Deletion Mutagenesis Independent of Recombination in Bacteriophage T7

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Deletion between directly repeated DNA sequences in bacteriophage T7-infected *Escherichia coli* was examined. The phage ligase gene was interrupted by insertion of synthetic DNA designed so that the inserts were bracketed by 10-bp direct repeats. Deletion between the direct repeats eliminated the insert and restored the ability of the phage to make its own ligase. The deletion frequency of inserts of 85 bp or less was of the order of 10^{-6} deletions per replication. The deletion frequency dropped sharply in the range between 85 and 94 bp and then decreased at a much lower rate over the range from 94 to 900 bp. To see whether a deletion was predominantly caused by intermolecular recombination between the leftmost direct repeat on one chromosome and the rightmost direct repeat on a distinct chromosome, genetic markers were introduced to the left and right of the insert in the ligase gene. Short deletions of 29 bp and longer deletions of approximately 350 bp were examined in this way. Phage which underwent deletion between the direct repeats had the same frequency of recombination between the left and right flanking markers as was found in controls in which no deletion events took place. These data argue against intermolecular recombination between direct repeats as a major factor in deletion in T7-infected *E. coli*.

Deletions often arise between directly repeated DNA sequences (1, 7, 11, 18, 33, 38, 42, 44, 48). Short regions of homology probably play a crucial role in the mechanism(s) responsible for this type of spontaneous deletion. Among the several possible mechanisms (18, 20, 21, 25) suggested as explanations for deletions is a form of copy choice by the DNA replication enzymes whereby newly replicated DNA synthesized complementary to one direct repeat slips into alignment with the other direct repeat so that the template between the direct repeats is either eliminated or not copied (1, 45). The observation that deletion break points frequently occur at short direct repeats has also prompted the idea that recombination may figure prominently in the molecular events leading to deletion (2, 4, 10, 11, 35, 36, 42, 44). If recombination does contribute to deletion, the recombination events are probably of the illegitimate rather than the homologous type usually mediated by the *Escherichia coli* RecA protein. The small size of the repeats distinguishes illegitimate recombination events (such as those associated with transposition, slip mispairing, and deletion) from homologous recombination, which is dependent upon extensive regions of homology (2, 6, 16). However, some studies show that deletions are RecA dependent (1, 3, 4), while others show no RecA dependence (6, 7, 11, 14, 15, 33, 53). Since the question of what role recombination plays in deletion is an important issue, we have collected data specifically addressing the involvement of recombination in deletions occurring in a nonessential gene of bacteriophage T7.

Bacteriophage T7 provides several advantages as a tool with which to study deletions. These include a wealth of genetic information (46–48), the availability of a complete sequence for the T7 genome (9), and a relatively good understanding of the biochemistry of T7 DNA replication

(32, 37). In addition, deletions in T7 frequently occur between direct repeats (30, 33, 48). The T7 DNA replication system is simpler than those of most phage or bacterial systems. T7 DNA can be synthesized in vitro with only the products of T7 gene 2.5 (single-stranded-DNA-binding protein), gene 4 (a helicase/primase), and gene 5 (DNA polymerase) (22, 32, 37, 43). The only known host protein important to T7 DNA replication is thioredoxin, which associates with the phage gene 5 product and confers extensive processivity upon the DNA polymerase (37). T7 is a highly recombinogenic phage, with recombination frequencies between widely spaced markers reaching as high as 40% (46). An in vitro recombination system has been developed for T7 (27, 39). Moreover, the availability of an efficient in vitro T7 DNA packaging system allows in vivo expression of DNA subjected to prior in vitro replication or recombination (23, 27). The high processivity of the T7 DNA polymerase and the high frequency of T7 recombination are two features of this phage that may be especially relevant to deletion frequencies.

Homologous recombination in T7 does not require any known host protein, and *E. coli* RecA protein serves no known function in T7 recombination (34). Available data do not reveal whether host systems important in illegitimate recombination play any role in T7 DNA metabolism. Thus, we set out to characterize deletion in T7 in vivo with the hope of eventually exploiting properties of the T7 in vitro systems to examine aspects of the biochemical processes involved in deletions. As part of the characterization of T7 deletion, we examined how the distance between direct repeats affects deletion, and we were surprised to find an abrupt decrease in deletion frequency as the distance between the direct repeats was increased from 85 to 94 bp. Those observations, described below, suggest that more than one mechanism may contribute to deletion in T7-infected *E. coli* and raise the possibility that either the short- or long-range deletion mechanism may involve recombination. To determine how often deletion between direct repeats is accompanied by recombination, we placed inser-

