JOURNAL OF VIROLOGY, Feb. 1979, p. 716-725
0022-538X/79/02-0716/10$02.00/0

Vol. 29, No. 2

Interruption-Deficient Mutants of Bacteriophage T5

I. Isolation and General Properties†

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Received for publication 15 August 1978

Mutants of bacteriophage T5 were isolated which lack one or more of the natural single-chain interruptions that occur in the mature DNA of this virus. Interruption-deficient mutants were detected by screening survivors of hydroxylamine mutagenesis for altered DNA structure by electrophoresis in agarose slab gels. Over 60 independent mutants were isolated from a survey of approximately 800 phage particles. All of the mutants were viable and could be grouped into two classes. Mutants in one class lacked one of the localized sites where interruptions occur in T5 DNA. To date, mutants that affect five different sites have been obtained. Mutants in the other class were essentially free from interruptions or had a reduced frequency of interruptions throughout the genome. The members of this class included several amber mutants. Complementation tests indicated that at least two genes are required for the presence of interruptions in mature T5 DNA.

The nonpermuted genome of bacteriophage T5 is contained within a single linear, double-stranded DNA molecule (27). T5 DNA has a molecular weight of approximately 80×10^6 (14, 24) and contains an 8.3% terminal redundancy (20). T5 DNA is distinguished from the DNA of most other coliphages by the presence of a small number of single-chain interruptions (1). These sites can be repaired by the action of T4 DNA ligase and are largely confined to one strand of the duplex molecule (12). Although the interruptions appear to occur at fixed sites, the frequency of interruptions at the different sites is highly variable (9, 24). The positions of the four most frequent interruptions, which occur in nearly every molecule, have been determined by electron microscopy (2, 3, 13, 23, 24) and agarose gel electrophoresis (8, 19). These sites occur at approximately 8, 18, 32, and 65% from what is arbitrarily designated as the left end of the genome. Some of the less frequent interruptions have also been mapped (19, 24). The highest concentration of these sites occurs at the left end of the DNA, within the terminal repetition.

The significance of the single-chain interruptions in T5 DNA is not known. Although it has been suggested that one or more of the interruptions could influence genetic recombination or DNA injection (1, 2), there is no direct evidence linking these sites to any specific process. To investigate this question, we initiated a search for mutants of T5 that lack the normal complement of interruptions. This search was aided by the development of an electrophoretic assay that allows the genomes of mutagenized phage to be rapidly analyzed for structural alterations. Use of this screening procedure resulted in the isolation of two classes of mutants with altered patterns of single-chain interruptions. Mutants in one class lacked one of the sites where interruptions normally occur. Mutants in the other class had reduced levels of interruptions throughout the genome. Some members of this class appeared to be completely free from interruptions.

The present report describes the general properties of interruption-deficient mutants of T5 and the methods used for their isolation. The accompanying paper (21) describes the properties of a mutant that specifically lacks the interruption located at 8% from the left end of T5 DNA.

MATERIALS AND METHODS

Bacteria and bacteriophage. T5*st*(+) is the wild-type strain of Abelson and Thomas (1). T5*st*(102) is a heat-stable deletion mutant (25). *Escherichia coli* B40*su*1, B40*su*2, and B40*su*3 were obtained from H. Berger. *E. coli* F, a fast-adsorbing strain for T5, was obtained from D. Botstein. *E. coli* B23 was obtained from M. Bessman. All bacteriophage were grown by confluent lysis as described by Rhoades and Rhoades (20).

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Isolation of mutants. T5*st*(102) was treated with hydroxylamine for 72 h at 37°C as described by Tessman (26) to a final survival of 10^{-6}. During the same period, a control incubated without hydroxylamine showed a 50% loss in titer. Survivors were plated on *E. coli* B40*su*1 at 30°C. Individual plaques were suspended in 10 mM Tris-hydrochloride (pH 8.0) and replated on *E. coli* B40*su*1 to eliminate heterozygotes. Single plaques were then transferred with sterile toothpicks to 1-cm^2 grids on soft-agar overlays (6) seeded with *E. coli* B40*su*1. After 6 to 8 h at 30 or 37°C, the soft-agar grids were removed from the plates with a spatula and placed in 0.1 ml of 10 mM Tris-hydrochloride (pH 8.0). After 30 min at 25°C, a 0.03-ml sample of the resuspended phage was mixed with 5 μl of 2% sodium dodecyl sulfate–0.1 M EDTA (pH 9.0) and heated for 5 min at 60°C. Ten minutes before analysis of the DNA, 5 μl of a freshly prepared solution of 1 M NaOH was added to denature the DNA. The denatured samples were then subjected to electrophoresis in horizontal 0.7% agarose slab gels as described by Rogers and Rhoades (22).

Amber mutants were obtained by plating survivors of hydroxylamine treatment on the *su*⁻ strain *E. coli* B23. Single plaques were then directly tested for interruption-deficient phenotypes as described above. Phage which were either completely free from interruptions or which exhibited reduced levels of interruptions throughout the genome were then retested on *E. coli* B40*su*1, B40*su*2, and B40*su*3.

