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DNA Polymerase I and the Bypassing of RecA Dependence of Constitutive Stable DNA Replication in *Escherichia coli rnhA* Mutants

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In *Escherichia coli rnhA* mutants, several normally repressed origins (*oriK* sites) of DNA replication are activated. The type of DNA replication initiated from these origins, termed constitutive stable DNA replication, does not require DnaA protein or the *oriC* site, which are essential for normal DNA replication. It requires active RecA protein. We previously found that the *lexA71*(Def)::Tn*5* mutation can suppress this RecA requirement and postulated that the derepression of a LexA regulon gene(s) leads to the activation of a bypass pathway, Rip (for RecA-independent process). In this study, we isolated a miniTn*10spc* insertion mutant that abolishes the ability of the *lexA*(Def) mutation to suppress the RecA requirement of constitutive stable DNA replication. Cloning and DNA sequencing analysis of the mutant revealed that the insertion occurs at the 3′ end of the coding region of the *polA* gene, which encodes DNA polymerase I. The mutant allele, designated *polA25*::miniTn*10spc*, is expected to abolish the polymerization activity but not the 5′→3′ or 3′→5′ exonuclease activity. Thus, the Rip bypass pathway requires active DNA polymerase I. Since the lethal combination of *recA*(Def) and *polA25*::miniTn*10spc* could be suppressed by derepression of the LexA regulon only when DNA replication is driven by the *oriC* system, it was suggested that the bypass pathway has a specific requirement for DNA polymerase I at the initiation step in the absence of RecA. An accompanying paper (Y. Cao and T. Kogoma, J. Bacteriol. 175:7254–7259, 1993) describes experiments to determine which activities of DNA polymerase I are required at the initiation step and discusses possible roles for DNA polymerase in the Rip bypass pathway.

The *oriC* site is normally the only active origin for initiation of chromosome replication in *Escherichia coli* (38). Mutations in the *rnhA* gene, which encodes RNase HI, activate a set of normally repressed origins (*oriK* sites) of chromosome replication (6, 18). It has been speculated that a large DNA-RNA hybrid occasionally forms during transcription at certain sites on the chromosome and is stabilized in the absence of RNase HI activity and that the resulting R-loop serves as an origin of opportunistic DNA replication (37). This type of DNA replication is termed constitutive stable DNA replication (cSDR). Although the efficiency and regulation of cSDR initiation are not as strong as those of *oriC* initiation (37), cSDR can sustain chromosome replication and renders the *oriC* system dispensable. Therefore, the otherwise lethal effect of deletion of *oriC* or inactivation of *dnaA* (the initiator gene) by insertion mutations is suppressed by an *rnhA* mutation; Δ*oriC rnhA* or *dnaA*::Tn*10 rnhA* mutants are viable (18).

The *oriK* system, unlike the *oriC* system, requires *recA*$^+$ (17, 35). Therefore, *dnaA*::Tn*10 rnhA recA*(Ts) (temperature-sensitive) mutants exhibit a temperature-sensitive cSDR activity and are temperature sensitive for growth. We previously reported that a mutation, termed *rin* (for *recA* independent), can suppress the RecA requirement of cSDR (35) and allows *dnaA*::Tn*10 rnhA recA*(Ts) mutants to grow at high temperatures (36). Alternatively, the *lexA71*(Def)::Tn*5* mutation, which derepresses the LexA regulon, leading to constitutive expression of the SOS response (39), also suppresses the RecA requirement: cSDR can occur at 42°C in *rnhA recA*(Ts) *lexA*(Def) strains. Introduction of an *rnhA*$^+$ gene completely inhibits the cSDR phenotype. Thus, the cSDR seen in these strains is not a manifestation of yet another replication pathway. We hypothesized that derepression of a gene under the control of the LexA repressor leads to activation of a process (Rip) (for RecA-independent process) which allows initiation at *oriK* sites in the absence of active RecA protein (36). In order to analyze the Rip bypass pathway, we have sought, in this study, a mutation that blocks the Rip activity. We describe the isolation and characterization of one such mutant and demonstrate that the mutation is a *polA* mutation that affects DNA polymerase I (PolI) activity.

