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Genetic and Phenotypic Analyses Indicating Occurrence of the *recN262* and *radB101* Mutations at the Same Locus in *Escherichia coli*

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The *radB101* and *recN262* mutations showed essentially identical phenotypes when compared in isogenic *Escherichia coli* strains for their effects on gamma and UV radiation survival and on conjugal recombination in a *uvrA recB recC sbcB sbcC* strain. Complementation tests involving attempts to reconstitute a *radB*$^+$ *recN*$^+$ strain by transductions between *radB101* and *recN262* donors and recipients, and tests involving plasmids carrying *recN*$^+$ and *recN*::Tn*1000* inserts, indicated that the *radB* and *recN* genes are identical. We suggest that the *radB101* mutation now be referred to as *recN2001*.

The *radB101* mutation was isolated on the basis of its sensitization of *Escherichia coli* K-12 cells to gamma radiation (14). This mutation maps at 56.5 min (14) relative to the 1983 version of the genetic map for *E. coli* K-12 (1), and it sensitizes cells to both gamma and UV radiation and to treatment with the alkylating agent methyl methanesulfonate (14). The *radB* mutant is normal for gamma and UV radiation mutagenesis, it shows only a slight enhancement of gamma and UV radiation-induced DNA degradation, and it shows a 60% deficiency in recombination ability (14). For gamma or UV radiation-induced killing, the *radB* mutation does not sensitize a *recA* strain, but does sensitize a *polA* strain and a *uvrB* (UV radiation only) strain, which suggests that the *radB* gene functions in the "*recA* epistasis group" for the repair of damaged DNA (14). DNA repair studies indicate that the *radB* gene plays a major role in UV-irradiated cells in the *recB*-dependent repair of DNA double-strand breaks that arise at nonrepaired DNA daughter-strand gaps (15) and that the *radB* mutation blocks 90% of the repair of X-ray-induced DNA double-strand breaks (16).

Shortly after the description of the *radB101* mutant, Lloyd and colleagues described a *rec-259* Mu d(Ap *lac*) insertion mutation (8) which also maps at 56.5 min (10) relevant to the 1983 genetic linkage map (1). This mutation inactivates a DNA damage-inducible gene (8, 11), *recN* (10), that plays a major role in recombination and radiation survival in *recB recC sbcB sbcC* cells, i.e., the RecF pathway (8, 11), and a large role in recombination in *recB recC sbcA* cells, i.e., the RecE pathway (7). Tn*5* insertions (5) and two point mutations, *recN261* and *recN262* (10), have been isolated, and the latter mutation has been studied extensively. The *recN262* mutation in wild-type cells causes about a 60% deficiency in recombination, a slight sensitization to UV radiation, and a large sensitization to mitomycin C and gamma radiation, but it does not sensitize *uvrB* cells to UV radiation-induced killing (10). At least for ionizing irradiation, the sensitizing effect of the *recN262* mutation has been shown to result from a partial deficiency in the repair of DNA double-strand breaks (10, 16). The *recN* gene has been cloned (10), and the gene product has a molecular weight of 63,599 (3, 12, 13).

The *radB101* and the *recN* mutations have been the subject of several separate studies, and they show similar map locations and similar effects on the repair of DNA double-strand breaks. For these reasons, we were prompted to determine whether the *radB* and *recN* genes are identical.

The bacterial strains used are listed in Table 1. Plasmids pSP100, pSP100::Tn*1000*-1, and pHSG415 have been described (10, 12) and were supplied by Steven M. Picksley and Robert G. Lloyd. Plasmid DNA was prepared by the "large-scale isolation of *E. coli* plasmid DNA" procedure (2).

