

NOTES

RadC, a Misleading Name?^{∇†}

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The pfam04002 annotation describes RadC as a bacterial DNA repair protein. Although the *radC* gene is expressed specifically during competence for genetic transformation in *Streptococcus pneumoniae*, we report that *radC* mutants exhibit normal uptake and processing of transforming DNA. They also display normal sensitivity to DNA-damaging agents, providing no support for the *rad* epithet.

RadC is an ~220-residue widespread bacterial protein (COG2003). A conserved domain corresponding to the C-terminal moiety of the protein is called pfam04002 (or RadC; for an explanation of the truncation of pfam04002 compared to COG2003, see the supplemental material). Rad serves as a mnemonic for radiation, and pfam04002 is annotated as “a DNA repair protein. RadC plays a role in repair of DNA damage after UV and X-ray irradiation in prokaryotes. The *Escherichia coli radC* gene encodes a RecG-like DNA recombination/repair function. RadC may function specifically in recombinational repair that is associated with the replication fork.” However, this annotation is solely based on the study of the *radC102* mutation of *E. coli*. The mutation was reported to cause mild UV and X-ray sensitivity and was initially mapped near to *pyrE* and *recG* (5). The study of an *E. coli* strain carrying the very same mutation led to the conclusion that RadC was required to prevent deletions at chromosomal tandem repeats induced by replication fork defects (21). However, the *radC102* mutation was then unambiguously demonstrated to be an allele of *recG* by Lombardo and Rosenberg (11), who concluded that the function of the *E. coli* RadC protein and of its many bacterial homologs remains to be determined. Since then, no publication describing the inactivation of *E. coli radC* and its phenotypic consequence(s) has appeared.

Intriguingly, *radC* is expressed specifically in cells competent for genetic transformation in four divergent naturally transformable species: *Streptococcus pneumoniae* (14, 19), *Streptococcus gordonii* (24), *Haemophilus influenzae* (18), and *Bacillus subtilis* (3, 7, 13). In *S. pneumoniae*, the *radC* gene (*spd_0975* in D39, *spr0996* in R6, and *sp1088* in TIGR4) was concluded to be essential in two independent studies (20, 22). However, in a third report, *radC* was inactivated through replacement of the entire coding sequence by a kanamycin resistance cassette, and

the protein appeared individually dispensable for chromosomal transformation (14). In *B. subtilis*, *radC* (*ysxA*) is not essential, and its inactivation did not affect chromosomal transformation (13). These puzzling observations prompted us to determine whether *S. pneumoniae radC* could be inactivated and to investigate the possible role(s) of RadC in transformation, as well as in the repair of DNA damage. All of the strains and plasmids used in the present study are listed, together with the primers, in Table S1 in the supplemental material.

Inactivation of *radC*. In vitro *mariner* mutagenesis (16) was used to generate minitransposon insertions in the *radC1-radC2* PCR fragment (Fig. 1). Insertions were readily obtained. Of 10 randomly selected insertions analyzed and found to be evenly distributed in the targeted region, 9 were localized within *radC*, demonstrating that this gene is not essential, at least in the *S. pneumoniae* D39-R6 lineage. This result contradicts two previous reports describing *radC* as an essential gene of *S. pneumoniae* (20, 22) but is consistent with the data of Peterson et al. (14).

A transformation-dedicated function of RadC? We first investigated whether transformation with chromosomal point mutations was affected in the absence of RadC by using standard procedures for transformation (6). In full agreement with a previous report (14), chromosomal transformation frequency appeared to be similar to that in wild-type cells (Table 1). We then compared the frequency of integration of pR290, a non-replicative recombinant plasmid carrying a 1,596-bp insert of pneumococcal DNA (see Table S1 in the supplemental material). This recombination event involves integration of a long stretch of heterologous DNA (the vector moiety). Integration of pR290 occurred at a normal frequency in the absence of RadC (Table 1). Altogether, these data suggest that RadC is not required (or is individually dispensable) for the integration of homologous DNA, as well as for homology-dependent integration of nonreplicative plasmids.

