JOURNAL OF VIROLOGY, Sept. 1984, p. 595–603
0022-538X/84/090595-09$02.00/0

Vol. 51, No. 3

Herpes Simplex Virus Amplicon: Effect of Size on Replication of Constructed Defective Genomes Containing Eucaryotic DNA Sequences

ANN D. KWONG AND NIZA FRENKEL*

Department of Biology, The Marjorie B. Kovler Viral Oncology Laboratories, The University of Chicago, Chicago, Illinois 60637

Received 27 January 1984/Accepted 24 May 1984

Previous studies (R. R. Spaete and N. Frenkel, Cell 30:295–304, 1982) have documented the potential use of defective virus vectors (amplicons) derived from herpes simplex virus for the efficient introduction of foreign DNA sequences into eucaryotic cells. Specifically, cotransfection of cells with helper virus DNA and cloned amplicons (8 to 10 kilobases [kb]) containing bacterial plasmid DNA sequences linked to a set of herpes simplex virus *cis*-acting propagation signals (a replication origin and a cleavage-packaging signal) resulted in the generation of virus stocks containing packaged defective genomes that consisted of uniform head-to-tail reiterations of the chimeric seed amplicon sequences. The chimeric defective genomes could be stably propagated in virus stocks and could thus be used to efficiently infect cells. We now report on additional studies designed to propagate relatively large sets of eucaryotic DNA sequences within chimeric packaged defective genomes. These studies have utilized a 12-kb chicken DNA sequence encoding the chicken ovalbumin gene and cloned by Lai et al. (Proc. Natl. Acad. Sci. U.S.A. 77:244–248, 1980) in the plasmid pOV12. Virus stocks derived from cells cotransfected with helper virus DNA and chimeric amplicons (overall size of 19.8 kb, of which 12 kb corresponded to the chicken DNA) contained defective genomes composed of reiterations of the 19.8-kb seed amplicon sequences. However, in addition to the authentically sized repeat units, defective genomes in the derivative virus stocks contained smaller repeat units representing deleted versions of the seed 19.8-kb amplicons. The recombinational events leading to the formation of deleted repeats did not appear to occur at unique sites, as shown by comparative analyses of multiple, independently generated virus series propagated from separate transfections. In contast, seed amplicons ranging in size from 11 to 15 kb and containing subsets of the 12-kb chicken DNA sequences replicated efficiently and could be stably propagated in virus stocks. The results of these studies suggest the existence of size restrictions (up to 15 kb) on the efficient replication of seed herpes simplex virus amplicons.

We have previously reported (8, 31, 34) the initial characterization of cloning-amplifying defective virus vectors (amplicons) derived from herpes simplex virus (HSV). HSV amplicons can be constructed with any one of the three known replication origins of the HSV genome (1, 7, 17, 31–33). In addition, a functional HSV amplicon must contain a cleavage-packaging signal derived from the end of the S component or from the junction of the S and L components (reviewed in reference 27) of standard HSV DNA (35; R. R. Spaete and N. Frenkel, submitted for publication).

The use of HSV-derived vectors for the efficient introduction of foreign DNA sequences into eucaryotic cells was initially demonstrated with cloned amplicons (overall size 8 to 10 kilobases [kb]) containing the two HSV-derived *cis* replication functions (replication origin and cleavage-packaging signal) as well as bacterial plasmid DNA sequences (8, 31–33). Cotransfection of cells with helper virus DNA and such chimeric seed amplicons resulted in the generation of virus stocks containing defective virus genomes derived from the input chimeric amplicons in addition to the helper virus genomes. The packaged chimeric defective genomes were similar to the helper virus DNA in overall size (≈150 kb), but consisted of multiple reiterations of the seed amplicon sequences. Thus, the linkage of bacterial plasmid DNA sequences to the *cis*-acting HSV DNA replicaton functions resulted in their efficient incorporation within packaged defective genomes. We now report on additional studies designed to obtain the amplification of specific sets of eucaryotic DNA sequences within chimeric defective genomes. In these studies we have chosen to work with the chicken ovalbumin gene contained in the plasmid pOV12 (14) kindly provided for us by B. W. O'Malley (Baylor College of Medicine). The considerations guiding this choice were threefold. First, the structural features of the ovalbumin gene and its 5′ upstream sequences have been most thoroughly investigated (3, 6, 9, 10, 11, 13, 14, 23, 36). Second, the induction of ovalbumin gene expression in the chicken oviduct has been, in recent years, an exciting model system for study of developmental issues, including aspects of hormonal regulation of eucaryotic gene expression (2, 4, 15, 16, 20, 23, 26, 29). Finally, the pOV12 clone of O'Malley and co-workers contains a 12-kb chicken DNA insert that includes the entire ovalbumin gene (7.6 kb with 7 introns), 3.8 kb of upstream sequences, and 0.7 kb of downstream sequences (14, 36). When compared with other packaged defective virus vectors (e.g., from papovaviruses), the HSV amplicon appeared to be uniquely fitted for the amplification of this relatively large segment of eucaryotic DNA. The results of our attempts to determine the size limits for amplification of foreign DNA sequences within chimeric genomes are described below.

* Corresponding author.

MATERIALS AND METHODS

Cells and viruses. Human epidermoid-2 (HEp-2) cells and African Green Monkey kidney (Vero) cells were obtained from the American Type Culture Collection. Rabbit skin cells were obtained from B. Roizman. The HSV type 1 (HSV-1) strain KOS was obtained from P. A. Schaffer, and the HSV-1 strain Justin was obtained from A. Sabin. The vhs-1 virus is a host shutoff mutant derived from HSV-1 (KOS); its properties were previously described (24).

Preparation of plasmid DNA. Plasmids were propagated in the bacterial strain DH1 (obtained from D. Hanahan) or C600. Large- and small-scale preparations of plasmid DNA were made as previously described (31).

Types of amplicons and designations of clones and virus stocks. We have followed our previous scheme for designations of amplicons and derivative virus stocks (31). Specifically, to facilitate identification, amplicons have been designated by the first letter of the HSV strain used as the source for the replication origin, followed by a number designation for the type of origin. Of the three documented replication origins in HSV DNA (17, 22, 25, 31, 32–34; reviewed in reference 7), the two origins located within the right and left inverted repeat sequences of the S component have been designated as ori-1 and ori-1′, respectively, whereas ori-2 corresponds to the replication origin derived from the L component within map coordinates 0.418 to 0.423. By this scheme, pP2-103, pP2-501, etc., all correspond to cloned amplicons containing the ori-2 derived from the Patton strain of HSV-1. The clones pF1′-2, pF1′-501, etc., all designate amplicons containing ori-1′ derived from HSV-1 strain F.

Cotransfection-derived virus stocks are further designated by the first letter of the strain used as helper virus followed by the seed amplicon designation. For example, JP2-501 and KP2-501 describe virus stocks derived by cotransfection of cells with the pP2-501 seed amplicons and helper virus DNAs from the Justin and KOS strains of HSV-1, respectively.

The construction of the pP2-103 amplicon was previously described (31). pF1′-2 is an amplicon derived (L. P. Deiss and N. Frenkel, manuscript in preparation) with HSV-1 (F) segments originally cloned by Mocarski and Roizman (22). Specifically, the *Pvu*II segment from pRB373 (22) was introduced into the *Pvu*II site of pBR322 to generate the plasmid pF1′-2 shown in Fig. 1. The pP2-501 and pP2-502 amplicons were constructed by inserting the 12.0-kb partial *Hin*dIII chicken DNA fragment from pOV12 (obtained from B. W. O'Malley [14]) into the *Hin*dIII site of pP2-103. The 12.0-kb DNA fragment contained the entire genomic chicken ovalbumin gene with flanking sequences and yielded 3.2-, 4.0-, and 4.8-kb fragments when fully digested with *Hin*dIII. These *Hin*dIII fragments were subcloned into pP2-103 and pF1′-2 to yield the chimeric amplicons listed in Fig. 1.

