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# Isolation, characterization, and comparison of recombinant DNAs derived from genomes of human hepatitis B virus and woodchuck hepatitis virus

(virology/molecular cloning/restriction endonuclease/bacteriophage λ/plasmids)

IAN W. CUMMINGS*, JEFFERY K. BROWNE*, WINSTON A. SALSER*†, GAIL V. TYLER‡, ROBERT L. SNYDER‡, JO MARIE SMOLEC§, AND JESSE SUMMERS§

*Molecular Biology Institute and †Department of Biology, University of California, Los Angeles, California 90024; ‡Penrose Research Laboratory, Zoological Society of Philadelphia, and Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104; and §The Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111

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**ABSTRACT** **The human hepatitis B virus (HBV) and the woodchuck hepatitis virus (WHV) are closely related by several criteria and belong to the same class of DNA viruses. The DNA genomes from these viruses are difficult to obtain in quantities required for biochemical analysis. We have, therefore, cloned these two DNAs in the vector λgt*WES* and subcloned into the kanamycin resistance plasmid pA01. Comparison of the recombinant DNAs with authentic viral DNAs by specific hybridization, size, and restriction enzyme analysis suggests that the recombinants contain the complete genome of each virus. The nominal size of the cloned HBV genome was 3150 base pairs, compared to 3200 base pairs for the cloned WHV genome. The small amount of nucleic acid homology previously reported between the HBV and WHV DNAs could be demonstrated between the cloned viral DNAs.**

Recently Summers *et al.* (1) demonstrated that the human hepatitis B virus (HBV) and woodchuck hepatitis virus (WHV) share many important characteristics. In WHV and HBV infections, 25-nm spherical, 25-nm tubular, and 40- to 50-nm spherical particles are observed by electron microscopy. Circular partially double-stranded DNA molecules isolated from the 40- to 50-nm spherical particles of both viruses appear to be the templates for an endogenous DNA polymerase. In addition, WHV and HBV have common antigens associated with the surfaces and the cores of the virus particles as well as a common sequence of 100–150 nucleotides in their DNA genomes (2). The strong correlation between the properties of HBV and WHV suggests that they are members of a unique family of viruses.

HBV and WHV have not been grown in tissue culture. For that reason, the only source of viral DNAs has been infectious serum. The yield of viral DNA obtained from highly infectious serum is of the order of 1–10 ng per ml, however, and lack of a suitable source for large amounts of pure viral DNA has precluded using many modern techniques in the study of these viruses. In order to facilitate large-scale preparation of the viral DNAs, recombinant λ bacteriophages containing the HBV and WHV genomes were isolated. Both circular viral DNAs contain unique *Eco*RI restriction endonuclease sites that after cleavage provide cohesive termini for cloning. *Eco*RI linear HBV and WHV DNAs were inserted into the EK2 λ phage cloning vector λgt*WES*·λB. The viral recombinant DNAs were then compared to virion DNAs by restriction enzyme analysis. Nucleic acid homologies between the cloned HBV and WHV DNAs were detected by Southern gel hybridization (3). Cloning of the complete HBV DNA genome by using λgt*WES*·λB and subsequent studies using this clone have been reported (4–7).

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## MATERIALS AND METHODS

**Enzymes.** *Escherichia coli* DNA polymerase 1, phage T4 DNA polymerase, *Sac* I, *Hin*dIII, *Bam*HI, *Bgl* II, and *Ava* I were purchased from commercial sources. T4 ligase was a gift of Forrest Fuller (Harvard). *Eco*RI was a gift of Mike Komaromy (University of California, Los Angeles).

**DNAs.** λgt*WES*·λB was a gift of P. Leder (National Institutes of Health). pA01 was a gift of Anthony Ohtsuka (University of California, San Diego). Wild-type λ DNA and φX174 am3 replicative form DNA were purchased from commercial sources.

**Bacterial Strains.** *E. coli* HB101 was a gift of Herbert Boyer (University of California, San Francisco). DP50 SupF and LE392 were a gift of P. Leder.

