JOURNAL OF VIROLOGY, May 1982, p. 395–401
0022-538X/82/050395-07$02.00/0

Vol. 42, No. 2

Two Defective DNAs of Human Polyomavirus JC Adapted to Growth in Human Embryonic Kidney Cells

KUNITO YOSHIIKE,† TATSUO MIYAMURA,† HARDY W. CHAN,‡ AND KENNETH K. TAKEMOTO*

Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20205

Received 9 November 1981/Accepted 14 January 1982

Human polyomavirus JC (JCV) adapted to growth in human embryonic kidney (HEK) cells contains two or more species of shorter-length viral DNA even after two cycles of plaque purification in HEK cells. We have molecularly cloned JCV DNA from one plaque isolate and determined the physical map of its DNA. Using gel electrophoresis and electron microscopy, we found that the cloned DNA consisted of two classes of JCV DNA. One class of DNA had a deletion of 30% (between 0.7 and 1.0 map units from the *Eco*RI site) and an insertion of 1% at the same site in the late region. The other had a deletion of 35% (0.18 to 0.53 map units) and an insertion of 12% at the same site in the early region of the JCV genome. Both DNAs had added sequences near the origin of DNA replication. From their structure, the two DNAs appear to complement each other. These results indicate that the HEK-adapted JCV may be replicating by complementation between two defective mutants.

Human polyomavirus JC (JCV), which was isolated from human brain tissue of a patient with progressive multifocal leukoencephalopathy (17), has a highly restricted host range and grows efficiently only in primary human fetal glial cells rich in spongioblasts (16). In search of more readily available host cells, JCV was adapted to growth in human amnion cells (23) and human embryonic kidney (HEK) cells (15). We have observed that after adaptation JCV grew almost as efficiently in HEK cells as in primary human fetal glial cells. Since the prototype JCV(Mad-1) grows poorly in HEK cells, it appeared that a new host range mutant of JCV, designated as JC-HEK, had been selected during serial passage in HEK cells.

The DNA from JC-HEK was found to be heterogeneous and of shorter length than the wild-type Mad-1 DNA (15). Although the DNA from JC-HEK plaque isolates was less heterogeneous, it was still composed of two or more species of viral DNA (15). From these observations we have postulated that JC-HEK is either a viable deletion mutant or a mixture of two complementing defective mutants.

In the present study we have molecularly cloned DNA from a JC-HEK plaque isolate. The cloned DNA was found to consist of two classes of DNA, each with deletions. One class of DNA had a deletion in the late region and the other had a deletion in the early region of the JCV genome. Thus, JC-HEK appears to grow by complementation between two defectives.

† Present address: Department of Enteroviruses, National Institute of Health, Shinagawa-ku, Tokyo 141, Japan.
‡ Present address: Syntex Research, Palo Alto, CA 94304.

MATERIALS AND METHODS

Viral DNA. Viral DNA was extracted by the method of Hirt (9) from HEK cells (Microbiological Associates, Rockville, Md.) infected with plaque-purified JC-HEK P1-O4 (15). Supercoiled circular DNA, isolated from the low-molecular-weight DNA fraction by the ethidium bromide-buoyant density method (19), was used as a source for molecular cloning. JC-HEK P1-O4 DNA contained more than two classes of viral DNA as described previously (15).

Restriction endonucleases. *Eco*RI, *Bam*HI, *Hin*dIII, and *Hin*cII were purchased from Bethesda Research Laboratories, Inc., Rockville, Md. The conditions for digestion of DNA were according to the supplier's instructions. The enzymes were used at concentrations at least four times higher than was necessary to digest the DNA completely.