MATERIALS AND METHODS

***E. coli* strains.** The *E. coli* strains used in this study are listed in Table 1. Three sets of isogenic strains were used: one set was derived from AQ634 (26), the second set was derived from JG108, and the third was derived from AQ377 (35). All strains were constructed by phage P1*vir*-mediated transduction (20), with the exceptions of AQ8876, which was constructed by Hfr-mediated conjugation; AQ8528, which was constructed by transformation with linear DNA (see below); and AQ8530, which was constructed by phage T4gt7-mediated transduction (42). The presence of *polA1* and *recA* mutations was verified by testing for sensitivity to UV radiation (UV^s). *polA25*::miniTn*10spc* strains were selected for Spc^r (resistance to spectinomycin) and confirmed by UV^s. AQ8867 was constructed from AQ8695 by transducing *rnhA*$^+$ with a linked Tn*10* insertion (Table 1). The presence of *rnhA*$^+$ was verified by the failure of pBR322 plasmid to transform the strain as

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TABLE 1. *E. coli* strains used in this study

Strain	Relevant genotype[a]					Other characteristic(s)	Source, reference, and/or construction information
	dnaA	*rnhA*	*recA*	*polA*	*lexA*		
AQ377[b]	+	*102*	*200*	+	+		35
AQ634[c]	+	+	+	+	+		26
AQ979	+	+	+	+	+	*zag*::Tn*10*	32
AQ1409	+	+	+	*1*	+	*zig*::Tn*10*	CM5280 (15)
AQ2652[d]	+	+	*200*	+	+		Derivative of JG108 (36)
AQ3364	*5*	+	+	+	+	*tnaA500*::Tn*10*	This laboratory
AQ3476[b]	+	*102*	*200*	+	*71*::Tn5	*sfiA11*	Derivative of AQ377 (36)
AQ3519	*850*::Tn*10*	*339*::*cat*	+	+	+		This laboratory
AQ3588	+	+	+	+	+		JM109 (45)
AQ4275	+	+	+	+	+		MC1000 (5)
AQ4286[b]	*850*::Tn*10*	*102*	*200*	+	*71*::Tn5	*sfiA11*	P1.AQ3519 × AQ3476 → Tc^{r}
AQ4791	+	+	+	+	+	*recF332*::Tn*3*	This laboratory
AQ5225[b]	*850*::Tn*10*	*102*	*200*	*25*::*spc*	*71*::Tn5	*sfiA11*	This work
AQ6375	+	+	+	+	+	*thy*$^{+}$, *Hfr*	CAG12200 (31)
AQ6384	+	+	+	+	+	Tn*10* at 2 min	FMJ201 (31)
AQ6466	+	+	+	+	+	Tn*10* at 87 min	CAG18495 (31)
AQ6471	+	+	+	+	+	Tn*10* at 91.5 min	CAG12164 (31)
AQ7763	+	+	+	+	+	*recD1903*::mini-*tet*	Constructed from DBP271 (1)
AQ8115	+	+	Δ*306*	+	+	mini*F-kan-recA*$^{+}$	This laboratory
AQ8528	+	+	+	*25*::*spc*	+	*recD1903*::mini-*tet*	This work (see Materials and Methods)
AQ8530	+	+	+	*25*::*spc*	+		T4gt7.AQ8528 × AQ6466 → select for Spc^{r} → screen for UV^{s}
AQ8534	+	+	+	*25*::*spc*	+		P1.AQ6466 × AQ8530 → select for Tc^{r} → screen for Spc^{r} and UV^{s}
AQ8555[d]	+	+	*200*	*1*	+		P1.AQ1409 × AQ2652 → Tc^{r}, screen for UV^{s} at 30°C
AQ8560[c]	+	+	+	*25*::*spc*	+		P1.AQ8534 × AQ634 → Tc^{r}, UV^{s}
AQ8619[b]	+	*102*	*200*	*25*::*spc*	*71*::Tn5	*sfiA11*	P1.AQ8534 × AQ3476 → Tc^{r}, screen for Spc^{r}
AQ8620[d]	+	+	*200*	*25*::*spc*	+		P1.AQ8534 × AQ2652 → Tc^{r}, screen for Spc^{r}, UV^{s} at 30°C
AQ8695[b]	+	*102*	*200*	*25*::*spc*	*71*::Tn5	*sfiA11*	As AQ8619 except Tc^{s} (by Bochner selection)
AQ8747[b]	*5*	*102*	*200*	*25*::*spc*	*71*::Tn5	*sfiA11*	P1.AQ3364 × AQ8695 → Tc^{r}, screen for temperature sensitivity
AQ8809[c]	+	+	+	*1*	+		P1.AQ1409 × AQ634 → Tc^{r}, UV^{s}
AQ8817[b]	*5*	*102*	*200*	*25*::*spc*	*71*::Tn5	*sfiA11*	As AQ8747 except Tc^{s} (by Bochner selection)
AQ8825[b]	*5*	*102*	*200*	+	*71*::Tn5	*sfiA11*	P1.AQ6466 × AQ8817 → Tc^{r}, screen for UV^{r}
AQ8867[b]	+	+	*200*	*25*::*spc*	*71*::Tn5	*sfiA11*	P1.AQ979 × AQ8695 → Tc^{r}, screen for rnhA^{+} (see Materials and Methods)
AQ8876[b]	*5*	*102*	+	*25*::*spc*	*71*::Tn5	*sfiA11 thy*$^{+}$	AQ8817 × AQ6375 → Thy^{+}, screen for temperature resistance
AQ8877[b]	+	+	*200*	*25*::*spc*	*71*::Tn5	*sfiA11*	As AQ8867 except Tc^{s} (by Bochner selection)
AQ8885[b]	+	+	*200*	*25*::*spc*	+	*sfiA11*	P1.AQ6471 × AQ8877 → Tc^{r}, screen for Km^{s}
AQ8886[b]	+	+	Δ	*25*::*spc*	*71*::Tn5	*sfiA11*	P1.AQ8115 × AQ8877 → Tc^{r}, screen for more UV^{s} at 30°C
AQ8937[b]	*5*	*102*	*200*	+	*71*::Tn5	*sfiA11*	As AQ8825 except Tc^{s} (by Bochner selection)
AQ9232	+	+	+	+	+	Δ*polB*::*spc*	SH2101 (M. F. Goodman)
AQ9239	+	+	+	+	+	Δ*polB*::*spc*	P1.AQ6384 × AQ9232 → Tc^{r} → screen for Spc^{r}
AQ9250[b]	*5*	*102*	*200*	+	*71*::Tn5	Δ*polB*::*spc*	P1.AQ9239 × AQ8937 → Tc^{r} → screen for Spc^{r}
AQ9298[b]	*5*	*102*	*200*	+	*71*::Tn5	*recF*::Tn*3*	P1.AQ4791 × AQ8937 → Ap^{r}, verified by UV^{s}

