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Human Polyomavirus JC Virus Genome

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The complete DNA sequence of the human JC virus, which was found to consist of 5,130 nucleotide pairs, is presented. The amino acid sequence of six proteins could be deduced: the early, nonstructural proteins, large T and small t antigens; the late capsid proteins, VP1, VP2, and VP3; and the agnogene product encoded within the late leader sequence, called the agnoprotein in simian virus 40. The extent of homology between JC virus DNA and the genomes of simian virus 40 (69%) and BK virus (75%) confirmed the close evolutionary relationship of these three polyomaviruses. The sequences showing the greatest divergence in these viral DNAs occurred within the tandem repeats located to the late side of the replication origins.

Exposure to JC virus (JCV) usually occurs during childhood and results in a subclinical infection (47); however, in a small number of immunodeficient individuals, an infection leads to the fatal demyelinating brain disease called progressive multifocal leukoencephalopathy (49). In addition to its pathogenic potential in humans, JCV has also proven to be a highly oncogenic virus in animals; inoculation of hamsters and primates with JCV results in a wide variety of tumors, some of which are among the more frequent types found in people (36, 50, 55, 71).

JCV is a member of the genus *Polyomavirus*, a group which includes simian virus 40 (SV40), polyomavirus and BK virus (BKV) (monkey, mouse, and human viruses, respectively). These latter three viruses have been studied intensively, and their structural and genetic organizations are found to be closely related (69). A similar understanding of JCV has been hampered by the lack of a readily available permissive cell system; however, available serological and biochemical evidence suggests that the organization of the JCV genome is similar to that of the other polyomaviruses (14, 15, 33, 42, 61, 71). In light of these results, an important question arises: what accounts for the diverse biological parameters (e.g., host range, tissue tropism, pathogenicity, and oncogenicity) exhibited by these viruses? Part of the answer may come from studies of the enhancer or activator element, a sequence usually found as a tandem repeat near the replication origin of each virus (1, 2, 7, 23, 34, 57, 58, 70). Hybridization and sequence data indicate that this is the region of the polyomavirus genomes which has diverged to the greatest extent (13, 14, 33, 54, 59, 76). Furthermore, the enhancer element has already been shown to influence the host range of polyomavirus (18, 29, 30) and the oncogenicity of BKV (73, 74).

To gain a better understanding of the organization of the JCV genome and the factors influencing its unique biology, we determined the nucleotide sequence (5,130 nucleotide pairs [np]) of the prototype Mad1 strain of JCV. Features of the JCV regulatory region, including the tandem repeats, are discussed in relation to the inefficient lytic and transforming activities of JCV in vitro. The probable primary structures of the six JCV proteins are also presented and are compared with the amino acid sequences of the SV40 and BKV proteins.

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MATERIALS AND METHODS

DNA preparation. Prototype JCV (Mad1) was passed in primary human fetal glial cells at low multiplicities of infection (<0.1 infectious unit per cell). DNA of homogeneous size was extracted by the method of Hirt (24) and used to construct the pMad1-TC clone used in all sequence analyses (15). The recombinant molecules represent full-length, biologically active DNA that is indistinguishable from DNA extracted directly from the original diseased brain material (14, 21; unpublished data).

DNA sequence analysis. Restriction endonuclease fragments were end labeled with the large fragment of *Escherichia coli* DNA polymerase 1 (Klenow reagent) and the appropriate [α-^{32}P]deoxynucleoside triphosphate. These DNAs were cleaved with a second restriction enzyme, and DNA fragments labeled at only one end were isolated from low-melting agarose gels, purified, and chemically cleaved by the method of Maxam and Gilbert (38). Electrophoresis on polyacrylamide gels (6%, 8%, or 12%) was carried out for various lengths of time. Gels were frozen and autoradiographed without intensifier screens for 0.5 to 3 days. Of the nucleotide sequence, ca. 80% was determined for both DNA strands; all determinations were repeated at least once.

RESULTS

The assignment of nucleotide numbers in this manuscript does not correspond to that used in previous studies presenting a portion of the JCV DNA sequence (14, 42). To facilitate comparisons with the other polyomavirus genomes, we began numbering within the presumed origin of DNA replication and proceeded clockwise toward the late gene region (Fig. 1). This is the system used by Fiers et al. (13) for SV40 and later adopted by Seif et al. (60) for the Dunlop strain of BKV [BKV(Dun)].

Origin of DNA replication. A number of studies have indicated that the replication origins of the JCV, BKV, and SV40 genomes are located near 0.67 map units (14, 21, 69). The organization of this region was highly conserved in the three viruses (Fig. 2). It included a true palindrome of 17 nucleotides (5074 to 5090 in JCV [Fig. 1]; 16 and 15 nucleotides shared with SV40 and BKV, respectively) and two sets of shared dyad symmetries to the late side of the palindrome (5096 to 5114 and 5118 to 12 in JCV [Fig. 1]). The second symmetry, which is the most highly conserved,

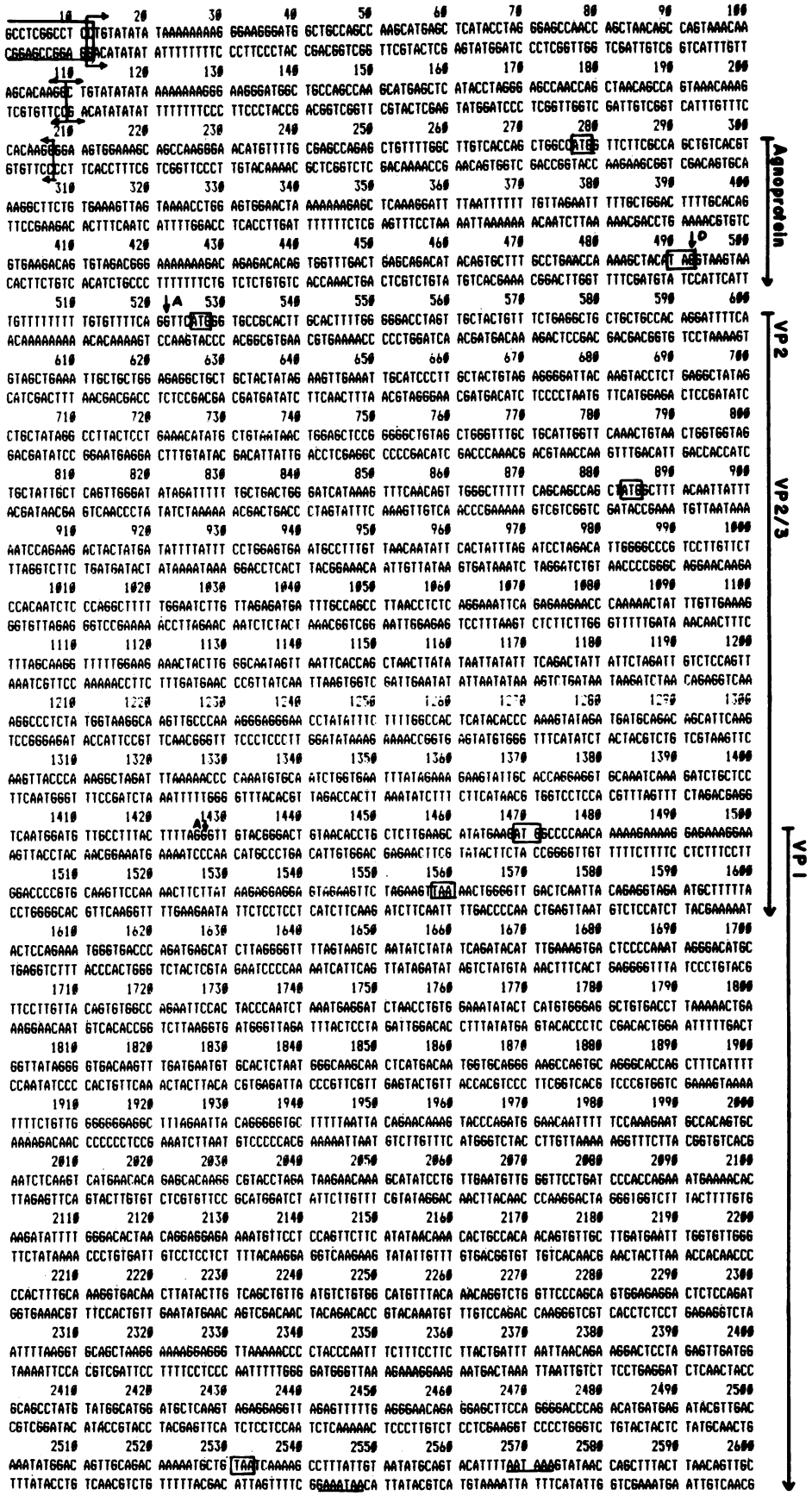

FIG. 1. Nucleotide sequence of JCV (Mad1). The circular genome of JCV consists of 5,130 np. Numbering begins near the center of the presumed origin of replication (within box) and proceeds toward the late region. The upper strand in each pair, read left to right, has the polarity and sequence of the late mRNAs. The lower strand in each pair, read right to left, has the polarity and sequence of the early mRNAs. The proposed coding regions for the JCV proteins are shown to the right of the sequence. Initiation and termination codons are indicated by boxes. Donor and receptor splice sites for the early and late messages are denoted by an arrow and a D or an A. The potential polyadenylation signals near the 3′ ends of the early and late coding regions are underlined. The 98-np tandem repeat is indicated by brackets with arrows.