Procedure for crosses. *E. coli* B40*su*1 or *E. coli* F was grown to 2×10^8 cells/ml in T-MGM (15) with aeration at 37°C. After centrifugation, the cells were resuspended at 10^9/ml in TMB (T-MGM without glucose or NH_4Cl) containing 1 mM $CaCl_2$ and were starved for 20 min at 37°C with aeration. The cells were then diluted with an equal volume of TMB containing 1 mM $CaCl_2$ and were infected at a multiplicity of 7.5 of each parent. After 20 min at 37°C without aeration, the infected cells were centrifuged to remove unadsorbed phage and were gently resuspended at 5×10^8/ml in prewarmed T-MGM containing 1 mM $CaCl_2$. After 5 min at 37°C, aeration was started and continued for 60 to 90 min. Lysis was completed by addition of $CHCl_3$. Progeny were plated on an appropriate indicator, and the nature of the DNA of individual plaques was tested as described above.

In crosses where one parent carried the *st*(102) deletion mutation, the progeny were fractionated either by plating on *E. coli* F, where the *st*(102) mutation confers a small-plaque phenotype, or by banding in CsCl density gradients. In the latter case, a portion of the lysate was made 45% (wt/wt) CsCl and centrifuged in a Beckman SW50.1 rotor at 25,000 rpm for 16 h at 4°C.

Complementation analysis and dominance tests. Lysates of mixedly infected bacteria were prepared as described above, except that *E. coli* B23 was used for all experiments involving amber mutants. Electrophoresis was carried out directly on the progeny present in the lysates. In some cases, particularly if one parent carried the *st*(102) deletion, the progeny were first fractionated by centrifugation in CsCl density gradients. After dialysis against 10 mM Tris-hydrochloride (pH 8.0)–1 mM $MgCl_2$–1 mM $CaCl_2$, the CsCl-purified phage particles were analyzed by electrophoresis.

RESULTS

Isolation of interruption-deficient mutants. The presence of single-chain interruptions in T5 DNA is not known to confer any phenotypic properties upon the virus. Interruption-deficient mutants were therefore obtained by direct screening of mutagenized phage preparations. To survey rapidly a large number of potential mutants, we used a procedure that permits the DNA present in a single T5 plaque to be analyzed, after alkaline denaturation, by agarose gel electrophoresis. Since bacterial DNA is efficiently degraded during the early stages of T5 infection (5), virtually all of the DNA in a T5 plaque consists of phage DNA, either packaged or unpackaged. The DNA content of a wild-type T5 plaque is sufficiently high (0.01 to 0.1 μg) to permit electrophoretic analysis with the use of ethidium bromide staining. In practice, however, more satisfactory results are obtained if each plaque is replica plated to form a 1-cm^2 area of lysis as described in Materials and Methods.

Mutagenesis was carried out by treating T5*st*(102) phage particles with hydroxylamine until the surviving fraction was 10^{-6}. T5*st*(102), a heat-stable deletion mutant that lacks 10% of the wild-type genome (25), was chosen to minimize any loss of viable phage not associated with the action of hydroxylamine. The surviving phage particles were analyzed by two procedures. In the initial stages of this investigation, survivors were plated at 30°C on *E. coli* B40*su*1 to avoid loss of conditionally lethal mutants. Heterozygotes were eliminated by replating individual plaques under identical conditions. Several plaques derived from each original isolate were then analyzed for loss of interruptions.

The results of a typical analysis of 22 preparations, derived from 10 independent survivors of hydroxylamine mutagenesis, are shown in Fig. 1. With the exception of slot 23, which received an insufficient amount of DNA, all of the samples exhibited a distinct pattern of single-chain fragments. A mutant pattern was clearly evident in slots 7 and 8. Both of these samples, which were derived from the same isolate, HA99, lacked two of the prominent single-chain fragments that were present in all of the other samples and in purified T5*st*(102) DNA (slot 1). As described below, this particular mutant pattern indicates loss of the interruption at 18.5%.

Of approximately 150 survivors of hydroxylamine treatment that were screened in this manner, 16 were found to contain at least one mutation which caused an interruption-deficient