[a] + indicates a wild-type gene, a number indicates an allele, and Δ indicates a deletion.
[b] Other genotype: *metE90 trpA9605 thy-708 deo-29 lacZ118 lacI22.*
[c] Other genotype: *ilv metB his-29 trpA9605 thyA deo proB.*
[d] Other genotype: *thyA36 deoC2 rha-5 lacZ53 lacY14 metE70 rpsL151.*

described previously (16). Isolation of Tc^{s} strains from Tc^{r} strains was done by the method of Bochner et al. (2). The Δ*polB*::*spc* strain was a generous gift from Myron Goodman. The allele was constructed by substituting parts of *araD* and *polB* genes with the omega fragment conferring Spc^{r}.

Chemicals and radioisotopes. Isopropyl-β-D-thiogalactoside (IPTG), antibiotics, and all other chemicals were from Sigma Chemical (St. Louis, Mo.). [*methyl*-^{3}H]thymine was from New England Nuclear Corp. (Boston, Mass.). Restriction enzymes were from GIBCO BRL Life Technologies, Inc. (Gaithersburg, Md.).

Media and growth conditions. Unless otherwise stated, cells were grown at 37°C with aeration by shaking either in Luria broth (LB) or M9 salts-glucose minimal medium supplemented with Casamino Acids (CAA medium) (0.2%; Difco Laboratories, Detroit, Mich.) and with specifically required amino acids (50 μg/ml) and thymine (8 μg/ml). When needed, antibiotics were added at the following concentrations: ampicillin, 40 μg/ml; chloramphenicol, 25 μg/ml; kanamycin, 55 μg/ml; and tetracycline, 20 μg/ml.

Construction of miniTn*10kan*::*spc*. The omega fragment conferring resistance to spectinomycin (27) was inserted at the

SmaI site of the *kan* gene of pNK862 (41). The resulting transposon-carrying plasmid was designated pBR862.