Molecular cloning. The procedure for molecular cloning was essentially as described by Israel et al. (11). The mixture of JC-HEK P1-O4 DNA and bacterial plasmid pBR322 (1) DNA was digested with either *Bam*HI or *Eco*RI. The cleaved linear DNAs were incubated at 12°C for 14 h with T4 ligase (Bethesda Research Laboratories) in a solution containing 66 mM Tris-hydrochloride (pH 7.6), 6.6 mM $MgCl_2$, 0.4 mM ATP, and 10 mM dithiothreitol. The products of the ligation reaction were used for transformation of *Escherichia coli* K-12 (strain HB101) bacteria under conditions described by Cohen et al. (3), and ampicillin-resistant transformants were selected for further screening. Bacterial colonies transformed by the *Bam*HI ligation mixture were screened for tetracycline sensitivity. Those transformed by *Eco*RI ligation mix-

ture were screened for JCV DNA sequences by the colony filter hybridization technique (7) with JCV DNA labeled in vitro by nick translation (20). Selected bacterial colonies were grown in 10 ml of L broth, and plasmid DNA was extracted by the rapid isolation method (5). For characterization, DNA (undigested or digested with restriction endonucleases) was subjected to agarose gel electrophoresis. Two recombinant plasmids, pJC(1-4) and pJC(2-7), used for comparison were generous gifts from Peter Howley, National Cancer Institute, Bethesda, Md. pJC(1-4) is pBR322 containing a prototype JCV Mad-1 DNA insert at the *Bam*HI site, and pJC(2-7) is pBR322 containing two JCV Mad-1 inserts at the *Eco*RI site.

Extraction of recombinant DNA. Plasmid DNA was prepared from chloramphenicol-amplified bacterial cultures (2). Supercoiled circular DNA was extracted by a modification of the clear lysate procedure (8) and was purified by the dye-buoyant density method (19).

Agarose gel electrophoresis. DNA samples were adjusted to 8% sucrose and 0.025% bromophenol blue and subjected to electrophoresis in either horizontal 0.6% agarose or vertical 1.2% agarose slab gels. The running Tris-borate buffer (pH 8.2) contained 89 mM Tris, 89 mM boric acid, and 2.5 mM EDTA. After running, gels were stained in buffer containing 0.5 μg of ethidium bromide per ml. DNA bands were visualized and photographed on a short-wavelength UV transilluminator.

Electron microscopy. The electron microscope heteroduplex method was essentially that of Davis et al. (4), and the conditions were described previously (24, 25). For electron microscopy, recombinant DNA was digested with either *Bam*HI or *Eco*RI and purified by the dye-buoyant density method (19).

Biological assay of DNA. For assay of antigen-forming activity, linear JC-HEK DNA was released from recombinant plasmid by digestion with *Eco*RI. HEK monolayer cultures were infected with DNA by the DEAE-dextran method (6) and were examined by the indirect immunofluorescence method. The antisera used were described previously (15).

RESULTS

Molecular cloning of JC-HEK DNA. The JC-HEK P1-4 DNA sample used for cloning was heterogeneous, but the major proportion of the molecules was composed of two classes of DNA, one of which was sensitive and the other of which was resistant to digestion with *Bam*HI. Nine of 12 ampicillin-resistant, tetracycline-sensitive bacterial colonies which were transformed by the *Bam*HI ligation mixture had recombinant plasmids containing one pBR322 and one JCV DNA ligated at the *Bam*HI site. Digestion of recombinant DNA with *Bam*HI released linear DNA molecules of the two. Digestion with *Eco*RI showed that there were two types of recombinant molecules with the opposite orientation of insertion. After combined digestion with *Bam*HI, *Hin*dIII, and *Hin*cII, the cleavage patterns of viral DNA from these nine clones were identical (data not shown).

About 500 bacterial colonies transformed by the *Eco*RI ligation mixture were screened for JCV DNA sequences by the colony hybridization method. JC-HEK DNA labeled in vitro hybridized to DNA from about 100 colonies. Plasmid DNA from 24 bacterial colonies with JCV DNA sequences were characterized by digestion with *Eco*RI, *Bam*HI, *Hin*dIII, and *Hin*cII. Seventeen of 24 bacterial colonies contained recombinant plasmids consisting of pBR322 molecules inserted with one JCV DNA molecule at the *Eco*RI site. Ten of 17 colonies had JCV DNA sensitive to *Bam*HI digestion. The cleavage patterns of JC-HEK DNA cloned at *Eco*RI and sensitive to *Bam*HI were identical to those of JC-HEK DNA cloned at the *Bam*HI site. These molecules were designated JC-HEK-A.

