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In Vitro Repair of Double-Strand Breaks Accompanied by Recombination in Bacteriophage T7 DNA

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A double-strand break in a bacteriophage T7 genome significantly reduced the ability of that DNA to produce viable phage when the DNA was incubated in an in vitro DNA replication and packaging system. When a homologous piece of T7 DNA (either a restriction fragment or T7 DNA cloned into a plasmid) that was by itself unable to form a complete phage was included in the reaction, the break was repaired to the extent that many more viable phage were produced. Moreover, repair could be completed even when a gap of about 900 nucleotides was put in the genome by two nearby restriction cuts. The repair was accompanied by acquisition of a genetic marker that was present only on the restriction fragment or on the T7 DNA cloned into a plasmid. These data are interpreted in light of the double-strand gap repair mode of recombination.

A popular model for repair of a double-strand break, based upon the recombination of the broken molecule with an intact portion of a homologous region of duplex DNA, is supported by considerable experimental evidence (23, 36–39). In this model, the double-strand break is first widened to form a gap so that each strand of both pieces of the broken DNA can establish base pairing with complementary strands on the intact homolog. Repairlike DNA synthesis with the unbroken donor molecule as template would fill the gap. The recipient DNA molecule which had suffered a strand break thereby acquires genetic information present on the donor molecule. There is also considerable evidence that phage T4 recombination is stimulated at the ends of DNA molecules (17). This paper describes repair of a double-strand break in the highly recombinogenic (34, 35) bacteriophage T7. T7 recombination is independent of the host RecA recombinase (21), and T7 is known to inactivate exonuclease V, the product of the *Escherichia coli recBCD* genes (43). It has been suggested that T7 single-strand-binding protein may play a central role in promoting the annealing of complementary single strands of phage DNA (28). T7 endonuclease I (gene 3), helicase-primase (gene 4), DNA polymerase (gene 5), and exonuclease (gene 6) play roles in T7 recombination (5, 7, 11, 12, 22, 26, 40, 41). The precise molecular mechanisms responsible for T7 recombination are poorly understood. Therefore, possible involvement of strand breaks in this phage's recombination mechanism(s) remains an open question.

Other workers have reported in vitro recombination of T7 DNA (6, 9, 25–29, 42). Recombination has also been observed with an in vitro system designed to maximize DNA replication (14, 15). Conditions that promote DNA replication may be important to recombination by stabilizing the exogenous DNA or by altering the mechanisms contributing to recombination. There is no significant degradation of exogenous DNA during in vitro DNA replication (15). Furthermore, both the observed high frequency of genetic exchange and the observed dependence on marker position are compatible with what is known about in vivo recombination (14). Also, recombination contributes to recovery of damaged DNA into viable phage in this system (14). These results have sparked interest in knowing whether repair of double-strand breaks would occur in the in vitro DNA replication system and whether this repair would be associated with recombination. The results of these measurements are presented below.

MATERIALS AND METHODS

Bacteria and phage. Bacteria used in this study were the wild-type, suppressor-free *E. coli* strain W3110, *E. coli* TB-1 [*ara* Δ(*lac-proAB*) *rpsL hsdR* Φ80 *lacZM15*], the suppressor-free *Shigella sonnei* strain ShD2 371-48, and *S. sonnei* Sh3-18, which contains an amber suppressor. Both *S. sonnei* strains and the ss$^-$ ("suicide in *Shigella*") T7 strain were gifts from R. Hausmann. Wild-type T7 and T7 with amber mutation *am*29 in gene 3, *am*20 in gene 4, *am*28 in gene 5, or *am*147 in gene 6 were from W. Studier. Our previous studies had shown that a single A-to-C transversion at position 23150 in the T7 genome is responsible for the ss$^-$ mutation (20). Plasmid pJP7 is a derivative of pUC19, into which a 620-bp *Tha*I fragment of T7 with the ss$^-$ mutation had been cloned. The 3,306-bp pJP7 plasmid has previously been described (20). Bacteria and phage were grown by using L broth and agar plates made from T broth (16).

