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Comparison of the *rep-38* and *mmrA1* mutations of *Escherichia coli*

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Summary

The *rep-38* and *mmrA1* mutations are located very close to each other (~ 85 min), and have been suggested to be allelic. To address this question we have compared the phenotypes of the *mmrA1* and *rep-38* mutants. Both the *mmrA1* and *rep-38* mutations blocked the enhanced killing and inhibition of postreplication repair by rich growth medium that occurs in UV-irradiated *Escherichia coli* K-12 *uvrA* cells, i.e., the *mmrA1* and *rep-38* strains did not show minimal medium recovery (MMR). However, ΦX174 bacteriophage propagated well in *mmrA1* strains, but not in *rep-38* strains; a *rep* mutation sensitized a *uvrA* strain to UV irradiation, but a *mmrA* mutation did not. During chloramphenicol treatment, the *rep-38* strain showed a larger amount of residual DNA synthesis than observed in the *mmrA1* strain. The *mmrA1* mutation appears to be a dominant mutation. This was determined by the failure of either plasmid pLC44-7 or episome F′KLF11, both of which carry the *mmrA*$^+$ gene, to complement the Mmr$^-$ phenotype of a *uvrA mmrA* strain. Plasmid pLC44-7 is known to complement the *rep-38* mutation, suggesting that *rep-38* is a recessive mutation. Although certain of the phenotypes of the *rep* and *mmrA* mutants are similar, a number are quite different. These differences suggest that these two mutations are not allelic.

The survival of UV-irradiated *uvrA* strains of *Escherichia coli* K-12 is higher when the cells are plated on minimal medium (MM) rather than on rich growth medium (e.g., yeast extract-nutrient broth; YENB) (Ganesan and Smith, 1968a; Sharma et al., 1982). This phenomenon has been referred to as 'minimal medium recovery' (MMR) (Ganesan and Smith, 1968b; Smith, 1971), and is due to the partial inhibition of postreplication repair in UV-irradiated *uvrA* strains by rich growth medium (Sharma et al., 1982; Sharma and Smith, 1986).

Recently we have isolated and mapped a mutation (*mmrA1*), which blocks the detrimental effects of rich growth medium on survival and on postreplication repair in UV-irradiated *uvrA* cells (Sharma et al., 1983). Originally, the *mmrA* gene was located at 84.3 min on the genetic linkage map of *E. coli* K-12 (Sharma et al., 1983). However, consistent with the most recent modification

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of the *E. coli* K-12 linkage map (Bachmann, 1983), we now conclude that the *mmrA1* mutation maps at 84.8 min, very close to the *rho* gene. However, *mmrA* is not an allele of the *rho* gene. This is supported by the fact that the *rho ssb-113* double mutant is nonviable (Fassler et al., 1985), and we have constructed a *mmrA1 ssb-113* double mutant (unpublished data).

The *rep* gene is located (at 84.7 min) very close to the *mmr* gene on the *E. coli* K-12 linkage map (Fig. 1). The similarities in map positions raised the possibility that *mmrA1* may be an allele of the *rep* gene (Bialkowska-Hobrzanska and Denhardt, 1984).

To address this question, we have made a comparative study of the phenotypes of the *mmrA1* and *rep-38* mutants. Our earlier studies showed that the rich growth-medium killing of UV-irradiated *uvrA* cells correlated with the lack of an abrupt inhibition of DNA synthesis in rich growth medium and, furthermore, the slowing of DNA synthesis immediately after UV irradiation (e.g., by growth in the presence of valine) seemed to help UV-irradiated *uvrA* cells to do more postreplication repair (Sharma and Smith, 1985). The presence of a *rep* mutation is known to reduce the rate of DNA replication fork movement (Lane and Denhardt, 1974). On the basis of these observations, we hypothesized that the *uvrA rep* strain, having a slower rate of fork movement, would not show enhanced killing that is observed for UV-irradiated *uvrA* cells plated on rich growth medium (Sharma et al., 1982). To test this, we have used isogenic *uvrA* and *uvrA rep* strains, which were grown as described earlier (Sharma and Smith, 1986). After UV irradiation, *uvrA* cells showed a higher survival on MM than on YENB (i.e., they showed MMR, Fig 2A), however, this media effect was not observed for *uvrA rep* cells (i.e., the survival of MM-grown, UV-irradiated

