JOURNAL OF BACTERIOLOGY, Mar. 1986, p. 1023–1025
0021-9193/86/031023-03$02.00/0
Copyright © 1986, American Society for Microbiology
Vol. 165, No. 3

Inviability of *dam recA* and *dam recB* Cells of *Escherichia coli* Is Correlated with Their Inability to Repair DNA Double-Strand Breaks Produced by Mismatch Repair

TZU-CHIEN V. WANG* AND KENDRIC C. SMITH

Department of Radiology, Stanford University School of Medicine, Stanford, California 94305

Received 20 September 1985/Accepted 24 December 1985

The molecular basis for the inviability of *dam-3 recA200*(Ts) and *dam-3 recB270*(Ts) cells was studied. The *dam-3 recA200*(Ts) cells were inviable in yeast extract-nutrient broth or in minimal medium at 42°C. Although the *dam-3 recB270*(Ts) cells were inviable in yeast extract-nutrient broth at 42°C, they were viable at 42°C in minimal medium, in which the high salt content suppresses the mutant phenotype caused by the *recB270*(Ts) mutation at 42°C. Under the growth conditions rendering *dam rec* cells inviable, the cells accumulated double-strand breaks in their DNA. Introduction of a *mutL* or *mutS* mutation restored the viability of *dam-3 recB270*(Ts) cells grown in yeast extract-nutrient broth at 42°C and eliminated the formation of DNA double-strand breaks in these cells. We conclude that the inability to repair DNA double-strand breaks produced by the mismatch repair process accounts for the inviability of the *dam recA* and *dam recB* cells.

The *dam* gene of *Escherichia coli* K-12 codes for DNA adenine methylase (10), which methylates -GATC- sequences in newly replicated daughter strands of DNA (5, 9). The *dam* mutant lacks DNA adenine methylase activity and displays a pleiotropic phenotype that includes increased spontaneous mutation rates, increased levels of recombination, and increased sensitivities to base analogs, alkylating agents, and UV radiation (2, 12). It has been suggested that DNA methylation may serve as one possible means of discriminating between parental strands (methylated) and daughter strands (unmethylated), so that the mismatch repair system can excise mismatched bases exclusively from the unmethylated DNA strands (see reference 15 for a review).

Double mutants of *dam recA*, *dam recB*, *dam lexA*, and *dam polA* are apparently inviable (11–13). However, the inviability of *dam recA* and *dam recB* cells can be suppressed by an additional mutation affecting mismatch repair, i.e., *mutH*, *mutL*, or *mutS* (4, 13), indicating that some action of the mismatch repair process is responsible for the lethal events in these double mutants. It has been suggested that DNA double-strand breaks may be produced in *dam* mutants by the operation of mismatch repair (4, 15). The formation of double-strand breaks in DNA is expected to be a lethal event in *recA* and *recB* cells, since these strains are deficient in the repair of DNA double-strand breaks (8, 19). Therefore, we have investigated whether the lethality of *dam rec* cells is due to an enhanced production of unrepairable DNA double-strand breaks and whether this production can be prevented by mutations that block mismatch repair.