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FIG. 1. Scheme for detecting direct repeat-mediated deletion events. Shown is a small portion of the T7 genetic map centered on the ligase gene, which extends from position 6475 to 7552. The unique *Xho*I restriction site introduced into the T7 wild type by directed mutagenesis (33) is shown by the dark bar at bp 6663. Also shown are the T7 RNA polymerase promoter 1.3 (ϕ 1.3), and the *E. coli* RNA polymerase termination site (TE). The solid boxes represent direct repeats, one of which was designed into the synthetic DNA insert to match a sequence already present in the T7 wild type. Asterisks mark the positions of stop codons. Reversion to ligase proficiency (bottom line) requires a deletion event between the direct repeats to remove the insert.

tions of DNA bracketed by direct repeats in the T7 genome to interrupt the coding sequence of the nonessential ligase gene (gene 1.3; Fig. 1). Deletion was revealed by the restoration of function to the disabled gene. We measured the frequency with which deletion was accompanied by recombinational exchanges between the regions flanking the deleted sequence.

MATERIALS AND METHODS

Bacterial strains and bacteriophage. The bacterial strains of *E. coli* K-12 are listed in Table 1. Bacteria were grown in tryptone broth medium and plates or L-broth medium as described by Miller (29). Wild-type bacteriophage T7 and T7 temperature-sensitive (ts) 101 (ts in gene 4) were obtained from F. W. Studier. T7_{HS7} (with an opal mutation in gene 1.2) was obtained from C. C. Richardson. Wild-type T7 was mutated to create a unique *Xho*I restriction site at position 6663 in the ligase gene (1.3) without changing the wild-type

amino acid sequence (33). This phage mutant was designated T7X. T7 phage that are ligase deficient because of an insertion in the new *Xho*I site are referred to as T7X followed by a number corresponding to the length of the insert in base pairs. Thus, in the text "T7X29" refers to a phage that carries the insert identified as X29 (see below; Fig. 1). Strain N2668 contains a ts mutation in its ligase gene and is unable to support the growth of T7 gene 1.3 mutant phage which do not produce functional phage ligase (26, 47).

Construction of T7 phage that contain inserts. The construction of T7 containing inserts has been previously described (33). Briefly, synthetic oligonucleotides were made on an Applied Biosystems 380B DNA synthesizer and purified by reverse phase high-performance liquid chromatography. Complementary oligonucleotides were treated with kinase and annealed together to generate a small duplex DNA molecule that contains an easily detectable restriction site (usually for *Bam*HI) and *Xho*I sticky ends at each terminus. Genomic DNA from T7X was digested with *Xho*I and then ligated to the synthetic insert. After phenol-chloroform extraction and precipitation with ethanol, the DNA was resuspended in 10 mM Tris-0.1 mM EDTA (pH 7.5) and incubated in an in vitro T7 packaging reaction (23). Phage containing inserts were isolated by plating on a lawn of wild-type bacteria which was overlaid with a lawn of the ligase-deficient host. After overnight incubation at 32°C, cloudy plaques were isolated and analyzed for the presence of an insert in gene 1.3. In all cases, the identity of the phage was confirmed by DNA sequencing. The sequences of the inserts except X350 and X900 are shown in Fig. 2.

T7 phage containing inserts of 350 and 900 bp were made in a slightly different manner. We took advantage of a pseudorevertant of T7X76-I that contains 63 bp of nonsense DNA in the T7 ligase gene yet still scores as ligase positive on phenotypic tests. This phage also contained the *Bam*HI site, which is bracketed by 10-bp direct repeats. DNA was isolated as a *Bam*HI fragment from plasmids that contained rat or human DNA. This DNA was inserted into the *Bam*HI site of the T7X76-I revertant and characterized as described for the synthetic inserts. T7X350 was made from an ~300-bp

TABLE 1. Bacterial strains

Strain	Genotype	Reference, source, or comment
W3110	F ⁻ <i>sup</i> ⁰	Wild type
011'	F ⁻ <i>supE</i>	
N2668	F ⁻ <i>thyA</i> Str ^r <i>lig-7</i> (Ts)	Ts for ligase
WM295	F ⁻ <i>recA56</i>	JC5088(Hfr <i>recA56</i>) × W3110 <i>thyA</i> derivative, <i>recA56</i> (51)
AB1157	F ⁻ <i>thr leu arg his pro thi ara lac gal mtl xyl</i> Str ^r <i>supE</i>	Wild type for relevant markers
BW9109	F ⁻ <i>thr leu arg his pro thi ara lac gal mtl xyl</i> Str ^r <i>supE</i> Δ(<i>xth-pnc</i>)	Demple et al. (8)
HR44	<i>thr leu lys argH thi ara lacY gal malA mtl xyl</i> Str ^r <i>tonA sup</i> ⁰ <i>optA1</i>	Does not support growth of T7 with a mutation in gene 1.2; Saito and Richardson (40)

