JOURNAL OF BACTERIOLOGY, Nov. 1987, p. 5241–5246
0021-9193/87/115241-06$02.00/0

Vol. 169, No. 11

Bacterial Genes *mutL*, *mutS*, and *dcm* Participate in Repair of Mismatches at 5-Methylcytosine Sites

M. LIEB

Department of Microbiology, University of Southern California School of Medicine, Los Angeles, California 90033

Received 30 March 1987/Accepted 12 August 1987

Certain amber mutations in the *c*I gene of bacteriophage lambda appear to recombine very frequently with nearby mutations. The aberrant mutations included C-to-T transitions at the second cytosine in 5'CC(A/T)GG sequences (which are subject to methylation by bacterial cytosine methylase) and in 5'CCAG and 5'CAGG sequences. Excess *c*I$^+$ recombinants arising in crosses that utilize these mutations are attributable to the correction of mismatches by a bacterial very-short-patch (VSP) mismatch repair system. In the present study I found that two genes required for methyladenine-directed (long-patch) mismatch repair, *mutL* and *mutS*, also functioned in VSP mismatch repair; *mutH* and *mutU* (*uvrD*) were dispensable. VSP mismatch repair was greatly reduced in a *dcm Escherichia coli* mutant, in which 5-methylcytosine was not methylated. However, mismatches in heteroduplexes prepared from lambda DNA lacking 5-methylcytosine were repaired in *dcm*$^+$ bacteria. These results indicate that the product of gene *dcm* has a repair function in addition to its methylase activity.

A number of amber mutations in gene *c*I of bacteriophage lambda and in the *lacI* gene of *Escherichia coli* yield excess wild-type recombinants when crossed with nearby mutations (3, 14, 15). These "repair-prone" amber mutations are C-to-T transitions in 5'CC(A/T)GG, 5'CCAG, and 5'CAGG sequences (16; Fig. 1). The excess recombinants result from the correction of mutant–wild-type mismatches at the sites of these mutations; such mismatches occur in the DNA heteroduplexes that accompany recombination (14). The absence of corepair in crosses of repair-prone mutations with markers as close as 10 base pairs indicates that a very-short-patch (VSP) repair system is responsible for specific mismatch repair (15). There is disparity in VSP mismatch repair during lambda crosses: repair always restores the C · G base pair (15). Transfection experiments with heteroduplexes containing a T · G or C · A mismatch at the site of a repair-prone mutation revealed that VSP repair corrects only the T · G mismatch (11). Correction by VSP repair probably accounts for much of the "high negative interference" observed in studies of recombination between close markers in *E. coli*. It has been suggested that VSP repair may prevent mutations caused by the spontaneous deamination of 5-methylcytosine (5-meC) (11, 16, 30).

A different system of mismatch repair was identified in transfection experiments with lambda DNA heteroduplexes (23, 26; M. Meselson, *in* K. B. Low, ed., *The Recombination of Genetic Material*, in press). This "long-patch" repair system corrects mismatches by removing tracts of up to several thousand nucleotides. Repair is triggered by a mismatch, but nucleotide excision occurs only on DNA strands that contain nonmethylated 5'GATC sequences. Methylation of adenine in these sequences by the product of bacterial gene *dam* occurs after DNA replication. Thus, methyladenine (meA)-directed repair provides a mechanism for correcting errors in newly synthesized DNA chains, in which GATC sequences are undermethylated. Certain mispairs, e.g., transition mismatches, are corrected more efficiently than most transversion mismatches (4, 12). Repair efficiency is also affected by the guanine-plus-cytosine content of the surrounding sequence (10). However, there is no evidence that the specific sequence in which a mispair occurs affects the frequency of meA-directed repair.

Since VSP repair and meA-directed repair differ in several respects, it was of interest to determine whether these systems were independent. I have studied the effects on VSP repair of mutations in bacterial genes *mutH*, *mutL*, *mutS*, and *uvrD* (*mutU*), all of whose functions are required for meA-directed mismatch repair (17). Bacteriophage crosses and transfection with lambda DNA heteroduplexes gave identical results: VSP repair is dependent on *mutL* and *mutS* but not on *mutH* or *uvrD*. The presence of meA at all GATC sequences in both DNA chains, which prevents long-patch repair, did not interfere with VSP repair (see also reference 11).

In an earlier paper, I reported that VSP repair was reduced only slightly in a *dam dcm* strain (15). Subsequently, I found that DNA extracted from phage grown on this strain (RB404) was only partially digested by *EcoR*II (data not shown). The studies presented here show that although 5meC in the DNA containing a mismatch is not a requirement for VSP repair, specific repair occurs only in *dcm*$^+$ bacteria.

After submission of this paper, a report describing studies of mismatch repair of constructed mutations in gene *lacZ* appeared (30). The results of these experiments by Zell and Fritz, who used heteroduplexes of phage M13 DNA, are in complete accord with the findings reported here.

