing the endpoint at \(-365\) produced a threefold increase in activity between the two cell types (Fig. 3 B). These data suggest the participation of at least two enhancer elements in the differential regulation of the K\(^b\) gene in D122 cells, one between \(-142\) and \(-190\) and the other between \(-190\) and \(-365\).

**K\(^b\) Promoter DNA Binding Proteins in Nuclear Extracts of A9 and D122 Cells.** To examine the nuclear factors binding to the K\(^b\) promoter, nuclear proteins were prepared from A9 and D122 cells, and examined by electrophoresis mobility shift assay (EMSA),\(^1\) using as a probe the domain \(-365\) to \(+10\). Three main complexes were detected (Fig. 4, B and C). The fastest migrating complex binds to enhancer A only, and is competed efficiently with excess enhancer A (\(-205\) to \(-154\)), AP2X (\(-188\) to \(-169\)), CREX (class I regulatory element) (\(-175\) to \(-154\)), and CRE (\(-172\) to \(-160\)), (Fig. 4, B and C, lanes 1, 8, 4, and 3, respectively), indicating that this complex binds to the NF-\(\kappa B\) domain, CRE. This complex exists at higher levels in nuclear extracts from D122 cells (Fig. 4 C) than extracts from A9 cells (Fig. 4 B). The intermediate band represents proteins binding to enhancer B only, because this protein–DNA complex is competed with an oligomer of enhancer B (\(-123\) to \(-58\)) (Fig. 4, B and C, lane 2) and is similarly expressed in both cell lines. The slowest migrating complex seems to be able to bind both enhancers as it is competed with oligomers comprising either enhancer A or B (Fig. 4, B and C, lanes 1 and 2). This complex is also slightly more abundant in D122 extracts.

To further analyze protein binding to subdomains of the promoter, analysis was repeated with enhancer A as a probe. Six complexes were identified by EMSA (Fig. 4, E and F). Complexes A1 to A5 but not A6 were more abundant in D122 extracts (compare Fig. 4 E, lane 1, to F, lane 8). Competition analysis indicated that A3 and A4 bind to the NF-\(\kappa B\) binding domain and are competed by the minimal 13-bp CRE (Fig. 4 E, lane 5, and F, lane 4); moreover, when CRE is used as a probe, it binds only complexes A3 and A4 (Fig. 4 F, lane 8, and F, lane 1). Complexes A1, A2, and A6 are bound at the domain partially overlapping CRE and AP2, since they are competed by CREX (Fig. 4 E, lane 2, and F, lane 7), by AP2X (Fig. 4 E, lane 4, and F, lane 5), and to some extent with CRE (Fig. 4 E, lane 5, and F, lane 4). A5 binds at the APIA domain and is competed mainly by APIAX (Fig. 4 E, lane 3, and F, lane 6) and APIA (Fig. 4 E, lane 6, and F, lane 3). Analysis of protein binding to enhancer B (\(-123\) to \(-58\)) reveals similar binding proteins in extracts of both clones (Fig. 4 D, lanes 2 and 3). The slowest migrating proteins are competed with oligomers comprising either enhancer A or B (Fig. 4 F, lane 8, and F, lane 1). Complexes A1, A2, and A6 are bound at the domain partially overlapping CRE and AP2, since they are competed by CREX (Fig. 4 E, lane 2, and F, lane 7), by AP2X (Fig. 4 E, lane 4, and F, lane 5), and to some extent with CRE (Fig. 4 E, lane 5, and F, lane 4).

---

Footnote:
---

1 Abbreviation used in this paper: EMSA, electrophoresis mobility shift assay.
quantities and batches of nuclear extracts were used for binding of the different probes, these data indicate that a number of enhancer A binding proteins are more abundant in D122 cells than in A9 cells. Analysis of nuclear extracts with CRE as a probe showed that levels of complex A3 and A4 (NF-κB and KBF1) are similar in A9 cells while D122 cells higher levels of A4 than A3 are detected (Fig. 4 G).

**Characterization of Proteins Bound to κB Motif in the H-2Kb Promoter.** The NF-κB motif in the H-2Kb promoter was shown to bind NF-κB (p50-p65 heterodimer), KBF1 (p50-p50 homodimer), and other related and nonrelated factors. To test whether complexes A3 and A4 comprise one of the known factors, we used anti-KBF1-specific antibodies nos. 2 and 3, which are specific for the p50 homodimer (44). Fig. 5 A shows that incubation of nuclear extracts with antibody no. 3 and to a lesser extent no. 2 shifted the p50 dimer-DNA complex to a slower mobility antibody–KBF1–DNA complex, and eliminated band A4 in EMSA (Fig. 5 A, lanes 3, 4, 7, and 8). An unrelated antibody did not change the mobility of the p50 homodimers (Fig. 5 A, lanes 2 and 6). Again the differential quantitative levels of KBF–DNA binding activity are obvious between the two cell types. Further support for the A4 complex containing a p50 homodimer is shown in Fig. 5 B. Recombinant shortened p50, which migrates slightly faster than p50 from D122 cells, reacted similarly with the no. 3 antibody to the cellular p50 homodimer (Fig. 5 B, compare lanes 5 and 4 to 2 and 1). Complex A4 was unaffected by addition of human recombinant MAD-3 (Fig. 5 B, lane 6), an IκB protein cloned from human macrophages (66). This indicates the absence of p65 or c-Rel from the A4 complex.

