JOURNAL OF VIROLOGY, Apr. 1996, p. 2387–2393 Vol. 70, No. 4
0022-538X/96/$04.00+0

Transcription of the JC Virus Archetype Late Genome: Importance of the κB and the 23-Base-Pair Motifs in Late Promoter Activity in Glial Cells

RAVI P. REDDY MAYREDDY, MAHMUT SAFAK, MARJANEH RAZMARA, PHILIP ZOLTICK, AND KAMEL KHALILI*

Molecular Neurovirology, Jefferson Institute of Molecular Medicine, Department of Biochemistry and Molecular Biology, Thomas Jefferson University, Philadelphia, Pennsylvania 19107

Received 14 September 1995/Accepted 12 January 1996

The transcription control region of the archetype strain of the human polyomavirus JC virus (JCV_{Cy}), unlike its neurotropic counterpart (JCV_{Mad-1}), contains only one copy of the 98-bp enhancer/promoter repeat with the 23-bp and the 66-bp insertion blocks. Early studies by us and others have indicated that the structural organization of JCV_{Mad-1} is critical for glial cell-specific transcription of the viral genome. In addition, the κB regulatory motif found in the JCV_{Mad-1} genome, which also exists in JCV_{Cy}, confers inducibility to the JCV_{Mad-1} early and late promoters in response to extracellular stimuli. In this study, we have investigated the regulatory role of the 23- and the 66-bp blocks and their functional relationship to the κB motif in stimulating transcription of the Cy early and late promoters in glial cells. We demonstrate that mutations in the κB motif reduce the basal activity of the Cy early promoter and decrease the levels of its induction by phorbol myristate acetate or factors derived from activated T cells. Under similar circumstances, mutation in the κB motif completely abrogated the basal and the induced levels of transcription of the viral late promoter. Using deletion and hybrid promoter constructs, we have demonstrated that the 23-bp block of the Cy promoter plays a critical role in the observed inactivation of Cy late promoter transcription in glial cells. Results from DNA binding studies have indicated the formation of a common nucleoprotein complex with the 23-bp sequence, mutant κB ($κB^{mut}$), and wild-type κB ($κB^{wt}$). Analysis of this complex by UV cross-linking has identified a 40-kDa protein which binds to the 23-bp sequence and the κB motif. The importance of these findings for the activation of JCV_{Cy} under various physiological conditions is discussed.

JC virus (JCV), a papovavirus which is widespread in the human population, is the etiologic agent of the demyelinating disease progressive multifocal leukoencephalopathy (PML) (26, 31; for reviews, see references 11 and 18). PML is an opportunistic disease that affects patients with impaired immune systems due to various illnesses, immunosuppressive therapeutic treatments, and genetic disorders. The structural organization of the JCV genome, isolated from the brains of PML patients, exhibits striking similarity to that of the well-characterized simian virus 40 (10). The viral early gene encoding the regulatory protein, T antigen, is separated from the late genes encoding the structural capsid proteins by noncoding sequences which contain the control sequences for transcription of the viral genome and replication of viral DNA. The greatest diversity between the nucleotide composition of JCV and that of the genomes of other papovaviruses rests in their regulatory regions, of which that in JCV encompasses two 98-bp tandem repeats, each containing multiple overlapping and nonoverlapping regulatory modules (23). In addition, there exists a functional κB regulatory motif located approximately 75 nucleotides further upstream from the 98-bp repeat on the early side of the viral genome which, in a manner similar to that of the classical κB element, confers inducibility to the viral promoter activity in response to phorbol esters (24). Earlier in vivo and in vitro studies strongly suggested that the productive infection of glial cells with JCV in the brains of PML patients is mediated, at least in part, by efficient transcription of the viral early and late promoters in these cells. The search for regulatory proteins which mediate tissue-specific transcription of JCV in glial cells has led to the identification of several nuclear proteins from brain and glial cells which, upon binding to the viral regulatory sequences, augment the activity of the JCV promoter (23).

In addition to its replication in the brain, it is also suspected that JCV persists and, under certain physiological conditions, replicates in the kidneys. This speculation is based on several studies indicating the presence of JCV particles in the urine of PML patients and non-PML individuals experiencing severe or mild immunosuppression, as well as in a large group of pregnant women (1, 5, 6, 9, 12, 19, 21, 28, 30). Of particular interest is the notion that the regulatory region of JCV obtained from urine, which is closely related to the JCV archetype (JCV_{Cy}) sequence, is distinct from those isolated from the brains (JCV_{Mad-1}) of PML patients (8, 20, 29, 30). As in JCV_{Cy}, the control region of the kidney isolates contains only one copy of the 98-bp enhancer/promoter found in Mad-1 with insertions of 23 and 66 nucleotides (Fig. 1). It appears that deletion of the 23- and 66-bp sequences from JCV_{Cy} and duplication of the remaining 98 bp could create a structure which is found in JCV_{Mad-1}. On the basis of this notion, it has been postulated that the kidney isolate JCV_{Cy} undergoes a DNA rearrangement process and gains a structure (JCV_{Mad-1}) which is adapted to the brain and through cytolytic infection of glial cells could cause PML in immunosuppressed individuals. Alternatively, it is possible that immunosuppressive conditions trigger DNA rearrangement and that the adapted virus thus

* Corresponding author. Mailing address: Molecular Neurovirology, Jefferson Institute of Molecular Medicine, Department of Biochemistry and Molecular Biology, Thomas Jefferson University, 233 S. Tenth St., Philadelphia, PA 19107. Phone: (215) 955-5534. Fax: (215) 923-5905.

