Some Features of Base Pair Mismatch and Heterology Repair in *Escherichia coli*

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ABSTRACT

We have used artificially constructed heteroallelic heteroduplex molecules of bacteriophage lambda DNA to transfect *Escherichia coli*, and *E. coli* mutants deficient in various functions involved in the adenine methylation-directed mismatch repair system, MutL, MutS, MutH, and UvrD (MutU). Analysis of the allele content of single infective centers shows that this repair system often acts on several mismatches, separated by as many as 2000 bp, on one of the strands of a heteroduplex molecule. When the methyl-directed mismatch repair system is disabled by *mutH* or *uvrD* mutations, localized mismatch repair becomes prominent. This prominent localized repair that can result in separation of very closely linked markers requires the functions MutL and MutS, is independent of adenine methylation, and appears to reflect another mechanism of mismatch repair. Heterology-containing heteroduplex molecules with a deletion in one strand often escape processing. However, when the heterology includes the stem and loop structure of a transposon, Tn*10*, the transposon is lost.

THE demonstration of *in vivo* processing of mismatched base pairs in the duplex structure of DNA has provided insight into features of the fidelity of replication as well as the clustering of genetic exchanges. In the case of replication fidelity, it has been suggested (WAGNER and MESELSON 1976) that mismatched bases introduced as replication errors on a newly synthesized DNA strand are preferentially excised and the gaps filled by repair synthesis. The incorrect nucleotide is thus removed. In the case of clustering of genetic exchanges, it has been shown that several closely linked parental markers can be included in a region of heteroduplex DNA created by recombination (WHITE and FOX 1974). Localized excision of mismatches and repair synthesis from the complementary strand template could result in products that display clustered coincident exchanges.

Evidence for a role for mismatch repair in replication fidelity comes from the characterization of mutations that simultaneously result in striking elevation of mutation frequency (GLICKMAN and RADMAN 1980) and defects in the repair process (LU, CLARK, and MODRICH 1983). The functions, so far recognized, that play roles in this mismatch repair system are MutL, MutS, MutH, and UvrD (MutU). The repair has been shown to be directed by the absence of adenine methylation at GATC *dam* methylation sites (PUKKILA *et al.* 1983) and can involve replacement of a thousand or more nucleotides. The efficiency with which repair occurs is dictated by the nature of the base pair mismatch (DOHET, WAGNER and RADMAN 1985). Since adenine methylation occurs after replication, errors in a newly synthesized strand of DNA would be subject to correction, thus contributing to replication fidelity. This subject has recently been reviewed by RADMAN and WAGNER (1986), CLAVERYS and LACKS (1986) and MESELSON (1987).

The DNA products of genetic recombination include regions in which the two strands of the duplex are contributed by different parental DNA molecules (FOX 1978). The presence of mutational sites distinguishing the two parents, within such heteroduplex regions, could result in either base pair mismatches (WHITE and FOX 1974) or regions of nonhomology (LICHTEN and FOX 1984) within the DNA duplex. Repair processes operating on such structures play a role in determining the final outcome of the recombination process.

In an effort to elaborate the rules that dictate the processing of base pair mismatches and regions of sequence nonhomology, we have examined the products of transfection of *E. coli* with bacteriophage lambda DNA molecules harboring such structures. The hosts for transfection included *mut*$^+$ bacteria as well as various mutants defective in methyl directed mismatch repair: *mutL*, *mutS*, *mutH*, and *uvrD*. The phage genotypes present among the products emerging from bacteria transfected with artificially constructed heteroduplex molecules reveal some of the features of the processing events that the DNA molecules have experienced.

Substantial differences are evident in the products of processing that are observed when various bacterial mutator functions are disabled. When the heterodu-

plex molecules harbor a set of three base pair mismatches covering a span of about 2000 bp, the pattern of allele loss evident in the products of single bursts from *mut*$^+$ bacteria seems to reflect frequent coincident loss of all three alleles from one or the other strand. With strains defective in *mutL* or *mutS*, little processing is evident; the phage products in most of the infective centers include all of the alleles present in the parental molecule. In contrast, with strains defective in the MutH or UvrD functions, much of the substantial processing that is evident appears to reflect the repair of mismatches one at a time so that most bursts are mixed for at least one of the allelic pairs present in the parental molecule. The localized processing that is prominent in *mutH* and *uvrD* strains appears to be independent of *dam*-directed adenine methylation and requires the activities of both the MutL and MutS functions. The residual repair that is evident in *mutL* and *mutS* strains remains to be accounted for. There appear, therefore, to be at least two distinct mechanisms that result in repair of mismatched base pairs (FOX and RAPOSA 1983; LIEB 1983).

For the case of heteroduplex molecules harboring a non-homology reflecting the presence of a deletion in one of the parental strands, bursts often include alleles from both parental phage. This is true in all of the bacterial strains examined. However, heteroduplex molecules harboring a heterology resulting from the presence of a transposon, Tn*10*, in one of the strands, experience a different fate. The transposon is lost in most or all of the products of heteroduplex transfection.