FIG. 2. DNA sequence of inserts used to interrupt the ligase gene. Shown is one strand of the pair of complementary oligonucleotides that were inserted into the *Xho*I restriction site in gene 1.3. The sequence is that of the DNA strand with the same sequence as the mRNA transcribed from the genome. Lowercase letters indicate the sequence in the T7 wild type before insertion of synthetic DNA; the uppercase letters indicate the sequence of the insert itself. The double lines above sequences mark the locations of direct repeats. Asterisks above sequences mark the positions of in-frame stop codons. Relevant restriction sites are underlined.

The strains used in the coinfection assays were constructed by standard recombination of the two markers, as described by Studier (46). For each insert used in these studies, two T7 strains carrying separate genetic markers which flank the insert on either the right or left side were constructed (Fig. 3). On the left of gene 1.3, an opal mutation in gene 1.2 was used. To the right of gene 1.3, a ts mutation in gene 4 served as a marker. Phage stocks were prepared as described by Studier (46) from single plaques grown on the *E. coli* strain to be used in the assay. These single plaque isolates were then concentrated by pelleting the phage at $100,000 \times g$ for 90 min and resuspending them in T7 diluent (10 mM Tris hydrochloride [pH 7.5], 10 mM $MgCl_2$, 500 mM NaCl) (49).

Fluctuations test. A test of deletion frequency, based on the Luria and Delbruck (24) fluctuations test, was performed. The advantage of this assay is that it measures the frequency of deletion independent from the rate of growth. This is important, since wild-type T7 strains grow significantly faster than ligase mutant T7 (data not shown). Cultures were grown to mid-log phase, and T7 was added at a multiplicity of infection of 0.1. The incubation was continued for 2 to 5 min to allow phage absorption. Then, the culture was diluted in warm broth and the diluted phage-infected cells were dispersed in 1-ml cultures. These cultures were incubated for 2 h to allow cell lysis to occur. A 10- μ l volume from five cultures was used to determine the total phage yield (N) on the appropriate wild-type *E. coli* strain. The entire 1-ml culture was plated on N2668, a strain which is ts for host ligase, on which only T7 with normal phage ligase (lig^+) will grow (26, 47). After overnight growth, the fraction of plates with no plaques was used to determine the probability (P) that the number of deletions (X) is zero. The frequency of deletion (d) was calculated as $P(X = 0) = e^{-dN}$.

Coinfection assay. This assay determines the amount of recombination between T7 phage strains in vivo and closely follows the Studier (46) method for measuring recombination frequency. *E. coli* 011' was grown in T broth (29) to mid-log phase. This bacterial culture was treated with 1/10 volume of 0.04 M KCN. The KCN-treated bacteria (0.4 ml) were added to a culture tube. We were careful to add an equal number of

FIG. 3. Strains used in recombination. Shown are diagrams of the construction of the pairs of $T7 \times \eta$ ($\eta = 29$ and 350 bp) strains which were used in the coinfection assay. One strain in each pair contained an *opal* mutation in gene 1.2 and cannot grow on *E. coli* HR44. The partner strain contained a *ts* mutation in gene 4 and cannot grow at 43°C. , Positions of the 10-bp direct repeats bracketing the insert (Fig. 1); , intercistronic regions.

each phage to the infection. The titers of phage stocks to be used were determined three times and were averaged. The two phage stocks were mixed together before infection, and a volume equal to the volume of bacteria (0.4 ml) was added to the bacteria, resulting in a multiplicity of infection of 10 (in some experiments 5) for each phage strain. After allowing 5 min for absorption, the phage-infected cells were diluted into fresh T broth by a factor of 6×10^{-5} . The culture was then split into separate 1-ml cultures. These were incubated for 1 h. A 10- μ l volume was used to determine the titers of each of five cultures on 011'. The titers on 011' were averaged to give a total phage yield. The remainder of each 1-ml culture was plated on N2668 and grown overnight. The plaques which grew on N2668 represented *lig*⁺ phage which had deleted the insertion and restored the proper reading frame to the ligase gene. To test for the presence of the genetic markers, 1.2 mutant and 4ts, only one plaque from each plate was toothpicked onto lawns of HR44 (restrictive for 1.2 mutant phage) and on 011'. The HR44 plate and one 011' plate were grown at 32°C, and a second 011' plate was grown at 43°C (restrictive for 4ts phage). These plaques were then categorized according to their phenotypes. The amount of recombination unrelated to deletion was measured similarly by using plaques from the plates used to determine the phage yield and which had not been selected for ligase activity.

