JOURNAL OF BACTERIOLOGY, Aug. 1989, p. 4494–4497
0021-9193/89/084494-04$02.00/0

Vol. 171, No. 8

NOTES

Saturation of Mismatch Repair in the *mutD5* Mutator Strain of *Escherichia coli*

VERONIQUE DAMAGNEZ, MARIE-PASCALE DOUTRIAUX,†* AND MIROSLAV RADMAN

Institut Jacques Monod, Centre National de la Recherche Scientifique, Université Paris 7 Tour 43, 2 Place Jussieu, 75251 Paris Cedex 05, France

Received 17 January 1989/Accepted 28 April 1989

The *mutD* (*dnaQ*) gene of *Escherichia coli* codes for the proofreading activity of DNA polymerase III. The very strong mutator phenotype of *mutD5* strains seems to indicate that their postreplicational mismatch repair activity is also impaired. We show that the mismatch repair system of *mutD5* strains is functional but saturated, presumably by the excess of DNA replication errors, since it is recovered by inhibiting chromosomal DNA replication. This recovery depends on de novo protein synthesis.

The mechanisms ensuring accuracy of DNA replication have been elucidated by study of mutator mutants affecting specific accuracy functions (for a review, see reference 4). In *Escherichia coli*, mutator mutations mapping in the *dnaE* gene presumably affect nucleotide selection by DNA polymerase III (28). Mutations mapping in the *dnaQ* gene (also called *mutD*) affect the proofreading 3′→5′ exonuclease function of the DNA polymerase III holoenzyme (6, 8), whereas *mutH*, *mutL*, *mutS*, and *mutU* mutations cause defects in methyl-directed postreplicative mismatch correction (for reviews, see references 21 and 24). *mutD5* and *dnaQ49* mutants are the most potent mutator strains known. All classes of transition, transversion, and frameshift mutations are increased up to 10^5-fold when *mutD5* strains are grown in rich medium (5, 10, 11). The *mutD5* gene of *E. coli* encodes the epsilon subunit, which carries out the 3′→5′ proofreading exonuclease function of the DNA polymerase III holoenzyme (6, 8). However, the high-mutation-rate effect of *mutD5* strains cannot be explained only by the defect in exonuclease activity, as this mutation rate is comparable to the error rate of in vitro replication with a polymerase devoid of proofreading activity (9, 16). The strong mutator phenotype of *mutD5* suggested that mismatch repair functions may be impaired as well. This prediction was supported by results from DNA heteroduplex transfection experiments (23, 25, 26). One hypothesis is that the high error rate in DNA replication saturates mismatch repair in *mutD5* mutants (23). Therefore, we have tested the mismatch repair capacity of *mutD5* strains under conditions in which chromosomal DNA replication was or was not allowed. Mismatch repair activity was determined by the extent of pure infective centers derived from infection with packaged hemimethylated heteroduplexes of lambda DNA. Under these conditions, bacteria proficient in mismatch repair produced essentially pure infective centers, whereas bacteria deficient in mismatch repair produced mostly mixed infective centers.

To evaluate mismatch repair activity, pure hemimethylated heteroduplexes of lambda DNA containing an A · C or a G · T mismatch were artificially constructed and introduced into *mutD5* and other appropriate strains. Hemimethylated heteroduplexes of lambda DNA with defined mismatches were prepared as previously described (19) by reannealing the separated DNA strands of a lambda mutant carrying a sequenced mutation in the *c*I gene of the lambda repressor and its wild-type allele. Lambda bacteriophages were obtained from Franklin Hutchinson (Yale University). The *c*I857 parental phage is wild type (turbid) at 32°C. The UV23 phage is derived from the *c*I857 phage by a G · C→A · T transition in position 43 of the lambda *c*I gene. The UV23 phage is mutant (clear) at 32°C. Unmethylated DNA was prepared from phages grown in *dam* (deficient in adenine methylation) bacteria, strain GM33 (18). Fully methylated DNA was prepared from phages grown in a Dam methylase overproducer strain, JC4588 (14). The heteroduplexes were then packaged into phage particles using Lambda Packaging Kits (Amersham Corp.).