MATERIALS AND METHODS

Bacteria and phage. Bacterial strains are listed in Table 1. Mutations in lambda gene *c*I were described previously (14, 16). Phage were propagated on *dam*$^+$ *dcm*$^+$ strains C600 or 594, unless otherwise stated, by the liquid culture method (18). Other media have been described previously (14).

Phage crosses. Crosses were performed essentially as in an earlier study (16). Bacteria were infected with a 1:1 mixture of *N*am53 and *O*am29 mutants to give a total multiplicity of

T = am6	T = am9	T = am10, am15	T = amB36
5'CCAGG	5'GCAGG	5'TCAGG	5'CCAGA

FIG. 1. Sites of amber mutations susceptible to VSP repair.

TABLE 1. *E. coli* strains used in this study

Strain	Relevant genotype[a]	Source or reference
594	*sup*^{0} *mut*$^+$	Lieb (14)
C600	*supE mut*$^+$	Bachmann (1)
Yme1	*supF*	Bachmann (1)
GM30	*dcm*$^+$	M. Marinus (19)
GM31	*dcm-6*	M. Marinus (19)
GM30(pDCM1)	*dcm*$^+$(p*dcm*$^+$)	This study
GM2290(pTP166)	*dam*$^+$(p*dam*$^+$)	Marinus et al. (21); M. Marinus
KL862	*mutH34 sup*^{0}	Feinstein and Low (5); J. Miller
KL864	*mutU4* (*uvrD*) *sup*^{0}	Feinstein and Low (5); J. Miller
KMBL3752	*mut*$^+$	Glickman and Radman (7); M. Radman
KMBL3760	*mutL101 dam-3*	Glickman and Radman (7); M. Radman
KMBL3773	*mutH101*	Glickman and Radman (7); M. Radman
KMBL3775	*mutS101*	Glickman and Radman (7); M. Radman

[a] All strains are *dam*$^+$ *dcm*$^+$ *supII* unless otherwise specified.

infection of 10. After a 15-min adsorption period at 37°C, broth was added and incubation was continued for a total of 100 min, followed by the addition of $CHCl_3$ and vortexing. Total progeny phage were assayed on strain C600, and N^+ O^+ recombinants were assayed on strain 594 unless otherwise indicated. The fraction of N^+ O^+ recombinants varied from about 0.3 to 1.2%.

Transfection experiments. Phage DNA was prepared by the method of Maniatis et al. (18). Phage in culture lysates were concentrated by using polyethylene glycol and purified on CsCl step gradients. DNA containing 5meC was extracted from lambda grown on strain GM30 (*dam*$^+$ *dcm*$^+$); phage grown on GM31 (*dam*$^+$ *dcm*) was the source of DNA lacking cytosine methylation. The absence of cytosine methylation was confirmed by digestion with *Eco*RII. DNA was extracted with phenol and phenol-chloroform and diluted to 10 μg/ml in TE (10 mM Tris chloride, 1 mM EDTA, pH 8.0). Equal volumes of DNAs containing different *c*I mutations were heated at 92.5°C for 2.5 to 3 min and chilled on ice. For renaturation, mixtures were kept at 56.5°C for 15 to 30 min. Bacteria were prepared by calcium chloride treatment as described by Maniatis et al. (18) and kept on ice for 2 to 6 h before the addition of DNA. Transfection mixtures contained about 10^6 lambda equivalents and 10^8 bacteria. Bacteria and phage were kept at 0°C for 30 min and then transferred to 43°C for 2 to 3 min. Agar and indicator bacteria were added, and the mixture was plated as an overlay on nutrient agar plates. About 2×10^3 to 2×10^4 plaques were obtained per microgram of DNA added. Denatured DNA retained less than 1% activity, and renaturation restored 10 to 60% of the original activity.

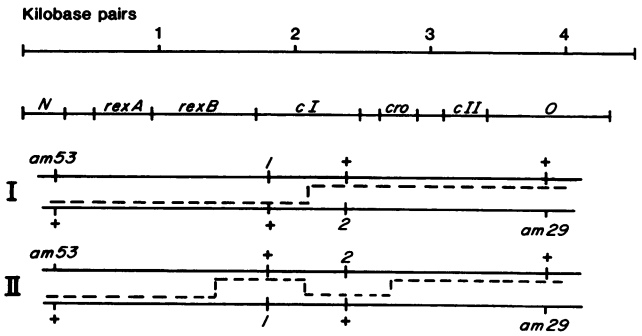

FIG. 2. Map of lambda genes *N* to *O*. Diagrams I and II illustrate reciprocal four-factor crosses. When crosses are made in configuration II, N^+ cI^+ O^+ recombinants are expected to require three crossovers and thus occur at a very low frequency.

