Proc. Natl. Acad. Sci. USA
Vol. 76, No. 9, pp. 4534–4538, September 1979
Genetics

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*

(recombination/marker rescue/inversion/inverted reiterations)

DAVID M. KNIPE†‡, WILLIAM T. RUYECHAN†§, ROBERT W. HONESS¶, AND BERNARD ROIZMAN†

†The Marjorie B. Kovler Viral Oncology Laboratories, The University of Chicago, Chicago, Illinois 60637; and ¶The Division of Virology, National Institute for Medical Research, London, England

Contributed by Bernard Roizman, May 21, 1979

ABSTRACT **In herpes simplex virus 1 (HSV-1) DNA, a small sequence, designated the *a* sequence, flanks the reiterated sequences at the ends of both the L and S components. The *a* sequence is the only sequence shared by the termini of all isomeric arrangements of HSV-1 DNA that arise from inversions of the covalently linked L and S components. We report that the *a* sequence, although present in both components, is a part of a structural gene mapping predominantly in the reiterated sequences of the S component. This conclusion is based on the observations that the mutant HSV-1(13)*ts*C75 is rescued by transfection of cells with the mutant DNA and any one of the four terminal or four L-S junction fragments of wild-type DNA. Furthermore, in doubly infected cells, this mutant shows little or no recombination or complementation with other *ts* mutants previously mapped within the reiterated sequences of the S component. Because it is otherwise difficult to explain the isolation of a mutant with several independent, equivalent mutations, the data argue for a mechanism that maintains the identity of the multiple copies of the *a* sequence.**

The paradox arising from the two observations that all termini rescue the *ts* mutant but that in coinfection tests the *ts* lesion is closely linked to the reiterated sequences of the S component could be accounted for by postulating that either recombination occurs while the DNA is in a circular form—in which case all *a* sequences would be adjacent to the reiterated sequence of the S component—or recombination can occur while the DNA is in a linear form. In this case the only effective substitution of the *a* sequence that is perpetuated is the one occurring at the L-S junction or in the S component.

In light of the observations that *ts*C75 and the other mutants tested in this study map in the reiterated sequences and fail to yield appreciable recombinational frequencies, it is unlikely that isomerization of the DNA occurs by intramolecular recombination between reiterated sequences.

In this paper we report that a temperature-sensitive (*ts*) mutant of herpes simplex virus 1 (human herpesvirus 1, HSV-1) designated as HSV-1(13)*ts*C75 is converted to ts$^+$ phenotype by transfection of cells with intact mutant DNA and any of the individual DNA fragments containing the terminal sequences of the HSV-1 genome. The following properties of HSV-1 DNA, reviewed in detail elsewhere (1), are pertinent to the understanding of the significance of these findings.

(*i*) HSV-1 DNA consists of two covalently linked components designated as L and S and comprising 82 and 18%, respectively, of the total DNA. Each component consists of unique sequences, U_L and U_S, bracketed by inverted repeats (2). The inverted repeats of the L component, designated *ab* and *b'a'*, each contains 6% of the viral DNA whereas the inverted repeats of the S component, designated *a'c'* and *ca*, each consists of 4.3% of the total DNA.

(*ii*) The *a* sequences are present in the inverted repeats of both the L and S components and are arranged in the same orientation at the ends of the molecule (Fig. 1). The size of the *a* sequence operationally defined by the minimal amount of exonuclease digestion required to obtain the maximal frequency of the circularization of the genome (3) is less than 400 base pairs (4) and may be as little as 280 base pairs (5).

(*iii*) The idealized sequence arrangement presented above is *ab*-U_S*b'a'a'c'*-U_S-*ca*. However, HSV-1 DNA molecules may deviate from this idealized sequence in three respects. First, as much as 50% of the L-S junctions contain a single *a* sequence (5). Second, the L terminal fragments are heterogeneous in that they contain from one to several 280-base-pair repeats (5). Finally, the *a* sequence is repeated in its entirety or in part in an inverted orientation several hundred base pairs away (3, 4).

