JOURNAL OF VIROLOGY, Oct. 1976, p. 211-221

Vol. 20, No. 1
Printed in U.S.A.

Sequence Arrangement in Herpes Simplex Virus Type 1 DNA: Identification of Terminal Fragments in Restriction Endonuclease Digests and Evidence for Inversions in Redundant and Unique Sequences

NEIL M. WILKIE* AND RITA CORTINI

*Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724, and Institute of Virology, Glasgow G11 5JR, Scotland**

Received for publication 10 May 1976

It has been proposed by Sheldrick and Berthelot (1974) that the terminal sequences of herpes simplex virus type 1 (HSV-1) DNA are repeated in an internal inverted form and that the inverted redundant sequences delimit and separate two unique sequences, S and L. In this study the sequence arrangement in HSV-1 DNA has been investigated with restriction endonuclease cleavage, end-labeling studies, and molecular hybridization experiments. The terminal fragments in digests with restriction endonucleases *Hind* III, *Hpa*-1, *Eco*RI and *Bum* were identified and shown to be consistent with the Sheldrick and Berthelot model. Inverted fragments which contain unique sequences as well as redundant sequences, and which the model predicts, were identified by DNA-DNA hybridization studies. Further cleavage of *Bum* fragments with *Hpa*-1 also revealed inversions of the terminal sequences that contained unique sequences. The results obtained showed that the unique sequences S and L are relatively inverted in different DNA molecules in the population, resulting in the presence of four related genomes with rearranged sequences in apparently equal amounts. The redundant sequences bounding S do not share complete sequence homology with those bounding L, but hybridization studies are presented which show that the terminal 0.3% of the genome is repeated in every redundant sequence.

The DNA of herpes simplex virus type 1 (HSV-1) is a linear duplex with a molecular weight of 100×10^6. A terminal redundancy of about 0.5×10^6 daltons was proposed for HSV-1 by Grafstrom et al. (4, 5), whereas Sheldrick and Berthelot (12), who also found terminal redundancy, showed that the terminal sequences are repeated in an internal inverted form. They proposed that two unique sequences of different molecular weights (S = 10^7 daltons; L = 75×10^6 daltons) are bounded by two sets of redundant sequences in the arrangement shown in Fig. 1a. Sheldrick and Berthelot (12) also pointed out that such an arrangement could lead, through recombination mechanisms, to the relative inversion of the unique sequences S and L in different HSV-1 DNA molecules.

Analysis of HSV DNA with restriction endonucleases showed that in limit digests various minor components can be discerned (10, 14, 18). Furthermore, the sum of the molecular weights of all fragments was more than the molecular weight of the intact genome (10, 18). Theoretical analyses of hypothetical cleavage products, by Clements et al. (2), assuming the presence of four related genomes with relative inversions of S and L, showed that the following situations could result. Model 1 (Fig. 1b): in the case of an enzyme for which there are no sites in any redundant region, four terminal fragments with molarities of 0.5 and four fragments with molarities of 0.25, arising from sequences spanning the internal redundant region, are generated. All other fragments are molar. Model 2 (Fig. 1b): in the case of an enzyme in which there are enzyme sites in only one set of redundant regions (assuming the redundant sets bounding S or L have different sequence homology) there would be a molar terminal fragment from that end which was cleaved, two 0.5 M fragments from the other terminus, and two 0.5 M fragments from internal sequences. The amount of DNA present in fragments of the genome after restriction endonuclease cleavage has been measured in the case of both HSV-1 (2, 6) and HSV-2 (2). The results suggested that the previously observed minor bands may be

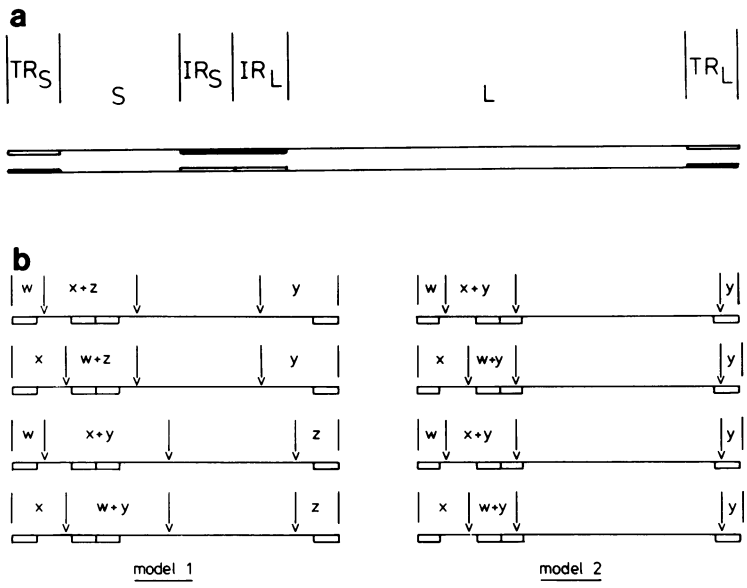


FIG. 1. *(a) Taken from the model of Sheldrick and Berthelot (12). In this figure the double-stranded molecule shown is intended to represent the molecular model published by Sheldrick and Berthelot (12), but has been drawn with S on the left-hand side. Throughout, inverted DNA is meant to refer to inversion of double-stranded segments and not the sequence polarity. The terminal sequence (TRS) delimiting S is redundant and repeated in an internal inverted form (IRS). S = 10 × 10⁶ daltons; TRS and IRS = 5 × 10⁶ daltons. Similarly, TRL and IRL (5 × 10⁶ daltons) delimit L (75 × 10⁶ daltons). (b) Two models showing the consequences for restriction endonuclease cleavage products, assuming that S and L can become relatively inverted and that TRS and IRS have different sequence homology than IRL and TRL. Model 1. 0.5 fragments: W, X, Y, and Z; 0.25 M fragments: W + Y, W + Z, X + Y, and X + Z. Model 2: terminal 1.0 M fragment: Y; terminal 0.5 M fragments: W and X; internal 0.5 M fragments: W + Y and X + Y. IRS = a restriction endonuclease site.*

0.25 M and 0.5 M fragments produced according to both of the models discussed above. Other models for restriction endonuclease digests are possible, e.g., where there are enzyme sites only in L or S (17) or where there are sites in every redundant sequence as well as in S and/or L (6). However, for the purpose of the present analysis only models 1 and 2 described above need be considered.