**Viruses.** DNA polymerase-positive human serum containing HBV surface antigen ($HB_sAg$) was obtained from the American Red Cross (sample ARC 2375). DNA polymerase-positive woodchuck serum containing WHV surface antigen ($WH_sAg$) was obtained from an animal at the Penrose Research Laboratory (sample HW60). Virus particles were purified, and DNA was $^{32}P$-labeled by the endogenous DNA polymerase reaction and extracted as described (8).

***Eco*RI Cleavage of λgt*WES*·λB, pA01 Recombinant DNAs, WHV DNA, and HBV DNA.** All *Eco*RI cleavages were carried out in 0.1 M Tris·HCl, pH 7.4/15 mM $MgCl_2$/50 mM NaCl/0.02% Nonidet P-40. Reactions were terminated by phenol extraction followed by ethanol precipitations or by treating with diethylpyrocarbonate followed by heat treatment.

**Ligation of HBV and WHV *Eco*RI Linear DNAs to λgt*WES*.** *Eco*RI linear λgt*WES* DNA was purified away from the λB *Eco*RI fragment by preparative electrophoresis on Seaplaque agarose gels (Marine Colloids, Rockland, ME). Four hundred nanograms of λgt*WES* arm DNA was separately ligated to 30–40 ng of *Eco*RI-linearized HBV and WHV DNAs in 66 mM Tris·HCl, pH 7.4/10 mM $MgCl_2$/1 mM ATP/1 mM spermidine/15 mM dithiothreitol/200 μg of gelatin per ml with T4 ligase at 4–6°C for two days. A small aliquot of each ligation was electrophoresed on a 0.8% agarose gel to verify that ligation was complete.

***In Vitro* Packaging and Transfection of Recombinant DNAs into DP50 SupF.** The ligation products were packaged *in vitro* by the method of Blatner and coworkers (9) and transfected into LE392. Characterized recombinant DNAs were grown in large cultures after transfection into DP50 SupF.

Abbreviations: HBV, hepatitis B virus; WHV, woodchuck hepatitis virus; kb, kilobase.

**Large-Scale Growth of λ Recombinants.** Recombinants were grown in 1- to 10-liter cultures by the methods of Enquist *et al.* (10).

**Identification of λ-HBV Clone.** Isolated plaques were picked from the initial plating and individually eluted in 1 ml of 20 mM Tris·HCl, pH 7.4/10 mM $MgSO_4$/0.01% gelatin. The eluted phages were then mixed with several drops of saturated DP50 SupF culture and plated in soft Seaplaque top agarose medium (0.8% Seaplaque agarose/1% NaCl/1% tryptone). Each resulting confluent lysate was processed by scraping the top agar into 1 ml of Tris/$MgSO_4$/gelatin and pelleting the agarose and cell debris at 6000 rpm in a Sorvall SS-34 rotor at 4°C. The phage were isolated from the resulting supernatant by precipitation with polyethyleneglycol 6000 at 10% (vol/vol) in 0.5 M NaCl/0.01 M $MgSO_4$. The phage precipitate was collected by centrifugation at 4000 rpm in a Beckman TJ-6 centrifuge with a swinging-bucket rotor at 4°C. The pellet was resuspended in 10 mM Tris·HCl, pH 7.0/1 mM EDTA extracted with phenol, and precipitated with ethanol. The recombinant phage DNA was then cleaved by *Eco*RI and electrophoresed on a 0.8% agarose gel in 40 mM Tris·HCl/5 mM sodium acetate/1 mM EDTA, pH 7.6, at 7 V/cm for 2 hr. Positive clones were tentatively identified on the basis of detection of an insert fragment of appropriate size as measured by electrophoresis and comparison with size markers provided by an *Eco*RI digest of wild-type λ DNA. Further characterization was carried out as described below.

**Identification of λ-WHV Clone.** DNAs from confluent plate lysates were prepared from several plaques obtained in the initial plating. Ten nanograms of the *Eco*RI linear viral DNA was labeled by a random copolymer-primed *E. coli* DNA polymerase I reaction, using [$\alpha$-$^{32}$P]dATP as label (11). The labeled DNA was then hybridized by the method of Gillespie and Spiegelman (12) to a nitrocellulose grid onto which had been applied a positive control of WHV viral DNA and the λ recombinant DNAs isolated as described above. Positive clones were identified by autoradiography and confirmed by electrophoresis of an *Eco*RI digest of the clone on agarose gels.