DNA. T7 DNA was purified as described by Richardson (24). Plasmid pJP7 DNA was prepared by alkaline lysis of strain TB-1 carrying that plasmid (30). The DNA sequence of bacteriophage T7 (2) was the source for identifying relevant sites on the T7 genetic map. Restriction digests were performed with enzymes from New England Biolabs or Bethesda Research Laboratories under the conditions recommended by the suppliers. In all cases, agarose gel electrophoresis showed that the restriction digests produced the pattern of DNA fragments expected from complete digestion at known sites. Restriction enzymes were inactivated by phenol-chloroform extraction followed by ethanol precipitation and resuspension in 10 mM Tris HCl (pH 7.5)–0.1 mM EDTA. Ethanol precipitation and resuspension sometimes reduced the ability of T7 DNA to be packaged efficiently, but this step was essential for removing glycerol (present in preparations of restriction enzymes), which inactivates the packaging reactions when present even in trace amounts (31).

In vitro DNA replication and recombination reactions. Extracts for in vitro replication and recombination were

TABLE 1. Effect of a double-strand break on in vitro recombination between T7 6^- ss^- DNA and wild-type DNA[a]

DNA		Titer on:			*Shigella sup*^{0} strain/W3110 (%)[b]	*Shigella sup*^{0} strain/*Shigella sup*$^+$ strain (%)[b]
Wild type	6^- ss^-	W3110	*Shigella sup*^{0} strain	*Shigella sup*$^+$ strain		
None	Present	1.7×10^4	8.0×10^3	9.1×10^6		
Control	None	4.3×10^6	2	7		
Cut	None	2.3×10^5	0	8		
Control	Present	3.0×10^6	3.5×10^5	1.1×10^7	11.6	3.2
Cut	Present	6.3×10^5	6.1×10^5	1.2×10^7	91	5.2

[a] Intact T7 6^- ss^- DNA was incubated together with wild-type T7 DNA that was either untreated (control) or digested with *Nhe*I restriction endonuclease (cut). After in vitro packaging, the phage were plated on the wild-type *E. coli* strain W3110 or on *S. sonnei* with (*sup*$^+$) or without (*sup*^{0}) an amber suppressor.
[b] Ratio of plaque counts on the indicated strains.

prepared with *E. coli* W3110 infected with T7 (4). Unless otherwise specified, the phage used to prepare the extracts had an amber mutation in gene 3. Reaction conditions have been described (14). A typical 0.05-ml in vitro recombination reaction mixture contained 4.5 nmol (nucleotide phosphorous equivalents) of T7 wild-type DNA plus either 9.0 nmol of a *Bst*XI restriction digest of 6^- ss^- DNA or 15.0 nmol of pJP7 DNA. Incubation was at 37°C for 30 min.

In vitro packaging. In vitro packaging was carried out as described elsewhere (8, 13, 31). In the present experiments, the extracts for packaging were made with strain W3110 infected for 18.5 min at 31°C with T7 that had amber mutations in genes 3, 5, and 6. A 0.036-ml portion of each DNA replication and recombination reaction mixture was added to 0.064 ml of packaging reaction mixture (8, 13), and 0.01 ml of that mixture was added to 0.02 ml of packaging extract. After 60 min of incubation at 31°C, the packaging reaction mixtures were diluted, resulting phage were plated on appropriate indicator bacteria, and the plates were incubated overnight at 30°C. The values in the tables below show the total phage present in the packaging reaction mixtures. A typical packaging reaction mixture contained 1.3×10^9 phage equivalents of exogenous (template) DNA.

RESULTS

To examine the association of recombination with the repair of double-strand breaks in bacteriophage T7, a double-strand cut was made in wild-type T7 genomes with *Nhe*I restriction endonuclease. This enzyme, which has a single recognition site on T7 DNA, puts a double-strand staggered break with a 4-base 5′ overhang at position 22969. The DNA was incubated under conditions that permit in vitro DNA replication and recombination, the product of the reaction was packaged, and the resulting phage were tested for viability. As seen in Table 1, the break reduced the ability of the DNA to make viable phage by a factor of about 20. The break was located 181 bp from the selectable genetic marker ss^- (Fig. 1). Wild-type T7 (ss^+) is unable to grow on *S. sonnei* (3). However, the ss^- mutation, an A-to-C transversion at position 23150 in gene 10, extends the host range of T7 to enable it to grow equally well on either *S. sonnei* or *E. coli* cells (3, 20). When DNA with an amber mutation in gene 6 and an ss^- mutation in gene 10 was incubated in the reaction mixtures, many more plaques were found on the *S. sonnei* with an amber suppressor. When both the 6^- ss^- DNA and the wild-type DNA were included in the reaction mixture and the product was packaged, about 12% of the phage were ss^- but free of amber mutations. When a double-strand break was put in the wild-type DNA, the cut increased the yield of phage able to grow on the *S. sonnei* suppressor-free host, so that essentially all of the phage recovered now carried the ss^- mutation but did not have the amber mutation in gene 6.

