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Enhanced Rate of Conversion or Recombination of Markers within a Region of Unique Sequence in the Herpes Simplex Virus Genome

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Insertion mutants of herpes simplex virus type 1, containing a second copy of the sequences of *Bam*HI fragment L (map coordinates 0.706 to 0.744) inserted in inverted orientation into the thymidine kinase gene (at map coordinate 0.315), have been further characterized. We reported previously that, as a result of intramolecular or intermolecular recombination between copies of the *Bam*HI-L sequence at the normal locus and inserted locus, a high proportion of progeny genomes exhibited either inversions of the unique sequence flanked by these inverted repeats or other rearrangements. Now we report that a genetic marker (*syn-1* or *syn-1*$^+$) originally present only in the inserted copy of *Bam*HI fragment L appears in progeny at both the normal and inserted loci, and vice versa, at high frequency. Because these phenomena have not been observed with other insertion mutants containing duplications of other sequences from unique regions of the genome, we conclude that *Bam*HI fragment L contains an element that enhances the rate of homologous recombination in adjacent sequences, resulting in genome rearrangements and gene conversion-like events.

The genome of herpes simplex virus (HSV) (Fig. 1) is a linear duplex DNA molecule of approximately 100×10^6 daltons and is composed of a long (L) and a short (S) component. Each component is flanked by inverted repeats, the ends of which (a sequence designated *a*) are common to both segments and are repeated in direct orientation at the ends of the genome (22, 27, 28). Recombination apparently occurs between the inverted repeats, resulting in inversion of the unique segments (U_L and U_S) flanked by the repeats. Populations of HSV genomes usually contain four isomers of viral DNA, because U_L and U_S can invert independently of each other (6, 23, 29).

Evidence has been presented that an element within the *a* sequence promotes the recombinations that result in inversions of the U_L and U_S components of the genome. Specifically, insertion of an additional *a* sequence into the thymidine kinase (*tk*) gene results in novel rearrangements of the genome, consistent with inversion of any genome segment bounded by inverted copies of the *a* sequence (2, 15–17, 24) and deletion of genome segments bounded by direct repeats (24). In addition, deletion of sequences containing *a* from the junction between the L and S components prevents inversions of the L and S components (18).

With one exception, duplication of other regions of the genome by insertion of an extra copy into the *tk* gene or elsewhere did not result in recombination between the repeated sequences at a frequency high enough to detect genomic rearrangements (deletions or inversions for direct or inverted repeats, respectively) that would have resulted (5, 12, 15). Duplication of sequences from *Bam*HI fragment L (map coordinates 0.706 to 0.744) in inverted orientation, however, resulted in inversions of unique sequences between the duplications and possibly other genomic rearrangements, apparently due to a high frequency of intramolecular or intermolecular recombination between the repeated sequences present at the normal and inserted loci (20).

The interactions between two copies of the *a* sequence that can result in recombination and genomic rearrangements can also result in elimination of any differences in nucleotide sequence in homologous sequences adjacent to the *a* sequences (3, 7, 10, 11, 26). The subject of this report is the finding that differences in nucleotide sequence between two copies of *Bam*HI fragment L, one inserted in the *tk* gene and the other at the normal locus, are eliminated at high frequency, presumably by recombinations between the two copies of the *Bam*HI-L sequence that also result in genomic rearrangements.

The cell fusion-inducing or syncytial (Syn) mutant, HSV type 1 strain MP [HSV-1(MP)], differs from the related wild-type strain HSV-1(mP) at several loci, one of which is contained within *Bam*HI fragment L. Mapping studies (1, 19) and nucleotide sequence analysis (Pogue-Geile and Spear, submitted for publication) have shown that the Syn phenotype of HSV-1(MP) results from a single nucleotide substitution which also eliminates a *Tha*I restriction endonuclease site present in HSV-1(mP) at map coordinate 0.737 (Fig. 1). Thus, the presence or absence of this *Tha*I site identifies the wild-type or mutant alleles, respectively, of the *syn-1* genes in HSV-1(mP) and HSV-1(MP). The evidence for this identification is that, within the 710-base-pair (bp) fragment to which the Syn mutation was mapped, nucleotide sequences for the two virus strains differed only at the *Tha*I site, and in recombinants of HSV-1(mP) and HSV-1(MP), all isolates expressing the Syn$^+$ phenotype had the *Tha*I site, whereas all isolates expressing the Syn phenotype lacked this site (Pogue-Geile and Spear, submitted).