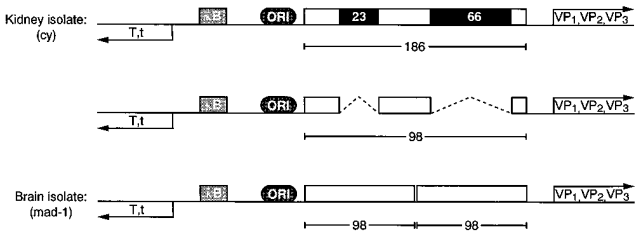

FIG. 1. Structural organization of regulatory regions from JCV isolates. (Top) The control region of kidney isolates of JCV, which is identical to the control region of archetype (Cy), is composed of κB, the origin of DNA replication (ORI), and a 186-bp sequence containing the 23- and 66-bp blocks. (Bottom) The control region of brain isolates of JCV (Mad-1) is composed of two 98-bp tandem repeats plus κB and the ORI on the early side of the repeats. (Middle) Structure of a hypothetical intermediate JCV promoter which is composed of the complete sequences of one copy of the 98-bp repeat seen in Mad-1 upon removal of the 23- and 66-bp blocks. The directions for the early genes (T and t) and the late genes (VP_1, VP_2, and VP_3) are shown by the arrows on the left and right, respectively.

efficiently replicates in the specific organ. Under any circumstances, results from the most recent studies suggest that the inactivity of the archetype relative to the rearranged forms is due to differences in the promoter/enhancer that controls transcription of the viral genome but not the origin of viral DNA replication (7).

During the past several years, our laboratory and others have been actively studying regulation of Mad-1 gene transcription in glial cells, but no major studies toward understanding the regulation of archetype gene transcription, particularly in glial cells, have been carried out. As a first step toward understanding the mechanism involved in expression of the JCV archetype genome, we have utilized a transient transfection assay and evaluated the transcriptional activity of the viral early and late promoters in glial cells under normal and phorbol 12-myristate 13-acetate (PMA)-induced conditions. We demonstrate that mutations in the κB motif enable the 23-bp sequence of the archetype control region to abrogate transcriptional activity of the viral late, but not early, promoter in glial cells.

MATERIALS AND METHODS

Cell culture and transfection. U-87MG, a cell line derived from a human glioblastoma, was obtained from the American Type Culture Collection and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum. CV-1 is a monkey kidney cell line obtained from the American Type Culture Collection and also maintained in DMEM plus 10% fetal calf serum. Transfections of the reporter constructs were carried out by the calcium phosphate precipitation method as described previously (14). For chloramphenicol acetyltransferase CAT assays, at 36 h after transfection of plasmids into subconfluent cells in 60-mm-diameter dishes, the cells were harvested and processed for CAT activity as described previously (13) by using equal amounts of protein from each lysate as determined with a Bio-Rad protein assay kit. Percent conversion was calculated by scintillation counting as described in the figure legends.

Plasmids. Cy_E-CAT and Cy_L-CAT are pBL-CAT3-based plasmids (17) containing the κB, ori, and 186-bp control regions of the virus (as depicted in Fig. 1) in the early and late orientations, respectively. For generation of κB mutants, we performed PCR-mediated site-directed mutagenesis (25) and altered the first two nucleotides of the κB motif, as described below. Integrity of all recombinant constructs was verified by direct sequencing. The various recombinant clones were generated by restriction enzyme digestion as denoted throughout the text.

Preparation of nuclear extract and band shift analysis. Nuclear extracts were prepared as previously described (2) from unstimulated U-87MG cells and from U-87MG cells that had been stimulated with 50 ng of PMA per ml for 1 h. The procedure for band shift analysis was essentially as described by Taylor et al. (27). Briefly, 10^6 cpm of ^{32}P-labeled probe was incubated with 5 μg of nuclear extract in a binding buffer containing 10 mM Tris HCl (pH 7.4), 50 mM NaCl, 1 mM NaEDTA, 5% glycerol, 1 mM dithiothreitol, 4 μg of poly(dI-dC), 0.5 μg of bovine serum albumin, and 3 mM GTP. Following a 30-min incubation on ice, the resulting complexes were resolved on a low-ionic-strength 6% native polyacrylamide gel electrophoresis (PAGE) gel containing 6.7 mM Tris HCl (pH 7.4), 3 mM Na acetate, and 1 mM NaEDTA. Electrophoresis was carried out at 4°C at a constant 140 V. The gel was then dried and autoradiographed at −70°C with an intensifying screen.

UV cross-linking. In UV cross-linking experiments, the protein-DNA binding reaction was performed in the same manner as for the DNA band shift assay. After complex formation, the reaction mixture was exposed to UV irradiation for 20 min as described previously (16). The complex was resolved on a 6% native gel, and the DNA-protein complex was excised from the gel and, after elution in a binding buffer at 37°C, mixed in a 1:1 ratio with sodium dodecyl sulfate (SDS)-protein sample buffer and analyzed by SDS-PAGE. The gel was then dried and autoradiographed at −70°C with an intensifying screen.

RESULTS AND DISCUSSION

Transcription of Cy early and late promoters in glial and kidney cells. In previous studies, we demonstrated that a stretch of 10 nucleotides, 5′-GGGAATTTCC-3′, located outside of the 98-bp regulatory region enhanced transcription of the Mad-1 early and late promoters when cells were treated with extracellular stimuli, including phorbol ester (24). To evaluate the importance of the κB motif in transcriptional regulation of the JCV archetype, hereafter referred to as Cy, reporter plasmids containing viral early (Cy_E) and late (Cy_L) promoters expressing the CAT gene were constructed and introduced into U-87MG human glial cells. The transfected cells were untreated or treated with PMA at various times. As shown in Fig. 2, treatment of cells with PMA substantially increased (by 20- to 30-fold) the levels of Cy_E (Fig. 2A) and Cy_L (Fig. 2B) promoter activity. The extent of induction of the viral promoters by PMA was decreased after 32 h (Fig. 2; compare lanes 2 with lanes 4). These results indicate that like those from Mad-1 (24), the promoters from Cy are responsive to PMA induction in glial cells.

