T-antigen-dependent transcriptional initiation and its role in the regulation of human neurotropic JC virus late gene expression

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The multifunctional protein of papovaviruses, T-antigen, regulates the virus lytic cycle partly by exerting transcriptional control over viral and cellular gene expression. In this study, the ability of the T-antigen of human neurotropic JC virus (JCV) to enhance expression from the virus late promoter has been further examined. By deletion analysis, a T-antigen-responsive region was mapped within the JCV 98 bp enhancer/promoter between nucleotides 139 and 168. Interestingly, T-antigen appears to mediate transactivation by increasing expression from a basal transcriptional initiation site and through a novel T-antigen-dependent initiation site (TADI). The TADI element contains a region homologous to initiator (Inr) sequences and is sufficient to confer T-antigen responsiveness to a homologous minimal promoter. Electrophoretic mobility shift and UV crosslinking analyses demonstrate that multiple cellular proteins interact with both single- and double-stranded forms of this sequence. Mutations within the TADI element which abolish T-antigen-mediated transcriptional activation also prevent the formation of specific nucleoprotein complexes. These data suggest that the ability of JCV T-antigen to regulate JCV late gene expression may be partly due to the formation of specific nucleoprotein complexes and transcriptional initiation from the TADI site on the viral promoter.

Introduction

Analysis of the interactions of DNA tumour viruses with their host cells has indicated that these viruses share a similar lytic cycle, with a carefully orchestrated temporal pattern of viral gene expression. Early in the lytic cycle, cellular transcription factors present in permissive cells enable the expression of virus-encoded regulatory proteins. These proteins then modify cellular control processes to optimize conditions for virus growth and multiplication, facilitating progression of the virus lytic cycle.

Papovaviruses, including simian virus 40 (SV40), have been extensively studied as they have a relatively small genome and require the multifunctional regulatory protein, the large tumour antigen (T-antigen). Specifically, the SV40 T-antigen, a 708 aa polypeptide, regulates the timing of the virus infection cycle, stimulates cellular proliferation, directly interacts with a number of cellular proteins, including the tumour suppressor p53, the retinoblastoma gene product pRb and DNA polymerase α, and regulates the expression of several cellular genes. Regulation of the lytic cycle by T-antigen involves transcriptional control exemplified by repression of its own gene expression, enhanced expression of the viral capsid proteins, and control of cellular gene expression. In addition, control over viral DNA replication, via its ability to bind, unwind and separate DNA strands and interact with DNA polymerase α and other replication protein complexes, and efficient virus assembly are among established roles for T-antigen (Conzen & Cole, 1994; Fanning & Knippers, 1992; Manfredi & Prives, 1994; Pipas, 1992).

The requirement of DNA binding for the ability of T-antigen to regulate transcriptional activity from viral and cellular promoters has been carefully studied. In vitro assays show that T-antigen, bound to its specific binding sites in the viral genomic control region, autoregulates its synthesis by sterically interfering with RNA polymerase binding or progression (Hansen et al., 1981; Rio et al., 1980; Rio & Tjian, 1983). In vivo, repression of viral early gene expression appears to be partially dependent on the ability of T-antigen to bind DNA and may require an additional interaction of T-antigen with auxiliary binding proteins (Mitchell et al., 1987).
Most studies have indicated that the ability of T-antigen to regulate late gene expression is independent of its ability to bind DNA. Of note, T-antigen mutants that are incapable of binding DNA can still transactivate the viral late promoter. Also, T-antigen can stimulate transcription of cellular promoters which do not contain T-antigen binding sites. Much attention has been focused on the identification of viral and cellular promoter regions involved in the regulation of transcription by T-antigen. Initial studies have pointed to the importance of multiple cis-acting regions of the viral promoter that play an important role in viral late gene transactivation (Gallo et al., 1988; Brady & Khoury, 1985). Several promoter regions [designated o, T, δ and TABS (T-antigen activatable site)] have been defined as required sites for SV40 late gene transactivation (Casaz et al., 1991; Gallo et al., 1988; Gruda & Alwine, 1991). The inclusion of the TABS site in a heterologous construct has been shown to confer responsiveness to T-antigen. Further, T-antigen has not been shown to bind to this site nor does it directly interact with the complexes that bind to this site. However, the presence of T-antigen modifies the binding characteristics of the complex by direct interaction with multiple components of the transcription complex, including the TATA binding protein and transcription enhancement factor-1 (Damania & Alwine, 1996; Gruda et al., 1993).

