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Mechanism and Control of Interspecies Recombination in *Escherichia coli*. I. Mismatch Repair, Methylation, Recombination and Replication Functions

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ABSTRACT

A genetic analysis of interspecies recombination in *Escherichia coli* between the linear Hfr DNA from *Salmonella typhimurium* and the circular recipient chromosome reveals some fundamental aspects of recombination between related DNA sequences. The MutS and MutL mismatch binding proteins edit (prevent) homeologous recombination between these 16% diverged genomes by at least two distinct mechanisms. One is MutH independent and presumably acts by aborting the initiated recombination through the UvrD helicase activity. The RecBCD nuclease might contribute to this editing step, presumably by preventing reiterated initiations of recombination at a given locus. The other editing mechanism is MutH dependent, requires unmethylated GATC sequences, and probably corresponds to an incomplete long-patch mismatch repair process that does not depend on UvrD helicase activity. Insignificant effects of the Dam methylation of parental DNAs suggest that unmethylated GATC sequences involved in the MutH-dependent editing are newly synthesized in the course of recombination. This hypothetical, recombination-associated DNA synthesis involves PriA and RecF functions, which, therefore, determine the extent of MutH effect on interspecies recombination. Sequence divergence of recombining DNAs appears to limit the frequency, length, and stability of early heteroduplex intermediates, which can be stabilized, and the recombinants mature via the initiation of DNA replication.

HOMOLOGOUS genetic recombination is required for DNA repair and for meiotic crossovers involved in chromosome disjunction (Kucherlapati and Smith 1988). However, crossovers between interspersed repeated sequences cause deleterious chromosomal rearrangements (for review see Radman 1991). A balance between the positive and negative effects of homologous recombination is kept by cellular mechanisms that control its frequency and fidelity. The key role of homologous recombination in the repair of DNA probably sets the limits to the extent of negative control of recombination. For example, a cellular Rec$^-$ phenotype could be favored to avoid chromosomal rearrangements, but it would lead to a great disadvantage because of the deficiency in DNA repair. The solution to this particular problem appears to be provided by the high fidelity of homologous recombination (*i.e.*, its strict requirement for sequence identity): DNA repair can proceed by unrestricted precise recombination between the identical sister chromatids, whereas the nonidentity of repeated sequences prevents their recombination (Rayssiguier *et al.* 1989; Petit *et al.* 1991; Radman 1991; Abdulkarim and Hughes 1996).

Thus, homologous genetic recombination is largely controlled at the DNA substrate level by the degree and length of sequence identity shared by the two recombining DNAs (for review see Radman 1991). The decrease in DNA homology through sequence divergence is much more efficient in preventing recombination than is the decrease in the length of homology (Shen and Huang 1986). Even a low divergence, *e.g.*, 1% or less, can severely inhibit homologous recombination in bacteria, yeast, and mammalian cells (de Wind *et al.* 1995; Datta *et al.* 1996, 1997; Vulic *et al.* 1997; Zahrt and Maloy 1997). This high fidelity of genetic recombination is caused not only by the intrinsic properties of recombination enzymes, but also by the editing of recombination by the mismatch repair system, the same system that controls also the fidelity of DNA replication through the correction of base pair mismatches caused by replication errors (for review see Radman and Wagner 1993a; Modrich and Lahue 1996).

Deficiency in any of the four mismatch repair proteins, MutS, MutL, MutH, and UvrD (MutU), has equal effect on the correction of DNA replication errors, reflecting the requirement of all four proteins in the successful error correction process (for review see Radman and Wagner 1986). This is generally not the case for the editing of homologous DNA recombination, where the effect of specific *mut* gene mutations depends on the nature of recombination substrates and events (Petit *et al.* 1991; Abdulkarim and Hughes 1996). Interspecies recombination between Salmonella and Escherichia in

M. Radman dedicates this paper to Jan Drake, whose editorial policy and sharp editorial pencil rejuvenated Genetics.

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conjugational and transductional crosses is increased ∼1000-fold by *mutS* and/or *mutL* mutations, ∼20-fold by *mutH*, and only about fivefold by a *mutU* mutation (Rayssiguier *et al.* 1989; see Table 2).

We have been studying gene exchange between two related species, *Escherichia coli* and *Salmonella typhimurium*, mediated by homologous recombination enzymes as a model system for the definition of the genetic barriers at the molecular level (Rayssiguier *et al.* 1989; Matic *et al.* 1995; Vulic *et al.* 1997). The principal genetic barrier among enterobacteria is the recombinational barrier, whose structural element is the genomic sequence divergence and whose enzymatic element is the mismatch repair system. So far, the basic molecular rules for this interspecies recombination appear to apply also to other homologous recombination systems involving similar sequences (homeologous recombination) in bacteria, yeast, and mammals (Radman and Wagner 1993a; de Wind *et al.* 1995; Datta *et al.* 1996, 1997; Vulic *et al.* 1997). Although the editing of homeologous strand exchange by the MutS and MutL proteins has been reproduced *in vitro* (Worth *et al.* 1994), the detailed mechanism of the editing of recombination by the mismatch repair system remains obscure.

This is a study of the roles of DNA methylation and MutS/L *vs.* MutH functions in recombinational editing. By studying the fidelity of interspecies recombination, we found that (i) this recombination often involves DNA synthesis initiated by a pairing between 16% (on average) diverged parental sequences and that requires the PriA primosome function and some RecF functions and (ii) editing of recombination by mismatch repair proteins occurs by two mechanisms: one is MutH independent and the other is MutH dependent.

MATERIALS AND METHODS

Bacterial strains and plasmids: The bacterial strains used are listed in Table 1. Figure 1 shows the position and direction of the transfer of the Hfr strains used in this study. SA965, SA977, SA534, and SU573 are *S. typhimurium* Hfr, while PK3 is *E. coli* Hfr. Plasmid pDDM6, which carries the gene coding for the Dam methylase enzyme (Guha and Guschlbauer 1992) under the control of the tac promoter, was propagated in mut^+ and *mutH* (AB1157) strains in the presence of 3 mm IPTG to induce Dam methylase.