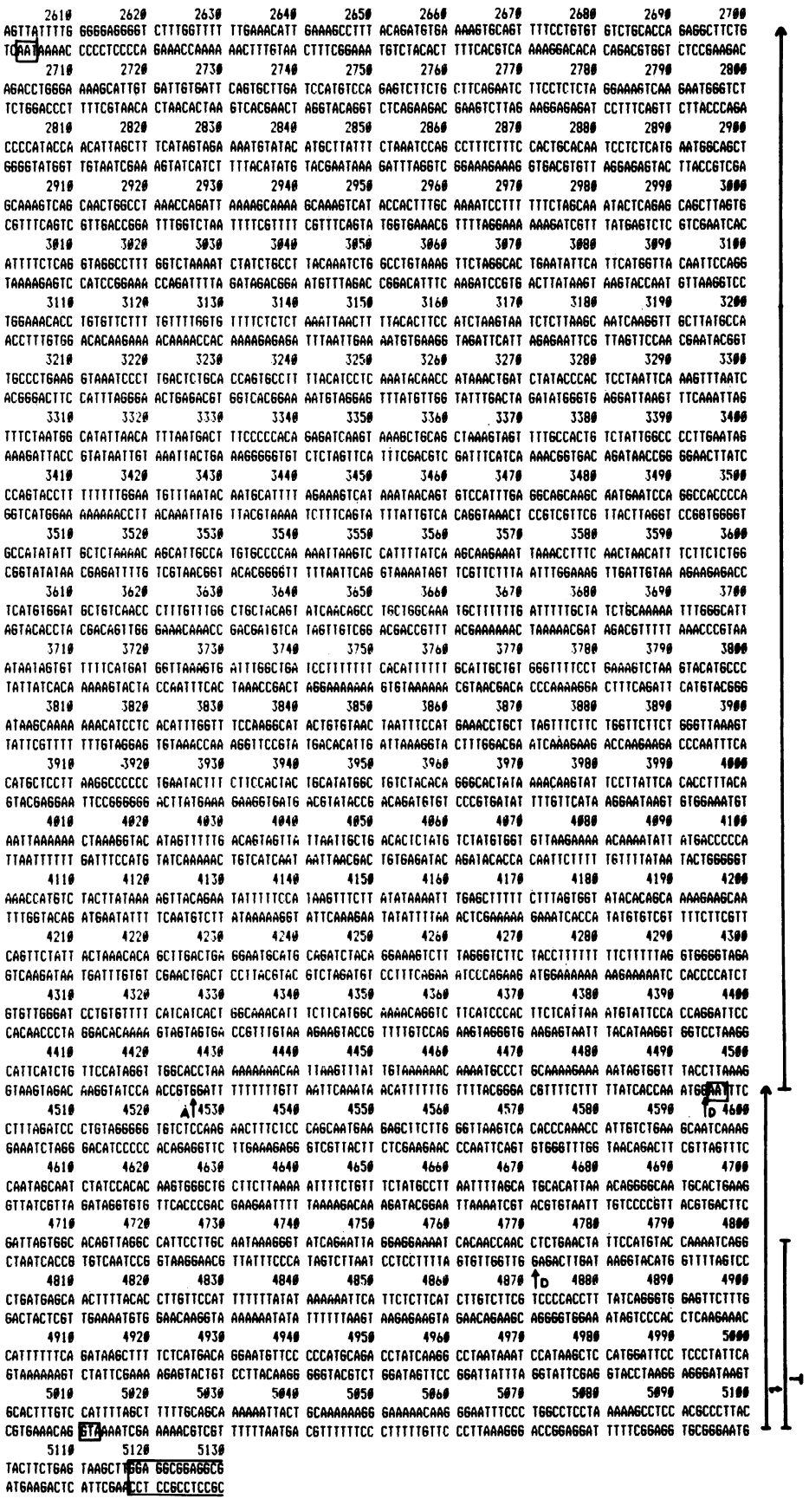

FIG. 1. *Continued*

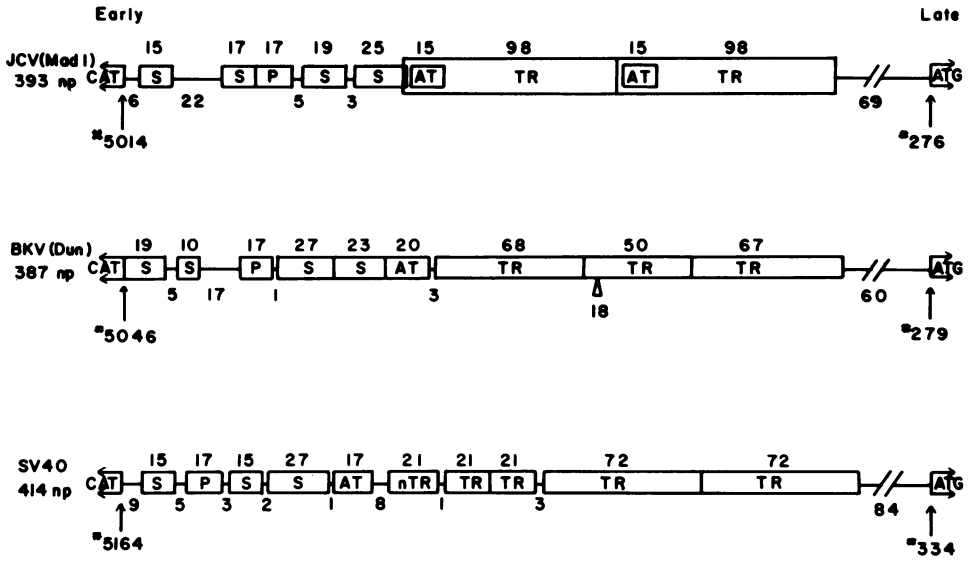

FIG. 2. Comparison of the JCV, BKV, and SV40 regulatory regions. The noncoding regions of the three polyomaviruses are shown. The letters CAT within the open box to the left represent the initiation codon (opposite strand and polarity) for the early proteins, large T and small t antigens. To the far right is the ATG initiation codon for the agnoprotein located within the late leader sequence. Comparisons among the three viral DNAs include dyad symmetries (S), true palindromes (P), TATA boxes (AT), tandem repeats (TR), and non-tandem repeats (nTR) (repeats which are not immediately adjacent to each other). Numbers above the linear arrangements refer to the sizes in np, of the indicated structures. Numbers below refer to the distances between the structures. The triangle underneath the middle tandem repeat of BKV indicates a deletion of 18 nucleotides. This set of nucleotides is present in the adjacent repeats.

probably includes the replication origin and the second T-antigen-binding site of each virus (6, 59, 62, 68).

A third symmetry, which was shared by JCV and BKV, was found to the early side of the 17-np palindrome (5057 to 5073 in JCV [Fig. 1 and 2]). This sequence lay within a stretch of DNA (31 nucleotides in JCV; 22 nucleotides in BKV) that is missing in the corresponding region of SV40 (1, 54, 59, 76; Fig. 2).

Located to the late side of the origin of SV40 are three copies of a 21-np repeat (Fig. 2). These repeats are required for efficient replication and transcription (4, 5, 10, 11) and include six copies of the sequence 5'-PyPyCCXCCC-3' (66). This sequence is also present in the regulatory regions of BKV, polyomavirus, several adenoviruses, and the herpes simplex virus type 1 thymidine kinase gene (4, 39; reviewed in 59) but is absent in the same region of JCV DNA. (However, one copy of the sequence 5'-TCCCTTCCC-3' was found in each 98-np repeat [Fig. 1].)

T-antigen-binding sites. The large T protein of SV40 and the related D2T protein of the adenovirus-SV40 hybrid virus, Ad2D2, interact with a specific pentanucleotide sequence located at three sites near the SV40 origin of replication (6, 68). The consensus sequence, 5'-(G>T)(A>G)GGC-3', is repeated three to six times within these binding sites. D2T protein also binds to the origin region of JCV (15). Two clusters of the pentanucleotide sequence are located here in JCV DNA, and their position and sequence correspond almost exactly with the first and second T-antigen-binding sites of SV40 (5069 to 5090 and 5118 to 14 in JCV [68; Fig. 1]). We were not able to identify a third site in the JCV or BKV sequence as has been proposed for SV40 (6, 62, 68).

