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Escherichia coli K-12 Mutants in Which Viability Is Dependent on *recA* Function

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A gene required for growth and viability in *recA* mutants of *Escherichia coli* K-12 was identified. This gene, *rdgB* (for Rec-dependent growth), mapped near 64 min on the *E. coli* genetic map. In a strain carrying a temperature-sensitive *recA* allele, *recA200*, and an *rdgB* mutation, DNA synthesis but not protein synthesis ceased after 80 min of incubation at 42°C, and there was extensive DNA degradation. The *rdgB* mutation alone had no apparent effect on DNA synthesis or growth; however, mutant strains did show enhanced intrachromosomal recombination and induction of the SOS regulon. The *rdgB* gene was cloned and its gene product identified through the construction and analysis of deletion and insertion mutations of *rdgB*-containing plasmids. The ability of a plasmid to complement an *rdgB recA* mutant was correlated with its ability to produce a 25-kilodalton polypeptide as detected by the maxicell technique.

The RecA protein of *Escherichia coli* has several enzymatic activities (24), is required for homologous recombination (24), and, in conjunction with the LexA protein, serves to control a complex set of events which occur after cells are exposed to agents which damage DNA, the SOS response (32). RecA protein is expressed at low levels under normal growth conditions, but its synthesis is greatly increased after SOS induction. Mutants deficient in RecA protein are deficient in homlogous recombination (18), are hypersensitive to many DNA-damaging agents (6), and exhibit spontaneous DNA degradation (5).

Although RecA protein is required for several aspects of DNA metabolism, mutants deficient in the protein are viable. Several conditionally lethal double mutations have been isolated in which a temperature-sensitive *recA* allele confers temperature sensitivity for growth and viability. Mutations in *polA* (23), *dam* (21), *rdg* (10), and *sdrT* (16) all confer conditional lethality on *E. coli* carrying the *recA200* mutation. In the case of *polA* and *dam*, DNA degradation appears to be the lethal event. In cells deficient in *rdg*, RNA synthesis is inhibited, while in *sdrT* mutants the initiation of stable DNA replication is blocked.

E. coli is capable of repairing several thousand apurinic-apyrimidinic (AP) sites per cell per generation which arise from the incorporation and subsequent removal of uracil from DNA. AP sites can be repaired by excision repair initiated by exonuclease III (30) or by a RecA-mediated event (17). A strain carrying both a temperature-sensitive allele for the exonuclease III gene and a temperature-sensitive allele for the *recA* gene is viable at 42°C and must have some other means for repairing AP sites. We therefore looked among the survivors of transposon Tn*5* mutagenesis of a strain which carried *xthA3* and *recA200* for clones that were temperature sensitive for growth. We expected to isolate mutants deficient in endonuclease IV, another AP endonuclease found in *E. coli*. Instead we found a mutation which conferred a temperature-sensitive phenotype on strains bearing *recA200* alone. We report the isolation and properties of strains which carry a mutation in a new locus in *E. coli*, *rdgB*.

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MATERIALS AND METHODS

Strains and growth conditions. The bacterial strains, plasmids, and phage used in this study are listed in Table 1. The media used for growth were tryptone yeast (TY; 10 g of tryptone, 5 g of yeast extract, and 10 g of NaCl per liter), VB minimal medium (35), M9 minimal medium (22), and nutritional supplements (35). M9 medium was sometimes supplemented with vitamin-free Casamino Acids (Difco Laboratories). For cell dilutions, buffered saline containing 0.9% NaCl and 50 mM Tris hydrochloride (pH 7.5) was used. Antibiotics were used at the following concentrations except where noted: tetracycline hydrochloride, 20 μg/ml; sodium ampicillin, 50 μg/ml; kanamycin sulfate, 50 μg/ml; streptomycin sulfate, 200 μg/ml. Cell growth was routinely monitored in a Klett-Summerson photocolorometer with a green 54 filter; 30 Klett units were equivalent to 10^8 cells/ml.

Strain construction. P1-mediated transductions were performed as described previously (34). *recA* alleles were introduced into appropriate recipients by cotransduction with the linked *srlC300*::Tn*10* allele (7). *rdgB2*::Tn*5* mutants were constructed by transducing the kanamycin resistance marker of Tn*5* associated with *rdgB* into a *recA200 srlC300*::Tn*10* recipient. Kanamycin-resistant transductants were then screened for temperature sensitivity. The wild-type *recA* gene was introduced into the chromosome by transduction of *recA200 srlC300*::Tn*10* to Srl^+, followed by screening for UV resistance. Transformations were performed as described previously (20).

Bacterial matings. Matings were performed in TY broth supplemented with 0.1% glucose. Fresh overnight cultures (0.1 ml) were diluted into 10 ml of fresh medium and grown with shaking at 37°C. When the cultures reached a cell density of 2×10^8/ml, they were mixed at a ratio of 1:10, F^- in excess, and incubated with gentle shaking for 1 h. Matings were interrupted by vigorous vortexing prior to plating on selective medium. Unmated Hfr and F^- bacteria were also plated as controls.