**Preparative Isolation of HBV and WHV Viral DNAs from Recombinants.** *Eco*RI-cleaved recombinant DNA was electrophoresed on a preparative (20 × 20 × 0.2 cm) 0.8% Seaplaque agarose gel in 50 mM Tris/acetate pH 7.6 buffer. The insert band was stained with ethidium bromide and cut out of the gel for extraction. The gel slice was melted at 65°C for 15 min, made 0.1 M in NaCl, equilibrated to 37°C, extracted with phenol saturated with 10 mM Tris·HCl, pH 7.0/1 mM EDTA, and quickly spun at 4000 rpm in a Beckman TJ-6 centrifuge at room temperature to separate phases. The aqueous phase was removed, concentrated with *n*-butanol, and ethanol precipitated.

**Ligation of HBV and WHV DNAs to pA01 *Eco*RI Linear DNAs.** HBV insert DNA (isolated from λ-HBV) and WHV insert DNA (isolated from λ-WHV) were each ligated to *Eco*RI linear pA01 (kanamycin resistance plasmid). Ligations were performed as described above.

**Transformation of Plasmid DNAs into *E. coli* HB101.** *E. coli* HB101 was transformed by adding ligated plasmid-insert DNAs to cells prepared by calcium shock treatments according to Cohen and Chang (13). Transformed cells were added to 5 ml of soft top agarose and plated on agar plates with kanamycin at 20 μg/ml. Colonies were allowed to grow overnight at 37°C before picking.

**Colony Hybridization to Identify Recombinants.** Colonies obtained above were screened for HBV or WHV virus-specific inserts by the method of Grunstein and Hogness (14), using probes prepared by the randomly primed DNA polymerase I reaction with [$\alpha$-$^{32}$P]dATP as label (see above). To prevent nonspecific binding to the nitrocellulose filter, Denhardt's coating solution was employed (0.02% Ficoll/0.02% bovine serum albumin/0.02% polyvinylpyrrolidone) (15) as a precoating and during hybridization. Positive colonies were identified in the pA01-HBV and pA01-WHV screenings by autoradiography.

**Large-Scale Growth of Plasmid Recombinant DNAs.** Plasmid-carrying bacteria were grown, and then the DNA was amplified with chloramphenicol overnight. Plasmid DNA was purified by a detergent lysis–cleared lysate protocol (16). Plasmid DNA was separated from *E. coli* RNAs by agarose A-5m gel (Bio-Rad) exclusion in 0.1 M NaCl/10 mM Tris·HCl, pH 7.0/1 mM EDTA or by cesium chloride/ethidium bromide density gradient centrifugation (17).

**Physical and Biological Containment Levels.** HBV and WHV recombinant DNAs were manipulated under P3 + EK1 (pA01 vector in HB101) and P2 + EK2 containment (λgt*WES* vector in DP50 SupF) under an approved memorandum of understanding and agreement as outlined in the National Institutes of Health Recombinant DNA Research Guidelines, Part VII (see *Federal Register*, Dec. 22, 1978).

## RESULTS

Serum containing HBV was obtained from a Red Cross donor positive for the HBV surface antigen ($HB_sAg$), subtype ad/w, who also tested positive for hepatitis B "e" antigen ($HB_eAg$). Serum containing WHV was from a 7-month-old woodchuck trapped in a study area in central New Jersey. Approximately 15% of all animals trapped in this area were found to be positive for the WHV surface antigen ($WH_sAg$) (1, 2) and had DNA polymerase-containing particles. The DNA polymerase-containing particles were purified from both sera by cesium chloride gradient centrifugation and the DNA was labeled with [$\alpha$-$^{32}$P]dTTP in an endogenous DNA polymerase reaction (1). [$^{32}$P]DNA was extracted from both types of viral particles (1) and cleaved at a unique *Eco*RI site for cloning in the λgt*WES*·λB bacteriophage. Fig. 1 is a representation of the structure of the HBV and WHV genomes and shows the approximate position of the *Eco*RI site relative to the cohesive ends.