MATERIALS AND METHODS

The *E. coli* and bacteriophage lambda strains that were used are described in Table 1. P1 transductions followed the procedures described by MILLER (1972). The *mut* double mutants were constructed by P1 transduction, selecting for the drug resistant markers of the appropriate mutator-inserted transposon. The presence of the second mutator mutation was confirmed by P1 back-crosses using the candidate double mutants as donors. Mutator activity was monitored by the frequency of trimethoprim-resistant (MILLER 1972) or lambda-resistant bacteria.

Standard methods for growing lambda in supplemented lambda broth and plating on trypticase agar have been described elsewhere (WHITE and FOX 1974). Phage stocks were grown in BNN45 *lac*Δ using the NZC broth method (BLATTNER *et al.* 1977). Phage concentration with polyethylene glycol and subsequent CsCl gradient purification have been described previously by LICHTEN and FOX (1983). In this case, however, the densities of the CsCl steps in the step gradient were 1.6, 1.4 and 1.3 g/ml.

DNA denaturation and strand separation with poly U, G were carried out by the method of DAVIS, BOTSTEIN and ROTH (1980). The CsCl gradients of denatured DNA were collected by puncturing the bottoms of polyallomer tubes and collecting 2-drop fractions into Eppendorf tubes. Five microliters of each fraction and an equal volume of ethidium bromide (1 mg/ml) were spotted on a piece of parafilm and photographed under UV light to determine the positions of the heavy and light peaks. Each fraction from the two peaks was diluted 50-fold in TE buffer (0.01 M Tris, 0.001 M EDTA) and the optical density at 260 nm measured.

To reduce contamination of material in the light peak by heavy strands, the light fractions were pooled, renatured and rerun on a second CsCl gradient according to the procedure of NEVERS and SPATZ (1975). The purity of each fraction was assessed by measuring biological activity in a transfection experiment before and after self-annealing. The yield of infective centers when complementary strands were annealed was 300 times greater than with unannealed or self-annealed DNA. Fractions from each peak were pooled and stored at 4°. Before transfection, equal volumes of the separated strands were hybridized in 50% formamide at 26° for 1 hr.

Undermethylated and hypermethylated DNAs were isolated from phage grown on a *dam*13::Tn*9* derivative of BNN45 and a BNN45 derivative harboring the Dam overproducing plasmid pTP166 (MARINUS, POTEETE and ARRAJ 1984), respectively. The level of methylation was confirmed by susceptibility to restriction by *Mbo*I.

For those experiments involving the transposon-harboring heteroduplex molecules and those examining the impact of adenine methylation, mixed random heteroduplex molecules were used. They were prepared by mixing purified phage in the appropriate ratios to give a total DNA concentration of 100 μg/ml in SM (50 mM Tris, pH 8, 100 mM NaCl, 10 mM $MgSO_4$). The mixture was made 20 mM with EDTA (pH 8.0) and heated to 65° for 15 min. NaOH was added to a final concentration of 0.1 M and the mixture allowed to stand at room temperature for 10 min. Tris-HCl (pH 7.2) was added to a final concentration of 0.2 M and the solution was mixed with an equal volume of formamide. The mixtures were incubated at 45° for 1 hr. Sham preparations were prepared by the same protocol, with the NaOH and Tris mixed before addition to the DNA.

Transfection was carried out using a modification of the method of MANDEL and HIGA (1970). Overnight cultures of bacteria in LB were diluted 100-fold in LB and incubated at 37°. When the cells reached a density of 3×10^8/ml, they were pelleted in a refrigerated centrifuge, suspended in an equal volume of cold 0.1 M $MgCl_2$, repelleted and suspended in ½ volume of cold 0.1 M $CaCl_2$, and held on ice for 20 min. The cells were pelleted again, resuspended in 1/20 volume of cold 0.1 M $CaCl_2$, and used immediately for transfection. An aliquot of 0.2 ml competent cells and 0.1 ml lambda heteroduplex DNA (1 μg/ml) was mixed and placed in ice water for 15 min, then at 37° for 20 min. Appropriate dilutions were plated as infective centers.

Isolated plaques were picked with a capillary tube and resuspended in 1 ml SM buffer. The contents of individual plaques were replated and the genotypes of phage present were determined in the following manner: the nonpermissive indicator, M182 (*sup*$^+$), was used to detect the presence of wild type (*P*$^+$) phage, since neither *Pam*3 nor *Pam*80 mutants can grow on this host. The *Pam*80 allele was detected on a plate with a lawn of QL (*suIII*) that is permissive for *Pam*80 mutants but not for *Pam*3 phage. Indicator plates with a lawn of D6431 (*suI*) were used to discriminate between *Pam*3 and *Pam*80 phage, since *Pam*80 mutants grow very poorly while *Pam*3 phage grow very well on this indicator. The double mutant, *Pam*3 *Pam*80, was not detectable by these assays.