In this report the restriction endonuclease analysis of HSV-1 DNA is extended, and the Sheldrick model of relative inversions is confirmed. Physical maps for three restriction endonucleases are presented in the accompanying paper (17).

MATERIALS AND METHODS

Enzymes. Restriction endonucleases *Hind* III, *Eco*RI, and *Hpa*-1 were prepared as described previously (2, 11; R. N. Yoshimori, Ph.D. thesis, Univ. of California, San Francisco, 1971). *Bum*, an enzyme from *Brevibacter umbrii*, was the kind gift of R. Roberts. Exonuclease III was the gift of B. Sugden and W. Keller, and lambda exonuclease (exonuclease V) was from T. Maniatis. The activities of exonucleases were determined by the digestion of ^{3}H-labeled T7 DNA. However, it was found that HSV-1 DNA was more resistant to exonuclease digestion than T7. The amount of enzyme necessary to digest HSV DNA sufficient to carry out the experiments described here was therefore determined arbitrarily. AMV reverse transcriptase was the kind gift of W. Keller.

Growth of virus and DNA. HSV-1 strain 17 was grown in confluent monolayers of BHK-21 cells in roller bottles essentially as described previously (16). Crude cell-released and cell-associated virions were combined, and after the addition of EDTA at 0.01 M final concentration and sodium dodecyl sulfate to 2%, nucleic acids were extracted twice with phenol and once with isoamyl alcohol/chloroform (1:24) and then dialyzed extensively against 0.01 M Tris-hydrochloride (pH 7.4). HSV-1 DNA was purified from the dialysate by isopycnic banding in CsCl, followed by rate-zonal sedimentation in preformed CsCl gradients. [G-^{32}P]DNA was extracted from virions grown in cells labeled with 40 μCi of carrier-free [^{32}P]orthophosphate per ml (New England Nuclear Corp.) present during viral growth. These cells had previously been starved for phosphate overnight.

End labeling of HSV-1 DNA. The method was essentially that described previously for adenovirus (Ad2) DNA (1). HSV-1 DNA (1 μg) was incubated at

37°C for 5 to 10 min with 1 μl of exonuclease III in a volume of 50 μl of 0.01 M Tris-hydrochloride (pH 7.9), 2 mM $MgCl_2$. The exonuclease was inactivated by 5 min of incubation at 65°C. The synthetic reaction was with 10 μl of AMV-reverse transcriptase in a volume of 100 μl of 0.06 M Tris-hydrochloride (pH 7.9), 10 mM $MgCl_2$, 50 mM KCl, 1 mM dithiothreitol, and 5 μM [α-^{32}P]deoxyribonucleotide triphosphate (specific activity approximately 100 Ci/mmol; New England Nuclear Corp.). The synthetic reaction was complete after 60 min at 37°C. The DNA was extracted with phenol and purified by gel filtration on Sephadex G-50. It was calculated that 0.3% of HSV-1 DNA nucleotides were replaced in this reaction.

Endonuclease digestion. Reaction mixtures contained, in a volume of 50 μl: 6 mM Tris-hydrochloride (pH 7.9), 6 mM $MgCl_2$, 6 mM β-mercaptoethanol, 0.5 μg of DNA, and sufficient endonuclease to produce a limit digest. When radioactive HSV DNA was digested, 0.5 μg of unlabeled Ad-2 DNA was included. Reactions were terminated after 3 to 4 h at 37°C by the addition of 10 μl of 0.1 M EDTA, 50% sucrose, containing 0.2% bromophenol blue.

Gel electrophoresis. DNA fragments were separated on slab gels of 0.3%, 0.5%, or 0.7% agarose, essentially as described previously (18) except that 3-mm spacers were used. Glass plates had sand-blasted surfaces to help support the low-concentration gels. Radioactive bands were located by autoradiography. In some experiments fragments were separated by electrophoresis on tube gels and located by UV fluorescence of ethidium bromide-stained DNA (11).

Purification of DNA fragments. DNA bands were excised from gels, and the agarose slices were dissolved in 5 M sodium perchlorate at 60°C. DNA was adsorbed to hydroxyapatite at 60°C. The hydroxyapatite was washed at 60°C with portions of 5 M sodium perchlorate and then 0.14 M phosphate buffer (pH 6.8). Finally, DNA was eluted with 0.4 M phosphate buffer (pH 6.8) and dialyzed extensively against 0.01 M Tris-hydrochloride (pH 7.4), 0.001 M EDTA. Where necessary, DNA was concentrated by ethanol precipitation and resolution in small volumes of 0.01 M Tris-hydrochloride (pH 7.4).

Hybridization procedures. The method described by Southern (15) was used. Ten to 20 μg of HSV-1 DNA cleaved by *Hind* III was applied uniformly across 0.3 or 0.5% agarose slab gels. After electrophoresis, the DNA in bands was denatured by immersing the slab in 0.3 M NaOH, 0.6 M NaCl. After 1 h the pH was returned to neutrality by immersion for a further hour in 1 M Tris-hydrochloride (pH 7.4), 0.6 M NaCl. Denatured DNA was then transferred to a sheet of nitrocellulose membrane filter (Schleicher and Schuell) by the method described by Southern (15). After transfer the sheet was baked in a vacuum oven at 70°C for at least 4 h and stored at room temperature. Strips of 3 mm in width were cut lengthways from the sheet so that each strip contained all the fragments of unlabeled DNA. The strips were preincubated at 80°C in Denhart solution (3) (0.02% Ficoll, 0.02% polyvinylpyrollidone, 0.02% bovine serum albumin in 3× SSC [0.15 NaCl plus 0.015 M sodium citrate]), and hybridization was carried out with 20,000 to 100,000 cpm of alkali-denatured ^{32}P-labeled HSV-1 DNA in a volume of 1 to 4 ml of the same buffers at 80°C. After about 12 h the strips were washed thoroughly with 6× SSC at 60°C and labeled DNA bands were located by autoradiography.

