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Escherichia coli DNA Repair Genes *radA* and *sms* Are the Same Gene

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***Escherichia coli* strains carrying *radA100* or *sms* mutations were identical in their sensitivities to either methyl methanesulfonate or UV radiation treatment and in their plasmid complementation patterns for UV radiation survival. DNA sequencing analysis of the *radA* mutant and *radA*$^+$ strains and comparison of their sequences with the published *sms* gene sequence showed the *radA* mutant to differ only by a G-to-A transition mutation, which is predicted to change a cysteine in a zinc-finger motif to tyrosine. The *sms* gene is concluded to be identical to the previously described *radA* gene.**

The *radA100* mutation (named for its role in radiation resistance) was isolated because it made *Escherichia coli* cells sensitive to gamma radiation (3). This mutation maps close to the *serB* locus (3) and has been placed at 99.5 min on the *E. coli* K-12 linkage map (1). Log-phase *radA* mutant cells show increased sensitivity to X rays, methyl methanesulfonate (MMS), and UV radiation when grown in rich medium but not when grown in minimal medium (3). Rich medium-grown *radA100* cells are also 30% deficient in their ability to repair X-ray-induced DNA double-strand breaks compared with *radA*$^+$ cells (15).

Ten years after the description of the *radA* gene, Neuwald et al. (11) described the *sms* gene (named for sensitivity to MMS), which is cotranscribed with the *serB* gene but is not required for serine biosynthesis. The *sms* gene is 1,380 bp long, and sodium dodecyl sulfate-polyacrylamide gel electrophoretic analysis shows a 55-kDa product (11). It is of general interest that certain Sms protein domains show substantial similarity to different motifs of Lon protease and RecA protein (8, 11), two proteins that have long been known for their roles in gene regulation and DNA repair (e.g., reference 6). The purpose of this work was to test the hypothesis that the *radA* and *sms* genes are identical.

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Effects of *radA100* and *sms* mutations on survival. Earlier studies of the *radA* and *sms* mutations involved distantly related *E. coli* strains. In this study, undesired strain differences were minimized by transducing both the *radA* and *sms* mutations into the same parental *uvr*$^+$ and *uvrA6* strains for phenotype comparison studies (all strains and plasmids used in this study are listed in Table 1). Although the original *sms-1* gene disruption mutant (AN1) has apparently been lost, George V. Stauffer kindly provided a similarly constructed mutant whose *sms* mutation was used in our studies. The *uvrA radA* and *uvrA sms* strains showed essentially identical sensitivities to MMS, and they required only 66 and 56% as much MMS treatment, respectively, as the *uvrA* control strain to yield a surviving fraction of 10%; i.e., their respective D_{10} ratios were 0.66 and 0.56 (Fig. 1a).

The *radA* mutation has been reported to sensitize a wild-type (*uvrA*$^+$) strain to UV radiation (3), while the *sms* mutation reportedly has no effect (11). We found that both the *radA* and *sms* mutations sensitized rich medium-grown cells to UV radiation to essentially identical levels; i.e., the respective D_{10} ratios were 0.60 and 0.50 in *uvrA*$^+$ strains (Fig. 1b) and 0.44 and 0.47 in *uvrA* mutant strains (Fig. 1c). Diver et al. (3) showed that the increased sensitivity of *radA* strains to X rays, UV radiation, and MMS depends on the cells being plated on rich medium after irradiation (as was done here for the experiments reflected in Fig. 1a and b). To determine whether the *sms* mutant shows a similar medium-dependent phenomenon, *uvrA*, *uvrA sms*, and *uvrA radA* cells were grown in minimal medium, UV irradiated, and plated on either minimal or rich medium. Both the *radA* and *sms* mutations showed the same medium-dependent effect on UV radiation survival, i.e., the D_{10} ratios for both mutants were 0.88 when plated on minimal medium versus 0.45 when plated on rich medium (Fig. 1c). Thus, the *radA* and *sms* genes played a larger role in resistance to DNA-damaging agents when cells were plated on rich medium versus minimal medium, and the close similarity of the phenotypes of the *radA* and *sms* mutations is consistent with the notion that these mutations affect the same gene.