Much less is known about the role of T-antigen during the lytic cycle of the human papovavirus JC virus (JCV). JCV is a neurotropic virus and the aetiological agent of the fatal human demyelinating disease, progressive multifocal leukoencephalopathy (Major et al., 1992). The study of JCV has been hampered by its narrow tissue- and host-specificity, and its inefficient and extended life-cycle (Frisque & White, 1992).

Like SV40, JCV has a small circular bidirectional genome, with an early coding region (encoding the viral T-antigen), late coding region (encoding structural proteins) and non-coding or regulatory region (containing the origin of viral DNA replication and the transcriptional control region) that lies between the two coding regions (Frisque & White, 1992). The transcriptional control region of JCV, which functions in opposite orientations to regulate early and late gene expression, contains two characteristic tandem 98 bp repeats. We have previously demonstrated that expression from the JCV promoter is significantly enhanced in the presence of JCV T-antigen in glial cells (Lashgari et al., 1989). Further, SV40 T-antigen, which shares a 72% amino acid homology with JCV T-antigen, can activate the same promoter (Lashgari et al., 1989).

Distinct transcription initiation sites have been detected from the viral promoter in the early and late phases of infection (Daniel & Frisque, 1983; Khalili et al., 1987; Vacante et al., 1989). At least seven major transcripts have been detected in glial cells (Kenney et al., 1986). Two of these transcripts map to the late side of the tandem repeats, four map within the 98 bp repeats, and one lies to the early side of the tandem repeats. Late gene transcription does not appear to be under the control of the viral TATA box, and this may account in part for the heterogeneous array of transcription start sites. This situation is analogous to that in SV40. Both viruses have a TACCTA sequence upstream of some of these late transcripts, an element that may function as a surrogate TATA box.

In this study, we have examined the ability of JCV T-antigen to activate its own late promoter and identified a T-antigen-dependent initiation (TADI) site within the JCV promoter. Additionally, we have characterized cellular proteins which interact with this TADI site.

**Methods**

**Cell lines.** All cell lines were maintained at 37 °C in an atmosphere containing 7% CO₂ in a humidified incubator. U-87MG, HeLa and HJC-15 cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% foetal bovine serum, 100 U/ml penicillin and 100 µg/ml streptomycin. BJAB cells were maintained in RPMI 1640 medium with the same supplements.

**Plasmids.** The reporter plasmid (pJCVCAT) contains the HindIII–Poul fragment representing the control region of the JCV late promoter cloned upstream of the chloramphenicol acetyltransferase (CAT) gene (Kenney et al., 1984). Deletion mutants were created by linearizing pJCVCAT at the unique Xho site in the polylinker upstream of the JCV promoter. Linear DNA was treated with Bsu31 nuclease for 1–5 min and the reaction was terminated by the addition of EDTA to a final concentration of 50 mM, followed by phenol–chloroform extraction. After repairing the ends of the DNA fragments with T4 polymerase, the treated DNAs were digested with NruI, which cleaves at a unique position within the vector so that each truncated promoter is co-terminated at the 5’ end. The DNA was subsequently self-ligated with T4 DNA ligase and the extent of the deletions was determined by direct DNA sequencing.

Small promoter constructs containing the JCV initiator region upstream of the minimal promoter and the CAT reporter gene were created by the direct insertion of oligonucleotides into the multiple cloning site of the pBLCAT3 reporter (pCAT) (Luckow & Schutz, 1987). Generation of the pCAT-TADI and pCAT-TADI constructs involved direct ligation of double-stranded TADI wild-type and mutant oligonucleotides into pCAT. The pCAT-D construct was generated by direct ligation of a double-stranded oligonucleotide containing the D-domain into pCAT. All plasmid constructs were verified by direct DNA sequencing.