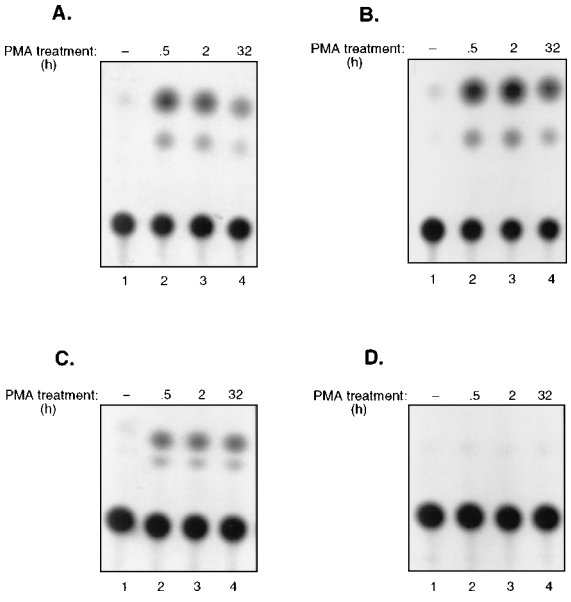

FIG. 2. Transcriptional activity of Cy promoters in glial cells. Approximately 5×10^5 U-87MG human astrocytic glial cells were transfected with 5 μg of the following plasmids by the Ca-phosphate precipitation method (14): Cy_E-CAT (A), Cy_L-CAT (B), Cy_E-$κB^{mut}$-CAT (C), and Cy_L-$κB^{mut}$-CAT (D). Cells were maintained in DMEM (lanes 1) or DMEM plus 50 ng of PMA for 0.5, 2, and 32 h (lanes 2 to 4, respectively). For CAT assays, at 36 h posttransfection glial cells were harvested and the CAT enzymatic activity was determined by the standard method (13). For quantitation, the percent conversion of chloramphenicol to its acetylated forms was determined by scintillation counting of the corresponding spots. Each experiment was performed at least twice. Representative data are shown.

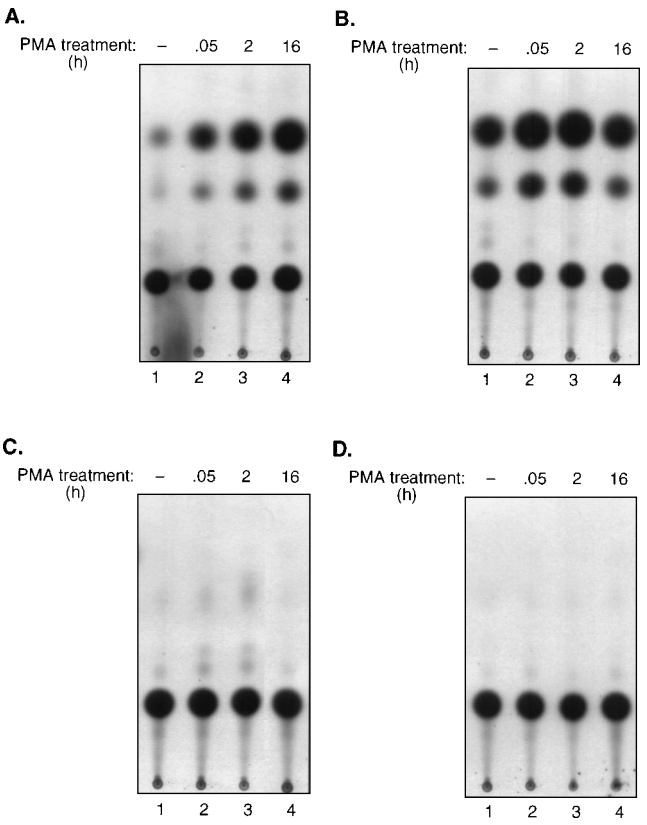

FIG. 3. Transcriptional activity of Cy promoters in kidney cells. Monkey kidney cell line CV-1 was transfected with the various Cy-reporter constructs containing the Cy early promoter with wild-type κB (A), the Cy late promoter with wild-type κB (B), the Cy early promoter with mutant κB (C), and the Cy late promoter with mutant κB (D), as described in the legend to Fig. 2. The experiments were repeated at least twice, and their representative data are illustrated.

Next, we employed site-directed mutagenesis within the κB sequence to convert the first two G residues of the κB motif to A residues (5′-AAGAATTTCC-3′). As expected, in a manner similar to that observed for Mad-1 early (Mad-1_E) promoter activity, mutation within the κB motif of the Cy genome decreased the extent of the induction of the Cy_E promoter by PMA and the conditioned media (Fig. 2C). Interestingly, in the context of the Cy_L promoter, the mutation in the κB motif showed a drastic effect and completely abrogated the ability of the Cy_L promoter to respond to PMA induction in glial cells (Fig. 2D). It is noteworthy that our previous study indicated that interference with κB function in the Mad-1 late gene decreased, but did not abolish, the activity of this gene in transfected glial cells (24). These observations suggest that in addition to κB, other elements within the viral regulatory region, such as the GC-rich sequence (22), may confer inducibility to the Cy_E, but not the Cy_L, promoter in glial cells. Also, it is likely that in the absence of the κB motif, the Cy_L promoter remains under negative regulation by the DNA sequences which are present in the Cy, but not in Mad-1, isolates.