Plasmid complementation studies. In an attempt to confirm that the *sms* and *rad* genes are identical, a complementation analysis was performed. Plasmids, kindly provided by George V. Stauffer, were introduced into the *uvrA*, *uvrA radA*, and *uvrA sms* strains and tested for their ability to protect their hosts from UV radiation-induced killing. Plasmid pserB59-1 (*serB*$^+$ *sms*$^+$) enhanced the UV radiation survival of both the *radA* and *sms* mutant strains, while plasmid pGS39 (*serB* negative and *sms* negative) failed to protect either strain from UV radiation (Table 2). Neither plasmid had a significant effect on the radiation survival rate exhibited by the *uvrA6* control strain (Table 2). Because of the construction of the complementing and noncomplementing plasmids used (Table 1) (11), one can conclude that the *radA* gene is identical to either the *sms* or the *serB* gene. The *serB* gene encodes phosphoserine phosphatase, which removes the phosphate group from phosphoserine to yield serine (13). Since the *radA* mutant was not auxotrophic for serine, as are typical *serB* mutants, it seems very reasonable to conclude that the *radA* and *sms* genes are identical.

Sequencing studies. If the *radA* and *sms* genes are identical, then the *radA* mutant should carry a mutation in its *sms* gene. To test this hypothesis, we selected primers flanking the se-

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

Strain or plasmid	Relevant characteristic(s)[a]	Source, derivation, or reference
Strains		
GS817	*sms* = Δ(*smp serB sms*)::*kan*	G. V. Stauffer
AB1886	*uvrA6*	S. Linn
SR776	*radA100* Thr^+	3
SR1120	*malE7*::Tn*5*	17
SR1267	Wild type	SR1120 × P1::Tn*9*cts·AB1886, Mal^+
SR1268	*uvrA6*	Same as for SR1267
YS1	Thr^+	SR1267 × P1vir·SR776, Thr^+
YS2	*radA100* Thr^+	Same as for YS1
YS3	*uvrA6* Thr^+	SR1268 × P1vir·SR776, Thr^+
YS4	*uvrA6 radA100* Thr^+	Same as for YS3
YS9	*uvrA6 sms* Thr^+	YS3 × P1vir·GS817, Kn^r
YS16	*sms* Thr^+	YS1 × P1vir·GS817, Kn^r
YS18	*uvrA6 radA100* Thr^+/pserB59-1	YS4, transformed with pserB59-1
YS19	*uvrA6 radA100* Thr^+/pGS39-4	YS4, transformed with pGS39-4
YS20	*uvrA6 sms* Thr^+/pserB59-1	YS9, transformed with pserB59-1
YS22	*uvrA6 sms* Thr^+/pGS39-4	YS9, transformed with pGS39-4
YS25	*uvrA6* Thr^+/pserB59-1	YS3, transformed with pserB59-1
YS26	*uvrA6* Thr^+/pGS39-4	YS3, transformed with pGS39-4
Plasmids		
pserB59-1	smp^+ $serB^+$ sms^+ Ap^r	G. V. Stauffer and reference 11
pGS39-4	Like pserB59-1 but Δ(*serB sms*)[b]	G. V. Stauffer and reference 11

[a] Unless stated otherwise, all bacterial strains, except GS817, also have the genotype *argE3 hisG4 leuB6* Δ(*gpt-proA*)*62 thr-1 thi-1 ara-14 galK2 lacY1 mtl-1 xyl-5 tsx-33 rfbD1 mgl-51 rpsL31 supE44 rac* F^- λ^-.

[b] Plasmid pGS39-4 was derived from pserB59-1 by a deletion which fused the distal part of the *serB* gene and the proximal part of the *sms* gene and inactivated both genes (11).

quence reported for the *sms* gene (11) and amplified the intervening DNA from both the *radA* mutant and $radA^+$ strains (i.e., strains YS3 and YS4, respectively). Chromosomal DNA was extracted from 1.5-ml overnight Luria-Bertani medium cultures in which the cells had been pelleted, resuspended in 500 μl of a mixture of 0.1 M NaCl, 10 mM Tris (pH 8), and 1 mM EDTA (14), and then pelleted and resuspended in 650 μl of water. The DNAs were extracted once each with phenol-$CHCl_3$ and $CHCl_3$ and then purified by ethanol precipitation and RNase A and T_1 (Sigma) treatment (generally as described in reference 10). The *sms* gene DNA sequence was amplified by thermocycling (model no. PTC-100 with a Hot

