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Escherichia coli mutator mutants deficient in methylation-instructed DNA mismatch correction

(2-aminopurine/*dam, mutH, mutL, mutS*/DNA strand discrimination/mismatch excision repair/high negative interference)

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ABSTRACT **Our approach to the isolation of DNA mismatch-correction-deficient mutants was based upon the isolation of 2-aminopurine-resistant second-site revertants of *Escherichia coli dam*$^-$ mutants. We isolated such second-site revertants which, when separated from the *dam*$^-$ mutation, have a mutator character of their own. These new mutators all mapped at three known mutator loci, *mutH, mutL,* and *mutS*, which exhibit the same mutagenic spectrum as the *dam*$^-$ mutator: increased levels of base substitution and frameshift mutations. The mutator potencies of double and triple *mut*$^-$ mutants suggest that these mutators are involved in the same general mismatch-repair pathway. All these mutations result in a hyper-recombination phenotype, but in four-factor crosses among λ phages, a specific loss of intragenic recombination (*Pam3* × *Pam80*) was found in *mutL* and *mutS* mutants, as would be predicted from the postulated role of mismatch correction in gene conversion and high negative interference phenomena.**

The existence of an excision-repair system acting upon mismatched base pairs in the DNA has been postulated in order to account for gene conversion (1, 2), high negative interference (3), and map expansion phenomena (4) (for review, see ref. 5). The possibility of an involvement of mismatch repair in the suppression of spontaneous mutations was indicated by the discovery that pneumococcus *hex*$^-$ and *Escherichia coli uvrE* mutants, which are probably deficient in the repair of some mismatched base pairs, appear to be spontaneous mutators as well (6, 7). Furthermore, mismatch repair has been implicated in the avoidance of mutagenesis by 5-bromouracil (5-BrUra) (8).

However, the existence of a mismatch repair system to efficiently correct replication errors implies that a strand discrimination mechanism must exist ensuring that the excision of the mismatched base occurs exclusively from the newly synthesized strand. Because DNA methylation is a postreplicative process [i.e., newly synthesized strands are undermethylated (9)], it has been suggested that DNA methylation is one possible means of discrimination between old (methylated) and new (undermethylated) strands (10–12). This suggestion is supported by the observation that *E. coli dam*$^-$ mutants deficient in general methylation of adenine residues (13) occurring within the 5′G-A-T-C3′ sequence (14) are also spontaneous mutators (15). Direct evidence in favor of the above hypothesis was obtained by using transfection assays with heteroduplex λ DNA differing in the degree of methylation and carrying different genetic markers.‡§ Furthermore, a role for *dam*-dependent methylation in DNA strand discrimination in the elimination of the mutagenic effects of base analogs has been indicated by the sensitivity and hypermutability of *E. coli dam*$^-$ mutants for the base analogs 2-aminopurine (2APur) and 5-BrUra (11, 12).

Our strategy to isolate mutants defective in adenine-methylation-instructed mismatch correction was based upon the sensitivity of the *dam*$^-$ mutants to 2APur (11). Fig. 1 illustrates the underlying hypothesis: relatively closely spaced, newly incorporated 2APur residues (or other mismatched bases) would result in overlapping excision repair tracts if excision occurred in both the parental and newly synthesized strands as postulated for the *dam*$^-$ mutants. Consequently, mutants deficient in mismatch correction (e.g., in endonucleolytic cleavage or exonucleolytic strand degradation) could be expected to restore resistance to 2APur in a *dam*$^-$ mutant while maintaining the mutator properties associated with the Dam$^-$ phenotype.

We have found such 2APur-resistant revertants of *dam*$^-$ strains which, when separated from the *dam* mutation, are mutators themselves. They all mapped to the locations of three known mutator loci. We have studied their phenotypes, all of which are consistent with the hypothesized mismatch-correction deficiency of these mutants. These same mutants have been identified by Rydberg (16) by screening for 5BrUra hypermutability. A brief account of our work was presented at the 1978 Cold Spring Harbor Symposium (12).

