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Identification of the Primate Papovavirus HD as the Stump-Tailed Macaque Virus

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The recently isolated primate papovavirus HD is shown to be indistinguishable from the stump-tailed macaque virus by immunofluorescent reactivity, by restriction endonuclease analysis, and by nucleic acid hybridization assay.

The recognized primate members of the papovavirus B genus (*Polyomavirus*) of the papovaviruses are simian virus 40 (SV40), simian agent 12 (SA12), stump-tailed macaque virus (STMV), human papovavirus BK (BK), and human papovavirus JC (JC). SV40 infects wild populations of several species of Asian macaques and was first discovered as an inapparent infectious agent of rhesus monkeys (16). Although SA12 was initially isolated from a vervet monkey kidney culture (6), serological evidence indicates that its natural host is the chacma baboon (17). Infection of stump-tailed macaques with STMV appears to be ubiquitous, and transmission of the virus seems to occur as a congenital infection (8, 14). Infection with the two known human papovaviruses appears to be limited to humans. In 1977 Waldeck and Sauer reported the isolation of a new primate papovavirus (HD virus) from Vero-76 cells, a continuous line derived from African green monkey kidney cells (18). Under stringent nucleic acid hybridization conditions they established that the HD viral genome shared no detectable homology with the genomes of SV40, BK, or the murine papovavirus polyoma. In this communication we report that HD and STMV are indistinguishable by immunofluorescent reactivity, by restriction endonuclease analysis, and by DNA-DNA hybridization assays. To eliminate the possibility of cross contamination, HD and STMV were each propagated and the respective DNAs were purified in separate laboratories. HD virus was purified from Vero-76 cells as previously described (18), and STMV was purified from infected primary rhesus kidney cell cultures (9).

The HD-infected Vero-76 cells were reacted in immunofluorescence tests with monospecific rabbit antisera to SV40, BK, JC, SA12, and STMV. Only the STMV antiserum was reactive, staining about 0.03% of the Vero cells, and the fluorescence was nuclear with sparing of nucleoli. The same small proportion of cells exhibited nuclear fluorescence when tested with a broadly cross-reactive serum, prepared against disrupted SV40 capsids, which contains antibodies to the antigenic determinant(s) common to all viruses of the polyomavirus subgroup (13). These data indicated that HD is an STMV-related papovavirus and that the viral antigen is expressed by a very small proportion of the Vero-76 cells.

To further assess the relationship between HD virus and STMV we examined the restriction enzyme cleavage patterns of the viral DNAs. Bosslet and Sauer reported that two size classes of circular DNAs could be purified from infected Vero-76 cells (1). The larger species of HD DNA, which is slightly smaller than the genome of SV40, is 6% larger than the smaller species of HD DNA. Digestion of HD DNA by endonuclease *Hae*II resulted in two size classes of full-length linear DNAs measuring 96 and 91% the size of full-length linear SV40 DNA (see Fig. 1A), indicating that each of the species of HD DNA contains one *Hae*II site. This enzyme also cleaves both STMV and SV40 DNAs once (Newell and Kelly, unpublished data; 12). The *Hae*II-cleaved linear STMV DNA comigrated with the: unit-length linear molecules of the smaller species of HD DNA (Fig. 1A). HD and STMV DNAs were then digested with either *Hpa*I or a mixture of *Hpa*I and *Hpa*II, and the resulting fragments were evaluated by electrophoresis in a 4% acrylamide gel (Fig. 1B). The restriction patterns for the two viral genomes were identical except that, in both digestions of HD DNA, an extra 1.7×10^5-dalton fragment