**Oligonucleotides.** The sequences of the oligonucleotides used are listed below. The underlined nucleotides in the mutant TADI oligonucleotide (TADIm) represent the mutated bases.

\[
\begin{align*}
\text{TADI}_{4'} & \quad 5'\text{CATCCGGTGGCTCCTAGTATGACCTATT} 3' \\
\text{TADI}_{5'} & \quad 5'\text{CTGAGATGACCTATCCTAGGAGAGCAAGC} 3' \\
\text{TADI} & \quad 5'\text{CTGAGATGACCTATCCTAGGAGAGCAAGC} 3' \\
& \quad 3'\text{TTACTCGAATGAGCTTCAAGGG} 5' \\
\text{TADI}_{5''} & \quad 5'\text{CTGAGATGACCTATCCTATAGGAGAGC} 3' \\
& \quad 3'\text{TTACTCGAATGAGCTTCAAGGG} 5' \\
\text{D-domain} & \quad 5'\text{CGGCCAGAGCTTCAAGGG} 3' \\
& \quad 3'\text{CTGTCGCAAGCGCACAAA} 5'.
\end{align*}
\]

**Transient transfections.** Transfection assays were performed according to the calcium phosphate precipitation technique (Graham & van der Eb, 1973). Briefly, 5 × 10⁶ cells were plated on 60 mm plates and
grown overnight. At 3 h prior to transfection, the cells were fed with new growth medium. The transfection mixture contained 2 µg reporter plasmid and 10 µg T-antigen-producing plasmid. The calcium phosphate precipitate was removed after 3–5 h and cells were treated with PBS containing 15% glycerol. After 2 min, cells were washed with PBS and maintained in DMEM containing 10% foetal calf serum. At 48 h post-transfection, cells were collected and a crude protein extract was prepared by repeated freeze–thaw. The extracts were quantitated by the Bio-Rad Bradford assay and equal amounts of protein extract (75–100 µg) were assayed for CAT activity (Gorman et al., 1982). The degree of transactivation was measured by scintillation counting of the spots cut from thin-layer chromatography plates. Each experiment was repeated at least four times with different plasmid preparations.

### Binding assays.
Nuclear extracts were prepared by a modification of the mini-extract protocol as described previously (Schreiber et al., 1989). Electrophoretic mobility shift assays, and one- and two-dimensional UV crosslinking analyses were performed as previously described (Raj & Khalili, 1994).

### RNA analysis.
RNA was extracted from transfected cells by the hot acid–phenol procedure as described previously (Queen & Baltimore, 1983). DNA was removed by treatment of the extracted RNA with 50 µg/ml DNase I. A labelled single-stranded M13-derived probe which spans the entire JCV promoter region and extends to the XhoI site present in the M13 phagemid was mixed with 20–50 µg purified RNA in 20 µl hybridization buffer [0.4 M NaCl, 0.04 M PIPES (pH 6.4), 1.25 mM EDTA, 80% formamide] and heat-denatured at 65 °C for 15 min, followed by gradual cooling to 37 °C. After incubation at 37 °C for 16 h, hybridizations were terminated by the addition of 180 µl 0.25 M NaCl, 0.03 M sodium acetate (pH 4.6), 1 mM ZnSO4, and 800 U/ml S1 nuclease. The reactions were incubated for 60 min at 37 °C. The S1-protected fragments were phenol–chloroform extracted, ethanol-precipitated and resolved on denaturing polyacrylamide–urea gels. Dideoxy sequencing with the same primer used to derive the S1 probe was run concurrently to accurately map the transcription initiation sites.

### Results

#### Identification of T-antigen-responsive regions on the viral late promoter

We have previously demonstrated that both JCV and SV40 T-antigens could stimulate the JCV late promoter (pJCV_L) (Lashgari et al., 1989). Here, we utilize a series of deletion mutants of the pJCV_L to further identify regions which are responsible for T-antigen transactivation (Fig. 1). Each reporter construct was transfected into the human astrocytic cell line U-87MG in the absence or presence of a plasmid expressing JCV T-antigen under the control of the cytomegalovirus early gene promoter.

