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Construction and Characterization of Hybrid Polyomavirus Genomes

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Several studies have suggested that certain unique features of the JC virus (JCV) regulatory region are responsible for the restricted lytic and transforming activities of this virus in vitro. To pursue this possibility, we have constructed hybrid polyomavirus genomes by exchanging the regulatory sequences of JCV, BK virus (BKV), and simian virus 40 (SV40). The host range of JCV was not expanded by the substitution of the BKV or SV40 regulatory signals; such hybrids were nonviable even in primary human fetal glial cells, the sole permissive cell for JCV. However, chimeric DNAs containing JCV regulatory sequences and BKV- or SV40-coding sequences were lytically active, indicating that the BKV and SV40 T proteins were capable of effectively interacting with the JCV replication and transcription signals to yield infectious hybrid viruses. Although JCV regulatory sequences and coding sequences both contribute to the restricted lytic activity of this virus, it appears that the latter sequences, most likely those encoding the T protein, have a greater influence on this behavior.

Our laboratory has been investigating the molecular basis for a number of unique biological features of the human polyomavirus JC virus (JCV). During our studies we have taken advantage of the wealth of information available for the closely related and better-characterized viruses simian virus 40 (SV40) and BK virus (BKV). Although the genomes of these three viruses share a common genetic organization and exhibit extensive sequence homology (69 to 75%; 19), these viruses are biologically distinct. Each virus frequently produces subclinical infections in its natural host (82); JCV also causes a fatal demyelinating brain disease, progressive multifocal leukoencephalopathy, in immunocompromised individuals (62). Each virus is highly tumorigenic in hamsters (82); JCV is also neurooncogenic in primates (45, 69). While most polyomaviruses are limited in the number of cells that they lytically infect, JCV displays an unusually narrow host range in vitro, growing well only in primary human fetal glial (PHFG) cells (58, 61). Finally, unlike SV40 and BKV, and in contrast to its own oncogenic behavior in vivo, JCV is highly restricted in its ability to transform cells in culture; transformation is an infrequent, prolonged event that has been demonstrated in only a few cell types (21, 32). In light of these observations, an important question arises: What accounts for the diverse biological properties (e.g., host range, tissue tropism, pathogenicity, and oncogenicity) exhibited by these viruses?

An obvious first step in answering this question was to identify sequence differences occurring in the three viral genomes. Hybridization studies had indicated that sequences near the replication origins at 0.67 map units had diverged to the greatest extent (41). Through direct nucleotide analysis these sequences were found to encompass a number of transcription control elements located to the late side of the JCV, SV40, and BKV replication origins (16, 17, 68, 75, 88), and comparisons of these signals have yielded important clues to the basis for JCV's unique properties. For example, (i) the arrangement of the JCV promoter signals are atypical when compared with those of SV40 and BKV and may contribute to the restricted activity of JCV in culture (Table 1; 19); (ii) differences in the JCV enhancer undoubtedly influence certain biological features of this virus, since the effects of viral enhancers on transcription are dependent upon the species of host cell in which the enhancement is assayed and upon the specific tissue from which the host cells are derived (6, 8, 9, 13, 22, 30, 34, 35, 37, 39, 40, 71, 72, 74); and (iii) in addition to the differences that occur among the JCV, SV40, and BKV enhancers, it has been shown that the promoter/enhancer sequences of JCV variants isolated from different progressive multifocal leukoencephalopathy patients are hypervariable (50), perhaps reflecting an adaptation of the virus to growth in brain tissue (in most individuals JCV appears to infect and to remain latent in kidney tissue). Small changes in promoter/enhancer elements are known to modify the host range and oncogenic properties of a virus (22, 30, 34, 35, 85, 86). Therefore, it was not surprising to find that, while the enhancer of prototype JCV (Mad 1) only functions well in PHFG cells (37), the altered enhancers of some JCV variants function in a wider range of cell types (M. T. Cannella and R. J. Frisque, unpublished results). Also consistent with these findings is the suggestion that the small deletion in the enhancer of the Mad 4 isolate of JCV is responsible for this variant's unusual propensity to cause pineocytomas in experimental animals (50).

To investigate the influence of JCV's regulatory sequences on its behavior in vitro, one of our approaches has been to construct and characterize chimeric polyomavirus genomes. A number of investigators have used a similar approach to study the function of various regulatory signals derived from other eucaryotic systems. In some of their experiments specific viral sequences (usually enhancer sequences) have been exchanged between two viruses (7–9, 13, 38, 40, 43, 71). In other instances control elements have been inserted into plasmids that contain a foreign, readily assayable gene (e.g., the chloramphenicol acetyltransferase, β-globin, or papovavirus T-antigen genes) (1, 2, 4, 14, 30, 37, 39, 55, 72, 84). Many of these studies have utilized transient expression systems to assess the activities of these regulatory sequences. Our experiments differ in that an intact regulatory region of one virus has been replaced by that of a second, closely related virus. The exchange includes not only enhancer sequences, but also early and late promoters, DNA

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TABLE 1. Comparisons of the regulatory regions[a] of the JCV, BKV, and SV40 genomes

Viral genome	% Homology (with JCV)		Size of regulatory region (np)	Origin: size of dyad symmetry (np)[b]	T Ag-binding sites (no. of copies)[c]		AT-rich sequence[d]		5'-PyPy-CCXCCC-3' sequence (no. of copies)[e]	CAAT box (5'-GGPy-CAATCT-3') (no. of copies)[f]	Size of enhancer (np)[g]	Core (5'-GTGG-TTTAAAG-3') (no. of copies)	Core (5'-AGGAAGTGAA-3') (no. of copies)
	Entire genome	Regulatory region			5'-(G>T)(A>G)-GGC-3'	5'-AAG-GC-3'	Size (np)	No. of copies					
JCV			393	25	2	0	15	2	0	4	2 × 98	2	1[h]
BKV(Dun)	75	55	387	23	2	1	20	1	2	3	3 × 68	3	3
BKV9		64	450	23	2	1	20	1	1	4	2 × 143	2	2
SV40	69	44	414	27	3	0	17	1	6	6	2 × 72	3[h]	0

[a] The regulatory region is considered to be all of those noncoding sequences present between the initiation codons for the early and late proteins.
[b] The symmetry is believed to be the center of the viral replication origins.
[c] Identified via binding studies and comparisons of sequence data. Two pentanucleotide consensus sequences have been determined (12, 73).
[d] The AT-rich sequence (also called the Goldberg-Hogness or TATA box) positions the start sites of the early viral mRNAs (3, 23, 26).
[e] Part of the recognition sequence for transcription factor Sp1 (15). JCV has two copies of the related sequence 5'-PyPyCCXXCCC-3'.
[f] The CAAT boxes in JCV and BKV show only partial homology with the consensus sequence.
[g] Except for BKV(Dun), the enhancer in each genome is a tandem duplication; in BKV(Dun) this signal is a tandem triplication (68, 50, 67 np; the middle repeat is a subset of the other two). Two core sequences have been identified in various enhancer sequences (28, 29, 87).
[h] One of the JCV and one of the SV40 core elements occur just to the late side of the tandem repeat.

replication origins, T-antigen-binding sites, and untranslated leader sequences of the early and late mRNAs. Because the coding regions have been left unaltered and the genome sizes are nearly identical, the possibility existed that viable hybrid viruses could be generated following transfection of permissive cells with these recombinant molecules.