YENB was nutrient broth (Difco) at 0.8% plus yeast extract (Difco) at 0.75%. YENB agar was nutrient agar (Difco) at 2.3% plus yeast extract at 0.75%. SMM and SMM agar were 0.4% glucose-salts medium (4) containing thiamine hydrochloride at 1 μg/ml, and, depending on each strain's nutritional requirements, SMM may have contained thymine at 10 μg/ml, D-biotin at 0.5 μg/ml, and L-amino acids at 1 mM. PB was Na_2HPO_4 at 5.83 g/liter plus KH_2PO_4 at 3.53 g/liter (pH 7.0).

Transductions and conjugations were accomplished generally as described by Miller (9). Transformations with plasmids were accomplished as described by Davis et al. (2).

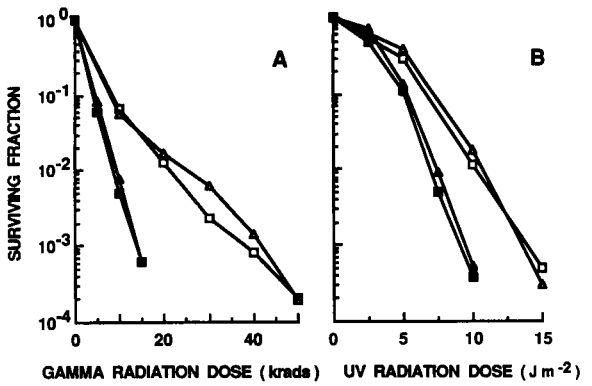

FIG. 1. Radiation survival of *radB* and *recN* mutants compared in isogenic *E. coli* strain backgrounds. Cells were grown to mid-logarithmic phase (1.2×10^8 CFU/ml) in SMM, gamma (A) or UV (B) irradiated in PB, and plated on SMM agar. Data points are averaged from duplicate experiments. Symbols: (A) □, SR1059 (+); ■, SR1060 (*radB*); △, SR1725 (+); ▲, SR1726 (*recN*); (B) □, SR1756 (*uvrB*); ■, SR1757 (*uvrB radB*); △, SR1509 (*uvrB*); ▲, SR1510 (*uvrB recN*).

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TABLE 1. *E. coli* K-12 strains used

Stanford Radiology no.	Genotype[a]	Source, derivation, or reference[b]
SR96	HfrH *thyA deo thi*	HfrH 1, F. Bonhoeffer
SR305	Same as SR1059, but Δ(*uvrB-chlA*) *recF143* Met^+ Bio^+	18
SR596	Same as SR1059, but Δ(*uvrB-chlA*) Met^+ Bio^+	18
SR655	Same as SR749, but *uvrB5 recN262*	SR1474 × P1 *vira* · SR749,[c] Tyr^+
SR749	*argE3 hisG4 leuB6* Δ(*gpt-proA*)*62 thr-1 thi-1 ara-14 galK2 lacY1 mtl-1 xyl-5 tsx-33 rfbD1 mgl-51 kdgK51 rpsL31 supE44* Δ(*rac*) F^- λ^-	AB1157, ECGSC
SR850	Hfr Cavalli *metB1 pyrE41 uhp-1 relA1 tonA22* $T2^r$	AT2243-11(c), ECGSC
SR991	Same as SR1059, but *pheA18*::Tn*10*	14
SR1026	Same as SR1059, but *radB101*	14
SR1059	*leuB19 metE70 thyA36 deo*(*C2?*) *bioA2 lacZ53 malB45 rha-5 rpsL151* IN(*rrnD-rrnE*) F λ	14
SR1060	Same as SR1059, but *radB101*	14
SR1075	Same as SR749, but *pheA18*::Tn*10*	16
SR1087	Same as SR749, but *radB101*	SR1075 × P1 *vira* · SR1026, Phe^+
SR1419	Same as SR749, but *uvrA6 recB21 recC22 sbcB15 sbcC201*	6, 19
SR1474	Same as SR749, but *uvrB5 recN262 tyrA16*::Tn*10*	SP264, S. M. Picksley
SR1484	Same as SR850, but Met^+	SR850 × P1 *vir* · SR749, Met^+
SR1507	Same as SR1419, but *tyrA16*::Tn*10*	SR1419 × P1 *vira* · SR1474, Tc^r
SR1508	Same as SR1419, but *recN262 tyrA16*::Tn*10*	Same as SR1507
SR1509	Same as SR1059, but Δ(*uvrB-chlA*) *tyrA16*::Tn*10* Met^+ Bio^+	SR596 × P1 *vira* · SR1474, Tc^r
SR1510	Same as SR1509, but *recN262*	Same as SR1509
SR1512	Same as SR1059, but Δ(*uvrB-chlA*) *recF143 recN262 tyrA16*::Tn*10* Met^+ Bio^+	SR305 × P1 *vira* · SR1474, Tc^r
SR1553	Same as SR749, but *recN262*	SR1075 × P1 *vira* · SR655, Phe^+
SR1702	Same as SR749, but *radB101 tyrA16*::Tn*10*	SR1087 × P1 *vira* · SR1507, Tc^r
SR1725	Same as SR1059	SR991 × P1 *vira* · SR655, Phe^+
SR1726	Same as SR1059, but *recN262*	Same as SR1725
SR1741	Same as SR1059, but Δ(*uvrB-chlA*) *recF143 radB101 tyrA16*::Tn*10* Met^+ Bio^+	SR305 × P1 *vira* · SR1702, Tc^r
SR1744	Same as SR1419, but *tyrA16*::Tn*10*	SR1419 × P1 *vira* · SR1702, Tc^r
SR1745	Same as SR1419, but *radB101 tyrA16*::Tn*10*	SR1419 × P1 *vira* · SR1702, Tc^r
SR1756	Same as SR1509	SR596 × P1 *vira* · SR1702, Tc^r
SR1757	Same as SR1509, but *radB101*	Same as SR1756

