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Interruption-Deficient Mutants of Bacteriophage T5

II. Properties of a Mutant Lacking a Specific Interruption†

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An examination was made of the properties of T5HA4, a mutant of bacteriophage T5 that lacks the single-chain interruption that occurs at 7.9% from the left end of the genome. The DNAs of T5HA4 and the wild type were compared by electrophoresis in agarose gels of both single-stranded fragments produced by denaturation and duplex fragments generated by sequential treatment with exonuclease III and SI nuclease. These studies demonstrated that T5HA4 also lacks an interruption that occurs at 99.6% in wild-type DNA. The interruptions at 7.9 and 99.6% therefore occur within the 8.3% of T5 DNA that is terminally repetitious. Evidence on the location of other interruptions within the terminal repetition was also obtained. Analysis of T5HA4 DNA with a restriction endonuclease indicated that the interruption deficiency is not due to a deletion or addition mutation. The injection of T5HA4 DNA into a host bacterium was found to occur, as with the wild type, in a two-step manner. The interruption at 7.9% is therefore not required for stopping DNA transfer after the initial 8% segment has been injected.

The genome of bacteriophage T5 contains a number of site-specific single-chain interruptions within one strand of its linear double-stranded DNA. As described in the preceding paper (18), mutants of T5 have been isolated that either lack specific interruptions or are deficient for interruptions throughout the genome. The latter class of mutations appears to define two genes that participate in the formation of interruptions in T5 DNA. In contrast, the single-site mutants appear to have undergone alterations of the recognition sequences that mark the positions of the interruptions. Mutants have been obtained that lack each of the four principal interruptions, which occur at 7.9, 18.5, 32.6, and 64.8% from the left end of T5 DNA, as well as one secondary interruption, located at 45.3%.

The existence of single-site interruption-deficient T5 mutants permits direct testing of the functional significance of specific interruptions. The present report is concerned with the properties of a mutant that lacks the interruption at 7.9%. This site is of interest for several reasons. It occurs close to the boundary between the repeated and nonrepeated segments at the left end of the genome. The terminal repetition in T5 DNA has been estimated to comprise 8.3% of intact DNA (16, 17). The 7.9% interruption also occurs close to the boundary between the first- and second-step transfer segments of T5 DNA. T5 is unique in that injection of its genome into a host bacterium occurs by a two-step process. Initially, an 8.0 to 8.5% segment, the first-step transfer (FST) DNA, is injected (14). Expression of at least two FST genes is then required before the remaining 92% of the genome is transferred (11). The FST segment appears to be at the left end of the T5 genome (10, 20). Because of its location, the interruption at 7.9% is therefore a possible candidate for the signal that terminates the first stage of DNA transfer.

The results obtained in this study have confirmed that the 7.9% interruption lies within the terminal repetition in T5 DNA. Mutants that lack the 7.9% interruption are also deficient for an analogous site at 99.6%. In addition, these studies have provided evidence on the location of various secondary interruptions that occur within the terminal repetition. The interruption at 7.9%, however, does not appear to play a role in DNA transfer. In the absence of this interruption, injection occurs in the normal, two-step manner.

MATERIALS AND METHODS

Bacteria and bacteriophage. The bacteria and wild-type T5 phage used in this study have been described previously (18). T5*st*(102) is a viable, heat-

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stable deletion mutant that lacks 10.2% of T5*st*(+) DNA (22). T5HA4, which lacks the 7.9% interruption, was originally isolated in T5*st*(102) as described in the preceding paper (18). The original isolate was able to grow on *Escherichia coli* B23 and B40*su*1, but was not able to grow on *E. coli* F. The inability to grow on *E. coli* F was eliminated by backcrossing against T5*st*(+). The recombinant obtained, however, adsorbed poorly on *E. coli* F. A fast-adsorbing, HA4 recombinant was obtained by performing a second backcross against T5*st*(+). This stock was used for the experiments reported here.

Analysis of T5HA4 DNA. The procedures for electrophoretic analysis of T5 DNA have been described previously. Single-stranded DNA was analyzed in 5-mm-thick horizontal agarose slab gels (16, 19). Double-stranded DNA underwent electrophoresis similarly or in cylindrical vertical gels (16). Treatment of T5 DNA with λ exonuclease, *Hpa*I restriction endonuclease, or exonuclease III and SI nuclease was carried out as described (5, 16).

Measurement of two-step transfer of T5 DNA. The procedure used to measure two-step transfer of T5 DNA was that of Lanni (11), except that shear treatment of infected cells was carried out for 6 min at "HIGH" speed in a VirTis 45 homogenizer. ^{32}P-labeled phage were purified on CsCl step gradients.