RESULTS

Use of synthetic DNA inserts to permit quantitative measurement of deletion frequency. To measure deletion between direct repeats, we used a technique similar to ones used successfully in other biological systems (3, 44), in which a quantitative estimate of a specific type of deletion event at a well-defined genetic locus was achieved by monitoring the elimination of a piece of foreign DNA, bracketed by direct repeats, from a selectable nonessential T7 gene. T7 ligase is a dispensable phage enzyme (26, 47), since under normal conditions the phage can use the host ligase to carry out DNA metabolism. *E. coli* mutants deficient in ligase permit the growth of wild-type T7 but cannot support the growth of ligase-deficient phage. Thus, in the experiments reported here, insertions were placed into T7 gene 1.3, which encodes

T7 ligase, and selection of phage that had deleted the insert was achieved by examining the ability of the phage to grow on a host (strain N2668) which will not support the growth of T7 ligase mutants.

All deletion measurements in this study were made at the same position in the T7 genome, the unique *Xho*I site created in gene 1.3 (encoding ligase). As deletions in T7 typically occur at 8- to 13-bp direct repeats (33), inserts were designed to give 10-bp direct repeats when ligated into the *Xho*I site. Most of the inserts were designed to create stop codons in all three reading frames and to make a unique restriction site in those phage that carried the insert (Fig. 2). Because of the limitations imposed by its short length, the X16 insert had a stop codon in only the in-frame sequence. With inserts ranging between 16 to 94 bp, the presence of the insert was verified by sequencing a relevant segment of the phage genome. The presence of the larger inserts was verified by analysis of the pattern of the restriction digest. DNA from ligase-proficient revertants of phage that had contained an insert were examined for loss of the restriction site diagnostic of the presence of the insert. To be sure that the particular phage examined were not all siblings, experiments were done with at least three independent lysates produced by infection of phage with each one of the inserts. In the case of the X16 insert, one of eight revertants tested was found to still have the *Xba*I recognition sequence for the restriction site within the insert. One of 16 revertants of T7X85 phage was found to still have a *Bam*HI site. For all the rest of the inserts, there were no other instances of a revertant still containing a restriction site from the insert. In a previous study (33), revertants from T7X76 and T7X94D were sequenced and found to have been restored to wild type (T7X). Thus, we conclude that essentially all of the revertants isolated from phage that had carried inserts bracketed by 10-bp direct repeats arose via deletions between the direct repeats.

Effect of distance between direct repeats on deletion frequency. The data in Table 2 show that the deletion frequency between 10-bp direct repeats decreases as the length of the spacer region increases. The shorter inserts (16 to 85 bp) are excised from the ligase gene at a frequency of about 10^{-6} . Longer inserts are spontaneously deleted from the T7 ge-

TABLE 2. Measurements of deletion frequency as a function of spacer size by using fluctuations tests^a

Insert size (bp)	Expt no.	No. of cultures	Fraction of cultures without deletions	Deletion frequency (10 ⁻⁷)	Avg (±SD) deletion frequency (10 ⁻⁷)
16	1	72	0.46	7.9	3.6 ± 2.9
	2	72	0.68	1.2	
	3	72	0.33	2.4	
	4	39	0.69	2.8	
29	1	72	0.53	16.0	12.1 ± 3.9
	2	71	0.55	8.3	
	3	72	0.33	12.0	
76	1	50	0.34	6.5	7.4 ± 2.3
	2	72	0.63	10.0	
	3	70	0.37	5.6	
85	1	72	0.57	16.0	15.3 ± 3.6
	2	72	0.53	13.0	
	3	72	0.40	20.0	
	4	72	0.72	12.0	
94	1	46	0.37	0.38	0.62 ± 0.41
	2	72	0.51	1.1	
	3	72	0.24	0.39	
350	1	71	0.58	0.68	0.41 ± 0.24
	2	72	0.54	0.24	
	3	72	0.67	0.30	
900	1	50	0.45	0.13	0.13 ± 0.01
	2	49	0.37	0.14	
	3	49	0.47	0.13	

^a Fluctuations tests were carried out with 1-ml cultures of strain W3110 grown at 37°C as described in the text.

nome with an approximately 10-fold-lower frequency. The data show a rather striking reduction in deletion frequency in the range between 85 and 94 bp. There was a much more modest decrease in deletion frequency as the length of the spacer region was increased between 94 and 900 bp. This sharp change in deletion frequency could be due to the involvement of more than one mechanism of deletion, with a second mechanism augmenting or inhibiting the first for deletions above or below the 85- to 94-bp length.