5' end of mRNAs. Inspection of the promoter sequences of several eucaryotic genes transcribed by RNA polymerase II has led to the identification of certain consensus sequences located at similar distances upstream from the transcriptional start sites. The Goldberg-Hogness sequence (5'-TATAAATA-3', also called the TATA box or AT-rich region) plays a role in positioning the 5' ends of mRNAs and is usually found about 25 nucleotides from the cap site (19, 20). A second sequence, the CAT box (5'-GGPyCAATCT-3') is located ca. 80 nucleotides from the mRNA initiation site and is required for efficient promoter function in some systems (3, 9, 22, 40).

An AT-rich region was located upstream from the start sites of JCV, BKV, and SV40 early messages (Fig. 1 and 2). The TATA box in JCV, unlike those in BKV and SV40, was duplicated since it is part of the tandem repeat of JCV (see below).

Although several good candidates for the CAT sequence exist in the 21-np repeats of SV40 (3, 5), potential CAT boxes within the tandem repeats of JCV (5'-GCTCATGCT 3' and 5'-AGCCATCCCT-3' [Fig. 1]) and BKV (5'-GGTCA TGGT-3' [59, 76]) demonstrate only a partial homology with the consensus sequence.

Analysis of the early SV40 mRNAs suggests at least two major starts, located 22 and 28 nucleotides downstream from the Goldberg-Hogness sequence (53). Beginning at the first start site, the DNA sequence is GCCTCTGAGCTATTCCA.

The locations of the 5' termini for the early JCV and BKV messages have not been defined precisely; however, S1 nuclease analysis and comparisons with SV40 identify two likely start sites in these DNAs (14, 15, 53, 59). The sequence containing the JCV starts reads GCCTCCAAGC TTACTCA and is found 22 and 28 nucleotides from the TATA box (Fig. 1); for BKV the sequence is GCCTCCA CCCTTTCTC and is 19 and 25 nucleotides from the same landmark (59).

The 5' ends of late JCV mRNAs have not been determined, in part due to the difficulty in obtaining a suitable lytic system. Comparisons with the major start sites of late SV40 and BKV messages suggest a possible 5' terminus for

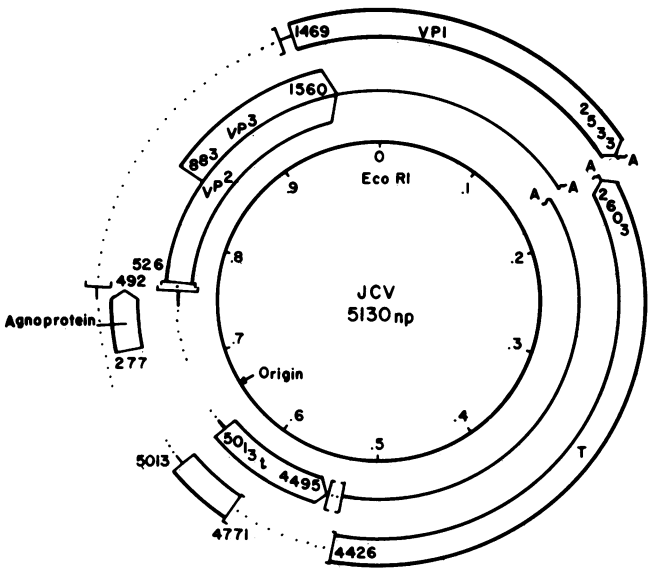

FIG. 3. Circular map of the JCV genome (Mad1 strain). The single *Eco*RI site is taken as map position 0.0 on the JCV genome. The map is divided into two nearly equal parts, depending on whether gene expression occurs primarily before (early) or after (late) viral DNA replication. Broad arrows depict the coding regions for the six proposed JCV proteins. The dots at the beginning of each arrow indicate uncertainty as to the exact 5′ end of the mRNAs. Brackets containing dots represent intervening sequences, and single lines indicate untranslated 5′ and 3′ portions of the early and late messages.

the late JCV mRNAs at nucleotide 163. Its position (within the repeat most distal from the origin) and the sequences surrounding it (7 of 8 nucleotides) are nearly identical to the BKV site (59). Within the distal repeat of JCV, there was a potential TATA box located 35 nucleotides upstream from nucleotide 163. However, as discussed below, several viable variants of JCV have been isolated which lack this second TATA box, suggesting it is not required for late transcription (J. D. Martin and R. J. Frisque, unpublished data). Furthermore, the Goldberg-Hogness sequence has not been found upstream from the late regions of BKV and SV40 (13, 54, 59, 76).

Tandem repeated sequences. The tandem repeated sequences located to the late side of the SV40 and BKV origins of replication have been identified as enhancer or activator elements because of their ability to increase transcription of associated genes (1, 2, 57). In SV40, the 72-np repeat occurs 78 nucleotides to the late side of the AT-rich region. An almost perfect 68-np triplication is found four nucleotides upstream from the same position in BKV DNA. Although these tandem repeats are similar in structure and location, at first glance they do not appear to share any sequence homology. However, a core sequence (5′-GTGG$^{TTT}_{AAA}$G-3′), identified in a number of viral and cellular enhancers, is found in both DNAs (5′-GTGGAAAG-3′ in SV40 and 5′-ATGGTTTG-3′ in BKV [75]). The 98-np tandem repeat of JCV (12 to 207 [Fig. 1 and 2]) included the TATA box and lay immediately adjacent to the 25-np symmetry thought to contain the replication origin. Again, the only apparent homology with other polyomavirus repeats resided in the core sequence (5′GTGCTTTG-3′ in JCV).

3′ ends of mRNAs. Following the termination codon for JCV large T protein was the polyadenylation signal, AAT AAA (2543 to 2548 [Fig. 1]), a sequence frequently positioned 10 to 20 nucleotides before the polyadenylate tract of most eucaryotic mRNAs (52). The dinucleotide CA is commonly found at the polyadenylation site itself. In JCV this dinucleotide was located within a palindrome (5′-TTA CAGCATT-3′) that lay 10 nucleotides beyond the polyadenylate signal sequence.

An AATAAA sequence (2568 to 2573 [Fig. 1]) also followed the termination codon for VP1. The dinucleotide CA occurred 8 and 21 nucleotides from the polyadenylation signal, and the latter CA also fell within a palindrome (5′-TTTACTTAACAGTT-3′).

As observed for the other polyomaviruses, the overlapping 3′ ends of the early and late messages of JCV included the polyadenylation signals.

Splicing. The consensus sequences for the donor and acceptor splice sites of eucaryotic mRNAs are 5′-AG$^{\downarrow}$GTAAGT-3′ and 5′-6PyXCAG$^{\downarrow}$-3′ ($^{\downarrow}$, cleavage site), respectively (35). Based upon these sequences and comparisons with SV40 and BKV, the donor sites for the large T and small t messages of JCV could be localized at nucleotides 4771 (5′-AGGTTGGT-3′) and 4494 (5′-AGGTAAAC-3′), respectively (Fig. 1 and 3). The shared acceptor site for the two mRNAs might be at nucleotide 4426 (5′-TTTTTTTT AGX-3′). Assigning these positions to the splice sites agreed with earlier S1 nuclease results in terms of sizes and numbers of early messages (15). A third RNA, representing a viral middle T message, has not been detected, and sequence data did not predict a middle T protein (Fig. 1).

A candidate for the donor splice site shared by the VP1, VP2, and VP3 messages occurred at nucleotide 492 (5′-AGGTAAGT-3′ [Fig. 1 and 3]). This site was located within the leader sequences; therefore, the late coding sequences were not interrupted by an intron. The potential acceptor splice sites were at nucleotides 522 (5′-TGTTTTCAGX-3′) for the VP2/3 message(s) and 1427 (5′-TTACTTTTAGX-3′) for VP1 (Fig. 1 and 3).

Viral proteins. A genetic map defining the probable locations of the six JCV proteins is shown in Fig. 3. A comparison of these proteins with those of BKV and SV40 emphasized the relatedness of these three viruses (Table 1). The homology was consistently greater between the two human viruses.

Early proteins. The probable primary structures of the JCV early proteins (Fig. 4 and 5) were deduced from an analysis of open reading frames, immunoprecipitation and S1 nuclease data (15, 17; Fig. 1), and comparisons with the same BKV and SV40 proteins. The T proteins of JCV and BKV exhibited the greatest homology (83% [Table 1]).