(*iv*) In addition, the L and S components can be inverted relative to each other. Thus, DNA extracted from virions consists of four equimolar populations of molecules differing in the orientation of the L and S components relative to each other. These populations have been designated as P (prototype), I_L (inversion of L component), I_S (inversion of S component), and I_{SL} (inversion of both components). A consequence of the inversion of L and S components relative to each other is that DNAs extracted from virions exhibit four different termini—two at the L and two at the S ends of the DNA—and four different L-S component junctions (see Fig. 1).

MATERIALS AND METHODS

Viruses. (HFEM)*ts*LB2 (6), HSV-1(17)*ts*D (7), HSV-1(13)*ts*F3 (8), HSV-1(MP) (9), and HSV-1(1061) (10) have been described elsewhere. The isolation of HSV-1 (13VB4)*ts*C75 (11) will be described elsewhere. The plating efficiency (38.5°C/34°C) of *ts*C75 was 1.2×10^{-5} in 13 independent determinations whereas the plating efficiency of the cloned parental HSV-1(13VB4) was 0.95 in 4 separate determinations.

Preparation of Viral DNA and Restriction Endonuclease DNA Fragments. The isolation of viral DNAs, purification of restriction endonucleases, conditions for digestion of DNA, electrophoresis of DNA fragments in agarose gels, and pre-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: *ts*, temperature sensitive; HSV-1, human herpes simplex virus 1.

* Paper no. 4 in a series. The preceding paper is ref. 12.

‡ Present address: Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115.

§ Present address: Department of Biochemistry, Uniformed Services University of the Health Sciences, Bethesda, MD 20014.

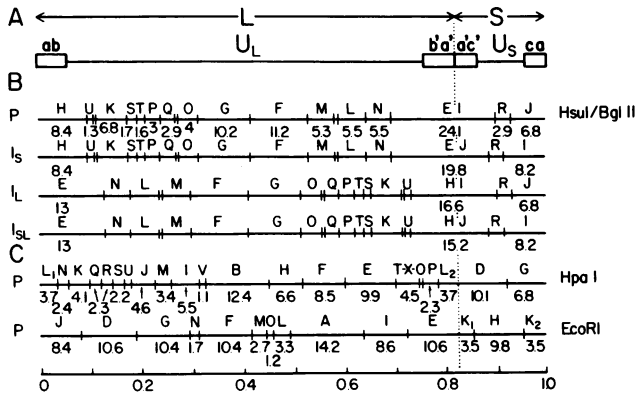

FIG. 1. Sequence and restriction endonuclease fragment arrangements in HSV-1 DNA used for the marker rescue mapping of HSV-1(13)*ts*C75. (*A*) Diagram of the structure of HSV DNA. *ab* and *b'a'* represent the inverted repeats bounding the L component, and *a'c'* and *ca* represent the inverted repeats bounding the S component. (*B*) *Hsu* I/*Bgl* II restriction endonuclease maps of the four isomers of HSV-1(1061) DNA. P, prototype; I_S, inverted S; I_L, inverted L; I_{SL}, inverted S and L. The number below each fragment denotes its molecular weight in millions. (*C*) Restriction endonuclease cleavage maps of HSV-1(MP) DNA in the P arrangement. Note that the 0.5 M L-S junction fragments are not shown as fused at the junction. The other forms of the DNA can be obtained by inverting the L component, the S component, or both.

parative purification of DNA fragments were as described (10, 12).

Marker Rescue Mapping. The genome location of the mutation in HSV-1 (13VB4)*ts*C75 was determined by the cotransfection marker rescue method as described (12).

Complementation and Recombination Tests. Complementation indices were determined according to Schaffer *et al.* (13). Replicate cultures of Vero cells were exposed either to individual mutants at multiplicities of 10 plaque-forming units per cell or to mixtures of two mutants at multiplicities of 5 plaque-forming units per cell of each. After 1 hr of adsorption at 39°C the cultures were replenished with mixture 199 supplemented with 1% inactivated fetal calf serum and further incubated for 17 hr at 39°C. Then, 5 ml of sterile skim milk was added to each dish and the cultures were frozen and thawed three times. Duplicate samples were pooled, sonicated, and titrated on Vero cells at 34°C.

Recombination tests were done in rabbit skin cell cultures at the multiplicities of infection described above. After 1 hr of adsorption at 34°C the cultures were replenished with the medium described above and further incubated at 34°C for 18 hr. The cultures were harvested as described above and titrated on Vero cells at 34 and 39°C.