The hemimethylated heteroduplexes were allowed to infect bacteria singly. The infected centers were plated before lysis under conditions that allowed discrimination between cells yielding only clear, only turbid, or both kinds of phages. Methyl-directed mismatch repair is expected to excise the mismatched base from the unmethylated strand, and the phage progeny of individual repaired molecules will consist of the genotype (pure turbid or pure clear infective centers) of the methylated strand. If no mismatch repair occurs, phages of both parental genotypes will be found in most infective centers (mixed infective centers) (for a review, see reference 24). Thus, mismatch repair activity is manifested by the decrease in mixed infective centers and by the bias preserving information on the methylated strand in pure infective centers. Transition mismatches (G · T and A · C) are well-repaired mismatches in wild-type *E. coli* (7). For the purpose of our study, a *dnaA*(Ts) mutation was P1 transduced into the *mutD5* strain so that mismatch repair efficiency could be determined under conditions in which *E. coli* DNA replication was allowed or prevented. P1 transductions were performed as described elsewhere (20). The *dnaA*(Ts) mutation does not affect lambda DNA replication

* Corresponding author.
† Present address: Institute of Molecular Biology, University of Oregon, Eugene, OR 97403.

TABLE 1. Bacterial strains

E. coli strain	Relevant genotype	Reference
C600	*supE thr leu thi lac*	1
C600 *mutL*	As C600 but *mutL*::Tn*5*	7
W3110	Prototroph	2
W3110 *dnaA*(Ts)	As W3110 but *tnaA*::Tn*10* *dnaA46*; W3110 × P1 (GC 2698)	This study (S. Rosenberg)
KD1079 *mutD5*	*mutD5 thr leu argE his* (*tonB-trpAB*)	5
KD1079 *mutD5* *dnaA*(Ts)	As KD1079 but *tnaA*::Tn*10* *dnaA46*; KD1079 *mutD5* × P1(GC2698)	This study (S. Rosenberg)

(22). Bacterial strains were all *E. coli* K-12 derivatives (Table 1).

Replication-allowed experiments were performed at 32°C. Fresh exponential culture (0.2 ml) grown in standard L broth (20) was incubated with the in vitro-packaged heteroduplex DNA for 15 min at 32°C to allow adsorption. Infected bacteria were then plated on L-broth plates and incubated at 32°C. Infected *mutD5* bacteria were diluted and plated with an excess of indicator bacteria (C600) so that infective centers would not develop on a mutator bacterial lawn. To avoid direct infection of indicator bacteria, the unadsorbed phages were first inactivated by incubating the infected bacteria for 20 min with 10 μl of extracted lambda receptor. (Lambda receptor was extracted as described elsewhere [12] from bacterial strain 6371, which produces high amounts of the LamB protein [27].) Infective centers were scored after being streaked on indicator bacteria at 32°C. The results of infection experiments with the two heteroduplexes are presented in Table 2. Wild-type *E. coli* C600 repaired mismatches efficiently at 32°C, producing 95 to 96% pure infective centers of the genotype carried by the methylated strand. *mutL* bacteria, which are deficient in mismatch repair, produced predominantly mixed infective centers at 32°C (86%). Mismatch repair in W3110 and W3110 *dnaA*(Ts) bacteria was similar to that of C600 bacteria. At 32°C the *mutD5* and *mutD5* *dnaA*(Ts) strains exhibited a low efficiency of mismatch repair for both heteroduplexes. The 39 to 52% pure infective centers indicate that mismatch repair was still active though inefficient, in these strains.