Tn*5* mutagenesis. For Tn*5* mutagenesis, RPC1 was grown to exponential phase in TY broth supplemented with 0.1% maltose at 37°C. Cells were harvested and suspended in 10 mM $MgSO_4$ for 1 h at room temperature. The culture was infected with λ *b*221 *ral*::Tn*5* *c*I857 *O*am29 at a multiplicity

of 10 for 2 h at room temperature, harvested, and plated on VB minimal glucose plates containing kanamycin sulfate (75 μg/ml) and 2.5 mM sodium PP_i. Four thousand colonies were picked and grown in individual wells of microwell plates. Samples of the 4,000 cultures were transferred to the surface of TY agar plates with a multipronged inoculator, and the plates were incubated at 30 and 42°C. Ten temperature-sensitive clones were obtained, and two of these, RPC102 and RPC104, were subsequently shown to be nonlysogenic by cross-streaking them against phage strains λ *C60* and λ *vir*. The other eight temperature-sensitive isolates were lysogens of unknown origin.

Enzyme assay. β-Galactosidase levels were determined by the method of Miller (22).

Measurement of mutation frequencies. Modified fluctuation tests were carried out by the procedure of Duncan and Weiss (9).

Measurement of recombination proficiency. Intrachromosomal recombination was measured by a modification of the procedure of Zieg et al. (37). Colonies grown for 20 h were picked from TY plates and grown in TY medium to a cell density of 10^8/ml. Cells were harvested, washed twice, and suspended in the original volume of buffered saline. Lac^+ recombinants were selected on VB minimal lactose plates supplemented with vitamin B_1, histidine, and arginine. Bacterial matings to determine conjugal proficiency were performed as described above. AB259 was crossed with AB1157 or RPC47, and Thr^+ and Str^r transconjugants were selected.

Analysis of plasmid-specific proteins. Maxicell analysis of plasmid-coded proteins was performed by the procedure of Sancar et al. (27) as described by Rodriguez and Tait (26).

Isolation and manipulation of DNA. The conditions for restriction endonuclease and BAL31 nuclease digestion were those suggested by the manufacturers. The addition of DNA linkers was performed as previously described (20). Ligation was done essentially by the procedure described in the International Biotechnologies, Inc., catalog. Plasmids were amplified with chloramphenicol and purified by a modified version of the alkaline lysis procedure of Birnboim and Doly (4). To construct an *E. coli* gene library, chromosomal DNA was extracted from AB1157 as described previously (26) and partially cleaved with *Sau*3AI. DNA fragments 5 to 15 kilobase pairs (kb) long were isolated by fractionation on 5 to 25% sucrose gradients (20), purified, and ligated into the *Bam*HI site of pBR322. Transformants of RPC72 were selected for ampicillin resistance and screened for temperature resistance by picking into microwell plates and replica plating at 30 and 42°C.

Measurement of sensitivity to DNA-damaging agents. Sensitivity to H_2O_2, UV irradiation, methyl methanesulfonate (MMS), and tertiary butyl hydroperoxide was determined as described previously (8).

RESULTS

Isolation of a mutation causing temperature-sensitive growth in conjunction with *recA200*. The rationale for our search for a mutation in the endonuclease IV gene, as described earlier, was to mutagenize a *recA*(Ts) *xthA*(Ts) strain and look for survivors whose growth was temperature dependent. Since *E. coli* can repair several thousand AP sites per cell per generation even in the absence of exonuclease III and RecA protein, we anticipated that endonuclease IV, another type II AP endonuclease, was capable of initiating repair at AP sites and that a mutation eliminating this repair would make a *recA xthA* strain inviable. Two clones which were temperature sensitive for growth were isolated after mutagenesis of RPC1 by Tn*5*. To determine whether the temperature sensitivity of these two strains was due to a Tn*5*-induced mutation, we prepared P1 lysates on these strains and transduced RPC1 to kanamycin resistance. The Tn*5* insertion from one of the two donors, RPC104, caused RPC1 to become temperature sensitive. To test whether the mutation in RPC104 required mutations in both *recA* and *xthA* to cause temperature-sensitive growth, we transduced the mutation into single *recA* and *xthA* mutants, RPC14 and BW299, respectively. The Tn*5* insertion in RPC104 conferred a temperature-sensitive phenotype on RPC14 but not on BW299. The Tn*5* insertion in RPC104 created a mutation which caused a defect in growth when RecA protein was inactive. This phenotype was not consistent with our assumption that an endonuclease IV mutation would cause inviability in a *recA*(Ts) *xthA*(Ts) strain, and the mutation did not alter the levels of endonuclease IV (unpublished results).

Genetic location of the mutation in RPC104 responsible for temperature-sensitive growth. The mutation in RPC104 could be in *dam*, *polA*, *rdg*, *sdrT*, or a new gene. We mapped the mutation to distinguish among these possibilities. An approximate map position was obtained for the mutation by conjugational analysis. Neither KL14, which transfers markers in a clockwise direction from an origin at 66 min, nor KL16, which transfers markers in a counterclockwise direction from an origin at 62 min, could transfer a wild-type copy of the gene to RPC48. This suggested that the mutation mapped between 62 and 66 min on the *E. coli* genetic map. Since none of the genes known to be required in the absence of RecA protein fall in this interval, the mutation we identified may be in a new gene. We designated the gene *rdgB* and the mutation *rdgB2*::Tn*5*. We will designate the gene called *rdg* by Froehlich and Epstein (10) as *rdgA*.