Mutagenesis with miniTn*10kan*::*spc*. An exponentially growing culture of MC1000 carrying pBR862 was incubated in the presence of IPTG (10^{-4} M) for 30 min to induce transposition. Cells were then concentrated 10-fold by centrifugation and subsequent resuspension with LB and infected with P1*vir*. The resultant P1 phage lysate was used to transduce AQ4286 to Spc^r. The Spc^r colonies were screened for temperature-sensitive growth by replica plating. Colonies that grew at 30°C but failed to grow at 42°C were purified and retested for temperature sensitivity.

Measurement of cSDR. The measurement of DNA synthesis in the presence of chloramphenicol was described previously (36).

Molecular techniques. DNA cloning and Southern hybridization were performed as described by Sambrook et al. (29). DNA sequencing was carried out with the M13 sequencing system from GIBCO BRL Life Technologies, Inc.

Transformation with linear DNA. A pUC8 derivative (pRRR25) containing a cloned *polA25*::miniTn*10spc* mutation was digested with *Pst*I. The digestion generated two linear fragments, a pUC8 vector containing an Ap^r marker (2.7 kb) and the insert that contained *polA25*::miniTn*10spc* (~11 kb). The linearized DNA fragments were used to transform a *recD* strain (AQ7763) with an electroporator (Invitrogen Corp., San Diego, Calif.). Spc^r transformants, candidates for *polA25*::miniTn*10spc* strains, were screened for Ap^s (plasmid free), and Spc^r was mapped at *polA* by cotransduction with linked Tn*10* markers.

Determination of sensitivities to DNA damage agents. The sensitivities to methyl methanesulfonate and UV light were determined by the methods of Karran et al. (14) and Lloyd and Barbour (21), respectively.

Determination of plating efficiencies at different temperatures. Cells were grown in LB medium to saturation. Total cell concentrations were determined with a particle counter (Particle Data Inc., Elmhurst, Ill.). Cells were then diluted with LB and plated on LB plates. The plates were incubated at 30, 37, 40, and 42°C for 36 h before surviving cells were counted. The surviving fraction (plating efficiency) was determined by dividing the number of survivors by the total number of cells plated.

RESULTS

Isolation of the *polA25*::miniTn*10spc* mutant allele. A previous study indicated that the requirement of cSDR (i.e., the *oriK* system) for RecA can be suppressed by inactivation of the LexA repressor by the *lexA71*(Def)::Tn*5* mutation (36). The result predicted that an *rnhA recA*(Ts) strain should be able to grow at high temperature in the absence of the *oriC* replication system if the LexA repressor is inactivated. We constructed such a strain [AQ4286 *rnhA102 recA200*(Ts) *lexA*(Def)::Tn*5* *dnaA*::Tn*10*] and found that it indeed exhibited the temperature-resistant (Tr) phenotype (Fig. 1). In this strain, because of *lexA*(Def), the *oriK* system was active at 42°C despite the *recA200*(Ts) mutation and replicated the genome in behalf of the *oriC* system, which had been inactivated by *dnaA*::Tn*10*. This strain was transduced to Spc^r with P1 phage that had been grown on cells mutagenized with miniTn*10spc* (see Materials and Methods). By screening a total of 7,375 Spc^r colonies, two temperature-sensitive mutants were found. One of them, AQ5225, was found to be very temperature sensitive (Fig. 1). The mutant was chosen as a candidate for the Rip mutation and characterized further. As will be described below, the

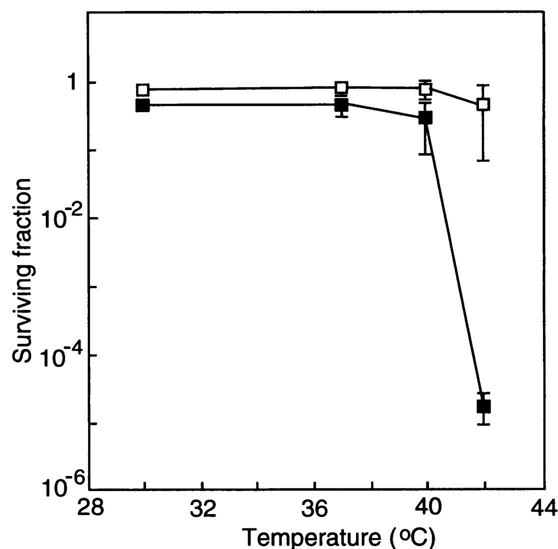

FIG. 1. Temperature sensitivity of AQ4286 and AQ5225. AQ4286 [*dnaA*::Tn*10* *rnhA* *recA200*(Ts) *lexA71*::Tn*5*] (□) and AQ5225 [*dnaA*::Tn*10* *rnhA* *recA200*(Ts) *lexA71*::Tn*5* *polA25*::miniTn*10spc*] (■) were grown in LB medium at 30°C to saturation. The surviving fraction at four different temperatures was determined as described in Materials and Methods. The data are averages for two independent determinations. The error bars indicate the standard errors of the means.

mutation is located within the *polA* gene; it is, therefore, designated *polA25*::miniTn*10spc*.