The large T antigen of SV40 is a multifunctional DNA-binding protein that is functionally and structurally related to the JCV and BKV proteins (15, 31, 43, 69, 71). It is thought that the protein mediates some of its functions through its specific binding to the origin region of the genome (45, 56, 64, 68). The results from several laboratories are consistent with the suggestion that a group of basic amino acids, located in the amino-terminal half of SV40 T antigen, may be involved in the binding (44; reviewed in 51). This peptide sequence lies within one of the most highly conserved regions of the JCV, BKV, and SV40 T proteins (19 of 20 amino acids were identical [Fig. 4, line 5]) and reads Pro-Pro-Lys-Lys-Lys-Lys-Lys in the JCV protein.

The carboxy terminus of the T protein showed the greatest divergence in the three viruses. Comparisons with the SV40 protein revealed that the proteins from both human viruses had acquired a stretch of 5 amino acids and deleted several

TABLE 1. Number of amino acids and nucleotides in the JCV, BKV, and SV40 proteins or regulatory regions and their degrees of homology

Protein or regulatory region	No. amino acids[a] in strain:			No. nucleotides[b] in strain:		
	JCV	BKV	SV40	JCV	BKV	SV40
VP1	354	362 (78)	362 (75)	1,065	1,089 (74)	1,089 (72)
VP2	344	351 (79)	352 (72)	1,035	1,056 (81)	1,059 (75)
VP3	225	232 (75)	234 (66)	678	699 (78)	705 (72)
T	688	695 (83)	708 (72)	2,067	2,088 (77)[c]	2,127 (71)[c]
t	172	172 (78)	174 (67)	519	519 (79)	525 (70)
Agnoprotein[d]	71	66 (59)	62 (46)	216	201 (75)	189 (63)
Regulatory region				393	387 (55)	414 (44)

[a] Numbers in parentheses indicate the percentage of amino acids shared with the corresponding JCV protein.
[b] Numbers in parentheses indicate the percentage of nucleotides shared with the corresponding regulatory sequences or coding sequences of JCV. The termination signal is included in the calculations.
[c] Numbers do not include intervening sequences of large T.
[d] Encoded within the leader sequences of late viral mRNAs.

segments of amino acids (3, 4, and 18 amino acids in JCV; 3 and 18 amino acids in BKV [Fig. 4]).

The probable amino acid sequence for the small t protein of JCV is shown in Fig. 5. The first 81 amino acids were shared with large T protein; the remaining 91 amino acids were unique to small t antigen due to differential splicing of the 2 early mRNAs (Fig. 3). The large degree of homology observed between the amino-terminal ends of both early proteins for all three viruses (89% for JCV × BKV; 82% for JCV × SV40) was significantly reduced beyond the large T donor splice site (69% for JCV × BKV; 53% for JCV × SV40).

Seif et al. (59) noted that the carboxy-terminal portion of the SV40, BKV, and polyomavirus t proteins contains six cysteines organized in the pattern CysXCysXXCys-(21 or 22 amino acids)-CysXCysXXCys. The identical pattern was in the JCV t protein and may represent a site(s) for protein-protein interaction (59).

There is no evidence that the JCV, BKV, or SV40 early regions encode a polyomavirus-like middle T protein. However, all four viruses do induce a related (or identical) host cell-specific middle T or Tau antigen in transformed cells (50,000 to 56,000 daltons) (12, 17, 26, 32, 65). In each case the viral large T protein appears to associate noncovalently with this cellular protein.

Late viral proteins. Three capsid proteins, VP1, VP2, and VP3, are produced late in polyomavirus lytic infections. A fourth protein, the agnoprotein, appears to interact in a specific way with VP1 during the late stages of SV40 development (27, 28, 37, 41). The proposed amino acid sequences for these four proteins in JCV (Fig. 6, 7, and 8) were based on comparisons with the other polyomaviruses; there have been no previous reports describing the number or sizes of the JCV structural proteins.

The VP1 polypeptide is the most highly conserved protein between JCV and SV40 and between BKV and SV40 (59; Table 1). VP1, presumably, is encoded within the large open reading frame at the 3′ end of the late region. As seen with SV40 and BKV, there are two potential initiation codons for the VP1 protein of JCV which occur in the same reading frame: ATG AAG ATG . . . . There is uncertainty over which codon is utilized; however, we followed the convention used for BKV (59) and specified the second ATG as the initiation codon (Fig. 6).

Near the amino terminus of the VP1 protein of SV40 and BKV is a stretch of eight identical amino acids which was missing in the JCV protein (Fig. 6). Since this part of VP1 overlaps with the VP2 and VP3 proteins, the deletion would also affect their sequences (Fig. 7).

We predict that the VP2 and VP3 proteins are encoded within the second open reading frame of the late region (Fig. 3 and 7). By analogy with SV40 and BKV, the VP3 sequence would be a subset of the VP2 sequence. At this time, we do not know if VP2 and VP3 are translated from the same or from different mRNAs.

There is some evidence that VP3 interacts with the SV40 genome (25). One particular stretch of basic amino acids that occurred in the shared VP2-VP3 sequences of JCV, BKV, and SV40 was similar to the potential binding site in the T protein discussed above; this stretch read Pro-Asn-Lys-Lys-Lys-Arg-Arg for JCV, Pro-Asn-Gln-Lys-Lys-Arg-Arg for BKV, and Pro-Asn-Lys-Lys-Lys-Arg-Lys for SV40.

The possibility of the SV40 and BKV genomes encoding a sixth protein was first suggested by sequencing data which identified an open reading frame within the late leader sequences (8, 59, 76). The agnoprotein of SV40 has been identified by genetic and biochemical analyses, and it defines a new complementation group, G (41). Approximately the first 50 amino acids of the JCV, BKV, and SV40 agnoproteins showed considerable homology; however, the remainder of the sequence of the proteins was completely different in each virus (Fig. 8).

As expected from its highly basic amino acid composition, the agnoprotein binds to nucleic acids (28). The longest stretch of basic amino acids in the JCV protein was found at the point where the sequences diverge (underlined) in the three viruses: Lys-Lys-Arg-Gln-Arg-His.

Sequence data have also suggested the presence of a second putative protein (59, 76, 77). The potential coding segment corresponds to the second open reading frame within the 3′ end of the SV40 and BKV early regions. Again, a JCV protein might also be encoded here, although it would only be about 75% the length of the proteins of the other two viruses. Its existence is in question since little homology was evident between the three viral sequences (Fig. 8 [SV40 is not shown because too few amino acids can be aligned with the JCV and BKV sequences]) and since a properly placed AUG was not found in all three viruses. (Methionine residue 1 of the putative JCV, BKV, and SV40 protein is at amino acid positions 3, 26, and 4, respectively.)

DISCUSSION

Numerous biochemical, immunological, and genetic studies (69) have predicted a close evolutionary relationship

