A Nuclear Protein Derived from Brain Cells Stimulates Transcription of the Human Neurotropic Virus Promoter, JCV\textsubscript{E}, \textit{in Vitro}\textsuperscript{*}

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The 98-base pair enhancer/promoter sequence is critical for cell type-specific transcription of the human neurotropic viral promoter, JCV\textsubscript{E}, in glial cells. Transcriptionally active extracts were prepared from glial cells and used to identify cis- and trans-acting regulatory elements that are involved in the glial-specific activation of the JCV\textsubscript{E} promoter. Results indicate that multiple regulatory sequences within the 98-base pair repeat specifically bind to nuclear proteins present in glial cells and positively regulate viral early RNA synthesis. The central region of this repeat, designated domain-B, interacts with a 45-kDa nuclear protein present in brain cells. This brain-specific protein was purified by conventional and DNA affinity chromatography. Complementation of the highly purified protein with HeLa extract significantly increased JCV\textsubscript{E} promoter activity. Thus, association of the novel glial-origin transcription factor with its target sequence increases transcription of the JCV\textsubscript{E} promoter in a non-glial context.

Demyelination in brain of patients with progressive multifocal leukoencephalopathy is caused by the destruction of oligodendrocytes, the myelin producing cells of the central nervous system (CNS)\textsuperscript{1} (1–5). The human papovavirus, JCV, has been repeatedly isolated from brain lesions of patients with progressive multifocal leukoencephalopathy and is thought to be the etiologic agent of this fatal demyelinating disease (3, 5–8). This virus preferentially infects oligodendroglial cells of the CNS and only propagates in glial cells in tissue culture (2, 7, 9). We (10) and others (9, 11) have shown previously that the highly restricted host range/tissue specificity of JCV to glial cells may rest in the expression of early viral genes that encode tumor (T) antigen (12). Using transgenic mice, Small et al. (13) have recently shown specific activation of the JCVE promoter and to identify the glial-specific regulatory protein(s) that determines viral promoter specificity. We demonstrate that the JCV control region is composed of multiple regulatory elements arrayed in a 98-bp tandem repeat, each of which interacts specifically with nuclear proteins present in glial extract. Among these nuclear proteins, we have identified and purified a 45-kDa brain-specific nuclear protein that, by binding to a central region of the 98-bp sequence, enhances viral RNA synthesis in non-glial extract. Furthermore, in vitro complementation studies suggest that the unique promoter specificity of JCVE may be determined, at least in part, by this novel 45-kDa transcription factor present in brain.

\textbf{EXPERIMENTAL PROCEDURES}

\textbf{Plasmids—}The plasmid pBJC was constructed by inserting the BamHI-digested JCV DNA into pBR322. The pMBP plasmid is a derivative of Pex-1 (obtained from A. Roach, Cornell Medical Center, New York, NY) containing 1.3 kilobases of MBP upstream regulatory region. The c-myc plasmid was obtained from D. Hall (Thomas Jefferson University). Plasmid pBJC-CAT contains the JCV\textsubscript{E} regulatory region in front of the CAT gene (9).

\textbf{Oligonucleotides and Labeled Fragments—}Oligonucleotides were synthesized by the phosphoramidite method on an automated syn-
The nucleotide composition of the oligonucleotides are:

\[ \text{Oligo-A1} \quad 5'\text{-CTGTTTCTACCCCTCC-3'} \]
\[ 3'\text{-GACGAGGGTGAAGGG-5'} \]
\[ \text{Oligo-A2} \quad 5'\text{-GCTGTTGCTCCTCCTAAGTATGCCT-3'} \]
\[ 3'\text{-CGACAAAGGGGAATCCATGCTGAG-5'} \]
\[ \text{Oligo-A3} \quad 5'\text{-CCTTGGCTTTGTTCATGCCTTAGT-3'} \]
\[ 3'\text{-GGAACGAGAAACATGACCGAAT-5'} \]
\[ \text{Oligo-A4} \quad 5'\text{-CCTTTGGCTGTCCCTCCTGCTTTG-3'} \]
\[ 3'\text{-GGAGCGGGAGGAACAGGAAACGAA-5'} \]
\[ \text{Oligo-B} \quad 5'\text{-GAGCTCGTCCGTGGCCAGCCTCCT-3'} \]
\[ 3'\text{-CTGGTACGCAGGCCTCGTGGAGA-5'} \]
\[ \text{Oligo-C} \quad 5'\text{-TTCCTCTTTTTTTATATATACAG-3'} \]
\[ 3'\text{-AAGGAGAAGAAAATATATGTC-5'} \]

Radiolabeled double-stranded oligonucleotide probes were prepared by 5'-end labeling using T4-polynucleotide kinase (27).