Recombinant p65 (Fig. 5 B, lane 3) could only weakly bind to the class I κB motif. Incubation of D122 nuclear extracts with recombinant MAD-3 eliminated complex A3 (Fig.
5 B, lanes 5 and 6). Although MAD-3 was shown to bind both p65 and c-rel, complex A3 was not shifted or eliminated by specific c-rel or v-rel antibodies (not shown), indicating that this complex represents the NF-κB p50-p65 heterodimer. In conclusion, higher levels of KBF1 are found in nuclear extracts of D122 cells as compared with A9 cells. In D122 nuclear extracts, KBF1 seems to be in excess over NF-κB while in A9 extracts NF-κB is in some excess over KBF1.

Stable Expression of p50 Suppresses Class I Expression while Stable Expression of p65 Increases Class I Expression. The data, so far, suggested that the ratio and relative amounts of KBF1 and NF-κB may regulate the expression of H-2K\(^b\) in the two cell lines. Northern blot analysis showed that p105 mRNA levels are lower in A9 than in D122 cells (Fig. 6 A). To test whether increased KBF1 levels affect K\(^b\) transcription, we have stably transfected A9 cells with CMV-pS01-s\(\alpha\) (53). By only expressing the NH\(_2\)-terminal 502 amino acids of the KBF1 precursor p105, we could circumvent the negative control imposed by the IxB\(\gamma\) contained in the COOH-terminal portion of p105. Clones expressing the truncated p105 mRNA showed, in addition to a novel shorter mRNA, elevated levels of the p105 mRNA (Fig. 6 B). This is in accordance with the observation that p50 can upregulate its own transcription (67). Thus, p50 may act as an inducer of transcription in variant κB sequences as those in the promoter of p105. The same clones, however, show the same p65 mRNA levels as parental A9 cells (Fig. 6 B), indicating that the balance between p50 and p65 subunits of NF-κB, rather than absolute amounts, controls class I expression. Three clones overexpressing p50 mRNA showed a threefold decrease in K\(^b\)-specific mRNA levels (Fig. 6 B) and revealed strongly increased KBF1 DNA binding activity in EMSA (Fig. 6 C). These cells also showed a two- to threefold decreased cell surface expression of the H-2K\(^b\) antigen (Fig. 6 D, left). Since the H-2D\(^b\) promoter also contains an NF-κB site, we tested whether A9-p50 transfectants are downregulated in H-2D\(^b\) expression; a 20–40% decrease was observed for the three (Fig. 6 D, right). To test whether overexpression of p65 might
Characterization of xB domain binding proteins. (A) A modified CRE probe (-172 to -148) was incubated with nuclear extracts of A9 (lanes 1-4) and D122 (lanes 5-8) in the presence of: no antibody (lanes 1 and 5); anti-H-2D\(^b\) (lanes 2 and 6); anti-p50 (c2) (lanes 3 and 7); anti-p50 (c3) (lanes 4 and 8). (B) CRE probe was incubated with nuclear extracts from D122 cells (lanes 2, 5, and 6) with recombinant p65 (lane 3); recombinant p50 (lanes 4) recombinant p50 and c~3 antibody (lane 1); nuclear extract and c~3 antibody (lane 2); and nuclear extract and recombinant IxB (MAD-3) (lane 6). The mixtures were analyzed by EMSA.

Discussion

The downregulation of MHC class I expression in malignant cells has been shown to be an important mechanism for tumor escape from immune surveillance. In this study the molecular basis underlying class I deficiency was investigated by comparing a high K\(^b\) expresser clone to a low expresser K\(^b\) clone. We have first demonstrated that the endogenous H-2K\(^b\) gene is structurally intact and can be expressed in the parental D122 cells similarly to a normal genomic clone, provided multiple copies of the gene are inserted (Fig. 2). A similar conclusion was reached in some other tumor systems. For example, the IC9 clone of the T10 sarcoma (H-2\(^d\) x H-2\(^k\)) does not express K\(^b\), K\(^k\), and D\(^k\) antigens, however, the cloned K\(^b\) gene from these cells could be expressed in Ltk\(^-\) cells and also in the original IC9 cell line from which it was derived (68). Similarly, the K\(^b\) gene isolated from nonexpressor cells, embryonal carcinoma PCC4-aza-R1, was functionally expressed when transfected into differentiated cells (69).