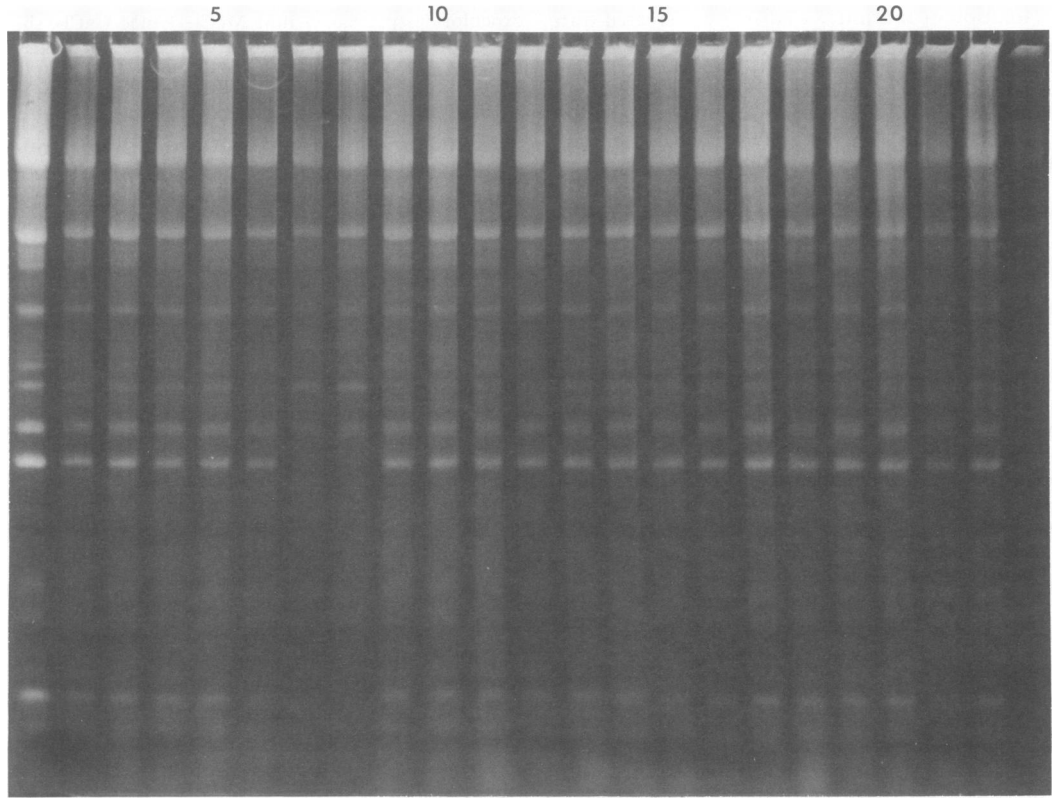

FIG. 1. *Example of isolation of interruption-deficient T5 mutants. The selection shown contained two or three descendants of isolates HA92 through HA101 from hydroxylamine mutagenesis of T5st(102). Both descendants of HA99 showed a mutant single-chain pattern in slots 7 and 8. Slot 1 contained purified T5st(102) DNA. The other 22 samples were prepared for electrophoresis from small-plate lysates as described in Materials and Methods. The direction of electrophoresis in this and all other figures is from top to bottom.*

phenotype. In one instance, HA104, two different mutant phenotypes segregated upon replating. Three different mutant phenotypes were recovered from a second isolate, HA73, by recombination (see Table 1).

Interruption-deficient mutants of T5 were also obtained by a more rapid procedure in which survivors of the hydroxylamine treatment described above were tested directly, without replating, for loss of interruptions. *E. coli* B23, an su^- host, was employed in these experiments in order to allow isolation of amber interruption-deficient mutants. Approximately 650 plaques were screened in this manner, with the isolation of 45 additional mutants. The phenotypes of four of these mutants were found to be suppressed by growth on su^+ strains.

Members of each of the categories of mutants obtained (see Table 1) were crossed with the wild type in an attempt to eliminate extraneous mutations. The original isolates, which carry the *st*(102) deletion, were crossed with T5*st*(+), and interruption-deficient *st*(+) recombinants were obtained. The interruption-deficient mutation was then crossed back and forth between T5*st*(+) and T5*st*(102) for, in some cases, a total of five backcrosses. Whenever possible, any detectable conditional lethal, plaque morphology, or host range mutations were eliminated by this procedure. However, as a result of the high level of mutagenesis employed in this study, it is likely that none of the strains are isogenic with the wild type.

Electrophoretic analysis. The various mutant patterns of single-chain fragments that were obtained are shown in Fig. 2 and 3. For convenience, all of the phage shown carry the *st*(102) deletion. A map showing the positions of the single-chain interruptions and fragments that are relevant for this study is shown in Fig. 4. The fragments are numbered as described by Rhoades (19).

The fragment patterns of the five mutants shown in Fig. 2 all differed from T5*st*(102) DNA in only a few instances. The simplest interpretation of each of these patterns is that they

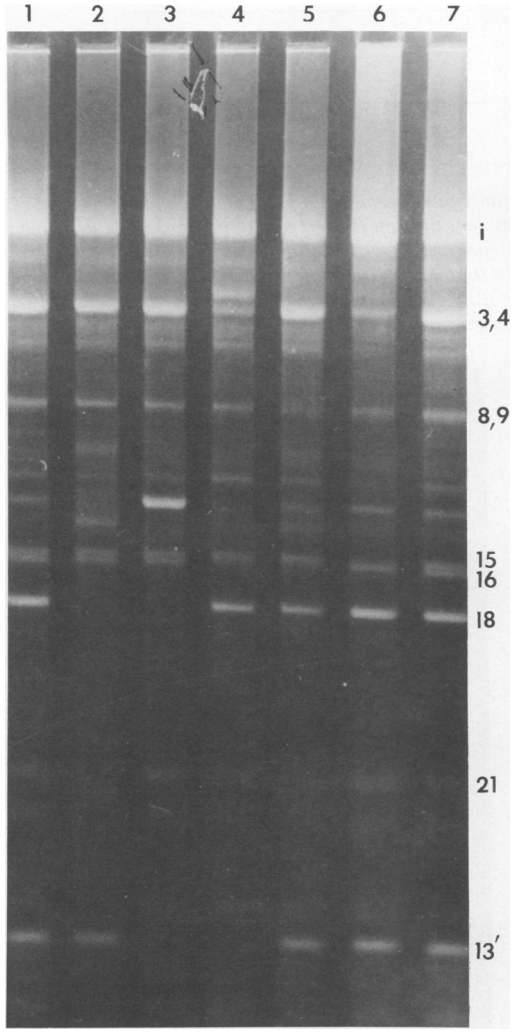

FIG. 2. *Electrophoretic patterns of five single-site interruption-deficient mutants. All slots contained 1 μg of alkali-denatured DNA from CsCl-purified phage particles. Slots 1 and 7, T5st(102); slot 2, HA4; slot 3, HA626; slot 4, HA65; slot 5, HA73-16; slot 6, HA214. The locations of the numbered fragments are shown in Fig. 4. The symbol "i" designates the intact strand of T5 DNA.*