Preparation and analyses of transfection derived viral stocks. Mixtures of plasmid DNA and HSV-1 helper virus DNA (KOS, Justin, or vhs-1) were used to transfect rabbit skin or Vero cell cultures (25-cm^2 dishes) by the calcium phosphate precipitation method (12, 28). The virus stocks derived from the cotransfections were serially passaged at 1:4 dilution in HEp-2 cells to generate subsequent passages.

Unlabeled DNA was prepared from Vero cells infected for 24 hours. ^{32}P-labeled DNA was prepared from infected Vero cells labeled with $^{32}P_i$ from 4 to 24 hours postinfection.

DNAs for restriction enzyme and blot hybridization analyses were prepared as previously described (31). Briefly, total infected cell DNA was digested with sodium dodecyl sulfate-proteinase K, extracted with phenol and chloroform-isoamyl alcohol, precipitated with isopropanol, and spooled on a glass rod. Helper virus DNA for use in transfections was prepared as previously described (18) by sodium dodecyl sulfate-proteinase K digestion followed by two rounds of equilibrium density centrifugations in CsCl.

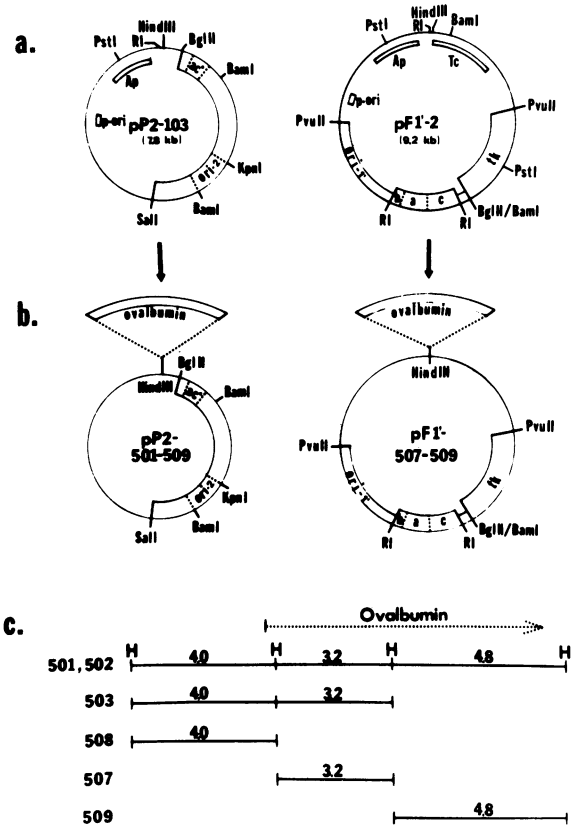

FIG. 1. Structure of the chimeric amplicons containing chicken DNA sequences (a). The amplicons pP2-103 (31) and pF1′-2 (L. P. Deiss and N. Frenkel, manuscript in preparation) were used as recipient vectors for the chicken ovalbumin DNA sequences. The pP2-103 amplicon contains the replication origin-2 of HSV-1 (Patton). *ac** denotes the location of the *a* sequence containing the cleavage-packaging signal and a small portion of the *c* inverted repeat sequence of S. The amplicon pF1′-2 contains the replication origin ori-1′ within the *Eco*RI to *Pvu*II segment shown—originally a segment of *Bam*I-N of HSV-1 (F) DNA (22). The cleavage-packaging signal is located within the *Eco*RI segment denoted as *bac* and constructed by Mocarski and Roizman (22) by the addition of *Eco*RI linkers to the *Hae*II S-L junction fragment of HSV-1 (F) DNA. The *Eco*RI segment was shown by Mocarski and Roizman (21) to contain 156 base pairs of *b*, 501 base pairs of *a*, and 618 base pairs of *c* sequences from HSV-1 (F) DNA. (b) Schematic representation of the amplicons pP2-501 through pP2-509 and pF1′-507 through pF1′-509 constructed to contain segments of the chicken ovalbumin DNA in the *Hin*dIII sites of pP2-103 and pF1′-2, respectively. (c) Chicken DNA segments that were inserted into the chimeric amplicons. The 12-kb chicken DNA segment, cloned by O'Malley and co-workers (14), consists of the three *Hin*dIII fragments as shown. The arrow denotes the domains of the gene (36). The amplicons pP2-501 through 509 and pF1′-507 through 509 contain the *Hin*dIII fragments shown. The clones pP2-501 and pP2-502 contain the entire 12-kb chicken DNA segment inserted in both possible orientations into the *Hin*dIII site of pP2-103.

Bacterial rescue of amplicons from infected cells. A Vero cell culture (25 cm^2) was infected with passage 3 (P3) of the KP2-501a series. Total infected cell DNA from this culture was prepared as described above, and 1/320 of it was

digested with *Sal*I. The digested DNA was circularized in a 300-μl ligation mixture, and one-third of the ligated DNA was used to transform DH1 bacteria. The plasmids from 12 of 135 resultant ampicillin-resistant colonies were analyzed by restriction enzyme analysis.

Southern blot hybridization. One-sixtieth of the DNA prepared from a 25-cm^2 infected Vero cell culture and approximately 0.01 μg of marker plasmid DNA were digested with restriction enzymes and applied to 0.65% agarose slab gels. The DNA was transferred to Gene Screen (New England Nuclear Corp.) or nitrocellulose (Schleicher & Schuell, Inc.) by the method of Southern (30). Radioactively labeled probes were prepared by nick translation of plasmid DNA with *Escherichia coli* DNA polymerase I. The 12-kb ovalbumin probe was prepared by digestion of pOV12 with the *Hin*dIII enzyme, elution of the resultant 3.2-, 4.0-, and 4.8-kb chicken DNA segments from a gel, and in vitro labeling of an equimolar mixture of these three fragments. This probe was slightly contaminated with pBR322 sequences as shown by the low level of hybridization to pP2-103 controls.

After DNA transfer and baking at 80°C for 4 h, the blots were preincubated at 68°C in 6× SSC (SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 1× Denhardt solution (5), and 0.2 mg of denatured salmon sperm DNA per ml. Hybridization was at 65°C for 24 h in 6× SSC, 1× Denhardt solution, 10% dextran sulfate, 30% formamide, 0.5% sodium dodecyl sulfate, 0.2 mg of carrier salmon sperm DNA per ml, and from 1 × 10^5 to 5 × 10^5 cpm of ^{32}P-labeled probe per ml. After hybridization, blots were incubated at 65°C in the hybridization buffer and at 70°C in 0.1× SSC–0.1% sodium dodecyl sulfate. The blots were then dried and exposed for autoradiography.

RESULTS

Amplification of a 19.8-kb amplicon containing the 12 kb-chicken DNA insert. The amplicon pP2-103 was derived as previously described (31) by cloning a 3.9-kb repeat unit from class II defective genomes present in a serially passaged virus stock propagated by B. Murray and co-workers from the Patton strain of HSV-1. This amplicon contains a replication origin (ori-2) and a functional cleavage-packaging signal within the locations shown in Fig. 1 (31; Spaete and Frenkel, submitted for publication).