The bacterial strains used in this work are listed in Table 1. The *recA200* and *recB270* mutants are temperature sensitive, i.e., they exhibit the Rec$^-$ phenotype at 42°C. Since a number of *E. coli* mutants are known to be inviable on complex growth medium but not on minimal growth medium (6, 17), the effect of growth medium on the viability of the *dam-3 recA200*(Ts) and *dam-3 recB270*(Ts) strains was examined. The cells were grown to logarithmic phase at 30°C in minimal medium (18) supplemented with histidine, leucine, arginine, proline, methionine, and threonine at 1 mM each and with thymine at 10 μg/ml. The ability of the cells to form colonies at 42 versus 30°C was tested on the same minimal medium solidified with 1.6% Noble agar (Difco Laboratories) and on 0.75% Difco yeast extract, 0.8% Difco nutrient broth, and 1.6% Difco Noble agar plates. When the colony-forming ability was assayed on yeast extract-nutrient broth agar, the *dam-3 recA200*(Ts) and *dam-3 recB270*(Ts) double mutants had a much reduced ability (10^3- to 10^4-fold) to form colonies at 42°C compared with those incubated at 30°C (Table 2). The *dam-3*, *recA200*(Ts), and *recB270*(Ts) single mutants had only a slightly reduced ability to form colonies at 42°C compared with those incubated at 30°C. These results confirm the observations of McGraw and Marinus (13) and indicate that *dam recA* and *dam recB* cells are inviable when grown in complex growth medium (brain heart infusion agar was used by these authors). However, when the colony-forming ability was assayed on minimal medium, we observed that only the *dam-3 recA200*(Ts) cells showed a reduced survival at 42 compared with 30°C; the *dam-3 recB270*(Ts) cells and the single mutants all had similar colony-forming ability at 42 and 30°C (Table 2).

A comparison of the composition of the two media suggested that the viability of *dam-3 recB270*(Ts) cells grown in minimal medium at 42°C may be due to the presence of salts, glucose, or both in the minimal medium; the absence of extra nutrients that are present in yeast extract-nutrient broth; or both. The addition of glucose to yeast extract-nutrient broth agar had no effect on inviability at 42°C, but the addition of NaCl at 0.1 M to yeast extract-nutrient broth agar was sufficient to restore the viability of *dam-3 recB270*(Ts) cells grown at 42°C. The presence of NaCl appeared to suppress the mutant phenotype caused by the *recB270*(Ts) mutation at 42°C, since the UV-radiation sensitivity of the *recB270*(Ts) mutant (SR1392) on yeast extract-nutrient broth agar at 42°C was suppressed by the presence of NaCl at 0.1 M (data not shown). Reducing the salt concentration of minimal medium to 1/10 of that normally used also rendered *dam-3 recB270*(Ts) cells inviable at 42°C (data not shown). These results suggest that the activity of exonuclease V, which contains the mutant *recB270*-protein subunit, is affected by

* Corresponding author.

TABLE 1. *E. coli* K-12 strains used[a]

Stanford radiology no.	Genotype	Source or derivation (strain no.)
SR1389	F^- *dam-3 sup-85*(Am)	M. G. Marinus (GM33)
SR1390	F^- *recA200*(Ts) *leuB6 proC32 metE70 hisF860 thyA54 thi-1 lacZ36 xyl-5 ara-14 mtl-1 rpsL109 tsx azi ton*	M. G. Marinus (GM821)
SR1391	F^- *recA200*(Ts) *dam-3 leuB6 proC32 metE70 hisF860 thyA54 thi-1 lacZ36 ara-14 mtl-1 tsx azi ton*	M. G. Marinus (GM84)
SR1392	F^- *recB270*(TS) *leuB6 his-6 metB1 argG6 lacX1 malA1 xyl-7 mtl-2 gal-6 tonA2 rpsL104 sup-59*	M. G. Marinus (GM868)
SR1393	F^- *recB270*(Ts) *dam-3 leuB6 thr-1 proA2 metB1 lacY1 galK2 ara-14 thi-1 thyA21 deoB14 tsx-33 supE44*	M.G. Marinus (GM56)
SR1689	F^- *mutS215*::Tn*10 thy metB1 lacY14*	R. Fowler (ES1481)
SR1690	F^- *mutL218*::Tn*10 tryA78 his leu arg thr*	R. Fowler (KD1073)
SR1704	As SR1393, but *mutS215*::Tn*10*	SR1393 × Plvira.SR1689, select Tc^r
SR1705	As SR1393, but *mutL218*::Tn*10*	SR1393 × Plvira.SR1690, select Tc^r

[a] Genotype symbols are those used by Bachmann (1).

the salt concentration, as are some other mutant enzymes in temperature-sensitive mutants (7, 16). The inviability of *dam-3 recB270*(Ts) cells at 42°C on yeast extract-nutrient broth agar can be suppressed by an additional *mutL* or *mutS* mutation (Table 2), confirming the earlier observations that the inviability of *dam rec* cells can be suppressed by an additional mutation affecting mismatch repair (4, 13).