In parallel, we performed a similar transfection experiment in monkey kidney cell line CV-1. As shown in Fig. 3A and B, the basal activities of JCV early and late promoters were increased upon treatment of the transfected cells with PMA. Introduction of mutations in the κB motif completely abrogated PMA responsiveness of the viral early and late promoters in CV-1 cells (Fig. 3C and D). Thus, it appears that in a manner similar to that of its effect in glial cells, the κB motif plays an important role in the transcriptional activity of the Cy late promoter in kidney cells. Interestingly, under similar conditions mutations in the κB motif of Cy_E exerted an effect on the Cy early promoter activity in CV-1 cells more drastic than those observed in U-87MG cells.

Induction of Cy_L activity via κB is not limited to PMA treatment. As shown in Fig. 4, treatment of the transfected cells with physiological reagents such as interleukin-2 and tumor necrosis factor alpha, both of which have been shown to induce NF-κB activity (3), enhanced transcriptional activity of the viral late promoter in glial cells (compare lane 1 with lanes 2 and 3). Under similar conditions, mutations in the viral κB motif completely abrogated viral transcription and its responsiveness to these inflammatory cytokines (Fig. 4, lanes 4 to 6).

Transcriptional activities of various regions of the Cy promoter sequences in glial cells. To obtain information regarding the regulatory elements responsible for the observed down-regulation of the Cy_L promoter in the κB mutant constructs, the Cy regulatory region was divided into two segments, one spanning the κB motif to the ori region (Cy_{SH}) and the other encompassing the remaining viral regulatory sequence (Cy_{HE}) (Fig. 5A). The transcriptional activity of each region in either the early or the late orientation was evaluated by transfection assay. Results shown in Fig. 5B clearly indicate that the region of the Cy promoter which lacks the κB sequence (Cy_{HE}) in the early and, more interestingly, in the late orientation was transcriptionally active and that its activity was increased by treatment of the cells with PMA. Additionally, the element containing the κB motif (Cy_{SH}) also exhibited transcriptional activity, although to a lesser extent, in treated and untreated cells (Fig. 5C). In a related approach, we have broadened the scope of these studies and have examined basal and induced transcriptional activities of the various regions of the Cy regulatory sequence in glial cells. As summarized in Fig. 6, results of these studies indicate that the Cy promoter is composed of several regulatory motifs which in either the early or the late orientation independently stimulate the basal and PMA-induced transcription of the viral promoter in glial cells. The results obtained for the constructs with deletions of κB in the late orientation are particularly interesting. It is evident that the Cy_L promoter sequence with a deletion in the κB motif is transcriptionally active; however, in the context of the full

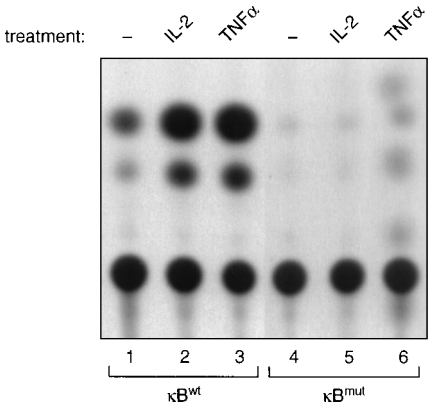

FIG. 4. Effects of interleukin-2 (IL-2) and tumor necrosis factor alpha (TNF-α) on transcription of the Cy_L promoter. Approximately 5 μg of the Cy_L reporter plasmid containing wild-type ($κB^{wt}$) or mutant κB motifs was introduced into U-87MG glial cells. Cells were untreated or were treated with IL-2 or TNF-α (5 ng/ml) for 36 h. Activity representing the percent conversion of chloramphenicol to its acetylated forms in transfected cells is shown.

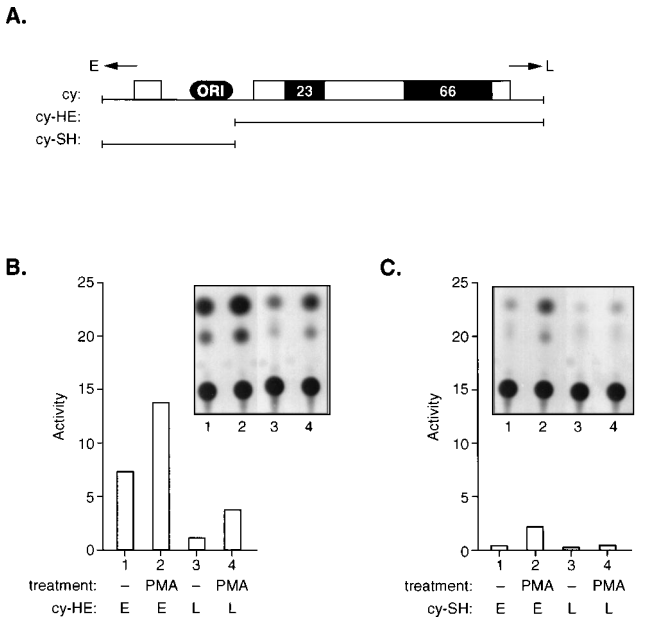

FIG. 5. Transcriptional activity of the Cy promoter region. (A) Structure of the Cy promoter depicting two DNA fragments of the viral genome, Cy_{HE} and Cy_{SH}, which were placed at the 5′ position of the CAT gene in the early and late orientations. Transcriptional activities of Cy_{HE} (B) and Cy_{SH} (C) in the early (E) and late (L) orientations in cells under normal and PMA-treated (32 h) conditions are also represented. The activity represents the average percent conversion of chloramphenicol to its acetylated forms obtained from two independent experiments. The inserts illustrate the results from the representative experiments.