Although our initial focus has been on the unusual features of the JCV regulatory region, it is clear that these sequences are not the sole determinants controlling the virus-cell interactions. Obviously, adaptation of a virus to growth in a particular host involves a number of factors. The polyomaviruses rely heavily on the host replication machinery; their own specialized proteins are responsible for the regulation of the complex series of events that occurs during the lytic cycle (early proteins) and for the assembly and infectivity of the intact virions (late proteins). The sequences encoding the six viral proteins of JCV, SV40, and BKV are highly conserved; however, differences can be readily detected in those sequences specifying the carboxy termini of the large and small T proteins, the agnoprotein, and the overlapping region of the VP1 and VP2/3 proteins (19). In this study we discuss our analyses of hybrid viral genomes and begin to assess the relative contributions made by the coding and noncoding regions of the polyomavirus genomes to virus-host interactions. Because problems associated with JCV's restricted host range and prolonged lytic cycle have severely limited the study of this human virus and have made many types of molecular analyses impractical, an understanding of the factors contributing to these phenomena is essential to our future investigations of JCV.

MATERIALS AND METHODS

Cells and viruses. CV-1 (African green monkey kidney cell line), WI-38 (human diploid lung cell line), and PHFG cells were propagated in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum, penicillin (99 U/ml), and streptomycin (73 U/ml). Cell cultures producing virus were frozen and thawed twice, and lysates were collected and stored at −70°C. Prior to their use in infectivity studies, lysates containing virus were sonicated (2 × 1 min; setting 30 on a Fisher sonic dismembrator, model 300).

Recombinant plasmids. All parental viral DNAs used to construct hybrid polyomavirus genomes were cloned into pBR322 with the exception of dl892 (77), a viable deletion mutant of SV40, which was inserted into pMK16 (23). Plasmid and viral DNAs were joined at their unique *Eco*RI sites. The pMad1-TC clone represents prototype JCV DNA isolated from virus that had been passaged in tissue culture. The parental BKV plasmid, pBKV9, was made by using viral DNA extracted from a preparation of the prototype Gardner strain of BKV, BKV(WT). Restriction enzyme and sequence analyses revealed that the cloned BKV DNA represents a viable variant of this virus. The pSV40 recombinant plasmid contains the DNA of the small-plaque variant of SV40 (strain 776). Transformation of *Escherichia coli* DH-1 with the recombinant DNAs and small- and large-scale preparations of plasmid DNAs have been described previously (46). The numbering systems used for the JCV, BKV, and SV40 genomes throughout this paper are those established by Frisque et al. (19), Seif et al. (76), and Fiers et al. (16), respectively.

Construction of hybrid viral genomes. To construct the pM-1(BKV) hybrid genome, the 482-nucleotide pair (np) *Nco*I fragment of BKV9 DNA (nucleotides 5075 to 341), containing all of the viral regulatory sequences, was isolated by partial *Nco*I digestion (this fragment contains additional *Nco*I sites) and ligated to the large *Nco*I fragment of pMad1-TC (nucleotides 275 to 4980 of JCV plus all of pBR322).

The first step in the construction of the pM-1(SV40) hybrid genome was to delete the single *Bam*HI site in pBR322 to facilitate isolation of one of the JCV DNA fragments required in a subsequent step. This was accomplished by digesting pMad1-TC with *Cla*I and *Sal*I, filling in the recessed ends by the action of the large fragment of DNA polymerase I in the presence of all four deoxynucleoside triphosphates, and recircularizing the molecule by ligation. This DNA [pMITC(Δ*Bam*)] was used to prepare a 7,770-np *Bam*HI-*Nco*I fragment (nucleotides 275 to 4307 of JCV plus all of pBR322) and a 678-np *Bam*HI-*Hin*fI fragment (nucleotides 4307 to 4985). All the SV40 regulatory sequences were present on a 422-np *Hin*fI-*Nco*I fragment (nucleotides 5135 to 333) produced by digesting pdl892 with these two enzymes. Ligation of the three fragments gave the hybrid DNA that contained protein-coding information of JCV and regulatory signals of SV40.

The construction schemes for the pSV40(M-1) and pBKV(M-1) hybrid DNAs are diagrammed in Fig. 1. Detailed descriptions of all of the constructions are available upon request.

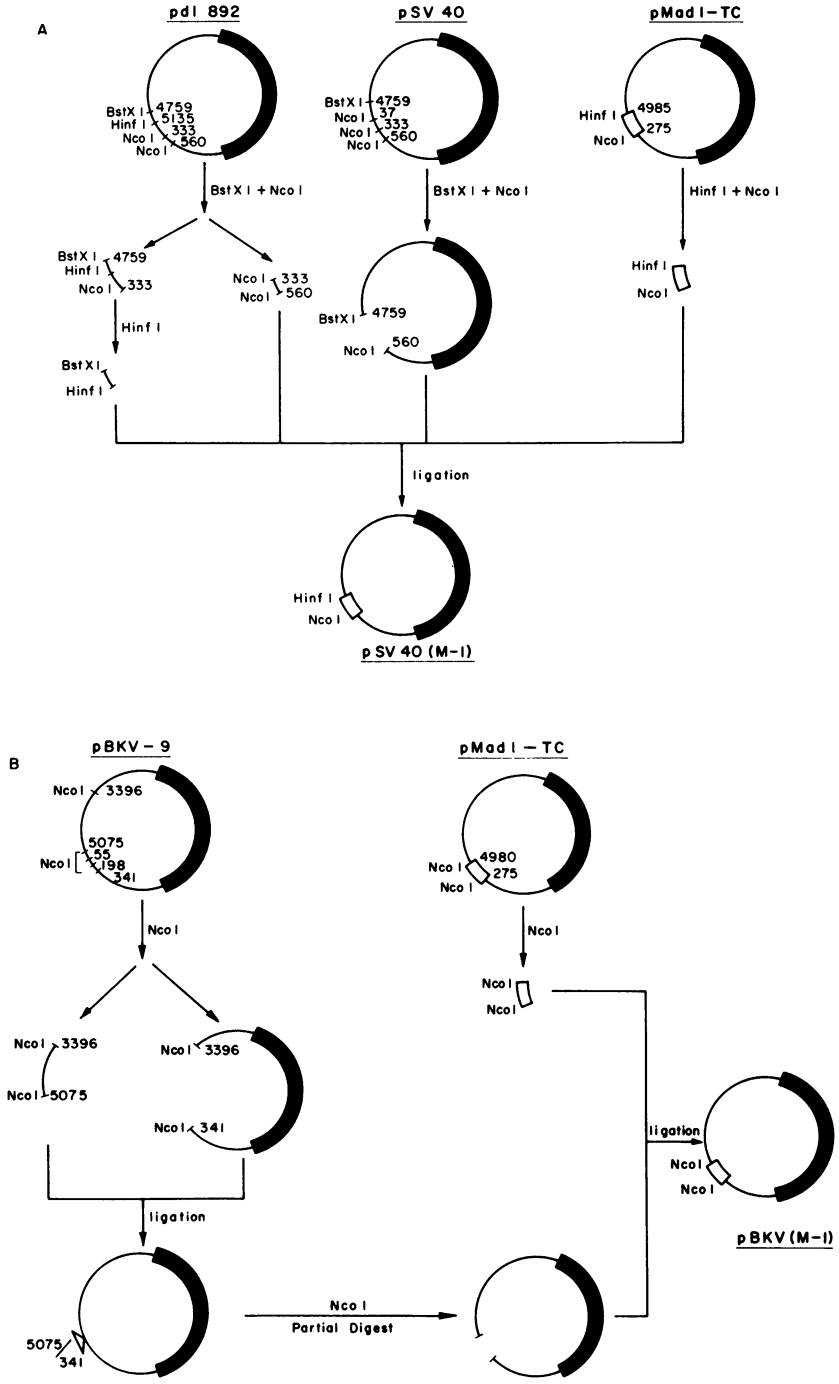

FIG. 1. Construction of hybrid polyomavirus genomes. Hybrids were constructed by exchanging regulatory seqences between JCV and SV40 (A) or JCV and BKV (B). The numbering schemes are based on earlier reports (16, 19, 76). The numbering for dl892 is identical to that for SV40 and does not reflect the 19-np deletion that occurs in the genome of this viable variant (nucleotides 35 to 53 of SV40 are missing in dl892 and one of the three SV40 *Nco*I restriction sites is lost). The numbering for BKV9 takes into account the differences found in its regulatory region (Fig. 2); the remainder of the molecule is numbered with the assumption that BKV9 and BKV(WT) are identical throughout the rest of their genomes (extensive restriction enzyme analysis has not revealed obvious differences outside the regulatory region). The thick lines represent plasmid sequences; the thin lines represent viral sequences.