Next we investigated whether DNA double-strand breaks were produced in *dam* mutants. When the cells were incubated at 42°C in minimal medium, very few DNA double-strand breaks were detected in *dam*, *recB*, *dam recB*, and *dam recB mutL* cells over a period of 4 h (Fig. 1). However, when the cells were incubated at 42°C in yeast extract-nutrient broth, the *dam recB* cells accumulated double-strand breaks in their DNA, but the *dam* and *recB* single mutants and the *dam recB mutL* triple mutant did not (Fig. 1). The *dam recA* cells, whether incubated in yeast extract-nutrient broth or in minimal medium, accumulated DNA double-strand breaks at 42°C, and this accumulation was accompanied by extensive DNA degradation, i.e., greater than 60% of the ^{3}H-labeled DNA was degraded to acid-soluble products by 3 h of incubation at 42°C (data not shown). Therefore, under the growth conditions that rendered the *dam rec* double mutants inviable, they accumulated DNA double-strand breaks. These results suggest that it is the formation of DNA double-strand breaks plus the failure to repair these breaks that accounts for the inviability in *dam rec* cells.

TABLE 2. Effect of growth medium on colony-forming ability of *dam-3 recA200*(Ts) and *dam-3 recB270*(Ts) strains[a]

Strain	Ratio of CFU at 42°C/CFU at 30°C in: Minimal medium	Yeast extract-nutrient broth
dam-3	0.96	0.50
recA200(Ts)	0.97	0.71
dam-3 recA200(Ts)	0.0017	0.00033
recB270(Ts)	0.96	0.70
dam-3 recB270(Ts)	1.0	0.00054
dam-3 recB270(Ts) *mutL218*::Tn*10*	0.92	0.90
dam-3 recB270(Ts) *mutS215*::Tn*10*	0.99	1.0

[a] Cells were grown in minimal medium at 30°C to logarithmic phase, diluted, and plated on minimal medium and yeast extract-nutrient broth plates. One set of plates was incubated at 30°C, and the other was plated at 42°C for 24 to 48 h before being scored for CFU. All cultures had the same CFU on the two media when incubation was at 30°C.

The formation of DNA double-strand breaks in *dam* mutants can be attributed to the mismatch repair process,