Six of seven clones of the JC-HEK DNA which were inserted to pBR322 DNA at the *Eco*RI site and resistant to *Bam*HI digestion had identical cleavage patterns after digestion with *Eco*RI and *Hin*dIII and with the same two enzymes and *Hin*cII (data not shown). The *Bam*HI-resistant DNA was designated JC-HEK-B. One bacterial colony had *Bam*HI-resistant JCV DNA slightly shorter than JC-HEK-B. Plasmid DNA containing JC-HEK-A or JC-HEK-B inserts was prepared from chloramphenicol-amplified cultures of the representative bacterial colonies and analyzed further as described below.

Characterization of cloned JC-HEK DNA. Figure 1 shows the cleavage maps of prototype JCV(Mad-1) DNA (14) and plasmid vector pBR322 DNA (22). Two recombinant plasmid DNAs containing JCV(Mad-1) DNA were used for comparison. JCV DNA was inserted into pBR322 DNA at the *Bam*HI site in recombinant

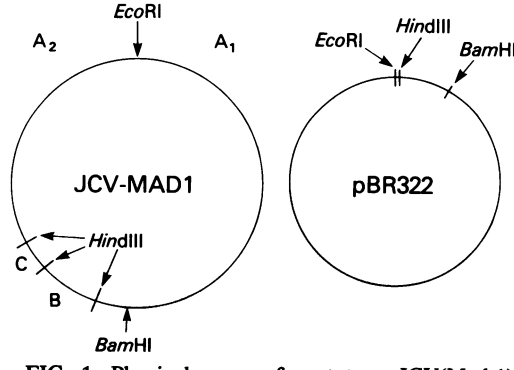

FIG. 1. Physical maps of prototype JCV(Mad-1) DNA and plasmid vector pBR322 DNA. The restriction endonuclease cleavage sites for JCV DNA (5.13 kilobases long) are based on the data reported by Martin et al. (14), and those for pBR322 (4.36 kilobases long) are based on the data reported by Sutcliffe (22).

plasmid pJC(1-4) (10). Two molecules of JCV DNA were ligated to pBR322 DNA at the *Eco*RI site (P. Howley, personal communication). Three representative plasmid DNAs (pJC010, pJC522, and pJC507) were used for characterization by restriction endonuclease cleavage followed by agarose gel electrophoresis. The *Bam*HI-sensitive JC-HEK-A DNA had been inserted into pBR322 DNA at the *Bam*HI site in pJC010 and at the *Eco*RI site in pJC522. The *Bam*HI-resistant JC-HEK-B DNA had been inserted into pBR322 DNA at the *Eco*RI site in pJC507.

The linear JC-HEK-A and JC-HEK-B DNAs, upon release from recombinant plasmids by *Bam*HI or *Eco*RI digestion, moved faster than JCV(Mad-1) DNA, which is 5.1 kilobases long (14), and more slowly than pBR322 DNA, which is 4.3 kilobases long (22), as shown in Fig. 2 (lanes 3, 4, and 5). Figure 2 also shows that JC-HEK-A DNA was shorter than JC-HEK-B DNA. JC-HEK-A DNA from pJC522 was cleaved once with *Bam*HI, yielding two fragments (Fig. 2, lane 7), whereas JC-HEK-B DNA from pJC507 was uncleaved with *Bam*HI (Fig. 2, lane 8).