JCV	Met	Asp	Lys	Val	Leu	Asn	Arg	Glu	Glu	Ser	Met	Glu	Leu	Met	Asp	Leu	Leu	Gly	Leu	Asp	Arg	Ser	Ala	Trp	Gly	Asn	Ile	Pro	Val	Met
BKV											Met	Glu								Glu		Ala					Leu		Leu	
SV40											Leu	Gln								Glu		Ser					Ile		Leu	
JCV	Arg	Lys	Ala	Tyr	Leu	Lys	Lys	Cys	Lys	Glu	Leu	His	Pro	Asp	Lys	Gly	Gly	Asp	Glu	Asp	Lys	Met	Lys	Arg	Met	Asn	Phe	Leu	Tyr	Lys
BKV						Arg					Phe									Asp				Arg			Thr			
SV40						Lys					Phe									Glu				Lys			Thr			
JCV	Lys	Met	Glu	Gln	Gly	Val	Lys	Val	Ala	His	Gln	Pro	Asp	Phe	Gly	Thr	N	Trp	Asn	Ser	Ser	Glu	Val	Pro	Thr	Tyr	Gly	Thr	Asp	Glu
BKV				Gln	Asp			Val								Thr	N		Ser	Ser	Ser		Val						Glu	
SV40				Asp	Gly			Tyr								Gly	Phe		Asp	Ala	Thr		Ile						Asp	
JCV	Trp	Glu	Ser	Trp	Trp	Asn	Thr	Phe	Asn	Glu	Lys	Trp	Asp	Glu	Asp	Leu	Phe	Cys	His	Glu	Glu	Met	Phe	Ala	Ser	Asp	Asp	Glu	Asn	Thr
BKV			Ser			Ser	Ser				Lys	Trp	Asp		Asp				His		Asp		Phe	Ala			Glu		Ala	
SV40			Gln			Asn	Ala				N	N	N		Asn				Ser		Glu		Pro	Ser			Asp		Ala	
JCV	Gly	N	Ser	Gln	His	Ser	Thr	Pro	Pro	Lys	Lys	Lys	Lys	Lys	Val	Glu	Asp	Pro	Lys	Asp	Phe	Pro	Val	Asp	Leu	His	Ala	Phe	Leu	Ser
BKV	Ala	Asp											Arg										Ser	Asp		His	Gln			
SV40	Ala	Asp											Arg										Ser	Glu		Leu	Ser			
JCV	Gln	Ala	Val	Phe	Ser	Asn	Arg	Thr	Val	Ala	Ser	Phe	Ala	Val	Tyr	Thr	Thr	Lys	Glu	Lys	Ala	Gln	Ile	Leu	Tyr	Lys	Lys	Leu	Met	Glu
BKV	Gln								Leu		Cys			Val								Gln	Ile					Leu		
SV40	His								Leu		Cys			Ile								Ala	Leu					Ile		
JCV	Lys	Tyr	Ser	Val	Thr	Phe	Ile	Ser	Arg	His	Gly	Phe	Gly	Gly	His	Asn	Ile	Leu	Phe	Phe	Leu	Thr	Pro	His	Arg	His	Arg	Val	Ser	Ala
BKV											Met	Cys	Ala	Gly				Ile												
SV40											Asn	Ser	Tyr	Asn				Leu												
JCV	Ile	Asn	Asn	Tyr	Cys	Gln	Lys	Leu	Cys	Thr	Phe	Ser	Phe	Leu	Ile	Cys	Lys	Gly	Val	Asn	Lys	Glu	Tyr	Leu	Phe	Tyr	Ser	Ala	Leu	Cys
BKV				Phe	Cys																			Leu						Thr
SV40				Tyr	Ala																			Met						Thr
JCV	Arg	Gln	Pro	Tyr	Ala	Val	Val	Glu	Glu	Ser	Ile	Gln	Gly	Gly	Leu	Lys	Glu	His	Asp	Phe	Asn	Pro	Glu	Glu	Pro	Glu	Glu	Thr	Lys	Gln
BKV		Asp		Tyr	His	Thr	Ile				Ile	Gln									Ser				Pro					
SV40		Asp		Phe	Ser	Val	Ile				Leu	Pro									Asn				Ala					
JCV	Val	Ser	Trp	Lys	Leu	Val	Thr	Gln	Tyr	Ala	Leu	Glu	Thr	Lys	Cys	Glu	Asp	Val	Phe	Leu	Leu	Met	Gly	Met	Tyr	Leu	Asp	Phe	Gln	Glu
BKV						Ile		Glu			Val					Glu			Phe			Leu				Glu			Tyr
SV40						Val		Glu			Met					Asp			Leu			Leu				Glu			Tyr
JCV	Asn	Pro	Gln	Gln	Cys	Lys	Lys	Cys	Glu	Lys	Lys	Asp	Gln	Pro	Asn	His	Phe	Asn	His	His	Glu	Lys	His	Tyr	Tyr	Asn	Ala	Gln	Ile	Phe
BKV	Asn	Val	Glu	Glu		Lys			Gln			Asp			Tyr		Phe	Lys	Tyr					Phe	Ala			Ile		
SV40	Ser	Phe	Glu	Met		Leu			Ile			Glu			Ser		Tyr	Lys	Tyr					Tyr	Ala			Ala		
JCV	Ala	Asp	Ser	Lys	Asn	Gln	Lys	Ser	Ile	Cys	Gln	Gln	Ala	Val	Asp	Thr	Val	Ala	Ala	Lys	Gln	Arg	Val	Asp	Ser	Ile	His	Met	Thr	Arg
BKV		Glu						Ser										Leu			Lys				Thr	Leu	His	Met		
SV40		Asp						Thr										Leu			Lys				Ser	Leu	Gln	Leu		
JCV	Glu	Glu	Met	Leu	Val	Glu	Arg	Phe	Asn	Phe	Leu	Leu	Asp	Lys	Met	Asp	Leu	Ile	Phe	Gly	Ala	His	Gly	Asn	Ala	Val	Leu	Glu	Gln	Tyr
BKV		Glu			Thr	Glu				His	Ile			Lys			Leu	Ile			Ala	His		Asn		Val	Leu		Gln	Tyr
SV40		Gln			Thr	Asn				Asp	Leu			Arg			Ile	Met			Ser	Thr		Ser		Asp	Ile		Glu	Trp
JCV	Met	Ala	Gly	Val	Ala	Trp	Ile	His	Cys	Leu	Leu	Pro	Gln	Met	Asp	Thr	Val	Ile	Tyr	Asp	Phe	Leu	Lys	Cys	Ile	Val	Leu	Asn	Ile	Pro
BKV							Leu						Lys			Ser		Ile	Phe				His		Ile		Phe		Val	
SV40							Leu						Lys			Ser		Val	Tyr				Lys		Met		Tyr		Ile	
JCV	Lys	Lys	Arg	Tyr	Trp	Leu	Phe	Lys	Gly	Pro	Ile	Asp	Ser	Gly	Lys	Thr	Thr	Leu	Ala	Ala	Ala	Leu	Leu	Asp	Leu	Cys	Gly	Gly	Lys	Ser
BKV		Arg																			Gly			Asp						Ala
SV40		Lys																			Ala			Glu						Ala
JCV	Leu	Asn	Val	Asn	Met	Pro	Leu	Glu	Arg	Leu	Asn	Phe	Glu	Leu	Gly	Val	Gly	Ile	Asp	Gln	Phe	Met	Val	Val	Phe	Glu	Asp	Val	Lys	Gly
BKV					Leu		Met	Glu			Thr						Ala				Tyr	Met								
SV40					Leu		Leu	Asp			Asn						Ala				Phe	Leu								
JCV	Thr	Gly	Ala	Glu	Ser	Arg	Asp	Leu	Pro	Ser	Gly	His	Gly	Ile	Ser	Asn	Leu	Asp	Cys	Leu	Arg	Asp	Tyr	Leu	Asp	Gly	Ser	Val	Lys	Val
BKV			Ala			Lys						His			Asn				Ser										
SV40			Gly			Arg						Gln			Asn				Asn										
JCV	Asn	Leu	Glu	Arg	Lys	His	Gln	Asn	Lys	Arg	Thr	Gln	Val	Phe	Pro	Pro	Gly	Ile	Val	Thr	Met	Asn	Glu	Tyr	Ser	Val	Pro	Arg	Thr	Leu
BKV				Lys			Leu						Ile					Leu							Pro			Lys		
SV40				Lys			Leu						Ile					Ile							Ser			Lys		
JCV	Gln	Ala	Arg	Phe	Val	Arg	Gln	Ile	Asp	Phe	Arg	Pro	Lys	Ala	Tyr	Leu	Arg	Lys	Ser	Leu	Ser	Cys	Ser	Glu	Tyr	Leu	Leu	Glu	Lys	Arg
BKV						Arg								Ile			Arg	Lys	Ser		Gln	Asn			Phe					
SV40						Lys								Asp			Lys	His	Cys		Glu	Arg			Phe					
JCV	Ile	Leu	Gln	Ser	Gly	Met	Thr	Leu	Leu	Leu	Leu	Leu	Ile	Trp	Phe	Arg	Pro	Val	Ala	Asp	Phe	Ala	Ala	Ala	Ile	His	Glu	Arg	Ile	Val
BKV		Leu				Met	Thr				Leu				Phe					Asp			Thr	Asp		Gln	Ser		
SV40		Ile				Ile	Ala				Met				Tyr					Glu			Gln	Ser		Gln	Ser		
JCV	Gln	Trp	Lys	Glu	Arg	Leu	Asp	Leu	Glu	Ile	Ser	Met	Tyr	Thr	Phe	Ser	Thr	Met	Lys	Ala	Asn	Val	Gly	Met	Gly	Arg	Pro	Ile	Leu	Asp
BKV	Glu							Ser		Ile		Met	Tyr	Thr	Phe	Ser	Arg			Tyr		Ile	Cys			Lys	Cys	Ile		
SV40	Glu							Lys		Phe		Leu	Ser	Val	Tyr	Gln	Lys			Phe		Val	Ala			Ile	Gly	Val		
JCV	Phe	Pro	Arg	Glu	Glu	Asp	Ser	Glu	Ala	Glu	Asp	Ser	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
BKV	Ile	Thr		Glu	Glu		Ser	Glu	Thr				N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
SV40	Trp	Leu		Asn	Ser		Asp	Asp	Asp				Gln	Gln	Asn	Ala	Asp	Lys	Asn	Glu	Asp	Gly	Gly	Glu	Lys	Asn	Met	Glu	Asp	Ser
JCV	Gly	His	Gly	Ser	Ser	Thr	Glu	Ser	Gln	Ser	Gln	Cys	Phe	Ser	Gln	Val	Ser	Glu	Ala	Ser	Gly	N	Ala	N	Asp	Thr	Gln	Glu	N	N
BKV			Gly	Ser	Ser	Thr	Glu					Cys	Ser	Ser		Val	Ser	Asp	Thr	Ser	Ala	Pro	Ala	Glu	Asp	Ser		Arg	Ser	Asp
SV40			Glu	Thr	Gly	Ile	Asp					Gly	Ser	Phe		N	N	N	N	N	Ala	Pro	Gln	N	Ser	Ser		Ser	Val	His
JCV	N	N	Asn	Cys	Thr	Phe	His	Ile	Cys	Lys	Gly	Phe	Gln	Cys	Phe	Lys	Lys	Pro	Lys	Thr	Pro	Pro	Pro	Lys	N	N	N			
BKV	Pro	His	Ser	Gln	Glu	Leu		Leu		Lys			Gln				Arg		Lys					Lys	N	Pro	Glu	Thr		
SV40	Asp	His	Asn	Gln	Pro	Tyr		Ile		Arg			Thr				Lys		Pro					Glu	N	Pro	Glu	Thr		

FIG. 4. Comparison of the large T proteins of JCV (Mad1), BKV(Dun), and SV40. The proposed sequences for the large T proteins of the three polyomaviruses are aligned for maximum homology. In those instances when the amino acid is the same for all three T proteins, only the JCV sequence is shown.