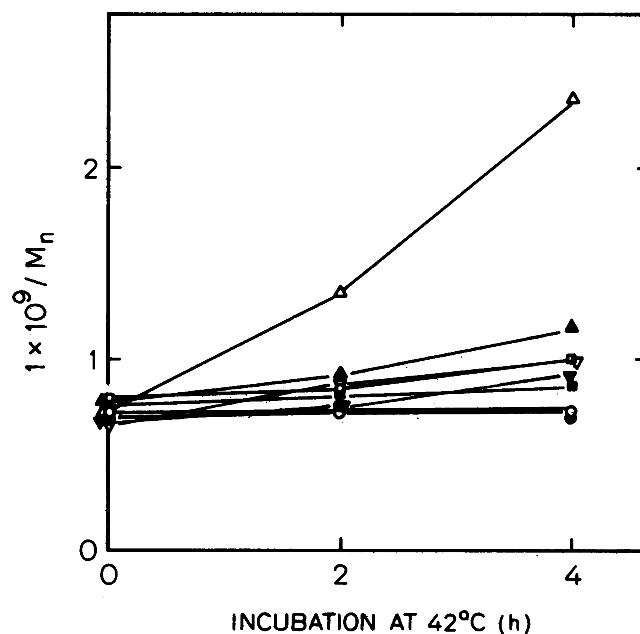

FIG. 1. Formation of double-strand breaks in the DNA of *dam-3* and *recB270*(Ts) mutants. Cells were grown in minimal medium at 30°C and labeled with [^{3}H]thymidine at 20 μCi/ml for 2 h. The ^{3}H-labeled cells were harvested by filtration, washed with phosphate buffer, and suspended in either minimal medium (solid symbols) or yeast extract-nutrient broth (open symbols). Then the cells were incubated at 42°C for different lengths of time before they were converted to spheroplasts and lysed on neutral sucrose gradients for the analysis of DNA double-strand breaks (19). The M_ns of DNAs were calculated relative to a bacteriophage T2 DNA marker (3). An increase in the values of $1/M_n$ during incubation at 42°C indicates the presence of DNA double-strand breaks. Symbols: ● and ○, *dam-3* (SR1389); ■ and □, *recB270*(Ts) (SR1392); ▲ and △, *dam-3 recB270*(Ts) (SR1393); and ▼ and ▽, *dam-3 recB270*(Ts) *mutL218*::Tn*10* (SR1705). Data for strains SR1389 and SR1392 are from one experiment, and data for strains SR1393 and SR1705 are the average of two experiments. The data for strain SR1704 [*dam-3 recB270*(Ts) *mutS215*::Tn*10*] (not shown) are similar to those for SR1705.

since mutations in *mutL* and *mutS* suppress the accumulation of DNA double-strand breaks (Fig. 1) and the inviability of *dam recB* cells (Table 2). Radman and Wagner (15) suggested that double-strand breaks should be produced by the mismatch repair system operating on the unmethylated DNA of *dam* cells either as a result of simultaneous attack on both strands at a single mismatch or as a result of overlapping excision events on opposite strands at two relatively close mismatches. Alternatively, when a gap produced by mismatch excision is not promptly repaired, it may expose the single-stranded DNA opposite the gap to attack by single-strand DNA endonucleases, thereby producing a DNA double-strand break, as we have observed for unrepaired DNA daughter strand gaps (19, 21).

Another factor determining the viability of *dam* mutants is the ability to repair DNA double-strand breaks, which is known to require functional *recA* (8), *recB* (19), and *recN* (14) genes. The failure to detect an accumulation of DNA double-strand breaks in the *dam* single mutant (Fig. 1) and the fact that *dam* cells are viable suggest that the infrequent DNA double-strand breaks produced in *dam* mutants are repaired. In support of this conclusion is the observation that the inviability of *dam recB(C)* cells can also be suppressed by an *sbcB* mutation (13). The *sbcB* mutation has been shown to restore the ability of *recB recC* cells to repair DNA double-strand breaks (20).

We thank M. G. Marinus and R. Fowler for providing bacterial strains used, Neil J. Sargentini and Rakesh C. Sharma for valuable discussions, and Carmencita Nicolas for technical assistance.

This work was supported by Public Health Service grant CA-02896 awarded by the National Cancer Institute, U.S. Department of Health and Human Services.

