reca Gene of Escherichia coli Complements Defects in DNA Repair and Mutagenesis in Streptomyces fradiae JS6 (mcr-6)

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Streptomyces fradiae JS6 (mcr-6) is a mutant which is defective in repair of DNA damage induced by a variety of chemical mutagens and UV light. JS6 is also defective in error-prone (mutagenic) DNA repair (J. Stonesifer and R. H. Baltz, Proc. Natl. Acad. Sci. USA 82:1180–1183, 1985). The reca gene of Escherichia coli, cloned in a bifunctional vector that replicates in E. coli and Streptomyces spp., complemented the mutation in S. fradiae JS6, indicating that E. coli and S. fradiae express similar SOS responses and that the mcr-6 gene product of S. fradiae is functionally analogous to the protein encoded by the reca gene of E. coli.

We are interested in understanding the fundamental mechanisms of mutation, DNA repair, and recombination in Streptomyces fradiae, a filamentous actinomycete that produces the macrolide antibiotic tylosin (4, 5). To aid these studies, we have isolated mutants of S. fradiae M1 which were defective in repair of damage induced by mitomycin C (MC) and characterized their responses to treatment with a variety of mutagenic agents (2, 3, 6, 25). One mutant, JS6 (mcr-6), is defective in the repair of potentially lethal damage induced by a variety of agents, including MC, UV light, 4-nitroquinoline-1-oxide (NQO), methyl methanesulfonate (MMS), hydroxyamine (HA), and N-methyl-N-nitro-N-nitrosoguanidine (MNG), and is less mutable by MNG, MMS, NQO, UV light, HA, and ethyl methanesulfonate (EMS) than is the wild-type strain (2, 25). A spontaneous MC-resistant mutant of JS6 expresses normal levels of resistance to all of the agents and shows wild-type levels of induced mutagenesis, indicating that a single mutation causes the multiple defects in JS6 (25). The mcr-6 gene product thus appears to control an SOS response in S. fradiae and to be functionally analogous to the RecA protein in Escherichia coli (28–30) and the RecE protein in Bacillus subtilis (24, 32).

One difference in the error-prone DNA repair systems expressed in S. fradiae and in E. coli or Salmonella typhimurium is that S. fradiae JS6 (mcr-6) is 10- to 100-fold less mutable by EMS than is its parent strain M1 (2, 25), whereas E. coli and S. typhimurium reca mutants express normal levels of EMS-induced mutagenesis (8, 12, 24). The dependence of EMS mutagenesis on an error-prone repair system suggested that S. fradiae expresses repair avoidance mechanisms more stringent than those observed in E. coli and similar to those expressed in the lower eucaryote Saccharomyces cerevisiae (25).

In B. subtilis, reca mutants display multiple defects in DNA repair, recombination, and mutagenesis similar to those observed in E. coli reca mutants (15, 16, 32). Like S. fradiae JS6 (mcr-6), B. subtilis reca mutants show a reduced level of induced mutagenesis by EMS (32). The multiple defects in a B. subtilis reca mutant have been complemented by a cloned reca gene from E. coli (16), indicating that the reca gene of E. coli and the recaE gene of B. subtilis encode analogous proteins. Since the reca promoter from E. coli functions in Streptomyces lividans (7), we surmised that the cloned reca gene of E. coli might complement the mcr-6 mutation in S. fradiae if the mcr-6 mutation was located in an analogous gene. To explore this possibility, we cloned the reca gene of E. coli into a vector which was bifunctional for E. coli and Streptomyces spp. and transformed S. fradiae JS6 (mcr-6). Plasmid pJC859, a derivative of pBR322 containing the reca gene of E. coli inserted on a 3.3-kilobase pair BamHI fragment originally cloned in pBEUI4 (27), was isolated from E. coli JC14773 by the method of Kieser (13), cut with BamHI, and ligated (17) with BamHI-digested pOJ160, a bifunctional vector containing the pUC19 origin of replication for E. coli and the SCP2* origin of replication for Streptomyces spp. (20; R. Stanzak and B. E. Schoner, manuscript in preparation). The ligation mix was used to transform E. coli JM109 (31) to apramycin resistance (23). Plasmid pRHB300 containing the appropriately sized BamHI fragment inserted in pOJ160 was transformed into S. fradiae PM73, a mutant defective in several restriction systems (19), by the method of Matsushima and Baltz (18). One transformant containing an appropriately sized plasmid was retained, and DNA was isolated and transformed into E. coli HB101 (recA13). pJC859 was also transformed into HB101, and transformants containing pRHB300 or pJC859 were tested for restoration of resistance to the lethal effects of UV light as previously described (17). Both transformants were resistant to UV light, whereas HB101 was sensitive, indicating that pRHB300 contained a functional reca gene. Plasmid pRHB300 isolated from PM73 was transformed into S. fradiae JS6, and several normally growing and slow-growing colonies were obtained. All of the normally growing colonies contained plasmids smaller than pRHB300, whereas the slow-growing colonies contained appropriately sized plasmid DNA. One transformant (PM74) that grew relatively slowly and contained pRHB300 was retained. (Presumably, the expression of the reca gene in PM74 caused the poor growth, but this theory has not been further studied.) Since JS6 is defective in the repair of damage induced by MC, UV light, and MMS (25), we determined the levels of resistance to these agents in PM74, JS6, and M1. PM74 was substantially more resistant to the potentially lethal effects of MMS than was JS6, but it was not as resistant as the wild-type strain M1 (Fig. 1). PM74 was completely resistant.