RESULTS

Rescue of *ts*C75 with Restriction Endonuclease Fragments. Three sets of rescue experiments were done. In the first set, rabbit skin cells were transfected with mixtures of intact *ts*C75 DNA and the individual restriction endonuclease fragments of HSV-1(1061) DNA were generated by digestion with *Hsu* I and *Bgl* II enzymes. The results (Table 1) indicate that the mutant was rescued by fragments *Hsu* I/*Bgl* II E, H, I, and J—i.e., by all four terminal fragments.

The second set of experiments involved the cotransfection of *ts*C75 DNA and the L-S junction fragments of HSV-1(1061) DNA. All four junction fragments rescued *ts*C75 (Table 1). Therefore, the sequences rescuing *ts*C75 are present in both the termini and the L-S junction.

The third set of rescue experiments was designed to verify

Table 1. Marker rescue of HSV-1(13)*ts*C75

Fragment added	Ratio of titers, 39°C/33°C × 10^4
Rescue with 0.5 and 1.0 M fragments of HSV-1(1061) DNA: *ts*C75 DNA + *Hsu* I/*Bgl* II	
None	1.0
E	125
F	2.5
G	9.5
H	237
I	36
J	235
K	2.3
L, M	2.4
N	5.8
O	1.7
P	4.7
Q	0.8
R	3.4
Rescue with 0.25 M fragments of HSV-1(1061) DNA: *ts*C75 DNA + *Hsu* I/*Bgl* II	
None	0.5
E–I	44
E–J	33
H–I	41
H–J	13
Rescue with fragments contained within the reiterated sequences of HSV-1(MP): *ts*C75 DNA + MP DNA	
None	<0.014
Hpa I-L_1, L_2	7.8
Hpa I-N, O, P	<0.022
*Eco*RI-K_1, K_2	1.0

the conclusion that the wild-type sequences rescuing *ts*C75 were within the inverted repeats. *ts*C75 was rescued with the mixture of fragments *Hpa* I-L_1, L_2 of HSV-1(MP) DNA which contained the *a* sequence and a portion of the *b* sequence of both copies of the inverted repeats of the L component. A mixture containing *Hpa* I fragments N, O, and P failed to rescue the mutant. Moreover, *ts*C75 was also rescued by fragments *Eco*RI-K_1, K_2 of HSV-1(MP) DNA containing the *a* sequence and a portion of the *c* sequence of both copies of the inverted repeats of the S component. The frequency of ts^+ recombinants was lower in this set of experiments because the level of rescue decreases nearly exponentially as the fragment size decreases (12). We conclude from these series of experiments that the sequences capable of rescuing *ts*C75 are present within the reiterated sequences of both the L and S components and more specifically within the *a* sequences of the wild-type DNA.

Recombination and Complementation of *ts*C75 with Other Mutants. Recombination and complementation tests between *ts*C75 and other physically mapped mutations were done to define the genetic relationship of the terminal *a* sequences to the rest of the genome. The mutants utilized for these studies were *ts*D and *ts*LB2 [known to map within the reiterated sequences bounding the S component, positions 0.83–0.865 and 0.965–1.0 map units (14)] and HSV-1(13)*ts*F3 [which maps between 0.30 and 0.42 map unit (unpublished data)]. The mutant *ts*C75 showed significant complementation and recombination with *ts*F3 but failed to show any recombination with either *ts*D or *ts*LB2 (Table 2). The mutant *ts*C75 does show slightly higher recombinational frequencies when the crosses are performed in baby hamster kidney cells, but the recombinational frequencies observed with *ts*D, *ts*LB2, and several other members of the complementation group I-2 (13) are the lowest values observed among all crosses performed

Table 2. Recombination and complementation between pairs of *ts* mutants

Cells infected with	Recombination index*	*n*	Complementation index†
*ts*C75	0.003	2	
*ts*LB2	0.001	3	
*ts*F3	0.008 ± 0.001	3	
*ts*D	0.002 ± 0.001	3	
*ts*C75 + *ts*LB2	0.03 ± 0.01	4	0.86
*ts*C75 + *ts*F3	11.4 ± 3.2	4	7.5
*ts*C75 + *ts*D	0.05 ± 0.01	4	0.67
*ts*LB2 + *ts*F3	6.9 ± 1.9	4	3
*ts*LB2 + *ts*D	0.25 + 0.07	4	0.35
*ts*F3 + *ts*D	9.9 ± 1.9	4	6.2

* (39°C titer/33°C titer) × 10^2. *n* is number of independent determinations.