DNA sequencing. Restriction endonuclease fragments that spanned either of the two junctions between the regulatory and coding sequences of the four hybrid viral genomes were isolated, purified, and labeled at their 3′ ends with the large fragment of *E. coli* DNA polymerase I and the appropriate α-^{32}P-labeled deoxynucleoside triphosphate. These DNAs were converted to single end-labeled fragments by the action of a second restriction enzyme. Nucleotide sequences were determined by the Maxam-Gilbert procedure (53); chemical cleavage products were analyzed on polyacrylamide gels (8 and 12%). Gels were frozen and autoradiographed for 2 to 3 days without the aid of an intensifier screen.

DNA transfection. All transfections were done by a modification of the DEAE-dextran technique (78). Cells were seeded onto 60-mm dishes or 24-well cluster plates (each containing 12-mm cover slips) and were approximately 75% confluent at the time of transfection. DEAE-dextran (25 mg; molecular weight, 500,000) was dissolved in 95 ml of Dulbecco modified Eagle medium and 5 ml of 1.0 M Tris hydrochloride, pH 7.5. This mixture (containing viral DNA) was added to cells that had been washed two times with serum-free Dulbecco modified Eagle medium. Plates were incubated at 37 or 39°C in a 10% CO_2 atmosphere for 2 to 4 h. At this time the cells were washed and refed with Dulbecco modified Eagle medium supplemented with 3 to 10% fetal calf serum. The viral DNAs used in these experiments had been separated from plasmid sequences and recircularized by ligation. Each DNA was electrophoresed on a 1% agarose gel prior to transfection to check that equivalent amounts of ligated DNA would be used.

Immunofluorescent staining. At various times after transfection, cells growing on 12-mm cover slips were fixed for 90 s in a 1:1 mixture of acetone and methanol. To detect T antigen, serum from a hamster bearing an SV40-induced tumor was added to cells for 30 min at 37°C (SV40 anti-T serum gave the most intense staining for all three viral T proteins). After rinsing the cells with phosphate-buffered saline, fluorescein-conjugated goat anti-hamster immunoglobulin G serum was added. Thirty minutes later the cover slips were rinsed with phosphate-buffered saline and distilled water and were mounted in buffered Gelvutol (10 g of polyvinyl alcohol in 40 ml of phosphate-buffered saline and 20 ml of glycerol) on microscope slides. The same procedure was followed for visualizing V antigen, except rabbit anti-V serum, active against the capsid proteins of all three viruses, was used as the first antibody and fluorescein-conjugated goat anti-rabbit immunoglobulin G serum was the second antibody.

HA titers. The hemagglutination (HA) test was conducted as described by Padgett and Walker (59). Briefly, doubling dilutions of the virus preparations were made in phosphate-buffered saline. An equal volume of 0.5% human type O erythrocytes from donors lacking antibodies to JCV was added, and the mixture was incubated for 3 h at 4°C. The HA titer was taken as the reciprocal of the highest dilution of the virus suspension showing complete agglutination of erythrocytes.

Isolation and analysis of viral DNA from infected cells. Viral DNA was extracted from infected PHFG cells by the method of Hirt (31). This DNA was purified by phenol and chloroform extractions and precipitated with 2 volumes of 95% ethanol. The DNA was suspended in TE (10 mM Tris hydrochloride, pH 7.5, 1 mM EDTA) and digested with various restriction endonucleases. The DNA fragments were separated on horizontal 1% agarose slab gels, denatured, and transferred to nitrocellulose paper as described by Southern (79). The appropriate plasmid DNAs were labeled with all four α-^{32}P-deoxynucleoside triphosphates by nick translation (70) and used as hybridization probes against the DNA bound to the nitrocellulose paper. Bands were visualized by autoradiography, using XAR-5 film.

RESULTS

Construction of hybrid polyomavirus genomes. To determine the contributions made by the JCV regulatory and coding sequences to the unique biology of this virus, we have exchanged the regulatory regions of JCV, BKV, and SV40. These sequences include all noncoding sequences present between the initiation codons for the agnoprotein and the T/t proteins of the viruses. Table 1 lists a number of differences in the regulatory signals of these viruses. The construction schemes for the pBKV(M-1) and pSV40(M-1) hybrid genomes are shown in Fig. 1; descriptions of the pM-1(BKV) and pM-1(SV40) constructs are given in Materials and Methods. The sequences donated by each viral genome to yield the hybrid molecule can be deduced from the naming scheme. For example, the name of the hybrid pBKV(M-1) indicates that the DNA was cloned into pBR322 (p) and that the coding sequences were donated by BKV and the regulatory sequences were donated by prototype JCV(Mad-1).

To facilitate the construction of the pM-1(SV40) and pSV40(M-1) hybrids, the viable SV40 deletion mutant dl892 (77) was used. The deletion in dl892 (nucleotides 35 to 53 of SV40) removes an *Nco*I restriction endonuclease site (*Nco*I was used in the cloning protocol) found at nucleotide 37 in SV40 strain 776. In addition, the first of six copies of the SV40 GC boxes plus one nucleotide of the second box is removed via this deletion. The start sites of the early and late dl892 mRNAs are the same ones utilized by the wild-type SV40 mRNAs (23). In each of our experiments dl892 DNA was used in parallel with SV40 DNA, and in several cell types the only difference detected between the two viral DNAs was a slight delay in the lytic cycle of the mutant. In the construction of the pM-1(BKV) and pBKV(M-1) hybrids, the variant BKV9 DNA, isolated and cloned from a preparation of BKV(WT), was used. The regulatory regions of the wild-type and variant BKV genomes differ only in the enhancer sequences (Table 1; Fig. 2); the 143-np tandem duplication of BKV9 appears to be derived from part of one of the 68-np repeats (last 36 np of the repeat) found in BKV (WT and Dun) plus 107 nucleotides located immediately to the late side of the BKV(WT) enhancer. BKV9 is lytically active in WI-38 cells (see below) and efficiently transforms BHK-21 and Rat-2 cells (B. Bollag, W. F. Chuke, and R. J. Frisque, manuscript in preparation).

The hybrid DNAs were made by exchanging the sequences located between the initiation codon for the agnoprotein and the first 29 (in JCV-SV40 hybrids) or 34 (in JCV-BKV hybrids) nucleotides of the early coding region. Since the first 10 amino acids of the JCV and SV40 early proteins and the first 11 amino acids of the JCV and BKV early proteins are identical (19), the large and small T-antigen-coding sequences are not altered.

To confirm that each recombinant clone was constructed properly, all hybrid DNAs were subjected to extensive restriction enzyme and sequence analyses (Fig. 3 and 4; data not shown). An example of the restriction enzyme digests for the pBKV(M-1) chimeric DNA is shown in Fig. 3. The restriction patterns demonstrate that all fragments are joined in the proper orientation and that tandem insertions of fragments have not occurred. Figure 4 depicts a linear representation of the junction regions of the four hybrid genomes. The positions of a number of regulatory signals relative to the coding information are indicated. The nucleotide sequence at each junction is also shown; approximately 100 nucleotides of sequence have been determined at each of the eight junctions, and these are identical to the published sequences (data not shown).