An experiment in which the DNA that carried the ss^- marker could not by itself be packaged into viable phage was done. Detection of transfer of the ss^- mutation could result only if the originally ss^+ T7 genome acquired an ss^- muta-

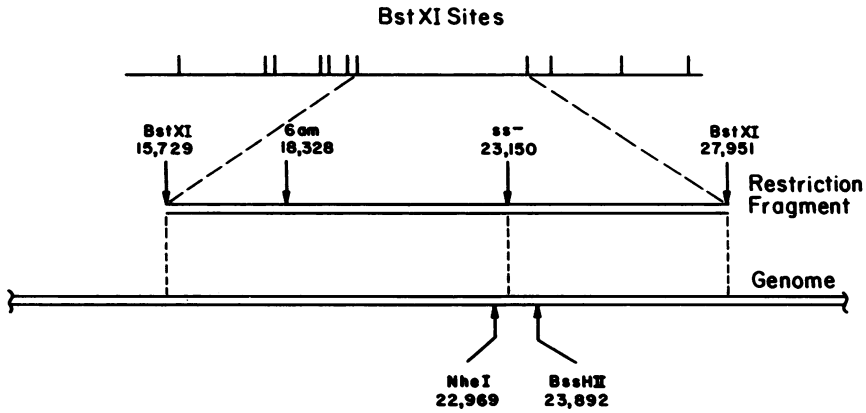

FIG. 1. Recombination between a T7 genome and a T7 restriction fragment. The top line shows the *Bst*XI restriction sites on the T7 genome. The positions of the amber 147 mutation (in gene 6) and of the ss^- mutation (in gene 10) on the 12,222-bp *Bst*XI restriction fragment are shown on the second line. The third line represents the T7 genome, with the single *Nhe*I and *Bss*HII restriction sites indicated. The information in the figure is based upon the sequence reported by Dunn and Studier (2).

TABLE 2. Recombination between a restriction fragment and T7 genomic DNA[a]

*Bst*XI restriction fragment	Genomic DNA[b]	Titer on:		% Recombination
		W3110	*Shigella* sup^0 strain	
None	Uncut	8.4×10^7	0	
None	*Nhe*I cut	6.7×10^6	0	
None	*Bss*HI cut	3.3×10^6	0	
None	Double cut	2.1×10^6	0	
+	None	7.8×10^3	5.9×10^2	
+	Uncut	9.4×10^7	1.3×10^6	1.4
+	*Nhe*I cut	1.4×10^7	1.1×10^7	78.6
+	*Bss*HI cut	1.1×10^7	9.9×10^6	90.0
+	Double cut	1.7×10^7	1.3×10^7	76.5

[a] Recombination was measured after in vitro reaction of T7 6^- ss^- DNA digested with *Bst*XI (+) or without ss^- DNA (none).
[b] The T7 genomic DNA was undigested (uncut), treated with either *Nhe*I or *Bss*HI alone, or treated with both of those enzymes (double cut).

