JOURNAL OF BACTERIOLOGY, Nov. 1986, p. 762–768
0021-9193/86/110762-07$02.00/0

Vol. 168, No. 2

In Vitro Packaging of Heteroduplex Bacteriophage T7 DNA: Evidence for Repair of Mismatched Bases

WARREN MASKER

Department of Biochemistry, Temple University School of Medicine, Philadelphia, Pennsylvania 19140

Received 15 May 1986/Accepted 5 August 1986

Heteroduplex DNA molecules that were wild type or contained combinations of amber, missense, and temperature-sensitive mutations were prepared from bacteriophage T7. These DNA molecules were then encapsulated in in vitro packaging reactions so as to produce infective T7 phage. The genotypes of the phage were examined to determine the degree to which mismatched base pairs in the heteroduplex had been corrected. The data show that conversion of the mismatches took place either during in vitro packaging or immediately after infection of either an *Escherichia coli* or *Shigella sonnei* host. The mode of mismatch conversion observed in these experiments was independent of the host *mutH*, *mutL*, *mutS*, and *uvrD* genes. There was no significant amount of discrimination between markers on either of the two complementary strands. The observed frequency of conversion of a mismatch depended on the genetic marker being monitored and on experimental conditions but was generally in the range between 5 and 30%.

Mispaired bases in a duplex DNA molecule can arise in a number of ways, including circumstances that compromise the normally high fidelity with which DNA metabolic processes are carried out (15). Unless the mispair is corrected before the DNA segment containing the mismatch is replicated, it will be permanently fixed as a mutation. The existence of a cellular mechanism for elimination of postreplicational mismatches was first inferred from genetic studies on recombination frequencies (10, 24–26, 36). More recently, biochemical studies have provided a better definition of the mismatch repair system in *Escherichia coli* (16, 17). Evidence has accumulated that mismatch correction involves the *E. coli* mutator genes *mutH*, *mutL*, and *mutS*, as well as *uvrD*, the structural gene for DNA helicase II (8, 11, 16, 22, 26, 33, 35). In this system the discrimination that determines which of the two DNA strands is to be corrected to reestablish complementarity with the other appears to be based on the methylation pattern present on the DNA (8, 16–18, 24, 26, 35).

We have examined in vitro mutagenesis in bacteriophage T7 DNA caused by incorporation of an altered nucleoside monophosphate during in vitro DNA replication (5) or by damage to the DNA itself resulting from exposure to a direct-acting mutagen (19). In these studies we monitored the biological activity and genetic makeup of the T7 DNA by encapsulating the DNA into phage heads with an in vitro packaging system and examining the growth of the resulting phage on appropriate indicator bacteria. The design of some of these experiments was such that mispaired (but otherwise undamaged) bases were likely to be introduced into the DNA during the in vitro manipulations before encapsulation. Thus, it is possible that the mutation yield could have been strongly influenced by mismatch correction. Previous studies pointed to the existence of mismatch correction in T7-infected *E. coli*. One group of workers examined in vitro packaging of heteroduplex DNA and of T7 DNA molecules formed after in vitro recombination and evoked the presence of a mismatch correction system to explain the genetic distribution of the phage recovered from their experiments (29). An additional demonstration of mismatch correction was achieved by transfection of *E. coli* with double-stranded heteroduplex T7 DNA. The mismatch repair observed in these experiments seemed to involve the methyl-directed mismatch repair system of *E. coli*, since resolution of the heteroduplexes appeared to depend on functional copies of the host genes *mutH*, *mutL*, and *mutS* (3). This observation was especially puzzling because of the lack of methylation of T7 DNA (1).

In this paper, I report experiments in which heteroduplex DNA molecules were prepared from T7 DNA with amber, missense, or temperature-sensitive mutations in a number of essential genes. By incubating this DNA in an in vitro packaging reaction mixture it was possible to monitor the fate of the heteroduplexes by examining the genotypes of the resulting phage. The analysis of these phage indicated that mismatch correction did occur either during the packaging reaction itself or immediately after infection of either an *E. coli* or *Shigella sonnei* host. The genetic markers were converted without a detectable level of discrimination between one or the other of the two DNA strands in the heteroduplex. Moreover, there was no dependence on the host *mutL*, *mutH*, or *mutS* gene. These data are consistent with the interpretation that mismatched base pairs are replaced or corrected by some mechanism. This mechanism is apparently distinct from the usual methyl-directed mismatch repair system used by *E. coli*.