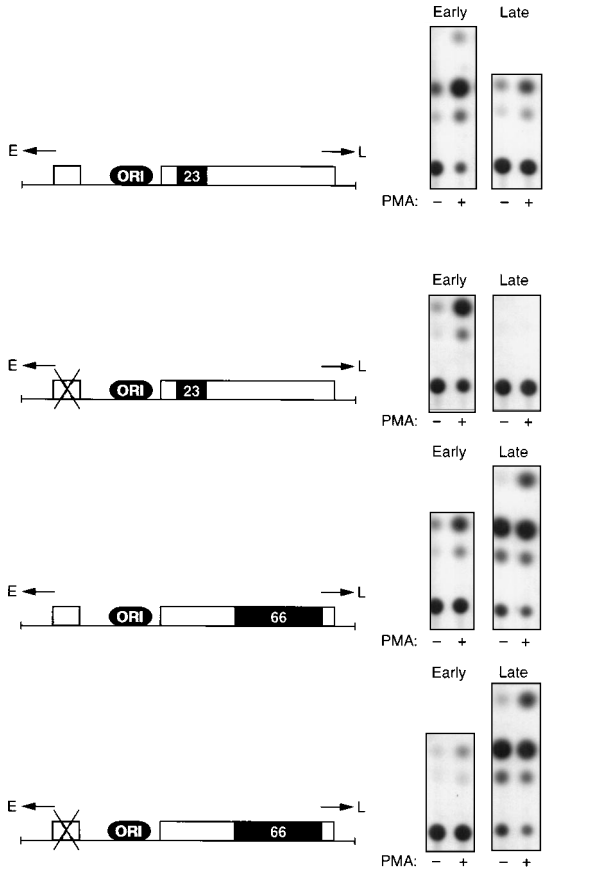

FIG. 7. Functional interaction of the κB motif with the 23- and 66-nucleotide blocks. Five micrograms of the reporter construct containing the chimeric promoter with either the 23-bp or the 66-bp block of Cy in the context of wild-type or mutant κB (as shown on the left) was introduced into glial cells, and the levels of its activity in untreated and treated cells were evaluated after 36 h. A series of representative CAT data indicating basal and induced transcriptional activity of each construct is shown on the right. E, early; L, late.

promoter, this activity could be modulated by the κB motif, since mutations in the κB region have a negative effect on the overall Cy_L promoter activity.

Functional relation of κB with the 23- and 66-bp sequences of the Cy promoter. In the next series of studies, we investigated the functional relation of the κB motif with the 23- and 66-nucleotide blocks of the Cy promoters. As shown in Fig. 7, the recombinants containing the κB and the 23-bp regions, in either orientation, were transcriptionally active and responded to PMA induction. The introduction of mutations within the κB region abrogated late gene transcription and reduced early promoter activity. The viral early and late promoters containing the wild-type or mutant κB in the context of the 66-bp region were transcriptionally active and responded to the inductions by PMA. These data indicate that mutations in the κB motif enable the 23-bp region, but not the 66-bp region, to suppress transcription of the Cy_L promoter. Of importance, it appears that mutations in the κB motif may not create a silencer element in this region, as similar mutations in the Mad-1 strain or a recombinant containing the 66-bp region remained transcriptionally active, although at a lower level.

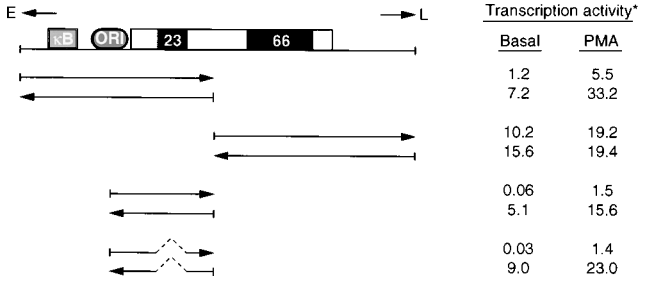

FIG. 6. Transcriptional activities of various regions of the Cy promoter regulatory sequence. Various DNA fragments spanning the regions from the early gene initiation site to the unique *Sac*I site which is located between the 23- and 66-bp blocks, the *Sac*I site to the late gene initiation site, and the ori regions to the *Sac*I site with and without the 23-bp motif were prepared and cloned in CAT reporter plasmids in the early (E) and late (L) orientations. The recombinants were introduced into glial cells, and CAT activities of the extracts from cells which were grown in the absence or the presence of PMA were determined. The transcriptional activity represents the average percent conversion of at least two independent experiments.

In a different set of studies we removed the entire viral promoter spanning the region between the 23-bp element and the CAT gene and evaluated the activity of the 23-bp motif in the context of the wild-type or mutant κB region. As illustrated in Fig. 8A, mutation in the κB motif drastically affected the basal and PMA-induced activity of the late promoter. Mutation in the κB motif of the early promoter showed no deleterious effect on transcription of the CAT gene (Fig. 8B).

Nuclear proteins associated with the κB and the 23-bp regulatory motifs. These results clearly indicate that cross-communication between the 23-bp sequence and the κB mutant switches off transcription of the Cy_L, but not the Cy_E, promoter. Since cross-interaction between two *cis*-acting elements, in general, is mediated by DNA-binding proteins, we performed a series of band shift analyses to identify the cellular