tion. 6^- ss^- DNA was digested with *Bst*XI restriction endonuclease, which has 11 recognition sites on T7. The fragment, which extends from position 15729 to position 27951, carries both the 6^- amber and the ss^- mutations. Because reassembly of a functional T7 genome from 12 pieces is a very unlikely event, the DNA with the ss^- mutation that is introduced into the reaction mixtures does not contribute to the total yield of phage. T7 able to grow on *Shigella* spp. can arise only when the wild-type T7 genome acquires the ss^- mutation from the *Bst*XI digest. When the DNA from the restriction digest was incubated in the in vitro replication and recombination reactions, essentially no biologically active DNA was produced (Table 2). The few viable phage that were found when only the *Bst*XI-digested DNA was present may come from contamination of an extract or from wild-type DNA produced by recombination between endogenous 3^- DNA (present in the extract) and the *Bst*XI digest of 6^- ss^- DNA. Intact T7 genomes produce viable phage particles, but essentially none of these are ss^- (Table 2). About 1.4% of the phage produced after incubation of wild-type DNA together with *Bst*XI-digested 6^- ss^- DNA were able to grow on suppressor-free *S. sonnei*. A double-strand break produced with *Nhe*I endonuclease reduced by about an order of magnitude the potential of the wild-type DNA to form viable phage. The *Nhe*I digestion appeared complete, as judged by electrophoresis of the products (data not shown). The higher-than-expected yield of phage produced from the *Nhe*I-cut DNA may be due to rescue by recombination with the endogenous (ss^+) DNA present in the extracts for DNA synthesis. When the restriction fragment from 6^- ss^- DNA was present, the *Nhe*I strand break in the T7 genome substantially increased the number of phage able to grow on *S. sonnei* beyond the number which was found without the strand break, so that most (79%) of the DNAs with potential to form viable phage carry the ss^- mutation (Table 2).

An experiment was done to see whether a double-strand gap lacking the site of the ss^- mutation would be repaired. Wild-type T7 DNA was cut either with the restriction enzyme *Nhe*I or *Bss*HI alone or with both endonucleases. The enzyme *Bss*HI has a single recognition site at position 23892. Treatment with both enzymes introduces a 923-bp gap in the T7 chromosome, and the site of the ss^- mutation is located within this gap (Fig. 1). Digestion with one or both of the restriction enzymes reduced the ability of the wild-type DNA to form phage after in vitro packaging (Table 2). When a *Bst*XI digest of 6^- ss^- DNA was included as a source of the ss^- mutation, a double-strand break at either restriction site or a double-strand gap markedly increased the yield of ss^- phage. As seen from the last three lines of Table 2, most of the phage produced under these conditions carried the ss^- marker. Thus, it does not matter whether the double-strand break is to the left or right of the ss^- marker or whether a gap is present.

If there is physical exchange of DNA, initiated at the point of the double-strand break, between the wild-type T7 genome and the *Bst*XI fragment of 6^- ss^- DNA, then a break in the same DNA molecule that has the ss^- marker should also stimulate recombination. Double-strand breaks were introduced at the *Nhe*I restriction site in both the wild-type T7 genome and in the 6^- ss^- restriction fragment. All combinations of those DNA molecules were tested for yield of viable phage with an ss^- genotype. The data in Table 3 show that when restriction fragments with *Nhe*I endonuclease cuts were incubated with intact genomes, recombination frequency decreased relative to the value for the control, in which neither partner had a double-strand break. When both types of DNA molecules were digested with *Nhe*I endonuclease, the yield of viable phage was much lower than the yield that was found when the genomic DNA was cut and the restriction fragment was intact. Thus, an *Nhe*I endonuclease break in the restriction fragment with the ss^- marker reduces the ability of that DNA molecule to restore viability to a wild-type T7 genome that also has a matching double-strand break. (When both types of DNA molecules were cut, some of the few viable phage recovered did show an ss^- phenotype.) Table 3 shows that when the T7 genomes were cut, the titer of recovered ss^- phage was essentially the

TABLE 3. Effect of double-strand breaks in both recombining partners[a]

6^- ss^- DNA	Genomic DNA	Titer on:			% Recombination[b] on:	
		W3110	*Shigella* sup^0 strain	*Shigella* sup^+ strain	*Shigella* sup^0 strain	*Shigella* sup^+ strain
Control	Control	1.7×10^8	1.7×10^6	3.9×10^6	1.0	2.3
Control	Cut	3.2×10^7	2.6×10^7	3.0×10^7	81.1	93.8
Cut	Control	1.7×10^8	7.8×10^4	1.4×10^5	0.046	0.082
Cut	Cut	4.4×10^6	1.2×10^6	1.2×10^6	27.0	27.3

[a] A *Bst*XI digest of 6^- ss^- DNA was recombined with wild-type T7 chromosomes. Both partners were either digested with *Nhe*I (cut) or retained as untreated controls. After in vitro DNA replication and recombination, the DNA was packaged and plated on strain W3110 or on *S. sonnei* with (sup^0) or without (sup^+) an amber suppressor.
[b] Values for recombination on sup^0 and sup^+ strains were obtained by dividing the number of plaques on the *Shigella* strains by the number on *E. coli* W3110.