† (Yield of A + B)/(yield of A + yield of B).

(unpublished data). The mutants *ts*D and *ts*LB2 showed no complementation between themselves but both showed significant recombination and complementation with *ts*F3. On the basis of these experiments, *ts*C75 appears to be in complementation group I-2—i.e., the same as *ts*LB2 and *ts*D (13)—and although in the wild-type DNA the sequences rescuing *ts*C75 occur in at least four copies distributed in the inverted repeated sequences at the three noncontiguous locations (Fig. 1), the sequence itself is part of a structural gene mapping in fewer locations.

The Phenotype of Mutant *ts* C75. Fig. 2 shows an autoradiogram of electrophoretically separated polypeptides specified by *ts*C75, its parent HSV-1(13), *ts*LB2, and its parent HSV-1(HFEM) labeled at the permissive (33°C) and nonpermissive (39°C) temperatures. *ts*LB2 expressed only the α polypeptides ICP 4, 0, and 27 at 39°C but resembled its parent at 33°C. The mutant *ts*C75 expressed large amounts of α polypeptides at 39°C plus small amounts of some β polypeptides ICP 6 and 8 and a trace of the γ polypeptide ICP 5. At 33°C this mutant also expressed a pattern of polypeptides similar to that of its parent. Both parental viruses showed virtually identical patterns at 33 and 39°C.

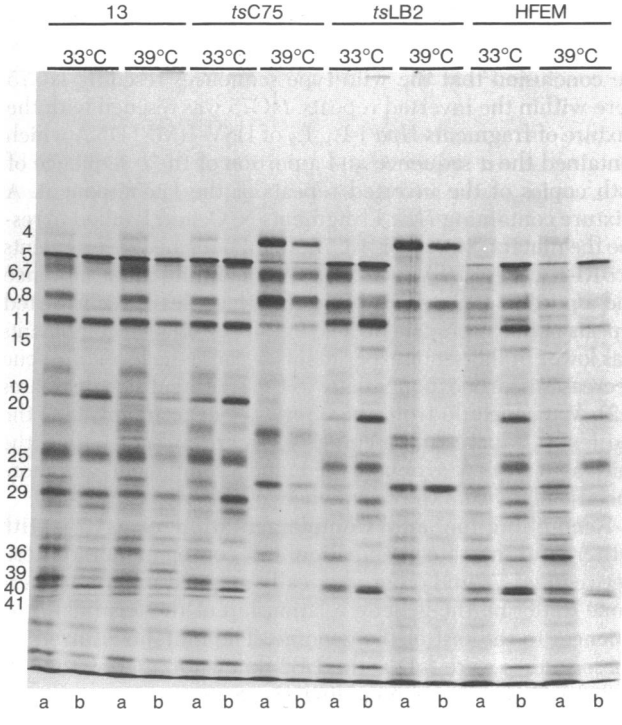

FIG. 2. Autoradiograms of electrophoretically separated polypeptides specified by HSV-1(13VB4)*ts*C75, HSV-1(HFEM)*ts*LB2, and their wild-type parents. HEp-2 cultures were infected with the respective viruses [13 = HSV-1(13); *ts*C75 = HSV-1(13)*ts*C75; *ts*LB2 = HSV-1(HFEM)*ts*LB2; HFEM = HSV-1(HFEM)] at 20 plaque-forming units per cell and incubated at 34 or 39°C as indicated. The lanes designated a carried samples from infected cells labeled with [^{14}C]isoleucine, [^{14}C]leucine, and [^{14}C]valine at 5–7 hr after infection. The lanes designated b carried samples labeled at 10–12 hr after infection. The polypeptides were subjected to electrophoresis in 9.25% polyacrylamide gels in the presence of sodium dodecyl sulfate.

Therefore, both mutants showed similar restricted patterns of polypeptide expression at 39°C, a result consistent with *ts*C75 being in the same complementation group as *ts*LB2.