[Debroy et al. (4) recently reported the nucleotide sequences, between the two rightmost *Pst*I sites indicated in Fig. 1, for HSV-1(MP) and, between map coordinates 0.732 and 0.745, for an unrelated wild-type strain designated HSV-1(KOS). Their sequence for HSV-1(MP) differs slightly

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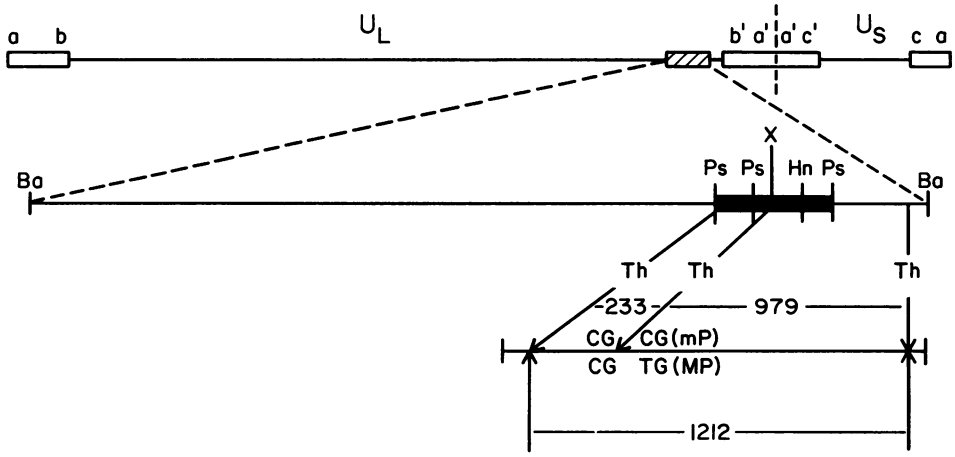

FIG. 1. Location of the mutation in HSV-1(MP) that causes the Syn phenotype and eliminates a *Tha*I site (Pogue-Geile and Spear, submitted). The *Bam*HI L fragment in the HSV-1 genome is represented as a crosshatched box and expanded below the genome, where the black bar represents the 710-bp fragment that contains the Syn mutation (×). In *Tha*I digests of mP DNA, two fragments (233 and 979 bp) would be detected by a probe prepared from the *Pst*I-*Hin*cII fragment in which the mutation is located. In *Tha*I digests of MP DNA, only one fragment (1,212 bp) would be detected. Ba, *Bam*HI; Ps, *Pst*I; Hn, *Hin*cII; Th, *Tha*I.

from ours, but not in the vicinity of the *Tha*I site (Pogue-Geile and Spear, submitted). They detected nine differences in sequence between HSV-1(MP) and HSV-1(KOS) within part of the region to which the Syn mutation was mapped. One difference is the same as described above for HSV-1(MP) and HSV-1(mP), and the others appears to reflect strain variability.]

We isolated mutants that contained *Bam*HI fragment L from HSV-1(mP) DNA inserted in inverted orientation into the *tk* gene of the HSV-1(MP) genome (Fig. 2) and vice versa. Construction of these insertion mutants was previously described along with evidence that, among progeny of these mutants, a large fraction had genomic rearrangements such as inversions of the unique squence between the inverted repeats of the *Bam*HI-L sequence (20). Multiple phenotypes with respect to plaque morphology were observed among progeny of the insertion mutants. For example, most of the mutants derived from HSV-1(MP) by insertion of *Bam*HI fragment L from HSV-1(mP) DNA initially formed plaques of mixed phenotype (partially Syn, partially Syn^+) as shown in Fig. 3A. Both Syn (Fig. 3B) and Syn^+ (Fig. 3C) variants, however, could be detected among the progeny of these mutants. Viruses isolated from the plaques of mixed phenotype (Fig. 3A) were heterogeneous and included both the Syn and Syn^+ variants, as well as viruses with mixed phenotype, whereas viruses isolated from the Syn or Syn^+ plaques were homogeneous and bred true.