Expression from the full-length JCV late promoter is dramatically enhanced (26-fold) upon expression of JCV T-antigen in the transfected cells. It is likely that the observed increased levels of late gene transcription result from transcriptional activation of the late promoter as well as amplification of the vector DNA by JCV T-antigen. Removal of DNA sequences spanning nt 4981–5112, which deletes the NF-κB motif (construct p[JCVA]), resulted in a slight decrease (26- to 20-fold) in the level of late promoter activity by JCV T-antigen. Similarly, deletion of the core origin of DNA replication (pJCVA13) produced decreased transactivation of the viral promoter by T-antigen in comparison to the full-length pJCV_L. Of note, although both of these constructs, pJCV_LAB and pJCV_AL, showed decreased levels of T-antigen-mediated transactivation, both were still responsive to T-antigen. These data suggest that, whereas the region encompassing the GC-rich sequence (GRS) that overlaps with the viral origin of DNA replication exerts some influence on late gene activation by T-antigen, the sequences positioned within the 98 bp repeats are also critical for the observed regulatory action. This is consistent with our previous observations demonstrating that sequences within the 98 bp repeats are important for T-antigen-mediated transactivation of the JCV late promoter (Chowdhury et al., 1990). Further, since these mutant constructs, i.e. JCV_LAB and JCV_AL, are incapable of replicating, the ability of T-antigen to activate the viral late promoter appears to be independent of its capacity to orchestrate viral DNA replication.

Sequential deletion analyses indicated that progressive deletions of the viral promoter region resulted in a gradual diminution in the extent of T-antigen-mediated activation. T-antigen was still able to enhance expression from the deletion promoter construct encompassing only part of one 98 bp repeat, pJCVA1 (Fig. 1). Removal of the sequence between nt 139 and 168 that created the pJCVA2 deletion mutant resulted in a loss of responsiveness of the promoter to JCV T-antigen. Further deletion constructs were also unresponsive to T-antigen (data not shown). These data indicate that the region spanning nt 139–168 of the JCV_L promoter is required for T-antigen-mediated transcriptional activation and that additional regions within the viral promoter enable maximal responsiveness of the late promoter to T-antigen. Similar data were observed in separate studies in which cells were co-transfected with the JCV_L deletion constructs and a plasmid expressing SV40 T-antigen (data not shown).

Additional support for this finding comes from experiments utilizing the HJC-15 cell line, a hamster glioblastoma cell line that constitutively expresses JCV T-antigen (Walker et al., 1973). In HJC-15 cells, we observed that the basal level of the JCV_L promoter activity in constructs containing the T-antiigen responsive region (pJCVA1 and larger constructs) was consistently several times higher over a wide range of reporter concentrations than those lacking the T-antigen-responsive region (pJCVA2 and smaller constructs) (G. V. Raj, C.-F. Chang & K. Khalili, unpublished observations).

Our follow-up studies have shown that the ability of T-antigen to activate the JCV_L promoter through the region spanning nt 139–168 is not restricted to glial cells and is seen in a wide variety of non-glial cells, including the epithelial HeLa and lymphoid BJAB cells. Taken together, these data indicate that the region between nt 139 and 168 of the viral promoter is required for T-antigen-mediated transactivation and the responsiveness is not restricted to human glial cells.
**T-antigen enhances transcriptional initiation**

Next, we sought to confirm the transactivational ability of T-antigen at the RNA level and map the transcriptional initiation sites within the JCV late promoter. To this end, U-87MG cells were transfected with the JCV \( \text{L}^{\Delta \kappa B} \) construct in the absence and presence of a JCV T-antigen-producing plasmid. RNA was harvested from these cells 60 h after transfection and subjected to S1 nuclease analysis. This single-stranded probe is derived from the early strand, and hence will hybridize only to RNA species produced by the late strand. The precise position of these transcription initiation sites was identified by dideoxy sequencing reactions run alongside the S1 analysis (not shown) utilizing the same primer used to derive the S1 probe.

In the absence of the viral T-antigen (\(-T\)), a series of low-intensity bands migrating near the bottom of the gel and corresponding to nt 240–247 (D-domain) were detected (Fig. 2a). These RNA species are likely to represent the basal level of expression.
of JCV late gene transcription. In the presence of the viral T-antigen, the viral late promoter activity was dramatically enhanced. Transcription initiation from the D-domain was increased in comparison to initiation from the same site in the absence of T-antigen (Fig. 2a, compare lane + T to lane − T). In addition, there appeared to be strong transcriptional initiation sites which mapped to nt 61 and 64 within the first 98 bp sequence and nt 159 and 162 within the second 98 bp sequence (Fig. 2a). Weaker transcription initiation sites mapped to nt 58 and 156 in the first and second 98 bp repeat, respectively. Evidently, transcription of these RNAs required T-antigen production, as no signals corresponding to these RNAs were observed in the absence of T-antigen. These TADI sites represent distinct initiation sites which overlap with a T-antigen-responsive region between nt 139 and 168 of the JCV late promoter (Kraus et al., 1996). Interestingly, the major transcripts initiated from TADI confers T-antigen responsiveness to a heterologous promoter. Oligo-}