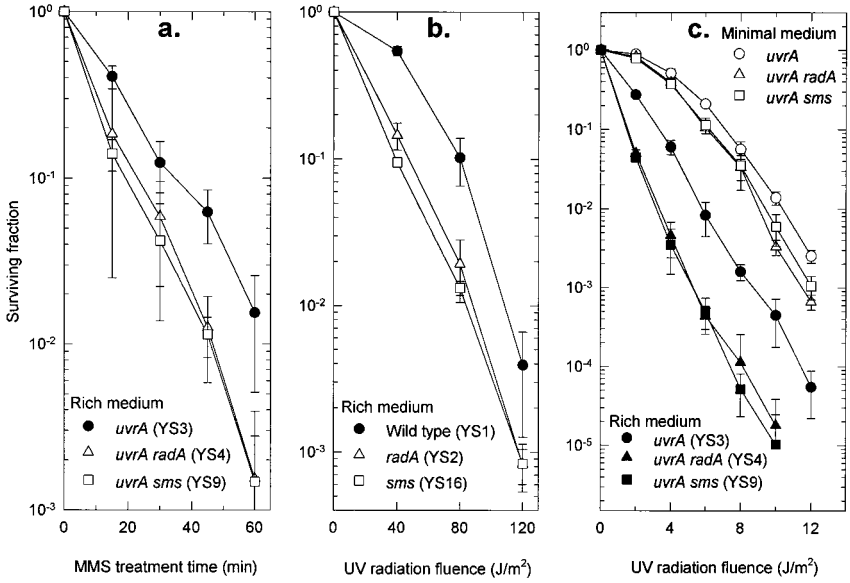

FIG. 1. Effects of *radA* and *sms* mutations on survival of *E. coli uvrA6* or $uvrA^+$ cells treated with MMS or UV radiation. Cells were grown to log phase in YENB (rich medium [0.75% Difco yeast extract and 0.8% Difco nutrient broth]), resuspended in phosphate buffer, treated with MMS at 0.05 M (in phosphate buffer with 10% dimethyl sulfoxide) for graded times at 37°C or with UV radiation (254 nm), and then plated on YENB agar (YENB solidified with 1.5% Difco Bacto Agar) as described previously (16). Cells treated with MMS were diluted 10-fold into 0.1% sodium thiosulfate (in phosphate buffer) before plating. For minimal medium data in panel c, cells were grown in and plated on glucose-minimal medium (17) supplemented with 1 mM (each) L-serine (for only the *uvrA sms* strain), L-histidine, L-leucine, L-arginine, L-proline, and L-threonine. Data were averaged from three to four experiments per strain. Error bars indicate 95% confidence intervals; those error bars that cannot be seen are covered by the symbol; missing lower parts of error bars indicate negative values. Strain names (Table 1) are given in parentheses.

TABLE 2. Effects of plasmids on UV radiation survival of *E. coli uvrA6* host strains

Host strain genotype	Avg surviving fraction after UV irradiation (6 J/m^2) ± SD[a]		
	No plasmid present	With plasmid pserB59-1	With plasmid pGS39-4
rad$^+$ (*sms*$^+$)	$3.7 \times 10^{-3} \pm 1.3 \times 10^{-3}$ (YS3)	$2.9 \times 10^{-3} \pm 1.1 \times 10^{-3}$ (YS25)	$3.5 \times 10^{-3} \pm 1.7 \times 10^{-3}$ (YS26)
radA100	$1.4 \times 10^{-4} \pm 0.2 \times 10^{-4}$ (YS4)	$1.7 \times 10^{-3} \pm 0.6 \times 10^{-3}$ (YS18)	$1.4 \times 10^{-4} \pm 0.8 \times 10^{-4}$ (YS19)
sms	$1.1 \times 10^{-4} \pm 0.7 \times 10^{-4}$ (YS9)	$1.4 \times 10^{-3} \pm 1.1 \times 10^{-3}$ (YS20)	$1.1 \times 10^{-4} \pm 1.2 \times 10^{-4}$ (YS22)

[a] Cells were grown to log phase in YENB (rich medium), UV irradiated (254 nm), and plated on YENB in duplicate as described previously (16). Data are averages from three to eight experiments per strain. The strain names (Table 1) are listed in parentheses.

