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Comparison of JC and BK Human Papovaviruses with Simian Virus 40: DNA Homology Studies

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Studies were performed to ascertain the relationship of human papovavirus JC to BK virus and to simian virus 40 (SV40) by further restriction endonuclease analysis and by DNA-DNA competition hybridization on membrane filters. Form I DNA extracted from two new isolates from cases of progressive multifocal leukoencephalopathy of human papovaviruses that were JC-like in their antigenic properties were found to yield restriction endonuclease fragmentation patterns similar to those of prototypic JC virus DNA and different from those of BK or SV40. Form I DNA preparations of JC and of BK viruses were found to be related to each other and to SV40 DNA to a similar extent, with JC and BK virus DNAs containing sequences homologous to both early and late regions of the SV40 genome. The relatedness in each comparison was less than 50%, and heterologous hybrids between either JC or BK and SV40 DNAs were found to be less stable than homologous SV40-SV40 hybrids in high concentrations of formamide, suggesting substantial mismatch within homologous regions, to the extent of 15 to 30%. The new JC-like isolates were also studied in competition hybridization reactions with SV40 DNA and yielded results similar to those obtained with JC virus.

In 1971 two human papovaviruses designated JC (19) and BK (8) were isolated that had antigenic characteristics quite distinct from each other and from simian virus 40 (SV40), although some antigenic relationship could be demonstrated among T antigens of the three viruses (B. L. Padgett and D. L. Walker, *In* J. L. Melnick [ed.], *Progress in Medical Virology*, vol. 22, in press). Shortly thereafter two other human isolates (designated as SV40-progressive multifocal leukoencephalopathy [PML]) were reported that had properties nearly identical to those of SV40, using as criteria both antigenic analysis (33) and restriction endonuclease mapping techniques (21). The possibility existed that, despite the marked differences between the JC and BK isolates and the SV40-like viruses, the former might have undergone only minor evolutionary divergence from SV40.

In a comparison between JC, BK, and prototypic SV40 genomes reported from this laboratory, analysis of restriction endonuclease mapping techniques yielded distinctive patterns for each of the three DNAs (17). Subsequent studies by others with BK and SV40 have extended the findings of major differences, using both nucleic acid hybridization and heteroduplexing techniques (9, 10, 12). JC virus presents considerable technical difficulty in cultivation, which has limited its study. However, some of the inherent technical problems have been overcome by means of in vitro labeling techniques, thus facilitating the studies reported here and allowing a threeway comparison of the primate papovaviruses by DNA-DNA hybridization.

MATERIALS AND METHODS

Virus propagation and DNA extraction. JC virus was inoculated onto primary human fetal glial cell cultures; at 21 to 28 days postinoculation, cultures were harvested and DNA was extracted from gradient-purified virion pools as previously described (17). Covalently closed circular (form I) DNA was separated from linear DNA by cesium chloride-ethidium bromide gradient technique.

New isolates from additional cases of PML tissue, designated Mad-2 and Mad-4 (B. L. Padgett, D. L. Walker, G. M. ZuRhein, A. E. Albert, and S. M. Chou, J. Infect. Dis., in press), were cultivated, and their DNA was handled in a manner identical to that of the original JC virus isolate.

BK virus was also cultivated in primary human fetal glial cells, and its DNA was similarly extracted.

SV40 virus, strain 776 (seed virus kindly supplied by D. Nathans), was grown in Vero cell cultures, inoculated at low multiplicity (1.6×10^{-2} PFU/cell), and incubated for 11 to 13 days, after which form I DNA was extracted as indicated above.

Labeling of viral DNA by nick translation. The form I papovavirus DNA preparations were labeled in vitro by the method of Schachat and Hogness (22) as follows. DNA (1 to 2 μg) was incubated with potassium phosphate buffer, pH 7.5, 50 mM; $MgCl_2$, 10 mM; EDTA, 1 mM; beta-mercaptoethanol, 1 mM; pancreatic DNase, 10 ng/ml; *M. luteus* DNA polymerase (kindly provided by R. D. Wells), 10 μl of a preparation containing 45 units/ml; dATP, dGTP, and dCTP, each 0.3 mM; and [^{3}H]dTTP, 0.01 mM (specific activity, 50,000 Ci/mol). The mixture was incubated for 8 h at 14°C. Reactions were stopped by the addition of equal volumes of 10 mM EDTA, and the products were purified by banding in neutral CsCl solution, $\rho_0 = 1.70$. The specific activity was 500,000 cpm per μg of DNA added to the reaction mixture.

Restriction enzyme preparation and incubation conditions. EcoRI endonuclease was prepared and used as previously described (29), except that incubation was carried out for 30 min at 37°C.