The *Eco*RI-cleaved DNAs were ligated with a preparation of λgt*WES* arms, and portions of the ligated samples were analyzed by electrophoresis through a 0.5% agarose gel and compared with viral DNAs before ligation. Both *Eco*RI-cleaved


FIG. 1. Structure of HBV and WHV DNAs. The arrowheads designate the 3′-OH end of the two strands of viral DNA. A large single-stranded region of variable length (indicated by the broken line) is found in the virion DNA, and is repaired by the endogenous DNA polymerase (8). The approximate position of the *Eco*RI site is shown. The numbers indicate the position of the *Eco*RI site measured in fractional genome length from either end of the complete strand (J. Summers and J. MacDowell, unpublished observations).

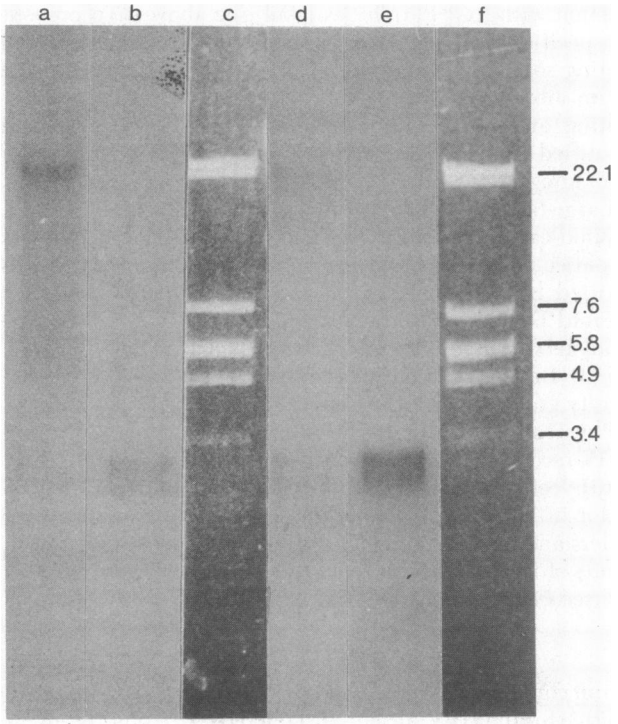


FIG. 2. Agarose gel electrophoresis autoradiograms of *Eco*RI-cleaved HBV and WHV [$^{32}$P]DNAs before and after ligation to λgt*WES* arms. Lane a, λgt*WES* arms ligated to WHV [$^{32}$P]DNA; lane b, WHV [$^{32}$P]DNA; lane d, λgt*WES* arms ligated to HBV [$^{32}$P]DNA; lane e, HBV [$^{32}$P]DNA; and lanes c and f, *Eco*RI-cleaved λ DNA. Sizes of λ *Eco*RI fragments in kilobases (kb) are marked adjacent to lane f. Lanes c and f were stained with ethidium bromide and photographed.

HBV and WHV DNAs migrated as heterogeneous components with maximum lengths of about 3100 and 3200 base pairs, respectively (Fig. 2, lanes b and e). Heterogeneity in the molecular weights of the labeled viral DNAs is consistent with the presence of a large and heterogeneous single-stranded region, as observed previously (1, 8). The maximum molecular weights are close to those reported earlier for fully double-stranded HBV and WHV DNAs (1). Both viral DNAs were efficiently ligated with the λ vector as indicated by the radioactivity migrating in the high molecular weight region of an agarose sizing gel (Fig. 2, lanes a and d).

The ligated λ-HBV and λ-WHV DNAs were then packaged *in vitro* and used to infect the bacterial host. Between 400 and 600 plaques were obtained from 50 ng of each recombinant DNA packaged. This efficiency was roughly 1/10th the expected value. (Such decreased efficiencies might arise because of the partially single-stranded DNA region within the viral DNAs).

Plate lysates were grown from several representative plaques of the λ-HBV and λ-WHV platings. A λ-HBV phage with an insert that was identical in size to the *Eco*RI linear HBV DNA was chosen for further characterization. A λ-WHV phage was identified by hybridization of nick-translated radioactive WHV DNA to plate lysate DNAs bound to nitrocellulose grids. A λ-WHV phage that hybridized strongly to probe and contained an *Eco*RI-excisable insert of the same size as *Eco*RI linear WHV DNA was chosen for further characterization.