Bonnet; MJ Research) with a 50-μl reaction mixture containing chromosomal DNA at 6 μg/ml, two oligonucleotide primers (Genosys Biotechnologies, Inc.) at 0.34 μM (each), 2.5 mM $MgCl_2$, 1.25 U of *Taq* DNA polymerase (Promega), and 1× *Taq* assay buffer (Promega); to provide a "hotstart," *Taq* was added after the first thermocycling step. The thermocycling conditions were 93°C for 5 min (step 1), 72°C for 3 min (step 2), and 93°C for 1 min (step 3), and then steps 2 and 3 were repeated 34 times. One microliter of the DNA amplification mixture produced above was cycle sequenced (with an *fmol* DNA Sequencing System kit; Promega) with 5′ end-labeled ([γ-^{32}P]ATP, >3,000 Ci/mmol; Andotek Co.) oligonucleotide primers and incubations of 95°C for 2 min (step 1), 95°C for 30 s (step 2), and 70°C for 30 s (step 3), and then steps 2 and 3 were repeated 29 times. The primers that elucidated the *radA100* mutation were CTGATGGGGGTATTCTGCATCC TC-3′ and ATCAGCTACCTGCTGTAGCGTGCA-3′ for DNA amplification and GAATCAGAAGTAATTGCTCGCC CG-3′ for cycle sequencing. DNA sequencing gel electrophoresis generally followed the method of Sambrook et al. (14) and employed 6% Long Ranger (FMC Bioproducts) polyacrylamide gels, a model STS-45 sequencer (International Biochemicals, Inc.), and 70-W constant power (with 2,200-V and 100-mA maxima). Sequences were visualized by autoradiography on Fuji RX film. The sequencing results for the *radA*$^+$ strain confirmed the published sequence (11) for the *sms* gene (data not shown). More importantly, with two different DNA amplification preparations, cycle sequencing of the *sms* gene in the *radA100* strain showed the same sequence as in the *radA*$^+$ strain except for a single transition mutation, i.e., a replacement of G by A at base number 146 (base numbering is as found in reference 11). Thus, the *radA100* mutant is predicted to carry a tyrosine residue in its RadA protein at amino acid position 28 (amino acid numbering is as found in reference 11) rather than the normal cysteine residue.

***sms* is the same as *radA*.** On the basis of survival, plasmid complementation, and DNA sequencing analysis, it is concluded that the *sms* and *radA* mutations studied here are alleles of the same gene, which we propose to call *radA* because this name preceded *sms* by 10 years and because it has been described in several publications (3, 5, 15), including the last version of the *E. coli* K-12 genetic linkage map (1).

Similarity of *radA* with other genes. Since the cysteine residue changed in the *radA100* mutant (Cys-28) is part of the metal-binding motif (2) noted by Neuwald et al. (11), i.e., Cys-11– –Cys-14– – – – –Cys-25– –Cys-28, our finding suggests that this motif plays a critical role in the DNA repair function performed by RadA protein. Such a metal-binding motif is often found to bind a Zn(II) ion and produce a zinc finger, which may allow the protein to bind to DNA and probe down into the major groove (7). While more than 200 different human genes have been shown to encode zinc fingers, relatively few examples have been found in *E. coli* (7), the best examples being the UvrA (18) and Fpg (12) DNA repair proteins. Obviously, more work needs to be done to validate a role for metal binding in the function of RadA protein in DNA repair.

In addition to the metal-binding motif involving amino acid residues 11 to 28 in RadA protein, Neuwald et al. (11) also observed that RadA protein shows substantial similarity to the RecA (RadA protein residues 59 to 151, as per reference 11) and Lon proteins (residues 357 to 456) and that it shows an ATP/GTP binding motif (residues 102 to 109). Since *recA* and *radA* mutations both inhibit the repair of DNA double-strand breaks (9, 15), it seems noteworthy that the *recA13* and *recA56* mutations, which completely inactivate RecA protein function, both occur in the RecA domain (4) that is highly similar to a domain in RadA protein; i.e., such mutations would affect RadA protein amino acid positions 88 and 96, respectively (cf. references 4 and 11). Our laboratory is currently testing the effect of *recA*-inactivating base substitutions in the *radA* gene to see if these also block the function of RadA protein.

Nucleotide sequence accession number. The nucleotide sequence of a 1,432-bp region containing the *radA* gene of *E. coli* has been submitted to the GenBank database under accession number U59449.

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