DISCUSSION

The prominent features of the data presented in this paper are that the mutant HSV-1(13)*ts*C75 can be converted to ts$^+$ phenotype by marker rescue with any of the fragments containing the reiterated sequences from either the L or S components of the viral DNA, even though by other tests the mutant sequences appear to be within the same gene as those of mutants that map in the reiterated sequences of the S component. The resolution of this paradox has important bearing on the structural and genetic organization of HSV DNA.

Implication of the Marker Rescue Studies. The observation that *ts*C75 is rescued only by all restriction endonuclease fragments containing the sequences *ab*, *b'a'*,*ac*, and *c'a'* leads to four conclusions. First, the mutation is likely to be in the *a* sequence inasmuch as this is the only region of the DNA known to be common to all of these DNA fragments (4). Second, all of the *a* sequences in the mutant DNA must be altered in some way inasmuch as *ts*C75 does not rescue itself by intermolecular or intramolecular recombination. Third, it is not likely that the temperature sensitivity of the mutant is due to several nonidentical mutations because each individual fragment rescues the mutant independently. Last, there must be a mechanism that in this instance ensured that the *ts* lesion appeared in all the *a* sequences of the DNA of *ts*C75. This conclusion is based on the following argument. If we assume for the sake of illustration that the *a* sequence is at most 400 base pairs long, which constitutes 0.25% of the viral genome, and that the probability of introducing a mutation in all regions of the genome is approximately equal, the probability of introducing a mutation in one copy of the *a* sequence is 0.0025. The probability of producing *n* independent mutations only in the *a* sequences is then $(0.0025)^n$. At the very least there would have to be three independent mutations, lowering the probability to 3.75×10^{-7}. Because there is at least one copy of the *a* sequence at each terminus of the DNA molecule and one or two copies at the L-S junction and in many cases tandem duplications at each of these locations, the probability of isolating such a mutant would be very low and in most cases this would occur after the entire genome was saturated with other *ts* mutations and lethal mutations.

The last conclusion is of particular interest because evidence for a mechanism that ensures the identity of the terminal portions of *a'c'* and *ca* reiterated sequences of the S component by copying of sequences from one end into the other has already been reported (14, 15). The studies on *ts*C75 demonstrate the identity of the sequences flanking the reiterated sequences of the L and S components and suggest that copying of sequence information from one end to the other may also be the mechanism for the maintenance of this identity.

Implications of the Complementation and Recombination Analysis in Doubly Infected Cells. The observations that

*ts*C75 does not complement *ts*LB2 or *ts*D and that the frequency of ts$^+$ recombinants in the progeny of doubly infected cells is very low or insignificant suggest that *ts*C75, *ts*LB2, and *ts*D all contain mutations in the same cistron. Previous studies have suggested that the mutations in *ts*D and *ts*LB2 are within the gene for α polypeptide 4, independently mapped in the *a'c'* and *ac* sequences of the S component (14–19). The complementation and recombination data and the proximity of the *ts*C75 mutations at the L-S junction and in the S component to the previously mapped location of the gene for α polypeptide 4 argue that this mutation is also in that structural gene.

A paradox becomes apparent from comparison of the results of the marker rescue by transfection and of genetic recombination in doubly infected cells. Specifically, the marker rescue studies indicate that *ts*C75 is converted to ts$^+$ phenotype independently by all terminal and L-S junction fragments and therefore the sequences containing the *ts* mutation are both in close proximity to and distant from the sequences containing the *ts*LB2 and *ts*D mutations. These results would predict that recombinants arising from a crossover in the L or S components (Fig. 3 *A* and *B*) would express a ts$^+$ phenotype and, because of the size of the L component, such recombinants should form at a high frequency. However, the low or insignificant frequency of the ts$^+$ recombinants in doubly infected cells (Table 2) indicates that the site of the *ts*C75 mutation that must be replaced by wild-type sequences to yield ts$^+$ recombinants is in close proximity to the *ts*LB2 and *ts*D mutations.