**Cells**—Hamster fetal glial cells, HJC (obtained from Dr. R. Frisque, Pennsylvania State University, State College, PA), were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. HeLa cells were grown in suspension in Eagle’s mini-Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. HeLa cells were grown in suspension in Eagle’s minimal essential medium supplemented with 10% serum.

**In Vitro Transcription**—Whole cell extracts were prepared from HJC and HeLa cells as described by Manley et al. (28). Transcription reactions (33 µl each) contained 12 mM Tris, pH 7.9, 12% glycerol, 60 mM KCl, 2 mM MgCl2, 0.6 mM CTP, 0.06 mM ATP, 0.6 mM GTP, 0.5 mM [32P]UTP, 0.5 µg of template DNA, and 1–5 µl of whole cell extract (approximately 50 µg of protein). After incubation for 60 min at 30 °C, run-off transcripts were analyzed by denaturing 6% polyacrylamide gel as described by Maxam and Gilbert (29). DNA templates were isolated by digestion of plasmids with SpAI (pBHC), Sau3A (c-myc), and NdeI (MB) to generate the 450, 405, and 201 nucleotides of the run-off transcripts, respectively.

**Band-shift Assay**—Band-shift assays were done as described (30) with several modifications. Protein samples were incubated with 200–500 ng/ml (2000 cpm/ng) of end-labeled double-stranded oligonucleotides in the presence of 300 µM of digoxigenin-11-dUTP (Pharmacia LKB Biotechnology Inc.) in a final volume of 30 µl. Incubations were carried out for 30 min in 17% glycerol, 12 mM Hepes, pH 7.9, 50 mM NaCl, 5 mM MgCl2, 4 mM Tris, pH 8.0, and 0.8 mM DTT. Samples were fractionated on low ionic strength 9% polyacrylamide gels for 3 h at 200 V in 0.3–0.5 X TBE at 4 °C. Gels were dried on the Whatman 3MM sheet overnight at 80 °C and bands detected by autoradiography.

**Chromatography**—Protein extracts were prepared from calf brain (Pelfreeze Laboratory) by modifying the procedure described by Dignam et al. (31). Briefly, minced tissue was homogenized with an electrical blender in two packed tissue volumes of phosphate-buffered saline for 3–5 min. The homogenate was washed with buffer A (50 mM Hepes, pH 7.9, 1.5 mM MgCl2, 10 mM KCl, 0.5 mM DTT, and 0.5 mM phenylmethylsulfonyl fluoride) and was resuspended in two packed tissue volume of buffer A. After gentle mixing, three packed tissue volumes of 1 x buffer B (1 x buffer B: 20 mM Hepes, pH 7.9, 0.42 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 25% glycerol, 0.5 mM DTT, 0.5 mM phenylmethylsulfonyl fluoride) was added and homogenized immediately. The subsequent steps were basically processed by the previously described method (31). The extract was stored in buffer containing 20 mM Tris, pH 7.9, 17% glycerol (v/v), 0.2 mM EDTA, 0.5 mM DTT, 0.1 mM KCl and 0.5 mM phenylmethylsulfonyl fluoride. Nuclear extracts prepared from 1 kg of brain were applied to a 500-ml phosphocellulose (PII) column (Bio-Rad), equilibrated with buffer A containing 10 mM Hepes, pH 7.9, 1.5 mM MgCl2, 10 mM KCl, 0.5 mM DTT, and 0.5 mM phenylmethylsulfonyl fluoride and was resuspended in two packed tissue volume of buffer A. After gentle mixing, three packed tissue volumes of 1 x buffer B (1 x buffer B: 20 mM Hepes, pH 7.9, 0.42 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 25% glycerol, 0.5 mM DTT, 0.5 mM phenylmethylsulfonyl fluoride) was added and homogenized immediately. The subsequent steps were basically processed by the previously described method (31). The extract was stored in buffer containing 20 mM Tris, pH 7.9, 17% glycerol (v/v), 0.2 mM EDTA, 0.5 mM DTT, 0.1 mM KCl and 0.5 mM phenylmethylsulfonyl fluoride. Nuclear extracts prepared from 1 kg of brain were applied to a 500-ml phosphocellulose (PII) column (Bio-Rad), equilibrated with buffer A. The column was washed with this buffer until no significant amount of protein was detected. Bound proteins were step-eluted with buffer A with 0.3, 0.5, and 1.0 M KCl. Peak protein fractions were pooled from each step and analyzed by band-shift assay. Active fractions were pooled, precipitated with 50% ammonium sulfate, and applied to a 500-ml DNA affinity column equilibrated with 25 mM Hepes, pH 7.8, 20% glycerol (v/v), 1 mM DTT, and 0.2 M KCl and stored at 70 °C.