λ-HBV and λ-WHV insert DNAs were purified after *Eco*RI cleavage on preparative Seaplaque agarose gels. The eluted DNA fragments were then ligated to *Eco*RI linear pA01 DNA. After transformation into HB101, 100 colonies were picked and put on nitrocellulose grids and replica plates. The colonies on nitrocellulose were prepared for colony hybridization by the method of Grunstein and Hogness (14). $^{32}$P-Labeled insert DNA probes with specific activities of $10^8$ cpm/μg were prepared by random priming (11). These probes were hybridized to the colony hybridization filters and roughly 30 positive colonies were detected in each cloning. One positive clone from pA01-HBV and one from pA01-WHV were chosen for further characterization.

The virus-sized inserts from the λ-HBV and λ-WHV clones were excised from the vector DNAs and compared with each other and standard molecular weight markers by electrophoresis through a 0.8% agarose gel (Fig. 3*A*). As expected, the HBV inserts derived from the λ and the plasmid vectors comigrated at a mobility corresponding to 3150 base pairs. Likewise, both WHV-derived inserts comigrated at a slightly lower mobility corresponding to 3200 base pairs. This electrophoretic behavior is consistent with an identity between the viral DNA segments carried in the plasmid vectors and those originally cloned into the λ vectors as well as with the sizes previously reported for authentic viral DNAs.

The DNA on the gel shown in Fig. 3*A* was denatured and transferred to a nitrocellulose filter sheet according to Southern (3). The DNA on half of the filter (lanes a–f) was hybridized with a DNA probe that was $^{32}$P-labeled in an endogenous DNA polymerase reaction of CsCl-purified WHV, whereas the DNA on the other half (lanes f–k) was hybridized with a [$^{32}$P]DNA HBV probe generated in the same way. After hybridization and washing, the filter was subjected to autoradiography (Fig. 3*B*). The λ-HBV and pA01-HBV inserts hybridized strongly to the

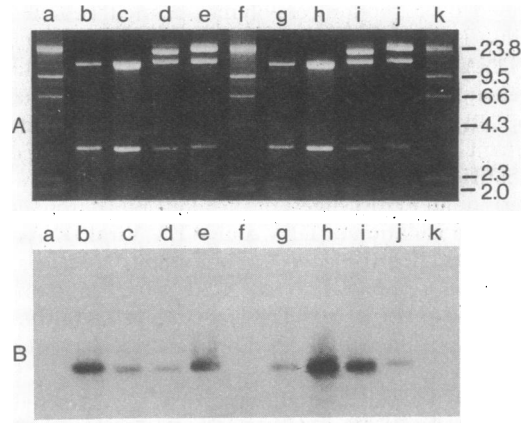


FIG. 3. Agarose gel electrophoresis and Southern blotting of cloned HBV and WHV DNAs. (*A*) Recombinant DNAs were digested with *Eco*RI and analyzed by electrophoresis through a 0.8% agarose gel. Samples were run in duplicate patterns for hybridization to WHV or HBV [$^{32}$P]DNA probes. Lanes a, f, and k, λ DNA partially digested with *Hin*dIII; lanes b and g, pA01-WHV; lanes c and h, pA01-HBV; lanes d and i, HBV; lanes e and j, λ-WHV. (*B*) The gel in lane a of Fig. 2 was soaked in 0.2 M NaOH for 30 min to denature the DNA, then neutralized, and the DNA was transferred to a nitrocellulose filter by the blotting method of Southern (3). The filter was then cut in half (through well f) and lanes a–e were hybridized with WHV [$^{32}$P]DNA while lanes g–k were hybridized with HBV [$^{32}$P]DNA. Both viral DNA probes were prepared from an endogenous reaction with cesium chloride-purified virus (1). The hybridization mixture contained 50% (wt/vol) formamide, 0.75 M NaCl, 0.075 M sodium citrate, 0.1% sodium dodecyl sulfate, 0.1% bovine serum albumin, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, and $2 \times 10^5$ cpm of $^{32}$P-labeled viral DNA. The hybridization was performed in an amount of hybridization mixture just sufficient to wet the filter. The wet filter was sealed in a plastic sandwich box and incubated at 43°C for 30 min. After extensive washing with 0.3 M NaCl/0.03 M sodium citrate plus 0.1% sodium dodecyl sulfate at 65°C, the filter was developed by autoradiography. Lanes a–k as in *A*. Sizes of λ *Hin*dIII fragments (in kb) are marked adjacent to lane k of gel *A*.