[a] Genotype nomenclature is that of Bachmann (1). As discussed in the text, *radB101* will henceforth be known as *recN2001*.
[b] ECGSC, *E. coli* Genetic Stock Center. Tc^r, Selected for tetracycline resistance.
[c] P1 *vira* is a reisolate of P1 *vir* that was obtained from A. J. Clark.

Cells were UV or gamma irradiated as described previously (14).

While the *recN262* and *radB101* alleles have been extensively described in several separate studies (see above), we have performed phenotypic comparisons on these two mutations in isogenic strains. Both mutations produced a very similar sensitization of SR1059 cells (Fig. 1A) and of AB1157 cells (16) to gamma radiation, and they produced a very similar sensitization of SR1059 Δ*uvrB* cells to UV radiation (Fig. 1B). Thus, the generally similar radiosensitization caused by the *radB* and *recN* mutations in separate studies becomes essentially identical when compared in isogenic strains in the same experiment. The fact that Picksley et al. (10) did not observe sensitization of their *uvrB* strain by the *recN262* mutation is explainable by their plating of the UV-irradiated cells on rich growth medium, whereas we have used minimal medium. We also found a much smaller effect of the *recN* mutation on the UV radiation survival of *uvrB* cells when cells were plated on rich rather than minimal medium (data not shown). That is, the *recN* gene must play a role in the phenomenon known as minimal medium recovery (reviewed in reference 17). Finally, the *recN262* and *radB101* mutations had essentially identical effects towards diminishing the conjugal recombination measured in *uvrA recB recC sbcB sbcC* cells (Table 2).