MATERIALS AND METHODS

Strains of bacteriophage and bacteria. Bacteriophage T7 with an ss$^-$ mutation was obtained from R. Hausmann (the designation ss$^-$ refers to suicide in shigella.) The ss$^-$ mutation allows the growth of T7$_{ss^-}$ on *S. sonnei*, a bacterium which does not support the growth of wild-type T7 (4, 9). The remainder of the bacteriophage T7 mutants and the wild type were from the collection of F. W. Studier. The mutations used in this study (the designations are those of Studier) included am29 (gene 3), am20 (gene 4), ts101 (gene 4), am28 (gene 5), and am147 (gene 6) (30, 31). The ss$^-$ mutation is known to be located in gene 10 (34). T7 phages with combinations of mutations were constructed by the recombination techniques described by Studier (30). The genetic map in Fig. 1 shows the positions of the T7 genes used in this study. In the text the mutant genes are designated by a subscript. Thus, a T7$_{3,6}$ phage has amber muta-

tions in genes 3 and 6. Temperature-sensitive mutations are designated ts.

The bacterial strains used in this study are listed in Table 1. Strains with Tn*5* transposition elements in the *mutH*, *mutL*, and *mutS* genes were received from G. Walker. Transduction mediated by P1 *vir* was used to transduce the *mut* mutations from an *E. coli* AB1157 genetic background to the suppressor-free strain W3110 (21). Selection was for kanamycin resistance. After purification, each transductant was tested for spontaneous mutation to resistance to 100 μg of rifampin per ml. Spontaneous mutator strains were identified by a higher frequency of mutation to rifampin resistance than what is normal in the wild-type parent. Typically, the frequency of spontaneous mutation in *mut* transductants was on the order of 10^3-fold greater than what was found with the W3110 parent. The *mutS*::Tn*5* derivative of suppressor-free *S. sonnei* $ShD_2$371-48 was constructed by transduction with P1 *vir* grown on *E. coli* GW3732 followed by selection for resistance to kanamycin. The resulting strain showed about a 10^3-fold higher frequency of mutation to rifampin resistance than did the parent $ShD_2$371-48 strain. The newly constructed strain will support the growth of $T7_{ss^-}$ but will not allow the growth of either the wild-type T7 or T7 with both an ss^- mutation and an amber mutation in an essential gene. This phenotype is interpreted to mean that *S. sonnei* WM377 contains a Tn*5* insertion in its *mutS* gene. $T7_{ss^-}$ phage (without an amber mutation) produces the same number of plaques regardless of whether it is grown on lawns of an *S. sonnei* strain with an amber suppressor (Sup^+), an *S. sonnei* strain without an amber suppressor (Sup^0), or *S. sonnei mutS* Sup^0.

Media and growth conditions. Tryptone broth and L broth were made as described by Miller (21). Phage titers were determined by using agar plates (15 g of agar per liter) and soft agar (6.5 g of agar per liter) made with tryptone broth. Agar, tryptone, and yeast extract were from Difco Laboratories. Phage-infected cells for use in the in vitro packaging experiments were prepared from strain W3110 infected with $T7_{3,4,6}$ or $T7_{3,5,6}$ at a multiplicity of infection of 3, as previously described (13).

DNA. DNA from bacteriophage T7 was purified from phage grown on strain 011′, as previously described (27). DNA concentrations are given as nucleotide phosphorus equivalents. For reference, 1 nmol of T7 DNA is equivalent to the DNA content of 7.5×10^9 phage particles.

Formation of heteroduplex DNA. Separation of DNA strands and formation of heteroduplex T7 DNA molecules were performed by the procedures described by Szybalski et al. (32) and Bauer et al. (3), with minor modifications.

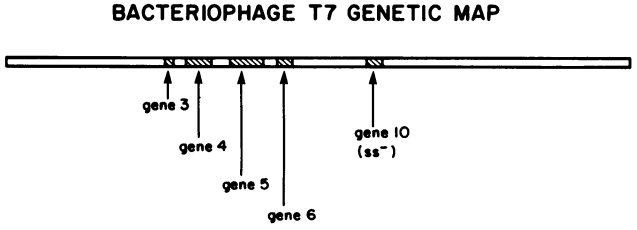

FIG. 1. Genetic map of bacteriophage T7. The locations and approximate sizes of the T7 genes important to this study are shown. The product of gene 3 is an endonuclease. Gene 4 codes for a primase and DNA unwinding activity. Gene 5 codes for a subunit of DNA polymerase. The product of gene 6 is an exonuclease. The ss^- mutation is in gene 10, which codes for a head protein (28, 31, 34). Information to construct this map was taken from reference 6.