When the infection experiments were performed at 42°C, bacteria were first grown to exponential phase at 32°C, as described above, and then shifted to 42°C for 2 h. L broth was added so that the growing bacteria remained physiologically in the exponential phase. Phages were adsorbed for 15 min at 42°C. Infected bacteria were plated on prewarmed plates and incubated at 42°C. Growth-restricted [*dnaA*(Ts)] and *mutD5* bacteria were plated in the presence of indicator bacteria after inactivation of unadsorbed phages with extracted lambda receptor. All strains tested, except the *mutD5* *dnaA*(Ts) strain, showed similar mismatch repair efficiency at 42 and 32°C (Table 2). The *dnaA*(Ts) mutation

TABLE 2. Genetic analysis of infective centers[a] derived from two hemimethylated heteroduplexes with a single G · T mismatch

E. coli strain	Culture conditions (temp[°C] and supplement[b])	Frequency of infective centers (%)					
		c^+——G——r me^+ / c——T——l me^-			c^+——G——r me^- / c——T——l me^+		
		Turbid (c^+)	Mixed (c/c^+)	Clear (c)	Turbid (c^+)	Mixed (c/c^+)	Clear (c)
C600	32	96	3	1	1	4	95
	32 + CM	97	2	1			
	42	93	7	0	1	9	90
	42 + CM	91	8	1			
C600 *mutL*	32	13	86	1	1	86	13
	32 + CM	7	92	1			
	42	12	87	1	1	92	7
	42 + CM	15	84	1			
W3110	32	96	3	1	0	4	96
	32 + CM	99	0	1			
	42	97	2	1	1	4	95
	42 + CM	92	8	0			
W3110 *dnaA*(Ts)	32	95	5	0	0	3	97
	32 + CM	80	20	0			
	42	97	2	1	0	3	97
	42 + CM	96	4	0			
KD1079 *mutD5*	32	40	60	0	0	48	52
	32 + CM	35	64	1			
	42	40	60	0	1	49	50
	42 + CM	41	59	0			
KD1079 *mutD5* *dnaA*(Ts)	32	39	61	0	1	48	51
	32 + CM	38	61	1			
	42	79	20	1	0	10	90
	42 + CM	41	59	0			

[a] One hundred infective centers were analyzed for each experimental point.
[b] CM, Chloramphenicol.

did not affect mismatch repair activity in the W3110 strain. Unlike its activity at 32°C, the *mutD5 dnaA*(Ts) strain repaired mismatches at 42°C as efficiently as the wild-type strains (79 to 90% pure infective centers) after replication was inhibited for 2 h. In the *mutD5 dnaA*(Ts) bacteria, the heteroduplex methylated on the strand carrying the c^+ genotype showed 79% pure turbid infective centers, whereas the same heteroduplex methylated on the strand carrying the *c*I genotype produced 90% pure clear infective centers. This difference may be related to the appearance of forward mutations due to the *mutD5* mutator effect. (Under the same experimental conditions, *mutD5* bacteria infected with c^+ phages produced only 90% pure turbid infective centers, whereas 100% pure clear infective centers were obtained when *mutD5* bacteria were infected with *c*I phages [data not shown].) Results similar to those described in Table 2 were obtained with the two reciprocal heteroduplexes carrying an A · C mismatch (data not shown). Thus, only the *mutD5* strain with ongoing chromosomal DNA replication showed deficiency in mismatch repair at 42°C. This suggests that mismatch repair capacity was saturated by the large number of mismatches occurring during DNA replication in the *mutD5* strain.

Since the 3′→5′ proofreading exonuclease function is defective in *mutD5* strains, the effect of chromosomal replication on mismatch repair efficiency in these mutants may be related to the high frequency of mismatches left after replication; e.g., a large number of mismatches could saturate the Mut system, allowing mismatches to escape repair (23). Indeed, *lacZ* fusions to the promoters of the *mutL* and *mutS* genes showed that these genes are expressed at a low level. An *E. coli* cell may contain only 10 to 30 MutL and MutS molecules (A. Brandenburger, J. M. Franssen, M. Faelen, and M. Radman, unpublished data).