HBV probe (lanes h and i) and weakly to the WHV probe (c and d). Similarly, the λ-WHV and pA01-WHV inserts hybridized strongly to WHV probe (lanes b and e) and weakly to HBV probe (lanes g and j). Lack of hybridization in the marker wells indicates a high degree of specificity in the reaction and suggests that there was no leakage between the wells. This "cross-hybridization" is consistent with a small amount of nucleic acid homology (3–5% in liquid hybridization) previously observed between HBV and WHV DNAs (2).

λ-WHV and λ-HBV DNAs were cleaved with *Eco*RI and the inserts were separated on a preparative agarose gel. Purified inserts, extracted from the gel, were labeled by nick translation and used for further characterizations. Portions of HBV and WHV DNAs not used in the ligation reaction were converted to fully double-stranded DNAs by incubation with T4 DNA polymerase in the presence of $\alpha$-$^{32}$P-labeled nucleoside triphosphate. The resulting $^{32}$P-labeled viral DNAs and cloned inserts were compared for identity by restriction enzyme cleavage and gel electrophoresis.

Fig. 4 shows a comparison of the sizes of the linear molecules produced by cleavage of the WHV DNA with *Eco*RI (lane b) or *Sac* I (lane d) with the cloned λ-WHV insert (lane c). All three products appear to be close in size at about 3200 base pairs, in agreement with the size previously reported (1). The *Eco*RI- and the *Sac* I-digested virion DNA, however, migrated slightly faster on the gel than the insert. This result might be best explained by the continued presence of a small single-stranded region in the virion DNA that was not repaired by the T4 DNA polymerase. The WHV and the cloned λ-WHV DNAs were digested with *Eco*RI and *Sac* I to determine the relative positions of the cleavage sites in the two DNAs (Fig. 4, lanes f and e). Two fragments of 2170 and 1030 base pairs were produced by cleavage of the λ-WHV DNA. In the case of the virion DNA, although the 2170-base-pair fragment is readily visible, the 1030-base-pair fragment is seen only after long exposure. Because the WHV [$^{32}$P]DNA is labeled only in the region of the genome that is single stranded in the intact virion and is repaired by the T4 DNA polymerase (8), it is expected that each fragment will contain label not only in proportion to its length, but also according to its position on the genome. Thus, the 1030-base-pair fragment is probably derived from a part of the genome outside the repaired single-stranded region. The results are consistent with the cloned λ-WHV insert representing the major molecular species extracted from the purified WHV, and consisting of the complete WHV genome.