More precise mapping of the *rdgB* mutation was accomplished by a series of two-factor crosses mediated by P1. We found that *rdgB2*::Tn*5* was linked to both *serA* and *metC* at 62.8 and 65 min, respectively, on the *E. coli* genetic map. Our initial data (Fig. 1A) placed *rdgB* between 63.6 and 64.2 min. Because we observed that Tn*5* could undergo transposition when introduced into a recipient cell during transduction, all transductions in which we selected for *rdgB2*::Tn*5* by resisitance to kanamycin were performed with recipients which carried the *recA200* allele. Kanamycin-resistant transductants were then screened for temperature-sensitive growth to confirm that a transductional event had occurred. Since the *recA200* allele might influence recombination frequencies, all transductions were performed with recipients carrying the *recA200* allele. This could account for the disagreement in map position when we used cotransduction frequencies obtained from crosses with *serA* and *metC*. We created another mutation by in vitro mutagenesis, *rdgB3*::*kan* (see below), which could be mapped in a $recA^+$ background. Averaging the distances obtained from cotransduction data for this mutation (Fig. 1B) placed *rdgB* at approximately 63.9 min on the *E. coli* map.

Cloning the *rdgB* gene. We isolated 11 clones from a plasmid library constructed from DNA from wild-type *E. coli* which complemented the double mutant RPC72. Since a clone of either the *recA* gene or the *rdgB* gene might complement the double mutant, we screened the 11 isolates for sensitivity to UV irradiation at 42°C. A wild-type *recA* gene would be expected to complement this defect. Two of the clones conferred resistance to UV irradiation. Restric-

TABLE 1. *E. coli* strains, bacteriophages, and plasmids

Designation	Description	Source (reference)[a]
E. coli strains[b]		
AB259	HfrPO1 *thi-1 relA1 spoT1*	B. Weiss (19)
AB1157	*thr-1 leuB6* Δ(*gpt-proA*)*2 hisG4 argE3 thi-1 ara-14 lacY1 galK2 xyl-5 mtl-1 rpsL31 tsx-33 supE44 rac*	B. Weiss (1)
BW299	KL16 *xthA3*	B. Weiss
BW9113	*argE3 his-4 thi-1 Spc*^{r} *xyl-5 mtl-1 tsx-29 supE44 zdh-201*::Tn*10* (*lacMS286* φ80dII *lacBK1*)	B. Weiss
DR1984	AB1157 *uvrC34 recA1*	CGSG (28)
JC158	*thi-1 serA6 relA1 lacI22*	CGSC
JC10240	HfrPO45 *recA56 srlC300*::Tn*10 thr-300 ilv-318 rpsE300*	B. Weiss (7)
KL14	HfrPO68 *thi-1 relA1 spoT1*	B. Weiss (3)
KL16	HfrPO45 *thi-1 relA1 spoT1*	B. Weiss (3)
KL268	HfrPO45 *thi-1 relA1 spoT1 deoB13 argA21 recA200*	B. Weiss
MM383	*lacZ53 rpsL151 thyA36 rha-5 deoC2* Δ(*rrnD-rrnE*) *polA12*	R. McMacken (23)
NK6027	HfrPO1 (*lac-pro*)*XIII thi-1 metC*::Tn*10*	N. Kleckner
NO120	Δ(*argF-lac*)*205 relA1 thi-1 sulA*::Mu d(Ap *lac* Xcam^r) *cps*	N. Ossanna
RPC0	BW299 *srlC300*::Tn*10*	BW299 × P1/JC10240
RPC1	BW299 *recA200*	RPCO × P1/KL268
RPC8	KL16 *srlC300*::Tn*10*	KL16 × P1/BW327
RPC10	KL16 *recA200*	RPC8 × P1/KL268
RPC13	RPC10 *rdgB2*::Tn*5*	RPC10 × P1/RPC104
RPC14	RPC10 *srlC300*::Tn*10*	RPC10 × P1/JC10240
RPC38	RPC14 *rdgB2*::Tn*5*	RPC14 × P1/RPC13
RPC39	KL16 *rdgB2*::Tn*5*	RPC38 × P1/KL16
RPC44	AB1157 *recA200 srlC300*::Tn*10*	AB1157 × P1/RPC14
RPC46	RPC44 *rdgB2*::Tn*5*	RPC44 × P1/RPC13
RPC47	AB1157 *rdgB2*::Tn*5*	RPC46 × P1/KL16
RPC48	AB1157 *rdgB2*::Tn*5 recA200*	RPC46 × P1/KL16
RPC52	RPC48 *metC*::Tn*10*	RPC48 × P1/NK6027
RPC53	RPC48 *metC*::Tn*10 rdgB*$^+$	RPC48 × P1/NK6027
RPC54	JC158 *recA200 srlC300*::Tn*10*	JC158 × P1/RPC14
RPC55	RPC54 *rdgB2*::Tn*5*	RPC54 × P1/RPC13
RPC58	KL16 *rdgB2*::Tn*5 recA200*	RPC10 × P1/RPC104
RPC63	KL16 *metC*::Tn*10*	KL16 × P1/NK6027
RPC70	AB1157 *srlC300*::Tn*10*	AB1157 × P1/RPC8
RPC71	AB1157 *recA200*	RPC70 × P1/RPC10
RPC72	RPC71 *rdgB2*::Tn*5*	RPC71 × P1/RPC47
RPC92	RPC96 *recA200 srlC300*::Tn*10*	RPC96 × P1/RPC14
RPC96	RPC98 *rdgB2*::Tn*5*	RPC98 × P1/RPC47
RPC98	AB1157 *thyA deo*	Spontaneous mutations[c]
RPC99	RPC98 *recA200 srlC200*::Tn*10*	RPC98 × P1/RPC14
RPC104	BW299 *rdgB2*::Tn*5*	This work
RPC130	MM383 *rdgB3*::*kan*	This work
RPC133	AB1157 *rdgB3*::*kan*	AB1157 × P1/RPC130
RPC134	BW9113 *rdgB2*::Tn*5*	BW9113 × P1/RPC47
RPC165	NO120 *rdgB3*::*kan*	NO120 × P1/RPC130
Bacteriophages		
P1 *vir*		CSH
λ *c60*		CSH
λ *vir*		CSH
λ *b221*		N. Kleckner
ral::Tn*5*		
*c*I857		
*O*am29		
Plasmids		
pBR322		CSH
pUC4-K		31