We have further shown that suppression of the K\(^b\) gene is at the transcriptional level; low RNA levels correlated with the low amount of H-2K\(^b\) protein in the cytosol and on the cell surface of nonexpressor cells (Fig. 1). CAT assays, using various deletions mutants of the K\(^b\) promoter region, indicated that the domain downstream to nucleotides -142, which encompasses enhancer B (containing TATA and CAAT boxes), is similarly activated in expresser and in nonexpresser cells (Fig. 3). Moreover, nuclear extracts of the two cell lines revealed similar factors binding to enhancer B at similar concentrations (Fig. 4, B-D). This region, however, shows very low activity in A9 and D122 cells. Similar results were obtained with constructs containing enhancer B alone in BALB/c 3T3 cells and in Ltk\(^-\) cells (25, 52). CAT constructs containing enhancers A and B show differential activities in A9 and D122 cells; a twofold activation is observed in the p190 CAT that contains the NF-\(\kappa B\) binding motif, and a threefold activation is observed in the activity of the p365 CAT construct between the two clones (Fig. 3). The active element upstream of the NF-\(\kappa B\) is probably a CREB/ATF/AP1-like binding motif between -210 and -188. Purified AP1, cyclic adenosine monophosphate-inducible transcription factors, as well as a member of the nuclear hormone receptor superfamily, H-2RIIBP, were shown to bind this motif (24, 36, 70). We have shown before that A9 cells expressed higher levels of c-fos and c-jun than D122 cells and that stable transfectants of either c-fos alone or c-fos + c-jun expression vectors increased H-2 expression (71, 72). Transient transfections of CAT constructs into c-fos + c-jun D122 over expressers showed high activation of the p365 and low activation of the p190 constructs, indicating also participation of an API complex in upregulation of class I in these cells (Yamit-Hezi, A., D. Plaksin, and L. Eisenbach, manuscript submitted for publication).

Gel shift analysis indicated that some enhancer A binding proteins were more abundant in nuclear extracts from D122 as compared with nuclear extracts from A9 cells (Fig. 4, E and F). Most prominent were complexes A3 and A4, which bind specifically to minimal NF-\(\kappa B\) domain -172 to -160 (Fig. 4 E, lane 8, and F, lane 1). By the use of specific anti-p50 antibodies, which react only with the p50 homodimer KBF1, and recombinant IxB (MAD-3), which binds to the
Figure 6. Effect of p50 transfection on K b expression in A9 cells. (A) Endogenous mRNA levels of p50 precursor (p105), in D122 (lane 1) and A9 (lane 2) cells. β-actin was used as control for equal amounts of RNA. (B) mRNA levels of p65, p50, p105, K b, and β-actin of A9 (lane 1) and A9-p50 transfectant. (lane 2) (C) A 32p CRE probe was incubated with nuclear extracts of A9 (lane 1) and A9-p50 transfectants (lanes 2–4). The mixtures were analyzed by EMSA. (D) H-2K b and H-2D b cell surface expression of A9 and A9-p50 transfectants: H-2K b (left) and H-2D b (right). Cell surfaces were analyzed by RIA as described in Materials and Methods.

p65 subunit and inhibiting binding of NF-κB complex to the DNA, we found that complex A3 is NF-κB and complex A4 represents KBF1 (Fig. 5). c-rel and v-rel antibodies did not react with any of the complexes, indicating that p65 and p50 are probably the only subunits in A3 and A4 complexes. The fact that both NF-κB and KBF1 activities are increased in D122 cells is in apparent discrepancy with a number of studies that showed a correlation between class I expression and activity of both KBF1 and NF-κB (73, 74). However, recently it was demonstrated that the presence of NF-κB but not KBF1 seems necessary for class I expression in a variety of human tumor cell lines (75), suggesting that it is the balance between NF-κB and KBF1 binding that might be the determinant for expression of K b in 3LL clones. In this study, we therefore transfected K b expresser A9 cells with a p50 expression vector, and low expresser (D122) cells with a p65 expression vector. Elevated K b mRNA and cell surface protein in D122-p50 transfectants correlated exclusively with binding of NF-κB to the A enhancer motif (Fig. 7). On the other hand, increased KBF1 binding in A9-p50 transfectants correlated with decreased K b mRNA and cell surface expression (Fig. 6).

Controversial results were obtained in various studies as to the role of KBF1 in activation of κB-controlled promoters. KBF1 was implicated in the constitutive basal expression of the MHC class I (36) and TNF-α gene in murine macrophages (76). Recombinant p50 and p65 subunits of NF-κB were shown to independently activate transcription in vitro from various κB motifs (56, 57). In contrast, transient coexpression of p50 with κB-dependent reporter constructs in transient CAT assays indicated that (p50) 2 suppresses the transactivation by NF-κB in monkey COS and mouse L cells (53). Similarly, transient cotransfection of excess p50 with low amounts of p65 expression vectors decreased expression of HIV-κB CAT reporter (77). In nontransformed CD4 + T lymphocytes, antigenic stimulation, which upregulates IL-2 gene expression, decreased the levels of KBF1 and increased the levels of NF-κB, indicating a physiological function for (p50) 2 homodimers in suppression of IL-2 expression (55). Another study using cell hybrids containing only KBF1 but not active NF-κB did not activate the IL-2 receptor κB enhancer (78). Similarly, coexpression of (p50) 2 with a reporter plasmid containing HIV-1 κB motif caused no activation of the reporter gene (54). Unlike KBF1, homodimers of p65 clearly have trans-activating function although it is not clear whether p65 homodimers actually exist in intact cells (53). Moreover, (p65) 2 homodimers have a reduced affinity for all κB motifs tested so far (56). Also, a heterodimer of p50 and
p65 (NF-κB) showed activating properties both in vitro and in vivo (53, 55–57). It is surprising, however, that the low expresser clone D122 shows higher levels of NF-κB than the high expresser clone A9. A possible explanation may reside in the relative affinities of NF-κB and KBF1 in the H-2 κB motif. Recently it was shown that KBF1 has a higher affinity to the H-2-κB motif than NF-κB, with $K_d$ values of 6.2 and 10.9 pM, respectively (56). KBF1, which is highly expressed in D122 nuclear extracts, might therefore prevent binding of trans-activating NF-κB by occupying κB binding sites. Transfection of multiple copies of the κB promoter may dilute the excess of KBF1 and enable activation of κB expression. Elevating the concentration of NF-κB by overexpressing p65 has a similar effect on activation of κB expression.