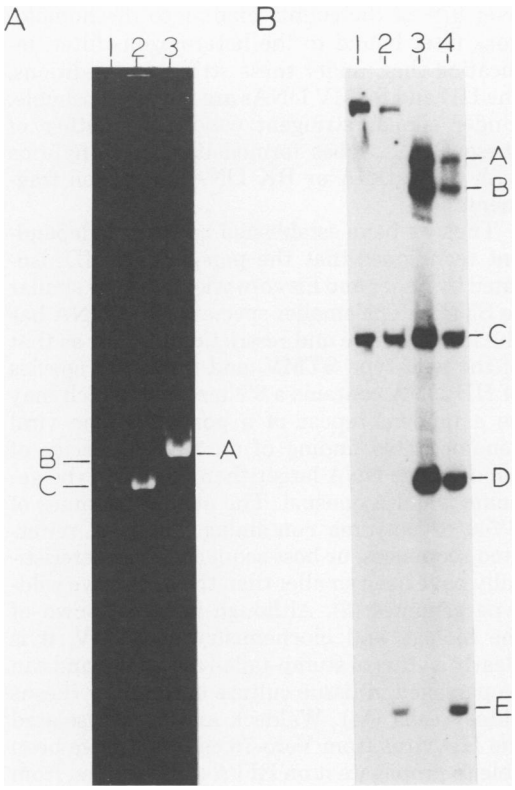

FIG. 1. *Restriction endonuclease digestions of HD and STMV DNAs. (A) HaeII digestion of HD DNA (1), STMV DNA (2), and SV40 DNA (3). Samples (50 μl) containing 0.1 to 0.3 μg of DNA were digested for 2 h at 37°C with HaeII in 0.006 M Tris-hydrochloride (pH 7.4), 0.006 M $MgCl_2$, and 0.006 M β-mercaptoethanol. The samples were analyzed by electrophoresis on a 1.4% agarose gel at 2 V/cm. The gel was stained with ethidium bromide (0.5 μg/ml) and visualized under shortwave UV light. HD DNA contained two size classes of DNA; the larger is 96% of SV40 length, and the smaller is 91% of SV40 length. STMV DNA is 91% of SV40 length. (B) HpaI digestion of STMV DNA (1) and HD DNA (2). Samples (25 μl) containing 10 to 30 ng of DNA were digested for 4 h at 37°C with HpaI in 0.01 M Tris-hydrochloride (pH 7.5), 0.01 M $MgCl_2$, 0.005 M β-mercaptoethanol, and 0.05 M NaCl. HpaI + HpaII digestion of STMV DNA (3) and HD DNA (4). Samples (25 μl) containing 10 to 30 ng of DNA were digested at 37°C with HpaII in 0.01 M Tris-hydrochloride (pH 7.5), 0.01 M $MgCl_2$, and 0.005 M β-mercaptoethanol. After 4 h, the reaction was made 0.05 M NaCl, HpaI was added, and the incubations were allowed to continue for another 4 h. The HpaI and the HpaI + HpaII digestions were made 20 mM Tris-hydrochloride, pH 7.8, and 50 mM NaCl and were incubated with 1/20 volume of a bacterial alkaline phosphatase (1 mg/ml in 0.5 M NH_4HCO_3, 25 mM $MgSO_4$, and 0.1 mM $ZnSO_4$ and heated to 65°C for 30 min) to remove the terminal phosphate groups (10, 19). The reactions were stopped after 30 min at 37°C by the addition of EDTA to a final concentration of 50 mM. They were incubated at room temperature for 10 min, extracted twice with buffer-saturated phenol, and dialyzed against 0.01 M Tris-hydrochloride (pH 8.6) and 0.001 M EDTA. The 5′ termini of the DNA were labeled by using T4 polynucleotide kinase in the presence of [γ-^{32}P]ATP (synthesized according to the Maxam and Gilbert [7] modification of the method of Glynn and Chappell [4]) by the method of Richardson (10) and Weiss et al. (19). The DNA was added to the[γ-^{32}P]ATP which had been dried under vacuum in a siliconized tube. The quantity of [γ-^{32}P]ATP was sufficient to make the final concentration at least 1 μM. The reaction was made 50 mM Tris-hydrochloride (pH 9.5)–10 mM $MgCl_2$–5 mM dithiothreitol, and spermidine was added to 1 mM (5). A 1- to 2-unit amount of T4 polynucleotide kinase per 100 μl was added, and the reaction was incubated at 37°C for 30 min. The reaction was dialyzed extensively against a solution of 10 mM Tris-hydrochloride (pH 7.5), 5 mM EDTA, and 1 M NaCl to remove the free ATP. The samples were analyzed by electrophoresis on a 4% acrylamide gel at 3.75 V/cm. The gel was dried and placed in close contact with a sheet of Kodak X-ray film (RP/S-54) for 12 h to 4 days. The masses (in daltons) of the four major fragments observed with HD and STMV DNAs are (A) 1.2, (B) 1.0, (C) 0.56, and (D) 0.35 × 10^6. Fragment E is seen in HD DNA, but not in STMV DNA. Its molecular weight is 0.17 × 10^6. These molecular-weight estimates are based on a genomic molecular weight of 3.45 × 10^6 for SV40.*

was present. Since the size of this extra fragment in the HD DNA digestions was exactly equal to the difference in mass between the two HD DNA species, it seems that the larger species contains a 1.7 × 10^5-dalton insertion bounded by *Hpa*I sites. These data are consistent with the finding of Bosselet and Sauer of a 6% DNA insertion bounded by *Hin*dIII sites in the larger species of HD DNA (1). One interpretation of these results is that the larger species of HD DNA contains an insertion of a tandem repeat of a portion of the viral genome. Cleavage of the larger species of HD DNA with a restriction enzyme recognizing a sequence present in this insertion would therefore result in the generation of an extra 1.7 × 10^5-dalton fragment. The smaller 6% fragment is generated only from the larger HD DNA species, thus the *Hpa*I and *Hpa*I plus *Hpa*II cleavage patterns of the smaller species of HD DNA and STMV are therefore identical.