[a] Abbreviations: CGSC, Coli Genetic Stock Center, Yale University School of Medicine (New Haven, Conn.); CSH, Cold Spring Harbor Laboratory (Cold Spring Harbor, N.Y.): P1 transductions, recipient × P1/donor.

[b] All bacterial strains were F$^-$ λ$^-$ derivatives of *E. coli* K-12 unless otherwise indicated.

[c] Selection for trimethoprim resistance (*thyA*) was followed by selection for a low thymine requirement (*deo*) as described by Miller (22).

tion analysis of the recombinant plasmids from these two clones showed that both had inserts which contained restriction sites identical to those determined for the *recA* gene (29). The remaining nine isolates were as sensitive to UV irradiation at 42°C as a *recA200* strain. Restriction analysis of the recombinant plasmids from these clones revealed that two regions of DNA had been cloned. Three clones contained recombinant plasmids with inserts containing two identical *Eco*RI fragments, and six clones contained plasmids with inserts lacking any *Eco*RI sites but containing two

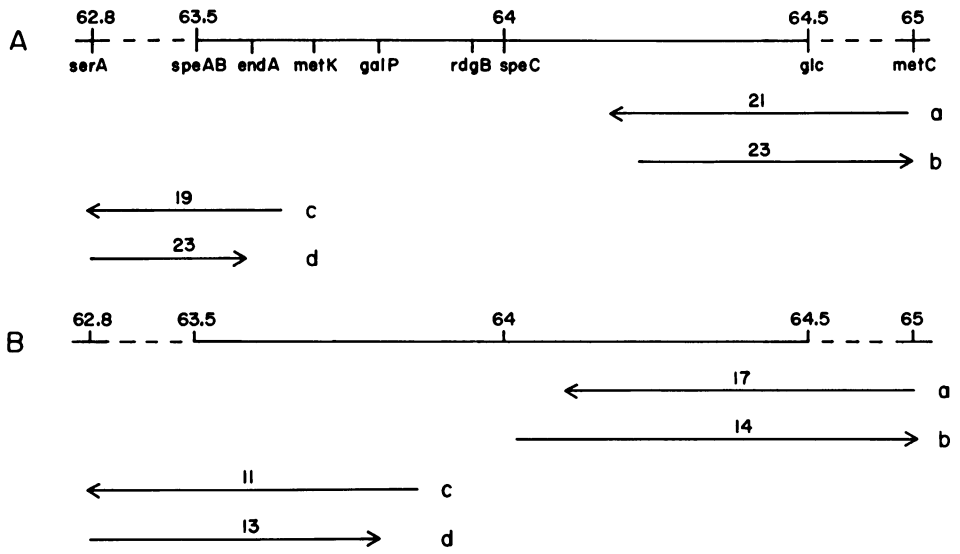

FIG. 1. Transductional mapping of the *rdgB* gene. A segment of the *E. coli* genetic map (2) is shown, to which we have added the *rdgB* gene. P1 transductional crosses are indicated by an arrow extending from the unselected marker toward the selected marker; values are cotransduction frequencies in percent; cotransductional frequencies were converted to map distances by the formula of Wu (36), with the assumption that the effective length of the P1 transducing fragment is 2 min. (A) Map position of *rdgB2*::Tn*5* based on crosses in which the recipients carried the *recA200* allele. The crosses (recipient × donor) (number of recombinants scored) are as follows: (a) AB1157 × RPC52 (90), (b) RPC13 × RPC53 (84), (c) AB1157 × RPC55 (104), and (d) RPC13 × RPC54 (145). (B) Map position of *rdgB3*::*kan* based on crosses in which the recipient carried the *recA*$^+$ allele. (a) RPC133 × RPC63 (94), (b) RPC133 × RPC63 (94), (c) RPC133 × JC158 (94), (d) RPC133 × JC158 (94).

identical *Eco*RV fragments. One plasmid from each class was chosen for further study. To determine whether either plasmid carried the *rdgB* gene, we determined the map position of each insert with a drug resistance gene as a genetic marker. We inserted a gene for kanamycin resistance derived from pUC4-K into an *Eco*RI site of pRPC200 linearized by partial digestion to create pRPC252 and into a *Sal*I site of pRPC200 to create pRPC201. These plasmids were transformed into strain MM383, which has a temperature-sensitive DNA polymerase I. The transformed cells were grown at 42°C in the presence of kanamycin to select for chromosomal integration of the plasmid. Subsequent incubations at 32 and 42°C in the presence of kanamycin were used to select haploid segregants bearing the kanamycin resistance gene integrated into the chromosome at the site of the insert (8). Phage P1 was then used to map the kanamycin resistance gene. The insert derived from pRPC200 was linked to both *metC* and *serA*, while the insert from pRPC245 was linked to *ampC* and *purA*. The plasmid pRPC200 carries a copy of the *rdgB* gene, as determined by its ability to complement the double mutant and its map position. The plasmid pRPC248 contains a gene or sequence of DNA which can suppress the defect of the double mutant. It remains to be tested whether plasmid copy number has an effect on suppression.