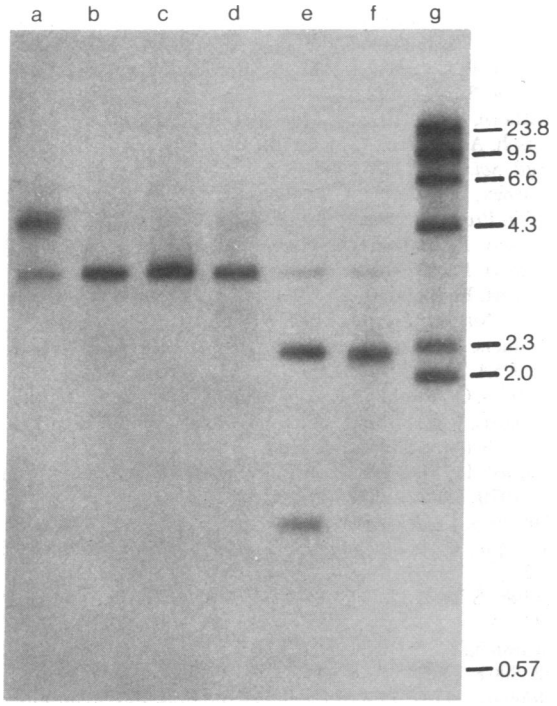


FIG. 4. Gel electrophoresis of WHV DNA and the cloned λ-WHV insert. WHV DNA used for cloning was made fully double-stranded before use by incubation in a reaction mixture containing 10 mM Tris·HCl at pH 8.0, 20 mM magnesium acetate, 10 μM each dATP, dGTP, and dCTP, 1 μM [$^{32}$P]dTTP (300 Ci/mmol; 1 Ci = $3.7 \times 10^{10}$ becquerels), 1 mM dithiothreitol, and 1 unit of T4 DNA polymerase (Worthington) in a final volume of 0.01 ml. After 4 hr at 37°C the reaction was terminated by the addition of 0.05 ml of 10 mM EDTA, pH 7.2/0.1% sodium dodecyl sulfate. Carrier ribosomal RNA (10 μg) was added and the nucleic acids were precipitated with 2 vol of ethanol. WHV insert was $^{32}$P-labeled by nick translation. WHV DNA (1 μg) was incubated in the same reaction mixture as above except with 4 units of *E. coli* DNA polymerase I (Worthington) instead of the T4 DNA polymerase. After 20 min at 22°C the reaction was terminated as above and the nucleic acids were precipitated with ethanol. WHV and λ-WHV [$^{32}$P]DNAs were dissolved in $H_2O$ and portions were digested with *Eco*RI or *Sac* I. The digested DNAs were analyzed by electrophoresis through a 1.5% agarose gel followed by autoradiography. Lane a, WHV DNA; lane b, WHV DNA + *Eco*RI; lane C, λ-WHV insert; lane d, WHV DNA + *Sac* I; lane e, λ-WHV insert + *Sac* I; lane f, WHV DNA + *Eco*RI + *Sac* I; lane g, λ DNA + *Hin*dIII. Sizes of λ *Hin*dIII fragments (in kb) are marked adjacent to lane g.

Linear molecules produced by *Eco*RI cleavage of the HBV DNA were compared with the purified cloned λ-HBV insert by agarose gel electrophoresis (Fig. 5, lanes b and c). Both molecules are close in size at about 3150 base pairs, but the *Eco*RI-digested HBV DNA migrated slightly faster than the λ-HBV insert. As in the case of the WHV DNA, this result might be explained by a small single-stranded region remaining in the HBV DNA.

The restriction fragments generated by double digestion of the HBV DNA with *Eco*RI + *Bam*HI, *Eco*RI + *Bgl* II, and