TABLE 4. Effect of a double-strand cut on plasmid-genome recombination[a]

DNA		Titer on:		% Recombination
Plasmid	Genomic	W3110	*Shigella sup*^{0} strain	
None	None	3.6×10^3	0	
None	Control	5.8×10^6	0	
None	Cut	4.4×10^4	0	
Present	None	5.2×10^3	0	
Present	Control	4.6×10^6	2.9×10^4	0.63
Present	Cut	4.0×10^5	7.1×10^4	17.8

[a] The ratios of ss$^-$ phage to total phage, defined as recombination, were measured after packaging the products of in vitro reactions that included DNA from the plasmid pJP7, which carries the ss$^-$ mutation in gene 10, and wild-type T7 DNA that was either intact (control) or digested with the enzyme *Nhe*I (cut).

same with or without a suppressor in the *S. sonnei* host, suggesting that in most cases, the 6$^-$ amber marker was not transferred together with ss$^-$. Data from experiments without strand breaks in the full-length genomes (Table 3) indicate that some recombinants probably also picked up the amber marker in gene 6, since the phage yield on the *Shigella* strain with the suppressor was about double the phage yield on the strain without a suppressor.

Intact T7 genomes are able to undergo recombination with homologous T7 DNA sequences cloned into plasmids (10, 19, 20, 32, 33). When plasmid DNA is included in the in vitro DNA replication-packaging reaction mixtures, T7 DNA present on a plasmid will not be packaged and thus cannot contribute to the total phage yield. However, recombination between the plasmid and genome could affect the genotypes of the phage that are produced. A pUC19 derivative, pJP7, into which a 620-bp region of T7 gene 10 (between *Tha*I-cut sites at positions 22681 and 23301) with the ss$^-$ mutation (at position 23150) had been cloned (20) was used to see how the presence of plasmid DNA would affect repair of double-strand breaks. Plasmid DNA was incubated with wild-type T7 chromosomes with or without a restriction cut, under conditions that would permit DNA replication and recombination. After in vitro packaging, the products of the reactions were examined for the ss$^-$ phenotype. Table 4 shows the effect of a strand break in the genome near the ss$^-$ mutation, together with relevant controls. As seen in Table 4, the level of recombination is lower than the level that was measured in other experiments (Tables 1 to 3). This is probably because the region of homology available on the plasmid is more limited than the region that was available on the *Bst*XI restriction fragment. A break in the chromosomes increased the number of ss$^-$ phage produced when the plasmid was available for recombination (Table 4).

To examine the effect of plasmid supercoiling, pJP7 was linearized by digestion with *Ssp*I, which cuts pUC19 at position 2501 (over 2,000 bp from the multiple cloning site into which T7 DNA was placed). Digestion was sufficient to completely linearize the pJP7 DNA, as judged by gel electrophoresis. Wild-type DNA was either cut with *Nhe*I or kept as a control before incubation with the plasmid DNA and packaging. Table 5 shows that linearizing the plasmid reduced the frequency of recombination by a factor of about 10. Thus, the supercoiling of the plasmid increased its ability to assist in the repair of double-strand breaks in the genome. The supercoiling may favor strand separation and thereby invite invasion from a recombinogenic partner DNA molecule.

TABLE 5. Recombination between T7 genomic DNA and closed circular or linear plasmid DNA[a]

DNA		Titer on:		% Recombination	Ratio[b]
Plasmid	Genomic	W3110	*Shigella sup*^{0} strains		
Uncut	Control	1.5×10^6	1.2×10^4	0.80	1
Uncut	Cut	2.5×10^5	4.8×10^4	19.2	24.0
Linear	Control	7.9×10^5	5.3×10^2	0.067	0.084
Linear	Cut	4.3×10^4	7.4×10^2	1.7	2.2 (25.0[c])

[a] Recombination was measured as the ratio of ss$^-$ phage to wild-type phage after in vitro reactions of plasmid pJP7 DNA that was either untreated (uncut) or linearized by digestion with the enzyme *Ssp*I and of T7 genomic DNA that was either untreated (control) or digested with *Nhe*I (cut).

[b] Ratio of percent recombination relative to recombination in the experiment in which both DNAs were uncut is shown.

[c] Ratio of recombination with or without an *Nhe*I cut in the genomic T7 under conditions in which the plasmid was linearized.