**RESULTS**

**Transcription of JCV F Promoter in Gliial Cell-free Extract**—Earlier studies by us (10) and others (11) have indicated that the hamster fetal glial cell line, HJC, provides a convenient tissue culture system to investigate the cell type-specific transcription of the JCV F promoter.

Based on those observations, we used cell-free transcriptionally active extracts from HJC cells to analyze transcription of the JCV F promoter in vitro. In parallel, whole cell extracts prepared from HeLa cells were used to examine the JCV F promoter activity in the non-glial cells. The template DNA used in this experiment, pBJC, contains the entire 5130-nucleotide JCV genome cloned into pBR322. Template pBJC DNA was digested with the endonuclease SpAI 450 bp downstream from the major early RNA initiation site (35) and incubated separately with 12 µg of glial and non-glial extracts (28). The results in Fig. 1A indicate that the glial extract efficiently transcribes JCV F promoter in vitro. Unlike the in vivo transient transfection assay (9, 11), the in vitro assay revealed significant levels of the promoter activity in the HeLa extract (Fig. 1A, top panels), suggesting that the DNA and/or chromatin structure may, in addition, play a role in restricting transcription of JCV F promoter to glial cells. The

**Fig. 1. In vitro transcription of JCV F promoter in glial and non-glial cells. A, run-off transcription was carried out as described (28). The arrow indicates the position of the 450 nucleotide in vitro synthesized RNAs in glial (lane 1) and HeLa (lane 3) extracts. Lanes 2 and 4 represent transcription of the JCV F promoter in the presence of 1 µg/ml of a-amanitin in glial and non-glial extracts, respectively. B, bottom: transcription of the c-myc promoter in glial and non-glial extracts. Arrow indicates the position of the 405-nucleotide run-off transcribed. Lanes 2 and 4 represent transcription in the presence of a-amanitin. B, in vitro transcription of JCV F when 500 ng of pBJC template were incubated with 1, 3, 6, and 9 µl of extracts (4.5–40.5 µg of protein, respectively) B, bottom: illustrates transcription of 500 ng of c-myc promoter in 1, 3, and 6 µl of extracts. The exposures were for 48 and 16 h at −70 °C with intensifying screens for panel A (top) and panel B (top), respectively.**
level of *in vitro* transcription in the glial extract, however, was consistently more than (5–8-fold) in HeLa extract, as measured by laser densitometry of the bands (data not shown) (Fig. IA, compare lanes 1 and 3). The *in vitro* synthesized transcripts were sensitive to 1 μg/ml of α-amanitin, indicating that they were transcribed by RNA polymerase II (Fig. 1A, lanes 2 and 4). Transcription efficiency of the control c-myc promoter revealed higher levels of transcription in the HeLa extract than in the glial extract (Fig. 1A, bottom panels).