We also examined whether RadC is important for the stability of incoming single-stranded DNA (ssDNA). The fate of transforming DNA was compared in wild-type and *radC* mutant cells using as the donor a 7,771-bp *S. pneumoniae* fragment uniformly labeled with ³²P. After 3 min of contact between competent cells and DNA and a further incubation of

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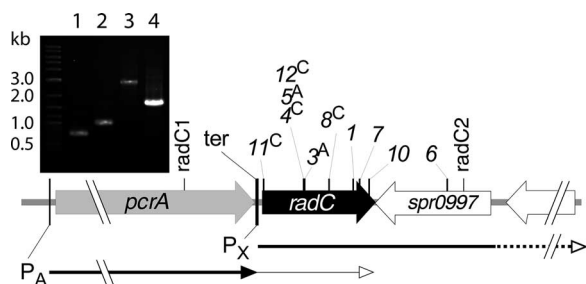


FIG. 1. Genetic organization of the *pcrA-radC* chromosomal region and location of *mariner* minitransposon insertion mutants of *radC*. Transcripts previously identified in the region are shown by horizontal arrows. Expression from P_A (σ^A promoter) is much lower for *radC* than for *pcrA*, most likely because of the presence of a terminator sequence, *ter* (20); only *radC* is induced at competence (14, 19). The dotted line indicates that transcripts initiated at P_X (σ^X competence-specific promoter) extend into the inversely oriented flanking genes (14). The location and orientation of *spc* cassette insertions were determined as previously described (16) through PCRs using primers MP127 or MP128 (see Table S1 in the supplemental material) in combination with either one of the two primers used to generate the *radC1-radC2* PCR fragment. Cassette-chromosome junctions were determined by DNA sequencing at position +9 (with respect to the first nucleotide of *radC* taken as +1) for *spc*^{12C}, +248 for *spc*^{12C}, *spc*^{5A}, and *spc*^{4C}, and +255 for *spc*^{3A}. The inset shows the control PCR of insertion *spc*^{4C} (strain R1966) retained for the present study, confirming the loss of the wild-type *radC1-radC2* fragment (shown in lane 4) and its replacement by a fragment (lane 3), the size of which was fully consistent with a simple minitransposon insertion in *radC*, and the *spc*^{4C} location with the primer pairs MP127-*radC1* (lane 1) and MP127-*radC2* (lane 2).

cells for 1 min in the presence of DNase I to terminate uptake, ssDNA was recovered from transformed cells by using a previously described method (2), with only minor modifications (see the supplemental material). No effect of the inactivation of *radC* on the amount of intact ssDNA recovered was detected (Fig. 2), suggesting that RadC does not affect the stability of incoming ssDNA. Inactivation of *dprA* (or *recA*) was previously shown to destabilize completely incoming ssDNA (1). To determine whether RadC could be responsible for ssDNA degradation when DprA is missing, we compared the fate of internalized transforming DNA in *dprA* and *radC dprA* mutant cells (Fig. 2). No stabilization of incoming ssDNA was detected in the latter, ruling out the possibility that RadC is a

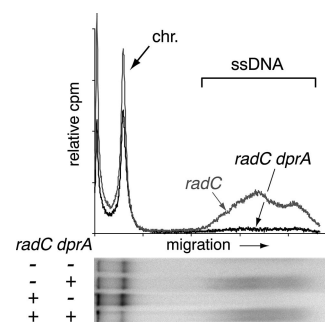


FIG. 2. Inactivation of *radC* has no detectable effect on the stability of internalized ssDNA in both wild-type and *dprA* mutant genetic backgrounds. Total DNA extracted from cells transformed with ³²P-labeled DNA was fractionated by gel electrophoresis (see the supplemental material). The top of the figure shows densitometer tracings of electropherograms used to calculate the amount of donor label recovered in each lane (expressed as relative cpm). The bottom of the figure shows electropherograms from *radC dprA* double mutant (R1939), *radC* mutant (R1933), *dprA* mutant (R1750), and wild-type (R1928) cell extracts. The positions of chromosomal DNA (chr.) and of internalized ssDNA are indicated by an arrow and a bracket, respectively.

nuclease responsible for ssDNA degradation in the absence of DprA (or RecA).

A role for RadC in competence regulation? In the experiments described above, transformation was induced with synthetic CSP (8), and competence was monitored using an *ssbB::luc* fusion (17). The kinetics of *ssbB* expression of wild-type and *radC* mutant cells appeared indistinguishable, suggesting that RadC plays no role in the response to CSP and in the decay of CSP-induced competence (see Fig. S1 in the supplemental material). In addition, a comparison of the spontaneous induction of competence in wild-type and *radC* mutant cultures revealed no significant difference (see Fig. S2 in the supplemental material). Altogether, these data indicate that RadC is either individually dispensable or plays no role in the regulation of competence.