reflect loss of one of the sites where interruptions normally occur in T5 DNA. In the case of T5HA4 (slot 2), the absence of fragment 18 and some of the smaller fragments suggests loss of the interruption at 7.9%. Moreover, several new fragments which span the site of the missing 7.9% interruption are visible (21). The absence of fragments 13′ and 18 in T5HA626 (slot 3), and the increased intensity of fragment 7′, both indicate loss of the interruption at 18.5%. Similarly, the absence of fragments 13′, 16, and what is probably fragment 4 in T5HA65 (slot 4) suggests loss of the interruption at 32.6%. A new fragment that presumably represents the fusion of fragments 4 and 13′ is visible just behind fragment 3. The interpretation of T5HA214 (slot 6) is less clear; however, the diminished intensity of the band where fragments 3 and 4 normally comigrate and the absence of fragment 15 suggest that the interruption at 64.8% has been lost. The faint band remaining at the position of fragments 3 and 4 probably represents a fragment that extends from 45% to an interruption

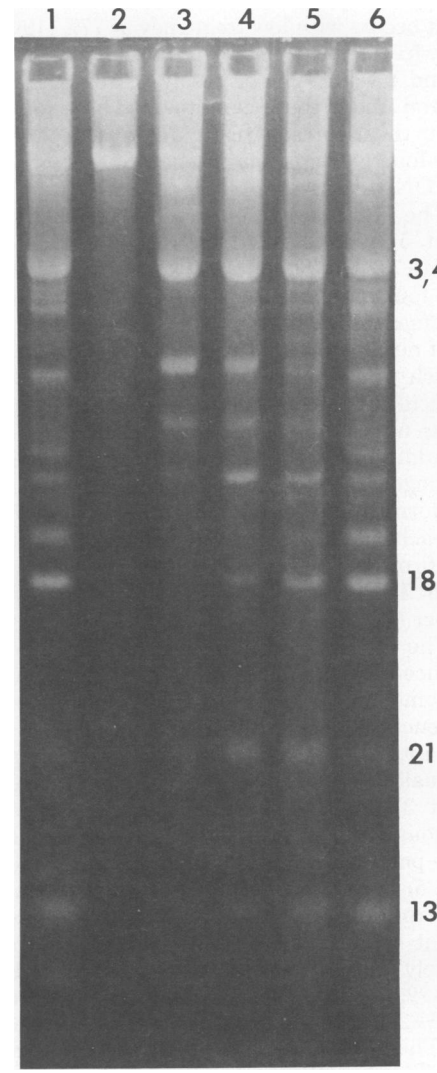

FIG. 3. *Electrophoretic patterns of four mutants that exhibited reduced levels of interruptions throughout the genome. All slots contained 0.5 μg of alkali-denatured DNA from CsCl-purified phage particles. Slots 1 and 6, T5st(102); slot 2, HA23; slot 3, HA104S; slot 4, HA90; slot 5, HA229.*

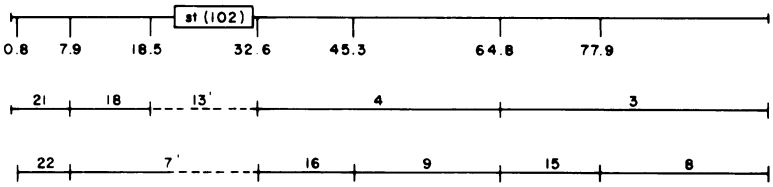

FIG. 4. *Maps of single-chain interruptions and single-chain fragments of T5 DNA. The locations of seven interruptions, expressed as a percentage of the wild-type length from the left end of the molecule, are given on the top line. The locations of the more prominent fragments of T5 DNA that define these sites are given in the lower part of the figure. Only the sites and fragments that are relevant for this study are shown. A more detailed map is available in the accompanying paper (21). The st(102) deletion extends from 21.6 to 32.0%. Fragments 7' and 13' are shortened versions of fragments 7 and 13 that occur in T5st(+) DNA.*

that occurs with low frequency at 77% (19). The new fragment formed by the fusion of fragments 3 and 4 would not be resolved from the intact strand under these conditions. These four mutants thus represent, individually, loss of each of the four principal single-chain interruptions in T5 DNA.

The fifth mutant shown in Fig. 2, T5HA73-16 (slot 5), was isolated by recombination from T5HA73 (see Table 1). Although HA73-16 has the four principal interruptions, the absence of fragment 16 suggests loss of the interruption that normally occurs at 45%. Loss of fragment 9, which would also be expected in HA73-16, was difficult to detect since fragments 8 and 9 comigrate under these conditions.

Additional electrophoretic studies, involving analogous sets of duplex fragments (19), were performed on the five mutants shown in Fig. 2. In each case, the interpretation given above was confirmed. The results of this kind of analysis with T5HA4 are given in the accompanying paper (21).