Whether the reduced level of c-myc promoter activity is the property of HJC glial extract or it is fairly common to all glial extracts and cells remains to be elucidated. It is perhaps noteworthy that the level of c-myc RNA in brain is extremely low (estimated 5–10 molecules/cell).²

To further define the optimum protein concentration for JCV₅ transcription, 500 ng of template DNAs were separately incubated with 1–9-μl extracts (4.5–40 μg protein/assay). In general, transcription of the JCV promoter in glial extract occurred in a wider range of protein concentration (13.5–40 μg protein/assay). In the HeLa extract, low levels of full-length transcript were detected when the template DNA was incubated with 13.5 μg of protein (Fig. 1B). Virtually no transcription off the c-myc promoter was detected with various concentrations of DNA and/or protein in the glial extract (Fig. 1B, bottom panels).

Results obtained from these experiments indicated that the glial extract efficiently transcribes the neurotropic viral promoter *in vitro*. The differences in JCV₅ promoter activity between the glial and non-glial extract suggest the presence of a positively acting factor(s) in glial cells and their absence in non-glial cells. Alternatively, a repressor(s) might exist in non-glial cells to exert negative regulations.

Multiple cis-acting Transcriptional Elements Participate in Expression of the JCV₅ Promoter—In previous studies we demonstrated that the JCV 98-bp regulatory sequence contains multiple protein-binding domains that may influence viral early promoter activity (26). Recently, we performed *in vivo* transient transfection assays where each binding domain was fused separately to the heterologous SV40 early reporter promoter upstream of the CAT gene. The results from those experiments indicated that the three binding regions, A₄, B, and C, independently and positively contribute to CAT gene expression in glial cells (10). To examine the role of these protein-binding domains in transcription of the JCV₅ promoter in glial extract, we performed competition experiments in which the specific DNA-binding proteins were sequestered from the extract by preincubation with an excess amount of oligonucleotides containing the target sequence for binding of the proteins (Fig. 2A). The pretreated extract (12 μg) was subsequently programmed to transcribe the JCV₅ promoter by addition of 500 ng of the linearized template DNA. Addition of 1 μg of oligo(A₁) showed only a 40% inhibition of JCV₅ transcription (Fig. 2B, lane 2), whereas preincubation with 1 μg of oligo(A₂), oligo(A₃), or oligo(B) abolished viral early RNA synthesis (Fig. 2B), lanes 3–5, respectively. Addition of oligo(C) and oligo(A₄) reduced the rate of JCV₅ transcription 8–10-fold (Fig. 2B, lanes 6 and 7, respectively). Neither of these competitor DNAs showed any effect on the transcription of a heterologous c-myc promoter in the HeLa extract, nor on the activity of glial specific promoter (myelin basic protein) in the glial extract.³

These results, together with our earlier observations (10), indicate that the protein-binding sequences, positioned within the JCV control region, differ in their ability to stimulate transcription of the JCV₅ promoter in glial cells.

To determine whether the JCV cis-acting elements exert their effect on the *in vitro* transcription of JCV₅ promoter by direct binding to nuclear proteins present in the glial extract, band-shift assay was performed. The [³²P]-labeled oligonucleotides representing these elements (oligo(A), and its derivatives, oligo(B) and oligo(C)) were incubated with the glial extract, and protein-DNA complexes fractionated by gel electrophoresis (30, 36, 37). With the exception of oligo(A₁) (Fig. 3, panel A), which showed no detectable binding activity, the DNA fragments formed multiple complexes by binding to protein components present in glial extract, and these complexes migrated with slower mobilities (Fig. 3, lane 1). When the glial extract was preincubated with 1 μg of the homologous

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² S. Astrin, personal communication.
³ S. Ahmed, unpublished observations.
oligonucleotide and used as a source of binding protein, the specific DNA-protein complex was abolished (Fig. 3, compare lanes 2 and 3). Thus, preincubation of glial extract with 1 μg of competitor DNA fragments effectively depletes specific DNA-binding protein(s) and may result in observable decreases in levels of JCV_E promoter activity.