MATERIALS AND METHODS

Bacterial Strains. The strains of *E. coli* K-12 used in this study are given in Tables 1 and 3.

Media and General Methods. The media used were as described earlier (17). Where specified, the plates were supplemented with 2APur from Sigma (200 μg/ml), L-valine (40 μg/ml), nalidixic acid (40 μg/ml), rifampicin (100 μg/ml), or streptomycin (100 μg/ml). P1.kc-mediated transductions were carried out as described (17); conjugations were performed as described by Miller (18).

Phages and Crosses. Phage crosses were performed with λ*imm434 cI Pam3 Rts* and λ*c*$^+$ *Pam80 R*$^+$ constructed by recombination with phages from the collection of R. Thomas (University of Brussels). Bacteria were grown in enriched medium to a concentration of 3×10^8 cells per ml, centrifuged, and resuspended in 10 mM $MgSO_4$. Infection was carried out at a multiplicity of infection of 5 for each parental type at 37°C for 10 min. Unadsorbed phage were eliminated by centrifu-

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Abbreviations: 2APur, 2-aminopurine; *mut*, mutator.

‡ M. Rykowsky, P. Pukkila, M. Radman, R. E. Wagner, and M. Meselson, unpublished data.

§ C. Dohet, M. F. Bourguignon-Van Horen, and M. Radman, unpublished data.

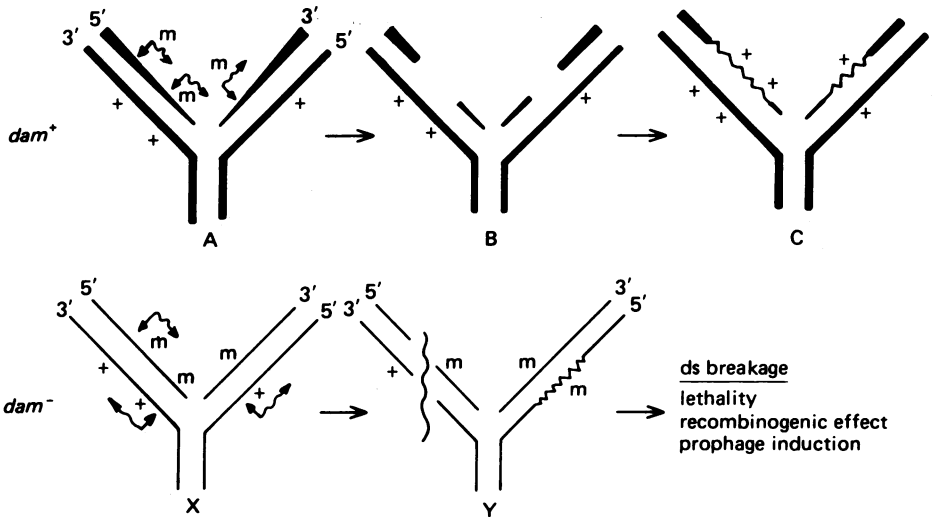

FIG. 1. Interpretation of the sensitivity of a *dam* mutant to the base analog 2APur: hypothesized lethal lesion provoked by mismatch correction under conditions where strand discrimination is defective. The thickness of the DNA strands indicates their degree of methylation. A replicational error or a base-analog-induced mismatch is symbolized by m. Arrows perpendicular to the DNA strands symbolize endonucleolytic attack at mismatched sites; wavy lines parallel to the DNA strand indicate the 5′ → 3′ direction of the excision repair tract (10). The wavy portion of the single-stranded DNA indicates an excision repair tract. Wild-type situation: (*A*) Endonuclease/exonuclease-mediated mismatch removal occurs specifically from the undermethylated, newly synthesized strands. (*B*) Exonucleolytic degradation and resynthesis leads to the intact, mutation-free molecule shown as *C*. *dam*⁻ situation: (*X*) Endonuclease/exonuclease can act on both the newly synthesized and the parental DNA strands due to the absence of methylation. (*Y*) The left arm depicts the production of a double-strand (ds) break due to overlapping excision tracts extending over several thousand DNA bases (2). The right arm illustrates a situation where mismatch repair results in error fixation rather than error avoidance, thus illustrating the mutator phenotype of *dam* mutants. The creation of double-strand breaks can account for several pleiotropic effects of the *dam*⁻ mutations: sensitivity to base analogs, hyper-Rec activity, increased levels of spontaneous prophage induction, and the lethality of the *dam-recA* and *dam-recB* combinations (see text). A consequence of this scheme is that a deficiency in an early step in mismatch correction would reverse many, perhaps all, of the *dam*-specific effects, except for the mutator effect.