A permissive indicator plate with D6432 (*suII*) was also spotted with the resuspended phage to score for the *cI* allele (*i.e.*, clear, turbid and mottled spots). As a confirming check,

TABLE 1

Bacterial and phage strains

Strain	Relevant genotype	Source
A. Bacteria		
M182	*sup*$^+$ *lac*ZΔX74	J. BECKWITH
M182 (λ*c*$^+$ *Pam*80)		Lysogen of M182
D6431	*suI*	J. MILLER
QL	*suIII*	M. MALAMY
Q5151	*groP*	E. SIGNER
ES1301	*mutS*201::Tn*5*	E. SIEGEL
ES1481	*mutS*215::Tn*10*	E. SIEGEL
ES1484	*mutL*218::Tn*10*	E. SIEGEL
AB1157 *mutH*::Tn*5*	*mutH*471::Tn*5*	G. WALKER
AB1157 *uvr*D::Tn*5*	*uvrD*260::Tn*5*	G. WALKER
D6432	*suII*	J. MILLER
D6432 *mutL*::Tn*10*	*mutL*218	Tet^R transductant of D6432 by P1 grown on ES1484
D6432 *mutS*::Tn*10*	*mutS*215	Tet^R transductant by P1 grown on ES1481
D6432 *mutH*::Tn*5*	*mutH*471	Kan^R transductant of D6432 by P1 grown on AB1157 *mutH*::Tn*5*
D6432 *uvrD*::Tn*5*	*uvrD*260	Kan^r transductant of D6432 by P1 grown on AB1157 *uvrD*::Tn*5*
D6432 *mutL*::Tn*10* *mutS*::Tn*5*	*mutL*218 *mutS*201	Kan^R transductant of D6432 *mutL*::Tn*10* by P1 grown on ES1301
D6432 *mutL*:Tn*10* *mutH*::Tn*5*	*mutL*218 *mutH*471	Tet^R transductant of D6432 *mutH*::Tn*5* by P1 grown on D6432 *mutL*::Tn*10*
D6432 *mutS*::Tn*10* *mutH*::Tn*5*	*MutS*215 *mutH*471	Tet^R transductant of D6432 *mutH*::Tn*5* by P1 grown on D6432 *mutS*::Tn*10*
D6432 *mutL*::Tn*10* *uvrD*::Tn*5*	*mutL*218 *uvrD*260	Tet^R transductant of D6432 *uvrD*::Tn*5* by P1 grown on D6432 *mutL*::Tn*10*
GM2150 *dam*::Tn*9*	*dam*13	M. MARINUS
GM2290 (W3110/ pTP166)	*dam*$^+$ plasmid	M. MARINUS
BNN45 *lac*Δ	*suII suIII*	*proC*::Tn*5* *lac*ΔU169 transductant of BNN45 (R. DAVIS)
BNN45 *lac*Δ dam::Tn*9*	*suII suIII dam*13	Cam^R transductant of BNN45 *lac*Δ by P1 grown on GM2150
BNN45 *lac*Δ/ pTP166	*suII suIII dam*13	Amp^R transformant of BNN45 *lac*Δ with plasmid from GM2250
B. Phage		
Lambda gt7 *cI*$^+$ *Pam*80		Lambda gt7 from DAVIS, BOTSTEIN and ROTH (1980)
Lambda gt7 *cI*Δ *Pam*3		Lambda gt7 from DAVIS, BOTSTEIN and ROTH (1980)
Lambda gt7 *cI*857 *Pam*3		Lambda gt7 from DAVIS, BOTSTEIN and ROTH (1980)
Lambda *cI*$^+$ b221		M. LICHTEN
Lambda *cI*857 b221 *P/Q*::Tn*10*		#471 N. KLECKNER (1979)
Lambda *cI*::Tn*10* b221		#473 N. KLECKNER (1979)

TABLE 2

Products of transfection with heteroduplexes of opposite strand orientation

	3′*cI*857 *Pam*3 5′(h) / 5′*cI*⁺ *Pam*80 3′(l)					3′*cI*⁺ *Pam*80 5′(h) / 5′*cI*857*Pam*3 3′(l)				
	mut⁺	*mutL*	*mutS*	*mutH*	*uvrD*	*mut*⁺	*mutL*	*mutS*	*mutH*	*uvrD*
c⁺/*cI*857 *P*3/*P*80	8	73	80	15	11	11	74	86	51	47
c⁺/*cI*857 *P*3	2	1	0	11	8	1	0	0	0	1
c⁺/*cI*857 *P*80	0	1	0	1	0	2	0	1	4	11
c⁺/*cI*857 *P*⁺	2	1	0	7	20	0	1	0	0	2
c⁺/*cI*857 *P*⁺/*P*3	1	4	3	27	32	1	0	1	0	0
c⁺/*cI*857 *P*⁺/*P*80	0	2	5	3	1	2	2	1	6	13
c⁺ *P*3/*P*80	0	0	1	0	0	7	0	2	11	2
c⁺ *P*3	5	0	0	0	0	4	0	0	2	0
c⁺ *P*80	23	11	9	4	2	42	19	8	19	9
c⁺ *P*⁺	3	2	0	7	15	0	2	0	0	3
c⁺ *P*⁺/*P*3	2	0	0	1	0	0	0	0	0	0
c⁺ *P*⁺/*P*80	1	0	0	0	0	1	0	0	1	1
*cI*857 *P*3/*P*80	3	1	0	0	0	3	1	0	4	2
*cI*857 *P*3	42	3	1	21	4	17	1	1	2	2
*cI*857 *P*80	1	0	0	1	0	7	0	0	0	1
*cI*857 *P*⁺	3	0	0	0	0	2	0	0	0	3
*cI*857 *P*⁺/*P*3	4	0	0	2	7	0	0	0	0	1
*cI*857 *P*⁺/*P*80	0	1	1	0	0	0	0	0	0	2

the appearance of each spot on all of the other indicator plates was also recorded.