Isolation of intracellular DNA from FST complexes. FST complexes (bacteria to which T5 phage have been adsorbed and have injected only the FST DNA), prepared and sheared as above, were centrifuged, washed, and resuspended in 0.1 M Tris-0.1 M NaCl-0.1 M EDTA, pH 8.0. DNA was extracted from the complexes with sodium dodecyl sulfate (SDS) and Pronase (2) and was dialyzed against 10 mM Tris-hydrochloride-0.1 mM EDTA (pH 8.0). In one experiment an attempt was made to separate the FST DNA from the host DNA by using the lysozyme-EDTA-Triton X-100 procedure used for plasmid DNA (9). The FST DNA, however, was found associated with the membrane-host DNA fraction. The FST DNA was released from this complex by the SDS/Pronase procedure used for intact cells (2) and was separated from the bulk of the host DNA by sedimentation through 6 to 20% neutral sucrose gradients. The pooled fractions were concentrated by pressure dialysis against 10 mM Tris-hydrochloride-0.1 mM EDTA (pH 8.0) before electrophoretic analysis.

RESULTS

The search for interruption-deficient mutants of T5 described in the preceding paper resulted in the isolation of seven mutants that lacked the single-chain interruption at 7.9% (18). These mutants were identified because, after denaturation, their DNAs lacked the single-chain fragments that have one end at 7.9%. These include fragments 18, 21, and 22 (Fig. 1a and b). The first mutant isolated in this category, T5HA4, was chosen for further study. The HA4 mutation has already been shown to be neither dominant nor recessive to the wild type (18). Phage carrying this mutation are viable and have the same average burst size as the wild type.

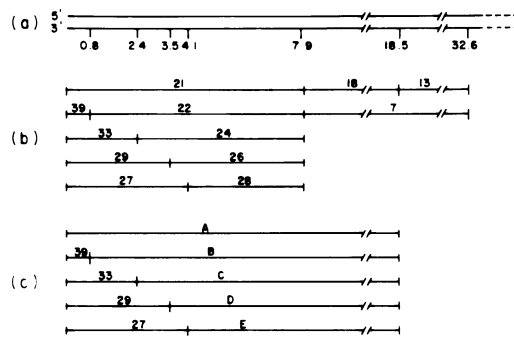

FIG. 1. *Maps of single-chain interruptions and fragments at the left end of wild-type T5 and T5HA4 DNAs. (a) Positions of interruptions in T5st(+) DNA, expressed as a percentage of wild-type length from the left end of the molecule. The strand polarities and interruptions are shown as determined in reference 16. (b) Locations of single-chain fragments at the left end of T5st(+) DNA. With the exception of fragment 30, which occurs between 0 and 3.3%, all previously mapped (16) fragments are shown. The st(102) deletion occurs between the 18.5 and 32.6% interruptions. (c) Locations of single-chain fragments at the left end of T5HA4 DNA. The positions of the left ends of fragments A through E were calculated by subtracting their molecular weights, given in Fig. 3, from the molecular weight of the interval from 0 to 18.5%, as determined electrophoretically (16). There is a small discrepancy between this value, 18.0% of T5st(+) DNA, and the value of 18.5% determined by electron microscopy (21).*

Analysis of single-stranded fragments of T5HA4 DNA. As described below and in the preceding paper (18), loss of fragment 18 and many of the small fragments at the left end of the genome clearly indicates that T5HA4 lacks the interruption at 7.9%. However, since the terminal repetition in T5 equals 8.3% of intact DNA, the sequence that contains the 7.9% interruption should also occur close to the right end of the molecule. Evidence has been obtained that interruptions occur at analogous sites within the two copies of the terminal repetition in T5 (16). Thus, if the previous analyses are correct, T5HA4 should lack two interruptions, one at 7.9% and one at approximately 99.6%.