In conclusion, this study demonstrates that KBF1 might have an inhibitory function on class I promoters containing κB motif in vivo, and that the balance between the p50 and p65 subunits of NF-κB is crucial for class I expression.

Although the K\textsuperscript{b} gene is barely expressed in D122 cells, the H-2D\textsuperscript{b} gene is expressed at detectable levels. In A9 cells the D\textsuperscript{b} gene is expressed at approximately threefold the density than it is on D122 cells. How can these allele-specific differences between K\textsuperscript{b} and D\textsuperscript{b} expression in D122 cells be explained? Comparison of promoter sequences in the two alleles showed that enhancer A is highly homologous between the two promoters. Indeed, transcription by p65 elevated also D\textsuperscript{b} expression and transfection by p50 suppressed partially D\textsuperscript{b} expression. Enhancer B on the other hand shows 14/66 differences in nucleotide sequences (25, 79). It is conceivable that different factors may bind to enhancer B and affect differentially basal level expression of class I alleles. Such differences were observed in EMSA using enhancer B sequences of K\textsuperscript{b} and D\textsuperscript{b} and nuclear extracts from A9 and D122 cells (R. Goodenow, personal communication). Allele-specific down-regulation of class I alleles was reported in several human tumors (80–84). It will be of interest to further investigate mechanisms involved in allele-specific regulation of class I genes.
We thank Professor Michael Feldman for his continuous advice and encouragement; D. Baltimore for the kind gifts of recombinant p50 and p65; S. Ruben for generously providing pBL50 and pBL65 constructs; A. Israel for supplying the pH-2-CAT construct and antibodies to KBFI; A. Baldwin for the p138H-2K-CAT, p38H-2K-CAT, and p190H-2K-CAT constructs; I. Verma for generously providing antibodies to c-myc; and M. Hannink for providing the antibodies to v-rel.

This work was supported by the Mildred Schell foundation (Bonn, Germany), and by a grant from the National Cancer Institute (CA-28139).

Address correspondence to Lea Eisenbach, Department of Cell Biology, Weizmann Institute of Science, Rehovot 76100, Israel.

Received for publication 4 December 1992 and in revised form 16 February 1993.