To further locate the *rdgB* gene in pRPC200, which has a 10.1-kb insert, we created a series of subclones and screened them for the ability to complement a double mutant. The restriction map of pRPC200 and the subclones we constructed are shown in Fig. 2. The *rdgB* gene was localized to a 4.7-kb segment of DNA in pRPC212 by subcloning an *Hin*dIII-*Sal*I fragment into pBR322. A mutant plasmid, pRPC231, was constructed by cloning the kanamycin resistance gene from pUC4-K into one of the *Eco*RV sites of pRPC212. The gene was further localized to a 1.3-kb segment of DNA on a *Pvu*I-*Hin*cII fragment in pRPC238. Deletions generated by BAL31 nuclease further localized the *rdgB* gene to a 0.85-kb segment of DNA in pRPC240. Insertion of the kanamycin resistance gene into the EcoRV site on the insert in pRPC240 inactivated the *rdgB* gene in the mutant plasmid pRPC243.

***rdgB* gene product is a 25-kilodalton polypeptide.** We identified an M_r-25,000 (25K) protein encoded by the *rdgB*$^+$ plasmid pRPC240, which was absent in pRPC243, that had an insertion that eliminated the ability to complement a double mutant. We identified this polypeptide by the maxicell technique, which specifically labels plasmid-coded proteins. Figure 3 shows the autoradiogram of a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel displaying the plasmid-coded proteins. The plasmid pRPC56 (lane 4) expressed β-lactamase and aminoglycoside phosphotransferse II, both at 31K. Plasmid pRPC240 expressed β-lactamase and a 25K protein (lane 2). When insertion of the kanamycin resistance gene eliminated *rdgB* activity in pRPC243 (lane 3), the 25K polypeptide was no longer detectable. A 0.85-kb fragment of DNA was sufficient to code for a 30K protein. Thus, we have subcloned the *rdgB* gene with little extra DNA sequences.

Isolation of a second *rdgB* mutant. A chromosomal *rdgB* mutation derived from the plasmid pRPC231 was obtained through genetic recombination with the plasmid as described above. The new mutation, *rdgB3*::*kan*, conferred a temperature-sensitive phenotype on strains bearing *recA200*. Since the kanamycin resistance gene was 100% linked to *rdgB3*::*kan*, genetic manipulations with this mutation were easily accomplished, and we mapped this new mutation to between 63.9 and 64.1 min on the *E. coli* map (Fig. 1).

Growth defect in *rdgB recA*(Ts) strains. Since recA protein is required for several aspects of DNA metabolism in *E. coli*, we examined the kinetics of DNA synthesis and protein synthesis at the nonpermissive temperature in mutant strains by measuring the cumulative incorporation of [^{3}H]thymine

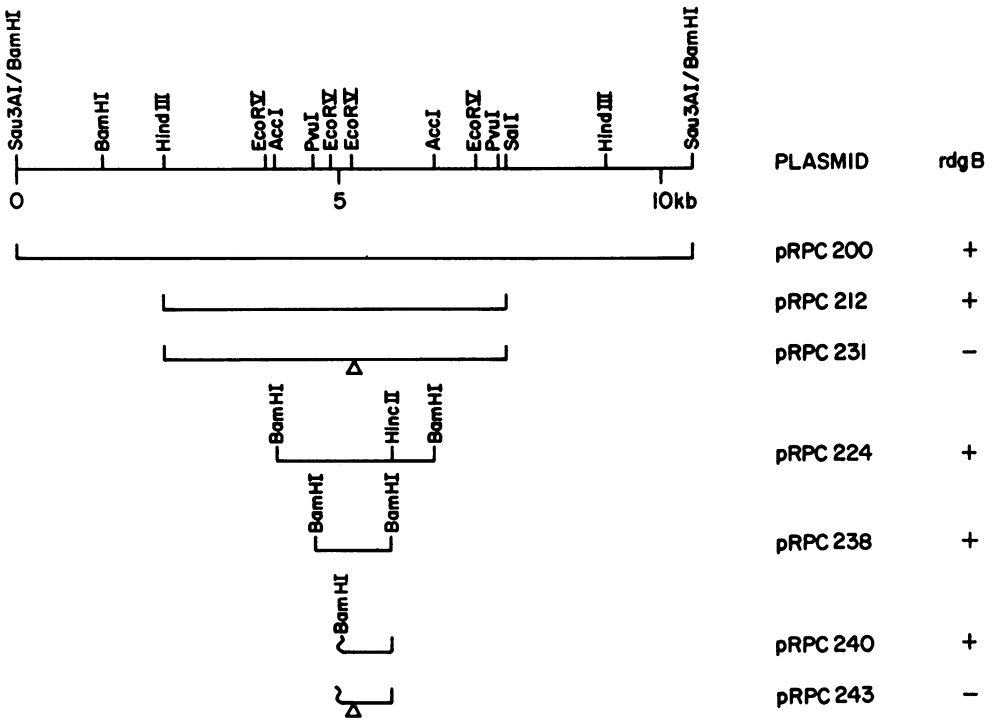

FIG. 2. Physical map of pRPC200 and its derivatives. The top line is a restriction map of the chromosomal DNA obtained from a *Sau*3AI partial digest cloned into the *Bam*HI site of pBR322. Vertical lines represent restriction sites used for subcloning. For plasmids pRPC234 and pRPC238, vertical lines with new restriction sites indicate that *Bam*HI linkers were used for subcloning. For plasmid pRPC224 a *Hinc*II site not mapped in pRPC200 is included. Wavy vertical lines indicate the endpoint of a deletion created by BAL31 nuclease digestion. A triangle represents an inserted kanamycin resistance gene. The plasmids and their properties are tabulated on the right. A plus indicates the ability of the plasmid to confer temperature resistance on a *recA200 rdgB2*::Tn*5* host at 42°C.