All resuspended plaques containing wild type (*P*⁺) phage were also spotted on Q5151 (*groP*) to test for the presence of *Pam*3 or *Pam*80 phage or both. This strain allows phage carrying the *Pam*3 or *Pam*80 alleles to grow while restricting the growth of wild type phage. Phage from spots scoring positive for presence of *Pam* phage were resuspended in 1 ml of SM buffer. These resuspended phage were plated for single plaques on Q5151 and then picked and stabbed with a toothpick onto indicator plates seeded with D6431 (*suI*) and QL (*suIII*) cells. Presence of both *Pam*3 and *Pam*80 phage in *P*⁺-containing plaques was considered evidence for a recombination event involving the replicated products of the initial transfection and was, therefore, scored as a *Pam*3/*Pam*80 burst. The absence of phage with one or both of the *Pam* alleles was considered evidence for formation of *P*⁺ recombinants by mismatch correction.

To detect the presence of Tn*10* in phage, 0.5 ml of an overnight culture of M182 (λ *c*⁺ *Pam*80) were overlaid on a LB + tetracycline (20 μg/ml) plate. The phage to be tested were then spotted. Tetracycline-resistant colonies were abundant in the spot if the phage contained a functional Tn*10*.

RESULTS

Point mutations and a deletion: Heteroduplex molecules in each of the two strand orientations were constructed with separated strands of DNA isolated from phage lambda *cI*857 *Pam*3 or *cI*ΔHindIII *Pam*3 and the complementary DNA strand isolated from lambda *c*⁺ *Pam*80. Each of these four preparations of heteroduplex molecules was used to transfect *suII* bacteria that were either *mut*⁺ or mutant in the *mutL*, *mutS*, *mutH* or *uvrD* loci. The transfected bacteria were plated for infective centers. A one-step growth curve of the phage in transfected bacteria showed that, on the average, each infective center yielded a burst of about 100 phage. For each transfection, the contents of 100 random infective centers were resuspended and the resuspended phage tested to identify the alleles that were represented in the contents of each plaque. This method permitted the detection of all of the possible genotypes that could be present, with the exception of those mutant at both *P* loci, *i.e.*, *Pam*3 *Pam*80 phage (Tables 2 and 3).

Transfection of *mut*⁺ bacteria gave rise to few infective centers in which all of the parental alleles were evident (7–12%) (Table 4). In contrast, with either *mutL* or *mutS* strains, all of the parental alleles were evident in most of the infective centers (about 80%). On transfection of *mutH* or *uvrD* bacteria, infective centers with all of the parental alleles present occurred at an intermediate frequency (11–55%). It would appear that the repair processing of heteroduplex molecules, reflected as allele loss, occurs frequently in the *mut*⁺ host, rarely in *mutL* and *mutS* bacteria, and at an intermediate frequency in *mutH* and *uvrD* hosts.

In order to describe the distribution of products, the fate of the *cI*⁻/*c*⁺ heteroduplexes will be commented on first. The most striking feature of the data on the *cI* markers is the observation that in a *mut*⁺ host, although both alleles survive only infrequently (13–17%) when the mutant allele is *cI*857, both survive very frequently when the mutant allele is a deletion (58 and 59%) (Table 5). It would appear that a molecule harboring a substantial region of sequence nonhomology, a single strand loop of about 700 base pairs, can replicate without experiencing repair processing.

Additional features of repair processing are evident from examination of the fate of the closely linked *P* alleles. Plaques in which representatives of all four

TABLE 3

Products of transfection with heteroduplexes of opposite strand orientation

	3′ *cI*ΔPam3 5′(h) / 5′ *cI*⁺ Pam80 3′(1)					3′ *cI*⁺ Pam80 5′(h) / 5′ *cI*ΔPam3 3′(1)				
	mut⁺	*mutL*	*mutS*	*mutH*	*uvrD*	*mut*⁺	*mutL*	*mutS*	*mutH*	*uvrD*
c⁺/*cI*Δ *P3*/*P80*	7	80	79	22	21	12	88	84	47	55
c⁺/*cI*Δ *P3*	28	0	1	10	6	10	1	0	5	3
c⁺/*cI*Δ *P80*	15	0	0	1	6	35	1	1	5	6
c⁺/*cI*Δ *P*⁺	6	0	0	10	14	0	0	0	1	1
c⁺/*cI*Δ *P*⁺/*P3*	2	4	4	36	29	0	0	0	1	0
c⁺/*cI*Δ *P*⁺/*P80*	0	2	1	0	1	2	0	0	19	11
c⁺ *P3*/*P80*	1	0	0	0	0	0	0	0	1	0
c⁺ *P3*	3	0	0	0	0	0	0	0	0	0
c⁺ *P80*	6	2	6	2	0	21	3	10	6	13
c⁺ *P*⁺	0	0	0	1	8	0	0	1	0	1
c⁺ *P*⁺/*P3*	0	0	0	1	1	0	0	0	0	0
c⁺ *P*⁺/*P80*	0	0	0	0	0	0	0	0	0	0
*cI*Δ *P3*/*P80*	0	5	6	3	2	1	2	1	5	3
*cI*Δ *P3*	28	3	2	9	9	16	2	3	8	4
*cI*Δ *P80*	3	0	0	0	0	2	3	0	0	2
*cI*Δ *P*⁺	1	0	0	0	1	1	0	0	0	0
*cI*Δ *P*⁺/*P3*	0	2	0	5	2	0	0	0	0	0
*cI*Δ *P*⁺/*P80*	0	2	1	0	0	0	0	0	2	1