References
1. Maryanski, J.L., P. Pala, G. Corradin, B.R. Jordan, and J.C. Cerrutini. 1986. H-2 restricted cytolytic T cells specific for HLA can recognize a synthetic HLA peptides. Nature (Lond.) 324:578.
2. Townsend, A., J. Rothbard, F. Gotch, G. Bahadur, D. Wraith, and J. McMichael. 1986. The epitopes of influenza nucleoprotein recognized by cytotoxic T lymphocytes can be defined with short synthetic peptides. Cell. 44:959.
3. Isakov, N., S. Katzav, M. Feldman, and S. Segal. 1983. Loss of expression of transplantation antigens encoded by the H-2 locus on Lewis lung carcinoma cells and its relevance to the tumor’s metastatic properties. J. Nall. Cancer Inst. 71:139.
4. Eisenbach, L., S. Segal, and M. Feldman. 1983. MHC imbalance and metastatic spread in Lewis lung carcinoma clones. Int. J. Cancer. 32:113.
5. De Batselier, P., S. Katzav, E. Gorelik, M. Feldman, and S. Segal. 1980. Differential expression of H-2 gene products of tumor cells in tumor cells is associated with their metastatic properties. Nature (Lond.) 280:179.
6. Bernards, R., P.I. Schrier, A. Houweling, J. Bos, A.J. Van der Eb, M. Zijlstra, and C.J.M. Melief. 1983. Tumorigenicity of cells transformed by adenovirus type 12 by evasion of T-cell immunity. Nature (Lond.). 305:776.
7. Schrier, P.I., R. Bernards, R. Vaessen, A. Houweling, and A.J. Van der Eb. 1983. Expression of class I major histocompatibility antigens switched off by highly oncogenic adenovirus type 12 in transformed rat cells. Nature (Lond.). 305:771.
8. Schmidt, W., and H. Festenstein. 1982. Resistance to cell-mediated cytotoxicity is correlated with reduction of H-2K gene products in AKR leukemia. Immunogenetics. 16:257.
9. Doyle, A., W. Martin, K. Funa, A. Gazdar, O. Carney, E. Martin, I. Linnoila, F.F. Cuttitta, J. Mushein, P. Brun, and L. Minna. 1985. Markedly decreased expression of class I histocompatibility antigens, protein, and mRNA in human small cell lung cancer. J. Exp. Med. 161:1135.
10. Lampson, A.L., C.A. Fisher, and J. Whelan. 1983. Striking paucity of HLA-A, B, C and β2-microglobulin on human neuroblastoma cell lines. J. Immunol. 130:2471.
11. Holden, C.A., A.R. Sanderson, and D. MacDonald. 1983. Absence of human leukocyte antigen molecules in skin tumors and some cutaneous appendages: evidence using monoclonal antibodies. J. Am. Acad. Dermatol. 9:867.
12. Sanderson, A.R., and P.C.L. Beverly. 1983. Interferon, β2-microglobulin and immunoselection in the pathway to malignancy. Immunol. Today. 4:211.
13. Masucci, M.G., S. Torstein dotter, J. Colombani, E. Klein, and G. Klein. 1987. Down regulation of class I HLA antigens of the Epstein Barr virus-encoded latent membrane protein in Burkitt lymphoma lines. Proc. Natl. Acad. Sci. USA. 84:4567.
14. Cordon-Cardo, C., Z. Fuks, M. Drobnjak, C. Moreno, L. Eisenbach, and M. Feldman. 1991. Expression of HLA-A, B, C antigens on primary and metastatic tumor cell populations of human carcinomas. Cancer Res. 51:6372.
15. Eisenbach, L., N. Hollander, L. Greenfeld, H. Yakor, S. Segal, and M. Feldman. 1984. The differential expression of H-2K versus H-2D antigens distinguishing low-metastatic from high metastatic clones is correlated with the immunogenic properties of the tumor cells. Int. J. Cancer. 34:567.
16. Plaksin, D., C. Gelber, and L. Eisenbach. 1992. H-2Dδ gene transfer into high metastatic D122 cells results in tumor rejection in allogeneic recipients, but does not affect metastasis in syngeneic recipients. Implications to mechanisms of alloregulation. Int. J. Cancer. 52:771.
17. Eisenbach, L., and M. Feldman. 1985. Genes and antigens controlling tumor metastases. In Hematology and Blood Transfusion: Modern Trends in Human Leukemia IV. Vol. 29. R. Neth, R. Calla, M. Greaves, and A. Jenkins, editors, Springer-Verlag, Berlin. 449–507.
18. Eisenbach, L., N. Hollander, S. Segal, and M. Feldman. 1985. The differential expression of class I MHC antigens controls the metastatic properties of tumor cells. Transplant. Proc. 17:729.
19. Eisenbach, L., S. Katzav, and M. Feldman. 1986. Immunomodulation of human metastases. In New Experimental Modalities in the Control of Neoplasia. P. Chandra, editor. Plenum Publishing Corporation. New York. 57–70.
20. Eisenbach, L., G. Kushtai, D. Plaksin, and M. Feldman. 1986. MHC genes and oncogenes controlling the metastatic phenotype of tumor cells. Cancer Res. 51:1.
21. Plaksin, D., C. Gelber, M. Feldman, and L. Eisenbach. 1988. Reversal of the metastatic phenotype in Lewis lung carcinoma cells after transfection with syngeneic H-2Kδ gene. Proc. Natl. Acad. Sci. USA. 85:4463.
22. van Bleek, G.M., and S.G. Nathenson. 1992. Presentation of antigenic peptides by HLA class I molecules. Trends Cell Biol. 2:202.
23. Braciale, T.J., and V.L. Braciale. 1991. Antigen presentation: structural themes and functional variations. Immunol. Today. 12:124.
24. Korber, B., N. Mermod, L. Hood, and I. Stroynowski. 1988.

1660 KBFI Acts as a Repressor of H-2Kδ Gene Expression
Regulation of gene expression by interferons: control of H-2 promoter responses. Science (Wash. DC). 239:1302.

25. Kimura, A., A. Israel, O. Le Bail, and P. Kourilsky. 1986. Detailed analysis of the mouse H-2K\textsuperscript{b} promoter: enhancer-like sequences and their role in the regulation of class I gene expression. Cell. 44:261.

26. Israel, A., A. Kimura, A. Fournier, M. Fellous, and P. Kourilsky. 1986. Interferon response sequence potentiates activity of an enhancer in the promoter region of a mouse H-2 gene. Nature (Lond.). 322:743.

27. Yano, O., J. Kannellopoulos, M. Kieran, O. Le Bail, A. Israel, and P. Kourilsky. 1987. Purification of KBF1, a common factor binding to both H-2 and \( \beta_{2} \)-microglobulin enhancers. EMBO (Eur. Mol. Biol. Organ.) J. 6:3317.

28. Israel, A., O. Yano, F. Logeat, M. Kieran, and P. Kourilsky. 1989. Two purified factors bind to the same sequence in the enhancer of mouse MHC class I genes: one of them is a positive regulator induced upon differentiation of teratocarcinoma cells. Nucleic Acids Res. 17:5245.