A role in mismatch repair? Mismatch repair proficiency of *radC* mutant cells was also analyzed both during and outside competence. During chromosomal transformation, mismatch repair is known to be responsible for the low efficiency of transformation of the *rif23* marker compared to the *str41* marker. The Hex system (4) rejects potential rifampin-resistant

TABLE 1. Comparison of chromosomal transformation, mismatch repair, plasmid installation, and gene conversion in wild-type and *radC* mutant cells

Strain (genotype)	Transforming DNA						Gene conversion ^d (Sm ^r cells/ 10,000 cells ± SD)
	Chromosomal			Plasmid			
	<i>str41</i> (%)	<i>rif23</i> (%)	Rif/Str ± SD	pR290 (%) ^a	pLS1 (%) ^b	pLS70 (%) ^c	
R1502 (wild type)	4.94	0.59	0.119 ± 0.034	0.70	0.046	0.43	0.117 ± 0.076
R990 (<i>rpsL</i> ⁺ - <i>rpsL1</i> , Sm ^s)							
R1969 (<i>radC</i>)	2.67	0.23	0.088 ± 0.029	0.71	0.041	0.42	
R2327 (<i>rpsL</i> ⁺ - <i>rpsL1</i> , Sm ^s ; <i>radC</i>)							

^a Nonreplicative plasmid carrying a 1,596-bp chromosomal insert (see Table S1 in the supplemental material).

^b Replicative plasmid (rolling-circle type; pMV158 derivative) (see Table S1 in the supplemental material).

^c Replicative plasmid (pLS1 derivative) carrying a 3,486-bp chromosomal insert (see Table S1 in the supplemental material).

^d Calculated from four to five independent cultures inoculated from individual colonies resuspended in 2 ml of C+Y medium and grown to an OD₅₅₀ of ~0.4 before plating.

transformants following recognition of the *rif23/rif⁺* mismatch at the heteroduplex stage in transformation. Mismatch repair was unaffected in *radC* mutant cells (Table 1).

To evaluate mismatch repair proficiency outside competence, we took advantage of a previously constructed duplication of the *rpsL* gene with the two alleles, *rpsL1* and *rpsL⁺* (23). Gene conversion can occur between *rpsL1*, which confers streptomycin resistance (*Sm^r*), and *rpsL⁺*, which confers *Sm^s*. Since the sensitive allele is dominant over the resistant allele, conversion of the former allele is revealed by the appearance of *Sm^r* colonies. This process was previously shown to depend on RecA and to be susceptible to mismatch repair (23) because the transient heteroduplex structure formed between the two *rpsL* genes creates a mismatch (*rpsL⁺/rpsL1*) recognized by the Hex system. Hex-dependent mismatch correction reduces spontaneous conversion to *Sm^r* by ~18-fold (23). The conversion frequency measured with *radC* mutant cells did not differ significantly from that of the wild-type parent (Table 1). We concluded that RadC plays no role in Hex-dependent generalized mismatch repair.

Sensitivity to DNA damages. To assess a possible involvement of RadC in the repair of DNA damages outside competence, we compared the sensitivity to UV irradiation, to mitomycin C, and to methyl methanesulfonate (MMS) of a wild-type and a *radC* mutant strain (as well as a *recA* mutant as a control) as follows. Stocks of bacteria grown in THY medium (or C+Y medium for the *recA* mutant) to an optical density at 550 nm (OD_{550}) of 0.4 were diluted 100-fold in C+Y medium (pH 6.8 to 7.0) and incubated at 37°C to an OD_{550} of 0.2. Then, 20 μ l of each culture and of 10-fold serial dilutions were spotted on 1-day-old D-agar plates containing horse blood and catalase (500 U/ml). To assay UV sensitivity, plates were then UV irradiated at 2.5 J/m² for 5, 10, 15, or 25 s. To measure sensitivity to mitomycin C and to MMS, plates contained variable concentrations of mitomycin C (5, 10, 15, or 20 ng/ml) or MMS (0.02, 0.04, 0.06, or 0.08%). In all cases, plates were incubated overnight at 37°C. The *radC* mutant strain exhibited sensitivity to UV (Fig. 3A) and to mitomycin C or MMS (Fig. 3B) similar to that of the wild-type strain, providing no support to the hypothesis that RadC plays a role in the repair of DNA damages in *S. pneumoniae*.