RESULTS

Figure 2 represents the cleavage profiles found for HSV-1 strain 17 DNA with *Hind* III and *Hpa*-1. The assignation of letters to the fragments and their molecular weights are given. The assignments of letters to bands of DNA is different from the data presented in reference 7, because our more recent experiments have allowed the number of fragments present in over-molar bands to be calculated (see reference 9). Wherever possible, individual fragments have received separate letters but *Hpa*-1 *o* contains several fragments (2), which for the purposes of this communication remain grouped under the letter "*o*." It will be appreciated that groups of fragments such as *Hind* III *hi* or *Hpa*-1 *dce* and *gh* have not been successfully separated on any gel system so far attempted (2, 18). In an earlier publication (2) it was proposed that the group *Hpa*-1 *cde* contained only two fragments and that the group *Hind* III *hi* contained three fragments, but subsequent mapping data (17) showed that the corrections indicated should be applied. The postulated molarity of each minor fragment is shown according to the Clements et al. (2) interpretation of the Sheldrick inversion model (12). In the case of other enzyme digests shown, each identifiable band observed after gel electrophoresis has been assigned a provisional letter, but this does not necessarily mean that each band contains only one fragment of the genome.

Identification of fragments from the termini. Terminal fragments in enzyme digests were determined directly through end-labeling studies. Intact HSV-1 DNA was treated with exonuclease III, and the gaps so produced were filled by using reverse transcriptase and α-^{32}P-labeled deoxyribonucleotide triphosphates. The product was then subjected to restriction endonuclease analysis. In an intact duplex DNA, *exo* III can only initiate at the 3′ termini and only the 3′ termini becomes labeled in this reaction. However, HSV DNA generally contains a number of single-strand, alkali-labile interruptions, some of which may be nicks or gaps (9, 13, 16). Since *exo* III can initiate at nicks, it was not surprising that in most experiments with HSV DNA all restriction endonuclease fragments became labeled. However, in

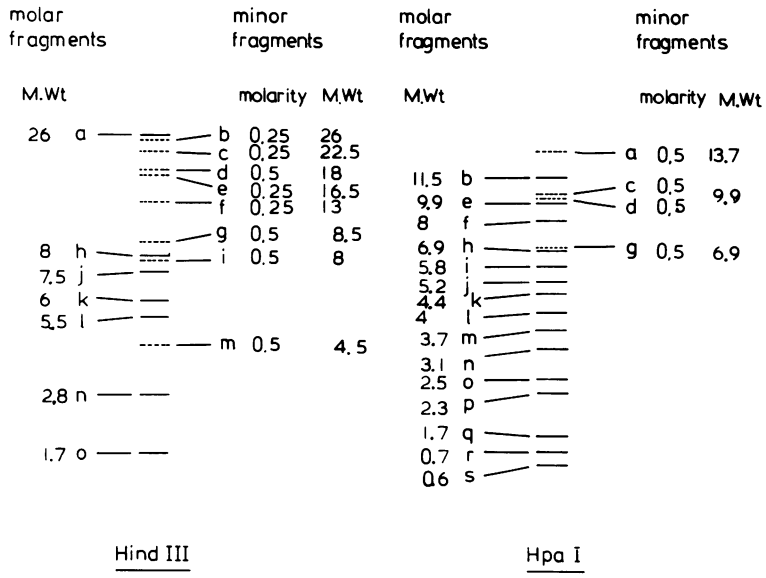


FIG. 2. *Schematic representation of Hind III and Hpa-1 digests of HSV-1 DNA. The molecular weight and molarity of each fragment is given. (——) Molar fragments; (- - -) minor fragments.*

the experiment shown in Fig. 3A, the terminal fragments could readily be identified as being the most heavily labeled bands.

In *Hind* III digests, *d, g, i,* and *m* were heavily labeled and, on that basis, are from the ends of HSV-1 DNA. They correspond to the four 0.5 M fragments identified previously (2) and clearly arise from a model 1 digest. In the case of *Bum* and *Eco*RI, three bands (*b, d,* and *j* and *b, e,* and *f*) were clearly the most strongly labeled. No molar quantitations are available for these digests, but the results clearly indicate that the fragments observed arise from a model 2 cleavage pattern with two 0.5 M end fragments (*Bum b* and *d*; *Eco*RI *b* and *e*) and one molar end arising from a cleavage site within a terminal repeat (*Bum j* and *Eco*RI *f*). In the case of *Hpa*-1 it has already been proposed that the cleavage profile arises in the same way (2). In experiments not shown, three heavily labeled bands could be identified in *Hpa*-1 *cde, gh,* and *m*. These groups all contain fragments with a postulated molarity of 0.5 (2), which would not fit the model. However, subsequent mapping data (17) indicate that *Hpa*-1 *m* is not a half-molar but a molar end from a cleavage site within the terminal redundant sequence. In this case the data fit the model well.

Certain aspects of these results were confirmed independently by treating duplex DNA with *exo* III or lambda exonuclease and then digesting with *Hind* III or *Hpa*-1 directly. In this case, *Hind* III *g* and *m* and *Hpa*-1 *m* could be shown to have disappeared from the profiles. Similar results have been reported for HSV-1 DNA cleaved with *Hind* III and *Eco*RI by Hayward et al. (7). Changes in other terminal fragments would be difficult to observe since they either migrate in bands containing more than one fragment or have a very large molecular weight.

Alignment of ends. In some experiments not all of the terminal fragments became heavily labeled with ^{32}P in the *exo* III-reverse transcriptase reaction. In the experiments shown in Fig. 3B, only *Hind* III *g* and *m*, *Hpa*-1 *cde* and *gh*, and *Eco*RI *f* became overlabeled. In a similar series of experiments, it could be seen similarly that only *Bum b* and *de* became heavily labeled. The most attractive explanation (see Discussion) would be that only one end of the duplex was being filled in with reverse transcriptase. In this case the terminal fragments *Hind* III *d* and *i*, *Bum b* and *d*, *Eco*RI *b* and *e*, and *Hpa*-1 *m* all must come from one end of the genome, and *Hind* III *g* and *m*, *Bum j*, RI *f*, and *Hpa*-1 *cde* and *fg* must come from the other end. Analysis of thymidine kinase mutants of HSV-1 which contained small deletions confirmed this (R. Cortini, unpublished observations). The mutants had deletions of about 150,000 to 200,000 daltons in the terminal redundant region of the genome that altered the mobility of the terminal fragments *Hind* III *m* and *Eco*RI *f* (4.5×10^6 and 3.5×10^6 daltons, respectively).