29. Kanno, M., C. Fromental, A. Staub, P. Ruffenach, I. Davidson, and P. Chambon. 1989. The SV40 TC-II(KB) and the related H-2K\textsuperscript{b} enhancers exhibit different cell type specific and inducible proto-enhancer activities, but the SV40 core sequence and the AP-2 binding site have no enhancer properties. EMBO (Eur. Mol. Biol. Organ.) J. 8:4205.

30. Macchi, M., J.M. Borrett, I. Davidson, M. Kanno, R. Rosales, M. Vigneron, J.H. Xiao, C. Fromental, and P. Chambon. 1989. The SV40 TC-II(KB) enhancer binds ubiquitous and cell type specifically inducible nuclear proteins from lymphoid and non-lymphoid cell lines. EMBO (Eur. Mol. Biol. Organ.) J. 8:4215.

31. Singh, H., J.H. LeBowitz, A.S. Baldwin, and P.A. Sharp. 1988. Molecular cloning of an enhancer binding protein: isolation by screening of and expression library with a recognition site DNA. Cell. 52:415.

32. Katoh, S., K. Ozawa, S. Kondoh, E. Soeda, A. Israel, K. Shirokii, K. Fujinaga, K. Itsuka, G. Gachelin, and K. Yokoyama. 1990. Identification of sequences responsible for positive and negative regulation by EIA in the promoter of H-2K\textsuperscript{b} class I MHC gene. EMBO (Eur. Mol. Biol. Organ.) J. 9:1217.

33. Hamada, K., S.L. Gleason, B.Z. Levi, S. Hirschfeld, E. Appella, and K. Ozato. 1989. H-2RJIBP, a member of the nuclear hormone receptor superfamily that binds to both the regulatory element of major histocompatibility class I genes and the estrogen response element. Proc. Natl. Acad. Sci. USA. 86:8289.

34. Korber, B., L. Hood, and I. Stroyownik. 1987. Regulation of murine class I genes by interferons is controlled by regions located both 5' and 3' to the transcription initiation site. Proc. Natl. Acad. Sci. USA. 84:3380.

35. Israel, A., A. Kimura, M. Kieran, O. Yano, J. Kannellopoulos, O. Le Bail, and P. Kourilsky. 1987. A common positive transacting factor binds to enhancer sequences in the promoters of mouse H-2 and \( \beta_{2} \)-microglobulin genes. Proc. Natl. Acad. Sci. USA. 84:2653.

36. Israel, A., O. Le Bail, D. Hatat, J. Piette, M. Kieran, F. Logeat, D. Wallach, M. Fellous, and P. Kourilsky. 1989. TNF stimulates expression of mouse MHC class I genes by inducing an NFkB-like enhancer binding activity which displaces constitutive factors. EMBO (Eur. Mol. Biol. Organ.) J. 8:3793.

37. Baldwin, A.S., and P.A. Sharp. 1988. Two transcription factors, NF-\( \kappa \)B and H2TIF1, interact with a single regulatory sequence in the class I major histocompatibility complex promoter. Proc. Natl. Acad. Sci. USA. 85:723.

38. Baeuerle, P.A., and D. Baltimore. 1989. A 65-kD subunit of active NF-\( \kappa \)B is required for inhibition of NF-\( \kappa \)B by IxB. Genes & Dev. 3:1689.

39. Urban, M.B., R. Schreck, and P.A. Baeuerle 1991. NF-\( \kappa \)B contacts DNA by a heterodimer of the p50 and p65 subunit. EMBO (Eur. Mol. Biol. Organ.) J. 10:1817.

40. Nolan, G.P., S. Ghosh, H.C. Liou, P. Tempst, and D. Baltimore. 1991. DNA binding and IxB inhibition of the cloned p65 subunit of NF-\( \kappa \)B, a rel-related polypeptide. Cell. 64:961.

41. Baeuerle, P.A., and D. Baltimore. 1988. I kappa B: a specific inhibitor of the NF-kappa B transcription factor. Science (Wash. DC). 242:540.

42. Baeuerle, P.A. 1991. The inducible transcription activator NF-\( \kappa \)B: regulation by distinct protein subunits. Biochim. Biophys. Acta. 1072:63.

43. Blank, V., P. Kourilsky, and A. Israel. 1992. NF-kappa B and related proteins: rel/dorsal homologues meet ankyrin-like repeats. Trends Biochem. Sci. 17:135.

44. Kieran, M., V. Blank, F. Logeat, J. Vandekerckhove, F. Lottepeich, O. Le Bail, P.A. Baeuerle, and A. Israel. 1990. The DNA binding subunit of NF-\( \kappa \)B is identical to factor KBF1 and homologous to the rel oncogene protein. Cell. 62:1007.

45. Ghosh, S., A.M. Gifford, L.R. Riverie, P. Tempst, G.P. Nolan, and D. Baltimore. 1990. Cloning of the p50 DNA binding subunit of NF-\( \kappa \)B: homology to rel and dorsal. Cell. 62:1019.