The existence of an interruption at 99.6% in wild-type T5 DNA, and its absence in T5HA4 DNA, can be demonstrated by comparing the single-chain fragments of the two DNAs. Only the wild type should have a short fragment of approximately 450 bases that occurs at the right end of the interrupted strand of T5 DNA. To resolve the smaller single-chain fragments, we subjected denatured T5*st*(+) and T5*st*(+) HA4 DNAs to electrophoresis in 1.4% agarose slab

gels. The results (Fig. 2) show that, for fragments of less than 4% of an intact strand, the single chains of the two DNAs were extremely similar. However, the fastest migrating of the detectable wild-type fragments appeared to be absent in HA4 DNA. Since the external 5′ end of the interrupted strand occurs at the right end of the molecule (16), the terminal location of this fragment can be demonstrated by limited digestion of wild-type DNA with λ exonuclease, an enzyme that is specific for external 5′ termini (4, 15). The result of digestion of T5*st*(+) DNA to 1% acid-soluble material with λ exonuclease is shown in slot 1 of Fig. 2. The only effect of exonuclease treatment was loss of the small fragment that is missing in T5HA4 DNA. This fragment therefore occurs at the external 5′ end of the interrupted strand. Digestion to 0.5% with λ exonuclease was sufficient to yield the results shown in Fig. 2. Identical electrophoretic patterns were also obtained with up to 5% digestion. The results with higher levels of digestion were not surprising, since, with the exception of the small terminal fragment, all of the fragments that occur within the right terminal repetition also occur at the left end of the DNA. The size of the 5′-terminal fragment identified in Fig. 2 has not been accurately measured. Its electrophoretic mobility, however, is compatible with the previous estimates of 450 bases.

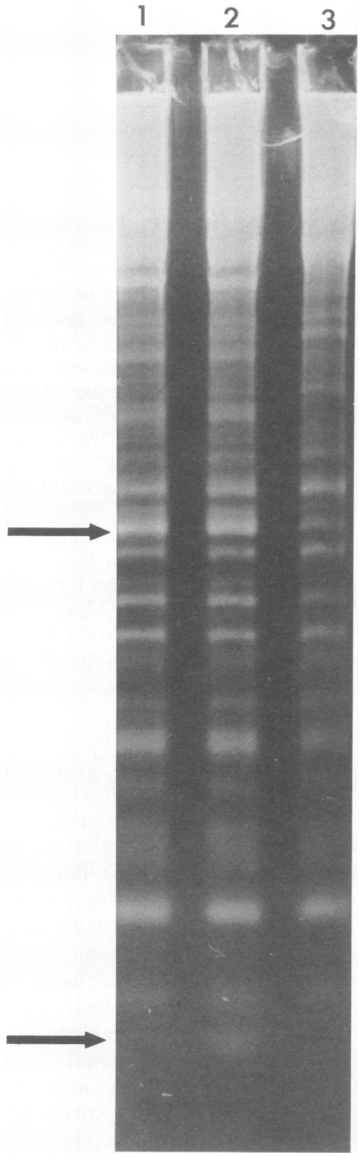

FIG. 2. *Electrophoresis of the small single-chain fragments of T5 DNA. Electrophoresis was carried out in a horizontal 1.4% agarose slab gel. The length of the gel was 15 cm. Each slot contained 15 μg of alkali-denatured DNA. Slot 1, T5st(+) DNA after 1% digestion with λ exonuclease; slot 2, T5st(+) DNA; slot 3, T5st(+)HA4 DNA. The upper arrow indicates the position, in gels 1 and 2, of fragment 28, which equals 3.8% of an intact strand. The lower arrow indicates the 5′-terminal fragment that is missing in T5HA4 DNA.*

Analysis of duplex fragments of T5HA4 DNA. Loss of the interruptions at 7.9 and 99.6% should result in alterations of many of the single-chain fragments produced by denaturation of T5 DNA. As noted above, fragments that have one terminus at either interruption should disappear while new fragments that span these sites should become evident. Analysis of these new fragments, many of which will have one terminus at a secondary interruption, can provide further information on the structure of T5 DNA. In principle, this information can be obtained from analyses of single-chain fragments as shown in Fig. 2. However, the clearest results have been obtained from analysis of the analogous duplex fragments generated by sequential treatment with exonuclease III of *E. coli* and SI nuclease of *Aspergillus oryzae*. Limited hydrolysis by exonuclease III at the 3′ side of each interruption, followed by digestion of the single-stranded regions by SI nuclease, results in cleavage of the duplex molecule at the site of each interruption (16). Since the electrophoretic mobilities of duplex molecules are almost entirely dependent on molecular weight in agarose gels (24), analysis of these fragments yields an accurate map of the interruptions.