A helicase-related function of RadC? *radC* was previously shown to be coexpressed with *pcrA* (20) (Fig. 1). PcrA is a DNA helicase involved in the replication of rolling-circle replicating plasmids (20). This coexpression prompted us to investigate whether RadC could be required for the establishment of a rolling-circle replicon plasmid (pLS1; see Table S1 in the supplemental material). Plasmid establishment frequencies were indistinguishable in *radC* mutant and wild-type cells (Table 1). We also investigated homology-facilitated plasmid establishment (12) using as donor plasmid pLS70, a pLS1 derivative carrying a 3,486-bp long chromosomal insert (see Table S1 in the supplemental material). The presence of a chromosomal insert is known to increase 10-fold or more the frequency of plasmid establishment by transformation in *S. pneumoniae* (12). The facilitation mechanism is likely to depend on synapsis of the internalized ssDNA plasmid fragment with the recipient chromosome, which favors reconstitution of an intact replicon. The frequency of pLS70 establishment was 10-fold higher than that of pLS1 in both wild-type and *radC* mutant cells (Table 1), indicating that RadC plays no role in the

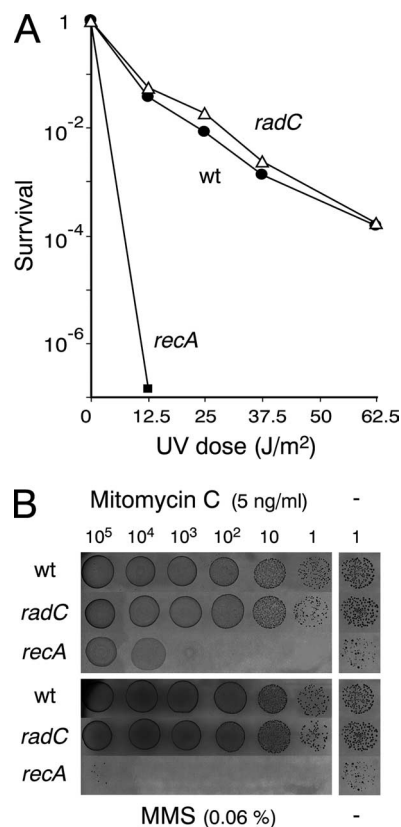


FIG. 3. Inactivation of *radC* has no detectable effect on resistance to UV irradiation (A) and mitomycin C and MMS (B). The strains were the wild type (R1501), the *radC* mutant strain (R1966), and the *recA* mutant strain (R1409).

establishment of rolling-circle replicons via transformation. These data provide no support to the hypothesis that RadC has a helicase-related function in connection with PcrA.

Concluding remarks. In the facultative phototrophic bacterium *Rhodobacter capsulatus*, *radC* was shown to be relatively highly expressed under both chemotrophic and phototrophic growth conditions (10). The expression of *radC* was reported to increase about fivefold after UV irradiation. However, the survival rate of *radC* mutant cells to mild UV treatment was not significantly different from that of the wild-type parent (10). In *B. subtilis*, *radC* belongs to the σ^M -responsive genes (9). As such, it is induced in response to environmental stresses, for example, stress generated by cationic antimicrobial peptides (15). During competence *radC* is specifically coinduced with six flanking genes: *maf-(radC)-mreBCD-minCD*. These genes encode proteins involved in cell shape determination and septum placement, suggesting that RadC could have a function related to cell envelope metabolism. However, neither of these observations provides a real clue as to the role of RadC since the genomic context of *radC* orthologues varies between species (data not shown). Thus, both the function of RadC proteins in nontransformable bacteria and their specific role during competence in naturally transformable species remain to be determined.

In view of the observations concerning *radC102* of *E. coli* (11) and those on *radC* of *S. pneumoniae* (the present study),

the pfam Group (<http://pfam.sanger.ac.uk/>) has followed our suggestion to rename the RadC family (to DUF2466; DUF stands for domain of unknown function), thereby avoiding the systematic annotation of newly sequenced bacterial genomes with a potentially misleading gene name.

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