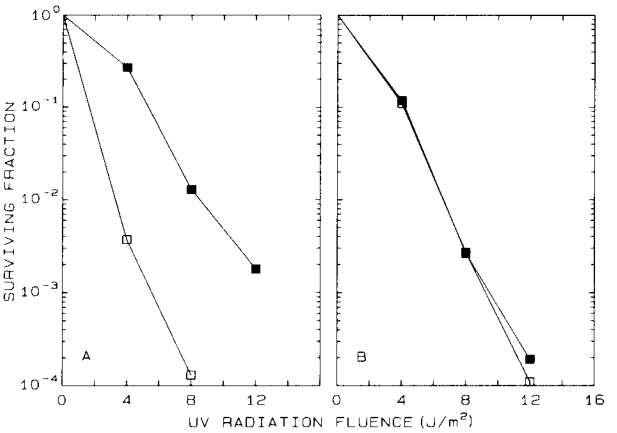

Fig. 2. Effect of plating medium on the UV-radiation survival of *E. coli* cells that were grown to logarithmic phase in MM, UV irradiated in MM, and plated on MM (■) and on YENB (□). (A) Strain SR1687 (*uvrA*); (B) strain SR1569 (*uvrA rep*). Results are the average of 2 Expts.

uvrA rep cells was the same on MM and YENB plates, Fig. 2B). Consistent with earlier data (Denhardt et al., 1967), the *rep* mutation sensitized *uvrA* cells to UV irradiation (compare Fig. 2A and B). However, the presence of the *mmrA1* mutation did not reduce the survival of UV-irradiated *uvrA* cells plated on MM (Sharma et al., 1983).

Rich growth medium has been shown to inhibit the repair of DNA daughter-strand gaps in UV-irradiated *uvrA* cells (Sharma et al., 1983), but the presence of the *mmrA1* mutation increases the repair of DNA daughter-strand gaps in rich growth medium (Sharma et al., 1983). Therefore, we tested whether the presence of a *rep* mutation would also eliminate the inhibition of the repair of DNA daughter-strand gaps by rich growth medium. MM-grown *uvrA* and *uvrA rep* cells were UV irradiated (2 J/m^2), pulse-labeled with [^{3}H]thymidine for 10 min, and then were incubated either in non-radioactive MM or YENB for 120 min at

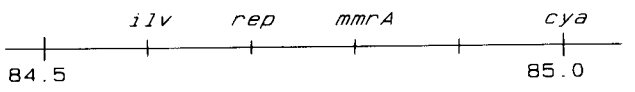

Fig. 1. A physical map of the *mmrA* and *rep* region of the *E. coli* K-12 chromosome. The map locations of genes shown are taken from Bachmann (1983).