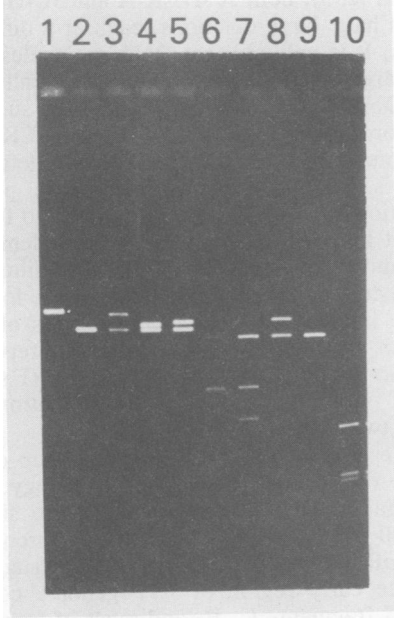

FIG. 2. Cloned JC-HEK DNA. Electrophoresis was done in a 0.6% agarose slab gel at 100 V for 3 h. pJC(1-4) and pJC(2-7) are pBR322 plus JCV(Mad-1) DNA. pJC010 and pJC522 are pBR322 plus JC-HEK-A DNA. pJC507 is pBR322 plus JC-HEK-B DNA. Lanes: 1, simian virus 40 DNA digested with *Eco*RI; 2, pBR322 digested with *Eco*RI; 3, pJC(1-4) digested with *Bam*HI; 4, pJC522 digested with *Eco*RI; 5, pJC507 digested with *Eco*RI; 6, pJC(2-7) digested with *Eco*RI plus *Bam*HI; 7, pJC522 digested with *Eco*RI plus *Bam*HI; 8, pJC507 digested with *Eco*RI plus *Bam*HI; 9, pBR322 digested with *Eco*RI plus *Bam*HI; 10, simian virus 40 digested with *Hin*dIII.

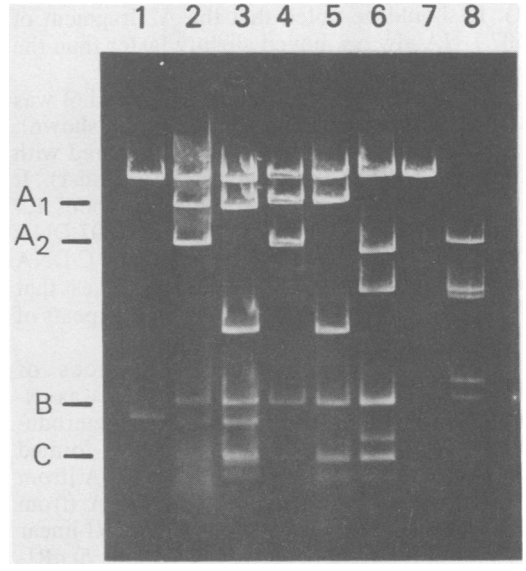

FIG. 3. Restriction endonuclease cleavage patterns of cloned JC-HEK DNA. Electrophoresis was done in a 1.2% agarose slab gel at 100 V for 3 h, 10 min. pJC(1-4) and pJC(2-7) are pBR322 inserted with JCV(Mad-1) DNA at the *Bam*HI and *Eco*RI site, respectively. pJC010 and pJC522 are pBR322 inserted with JC-HEK-A at the *Bam*HI and *Eco*RI site, respectively. pJC507 is pBR322 inserted with JC-HEK-B at the *Eco*RI site. Lanes: 1, pBR322 digested with *Bam*HI plus *Eco*RI plus *Hin*dIII; 2, pJC(1-4) digested with *Bam*HI plus *Eco*RI plus *Hin*dIII; 3, pJC010 digested with *Bam*HI plus *Eco*RI plus *Hin*dIII; 4, pJC(2-7) digested with *Eco*RI plus *Hin*dIII; 5, pJC522 digested with *Eco*RI plus *Hin*dIII; 6, pJC507 digested with *Eco*RI plus *Hin*dIII; 7, pBR322 digested with *Eco*RI plus *Hin*dIII; 8, simian virus 40 digested with *Hin*dIII.

As shown in Fig. 1, digestion of JCV(Mad-1) DNA with *Hin*dIII results in the production of three fragments: A, B, and C. *Eco*RI cleaves the *Hin*dIII-A fragment once, yielding A1 and A2 fragments. The *Bam*HI site is opposite (0.51 map units [m.u.]) the *Eco*RI site and within the *Hin*dIII-A fragment. *Eco*RI, *Hin*dIII, and *Bam*HI cleave pBR322 DNA once (Fig. 1). Digestion of pJC(1-4) and pJC(2-7) plasmids [containing pBR322 and JCV(Mad-1) DNA] with appropriate enzymes generated the cleavage patterns shown in Fig. 3 (lanes 2 and 4), which are consistent with the results reported by Martin et al. (14). Cleavage of pJC010, pJC522, and pJC507 DNAs revealed that JC-HEK-A and JC-HEK-B DNAs lacked fragments A2 and A1, respectively (Fig. 3, lanes 3, 5, and 6). From these results the changes of JC-HEK-A and JC-HEK-B DNAs were located in the A2 and A1 regions, respectively, of the JCV genome (Fig.