TABLE 1. Bacterial strains

Species and strain	Relevant genotype or phenotype[a]	Source (reference)
E. coli		
W3110	Wild type, Sup^0	C. Richardson (2)
011′	*thyA*	C. Richardson (30)
GW3732	*thr leu arg his pro thi ara lac gal mtl xyl mutS201*::Tn*5*	G. Walker (23)
GW3734	As GW3732, except *mutL211*::Tn*5*	G. Walker (23)
GW3821	As GW3732, except *mutH471*::Tn*5*	G. Walker (23)
WM372	W3110 with *mutS201*::Tn*5*	This study
WM374	W3110 with *mutH471*::Tn*5*	This study
WM375	W3110 with *mutL211*::Tn*5*	This study
WM280	*thy trpA33 rha uvrD101*	(12)
S. sonnei		
Sh_3-18	Amber suppressor	R. Hausmann (9)
$ShD_2$371-48	Sup^0	R. Hausmann (9)
WM377	$ShD_2$371-48 with *mutS201*::Tn*5*	This study

[a] Sup^0, Free of any amber suppressor.

Approximately 300 pmol of purified T7 DNA in 10 mM Tris hydrochloride (pH 7.5)–1 mM EDTA was made 0.1 N in NaOH and incubated at 37°C for 10 min. The solution was neutralized with 2 M KH_2PO_4, and 100 μg of poly(G · U) and 0.1% (wt/vol) sarcosyl were added. The 0.45-ml mixture was diluted by the addition of 1 ml of 10 mM Tris (pH 7.5)–0.1 mM EDTA and then heated to 100°C for 10 min. After cooling, the entire sample was mixed with enough 10 mM Tris (pH 7.5)–0.1 mM EDTA to give a total volume of 6 ml and then added to 8.0 g of CsCl. The solution was centrifuged at 37,000 rpm at 20°C for 40 h in a Spinco Ti 50 rotor to form a CsCl gradient. Fractions were collected, and optical density at 260 nm was used to determine the positions of the peaks of r and l strands of DNA in the gradient.

Various combinations of r and l DNA were mixed to give approximately equal concentrations of each DNA species. The mixture was made 0.1 N in NaOH and incubated at 37°C for 10 min. These solutions were neutralized with 2 M KH_2PO_4, and CsCl was added to give a final concentration of 2.5 M. The mixture was heated at 65°C for more than 3 h and then slowly cooled to room temperature. This reannealing step was followed by overnight dialysis against 10 mM Tris (pH 7.5)–0.1 M EDTA. After dialysis, the DNA was introduced into the in vitro packaging reaction mixtures without further manipulation.

In vitro packaging of DNA. In vitro packaging of DNA was performed as previously described (13). The extracts used were prepared from *E. coli* W3110 infected with either $T7_{3,4,6}$ or $T7_{3,5,6}$. To minimize in vitro recombination, T7 gene 6 exonuclease was not added to the reaction mixtures.

RESULTS

Individual strands from purified T7 DNA with an ss^- mutation were separated from one another. Separated strands are designated r or l. This DNA was reannealed by incubating the r and l strands together or was included in control reactions with twice the amount of either the r or l