Bacillus amyloliquefaciens strain H restriction endonuclease (Bam I) was prepared by the method of Wilson and Young (34). Incubation mixtures included Tris-hydrochloride, pH 7.4, 7 mM; $MgCl_2$, 9 mM; NaCl, 35 mM; DNA, 0.5 to 1 μg; and enzyme, 5 μl, in a total volume of 50 μl. When double digestions were performed, the first enzyme was allowed to incubate at 37°C for 30 min; the reaction mixture was then heated to 68°C for 5 min to inactivate the first enzyme and returned to 37°C for an additional 30 min after addition of the second enzyme. Finally, the second reaction was again stopped by incubation at 68°C for 30 min. The products of such digestion were analyzed on gels; λcb2 DNA at 20 μg/ml (kindly supplied by W. Szybalski) cut by EcoRI was used as marker.

Kpn endonuclease was prepared from *Klebsiella pneumoniae* (kindly supplied by J. Davies) using conditions similar to those for the preparation of EcoRI. Some residual exonuclease activity was removed by passing the phosphocellulose fraction over DNA agarose, prepared by the method of Schaller et al. (23) in a buffer composed of Tris-hydrochloride pH 7.4, 10 mM; NaCl, 100 mM; EDTA, 1 mM; and beta-mercaptoethanol, 7 mM. Incubation conditions for Kpn digestion were identical to those used for EcoRI, employing 2 μl of enzyme per 50-μl reaction.

Hin III endonuclease was prepared and used as described by Tanaka et al. (30). Incubation conditions were the same as those described for Bam I.

For separation of DNA fragments obtained by digestion with restriction endonucleases, the electrophoresis buffer contained Tris-acetate, 40 mM, pH 8.0; sodium acetate, 20 mM; EDTA, 1 mM; and ethidium bromide, 0.5 μg/ml. A sample volume of 50 μl with 30 μl of bromophenol blue dye solution (30% sucrose [wt/wt]-50 mM EDTA-0.02% bromophenol blue in electrophoresis buffer lacking ethidium bromide) was applied to 1.5% agarose gel (13 by 0.6 cm) and subjected to electrophoresis at 50 V for 3 h at room temperature by the method of Sharp et al. (26). Bands were identified with UV light.

Hybridization on membrane filters. To prepare supercoiled DNA for adsorption onto membrane filters, it was first digested with EcoRI to unit linear molecules and then denatured and immobilized on membrane filters (Millipore Corp.; type HAWP; 2.5 cm or 4.7 cm) in 6× SSC (0.90 M NaCl plus 0.090 M sodium citrate) as described by Denhardt (5). When 4.7-cm filters were used, up to seven 11-mm-diameter circles were cut from each with a no. 6 cork borer. Incubations were performed in vials with Teflon-lined caps. Double-stranded DNA for annealing was denatured by heating at 100°C for 5 min in 6× SSC and quenched on ice before addition to the incubation vials containing filters. These were then incubated at 65°C for 16 h. In competition hybridization experiments, competing DNAs were degraded to an average size of 5×10^5 daltons by sonic treatment prior to thermal denaturation in 6× SSC.

Hybridization to agarose gel fractions. After Bam-Kpn sequential endonuclease digestion and subsequent separation of restriction fragments on agarose gels, DNA was transferred from gel slices to membrane filters as follows. Ten sequential 1.5-mm slices from a segment of the gel bracketing the two Bam-Kpn fragments visualized with UV light were prepared. Each slice was boiled separately for 10 min in 10 ml of 6× SSC, cooled quickly in an ice bath, and stored overnight at −20°C, and the contents (including both DNA and agarose) were immobilized on membrane filters. In separate reconstruction experiments with labeled DNA and agarose, it was established that at least 90% of the input DNA could be adsorbed to the membrane filter by this method; overnight storage at −20°C was found to be essential for this step.

RESULTS

Comparison of JC virus DNA with DNA from other PML isolates. The first question addressed was the extent to which the initial JC isolate was representative of other antigenically similar isolates recovered from tissue of PML patients. Therefore two other JC-like papovavirus isolates, designated Mad-2 and Mad-4 (Padgett et al., in press), were grown, and their DNA was extracted and analyzed after digestion with Hin III and EcoRI enzymes. After digestion with Hin III, six fragments (Fig. 1a) were obtained with SV40 DNA, whereas a distinctly different set, consisting of four bands (Fig. 1b), was obtained with BK DNA. These patterns are in agreement with earlier studies (17). Digestion of viral DNA from the three PML isolates with Hin III yielded sets of three fragments (Fig. 1c–e) that were similar to each other and distinctly different from those of SV40 or BK. The three fragments, labeled Hin III A, B, and C, have molecular weights corresponding to 88, 8, and 4% of SV40 if one uses the data of Danna et al. (4) as a standard. The Hin III A fragments of both JC and Mad-2 show distinct doubling which, on the basis of experiments shown below, probably reflects the presence of a high level of size-defec-