To derive the amplicons pP2-501 and pP2-502 (Fig. 1), the 12-kb chicken DNA segment was excised from pOV12 (14) by partial *Hin*dIII digestion and was inserted (in both possible orientations) into the *Hin*dIII site of pP2-103. To test whether the chicken DNA sequences could be incorporated into defective HSV genomes, the pP2-501 and pP2-502 clones were each used to transfect a rabbit skin cell culture along with HSV-1 (KOS) helper virus DNA. Control transfections received the HSV-1 (KOS) helper virus DNA alone or a mixture of helper virus DNA and the pP2-103 amplicon. After serial propagation of resultant virus stocks, ^{32}P-labeled DNA from cells infected with P3 of each of the derivative series was analyzed by restriction enzymes. Figure 2 (lanes 1 through 4) shows representative digestion patterns of the ^{32}P-labeled DNAs with the *Bam*I enzyme. This enzyme releases a common 2.2-kb fragment from the pP2-103, pP2-501, and pP2-502 amplicons (a fragment that is equivalent to *Bam*I-V of the helper virus DNA—the segment containing the HSV replication origin, ori-2). The results of these analyses can be summarized as follows. (i) P3 of the control series, which was derived from a transfection with helper virus DNA alone (KOS transfection; Fig. 2, lane 1), did not

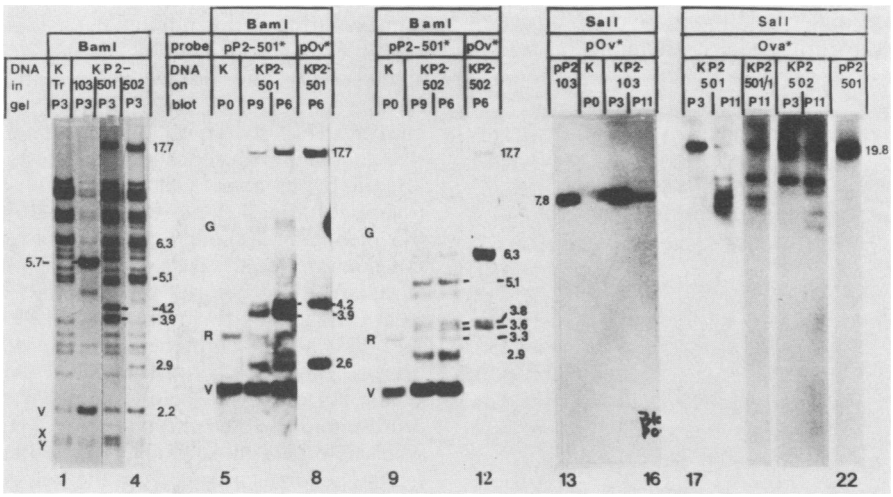

FIG. 2. Analyses of DNA from cells infected with transfection-derived virus stocks. Lanes 1 through 4 contain the *Bam*I digests of ^{32}P-labeled DNAs from cells infected with P3 of the K-Tr, KP2-103a, KP2-501a, and KP2-502a series. Serially passaged virus stocks were made by 1:4 propagation of virus derived from transfected rabbit skin cell cultures (in 25-cm^2 dishes) receiving 5 μg of helper HSV-1 (KOS) DNA alone (K-Tr) or 5 μg of helper KOS DNA and 1 μg of the plasmid amplicon DNAs (KP2-103a, KP2-501a, and KP2-502a). In lane 2, the 5.7- and 2.2-kb (*Bam*I-V) bands represent the *Bam*I fragments of authentically sized pP2-103-derived repeat units. In lanes 3 and 4, the 17.7- and 2.2-kb bands represent the *Bam*I fragments of authentically sized pP2-501- and pP2-502-derived repeat units. The 4.2- and 3.9-kb new bands (lane 3) and the 6.3-, 5.1-, and 2.9-kb bands (lane 4) represent major species of new types of repeat units. The *Bam*I fragments X and Y, which are amplified in P3 of KP2-501a (lane 3), are typical of class I endogenous defective genomes. Lanes 5 through 12 and 14 through 21 show Southern transfer blots containing the *Bam*I or *Sal*I digests of DNAs from cells infected with plaque purified HSV-1 (KOS) (K, PO), or infected with different passages (P3, P6, P9, and P11) of the series KP2-103a, KP2-501a, KP2-502a, and KP2-501/1. The series Kp2-501/1 (lane 19) was propagated starting from P2 of the KP2-501a. Lanes 13 and 22 contain the *Sal*II digests of the bacterially cloned amplicons pP2-103 and pP2-501 as size markers. The blots were hybridized with ^{32}P-labeled pP2-501 (pP2-501*), pOV12 (pOV*), or the probe enriched for the 12-kb chicken DNA insert in pOV12 (Ova*), prepared as described in the text.

contain significant amounts of defective genomes, although helper virus-derived defective genomes arose in later passages of the series (data not shown). (ii) The KP2-103a series, which was derived from a cotransfection with KOS helper virus DNA and the 7.82-kb pP2-103 amplicon, contained significant proportions of defective genomes of overall size indistinguishable from standard HSV DNA (as judged by migration of undigested DNA; data not shown). As shown in Fig. 2 (lane 2), these defective genomes consisted of repeat units closely resembling the input seed amplicons (generating 5.7- and 2.2-kb *Bam*I fragments). (iii) The KP2-501a and KP2-502a series, which were derived from transfections with the KOS helper virus DNA and the pP2-501 and pP2-502 amplicons, respectively, contained defective genomes consisting of repeat units similar to the input 19.8-kb seed amplicons (2.2 kb plus 17.7 kb in the *Bam*I digests; Fig. 2, lanes 3 and 4). However, the proportions of these defective genomes in the KP2-501a and KP2-502a (P3) virus stocks were relatively low. Furthermore, in addition to the "authentically replicated" chimeric genomes, the derivative virus stocks contained defective genomes with repeat units smaller than the 19.8-kb seed amplicons. As shown below, some of these species arose by deletion of the input pP2-501 and pP2-502 seed amplicons.

Further characterization of the generated chimeric defective genomes. Additional studies of the KP2-501a and KP2-502a series were designed to (i) further analyze the structure of derivative chimeric defective genomes and (ii) determine the stability of the generated genomes over additional virus passages. In the first set of experiments an attempt was made to shuttle the replicating defective genomes from the KP2-501a virus stocks back into bacteria. Because the seed pP2-501 amplicon contained a single *Sal*I site, unlabeled DNA from cells infected with P3 of the KP2-501a series was digested with the *Sal*I enzyme (to generate repeat unit monomers), self-ligated under conditions favoring circularization, and used to transform bacteria to ampicillin resistance. Eleven of 12 rescued bacterial plasmid clones that were anlyzed were found to be indistinguishable from the input pP2-501 amplicon (Fig. 3), whereas one of the clones (no. 6) had an altered restriction enzyme pattern. Thus, the 19.8-kb repeat units present within defective genomes in P3 of the KP2-501a series represented authentically replicated input seed amplicons. In contrast, the most abundant smaller repeat units within this virus series apparently lacked one or both functions (plasmid replication origin or the drug resistance gene) needed for propagation in bacteria.

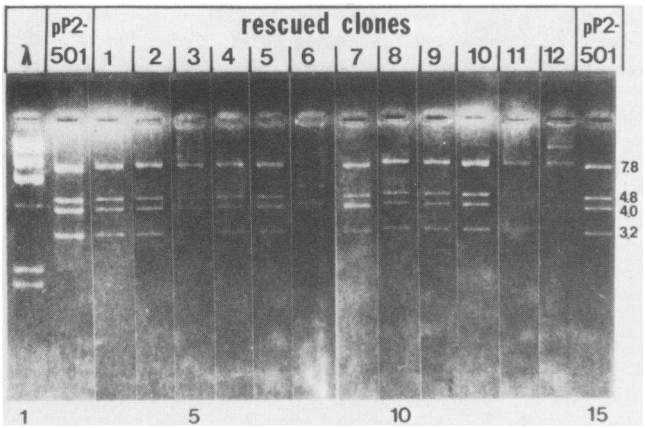

FIG. 3. *Hin*dIII digests of plasmids rescued from KP2-501a defective genomes. The gels were stained with ethidium bromide and photographed under UV light. Lanes: 1, *Hin*dIII-digested bacteriophage λ DNA serving as a size marker; 2 and 15, *Hin*d III pattern of the original pP2-501 seed amplicon; 3 through 14, clones from bacteria transformed with DNA from cells infected with P3 of the KP2-501a series. The infected cell DNA was digested with *Sal*I, and the resultant monomeric repeat units were circularized by ligation before the bacterial transformation. All clones but no. 6 (lane 8) show restriction patterns similar to that of the input pP2-501 seed amplicon. Clone no. 6 appears to contain a deletion within the pP2-103 portion of the pP2-501 chimeric amplicon.