Figure 2 shows that cSDR in AQ5225 (*polA25*::mini Tn*10spc*) was seen only at 30°C and not at 42°C, whereas the parental strain AQ4286 (*polA*$^+$) exhibited cSDR at both temperatures. AQ5225 was at least 10^4-fold more sensitive to UV irradiation at 30°C than the parent (data not shown).

Cloning and mapping of *polA25*::miniTn*10spc*. The transposon miniTn*10spc* has no *Pst*I site (Fig. 3A). In order to clone a chromosomal DNA fragment containing the *polA25*::mini Tn*10spc* gene, chromosomal DNA was isolated from AQ5225, digested with *Pst*I, and ligated with *Pst*I-digested pUC8. The ligated DNA was used to transform JM109, with selection for Spc^r. One Spc^r transformant contained a plasmid carrying an 11-kb insert which could be cleaved out by *Pst*I digestion. This plasmid was designated pRRR25. A physical map was constructed for the fragment by digesting it with several restriction enzymes. The resulting restriction map was found to match the pattern of restriction sites in the region of the *E. coli* chromosome around 87 min (19). The location of the insert was confirmed by positive hybridization of the fragment to λ clones 546, 547, and 548 and negative hybridization to clones 544 and 545 of the Kohara library (data not shown). Further restriction analysis localized the insertion near the end of the *polA* gene (Fig. 3A).

Deoxyribonucleotide sequence of *polA25*::miniTn*10spc*. The cloned fragment had an *Sst*I site about 1 kb from the end of the *polA* gene and a *Bam*HI site about 70 bp into the transposon (Fig. 3A). The *Sst*I-*Bam*HI segment was cloned into M13 RF and sequenced from the *Bam*HI end. The sequence obtained revealed that the transposon was inserted between coordinates 2612 and 2613 of the *polA* coding sequence (Fig. 3B). The insertion occurred such that the six translational stop codons at the left end of IS*10*(Left) are in frame; therefore, the polypeptide is most likely to be truncated at the site of the insertion.

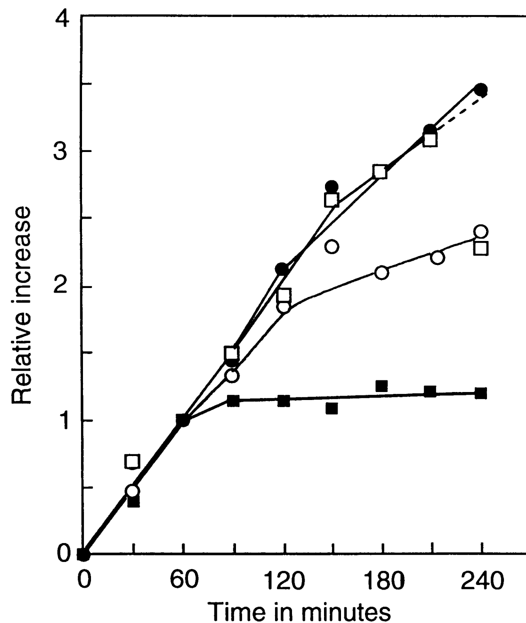

FIG. 2. DNA synthesis in the presence of chloramphenicol in AQ4286 and AQ5225. AQ4286 (circles) and AQ5225 (squares) were grown at 30°C in CAA medium to saturation, diluted 100-fold with fresh CAA medium, and grown to exponential phase. Cultures were split into two halves, and a mixture of [^{3}H]thymine and chloramphenicol was added to both. One half was incubated at 30°C (open symbols), and the other was incubated at 42°C (shaded symbols). DNA synthesis in the presence of chloramphenicol was determined as described previously (36).