The results of sequential exonuclease III-SI nuclease digestion of T5*st*(+) and T5*st*(102) DNA in the presence and absence of the HA4 mutation are shown in Fig. 3. Electrophoresis was carried out in 0.5% agarose gels to resolve the larger duplex fragments. The fragments are

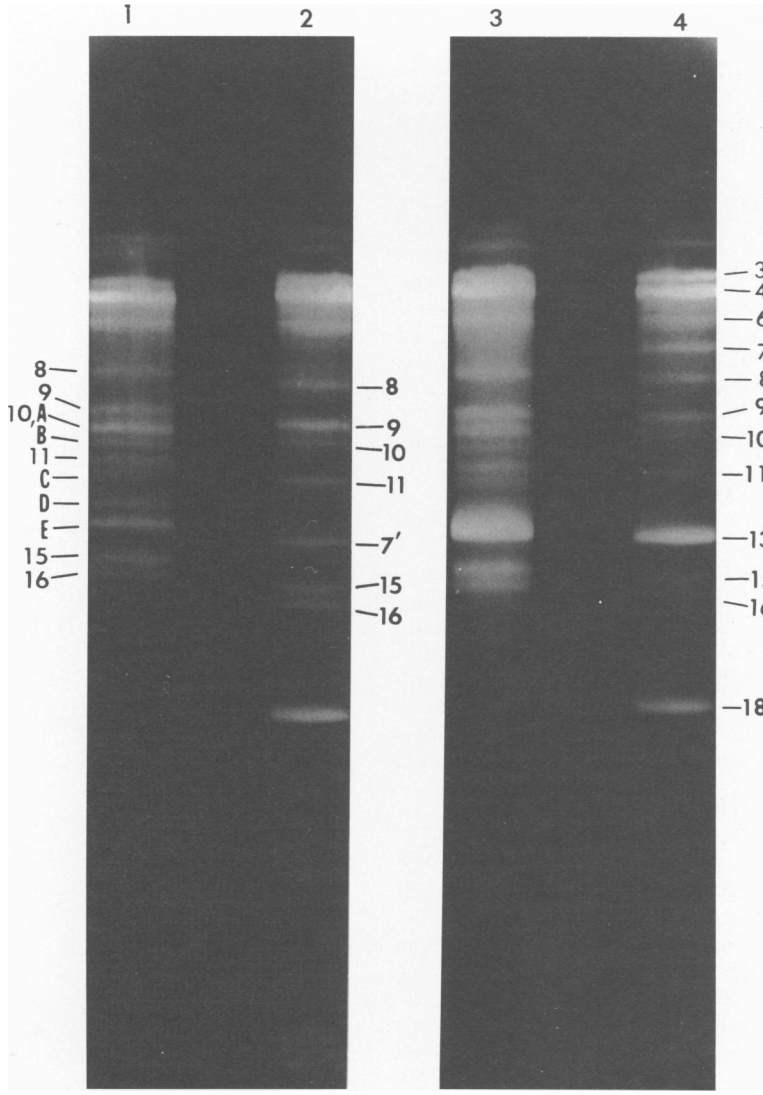

FIG. 3. *Electrophoresis of the large duplex fragments generated by exonuclease III-SI nuclease treatment of T5 DNA. Electrophoresis was carried out in cylindrical 0.5% agarose gels. Gel 1, T5st(102)HA4 DNA; gel 2, T5st(102) DNA; gel 3, T5st(+)HA4 DNA; gel 4, T5st(+) DNA. The fragments that occur in T5st(+) DNA are numbered in order of decreasing size (see 16). Electrophoresis in the presence of reference molecules was employed to identify the numbered fragments in the other samples. The letters A through E denote five new fragments that appear in T5st(102)HA4 DNA. Their molecular weights, expressed as a percentage of T5st(+) DNA, are 17.4% for B, 15.8% for C, 14.6% for D, and 14.0% for E. Fragment A comigrates with fragment 10, and therefore equals approximately 18.4% of T5st(+) DNA.*

numbered in order of decreasing size as they occur in T5*st*(+) DNA (gel 4). The deletion in T5*st*(102) DNA does not remove an interruption. Therefore, the only detectable alteration in this DNA is loss of two fragments, 7 and 13, that span the deletion. Fragment 7′, the shortened version of fragment 7, can be seen in Fig. 3 (gel 2). Fragment 13′ is too small to be seen in this experiment.

The HA4 mutation results in loss of fragments 7, 18, 21, and 22 of those visible in Fig. 3. A number of new fragments are evident. Analysis of T5*st*(102) DNA is most useful in this regard since several of the new fragments are obscured by fragment 13 in T5*st*(+) DNA. The five new fragments detectable in T5*st*(102)HA4 DNA are labeled A through E in Fig. 3 (gel 1). The existence of fragment A is implied because of the

increased intensity of the band containing fragment 10. Since no interruptions have been located between the principal interruptions at 7.9 and 18.5% (16, 21), fragments A through E presumably extend leftward from 18.5% to various sites near the left end of the molecule. The positions of these fragments, based on the above assumption and their molecular weights, are shown in Fig. 1. Fragment A appears to extend from the interruption of 18.5% to the left end of the molecule. The other four fragments terminate at 0.6, 2.2, 3.4, and 4.0%. These positions agree well with the locations of 0.8, 2.4, 3.5, and 4.1% previously determined for secondary interruptions within the left terminal repetition in T5 DNA (16).