Lytic activity of hybrid polyomavirus genomes in PHFG cells. Initial characterization of the hybrid DNAs involved their introduction into PHFG cells, which are permissive for the lytic growth of all three parental viruses. Viral DNA was

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BKV(WT)  GCCTCGGCCT CTTATATATT ATAAAAAAAA AGGCCACAGG GAGGAGCTGC
BKV9     GCCTCGGCCT CTTATATATT ATAAAAAAAA AGGC
         10        20        30        40        50

BKV(WT)  TTACCCATGG AATGCAGCCA AACCATGACC TCAGGAAGGA AAGTGCATGA
BKV9
         60        70        80        90        100

BKV(WT)  CTCACAGGGG AATGCAGCCA AACCATGACC TCAGGAAGGA AAGTGCATGA
BKV9
         110       120       130       140       150

BKV(WT)  CTCACAGGGA GGAGCTGCTT ACCCATGGAA TGCAGCCAAA CCATGACCTC
BKV9      CACAGGGA GGAGCTGCTT ACCCATGGAA TGCAGCCAAA CCATGACCTC
          40        50        60        70        80

BKV(WT)  AGGAAGGAAA GTGCATGACT GGGCAGCCAG CCAGTGGCAG TTAATAGTGA
BKV9     AGGAAGGAAA GTGCATGACT GGGCAGCCAG CCAGTGGCAG TTAATAAGCA
         90        100       110       120       130

BKV(WT)  AACCCCGCCG ACAGACATGT TTTGCGAGCC TAGGAATCTT GGCCTTGTCC
BKV9      GCCA G   ACAGACATGT TTTGCGAGCC TAGGAATCTT GGCCTTGTCC
                  140       150       160       170

BKV(WT)  CCAGTTAAAC TGGACAAAGG CCATGGTTCT GCGCCA
BKV9     CCAGTTAAAC TGGACAAAGG CCATGGTTCT GCGCCAAACC ATGACCTCAG
         180       190       200       210       220

BKV(WT)
BKV9     GAAGGAAAGT GCATGACTGG GCAGCCAGCC AGTGGCAGTT AATAAGCAGC
         230       240       250       260       270

BKV(WT)
BKV9     CAGACAGACA TGTTTTGCGA GCCTAGGAAT CTTGGCCTTG TCCCCAGTTA
         280       290       300       310       320

BKV(WT)                                 G CTGTCACGAC AAG...
                                        340       350
BKV9     AACTGGACAA AGGCCATGGT TCTGCGCCAG CTGTCACGAC AAG...
         330       340       350       360       370
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FIG. 2. Comparison of BKV(WT) and BKV9 regulatory sequences. BKV9 DNA was isolated from a preparation of BKV(WT) and cloned into the unique *Eco*RI restriction site of pBR322. The *Hin*dIII restriction fragment [nucleotides 4989 to 348 in BKV(WT)] that spans the regulatory region of the BKV9 genome was sequenced by the method of Maxam and Gilbert (53). The numbering system is that used by Seif et al. (76) for the Dunlop strain of BKV. The sequences shown represent the late strand of each DNA, and numbering begins at the center of the origin of replication. Approximately 200 nucleotides of sequence to the early side of each origin were found to be identical and are not shown. Brackets with arrows denote the boundaries of the large tandem repeats (enhancers) of the two BKV strains. The underlined sequence represents the 43 nucleotides in BKV(WT) that are absent in BKV(Dun) (75). The initiation codon for each agnoprotein is indicated by a box. The first 10 nucleotides encoding the BKV9 agnoprotein form part of the large tandem duplication, and therefore a second ATG is found at nucleotide 200.

separated from plasmid sequences, recircularized, and transfected (0.2 μg) into the cells (~1×10^5 to 2×10^5/16-mm well) by the modified DEAE-dextran method (78). To follow the events that occurred during the interaction of these polyomaviruses with their host cell, a number of parameters were investigated, including: the expression of T (early protein) and V (late proteins) antigens (determined 2 to 35 days post-transfection), the appearance of cytopathic effects (CPE), the production of hemagglutinating activity, and the presence of infectious virions in lysates of the transfected cells (Table 2). This basic biological characterization of the hybrid DNAs would allow us to assess whether viable hybrid viruses were produced or, if they were not, the stage at which the infection was blocked. While S1 nuclease analysis and quantitation of the viral mRNAs in transfected cells would provide additional comparative data of the parental and hybrid DNA lytic activities, such experiments are not feasible at this time due to our inability to obtain sufficient quantities of PHFG cells.

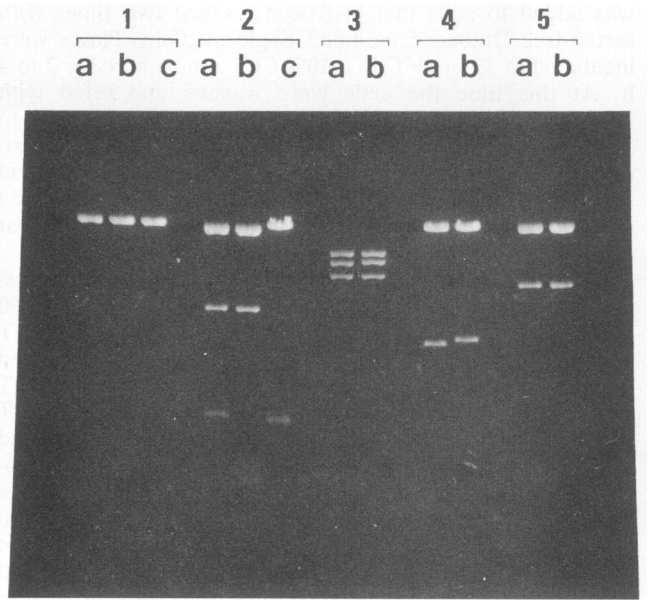

FIG. 3. Restriction enzyme analysis of the pBKV(M-1) hybrid genome. To be sure that DNA fragments were joined in the proper orientation and without tandem insertions, pBKV(M-1) DNA (a) was cleaved with a number of restriction enzymes and electrophoresed on 1% agarose gels. Reference DNAs included pBKV9 (b) and pMad1-TC (c). Five restriction enzymes were used, and expected fragment sizes are given below. For pBKV(M-1), fragment sizes for the correct as well as potentially incorrect structures are indicated; in each case the correct construct was verified by the digest. (1) *Cla*I digest: Each DNA is linearized by *Cla*I, yielding 9,521-, 9,578-, and 9,492-np fragments for pBKV(M-1), pBKV9, and pMad1-TC DNAs, respectively. (2) *Nco*I digest: This enzyme was used in the cloning protocol for pBKV(M-1) and generates three fragments for pBKV(M-1) (7,417, 1,679, and 425 np), five fragments for pBKV9 (7,417, 1,679, 196, 143, and 143 np), and two fragments for pMad1-TC (9,067 and 425 np). (3) *Pvu*II digest: To determine whether the pBKV9 coding and pMad1-TC regulatory sequences were joined in the proper orientation, *Pvu*II was used. If the pBKV(M-1) was made correctly, its *Pvu*II digestion pattern (3,736, 3,187, 2,577, and 21 np) would be indistinguishable from that of pBKV9 (3,736, 3,265, and 2,577 np) on the agarose gel; insertion in the opposite (incorrect) orientation would yield 3,736-, 2,776-, 2,577- and 432-np fragments after *Pvu*II digestion of the hybrid genome. This digestion would also alert us to the presence of a tandem duplication of the JCV regulatory fragment [an extra band(s) would be detected on the gel having a size of 836 np or 425 or 432 np or both). (4) *Bst*XI digest: To check further for a tandem insertion of the JCV DNA in pBKV(M-1), *Bst*XI was used. This restriction enzyme cleaves both pBKV(M-1) and pBKV9 DNAs two times (8,412 and 1,109 np and 8,412 and 1166 np, respectively). Insertion of two copies of the JCV DNA would have yielded a 1,534-np fragment rather than the 1,109-np fragment following *Bst*XI digestion of the hybrid DNA. (5) *Bgl*II digest: It was also necessary to check the orientation of the two largest pBKV9 *Nco*I fragments joined together in the initial cloning step of pBKV(M-1). Digestion of pBKV(M-1) and pBKV9 DNAs with *Bgl*II results in the production of two fragments each, 7,319 and 2,202 np versus 7,376 and 2,202 np, respectively. If the two pieces were put together in the wrong orientation, *Bgl*II digestion of the hybrid DNA would have given 8,045- and 1,476-np fragments.