There are two plausible explanations for this paradox. The first explanation is based on the assumption that recombination occurs in a linear form and that the *a* sequences are not genetically equivalent even though they are structurally identical (Fig. 3). According to this hypothesis replacement of the mutated sequences at the termini of the DNA molecule by wild-type sequences does not convert the mutant to ts$^+$ phenotype because the terminal *a* sequence is not transferred from the terminal position to the L-S junction. This hypothesis is consistent with the current model of replication of HSV-1 DNA. The model, described elsewhere in detail (15, 20), envisions that the DNA circularizes after digestion of the terminal *a* sequence by a processive exonuclease, that a head-to-tail concatemer is generated as a consequence of the replication and, finally, that the missing terminal *a* sequence is regenerated by repair synthesis using the internal inverted repeat as a template. Moreover, replacement of a mutated terminal *a* sequence by re-

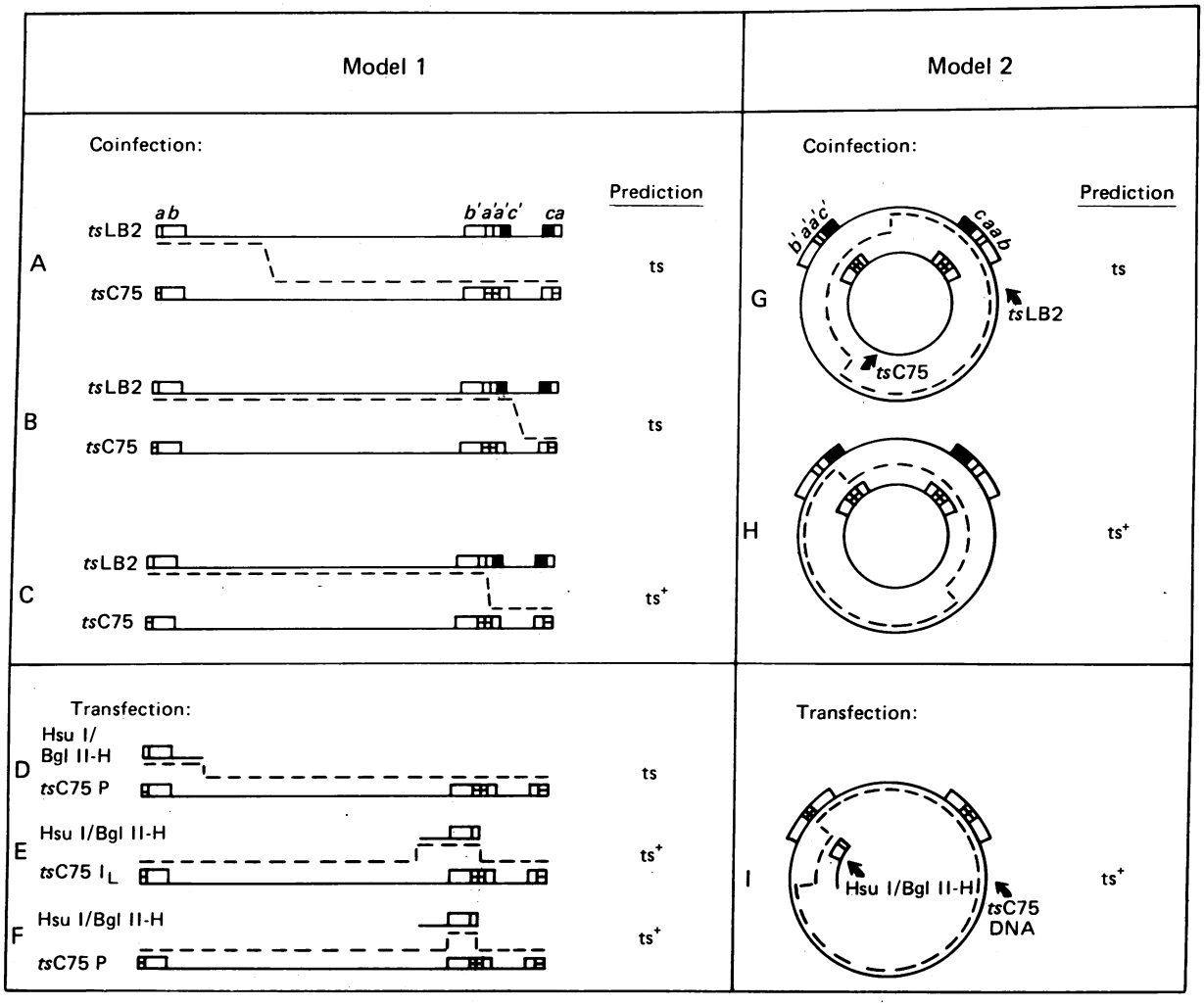

FIG. 3. Diagrams of two models explaining the unusual genetic behavior of HSV-1(13)*ts*C75. The blocks on top of each line represent the repeated regions of the genome: solid blocks represent a *ts*LB2 mutated sequence and blocks with a horizontal line represent a *ts*C75 mutated sequence. (*Left*) Model 1 predicts that recombination takes place between linear forms of the HSV DNA but that replacement of reiterated sequences at the ends of the molecule would not result in the expression of the ts$^+$ phenotype because repair copying from the internal repeats obliterates the rescuing sequences. Therefore, only the recombinational events in *C*, *E*, and *F* would lead to ts$^+$ progeny. (*Right*) Model 2 predicts that recombination takes place between circular forms of the HSV DNA and only a crossover event between an *a* and an *ac* sequence will lead to ts$^+$ progeny, as shown in *H* and *I*.

combination would not lead to its appearance in the internal inverted repeat because it would be lost during replication and subsequent repair synthesis. In order for the replaced *a* sequence to become established in the DNA molecule, the recombinational event would have to occur at the internal inverted repeat as illustrated in Fig. 3C. According to this hypothesis, in marker rescue experiments the mutated *a* sequences were replaced in all sites. However, the successful conversion to ts^+ phenotype resulted only from initial substitution of the mutated *a* sequence at the internal inverted repeat (Fig. 3 *E* and *F*).

An alternative nonexclusive hypothesis is based on the assumption that, in doubly infected cells, recombination occurs chiefly while the DNA is in a circular form. As illustrated in Fig. 3 *G* and *H*, the recombination between two circular DNA molecules requires that one of the two crossover sites must occur between the nearest *ts*C75 and *ts*LB2 or *ts*D mutations. Inasmuch as the distance between two adjacent sites is small, the expected recombination frequency should