Conjugational crosses: Donor and recipient strains were grown in rich medium or minimal medium where indicated. M63 minimal medium contained glucose (0.4%), histidine, leucine, proline, arginine, threonine (100 μg/ml), and thiamine (30 μg/ml). Log-phase bacteria were grown to 2–4 × 10^8 cells/ml, mixed in a 1:1 Hfr:F^- ratio, and filtered through a 0.45-μm pore size filter (Schleicher & Schuell, Keene, NH) that was incubated on prewarmed, rich medium agar for 40 or 60 min. Filters were resuspended in 10^{-2} m $MgSO_4$, and bacteria were separated by vortexing. Bacteria were plated on M63 minimal medium supplemented with histidine, leucine, proline, and threonine (100 μg/ml), thiamine (30 μg/ml), glucose (0.4%), and nalidixic acid (40 μg/ml) to counterselect donor cells, but without arginine to select for transconjugants in crosses involving SA965 and SA977 donors. Resuspension of filters on which crosses with SU573 and SA534 donors were plated on similar plates, but to select for leucine prototrophy. In interspecies crosses where the recipients used were *S. typhimurium* and donor *E. coli* PK3, selection was on M63 minimal medium containing glucose (0.4%), thiamine (30 μg/ml), isoleucine, and valine (100 μg/ml), and Streptomycin (100 μg/ml) to counterselect donor cells. Colonies were counted after 4 days or more if scoring for the frequency of recombinants of *priA* cells was performed. In an intraspecies cross where the donor used was PK3, selection was performed for arginine marker.

To minimize the appearance of suppressor mutations because of the mutator effect of *mutS* and *mutH* recipients used, the *priA* and *recF* strains were grown in M63 minimal medium containing glucose (0.4%, McPhee 1993), histidine, leucine, proline, arginine, threonine (100 μg/ml), and thiamine (30 μg/ml) until their midexponential phase of regrowth, and were then agitated for 3 hr in rich medium before conjugation. Recombination frequency is expressed as the number of recombinants per Hfr donor. Values represent the means and standard deviations of the recombination frequencies obtained in independent crosses.

RESULTS AND DISCUSSION

MutS *vs.* MutH effects on interspecies recombination: Presumably because of their mismatch recognition and binding activities (for review see Modrich and Lahue 1996), the MutS and MutL functions appear indispensable for all modes of the editing of DNA recombination and replication (this paper and unpublished results from our laboratory). The roles of MutH and UvrD in the editing of recombination are poorly understood. The key role of MutH in the mismatch repair process is to produce a single-strand scission (nick) 5′ to an unmethylated GATC sequence. This MutH activity is somehow stimulated by the binding of MutS protein to a nearby mismatch (mispaired or unpaired bases in duplex DNA) followed by binding of MutL. The *uvrD* gene encodes DNA helicase II, whose role in mismatch repair is supposedly to peel off the nicked strand in the direction of the mismatch and expose the free single strand for destruction by single-strand-specific nucleases (for review see Modrich and Lahue 1996).

To determine the epistatic relationships of such mutations, we have studied the effect of a series of single and double mutations that affect mismatch repair and/or recombination machinery. Such analysis is useful for pathway assignments of genes that control the fidelity of genetic recombination. A large number of mutations studied makes the epistasis analysis notoriously difficult. Therefore, we chose to express the effects of *mutS* and *mutH* mutations in different mutational backgrounds (Table 2). Because the MutS function is involved in all whereas MutH is involved in only some aspects of editing of recombination by mismatch repair, the ratio of MutS to MutH effects shows the extent of MutH-independent editing (Table 2).

The ratio of the MutS to MutH effects is significantly

TABLE 1

***E. coli* and *S. typhimurium* strains and plasmids used in this study**

Strain or plasmid	Relevant genotype	Source of strain or allele
FR186	*mutH471*::Tn*5*	Pang *et al.* (1985)
FR188	*mutS215*::Tn*10*	G. Walker's collection
FR189	*uvrD260*::Tn*5*	Pang *et al.* (1985)
MG1655	*recD*::Tn*10*	D. Touati's collection
AQ8845	*priA2::kan*	Masai *et al.* (1994)
JC10990	*recF*::Tn*3*	A. J. Clark's collection
ES548	*mutH thy*$^+$	Vaccaro and Siegel (1977)
GC2277	*sfiAII pyrD*:: Tn*5*	R. D'ari's collection
GY4375	*dam13*::Tn*9*	Marinus *et al.* (1983)
TT11691[a]	*dam-101*::Tn*10*	K. E. Sanderson's collection
FR146[a, c]	*mut*$^+$ *dam*$^+$	K. E. Sanderson's collection
FR147[a, c]	*mutH 101*::Tn*5*	Rayssiguier *et al.* (1989)
FR826[a, c]	*dam-101*::Tn*10*	This study
FR827[a, c]	*mutH101*::Tn*5 dam-101*::Tn*10*	This study
FR266[b]	*recA*$^+$ *mut*$^+$	Matic *et al.* (1995)
FR828[b]	*mutH471*::Tn*5*	This study
FR559[b]	*mutS201*::Tn*5*	Matic *et al.* (1995)
FR829[b]	*uvrD260*::Tn*5*	This study
FR830[b]	*uvrD260*::Tn*5 mutH thy*$^+$	This study
FR831[b]	*uvrD260*::Tn*5 mutS215*::Tn*10*	This study
FR483[b]	*recD1009*	Matic *et al.* (1995)
FR832[b]	*recD*::Tn*10 mutH thy*$^+$	This study
FR470[b]	*recD1009 mutS215*::Tn*10*	Matic *et al.* (1995)
FR833[b]	Δ*recG263* Km^R	Matic *et al.* (1995)
FR834[b]	Δ*recG263* Km^R *mutH thy*$^+$	This study
FR835[b]	Δ*recG263* Km^R *mutS215*::Tn*10*	This study
FR836[b]	*ruvA60*::Tn*10*	Matic *et al.* (1995)
FR837[b]	*ruvA60*::Tn*10 mutH471*::Tn*5*	This study
FR838[b]	*ruvA60*::Tn*10 mutS201*::Tn*5*	Matic *et al.* (1995)
FR358[b]	*lexA1 malB*::Tn*9*	Matic *et al.* (1995)
FR839[b]	*lexA1 malB*::Tn*9 mutH471*::Tn*5*	This study
FR840[b]	*lexA1 malB*::Tn*9 mutS201*::Tn*5*	Matic *et al.* (1995)
FR841[b]	*recF*::Tn*3*	This study
FR842[b]	*recF*::Tn*3 mutH471*::Tn*5*	This study
FR843[b]	*recF*::Tn*3 mutS215*::Tn*10*	This study
FR844[b]	*priA2::kan sfiAII*	This study
FR845[b]	*priA2::kan mutH thy*$^+$ *sfiAII*	This study
SA977[a]	*leuBCD39 ara7* P22^R Rif^R	K. E. Sanderson's collection
SA965[a]	*leuBCD39 ara7* Rif^R	K. E. Sanderson's collection
SU573[a]	*purC7*	K. E. Sanderson's collection
SA534[a]	*serA13 rfa-3058*	K. E. Sanderson's collection
PK3	*lacY thi- leuB6 azi 15 tonA21 supE44* Rif^R	Kahn (1968)
PK3 *dam*	*lacY thi- leuB6 azi15 tonA21 supE44 dam13*:: Tn*9*	This study
pDDM6	Dam methylase	Guha and Guschlbauer (1992)