Deletion frequency is independent of recombination. Deletions occurring between directly repeated sequences could result from intermolecular recombinational crossing over between the leftmost direct repeat on one DNA molecule and the rightmost direct repeat on another molecule. In molecules that had undergone deletion, the region to the left of the deleted sequence would be donated by one partner in the exchange while the region to the right of the rightmost direct repeat derives from the other partner. This intermolecular homologous recombination should depend upon the probability with which the direct repeats are brought into alignment. We expect this juxtaposition to be insensitive to the distance between direct repeats (similar to the relative insensitivity of deletion frequency to the increase in insert length from 94 to 900 bp; Fig. 4). If deletions arise frequently by such an intermolecular recombinogenic mechanism, this would predict that the population of phage with deletions would be disproportionately enriched with recombinants vis a vis the population of phage without deletions. To test the dependence on recombination, four phage stocks were constructed. One of these carried a 350-bp insert located between two 10-bp direct repeats in gene 1.3 and a mutation in gene 1.2 (Fig. 3). The partner to this phage also carried the

same 350-bp insert but was wild type for gene 1.2 and had a *ts* mutation in gene 4 to the right of the ligase-encoding sequence. A similar set of phage stocks was constructed for the 29-bp insert.

Bacteria were infected at a high multiplicity of infection with equal numbers of the two phage types, ligase-proficient

FIG. 4. Deletion frequency as a function of distance between 10-bp direct repeats. Fluctuations tests were performed to establish the frequency with which several of the inserts shown in Fig. 2 were deleted from the T7 genome. Shown are the average values of at least three separate fluctuation tests performed on phages that carried each of the inserts.

TABLE 3. Recombination with 29- and 350-bp deletions

No. of base pairs deleted ^a	Plaque type	Amt recombination					Total no. of plaques tested
		Parental type		Recombinant type		% Recombination	
		4ts	1.2	WT ^b	1.2, 4ts		
29	Ligase positive	88	1.5	7.5	3	10.5	67
		93	4	3	1	4	80
		55	29	16	0	16	31
	Unselected	78	15	5	2	7	85
350	Ligase positive	64	31	5	0	5	84
	Unselected	42	46	6	6	12	50

^a *E. coli* 011' was infected at 30°C to optical density at 590 nm (OD₅₉₀) values of 0.4 and 0.6 for X29 and X350, respectively.

^b WT, Wild type.

phage were recovered, and the recovered phage were tested for phenotype with regard to 1.2 and 4ts. The numbers of both 1.2 mutant 1.3⁺ 4ts phages and wild-type phages were determined in order to establish the number of recombinants. For comparison, unselected phage (with a reversion frequency to lig⁺ of 10⁻⁷ to 10⁻⁶) were also recovered from the same infection, and these were tested for phenotype. To eliminate concern that wild-type phage presumed to have arisen from recombinogenic crosses might simply be revertants that had lost either the 1.2 mutation or 4ts markers, the stocks of phage used to initiate the infection were chosen to be very low in reversion of the relevant genetic marker. Approximately 60 phage were recovered from each bacteria in the culture, thus assuring that replication had taken place. To relieve the concern that the lig⁺ phage recovered from the experiments might be siblings reflecting only a few, perhaps nonrepresentative, deletion events, a large number of cultures of phage-infected cells were examined under conditions that assured that only a few deletion events occurred during lysis of the culture. *E. coli* isolates were infected with a phage mixture, and the infected cells were distributed to a large number of separate culture tubes. After lysis of the bacteria, a small (10 µl) portion was removed from several cultures, titers were determined, and the phage were plated on an indicator bacterium (011') that would not discriminate between lig⁺ and lig mutant phage. The entire contents of the culture tube were plated on the selective host (N2668) in the manner of the Luria and Delbruck fluctuations test (24). Only one plaque was examined from each of the 30 to 100 cultures plated in an experiment. Thus, each plaque examined must result from an independent deletion event.