An explanation for the multiple plaque phenotypes emerged from Southern blot analyses of *Tha*I digests done to determine which alleles of the *syn-1* gene were present in the insertion mutants. The mutants as constructed should have

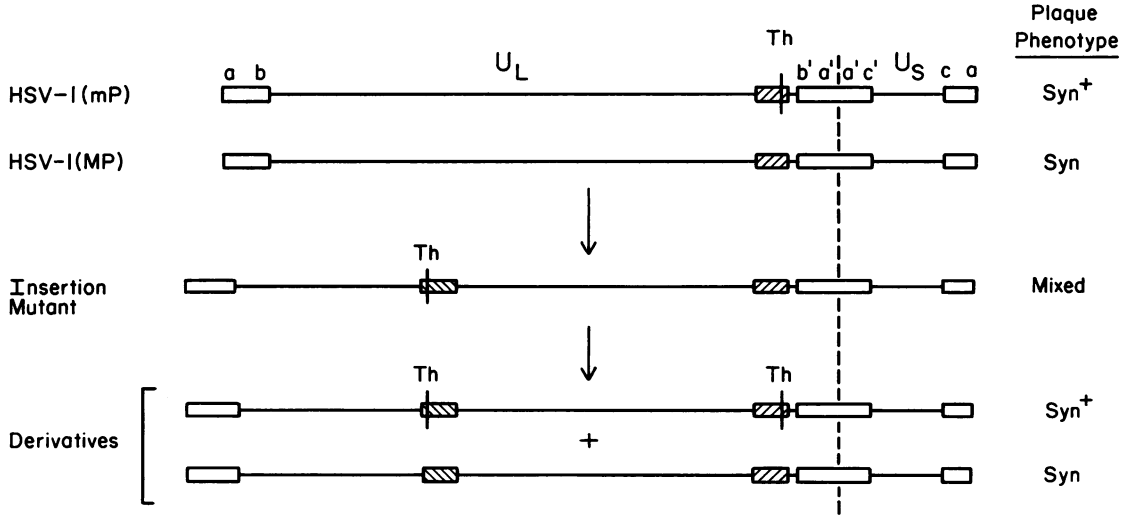

FIG. 2. Location and orientation of *Bam*HI-L sequences (crosshatched boxes) in the genomes of HSV-1(mP), HSV-1(MP), and insertion mutants obtained by inserting the *Bam*HI L fragment of HSV-1(mP) DNA into the *tk* gene of HSV-1(MP) DNA (20). The *Tha*I site (Th) is a marker for the wild-type allele of the *syn-1* gene. This *Tha*I site is eliminated by the mutation responsible for the Syn plaque phenotype of HSV-1(MP).

the mutant allele at one position (normal or inserted locus) and the wild-type allele at the other; *Tha*I digests of their DNAs should contain three fragments capable of hybridizing to the *Pst*I-*Hin*cII probe described in the legend to Fig. 1 (the 1,212-bp fragment characteristic of the mutant allele and the 979- and 233-bp fragments characteristic of the wild-type allele). If the differences in nucleotide sequences between the mutant and wild-type alleles had been eliminated in progeny of the insertion mutants, then only the 1,212-bp fragment or two smaller fragments (979 and 233 bp) would be detected in the *Tha*I digests. The results (Fig. 4) demonstrated that DNAs of all the Syn variants isolated had only the *Tha*I fragment characteristic of the mutant allele (1,212 bp), DNAs of the Syn$^+$ variants had only the fragments characteristic of the wild-type allele (979 and 233 bp), and DNAs from the viruses of mixed phenotype had all three fragments.