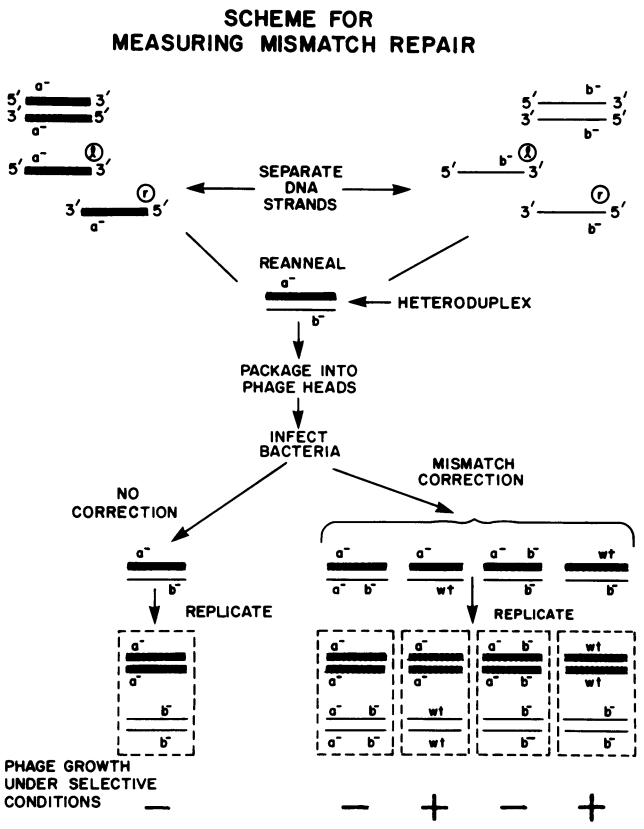

FIG. 2. Scheme for monitoring mismatch repair. The protocol used to examine mismatch correction after in vitro packaging of T7 heteroduplex DNA is shown. Also shown is the expected genetic distribution after a growth cycle following infection of a host with phages that contain heteroduplex DNA that has or has not been subjected to mismatch correction. Not all of the possible genotypes resulting from mismatch correction are shown. Only single mismatch correction events are depicted.

strand. The DNA recovered from these incubations was introduced into an in vitro DNA packaging reaction mixture that included a packaging extract prepared from suppressor-free *E. coli* infected with $T7_{3,5,6}$. The three amber mutations in the phage used to make the packaging extract blocked DNA replication and served to inhibit in vitro recombination during packaging. Moreover, any residual recombination that might have occurred would have been likely to introduce amber mutations from the endogenous DNA into the region of the T7 chromosome between genes 3 and 6. Phage produced by packaging this DNA would not form plaques on suppressor-free hosts. The data (not shown) indicate that the reannealed DNA could in fact be encapsulated in vitro to form infective DNA molecules. Separated r and l DNA strands each produced only about 2% of the number of phage obtained when both complementary strands were incubated together, indicating only a small amount of cross contamination in the separated DNA. The overall efficiency with which the reannealed DNA was packaged into infective phage was lower than that measured with virion duplex DNA, but the phage yield was still more than sufficient to allow analysis of the genotype of the product phage.

To monitor mismatch repair, the general procedure outlined in Fig. 2 was followed. In the first experiment heteroduplex DNA molecules were formed from wild-type T7 and T7 with a temperature-sensitive mutation in gene 4 (ts101). (In Fig. 2, a is mutation ts101 and b is the wild type.) This DNA was encapsulated in vitro, and the resulting phages were grown on suppressor-free *E. coli* at a permissive temperature. Some of the phages recovered from this experiment were tested by introducing phages from the original plaques onto lawns of *E. coli* followed by incubation at 30 and 43°C. The data (Table 2) show that 15 to 30% of the original phages were unable to grow at the restrictive temperature and therefore represented pure bursts of $T7_{ts101}$ phage. Portions of each of 15 separate plaques were individually dispersed in diluent and then plated on *E. coli* and grown at 30 and 43°C to determine the ratio of temperature-sensitive phage to wild-type phage in each plaque. Of the 15 plaques tested, 3 were found to contain temperature-sensitive phage, 5 contained only wild-type phage, and 7 were mixtures of both types. Since the presence of a wild-type copy of gene 4 on one DNA strand would cause an initial burst of phage that could subsequently propagate at 43°C, the data in Table 2 indicate that a substantial portion of the heteroduplex DNA was converted to a temperature-sensitive phenotype during in vitro packaging or immediately after infection of the *E. coli* host. The in vitro packaging was performed with extracts that were made from cells infected with T7 that had the wild-type gene 4. Since there were no copies of the ts101 mutation on any endogenous DNA present in the packaging extract, recombination could not have been responsible for the results presented in Table 2.