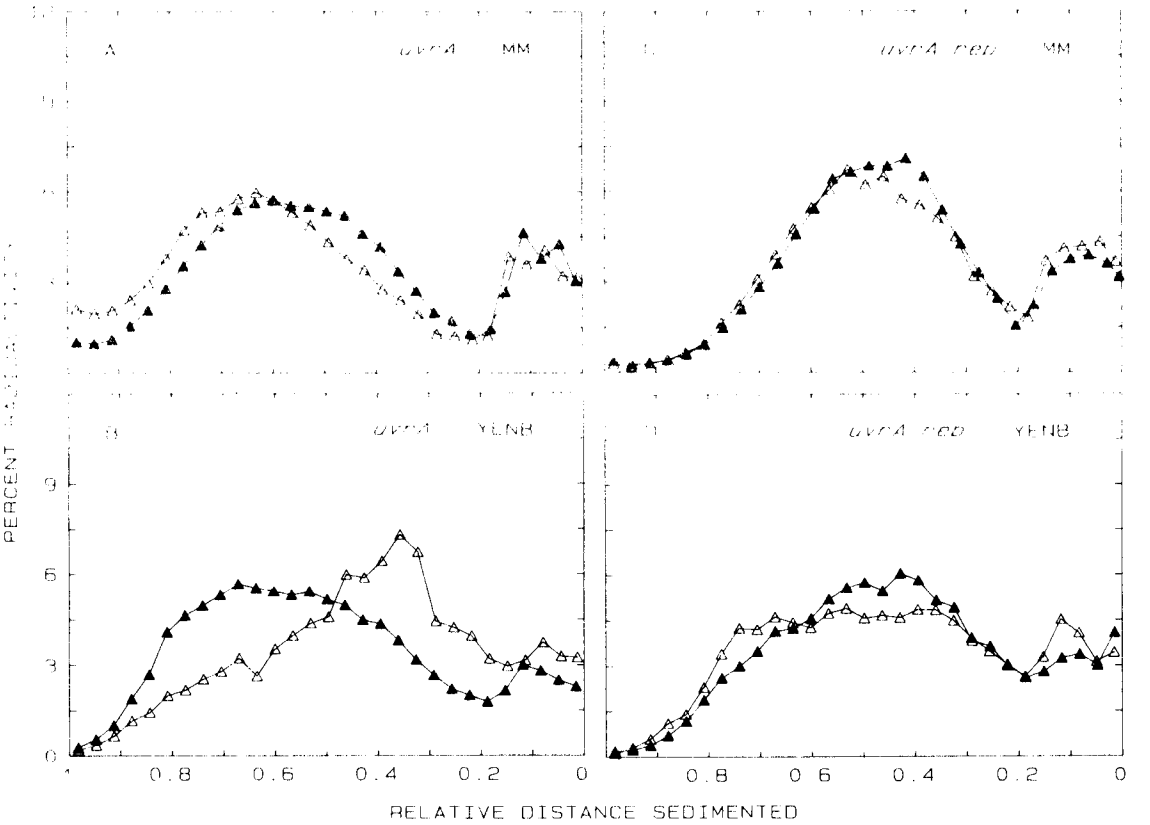

Fig. 3. Effect of rich growth medium on the repair of DNA daughter-strand gaps in UV-irradiated *E. coli* cells. Cells were grown to logarithmic phase in MM, UV irradiated (2 J/m^2) in MM, and pulse-labeled with [^{3}H]thymidine for 10 min in MM. Immediately after pulse-labeling, cells were allowed to repair in MM (A, C, open symbols) or in YENB (B, D, open symbols) for 120 min at 37 °C. For experimental details, see Sharma and Smith (1986). Unirradiated cells were treated similarly (closed symbols). (A, B) Strain SR1687 (*uvrA*); (C, D) strain SR1569 (*uvrA rep*). The DNA sedimentation profiles from alkaline sucrose gradients are from a representative experiment.

37 °C. The *uvrA* cells that were incubated in MM showed the complete repair of DNA daughter-strand gaps (Fig. 3A). However, *uvrA* cells that were incubated in YENB showed a large deficiency in the repair of DNA daughter-strand gaps (Fig. 3B). In contrast, the *uvrA rep* cells showed no effect of postirradiation-incubation medium on the repair of DNA daughter-strand gaps (compare Fig. 3C and D). These results for DNA repair are consistent with the UV-radiation survival data shown in Fig. 2, and indicate that a mutation in the *rep* gene, which is known to reduce the rate of DNA replication fork movement, allows UV-irradiated *uvrA* cells to perform more postreplication repair when incubated in rich growth medium. This is consistent with our earlier observation that the slowing down of DNA synthesis by growth in the presence of valine allows UV-irradiated *uvrA* cells to do more post-replication repair (Sharma and Smith, 1985).

The *rep* mutants of *E. coli* are unable to support the growth of bacteriophage ΦX174 (Denhardt et al., 1967). Therefore, the *uvrA mmrA* strain was checked for this Rep phenotype using bacteriophage ΦX174 (Denhardt et al., 1972). Bacteriophage ΦX174 grew well on both *uvrA* and *uvrA mmrA* strains, but not on the *uvrA rep* strain (data not shown).