The mutant patterns shown in Fig. 3 reflect reduced levels of interruptions throughout the genome. In the case of T5HA23 (slot 2), the absence of detectable fragments smaller than the intact strand indicates that this genome is virtually free from interruptions. T5HA104S (slot 3) appears to retain some interruptions although the overall level is greatly reduced. The patterns of T5HA90 and T5HA229 (slots 4 and 5) both suggest that most of the principal interruptions are present, but that the less frequent secondary interruptions are either completely absent (HA90) or greatly reduced (HA229). The most prominent feature of T5HA229 is the increased intensity of fragment 21. The new bands that can be seen in T5HA90 and T5HA104S are probably derived from molecules that lack either or both of the principal interruptions at 7.9 and 18.5%.

The patterns of suppression of the four amber interruption-deficient mutants are shown in Fig. 5. Two of these mutants, T5*am*HA676 and

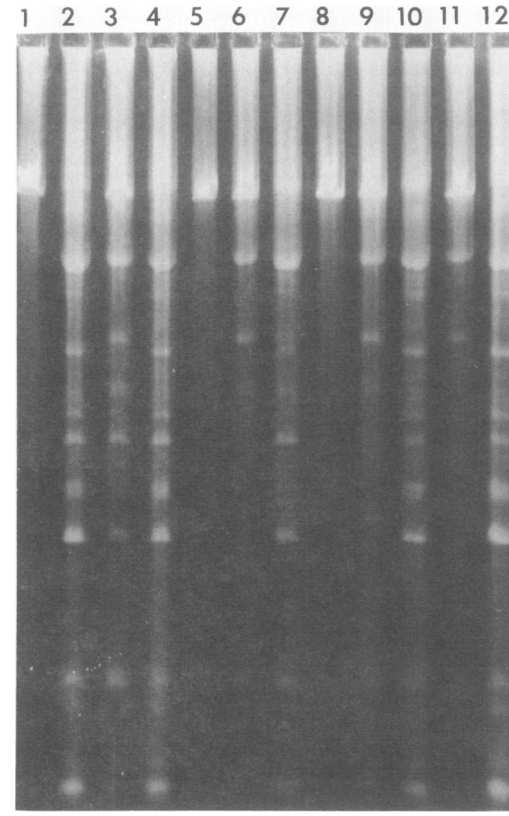

FIG. 5. *Electrophoretic analysis of amber interruption-deficient mutants after growth on su⁻ and su⁺ strains. Slots 1 to 4 contained T5amHA676 grown on E. coli B23, B40su1, B40su2, and B40su3, respectively. Slots 5 to 8 contained T5amHA911 grown on the same hosts. Slots 9 and 10 contained T5amHA346 grown on B23 and B40su1. Slots 11 and 12 contained T5amHA910 grown on B23 and B40su1. All slots contained alkali-denatured DNA from CsCl-purified phage particles.*

T5*am*HA911, were free from interruptions when grown on *E. coli* B23. After growth on *E. coli* B40*su*1 and B40*su*3, *am*HA676 was suppressed to the wild type (slots 2 and 4); however, growth

on B40*su*2 resulted in a pattern similar to that of T5HA90 (see Fig. 3) in which only the principal interruptions are present (slot 3). In contrast, *am*HA911 was fully suppressed only by *E. coli* B40*su*2 (slot 7). Growth on B40*su*1 resulted in a reduced level of interruptions (similar to T5HA104S), whereas no suppression occurred on B40*su*3. The two other amber mutants, T5*am*HA346 and T5*am*HA910, exhibited a reduced level of interruptions on *E. coli* B23 (slots 9 and 11) that resembles HA104S. Both of these mutants were suppressed to the wild type on *E. coli* B40*su*1 (slots 10 and 12) and on the *su*2 and *su*3 strains (data not shown). In addition, all of the mutants that had a phenotype on *E. coli* B40*su*1 that was intermediate between the wild type and completely interruption deficient (like HA90 and HA104S) were tested on *E. coli* B23. None of the patterns were found to change.

A list of the categories of interruption-deficient T5 mutants obtained in this study is given in Table 1.

Dominance tests. Dominance tests were performed by analyzing the single-chain fragments present in the unreplicated progeny of mixedly infected bacteria. In the case of the mutants that lacked a single interruption, mixed infection with the wild type gave bursts that appeared to contain both mutant and wild-type genomes. The results obtained for mixed infection with T5*st*(+)HA4, which lacks the interruption at 7.9%, and T5*st*(102) are shown in Fig. 6. In

TABLE 1. *Categories of interruption-deficient mutants*

Mutant phenotype[a]	No. isolated[b]	Representative alleles[c]
8^-	7 (2)	HA4, HA73-17, HA336
18^-	11 (1)	HA99, HA626, HA682
32^-	16 (4)	HA65, HA77, HA435
45^-	2 (1)	HA73-16, HA772
65^-	5 (3)	HA83, HA214, HA392
Interruption-free	9 (4)	HA23, HA34, HA54, HA104L, HA363, HA663, *am*HA676, HA883, *am*HA911
Reduced interruptions	9 (2)	HA73, HA104S, *am*HA346, HA645, HA771, HA776, HA896, *am*HA910, HA935
Principal interruptions only	3 (1)	HA90, HA599, HA835
Reduced secondary interruptions	1 (1)	HA229

[a] The designations "8^-," "18^-," etc., refer to phage specifically lacking interruptions at 8%, 18%, etc., from the left end of T5 DNA. The last four categories describe mutants with generally reduced levels of interruptions throughout the genome, as shown in Fig. 3.