TABLE 2. Identification of lambda *c*I mutations by plaque phenotype

Host	Temp (°C)	Phenotype[a] of plaque produced by the following mutation(s):					
		*c*I$^+$	am6	am9 am10	am302 amB36	am6 am302	ts857
sup^0 (594)	32	T	C	C	C	C	T
sup^0 (594)	37	T	C	C	C	C	
supE (C600)	37	T	T	T	C	C	
supE (C600)	41	T	T	T	C	C	C
supF (Yme1)	37	T	C	T	T	C	

[a] T, Turbid; C, clear.

Plaques formed by transfected bacteria were chosen at random; when possible, every plaque on a plate was isolated. Agar plugs containing individual plaques were transferred with Pasteur pipettes from the plates to tubes containing 1 ml of phage diluent and 1 drop of $CHCl_3$. The phage were allowed to elute and were diluted about 1:50; aliquots were spotted on petri plates containing nutrient medium and an overlay of indicator bacteria. The genotypes of the phage eluted from each plaque were revealed by the plaque phenotypes on two or more hosts. For example, a mixture of mutant am302 and wild-type phages produced clear and turbid plaques on strains 594 (*sup*^{0}) and C600 (*supE*) but only turbid plaques on strain Yme1 (*supF*) (Table 2).

RESULTS

Requirement of *mutL* and *mutS* for VSP repair in lambda crosses. Four-factor crosses (Fig. 2) are used in studies of intragenic recombination in gene *c*I. In each cross, one parent carries an amber mutation in gene *N*, to the left of *c*I, and the other parent carries a mutation in gene *O*, to the right of *c*I. All the *c*I mutants form clear plaques on Sup^0 hosts, which lack a suppressor for amber mutations. Recombinants that are N^+ O^+ are selected by plating the phage progeny from bacteria infected with two parents on a lawn of Sup^0 bacteria; N^+ O^+ phage that are also cI^+ form turbid plaques. Dividing the number of turbid plaques by the total number of plaques yields the frequency of cI^+ recombinants.

Mutations *c*I am6 and *c*I am10, both of which are subject to VSP repair, were each crossed with nearby mutations whose locations are shown in Fig. 3. Mutation 323 (adjacent to am6) was used in control crosses to determine the expected frequency of recombinants in crosses of am6 with neighboring mutations (16). The expected frequency of recombinants in crosses with am10 was based on the results of crosses with mutation 103 (data not shown). Crosses were made in either configuration I, in which the production of N^+ cI^+ O^+ recombinants requires only one crossover, or in configuration II, in which such recombinants are expected to be extremely rare, since they require three crossovers (Fig. 2).

In a wild type (*mut*$^+$) host, crosses in which one parent carried *c*I mutation am6 or am10 produced up to 30 times as many cI^+ recombinants as expected (Fig. 4, crosses B, C, D, and E). The excess recombinants are the result of VSP

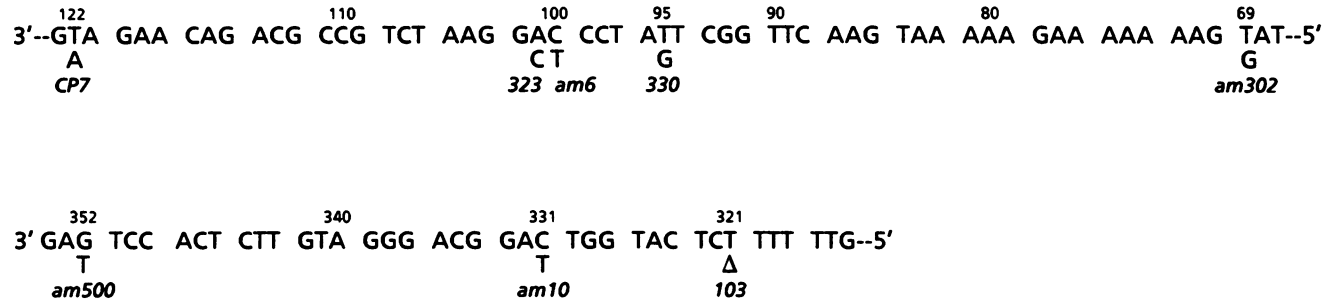

FIG. 3. Location of mutations in gene *cI*. The sequences are arranged 3′ to 5′ so that the mutations are oriented correctly relative to genes *N* and *O* (Fig. 1).

mismatch repair (14). The percentage of cI^+ recombinants in crosses made in a *mutH* host did not differ consistently from the percentage of cI^+ recombinants in mut^+ crosses. Significantly fewer recombinants were obtained when crosses involving am6 or am10 were performed in *mutS* or *mutL* host bacteria. However, there was no reduction in the frequency of cI^+ recombinants when two mutations not subject to VSP repair were crossed in *mutL* or *mutS* bacteria (cross A and unpublished results). The participation of *mutL* and *mutS* products in VSP repair was confirmed by crosses made in other *mutL* and *mutS* strains, including a *mutH mutS* strain which contains a Tn*5* insertion in *mutS* (data not shown).