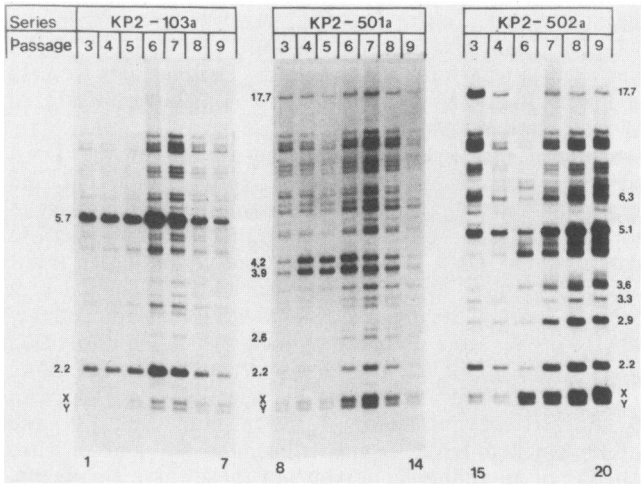

FIG. 4. Stability of defective virus genomes in the KP2-103a, KP2-501a, and KP2-502a series. *Bam*I digests of ^{32}P-labeled DNAs from cells infected with P3 through P9 of the series shown. Band notations are as in Fig. 2.

The second set of experiments was designed to monitor the stability and abundance of the chimeric defective genomes during serial undiluted passaging. Specifically, the KP2-103a, KP2-501a, and KP2-502a series were each propagated serially, and ^{32}P-labeled DNAs from cells infected with P3 through P9 of each series were analyzed by restriction enzymes (Fig. 4). These analyses have revealed that defective genomes with repeats closely resembling the seed 19.8-kb amplicons (and yielding the 17.7- and 2.2-kb *Bam*I fragments; Fig. 4, lanes 8 through 20) persisted throughout this passaging, although they did not reach high proportions in any of the serially passaged virus stocks. In addition to the authentically structured repeat units, the digests of DNAs from cells infected with P3 through P9 of the KP2-501a and KP2-502a stocks contained varied proportions of fragments arising from repeat units of sizes smaller than the input seeds. As discussed below, some of these new fragments represented deleted derivatives of the seed amplicons, whereas others most likely represented "endogenous" defective genomes arising from the helper virus (see legend to Fig. 2). In contrast to the apparent heterogeneity of the KP2-501a and KP2-502a virus populations, the KP2-103a series, which was derived from the transfection receiving the 7.82-kb pP2-103 amplicon (devoid of chicken DNA sequences), contained homogenously sized repeat units closely resembling the seed amplicons and only small proportions of "endogenous" defective genomes. Thus, as previously reported (31), defective genomes arising from pP2-103 reached relatively high proportions and could be stably propagated in serially passaged virus stocks.

Finally, several sets of hybridization studies were performed to further characterize the structure of defective genomes arising by replication of the seed amplicons. In the

first, ^{32}P-labeled pP2-501 and pOV12 probes were each hybridized to Southern-type blots containing the *Bam*I fragments of DNAs from cells infected with plaque-purified HSV-1 (KOS) or with P6 and P9 of the KP2-501a and KP2-502a series. Analyses of the resultant hybridization patterns (Fig. 2, lanes 5 thorugh 12) revealed the following. (i) The pP2-501 probe, but not the pOV12 probe, hybridized to several helper virus fragments (*Bam*I-G, -R, and -V) spanning the L component sequences that are included in the pP2-103 amplicon. (ii) As expected, the pP2-501 and pOV12 probes both hybridized to the 17.7-kb *Bam*I fragment arising from the authentically replicated pP2-501 and pP2-502 seeds, whereas only the pP2-501 probe hybridized to the 2.2-kb segment (*Bam*I-V) corresponding to the viral DNA sequences containing the replication origin of the pP2-501 and pP2-502 amplicons. However, the ratio of the 17.7- and 2.2-kb hybridization bands was not equimolar, indicating that the majority of defective genomes containing the *Bam*I-V fragment did not contain the "authentic" 17.7-kb chicken DNA segment. (iii) The *Bam*I-digested KP2-501a and KP2-502a DNAs contained new fragments that hybridized both labeled probes (e.g., the 4.2- and 2.6-kb fragments in the KP2-501a digest and the 6.3-, 3.8-, and 3.6-kb fragments in the KP2-502a digest). Therefore, these fragments arose by deletions of the input seed amplicons. In addition, the KP2-501a and KP2-502a DNAs contained fragments that hybridized solely with the pP2-501 probe (e.g., the 3.9-kb fragment of KP2-501a DNA and the 5.1-, 3.3-, and 2.9-kb fragments of KP2-502a DNA). These new fragments may represent portions of input amplicons that have undergone rearrangements to delete altogether the bacterial plasmid and chicken DNA portions. Alternatively, they may represent "endogenous" defective genomes that arose from the helper virus DNA.

A second set of hybridization experiments was designed to determine whether the rearrangements that were observed in the chimeric defective genomes took place at early or late stages of the serial undiluted propagation. Specifically, a blot containing *Sal*I-digested DNAs from cells infected with P3 and P11 of the KP2-103a series was hybridized with a ^{32}P-labeled pOV12 probe (Fig. 2, lanes 15 and 16). In addition, blots containing *Sal*I-digested P3 and P11 DNA of the KP2-501a and KP2-502a series were hybridized with a probe greatly enriched for the 12-kb chicken ovalbumin DNA portion of pOV12 prepared as described above (Fig. 2, lanes 17 through 21). Also included in this last hybridization (Fig. 2, lane 19) was the *Sal*I digest of DNA from passage 11 of KP2-501/1—a subseries derived in parallel to the KP2-501a series, starting at the common passage 2. Because the *Sal*I enzyme cleaves the seed pP2-103, pP2-501, and pP2-502 amplicons at a single location, we expected each of the hybridization bands in the *Sal*I blots to represent a different sized repeat unit. The results of these hybridizations further established the presence of new types of smaller repeat units that hybridized strongly with the chicken DNA probe. Therefore, these new chimeric defective genomes must have arisen by deletion of the input chimeric amplicons. Furthermore, the data also showed that significant generation of altered chimeric repeat units had occurred during the initial stages of the serial propagation, because the majority of the new repeat units (represented by the *Sal*I bands) in P11 DNA had their counterparts in P3 DNA and because the hybridization pattern of P11 KP2-501/1 resembled that of P11 KP2-501a DNA. During the serial passaging, however, the relative ratios of the different-sized repeat units fluctuated, with a generally notable increase in the proportion of defective genomes containing the smaller repeat units. A minority of smaller novel types of repeat units was detected in P11 DNA. These species could have arisen during the propagation of the series or could have been present in P3 DNA in amounts below the detection level of the hybridization shown in Fig. 2.

Due to the complexity of the DNA species present in the serially passaged virus stocks, a more thorough analysis of the detailed structure of the KP2-501a and KP2-502a defective genome species was not undertaken. However, the results of the hybridization studies discussed above and additional hybridization analyses with the *Hpa*I and *Kpn*I enzymes (data not shown), taken together with the failure to shuttle the most abundant altered repeats of pP2-501a (yielding the 4.2- and 3.9-kb *Bam*I fragments; Fig. 2 and 4) back into bacteria, suggested that the recombinational events leading to the generation of new types of repeat units could affect both the chicken and bacterial plasmid DNA portions of the input seed amplicons.