TABLE 4

Percent of bursts with all of the parental alleles evident[a]

	cI857P3 (h) / *c*⁺ *P80* (1)	*cIΔP3* (h) / *c*⁺ *P80* (1)	*c*⁺ *P80* (h) / *cI857P3* (1)	*c*⁺ *P80* (h) / *cIΔP3* (1)
mut⁺	8	7	11	12
mutL	73	80	74	88
mutS	80	79	86	84
mutH	15	22	51	47
uvrD	11	21	47	55

[a] Compiled from data in Tables 2 and 3.

TABLE 5

Percent of bursts with *cI*/*c*⁺, *cI*, or *c*⁺ phage[a]

	cI857P3 (h) / *c*⁺ *P80* (1)					*c*⁺ *P80* (h) / *cI857P3* (1)				
	mut⁺	*mutL*	*mutS*	*mutH*	*uvrD*	*mut*⁺	*mutL*	*mutS*	*mutH*	*uvrD*
cI/*c*⁺	13	82	88	64	72	17	77	89	61	74
c⁺	34	13	10	12	17	54	21	10	33	15
cI	53	5	2	24	11	29	2	1	6	11
	cIΔP3 (h) / *c*⁺ *P80* (1)					*c*⁺ *P80* (h) / *cIΔP* 3 (1)				
	mut⁺	*mutL*	*mutS*	*mutH*	*uvrD*	*mut*⁺	*mutL*	*mutS*	*mutH*	*uvrD*
cI/*c*⁺	58	86	85	79	77	59	90	85	78	76
c⁺	10	2	6	4	9	21	3	11	7	14
cI	32	12	9	17	14	20	7	4	15	10

[a] Compiled from data in Tables 2 and 3.

parental alleles are evident are, as shown in Tables 2 and 3, abundant among the products of *mutL* and *mutS*, less frequent among the products of *mutH* and *uvrD*, and uncommon among the products of *mut*⁺ transfections.

The remaining plaques, representing loss of one or more of the parental alleles, reflect the products of repair of heteroduplex molecules prior to replication. In this case, the data from both kinds of *cI* heteroduplex have been pooled (Table 6), since the two subsets show no evidence of substantial difference for any given class. In the *mut*⁺ transfection, the most abundant class is that in which both alleles from one of the parental strands are lost (Table 6, lines 2 and 3). Those products of transfection that reflect the loss of only one or both mutant *Pam* alleles (Table 6, lines 4–7) represent the formation of wild type recombinants as a consequence of repair of only a portion of the heteroduplex.

For a given strand orientation, the frequency of such P^+-containing bursts is similar in *mut*⁺, *mutL* and *mutS* hosts (Table 6, line 7). Their frequency in *mutH* and *uvrD* hosts is substantially greater. Furthermore, for any given host, the frequency of such P^+ containing plaques is substantially higher in one strand orientation than the other.

Methylation: The role of adenine methylation in the formation of recombinants by mismatch repair of heteroduplex molecules has been examined. Heteroduplex DNA was prepared from phage that had experienced different degrees of methylation and used to transfect the various strains of *E. coli*. The yield of mismatch corrected P^+ products arising from *Pam*3/*Pam*80 heterozygotes was determined.

DNA with little or no adenine methylation was isolated from phage grown on a *dam*⁻ host. DNA harboring normal levels of methylation was isolated from phage grown on wild type bacteria, and DNA

TABLE 6

Distribution of bursts with various combinations of *P* alleles[a]

	P3+ (h) / *+ P80* (l)					*+ P80* (h) / *P3+* (l)				
	mut$^+$	*mutL*	*mutS*	*mutH*	*uvrD*	*mut*$^+$	*mutL*	*mutS*	*mutH*	*uvrD*
1) *P3+* / *+ P80*	19	159	166	40	34	34	165	173	119	109
2) *P3 +*	108	7	4	51	27	48	4	4	17	10
3) *+ P80*	48	14	15	9	8	109	26	20	34	42
4) *P3 +* / *+ +*	9	10	7	72	71	1	0	1	1	1
5) *+ P80* / *+ +*	1	7	8	3	2	5	2	1	28	28
6) *+ +*	15	3	0	25	58	3	3	1	1	10
7) Total of lines 4, 5, 6	25	20	15	100	131	9	5	3	30	39

[a] Compiled from data in Tables 2 and 3.

with more complete methylation was isolated from phage grown on bacteria harboring the adenine methylase overproducing plasmid pTP166. Heteroduplex DNA was prepared by denaturation and annealing of mixtures of DNA harboring the appropriate markers with the appropriate degrees of methylation. Such preparations include a mixture of the two possible heteroduplex orientations as well as the two homoduplex parental products.