Purification of a Brain-specific Transcriptional Factor That Stimulates JCV_E Promoter Activity—The dissimilarity in the electrophoretic mobilities of glial and nonglial nuclear proteins that bind to the central region of the 98-bp repeat (26), and the effect of this binding sequence in transcription of the JCV_E promoter in vivo (10) and in vitro (Fig. 2B, lane 5) suggest that the DNA sequences spanning this region, i.e. domain B, play a key role in the strong, glial-specific transcription of the JCV_E promoter. Thus, our further investigations have focused on this domain. Using a combination of conventional and DNA affinity chromatography, we partially purified this nuclear protein with binding activity for this domain. Calf brain nuclear extract was found to contain high levels of this DNA-binding protein and was, therefore, used as a source for protein purification. Frozen calf brain stored at –80 °C was slowly thawed at 4 °C and total protein extract was prepared (see “Experimental Procedures”). Fig. 4A shows the purification scheme. The amount of extract from 1 kg of calf brain (approximately 1200 mg of protein) was applied to a phosphocellulose (P11) column with buffer “A” containing 0.1, 0.3, 0.5, and 1 M KCl. The active fractions were pooled, and the proteins were precipitated with ammonium sulfate (Cf = 50%). The precipitated proteins were applied to a 3-ml DNA affinity column coupled with oligo(B) (32). The active fractions from the DNA affinity column (first run) were eluted with a buffer containing 0.1, 0.5, and 1 M KCl. Fig. 4B illustrates the binding activities obtained from the phosphocellulose and the affinity column fractionation.

The complex formation was abolished when the preheated extract (90 °C) or the proteinase K-treated extract was used. Use of the oligonucleotide-affinity column markedly increased the purity and specificity of the DNA binding activity (See Fig. 4, legend).

Characterization of the JCV_E Brain-specific Enhancer/Pro-moter-Binding Protein—Fractions containing DNA binding activity from the phosphocellulose and the DNA affinity columns were subjected to SDS-polyacrylamide gel electrophoresis and silver staining. The active fractions eluted from the DNA affinity column (first run) containing 0.5 M KCl elution buffer (see “Experimental Procedures”). Approximately 200 mg protein of the specific B domain-binding protein were pooled after washing with 0.5 M KCl elution buffer (see “Experimental Procedures”). Approximately 40–60-fold purity over the crude extract was estimated at this stage. The determinations were done by measuring total protein concentration (Bio-Rad Protein assay) in the fractions containing the binding activity and band shift assay in the presence of carrier poly(dI-dC). The pooled fractions from phosphocellulose column were collected and applied on a double-stranded oligonucleotide affinity column containing the B domain sequences (see “Experimental Procedures”). The pooled proteins with binding activities to oligo(B) were pooled from the first and second round of chromatography, respectively. These steps significantly enhanced the purity of the DNA-binding protein (about 5000-fold). B, band shift assay. A representative band shift assay illustrates purification of the DNA-binding protein. Binding of the protein components from the crude calf brain protein extract, fractions eluted from the phosphocellulose and the affinity columns are shown. In the last two lanes, the probe was mixed with the preheated (90 °C for 5 min), or proteinase K–(50 μg/ml) treated extract.

As a first step toward analyzing the function of this brain-specific DNA-binding protein, we tested the ability of this protein to stimulate transcription of the JCV_E promoter in non-glial HeLa extract. In this experiment, 1 and 3 μl of the purified protein was preincubated with the DNA template pBJC. After 15 min, nucleotide triphosphates and crude HeLa extract were added. The reaction mixture was incubated for an additional 60 min at 30 °C. Mixing of 1 and 3 μl of the purified protein (approximately 0.1–0.5 μg of protein, respectively) to the HeLa extract resulted in a modest stimulation (2–3-fold) of JCV_E transcription (Fig. 6A, compare lanes 1 and 3). Increasing the amount of the purified protein resulted in no further stimulation of the JCV_E basal transcriptional activity (not shown). Supplementation of the HeLa extract with purified protein did not increase the transcription of the c-myc or glial-specific promoter such as that for myelin basic protein (MB) (Fig. 6A). Fig. 6B illustrates the quantitative effect of the purified protein on transcription of the JCV_E, c-
myc, and myelin basic protein promoters in vitro. This experiment suggests that the partially purified 45-kDa protein derived from brain represents a novel regulatory factor that activates transcription of the JC virus promoter by interacting with a specific DNA sequence.