Effect of size on the replication of seed pP2- amplicons. The apparent instability of the 19.8-kb chimeric amplicons in the cotransfection-derived virus stocks could reflect the presence of specific chicken DNA sequences promoting this structural instability, e.g., due to the presence of (homologous or nonhomologous) sequences leading to efficient recombinations between the chicken, HSV, or bacterial plasmid DNA sequences. Alternatively, the observed heterogeneity of the progeny-defective genomes could reflect a more random pattern of deletions coupled with a relatively low replication efficiency of defective genomes containing large repeat units. Two sets of studies were done to test these hypotheses. In the first study, 16 separate cotransfections were performed in parallel under different ratios of helper virus DNA to seed amplicon DNA. In addition, three different types of helper virus DNA were used in these transfections—HSV-1 (KOS), HSV-1 (Justin), and the virion associated host shutoff (vhs) mutant derived from the KOS virus strain (24). *Hin*dIII-digested DNAs from cells infected with serially passaged virus of the resultant series were hybridized with ^{32}P-labeled pOV12 probe. The blot hybridization patterns (Fig. 5) have revealed the presence of the four *Hin*dIII fragments (7.8, 4.8, 4.0, and 3.2 kb) comprising together the pP2-501 seed in the majority of the transfection-derived virus stocks. However, comparison of the relative intensities of the four hybridization bands indicated that many of the series contained rearranged repeat units that included only portions of the input amplicon sequences. Furthermore, digestion of DNAs from different virus series yielded different sets of new bands, indicating the existance of multiple, nonunique, patterns in the rearrangements of the 19.8-kb seed amplicon sequences.

In a second set of studies, we derived the additional pP2 amplicons listed in Fig. 1 by cloning one or two of the three *Hin*dIII fragments of the 12-kb chicken DNA (from pOV12) into the *Hin*dIII site of pP2-103. The resultant amplicons, pP2-507 (11 kb), pP2-508 (11.8 kb), pP2-509 (12.6 kb), and pP2-503 (15 kb), as well as pP2-501 (19.8 kb), were each employed in cotransfection tests along with HSV-1 (KOS) or HSV-1 (Justin) helper virus DNAs. Restriction enzyme analyses of ^{32}P-labeled DNA From cells infected with P2 or P3 of the resultant series (see Fig. 6 for the Justin helper series) and blot hybridizations of unlabeled P3 DNA with ^{32}P-labeled pOV12 probe (see Fig. 7 for the KOS helper series) revealed the following. (i) Transfections with amplicons ranging in size from 11 to 15 kb (pP2-507, pP2-508, pP2-509, and pP2-503) yielded, in most cases, homogeneous

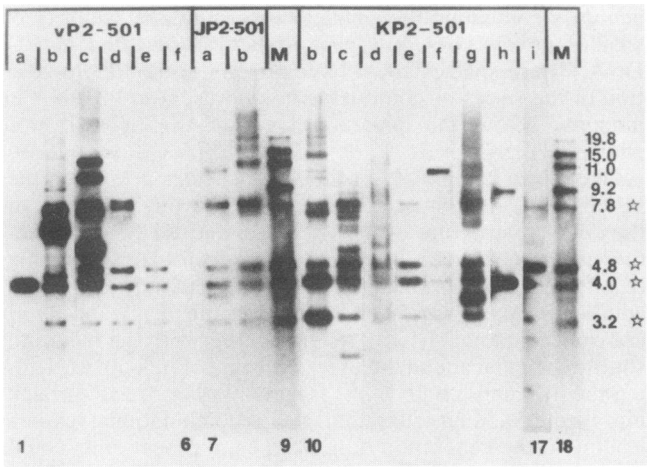

FIG. 5. Defective genomes arising from the pP2-501 seed amplicon. Blots containing *Hin*dIII digests of DNAs from cells infected with P2 of the 16 series shown were hybridized with a ^{32}P-labeled pOV12 probe. Lanes 9 and 18 contained a mixture of molecular weight markers (M) consisting of *Sal*I-digested pP2-501 (19.8 kb), *Sal*I-digested pP2-503 (15 kb), *Sal*I-digested pP2-507 (11 kb). *Sal*I-digested pF1′-2 (9.2 kb), and *Hin*dIII-digested pP2-501 (7.8, 4.8, 4.0, and 3.2 kb). The quantities of input DNAs in the transfections were as follows: vP2-501a and vP2-501b, 2 μg of vhs-1 helper and 5 μg of plasmid DNA; vP2-501c and vP2-501d, 2 μg of helper and 2 μg of plasmid DNA; vP2-501e, 0.5 μg of helper and 0.5 μg of plasmid DNA; vP2-501f, 0.5 μg of helper and 1 μg of plasmid DNA; JP2-501a and JP2-501b, 5 μg of Justin helper and 1 μg of plasmid DNA; KP2-501b, KP2-501e, and KP2-501f, 2.4 μg of KOS helper and 2 μg of plasmid DNA; KP2-501c and KP2-501d, 2.4 μg of helper and 5 μg of plasmid DNA; KP2-501g and KP2-501i, 0.6 μg of helper and 0.5 μg of plasmid DNA; KP2-501h, 2.4 μg of helper and 1 μg of plasmid DNA. The hybridization bands with stars represent the *Hin*dIII fragments of sizes similar to those of the input pP2-501 seed amplicon.

defective genome populations with repeat units similar to the input amplicons. A comparison of the relative ratios of the amplicon-derived defective virus DNA to helper virus DNA (at P3) revealed that defective genomes derived from the larger repeat units did not reach as high proportions as those derived from smaller seed amplicons. (ii) In contrast to the relative homogeneity of defective genomes derived from the smaller amplicons, the JP2-501 and KP2-501 virus stocks contained significant proportions of defective genomes with deleted chimeric repeat units in addition to the 19.8-kb authentically sized repeats.

Because the *Hin*dIII fragments in the smaller amplicons account for the entire 12-kb chicken DNA insert in pP2-501, it appears that the presence of specific chicken DNA sequences in seed amplicons cannot by itself account for the observed rearrangements in the 19.8-kb pP2-501-derived defective genomes. These results are in agreement with the observations described above concerning the nonunique patterns of sequence rearrangements in the 16 independently derived pP2-501 series. We conclude on the basis of these studies that the increase in size of the input seed repeat from 15 to 19 kb is the major factor in the accumulation of smaller rearranged units of the chimeric defective genomes.

Effect of size on the replication of seed pF1′ amplicons. The studies described thus far have all utilized chimeric derivatives of pP2-103—a class I amplicon containing the ori-2 replication origin of HSV-1 DNA. To test whether repeat units of sizes up to 14 kb could be efficiently propagated with the replication origin of class II seed amplicons, we introduced the 3.2-, 4.0-, and 4.8-kb chicken DNA inserts into pF1′-2. This 9.2-kb amplicon (L. P. Deiss and N. Frenkel, manuscript in preparation) contained the replication origin ori-1′, derived from the left inverted repeat of the S component, and the cleavage-packaging signal derived from the S-L junction of standard HSV (F) DNA (Fig. 1). The derivative chimeric amplicons pF1′-507 (12.4 kb), pF1′-508 (13.2 kb), and pF1′-509 (14 kb), as well as the original pF1′-2 amplicon, were each used in cotransfection experiments with HSV-1 (Justin) helper virus DNA. Restriction enzyme analyses of ^{32}P-labeled DNAs from cells infected with P3 of the resultant series (Fig. 8) and blot hybridization analyses with pOV12 as the hybridization probe (data not shown) revealed a similar pattern to that described above for the class II amplicons. Thus, each of the pF1′ seed amplicons described above was successfully propagated in the cotransfection-derived virus stocks.