The observation that heterologies resulting from the presence of a deletion usually remain unprocessed following transfection (Table 5B, line 1) permitted us to enrich our sample for infective centers resulting from heteroduplex DNA transfection. One of the DNA strands in each heteroduplex harbored the 700-bp *Hin*dIII deletion in *cI* and was present in a fourfold excess over *cI*$^+$ strands in the annealing mixture. Due to random pairing of strands during reannealing, turbid and mottled plaques resulting from such a transfection are, for the most part, products of heteroduplex molecules.

The turbid and mottled products of such transfections were tested for the presence of a large abundance of P^+ phage by spot tests. Analysis of the other alleles present in plaques that display such a large abundance of P^+ phage show that, for nearly all, either one or both of the *Pam* alleles are absent. These P^+ phage are therefore products of mismatch repair, and not recombination.

The results of such transfections are assembled in Table 7. They include transfection with heteroduplex molecules that are under-methylated, hyper-methylated and methylated at the level present in normal phage preparations. In addition we include the results of transfection with heteroduplex molecules harboring one strand that is under-methylated and one that is hyper-methylated. The array of bacterial hosts for transfection include *mut*$^+$, *mutL*, *mutS*, *mutH*, *uvrD*, and the double mutants *mutH mutL*, *mutH mutS*, *uvrD mutL* and *mutL mutS*.

Transfection of the *mut*$^+$ strain reveals the formation of very few bursts with P^+ products unless both strands are hyper-methylated (Table 7, line 1). The complete methyl directed mismatch repair system would be expected to repair all of the base pair mismatches on the under-methylated strand. The elevated level of P^+ products with hyper-methylated DNA could reflect the persistance of alleles of both parental strands, thus allowing for localized repair.

In all of the cases of transfection of *mutL*, *mutS* or *mutL mutS* strains, the yield of P^+ products is low (Table 6, lines 2–4). In these strains methyl-directed mismatch repair is disabled and localized mismatch repair is reduced.

As in the earlier experiments, P^+ products are frequent in strains that are defective in *mutH* or *uvrD* (Table 7, lines 5 and 8). Their yield seems to be independent of the degree of adenine methylation. The localized mismatch repair evident in these strains requires both the MutL and MutS functions (Table 7, lines 6, 7 and 9).

A transposon heterology: The transfection products of heteroduplex molecules harboring the stem and loop structure of a transposon, Tn*10*, in one of the strands have been examined. Mixtures of DNA from the two parental phage were denatured and reannealed, and the mixture of random products used to transfect the various bacterial hosts.

Table 8 displays the results of one such transfection experiment. The phage from which DNAs were isolated differ with respect to the presence of a *cI* mutation and the transposon. Tn*10* is located between the genes *P* and *Q* of one parent, within the 1200-bp *Acc*I/*Hin*dIII fragment (KLECKNER 1979). All plaques resulting from transfection were tested for the presence of phage producing clear plaques, turbid plaques, or a mixture. The expected relative yield of such products in the absence of mismatch correction is calculated on the assumption that the denatured strands of

TABLE 7

P^+ products among *cI* heteroduplex products of transfection

	me⁻	me⁺	me⁺/⁻	*Pam3 me⁺* / *Pam80me⁻*	*Pam3 me⁻* / *Pam80me⁺*
mut⁺	4/43	34/127	2/44	1/34	1/41
mutL	5/51	3/44	2/42	3/29	2/28
mutS	8/42	5/34	5/34	5/40	5/39
mutL mutS	2/44	4/47	1/34	4/47	1/39
mutH	38/59	29/59	30/60	36/79	38/78
mutH mutL	3/67	6/66	6/73	8/51	8/44
mutH mutS	3/37	3/38	6/39	3/39	0/39
uvrD	30/32	42/47	21/30	26/33	29/37
uvrD mutL	5/45	5/38	6/31	1/43	7/49

TABLE 8

Distribution of products of transfection with heteroduplexes containing Tn*10* between *P* and *Q*

		cI	*c*⁺	*cI*/*c*⁺
A)				
	Expected	0.69	0.027	0.28
	mut⁺ (176)	0.85	0.14	0.01
	mutL (175)	0.74	0.07	0.18*
	mutS (176)	0.78	0.08	0.14
	mutH (176)	0.75	0.07	0.18
	uvrD (176)	0.77	0.13	0.10*
B)				
	Expected	0.20	0.30	0.50
	mut⁺ (264)	0.36	0.62	0.015
	mutL (210)	0.33	0.52	0.15
	mutS (264)	0.33	0.48	0.19
	mutH (264)	0.34	0.47	0.19
	uvrD (264)	0.29	0.59	0.12

(A) and (B) show results from experiments with different ratios of parental DNAs.

The number of infective centers screened is in parentheses, and asterisks indicate the infective centers from which clear plaques were screened for the transposon.

the two phage DNA's assorted at random.

The presence of transfection products, with the various *mut* strains, that include both clear and turbid plaque formers show that biologically active heteroduplex products were present in the annealed mixture. The rarity of such products following transfection of the *mut*⁺ strain is to be expected in view of the activity of the methyl-directed mismatch repair system operating on the *cI*/*c*⁺ mismatch. Five to ten clear plaques were picked from each of 25 *cI*/*c*⁺ products that resulted from transfection of *mut* strains with heteroduplex molecules. Phage from each plaque were tested for the presence of the transposon by the capacity to induce tetracycline resistant colonies in a tetracycline sensitive lambda lysogen. None showed evidence of the transposon. The absence of the transposon in six of these products was confirmed by restriction analysis. For all six, the 1200-bp *Acc*I/*Hin*dIII fragment was present and transposon fragments were absent.