To reduce concerns about recombination events producing results that only appear to arise from mismatch correction, I performed an experiment using heteroduplex DNA with each strand carrying a separate mutation in a single gene that is essential to T7 DNA replication. The complementary strands of DNA from T7 with an amber mutation in gene 4, a gene essential for DNA replication (28, 31), were separated from one another. In the same experiment the strands of DNA from a T7 phage with a distinct temperature-sensitive mutation in gene 4 (ts101) were also separated. Heteroduplexes were formed as indicated in Table 2, and the resulting DNA was encapsulated in vitro by using an extract made from suppressor-free *E. coli* that had been infected with $T7_{3,4,6}$. The use of this phage to prepare the packaging extract greatly reduced the likelihood of in vitro recombination because the amber mutations in the phage used for the packaging extract was the same as the 4am mutation in the exogenous DNA were the same and 4am and 4ts mutations are closely linked by virtue of being in the same gene. Since the product of gene 4 is essential to DNA synthesis in vivo, recombination was also effectively eliminated because there

TABLE 2. Packaging of heteroduplex wild-type and temperature-sensitive DNA[a]

Relevant genotype of DNA strand:		PFU (10^3)	No. of plaques tested	No. (%) of plaques temperature sensitive
r	l			
4ts	wt	3.8	194	28 (14.1)
wt	4ts	2.5	147	42 (29.0)

[a] Heteroduplex molecules were formed from wild-type (wt) T7 and T7 with a temperature-sensitive mutation in gene 4. The DNA was packaged in vitro by using an extract from $T7_{3,5,6}$-infected *E. coli* W3110. The resulting phages were plated on strain W3110 at 30°C. A number of plaques were tested for temperature-sensitive phage by inserting a sterile toothpick in the plaque and then inserting the toothpick into lawns of strain W3110 that were subsequently grown at 30 or 43°C. The number of phage unable to grow at the restrictive temperature is shown.

is no mechanism available for postinfection formation of both 4am and 4ts duplex DNA molecules from the original heteroduplex. The packaged phages were plated on suppressor-free *E. coli* that was either wild type or carried mutations on the *mutL*, *mutH*, or *mutS* gene. The phage-infected cells were grown at a permissive (30°C) or restrictive (43°C) temperature (Table 3). No phages were recovered when both the r and l DNA strands contained an amber mutation. When both strands were made from the ts101 phage, the number of plaques formed at the restrictive temperature was about 0.2% of that formed at the permissive temperature. This probably represented a low level of in vitro recombination between the 4ts exogenous DNA and the 3am 4am 6am endogenous DNA present in the packaging extract. Conversion of ts101 to wild type occurred at a frequency of 3 to 5% of the total phage production regardless of whether the r or l DNA strand contained the ts101 mutation (Table 3). Also, *mutL*, *mutH*, and *mutS* mutations in the *E. coli* mutator genes had no effect.

It was desirable to perform an experiment in which phage produced from uncorrected mismatches in heteroduplexes could be measured and distinguished from phage whose genotype was altered by mismatch correction and in which any phage produced from unseparated DNA strands would not be a problem. Since T7 will not grow on *S. sonnei* unless it carries an ss^- mutation, this marker was used to positively identify exogenous DNA introduced into the packaging system. The correction of heteroduplex amber markers in combination with the ss^- missense mutation was monitored by the ability of T7 to grow on *S. sonnei* with and without an amber suppressor. Two T7 mutants, one which contained an amber mutation in gene 5, as well as the ss^- missense mutation, and one which contained an amber mutation in gene 6 together with the ss^- mutation, were used in another experiment. The complementary strands of DNA from these phages were separated and then hybridized together to form heteroduplexes or appropriate controls (Table 4). This DNA was then incubated in an in vitro packaging reaction mixture that used an extract from $T7_{3,5,6}$-infected cells. Since any endogenous DNA in the extracts contained the same amber mutations in genes 5 and 6 that were present on the exogenous DNA, in vitro recombination could not contribute to the rescue of either amber genetic marker. The phages recovered after in vitro packaging were plated on *S. sonnei* indicator bacteria with and without an amber suppressor to provide a measurement of the total phage yield and the percentage of conversion of the amber mutation to the wild type. Approximately 10% of the heteroduplex molecules yielded phage without an amber mutation (Table 5). This percentage did not change when *S. sonnei* with a *mutS* mutation was used as the indicator strain. The controls showed no evidence of phage without an amber mutation among those recovered after packaging either of the

TABLE 3. Packaging of heteroduplex T7 DNA with mutations in gene 4[a]