Because a large amount of endogenous DNA is synthesized after infection with wild-type T7 and because some of it remains in the extracts after centrifugation, it was not possible to perform experiments with crude extracts made from *E. coli* cells infected with wild-type T7. To avoid this problem, extracts were made from phage with the amber mutation in gene 4 and were mixed with similar extracts made from phage with the amber mutation in gene 5. Data obtained with these mixed extracts gave results similar to those found with extracts made from gene 3-deficient phage (Table 6). The level of recombination was lower when extracts lacking the products of both gene 3 and gene 6 were used.

DISCUSSION

When a chain break is present in T7 DNA, most of the DNAs capable of forming viable phage have apparently repaired the break by recombination with the physically intact partner. It is possible that recombination could take place by joining the DNA molecule that already has a break with a new break introduced into the partner in the exchange. However, the use of restriction fragments or plasmid DNA makes that explanation very unlikely. A simpler and more attractive hypothesis is that double-strand breaks may be widened (perhaps by using gene 6 exonuclease) and

TABLE 6. Effect of different extracts on recombination[a]

Extract[b]	Genomic DNA	Titer on:		% Recombination
		W3110	*Shigella sup*^{0} strain	
None	Control	3.3×10^7	4×10^2	
None	Cut	4.7×10^5	1×10^4	
3$^-$	Control	4.6×10^7	1.1×10^5	2.4
3$^-$	Cut	1.2×10^7	9.4×10^6	78.3
3$^-$ 6$^-$	Control	5.3×10^6	8.1×10^1	0.0016
3$^-$ 6$^-$	Cut	2.3×10^5	3.7×10^3	1.6
4$^-$ + 5$^-$	Control	9.3×10^6	4.9×10^5	5.3
4$^-$ + 5$^-$	Cut	4.4×10^6	3.1×10^6	70.5

[a] Recombination was measured between a *Bst*XI fragment of T7 6$^-$ ss$^-$ DNA and genomic T7 DNA that was intact (control) or digested with *Nhe*I (cut).

[b] In vitro reactions were carried out without extract or with extracts from 3$^-$ phage, from 3$^-$ 6$^-$ phage, or from a 1:1 mixture of extracts from 4$^-$ and 5$^-$ phage (4$^-$ + 5$^-$).

the resulting gaps may be filled in with DNA synthesis that uses the other recombining partner as template. This is essentially the model that has been suggested to account for double-strand gap repair in yeast and in bacteriophage lambda (37, 39). The data in Table 2 are in accord with this model, since a gap was repaired almost as effectively as a chain break and since in both cases, nearly all of the viable phage were recombinants. In a minor variation of this model, independent invasions of either piece of the broken duplex with the same or different intact homologs could provide templates for synthesis of relatively extensive 3′ tails on each of the two pieces of the broken chromosome. Since these tails would be complementary to one another, annealing of the tails could restore the broken duplex (23).

Gene 3-deficient phage were initially chosen for the DNA replication system to minimize endogenous DNA in the system (4, 15). Our earlier evidence for recombination in this system (14) prompted the choice of the same phage mutant as the source for extracts in the present study. However, in vivo studies have indicated a role for the gene 3 endonuclease in general homologous recombination in T7 (7, 12, 22, 26, 41). The properties of the gene 3 endonuclease are compatible with a role in facilitating resolution of the Holliday junctions formed during recombination (1, 18). A trivial explanation for the apparent lack of dependence on gene 3 derives from the likelihood that some gene 3 endonuclease is probably present in these extracts because of wild-type contamination in preparations of the gene 3-deficient phage used to make extracts or because of amber read-through. Alternatively, other activities present in the extracts might effectively substitute for the gene 3 product. Another alternative is that T7 uses more than one mode of recombination and that the gene 3 product is not normally involved in the mechanism responsible for double-strand gap repair. For example, there is no apparent need for an endonuclease in a mechanism involving annealing of homologous 3′ tails, each resulting from independent synthesis with ss^- DNA as a template.