TABLE 9

Distribution of products of transfection with heteroduplexes containing *cI*::Tn*10*

		cI	*c*⁺	*cI*/*c*⁺
A)				
	Expected	0.03	0.69	0.28
	mut⁺ (140)	0.02	0.98	0
	mutL (319)	0.05	0.95	0
	mutS (284)	0.06	0.94	0
	mutH (381)	0.03	0.97	0
	uvrD (242)	0.04	0.96	0
B)				
	Expected	0.69	0.03	0.28
	mut⁺ (103)	0.84	0.15	0
	mutL (202)	0.79	0.21	0
	mutS (195)	0.84	0.16	0
	mutH (301)	0.80	0.20	0
	uvrD (172)	0.84	0.16	0

(a) and (b) show results from experiments with different ratios of parental DNAs.

The numbers of infective centers screened is in parentheses.

In an additional experiment heteroduplex DNA was prepared by denaturation and annealing of a mixture of DNA from *cI*⁺ phage and phage harboring the transposon in the *cI* gene (KLECKNER 1979). There was no evidence (less than 1%) on any of the bacterial hosts of transfection products that harbored a mixture of clear and turbid plaque formers (Table 9). It would appear that transfection with heteroduplex molecules that harbor a transposon in one strand results in loss of the transposon.

If only the transposon were lost, the remaining nine base pair repeat that is characteristic of Tn*10* insertion could leave a product that was still defective in *cI*. The failure to detect a significant number of mixed *cI* and *cI*⁺ plaque formers suggests the possibility that even the repeat sequence is lost, and that the resulting product resembles that of precise excision.

DISCUSSION

Transfection of *mut*⁺ bacteria with heteroduplex molecules containing only base pair mismatches often gives rise to infective centers in which only one of the parental strands is represented (Table 2, lines 9 and 14). Such plaques are relatively uncommon in the products of transfection of mutator strains. It seems likely, therefore, that a function that is defective in the mutator strains is responsible for the frequent coincident loss of all three alleles resident on one of the strands of the parental heteroduplex and that the extent of this coincident loss is likely to exceed 2000 bp (the distance between the *cI* and *P* loci).

WAGNER and MESELSON (1976) argued for strand loss in about two-thirds of the infective centers pro-

duced by transfection. Such apparent strand loss could be the consequence of repair of all of the mismatches on one of the parental strands. Their experiments differ from those reported here in that the transfection was helper-mediated, and that the phage were *red*$^-$ and the bacterial hosts *recA*$^-$. It seems likely that the disabled recombination functions, resulting in a substantial reduction in burst size, could also contribute to the apparent strand loss they reported. In the experiments reported here, the large fraction of infective centers harboring allelic contributions from both parents, following transfection of *mut* strains, and the large burst size that we have observed from transfected bacteria, argue that strand loss does not occur very frequently.

The methyl-directed mismatch repair appears to be responsible for the frequent formation of the single-parent infective centers in the *mut*$^+$ host. This could be the consequence of very long patches of repair by this system or multiple repair events acting on the "younger" and therefore less methylated strand of any given heteroduplex. The absence of this repair system in *mutH*, *mutL*, *mutS* and *uvrD* strains would account for the abundance of infective centers harboring phage with alleles from both parents after transfection of those hosts (Table 4). Nevertheless, mismatch repair is evident in the *mut*$^-$ strains, particularly in the *mutH* and *uvrD* strains. Since the *mut* mutations are the consequence of insertion of transposable elements, the mutants are not likely to be leaky.

The mismatch repair activity that is evident in *mutH* and *uvrD* strains appears to be the consequence of a different mechanism for mismatch repair. If methyl-directed mismatch repair functions were to act by replacement synthesis of long tracts of polynucleotide initiating at an unmethylated or hemi-methylated GATC site, the alleles on one of the parental strands could all be lost. Such replacement synthesis would be unlikely to separate closely linked markers to form P^+-containing plaques.

We suggest that the P^+ containing infective centers can result from a second mechanism of mismatch repair operating by repair of either a single nucleotide or a sequence of nucleotides so short that the tract is often shorter than the interval between the two *P* alleles (Fox and Raposa 1983), reported to be separated by 27 nucleotides (Reiser 1983). A similar mechanism has been suggested by Lieb (1983). The observations reported here suggest that the absence of the methyl-directed repair system in *mut* strains could result in the persistence of heteroduplex substrates, which therefore remain candidates for the kind of repair that results in the formation of P^+ products. Such would be the case in *mutH* and *uvrD* strains in which P^+ products are abundant.

The relative rarity of P^+ products in *mutL* and *mutS* strains (Table 6, lines 4–7) could be accounted for if the functions defective in these strains, in addition to playing a role in methyl-directed mismatch repair, were also required in the proposed second mechanism. The transfection experiments displayed in Table 7, which include the double mutants *mutL mutH*, *mutS mutH*, *mutL uvrD* and *mutL mutS*, provide support for this suggestion. *mutL* and *mutS* are epistatic to *mutH* and *uvrD* with respect to formation of P^+ products by localized mismatch repair. In addition, these observations show that the level of *dam*-directed adenine methylation plays no detectable role in this localized repair.