The mobility of *Hpa*-1 (3.4×10^6 daltons) was unchanged, suggesting that this came from the opposite end of the molecule. No conclusions could be drawn about the mobilities of the other terminal fragments in the digests since they either had molecular weights too large to expect a small deletion to affect the mobility (8×10^6 to 18×10^6 daltons as opposed to 3.5×10^6 to 4.5×10^6 daltons) or else were present in a complex area of the digest where small differences would be difficult to detect (e.g., *Bum j*, *Hpa*-1 *cde*, etc.).

Confirmation of inversions of unique sequences by further cleavage. Generally ^{32}P-labeled HSV-1 DNA was cleaved with *Bum*, and fragments *a*, *b*, *c*, and *d* were isolated. Each fragment was further cleaved with *Hpa*-1 and then analyzed by gel electrophoresis. The results are shown in Fig. 4. The cleavage patterns for *Bum a* and *b* were identical, except that the largest product from *Bum a* was missing in *Bum b*, and a new and smaller fragment had appeared. A similar situation was true for *Bum c* and *d*, although the overall pattern was different. The fragments that changed in the *Bum c* and *d* digests had the same mobility as those that changed in the *Bum a* and *b* set. *Bum b* and *Bum d* are terminal fragments (see above). The experiment shown in Fig. 4a can therefore be interpreted as showing relative inversions of the same sequence directly. The theoretical analysis of this situation is shown in Fig. 4b. In a model 2 situation the further cleavage profiles of a terminal fragment and its internal inversion should be identical, with the exception that the terminal fragment *a* in the second digest is replaced in the inverted form by $a + x$. This experiment offers strong evidence in support of the model for relative inversions of the unique sequences in HSV-1.

Hybridization experiments. Generally ^{32}P-labeled HSV-1 DNA was cleaved with *Hind* III, and individual restriction bands were isolated. HSV-1 DNA was also end labeled with [α-^{32}P]dATP (using cold dTTP, dGTP, and dCTP) as described and cleaved with *Eco*RI, and the heavily labeled *Eco*RI *f* terminal fragment was isolated (see Fig. 3).

Using the Southern blot hybridization method described under Materials and Methods, the labeled fragments were hybridized to unlabeled *Hind* III fragments of HSV-1 DNA. The results are presented in Fig. 5. Unique fragments such as *Hind* III *j*, *k*, or *n* hybridize only to themselves. However, end-labeled terminal fragment *Eco*RI *f* annealed to *Hind* III *ab*, *c*, *de*, *f*, *g*, *hi*, and *m*, i.e., all of the minor bands in the *Hind* III cleavage pattern. In this experiment the same result was found for hybridization of [^{32}P]*Hind* III *de* (a 0.5 M fragment plus a 0.25 M fragment), except that in this experiment the hybridization to *Hind* III *m* is difficult to see. It is not easy to interpret this result in terms other than the Sheldrick inversion model.

DISCUSSION

Sheldrick and Berthelot (12) proposed the model for HSV DNA shown in Fig. 1a. They further proposed that the unique sequences S and L might become inverted due to recombination. It has been shown (2) that depending on the presence of restriction endonuclease cleavage sites in the redundant sequences bounding the S and L sequences, minor fragments would be generated with molarities of 0.5 and 0.25 in the case of model 1 and minor fragments with molarities of only 0.5 in the case of model 2 (see Fig. 1). Furthermore, the molarities and molecular weights of the minor fragments actually found in digests of HSV DNA corresponded closely to that predicted for models 1 and 2 for HSV-1 and HSV-2 DNA (2, 17).

It could be predicted, therefore, that for model 1 each 0.5 M fragment must arise from an end. The results presented here show that this is indeed the case. Each 0.5 M fragment identified in *Hind* III digests becomes overlabeled in end-labeling reaction. Furthermore, in *Hpa*-1 digests, proposed to be due to a model 2 cleavage (2, 17), only three overlabeled bands could be identified. Two of these contained 0.5 M fragments previously identified. The remaining fragment, originally thought to be a 0.5 M fragment, was subsequently shown to be a molar end, as fits the model (17). The results also suggest that the three end-labeled bands observed in *Eco*RI and *Bum* digests also arise from model 2 cleavage patterns. The experiments describing alignment of the ends allowed us to group the terminal fragments in *Hind* III *d* and *i*, *Hpa*-1 *m*, *Bum b* and *d*, and *Eco*RI *b* and *e* to one terminus and those in *Hind* III *g* and *m*, *Hpa*-1 *cde* and *gh*, *Bum j*, and *Eco*RI *f* at the other.

The identification of terminal fragments in digests of HSV-1 strain 17 DNA presented in this study are consistent by the assignment of terminal fragments in strain MP DNA by Hayward et al. (6) using lambda exonuclease digestion followed by *Hind* III or *Eco*RI cleavage. The cleavage profiles of strain MP DNA by these two enzymes show minor bands similar to those obtained with strain 17 DNA but with somewhat different relative mobilities. In each case, however, four terminal 0.5 M bands were

obtained with *Hind* III and three terminal fragments were obtained with *Eco*RI. The advantage of using end-labeling studies is that termini can be identified even when they are present in a group of fragments with identical relative mobilities.