All constructions were done by P1 transduction.
[a] *Salmonella typhimurium* LT2 strains.
[b] AB1157 Nal^R (*thr-1 leuB6 proA2 his4 thi-1 argE3 lacY1 galK2 ara14 xy15 mtl-1 tsx-33 str31 supE44*).
[c] SL4213 [*galE496 metA22 metE551 rpsl120 xyl-404* (Fels2) *H1b rn*ml H2-enx ilv-452 hsdSA29 hsdL6].

decreased in *uvrD*, *recD*, *recG*, and *recF* mutants. *LexA1* (a dominant mutation preventing the induction of SOS response), *uvrD*, *recD*, *recG*, and *recF* show a pronounced decrease in the MutS effect, whereas *priA*, *recF*, and *lexA1* also show a decreased effect of MutH. The *mutH* effect on interspecies recombination is increased from ∼20-fold in the wild type to ∼120-fold in *recD*, 50-fold in *uvrD*, and 45-fold in *recG* (Table 2). Because the interspecies recombination frequencies are similar in the *lexA1*, *recF*, and *priA* mutants, it may be that the effects of RecF and PriA functions and/or of some components active in the same pathway are inducible because of the SOS response that is activated in *E. coli* during the course of conjugation with Salmonella (Matic *et al.* 1995). The significance of these findings will be discussed below.

MutH-dependent editing of interspecies recombina-

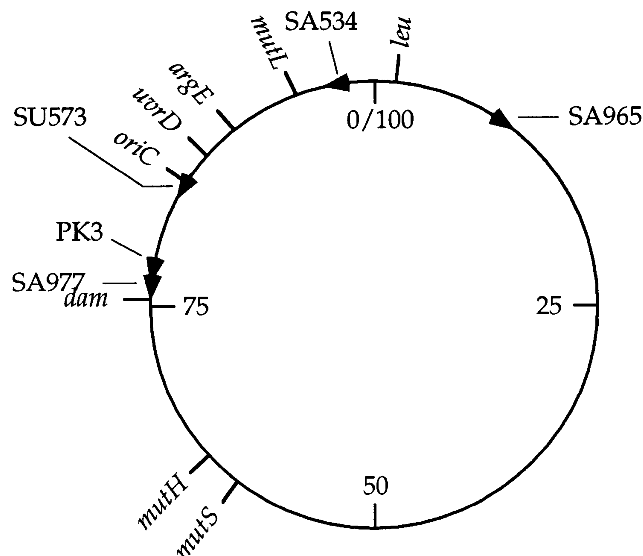

Figure 1.—The schematic representation of the position and direction of transfer of Hfr donors used in this study (see materials and methods). *E. coli* Hfr strain is PK3, while *S. typhimurium* Hfr strains are SA977, SA965, SA534, and SU573.

tion requires unmethylated GATC sequences: Methylation of the adenine moiety in the GATC sequence to 6-methyl-adenine by the Dam methylase prevents the MutH activity and, thus, prevents mismatch repair (for review see Modrich and Lahue 1996). However, internal free ends, such as nicks, can substitute for both the GATC sequence and MutH function (Lahue *et al.* 1987; Längle-Roualt *et al.* 1987).

Table 3 shows that the overexpression of Dam methylase (in the F^- cells) during the course of *S. typhimurium* Hfr × *E. coli* F^- conjugational crosses increases interspecies recombination to the same extent as the *mutH* mutation. Furthermore, Dam overexpression has no effect on recombination in a *mutH* mutant (Table 3). This result suggests that the MutH-dependent editing of recombination requires unmethylated GATC sequences in one or both strands of the DNA heteroduplex, just as in the case of standard mismatch repair.

What is the origin of unmethylated GATC sequences in recombination: parental Hfr DNA, parental newly synthesized F^- DNA, or some particular DNA synthesis associated with the recombination process?

Methylation of parental DNAs does not determine the MutH effect on interspecies recombination: To explore the effect of Dam methylation of parental DNAs on the MutH-dependent editing of recombination, the following interspecies crosses between *E. coli* Hfr and *S. typhimurium* F^- were carried out: Hfr (Dam^+) × F^- (Dam^+), Hfr (Dam^+) × F^- (Dam^-), Hfr (Dam^-) × F^- (Dam^+), and Hfr (Dam^-) × F^- (Dam^-). Note that the crosses in Table 4 are exceptions in this article in that *E. coli* is the Hfr. These crosses cannot be compared with those in the article by Rayssiguier *et al.* (1989), which were performed too long ago. The results in Table 4 show that the effect of MutH on interspecies recombination is not affected by the methylation status of parental DNAs in a way that could be expected from the known roles of the MutH protein and unmethylated GATC sequences. For example, in the interspecies cross between *S. typhimurium* F^- (Dam^-) and *E. coli*, Hfr (Dam^+ or Dam^-), one would expect the highest effect of MutH-dependent rejection of the Hfr DNA, *i.e.*, the highest ratio of recombination in *mutH* compared with *mutH*$^+$ crosses. In the total absence of Dam methylation, even the inactivation of mismatched intermediates by the MutS, L, and H activities can be expected (Doutriaux *et al.* 1986). However, this was not found: when both parents are Dam^+, the MutH effect on recombination is 10-fold (line 1 *vs.* line 3), as compared to 13-fold when only the Hfr DNA is unmethylated (line 5 *vs.* line 7); when both DNAs are unmethylated, the MutH effect is also 10-fold (line 6 *vs.* line 8). Note that independently of the donor's DNA methylation, recombination frequency is about five times higher in *dam* recipients, presumably because of their constitutive expression of SOS response (Peterson and Mount 1993).

***PriA*, *recF*, and *lexA1* mutations affect MutH-dependent editing of interspecies recombination:** Because the MutH-dependent editing of recombination intermediates requires unmethylated GATC sequences (Table 3), which are probably not carried by the parental DNAs (Table 4), it may be that a DNA synthesis mechanistically associated with genetic recombination produces such sequences. The functions of two proteins are supposed to be involved in both DNA recombination and replication: the primosome assembly protein PriA, which is apparently required for replication dependent recombination (Kogoma *et al.* 1996; Sandler *et al.* 1996), and the RecF recombination protein, which was claimed to be required for resumption of replication forks disrupted by radiation damage to DNA (Courcelle *et al.* 1997). PriA protein is hypothesized to recognize sites for loading of DnaB helicase onto the single-stranded DNA within a D loop structure produced by the invading DNA strand and, in that way, permit the primosome assembly system to initiate replication from the invading DNA strand (Kogoma 1996; McGlynn *et al.* 1997).