Several points about the results presented in Fig. 3 and 4 should be emphasized. First, with one exception, all the mutants analyzed in Fig. 4 actually contain insertions of the *Bam*HI-L sequence in the *tk* gene, as determined by Southern blot analyses published elsewhere (20) or not shown in the case of mP-TK133.5. Therefore, those mutants having only one kind of allele of the *syn-1* gene must have two copies of this allele. Second, some of the insertion mutants isolated contained two identical alleles of the *syn-1* gene (probably due to marker transfer at the normal or inserted locus having occurred prior to isolation of the mutant). However, most of the insertion mutants as originally isolated contained two different alleles of the *syn-1* gene. These mutants exhibited mixed plaque phenotype and, through several rounds of plaque purification, continued to throw off both Syn and Syn$^+$ variants. Third, because both Syn and Syn$^+$ variants are produced during development of the plaques of mixed phenotype, it is not possible to determine the plaque phenotype of the original insertion mutant. Fourth, in plaques producing both Syn and Syn$^+$ variants of the insertion mutants derived from strain MP, the mutant and wild-type phenotypes appear to be codominant, in contrast to the Syn$^+$ phenotype of infectious centers producing both HSV-1(mP) and HSV-1(MP) (9, 13, 14, 21). Possibly some of the other genetic differences between strains MP and mP (19) influence the results obtained in the latter case.

We conclude that, during propagation of insertion mutants of the kind described above, differences in nucleotide sequences between the inserted and normal copies of the duplicated sequence are eliminated at high frequency. The genomic rearrangements described earlier for these insertion mutants (20) provided evidence that intermolecular or intramolecular recombination between inserted and normal copies of the *Bam*HI-L sequence occurs at high frequency. The elimination of nucleotide sequence differences could be another consequence of these recombinations, provided that multiple intermolecular recombinations occurred within the *Bam*HI-L sequence or that gene conversion occurred.

We conclude further that an element within the *Bam*HI L fragment must enhance the rate of homologous recombination (and perhaps also gene conversion) in adjacent sequences. Although a number of HSV-1 insertion mutants containing duplications of other regions of the genome have been analyzed (5, 12, 15), none except those having additional copies of the *Bam*HI-L sequence (20) or the *a* sequence (2, 15–17, 24) have given evidence of high frequencies of recombination between the repeated sequences. The recombination-enhancing elements proposed to exist within