1). It should be noted that the A2 fragment of 507 DNA always moved slightly faster than the A2 fragment of JCV(Mad-1).

The negative of Fig. 3 (lanes 4, 5, and 6) was traced with a densitometer (data not shown), and the areas under bands were compared with those of *Hin*dIII fragments of JCV(Mad-1). It was found that 522 DNA contained about 1.4 times as much fragment C DNA, and 507 DNA contained twice as much fragment B and C DNA as JCV(Mad-1) DNA. These results suggest that JC-HEK-B DNA may contain tandem repeats of fragments B and C.

The homology of base sequences of JCV(Mad-1) DNA and JC-HEK DNAs was examined by the electron microscope heteroduplex method. Heteroduplex molecules formed between *Bam*HI-linear JCV(Mad-1) DNA [from pJC(1-4)] and *Bam*HI-linear JC-HEK-A (from pJC010) and those formed between *Eco*RI-linear JCV(Mad-1) DNA [from pJC(2-7)] and *Eco*RI-linear JC-HEK-B (from pJC507) were photographed, and each section of the molecule was measured on magnified images of the negatives. Figure 4A shows a summary of measurements on 58 heteroduplexes between *Bam*HI-linear Mad-1 and JC-HEK-A DNAs, some of which are shown in Fig. 4B, C, D, and E. Figure 4F shows a summary of the measurements on 28 heteroduplexes between *Eco*RI-linear Mad-1 and JC-HEK-B DNAs, some of which are shown in Fig. 4G, H, and I. JC-HEK-A DNA had an inserted DNA of 0.16 m.u. and a deletion of 0.31 m.u. JC-HEK-B DNA had a deletion of 0.35 m.u. (with an insertion of 0.12 m.u. at the same site) and an added DNA of 0.13 m.u. From the cleavage patterns produced by restriction endonucleases (Fig. 3) and the measurements on the heteroduplexes (Fig. 4), the deletions and insertions of JC-HEK-A and JC-JEK-B DNAs were mapped with reference to Mad-1 DNA (Fig. 5).

The location of the insertion loop of 0.13 m.u. on 28 JC-HEK-B heteroduplexes (Fig. 4F) varied between 0.61 and 0.74 m.u., as indicated by relatively large standard deviations for the double-stranded sections adjacent to the loop. Since the densitometer tracing showed that JC-HEK-B DNA contained two B and two C fragments, we concluded that there is a tandem repeat of the region from B to C in JC-HEK-B DNA.

Biological activity of cloned JC-HEK DNA. HEK cover slip (10 by 30 mm) cultures were infected with 0.4 μg of *Eco*RI-digested pJC522 (linear JC-HEK-A) or pJC507 (linear JC-HEK-B) in the presense of 100 μg of DEAE-dextran per ml. Four days after infection 0.05 to 0.1% of the cells were T antigen positive in the cultures infected with 522 DNA, but no V antigen-positive cells were found even 7 days after infection. Neither T- nor V-antigen production was detected in the HEK cultures infected with 507 DNA on days 4 and 7 after infection. The results of these experiments indicate that JC-HEK-A DNA had the intact early region of JCV genome, whereas the JC-HEK-B DNA was defective in the early function.

DISCUSSION

The present study has demonstrated that DNA from JC-HEK P1-O4 contains two types of DNA, JC-HEK-A and JC-HEK-B, as the major species. DNAs from JC-HEK P1-O3, P1-O4, P1-O5, P1-O6, and P1-O8 (15) were digested with *Eco*RI and *Hin*dIII and subjected to electrophoresis in an agarose slab gel, together with similarly digested pJC522 and pJC507 DNAs (data not shown). The results showed that all the plaque-isolates examined appeared to generate fragments originating from JC-HEK-A and JC-HEK-B DNAs. It is likely, therefore, that the virus particles containing these two DNAs are common to these plaque isolates.

As shown in the physical maps of JC-HEK DNA (Fig. 5), both JC-HEK-A and JC-HEK-B DNAs have large deletions, each at a different region. It is unlikely that DNA molecules with such large deletions are viable. Structurally, the two DNAs are complementary. We suggest, therefore, that JC-HEK may grow in HEK cells by complementation between the two defective mutants. In our previous study (15), plaque formation by JC-HEK did not appear to follow two-hit kinetics as expected for complementation, and plaques developed at lower dilutions. We postulate that perhaps plaques were formed by the initial infection with aggregates of two kinds of virus particles. It was recently reported that another human polyoma virus, the RF strain of BK virus, grows by complementation between two defective viruses (18).