Even in *mutL* and *mutS* hosts, crosses with am6 or am10 yielded more cI^+ recombinants than expected from similar crosses with "ordinary" point mutations. It could be argued that since N^+ O^+ recombinants were selected on mut^+ plating bacteria, unrepaired heteroduplexes that had been packaged into phage capsids might have been repaired in the bacteria used for plating (9). The resulting plaques would contain both cI^+ and mutant phages and would have a mottled appearance. Such mottled plaques were not scored as turbid in this study. In some experiments, duplicate platings of cross lysates were made on mut^+ and *mutS* hosts. There was no significant difference in the frequency of turbid plaques on the two hosts. It would appear, therefore, that some VSP repair occurs in the absence of MutL or MutS.

In transfection experiments with lambda heteroduplexes, Nevers and Spatz (24) and Rydberg (27) observed a deficiency of mismatch repair in *uvrD* and *uvrE* mutants. The product of *uvrE* is also required in an in vitro system that performs meA-directed (long-patch) repair (16). Mutations *uvrD*, *uvrE*, *recL*, and *mutU* are now known to represent a single gene, whose product is helicase II (8, 13).

To study the requirement for *uvrD* function in VSP repair, I made crosses in a *uvrD* strain and a $uvrD^+$ strain that was isogenic with the exception of a *mutH* mutation. Although frequencies of recombinants attributable to VSP repair were consistently higher in the *uvrD* strain, the differences between crosses made in *uvrD* and $uvrD^+$ hosts were not significant (Table 3).

VSP mismatch repair after transfection. When lambda phage is grown on a dam^+ dcm^+ host, some adenines in 5′GATC sequences remain unmethylated. In transfected bacteria, base-pair mismatches in DNA heteroduplexes prepared from such phage are corrected efficiently by meA-directed long-patch repair (25). To study VSP mismatch repair after transfection, I prepared heteroduplexes of lambda DNA containing two closely linked mutations in one strand and the wild-type equivalents in the other. I denatured and renatured 1:1 mixtures of the mutant and wild-type DNAs; therefore, only 50% of renatured molecules were heteroduplexes. Since VSP repair appears to act only on T · G mismatches (11), only 25% of the transfected bacteria received a heteroduplex containing the correctable mismatch. Plaques produced by progeny of individual transfected bacteria were chosen at random, and the genotypes of eluted phage were characterized by plating on appropriate hosts.

Only one phage genotype was present in 95% of the

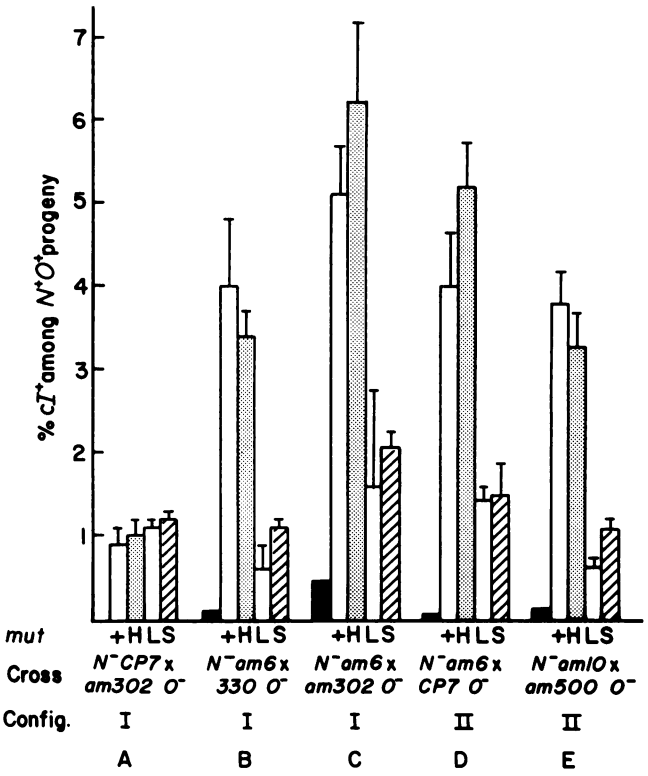

FIG. 4. Effects of mutations in *mutH*, *mutL*, and *mutS* on the frequency of cI^+ recombinants. Crosses A to E were performed in isogenic mut^+ (KMBL372) (+), *mutH* (KMBL3773) (H), *mutL* (KMBL3760) (L), or *mutS* (KMBL3775) (S) strains. In configuration (Config.) I crosses, N^+ cI^+ O^+ recombinants require only one crossover; in configuration II, N^+ cI^+ O^+ recombinants are expected to require three crossovers. The bars indicate the frequency of cI^+ phage among N^+ O^+ progeny. Standard deviations are shown as vertical lines above the bars. Black bars indicate the expected frequency of cI^+ recombinants based on comparable crosses between two markers that are not susceptible to VSP repair. Distances (in base pairs) between *cI* markers: cross A, 53; cross B, 5; cross C, 31; cross D, 22; and cross E, 21.