gation through 5% (vol/vol) glycerol/10 mM $MgSO_4$. Adsorbtion was 99% or greater in each case. Infected bacteria were allowed to grow for 70 min and were then treated with chloroform. After centrifugation to remove bacterial debris, free phage were plated together with the appropriate indicator strains. Phage × prophage crosses were performed by superinfecting (λ^+) lysogens, grown as above, with *λimm434 cI Ram216 red⁻ int⁻* at a multiplicity of infection of 5 and terminating the cross as described for phage crosses. The frequency of *λimm434* R^+ recombinants was determined by plating the lysate on KMBL 241 (*λ c1857 red⁻ Ram5*) *suII⁺* and N100 (*λ c1857 red⁻ Ram5*) *su⁻* at 32°C.

Calculation of Mutation Rate. Mutation rates, expressed as mutations per cell per generation, were calculated as described earlier (11) by the method of Stahl (19). Fluctuation was usual with the mutation rates seen for Nal^R, Val^R, and Rif^R so that only differences of a factor of 2 or more can be considered significant.

Table 1. Strains of *E. coli* K-12 used

KMBL 241	*recA36, cys, thr, leu, thi, lac, pyr, arg, ile*
KMBL 1385	*bio87, thyA301, metE116, uvrE502*
KMBL 3701	HfrG61, *his-136, dam-4*
KMBL 3702	HfrG61, *his-136, dam-3*
KMBL 3731	*thr-46, leu-46, proA46, thi-46, his-46, arg-46, thyA, lacY46, galK46, ara-46, mtl-46, strA46, dam-4*
KMBL 3751	*thyA306, lysA65, argA103, bio-87, metE72, pheA97, purA, aroB, endA101*
KMBL 3752	*thyA306, lysA65, argA103, bio-87, metE72, pheA97, purA, aroB, cysC, endA101*
KA 839*	*thi, argA21, lysA22, mutS3*
KA 841†	*leu, lacZ32, trp, his, argA, ilv, str, mutR34*
C 600	*suII⁺*
C 594	*su⁻*
QR 48	*suII⁺, recA*
N 100	*su⁻, recA*

* Received from E. Siegel as ES 455.
† Received from E. Nestman as RH 213.

RESULTS AND DISCUSSION

Genetic Characterization of Second-Site 2APur-Resistant Revertants of *dam⁻* Strains. The 32 independent derivatives of *dam⁻* strains resistant to 200 μg of 2APur per ml obtained in KMBL 3701 and KMBL 3702 were all shown to be second-site revertants by the recovery of 2APur-sensitive *dam⁻-aroB⁺* transductants of KMBL 3751. In addition, all 2APur-resistant *dam⁻* derivatives showed spontaneous mutation rates towards streptomycin and nalidixic acid resistance higher than the original *dam⁻* strain (data not shown).

To localize these second-site mutations, bacteriophage P1 grown on these strains was used to transduce KMBL 3751 to *purA⁺* and *thyA⁺-lysA⁺* to check for the presence of the known mutators *mutL* and *mutH*, which cotransduce with these markers (20). Eight revertants were found to have mutator mutations 100% cotransducible with *thyA-lysA* and therefore likely to be *mutH* (21); 13 were found to be tightly linked with *purA* and therefore presumably *mutL* mutations. The 11 unmapped mutators were then localized by conjugation with KMBL 3731 (*dam⁻*) to *mutS*. From the transductions which are summarized in Table 2 it can be concluded that the intro-