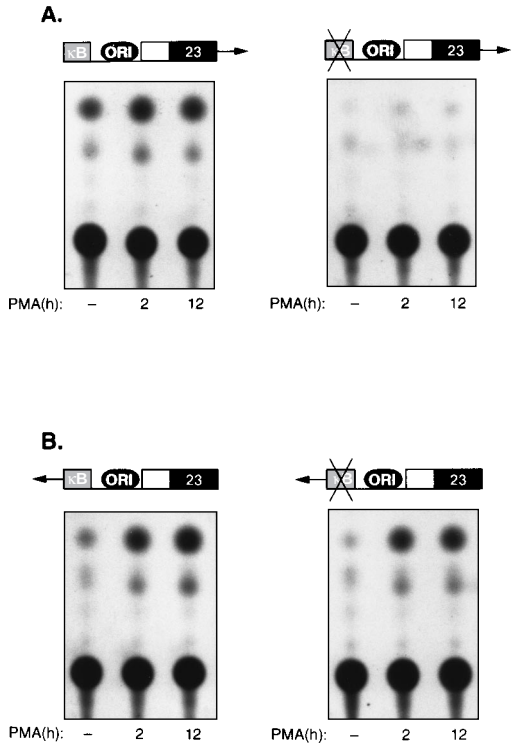

FIG. 8. Transcriptional activity of the truncated Cy promoter containing κB and the 23-bp motifs. Five micrograms of recombinant constructs containing either wild-type or mutant κB sequence and the DNA sequences spanning the 23-bp motif in the early or late orientation was introduced into glial cells. The transfected cells were untreated or were treated for 2 and 12 h with 50 ng of PMA per ml. After 36 h, cells were harvested and the CAT enzymatic activity of the extracts prepared from the cells was determined.

protein(s) which is associated with the 23-bp motif and the κB sequence of the Cy promoter. In this respect, nuclear extracts were prepared from PMA-stimulated glial cells and reacted with ^{32}P-labeled DNA probes representing the 23-bp sequence, κB, and a mutant κB sequence (κB^{mut}). As shown in Fig. 9A, the κB probe formed three classes of complexes, a, b, and c, which were not detected upon addition of unlabeled wild-type κB as a competitor. The addition of the mutated κB and 23-bp competitors effectively competed for formation of the b complex, but the other two classes of complexes remained unaltered. These observations suggest that the more slowly migrating complex a, shown by the arrowhead, may represent binding of the classical heterodimer p50:p65 κB-binding proteins and the faster-migrating complex c may indicate binding of the p50 or the p65 in a monomer form to the DNA (15). The κB^{mut} probe, by binding to the cellular proteins, formed a major complex which exhibited an electrophoretic mobility similar to that observed for the b complex with the wild-type κB (Fig. 9B, lane 1). This complex was eliminated by the addition of unlabeled wild-type and κB^{mut} oligonucleotides. Again, the 23-bp unlabeled competitor was able to efficiently inhibit the formation of this nucleoprotein complex (Fig. 9B). Similarly, the 23-bp probe, by binding to the cellular proteins, formed a major complex which was not observed upon inclusion of the unlabeled 23-bp DNA competitors (Fig. 9C). The intensity of this complex was decreased by addition of the mutant oligonucleotide and to a lesser degree by addition of the wild-type κB oligonucleotide. Note that these studies were performed under conditions in which the probes were in excess.

In the next series of studies, we performed supershift analysis of nucleoprotein complexes associated with JCV κB and the 23-bp motif. Extracts from untreated and PMA-treated cells were reacted with the ^{32}P-labeled κB, κB^{mut}, and 23-bp probes. As shown in Fig. 9D, treatment of cells with PMA induced the intensity of the band corresponding to the a complex. Addition of anti-p65 monoclonal antibody to the binding reaction mixture containing the κB probe supershifted a complex with no effect on the migration of the b complex. No alterations in the electrophoretic mobilities of the complexes associated with the κB^{mut} (Fig. 9E) and 23-bp (Fig. 9F) probes were obtained upon inclusion of the antibody in the reaction mixture. Also, addition of anti-p50 antibody showed no effect on the migration of the b band or the complexes seen with the κB^{mut} and 23-bp probes. Thus, it is likely that the proteins participating in the formation of the b complex or associated with the κB^{mut} and 23-bp sequences are distinct, and least antigenically, from the classical NF-κB subunits.

To further characterize nuclear proteins which are associated with the major complex seen with the 23-bp and κB^{mut} sequences, we performed two-dimensional UV cross-linking experiments (2). In this study, the major nucleoprotein complexes obtained with the 23-bp and κB^{mut} probes, as shown in Fig. 9B and C, respectively, were eluted from the native acrylamide gel and, after UV irradiation for 10 min, analyzed by