The system described here currently offers the best available means of producing well-defined DNA substrates for T7 recombination and quantitatively controlling the numbers of these molecules available for exchange with one another. The experiments described above focus only on the initial structures of the DNA molecules that engage in recombination. Processing of these substrates by other enzymes may take place before recombination begins or as part of the resolution of recombination intermediates. Also, it must be kept in mind that more than one mechanism of recombination might operate in T7 (9, 26) and that double-strand breaks may not stimulate all modes of genetic exchange available within a T7-infected cell. However, it does seem clear that a double-strand break in a T7 genome will be repaired more efficiently if even a short unbroken homologous piece of DNA is available and that the frequency of genetic rearrangement near the site of the strand break will be markedly elevated.

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REFERENCES

1. **De Massay, B., R. A. Weisberg, and F. W. Studier.** 1987. Gene 3 endonuclease of bacteriophage T7 resolves conformationally branched structures in double-stranded DNA. J. Mol. Biol. **193**:359–376.
2. **Dunn, J. H., and F. W. Studier.** 1983. The complete nucleotide sequence of bacteriophage T7 DNA and the locations of T7 genetic elements. J. Mol. Biol. **166**:477–535.
3. **Hausmann, R., B. Gomez, and B. Moody.** 1968. Physiological and genetic aspects of abortive infection of a *Shigella sonnei* strain by coliphage T7. J. Virol. **2**:335–345.
4. **Hinkle, D. C., and C. C. Richardson.** 1974. Bacteriophage T7 deoxyribonucleic acid replication in vitro. Requirements for deoxyribonucleic acid synthesis and characterization of the product. J. Biol. Chem. **249**:2974–2984.
5. **Kerr, C., and P. D. Sadowski.** 1972. Gene 6 exonuclease of bacteriophage T7. II. Mechanism of the reaction. J. Biol. Chem. **247**:311–318.
6. **Kerr, C., and P. D. Sadowski.** 1974. Packaging and maturation of bacteriophage T7 in vitro. Proc. Natl. Acad. Sci. USA **71**:3545–3549.
7. **Kerr, C., and P. D. Sadowski.** 1975. The involvement of genes 3, 4, 5, and 6 in genetic recombination in bacteriophage T7. Virology **65**:281–285.
8. **Kuemmerle, N. B., and W. E. Masker.** 1977. In vitro packaging of UV radiation-damaged DNA from bacteriophage T7. J. Virol. **23**:509–516.
9. **Lee, D., and P. D. Sadowski.** 1983. In vitro recombination of bacteriophage T7 DNA detected by a direct physical assay. J. Virol. **48**:647–653.
10. **Lee, D., D. Vetter, L. Beatty, and P. Sadowski.** 1985. In vitro recombination of bacteriophage T7 DNA: further characterization of the reaction using plasmid DNA. Can. J. Biochem. Cell Biol. **63**:243–248.
11. **Lee, M., and R. C. Miller, Jr.** 1974. T7 exonuclease (GENE 6) is necessary for molecular recombination of bacteriophage T7. J. Virol. **14**:1040–1048.
12. **Lee, M., R. C. Miller, Jr., D. Scraba, and V. Paetkau.** 1976. The essential role of bacteriophage T7 endonuclease (gene 3) in molecular recombination. J. Mol. Biol. **104**:883–888.
13. **Masker, W. E.** 1982. In vitro packaging of bacteriophage T7 DNA requires ATP. J. Virol. **43**:365–367.
14. **Masker, W. E., and N. B. Kuemmerle.** 1980. In vitro recombination of bacteriophage T7 DNA damaged by UV radiation. J. Virol. **33**:330–339.
15. **Masker, W. E., N. B. Kuemmerle, and D. P. Allison.** 1978. In vitro packaging of bacteriophage T7 DNA synthesized in vitro. J. Virol. **27**:149–163.
16. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
17. **Mosig, G.** 1988. Mapping and map distortions in bacteriophage crosses, p. 141–167. *In* R. Kucherlapati and G. R. Smith (ed.), Genetic recombination. American Society for Microbiology, Washington, D.C.
18. **Muller, B., C. Jones, and S. C. West.** 1990. T7 endonuclease I resolves Holliday junctions formed in vitro by RecA protein. Nucleic Acids Res. **18**:5633–5636.
19. **Pierce, J. C., and W. Masker.** 1989. Genetic deletions between directly repeated sequences in bacteriophage T7. Mol. Gen. Genet. **217**:215–222.
20. **Pierce, J. C., and W. E. Masker.** 1988. A single-base change in gene 10 of bacteriophage T7 permits growth on *Shigella sonnei*. J. Virol. **62**:4369–4371.
21. **Powling, A., and R. Knippers.** 1974. Some functions involved in bacteriophage T7 genetic recombination. Mol. Gen. Genet. **134**:173–180.
22. **Powling, A., and R. Knippers.** 1976. Recombination of bacteriophage T7 in vivo. Mol. Gen. Genet. **149**:63–71.
23. **Resnick, M.** 1976. The repair of double strand breaks in DNA: a model involving recombination. J. Theor. Biol. **59**:97–106.
24. **Richardson, C. C.** 1966. The 5′-terminal nucleotides of bacteriophage T7 deoxyribonucleic Acid. J. Mol. Biol. **15**:49–61.
25. **Roberts, L., R. Sheldon, and P. D. Sadowski.** 1978. Genetic recombination of bacteriophage T7 DNA in vitro. IV. Asymmetry of recombination frequencies caused by polarity of DNA packaging in vitro. Virology **89**:252–261.