Table 2. Acquirement of 2APur resistance by introduction of known mutator mutations into *dam⁻* strain KMBL 3752

Donor	Marker selected	Fraction $2APur^R$	Correlation of *mut⁻* and $2APur^R$
KA 841 (*mutR34*)	*thyA⁺-lysA⁺*	100/100	100/100
KA 840 (*mutL13*)	*purA⁺*	94/100	94/94
KA 839 (*mutS3*)	*cysC⁺*	44/100	44/44

duction of these mutator mutations into a *dam*$^-$ strain does indeed result in 2APur resistance. Although a wild-type strain grows normally in the presence of 1 mg of 2APur per ml in agar, the limiting concentrations allowing colony formation of *dam*$^-$, *dam*$^-$ *mut*$^-$, and *mut*$^-$ strains are about 10, 400, and 750 μg/ml, respectively.

Mutation Rates in Multiple Mutator Mutants. We examined the spontaneous mutation rates of a series of otherwise isogenic strains carrying mutations *mutH*, *mutL*, and *mutS* (Table 3). It appears that *mutH101* and *mutL101* are stronger mutators for these markers than *mutS101* (Table 3). The presence of the *dam-3* mutation in the MutH, MutL, or MutS strains did not clearly affect the mutator activities of the more powerful mutators *mutH* and *mutL;* nor was there a great alteration in mutation rates in strains carrying multiple mutator mutations in either the presence or absence of the *dam*$^-$ mutation. Although additive mutator effects cannot be excluded, these results, and the fact that the *mutH*, *mutL*, *mutS*, and *dam*$^-$ mutations result in the same mutational spectrum [i.e., increased transition and frameshift mutagenesis (20, 22)], are interpreted to mean that *mutH*, *mutL*, *mutS*, and *dam* are involved in the same error-avoidance mechanism. However, the significance of the apparent reduction of the mutation rates in some *mut* combinations cannot be ascertained.

The possible involvement of *uvrE* (*mutU*) in the hypothesized error-avoidance pathway is indicated by the failure of *uvrE* mutants to correct some heteroduplex phage λ DNA (7). We transduced the *uvrE504* mutation into *mutH*, *mutL*, *mutS*, and *dam*$^-$ strains. Its introduction into a *dam*$^-$ strain did not result in 2APur resistance, nor were 2APur-resistant *dam mut* strains sensitized to 2APur by the introduction of the *uvrE* mutation. The *uvrE* mutation, itself a mutator, did not enhance the mutation rates in strains carrying the *mutH*, *mutL*, or *mutS* mutations (Table 3), suggesting that the *uvrE* mutation may affect the same error-avoidance pathway. This is especially likely because *uvrE* also shares the same mutational specificity as the *mutH*, *mutL*, *mutS*, and *dam* mutations (20, 22).

We realize that the additivity of mutator effects cannot be ascertained from the data in Table 3; therefore, a forthcoming comparative analysis of the mutational spectra in single and multiple mutants should be more informative about single versus multiple pathways. At present, we have no explanation for the observation (Table 3 and data not shown) that the combination *dam uvrE* results in a lower mutation rate than found for either *dam*$^-$ or *uvrE* alone.

Restored Viability of *dam recA* Mutants by Addition of a *mutH*, *mutL*, or *mutS* Mutation. The lethality of the *dam recA* double mutant (15) may reflect a requirement for the *recA* gene to repair double-strand breaks hypothesized in Fig. 1. Hence, an additional deficiency in mismatch correction should prevent the occurrence of the double-strand breaks and alleviate the need for the *recA* gene in the maintenance of viability in a *dam*$^-$ background. We constructed KMBL 3854, a *dam-3* strain carrying the temperature-sensitive mutation *recA200*, and found that, as predicted, the introduction of *mutH*, *mutL*, or *mutS* mutations into this strain restored colony-forming ability on nutrient agar plates at the restrictive temperature.