and [^{3}H]leucine (Fig. 4). In an *rdgB2*::Tn*5* *recA200* double mutant, incorporation of [^{3}H]thymine declined after 60 min at the nonpermissive temperature and stopped by 80 min. DNA synthesis in an *rdgB2*::Tn*5* mutant was normal, while

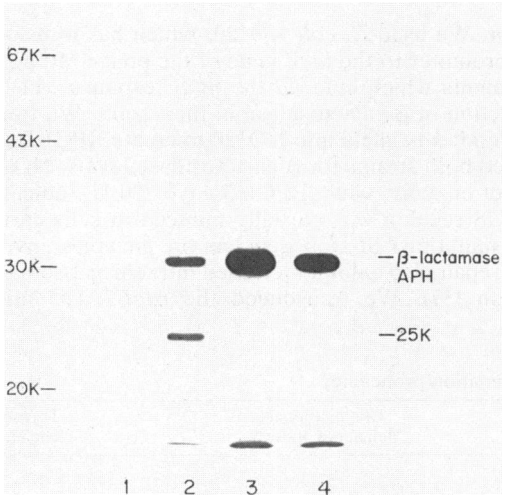

FIG. 3. SDS-PAGE of proteins produced by plasmids bearing wild-type and disrupted *rdgB* genes in strain DR1984. Proteins were labeled with [^{35}S]methionine by the maxicell technique. Lanes: 1, no plasmid; 2, pRPC240 (wild-type *rdgB* gene); 3, pRPC243 (disrupted *rdgB* gene); 4, pRPC56 (vector with a cloned kanamycin resistance gene). APH, Aminoglycoside 3′-phosphotransferase.

DNA synthesis in a *recA200* mutant was slightly reduced. Protein synthesis in the double mutant did not decline until 80 min after the temperature shift and was only slightly reduced at 160 min. This suggests that the defect in DNA synthesis was not caused by a general defect in RNA or protein metabolism.

Since *recA* mutants spontaneously degrade their DNA, it is possible that the defect in DNA synthesis seen by measuring cumulative incorporation of [^{3}H]thymine into DNA is a reflection of increased DNA degradation in the double mutant. To determine whether DNA degradation did occur in the double mutant, we added a large excess of cold thymine to labeled cells and monitored the level of acid-insoluble radioactivity. The double mutant showed substantial degradation of chromosomal DNA (Fig. 5), while the *recA200* mutant showed moderate levels of degradation. Neither the wild-type strain nor an *rdgB* strain exhibited degradation of chromosomal DNA. These data suggest that the defect in DNA synthesis seen in Fig. 4 was due to degradation of DNA rather than to a defect in polymerization. The extensive degradation of DNA in the double mutant might be expected to cause inviability at the nonpermissive temperature. We grew RPC98 and RPC92 at the nonpermissive temperature for various periods of time and plated the cells at the permissive temperature to quantitate the number of viable cells (Fig. 6). The double mutant continued to grow and produce viable cells for 60 min at 42°C. After 1 h, however, the double mutant rapidly lost viability. The defect in a *recA*(Ts) *rdgB* double mutant appears to be increased degradation of DNA, which leads to cell death.

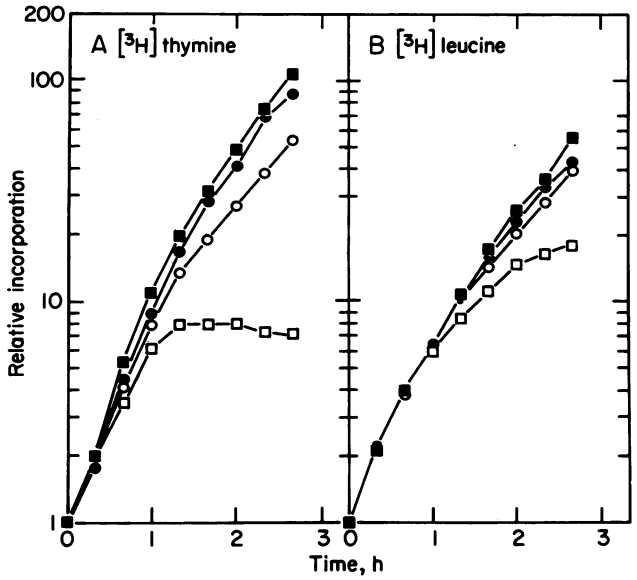

FIG. 4. DNA synthesis (A) and protein synthesis (B) in mutant strains at the nonpermissive temperature. Strains RPC98 (wild-type) (■), RPC99 (*recA200*) (○), RPC96 (*rdgB2*::Tn*5*) (●), and RPC92 (*recA200 rdgB2*::Tn*5*) (□) were grown at 30°C in M-9 medium containing 5 μg of thymine per ml and 20 μg of each required amino acid per ml. When the cell densities were approximately 2×10^8/ml, the cultures were diluted 20-fold into the same medium containing [*methyl*-^{3}H]thymine at 10 μCi/ml (A) or L-[4,5-^{3}H]leucine at 10 μCi/ml. After 1 h the cultures were shifted to 42°C (zero time). Samples (50 μl) were removed periodically and applied to a filter, treated with acid, and washed as previously described (34). The data reported are relative to the following values at zero time: (A) 519 cpm for RPC99, 375 cpm for RPC98, 414 cpm for RPC96, and 404 cpm for RPC92; (B) 733 cpm for RPC99, 885 cpm for RPC98, 605 cpm for RPC96, and 641 cpm for RPC92.