Little is known about the genetic map of JC virus. From base sequence homology, the JCV(Mad-1) DNA has been aligned to simian virus 40 DNA and BKV DNA with reference to the *Eco*RI cleavage site (13). The study suggests that the early region of JCV genome covers *Hin*dIII fragments C, B, and most of fragment A1 (Fig. 5). Since linear JC-HEK-A DNA could induce T-antigen production in HEK cells despite its large deletion of 0.3 m.u., it must have the intact early region. Results of the biological assay support the relationship between the physical and genetic maps of JCV as presented by Law et al. (13).

JC-HEK virus can grow efficiently in HEK cells, whereas the prototype Mad-1 cannot. Since JC-HEK is a host range mutant, its DNA must be different from JCV(Mad-1) DNA. The possible site of mutation responsible for the

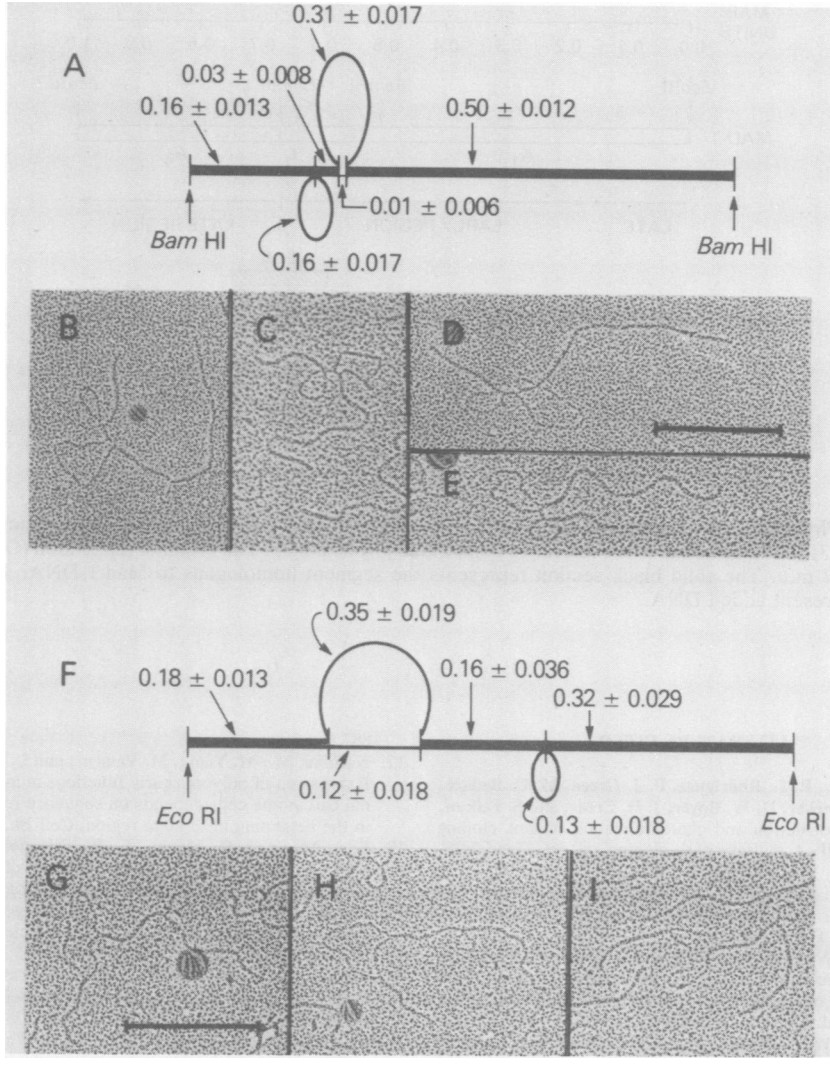

FIG. 4. Heteroduplex molecules between JCV(Mad-1) and JC-HEK DNAs. (A) Summary of measurements on 58 molecules consisting of *Bam*HI-linear JC-HEK-A (from pJC010) and *Bam*HI-linear JCV(Mad-1) DNAs. The thick line represents the double-stranded section, and the thin line represents the single-stranded section. Measurement shows that JC-HEK-A DNA is 14% shorter than JCV(Mad-1) DNA. (B, C, D, E) Electron micrograph of heteroduplex for (A). Bar, 0.5 μm. (F) Summary of measurements on 28 molecules consisting of *Eco*RI-linear JC-HEK-B (from pJC507) and *Eco*RI-linear Mad-1 DNAs. JC-HEK-B DNA was 9% shorter than JCV(Mad-1) DNA. (G, H, I) Heteroduplex for (F).