Hyper-Recombination Phenotype of *dam*, *mutH*, *mutL*, and *mutS* Mutants. In crosses between the wild-type prophage λ residing in KMBL 3752, KMBL 3754 (*dam-3*), KMBL 3755 (*dam-3 mutH101*), KMBL 3760 (*dam-3 mutL101*), and KMBL 3767 (*dam-3*, *mutS101*) and the superinfecting phage λ *imm434 cI Ram216 red*$^-$ *int*$^-$, marker rescue of the R^+ allele from the λ$^+$ prophage was measured (see *Materials and Methods*). The average frequencies of λ *imm434* R^+ recombinants were 0.004, 0.011, 0.4, 0.48, and 0.068 for the wild-type, *dam*$^-$, *dam*$^-$ *mutH*, *dam*$^-$ *mutL*, and *dam*$^-$ *mutS* strains, respectively. The moderate hyper-Rec phenotype of the *dam*

Table 3. Spontaneous mutation rates of *E. coli* derivatives carrying various combinations of *dam*, *mutH*, *mutL*, *mutS*, and *uvrE* mutations

Strain	Relevant genotype	Mutation rates per cell per generation × 10^{10}			
		Str^R	Val^R	Nal^R	Rif^R
dam-mutator combinations					
KMBL 3752	Wild type	0.54	45	3.0	25
KMBL 3754	*dam-3*	8.3	2,600	250	930
KMBL 3755	*dam, mutH101*	28	21,000	2700	15,000
KMBL 3773	*mutH101*	79	60,000	1500	4,900
KMBL 3760	*dam, mutL101*	74	69,000	6100	13,000
KMBL 3774	*mutL101*	89	21,000	1300	4,900
KMBL 3767	*dam, mutS101*	3.4	3,300	1980	2,600
KMBL 3775	*mutS101*	4.0	2,800	880	2,600
Multiple mutator combinations					
KMBL 3850	*mutL101, mutS101*	33.0	—	4400	2,960
KMBL 3846	*mutH101, mutS101*	6.0	—	1580	6,230
KMBL 3848	*mutH101, mutL101*	14.9	—	3000	8,130
KMBL 3852	*mutH101, mutL101, mutS101*	99.2	—	3800	7,880
Combinations with *uvrE*					
KMBL 3789	*uvrE502*	5.8	2,400	196	—
KMBL 3790	*dam-3, uvrE502*	1.3	400	140	—
KMBL 3791	*dam-3, mutH101, uvrE502*	17.0	21,000	2700	—
KMBL 3792	*dam-3, mutL101, uvrE502*	18.6	37,000	2100	—
KMBL 3793	*dam-3, mutS101, uvrE502*	8.6	3,200	2100	—

The strains listed are derivatives of KMBL 3752; the various markers were introduced by P1-mediated transductions. The mutations *dam*$^-$, *mutL*, *mutS*, and *uvrE* were introduced by selection for *aroB*$^+$, *purA*$^+$, *cysC*$^+$, and *metE*$^+$ transductions, respectively, and screening for the appropriate characteristics. The mutation *mutH* was introduced by the simultaneous selection of *thyA*$^+$-*lysA*$^+$ transductants with the appropriate P1 phage. These results are the mean of not less than 38 independent cultures. This mean was determined by excluding mutational "jackpots," which were defined as cultures having more than 5 times the mean number of mutants.

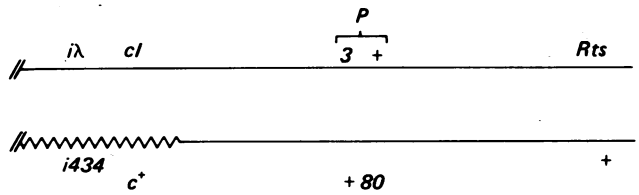

FIG. 2. Arrangement of markers in the bacteriophage λ crosses described in Table 4. *Pam3* and *Pam80* are two intragenic markers probably separated by only a few hundred nucleotides, whereas *cI* and *Rts2* are flanking markers. Marker distances are not proportional to physical distance (i.e., *cI–P* and *P–R* gene distances are roughly 2000 and 8000 nucleotides, respectively). Note that the immunity regions are nonhomologous.

mutant confirms other reports (23). A moderate hyper-Rec phenotype of *mutH*, *mutL*, and *mutS* mutants were also detected in Hfr crosses by S. Feinstein and B. Low (personal communication). The presence of *mutH*, *mutL*, and *mutS* mutations in a *dam*$^-$ background not only does not suppress the hyper-Rec phenotype of the *dam*$^-$ mutation, but leads to a strong hyper-Rec phenotype in our crosses, suggesting that unrepaired mismatched base pairs may initiate recombinational exchanges.