If the MutH editing step of recombination requires *de novo* DNA synthesis associated with recombination, and if such DNA synthesis requires PriA and/or RecF functions, then the MutH effect on interspecies recombination should be diminished in *priA* and *recF* mutants. This was indeed observed (Table 2, lines 19–28): the *mutH* mutation increased interspecies recombination only twofold in a *priA* mutant (compared to the 11-fold increase in the corresponding wild-type background). Because the MutH effect is also similar in an intraspecies *E. coli priA* cross (twofold; data not shown), it appears that most recombination-associated DNA synthesis re-

TABLE 2

Interspecies (*S. typhimurium* Hfr × *E. coli* F) recombination in different recombination and/or mismatch repair-deficient mutants

	E. coli F^- × *S. typhimurium* Hfr				
Recipient	Recombination frequency	Experiment no.	*mutH* effect	*mutS* effect	Ratio of *mutS* to *mutH* effects
	Arg^+/SA977 Hfr				
1. *mut*$^+$ *rec*$^+$	$6.8 \times 10^{-6} \pm 1.5 \times 10^{-6}$	11			
2. *mutH*	$1.5 \times 10^{-4} \pm 0.4 \times 10^{-4}$	5	22		
3. *mutS*	$5.0 \times 10^{-3} \pm 2.2 \times 10^{-3}$	5		735	33
4. *uvrD*	$3.1 \times 10^{-5} \pm 0.6 \times 10^{-5}$	3			
5. *uvrD mutH*	$1.6 \times 10^{-3} \pm 0.6 \times 10^{-3}$	5	52		
6. *uvrD mutS*	$7.6 \times 10^{-3} \pm 3.6 \times 10^{-3}$	4		245	5
7. *recD*	$3.1 \times 10^{-5} \pm 1.3 \times 10^{-5}$	5			
8. *recD mutH*	$3.7 \times 10^{-3} \pm 1.8 \times 10^{-3}$	4	119		
9. *recD mutS*	$7.1 \times 10^{-3} \pm 2.6 \times 10^{-3}$	3		229	2
10. *recG*	$3.1 \times 10^{-6} \pm 1.5 \times 10^{-6}$	6			
11. *recG mutH*	$1.4 \times 10^{-4} \pm 0.4 \times 10^{-4}$	3	45		
12. *recG mutS*	$3.3 \times 10^{-4} \pm 2.3 \times 10^{-4}$	10		106	2
13. *ruvA*	$6.3 \times 10^{-7} \pm 0.3 \times 10^{-7}$	3			
14. *ruvA mutH*	$1.3 \times 10^{-5} \pm 0.9 \times 10^{-5}$	7	21		
15. *ruvA mutS*	$5.7 \times 10^{-4} \pm 3.7 \times 10^{-4}$	3		905	44
16. *lexA1*	$3.8 \times 10^{-7} \pm 0.3 \times 10^{-7}$	5			
17. *lexA1 mutH*	$4.0 \times 10^{-6} \pm 0.9 \times 10^{-6}$	7	11		
18. *lexA1 mutS*	$4.2 \times 10^{-5} \pm 1.0 \times 10^{-5}$	4		111	11
	Arg^+/SA965 Hfr				
19. *mut*$^+$ *rec*$^+$	$3.3 \times 10^{-6} \pm 1.0 \times 10^{-6}$	3			
20. *mutH*	$7.9 \times 10^{-5} \pm 1.1 \times 10^{-5}$	4	24		
21. *mutS*	$1.6 \times 10^{-3} \pm 0.06 \times 10^{-3}$	3		485	20
22. *recF*	$5.7 \times 10^{-7} \pm 0.06 \times 10^{-7}$	3			
23. *recF mutH*	$5.1 \times 10^{-6} \pm 2.7 \times 10^{-6}$	6	9		
24. *recF mutS*	$2.9 \times 10^{-5} \pm 0.2 \times 10^{-5}$	4		51	6
	Leu^+/SU573 Hfr				
25. *mut*$^+$ *rec*$^+$	$1.2 \times 10^{-6} \pm 0.3 \times 10^{-6}$	3			
26. *mutH*	$1.3 \times 10^{-5} \pm 0.3 \times 10^{-5}$	3	11		
27. *priA*	$3.9 \times 10^{-7} \pm 2.5 \times 10^{-7}$	4			
28. *priA mutH*	$8.8 \times 10^{-7} \pm 5.5 \times 10^{-7}$	4	2		

Conjugational crosses are described in materials and methods. The results are the median values of at least three independent experiments. The analysis is focused on the relative effects of *mutH* and *mutS* mutations (see text). *Mut*$^+$ *rec*$^+$ strain is *E. coli* K-12; AB1157. Conjugation time was 40 min for crosses involving SA977 and 60 min for SA573 and SA965 donors.

quired for editing by MutH depends on PriA activity. The effect of RecF on editing by MutH protein is less pronounced (MutH effect is still ninefold). Perhaps by coincidence, it is quantitatively closer to the effects of *lexA1* (11-fold).

Table 2 shows that a *priA* null mutation and a *recF* mutation inhibit interspecies recombination by ~15-fold in *mutH* background, while having a twofold and negligible effect, respectively, on intraspecies recombination (data not shown). Thus, PriA and RecF functions are more limiting for interspecies than intraspecies recombination in an otherwise wild-type genetic background. This effect is more pronounced in MutH-deficient bacteria, presumably because of the efficient MutH-dependent prevention of interspecies recombination involving DNA synthesis. The significance of this finding and the observation that the *lexA1* and *ruvA* mutations also decrease interspecies more than intraspecies recombination (Table 2; Matic *et al.* 1995; data not shown) are discussed below.

The difference between these results and those of a previous study on the implication of the *recF* gene product in interspecies recombination (Matic *et al.* 1995) derives from the difference in leakiness of the two *recF* alleles used. It has been reported that the *recF143* substitution mutation used in the previous study, among its other leaky phenotypes tested, exerts an effect in conjugational recombination in *recBCsbcBC* recipients 10 times weaker than that of the *recF349* deletion allele used in this study (Sandler and Clark 1993).