46. Urban, M.B., and P.A. Baeuerle. 1991. The role of the p50 and p65 subunits of NF-\( \kappa \)B in the recognition of cognate sequences. New Biol. 3:279.

47. Liou, H.C., G.P. Nolan, S. Ghosh, T. Fujita, and D. Baltimore. 1992. The NF-\( \kappa \)B p50 precursor, p105, contains an internal IxB-like inhibitor that preferentially inhibits p50. EMBO (Eur. Mol. Biol. Organ.) J. 11:3003.

48. Henkel, T., U. Zabel, K. van Zee, J.M. Muller, E. Fanning, and P.A. Baeuerle. 1992. Intramolecular masking of the nuclear location signal and dimerization domain in the precursor for the p50 NF-\( \kappa \)B subunit. Cell. 68:1121.

49. Inoue, J., L.D. Kerr, A. Kakizaka, and J.M. Verma. 1992. IxB\( \alpha \), a 70 kd protein identical to the c-terminal half of p10 NF-\( \kappa \)B: a new member of the IxB family. Cell. 68:1109.

50. Burke, P.A., S. Hirschfeld, Y. Shirayoshi, J.W. Kasik, K. Hamada, E. Appella, and K. Ozato. 1989. Developmental and tissue-specific expression of nuclear proteins that bind the regulatory element of the major histocompatibility complex class I gene. J. Exp. Med. 169:1309.

51. Zachow, K.R., and H.T. Orr. 1989. Regulation of HLA class I transcription in T cells. J. Immunol. 143:3385.

52. Baldwin, A.S., and P.A. Sharp. 1987. Binding of a nuclear factor to a regulatory sequence in the promoter of the mouse H-2K\textsuperscript{b} class I major histocompatibility gene. Mol. Cell. Biol. 7:305.

53. Schmitz, M.L., and P.A. Baeuerle. 1991. The p65 subunit is a positive and negative regulation by E1A in the promoter of the mouse H-2K\textsuperscript{b} class I MHC gene. EMBO (Eur. Mol. Biol. Organ.) J. 10:3805.

54. Schmid, R.M., N.D. Perkins, C.S. Duckett, P.C. Andrews, and G.J. Nabel. 1991. Cloning of an NF-KB subunit which regulates the expression of nuclear proteins that bind the regulatory element of the major histocompatibility complex class I gene. J. Immunol. 143:3385.

55. Kang, S.M., A.C. Tran, M. Grilli, and M.J. Lenardo. 1992. NFkB subunit regulation in nontransformed CD4\textsuperscript{+} T lymphocytes. Science (Wash. DC). 256:1452.

56. Fujita, T., G.P. Nolan, S. Ghosh, and D. Baltimore. 1992. Independent modes of transcriptional activation by the p50 and p65 subunits of NF-\( \kappa \)B. Nature (Lond.). 352:733.
p65 subunits of NF-κB. *Genes & Dev.* 6:775.

57. Kretzschmar, M., M. Meisterernst, C. Scheiderer, G. Li, and R.G. Roeder. 1992. Transcriptional regulation of the HIV-1 promoter by NFκB in vitro. *Genes & Dev.* 6:761.

58. Chirgwin, J.M., A.G. Przyblyla, R.J. MacDonald, and W.J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonucleic acid. *Biochemistry.* 18:5294.

59. Ruben, S.M., P.J. Dillon, D. Schrecker, T. Henkel, C. Chen, W.M. Major, P.A. Baeuerle, and C.A. Rosen. 1991. Isolation of a rel-related human cDNA that potentially encodes the 65-kD subunit of NFκB. *Science (Wash. DC).* 251:1490.

60. Nudel, U., R. Zakut, M. Shani, S. Neuman, Z. Levi, and D. Yaffe. 1983. The nucleotide sequence of the rat cytoplasmic β actin gene. *Nucleic Acids Res.* 11:1759.

61. Daniel-Vedele, F., A. Israel, C. Benicourt, and P. Kourilsky. 1985. Functional analysis of the mouse H-2Kb gene promoter in embryonal carcinoma cells. *Immunogenetics.* 21:601.

62. Ozato, K., and D.H. Sachs. 1981. Monoclonal antibodies reacting to antigens of the H-2 haplotypes reveal genetic control of isotype expression. *J. Immunol.* 125:317.

63. Schütte, R., M. Muller, C. Kaltschmidt, and R. Renkawitz. 1988. Many transcription factors interact synergistically with steroid receptors. *Science (Wash. DC).* 242:1418.

64. Nielsen, D.A., J. Chou, A.J. Mackrell, M.J. Casabdown, and D.F. Steiner. 1983. Expression of a preinsulin β-galactosidase gene fusion in mammalian cells. *Proc. Natl. Acad. Sci. USA.* 80:5198.

65. Schreiber, E., P. Matthias, M. Muller, and W. Schaffner. 1989. Rapid detection of octamer binding proteins with 'mini-extracts', prepared from a small number of cells. *Nucleic Acids Res.* 17:6419.

66. Haskill, S., A.A. Beg, S.M. Tompkins, J.S. Morris, A.D. Yurochko, A. Sampson-Johannes, K. Mondal, P. Ralph, and J.A.S. Baldwin. 1991. Characterization of an immediate-early gene induced in adherent monocytes that encodes IkB-like activity. *Cell.* 65:1281.