Recombination Between Close and Well-Separated Markers in Mutator Mutants: λ *cI Pam3 Rts2* × λ *imm434 C*$^+$ *Pam80 R*$^+$ Crosses. Intragenic recombination in eukaryotes is often refered to as "gene conversion" because the majority of recombinants between two or more closely spaced markers are produced not by crossing over between the markers, but by the formation of hybrid DNA involving one or more markers (molecular heterozygosity) followed by mismatch correction events in such heteroduplex regions which create a variety of recombinant genomes (ref. 3; for a review, see ref. 5). An excess of recombinants between tightly linked markers in the neighborhood of a selected event is called "negative interference" and has been extensively studied in bacteriophage λ (3, 24). Because recombination in a *rec*$^+$ *E. coli* between well-separated markers in phage λ is usually due to reciprocal exchanges (25), one would predict that mismatch-correction-deficient mutants should decrease the frequency of intragenic recombination with little effect upon recombination between well-separated markers.

Fig. 2 shows the order and relative distances of the two closely linked markers *Pam3* and *Pam80* (24) and the two well-separated "outside" markers *cI* (clear plaque morphology) and *Rts2* (temperature-sensitive plaque formation). Because the λ *P* gene is required for λ DNA replication and our *E. coli* hosts carry no suppressors of amber mutations, the crosses were performed under nonreplicative conditions where only recombined oligomeric λ DNAs are maturable.

Of the mutator mutants, *mutL* and *mutS* decreased intragenic recombination (see Table 4, column A) without any significant effect on intergenic recombination (Table 4, column B). *mutH*, however, consistently resulted in a very high frequency of *P*$^+$ recombinants, for which we have no satisfying interpretation. Despite the hyper-Rec phenotype of all three mutators, enhanced recombination was not detected in these crosses (see column B), perhaps because our phages carry an intact autonomous recombination system (Red$^+$). The *P*$^+$ recombinants arising in the *mutS* and *mutL* strains, if not due to mismatch correction, must represent either real crossovers between *am3* and *am80* or the termination of single-strand assimilation between the two markers during heteroduplex formation. The occurrence of crossovers between these markers should increase the proportion of *c*$^+$ phage among the *P*$^+$ progeny (see Fig. 2). Only *mutS*, which was most efficient in suppressing *P*$^+$ recombinants, exhibited an increase in the *c*$^+$/*cI* ratio (Table 4, column F). The two other mutators gave the same quite high *c*$^+$/*cI* ratios as the wild-type (compare columns E and F of Table 4), indicating a bias in the formation of *P*$^+$ recombinants (i.e., the λ *imm434 c*$^+$ genome is more susceptible to *P*$^+$ conversion events than is the λ *cI* genome). Similar to observations made in eukaryotic organisms (for review, see ref 5), the occurrence of a gene conversion event is often accompanied by crossovers of outside markers, in our case from about 7% without selection (column B of Table 4) to around 30% among the selected *P*$^+$ progeny (column C). We understand neither the quantitative differences between the mutators *mutL* and *mutS* in the suppression of *Pam*$^+$ recombinants, nor the opposite (hyper-Rec) effect of *mutH*. The quantitative differences between the results shown in Table 4 and the related published experiments (11) could, however, be explained by the acquisition of a recombinational hot spot during construction of λ *cI Pam3 Rts2* phage.