DISCUSSION

We have described in this paper our studies of HSV amplicons containing different-sized subsets of a 12-kb chicken DNA sequence encoding the ovalbumin gene. These studies have shown that although the 19.8-kb chimeric amplicon pP2-501 replicated in transfection-derived virus stocks, it was not homogeneously propagated, since the derivative virus stocks also contained significant proportions of defective genomes with deleted repeat units. In contrast, the cotransfection of cells with helper virus DNA and seed amplicons ranging in size from 8 to 15 kb yielded virus stocks containing relatively high proportions of chimeric defective genomes with repeat units closely resembling the input seeds. In this respect, it is noteworthy that we have recently constructed amplicons less than 5 kb in size that contain the necessary functions for dual propagation in bacteria and in HSV stocks. These amplicons should be useful for the efficient and homogeneous propagation of foreign DNA sequences up to 10 kb in size. Thus, cotransfection of cells with helper virus DNA and such 15-kb chimeric seed amplicons should result in the formation of relatively homogeneous populations of defective virus DNA molecules, each containing 10 tandem reiterations of the chimeric seed repeats. Furthermore, because the chimeric defective virus genomes are packaged in virions, the transfection-derived virus stocks could be used to synchronously and efficiently introduce the foreign DNA sequences into a large variety of host cell species that are known to be susceptible to HSV infection.

Although the molecular events leading to the formation of deletions within the seed repeat units are not understood, there are several points that can be made concerning the observed sequence rearrangements. First, the data presented above concerning the structural complexity of deleted-rearranged defective genomes in different stocks derived from pP2-501 and pP2-502 transfections, as well as the results of our experiments with different-sized seed amplicons, strongly suggest that the major determinant in the frequency of observed deletion rearrangements is the overall size of the input amplicon, rather than the nature of the cloned foreign DNA sequences. Although we have only described our studies concerning the chicken ovalbumin DNA sequences in this paper, we have observed similar patterns of deletion rearrangements in virus stocks derived from cotransfections with amplicons exceeding 15 kb in overall size and containing human DNA or additional sets of HSV DNA sequences (L. P. Deiss, R. R. Spaete, R. Danovich, and N. Frenkel, unpublished results). It should be noted however, that in the absence of data regarding the fine structure of the deleted repeat units, we cannot exclude the

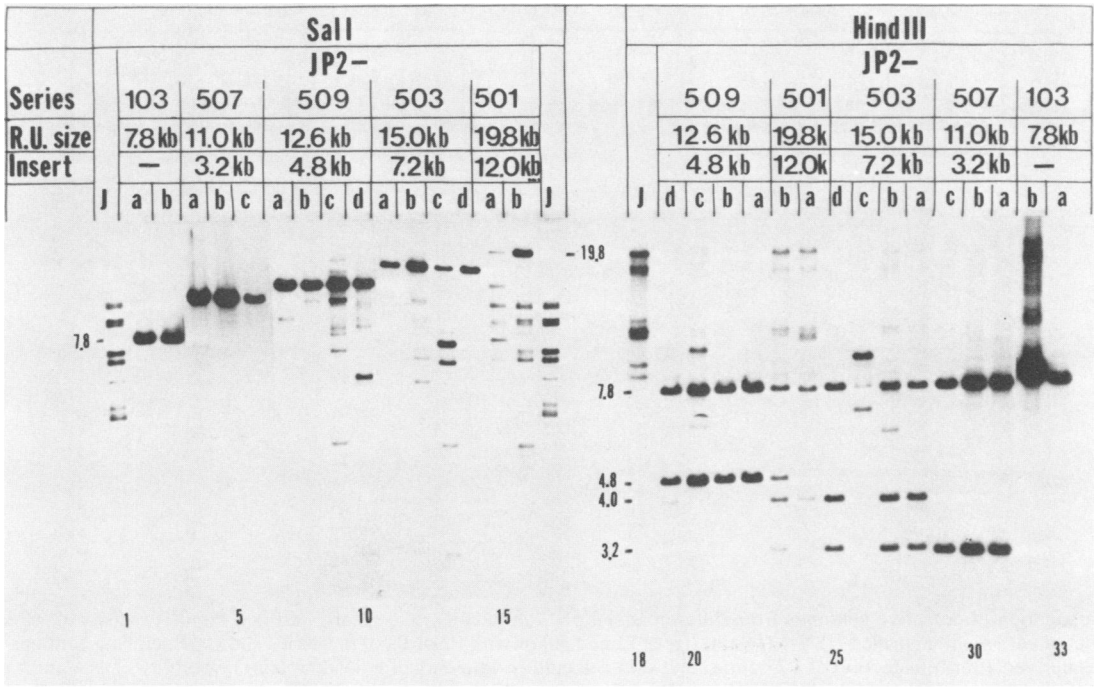

FIG. 6. Propagation of defective genomes from different-sized pP2 amplicons. Shown are *Sal*I and *Hin*dIII digests of ^{32}P-labeled DNA from cells infected with P2 or P3 of the series shown. Each lane contains DNA from a separate series derived after transfection of a 25-cm^2 rabbit skin cell cutlure with 5 μg of HSV-1 (Justin) helper virus DNA and 1 μg of the test amplicon. Lanes 1, 17, and 18 contain ^{32}P-labeled DNA from cells infected with plaque-purified HSV-1 (Justin). The *Sal*I enzyme cleaves at a single site within each of the input seed amplicons. Cleavage of the amplicons with the *Hin*dIII enzyme yields the 7.8-kb pP2-103 portion and the additional 3.2-kb (in pP2-507), 4.8-kb (in pP2-509), 3.2- plus 4.0-kb (in pP2-503), and 3.2- plus 4.0- plus 4.8-kb (in pP2-501) chicken DNA fragments.

hypothesis that recombinational events leading to the observed rearrangements had occurred within short stretches of homologous sequences distributed in multiple locations within the seed amplicons.

Second, because our analyses of the serially passaged virus stocks only allowed detection of DNA species that underwent amplification, it is not clear whether genomic rearrangements also occurred in the virus stocks generated with the smaller-sized amplicons. In fact, it is reasonable to suggest that recombinational events leading to deletions

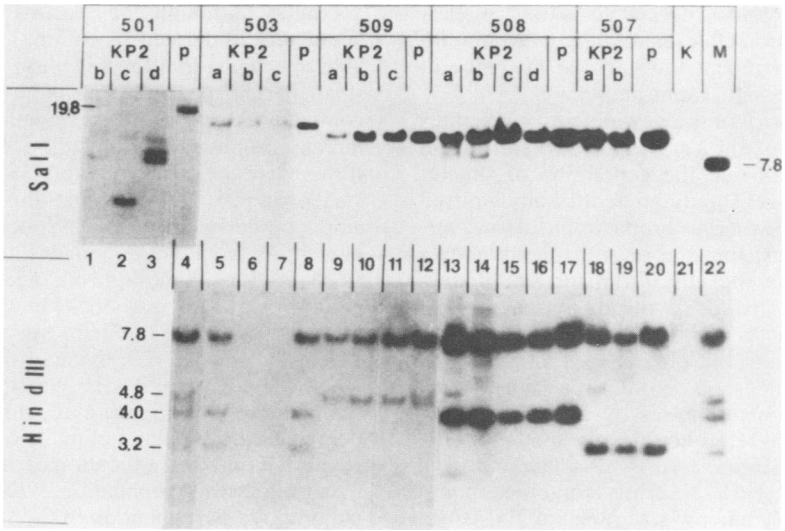

FIG. 7. Effect of amplicon size on the propagation of derivative-defective genomes. Southern-type blots containing *Sal*I- and *Hin*dIII-digested DNA from cells infected with plaque-purified HSV-1 (KOS) (lane 21) or P3 of the series shown were hybridized with ^{32}P-labeled pOV12 probe. The *Sal*I- and *Hin*dIII-digested amplicons (p) serving as seeds in the transfections were included in adjacent lanes (lanes 4, 8, 12, 17, and 20) as size markers. Also included were the markers pP2-103 cleaved with *Sal*I (lane 22, top) and the pP2-501 cleaved with *Hin*dIII (lane 22, bottom). Each of the series shown was derived from a cotransfection of a 25-cm^2 culture of rabbit skin cells with 5 μg of HSV-1 (KOS) DNA and 1 μg of the seed amplicon.