The smaller fragments generated by exonuclease III-SI nuclease treatment of T5*st*(+)HA4 DNA were analyzed in 1.0% agarose gels (Fig. 4). T5HA4 DNA would be expected to lack all short fragments that extend leftward from 7.9% in wild-type DNA. Fragments that extend rightward from the left end of the DNA to secondary interruptions in the terminal repetition should not be altered by the HA4 mutation. As indicated in Fig. 4, T5*st*(+)HA4 DNA lacks fragments 21, 22, 24, 26, and 28 and retains fragments 27, 29, and 30. This result agrees with the previously determined locations of these fragments (Fig. 1).

T5*st*(+)HA4 DNA contains a number of new small duplex fragments (Fig. 4). These are presumably derived from the right end of the DNA where loss of the 99.6% interruption will result in fusion of the short terminal fragment described above (Fig. 2) to the fragments contained within the right terminal repetition. Thus, slightly longer versions of fragments 22, 24, 26, and 28 should be present in T5HA4 DNA. Bands that could correspond to most of the predicted fragments were seen; however, it was difficult to analyze the right end of T5 DNA by use of the exonuclease III-SI nuclease procedure. Since the 3′ end of the intact strand occurs at the right end of the DNA, fragments that extend to the right end will be degraded from both sides by exonuclease III. This effect, especially if exonuclease III acts preferentially at duplex 3′ termini, precludes accurate measurement of molecular weights.

Analysis with a restriction endonuclease. T5HA4 was isolated after hydroxylamine mutagenesis, which induces point mutations. However, the procedure used for mutagenesis involved heating in the presence of a chelating agent (23). Since T5 deletion mutations are selected in a similar manner (8), the interruption deficiency of T5HA4 could be due to loss of the region containing the 7.9 and 99.6% interruptions. Although a deletion in HA4 DNA might have been detected by the studies described above, this possibility can be directly tested by restriction enzyme analysis. The most detailed restriction map of T5 currently available is that obtained by cleavage with an enzyme from *Haemophilus parainfluenzae, Hpa*I. The 26 fragments generated by *Hpa*I cleavage of T5*st*(+) DNA have been ordered (5). The 11th and 17th largest fragments are known to contain the 7.9 and 99.6% interruptions, respectively. The results of *Hpa*I digestion of T5*st*(+) and

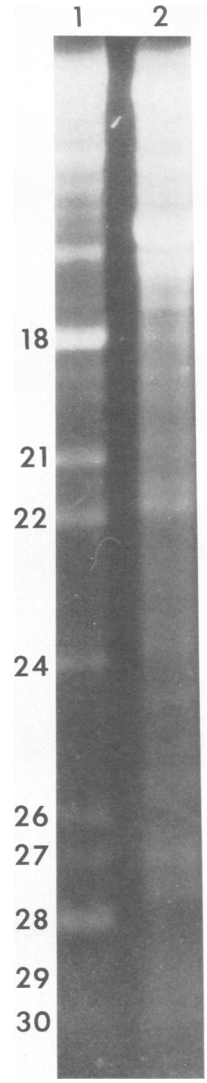

FIG. 4. *Electrophoresis of the small duplex fragments generated by exonuclease III-SI nuclease treatment of T5 DNA. Electrophoresis was carried out in a horizontal 1.0% agarose slab gel. The length of the gel was 8.5 cm. Slot 1, wild-type T5 DNA; slot 2, T5HA4 DNA.*

T5*st*(+)HA4 DNA are shown in Fig. 5. Each digest was analyzed in 0.7 and 1.5% agarose gels to resolve the entire spectrum of fragments. The mobilities of all 26 *Hpa*I fragments, including 11 and 17, did not appear to be altered in T5HA4 DNA. A small alteration in fragment 11 might be difficult to detect since this fragment comigrates with fragments 12 and 13. Fragment 17, however, is well separated from fragment 16, which is only 50 base pairs larger. Consequently, a deletion of 20 to 30 base pairs from fragment 17 should have been easily detected. It is therefore likely that the HA4 mutation is a single, hydroxylamine-induced base transition.