67. Ten, R.M., C.V. Paya, N. Israel, O. Le Bail, M.G. Mattei, J.L. Virelizier, P. Kourilsky, and A. Israel. 1992. The characterization of the promoter of the gene encoding the p50 subunit of NFκB indicates that it participates in its own regulation. *EMBO (Eur. Mol. Biol. Organ.)* J. 11:195.

68. Maschek, U., W. Pulm, S. Segal, and G.J. Hammerling. 1989. Major histocompatibility complex class I genes in murine fibrosarcoma IC9 are down regulated at the level of the chromatin structure. *Mol. Cell. Biol.* 9:3136.

69. Daniel-Vedele, F., D. Morello, C. Benicourt, C. Transy, O. Le Bail, F. Plata, and P. Kourilsky. 1984. Functional expression of a mouse H-2Kb gene isolated from non-expressing teratocarcinoma cells. *EMBO (Eur. Mol. Biol. Organ.)* J. 3:597.

70. Nagata, T., J.H. Segars, B.Z. Levi, and K. Ozato. 1992. Retinoic acid-dependent transactivation of major histocompatibility complex class I promoters by the nuclear hormone receptor H-2RIIBP in undifferentiated embryonal carcinoma cells. *Proc. Natl. Acad. Sci. USA.* 89:937.

71. Kushtai, G., J. Barzilay, M. Feldman, and L. Eisenbach. 1988. The c-fos proto-oncogene in murine 3LL carcinoma clones controls the expression of MHC genes. *Oncogene.* 2:119.

72. Kushtai, G., M. Feldman, and L. Eisenbach. 1990. c-fos Transfection of 3LL tumor cells turns on MHC gene expression and consequently reduces their metastatic competence. *Int. J. Cancer.* 45:1131.

73. Blanchet, O., J.F. Bourge, H. Zinzsnzer, Z. Tetali, L. Degos, and P. Paul. 1991. DNA binding of regulatory factors interacting with MHC-class-I gene enhancer correlates with MHC-class-I transcriptional level in class-I-defective cell lines. *Int. J. Cancer.* 6:138.

74. Hesseling, U., W. Schmidt, H.R. Scholer, P. Gruss, and A.K. Hatzopoulos. 1990. A transcription factor interacting with the class I gene enhancer is inactive in tumorigenic cell lines which suppress major histocompatibility complex class I genes. *Mol. Cell. Biol.* 10:4100.

75. Collart, M.A., P.A. Baueurle, and P. Vassali. 1990. Regulation of tumor necrosis factor alpha transcription in macrophages: involvement of four κB-like motifs and constitutive and inducible forms of NF-κB. *Mol. Cell. Biol.* 10:1498.

76. Franzoso, G., V. Bours, S. Park, M. Tomita-Yamaguchi, K. Kelly, and U. Siebenlist. 1992. The candidate oncoprotein Bel-3 is an antagonist of p50/NF-κB-mediated inhibition. *Nature (Lond.).* 359:339.

77. Hemar, A., P. Barrand, M. Ferrer, V. Cornet, G. Buttin, and A. Dautry-Varsat. 1991. Activity of the κB enhancer of the interleukin-2 receptor a chain in somatic cell hybrids is accompanied by the nuclear localization of NF-κB. *New Biol.* 3:1097.

78. Wats, S., J.M. Vogel, W.D. Harriman, T. Itoh, H.J. Stauss, and R.S. Goodenow. 1987. DNA sequence analysis of the C3H H-2Kb and H-2Dd loci. *J. Immunol.* 139:3878.

79. Natali, P.G., M.R. Nicotra, A. Bigotti, I. Venturo, L. Marcenaro, P. Giacomini, and C. Russo. 1989. Selective changes in expression of HLA class I polymorphic determinants in human solid tumors. *Proc. Natl. Acad. Sci. USA.* 86:6719.

80. Rees, B.C., A.M. Buckle, K. Gelsthorpe, V. Janes, C.W. Potter, K. Roger, and G. Jacob. 1988. Loss polymorphic A and B locus HLA antigens in colon carcinoma. *Br. J. Cancer.* 57:374.

81. Smith, M.E.F., W.F. Bodmer, and J.G. Bodmer. 1988. Selective loss of HLA-A, B, C locus products in colorectal adenocarcinoma. *Lancet.* 8589:824.

82. Momburg, F., A. Ziegler, J. Harpecht, P. Moller, G. Moldenhauer, and G.J Hammerling. 1989. Selective loss of HLA-A or HLA-B antigen expression in colon carcinoma. *J. Immunol.* 142:352.

83. Lopez-Nevo, M.A., F. Esteban, A. Ferrone, J. Gutierrez, M.R. Oliva, C. Romero, C. Huelin, F. Ruiz-Cabello, and F. Garrido. 1989. HLA class I gene expression on human primary tumors and autologous metastases: demonstration of selective losses of HLA antigens on colorectal, gastric and laryngeal carcinomas. *Br. J. Cancer.* 59:221.