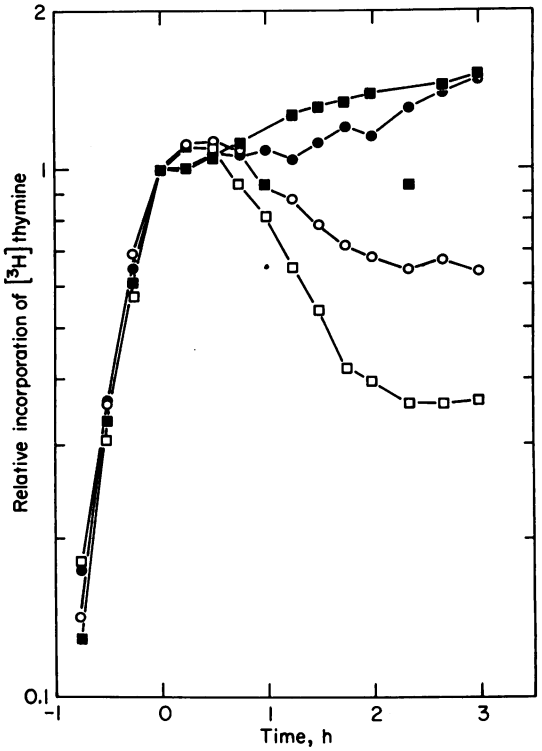

FIG. 5. Degradation of chromosomal DNA in mutant strains after a shift to the nonpermissive temperature. Cultures of KL16 (wild-type) (■), RPC10 (*recA200*) (○), RPC39 (*rdgB2*::Tn*5*) (●), and RPC58 (*recA200 rdgB2*::Tn*5*) (□) were grown in M-9 medium containing 1% Norit-treated Casamino Acids and 1.5 mM deoxyadenosine at 30°C. When the cell densities were about 2×10^8/ml, the cultures were diluted 20-fold into the same medium containing [*methyl*-^{3}H]thymine at 10 μCi/ml. After 1 h, thymine was added to 400 μg/ml, and the cultures were shifted to 42°C. Portions (50 μl) were sampled as described in the legend to Fig. 4. Acid-precipitable radioactivity was recorded relative to values at zero time: 17,443 cpm for KL16, 12,599 cpm for RPC10, 11,964 cpm for RPC39, and 3,017 cpm for RPC58.

Properties of *rdgB* strains. Strains bearing an *rdgB* mutation grow normally, exhibit normal DNA synthesis, and do not show degradation of their DNA. Strains bearing *recA* and *rdgB* mutations degrade their DNA, leading to cell death. The increased instability of DNA in the double mutant suggests that the *rdgB* gene product might share some overlapping role with RecA protein in DNA repair. We examined several properties of *rdgB* mutants to determine whether they exhibited some deficiency in repair. *rdgB* mutants were not significantly sensitive to UV irradiation, MMS, hydrogen peroxide, nitrofurantoin, or tertiary butyl hydroperoxide (data not shown). We also examined several other phenotypes that might indicate a role in repair. One cellular response to DNA damage is induction of the SOS system; thus, we determined whether a gene known to be part of the SOS regulon, *sulA* (11), was induced in an *rdgB* mutant. We used *E. coli* NO120, which has a fusion of the *sulA* promoter to the *lacZ* gene of the phage Mu d(Ap^r *lac*). Treatments which induced the SOS response caused overproduction of β-galactosidase in this strain. We transduced the *rdgB3*::*kan* allele into NO120 to create RPC165 and then assayed both strains for β-galactosidase levels. NO120 gave 57 U of enzyme, while RPC165 gave 270 U, indicating that the SOS regulon was partially induced in cells carrying an *rdgB* mutation. Strains deficient in enzymes involved in DNA repair also exhibit increased intrachromosomal recombination (37). We transduced the *rdgB2*::Tn*5* allele into

TABLE 2. Effect of *rdgB* on recombination proficiency

Strain	Relevant genotype	No. of Lac$^+$ colonies/plate	Conjugational deficiency index[a]	Transductional deficiency index[b]
BW9113	Wild type	59	—[c]	—
RPC134	*rdgB2*::Tn*5*	322	—	—
AB1157	Wild type	—	1.0	1.0
RPC47	*rdgB2*::Tn*5*	—	1.1	1.5

[a] Conjugal deficiency indexes represent wild-type frequencies divided by mutant frequencies for 40-min matings with AB259 selecting Thr$^+$ Str$^+$ recombinants.
[b] Transductional deficiency indexes represent wild-type frequencies divided by mutant frequencies for transductions selecting for His$^+$ transductants. Transductions were carried out with P1 *vir* grown on KL16. Transductions were carried out at a multiplicity of infection of 0.67.
[c] —, Not determined.