LITERATURE CITED

1. **Bachmann, B. J.** 1983. Linkage map of *Escherichia coli* K-12, edition 7. Microbiol. Rev. **47**:180–230.
2. **Bale, A., M. D'Alarcao, and M. G. Marinus.** 1979. Characterization of DNA adenine methylation mutants of *Escherichia coli* K12. Mutat. Res. **59**:157–165.
3. **Bonura, T., C. D. Town, K. C. Smith, and H. S. Kaplan.** 1975. The influence of oxygen on the yield of DNA double-strand breaks in X-irradiated *Escherichia coli* K-12. Radiat. Res. **63**:567–577.
4. **Glickman, B. W., and M. Radman.** 1980. *Escherichia coli* mutator mutants deficient in methylation-instructed DNA mismatch correction. Proc. Natl. Acad. Sci. USA **77**:1063–1067.
5. **Hattman, S., T. Keister, and A. Gottehrer.** 1978. Sequence specificity of DNA methylases from *Bacillus amyloliquefaciens* and *Bacillus brevis*. J. Mol. Biol. **124**:701–711.
6. **Joyce, C. M., and N. D. F. Grindley.** 1984. Method for determining whether a gene of *Escherichia coli* is essential: application to the *polA* gene. J. Bacteriol. **158**:636–643.
7. **Kohno, T., and J. Roth.** 1979. Electrolyte effects on the activity of mutant enzymes *in vivo* and *in vitro*. Biochemistry **18**:1386–1392.
8. **Krasin, F., and F. Hutchinson.** 1977. Repair of DNA double-strand breaks in *Escherichia coli*, which requires *recA* function and the presence of a duplicate genome. J. Mol. Biol. **116**:81–98.
9. **Lacks, S. A., and B. Greenberg.** 1977. Complementary specificity of restriction endonucleases of *Diplococcus pneumoniae* with respect to methylation. J. Mol. Biol. **114**:153–168.
10. **Marinus, M. G.** 1973. Location of DNA methylation genes on the *Escherichia coli* K-12 genetic map. Mol. Gen. Genet. **127**:47–55.
11. **Marinus, M. G., and N. R. Morris.** 1974. Biological function for 6-methyladenine residues in the DNA of *Escherichia coli* K12. J. Mol. Biol. **85**:309–322.
12. **Marinus, M. G., and N. R. Morris.** 1975. Pleiotropic effects of a DNA adenine methylation mutation (*dam-3*) in *Escherichia coli* K12. Mutat. Res. **28**:15–26.
13. **McGraw, B. R., and M. G. Marinus.** 1980. Isolation and characterization of Dam$^+$ revertants and suppressor mutations that modify secondary phenotypes of *dam-3* strains of *Escherichia coli* K-12. Mol. Gen. Genet. **178**:309–315.
14. **Picksley, S. M., P. V. Attfield, and R. G. Lloyd.** 1984. Repair of DNA double-strand breaks in *Escherichia coli* K12 requires a functional *recN* product. Mol. Gen. Genet. **195**:267–274.
15. **Radman, M., and R. Wagner.** 1984. Effects of DNA methylation on mismatch repair, mutagenesis, and recombination in *Escherichia coli*. Curr. Top. Microbiol. Immunol. **108**:23–28.
16. **Tessman, E. S., and P. K. Peterson.** 1982. Suppression of the *ssb-1* and *ssb-113* mutations of *Escherichia coli* by a wild-type *rep* gene, NaCl, and glucose. J. Bacteriol. **152**:572–583.
17. **Torrey, T. A., T. Atlung, and T. Kogoma.** 1984. *dnaA* suppressor (*dasF*) mutants of *Escherichia coli* are stable DNA replication (*sdrA/rnh*) mutants. Mol. Gen. Genet. **196**:350–355.
18. **Wang, T. V., and K. C. Smith.** 1981. Effect of *recB21*, *uvrD3*, *lexA101* and *recF143* mutations on ultraviolet radiation sensitivity and genetic recombination in *uvrB* strains of *Escherichia coli*. Mol. Gen. Genet. **183**:37–44.
19. **Wang, T. V., and K. C. Smith.** 1983. Mechanisms for *recF*-dependent and *recB*-dependent pathways of postreplication repair in UV-irradiated *Escherichia coli uvrB*. J. Bacteriol. **156**:1093–1098.
20. **Wang, T. V., and K. C. Smith.** 1985. Mechanism of *sbcB*-suppression of the *recBC*-deficiency in postreplication repair in UV-irradiated *Escherichia coli* K-12. Mol. Gen. Genet. **201**:186–191.
21. **Wang, T. V., and K. C. Smith.** 1986. Postreplicational formation and repair of DNA double-strand breaks in UV-irradiated *Escherichia coli uvrB* cells. Mutat. Res. **165**:39–44.