[b] The values in parentheses are the number of mutants obtained in the initial phase of this study when each isolate was replated before testing. All allele numbers below HA300 are members of this group.

[c] Mutants HA73-17 and HA73-16 were isolated from HA73 by recombination. HA104L and HA104S are segregants of isolate HA104. Amber mutants are denoted by the prefix "*am*."

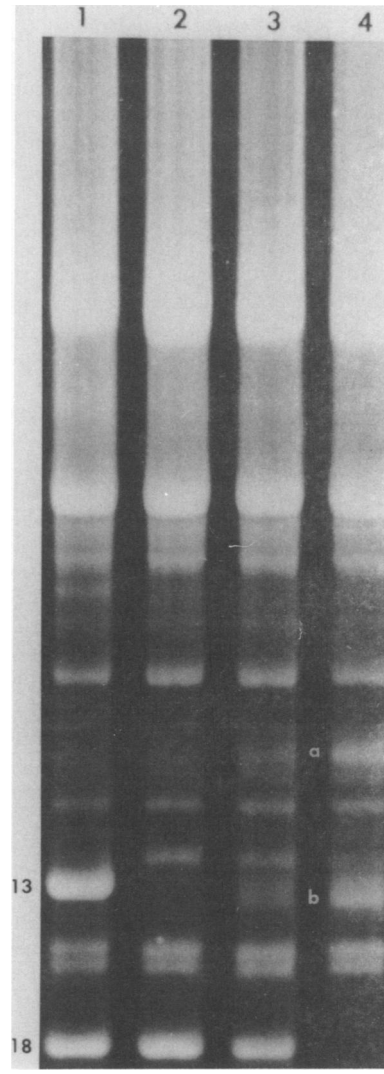

FIG. 6. *Electrophoretic analysis of the progeny of mixed infection with T5st(+) and T5st(102)HA4. The progeny were fractionated in CsCl to separate the st(+) and st(102) phage before analysis. Slot 1, T5st(+) DNA; slot 2, T5st(102) DNA; slot 3, mixed infection progeny carrying the st(102) deletion; slot 4, T5st(102)HA4 DNA. The symbols "a" and "b" denote two new fragments that appeared in T5st(102)HA4 DNA.*

addition to lacking fragment 18, T5HA4 contained several new fragments that distinguish it from the wild type (Fig. 6, slot 4). As described in the accompanying paper (21), the new fragments extended between interruptions located close to the left end of genome and the interruption at 18.5%. The presence of both mutant and wild-type genomes was therefore demonstrated by the presence of both fragment 18 and the new fragments in the mixed infection progeny. Similar results were obtained for T5HA99, which lacks the interruption at 18.5%, and for T5HA65, which lacks the interruption at 32.6%. Analysis of the mutants that lack interruptions at 45.3 and 64.8% was more difficult since, under the electrophoretic conditions employed here, neither of these mutants possesses fragments that are absent in the wild type.

These results indicate that loss of a single interruption is not due to the absence of a diffusible gene product. Instead, it appears that in each mutant one of the sites where interruptions normally occur has been altered so that the site is no longer recognized by the mechanism that specifies the interruptions.

In contrast, all of the mutations that result in a reduced level of interruptions throughout the genome appear to be recessive to wild type. These tests were carried out on the 21 mutants listed in Table 1 that resemble either T5HA23 (no interruptions) or T5HA104S and T5HA90 (reduced levels of interruptions). In all cases, the progeny of mixedly infected bacteria were found to possess normal patterns of single-chain fragments. The results of mixed infection with T5*st*(+)HA23 and T5*st*(102) are shown in Fig. 7. T5HA229 also appears to be recessive; however, the phenotype of this mutant is so similar to that of the wild type (see Fig. 3) that an intermediate result would be difficult to recognize.

Complementation analysis. The results presented above suggest that absence of all or most of the single-chain interruptions in T5 DNA can be due to the lack of a diffusible gene product. To determine the number of genes involved in this process, we carried out complementation tests between various pairs of interruption-free and reduced-interruption mutants. The results of these experiments, which are summarized in Table 2, can be stated as follows. In all of the tests involving pairs of interruption-free mutants, no complementation was observed. The progeny, like the parents, were free from interruptions. Several of the mutants that had reduced levels of interruptions (HA346, HA645, HA771, *am*HA910, and HA935) also failed to complement the interruption-free mutants. In these cases, the progeny resembled the reduced-interruption parent. However, three of the reduced-interruption mutants (HA73, HA104S, and HA896) were found to partially complement the interruption-free mutants and the other reduced-interruption mutants. The results of mixed infections with T5HA104S and several of the interruption-free mutants are shown in Fig. 8. In each case, the mixed-infection progeny had all of the major single-chain fragments, but the minor fragments were barely visible. These two groups of mutants can, therefore, cooperate to produce progeny that have most but not all of the interruptions present in the wild type. One interpretation of this result is