The *rep* mutant showed a larger amount of residual DNA synthesis than did the wild-type strain, when initiation of new rounds of replication at *oriC* was blocked by chloramphenicol

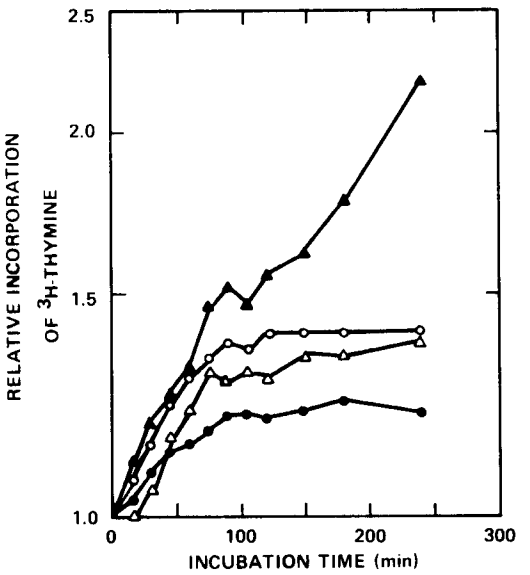

Fig. 4. Effect of chloramphenicol treatment on DNA synthesis kinetics in *E. coli* cells. DNA was labeled by growing cells in MM containing 2 μCi/ml of [^{3}H]thymine. Chloramphenicol (200 μg/ml, final concentration) was added to the cultures at time zero and DNA synthesis was followed. ○, SR1097 (*uvrA*); ●, SR1098 (*uvrA mmrA*); △, SR1687 (*uvrA*); ▲, SR1569 (*uvrA rep*). The data are from a representative experiment.

treatment (Lane and Denhardt, 1974). This suggests that the chromosome of the *rep* mutant has a larger number of replication forks (Lane and Denhardt, 1974). To find out if the chromosome of the *mmrA* mutant has more growing forks than does the chromosome of the wild-type strain, the DNA of *uvrA, uvrA mmrA* and *uvrA rep* cells was prelabeled as described earlier (Sharma and Smith, 1985). Chloramphenicol (200 μg/ml, final concentration) was added to the cultures in radioactive minimal medium, and the kinetics of DNA synthesis was followed (Fig. 4). After 90 min of chloramphenicol treatment, the *uvrA rep* and *uvrA* strains showed 52% and 27% increases in the DNA content, respectively. These results are consistent with the earlier observation showing the higher amount of residual DNA synthesis in the *rep* strain (Lane and Denhardt, 1974). In fact, after about a 20-min lag in DNA synthesis after 90 min, the *uvrA rep* strain resumed DNA synthesis at about the normal rate (tested up to 240 min). This suggests that the *uvrA rep* strain may be constitutive for stable DNA replication (Kogoma and Lark 1975). However, after a similar chloramphenicol treatment, the *uvrA mmrA* and *uvrA* strains showed 21% and 37% increases in DNA content, respectively. The smaller amount of residual DNA synthesis that is observed in the *mmrA* strain suggests that the chromosome of the *mmrA* strain has fewer active replication forks than does the *rep* strain.

Complementation of the *rep-38* mutation by plasmid pLCL44-7 (Clarke and Carbon, 1979) suggests that *rep-38* is a recessive mutation (Bialkowska-Hobrzanska and Denhardt, 1984). Plasmid pLC44-7, which complements mutations in the *ilv GEDAC* (84.6 min), *rep* (84.7 min) and *cya* (85.0 min) genes (Bialkowska-Hobrzanska and Denhardt, 1984; Neidhardt et al., 1983), should also carry the *mmr* gene (84.4 min). The plasmid was isolated from strain SR1313 as described by Holmes and Quigley (1981). Strain SR1313 and other strains used in this study are listed in Table 1. Plasmid DNA was used to transform strain SR1293 (*uvrA6 mmrA1 ilv*::Tn*5*) according to the method of Mandel and Higa (1970), and the transformants were selected for isoleucine–valine prototrophy (Ilv$^+$) on minimal medium plates. The recipient strain (SR1293) has a kanamycin-resistance (Km^r) marker inserted into the *ilv* gene; therefore the purified transformants that became Ilv$^+$ were screened for Km^r. The transformants that were Ilv$^+$ Km^r were considered to carry plasmid pLC44-7. 24 transformants were then screened for the Mmr phenotype, all were found to be Mmr$^-$ (i.e., the UV-radiation survival of these strains was the same on MM or YENB plates) (data not shown). These results suggest that the *mmrA1* mutation is dominant.