Thus, the expected effect of the insertion on DNA PolI polypeptide is twofold: the change of Gln (CAA) to His (CAC) at the 871 residue and the shortening of the polypeptide by 57 amino acid residues at the C terminus.

The combination of *recA* and *polA25*::miniTn*10spc* is lethal. The combination of the *recA*(Def) and *polA* mutations is known to be lethal (8). It was possible, therefore, that the temperature-sensitive phenotype of AQ5225 was due to the combination of the *recA200*(Ts) and *polA25*::Tn*10spc* mutations that the strain carried. In order to characterize the *polA25*::miniTn*10spc* mutation, it was necessary to move the mutation to other strains. The inability of the strain to propagate P1 phage, due presumably to the presence of a P1-like plasmid in the strain (7a), prompted us to construct a *polA25*::miniTn*10spc* strain with a different genetic background by transformation (see Materials and Methods). The *polA25*::miniTn*10spc* mutation was subsequently transduced into other strains by linkage to a nearby Tn*10* marker (see Materials and Methods). The *polA25*::miniTn*10spc* mutation alone rendered cells as sensitive to methyl methanesulfonate and to UV irradiation as the *polA1* mutation did (Fig. 4). The result suggested that the *polA25*::miniTn*10spc* mutant is defective in excision repair. When the *polA25*::miniTn*10spc* mutant allele was combined with *recA*(Ts), the double mutant (AQ8620) was indeed as temperature sensitive as a *recA200* (Ts) *polA1* double mutant (AQ8555) (data not shown). Thus, the combination of *recA*(Def) and *polA25*::miniTn*10spc* is lethal.

***lexA*(Def) can suppress the lethality of the *recA*(Def) *polA25*::miniTn*10spc* combination.** Figure 5 shows that the *lexA*(Def) mutation, which derepresses the LexA regulon genes, permitted *recA200*(Ts) *polA25*::miniTn*10spc* mutants (AQ8877) to grow at 42°C. The *recA* gene is one of the LexA regulon genes that can be derepressed by the *lexA*(Def) mutation (39). Therefore, it was possible that the temperature-resistant growth of the triple mutant came about because of the overproduction of RecA200(Ts) protein, which might have a residual activity at 42°C. This possibility was ruled out because the *recA200*(Ts) allele could be replaced with the Δ*recA306* allele (AQ8886 in Fig. 5). Thus, derepression of the LexA regulon suppresses the lethality of the *recA*(Def) *polA25*::miniTn*10spc* combination.

***polA*$^+$ is essential for the Rip pathway.** When *polA25*::mini Tn*10spc* was introduced into AQ3476 [*dnaA*$^+$ *rnhA recA*(Ts) *lexA*(Def)], the resulting strain (AQ8619) was not temperature sensitive, despite the *recA*(Ts) *polA25*::miniTn*10spc* combination (Fig. 5). This indicated that in a strain in which the *oriC* system is in operation (as in *dnaA*$^+$ strains), the conditional lethality of *recA*(Ts) *polA25*::miniTn*10spc* can be suppressed by *lexA*(Def), as demonstrated above. In order to determine whether the *oriK* system was operating under this condition, a *dnaA*(Ts) mutation which inactivates the *oriC* system at 42°C was introduced. The resulting strain (AQ8747) was temperature sensitive (Fig. 5), indicating that neither the *oriC* nor the *oriK* system was active at the restrictive temperature. The temperature sensitivity was due to the inactivation of RecA(Ts) [and also DnaA(Ts)] at 42°C because replacement of *recA*(Ts) with a *recA*$^+$ allele restored temperature-resistant growth (AQ8876 in Fig. 5). The effect of RecA(Ts) inactivation was not manifested when *polA* was a wild type (AQ8825). These results indicate that the *polA25*::miniTn*10spc* mutation abolishes the ability of *lexA*(Def) to suppress RecA dependence of the *oriK* system. We conclude that *polA*$^+$ is required for the Rip pathway to operate in the absence of active RecA.

Effects of Δ*polB*::*spc* and *recF*::Tn*3* mutations. The Δ*polB*::*spc* and *recF322*::Tn*3* mutations were tested for their possible effects on the activation of Rip by *lexA*(Def) (see Discussion for the rationale). Thus, the mutations were introduced into AQ8937 (Table 1), in which the *recA*(Ts) defect was suppressed by *lexA*(Def), and the derivatives were tested for temperature sensitivity. The Δ*polB*::*spc* or *recF322*::Tn*3* derivative of AQ8937 could grow at the restrictive temperature as well as the parental strain (data not shown). Thus, neither *polB*$^+$ nor *recF*$^+$ is essential for the Rip bypass pathway.