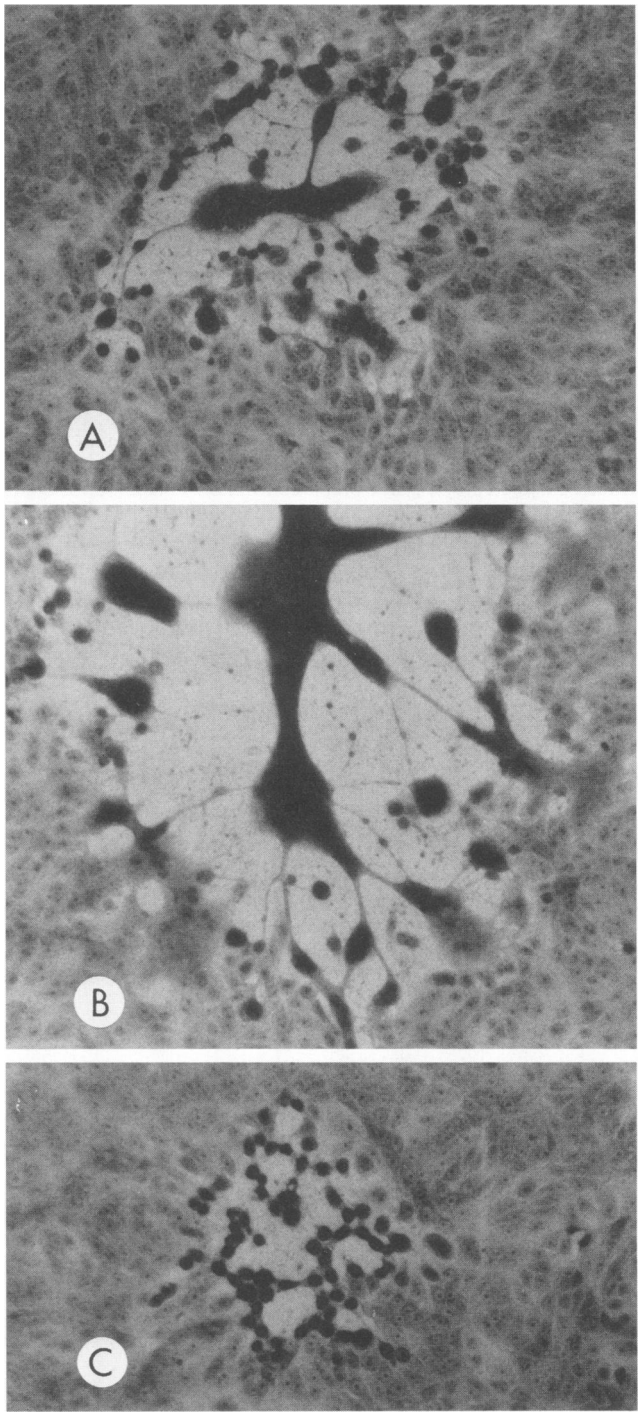

FIG. 3. Photomicrographs of plaques produced on Vero cells by insertion mutants of the kind depicted in Fig. 2. Viruses isolated from plaques of the kind shown in panel A were of three types with respect to plaque phenotype: mixed (A); Syn (B), for derivative MP-TK106.2H; or Syn$^+$ (C), for MP-TK106.2A. Both of these derivatives were isolated from stocks of MP-TK106.2. The Syn and Syn$^+$ derivatives breed true with respect to plaque phenotype.