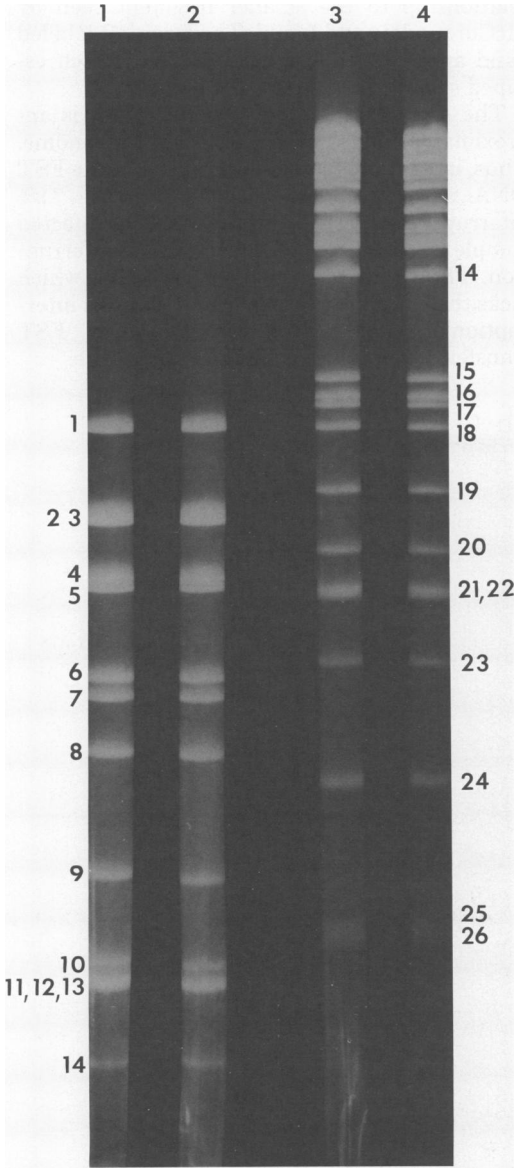

FIG. 5. *Digestion of wild-type T5 and T5HA4 DNAs with HpaI endonuclease. Electrophoresis was carried out in 0.7% (gels 1 and 2) and 1.5% (gels 3 and 4) cylindrical agarose gels. Gels 1 and 3, T5st(+) DNA; gels 2 and 4, T5st(+) HA4 DNA. Fragment 11, visible in gels 1 and 2, spans the 7.9% interruption. Fragment 17, visible in gels 3 and 4, spans the 99.6% interruption.*

Two-step DNA transfer by T5HA4. The interruption at 7.9% has been proposed to be a signal or mechanical barrier that prevents injection beyond the FST (first-step transfer) segment of T5 DNA (1, 3). This hypothesis has been questioned since most T5 DNA molecules have additional interruptions between 0 and 7.9% (13, 16). It is also possible that, although the 7.9% interruption has no role in termination of further DNA transfer, it is the preferential site of breakage when infected cells are subjected to shear and therefore represents one end of the FST segment as isolated. The HA4 mutation provides a direct means to test both of these hypotheses.

Two-step injection was demonstrated by infecting *E. coli* F cells at high concentration in nutrient-free buffer. Under these conditions only the FST segment is transferred to the host cell (14). One portion of the FST complexes was then subjected to shear forces to remove the phage capsid and the remaining 92% of the genome. Only a small fraction of the infective centers survived the shear treatment. Table 1 shows that the fraction of infective centers surviving shearing of T5HA4 FST complexes was identical to that obtained for wild-type complexes. A second portion of the FST complexes was not immediately sheared, but was diluted into warm nutrient medium and aerated for 15 min to allow transfer of the total DNA. With this treatment both the infective centers and the

TABLE 1. *Transfer of T5 wild-type and HA4 DNA into host cells*

Expt no.	Phage genotype	Conditions for transfer of:			
		FST DNA		Total DNA	
		IC%[a]	^{32}P%[b]	IC%[a]	^{32}P%[b]
1	Wild type	7.5	—	100	—
	HA4	5.3	—	111	—
2	Wild type	11.7	10	90	63
	HA4	7.3	16	79	66
3	Wild type	0.2	14	106	71
	HA4	2.4	12	100	67

[a] Infective centers after blending, expressed as a percentage of infective centers in an unblended control.

[b] ^{32}P associated with the host cells after blending, expressed as a percentage of ^{32}P initially adsorbed.

majority of the ^{32}P became resistant to shear in both wild-type and HA4 infection.