JCV	Met	Asp	Lys	Val	Leu	Asn	Arg	Glu	Glu	Ser	Met	Glu	Leu	Met	Asp	Leu	Leu	Gly	Leu	Asp	Arg	Ser	Ala	Trp	Gly	Asn	Ile	Pro	Val	Met
BKV											Met	Glu								Glu		Ala					Leu		Leu	
SV40											Leu	Gln								Glu		Ser					Ile		Leu	
JCV	Arg	Lys	Ala	Tyr	Leu	Lys	Lys	Cys	Lys	Glu	Leu	His	Pro	Asp	Lys	Gly	Gly	Asp	Glu	Asp	Lys	Met	Lys	Arg	Met	Asn	Phe	Leu	Tyr	Lys
BKV						Arg					Phe									Asp				Arg			Thr			
SV40						Lys					Phe									Glu				Lys			Thr			
BKV	Lys	Met	Glu	Gln	Gly	Val	Lys	Val	Ala	His	Gln	Pro	Asp	Phe	Gly	Thr	N	Trp	Asn	Ser	Ser	Glu	Val	Gly	Cys	N	Asp	N	Phe	Pro
JCV				Gln	Asp			Val								Thr	N		Ser	Ser	Ser			N	Cys	Ala	Asp	N	Phe	Pro
SV40				Asp	Gly			Tyr								Gly	Phe		Asp	Ala	Thr			N	Phe	Ala	Ser	Ser	Leu	Asn
JCV	Pro	Asn	Ser	Asp	Thr	Leu	Tyr	Cys	Lys	Glu	Trp	Pro	Asn	Cys	Ala	Thr	Asn	Pro	Ser	Val	His	Cys	Pro	Cys	Leu	Met	Cys	Met	Leu	Lys
BKV	Leu	Cys	Pro		Thr	Leu				Glu			Ile		Ser	Lys	Lys	Pro		Val	His		Pro		Met	Leu		Gln		Arg
SV40	Pro	Gly	Val		Ala	Met				Gln			Glu		Ala	Lys	Lys	Met		Ala	Asn		Ile		Leu	Leu		Leu		Arg
JCV	Leu	Arg	His	Arg	Asn	Arg	Lys	Phe	Leu	Arg	Ser	Ser	Pro	Leu	Val	Trp	Ile	Asp	Cys	Tyr	Cys	Phe	Asp	Cys	Phe	Arg	Gln	Trp	Phe	Gly
BKV	Leu	Arg		Leu				Phe	Leu		Lys	Glu					Ile					Ile				Thr	Gln			
SV40	Met	Lys		Glu				Leu	Tyr		Lys	Asp					Val					Phe				Arg	Met			
JCV	Cys	Asp	Leu	Thr	Gln	Glu	Ala	Leu	His	Cys	Trp	Glu	Lys	Val	Leu	Gly	Asp	Thr	Pro	Tyr	Arg	Asp	Leu	Lys	Leu					
BKV	Leu			Thr	Glu	Glu	Thr		Gln	Trp		Val	Gln	Ile	Ile		Glu		Pro	Phe									
SV40	Leu			Cys	Glu	Gly	Thr		Leu	Leu		Cys	Asp	Ile	Ile		Gln		Thr	Tyr									

FIG. 5. Comparison of the small t proteins of JCV (Mad1), BKV(Dun), and SV40. The proposed sequences for the small t proteins of the three polyomaviruses are aligned for maximum homology as described in the legend to Fig. 4.

between JCV, BKV, and SV40; nucleotide sequence analysis confirms these predictions. However, a number of differences do exist among these viruses, particularly in the sequences lying to the late side of the origin, and it is these differences which might begin to explain the unique biology of JCV (e.g., its restricted lytic and transforming abilities in vitro). In the laboratory, JCV exhibits an extremely narrow host range. In its sole permissive cell type, primary human fetal glial cells, replication is inefficient; in most cells it rarely occurs at all. Unlike SV40, which is expressed in a

JCV	Met	Ala	Pro	Thr	Lys	Arg	Lys	Gly	Glu	Arg	N	N	N	N	N	N	N	N	Lys	Asp	Pro	Val	Gln	Val	Pro	Lys	Leu	Leu	Ile	Arg
BKV									Glu	Cys	Pro	Gly	Ala	Ala	Pro	Lys	Lys	Pro		Glu								Leu		Lys
SV40									Ser	Cys	Pro	Gly	Ala	Ala	Pro	Lys	Lys	Pro		Glu								Val		Lys
JCV	Gly	Gly	Val	Glu	Val	Leu	Glu	Val	Lys	Thr	Gly	Val	Asp	Ser	Ile	Thr	Glu	Val	Glu	Cys	Phe	Leu	Thr	Pro	Glu	Met	Gly	Asp	Pro	Asp
BKV			Val				Glu							Ala	Ile								Asn		Glu			Asp		
SV40			Ile				Gly							Ser	Phe								Asn		Gln			Asn		
JCV	Glu	His	Leu	Arg	Gly	Phe	Ser	Lys	Ser	Ile	Ser	Ile	Ser	Asp	Thr	Phe	Glu	Ser	Asp	Ser	Pro	Asn	Arg	Asp	Met	Leu	Pro	Cys	Tyr	Ser
BKV		Asn	Leu	Arg		Phe		Leu	Lys	Leu	Ser	Ala	Glu	Asn	Asp		Ser	Ser				Glu	Arg	Lys	Met				
SV40		His	Gln	Lys		Leu		Lys	Ser	Leu	Ala	Ala	Glu	Lys	Gln		Thr	Asp				Asp	Lys	Glu	Gln				
JCV	Val	Ala	Arg	Ile	Pro	Leu	Pro	Asn	Leu	Asn	Glu	Asp	Leu	Thr	Cys	Gly	Asn	Ile	Leu	Met	Trp	Glu	Ala	Val	Thr	Leu	Lys	Thr	Glu	Val
BKV	Thr																	Leu								Val	Gln			
SV40	Val																	Ile								Val	Lys			
JCV	Ile	Gly	Val	Thr	Ser	Leu	Met	Asn	Val	His	Ser	Asn	Gly	Gln	Ala	Thr	His	Asp	Asn	Gly	Ala	Gly	Lys	Pro	Val	Gln	Gly	Thr	Ser	Phe
BKV			Ile		Ser	Met	Leu		Leu		Ala	Gly	Ser		Lys	Val		Glu	His		Gly				Ile			Ser	Asn	
SV40			Val		Ala	Met	Leu		Leu		Ser	Gly	Thr		Lys	Thr		Glu	Asn		Ala				Ile			Ser	Asn	
JCV	His	Phe	Phe	Ser	Val	Gly	Gly	Glu	Ala	Leu	Glu	Leu	Gln	Gly	Val	Leu	Phe	Asn	Tyr	Arg	Thr	Lys	Tyr	Pro	Asp	Gly	Thr	Ile	Phe	Pro
BKV				Ala				Asp	Pro			Met					Met								Asp	Gly		Ile	Thr	
SV40				Ala				Glu	Pro			Leu					Ala								Ala	Gln		Val	Thr	
JCV	Lys	Asn	Ala	Thr	Val	Gln	Ser	Gln	Val	Met	Asn	Thr	Glu	His	Lys	Ala	Tyr	Leu	Asp	Lys	Asn	Lys	Ala	Tyr	Pro	Val	Glu	Cys	Trp	Val
BKV			Pfo		Ala	Gln			Val				Asp				Tyr				Asn	Asn								
SV40			Ala		Val	Asp			Gln				Asp				Val				Asp	Asn								
JCV	Pro	Asp	Pro	Thr	Arg	Asn	Glu	Asn	Thr	Arg	Tyr	Phe	Gly	Thr	Leu	Thr	Gly	Gly	Glu	Asn	Val	Pro	Pro	Val	Leu	His	Ile	Thr	Asn	Thr
BKV				Ser	Arg				Ala						Phe											Val			
SV40				Ser	Lys				Thr						Tyr											Ile			
JCV	Ala	Thr	Thr	Val	Leu	Leu	Asp	Glu	Phe	Gly	Val	Gly	Pro	Leu	Cys	Lys	Gly	Asp	Asn	Leu	Tyr	Leu	Ser	Ala	Val	Asp	Val	Cys	Gly	Met
BKV									Gln								Ala		Ser			Val			Ala		Ile			Leu
SV40									Gln								Ala		Ser			Val			Val		Ile			Leu
JCV	Phe	Thr	Asn	Arg	Ser	Gly	Ser	Gln	Gln	Trp	Arg	Gly	Leu	Ser	Arg	Tyr	Phe	Lys	Val	Gln	Leu	Arg	Lys	Arg	Arg	Val	Lys	Asn	Pro	Tyr
BKV				Ser			Thr				Arg			Ala					Ile	Arg					Ser				
SV40				Thr			Thr				Lys			Pro					Ile	Thr					Ser				
JCV	Pro	Ile	Ser	Phe	Leu	Leu	Thr	Asp	Leu	Ile	Asn	Arg	Arg	Thr	Pro	Arg	Val	Asp	Gly	Gln	Pro	Met	Tyr	Gly	Met	Asp	Ala	Gln	Val	Glu
BKV							Ser								Gln								Tyr			Glu	Ser			
SV40							Ser								Gln								Ile			Ser	Ser			
JCV	Glu	Val	Arg	Val	Phe	Glu	Gly	Thr	Glu	Glu	Leu	Pro	Gly	Asp	Pro	Asp	Met	Met	Arg	Tyr	Val	Asp	Lys	Tyr	Gly	Gln	Leu	Gln	Thr	Lys
BKV					Phe	Asp	Gly			Arg								Ile			Ile		Lys	Gln			Leu	Gln		Lys
SV40					Tyr	Glu	Asp			Glu								Ile			Ile		Glu	Phe			Thr	Thr		Arg
JCV	Met	Leu																												
BKV		Leu																												
SV40		Gln																												