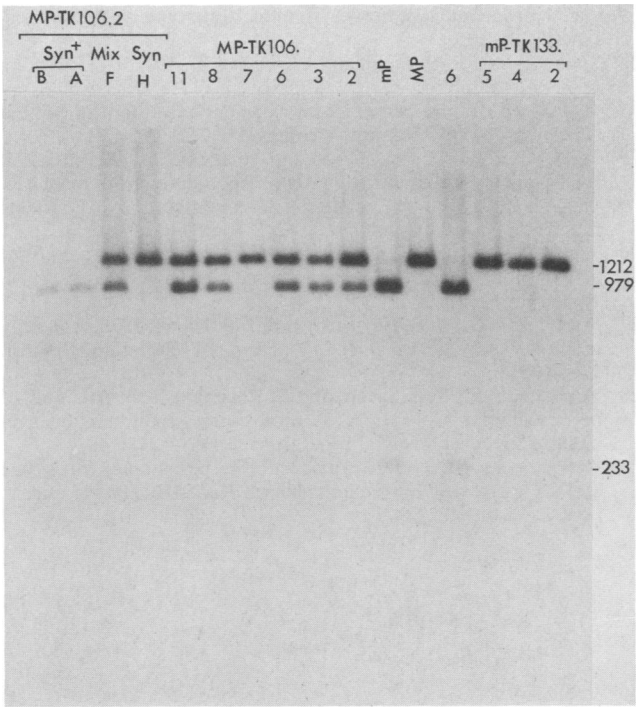

FIG. 4. Southern blot analyses of *Tha*I-digested DNAs from insertion mutants and the parental viruses, HSV-1(mP) and HSV-1(MP). The DNA fragments were fractionated by electrophoresis on a 2% agarose gel and transferred to Nytran. The single-stranded radioactive probe was produced by synthesis in vitro, by using as template the opposite strand of the *Pst*I-*Hin*cII fragment indicated in the legend to Fig. 1. The insertion mutants MP-TK106.2, MP-TK106.3, MP-TK106.6, MP-TK106.7, MP-TK106.8, and MP-TK106.11 were produced by cotransfection of HSV-1(MP) genomic DNA with a plasmid containing the *Bam*HI L fragment of HSV-1(mP) DNA inserted into a cloned copy of the HSV-1 *tk* gene. The derivatives designated B, A, F, and H were all plaque purified from MP-TK106.2. The insertion mutants mP-TK133.2, mP-TK133.4, and mP-TK133.5 were produced by cotransfection of HSV-1(mP) genomic DNA with a plasmid containing the *Bam*HI L fragment of HSV-1(MP) DNA inserted into a cloned copy of the HSV-1 *tk* gene. The mutant designated 6 was also isolated from progeny obtained after this cotransfection; it proved to be a spontaneous TK^- mutant, however, and not an insertion mutant. The insertion mutants designated MP-TK106.2, MP-TK106.5, MP-TK106.8, and MP-TK106.11 and the derivative designated MP-TK106.2F all produced the three types of plaques shown in Fig. 3. The insertion mutants mP-TK133.2, mP-TK133.4, mP-TK133.5, and MP-TK106.7 and the derivative MP-TK106.2H produced only Syn plaques (Fig. 3B). The insertion mutant derivatives MP-TK106.2B and MP-TK106.2A, as well as the spontaneous TK^- mutant 6, produced only Syn^+ plaques (Fig. 3C). Sizes of DNA fragments detected by the radiolabeled probe are given in base pairs. The 979- and 233-bp fragments were consistently found together. Overexposure of the autoradiogram (not shown) was necessary to detect the 233-bp fragment in every instance.

the *Bam*HI L fragment and the *a* sequence could be in some ways analogous to other elements shown to enhance rates of recombination in their vicinity, in both procaryotic and eucaryotic systems (see, for example, references 8 and 25).

The question arises whether the *Bam*HI L fragment of HSV-1 DNA contains the same element causing enhanced rates of recombination as does the *a* sequence. Lack of recombination between the *a* and *Bam*HI-L sequences may signify only that homology is too limited and not that the mechanisms are different. Only a small portion of the *Bam*HI L fragment has been sequenced, and, within this region, there is no evident homology with the *a* sequence.

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LITERATURE CITED

1. **Bond, V. C., and S. Person.** 1984. Fine structure physical map locations of alterations that affect cell fusion in herpes simplex virus type 1. Virology **132**:368–376.
2. **Chou, J., and B. Roizman.** 1985. Isomerization of herpes simplex virus 1 genome: identification of the *cis*-acting and recombination sites within the domain of the *a* sequence. Cell **41**:803–811.
3. **Davidson, A., and N. Wilkie.** 1983. Inversion of the two segments of the herpes simplex virus genome in intertypic recombinants. J. Gen. Virol. **64**:1–18.
4. **Debroy, C., N. Pederson, and S. Person.** 1985. Nucleotide sequence of a herpes simplex virus type 1 gene that causes cell fusion. Virology **145**:36–48.
5. **Gibson, M. G., and P. G. Spear.** 1983. Insertion mutants of herpes simplex virus have a duplication of the glycoprotein D gene and express two different forms of glycoprotein D. J. Virol. **48**:396–404.
6. **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 segments. Proc. Natl. Acad. Sci. USA **72**:4243–4247.
7. **Hubenthal-Voss, J., and B. Roizman.** 1985. Herpes simplex virus 1 reiterated S component sequences (c_1) situated between the *a* sequence and α4 gene are not essential for virus replication. J. Virol. **54**:509–514.
8. **Keil, R. L., and G. S. Roeder.** 1984. Cis-acting, recombination-stimulating activity in a fragment of the ribosomal DNA of S. cerevisiae. Cell **39**:377–386.
9. **Keller, J. M.** 1976. The expression of the syn^- gene of herpes simplex virus type 1. I. Morphology of infected cells. Virology **69**:490–499.
10. **Knipe, D. M., W. T. Ruyechan, R. W. Honess, and B. Roizman.** 1979. Molecular genetics of herpes simplex virus: the terminal *a* sequences of the L and S components are obligatorily identical and constitute a part of a structural gene mapping predominantly in the S component. Proc. Natl. Acad. Sci. USA **76**:4534–4538.
11. **Knipe, D. M., W. T. Ruyechan, B. Roizman, and I. W. Halliburton.** 1978. Molecular genetics of herpes simplex virus: demonstration of regions of obligatory and nonobligatory identity within diploid regions of the genome by sequence replacement and insertion. Proc. Natl. Acad. Sci. USA **75**:3896–3900.
12. **Lee, G. T.-Y., K. L. Pogue-Geile, L. Pereira, and P. G. Spear.** 1982. Expression of herpes simplex virus glycoprotein C from a DNA fragment inserted into the thymidine kinase gene of this virus. Proc. Natl. Acad. Sci. USA **79**:6612–6616.
13. **Lee, G. T.-Y., and P. G. Spear.** 1980. Viral and cellular factors that influence cell fusion induced by herpes simplex virus. Virology **107**:402–414.
14. **Manservigi, R., P. G. Spear, and A. Buchan.** 1977. Cell fusion induced by herpes simplex virus is promoted and suppressed by different viral glycoproteins. Proc. Natl. Acad. Sci. USA **74**:3913–3917.
15. **Mocarski, E. S., L. E. Post, and B. Roizman.** 1980. Molecular engineering of the herpes simplex virus genome: insertion of a second L-S junction into the genome causes additional genome inversions. Cell **22**:243–255.
16. **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. USA **78**:7047–7051.