Failure to label some of the end fragments with *exo* III-reverse transcriptase in some ex-

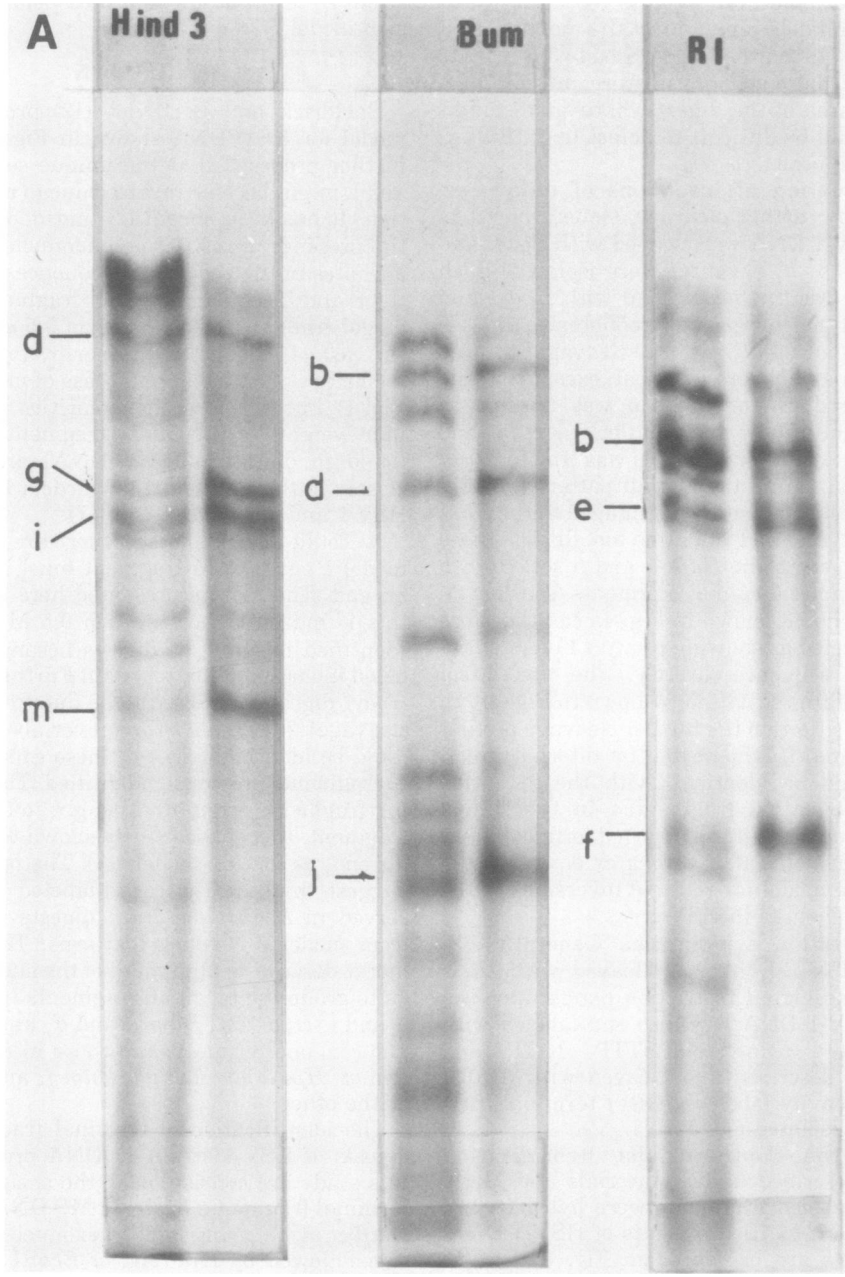


FIG. 3. *End-fragment analysis. (A) HSV-1 DNA was end labeled with α-^{32}P-labeled deoxyribonucleotides as described and then cleaved with Hind III, Bum, and EcoRI. The cleaved DNA was separated on 0.3% agarose slab gels with [G-^{32}P]DNA run in parallel slots. Labeled bands were identified by autoradiography. (B) As for Fig. 3, but DNA cleaved with Hind III, EcoRI, and Hpa-1. Dots indicate the heavily labeled terminal fragments.*

periments could, of course, have a number of explanations. On the basis of electron microscope studies of HSV-1 DNA treated with lambda exonuclease, Hyman et al. (8) proposed that within at least one of the terminal redundant regions sequences close to the terminus are repeated some distance away in an inverted form. After exonuclease digestion, the strand could loop back on itself and allow the complementary redundant sequences to base pair. A looped-back strand base paired to itself would provide a poor substrate for reverse transcriptase. Because *Hpa*-1 *m* was sensitive to exonucleases and disappeared from the cleavage pattern when the DNA was first digested with *exo* III/lambda exonuclease and yet was not labeled with reverse transcriptase, this observation offers the most likely explanation of our failure to label some end fragments in some experiments. Our results suggest that this structure might be confined to only one of the terminally redundant regions, and the mapping data presented