Because the observed activation of the JC virus promoter upon addition of the purified proteins to the HeLa extract might be obscured by endogenous non-glia factors that recognize the same region of the JC virus template DNA (26), the endogenous binding proteins were sequestered by preincubation of the extract with oligonucleotide B. The template DNA and the purified protein were subsequently added to the reaction mixtures. To determine the optimum concentration of the specific oligonucleotide to effectively bind to all endogenous factors, transcription of JC virus promoter in the HeLa extract was carried out in the presence of increasing amounts of oligo(B) DNA. Incubation of 0.01 and 0.1 μg of oligo(B) in the HeLa extract significantly reduced the rate of JC virus RNA synthesis in vitro; with 0.5 μg, transcription of the viral promoter was virtually blocked (Fig. 7A, lane 2). Based on this result, we used 0.1 μg of oligo(B) to absorb the endogenous binding protein. Addition of 1 and 3 μl of the purified protein to the pretreated HeLa cell extract increased the level of JC virus transcription (Fig. 7B, compare lane 1 with lanes 2 and 3). Thus, the 45-kDa brain-specific DNA-binding protein, in concert with other transcriptional factors present in HeLa cells, stimulates in vitro transcription of the viral promoter in these extracts.

In the above experiment, the purified factor might have increased transcription of the JC virus promoter indirectly by releasing the bound endogenous protein to the competitor oligonucleotide through exchange reactions. In particular, the binding interchanged might occur when these proteins have distinct binding affinities for the cognate DNA sequence. The factor present in the HeLa extract is distinct from its counterpart in brain extract and generates discernible DNA-protein complexes (Fig. 8A, compare lanes 2 and 3). Therefore, band shift experiments were performed to determine whether the HeLa and brain proteins are exchanged in the DNA-protein complex during the incubation period. Limiting amounts of the oligo(B) probe were preincubated with the HeLa extract, and the purified factor was added at 0, 5, and 30 min postincubation. Simultaneous incubation of the probe with the HeLa and brain proteins (0 min) generated two groups of brain and HeLa complexes (Fig. 8B, lane 2). When the purified factor was added at 5 or 30 min, the HeLa complexes were predominant (Fig. 8A, lanes 2 and 3, respectively). These results suggest that after formation of the HeLa/oligo(B) complexes no appreciable exchange occurs upon addition of the purified brain factor. Furthermore, these experiments suggest that the binding affinity of the HeLa factors to the oligo(B) sequence is greater than that of the 45-kDa brain factor.

**DISCUSSION**

Progressive multifocal leukoencephalopathy is characterized by a degenerative demyelination in the CNS of immunocompromised patients (1, 2). Neuropathological studies of the CNS of expired patients indicate the presence of abnormally giant glial cells and focal lesions with demyelination (1,
neurotropic viral promoter, JCV

Fig. 7. Effect of purified protein from calf brain extract on transcription of JCV promoter in HeLa extract. A, 500 ng of SphI-digested pBjc DNA was transcribed in vitro in the presence of decreasing amounts of oligo(B). The amount of oligonucleotide used is indicated in each lane. Arrow indicates run-off transcription products representing JCV. B, 500 ng of pBjc template DNA was incubated with the HeLa extracts containing 100 ng of oligo(B) under transcription reaction conditions. Lane 1, no protein added; lanes 2 and 3, 1 and 3 µl of purified proteins added to each reaction. The level of activity in panels A and B was measured by densitometric scanning of the bands and rated arbitrarily on a scale of 0–7.

Fig. 8. Interaction of brain and HeLa factors with JCV enhancer/promoter sequences in vitro. A, end-labeled double-stranded oligo(B) fragment (2000 cpm) was incubated with HeLa (lane 2) and brain (lane 3) extracts, and the resulting complexes were separated from unbound fragment by electrophoresis. Brackets indicate positions of the HeLa and the brain DNA-protein complexes. B, limiting amounts of end-labeled oligo(B) (800–1000 cpm) were incubated with HeLa extract, the protein purified from brain extracts was added at time 0 (lane 1), 10 min (lane 2), and 30 min (lane 3) postincubation, and the mixture was incubated for an additional 30 min.