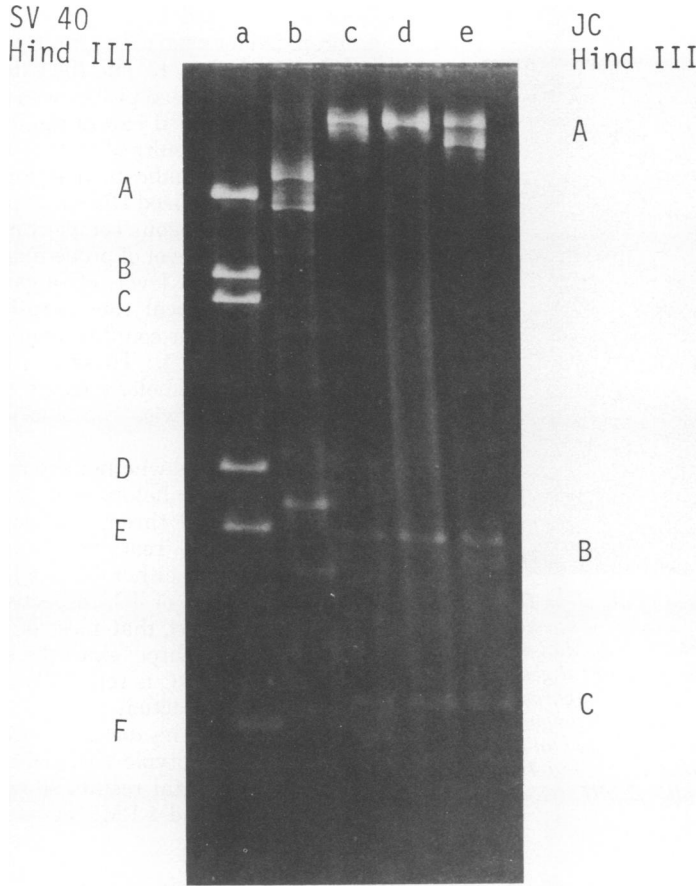


FIG. 1. *Comparison of SV40, JC, and BK virus DNA with viral DNA from two other PML isolates by digestion with HinIII. DNAs from SV40, JC, and BK papovaviruses as well as from two recent PML isolates were digested with Hin III restriction endonuclease, and the resultant fragments were fractionated by electrophoresis in an acrylamide gradient (3.5 to 10%) gel. (a) SV40, (b) BK, (c) JC, (d) Mad-4, and (e) Mad-2.*

tive viral DNAs. Digestion with additional enzyme or for longer incubation times was not found to alter these patterns in a significant manner.

The three PML isolates, upon digestion with EcoRI, are all converted to the respective unit linears, indicating the presence of a single EcoRI cut. Analysis of the Mad-4 virus DNA preparation (Fig. 2a) reveals the presence of form I DNA (faster fraction) and form II DNA (slower fraction). Upon digestion with EcoRI, the preparation is converted to unit linears with intermediate mobility. Similarly, the Mad-2 and JC viral DNA preparations consisting of both form I and form II DNAs (Fig. 2c and e, respectively) are also converted to unit linears (Fig. 2d and f, respectively). In contrast to the Mad-4 viral DNA preparation, a multiplicity for each of the three forms is seen in both the Mad-2 and JC preparations. The reason for such variation in DNA size homogeneity seen the several preparations is not clear.

Figure 3 shows that the distinctive single Hpa I cut in JC DNA is shared by the other two PML isolates (Fig. 3b, d, and e); again the multiplicity of bands, most pronounced in the Mad-2 preparation (Fig. 3e), is seen. For comparison, SV40 has the expected three fragments (Fig. 3a), and BK shows no evidence of specific cleavage (Fig. 3c). Although mapping restriction endonucleases as a basis for comparison of viral DNAs only permits us to examine a small number of bases, we infer from these findings that JC virus DNA could be considered prototypic of a distinct papovavirus class. On the basis of this inference we proceeded to compare DNA homologies among the three prototypic virus strains, JC, BK, and SV40.

DNA-DNA competition hybridization on membrane filters. Homologous combinations

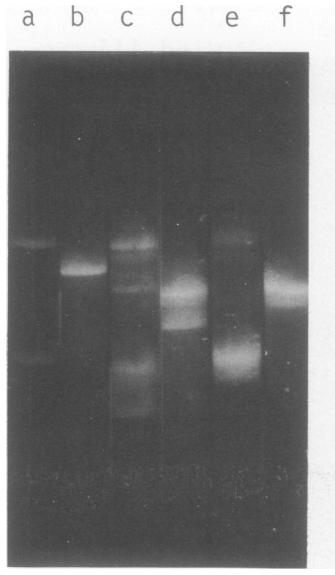


FIG. 2. *Comparison of JC virus DNA with DNA from two other PML isolates by digestion with EcoRI. DNAs from JC, Mad-2, and Mad-4 were digested with EcoRI, and the resultant digest was fractionated by electrophoresis in 1.0% agarose. For comparison, the undigested viral DNA preparations containing a mixture of covalently closed and relaxed circles were included. (a) Mad-4, (b) Mad-4 plus EcoRI, (c) Mad-2, (d) Mad-2 plus EcoRI, (e) JC, and (f) JC plus EcoRI.*

of immobilized DNA and labeled probe were prepared for each of the three prototypic viruses JC, BK, and SV40, and the extent of hybridization of the labeled probes was determined as a function of added excess unlabeled DNA competitor. The results of testing all nine possible combinations indicate that for each combination of immobilized DNA and probe, excess unlabeled homologous competitor was capable of reducing the level of probe fixation to the filter to a plateau level of approximately 10%, whereas identical concentrations of heterologous competitor could not reduce this level below 50% (Fig. 4). These results indicate that comparable homology exists between each of the three pairwise combinations of these viruses.