FIG. 6. Comparison of the VP1 proteins of JCV (Mad1), BKV(Dun), and SV40. The amino acid sequences of the VP1 capsid proteins of the three polyomaviruses are aligned for maximum homology as described in the legend to Fig. 4. In each viral DNA, there are two potential initiation codons for the VP1 protein which occur in the same reading frame. We have used the second methionine residue as the first amino acid in the protein sequence.

JCV	Met	Gly	Ala	Ala	Leu	Ala	Leu	Leu	Gly	Asp	Leu	Val	Ala	Thr	Val	Ser	Glu	Ala	Ala	Ala	Ala	Thr	Gly	Phe	Ser	Val	Ala	Glu	Ile	Ala
BKV						Ala						Val		Ser																
SV40						Thr						Ile		Thr																
JCV	Ala	Gly	Glu	Ala	Ala	Ala	Thr	Ile	Glu	Val	Glu	Ile	Ala	Ser	Leu	Ala	Thr	Val	Glu	Gly	Ile	Thr	Ser	Thr	Ser	Glu	Ala	Ile	Ala	Ala
BKV							Ala				Gln	Ile			Leu						Ile		Ser							
SV40							Ala				Gln	Leu			Val						Leu		N							
JCV	Ile	Gly	Leu	Thr	Pro	Glu	Thr	Tyr	Ala	Val	Ile	Thr	Gly	Ala	Pro	Gly	Ala	Val	Ala	Gly	Phe	Ala	Ala	Leu	Val	Gln	Thr	Val	Thr	Gly
BKV						Gln	Thr					Ala				Gly		Ile							Ile				Ser	
SV40						Gln	Ala					Ser				Ala		Ile							Leu				Thr	
JCV	Gly	Ser	Ala	Ile	Ala	Gln	Leu	Gly	Tyr	Arg	Phe	Phe	Ala	Asp	Trp	Asp	His	Lys	Val	Ser	Thr	Val	Gly	Leu	Phe	Gln	Gln	Pro	Ala	Met
BKV	Ile		Ser	Leu			Val						Ser												Tyr			Ser	Gly	
SV40	Val		Ala	Val			Val						Ser												Tyr			Pro	Gly	
JCV	Ala	Leu	Gln	Leu	Phe	Asn	Pro	Glu	Asp	Tyr	Tyr	Asp	Ile	Leu	Phe	Pro	Gly	Val	Asn	Ala	Phe	Val	Asn	Asn	Ile	His	Tyr	Leu	Asp	Pro
BKV		Leu	Glu		Phe	Asn		Asp	Glu										Asn	Thr			Asn	Asn	Ile	Gln			
SV40		Val	Asp		Tyr	Arg		Asp	Asp										Gln	Thr			His	Ser	Val	Gln			
JCV	Arg	His	Trp	Gly	Pro	Ser	Leu	Phe	Ser	Thr	Ile	Ser	Gln	Ala	Phe	Trp	Asn	Leu	Val	Arg	Asp	Asp	Leu	Pro	Ala	Leu	Thr	Ser	Gln	Glu
BKV						Ser			Ala	Thr					Leu		His	Val	Ile	Arg	Asp		Ile		Ser	Ile				
SV40						Thr			Asn	Ala					Phe		Arg	Val	Ile	Gln	Asn		Ile		Arg	Leu				
JCV	Ile	Gln	Arg	Arg	Thr	Gln	Lys	Leu	Phe	Val	Glu	Ser	Leu	Ala	Arg	Phe	Leu	Glu	Glu	Thr	Thr	Trp	Ala	Ile	Val	Asn	Ser	Pro	Ala	Asn
BKV	Leu	Gln				Glu	Arg	Phe	Phe	Arg	Asp												Thr	Ile	Val		Ala		Ile	
SV40	Leu	Glu				Gln	Arg	Tyr	Leu	Arg	Asp												Thr	Val	Ile		Ala		Val	
JCV	Leu	Tyr	Asn	Tyr	Ile	Ser	Asp	Tyr	Tyr	Ser	Arg	Leu	Ser	Pro	Val	Arg	Pro	Ser	Met	Val	Arg	Gln	Val	Ala	Gln	Arg	Glu	Gly	Thr	Tyr
BKV	Phe			Tyr	Ile	Gln	Gln				Asp				Ile			Ser						Glu				Thr	Arg
SV40	Trp			Ser	Leu	Gln	Asp				Thr				Ile			Thr						Asn				Leu	Gln
JCV	Ile	Ser	Phe	Gly	His	Ser	Tyr	Thr	Gln	Ser	Ile	Asp	Asp	Ala	Asp	Ser	Ile	Gln	Glu	Val	Thr	Gln	Arg	Leu	Asp	Leu	Lys	Thr	N	N
BKV	Val	His				Thr		N	N	Ser			Asp					Glu	Glu			Gln		Met	Asp	Leu	Arg	Asn	Gln	Gln
SV40	Ile	Ser				Thr		Asp	N	Asn			Glu					Gln	Gln			Glu		Trp	Glu	Ala	Gln	Ser	Gln	Ser
JCV	Pro	Asn	Val	Gln	Ser	Gly	Glu	Phe	Ile	Glu	Arg	Ser	Ile	Ala	Pro	Gly	Gly	Ala	Asn	Gln	Arg	Ser	Ala	Pro	Gln	Trp	Met	Leu	Pro	Leu
BKV	N	Ser		His							Lys	Thr	Ile									Thr								
SV40	Pro	Asn		Gln							Lys	Phe	Glu									Thr								
JCV	Leu	Leu	Gly	Leu	Tyr	Gly	Thr	Val	Thr	Pro	Ala	Leu	Glu	Ala	Tyr	Glu	Asp	Gly	Pro	Asn	Lys	Lys	Lys	Arg	Arg	N	N	N	N	N
BKV							Thr			Pro			Glu								Gln				Arg	Val	Ser	Arg	Gly	Ser
SV40							Ser			Ser			Lys								Lys				Lys	Leu	Ser	Arg	Gly	Ser
JCV	N	N	Lys	Glu	N	Gly	Pro	Arg	Ala	Ser	Ser	Lys	Thr	Ser	Tyr	Lys	Arg	Arg	Ser	Arg	Ser	Ser	Arg	Ser						
BKV	Ser	Gln		Ala	Lys		Thr	Arg			Ala		Thr	Thr	Asn				Ser											
SV40	Ser	Gln		Thr	Lys		Thr	Ser			Ala		Ala	Arg	His				Asn											

FIG. 7. Comparison of the late structural proteins VP2 and VP3 of JCV (Mad1), BKV(Dun), and SV40. The proposed sequences for the VP2 and VP3 proteins of the three polyomaviruses are aligned for maximum homology as described in the legend to Fig. 4. In each virus, VP3 is encoded by the carboxy-terminal sequences of VP2. The first methionine residue in the VP3 protein is underlined.

variety of eucaryotic cells, most cells do not even express T antigen after JCV infection (46). This restricted activity does not appear to involve an early step in the virus-cell interaction (i.e., adsorption, penetration, or uncoating [16]); one possible explanation is that JCV has a weak or defective regulatory signal(s) (e.g., the early promoter).