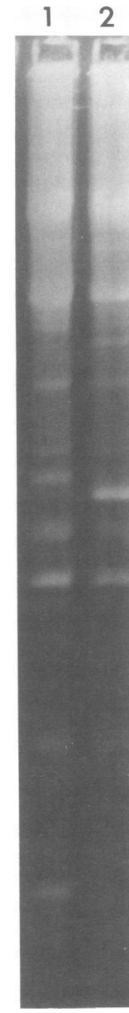

FIG. 7. *Electrophoretic analysis of the progeny of mixed infection with T5st(+)HA23 and T5st(102). The progeny were fractionated in CsCl to separate the st(+) and st(102) phage before analysis. Slot 1, mixed-infection progeny of st(102) density; slot 2, mixed-infection progeny of st(+) density.*

TABLE 2. *Complementation analysis of interruption-deficient mutants*[a]

Allele	Allele							
	HA23	HA34[b]	HA363[c]	*am*HA676	HA73	HA104S	*am*HA346[c]	HA896
HA896	+					−		−
*am*HA346[c]	−					+	−	
HA104S	+	+	+	+	−	−		
HA73	+				−			
*am*HA676	−		−	−				
HA363[c]	−		−					
HA34[b]	−	−						
HA23	−							

[a] +, Positive complementation as shown in Fig. 8; −, no complementation.

[b] Results identical to that shown for HA34 have been obtained with HA54 and HA104L. All pairwise tests within this group were negative.

[c] Results identical to that shown for *am*HA346 have been obtained with HA645, HA771, *am*HA910, and HA935. Results identical to that shown for HA363 have been obtained for HA663, HA883, and *am*HA911. Pairwise tests within these two groups have not been done.

that T5HA23 and T5HA104S define two genes whose functions are required for wild-type levels of interruptions. We have tentatively designated the gene represented by HA23 as *sciA* (single-chain *i*nterruption) and the gene represented by HA104S as *sciB*.

Other properties. The growth properties of T5HA23, which is free from interruptions, were tested for any detectable differences from the wild type. In addition to normal plaque morphology, the latent period and burst size of T5HA23 were indistinguishable from the wild type. The absence of interruptions also did not alter the survival of either T5*st*(+)HA23 or T5*st*(102)HA23 after heating to 60°C in citrate buffer (11).

DISCUSSION

This report describes the isolation of two classes of viable interruption-deficient mutants of bacteriophage T5. In one class, the interruption deficiency appears to be limited, in each mutant, to a single site. Since these mutations are neither dominant nor recessive to the wild type, they presumably represent alterations of critical sequences that specify the interruptions. Mutants in the second class are interruption-free or have reduced levels of interruptions throughout the genome. These mutations are recessive to wild type and appear to define two genes, *sciA* and *sciB*, whose products participate in formation of the interruptions in T5 DNA.

Both classes of interruption-deficient mutants were isolated from a stock of T5*st*(102) that had been heavily mutagenized with hydroxylamine. Although the resulting high frequency of interruption-deficient mutants was advantageous for this study, it is very unlikely that any of the surviving phage were free from additional hydroxylamine-induced mutations. We have attempted to reduce the level of extraneous mutations by backcrossing selected mutants with the wild type. The *st*(+) and *st*(102) alleles were employed as second markers in these crosses to insure that the mutant genomes were able to recombine. Since this procedure may never be completely successful, it is fortunate that multiple alleles were obtained for most of the mutant categories. Future studies of each interruption-deficient phenotype will therefore not be dependent upon the possibly aberrant properties of single mutants.

The occurrence of both classes of interruption-deficient mutants at nearly equal frequencies is of interest since the single-site mutants presumably represent alterations of sequences that are much smaller than a gene. Hydroxylamine, however, is known to mutate preferentially single-stranded DNA (7). Since the regions surrounding interruptions are more likely to be transiently single stranded than other regions (especially in a phage particle), hydroxylamine may preferentially mutate these sites in T5 DNA. If correct, this hypothesis offers a possible explanation for the observation that, with one apparent exception (M. Rhoades, unpublished data), no mutants were obtained with new interruptions.

Information on the sequences surrounding the interruptions in T5 DNA has recently been obtained by Nichols and Donelson (17, 18). A study of the 5′ sequences, done on intact DNA, indicates that pGpCpGpC- occurs at all of the interruptions and suggests that the octanucleotide pGpCpGpCpGpGpTpG- occurs at most or all of the principal interruptions. The sequences at the 3′ side are more diverse. Studies on isolated single-chain fragments have provided extensive