altered host range involves the region near the origin of DNA replication, as shown recently with the polyoma host range mutants (12, 21). JC-HEK-A and JC-HEK-B DNAs have inserted DNA sequences near the replication origin (Fig. 5). Apparently, the insert of 0.13 m.u. in JC-HEK-B DNA is the tandem repeat of the *Hin*dIII B to C region. Since JC-HEK-A DNA appears to be transcribed first in HEK cells to express the early genes, it should have the altered DNA segment associated with host range mutation. It is possible that the DNA insert of 0.16 m.u. near the origin of replication (Fig. 5) is responsible for the altered host range and that without a deletion in another region the DNA would be too large to be packaged into mature virions. Further characterization of this DNA insert and its adjacent region is now in progress.

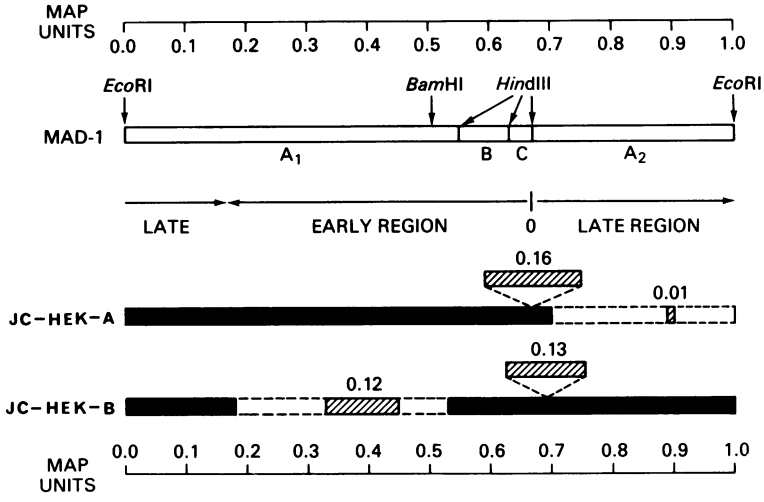

FIG. 5. Physical maps of cloned JC-HEK DNA. The alignment of the physical map to the genetic map is from Law et al. (13). The *Eco*RI site is the reference point in the physical map. The origin of replication (O) is believed to be at 0.67 m.u. The solid black section represents the segment homologous to Mad-1 DNA. The hatched sections represent added DNA.