17. **Mocarski, E. S., and B. Roizman.** 1982. Structure and role of the herpes simplex virus DNA termini in inversion, circularization and generation of virion DNA. Cell **31**:89–97.
18. **Poffenberger, K. L., E. Tabares, and B. Roizman.** 1983. Characterization of a viable, noninverting herpes simplex virus 1 genome derived by insertion and deletion of sequences at the junction of components L and S. Proc. Natl. Acad. Sci. USA **80**:2690–2694.
19. **Pogue-Geile, K. L., G. T.-Y. Lee, S. K. Shapira, and P. G. Spear.** 1984. Fine mapping of mutations in the fusion-inducing MP strain of herpes simplex virus type 1. Virology **136**:100–109.
20. **Pogue-Geile, K. L., G. T.-Y. Lee, and P. G. Spear.** 1985. Novel rearrangements of herpes simplex virus DNA sequences resulting from duplication of a sequence within the unique region of the L component. J. Virol. **53**:456–461.
21. **Roizman, B.** 1962. Polykaryocytosis. Cold Spring Harbor Symp. Quant. Biol. **27**:327–334.
22. **Sheldrick, P., and N. Berthelot.** 1974. Inverted repetitions in the chromosome of herpes simplex virus. Cold Spring Harbor Symp. Quant. Biol. **39**:667–678.
23. **Skare, J., and W. C. Summers.** 1977. Structure and function of herpesvirus genomes. II. EcoRI, XbaI and Hind III endonuclease cleavage sites on herpes simplex virus type 1 DNA. Virology **76**:581–595.
24. **Smiley, J. R., B. S. Fong, and W.-C. Leung.** 1981. Construction of a double-jointed herpes simplex viral DNA molecule: inverted repeats are required for segment inversion and direct repeats promote deletions. Virology **113**:345–362.
25. **Stahl, F. W., J. M. Crasemann, and M. M. Stahl.** 1975. Rec-mediated recombinational hot spot activity in bacteriophage lambda. III. Chi mutations are site mutations stimulating rec-mediated recombination. J. Mol. Biol. **94**:203–212.
26. **Varmuza, S. L., and J. R. Smiley.** 1984. Unstable heterozygosity in a diploid region of herpes simplex virus DNA. J. Virol. **49**:356–362.
27. **Wadsworth, S., G. S. Hayward, and B. Roizman.** 1976. Anatomy of herpes simplex virus DNA. V. Terminal reiteration. J. Virol. **17**:503–512.
28. **Wadsworth, S., R. J. Jacob, and B. Roizman.** 1975. Anatomy of herpes simplex virus DNA. II. Size, composition, and arrangement of inverted terminal repetitions. J. Virol. **15**:1487–1497.
29. **Wilkie, N. M.** 1976. Physical maps for herpes simplex virus type 1 DNA for restriction endonucleases *Hin*d III, *Hpa*-1, and *X. bad*. J. Virol. **20**:222–233.