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FIG. 1. Inactivation of *S. fradiae* M1 (○), JS6 (△), and PM74 (□) by MMS and MC. Cells were grown in tryptic soy broth, homogenized and sonicated to obtain single cells as previously described (1), treated with MMS or MC, and plated on AS-1 agar as previously described (3, 25).

FIG. 2. Inactivation of *S. fradiae* M1 (○), JS6 (△), and PM74 (□) by UV light. Cells were grown in tryptic soy broth, homogenized and sonicated as previously described (1), treated with UV light, and plated on AS-1 agar as previously described (3, 25).

FIG. 3. Inactivation and mutagenesis to spectinomycin resistance of *S. fradiae* M1 (○), JS6 (△), and PM74 (□) by EMS. Cells were grown in tryptic soy broth, homogenized and sonicated as previously described (1), and treated with EMS as previously described (3, 25). Surviving fractions and mutagenesis to spectinomycin resistance after segregation were determined as previously described (3, 25).

potentially lethal damage due to small lesions (MMS induced) and bulky lesions (UV light and MC induced). The *E. coli* RecA protein contains recombinase and protease activities (28, 29) and exerts its control of DNA repair pathways in *E. coli* first by becoming activated in response to treatment of cells with a mutagenic agent and then by participating in the proteolytic cleavage of LexA repressors. Thus, our data suggest that the genes encoding certain enzymatic pathways for repair of small and bulky lesions are present but not efficiently expressed in JS6 and that the RecA protein is able to induce them, presumably by participating in proteolytic cleavage of a protein repressor analogous to the LexA protein (28, 30).

Since EMS mutagenesis is mediated by an error-prone DNA repair pathway in *S. fradiae* (2, 25), and since efficient error-prone DNA repair in *E. coli* requires activated RecA protein to induce the umuCD system by cleavage of the LexA repressor and to participate directly in the process (9, 25, 28, 30), we also measured EMS-induced mutagenesis in PM74, JS6, and M1. It has been shown previously that comparable doses of EMS give 10- to 100-fold lower frequencies of spectinomycin-resistant mutants in JS6 than in M1 (2, 25). PM74 yielded a much higher frequency of induced mutants than did JS6, nearly equivalent to those obtained in M1 (Fig. 3).

The results of our study indicate that multiple defects in DNA repair and mutagenesis in *S. fradiae* JS6 (mcr-6) can be complemented by the recA gene of *E. coli* and that the mcr-6 mutation, therefore, is located in a gene analogous to the recA gene of *E. coli* and the recE gene of *B. subtilis*. These results support the notion that the functions of the recA gene in regulation of DNA repair and induced mutagenesis (parts of the SOS response) may be highly conserved within procaryotes (10, 11, 14, 16, 21, 22, 28). Our data also imply that the *E. coli* RecA protein is activated by an inducing signal in *S. fradiae* and participates in the cleavage of an analog of the *E. coli* LexA protein (28, 29) to induce an SOS response in *S. fradiae*.
NOTES

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LITERATURE CITED