To test whether de novo protein synthesis was necessary for the recovery of mismatch repair in the *mutD5 dnaA*(Ts) strain under nonreplicative conditions (42°C), we performed the heteroduplex infections in the presence of chloramphenicol, a protein synthesis inhibitor. Chloramphenicol (100 μg/ml, final concentration) was added to the medium at the end of the exponential growth phase. Twenty minutes later, cultures were either switched to 42°C for 2 h or left at 32°C for 2 h in the presence of chloramphenicol. Then phage adsorption was performed, and infective centers were plated on L-broth plates without chloramphenicol. The addition of chloramphenicol did not affect mismatch repair efficiencies at 32°C (Table 2); i.e., mismatch repair enzymes were still active after 2.5 h of protein synthesis block. However, at 42°C, the presence of chloramphenicol prevented *mutD5 dnaA*(Ts) bacteria from recovering their mismatch repair activity, indicating that de novo synthesis of at least one protein is necessary for recovery of efficient mismatch repair following saturation of its activity in replicating *mutD5* strains. The effect was probably not due to an unstable component of the mismatch repair system, since mismatch repair in wild-type bacteria is not affected by the inhibition of protein synthesis. It may be that at least one of the mismatch repair enzymes of *E. coli* is inactivated in the course of mismatch repair. Candidates are MutL and MutH proteins whose overproduction in *mutD5* cells fully restores the mismatch repair activity (R. M. Schaaper and M. Radman, submitted for publication). A precedent for protein suicide during DNA repair is already known: the O^6-methyl-transmethylase of *E. coli* is inactivated by the act of transmethylation (17).

Saturation of the Hex mismatch repair system during transformation of *Streptococcus pneumoniae* has been proposed (13, 29). An excess of either homologous DNA or heterologous DNA did not alter marker effects which reflect mismatch repair in transformation. The apparent loss of marker effects was observed with an excess of DNA from a nonisogenic strain, which was interpreted as the inhibition of mismatch repair by the excess of mismatches present in the hybrid DNA formed between the donor strand and the recipient strand.

Whatever the precise mechanism of saturation of inactivation of mismatch repair in fast-growing *mutD5* mutator strains, this and other studies (23, 25, 26) can now explain the excessive mutator effect of *mutD* (*dnaQ*) mutants as being due to the initial structural defect in the proofreading exonuclease and to the acquired, reversible defect in mismatch repair. The former defect increases the rate of DNA replication errors, and the latter decreases the efficiency of correction of replication errors. Their coincidence in the *mutD5* mutant results in an error amplification phenomenon.

We thank R. d'Ari, E. Sampson, S. Kulkarni, and F. Stahl for critical reading of the manuscript. R. d'Ari provided strain GC 2698 with the *dnaA*(Ts) mutation linked to Tn*10*. S. Rosenberg constructed the *mutD5 dnaA*(Ts) strain.

V.D. was the recipient of a bourse d'étude from Ministère de l'Industrie et de la Recherche. M.-P.D. was the recipient of a fellowship from the Ligue Nationale Française Contre le Cancer. This work was supported by research contracts 520013 from Institut National de la Santé et de la Recherche Médicale-CNAMTS and 6159 from l'Association pour la Recherche sur le Cancer.

LITERATURE CITED

1. **Appleyard, R. K.** 1954. Segregation of new lysogenic types during growth of a doubly lysogenic strain derived from *Escherichia coli* K12. Genetics. **39**:440–452.
2. **Bachmann, B. J.** 1972. Pedigrees of some mutant strains of *Escherichia coli* K-12. Bacteriol. Rev. **36**:525–557.
3. **Claverys, J.-P., and S. A. Lacks.** 1986. Heteroduplex deoxyribonucleic acid base mismatch repair in bacteria. Microbiol. Rev. **50**:133–165.
4. **Cox, E. C.** 1976. Bacterial mutator genes and the control spontaneous mutations. Annu. Rev. Genet. **10**:135–156.
5. **Degnen, G. E., and E. C. Cox.** 1974. Conditional mutator gene in *Escherichia coli*: isolation, mapping, and effector studies. J. Bacteriol. **117**:477–487.
6. **DiFrancesco, R., S. K. Bhatnagar, A. Brown, and M. J. Bessman.** 1984. The interaction of DNA polymerase III and the product of the *Escherichia coli* mutator gene, *mutD**. J. Biol. Chem. **259**:5567–5573.
7. **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.
8. **Echols, H., C. Lu, and P. M. J. Burgers.** 1983. Mutator strains of *Escherichia coli*, *mutD* and *dnaQ*, with defective exonucleolytic editing by DNA polymerase III holoenzyme. Proc. Natl. Acad. Sci. USA **80**:2189–2192.
9. **Fersht, A. R., J. W. Knill-Jones, and W. C. Tsui.** 1982. Kinetic basis of spontaneous mutation: misinsertion frequencies, proofreading specificities and cost of proofreading by DNA polymerases of *Escherichia coli*. J. Mol. Biol. **156**:37–51.
10. **Fowler, R. G., G. E. Degnen, and E. C. Cox.** 1974. Mutational specificity of a conditional *Escherichia coli* mutator, *mutD5*. Mol. Gen. Genet. **133**:179–191.
11. **Fowler, R. G., R. M. Schaaper, and B. W. Glickman.** 1986. Characterization of mutational specificity within the *lacI* gene for a *mutD5* mutator strain of *Escherichia coli* defective in 3′→5′ exonuclease (proofreading) activity. J. Bacteriol. **167**: 130–137.
12. **Gabay, J., and K. Yasunaka.** 1980. Interaction of the *lamB* protein with the peptidoglycan layer in *Escherichia coli* K12. Eur. J. Biochem. **104**:13–18.