When deficiencies in viral transcription and replication are discussed, attention is focused on certain features of the JCV regulatory region, specifically the tandem repeat (enhancer?), which shares little homology with the BKV or SV40 repeats; the duplicated TATA box; the absence of the sequence PyPyCCXCCC; and the presence of a CAT box which shows only partial identity with the consensus sequence.

Small changes in the enhancer sequences have dramatic effects on the host range and oncogenic properties of the polyomaviruses (18, 29, 30, 73, 74). It has been suggested that these sequences may have recently diverged in these viruses and perhaps represent modified enhancer elements of their hosts (57). If the tandem repeat of JCV represents an acquired or altered enhancer that only functions efficiently in brain tissue, then this would help to explain the apparent adaptation of JCV for growth in these cells. In the general population, JCV probably replicates in kidney or lung cells; strains of JCV isolated from diseased brain tissue do not grow in these cells in culture but instead show a distinct predilection for brain cells (both in their lytic and nonproductive cycles) (17, 36, 46, 48, 50, 55, 71). Significantly, these isolates tend to delete and insert (host?) sequences within their tandem repeats (J. D. Martin and R. J. Frisque, unpublished data).

The duplication of the TATA sequence represents a second feature of the JCV regulatory region which might alter the transcription of early mRNAs. Assuming the tandem repeat of JCV is an enhancer, then the relative positions of the TATA box and enhancer might preclude an efficient interaction involving the two sequences. Specifically, would the tandem repeat efficiently enhance transcription from the TATA sequence when the latter sequence lies within the enhancer? Furthermore, since enhancers appear to preferentially potentiate transcription from the most proximal promoter (72), might not the wrong TATA box of JCV be utilized? Based on position, we expect that the correct TATA box (the one that positions the proper 5′ termini of the early messages) is located nearest the early region.

The absence of the sequence PyPyCCXCCC might pose still another problem for the expression of the early genes of JCV. In the SV40 genome are found three copies of a 21-np repeat that are located to the late side of the replication origin. The repeats are required for efficient replication and transcription (4, 5) and include six copies of the sequence PyPyCCXCCC. This sequence is present in the regulatory regions of BKV, polyomavirus, several adenoviruses, and the herpes simplex virus type 1 thymidine kinase gene (4, 39, 59). Dynan and Tjian (11) have recently isolated a promoter-specific transcription factor Sp1 from whole-cell extracts

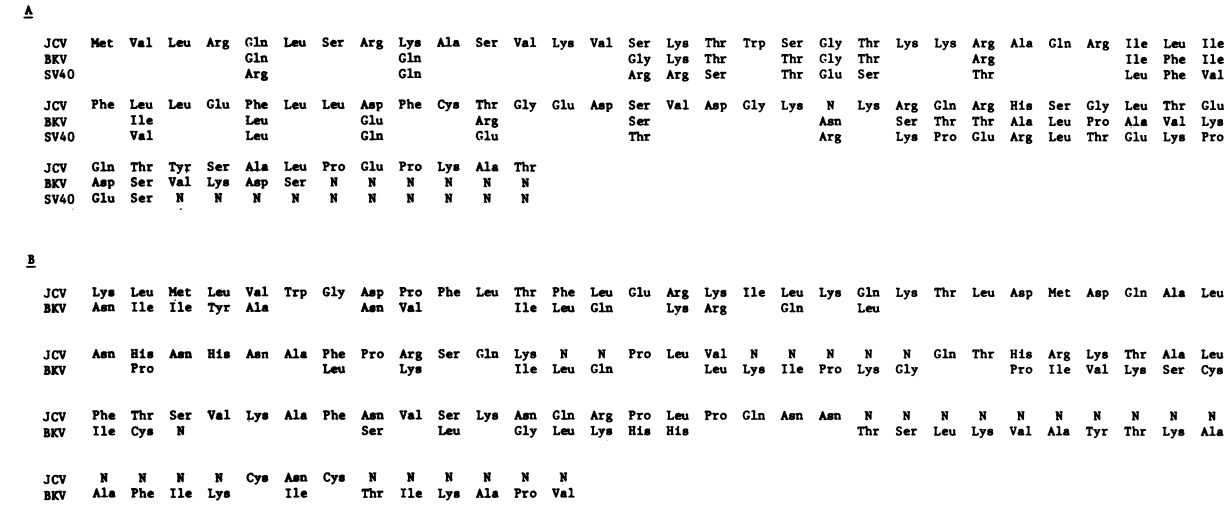

FIG. 8. Comparisons of two potential proteins that might be encoded within the late leader sequences (A) or the 3′ ends of the early regions (B) of JCV (Mad1), BKV(Dun), and SV40. (A) Comparison of the agnoproteins thought to be encoded by the late leader sequences of the three polyomaviruses. Agnoprotein has been identified in SV40. Amino acid sequences are aligned to show maximum homology as described in the legend to Fig. 4. (B) Comparison of polypeptide sequences which may be translated from the 3′ ends of the JCV and BKV early regions. The putative SV40 protein sequence (98 amino acids) is not included since little homology is evident with the two sequences of the human viruses.

that appears to both bind to the 21-np repeat region and stimulate transcription of the SV40 early and late promoters (but not of other promoters tested). A second factor, Sp2, was also identified and represents a general factor required for transcription of all promoters tested. Additional studies by these investigators have included BKV which lacks the 21-np repeats but does show partial homology with this region of SV40 (e.g., the sequence PyPyCCXCCC is shared). Transcription of the BKV early messages also depends on Sp1, although the stimulatory effect was reduced by a factor of 10 when compared with SV40 (10). Neither the 21-np repeats nor the PyPyCCXCCC sequence is found in the JCV regulatory region. Their absence is suggestive, especially in light of our recent analysis of a number of viable JCV variants. Preliminary sequence results for their regulatory regions reveal a consistent pattern, the loss of the upstream TATA box and the insertion of the sequence PyPyCCXCCC (J. D. Martin and R. J. Frisque, unpublished data). The biological properties of these variants are now being studied. It should be noted that prototype JCV DNA does contain the sequence PyPyCCXXCCC (TCCCTTCCC) located at a position that corresponds to the region containing the SV40 21-np repeats. This sequence may contribute to the lytic activity of Mad1 in primary human fetal glial cells.

The CAT box is a sequence thought to be involved in the binding of RNA polymerase II (3). Potential CAT boxes are found within the tandem repeats of JCV and BKV but are only partially homologous to the consensus sequence. Since BKV functions efficiently in vitro, it seems unlikely, however, that the restricted activity of JCV in tissue culture stems from a defective CAT sequence.

We have recently replaced the regulatory sequences of JCV (nucleotides 5015 to 275) with those of BKV to test whether one or more of the sequences discussed above are contributing to the inefficient lytic and transforming properties of JCV. Preliminary results show that this hybrid virus behaves like the parental BKV in its ability to efficiently induce T antigen and transform BHK-21 cells (B. Bollag, L. B. Peitzman, J. M. Slauch, and R. J. Frisque, unpublished data).

Our discussion has focused on differences in certain regulatory elements of the polyomaviruses and how these differences might affect biological parameters. Although the proteins of these viruses show a large degree of similarity, there are significant stretches of nonhomology which may contribute to the unique biology of each virus. Examples of these nonhomologous stretches include the following. (i) The carboxy termini of the large T protein and agnoprotein vary considerably in JCV, BKV, and SV40. The agnoprotein, like large T, may have regulatory functions located within these unrelated sequences. (ii) The unique coding sequences of the small t protein of these three viruses are less homologous than those sequences shared with the amino terminus of large T. One might speculate that, since small t is dispensable for the lytic growth of these viruses in vitro (63, 67), alterations in its unique coding sequences might be better tolerated than changes in sequences overlapping the multifunctional large T protein. Alternatively, small t might contribute to the host range phenotype of the polyomaviruses, and differences in its coding sequence might reflect a functional requirement in the various cells permissive for each virus. (iii) The most obvious difference in the capsid proteins is an eight-amino-acid deletion that affects all three JCV proteins. We do not know whether this alteration might interfere with the structural function of these proteins or whether it contributes to the immunological differences observed between the JCV, BKV, and SV40 capsids.

Certainly a more thorough analysis of both coding and noncoding sequences must be conducted before their influence on the biology of JCV can be fully assessed. The nucleotide sequence presented here suggests several regions on which to focus such studies.

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