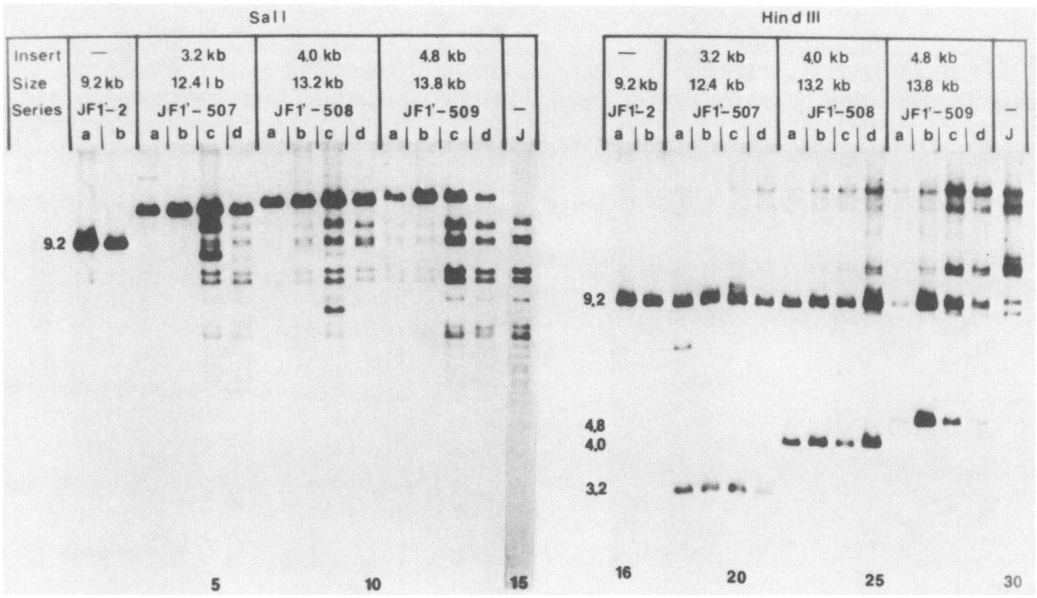

FIG. 8. Propagation of defective genomes from different sized pF1′ amplicons. Shown are *Sal*I and *Hin*dIII digests of ^{32}P-labeled DNA from cells infected with plaque purified HSV-1 (Justin) (lanes 15 and 30) or with P2 or P3 of the series shown. Each lane contains DNA from a separate series derived after transfection of a 25-cm^2 rabbit skin cell culture with 5 μg of HSV-1 (Justin) helper virus DNA and 1 μg of the test amplicon. The *Sal*I enzyme cleaves each of the JF1′ amplicons at a single site. Cleavage of the amplicons with *Hin*dIII yields the 9.2-kb pF1′-2 amplicon portion and the additional 3.2-kb (in pF1′-507), 4.0-kb (in pF1′-508), and 4.8-kb (in pF1′-509) chicken DNA fragments.

occurred in each of the virus series that we studied. However, the altered amplicon repeat unit(s) derived from these recombinational events might have been out-competed by seed amplicons of the appropriate size for efficient replication. In support of this selection model are two obsrvations: (i) the serial propagation of the KP2-501 and KP2-502 series resulted in a progressive increase in the proportions of defective genomes with smaller repeat units; and (ii) our observation of the significant presence of endogenous defective genomes (i.e., arising from helper virus DNA) in stocks derived from transfections with the large pP2-501 and pP2-502 seeds, but not in transfections receiving the smaller, efficiently replicating amplicons. These observations suggest the existence of a competition effect that inhibits the amplification of newly generated repeat units in the presence of higher proportions of successful input amplicons.

Third, because the majority of the new defective genomes species were already present in P2 or P3 of the KP2-501a and KP2-502a series, it appears that the generation of smaller repeat units from these larger input amplicons had occurred relatively early during the serial propagation. However, there are reasons to suggest that the rearrangement events are not merely artifacts of the DNA transfection process itself, but might rather (or also) occur during normal replication of the defective virus genomes. Of relevance to this hypothesis are previous structural analyses of naturally occurring defective genomes in serially passaged virus stocks. Specifically, previous analyses by us and by other investigators (reviewed in reference 7) revealed that the majority, if not all, of the defective virus series derived from a number of HSV-1 and HSV-2 strains contained major repeat units in the relatively narrow size range of 7 to 10 kb. This observation was all the more striking in a series that was derived from HSV-2 (G) (7) and in several series that were derived from HSV-1 (Justin) (U. Gompels and N. Frenkel, manuscript in preparation). Each of these series contained a heterogenous assembly of class I defective genomes with repeat units ranging in size from 7 to 25 kb. Nonetheless, in each of these virus populations, the major repeat units were in the narrow size range of 7 to 10 kb. Furthermore, in the case of the HSV-2 (G) series (7) and a series (19) derived from the *ts*LB2 mutant of HSV-1 (HFEM) there is evidence that suggests that smaller repeat units have evolved from their larger counterparts. Thus, several different-sized repeat units in these virus populations contained an identical small deletion within a defined area of the repeat unit. As is the case with the chimeric amplicons that we studied, the various-sized repeat units were present in early passages of the series and persisted at variable relative ratios through prolonged serial progagation.

Finally, although the reasons underlying the increased abundance of deleted repeat units are unknown, it is reasonable to suggest that the selection of defective genomes with smaller repeats does not merely reflect their ability to compete better for limited replication machinery due to the increased number of replication origins per DNA molecule. If this were the case, we would have expected progressively smaller repeat units arising during serial propagation of amplicon-derived or naturally occurring defective viral genomes. As already mentioned, this prediction is not supported by analyses of multiple passages of a number of "natural" serially passaged virus stocks in which repeat units 7 to 10 kb in size are the predominant species for more than 35 passages. Taken together, these observations suggest the existence of a structural size constraint in the replication of "constructed" or naturally occurring defective genome repeat units. On the basis of many observations (7), it has been suggested that the replication of defective genomes proceeds by a rolling circle replication, which is also thought to be the major mode of replication of the standard virus genome (27). If so, it remains to be seen whether the proposed advantageous size of the common defective genome repeat units compared with their 150-kb standard virus counterparts plays a role in the rapid selection of defective HSV genomes to become the predominant species in relatively early passages of serially propagated virus populations.

ACKNOWLEDGMENTS

These studies were supported by Public Health Service research grants AI-15488 and CA-19264 from the National Cancer Institute and by National Science Foundation grant PCM 8118303. A.D.K. is a predoctoral trainee supported by Public Health Service training grant AI 07182 from the National Institutes of Health.

We thank B. W. O'Malley for the gift of the pOV12 clone and Malera Traylor for her skillful technical help.