DISCUSSION

One of the unique features of the *oriK* system (cSDR) is the requirement for RecA protein. However, the role that RecA protein plays in the replication is not clear. An earlier flow cytometry study suggested that RecA acts at the initiation step of cSDR (17). In order to shed further light on the RecA requirement, we sought suppressor mutations that suppress the temperature sensitivity of *rnhA dnaA*::Tn*10 recA200*(Ts) triple mutants. One such suppressor has turned out to be *lexA*(Def) (36). It has been hypothesized that the derepression of a gene(s) in the LexA regulon activates a pathway (Rip) that permits initiation at *oriK* sites to occur in the absence of active RecA. Thus, *dnaA*::Tn*10 rnhA recA*(Ts) *lexA*(Def) mutants are temperature resistant despite the RecA(Ts) protein and despite the inactivation of the *oriC* system by the *dnaA* mutation. In this study, we have isolated a *polA* (*polA25*::miniTn*10spc*) mutant allele which renders the quadruple-mutant parent strain temperature sensitive. The *polA* mutation specifically inactivates the *oriK* system (i.e., cSDR) by blocking the Rip pathway, because the temperature-sensitive growth is seen

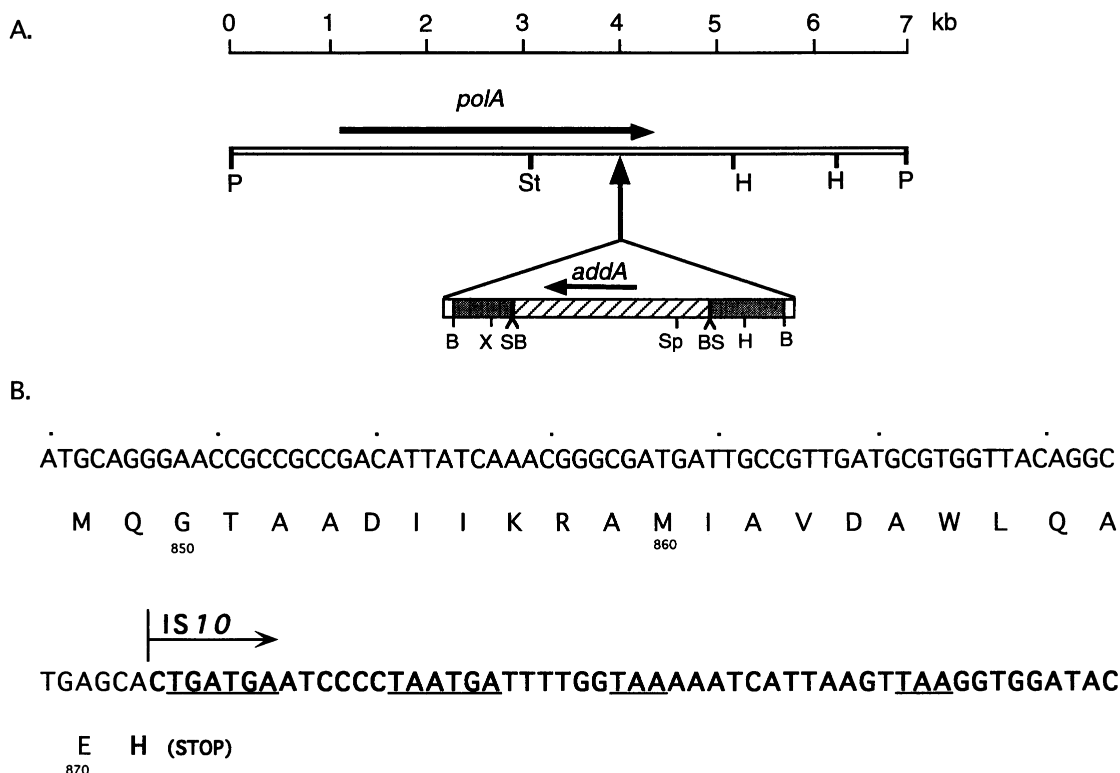

FIG. 3. Restriction map and partial nucleotide sequence of the *polA25*::miniTn*10spc* mutant allele. (A) The horizontal arrows represent the transcription directions and coding regions of the *polA* and *addA* genes, and the vertical arrow indicates the insertion position of transposon miniTn*10spc* in *polA*. The open, striped, and stippled segments in the transposon indicate parts of IS*10*, the omega fragment, and parts of Tn*903*, respectively. P, *Pst*I; H, *Hin*dIII; B, *Bam*HI; S, *Sma*I; X, *Xho*I; St, *Sst*I; Sp, *Sph*I. (B) DNA sequence (upper line) and corresponding protein sequence (lower line). The