Relevant genotype of *E. coli* indicator strain	Relevant genotype of DNA strand: r	l	PFU (10^2) at: 43°C	30°C	Correction of temp-sensitive mutation (%)
wt	4ts	4am	2.9	61.0	4.8
	4am	4ts	2.4	41.0	5.9
mutL	4ts	4am	1.7	64.0	2.7
	4am	4ts	2.1	42.0	5.0
mutH	4ts	4am	2.6	66.0	3.9
	4am	4ts	2.0	46.0	4.4
mutS	4ts	4am	2.2	66.0	3.3
	4am	4ts	1.5	41.0	3.7
wt	4am	4am	<0.01	<0.01	
	4ts	4ts	<0.27	120.0	<0.23

[a] Heteroduplex DNA was formed from T7 with a temperature-sensitive mutation in gene 4 (4ts) and T7 with an amber mutation in gene 4 (4am). The DNA was packaged by using an extract from $T7_{3,4,6}$-infected *E. coli* W3110 in which $T7_{3,4,6}$ carried the same gene 4 amber mutation (am20) that was present in the DNA used to prepare the heteroduplexes. The resulting phages were plated on suppressor-free *E. coli* that was wild type (wt) or carried the mutation *mutL*, *mutH*, or *mutS*, and the bacteria were incubated at 30 or 43°C to give the indicated number of phage.

TABLE 4. Packaging of T7 ss− heteroduplex DNA[a]

Species and relevant genotype or phenotype	Relevant genotype of DNA strand: r	l	PFU (10^3)
S. sonnei			
Sup^+	5am ss^-		0.8
Sup^0	5am ss^-		<0.01
Sup^+		5am ss^-	4.3
Sup^0		5am ss^-	<0.01
Sup^+		6am ss^-	0.5
Sup^0		6am ss^-	<0.01
Sup^+	6am ss^-	6am ss^-	16.0
Sup^0	6am ss^-	6am ss^-	<0.01
Sup^+	5am ss^-	5am ss^-	78.0
Sup^0	5am ss^-	5am ss^-	0.03
Sup^+	5am ss^-	6am ss^-	26.0
Sup^0	5am ss^-	6am ss^-	2.4
Sup^0 *mutS*	5am ss^-	6am ss^-	2.9
Sup^+	6am ss^-	5am ss^-	30.0
Sup^0	6am ss^-	5am ss^-	4.4
Sup^0 *mutS*	6am ss^-	5am ss^-	6.2
E. coli			
Wild type	5am ss^-	6am ss^-	1.4
mutS	5am ss^-	6am ss^-	1.3
mutL	5am ss^-	6am ss^-	2.3
uvrD	5am ss^-	6am ss^-	3.6
Wild type	6am ss^-	5am ss^-	5.1
mutS	6am ss^-	5am ss^-	5.7
mutL	6am ss^-	5am ss^-	9.7
uvrD	6am ss^-	5am ss^-	13.0

[a] DNA from T7 with 5am ss^- mutations or 6am ss^- mutations was separated into single strands and reannealed. The DNA was encapsulated by using an extract from $T7_{3,5,6}$-infected *E. coli* W3110. The resulting phages were grown on *S. sonnei* host bacteria as indicated or on suppressor-free *E. coli* that was either wild type or carried the mutation *mutS*, *mutL*, or *uvrD*.

TABLE 5. Packaging of heteroduplex T7 DNA with extracts made from mut^- *E. coli*[a]

Relevant genotype of *E. coli* (extract source)	Indicator bacteria (PFU[10^3]) Sup^+	*mutS* Sup^0	Correction of amber mutation (%)
Wild type	12.0	2.3	19.2
mutL	7.3	2.3	31.5
mutS	1.2	0.39	32.5
mutH	2.4	0.59	24.6

[a] Heteroduplex DNA from T7 6am ss^- (r strand) and T7 5am ss^- (l strand) was packaged in vitro using extracts from $T7_{3,5,6}$-infected suppressor-free *E. coli* with the mutation by *mutL*, *mutS*, or *mutH*. The phages were grown on *S. sonnei* that either carried an amber suppressor or was suppressor free and *mutS*.