TABLE 3. VSP repair in *mutH* and *uvrD* hosts

Expt	cI mutation in the following parental phage:		Configuration[a]	% cI+[b] (± SD) when crossed in:	
	N	*O*		*uvrD*+ *mutH* (KL862)	*uvrD* *mutH*+ (KL864)
1	am6	330	I	2.3 ± 0.4	4.1 ± 0.2
2	am6	am302	I	4.8 ± 0.5	5.6 ± 0.6
3	am6	CP7	II	3.6 ± 0.5	5.7 ± 0.5
4	am10	am500	II	2.3 ± 0.4	3.6 ± 0.8
5	CP7	am302	I	1.0 ± 0.2	1.1 ± 0.1

[a] See Fig. 2.
[b] Percentage of N^+ O^+ phage that were cI+.

plaques produced by transfected *mut*+ bacteria (Table 4, experiment 1). This is the result expected if mismatches in heteroduplex molecules are corrected by excision and resynthesis of a long tract of nucleotides. Among plaques produced by transfected *mutH* bacteria, about 15% contained progeny in which the am6 mutation had been repaired to am+ without the corepair of am302, which is located 31 base pairs from am6 (Table 4, experiments 2 and 3). This fraction of plaques with phage that had undergone VSP repair represents the majority of all transfections with heteroduplexes containing a correctable (T · G) mismatch. None of the plaques tested contained phage that had lost the am302 mutation but retained am6. Thus, in the *mutH* host, am6 was corrected by VSP repair.

When *mutL* or *mutS* bacteria were transfected with the same DNA, about 30% of the resulting plaques contained mixtures of am6 am302 and wild-type phages, indicating that over 50% of heteroduplexes were not corrected in these hosts (Table 4, experiments 4 and 5). Only one instance of mismatch repair of am6 was found after transfection of a *mutS* or *mutL* strain, confirming the importance of genes *mutS* and *mutL* in VSP repair.

Heteroduplexes were also prepared with DNA from phage with repair-prone amber mutations that arose in 5'CAGG (am9 and am10) and 5'CCAG (amB36) and were used to transfect *mutH* bacteria. Mutations am9, am10, and amB36 were corrected less efficiently than was mutation am6 (Table

TABLE 4. VSP repair of heteroduplex DNA

Expt	Heteroduplex[a]	5meC in DNA[b]	Host	Total no. of plaques tested	% of plaques with indicated genotypes:			
					am6 am302	+ +	am6 am302 and + +	+ am302 and + +
1	am6 am302 + +	+	*mut*+ (KMBL3752)	80	51	44	2.5	2.5[c]
2	am6 am302 + +	+	*mutH* (KMBL3773)	206	35	34	18	13
3	am6 am302 + +	−	*mutH* (KMBL3773)	128	32	26.5	26.5	15
4	am6 am302 + +	+	*mutL* (KMBL3760)	90	40	29	30	1
5	am6 am302 + +	+	*mutS* (KMBL3775)	90	51	23	26	0
					am9 +	+ ts857	am9 + and + ts857	+ + and + ts857
6	am9 + + ts 857	+	*mutH* (KMBL3773)	157	23	33	39	5[d]
					am10 ts1	+ +	am10 ts1 and + +	+ ts1 and + +
7	am10 ts1 + +	−	*mutH* (KMBL3773)	199	29.5	34	30	6.5[e]
					+ amB36	amQ18 +	+ amB36 and amQ18 +	+ + and amQ18 +
8	+ amB36 amQ18 +	−	*mutH* (KMBL3773)	212	34	36	24	6

[a] 1:1 mixtures of DNAs containing the markers indicated were heated and reannealed. Distances (in base pairs) between mutations were as follows: am6 and am302, 31; am9 and ts857, 50; am10 and ts1, ca. 100 and amQ18 and amB36, 5.
[b] All DNAs were extracted from phage grown on *dam*+ hosts.
[c] The plaque contained only + am302 phage.
[d] Includes plaques containing only + + phage.
[e] Includes plaques containing only + ts1 phage.