To determine whether the homology seen between pairwise heterologous combinations was common to all three, homologous SV40-SV40 hybridizations reactions were exhaustively competed with either JC or BK followed by the addition of BK or JC, respectively. The results (Fig. 5) suggest that most of the homology is shared by all three, since the subsequent addition of BK or JC is relatively ineffective in the competition reaction.

In view of the distinct differences between the three prototypic virus DNAs inferred from the experimental results shown in Fig. 4, the identity of Mad-4 PML isolate as JC-like was

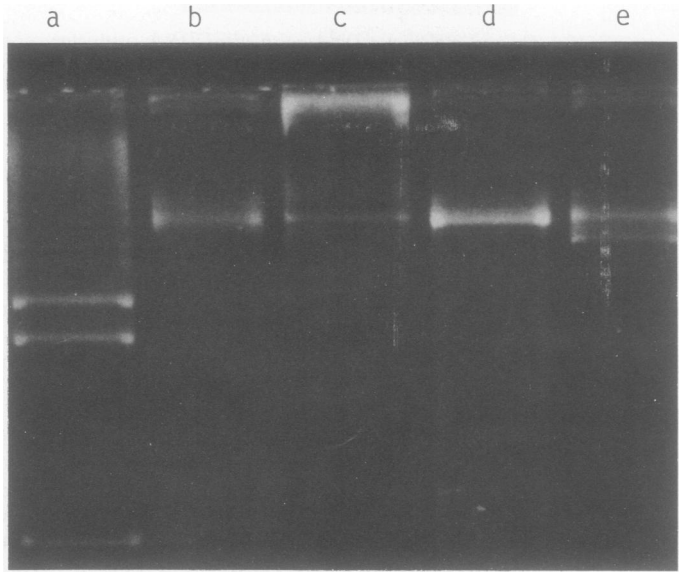


FIG. 3. *Comparison of SV40, JC, and BK virus DNAs with viral DNA from two other PML isolates by digestion with Hpa I. DNA from SV40, JC, and BK papovaviruses as well as from two new PML isolates were digested with Hpa I, and the resultant fragments were fractionated by electrophoresis in an acrylamide gradient (3.5 to 10%) gel. (a) SV40, (b) JC, (c) BK, (d) Mad-4, and (e) Mad-2.*

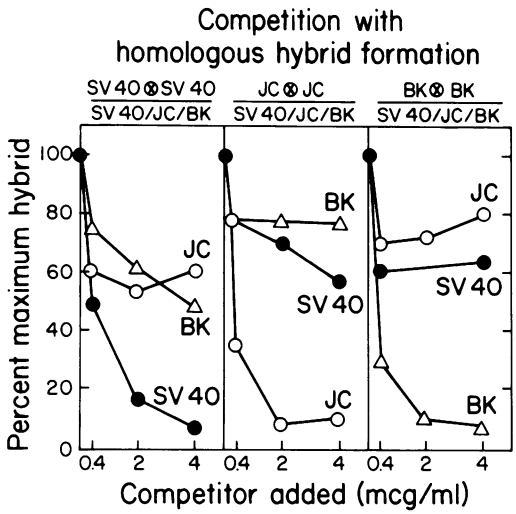
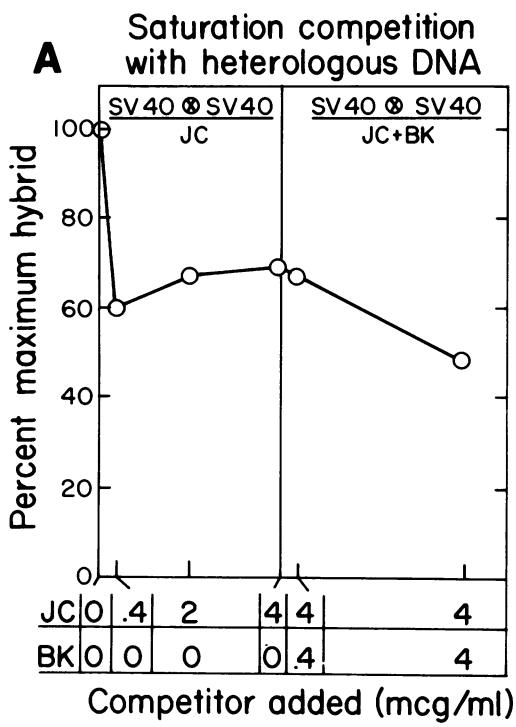


