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Escherichia coli K-12 Clones That Overproduce *dam* Methylase Are Hypermutable

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A strain of *Escherichia coli* K-12 that overproduces *dam* methylase 50-fold was found to be hypermutable, and mutations which resulted in loss of excess methylase activity restored mutation frequencies to wild-type levels. These results are consistent with involvement of this deoxyribonucleic acid methylase in mismatch correction.

A postreplication mismatch repair system could contribute significantly to the in vivo fidelity of DNA replication. To be effective in this process, an enzyme system must be able to differentiate newly synthesized DNA from template strands. It has been postulated that *dam* methylation of adenine residues in DNA at the sequence d(G-A-T-C) (5) may provide the necessary discriminatory mechanism in *Escherichia coli* (21). Since methylation is a postreplication process (3, 10, 14), parental strands will be methylated, but newly synthesized DNA will exist in a transiently undermethylated state for some period of time subsequent to synthesis.

Direct evidence that a methylation-directed repair process operates in *E. coli* has been obtained by Meselson and co-workers, who examined the effects of *dam* methylation on in vivo repair of transfecting λ heteroduplexes containing base mismatches (21; M. Meselson, P. Pukkila, M. Rykowski, J. Peterson, M. Radman, R. Wagner, G. Herman, and P. Modrich, J. Supramol. Struct. Suppl. 4:311, 1980). Heteroduplexes methylated on one strand or unmethylated on both strands were found to be subject to mismatch repair to the same extent in vivo; however, mismatch repair of the half-methylated heteroduplexes was markedly biased toward correction of the unmethylated strand, yielding predominantly the genotype of the methylated strand. In contrast, if both strands of the DNA were unmethylated, mismatch elimination occurred on either strand. Moreover, and of particular significance to this report, little if any in vivo correction was observed on either strand if both strands were fully methylated before transfection.

The existence of a methyl-directed mismatch repair system is consistent with the phenotype of mutants deficient in *dam* methylase activity. Such mutants exhibit increased spontaneous mutability (7, 15), as well as increased levels of genetic recombination (9) and increased sensitivity to some chemical mutagens, including the base analogs 2-aminopurine and 5-bromouracil (7, 15, 16). Since fully methylated DNA was not repaired in transfection assays, it might be expected that cells which contain increased levels of *dam* methylase would also be hypermutable as a consequence of more rapid methylation of newly replicated DNA.

We have recloned the *dam* locus of *E. coli* on a *Pst*I fragment into the plasmid vector pBR322 (4; G. Herman and P. Modrich, manuscript in preparation) and obtained a clone, JC4588 (pGG503), with a 50-fold elevation of in vitro methylase activity (Table 1). The *dam* methylase purified to apparent homogeneity from a strain harboring this hybrid plasmid is physically indistinguishable from that obtained from wild-type *E. coli*, and yields obtained are consistent with the level of in vitro activity, indicating that elevated activity is a result of enzyme overproduction.

Availability of such strains allowed us to test whether cells containing elevated levels of *dam* methylase are hypermutable, as are cells deficient in the enzyme. Thus, spontaneous mutation rates for three chromosomal markers were compared, utilizing *E. coli* strains JC4588-(pBR322) and JC4588(pGG503) (Table 1). All studies were performed in a *dam*$^+$ *recA* background to prevent recombination between plasmid and chromosome and to evaluate mutation frequencies in the absence of *recA*-dependent error-prone repair (22). The methylase-overproducing strain exhibited mutation rates for resistance to streptomycin, rifampin, or valine 10-, 30-, and 60-fold that of control cells containing the vector pBR322 alone. Although the *rpsL* (formerly *strA*) locus and *dam* are 10% cotransducible (13), the DNA sequence of *rpsL* contains an internal *Pst*I cleavage site (18); hence *rpsL* function cannot be specified by pGG503. Loci corresponding to rifampin and valine resistance are not linked significantly to *dam* (1, 17). There-

TABLE 1. *dam methylase activity and mutability of plasmid-containing strains of E. coli K-12*[a]

Strain	Relevant plasmid genotype	*dam* methylase activity (U/mg)	Mutation rate/cell per generation ($\times 10^{10}$)		
			Str^r	Val^r	Rif^r
JC4588(pBR322)		20–30	2.0 ± 0.8	50 ± 20	90 ± 30
JC4588(pGG503)	*dam*$^+$	1,100–1,300	20 ± 5	3,000 ± 400	3,000 ± 500
JC4588(pDHA301)	*dam*	20–50	0.9 ± 0.5	10 ± 3	100 ± 30

[a] Results are the average of two to four experiments, each with ten independent cultures. Cells were grown at 37°C on Luria broth without added glucose (17) supplemented with 2 μg of tetracycline per ml. Cells were plated on T plates (17) containing 1.5% (wt/vol) agar supplemented with 10 μg of tetracycline and 50 μg of streptomycin sulfate (Str) per ml or 100 μg of rifampin (Rif) per ml as indicated. To test for resistance to valine (Val), cells were washed with minimal A medium and plated on minimal A plates containing necessary supplements and 40 μg of L-valine per ml (17). The average number of mutants induced per culture, m, was determined as described by Lea and Coulson (11), and the mutation rate per cell per generation was calculated from the formula: $a = m/N$, where N is the population size (20). Data are reported as the average mutation rate ± standard error. Strain JC4588 (F$^-$ *endA recA56 gal his-322 thi*) contains a chromosomal *dam*$^+$ allele. Enzyme assays were performed as described previously (5) on cells harvested at an absorbance at 590 nm of 2.5.

fore, mutation frequencies measured reflect mutagenesis of chromosomal loci.