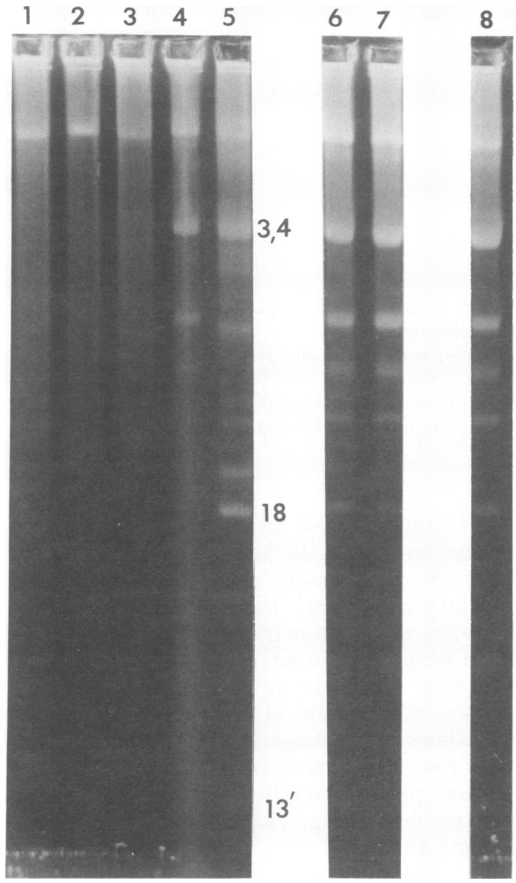

FIG. 8. *Electrophoretic analysis of complementation tests between T5HA104S (reduced interruptions) and several interruption-free mutants. Slot 1, denatured T5st(102)HA54 DNA; slot 2, denatured T5st(102)HA23 DNA; slot 3, denatured T5st(+)HA23 DNA; slot 4, denatured T5st(102)HA104S DNA; slot 5, denatured T5st(102) DNA; slot 6, progeny from mixed infection with T5st(102)HA104S and T5st(102)HA54; slot 7, st(102) density progeny from mixed infection with T5st(102)HA104S and T5st(+)HA34; slot 8, st(102) density progeny from mixed infection with T5st(102)HA104S and T5st(+)HA23.*

3′ sequences at two sites, 8 and 18%, where interruption-deficient mutants have been obtained. At the 18% site four of the first six base pairs on the 3′ side are GC, whereas at 8% one of the first six is a GC pair. There are thus numerous sites in the immediate vicinity of these two interruptions where hydroxylamine, which is specific for C, could act. The higher frequency of GC pairs at 18%, however, does not seem to have significantly increased the frequency of mutations at this site as compared to 8% (Table 1). It is possible, as suggested by Nichols and Donelson (18), that the critical 3′ sequences occur farther from the interruptions in a region where overlapping segments with twofold rotational symmetry are found.

The existence of viable T5 mutants that are apparently free from interruptions raises questions as to whether these sites play an essential role in the viral life cycle. The DNA of these mutants, however, has only been shown to lack interruptions in mature virus particles. The intracellular forms of the DNA of the interruption-deficient mutants have not been examined. It is possible that the defects seen in these mutants affect only the terminal stages of viral development. Therefore, at present, the only firm conclusions are that intact T5 phage heads can contain DNA that has no interruptions and that such DNA is infective.

The conclusion that at least two genes control the formation of interruptions in mature T5 DNA was based on the partial complementation observed after mixed infection with certain pairs of mutants. Since a full wild-type phenotype was never observed, these results might be due to intragenic complementation. However, all complementing pairs invariably produced patterns similar to those shown in Fig. 3, including infections on su^- hosts in which one parent carried an amber mutation. Thus, although the reason for partial complementation is not known, the present results favor a two-gene hypothesis.

The availability of interruption-deficient mutants of T5 should greatly facilitate further analyses of the origin and function of the interruptions in T5 DNA. In particular, the single-site mutants should permit evaluation of the role of interruptions in processes such as genetic recombination, transcription control, and DNA transfer. As described in the accompanying paper (21), T5HA4, which lacks the interruption at 8%, is not deficient in the two-step transfer process that is unique to T5. It should also be possible to recombine the various single-site mutations to produce multiple mutants. Since the defects in the single-site mutants appear to be permanent, the construction of recombinants that lack all of the principal interruptions should provide additional insights into the possible functions of these sites.

Analysis of the interruption-free mutants should provide some insight into the mechanism that generates interruptions in T5 DNA. Little is known about the nature of the T5 *sciA* and *sciB* genes, although bacteria infected with an *sciA* mutant, HA23, are not deficient for four previously isolated (22) site-specific endonucleases that are induced by T5 (S. Rogers, unpublished data). The availability of amber mutations in the *sciA* gene should permit identification and isolation of its gene product. Knowledge

of the location of the *sci* genes on the T5 genetic map may also provide some indications as to how, or when, they are expressed. Preliminary results (B. Lange-Gustafson, unpublished data) have located the HA23 mutation in a late gene region at the extreme right of the nonrepeated portion of the T5 genome. None of the mutations in the *sciB* gene has been mapped; however, recombination between HA23 and HA104S appears to be infrequent.

The T5 D15 gene, an essential early gene that specifies a 5′ exonuclease (10), has been implicated in generating interruptions in intracellular T5 DNA. Although D15 mutants are defective in initiating late gene expression (4), Moyer and Rothe (16) have obtained evidence that the D15 gene product directly participates in forming interruptions. These authors have also discovered that the purified D15 protein has endonucleolytic activity, although it is unable to introduce single-chain interruptions into duplex DNA. It is also not known whether the breaks generated by the D15 nuclease are those present in mature phage particles. Nevertheless, these results suggest that the process or processes that place interruptions into T5 DNA are complex and that their understanding will require analysis of a number of genes.

ACKNOWLEDGMENTS

This research was supported by grant PCM 76-24036 from the National Science Foundation. S.G.R. was supported by a training grant (HD-139) from the National Institutes of Health.

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