Characterization of the FST DNA from T5 wild-type and HA4 infections. Intracellular DNA isolated from shear-treated FST complexes was analyzed by electrophoresis (Fig. 6). T5*st*(+) *Hpa*I fragments were used as standards for molecular-weight determination. The identity of the FST DNA was confirmed by autoradiography. This analysis showed that the FST fragments from wild type- and HA4-infected cells are identical. The major ^{32}P-labeled fragment comigrated with *Hpa*I fragment 4, indicating a molecular weight of 6.0×10^6 (5) or 7.9% of T5*st*(+) DNA. This value agrees well with previously published values of 6.3×10^6 for duplex FST DNA (14) and 3.0×10^6 for FST single strands (7). There was also a less prominent ^{32}P-labeled band that migrated ahead of *Hpa*I fragment 8 and thus has a molecular weight of 3.8×10^6 (5). McCorquodale and Lanni (14) also observed a smaller fragment in DNA isolated from FST complexes, but estimated its size to be 1.5×10^6 by sedimentation in sucrose gradients. McCorquodale (13) has attributed this fragment to shearing of DNA extruded from phage heads. We do not know the significance or origin of the smaller fragment seen here or its relationship to the smaller fragment seen by McCorquodale and Lanni. The large ^{32}P-labeled band appears to be intact T5 DNA which escaped shear.

The size of the major FST fragment is approximately equal to 7.9% of the T5 genome. Thus, in wild-type infection, transfer of the FST DNA must terminate very near to the 7.9% interruption, and shear treatment of the infected complexes results in breakage near this interruption. The results obtained with T5HA4, which lacks the 7.9% interruption, show that this interruption neither signals termination of FST transfer nor provides a shear-sensitive site.

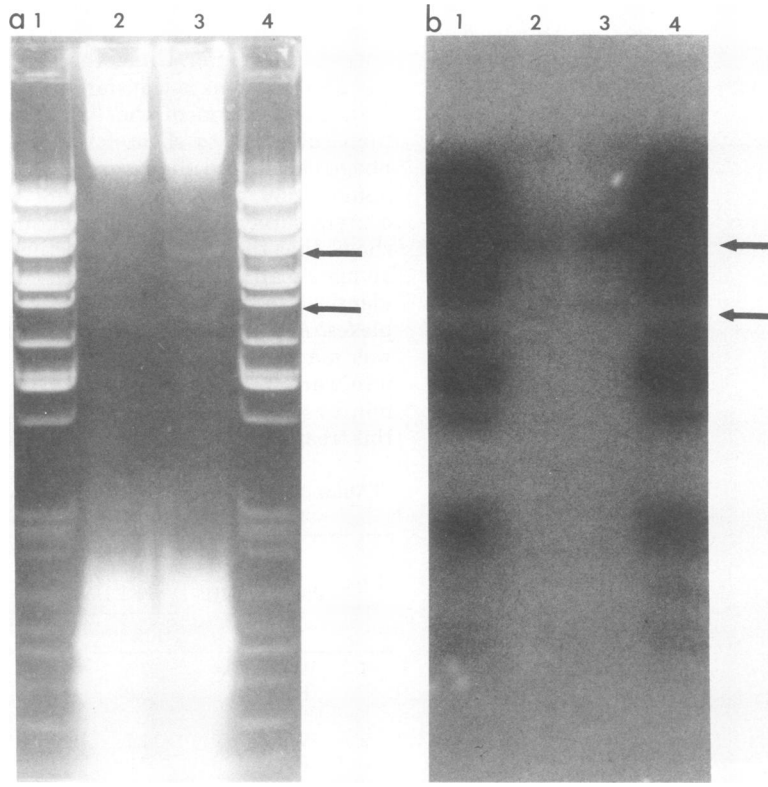

FIG. 6. *Electrophoretic analysis of intracellular DNA from FST complexes. (a) Appearance of gel stained with ethidium bromide; slots 1 and 4, ^{32}P-labeled HpaI-digested T5st(+) DNA; slot 2, ^{32}P-labeled DNA isolated from T5st(+)HA4 FST complexes; slot 3, ^{32}P-labeled DNA isolated from T5 wild-type FST complexes. (b) Autoradiograph of the same gel. The arrows indicate the positions of the 6.0×10^6- and 3.8×10^6-dalton phage DNA fragments. The gel was dried under vacuum on filter paper and then clamped against Kodak Royal Blue X-ray film for autoradiography.*

DISCUSSION

This report describes the properties of T5HA4, a mutant that lacks one of the specific sites where single-chain interruptions occur in T5 DNA. Analysis of the single-chain fragments produced by denaturation of T5HA4 DNA clearly indicates that this mutant lacks the principal single-chain interruption that occurs at 7.9%. Since this site occurs within the 8.3% of T5 DNA that is terminally repetitious, the HA4 mutation also results in loss of an analogous interruption at 99.6%.