FIG. 4. *Comparison of homology between SV40, JC, and BK virus DNA by competition hybridization. Three sets of hybridization reactions were assembled using in each case 0.2 μg of viral DNA fixed to the membrane filter and 5 ng of homologous DNA as probe (500 cpm/ng). Within each set of homologous hybridization reactions, excess unlabeled SV40, JC, or BK virus DNA was added as competitor, and the competition was measured as a function of concentration of unlabeled DNA added. The 100% level of binding corresponds to 780, 510, and 540 cpm for the SV40, JC, and BK uncompeted homologous reactions, respectively. These figures represent increments of 9-, 10-, and 14-fold, respectively, over the level of binding to blank filters. The composition of each hybridization reaction is summarized above this and following figures. In the convention used the hybridization, symbolized by a circled cross, is as follows. The viral DNA immobilized on the membrane filter is on the left, the source of labeled probe is on the right, and the source of competitor is in the denominator. If several competitors were used, respectively, they are separated by a slash. For example, SV40 ⊗ SV40 denotes that SV40 viral DNA attached to the membrane filter was hybridized with SV40-labeled probe, whereas the entry in the denominator, SV40/JC/BK, denotes that either SV40 or JC or BK was used in excess as unlabeled competitor as indicated directly on the curve.*

tested in a competition hybridization experiment. As shown in Fig. 6, Mad-4 DNA competed the homologous JC-JC reaction as effectively as did an equal concentration of unlabeled JC DNA; SV40 DNA as a negative control was relatively ineffective, as previously seen. In another experiment competition hybridization was performed using unlabeled Mad-2 and Mad-4 viral DNA competitors, respectively, with JC viral DNA on the membrane filter and JC viral ^{3}H-labeled DNA as probe, in 2× SSC and 50% formamide at 37°C; results similar to those shown in Fig. 6 were obtained. Moreover, Mad-2 was found to be as effective as either Mad-4 or JC in the competition (data not shown).

Preparation of fragments containing early and late SV40 genome functions. The enzymes EcoRI, Bam and Kpn convert the SV40 DNA preparation consisting of form I and form II DNAs (Fig. 7a) to unit linears (Fig. 7b, c, and d, respectively). The three respective sites of enzymatic cleavage occur at distinctly different positions, since digestion of SV40 sequentially by any pair of these enzymes results in two discrete fragments. Results of sequential digestion with Eco RI plus Bam, EcoRI plus Kpn, and Bam plus Kpn are shown in Fig. 7e, f, and g, respectively. The digestion shown in Fig. 7f contains unit linears (slowest band) owing to incomplete digestion by Kpn, in addition to the expected bands that migrate faster in the gel. By coelectrophoresis with internal standards, molecular weights ($\times 10^6$) of 2.5 plus 0.5, 2.1 plus 0.9, and 1.8 plus 1.2 were determined for the pairs of fragments obtained by EcoRI-Bam, EcoRI-Kpn, and Bam-Kpn double digestion, respectively. This allows us to locate the Bam and Kpm sites in SV40 at 0.16 and 0.70 map units, respectively, relative to the EcoRI site arbitrarily defined as origin. Of particular interest in connection with the present investigations are the two fragments obtained by Bam-Kpn digestion. The two sites of cleavage by these enzymes that occur at 0.16 and 0.70 map units results in resolution of the SV40 genome into two fragments, comprising approximately 55 and 45% of the genome, respectively. From studies of Khoury et al. (13), the larger (L) fragment contains all of the early function and in addition about 20% of the regions of the genome that specify late functions, whereas the smaller (S) fragment represents late functions only.

Localization of homologous sequences in early versus late genome fragments. The combined Kpn-Bam digest was fractionated; the agarose gels were sliced, and DNA was transferred directly to nitrocellulose membrane filters for hybridization studies as described. Probes prepared from each of the three viral DNAs were hybridized onto membrane filters containing the gel fractions under conditions where the amount of labeled probe bound was proportional to the concentration added. The results (Fig. 8) indicate that the observed homology fractionates with each of the fragments. An apparent difference in location of fragment peaks is probably related to technical variation in slices and therefore trivial. For the homologous reaction in which SV40 probe was used,



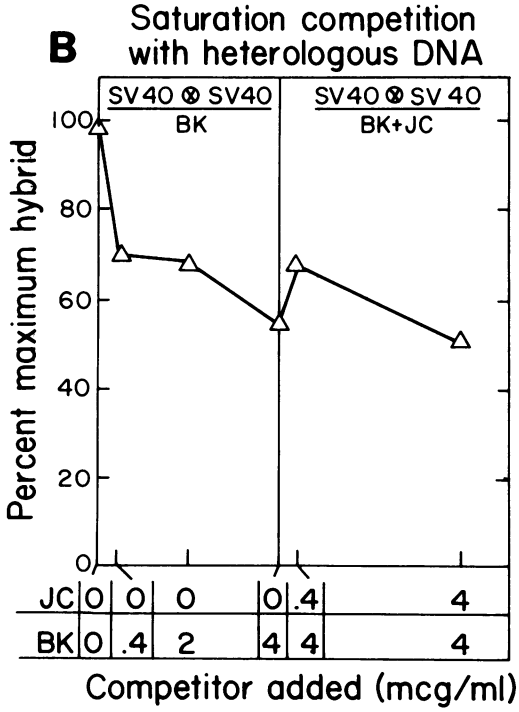


FIG. 5. *Competition of the homologous SV40 membrane filter-probe reaction with BK and JC virus DNA. Two series of homologous SV40 membrane filter-probe combinations were assembled, and JC or BK virus DNA was added as competitor, respectively. To determine the extent of overlap of homology between JC and BK with respect to SV40, additional BK and JC virus DNA was added to each of the two series, respectively, and the extent of additional competition of the homologous SV40 reaction was determined. The 100% hybridizations in the uncompeted reactions were 450 and 410 cpm, respectively; these values both represent increments of 10-fold over the level of binding to blank filters. (a) competition by JC DNA followed by BK, (b) competition by BK DNA followed by JC.*