In Table 3, we looked for recombination of the flanking markers concurrent with the deletion of the 29- and 350-bp inserts. We examined the deletion of X29 in three separate experiments, each of which started with over 90 separate cultures. We were able to test from 31 to 80 plaques per experiment. Among these, the number of recombinants was relatively low (4 to 16% of the total). This was not significantly different than the background value of 7% recombination seen among the 85 unselected plaques tested. We also measured the amount of recombination associated with deletion of X350. In seven separate experiments involving between 30 and 100 cultures each, we found 5% recombinants in a total of 84 plaques tested for phenotype. This compared well with the background value of 12% recombinants seen in the 50 unselected plaques. As can be seen from

the levels of recombination between genes 1.2 and 4 irrespective of deletion, the absence of T7 ligase (due to the presence of the inserts) does not eliminate recombination in this system. The host ligase is sufficient to give 7 to 12% homologous recombination in the unselected phage. The 1.2 marker was underrepresented among the lig⁺ and the lig mutant plaques. The reason for this is not clear but may be related to the ability of the 1.2 mutants to form normal plaques on strain HR44 after growth in the 011' strain used for recombination. Among the phage that have not undergone deletion, the yield of wild-type phage is reasonable considering the distance between the 1.2 and 4 genes (27). This provides added assurance that the bacteria were infected with both parents (5) and that both parents were available for recombination. In view of this, we believe that the yield of 4ts nonrecombinants provides a more reliable index of phage recovery and that the number of wild-type recombinants is a more reliable index of recombination frequency.

Effects of phage mutations on deletion frequency. On the basis of what is known about DNA metabolism in T7, there is no reason to suspect that either of the two marker genes used to assay recombination would by themselves affect recombination between direct repeats. But, since the products of both gene 1.2 and gene 4 are involved in DNA synthesis (22, 31, 32, 37, 43), it was important to ascertain whether either of these mutations might cause significant effects on the frequency of deletion of either the longer or shorter inserts. Therefore, we measured the deletion frequency of the two newly constructed strains of each of the insertions. Figure 5 shows the data from three separate measurements of deletion frequency in each of the strains used. The average deletion frequency of T7 1.2 X29 was 7.57×10^{-7} , approximately threefold lower than the average deletion frequency of T7 X29 4ts (25.1×10^{-7}). This trend was repeated with the average deletion frequency of T7 1.2 X350 (1.51×10^{-7}) and T7 X350 4ts (5.15×10^{-7}). This result is consistent with the low number of 1.2 mutant 4ts recombinants compared with wild-type recombinants shown in Table 3.

Effects of host mutations on deletion frequency. One explanation for the difference in the deletion frequency of the 29- and 350-bp inserts has to do with host functions that act preferentially to reduce the frequency of longer deletions. As noted above, several studies have suggested a relationship between the *recA* mutation and deletion frequency. Fluctu-

FIG. 5. Deletion frequency of strains used to measure recombination. Fluctuation tests were performed on the strains used in the coinfection assay. 011' was grown in T broth (29) at 30°C to an OD₅₉₀ of 0.4 for the X29 strains and an OD₅₉₀ of 0.6 for the X350 strains. 011' was infected at a multiplicity of infection of 0.1, dispersed into 1-ml cultures, and incubated 2 h to lysis. A 10-μl portion was removed from five cultures to determine the average total phage yield per culture. The remainder of each 1-ml culture was plated on N2668 to assay for deletions. The results were analyzed as outlined in Materials and Methods to determine the deletion frequency. Three separate determinations of deletion frequency with 95 cultures each were made for each strain.

ations tests were used to determine the dependence of T7 deletions of 29- and 350-bp inserts on *recA* (Table 4). The deletion frequency of T7 X350 was unaffected by the host *recA* mutation. In contrast, there was a fourfold reduction in the frequency with which T7 X29 was deleted when grown in a *recA56* strain, suggesting that this host mutation (or a closely associated gene) may affect the deletion of short inserts. However, in the data for the T7 X29 phage grown on

the *recA*⁺ strain, there is almost a twofold difference within the data set. Therefore, although *recA* appears to influence the deletion frequency of the X29 insert, this is not a major effect. Earlier studies using a simpler assay (33) did not reveal a significant effect due to *recA* upon excision of X76.

Yi et al. (53) have reported that *recA*-independent deletion events were increased 18- to 35-fold in BW9101, an *E. coli* strain with a deletion in the region between *xth* and *pnc*. That work indicated the presence of an uncharacterized gene which influences illegitimate recombination (deletion frequency) in this region. We examined the deletion frequency of X29 and X350 in the BW9101 background and the isogenous AB1157 wild type (Table 5). These data show that the as yet uncharacterized function missing in the *xth-pnc* deletion mutant has no effect on either class of deletion event in T7.