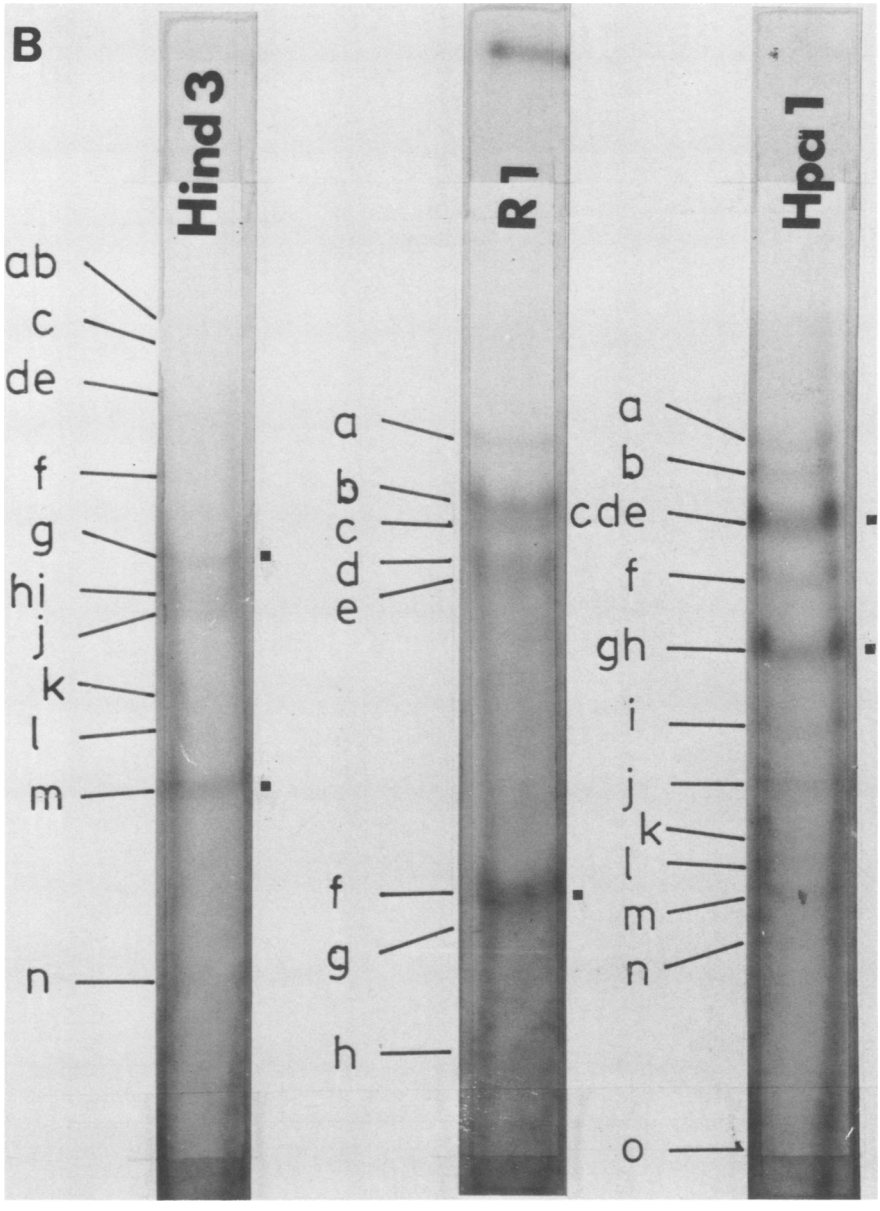
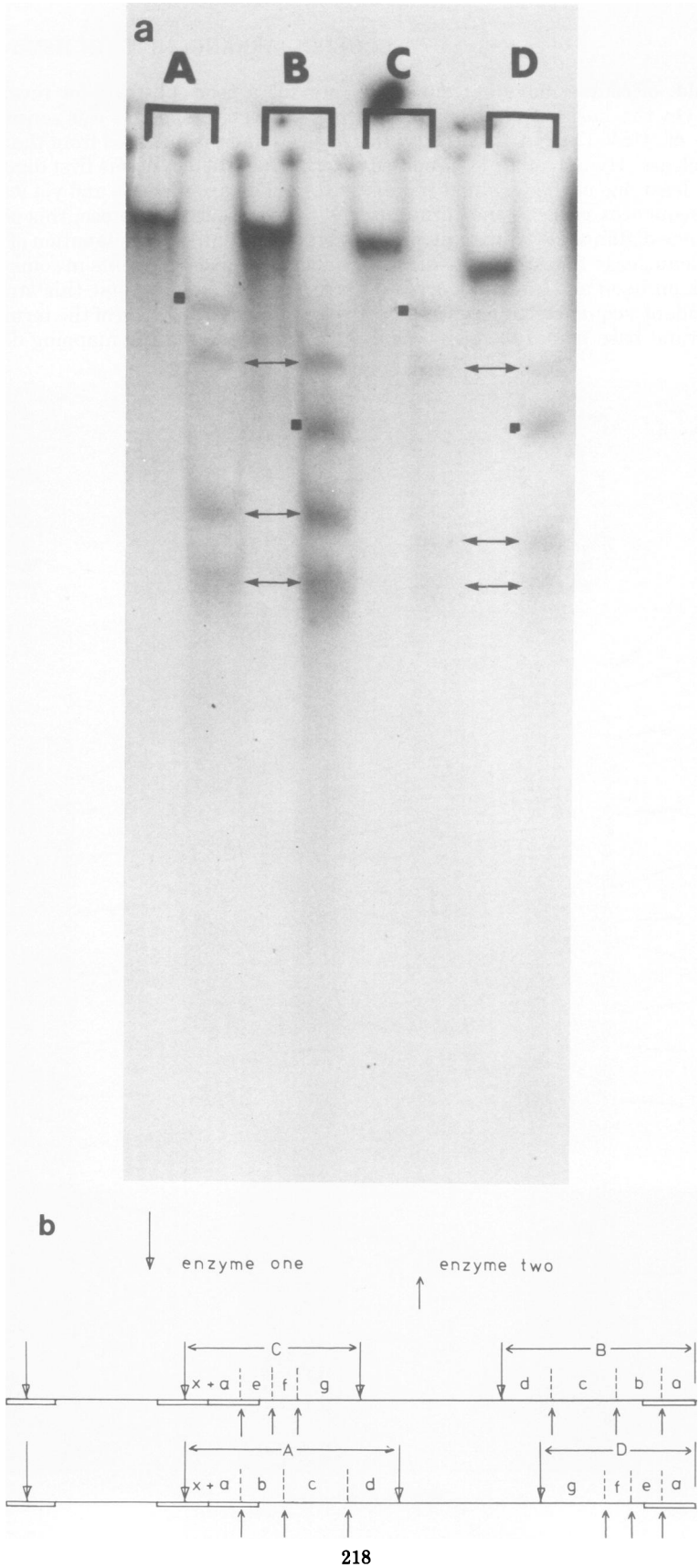


FIG. 3–*Continued*


a
A
B
C
D
b
enzyme one
enzyme two
C
x + a
e
f
g
B
d
c
b
a
A
x + a
b
c
d
D
g
f
e
a