JCV has consistently been isolated from these focal lesions and shown to be expressed in the glial cells around these lesions (7). It is established that the restriction of JCV propagation to glial cells is due to the expression of the JCV T-antigen which, in turn, is regulated by the 98-bp enhancer/promoter repeat. Each repeat contains multiple binding domains that interact specifically with nuclear proteins present in brain and HeLa extracts (26). The electrophoretic mobilities of these proteins, which recognize the 5' - and 3'-terminal regions of the repeat (designated by A and C), are similar in brain and HeLa extracts. The proteins which recognize the central region of the 98-bp enhancer/promoter, i.e. domain B, are distinct (26). Fusion of these binding elements, in particular the sequence containing domain B to the SV40 enhancerless promoter showed a profound stimulation of the SV40 early promoter in glial cells (10). These results suggest the presence of an intricate network of cis-responsive elements within the JCV 98-bp sequence which are required for the specificity of early gene transcription. In accord with the in vivo experiments, detailed analysis of the viral regulatory region using band-shift and run-off transcription assays established that the JCV 98-bp sequence encompasses multiple protein binding/transcriptional domains. Each domain distinctly influences transcription of the viral early promoter in glial extracts. Addition of synthetic oligonucleotides to the extract results in a concomitant depletion of a specific DNA-binding protein and reduction of JCV early promoter transcription in glial extract.

Which domain(s) plays a central role in the cell type-specific activation of the viral promoter in glial cells has yet to be identified. Domain B, as previously shown by UV-crosslinking, interacts with the 45- and 88-kDa protein components present in brain nuclear extracts. Interestingly, this domain contains the NF-1-binding sequence that may function as a target site for binding of the ubiquitous transcriptional factor (23). Whether the 45- and/or 88-kDa nuclear proteins are members of the NF-1 family present in brain cells remains to be determined. However, in earlier studies we have demonstrated that the 45-kDa protein specifically recognizes the tetranucleotide repeat, GCCA, within this domain.

The domain B-binding protein has been purified from calf brain by affinity chromatography on the oligo(B) column. Binding activity was localized to a 45-kDa protein. The affinity purified protein was shown to activate transcription of the JCV promoter in HeLa extract and is a good candidate for the cell type-specific factor that mediates transcription of the JCV promoter in glial cells. Our results, however, do not exclude the possibility that other binding domains are also involved in the cell type specificity of JCV transcription.

There are also two copies of CAAT box-like sequences in the middle of domain A, designated oligo(A2), that by binding to the CTF/NF-1 may increase transcription of the JCV promoter. Note that UV-cross-linking experiments demonstrated that the protein interacting with oligo(A2) has a molecular size of 82 kDa (in glial and non-glial cells), which is different from the size of the CTF/NF-1 protein (23, 38).

Domain C also functions as a transcriptional element in vivo and in vitro (shown in Fig. 2). This domain contains a TATA sequence and a tract of adenosines (A). The A/T-rich sequence may constitute a functional TATA box for a viral early promoter. It has recently been shown that dA-dT tracts present in the promoter region can stimulate transcription mediated by polymerase II (39). The poly(dA-dT) tracts bind to a 248-residue protein called “datin” found in yeast (40). Whether this novel regulatory domain plays a functional role in transcription of the brain-specific JCV promoter remains unknown.

Using Bal31 deletion mutagenesis, we have recently identified a silencer sequence positioned near the binding site of the 45-kDa protein within the 98-bp repeat. This silencer sequence interacts with a 56-kDa nuclear protein present in glial and non-glial cells. We speculate that binding of the 45-kDa glial-specific activator protein to JCV DNA may preclude the association of the repressor with the silencer, resulting in active transcription of the viral genome in the infected cells.

Results presented here demonstrate that transcription of the JCV early promoter is under a complex control mechanism...
that involves several cis- and trans-regulating signals. These elements include a 45-kDa protein present in brain that binds to its target site in the JCV enhancer/promoter sequence and increases transcription of the viral promoter in a non-glial extract. Whether this novel brain regulatory factor in concert with other ubiquitous regulatory proteins, stimulates the viral gene expression in lytically infected cells remains to be determined. Purification of nuclear proteins that bind to the other regulatory domains of JCV will help in better understanding the molecular mechanisms through which the JCV early promoter is actively expressed in glial cells.

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