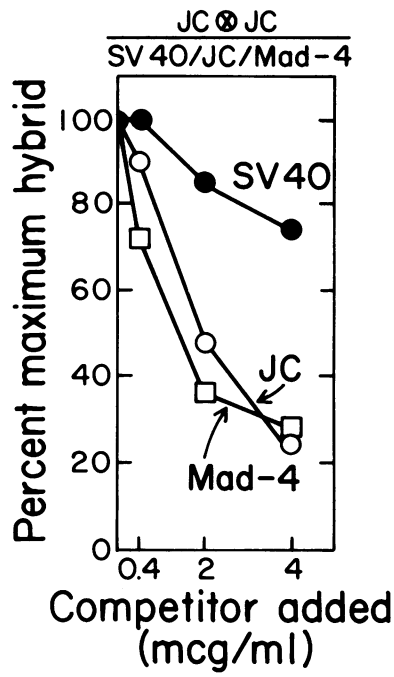


FIG. 6. *Comparison of homology between SV40, JC, and Mad-4 virus DNA by competition hybridization. Three sets of hybridization reactions were assembled using 0.2 μg of JC viral DNA fixed to the membrane filter and 5 ng of homologous JC DNA probe (500 cpm/ng). Excess unlabeled SV40, JC, or Mad-4 virus DNA was added as competitor, and competition was measured as a function of added unlabeled DNA. The 100% level of binding corresponds to 740 cpm, which represents an increment of 15-fold over the level of binding to blank filters.*

approximately 70% of the hybridized probe was associated with the larger fragment, whereas 30% was associated with the smaller fragment. This relationship parallels the 55:45 ratio between the molecular weights of the two fragments. In the case of JC or BK probe, more hybridized probe is seen associated with the smaller than with the larger fragment, which suggests a strong bias in favor of homology

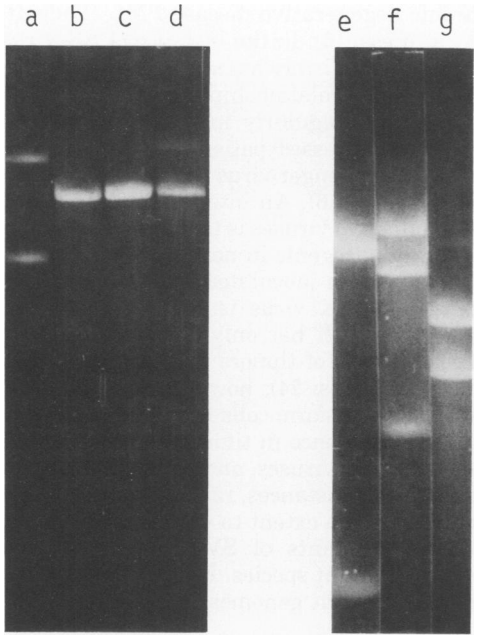


FIG. 7. *Analysis of Bam and Kpn restriction endonuclease fragments of SV40 DNA. SV40 DNA was digested with EcoRI, Bam or Kpn restriction endonucleases singly (yielding unit length linears) or sequentially (yielding two fragments), and the resultant mixture was fractionated by electrophoresis in 1.0% agarose. (a) Control (undigested), (b) EcoRI, (c) Bam, (d) Kpn, (e) EcoRI plus Bam, (f) EcoRI plus Kpn, and (g) Bam plus Kpn.*

with late functions. The degree of bias in the binding of JC or BK probe to the smaller Kpn-Bam SV40 fragment was calculated by first determining the ratio of JC (or BK) probe bound to the smaller fragment to that bound to the larger fragment (from inspection of Fig. 8, these ratios are already greater than 1). The ratios thus obtained were corrected for molecular weight, using as correction factor the ratio of SV40 probe bound to the larger fragment to that bound to the smaller fragment. Thus for JC and BK probes, bias factors of 5.78 and 3.25, respectively, were obtained by these calculations.

Stability of hybrids. Hybrids were prepared between unlabeled SV40, JC or BK DNA fixed on membrane filters, and labeled SV40 probe. These complexes were then denatured in 2× SSC at 37°C with 10% increments of formamide over the range of concentrations from 50 to 90%. The homologous (SV40-SV40) complex showed relatively greater stability than did the heterologous JC-SV40 or BK-SV40 combinations, which denatured at a significantly lower formamide concentration (Fig. 9). Although relatively few counts were involved, it appeared in this experiment that the least stable complex was between JC and SV40. This relatively greater instability was again seen when thermal denaturation of hybrids was tested (data not shown). Using the figure of 0.8% mismatch for each percent formamide decrement in hybrid stability (9), this would then correspond to an average mismatch within regions of homology of 15 to 20% for BK SV40 and 25 to 30% for JC SV40.