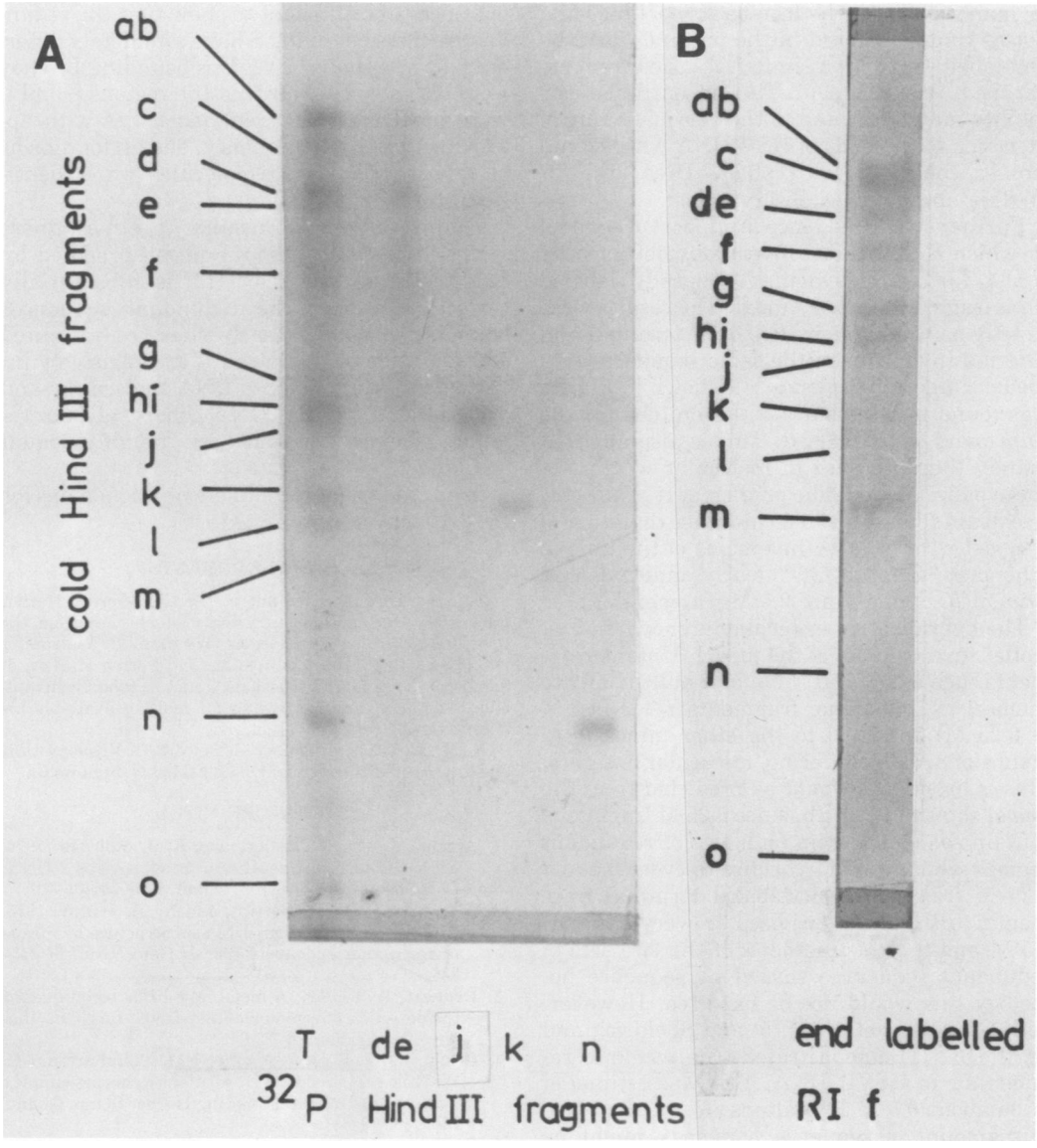


FIG. 5. *Hybridization of isolated ^{32}P-labeled fragments to unlabeled Hind III fragments of HSV-1 DNA. The technique was that described in Materials and Methods. (A) Hybridization of generally ^{32}P-labeled Hind III de, j, k, and n. The position of the cold fragments on the strips of nitrocellulose membrane was determined by hybridization with fragmented, unfractionated [^{32}P]HSV-1 DNA. (B) Hybridization of end-labeled EcoRI f.*

FIG. 4. *(a) Further cleavage of Bum a, b, c, and d. ^{32}P-labeled Bum a, b, c, and d were isolated as described and recleaved with Hpa-1. Intact and cleaved fragments Bum a (A), Bum b (B), Bum c (C), and Bum d (D) were separated by electrophoresis on a 0.7% slab gel, and the position of DNA bands was determined by autoradiography. Arrows indicate fragments identical in the pairs of digests and dots indicate those fragments that vary. In the internal inversion, a is replaced by a + x. (b) Diagrammatic representation of the further cleavage products from related, relatively inverted 0.5 M fragments from a model 2 cleavage pattern.*

in the accompanying paper shows that this would be from the L region of the genome. In Hyman's study (8) the loop-back reaction could not be controlled, and in the present work the end-labeling reaction similarly varied from experiment to experiment. This uncontrolled variability may be related to the very slow rate of digestion of the ends of HSV DNA with exonucleases compared with phage DNA like T7 (Wilkie, unpublished observations).

Further strong evidence in support of a model in which S and L can invert was obtained by *Hpa*-1 further cleavage of generally labeled *Bum* fragments *a*, *b*, *c*, and *d*. The results were exactly as would be predicted for terminal and internal inversions of the same sequences in a model 2 digest. The distance *x* shown in Fig. 4a was found to be about 2×10^6 daltons for the *Bum* pairs *a*, *b* and *c*, *d*. This is also approximately the difference in molecular weights of these pairs, as would be predicted. It is difficult to explain this result in terms other than that of a model with relative inversions of the unique sequences. The results clearly indicate that *Bum a*, *b*, *c*, and *d* are 0.5 M fragments.