TABLE 6. Packaging of heteroduplex DNA from 5am and 6am ss⁻ T7[a]

Relevant genotype of DNA strand:		Indicator bacteria (PFU[10^2])			Correction to ss⁻ without amber mutation (%) (5)
r	l	*E. coli* W3110	*S. sonnei* Sup⁺	*S. sonnei* Sup⁰	
5am	5am	0			
6am ss⁻	6am ss⁻		40.0	<0.01	
6am ss⁻	5am	5.7	19.0	1.7	8.9
5am	6am ss⁻	1.4	4.0	0.3	7.8

[a] Heteroduplex DNA was formed from T7 carrying an amber mutation in gene 5 or an amber mutation in gene 6 plus an ss⁻ mutation. The DNA was packaged in vitro by using an extract from $T7_{3,5,6}$-infected *E. coli* W3110.

homoduplex DNAs made from 5am ss⁻ DNA or 6am ss⁻ DNA. The phages that were recovered from this experiment were also plated on *E. coli* indicator bacteria that were suppressor free but contained mutations in genes thought to affect mismatch repair (Table 4). Although some variation (within a factor of 2) was seen among the yield of phage without an amber mutation, measured with the *mutS*, *mutL*, and *uvrD* indicator bacteria, these data confirmed the results presented in Table 3 and showed that wild-type phage was produced from heteroduplex DNA with mutations in genes 5 and 6 in spite of the presence of host mutations known to affect methyl-directed mismatch correction.

Packaging extracts were prepared from $T7_{3,4,6}$-infected *E. coli* that was either wild type or carried the mutation *mutH*, *mutL*, or *mutS*. These extracts were used to package heteroduplex DNA made of combined strands of 5am ss⁻ and 6am ss⁻ DNA. The resulting phages were plated on *S. sonnei* with an amber suppressor and on suppressor-free *S. sonnei* with a *mutS* mutation to measure the amount of conversion to phage without an amber mutation. Phage without an amber mutation were recovered at about the same frequency irrespective of the *mut* mutation in the bacteria used to prepare the extracts for the packaging reaction (Table 5).

To determine whether the genetic information on either the r or l strand would be selected in preference to its complementary partner after infection of *S. sonnei*, heteroduplex DNA was formed between T7 DNA molecules that were either 5am ss⁺ or 6am ss⁻. Suppressor-free *S. sonnei* was used as an indicator strain, and therefore any phage that arose from the 5am DNA strand would be unable to propagate even if the amber mutation was converted to the wild type. Although two correction events on the 5am DNA strand could produce phage that would grow on suppressor-free *S. sonnei*, only a single correction event was needed on the 6am ss⁻ strand. The data (Table 6) indicate that the fraction of phage converted from 6am ss⁻ to ss⁻ without an amber mutation was the same irrespective of whether the ss⁻ mutation was present on the r or l strand.

DISCUSSION

The data presented in this paper show that progeny phages produced by infection of either *E. coli* or *S. sonnei* with T7 containing a heteroduplex genome have a genetic make up consistent with the correction of mismatched base pairs in the DNA. The results do not reveal the mechanism by which the observed distribution of T7 genotypes arose from packaging heteroduplex DNA and do not establish whether the elimination of mismatches was due to phage or bacterial proteins. One simple possibility is that a repairlike mechanism distinct from the *E. coli* methyl-directed mismatch repair system operates in T7-infected cells. There is, however, no direct evidence to substantiate this possibility. Also, our incomplete understanding of both the T7 recombination process and the mechanism by which phage DNA is encapsulated dictates a cautious approach.

The results of several control experiments argue against some mechanisms that might produce genotypes that could appear to have arisen from mismatch repair. The data do not favor the selective replication of one strand of the DNA heteroduplex. The data presented in Table 2 eliminate the possibility that recombination between the heteroduplex DNA and endogenous DNA present in the extract used for in vitro packaging might have affected the results presented here. The observed yield of pure bursts of ts101 phage could not have arisen through recombination since that genetic marker was not present on the phage used to prepare the extracts used for in vitro packaging. The yield of ts101 phage was much higher than could be accounted for by failure to completely separate DNA strands during the formation of the heteroduplex DNA. Also, the data in Table 2 show that repair of mismatches can occur on either the r or l strand. Replication of the heteroduplex DNA molecules could yield daughter phage chromosomes that would each carry the genetic information originally present on one strand of the heteroduplex. Recombination between these progeny molecules could then generate wild-type phage or other rearrangements of genetic markers that might appear to have resulted from excision of mismatches. The data in Table 3 do not support such an interpretation for the results presented here. Each strand of the heteroduplex carried a deficiency in gene 4. Since the gene 4 protein is essential to DNA replication, the absence of functional copies of that protein would block DNA replication and thereby preclude recombination between progeny. Other experiments (Tables 3 through 6) also used mutations in genes that code for products essential to DNA replication and recombination. Although it is impossible to unequivocally rule out effects due to recombination, it is very unlikely that either in vitro or in vivo recombination played a significant role in the results described here.