### Table 1. The JCV transcription start site has homology with promoters containing a functional initiator element

<table>
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<th>Source</th>
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of JCV late gene transcription. In the presence of the viral T-antigen, the viral late promoter activity was dramatically enhanced. Transcription initiation from the D-domain was increased in comparison to initiation from the same site in the absence of T-antigen (Fig. 2a, compare lane + T to lane − T). In addition, there appeared to be strong transcriptional initiation sites which mapped to nt 61 and 64 within the first 98 bp sequence and nt 159 and 162 within the second 98 bp sequence (Fig. 2a). Weaker transcription initiation sites mapped to nt 58 and 156 in the first and second 98 bp repeat, respectively. Evidently, transcription of these RNAs required T-antigen production, as no signals corresponding to these RNAs were observed in the absence of T-antigen. These TADI sites represent distinct initiation sites which overlap with a T-antigen-responsive region between nt 139 and 168 of the JCV late promoter (Kraus et al., 1996). Interestingly, the major transcripts initiated from TADI confers T-antigen responsiveness to a heterologous promoter. Oligo-
Fig. 3. Effect of JCV T-antigen on minimal promoters containing T-antigen-responsive regions. The average fold effect (with SD) of increasing concentrations of JCV T-antigen (0, 2.5, and 10 µg) on the expression of 2 µg pBLCAT3-derived reporter constructs containing TADI, D or TADI\textsuperscript{mt} oligonucleotides in U-87MG cells is shown.

Discussion

We have previously demonstrated that both JCV and SV40 T-antigens are capable of activating the JCV late promoter (Lashgari et al., 1989). In this study, we have further examined the ability of JCV T-antigen to enhance expression from the JCV late promoter and by serial deletion analysis, we have identified several T-antigen-responsive regions within the JCV promoter. Interestingly, we have shown that a region within the JCV promoter located between nt 139 and 168 is necessary for T-antigen-mediated transactivation of the JCV late promoter. Moreover, we have identified a distinct TADI site within this region and sequence analysis of this site reveals a homology with the consensus initiator (Inr) element. Further, the TADI region is sufficient to confer T-antigen responsiveness to a heterologous reporter construct. In contrast, although S1 nuclease analysis showed that increased transcriptional initiation is noted from the D-domain, the D-domain by itself is insufficient to confer T-antigen-mediated transactivation. Electrophoretic mobility shift and UV crosslinking analyses demonstrate that multiple DNA–protein complexes are formed with the TADI probes. Mutation of the TADI site alters the binding of some of these DNA–protein complexes and abolishes the ability of T-antigen to activate expression from the initiator site. Taken together, these data support a model where the ability of the JCV T-antigen to activate the JCV late promoter may be partly accounted for by its ability to interact with specific protein complexes formed with the Inr element on the viral promoter. Further analysis of the ability of the T-antigen to activate the JCV late promoter with the TADI mutation \textit{in situ} may be instructive, especially considering the presence of the other T-antigen-responsive site on the viral promoter, the D-domain. Perhaps, the presence of auxiliary elements may allow for increased transcription from the D-domain mediated by T-antigen. These intriguing possibilities remain to be explored.
Transcription regulation of JCV in CNS cells

Fig. 4. Characterization of TADI nucleoprotein complexes. (a) Nuclear extract (5 µg) from U-87MG cells was incubated with end-labelled single- and double-stranded TADI probes and examined for binding by gel shift analysis. TADI_E and TADI_L are single-stranded oligonucleotides corresponding to the early and late strands, respectively, of the double-stranded TADI probe (TADI_ds). Alphabetic designations of various nucleoprotein complexes are also shown. (b) UV crosslinking data from one-dimensional analysis listing the sizes of the nucleoprotein complexes formed with specific TADI probes and from two-dimensional analysis tabulating the sizes of proteins within each specific complex. Alphabetic designations refer to the specific complexes named in (a).