TABLE 3

The effects of *mutH* mutation and of Dam methylase overexpression on interspecies recombination between *S. typhimurium* Hfr and *E. coli* F^-

E. coli F^- × *S. typhimurium* Hfr		
Recipient	Recombination frequency	Experiment no.
	Arg^+/SA977 Hfr	
mut$^+$ *rec*$^+$	$7.5 \pm 0.4 \times 10^{-6}$	4
mutH	$1.5 \pm 0.4 \times 10^{-4}$	5
mut$^+$ pDam	$1.2 \pm 0.1 \times 10^{-4}$	3
mutH pDam	$1.6 \pm 1.2 \times 10^{-4}$	5
	Leu^+/SA534 Hfr	
mut$^+$ *rec*$^+$	$3.9 \pm 1.9 \times 10^{-7}$	3
mutH	$1.1 \pm 0.1 \times 10^{-5}$	3
mut$^+$ pDam	$1.3 \pm 0.1 \times 10^{-5}$	2
mutH pDam	$1.2 \pm 0.1 \times 10^{-5}$	3

The *mut*$^+$ *rec*$^+$ strain is *E. coli* K-12; AB1157. Conjugation time was 40 min.

A model for the editing of interspecies recombination: These results can be organized within the frame of a model involving two mechanisms for editing of interspecies recombination in *E. coli*: one is MutH independent and the other is MutH dependent (Figure 2). These two mechanisms could act in parallel as two alternative mechanisms: one is DNA end dependent but MutH independent, and the other is dependent on MutH, unmethylated GATC sequences, and *de novo* DNA synthesis. However, because of the requirement of DNA strand transfer for *de novo* DNA synthesis, we hypothesize that the MutH-independent editing is an early-stage editing, whereas the MutH-dependent process is a late-stage editing. The key features of the model are as follows:

1. The recombination between diverged DNAs is limited by the number of minimum efficient processing segments (MEPS), *i.e.*, minimal blocks of sequence identity that are sufficient for the efficient RecA-catalyzed initiation of the DNA strand transfer or exchange process (Shen and Huang 1986; Vulic *et al.* 1997). The outcome of the initiated strand invasion process (including its reversibility, Rayssiguier *et al.* 1989) will depend on several recombination and repair proteins acting on intermediates of genetic recombination.
2. Because the sequence divergence between the recombining DNAs limits the length and/or stability of the initial heteroduplex intermediate, the interspecies recombination becomes more dependent on recombination-associated DNA replication than the isogenic intraspecies recombination. The PriA- and RecF-dependent *de novo* initiation of DNA synthesis upon the early heteroduplex intermediates appears to prevent the dissociation of such unstable recombination initiation events, particularly when MutH is not active (Table 2; Figure 2e). Recombination-associated DNA synthesis has been postulated for some systems of homeologous recombination in yeast (Porter *et al.* 1996; Tran *et al.* 1997).
3. Direct maturation of the recombination process can be provided by branch migration of the Holliday junction away from the invading end into the domain of diverged parental sequences (Figure 2d). About a 10-fold decrease of interspecies recombination by the *ruvA* mutation (Table 2, lines 13–15) suggests that RuvAB provides the branch migration function (Matic *et al.* 1995). This activity may be SOS inducible (Shurvinton and Lloyd 1982; see the effect of the *lexA1* mutation in Table 2, lines 16–18) because interspecies conjugation induces SOS response (Matic *et al.* 1995).
4. MutH-independent or early-stage editing of interspecies recombination corresponds to the dissociation of the earliest RecA-catalyzed heteroduplex intermediate (Figure 2c) because of mismatch formation. This editing requires the key mismatch recognition and repair functions, MutS and MutL, as well as the UvrD (helicase II) function. Helicase II can become dispensable at very high concentrations of the MutS and MutL proteins (S. Štambuk and M. Radman, unpublished results). This mechanism was probably reproduced *in vitro* in the study by Worth *et al.* (1994). The effect of the *recG* mutation on interspecies recombination in the *mutS* mutant (Table 2, lines 10–12) suggests that the RecG protein may be recombinogenic only in the *mutS* mutant (compare lines 3 and 12 in Table 2) or that the MutS-mediated,

TABLE 4

Interspecies recombination (*E. coli* Hfr × *S. typhimurium* F^-) and the *mutH* mutation effect in crosses involving parents with different Dam methylation status (see text for explanations)

S. typhimurium F^- × *E. coli* Hfr		
Recipient	Recombination frequency	Experiment no.
	Met^+/PK3 Hfr	
1. *mut*$^+$ *rec*$^+$	$4.5 \pm 1.1 \times 10^{-6}$	2
2. *dam*	$2.5 \pm 0.3 \times 10^{-5}$	2
3. *mutH*	$4.7 \pm 1.1 \times 10^{-5}$	3
4. *dam mutH*	$1.1 \pm 0.4 \times 10^{-4}$	3
	Met^+/PK3*dam* Hfr	
5. *mut*$^+$ *rec*$^+$	$3.8 \pm 0.4 \times 10^{-6}$	2
6. *dam*	$2.7 \pm 0.6 \times 10^{-5}$	2
7. *mutH*	$5.0 \pm 3.6 \times 10^{-5}$	3
8. *dam mutH*	$2.8 \pm 2.4 \times 10^{-4}$	3

The *mut*$^+$ *rec*$^+$ strain is *S. typhimurium* LT2; SL4213. Conjugation time was 40 min.