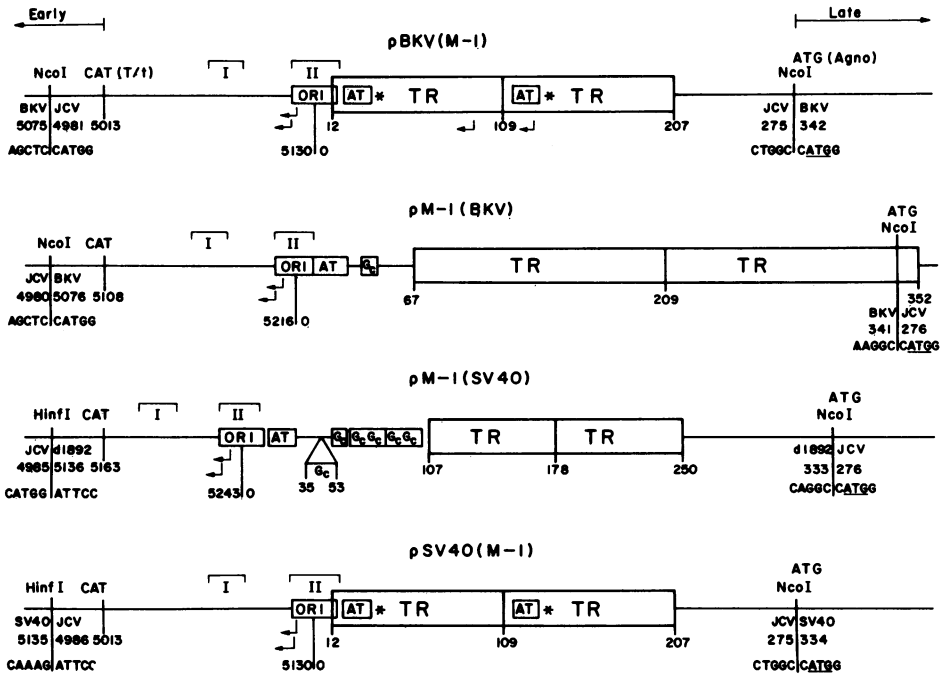

FIG. 4. Linear representation of sequences encompassing the regulatory regions of four hybrid polyomavirus DNAs. The positions of regulatory elements relative to the junction sites and to the initiation codons of the early (CAT) and late (ATG) regions are shown (drawn to scale). Included are the viral enhancers (TR), GC boxes (GC; in JCV the asterisks indicate two potential Sp1 binding sites), AT-rich or TATA sequences (AT), centers of the presumed DNA replication origins (ori), T-antigen-binding sites 1 and 2 (I, II), and 5′ termini of early mRNAs (indicated by arrows; in JCV the start sites are positioned by both TATA boxes in transformed cells [45a] and by the upstream TATA box in lytically infected cells [36]). Restriction enzymes used to join the DNAs together are positioned above the junction sites (long vertical lines). A short stretch of the two viral DNA sequences is given at each boundary. All sequences shown represent the late strand of each DNA. Numbering is according to Fiers et al. (16; SV40), Seif et al. (76; BKV) and Frisque et al. (19; JCV) and begins within the replication origins and proceeds toward the late region. The deletion in dl892 is indicated by the triangle and results in the removal of one complete GC box. The tandem repeat of our BKV strain extends seven nucleotides past the initiation codon for the agnoprotein. Therefore, in the pM-1(BKV) hybrid 11 nucleotides at the 3′ end of the BKV enhancer are replaced by JCV sequences; 10 of these 11 nucleotides are identical to the two viruses.

The hybrid molecules containing JCV proteins and SV40 or BKV regulatory signals could, at best, only induce transient expression of T antigen in PHFG cells. Quite unexpectedly, however, the hybrids containing JCV regulatory elements [i.e., BKV(M-1) and SV40(M-1)], like the parental DNAs, proceeded through a complete lytic cycle; T and V antigens were expressed and progeny virus was produced. Vacuolization and cell death were observed in all cultures that had expressed V antigen except those transfected with prototype JCV DNA, which fails to cause readily identifiable damage to PHFG cells (60). CPE was detected much earlier in the cultures transfected with SV40 or dl892 DNA (8 to 10 days post-transfection) than in those transfected with BKV, BKV(M-1), or SV40(M-1) DNAs (~3 weeks post-transfection). The virus present in these cell lysates was tested for the ability to agglutinate erythrocytes. Since SV40 does not possess hemagglutinating activity, only the JCV and BKV samples capable of inducing V antigen contained hemagglutinating virus; the BKV and BKV(M-1) samples had significantly higher HA titers than the Mad 1 sample (1,024 versus 32). To demonstrate that infectious virions were produced in these experiments, cell lysates from the original transfection were added to fresh PHFG cells. The induction of T antigen and the presence of viral DNA in the secondarily infected cells indicated that viable virus was present.

Furthermore, it was confirmed by restriction enzyme analysis that viral DNA extracted from these cells was the same DNA used in the initial transfection (Fig. 5). The samples representing M-1(BKV) (Fig. 5A) and M-1(SV40) (Fig. 5B) did not contain viral DNA, in agreement with the findings outlined in Table 2 that suggested that these DNAs were lytically inactive. All other samples [Mad 1-TC, BKV, SV40, dl892, SV40(M-1), and BKV(M-1)] did contain viral DNAs, and their digestion with *Nco*I gave the predicted sizes of DNA fragments (Fig. 5A and B). Because the dl892 and SV40(M-1) DNAs could not be distinguished from one another by cleavage with *Nco*I (each DNA yielded two fragments of nearly identical size), these DNAs were also cleaved with *Hpa*II plus *Bgl*I (Fig. 5B) to generate two fragments of dl892 DNA (4,893 and 331 np) versus one fragment of SV40(M-1) DNA (5,222 np; a *Bgl*I site located within the dl892 replication origin is missing in the JCV regulatory sequences). It will be noted in Fig. 5A that, in addition to observing the three expected fragments (3,055, 1,679, and 425 np) following *Nco*I digestion of the BKV(M-1) DNA sample, extra fragments were detected. Additional minor fragments were also present in digests of some of the other samples (e.g., see SV40 cleaved with *Nco*I in Fig. 5B). The extra BKV(M-1) DNA fragments appeared due to the presence of a variant BKV(M-1) genome in the sample which presumably arose after passage of the undiluted lysate in PHFG cells. This variant DNA was recovered by recombinant DNA methods and tested for its ability to induce T antigen in PHFG cells. Although this deleted molecule appeared to replicate in the presence of BKV(M-1) DNA