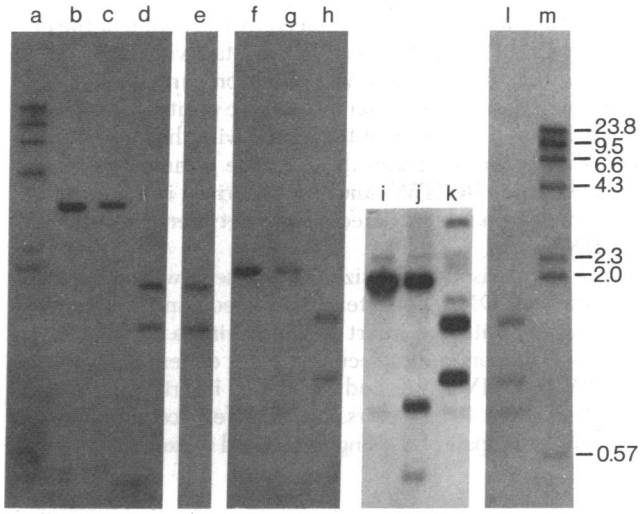


FIG. 5. Gel electrophoresis of HBV DNA and the cloned λ-HBV insert. HBV and λ-HBV insert DNAs were labeled with $^{32}$P as described for Fig. 4, digested with restriction enzymes, and analyzed by agarose gel electrophoresis. Lane a, λ DNA + *Hin*dIII; lane b, HBV DNA + *Eco*RI; lane c, λ HBV insert; lane d, HBV DNA + *Eco*RI + *Bam*HI; lane e, λ HBV DNA insert + *Bam*HI; lane f, HBV DNA + *Eco*RI + *Bgl* II; lane g, λ HBV insert + *Bgl*II; lane h, HBV DNA + *Eco*RI + *Ava* I. Lanes i, j, and k are longer exposures of lanes f, g, and h. Lane l, λ-HBV insert + *Ava* I; lane m, λ DNA + *Hin*dIII. The λ *Hin*dIII markers in lane a were run on the same gel as the samples in lanes b–d and f–h. The markers in lane m were on the same gel as the samples in lanes e and l. Sizes of λ *Hin*dIII fragments (in kb) are marked adjacent to lane m.

*Eco*RI + *Ava* I were compared with those from the corresponding digestions of the cloned λ-HBV insert. The two *Eco*RI + *Bam*HI fragments of 1770 and 1330 base pairs were easily visualized in the cleavage products of either DNA (Fig. 5, lanes d and e). *Eco*RI + *Bgl* II digestion of the λ-HBV insert produced fragments of 1950, 770, and 480 base pairs (lanes g and j). Only the 1950-base-pair fragment was easily visualized in the digests of the HBV DNA (lanes f and i), with the 770- and 480-base-pair fragments appearing only after long exposures of the autoradiogram (lane i). *Eco*RI + *Ava* I digestion of the λ-HBV insert produced fragments of 1480, 980, and 790 base pairs (lane l), whereas in the digestion of the HBV DNA only the 1480- and 980-base-pair fragments were readily visible (lane h). After longer exposures the 790-base-pair fragment could be visualized (lane k). This disproportionate distribution of label among the restriction fragments generated from HBV DNA has been repeatedly observed and is due to the nonrandom localization of the repaired single-stranded region. The close molecular sizes of the intact HBV DNA and the cloned λ-HBV insert, and the identical restriction patterns, are consistent with the cloned λ-HBV insert representing the major molecular species of DNA extracted from purified HBV and probably consisting of the entire HBV genome.

## DISCUSSION

We have attempted to produce recombinant DNAs that contain the entire DNA genome of HBV WHV. In evaluating the resulting clones, we have used three criteria: hybridization of the inserted DNAs to authentic [$^{32}$P]DNA produced in an endogenous DNA polymerase reaction of purified virus, comparison of the size of the inserts with that of authentic viral DNA, and comparison of restriction enzyme cleavage sites in the inserts with those in the authentic viral DNAs.

Specific hybridization of the insert DNA on Southern blots (Fig. 3*B*) indicates that the inserted DNAs are of viral origin. The $^{32}$P probes used for these hybridizations are highly virus-specific, because they were produced by the action of the endogenous viral DNA polymerase in the intact viral cores. Thus contamination of the virus or viral DNA preparation with host DNA would have no effect on the isotopic purity of the probe. In addition, hybridization of the inserts with the heterologous viral DNA probes indicates the presence of nucleic acid homology between the HBV- and WHV-derived inserts. Nucleic acid homology has been detected also between the authentic viral DNAs.

Close comparison of the size of the inserts with that of the authentic viral DNAs consistently revealed a small difference in migration, with the insert DNAs having a slightly lower mobility. The nominal molecular weight difference between the HBV or WHV DNAs and the cloned inserts as judged by mobility is about 40 base pairs. Such a difference might be due to incomplete repair of the single-stranded region, with a small remaining gap in the authentic viral DNAs. Alternatively, the authentic viral DNAs may contain structural features that affect its migration through the gel (8). A small duplication occurring during the cloning or growth in λ, might explain the difference in mobilities; however, such anomalies have not, to our knowledge, been reported to trouble this cloning vector system. Polyoma viral DNA cloned in λgt*WES* is a sufficiently faithful replica of the viral DNA to be fully infectious (18).

Finally, restriction enzyme analysis of the authentic viral DNAs suggests that the preparation of viral DNA used for cloning consisted of a single major molecular species, because the total complexity of the fragments was close to the molecular weight of the intact DNA. The cloned DNA inserts appear to be derived from the major molecular species in each case and probably represent the entire genome of each virus.

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