LITERATURE CITED

1. **Bolivar, F., R. L. Rodriguez, P. J. Green, M. C. Betlach, H. L. Heyneker, H. W. Boyer, J. H. Crosa, and S. Falkow.** 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene **2**:95–113.
2. **Clewell, D. B.** 1972. Nature of Col E1 plasmid replication in *Escherichia coli* in the presence of chloramphenicol. J. Bacteriol. **110**:667–676.
3. **Cohen, S. N., A. C. Y. Chang, and L. Hsu.** 1972. Nonchromosomal antibiotic resistance in bacteria: genetic transformation of *Escherichia coli* by R-factor DNA. Proc. Natl. Acad. Sci. U.S.A. **69**:2110–2114.
4. **Davis, R. W., M. Simon, and N. Davidson.** 1971. Electron microscope heteroduplex methods for mapping regions of base sequence homology in nucleic acids. Methods Enzymol. **21**:413–428.
5. **Davis, R. W., M. Thomas, J. Cameron, T. P. St. John, S. Scherer, and R. A. Padgett.** 1980. Rapid DNA isolations for enzymatic and hybridization analysis. Methods Enzymol. **65**:404–411.
6. **Frisque, R. J., J. D. Martin, B. L. Padgett, and D. L. Walker.** 1979. Infectivity of the DNA from four isolates of JC virus. J. Virol. **32**:476–482.
7. **Grunstein, M., and D. S. Hogness.** 1975. Colony hybridization: a method for the isolation of cloned DNAs that contain a specific gene. Proc. Natl. Acad. Sci. U.S.A. **72**:3961–3965.
8. **Guerry, P., D. J. LeBlanc, and S. Falkow.** 1973. General method for the isolation of plasmid deoxyribonucleic acid. J. Bacteriol. **116**:1064–1066.
9. **Hirt, B.** 1967. Selective extraction of polyoma DNA from infected mouse cell cultures. J. Mol. Biol. **26**:365–369.
10. **Howley, P. M., F. Rentier-Delrue, C. A. Heilman, M.-F. Law, K. Chowdhury, M. A. Israel, and K. K. Takemoto.** 1980. Cloned human polyomavirus JC DNA can transform human amnion cells. J. Virol. **36**:878–882.
11. **Israel, M. A., H. W. Chan, W. P. Rowe, and M. A. Martin.** 1979. Molecular cloning of polyoma virus DNA in *Escherichia coli*: plasmid vector system. Science **203**:883–887.
12. **Katinka, M., M. Yaniv, M. Vasseur, and D. Blangy.** 1980. Expression of polyoma early functions in mouse embryonal carcinoma cells depends on sequence rearrangements in the beginning of the late region. Cell **20**:393–399.
13. **Law, M.-F., J. D. Martin, K. K. Takemoto, and P. M. Howley.** 1979. The colinear alignment of genomes of papovaviruses, JC, BK, and SV40. Virology **96**:576–587.
14. **Martin, J. D, R. J. Frisque, B. L. Padgett, and D. L. Walker.** 1979. Restriction endonuclease cleavage map of the DNA of JC virus. J. Virol. **29**:846–855.
15. **Miyamura, T., K. Yoshiike, and K. K. Takemoto.** 1980. Characterization of JC papovavirus adapted to growth in human embryonic kidney cells. J. Virol. **35**:498–504.
16. **Padgett, B. L., C. M. Rogers, and D. L. Walker.** 1977. JC virus, a human polyomavirus associated with progressive multifocal leukoencephalopathy: additional biological characteristics and antigenic relationships. Infect. Immun. **15**:656–662.
17. **Padgett, B. L., D. L. Walker, G. M. ZuRhein, R. J. Eckroade, and B. H. Dessel.** 1971. Cultivation of papova-like virus from human brain with progressive multifocal leukoencephalopathy. Lancet **i**:1257–1260.
18. **Pater, A., M. M. Pater, and G. di Mayorca.** 1980. Arrangement of the genome of the human papovavirus RF virus. J. Virol. **36**:480–487.
19. **Radloff, R., W. Bauer, and J. Vinograd.** 1967. A dye-buoyant-density method for the detection and isolation of closed circular duplex DNA: the closed circular DNA in HeLa cells. Proc. Natl. Acad. Sci. U.S.A. **57**:1514–1521.
20. **Rigby, R. W., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labeling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
21. **Sekikawa, K., and A. J. Levine.** 1981. Isolation and characterization of polyoma host range mutants that replicate in nullipotential embryonal carcinoma cells. Proc. Natl. Acad. Sci. U.S.A. **78**:1100–1104.
22. **Sutcliffe, J. G.** 1979. Complete nucleotide sequence of the *Escherichia coli* plasmid pBR322. Cold Spring Harbor Symp. Quant. Biol. **43**:77–90.

23. **Takemoto, K. K., P. M. Howley, and T. Miyamura.** 1979. JC human papovavirus replication in human amnion cells. J. Virol. **30**:384–389.
24. **Watanabe, S., K. Yoshiike, A. Nozawa, Y. Yuasa, and S. Uchida.** 1979. Viable deletion mutant of human papovavirus BK that induces insulinomas in hamsters. J. Virol. **32**:934–942.
25. **Yoshiike, K., and V. Defendi.** 1977. Addition of extra DNA sequences to simian virus 40 DNA in vivo. J. Virol. **23**:323–337.