TABLE 2. Transfection of PHFG cells[a] with hybrid polyomavirus DNAs

DNA	T Ag expression[b]		V Ag expression[c]	CPE (day)[d]	HA titer[e]	Passage of lysate[f]	
	10 days	21 days				T Ag	Viral DNA
Control	0, 0	0, 0	0, 0	−	0	−	−
M-1(Δ*Nco*)	0, 0	0, 0	0, 0	−	0	−	−
M-1	0, 302	960, TNC[g]	25, TNC	−	32	+	+
M-1(BKV)	0, 19	4, 10	0, 0	−	0	−	−
BKV(M-1)	21, TNC	TNC, TNC	100, TNC	+ (24)	1,024	+	+
BKV	3, 376	TNC, TNC	TNC, 150[h]	+ (21)	1,024	+	+
M-1(SV40)	0, 0	0, 0	0, 0	−	0	−	−
SV40(M-1)	NT,[i] TNC	NT, TNC	NT, 25[h]	+ (20)	0	+	+
SV40	NT, TNC	NT, TNC	NT, TNC	+ (8)	0	+	+
dl892	776, TNC	TNC, TNC	TNC, TNC	+ (10)	0	+	+

[a] PHFG cells were transfected with 0.2 μg of each DNA, using the modified DEAE-dextran technique (78). PHFG cells are permissive for JCV, BKV, and SV40.

[b] The number of cells showing nuclear fluorescence on one-half of a 12-mm cover slip were determined for two different experiments. Variability in the numbers for a particular DNA reflects the variability in the condition of this heterogeneous population of cells from experiment to experiment (20, 58). Control and M-1(Δ*Nco*) DNAs are negative controls: the former represents a mock transfection; the latter is a JCV clone lacking sequences from nucleotides 4980 to 275.

[c] V antigen expression was determined at several times; day 21 post-transfection results are shown.

[d] The day that CPE was first observed is given in parentheses, and the number represents the average of three experiments [one for SV40(M-1) and two for SV40].

[e] HA titers were determined 28 days post-transfection.

[f] Induction of T antigen indicates the presence of infectious virus in the lysate. Detection of viral DNA and its analysis by restriction enzymes would confirm that the expected parental or hybrid DNA was present.

[g] TNC, Too numerous to count (>1,000 fluorescent nuclei). In these instances, focal areas of T- or V-antigen-positive cells could usually be discerned, suggesting that infection of a single cell resulted in the spread of virus to adjacent cells.

[h] These numbers represent a minimum value since the immunofluorescence was weak and positive cells were difficult to count. CPE was apparent in these cultures at this time.

[i] NT, Not tested.

(Fig. 5A), it did not produce T antigen or replicate by itself (data not shown) and was therefore a nonviable mutant. The occurrence of such mutant DNAs in these experiments was not totally unexpected; altered, nonviable species of viral DNA have frequently been observed during brief passage of papovaviruses in culture at both high and low multiplicities of infection (5, 42, 51, 56, 57).

Activity of hybrid polyomavirus genomes in WI-38 cells. WI-38 cells, a human diploid lung cell line, are permissive, semipermissive or nonpermissive for the lytic growth of BKV, SV40, or JCV, respectively (58, 82). These cells represented a convenient culture system for determining whether the hybrid DNAs exhibited altered host range properties when compared with the parental genomes. Following their transfection with the DNAs listed in Table 3, WI-38 cells were maintained at either 37 or 39°C, since JCV and BKV, under some conditions, replicate more efficiently at the higher temperature (27, 52, 54). T-antigen expression was monitored at several times after transfection (up to 6 weeks), and the day on which CPE were first observed was recorded. BKV9, like other BKV strains, was lytically active in these cultures, with CPE occurring at about the same time as in PHFG cells. When WI-38 cells were transfected with the SV40 and dl892 DNAs, the number of cells expressing T antigen increased with time, and many began dying 12 days after transfection. However, only a subpopulation of the cells appeared to support a complete lytic cycle; most cells survived and eventually exhibited a transformed phenotype (e.g., rapid growth, altered morphology, T-antigen expression). JCV DNA and all four hybrid DNAs induced low levels of transient T-antigen production in WI-38 cells. With time, T-antigen-positive cells disappeared from these cultures, and CPE was absent in each case.

Activity of hybrid polyomavirus genomes in CV-1 cells. The CV-1 cell line offered us an additional opportunity to investigate the host range properties of the hybrid DNAs. CV-1 cells are permissive, semipermissive, or nonpermissive for the lytic growth of SV40, BKV, or JCV, respectively (58, 82). Results of the transfection of these cells with the parental and hybrid DNAs are shown in Table 4. The values listed under T-antigen expression are given as percentages rather than direct counts of fluorescent cells. Presenting the data in this way was feasible because (i) CV-1 cells formed a uniform monolayer (in contrast to PHFG and WI-38 cells), which made it easier to determine the number of positive nuclei per microscopic field, and (ii) all DNAs induced early viral proteins in a significant number of CV-1 cells, thereby increasing the likelihood of finding T-antigen-containing cells in randomly selected microscopic fields. The parental JCV and BKV DNAs and all four hybrid DNAs gave values between 0.1 and 0.5%, and, with the exception of SV40(M-1), expression of T antigen was transient. SV40 and dl892 DNAs were more efficient at producing T antigen in CV-1 cells, and transfection with these DNAs, as well as with SV40(M-1), resulted in the production of viable virus. The SV40(M-1) DNA was less efficient than the other two DNAs in this last regard; foci of T-antigen-containing cells appeared slower, CPE was not observed, and lysates of the transfected cells contained fewer infectious virions [<0.1% of those cells secondarily infected with the SV40(M-1) sample produced T antigen 2 days post-transfection]. A comparison of the plaque-forming ability of the SV40 and SV40(M-1) DNAs (viral DNA separated from plasmid sequences and recircularized by ligation) reflected the inefficient lytic behavior of the hybrid DNA; SV40 DNA gave 6.0×10^4 PFU/μg of DNA, while SV40(M-1) DNA failed to form plaques on CV-1 cells (data not shown).

The abilities of the three parental and four hybrid polyomavirus DNAs to induce T-antigen expression and produce viable virus in three cell types are summarized in Table 5. The activities of the parental DNAs agree with published results with one exception: earlier studies had indicated that the induction of T antigen in CV-1 cells by JC