DISCUSSION

Under the experimental conditions used here, deletion frequency was not a smooth and continuous function of the distance between 10-bp direct repeats. Deletion frequency fell rapidly as the length of the insert increased from 85 to 94 bp. Beyond this, the deletion frequency fell off slowly as a nearly linear function of the distance between direct repeats as the insert length was increased by an order of magnitude. The pronounced difference in deletion frequency between the 85- and 94-bp inserts suggests that excision of the shorter inserts from the chromosome may involve a fundamentally different mechanism that acts in addition to the mechanism responsible for longer deletions. If this interpretation is correct, it means that there is a well-defined upper boundary to the distance at which the short-range mechanism acts. The reason for the abrupt transition in deletion frequency as insert lengths are increased beyond 85 bp is not clear, but it may mean that the short-range deletions occur very near the replication fork at which DNA is in immediate contact with replication enzymes. Thus, the length of protein-DNA contacts might be important, or wrapping of the DNA around certain structures or writhing of the DNA itself might tend to bring direct repeats separated by a specific distance into juxtaposition. The observation that the deletion frequency did not increase when the spacer length decreased below 85

TABLE 4. Effect of *recA* on deletion frequency^a

Insert size (bp)	Strain	No. of cultures	Fraction of cultures without deletions	Titer (±SD) of cultures (10 ⁶)	Deletion frequency (10 ⁻⁸) ^b	Avg (±SD) deletion frequency (10 ⁻⁸)
29	W3110 <i>recA</i> ⁺	76	0.29	1.40 ± 0.19	88.5	110.8 ± 29
		95	0.78	0.25 ± 0.14	99.9	
		96	0.63	0.32 ± 0.12	144.0	
	WM295 <i>recA</i> mutant	40	0.48	2.69 ± 0.25	27.8	27.0 ± 6.3
		72	0.63	2.32 ± 0.64	20.3	
		72	0.40	2.76 ± 0.28	32.9	
	W3110 <i>recA</i> ⁺	72	0.51	37.4 ± 6.2	1.8	1.66 ± 0.34
		40	0.58	43.2 ± 6.5	1.28	
		96	0.55	31.1 ± 3.8	1.91	
350	WM295 <i>recA</i> mutant	96	0.56	27.3 ± 2.5	2.11	2.47 ± 0.50
		96	0.78	12.0 ± 9.7	2.06	
		40	0.68	16.6 ± 2.8	2.38	
	W3110 <i>recA</i> ⁺	72	0.64	13.4 ± 1.9	3.34	

^a Bacteria were grown at 37°C in L broth (21) to an OD₅₉₀ of 0.6.

^b The deletion frequency was determined with the fluctuations test as described in Materials and Methods.

TABLE 5. Effect of *E. coli xth-pnc* on T7 deletion frequency^a

Insert size (bp)	Strain	No. of cultures	Fraction of cultures without deletions	Avg ($\pm$ SD) titer of cultures (10^6)	Deletion frequency (10^{-7}) ^b	Avg deletion frequency (10^{-7})
29	AB1157	40	0.30	2.6 ± 0.9	4.6	4.8 ± 1.1
		40	0.30	3.4 ± 7.0	3.5	
		40	0.68	0.6 ± 0.3	6.1	
		72	0.40	1.9 ± 0.9	4.8	
	BW9109	40	0.45	1.6 ± 0.7	5.0	3.3 ± 1.3
		40	0.25	7.2 ± 2.6	1.9	
		40	0.68	1.2 ± 0.5	3.0	
		40	0.30	3.6 ± 1.5	3.3	
350	AB1157	72	0.53	5.6 ± 0.3	1.1	0.91 ± 0.27
		72	0.63	6.5 ± 3.3	0.71	
	BW9109	40	0.63	3.8 ± 1.1	1.2	0.94 ± 0.40
		72	0.38	14.7 ± 3.6	0.7	

^a The bacteria were grown at 37°C in L broth (21) to an OD₅₉₀ of 0.6.

^b The deletion frequency was determined by using the fluctuations test as described in Materials and Methods.

bp could mean that there is an intrinsic limitation in the short-range deletion mechanism that will not allow deletions to accumulate at a higher rate. For example, constraints imposed by the replication proteins or by primer-template configurations at the replication fork may limit the deletion frequency. Alternatively, a repairlike mechanism could eliminate some deletions.