Because of the similar map positions and phenotypes for the *radB101* and *recN262* mutations, we have performed genetic complementation tests to determine whether they occur in the same gene. With bacteriophage P1-mediated transduction, both the *radB101* and *recN262* mutations show

TABLE 2. Effect of *recN* and *radB* mutations on conjugal recombination in *E. coli uvrA recB recC sbcB sbcC* recipient strains[a]

Recipient strain	Hfr strain SR96		Hfr strain SR1484	
	Leu^+ recombinants/ml	Recombination efficiency versus wild type	Arg^+ recombinants/ml	Recombination efficiency versus wild type
SR1507 (+)	1.10×10^5	1.0	3.35×10^5	1.0
SR1508 (*recN262*)	7.31×10^3	0.066	5.65×10^4	0.17
SR1744 (+)	1.04×10^5	1.0	2.18×10^5	1.0
SR1745 (*radB101*)	6.10×10^3	0.059	4.83×10^4	0.22

[a] Recipients were isogenic (Table 1) and were all mated simultaneously with a given Hfr culture for 45 min before plating on SMM medium deficient in either leucine or arginine, as appropriate, and containing streptomycin at 200 μg/ml.

TABLE 3. Effect of plasmids on gamma radiation survival of *E. coli* host strains[a]

Plasmid host strain	Cell survival after 30 krads			
	No plasmid (control)	pSP100 (*recN*$^+$)	pSP100::Tn*1000*-1 (*recN*)	pHSG415 (no insert)
SR749 (*rec*$^+$)	1.9×10^{-1}	NT[b]	NT	NT
SR1553 (*recN262*)	3.8×10^{-4}	2.7×10^{-1}	5.2×10^{-4}	3.6×10^{-4}
SR1087 (*radB101*)	2.3×10^{-5}	9.4×10^{-2}	2.7×10^{-5}	1.4×10^{-5}

[a] Strains were shaken overnight at 30°C in YENB (ampicillin was included at 50 μg/ml when cells carried a plasmid). Cells were diluted 1/500 with fresh medium, shaken to mid-logarithmic phase (optical density at 650 nm = 0.4, i.e., 4×10^7 CFU/ml), filter harvested, suspended in PB, gamma irradiated, and then plated in duplicate on YENB agar. Data are taken from triplicate survival curves with four dose points.

[b] NT, Not tested.

about a 50% linkage with the *tyrA* gene (8, 14). To test whether the *radB101* and *recN262* mutations can complement each other, about 800 tyrosine prototrophic isolates each were obtained from two transductions: SR1512 (*uvrB recF recN262 tyr*) × P1 *vir*a · SR1087 (*radB101*) (see Table 1) and SR1741 (*uvrB recF radB101 tyr*) × P1 *vir*a · SR1553 (*recN262*). (UV irradiation and the *uvrB recF* strain background were employed because of the high sensitivity these conditions provide for scoring the presence of the *recN* and *radB* mutations.) While a *uvrB recF recN*$^+$ *radB*$^+$ strain was much more resistant to UV radiation than the *recN262* (SR1512) or *radB101* (SR1741) derivatives, none of the 1,658 Tyr$^+$ transductants showed significantly greater resistance to UV radiation than the Tyr$^-$ recipient strains (SR1512 and SR1741) (data not shown). These data indicate that the *radB101* and *recN262* mutations are located very close to each other and are likely to affect the same gene.

For a more definitive test, plasmids carrying the *recN*$^+$ gene or an inactivated *recN* gene were tested for their ability to restore resistance to gamma radiation to the *radB* and *recN* strains. The plasmid carrying the functional *recN* gene in a 5.6-kilobase bacterial DNA insert (pSP100) was able to restore resistance to gamma radiation to both the *radB* and *recN* strains, whereas this effect was not produced either by the plasmid carrying the inactivated *recN* gene (pSP100::Tn*1000*-1) or by the parental plasmid without an insert (pHSG415) (Table 3).

Good evidence that the *radB* and *recN* genes are identical is provided by the inability of plasmid pSP100::Tn*1000*-1, in contrast to plasmid pSP100, to protect the *radB* strain and by the inability to produce a UV radiation-resistant transductant from *radB* and *recN* donors and recipients. Since the *recN*/*radB* gene has been more extensively studied in association with the *recN* terminology, we suggest that the *radB* nomenclature be dropped in favor of *recN*. Henceforth we will refer to *radB101* as *recN2001* (*rec* allele number selected with permission from A. J. Clark, University of California, Berkeley).