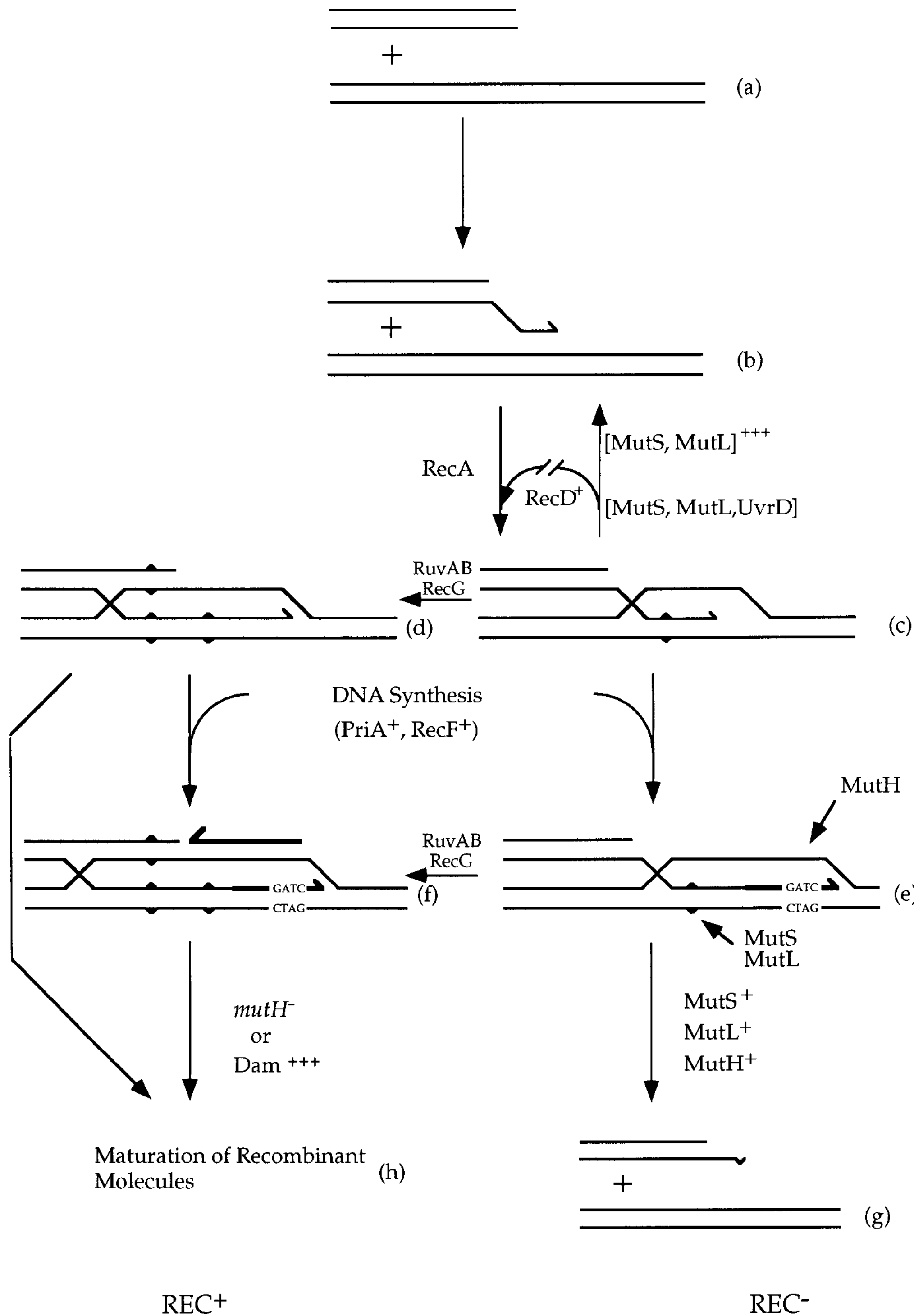

Figure 2.—(a–g) A model for two mechanisms of editing of homologous recombination by the mismatch repair proteins. The MutH-independent (early-stage) editing of homologous recombination during the course of bacterial conjugation is directly related to the degree and pattern of sequence divergence between the two recombining DNAs. This editing (c–b) occurs before extensive branch migration and/or recombination-initiated DNA replication. The MutH-dependent (late-stage) editing (e to g) occurs after the initiation of DNA synthesis. Both mechanisms of editing by the mismatch repair components require MutS and MutL proteins: the early-stage editing requires specifically UvrD helicase and RecBCD nuclease, and the late-stage editing requires specifically the MutH protein (see text for details). Newly synthesized DNA strands are presented by thick lines, and mismatches are represented by triangular bumps in the duplex DNA. REC^+ and REC^- refer to high and low recombination frequencies, respectively. (+++) Overexpression of MutS, MutL, and Dam proteins.

MutH-independent editing is ~10 times less efficient in the *recG* mutant (Table 2, line 12). Curiously, a functional interaction was postulated between a RecG homolog of *S. pneumoniae* and some undefined mismatch repair function (Martin *et al.* 1996).

This (early) stage of editing presumably occurs before the initiation of DNA synthesis (and/or RuvAB-catalyzed branch migration) and does not involve the MutH function (Table 2; Figure 2; Worth *et al.* 1994). The RecA protein could use the aborted single-stranded end to reinitiate the strand invasion process that leads eventually to the repeated (and therefore more successful) attempts to initiate recombination in the rare regions of sufficient sequence identity (MEPS, see Shen and Huang 1986). Inspired by the results of Zahrt and Maloy (1997), F. W. Stahl suggested to us that the RecBCD nuclease activity renders this early editing event at a given

site irreversible by the breakdown of the dissociated single-stranded end (see below). (Note that unlike events in isogenic recombination, the destruction of a donor MEPS sequence condemns the RecABCD system to search for the next MEPS within the double-stranded Hfr DNA, which, at 16% divergence from the recipient chromosome, may be many kilobases away from the Hfr DNA end). Consequently, in a *recD* null mutant, the repeated strand invasions would decrease the global effect of MutS on recombination as observed (Table 2, lines 1–3 *vs.* 7–9). In this scheme, the RecBCD nuclease is required to improve the efficiency of the early-stage editing. Therefore, the frequency and stability of the intermediate (c) should increase in the *recD* mutant, and the impact of the late-stage editing (the MutH effect) is expected to increase, which was found to be the case (see the next paragraph). The role of RecBCD nuclease in early-stage editing is also reflected in the observation that the contribution of this mechanism is not significant in the RecF and RecE recombination pathways (S. Štambuk and M. Radman, unpublished results).

5. Late-stage editing is defined operationally as the MutH-dependent editing that appears to act on the DNA strand that contains a mismatch in the heteroduplex region derived from the parental sequences and an unmethylated GATC sequence in the newly synthetized extension of the same strand (Figure 2e). The results in Tables 2–4 support this conclusion. Curiously, UvrD (helicase II) function is not required for this editing step. In fact, the increase of interspecies recombination caused by the *mutH* mutation is even higher in the *uvrD* mutant (52-fold) than in the wild-type strain (22-fold; Table 2, lines 4–6). This difference may result from the higher incidence of the late-stage intermediates caused by the inefficient early-stage editing in the *uvrD* mutant and, hence, more impact of the MutH-dependent editing. [The same holds for the *recD* and *recG* mutants (Table 2, lines 7–12).] Alternatively, another helicase may replace the missing helicase II and be even more efficient in this late-stage editing step. This editing step resembles an incomplete mismatch repair process that leads to the separation of the two parental molecules (Figure 2g) involved in the attempted recombination event that has already initiated DNA synthesis. We postulate that the unwinding/excision tract extends from the unmethylated GATC to the Holliday junction, thus interrupting the recombination process by separating the two parental DNA molecules (Figure 2, e–g).
6. Coincident branch migration and DNA replication (Figure 2f) should lead to the productive one-end recombination event associated with an unscheduled replication fork. Equal and nonadditive effects of the *mutH* mutation and of the Dam methylase overexpression (Tables 3) are well accounted for by this model (Figure 2h).