26. **Roeder, G. S., and P. D. Sadowski.** 1979. Pathways of recombination of bacteriophage T7 DNA in vitro. Cold Spring Harbor Symp. Quant. Biol. **43**:1023–1032.
27. **Sadowski, P. D.** 1977. Genetic recombination of bacteriophage T7 DNA in vitro. II. Further properties of the in vitro recombination-packaging reaction. Virology **78**:192–202.
28. **Sadowski, P. D., W. Bradley, D. Lee, and L. Roberts.** 1980. Genetic recombination of bacteriophage T7 DNA in vitro, p. 941–952. *In* B. Alberts and C. F. Fox (ed.), Molecular mechanisms of replication and genetic recombination. Academic Press, Inc., New York.
29. **Sadowski, P. D., and D. Vetter.** 1976. Genetic recombination of bacteriophage T7 DNA in vitro. Proc. Natl. Acad. Sci. USA **73**:692–696.
30. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
31. **Serwer, P., W. E. Masker, and J. L. Allen.** 1983. Stability and in vitro DNA packaging of bacteriophages: effects of dextrans, sugars, and polyols. J. Virol. **45**:665–671.
32. **Smith, R. D., and R. C. Miller, Jr.** 1981. Replication and plasmid-bacteriophage recombination. I. Marker rescue analysis. Virology **115**:223–236.
33. **Stone, J. C., and R. C. Miller, Jr.** 1984. Plasmid-phage recombination in T7 infected *Escherichia coli*. Virology **137**:305–313.
34. **Studier, F. W.** 1969. The genetics and physiology of bacteriophage T7. Virology **39**:562–574.
35. **Studier, F. W.** 1972. Bacteriophage T7. Science **176**:367–376.
36. **Symington, L. S.** 1991. Double-strand-break repair and recombination catalyzed by a nuclear extract of *Saccharomyces cerevisiae*. EMBO J. **10**:987–996.
37. **Szostak, J. W., T. L. Orr-Weaver, R. Rothstein, and F. W. Stahl.** 1983. The double-strand-break repair model for recombination. Cell **33**:25–35.
38. **Takahashi, N., and I. Kobayashi.** 1990. Evidence for the double-strand break repair model of bacteriophage lambda recombination. Proc. Natl. Acad. Sci. USA **87**:2790–2794.
39. **Thaler, D. S., and F. W. Stahl.** 1988. DNA double-chain breaks in recombination of phage lambda and of yeast. Annu. Rev. Genet. **22**:169–197.
40. **Tsujimoto, Y., and H. Ogawa.** 1977. Intermediates in genetic recombination of bacteriophage T7 DNA. J. Mol. Biol. **109**:423–436.
41. **Tsujimoto, Y., and H. Ogawa.** 1978. Intermediates in genetic recombination of bacteriophage T7 DNA. Biological activity and the roles of gene 3 and gene 5. J. Mol. Biol. **125**:255–273.
42. **Valachopoulov, P. J., and P. D. Sadowski.** 1977. Genetic recombination of bacteriophage T7 DNA in vitro. III. A physical assay for recombinant DNA. Virology **78**:203–215.
43. **Wackernagel, W., and U. Hermanns.** 1974. Inhibition of exonuclease V after infection of *E. coli* by bacteriophage T7. Biochem. Biophys. Res. Commun. **60**:521–527.