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LITERATURE CITED

1. **Bachmann, B. J.** 1983. Linkage map of *Escherichia coli* K-12, edition 7. Microbiol. Rev. **47**:180–230.
2. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
3. **Finch, P. W., P. Chambers, and P. T. Emmerson.** 1985. Identification of the *Escherichia coli recN* gene product as a major SOS protein. J. Bacteriol. **164**:653–658.
4. **Ganesan, A. K., and K. C. Smith.** 1968. Dark recovery processes in *Escherichia coli* irradiated with ultraviolet light. I. Effect of *rec*$^-$ mutations on liquid holding recovery. J. Bacteriol. **96**:365–373.
5. **Kolodner, R., R. A. Fishel, and M. Howard.** 1985. Genetic recombination of bacterial plasmid DNA: effect of RecF pathway mutations on plasmid recombination in *Escherichia coli*. J. Bacteriol. **163**:1060–1066.
6. **Lloyd, R. G., and C. Buckman.** 1985. Identification and genetic analysis of *sbcC* mutations in commonly used *recBC sbcB* strains of *Escherichia coli* K-12. J. Bacteriol. **164**:836–844.
7. **Lloyd, R. G., C. Buckman, and F. E. Benson.** 1987. Genetic analysis of conjugational recombination in *Escherichia coli* K12 strains deficient in RecBCD enzyme. J. Gen. Microbiol. **133**: 2531–2538.
8. **Lloyd, R. G., S. M. Picksley, and C. Prescott.** 1983. Inducible expression of a gene specific to the RecF pathway for recombination in *Escherichia coli* K12. Mol. Gen. Genet. **190**:162–167.
9. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
10. **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.
11. **Picksley, S. M., R. G. Lloyd, and C. Buckman.** 1984. Genetic analysis and regulation of inducible recombination in *Escherichia coli* K-12. Cold Spring Harbor Symp. Quant. Biol. **49**: 469–474.
12. **Picksley, S. M., S. J. Morton, and R. G. Lloyd.** 1985. The *recN* locus of *Escherichia coli* K12: molecular analysis and identification of the gene product. Mol. Gen. Genet. **201**:301–307.
13. **Rostas, K., S. J. Morton, S. M. Picksley, and R. G. Lloyd.** 1987. Nucleotide sequence and LexA regulation of the *Escherichia coli recN* gene. Nucleic Acids Res. **15**:5041–5049.
14. **Sargentini, N. J., and K. C. Smith.** 1983. Characterization of an *Escherichia coli* mutant (*radB101*) sensitive to γ and uv radiation, and methyl methanesulfonate. Radiat. Res. **93**:461–478.
15. **Sargentini, N. J., and K. C. Smith.** 1986. Role of the *radB* gene in postreplication repair in UV-irradiated *Escherichia coli uvrB*. Mutat. Res. **166**:17–22.
16. **Sargentini, N. J., and K. C. Smith.** 1986. Quantitation of the involvement of the *recA*, *recB*, *recC*, *recF*, *recJ*, *recN*, *lexA*, *radA*, *radB*, *uvrD*, and *umuC* genes in the repair of X-ray-induced DNA double-strand breaks in *Escherichia coli*. Radiat. Res. **107**:58–72.
17. **Sharma, R. C., and K. C. Smith.** 1986. Repair of DNA double-strand breaks in UV irradiated *Escherichia coli uvrB recF* cells is inhibited by rich growth medium. Mutat. Res. **166**:23–28.
18. **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.
19. **Wang, T. V., and K. C. Smith.** 1986. *recA*(Srf) suppression of *recF* deficiency in the postreplication repair of UV-irradiated *Escherichia coli* K-12. J. Bacteriol. **168**:940–946.