TABLE 5. Requirement of the *dcm* gene for VSP repair

Expt	cI mutation in the following parental phage[a]		Distance (base pairs)	Configuration[b]	% cI^{+}[c] (± SD) when crossed in:	
	N	*O*			GM30 (*dcm*$^{+}$)	GM31 (*dcm*)
1	am6	am330	5	I	1.7 ± 0.2[d]	0.29 ± 0.19
2	am6	cP7	22	II	2.2 ± 0.6	0.36 ± 0.15
3	am10	am500	21	II	2.0 ± 0.4	0.26 ± 0.09
4	am15	am505	27	II	0.85 ± 0.26	0.24 ± 0.15
5	101	*B*am36	25	II	1.1 ± 0.27	0.15 ± 0.05
6	cP7	am330	27	I	0.68 ± 0.04	0.63 ± 0.11
7	101	am500	49	I	0.90 ± 0.07	0.90 ± 0.18

[a] Phages were grown on C600 (*dam*$^{+}$ *dcm*$^{+}$).
[b] See Fig. 2.
[c] Percentage of *N*$^{+}$ *O*$^{+}$ progeny that were cI^{+}.

4, experiments 6, 7, and 8), reflecting a lower frequency of excess cI^{+} recombinants in phage crosses (16).

Requirement of the *dcm* gene product for VSP repair. Mutation am6, which is subject to VSP repair, arose at the site of a methylated cytosine in the sequence 5'CCAGG (Fig. 1). In *E. coli* K-12, the product of gene *dcm* methylates the internal cytosines of this sequence (reviewed by Marinus [20]). Mutations that are subject to VSP repair are also found in related sequences which are not known to be methylated (Fig. 1). Thus, a 5meC near the mismatch is not likely to be a requirement for mismatch repair. However, the similarity of the sequences in which VSP repair has been observed suggested the possibility that the *dcm* product plays a role in specific mismatch repair.

In all crosses in a *dcm* host, there were almost no excess recombinants. *dcm* function is required for mismatch repair of am10, am15, and amB36 as well as of am6 (Table 5). However, the absence of cytosine methylase had no effect on recombination between cI mutations that are not subject to VSP repair (Table 5, experiments 6 and 7). The presence or absence of 5meC in the DNA of infecting phage did not affect the frequency of cI^{+} recombinants (data not shown).

A requirement for *dcm* function in VSP repair was also seen in transfection experiments. Since a *dcm mutH* or *uvrD* host for transfection was not available, heteroduplexes were protected from meA-directed repair by methylation of all 5'GATC sequences in vivo. DNA was prepared from phage grown in a *dcm*$^{+}$ host containing a *dam*$^{+}$ plasmid; adenine methylation was confirmed by the resistance of the DNA to digestion by *Mbo*I (data not shown). A mixture of DNAs prepared from lambda cI ts857 and the double mutant am6 am302 was denatured, reannealed, and transfected into *dcm*$^{+}$ (p*dcm*$^{+}$) and *dcm* bacteria. VSP repair of am6 was very rare in the *dcm* host (Table 6). In the *dcm*$^{+}$ host, am6 was repaired to cI^{+} in 14% of transfections, which is equivalent to 56% of the heteroduplexes susceptible to VSP repair (see above).

DNA isolated from phage grown on a *dcm* host was used in some of the transfection experiments shown in Table 4. A comparison of experiments 2 and 3 shows that heteroduplexes lacking cytosine methylation were as susceptible to correction of am6 as were methylated heteroduplexes.

DISCUSSION

Although both meA-directed repair and VSP repair are efficient systems for correcting single-base-pair mismatches, they exhibit significant differences. VSP repair is limited to T · G mismatches in a specific context, while meA-directed repair corrects a number of mismatches irrespective of specific context. meA-directed repair replaces long tracts of nucleotides, while VSP repair removes fewer than 10 nucleotides. Removal of a long tract of nucleotides requires the presence, adjacent to the mismatch, of a 5'GATC sequence in which adenine is not methylated. The results of this study showed that the VSP system repairs mismatches in DNA in which all GATCs are methylated (Table 6). In addition, Meselson (in press) reported mismatch repair of a repair-prone *P*am mutation in fully adenine-methylated lambda heteroduplexes. The two repair systems also differ in that the functions of genes *mutH* and *uvrD* (*mutU*), which are required for meA-directed repair, are not needed for VSP repair.

Despite their differences, both mismatch repair systems appear to use the products of genes *mutL* and *mutS*. *mutS* protein binds to mismatched base pairs, with the highest affinity for a G · T mismatch (29), and may be indifferent to the context in which the mismatch occurs. The function of *mutL* is not yet known, but its product is presumed to interact with that of *mutS*. In the case of VSP repair, repair specificity is almost certainly provided by cytosine methylase, the product of gene *dcm*. *mutH* apparently cleaves a DNA strand 5' to an unmethylated GATC sequence (23), supplying strand specificity in meA-directed mismatch repair. *uvrD* (DNA helicase), which, like *mutH*, is not required in VSP repair, may participate in the removal or resynthesis of long tracts of nucleotides.