Salt dependence. Since the observed homology between SV40 and either JC or BK amounted to less than 50% of the SV40 genome, it was important to establish the degree of stringency of the hybridization conditions used. SV40 membrane filters were hybridized with each of the three probes under conditions of increasing salt concentration (Fig. 10). Viral

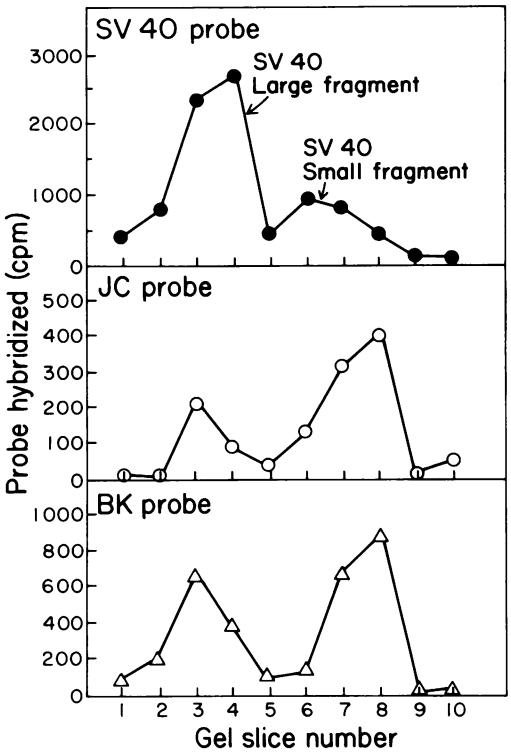


FIG. 8. *Localization of homology between SV40 and JC or BK virus DNA. SV40 DNA digested sequentially with Bam and Kpn was fractionated as shown in Fig. 7g, gels were sliced, and DNA was adsorbed to membrane filters and hybridized with ^{3}H-labeled SV40, JC, or BK DNA probes. Gel slices were numbered sequentially beginning at a point, not the gel origin, which permitted bracketing the two DNA bands with regions free of DNA. (a) SV40 probe (5,000 cpm); (b) JC probe (5,000 cpm); (c) BK probe (5,000 cpm).*

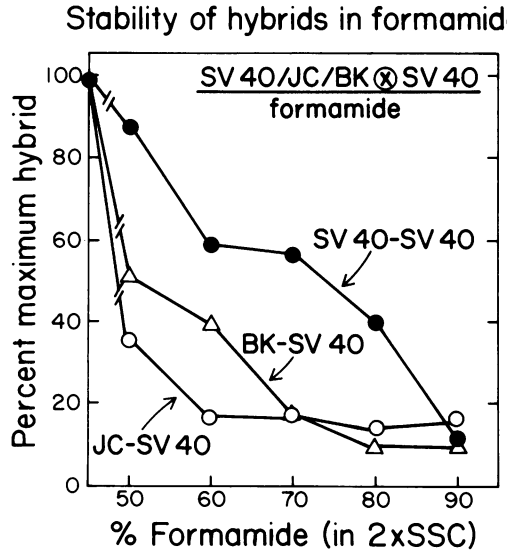


FIG. 9. *Stability of homologous and heterologous hybrids. Total SV40 or JC or BK DNA was fixed on membrane filters and hybridized with ^{3}H-labeled SV40 DNA probe as in Fig. 4. After incubation the filters were placed in fresh incubation medium containing 10% increments of formamide in 2 × SSC and incubated at 37°C for 15 min. Labeled DNA remaining on the filter was determined. The 100% hybridizations correspond to 570 cpm for SV40 whole virus DNA, 310 cpm with JC virus DNA, and 490 cpm with BK virus DNA. These values represent increments of 13-, 7-, and 11-fold, respectively, over the level of binding to blank filters. The respective combinations of membrane filter and DNA probe were: (a) SV40-SV40; (b) JC-SV40; and (c) BK-SV40.*

DNA of similar complexity (obtained from φX174, kindly provided by R. D. Wells) was included for comparison. The results show that the observed homology can be attributed to DNA-DNA hybrid formation rather than to entrapment of DNA probe by the membrane filter, because less than 50 cpm out of 5,000 cpm added were hybridized in the φX174-SV40 and SV40-φX174 negative controls over a range of 1 to 8× SSC. Over the same range of salt concentration, hybridization was consistently observed in the papovavirus systems and found to be salt dependent.