Since the cloned DNA segment containing the *dam* locus has a molecular weight of $>10 \times 10^6$, it was possible that another locus was responsible for increased spontaneous mutability. Accordingly, a *dam* derivative of pGG503 (pDHA301) was isolated under conditions such that single mutations were induced in the plasmid. Plasmid pGG503 DNA was mutagenized with hydroxylamine at 60°C for 60 min as described previously (8), and DNA was transformed (12) into the *dam-4* strain SK1036 (F$^-$ *mtl-1 xyl-7 lacMS286* φ80dII*lac*BK1 *argH1 his-4 ilvD188 metE46 thi* Spc^r *tsx-3 dam-4*). Tetracycline-resistant transformants (10 to 15% of the level of nonmutagenized controls) were screened for sensitivity to 2-aminopurine since *dam* cells grow poorly in the presence of high levels of this base analog (7). *dam* methylase activity in a *dam-4* strain harboring one mutant plasmid [SK1036(pDHA301)] was less than 1% of that of the same strain harboring pGG503 (data not shown), and products of restriction enzyme hydrolysis of this DNA were identical to those obtained from the parent plasmid pGG503. Plasmid DNA was extracted (2) from this strain and transformed into a *dam*$^+$ *recA* recipient [JC4588(pDHA301)]. A decrease in in vivo mutation rates to near wild-type levels accompanied loss of excess methylase activity (Table 1). (The *dam* locus, as defined by mutations resulting in deficiency in DNA methylase activity [9, 13], has not been shown to be the structural gene for the *dam* methylase. Consequently, it is possible that *dam* mutants define a regulatory element which coordinately controls DNA methylase activity and a second locus involved in mutagenesis. Whereas this cannot be excluded on the basis of available evidence, it seems unnecessarily complex in view of the transfection experiments of Meselson et al. [J. Supramol. Struct. Suppl. 4:311, 1980], which clearly demonstrate that patterns of DNA methylation can direct the process of mismatch correction.)

Results presented here are compatible with a role for the *E. coli dam* methylase in repair of mismatches arising during DNA replication. In particular, they are perfectly consistent with results of transfection experiments which have indicated that DNA methylated on both strands is not subject to mismatch repair (Meselson et al., J. Supramol. Struct. Suppl. 4:311, 1980). Since either deficiency (7, 15) or overproduction of *dam* methylase results in an increased spontaneous mutation rate, in vivo levels of enzyme appear to be regulated. It thus seems plausible that methylase activity is controlled to allow transient undermethylation of newly replicated DNA. It is noteworthy in this respect that adenine methylation of newly synthesized nascent DNA chains is less than that of bulk DNA (14).

An alternate explanation is that the hypermutable phenotype of overproducing strains is due to methylation of sites which remain unmodified in the wild-type background. Although we cannot exclude this possibility, we have previously found that plasmid ColE1 appears to be fully methylated in wild-type *E. coli* K-12 hosts (5). Less than 1% of the 15 *dam* sites of this plasmid are unmethylated on one or both strands. Similarly, restriction endonuclease digestion patterns have led Razin and co-workers to conclude that DNA of *E. coli* strains B and C is methylated symmetrically on both strands at most, if not all, d(G-A-T-C) sequences in vivo (19). Thus, if unmethylated sites exist in nonreplicating DNA in wild-type *E. coli*, they would appear to be extremely rare.

Glickman et al. (7) have postulated that there are at least two modes of mutagenesis in *E. coli*: "direct mutagenesis" yields point mutations and

results from spontaneous incorporation of incorrect bases during replication or as a result of treatment with chemical mutagens including base analogs; "indirect mutagenesis" is presumed to result from induction of a *recA*-dependent error-prone repair system (22), which responds to extensive DNA damage to allow cell survival at the expense of replication fidelity. Glickman and Radman (6) have suggested that the spontaneous mutator phenotype of *dam* mutants is a direct mutagenic effect resulting from occasional fixation of incorrect bases during random mismatch repair. In contrast, they postulate that many of the pleiotropic effects of *dam* mutants, including a hyper-recombinational phenotype (9), inability to grow in the presence of several base analogs (7), increased levels of prophage induction (16), and lethality in conjunction with nonlethal *recA* or *recB* mutations (15), result from production of DNA double-strand breaks or induction of error-prone repair. Although these workers concluded that hypermutability of *dam* mutants is a direct consequence of undermethylation (6), a role for error-prone repair could not be excluded due to inviability of *dam recA* double mutants. However, in work reported here the hypermutable phenotype was observed in a *recA* host; hence, it presumably cannot be a consequence of the error-prone repair pathway.

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