To further test this conclusion, F′KLF11, which carries the *mmrA* locus and flanking regions of about 3 and 4.2 min (Bachmann and Low, 1980; Bachmann, 1983), was transferred by mating strain SR601 (F′KLF11) with strain SR1293 [*uvrA6 mmrA1 ilv*::Tn*5* (Km^r)]. The Ilv$^+$ colonies were cloned and screened for Km^r. The Ilv$^+$ Km^r merodiploids were screened for the Mmr phenotype. All of the 9 merodiploids tested were found to be Mmr$^-$ (data not shown), suggesting that *mmrA1* is a dominant mutation. In order to rule

TABLE 1

LIST OF STRAINS OF *E. coli* [a]

Strain No.	Relevant genotype	Other characteristics	Source, derivation, reference
SR349	*uvrA6*	*thyA36 deo(C2?) leuB19 metE70 lacZ53 rha-5 bioA2 rpsL151*	Youngs and Smith (1978)
SR601	*recA1*	F′KLF11/*argG his leu metB gal lacY malA mtl xyl tonA tsx rpsL supE*	KLF11/JC1553, ECGSC
SR960	+	*ilvA700*::Tn*5 thyA deo* λ^r	K.J. Shaw (CBK007)
SR1098	*uvrA6 mmrA1*	*thyA36 deo(C2?) leuB19 lacZ53 rha-5 bioA2 rpsL151*	Sharma et al. (1983)
SR1178	*uvrA6*	*ilvA700*::Tn*5 thyA36 deo (C2?) leuB19 metE70 lacZ53 rha-5 bioA2 rpsL151*	P1::Tn*9c*ts·SR960 ×SR349; select Km^r
SR1293	*uvrA6 mmrA1*	*ilvA700*::Tn*5 thyA36 deo (C2?) leuB19 lacZ53 rha-5 bioA2 rpsL151*	P1::Tn*9c*ts·SR960 ×SR1098; select Km^r
SR1313	+	pLC44-7 (*ilv*$^+$ *trxA*$^+$ *rep*$^+$)/*argH his*-4 *lacMS*286 *Φ80dII lacBK1 malA1 mtl-1 xyl-7 rpsL supE44*	S.R. Kushner (SK3968)
SR1569	*uvrA rep-38(am)*	*thy*(Ts) Su^- ΦX174^r $P2^r$	Bialkowska-Hobrzanska et al. (1985) (D94)
SR1687	*uvrA*	*thy*(Ts) Su^- ΦX174^s $P2^s$	Bialkowska-Hobrzanska et al. (1985) (D92)

[a] Genotype symbols are those used by Bachmann (1983). All strains are F^- and λ^- unless noted. Km^r means that cells became resistant to kanamycin. ECGSC is the *E. coli* Genetic Stock Center. Strains SR1569 and SR1687 are derivatives of *E. coli* C (D94 = HF4704).

out the possibility that F′KLF11 carries a mutant allele of the *mmr* gene, a similar conjugation experiment was performed using *uvrA6* (SR1178) as the recipient strain. All of the 10 merodiploids screened were Mmr^+ (data not shown), suggesting that F′KLF11 does carry the wild-type *mmr* gene.

In summary, although certain of the phenotypes of the *rep* and *mmrA* mutants are similar, a number are quite different. These differences suggest that these two mutations are not allelic.

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