The experiments for which results are presented in Table 4 made use of a missense ss⁻ mutation that extends the T7 host range to include *S. sonnei*. Because ss⁺ DNA will not permit growth on this host, all phages recovered from these experiments must have arisen from the exogenous ss⁻ DNA. The use of this mutation reduced concern about in vitro recombination (since any contaminant DNA would be ss⁺). Moreover, use of a host with an amber suppressor allowed determination of the total phage yield, as well as the number of corrections of the amber markers. The data showed a 10 to 15% correction of the amber marker to the wild type. There was no measurable dependence on the host *mutH*, *mutL*, or *uvrD* gene (Table 4). Also, very little effect was seen when *mut*⁻ strains were used to prepare the extracts for in vitro packaging (Table 5). Thus, it is unlikely that the products of the *mut* genes acted to alter the genotype of the heteroduplex DNA before or during encapsulation. Therefore, the phenomenon is apparently independent of the *E. coli* methyl-directed mismatch repair system. If both methyl-directed and methyl-independent mismatch correction systems were operating it seems likely that at least those DNA molecules that remained as heteroduplexes after in vitro

packaging (Table 3) would have been subject to further mismatch repair after in vivo infection of the host. The absence of any measurable dependence on the mismatch repair genes suggests that the *E. coli* methyl-directed mismatch correction system probably does not operate after T7 infection. The physiological differences between the apparently normal infections by phage made by in vitro packaging and the infectivity achieved after transfection of spheroplasts with duplex DNA may, however, allow host systems greater opportunity to act on transfected DNA. This could account for the report of reduced mismatch correction after transfection of *mutH*, *mutL*, and *mutS E. coli* with heteroduplex T7 DNA (3).

The data presented in this paper show considerable variation in the amount of mismatch correction. Since the mechanism(s) involved in the correction of the mispairs is not known, it is impossible to determine whether some mispaired bases are repaired more easily than others in T7-infected cells. In some cases the location of mispaired bases may affect the apparent amount of correction. For example, the close proximity of the 4ts and 4am genetic markers may have been responsible for the unusually low correction to wild type indicated by the data in Table 3. Even a moderate amount of repairlike DNA synthesis could convert the ts101 marker to the wild type while introducing a complementary copy of the second (amber) marker into the DNA strand that was undergoing correction. In Table 6 the presence of the ss^- marker on the coding (sense) r strand of DNA favored the growth of a 6am ss^- and 5am ss^+ heteroduplex on a *Shigella* host. This effect was relatively small and could have been at least partially due to the dominant nature of the ss^- mutation (4, 9). In this experiment correction of the gene 6 amber marker to the wild type was the same irrespective of whether the 6am ss^- genotype was on the r or l DNA strand (Table 6). Thus, in the system described here there is no major difference in the degree of repair of the r or l DNA strand. The results obtained with whole phage made by in vitro packaging of heteroduplex DNA are distinct from what was reported (3) after transfection of *E. coli* spheroplasts with heteroduplex T7 DNA molecules.

It is not clear what mechanism is used to discriminate between the "correct" and "incorrect" partner in the mismatch. As discussed above, models evoking strand discrimination based on DNA methylation patterns would not seem to apply to the data presented here. There is however data to support the existence of a separate system of methyl-independent mismatch repair (7). It is easier to reconcile the results of the present study with models (14) in which the presence of nonmethyl signals such as single strand interruptions in the DNA molecules serves to dictate the choice of which DNA strand is corrected to match the other. If the observed correction is a bacterial process then, although some mechanism of strand discrimination might operate on the bacterial DNA, the absence of proper recognition signals on the phage DNA might make the correction of mismatches on that DNA an essentially random process. In any event the mechanism that alters the mismatches present on the T7 heteroduplexes almost certainly affects the way the phage reacts to mutagenic DNA damage and may also affect the overall fidelity of T7 DNA replication. Furthermore, the observations with T7 may reflect the presence of alternative mismatch correction pathways in the host, and the availability of in vitro DNA replication, DNA repair, and DNA packaging systems may aid in the study of the molecular mechanisms of these pathways.

ACKNOWLEDGMENTS

I thank S. McAfee for technical assistance and G. Walker for the gift of bacterial strains.

This work was supported by Public Health Service grant GM-34614 from the National Institutes of Health.

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