The lack of a coherent effect of Dam methylation of parental DNAs on MutH- (and unmethylated GATC-) dependent editing of recombination (Table 4) leads to the prediction that the functional interaction between the mismatch recognition by MutS/L and the GATC recognition by MutH cannot cross the Holliday junction (see Figure 2 and imagine a GATC sequence to the left of the Holliday junction). If it could, then the MutH effect should be at least as efficient in the case of unmethylated parental GATCs as in the case of newly synthesized GATCs because the mismatch repair process should necessarily destroy the three-stranded junction in the former case. This implies that for efficient MutS, L, and H activity, the mismatch and the GATC sequence must be strictly "in *cis*" on the same strand of a heteroduplex DNA. Experiments with structural barriers between the mismatch and the GATC sequence should test this prediction.

Comparison with homeologous recombination in other systems: The conspicuous resemblance in the genetic requirements between the interspecies recombination and the adaptive frameshift mutagenesis in *E. coli* (Foster 1998; Rosenberg *et al.* 1998) may be more than accidental. The key intermediates in the adaptive mutagenesis proposed by Rosenberg and colleagues (Harris *et al.* 1996) are identical to the intermediates in Figure 2. Could it be that the privileged sites for adaptive mutations are created by recombination-associated DNA synthesis? Such a process seems to occur in yeast and involves the *REV3* DNA polymerase (Strathern *et al.* 1995; Holbeck and Strathern 1997).

In yeast, meiotic interspecies (*S. cerevisiae* × *S. paradoxus*) recombination (Hunter *et al.* 1996) and several mitotic recombination systems showed effects of null mutations in gene homologs of bacterial *mutS* and *mutL* genes (Alani *et al.* 1994; Selva *et al.* 1995; Datta *et al.* 1996, 1997) similar to those in bacterial crosses. However, in some yeast homeologous recombination systems associated with transformation, the effects of mismatch repair mutations appear to range from weak to nonexistent (Mezard *et al.* 1992; Priebe *et al.* 1994; Porter *et al.* 1996).

Gene replacement by homologous recombination between a linear donor DNA fragment and the chromosome in the mouse embryonic stem cells is highly sensitive to the natural sequence divergence (0.6%), and this effect can be totally accounted for by the activity involving a *mutS* homolog gene (MSH2, de Wind *et al.* 1995). This result suggests that the practice of gene therapy by gene replacement will have to take into account the effects of DNA sequence divergence (polymorphism) that differentiate donor and recipient DNAs and those of mismatch repair systems. Last but not least, facilitated recombination between genes and/or entire

genomes of related species may provide a tool for fast experimental evolution of new genes/proteins and new metabolic pathways (for review see Radman and Wagner 1993b).

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LITERATURE CITED

Abdulkarim, F., and D. Hughes, 1996 Homologous recombination between the *tuf* genes of *Salmonella typhimurium.* J. Mol. Biol. **260:** 506–522.

Alani, E., R. Reenan and R. Kolodner, 1994 Interaction between mismatch repair and genetic recombination in *Saccharomyces cerevisiae.* Genetics **137:** 19–39.

Courcelle, J., C. Carswell-Crumpton and P. Hanawalt, 1997 *recF* and *recR* are required for the resumption of replication at DNA replication forks in *Escherichia coli.* Proc. Natl. Acad. Sci. USA **94:** 3714–3719.

Datta, A., A. Adjiri, L. New, G. F. Crouse and S. Jinks-Robertson, 1996 Mitotic crossovers between diverged sequences are regulated by mismatch repair proteins in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **16:** 1085–1093.

Datta, A., M. Hendrix, M. Lipsitch and S. Jinks-Robertson, 1997 Dual roles for DNA sequence identity and the mismatch repair system in the regulation of mitotic crossing-over in yeast. Proc. Natl. Acad. Sci. USA **94:** 9757–9762.

de Wind, N., M. Dekker, A. Berns, M. Radman and H. te Riele, 1995 Inactivation of the mouse Msh2 gene results in mismatch repair deficiency, methylation tolerance, hyperrecombination, and predisposition to cancer. Cell **82:** 321–330.

Doutriaux, M.-P., R. Wagner and M. Radman, 1986 Mismatch-stimulated killing. Proc. Natl. Acad. Sci. USA **83:** 2576–2578.

Foster, P. L., 1998 Adaptive mutation: Has the unicorn landed? Genetics **148:** 1453–1459.

Guha, S., and W. Guschlbauer, 1992 Expression of *Escherichia coli dam* gene in *Bacillus subtilis* provokes DNA damage response: N6-methyladenine is removed by two repair pathways. Biochim. Biophys. Acta **1132:** 309–310.

Harris, R. S., K. J. Ross and S. M. Rosenberg, 1996 Opposing roles of the Holliday junctions processing systems of *Escherichia coli* in recombination-dependent adaptive mutation. Genetics **142:** 681–691.

Holbeck, S. L., and J. N. Strathern, 1997 A role for *REV3* in mutagenesis during double-strand break repair in *Saccharomyces cerevisiae.* Genetics **147:** 1017–1024.

Hunter, N., S. R. Chambers, E. J. Louis and R. H. Borts, 1996 The mismatch-repair system contributes to meiotic sterility in an interspecific yeast hybrid. EMBO J. **15:** 1726–1733.

Kahn, P. L., 1968 Isolation of high frequency recombining strains from *Escherichia coli* containing the V colicinogenic factor. J. Bacteriol. **96:** 205–214.

Kogoma, T., 1996 Recombination by replication. Cell **85:** 625–627.

Kogoma, T., G. W. Cadwell, K. G. Barnard and T. Asai, 1996 The DNA replication priming protein, PriA, is required for homologous recombination and double-strand break repair. J. Bacteriol. **178:** 1258–1264.

Kucherlapati, R., and G. R. Smith, 1988 *Genetic Recombination.* American Society for Microbiology, Washington, DC.

Lahue, R. S., S.-S. Su and P. Modrich, 1987 Requirement for d(GATC) sequences in *Escherichia coli mutHLS* mismatch correction. Proc. Natl. Acad. Sci. USA **84:** 1482–1486.