be also small or insignificant. This hypothesis makes no assumptions about and neither supports nor contradicts the concept of obligatory identity of *a* sequences discussed in the preceding sections. However, it should be noted that, if all *a* sequences were genetically equivalent, the crossover generating the ts^+ recombinant could occur at either or both of the two L-S junctions in the circle. However, if one of the junctions were modified and subsequently the unit length molecule were excised at this modified junction, it would be expected, as described in the preceding paragraph, that only crossover events at the unmodified L-S junction would yield ts^+ recombinants. The experiments described in this paper do not distinguish between these two possibilities.

The first model does suffer from one potential problem. It further requires that there is no intermolecular recombination between a recombinant molecule generated as shown in Fig. 3*A* and a *ts*C75 DNA molecule that would transfer the wild-type *a* sequence to the L-S junction of the *ts*C75 DNA molecule because this type of recombinational event would generate a ts^+ recombinant. If replication and repair-mediated replacement of the terminal *a* sequences were to occur immediately after recombination, this type of event would not be possible, of course. Because of the simplicity of model 2 and recent results showing genetic linkage of markers at both ends of L to markers in the S component (unpublished data and ref. 15), we tend to favor the circular model of recombination as an explanation for the genetic behavior of *ts*C75.

Implications for the Mechanism of Isomerization of HSV DNA. Our results do permit a corollary conclusion concerning the mechanism by which DNA molecules exhibiting inversions of the L and S components arise. It has been suggested that the inversions arise as a result of legitimate recombination between the 5.8×10^6 molecular weight *ab* sequence at the terminus of the L component of the 4.1×10^6 molecular weight *ca* sequence and their internal inverted repeats (2). Because analyses of the DNA produced by clonal infections invariably yield all four arrangements of the DNA in approximately equimolar concentrations, intramolecular recombinational events would have to occur at high frequency. If this were the case, it would be expected that the recombinational event illustrated in Fig. 3 *A* or *G* could be followed by an intramolecular recombinational event, generating progeny with a ts^+ phenotype. Inasmuch as the frequency of ts^+ recombinants was insignificant, it follows that intramolecular recombination between the terminal sequences and their inverted repeat is probably not a common occurence and that the frequency of isomerization of HSV DNA resulting from this process cannot be very high. A current model of DNA replication (7, 15, 20) envisions that the isomerization of the DNA is a direct consequence of the obligatory repair of the terminal sequences of the unit length molecules from the concatemers and, by this model, isomerization could occur at a high frequency.