Further Discussion. We envision three simple possibilities. (*i*) The *mutH*, *mutL*, *mutS*, and *uvrE* genes might code for mismatch-repair enzymes of different recognition specificities. (*ii*) All four genes might code for different subunits of a complex multimeric mismatch-correction enzyme. Mutations in the different subunits could show quantitative but not qualitative differences. (*iii*) Some of these mutator genes might be regulatory whereas others might be structural genes. The latter two possibilities could account for the fact that the properties of the multiple mutants indicate a single error-avoidance pathway (Table 3) and that all four mutators appear to have similar mutagenic specificities (20, 22).

The evidence supporting the hypothesis of methylation-instructed mismatch correction (10–12) is the following: (*i*) The *dam*$^-$ mutation increased spontaneous mutagenesis and caused hypermutability to base analogs (11, 15). (*ii*) The transfection

Table 4. Recombination between close and well-separated markers: λ*cI Pam3 Rts2* × λ*imm434c*$^+$ *Pam80 R*$^+$ crosses in mutator strains

	Recombination frequencies					
Cross in	*P*$^+$/total (A)	*cIR*$^+$/total (B)	(*cIR*$^+$)/*P*$^+$ (C)	A/B (D)	*c*$^+$/*c* among total (E)	*c*$^+$/*c* among *P*$^+$ (F)
KMBL 3752	0.013	0.064	0.33	0.209	1.03	5.67
KMBL 3773 (*mutH*)	0.169	0.086	0.46	1.965	0.69	5.78
KMBL 3774 (*mutL*)	0.0054	0.060	0.20	0.090	0.84	5.83
KMBL 3775 (*mutS*)	0.0019	0.074	0.16	0.027	0.80	17.33
KMBL 3789 (*uvrE*)	0.068	0.049	0.42	1.387	0.49	6.71

Column A: phage titer on 594 Su$^-$/C600 Su$^+$ (average of platings at 32° and 42°C). Column B: clear-plaque titer on C600 Su$^+$ at 42°C/total titer on C600 at 32°C (crossover frequency in an unselected population). Column C: clear-plaque titer on 594 Su$^-$ at 42°C/total titer on 594 Su$^-$ at 32°C (crossover frequency among *am*$^+$ phage). Column E: ratio of turbid to clear plaques on C600 Su$^+$ at 32°C. Column F: ratio of turbid to clear plaques on 594 Su$^-$ at 32°C.

of *E. coli* wild-type strains with phage λ heteroduplex DNA with only one of the DNA strands methylated resulted in the preferential loss of genetic markers carried by the nonmethylated strand,[‡§] whereas such methylation-instructed strand discrimination is absent in our *mutH101*, *mutL101*, and *mutS101* strains.[§] These results cannot be accounted for by the preferential replication of the methylated DNA strands because the proliferative potential of the methylated and nonmethylated DNA was found to be equal. (*iii*) The loss of mismatch correction can also be inferred from the data on recombination of closely spaced markers in phage λ crosses (Table 4 and ref. 11). (*iv*) Finally, similar or identical mutational specificity is exhibited by the *mutH*, *mutL*, *mutS*, and *dam*$^-$ mutants (20, 22). These points, together with other results (refs. 10–12 and 22, and this work), are consistent with the existence of a methylation-instructed mismatch repair pathway acting as a postreplicative mutation-avoidance system. Insights into the intricacies of such a system, however, must await the biochemical characterization of the mismatch-repair enzymes.

If only the five genes considered in this report determined the general characteristics of postreplicative mismatch correction, then the strongest of these mutators or their combination should give an order-of-magnitude estimate of the fidelity of the *E. coli* replication machinery. The mutators *mutH* and *mutL* increase the spontaneous mutation rate in a forward-mutation system by 10^3- to 10^4-fold. Taking as a rough estimate an error rate of 10^{-9} per nucleotide replicated (20), we can estimate the *in vivo* replication fidelity in *E. coli* as 10^{-5}–10^{-6} mistakes per nucleotide per replication. This corresponds to the minimal error rates of DNA polymerase assayed *in vitro* with artificial homopolymers, alternating polymers, or primed single-stranded φX174 DNA as templates (26).

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