The hybridization experiments shown in Fig. 5 offer further proof of the model. Unique fragments such as *Hind* III *h*, *k*, or *n* anneal only to themselves, but minor fragments *d* + *e* (0.5 M + 0.25 M) annealed to the other minor fragments of widely differing molecular weights. This is exactly as would be predicted from the model shown in Fig. 1b, since 0.25 M fragments contain sequences from both sets of redundant regions which would hybridize to every 0.5 and 0.25 M fragment. End-labeled terminal fragment *Eco*RI *f* also hybridized to every *Hind* III 0.5 M and 0.25 M fragment. If the two sets of redundant sequences shared no sequence homology this would not be expected. However, both Grafstrom et al. (4, 5) and Sheldrick and Berthelot (12) demonstrated true terminal redundancy in HSV-1 DNA. This was estimated to be about 0.5×10^6 daltons (5). In that case this amount of sequence homology might be expected to be shared by the redundant sequence bounding S and L and the ability of end-labeled terminal fragments to anneal to all repeat sequences is to be expected. HSV nucleotides (0.3%) were replaced during the end-labeling reaction. The labeled sequences should therefore be mainly from the true terminal redundancy, and the experiment indicates that these sequences are repeated in the internal inverted forms of the redundant regions.

The results suggest that the redundant sequences bounding S and L do not share complete sequence homology. Delius and Clements (in press) have constructed complete partial denaturation maps for HSV-1 DNA. The detail obtained was sufficient to show that the redundant regions bounding S have completely different patterns than the regions bounding L. They were also able to show that the regions S and L were inverted in different molecules with approximately equal frequency, and all four forms of relatively inverted molecules were identified.

Taking all these results together, there seems little doubt that the model proposed by Sheldrick and Berthelot (12) is substantially correct. However, the redundant sequences bounding S and L clearly share only limited sequence homology. S and L are relatively inverted in different HSV DNA molecules, and preparations of HSV DNA consist of four related genomes present in roughly equal amounts.

In a second paper, complete physical maps for HSV-1 DNA are presented (17).

ACKNOWLEDGMENTS

This work was carried out at the Cold Spring Harbor Laboratory during temporary leave of absence from the Institute of Virology in Glasgow. We thank J. Watson for making available the facilities at Cold Spring Harbor, J. Sambrook for helpful discussions, Janet Arrand for invaluable assistance, and R. Hyman for making available his manuscript before publication.

N. M. Wilkie is a member of the M.R.C. Virology Unit and R. Cortini is the recipient of an E.M.B.O. Fellowship.

LITERATURE CITED

1. **Arrand, J. R., W. Keller, and R. J. Roberts.** 1974. Extent of terminal repetition in adenovirus 2 DNA. Cold Spring Harbor Symp. Quant. Biol. 39:401–407.
2. **Clements, J. B., R. Cortini, and N. M. Wilkie.** 1976. Analysis of herpesvirus DNA substructure by means of restriction endonucleases. J. Gen. Virol. 30:243–256.
3. **Denhart, D. T.** 1966. A membrane-filter technique for the detection of complementary DNA. Biochem. Biophys. Res. Commun. 23:641.
4. **Grafstrom, R. H., J. C. Alwine, W. L. Steinhart, and C. W. Hill.** 1974. Terminal repetitions in herpes simplex virus type 1 DNA. Cold Spring Harbor Symp. Quant. Biol. 39:679–681.
5. **Grafstrom, R. H., J. C. Alwine, W. L. Steinhart, C. W. Hill, and R. W. Hyman.** 1975. The terminal repetition of herpes simplex virus DNA. Virology 67:144–157.
6. **Hayward, G. S., N. Frenkel, and B. Roizman.** 1975. Anatomy of herpes simplex virus DNA: strain differences and heterogeneity in the locations of restriction endonuclease cleavage sites. Proc. Natl. Acad. Sci. U.S.A. 72:1768–1772.
7. **Hayward, G. S., R. J. Jacob, S. C. Wadsworth, and B. Roizman.** 1975. Anatomy of herpes simplex virus DNA: evidence for four populations of molecules that differ in the relative orientations of their long and short components. Proc. Natl. Acad. Sci. U.S.A. 72:4243–4247.
8. **Hyman, R. W., S. Burke, and L. Kudler.** 1975. A nearby inverted repeat of the terminal sequence of

herpes simplex virus DNA. Biochem. Biophys. Res. Commun. 68:609-615.
9. **Kieff, E. D., S. L. Bachenheimer, and B. Roizman.** 1971. Size, composition and structure of the deoxyribonucleic acid of subtypes 1 and 2 herpes simplex virus. J. Virol. 8:125-132.
10. **Roizman, B., M. Kozak, R. W. Honess, and G. Hayward.** 1974. Regulation of herpesvirus macromolecular synthesis: evidence for multilevel regulation of herpes simplex 1 RNA and protein synthesis. Cold Spring Harbor Symp. Quant. Biol. 39:687-701.
11. **Sharp, P. A., B. Sugden, and J. Sambrook.** 1973. Detection of two restriction endonuclease activities in hemophilus parainfluenzae using analytical agarose-ethidium bromide electrophoresis. Biochemistry 12:3055-3063.
12. **Sheldrick, P., and N. Berthelot.** 1974. Inverted repetitions in the chromosome of herpes simplex virus. Cold Spring Harbor Symp. Quant. Biol. 39:667-678.
13. **Sheldrick, P., M. Laithier, D. Lando, and M. L. Ryhiner.** 1973. Infectious DNA from herpes simplex virus: infectivity of double-stranded and single-stranded molecules. Proc. Natl. Acad. Sci. U.S.A. 70:3621-3625.
14. **Skare, J., W. P. Summers, and W. C. Summers.** 1975. Structure and function of herpesvirus genomes. I. Comparison of five HSV-1 and two HSV-2 strains by cleavage of their DNA with *Eco* RI restriction endonuclease. J. Virol. 15:726-732.
15. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98:503-518.
16. **Wilkie, N. M.** 1973. The synthesis and substructure of herpesvirus DNA: the distribution of alkali-labile single strand interruptions in HSV-1. J. Gen. Virol. 21:453-467.
17. **Wilkie, N. M.** 1976. Physical maps for herpes simplex virus type 1 DNA for restriction endonucleases *Hind* III, *Hpa*-1, and *X. bad*. J. Virol. 20:222-233.
18. **Wilkie, N. M., J. B. Clements, J. C. M. Macnab, and J. H. Subak-Sharpe.** 1974. The structure and biological properties of herpes simplex virus DNA. Cold Spring Harbor Symp. Quant. Biol. 39:657-666.