T5HA4 was isolated after hydroxylamine mutagenesis and appears to be free from detectable deletion or addition mutations. The interruption deficiency is therefore presumably due to a base substitution mutation that originally occurred at either 7.9 or 99.6%. As is the case for other terminal redundancy heterozygotes, segregation to homozygosity would be expected to occur during subsequent growth. Alternatively, it is possible that the HA4 mutation itself is not located at 7.9 and 99.6%. The observation that HA4 is neither dominant nor recessive to the wild type only indicates that this particular interruption deficiency is not due to lack of a diffusible gene product. Other possibilities, such as inactivation of a distal essential site, have not been eliminated. The only direct evidence on this question has been obtained with mutants that lack the interruption at 32.6% (18). Several mutations that result in loss of the 32.6% interruption have been found to be tightly linked to the nearby *st*(102) deletion (unpublished data).

Loss of the interruptions at 7.9 and 99.6% in T5HA4 DNA results in a number of changes in the fragments produced by denaturation or digestion with exonuclease III and SI nuclease. These changes reflect the loss of fragments that normally terminate at 7.9 and 99.6% and the appearance of new fragments that span these sites. T5HA4 is particularly useful for this kind of analysis since the terminally repeated segments of T5 DNA contain a high density of secondary interruptions. These sites appear to be clustered in the left half of the repeated region between 0 to 4.1% and 91.7 to 95.8%. Consequently, T5HA4 DNA contains virtually all of the fragments of less than 4% of an intact strand (Fig. 2). These fragments extend from the left end of the DNA to interruptions located between 0.8 and 4.1%. Numerous fragments which equal 4 to 8% of T5*st*(+) DNA are missing in T5HA4 DNA. These fragments normally extend leftward from 7.9% and 99.6% are replaced at the left end of HA4 DNA, by a new set of fragments that extend left from 18.5% (Fig. 3). The four secondary interruptions mapped by this set of fragments had been previously located by analysis of restriction fragments of T5 DNA (16). The redundant regions contain additional interruptions which could be mapped by more extensive analysis of T5HA4 DNA.

It should be noted, however, that at present none of the new fragments that appear in T5HA4 DNA have been unambiguously mapped. It is possible, for example, that some or all of fragments A through E (Fig. 3) do not extend as far rightward as the 18.5% interruption. The construction of double mutants that lack both the 7.9 and 18.5% interruptions should resolve this question.

The observation that T5HA4 transfers its DNA in a normal, two-step manner unambiguously excludes a role for the 7.9% interruption in this process. Because of its location, the 7.9% site was a candidate for the signal that interrupts transfer, provided that the left end is transferred first. Although direct evidence on the direction of T5 DNA injection has not been obtained, the available evidence is most consistent with initial transfer of the left end (10, 20). If the right end is transferred first, a role for interruptions can also be excluded since the region immediately around 92% is largely free from even secondary interruptions (21). Normal two-step transfer has also been observed with T5HA23, a mutant that is completely free from interruptions (S. Rogers, unpublished data).

The size of the FST segment isolated from sheared T5HA4-infected cells is identical to that obtained for wild-type T5. Moreover, the band containing the HA4 FST segment is as sharp as that seen for wild type. Thus, even though the FST fragment equals 7.9% of T5 DNA, the interruption at 7.9% does not provide an essential shear-sensitive site. This observation is surprising considering the known shear sensitivity of single-chain interruptions (6) and suggests that the actual size of the initially transferred segment equals 7.9% of intact DNA. If the 7.9% interruption had provided a shear-sensitive site, the actual size of the initially transferred segment could have been substantially larger or smaller than 7.9%.

A value of 7.9% for T5 FST DNA implies that a small portion of the terminal repetition is not included in the initially transferred segment. One of the functions of the FST DNA is extensive degradation of host DNA (12). Presumably, the FST DNA is protected from similar degradation during normal infection although the protective mechanism has not been identified. Even if such a mechanism exists, some degradation of FST DNA could occur. Therefore, by holding a portion of the repeated region in reserve, T5 has

the potential for restoring, by recombination, a continuous genome once DNA transfer has been completed.

ACKNOWLEDGMENTS

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