Mutations in bacterial genes *mutS* and *mutL* lead to a significant reduction in the frequency of VSP repair, but excess recombinants still appear in phage crosses made in such hosts (Fig. 4). A *dcm* mutation is more effective in reducing the frequency of excess recombinants than is either a *mutS* or a *mutL* mutation (compare Table 5 and Fig. 4). It is particularly interesting that gene *dcm* appears to have a direct function in VSP mismatch repair. Mismatches in heteroduplexes prepared from DNA lacking 5meC are repaired as efficiently as mismatches in DNA from phage grown in *dcm*$^{+}$ hosts (Table 4). Furthermore, two of the three sequences in which T · G mismatches are subject to

TABLE 6. Requirement of the *dcm* gene for VSP repair of am6 in heteroduplexes[a]

Expt	Host	Total no. of plaques tested	% of plaques containing:			
			+ am6 am302	ts857 + +	+ am6 am302 and ts857 + +	+ + am302 and ts857 + +
1	*dcm*$^{+}$(p*dcm*$^{+}$)	150	33	26	27	14[b]
2	*dcm* (GM31)	200	40.5	36.5	22[c]	1

[a] The heteroduplex used distances (in base pairs) between mutations were as follows: ts857 and am6, 99; am6 and am302, 31.
Phage DNA was extracted from lambda grown on GM2290(p*dam*$^{+}$) and shown to be resistant to digestion by *Mbo*I.
[b] Includes one plaque containing only + + am302 phage.
[c] Includes four plaques containing both ts857 + + and + + + phage plus one plaque containing only wild-type (+ + +) phage.

VSP repair are not expected to contain 5meC, assuming that the *dcm* product methylates only the central cytosines in 5'CC(A/T)GG sequences. The *dcm* product must be capable of recognizing sequences other than 5'CC(A/T)GG irrespective of its ability to methylate cytosines in such sequences.

At present, too little is known to warrant extensive speculation, but it will certainly be of interest to determine whether cytosine methylase has a nuclease activity. Although Bhagwat et al. (2) reported "no significant restriction activity" associated with cells containing cloned *dcm*, a nicking activity has not been excluded. 5meC presumably performs some (as-yet-unknown) function in *E. coli*. The repair activity of cytosine methylase may have evolved to remove the thymines that arise as the result of the spontaneous deamination of 5meC. This repair function appears to be lacking in the cytosine methylase associated with *Eco*RII (unpublished observations). The gene for *Eco*RII, which has the same methylation specificity as *dcm* methylase (22), was recently cloned and sequenced (28).

meA-directed repair competes very successfully with VSP repair for mismatches in transfected heteroduplexes prepared from partially adenine-methylated phage grown in a *dam*$^+$ host (Table 3), experiment 1. In transfection experiments, VSP repair is not observed unless the heteroduplexes are fully adenine methylated or a *mutH* or *uvrD* mutation disables the meA-directed system of the host. The repair of mismatches at *P*am3 and *P*am80 "one at a time" after transfection of heteroduplexes into *mutH* or *mutU* (*uvrD*) strains (6) can be attributed to VSP repair.

On the other hand, the meA-directed system does not appear to repair a large proportion of mismatches in gene *c*I when phage are crossed in a *mut*$^+$ strain. The removal of long tracts of nucleotides would lead to the corepair of close markers, thus reducing the frequency of excess *c*I$^+$ recombinants arising by VSP repair. Such recombinants should therefore be more frequent when crosses are made in *mutH* or *uvrD* bacteria. However, the absence of MutH and UvrD function in the host did not increase significantly the frequency of recombinants in crosses between closely linked markers (Fig. 4). Mismatch repair of bacteriophage lambda heteroduplexes transfected into bacteria may not precisely reflect the repair events accompanying recombination and cytosine deamination in bacteria.

ACKNOWLEDGMENTS

I thank M. Marinus, J. H. Miller, and M. Radman for providing bacterial strains and A. S. Bhagwat for the gift of pDCMI.

This research was supported by Public Health Service grant GM 25034 from the National Institutes of Health and by grant DMB-85 16062 from the National Science Foundation.