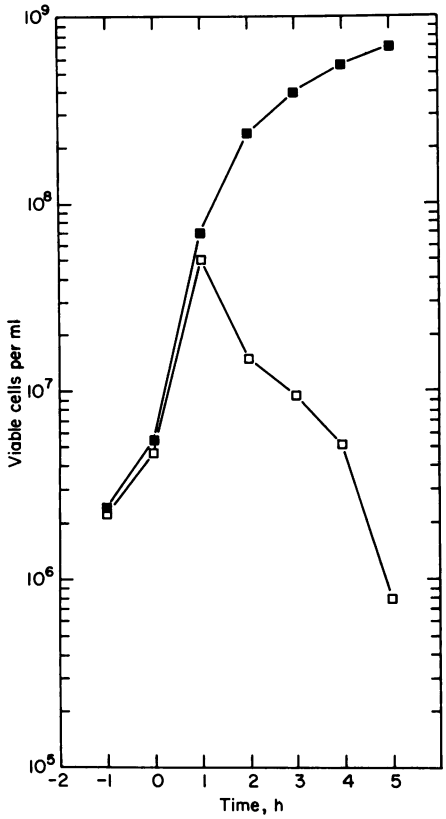

FIG. 6. Viability of a *recA200 rdgB2*::Tn*5* strain after a shift to the nonpermissive temperature. Cultures of RPC98 (wild-type) (□) and RPC92 (*recA200 rdgB2*::Tn*5*) (■) were grown to a cell density of approximately 2×10^7/ml in TY broth at 30°C. The cultures were diluted 20-fold into the same medium and grown at 30°C for 1 h and then shifted to 42°C (zero time). Viable counts were determined periodically by plating samples of the culture on TY plates which were incubated at 30°C for 24 h.

BW9113, which carries a nontandem duplication of two partially deleted lactose operons, to produce RPC134 and measured the ability of the two strains to produce Lac$^+$ progeny (Table 2). RPC134 exhibited a fivefold increase in Lac$^+$ progeny formation. We also examined the conjugational and transductional proficiency of an *rdgB* mutant (Table 2). The efficiency of neither transduction nor conjugation was appreciably affected in the mutant. Finally, we determined whether *rdgB* mutants exhibited an increase in spontaneous mutation rate (Table 3). Both the *his-4* allele and the *argE3* allele reverted at a slightly decreased rate in an *rdgB* mutant.

TABLE 3. Spontaneous mutation rates in an *rdgB* mutant

Strain	Relevant genotype	Allele tested	Reversion frequency/10^9 cells
AB1157	Wild type	*his-4*	3.2
RPC47	*rdgB2*::Tn*5*	*his-4*	1.0
AB1157	Wild type	*argE3*	1.9
RPC47	*rdgB2*::Tn*5*	*argE3*	0.6

DISCUSSION

The *rdgB2*::Tn*5* and *rdgB3*::*kan* mutations defined a new gene in *E. coli*. The *rdgB* gene expands a class of genes which are required for viability when RecA protein is absent: *dam*, *sdrT*, *rdgA*, and *polA*. The lethal defect in a *dam recA* mutant is the formation of double-strand breaks (33). Radman and Wagner (25) proposed that the mismatch repair system incises both strands at mismatches when DNA is not methylated, leading to the formation of overlapping excision events and strand breakage. Repair of double-strand breaks requires a functional *recA* gene (15). The *rdgB* mutation does not appear to affect methyl-directed mismatch repair. We have found that *dam* methylation sites, GATC, are normally methylated in *rdgB* mutants (unpublished results), and we have also shown that *rdgB* mutants do not have increased mutation frequencies, which would be expected if there were a defect in certain types of repair.

The lethal defect in *sdrT recA* mutants appears to be the constitutive rather than the inducible nature of stable DNA replication (cSDR) (16). A similar phenotype is seen in *rnh dnaA recA* mutants (12, 14). Initiation of replication in *rnh* mutants does not occur at *oriC* and does not require DnaA protein. The initiation event in these strains occurs at other sites, *oriK*, and requires RecA protein for initiation of chromosomal replication (12). The remainder of the replication cycle does not require RecA protein, and the cells contain integral chromosomes when RecA protein is inactivated (13). There is no DNA degradation of these completed chromosomes (13). Since *rdgB recA* mutants exhibit significant DNA degradation, it appears that the *rdgB* mutation may not alter replication and make the cells dependent on cSDR.

Transcription is blocked in *rdgA recA* strains when RecA function is lost, while DNA synthesis is not significantly affected and the cells remain viable for many hours (10). The difference in phenotype between *rdgA recA* and *rdgB recA* mutants suggests that these mutations do not affect the same process.

The reason for loss of viability in *recA polA* mutants is unknown (23). The double mutants exhibit extensive DNA degradation and loss of viability. It is possible that some essential repair event requires either RecA protein or DNA polymerase I.

We have not identified the function of the *rdgB* gene product; however, the properties of the single and double mutants suggest that it plays a role in maintaining the integrity of chromosomal DNA. The higher levels of intrachromosomal recombination and the induction of the SOS regulon suggest that some lesion in the chromosome requires repair by a RecA-mediated event in the absence of the *rdgB* gene product. In the absence of RecA protein and the *rdgB* gene product, this unrepaired lesion leads to inviability caused by degradation of the chromosome.

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