Längle-Rouault, F., G. Maenhaut-Michel and M. Radman, 1987 GATC sequences, DNA nicks and the MutH function in *Escherichia coli* mismatch repair. EMBO J. **6:** 1121–1127.

Marinus, M. G., M. Carraway, A. Z. Frey, L. Brown and J. A. Arraj, 1983 Insertion mutations in the *dam* gene of *Escherichia coli* K-12. Mol. Gen. Genet. **192:** 288–289.

Martin, B., G. J. Sharples, O. Humbert, R. G. Lloyd and J. P. Claverys, 1996 The *mmsA* locus of *Streptococcus pneumoniae* encodes a RecG-like protein involved in DNA repair and in three-strand recombination. Mol. Microbiol. **19:** 1035–1045.

Masai, H., T. Asai, Y. Kubota, K. Arai and T. Kogoma, 1994 *Escherichia coli* PriA protein is essential for inducible and constitutive stable DNA replication. EMBO J. **15:** 5338–5345.

Matic, I., C. Rayssiguier and M. Radman, 1995 Interspecies gene exchange in bacteria: the role of SOS and mismatch repair systems in evolution of species. Cell **80:** 507–515.

McGlynn, P., A. A Al-Deib, J. Liu, K. J. Marians and R. G. Lloyd, 1997 The DNA replication protein PriA and the recombination protein RecG bind D-loops. J. Mol. Biol. **270:** 212–221.

McPhee, D. G., 1993 Directed mutation: paradigm postponed. Mutat. Res. **285:** 109–116.

Mezard, C., D. Pompon and A. Nicolas, 1992 Recombination between similar but not identical DNA sequences during yeast transformation occurs within short stretches of identity. Cell **70:** 659–670.

Modrich, P., and R. Lahue, 1996 Mismatch repair in replication fidelity, genetic recombination, and cancer biology. Annu. Rev. Biochem. **65:** 101–133.

Pang, P. P., A. S. Lundberg and G. C. Walker, 1985 Identification and characterization of the *mutL* and *mutS* gene products of *Salmonella typhimurium* LT2. J. Bacteriol. **163:** 1007–1015.

Peterson, K. R., and D. W. Mount, 1993 Analysis of the genetic requirements for viability of *Escherichia coli* K-12 DNA adenine methylase (*dam*) mutants. J. Bacteriol. **175:** 7505–7508.

Petit, M. A., J. Dimpfl, M. Radman and H. Echols, 1991 Control of chromosomal rearrangements in *E. coli* by the mismatch repair system. Genetics **129:** 327–332.

Porter, G., J. Westmoreland, S. Priebe and M. Resnick, 1996 Homologous and homeologous intermolecular gene conversion are not differentially affected by mutations in the DNA damage or the mismatch repair genes *RAD1*, *RAD50*, *RAD51*, *RAD52*, *RAD54*, *PMS1* and *MSH2.* Genetics **143:** 755–767.

Priebe, S., J. Westmoreland, T. Nilsson-Tillgren and M. Resnick, 1994 Induction of recombination between homologous and diverged DNAs by double-strand gaps and breaks and role of mismatch repair. Mol. Cell. Biol. **14:** 4802–4814.

Radman, M., 1991 Avoidance of inter-repeat recombination by sequence divergence and a mechanism of neutral evolution. Biochimie **73:** 357–361.

Radman, M., and R. Wagner, 1986 Mismatch repair in *Escherichia coli.* Annu. Rev. Genet. **20:** 523–538.

Radman, M., and R. Wagner, 1993a Mismatch recognition in chromosomal interactions and speciation. Chromosoma **102:** 369–373.

Radman, M., and R. Wagner, 1993b DNA mismatch repair systems: mechanisms and applications in biotechnology. Biotechnol. Genet. Eng. Rev. **11:** 357–366.

Rayssiguier, C., D. S. Thaler and M. Radman, 1989 The barrier to recombination between *Escherichia coli* and *Salmonella typhimurium* is disrupted in mismatch-repair mutants. Nature **342:** 396–401.

Rosenberg, S. M., C. Thulin and R. S. Harris, 1998 Transient and heritable mutators in adaptive evolution in the lab and in nature. Genetics **148:** 1559–1566.

Sandler, S. J., and A. J. Clark, 1993 Use of high and low level overexpression plasmids to test mutant alleles of the *recF* gene of *Escherichia coli* K-12 for partial activity. Genetics **135:** 643–654.

Sandler, S. J., H. S. Samra and A. J. Clark, 1996 Differential suppression of *priA2*::*kan* phenotypes in *Escherichia coli* K-12 by mutations in *priA*, *lexA* and *dnaC.* Genetics **143:** 5–13.

Selva, E. M., L. New, G. F. Crouse and R. S. Lahue, 1995 Mismatch correction acts as a barrier to homeologous recombination in *Saccharomyces cerevisiae.* Genetics **139:** 1175–1188.

Shen, P., and H. V. Huang, 1986 Homologous recombination in *Escherichia coli*: dependence on substrate length and homology. Genetics **112:** 441–457.

Shurvinton, C. E., and R. G. Lloyd, 1982 Damage to DNA induces expression of the *ruv* gene of *Escherichia coli.* Mol. Gen. Genet. **185:** 352–355.

Strathern, J. N., B. K. Shafer and C. B. McGill, 1995 DNA synthesis errors associated with double-strand-break repair. Genetics **140:** 965–972.

Tran, H., N. Degtyareva, D. Gordenin and M. Resnick, 1997 Altered replication and inverted repeats induce mismatch repair-independent recombination between highly diverged DNAs in yeast. Mol. Cell. Biol. **17:** 1027–1036.

Vaccaro, K. K., and E. C. Siegel, 1977 The frameshift mutability of *polA1* and *recA1* derivatives of mutator strains of *Escherichia coli.* Mutat. Res. **42:** 443–446.

Vulic, M., F. Dionisio, F. Taddei and M. Radman, 1997 Molecular keys to speciation: DNA polymorphism and the control of genetic exchange in Enterobacteria. Proc. Natl. Acad. Sci. USA **94:** 9763–9767.

Worth, L., Jr., S. Clark, M. Radman and P. Modrich, 1994 Mismatch repair proteins MutS and MutL inhibit RecA-catalyzed strand transfer between diverged DNAs. Proc. Natl. Acad. Sci. USA **91:** 3238–3241.

Zahrt, T. C., and S. Maloy, 1997 Barriers to recombination between closely related bacteria: MutS and RecBCD inhibit recombination between *Salmonella typhimurium* and *Salmonella typhi.* Proc. Natl. Acad. Sci. USA **94:** 9786–9791.

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