Dual Role of Reiterated Sequences in HSV DNA. The data presented here and in published reports (14, 16, 18, 19) show that the repetitive sequences of both the L and S components of HSV serve as portions of structural genes for early viral gene products. It is likely that these reiterated sequences serve an additional role such as structural elements of the DNA for chromosome structure or DNA replication because it has been demonstrated that the virus does not need functional genes in both ends of the S component to be viable (14). We have instead argued that the structural genes constitute a part of the reiterated sequences to stabilize the reiterated sequence from genetic drift (1). The presence of reiterated sequences as portions of eukaryotic gene coding sequences (21) may also be explained by this hypothesis.

We thank Mayer Stein for his invaluable technical assistance. D.M.K. was a Special Fellow of the Leukemia Society of America. W.T.R. was a Postdoctoral Fellow of the National Cancer Institute. This research was supported by grants from the American Cancer Society (VC 103N) and the National Cancer Institute (CA 08494 and CA 19264).

1. Roizman, B. (1979) *Cell* **16,** 481–494.
2. Sheldrick, P. & Berthelot, N. (1974) *Cold Spring Harbor Symp. Quant. Biol.* **39,** 667–678.
3. Hyman, R. W., Burke, S. & Kudler, L. (1976) *Biochem. Biophys. Res. Commun.* **68,** 609–615.
4. Wadsworth, S. C., Hayward, G. S. & Roizman, B. (1976) *J. Virol.* **17,** 503–512.
5. Wagner, M. J. & Summers, W. C. (1978) *J. Virol.* **27,** 374–387.
6. Halliburton, I. W., Randall, R. E., Killington, R. A. & Watson, D. H. (1977) *J. Gen. Virol.* **36,** 471–494.
7. Brown, S. M., Ritchie, D. A. & Subak-Sharpe, J. H. (1973) *J. Gen. Virol.* **18,** 329–346.
8. Manservigi, R., Cassai, E., Tognon, M. & Jacob, R. J. (1979) *Microbiologica* **2,** 51–64.
9. Hoggan, M. D. & Roizman, B. (1959) *Am. J. Hyg.* **70,** 208–219.
10. Ruyechan, W. T., Morse, L. S., Knipe, D. M. & Roizman, B. (1979) *J. Virol.* **29,** 677–697.
11. Heine, J. W., Honess, R. W., Cassai, E. & Roizman, B. (1974) *J. Virol.* **14,** 640–651.
12. Knipe, D. M., Ruyechan, W. T. & Roizman, B. (1978) *J. Virol.* **29,** 698–704.
13. Schaffer, P. A., Carter, V. C. & Timbury, M. C. (1978) *J. Virol.* **27,** 490–504.
14. Knipe, D. M., Ruyechan, W. T., Roizman, B. & Halliburton, I. W. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 3896–3900.
15. Roizman, B., Jacob, R. J., Knipe, D. M., Morse, L. S. & Ruyechan, W. T. (1979) *Cold Spring Harbor Symp. Quant. Biol.* **43,** 808–826.
16. Stow, N. D., Subak-Sharpe, J. H. & Wilkie, N. M. (1978) *J. Virol.* **28,** 182–192.
17. Frenkel, N., Locker, H., Batterson, W., Hayward, G. & Roizman, B. (1976) *J. Virol.* **20,** 527–531.
18. Morse, L. S., Pereira, L., Roizman, B. & Schaffer, P. A. (1978) *J. Virol.* **26,** 389–410.
19. Preston, V. G., Davison, A. J., Marsden, H. S., Timbury, M. C., Subak-Sharpe, J. H. & Wilkie, N. M. (1978) *J. Virol.* **28,** 499–571.
20. Jacob, R. J., Morse, L. S. & Roizman, B. (1979) *J. Virol.* **29,** 448–457.
21. Lis, J. T., Prestidge, L. & Hogness, D. S. (1978) *Cell* **14,** 901–919.