If there are indeed two different mechanisms of mismatch repair, it becomes possible to suggest a way of accounting for the singular behavior of the *cIam6* class of mutations (Lieb 1985; Lieb, Allen and Read 1986). In crosses between *cI* mutants, the presence of such a mutation in one of the parents results in about a tenfold enhancement of the frequency of recombination for a given nucleotide distance between the *cI* markers (Lieb 1983). Pukkila *et al.* (1983) have shown that at least some base pair mismatches do not signal methyl directed mismatch repair. The second pathway could display a different specificity. If the *cIam6*/c^+ mismatch were not recognized by the methyl directed system and were a good substrate for the second pathway, the localized excision and repair by this pathway referred to by Lieb (1983) as very short patch repair, could give rise to an increased abundance of c^+ products of recombination.

The abundance of cI^-/c^+ products of transfection of *mut*$^+$ bacteria with heteroduplex molecules that include the deletion heterology in *cI* (Table 5B) suggests that such nonhomologies are not recognized by the methyl-directed repair system, and often remain uncorrected (Fox and Raposa 1983). A similar behavior of an IS*1* heterology has been reported by Dohet *et al.* (1987). Furthermore, if the methyl-directed repair system were to give rise to long tracts of replacement synthesis, some $cI\Delta/c^+$ heterozygotes could be lost adventitiously as a consequence of co-correction with the mismatches that are present in the *P* gene of the DNA molecules. This loss would be reduced in *mut* strains, thus giving rise to the observed greater yield of cI/c^+ products in those strains.

For the case of transfection with molecules harboring a heterology that reflects the presence of a deletion, there remains the possibility that some fraction of the molecules are processed in a manner that results in an inviable product. Preliminary investigation of the products of transfection with non-homology containing heteroduplexes formed by random annealing shows that as many as half of the transfection products may be lost. With base pair mismatch-containing heteroduplexes, the products of transfection of the *mutL*

and *mutS* strains show no evidence of loss of viability (PEARSON and FOX 1987).

In contrast to the limited processing of a heterology resulting from the presence of a deletion in one of the heteroduplex strands, the stem and loop structure of a transposon is lost in transfection. At least some of the heteroduplex molecules harboring such a structure give rise to infective centers. When the transposon-carrying strand also harbors a mutation in *cI*, and the complimentary strand is c^+, bursts mixed for *cI* are detected among transfection products (Table 8). Despite this evidence of transfection by heteroduplex molecules, these bursts show no evidence of the presence of the transposon. Furthermore, when the heteroduplex includes a c^+ strand and a strand with the transposon inserted into *cI*, few, if any, mixed bursts are evident (Table 9). These observations demonstrate that the stem and loop structure in such a heteroduplex is lost and it would appear that the loss is precise, reestablishing the wild type sequence. Such a loss could be the consequence of processing, *i.e.*, repair of the heteroduplex molecule, but it is also possible that the loss occurs in the course of replication of the molecule harboring the stem and loop structure.

One feature that merits comment is the asymmetry in the distribution and composition of P^+-containing products arising from the two strand orientations of heteroduplexes. The sequence data of REISER (1983) shows that both the *Pam*80 and the *Pam*3 mutations are the result of CG to TA transitions. *Pam*3 reflects a change from CCAGT to CTAGT and *Pam*80 a change from CCAGA to CTAGA transition. In each heteroduplex that we have constructed, one of the mismatches would be T/G similar to the mismatch that would be created by the *cIam*6 mutation LIEB, ALLEN and READ (1986). If the localized mismatch repair were responsible for correcting the T to C in such mismatches, as suggested by LIEB (1985) and MESELSON (1987), the high frequency of *P*3 +/++ in one strand orientation and + *P*80/++ in the other would be accounted for (Table 6, lines 4 and 5).

The presence of mismatch repair, evident at a diminished level in *mutL*, *mutS* and in the *mutL mutS* double mutant (Tables 4 and 7), remains to be accounted for. It seems unlikely that this activity is due to the leakiness of the mutants since in all cases they are the products of transposon insertion. The observation that the *mutL mutS* double mutant is not further reduced in the residual activity (Table 7, line 7) also argues against leakiness.

About 10% of the products of transfection of the mut^+ strain contain all the alleles present in the two parental strands (Table 4). These could include the products in which for one reason or another replication has preceded repair, or occasional heteroduplex molecules in which both strands were highly methylated at GATC sites and therefore poor substrates for the methyl directed mismatch repair. They could also include infective centers from bacteria that had taken up more than one molecule of transfecting DNA.

In conclusion, we have shown that the methyl-directed mismatch repair system often acts on several mismatches, separated by as many as 2000 bp, on one of the strands of a heteroduplex molecule. When the methyl-directed mismatch repair system is disabled by *mutH* or *uvrD* mutations, localized mismatch repair becomes prominent. This prominent localized repair that can separate very closely linked markers requires the functions MutL and MutS, is independent of adenine methylation, and appears to reflect another mechanism of mismatch repair.

Heterology-containing heteroduplex molecules that reflect the presence of a deletion in one strand often escape processing. However, when the heterology reflects the presence of the stem and loop structure of a transposon, Tn*10*, the transposon is lost.

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