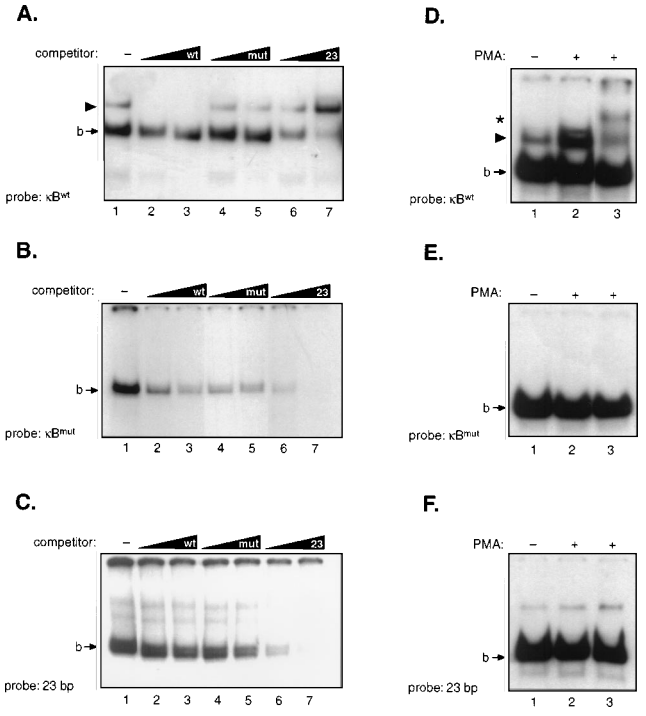

FIG. 9. Interaction of the κB and 23-bp motifs with cellular proteins. Samples (approximately 10 μg) of nuclear extracts from PMA-stimulated glial cells were prepared and mixed with ^{32}P-labeled wild-type κB (κB^{wt}) (A), mutated κB (B), or the 23-bp sequence (C) as described previously (2). The nucleoprotein complexes were separated on a 6% native acrylamide gel and visualized by autoradiography. In competition experiments, 10 and 50 ng of the unlabeled double-stranded DNAs, as indicated above each lane, were included in the binding reaction mixtures. The area of the gels which demonstrated a specific nucleoprotein complex is shown. For supershift analysis the nucleoprotein complexes with the wild-type κB (D), mutant κB (E), and 23-bp (F) probes were formed with untreated extract (lane 1) or with PMA-treated extract in the absence (lane 2) or the presence (lane 3) of anti-p65 antibody. The position of the b complex is shown by an arrow. The arrowheads denote the NF-κB complex, and the asterisk shows the supershift complex. wt, wild type; mut, mutant.

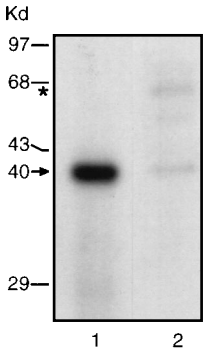

FIG. 10. UV cross-linking analysis of the nucleoprotein complex. The nucleoprotein complex shown in Fig. 9B and C was eluted from the native gel and, after cross-linking by UV irradiation (2), was analyzed by SDS–10% PAGE. Lane 1, complex formed with the 23-bp motif; lane 2, complex obtained with κB^{mut}.

SDS-PAGE. As shown in Fig. 10, a major 40-kDa band was detected in the sample corresponding to the protein associated with the 23-bp probe. Similarly, the κB^{mut} probe showed a band with a 40-kDa molecular mass and an additional minor band which corresponds to a 60-kDa protein. The 40-kDa protein complex was also detected in the b complex formed with the wild-type κB sequence (26a). Thus, it is evident that a common 40-kDa protein is associated with the 23-bp sequence and the κB^{mut} sequence. Identification of the 40-kDa cellular protein which binds to the κB and the 23-nucleotide insertion motifs of the Cy_L promoter is of particular interest, in light of our transfection studies indicating cross-interactions between these two regulatory elements.

Altogether, these observations are consistent with a model in which a cellular protein, such as the 40-kDa species, exhibits the ability to bind the 23-bp and the κB motifs of the Cy_L promoter. In the presence of the wild-type κB motif and classical NF-κB-binding proteins, including p50:p65, the 40-kDa protein or other related proteins may not effectively interact with the κB sequence. However, these proteins could bind to the 23-bp motif and exert their activity. Mutations in the κB motif, which interfere with the binding of p50:p65 activators to their targets, provide the opportunity for the 40-kDa protein to bind the κB domain. Under these circumstances, the interaction of the 40-kDa protein with the mutated κB and the 23-bp sequence may generate complexes which become nonproductive for Cy_L transcription. Accordingly, one could envision a similar role for the 40-kDa protein in regulating transcription of the Cy_L promoter with the wild-type κB under conditions whereby the p50/p65 activators are not expressed or are not fully active. Again, binding of the 40-kDa protein or the other related proteins to the κB and 23-bp motifs in the absence of p50:p65 may down-regulate Cy_L gene transcription. These are intriguing speculations in light of clinical observations indicating that the immune states of individuals and the physiological stature of cells are important for reactivation of the virus (for a review, see reference 4). Thus, parallel studies of transcription of the wild-type and mutant JCV genomes could provide an avenue to unravel the highly complicated regulatory pathways which involve participation of inducible regulatory proteins. Our future studies will focus on determining the role of the 40-kDa protein in reactivation of the Cy_L promoter in lytically infected cells and evaluating the possible interplay between the 40-kDa protein and other cellular proteins, including the NF-κB family.

ACKNOWLEDGMENTS

We thank Richard J. Frisque for support and for providing reagents used in these experiments. We also thank the past and present members of the Molecular Neurovirology Section of the Jefferson Institute of Molecular Medicine for sharing ideas and reagents and for support and insightful suggestions during the course of this study. We thank Cynthia Schriver and Roni Feldman for providing editorial assistance in the preparation of the manuscript.

This work was made possible by grants awarded by the NIH to K.K.