13. **Guild, W. R., and N. B. Shoemaker.** 1974. Intracellular competition for a mismatch recognition system and marker-specific rescue of transforming DNA from inactivation by ultraviolet irradiation. Mol. Gen. Genet. **128**:291–300.
14. **Herman, G. E., and P. Modrich.** 1981. *Escherichia coli* K-12 clones that overproduce *dam* methylase are hypermutable. J. Bacteriol. **145**:644–646.
15. **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.
16. **Kunkel, T. A.** 1988. Exonucleolytic proofreading. Cell **53**: 837–840.
17. **Lindahl, T., B. Demple, and P. Robins.** 1982. Suicide inactivation of the *E. coli* O^6-methylguanine-DNA methyltransferase. EMBO J. **1**:1359–1363.
18. **Marinus, M. G., and N. R. Morris.** 1975. Pleiotropic effect of a DNA adenine methylation mutation (*dam-3*) in *Escherichia coli* K12. Mutat. Res. **28**:15–26.
19. **Meselson, M., and R. Yuan.** 1968. DNA restriction enzyme from *E. coli*. Nature (London) **217**:1110–1114.
20. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. **Modrich, P.** 1987. DNA mismatch correction. Annu. Rev. Biochem. **56**:435–466.
22. **Monk, M., and J. Gross.** 1971. Induction of prophage 1 in a mutant of *E. coli* K12 defective in initiation of DNA replication at high temperature. Mol. Gen. Genet. **110**:299–306.
23. **Radman, M., C. Dohet, M.-F. Bourguignon, O. P. Doubleday, and P. Lecomte.** 1981. High fidelity devices in the reproduction of DNA, p. 431–445. *In* E. Seeberg, and K. Kleppe (ed.), Chromosome damage and repair. Plenum Publishing Corp., New York.
24. **Radman, M., and R. Wagner.** 1986. Mismatch repair in *Escherichia coli*. Annu. Rev. Genet. **20**:523–538.
25. **Schaaper, R. M.** 1988. Mechanisms of mutagenesis in the *Escherichia coli* mutator *mutD5*: role of DNA mismatch repair. Proc. Natl. Acad. Sci. USA. **85**:8126–8130.
26. **Schaaper, R. M.** 1989. *Escherichia coli* mutator *mutD5* is defective in the *mutHLS* pathway of DNA mismatch repair. Genetics **121**:205–212.
27. **Schwartz, M., and L. le Minor.** 1975. Occurrence of the bacteriophage lambda receptor in some enterobacteriaceae. J. Virol. **15**:679–685.
28. **Sevastopoulos, C. G., and D. A. Glaser.** 1977. Mutator action by *Escherichia coli* strains carrying *dnaE* mutations. Proc. Natl. Acad. Sci. USA. **74**:3947–3950.
29. **Tiraby, G., M. S. Fox, and H. Bernheimer.** 1975. Marker discrimination in deoxyribonucleic acid-mediated transformation of various *Pneumococcus* strains. J. Bacteriol. **121**:608–618.