LITERATURE CITED

1. **Barnett, J. W., D. A. Eppstein, and H. W. Chan.** 1983. Class I defective herpes simplex virus DNA as a molecular cloning vehicle in eurcaryotic cells. J. Virol. **48**:384–395.
2. **Bellard, M., M. T. Kuo, G. Dretzen, and P. Chambon.** 1980. Differential nuclease sensitivity of the ovalbumin and B-globin chromatin regions in erythrocytes and oviduct cells of laying hens. Nucleic Acids Res. **8**:2737–2750.
3. **Benoist, C., R. Breathnach, and P. Chambon.** 1980. The ovalbumin gene-sequence of putative control regions. Nucleic Acids Res. **8**:127–141.
4. **Colbert, D. A., B. J. Knoll, S. L. C. Woo, M. L. Mace, M. J. Tsai, and B. W. O'Malley.** 1980. Differential hormonal responsiveness of the ovalbumin gene and its pseudogenes in the chick oviduct. Biochemistry **19**:5586–5592.
5. **Denhardt, D. T.** 1966. A membrane filter technique for the detection of complementary DNA. Biochem. Biophys. Res. Commun. **23**:641–646.
6. **Dugaiczyk, A., S. L. Woo, D. A. Colbert, E. C. Lai, M. L. Mace, and B. W. O'Malley.** 1979. The ovalbumin gene: cloning and molecular organization of the entire natural gene. Proc. Natl. Acad. Sci. U.S.A. **76**:2253–2257.
7. **Frenkel, N.** 1981. Defective interfering herpesviruses, p. 91–120. *In* A. J. Nahmias, W. R. Dowdle, and R. S. Schinazy (ed.), The human herpesviruses—an interdisciplinary perspective. Elsevier-North Holland Inc., New York.
8. **Frenkel, N., R. R. Spaete, D. A. Vlazny, and H. Locker.** 1982. The herpes simplex virus amplicon—a novel animal-virus cloning vector, p. 205–209. *In* Y. Gluzman (ed.), Eukaryotic viral vectors. Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y.
9. **Gannon, F., J. M. Jeltsch, and F. Perrin.** 1980. A detailed comparison of the 5′ end of the ovalbumin gene cloned from chicken oviduct and eukaryotic DNA. Nucleic Acids Res. **8**:4405–4419.
10. **Gannon, F., K. O'Hare, F. Perrin, J. P. LePennec, C. Benoist, M. Cochet, R. Breatnach, A. Royal, A. Garapin, B. Carni, and P. Chambon.** 1979. Organization and sequences at the 5′ end of a cloned complete ovalbumin gene. Nature (London) **278**:428–434.
11. **Garel, A., and R. Axel.** 1976. Selective digestion of transcriptionally active ovalbumin genes from oviduct nuclei. Proc. Natl. Acad. Sci. U.S.A. **73**:3966–3970.
12. **Graham, F. L., and A. J. Van der Eb.** 1973. A new technique for the assay of infectivity of human adenovirus DNA. Virology **52**:456–467.
13. **Kuebbing, D., and C. D. Liarakos.** 1978. Nucleotide sequence of the 5′ end of ovalbumin messenger RNA from chicken. Nucleic Acids Res. **6**:2253–2266.
14. **Lai, E. C., S. L. C. Woo, M. E. Bordelon-Riser, T. H. Fraser, and B. W. O'Malley.** 1980. Ovalbumin is synthesized in mouse cells transformed with the natural chicken ovalbumin gene. Proc. Natl. Acad. Sci. U.S.A. **77**:244–248.
15. **Lawson, G. M., M. J. Tsai, and B. W. O'Malley.** 1980. Deoxyribonuclease I sensitivity of the nontranscribed sequences flanking the 5′ and 3′ ends of the ovomucoid gene and the ovalbumin and its related X and Y genes in hen oviduct nuclei. Biochemistry **19**:4403–4411.
16. **LeMeur, M., N. Glanville, J. L. Mandel, P. Gerlinger, R. Palmiter, and P. Chambon.** 1981. The ovalbumin gene family: hormonal control of X and Y gene transcription and mRNA accumulation. Cell **23**:561–571.
17. **Locker, H., and N. Frenkel.** 1979. Structure and origin of defective genomes contained in a serially passaged herpes simplex virus type 1 (Justin). J. Virol. **29**:1065–1077.
18. **Locker, H., and N. Frenkel.** 1979. The *Bam*HI, *Kpn*I, and *Sal*I restriction enzyme maps of the DNAs of herpes simplex virus strains Justin and F: occurrence of heterogeneities in defined regions of the viral DNA. J. Virol. **32**:429–441.
19. **Locker, H., N. Frenkel, and I. Halliburton.** 1982. Structure and expression of class II defective herpes simplex virus genomes encoding the infected cell polypeptide number 8. J. Virol. **43**:574–593.
20. **McKnight, G. S.** 1978. The induction of ovalbumin and conalbumin mRNA by estrogen and progesterone in chick oviduct explant cultures. Cell **14**:403–413.
21. **Mocarski, E. S., and B. Roizman.** 1981. Site-specific inversion sequence of the herpes simplex virus genome: domain and structural features. Proc. Natl. Acad. Sci. U.S.A. **78**:7047–7051.
22. **Mocarski, E. S., and B. Roizman.** 1982. Herpesvirus-dependent amplification and inversion of cell-associated viral thymidine kinase gene flanked by viral *a* sequences and linked to an origin of viral DNA replication. Proc. Natl. Acad. Sci. U.S.A. **79**:5626–5630.
23. **Mulvihill, E. R., J. P. LePennec, and P. Chambon.** 1982. Chicken oviduct progesterone receptors. Location of specific regions of high-affinity binding in cloned DNA fragments of hormone-responsive genes. Cell **24**:621–632.
24. **Read, G. S., and N. Frenkel.** 1983. Herpes simplex virus mutants defective in the virion-associated shut-off of host polypeptide synthesis and exhibiting abnormal synthesis of α (immediate early) viral polypeptides. J. Virol. **46**:498–512.
25. **Reyes, G. R., R. LaFemina, S. D. Hayward, and G. S. Hayward.** 1980. Morphological transformation by DNA fragments of human herpesviruses: evidence for two distinct transforming regions in herpes simplex virus types 1 and 2 and lack of correlation with biochemical transfer of the thymidine kinase gene. Cold Spring Harbor Symp. Quant. Biol. **44**:629–641.
26. **Robinson, S. I., B. D. Nelkin, and B. Vogelstein.** 1982. The ovalbumin gene is associated with the nuclear matrix of chicken oviduct cells. Cell **28**:99–106.
27. **Roizman, B.** 1979. The structure and isomerization of herpes simplex virus genomes. Cell **16**:481–494.
28. **Ruyechan, W. T., L. S. Morse, D. M. Knipe, and B. Roizman.** 1979. Molecular genetics of herpes simplex virus. II. Mapping of the major viral glycoproteins and of the genetic loci specifying the social behavior of infected cells. J. Virol. **29**:677–697.
29. **Senear, A. W., and R. D. Palmiter.** 1981. Multiple structural features are responsible for the nuclease sensitivity of the active ovalbumin gene. J. Biol. Chem. **256**:1191–1198.
30. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
31. **Spaete, R. R., and N. Frenkel.** 1982. The herpes simplex virus amplicon: a new eucaryotic defective-virus cloning-amplifying vector. Cell **30**:295–304.
32. **Stow, N. D.** 1982. Localization of an origin of DNA replication within the TR_S/IR_S repeated region of the herpes simplex virus type 1 genome. EMBO J. **1**:863–867.
33. **Stow, N. D., and E. C. McMonagle.** 1984. Propagation of foreign DNA sequences linked to a herpes simplex virus origin of replication, p. 199–204. *In* Y. Gluzman (ed.), Eukaryotic viral vectors. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
34. **Vlazny, D. A., and N. Frenkel.** 1981. Replication of herpes simplex virus DNA: localization of replication recognition signals within defective virus genomes. Proc. Natl. Acad. Sci. U.S.A. **78**:742–746.
35. **Vlazny, D. A., A. Kwong, and N. Frenkel.** 1982. Site specific cleavage/packaging of herpes simplex virus DNA and the selective maturation of nucleocapsids containing full length viral DNA. Proc. Natl. Acad. Sci. U.S.A. **79**:1423–1427.
36. **Woo, S. L. C., W. G. Beattie, J. F. Catterall, A. Dugaiczyk, R. Staden, G. G. Brownlee, and B. W. O'Malley.** 1981. Complete nucleotide sequence of the chicken chromosomal ovalbumin gene and its biological significance. Biochemistry **20**:6437–6446.