1-D UV crosslinking analysis

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<th>Probe</th>
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</tr>
<tr>
<td>TADI_L</td>
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<tr>
<td>TADI_ds</td>
<td>210, 115, 97, 54, 46, 30, 28</td>
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2-D UV crosslinking analysis

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<tr>
<td></td>
<td>C</td>
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<td>K</td>
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Fig. 5. Comparison of nucleoprotein complex formation between wild-type and mutant single- and double-stranded TADI probes. Nuclear extracts (5 µg) from U-87MG cells were examined for binding to wild-type and mutant (mt) single- and double-stranded TADI probes by gel shift assays. E, L and ds refer to the early and late single strands, and the double-stranded TADI oligonucleotides, respectively. The arrowheads and arrow indicate nucleoprotein complexes which are disrupted by mutations in the TADI_E and TADI_L probes, respectively. Asterisks indicate new nucleoprotein complexes formed in the TADI_E and TADI_L mutant probes.

A study by Kumar et al. (1996) demonstrated that JCV late gene transcription was dependent on the NF-1 sites present within the 98 bp repeats. Cluster mutations consisting of five mutated nucleotides within the NF-1 sites demonstrated reduced CAT activity compared to the wild-type sequence. Although three of the five mutations lie within the region that we mapped by deletion analysis to contain the minimal T-antigen-responsive region, the sequence we characterized is distinct from the one described by Kumar et al. (1996). We describe T-antigen-dependent transcription sites initiating from an Inr-like sequence in the JCV late promoter. An oligonucleotide containing this TADI sequence element is able to confer T-antigen responsiveness to a heterologous promoter. Of note, this oligonucleotide does not contain the adjacent NF-1 site, which Kumar et al. (1996) described as being essential for T-antigen-dependent late gene transactivation. However, the interaction or cooperation of factors
interacting with both of the sites to mediate optimal levels of JCV T-antigen-mediated late gene transactivation is also possible.

The JCV regulatory region contains two identical tandem 98 bp repeats. Thus, there are two copies of the JCV initiator-like sequence, one located in each 98 bp repeat, and transcription initiation is detected from both sites by S1 nuclease analysis (Fig. 2). Deletion analysis demonstrated that the TADI site located between nt 139 and 168 represents the minimal region of the JCV late promoter that is required for T-antigen-mediated transcriptional activation. Interestingly, deletion of the upstream TADI sequence resulted in only a slight decrease in reporter activity in response to T-antigen (compare pJCV\textsubscript{AT} and pJCV\textsubscript{AT}). Perhaps upon removal of the upstream TADI element, the downstream site can compensate. Further deletion and mutational analysis will be necessary to investigate this possibility. Nonetheless, the sequence identity of these TADI elements suggests that either element is able to confer T-antigen responsiveness.

The ability of TADI, but not TADI\textsuperscript{mut} oligonucleotides to confer T-antigen responsiveness to the minimal pBLCAT3 promoter is interesting. The data indicate that JCV T-antigen can activate transcription from a single transcriptional initiation site. Although these data stand in contrast to the SV40 promoter, where SV40 T-antigen requires both a transcriptional initiator and an upstream element (Casaz \textit{et al.}, 1991; Sceller \textit{et al.}, 1991), the possibility of upstream cryptic sites within pBLCAT3 has not been conclusively ruled out.

The ability of T-antigen to transactivate the viral late promoter is attributed to potential interactions between T-antigen and cellular proteins that interact with the viral promoter, which is similar to the case with the SV40 promoter (Damania \& Alwine, 1996; Gallo \textit{et al.}, 1990; Gruda \textit{et al.}, 1993). Indeed, JCV T-antigen has been demonstrated to cooperatively interact with cellular proteins, including YB-1, Pur \textalpha, and Tst-1 to regulate JCV late gene expression (Chen \& Khalili, 1995; Chen \textit{et al.}, 1995; Kerr \textit{et al.}, 1994; Renner \textit{et al.}, 1994). Further, the direct interaction between T-antigen and existing cellular transcriptional regulators, like p53, pRb and AP-2, and/or the basal transcriptional machinery may also contribute to the regulation of cellular and viral promoters by T-antigen.

The demonstration of a such a heterogeneous array of cellular proteins which bind to the TADI sequence underscores the potential complexity involving this region of JCV. More detailed mutational analysis of the JCV Inr region will help decipher the role these JCV initiator binding proteins have in the transcriptional initiation of JCV late gene expression. Moreover, the identification of specific cellular proteins which interact with this sequence will be equally as informative.

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