Our data argue that in strains of bacteriophage T7-infected *E. coli* a higher frequency of recombination between flanking markers does not accompany deletion between 10-bp direct repeats, for either the long- or short-range deletions. Our assay detects only recombination between distinct chromosomes. If these phage associate randomly, approximately half of the recombination would occur between phage of the same genotype and we could detect at most 50% of the recombinants. Exchanges involving newly replicated sister DNA molecules still attached to the parent strand by an active replication fork will also be genetically silent. Nonetheless, the assay is sufficiently sensitive to reveal redistributions of genetic markers among DNA molecules which had undergone deletion due to intermolecular or double-strand break recombination taking place between distinct parents. Our observation that the T7 phage which reverted to wild type because of deletion show the same amount of recombination as the phage which had not experienced deletion (Table 3) argues that intermolecular recombination between short repeated sequences does not drive deletion mutagenesis in either class of deletion events. These data do not eliminate the possibility that sister strand recombination or intramolecular recombination may predominate the deletion mechanism.

The possible influence of two host functions on deletion was examined. Table 4 shows that the deletion of X350 is independent of *recA*. The apparent involvement of *recA* in 29-bp deletions appears to contradict other data (Table 3), which give no indication of association between deletion and recombinogenic exchanges. But as previously noted by Albertini et al. (1), *RecA* is a pleiotropic protein, and dependence upon *recA* could be related to other properties. The $\Delta xth-pnc$ host mutation, previously shown to influence deletion frequency (53), also has no effect on deletion frequency in this system (Table 5).

In bacteriophage T4, Singer and Westlye (44) found a

significant level of recombinogenic exchange during the deletion of inserts between 24-bp direct repeats. The length of the direct repeats in our study corresponds to what is typical of spontaneous deletions in T7 (30, 33, 48), and this is less than half of the length of the direct repeats used in the T4 study. Thus, recombination may be more important in excisions mediated by long direct repeats. On the other hand, the data may simply reflect fundamental differences in the biochemistry of T4 and T7 DNA replication and recombination.

The data shown in Table 3 confirm the difference in deletion frequency when phage with inserts of 29- and 350-bp inserts are compared (Table 2). In the presence of the 4ts mutation, both the 29- and the 350-bp inserts were deleted with higher frequency than what was measured with the same two inserts when a 1.2 mutation was present. Gene 1.2 encodes an inhibitor of *E. coli* dGTP triphosphohydrolase (19). When T7 deficient in 1.2 infects *E. coli*, the dGTP pools do not increase as greatly as with a T7 wild-type infection. T7 DNA synthesis is decreased in the absence of gene 1.2 due either directly to the protein deficiency or to the induced dGTP deficiency (31). The gene 4 product is a primase and a helicase (22, 43). Even at the permissive temperature at which these experiments were done, this critical protein may not function the same as a truly wild-type gene 4 product. Although the function of the *ts* gene 4 product in these constructs is poorly defined, the *ts* mutation in gene 4 may affect the amount of single-stranded DNA, which, in turn, may be responsible for the altered deletion frequency. The relative paucity of 1.2 mutant 4ts recombinants as compared with wild-type recombinants (Table 3) may be due to a decrease in viability for two mutations affecting DNA replication. Since both the 1.2 and 4ts mutations may affect replication fork transit, the relative differences in deletion frequency seen in the presence of these mutations are compatible with the idea that replication intermediates are more important than recombination in causing deletion.

Our demonstration that under the experimental conditions employed recombinogenic exchange is not integral to deletion between direct repeats in T7-infected *E. coli* is not in accord with data from a number of different biological systems pointing to the involvement of recombination in deletion mutagenesis (1, 3, 4, 44). In addition, recombination

models of deletion mutagenesis are supported by evidence for gene duplication mediated by short homologies in *E. coli* (50). However, not all previous studies of deletion mechanisms support the involvement of recombinational exchange (2, 7, 15, 17). For example, some models invoke replicative mechanisms based on the Striesinger copy choice model of frameshift mutagenesis (45). Our data (Fig. 5) showing that mutations in T7 gene 1.2 and/or gene 4 affect deletion are also mostly easily explained by a mechanism involving a replicative intermediate. Neither homologous recombination (Table 3) nor at least one system known to affect illegitimate recombination (Table 5) can explain the sharp difference between the frequency of deletion of short and long inserts. This invites speculation that DNA rearrangements during the replicative process may sometimes cause deletion. If the intermediates to deletion involve DNA structures or protein-DNA interactions in the immediate vicinity of the replication fork and are thereby especially sensitive to the separation between direct repeats, this might explain why a narrow range of insert lengths strongly affects deletion frequency.

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