REFERENCES

1. **Andrews, C. A., R. Daniel, and K. Shah.** 1983. Serologic studies of papovavirus infections in pregnant women and renal transplant recipients, p. 133–141. *In* J. L. Sever and D. Madden (ed.), Polyomavirus and human neurological disease. Alan R. Liss, Inc., New York.
2. **Ausubel, F., R. Brent, R. E. Kingston, D. D. Moar, J. G. Siedman, J. A. Smith, and K. Struhl.** 1989. Current protocols in molecular biology. John Wiley & Sons, Inc., New York.
3. **Baeuerle, P. A., and T. Henkel.** 1994. Function and activation of NF-κB in the immune system. Annu. Rev. Immunol. **12:**141–179.
4. **Berger, J. R., and M. Concha.** 1995. Progressive multifocal leukoencephalopathy: the evolution of a disease once considered rare. J. NeuroVirol. **1:** 5–18.
5. **Chesters, P. M., J. Heritage, and D. J. McCance.** 1983. Persistence of DNA sequences of BK virus and JC virus in normal human tissues and diseases tissues. J. Infect. Dis. **147:**676–684.
6. **Coleman, D. V., S. D. Gardner, C. Mulholland, V. Fridiksdottir, A. A. Porter, R. Lilford, and H. Valdimarsson.** 1983. Human polyomavirus in pregnancy. A model for the study of defence mechanisms to virus reactivation. Clin. Exp. Immunol. **53:**289–296.
7. **Daniel, A. M., J. J. Swenson, R. P. R. Mayreddy, K. Khalili, and R. J. Frisque.** Sequences within the early and late promoters of archetype JC virus restrict viral DNA replication and infectivity. Virology, in press.
8. **Dorries, K.** 1984. Progressive multifocal leucoencephalopathy: analysis of JC virus DNA from brain and kidney tissue. Virus Res. **1:**25–38.
9. **Flaegstad, T., A. Sundsfjord, R. R. Arthur, M. Pedersen, T. Traavik, and S. Subramani.** 1991. Amplification and sequencing of the control regions of BK and JC virus from human urine by polymerase chain reaction. Virology **180:** 553–560.
10. **Frisque, R. J., G. L. Bream, and M. T. Cannella.** 1984. Human polyomavirus JC virus genome. J. Virol. **51:**458–469.
11. **Frisque, R. J., and F. A. White.** 1992. The molecular biology of JC virus, causative agent of progressive multifocal leukoencephalopathy, p. 25–158. *In* R. Roos (ed.), Molecular neurovirology. Humana Press, Totowa, N.J.
12. **Gardner, S. D., E. F. D. MacKenzie, C. Smith, and A. A. Porter.** 1984. Prospective study of the human polyomaviruses BK and JC and cytomegalovirus in renal transplant recipients. J. Clin. Path. **37:**578–586.
13. **Gorman, C. M., L. F. Moffat, and B. H. Howard.** 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol. Cell. Biol. **2:**1044–1051.
14. **Graham, F. L., and A. J. van der Eb.** 1973. A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology **52:**456–457.
15. **Grimm, S., and P. A. Baeuerle.** 1993. The inducible transcription factor NF-κB: structure-function relationship of its protein subunits. Biochemistry **290:**297–308.
16. **Khalili, K., J. Rappaport, and G. Khoury.** 1988. Nuclear factors in human brain cells bind specifically to the JCV regulatory region. EMBO J. **7:** 1205–1210.
17. **Luckow, B., and G. Schultz.** 1987. CAT constructions with multiple unique restriction sites for the functional analysis of eukaryotic promoters and regulatory elements. Nucleic Acids Res. **15:**5490–5498.
18. **Major, E. O., K. Amemiya, C. S. Tornatore, S. A. Houff, and J. R. Berger.** 1992. Pathogenesis and molecular biology of progressive multifocal leukoencephalopathy, the JC virus-induced demyelinating disease of the human brain. Clin. Microbiol. Rev. **5:**49–73.
19. **Markowitz, R.-B., B. A. Eaton, M. F. Kubik, D. Latorra, J. A. McGregor, and W. S. Dynam.** 1991. BK virus and JC virus shed during pregnancy have predominantly archetypal regulatory regions. J. Virol. **65:**4515–4519.
20. **Martin, J. D., D. M. King, J. M. Slauch, and R. J. Frisque.** 1985. Differences in regulatory sequences of naturally occurring JC virus variants. J. Virol. **53:** 306–311.
21. **Myers, C., R. J. Frisque, and R. R. Arthur.** 1989. Direct isolation and characterization of JC virus from urine samples of renal and bone marrow transplant patients. J. Virol. **63:**4445–4449.
22. **Raj, G. V., and K. Khalili.** 1994. Identification and characterization of a novel GGA/C-binding protein, GBPi, that is rapidly inducible by cytokines. Mol. Cell. Biol. **14:**7770–7781.
23. **Raj, G. V., and K. Khalili.** 1995. Transcriptional regulation: lessons from the human neurotropic polyomavirus, JCV. Virology **213:**283–291.
24. **Ranganathan, P. N., and K. Khalili.** 1993. The transcriptional enhancer

element, κB, regulates promoter activity of the human neurotropic virus, JCV, in cells derived from the CNS. Nucleic Acids Res. **21:**1959–1964.
25. **Rashtchian, A., C. G. Thornton, and G. Heidecker.** 1992. A novel method for site-directed mutagenesis using PCR and uracil DNA glycosylase. PCR Methods Applicat. **2:**124–130.
26. **Richardson, E. P., Jr.** 1961. Progressive multifocal leukoencephalopathy. N. Engl. J. Med. **265:**815–823.
26a. **Safak, M., et al.** Unpublished data.
27. **Taylor, J. P., R. J. Pomerantz, O. Bagasra, M. Chowdhury, J. Rappaport, K. Khalili, and S. Amini.** 1992. TAR-independent transactivation by Tat in cells derived form the CNS: a novel mechanism of HIV-1 gene regulation. EMBO J. **11:**3395–3403.
28. **Tominaga, T., Y. Yogo, T. Kitamura, and Y. Aso.** 1992. Persistence of archetypal JC virus DNA in normal renal tissue derived from tumor-bearing patients. Virology **186:**736–741.
29. **Yogo, Y., T. Kitamura, C. Sugimoto, K. Hara, T. Iida, F. Taguchi, A. Tajima, K. Kawabe, and Y. Aso.** 1991. Sequence rearrangement in JC virus DNAs molecularly cloned from immunosuppressed renal transplant patients. J. Virol. **65:**2422–2428.
30. **Yogo, Y., T. Kitamura, C. Sugimoto, T. Ueki, Y. Aso, K. Hara, and F. Taguchi.** 1990. Isolation of a possible archetypal JC virus DNA sequence from nonimmunocompromised individuals. J. Virol. **64:**3139–3143.
31. **ZuRhein, G. M.** 1967. Polyoma-like virions in a human demyelinating disease. Acta Neuropathol. **8:**57–68.