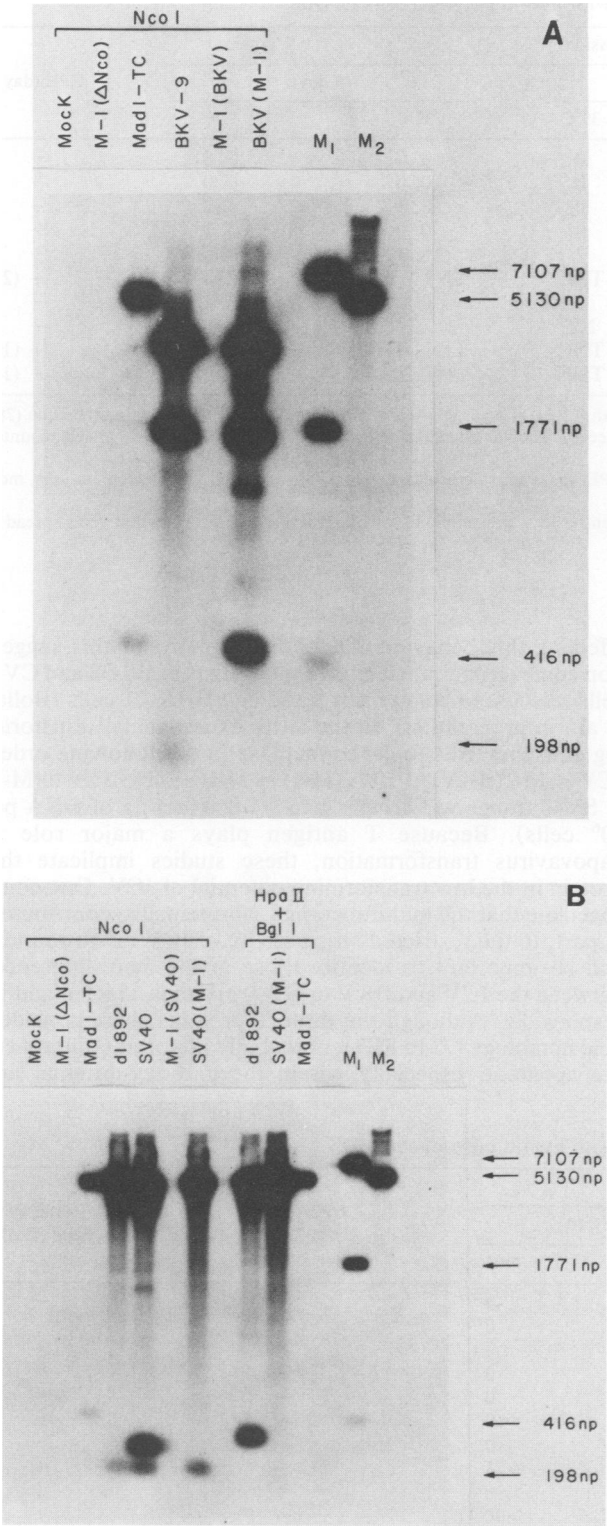

FIG. 5. Identification of viral DNAs present in secondarily infected PHFG cells. Lysates from PHFG cells transfected with the DNAs listed in Table 2 were used to infect fresh cultures of PHFG cells. Several days later, viral DNAs were extracted by the method of Hirt (31), purified, cleaved with restriction enzymes, and electrophoresed on 1% agarose gels. Following Southern transfer to nitrocellulose paper (79), the DNAs were hybridized to the appropriate nick-translated probes (70) and visualized by autoradiography. (A) JCV and BKV samples, distinguished on the basis of their different *Nco*I cleavage patterns. Viral DNA from equivalent numbers of cells were electrophoresed on these gels; the Mad1-TC samples (called M-1 DNA in Tables 2 to 5) consistently contained less DNA than the other samples that produced viable virus. For example, a comparison of the Mad1-TC and BKV(M-1) lanes clearly demonstrates that the band intensities for the shared 425-np fragment (corresponding to the JCV regulatory region), as well as for the larger DNA fragments, were greater for the BKV(M-1) sample. (B) JCV and SV40 samples. The *Nco*I digestion patterns are unique for all of the DNAs except dl892 and SV40(M-1). These two samples were distinguished by cleavage with *Hpa*II plus *Bgl*I. The Mad1-TC sample was also included, since its digestion pattern would be identical to that of SV40(M-1). Markers M1 and M2 represent *Hin*dIII-cleaved pMad1-TC DNA and linear Mad1-TC DNA, respectively, and the fragment sizes are given in nucleotide pairs (np).

virus usually was not detectable (58). The activities of the hybrid DNAs did not conform to our expectations. Replacement of JCV regulatory sequences with those of SV40 or BKV [M-1(BKV) or M-1(SV40)] did not generate viable virus, although expression of the JCV T antigen was observed. The hybrids containing the JCV regulatory elements and SV40- or BKV-coding sequences [BKV(M-1) or SV40(M-1)] were lytically active in PHFG cells and, in the case of SV40(M-1), in CV-1 cells as well.

DISCUSSION

The chimeric viral genomes described in this study represent the first hybrid DNA viruses to be constructed by exchanging intact viral regulatory regions while maintaining the integrity of the sequences encoding viral proteins. To determine what effects these exchanges have had on the biology of the viruses, one of our approaches had been to test each hybrid DNA for its ability to proceed through a lytic cycle in different cell culture systems. At the onset of these transfection experiments, it was not possible to predict whether viable virus would be produced; therefore, both early and late events in the lytic cycle were monitored.

The most efficient culture system for the propagation of JCV is PHFG cells. Even in these cells the lytic cycle of this virus is prolonged, CPE is difficult to recognize, and plaque formation is absent (58). BKV and SV40 are also lytically active in PHFG cells and, compared to JCV, cause more rapid and extensive damage to these cultures. Replacement of the JCV regulatory sequences with those of BKV or SV40 [M-1(BKV) or M-1(SV40)] did not generate a more cytolytic virus. In fact, these hybrids were nonviable and, at best, only induced T-antigen expression. On the other hand, joining a JCV regulatory region to BKV- or SV40-coding sequences [BKV(M-1) or SV40(M-1)] did yield viruses that replicated in PHFG cells. In these cells the hybrid DNAs were more lytically active than the parental JCV DNA; their growth cycle was shorter, CPE was more pronounced, and greater quantities of virus were produced. These studies are the first to demonstrate that the complete exchange of regulatory information between two DNA viruses will yield viable hybrid virus. The results are significant for two reasons. (i) They indicate that productive interactions can occur between the JCV regulatory sequences and the BKV and SV40 proteins. For example, the early promoter/enhancer of JCV must direct the initiation of transcription of the BKV and SV40 early regions to generate functional T antigens. These proteins must then recognize and bind to specific sequences near the JCV origin to mediate their effects on DNA replication and early and late transcription. It should be noted that, although three T-

TABLE 3. Transfection of WI-38 cells[a] with hybrid polyomavirus DNAs

DNA	T-antigen expression[b]						CPE (day)[c]
	2 days		10 days		14 days		
	37°C	39°C	37°C	39°C	37°C	39°C	
Control	0, 0, 0	0, 0, 0	0	0	0, 0	0, 0	–
M-1(ΔNco)	0, 0, 0	0, 0, 0	0	0	0, 0	0, 0	–
M-1	0, 0, 0	0, 3, 0	0	0	0, 5	4, 2	–
M-1(BKV)	0, 1, 0	0, 7, 0	0	0	0, 0	0, 1	–
BKV(M-1)	0, 0, 0	0, 0, 0	5	2	0, 12	0, 0	–
BKV	0, 4, 0	0, 11, 2	TNC[d]	TNC	NT,[e] TNC	TNC, TNC	+ (21)
M-1(SV40)	6, 19, 2	3, 28, 17	0	0	0, 0	0, 2	–
SV40(M-1)	10, 1	NT	1	NT	3	NT	–
SV40	83, 354, 27	114, 600, 40	TNC	TNC	TNC, TNC	TNC, TNC	+ (12)
dl892	32, 83, 5	42, 16, 25	TNC	TNC	NT, TNC	NT, TNC	+ (12)

[a] WI-38 cells (1.5 × 10^6/60-mm plate), a human lung cell line, were transfected with 0.5 μg of each DNA, using the modified DEAE-dextran technique (78).
[b] T-antigen expression was monitored at several times after transfection (up to 6 weeks). The values indicated the number of T-antigen-positive cells counted per one-half of a 12-mm cover slip. Each value represents a single experiment.
[c] The number in parentheses indicates the first day CPE was observed. Although CPE was readily detected early in SV40- and dl892-transfected cultures, most cells survived and appeared to be morphologically transformed.
[d] TNC, Too numerous to count (>1,000 fluorescent nuclei). Focal areas of T-antigen-positive cells could usually be discerned, suggesting that virus spread to adjacent cells.
[e] NT, Not tested.