DISCUSSION

Several properties of the newly identified human papovaviruses JC and BK make them particularly interesting biologically. One is their apparent ubiquity in human populations (Padgett and Walker, in press; 7, 18, 25). A second is their association with human disease states: in the instance of JC virus one can draw the strong inference that it causes the rare chronic degenerative disease PML (Padgett et al., in press; 16). In the instance of BK virus it has not been firmly associated with disease in an etiologic relationship but rather has occurred most regularly in the urinary tract of immunosuppressed patients as an apparently innocent passenger virus (Padgett and Walker, in press; 2, 3, 6). An interesting difference between the two viruses is that, whereas JC virus is highly oncogenic in newborn hamsters, particularly when inoculated by the intracerebral route (32), BK virus is relatively low in its oncogenicity. It has only occasionally induced the formation of tumors in hamsters (Padgett et al., in press; 24); however, it has been reported to transform cells in vitro (14, 20, 27).

The coincidence in timing of initial isolation of JC and BK viruses, and of SV40 from human brain in two instances, raised several questions concerning the extent to which the former represented variants of SV40 rather than distinctly different species. Howley et al. (9) have established that genomes from three BK virus

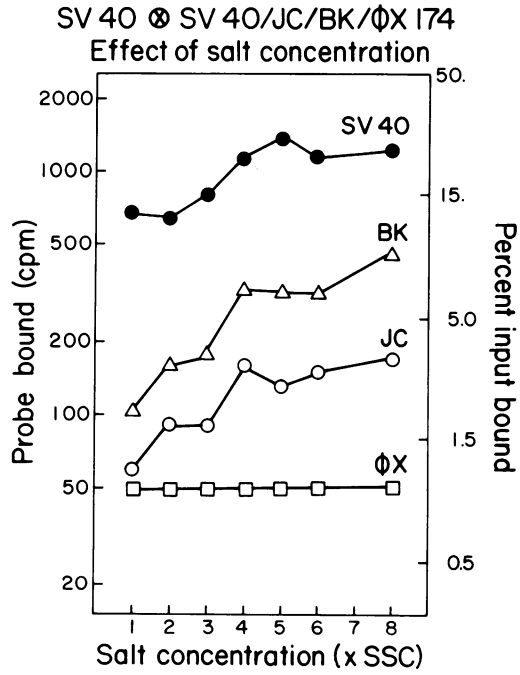


FIG. 10. *Salt concentration dependence of homologous and heterologous hybrid formation. SV40 DNA fixed to membrane filters was hybridized with ^{3}H-labeled SV40, JC, and BK DNA probes at 65°C and incubated in medium containing from 1 to 8× SSC; the extent of hybrid formation was determined. SV40 and φX174 were also compared to ascertain the extent of homology which the method might reveal between two unrelated virus DNAs of similar complexity.*

isolates resemble each other closely, and several investigators have shown that there are major differences between BK and SV40, both antigenically (1, 6, 28, 35) and by comparison of genomes (9, 10, 12).

The present studies establish a similar individuality for several JC virus isolates that resemble each other closely and are significantly different from both BK and SV40 by restriction fragment patterns and genome homology. Whereas a three-way relatedness is clearly demonstrable by competition hybridization, the degree of relatedness is apparently 50% at most, and of approximately the same degree in each comparison. The data further suggest that the shared regions are approximately the same for all three DNAs. This is of particular interest in that both JC and BK viruses hemagglutinate human and guinea pig erythrocytes, in marked contrast to SV40, and might have been supposed to be more closely related to each other than to SV40. It is of further interest that there is significant mismatch in homologous sequences and that greater mismatch is found between JC and SV40 than between BK and SV40.

Our findings confirm the rather surprising result with BK virus (12) that the areas of genome most homologous are those thought to specify late functions of SV40. The most easily demonstrable antigenic similarity is among the T antigens of the three viruses, and since SV40 codes for T antigen as an early function, one might expect substantial relatedness to be demonstrable in that region. However, both the comparison of BK with SV40 by Khoury et al. (12) and the present results suggest that the region of the SV40 genome carrying late functions is more closely related to JC and BK. It is possible that this conclusion may be even stronger than stated, in that the SV40 fragment (L) we have used to represent early functions does contain some late sequences. However, the fragment representing late functions (S) is clearly not mixed in the same way, and thus the stronger homology with that fragment is especially notable.

With these results we can draw certain conclusions concerning relatedness between the two hemagglutinating human papovaviruses and between either of them and SV40. It is clear that they are related, but only to the extent of about 50% or less of their genomes. Even in those regions, an average mismatch of 15 to 30% is found. Despite the fact that the most readily demonstrable antigenic relationship is with the T antigen, the genomic homology is located primarily in that segment of the SV40 DNA that specifies late functions. The phenomenon of shared antigenicity of protein products in the absence of demonstrable nucleic acid homology has been well documented in other viral systems (reviewed by Temin [31]) and probably pertains here as well.

These findings seem to support the conclusion that these papovaviruses, although quite possibly arising from a common ancestor, have diverged into three discretely different species (3). Their similarities and differences are made all the more biologically interesting by the fact that, although they seem to share a common T antigen, their potency as oncogenic agents in hamsters varies over a wide range. Whereas a role for T antigen in oncogenic processes is widely assumed, its precise functions in transformation are still unknown. Further studies of the similarities and differences between the three viruses in this regard may offer ways to test the importance of T antigen in papovavirus oncogenicity.

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