LITERATURE CITED

1. **Bachmann, B. J.** 1972. Pedigrees of some mutant strains of *Escherichia coli* K-12. Bacteriol. Rev. **36**:525–557.
2. **Bhagwat, A. S., A. Sohail, and R. J. Roberts.** 1986. Cloning and characterization of the *dcm* locus of *Escherichia coli* K-12. J. Bacteriol. **166**:751–755.
3. **Coulondre, C., and J. H. Miller.** 1977. Genetic studies of the *lac* repressor. III. Additional correlation of mutational sites with specific amino acid residues. J. Mol. Biol. **117**:525–575.
4. **Dohet, C., R. Wagner, and M. Radman.** 1985. Repair of defined single base-pair mismatches in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **82**:503–505.
5. **Feinstein, S. I., and K. B. Low.** 1986. Hyper-recombining recipient strains in bacterial conjugation. Genetics **113**:13–33.
6. **Fox, M. S., and S. Raposa.** 1983. Mismatch repair. UCLA Symp. Mol. Cell. Biol. New Ser. **11**:333–335.
7. **Glickman, B. W., and M. Radman.** 1980. *Escherichia coli* mutants deficient in methylation-instructed DNA mismatch correction. Proc. Natl. Acad Sci. USA **77**:1063–1067.
8. **Hickson, I. D., H. M. Arthur, D. Bramhill, and P. T. Emmerson.** 1983. The *E. coli uvrD* gene product is DNA helicase II. Mol. Gen. Genet. **190**:265–270.
9. **Huisman, O., and M. S. Fox.** 1986. A genetic analysis of primary products of bacteriophage lambda recombination. Genetics **112**: 409–420.
10. **Jones, M., R. Wagner, and M. Radman.** 1987. Repair of a mismatch is influenced by the base composition of the surrounding nucleotide sequence. Genetics **115**:605–610.
11. **Jones, M., R. Wagner, and M. Radman.** 1987. Mismatch repair of deaminated 5-methylcytosine. J. Mol. Biol. **194**:155–159.
12. **Kramer, B., W. Kramer, and H.-J. Fritz.** 1984. Different base/base mismatches are corrected with different efficiencies by the methyl-directed DNA mismatch-repair system of *E. coli*. Cell **38**:879–887.
13. **Kushner, S. R., J. Shepherd, G. Edwards, and V. F. Maples.** 1978. *uvrD*, *uvrE* and *recL* represent a single gene, p. 251–254, *In* P. C. Hanawalt, E. C. Friedberg, and C. F. Fox (ed.), DNA repair mechanisms. Academic Press, Inc., New York.
14. **Lieb, M.** 1983. Specific mismatch correction in bacteriophage lambda crosses by very short patch repair. Mol. Gen. Genet. **191**:118–125.
15. **Lieb, M.** 1985. Recombination in the λ repressor gene: evidence that very short patch (VSP) mismatch correction restores a specific sequence. Mol. Gen. Genet. **199**:465–470.
16. **Lieb, M., E. Allen, and D. Read.** 1986. Very short patch mismatch repair in phage lambda: repair sites and length of repair tracts. Genetics **114**:1041–1060.
17. **Lu, A. L., S. Clark, and P. Modrich.** 1983. Methyl-directed repair of DNA base-pair mismatches *in vitro*. Proc. Natl. Acad. Sci. USA **80**:4639–4643.
18. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
19. **Marinus, M. G.** 1973. Location of DNA methylation genes on the *Escherchia coli* K-12 genetic map. Mol. Gen. Genet **127**: 47–55.
20. **Marinus, M. G.** 1984. Methylation of procaryotic DNA, p. 81–109. *In* A. Razin, M. Cedar, and A. D. Riggs (ed.), DNA methylation. Springer-Verlag, New York.
21. **Marinus, M. G., A. Potecte, and J. A. Arraj.** 1984. Correlation of DNA adenine methylase activity with spontaneous mutability in *Escherichia coli* K-12. Gene **28**:123–125.
22. **May, M. S., and S. Hattman.** 1975. Deoxyribonucleic acid-cytosine methylation by host- and plasmid-controlled enzymes. J. Bacteriol. **122**:129–138.
23. **Modrich, P.** 1987. DNA mismatch correction. Annu. Rev. Biochem. **56**:435–466.
24. **Nevers, P. P., and H.-C. Spatz.** 1975. *Escherichia coli* mutants *uvrD* and *uvrE* deficient in gene conversion of λ-heteroduplexes. Mol. Gen. Genet. **139**:233–243.
25. **Pukkila, P., J. Peterson, G. Herman, P. Modrich, and M. Meselson.** 1983. Effects of high levels of DNA adenine methylation on methyl-directed mismatch repair in *Escherichia coli*. Genetics **104**:571–582.
26. **Radman, M., and R. Wagner.** 1986. Mismatch repair in *Escherichia coli*. Annu. Rev. Genet. **20**:523–538.
27. **Rydberg, B.** 1978. Bromouracil mutagenesis and mismatch repair in mutator strains of *Escherichia coli*. Mutat. Res. **52**: 11–24.
28. **Som, S., A. S. Bhagwat, and S. Friedman.** 1986. Nucleotide sequence and expression of the gene encoding the *Eco*RII modification enzyme. Nucleic Acids Res. **15**:313–332.
29. **Su, S. S., and P. Modrich.** 1986. *Escherichia coli mutS*-encoded protein binds to mismatched DNA base pairs. Proc. Natl. Acad. Sci. USA **83**:5057–5061.
30. **Zell, R., and H.-J. Fritz.** 1987. DNA mismatch-repair in *Escherichia coli* counteracting the hydrolytic deamination of 5-methyl-cytosine residues. EMBO J. **6**:1809–1815.