To examine more directly the relationship of STMV and HD, the nucleic acid homology between their two genomes was evaluated. Both HD and STMV DNAs were labeled in vitro to a specific activity of 1.0 × 10^7 cpm per μg (11) and annealed separately to *Hpa*I-*Hpa*II restriction fragments of HD or STMV DNA immobilized on nitrocellulose filters (15). Under the

standard stringent conditions of DNA-DNA hybridization (T_m-25°C), each of the radiolabeled viral probes was equally effective in forming stable hybrids with either HD or STMV DNA fragments immobilized on the filters (Fig. 2). After autoradiography, the amount of radiolabel binding to the nitrocellulose filter strips was determined. For each of the viral DNA probes, over 97% of the counts binding to the homologous filter bound to the heterologous filter, indicating that, under these stringent conditions, the HD and STMV DNAs are indistinguishable. Under similar stringent conditions neither of these DNA probes formed detectable hybrids with SV40 DNA or BK DNA restriction fragments.

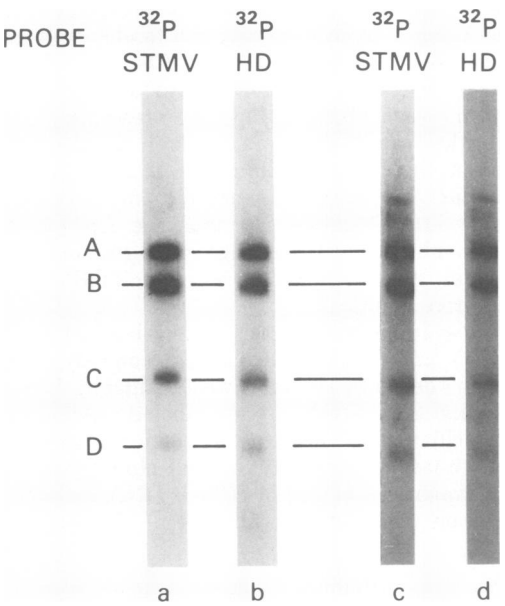

FIG. 2. *Nitrocellulose filter hybridization of HD and STMV DNAs following restriction endonuclease digestion and transfer from agarose gels. Viral DNAs were cleaved with HpaI and HpaII as described in the legend to Fig. 1. The DNA fragments were electrophoresed in 1.6% slab gels for 3 h at 120 V, denatured in situ, and transferred to a sheet of nitrocellulose (BA85, Schleicher and Schull) as described by Southern (15). The sheet was then incubated in 0.02% Ficoll, 0.02% bovine serum albumin, 0.02% polyvinyl pyrrolidine, and 4× SSC for 4 h at 60°C (2). Each 5-mm-wide strip of nitrocellulose paper contains approximately 0.01 μg of transferred HD or STMV DNA fragments. Hybridizations were carried out in reaction mixtures (1.0 ml) containing 1.0 M NaCl, 2 mM TES (pH 8.0), 0.5% SDS, 1 mM EDTA, 2 μg of yeast RNA per ml, 0.02% Ficoll, 0.02% bovine serum albumin, 0.02% polyvinyl pyrrolidine, 50% formamide, and 0.02 μg of denatured ^{32}P-labeled viral DNAs (specific activity approximately 10^7 cpm per μg) for 24 h at 34°C. The nitrocellulose strips were then extensively washed with 4× SSC at 68°C, dried, and subjected to autoradiography for 24 h using Kodak XR-2 X-ray film. The reactions shown in the left two columns are the unlabeled HD HpaI-HpaII DNA fragments hybridized to [^{32}P] STMV DNA (a) and [^{32}P] HD DNA (b). In the right two columns are the unlabeled STMV HpaI-HpaII DNA fragments hybridized to [^{32}P]-STMV DNA (c) and [^{32}P] HD DNA (d).*

Thus we have established by three independent techniques that the papovavirus HD, isolated by Sauer and his co-workers, is very similar to STMV. The smaller species of HD DNA has the identical size and restriction pattern as that of the wild-type STMV, and the larger species of HD DNA contains a 6% insertion which may be a tandem repeat of a portion of the viral genome. This finding of an altered species of papovavirus DNA larger than the wild-type genome is quite unusual. The altered genomes of SV40 or polyoma containing deletions, reiterated sequences, or host sequences characteristically have been smaller than the respective wild-type genomes (3). Although little is known of the biology and biochemistry of STMV, it is clearly a virus of stump-tailed macaques and can be passaged in tissue culture on primary rhesus kidney cells (14). Waldeck and Sauer isolated the HD virus from Vero-76 cells and have been able to propagate it on RITA cells, derived from the same species (*Cercopithecus aethiops*) (1). We have no explanation for the presence of this papovavirus in one line of Vero cells; Vero cells propagated in other laboratories are apparently free of this virus (1). We propose that in the future this isolate be referred to as the HD strain of STMV.

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