antigen-binding sites have been identified in the BKV and SV40 genomes (12, 73, 81), only two sites have been recognized in the JCV genome (19). Further evidence that specific interactions do occur between the SV40 T antigen and JCV sequences comes from recent binding (18) and DNA replication (43; W.-F. Chuke and R. J. Frisque, unpublished results) studies in vitro. Additional interactions involving the coding and noncoding sequences of the chimeric DNAs are required in the final stages of productive infections. These stages include transcription of late mRNAs utilizing JCV promoter sequences, post-transcriptional processing and translation of chimeric mRNAs, and packaging of hybrid genomes within intact capsids to yield infectious virions. (ii) Results from the lytic studies are also significant because they suggest that the JCV regulatory and coding sequences both contribute to the restricted activity of JCV in vitro and that the latter sequences play a larger role in effecting this behavior. Additional support for this suggestion comes from transfection experiments in WI-38 and CV-1 cells (discussed below) and Rat 2 and BHK-21 cells (Bollag et al., in preparation). In the latter experiments, transforming efficiency was found to increase in the following order: JCV < M-1(BKV) < BKV(M-1) ≈ M-1(SV40) < SV40(M-1) < SV40 (range was from <1 to >1,000 foci/μg of DNA per 10^6 cells). Because T antigen plays a major role in papovavirus transformation, these studies implicate this protein in the low transforming potential of JCV. One could speculate that the multifunctional T protein also contributes, in part, to the restricted lytic activity of JCV in vitro, and it will be important to identify those amino acid differences between the JCV and BKV or SV40 proteins which might be responsible. Although the three T proteins share considerable homology (72 to 83%), potentially important differences are apparent, especially within the carboxy-terminal and

TABLE 4. Transfection of CV-1 cells[a] with hybrid polyomavirus DNAs

DNA	T-antigen expression (%)[b]				CPE (day) at 37°C[c]	Passage of lysate (T Ag)[d]
	Day 2		Day 10			
	37°C	39°C	37°C	39°C		
Control	0	0	0	0	–	–
M-1(ΔNco)	0	0	0	0	–	–
M-1	0.20	0.25	0	0	–	–
M-1(BKV)	0.35	0.45	0	0	–	–
BKV(M-1)	0.15	0.10	0	0	–	–
BKV	0.30	0.50	0	0	–	–
M-1(SV40)	0.15	0.45	0	0	–	–
SV40(M-1)	0.30	NT[e]	>25[f]	>25	–	+
SV40	2.15	4.55	>90	>90	+ (6)	+
dl892	1.15	1.75	>90	>90	+ (6)	+

[a] CV-1 cells (1.5 × 10^6/60-mm plate), a monkey kidney cell line, were transfected with 0.5 μg of each DNA, using a modified DEAE-dextran procedure (78).
[b] Following transfection, cells were incubated at 37 or 39°C since BKV has been reported to function better at the higher temperature in CV-1 cells (52, 54) and because JCV also prefers the higher temperature in PHFG cells (27). Numbers given are the percentage of cells expressing T antigen 2 or 10 days post-transfection (20 to 40 random microscopic fields were counted per cover slip) and represent the average of two to three experiments.
[c] The number in parentheses indicates the first day CPE was observed. Although CPE was not detected in SV40(M-1)-transfected cultures, viable virus was produced.
[d] Lysates of the transfected cells were added to fresh cultures of CV-1 cells. Induction of T antigen indicates the presence of infectious virus in the lysate.
[e] NT, Not tested.
[f] Numerous foci of T-antigen-positive cells were observed; >25% of cells within a focus were positive. For SV40 and dl892, the >90% value refers to the number of positives on the entire cover slip, not just to the number of positives within a focus.

TABLE 5. Summary of the activity of hybrid polyomavirus DNAs in three cell types

DNA	T-antigen expression[a]			Lytic activity[b]		
	PHFG	WI-38	CV-1	PHFG	WI-38	CV-1
Control	−	−	−	−	−	−
M-1(Δ*Nco*)	−	−	−	−	−	−
M-1	+	+/−	+/−	+	−	−
M-1(BKV)	+/−	+/−	+/−	−	−	−
BKV(M-1)	+	+/−	+/−	+	−	−
BKV	+	+	+/−	+	+	−
M-1(SV40)	−	+/−	+/−	−	−	−
SV40(M-1)	+	+/−	+	+	−	+
SV40	+	+	+	+	+	+
dl892	+	+	+	+	+	+

[a] A minus indicates that T-antigen expression was never observed in that cell line when transfected with that particular DNA. A plus/minus indicates that only transient expression was observed. A plus indicates that T-antigen expression increased with that particular cell type and DNA over the course of the experiment.

[b] Lytic activity was detected via several parameters: by the appearance of foci of cells expressing viral antigens, CPE, and infectious virions in lysates of transfected cells.

origin-binding sequences (19). Mutations affecting these domains of the SV40 T antigen are known to restrict the lytic behavior of this virus (10, 11, 25, 33, 47, 49, 64–67, 80, 83).

There have been a number of reports suggesting that enhancer sequences alter the host range properties of a virus (6, 13, 40), with more recent studies indicating that these elements confer tissue rather than species specificity upon a virus (1, 8, 9, 24, 30, 63, 71, 72). Our transfections of WI-38 and CV-1 cells were conducted because these cells clearly differ from PHFG cells, and from one another, in their ability to support the growth of JCV, BKV, and SV40 (82). In our experiments the replacement of the intact JCV regulatory region with that of BKV or SV40 did not extend the host range of these viruses to WI-38 (permissive for BKV) or CV-1 (permissive for SV40) cells. This was not surprising given the PHFG cell results; M-1(BKV) and M-1(SV40) were nonviable even in cells permissive for JCV. The only hybrid to grow in cells other than PHFG cells was SV40(M-1) DNA. Although infectious virus was produced following transfection of CV-1 cells with this DNA, the efficiency of T-antigen expression and virus production was reduced compared with SV40-transfected cells, presumably because the JCV regulatory signals functioned less efficiently in CV-1 cells (Table 4) (37; Cannella and Frisque, unpublished results). Other investigators have obtained similar results by replacing the SV40 enhancer with that of Moloney murine sarcoma virus (40, 43); in monkey cells the lytic cycle of the chimeric virus was prolonged and plaque formation was not detected.

Based on the SV40(M-1) results in CV-1 cells, we had anticipated that BKV(M-1) might by lytically active in WI-38 cells. Our failure to demonstrate such activity suggested that the JCV regulatory elements functioned even less efficiently in WI-38 cells than in CV-1 cells or that the level of BKV T antigen produced was insufficient to support a BKV(M-1) lytic infection, or both.

It is clear that the adaptation of a virus to its host requires a number of complex interactions. These must occur between viral and cellular components as well as between regulatory and structural elements of the virus. The hybrid polyomavirus DNAs constructed in this study have allowed us to begin assessing the relative contributions of the regulatory and coding information of JCV to its unique biology. At the start of these experiments, our attention was focused on several unusual features of the JCV regulatory region: the duplication of its TATA sequence, the absence of the GC box, and the presence of an enhancer element that shared little sequence homology with those of other polyomaviruses. Our interest in this region of the JCV genome was heightened by reports that indicated that these signals influenced the host range of a virus and its oncogenic and pathogenic properties. The studies presented in this paper demonstrate that sequence alterations (i.e., altered compared to BKV and SV40 sequences) in both the JCV-coding and noncoding regions play a role in JCV's restricted lytic behavior in vitro. We are now investigating specific differences in the JCV, BKV, and SV40 T antigens to evaluate the effects these changes have had on the activity of this multifunctional protein and, in turn, on the biological properties of these viruses.

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