first A in the DNA sequence corresponds to coordinate +2542 of the *polA* sequence (12). The numbers in the protein sequence indicate the positions of the amino acid residues. The boldface letters represent parts of the IS*10*(Left), and the underlined letters represent in-frame stop codons.

only when the *oriC* system is absent (AQ8747 in Fig. 5). DNA PolI is therefore an essential enzyme for the Rip pathway.

It is interesting to note that a *polA* mutant that has a miniTn*10* insertion at a site identical to that of *polA25*::mini Tn*10spc* has recently been isolated (30). The mutant (*polA2099*) was isolated by screening for a completely different

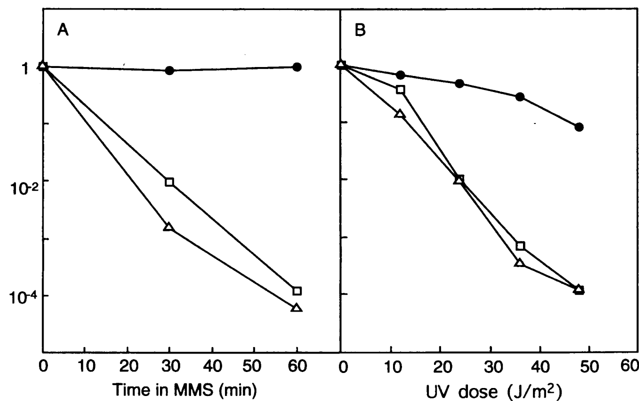

FIG. 4. Sensitivities of *polA* mutants to DNA-damaging agents. The sensitivity to methyl methanesulfonate (MMS) (0.025%) (A) and to UV irradiation (B) was determined for AQ634 (*polA*$^+$) (●), AQ8809 (*polA1*) (□), and AQ8560 (*polA25*::miniTn*10spc*) (▲).

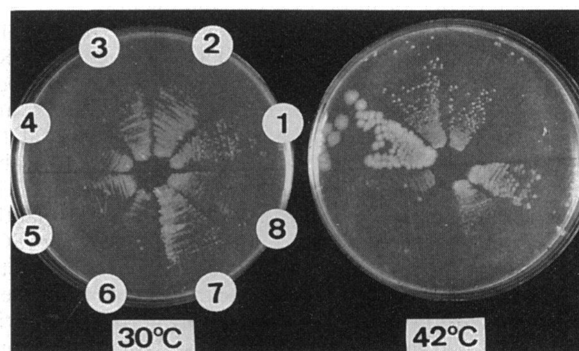

FIG. 5. Growth properties of *polA25*::miniTn*10spc* mutants at 30 and 42°C. Single colonies of *polA25*::miniTn*10spc* mutant strains were spread on LB plates with toothpicks, and the plates were incubated at 30 and 42°C for 30 h. The strains tested and their relevant genotypes are as follows. Sector 1, AQ8885 (*dnaA*$^+$ *rnhA*$^+$ *recA200 polA25 lexA*$^+$); sector 2, AQ8877 (*dnaA*$^+$ *rnhA*$^+$ *recA200 polA25 lexA71*::Tn*5*); sector 3, AQ8886 (*dnaA*$^+$ *rnhA*$^+$ Δ*recA polA25 lexA71*::Tn*5*); sector 4, AQ3476 (*dnaA*$^+$ *rnhA102 recA200 polA*$^+$ *lexA71*::Tn*5*); sector 5, AQ8619 (*dnaA*$^+$ *rnhA102*/*rnhS102 recA200 polA25 lexA71*::Tn*5*); sector 6, AQ8747 (*dnaA5 recA200 polA25 lexA71*::Tn*5*); sector 7, AQ8876 (*dnaA5 rnhA102 recA*$^+$ *polA25 lexA71*::Tn*5*); sector 8, AQ8825 (*dnaA5 rnhA102 recA200 polA*$^+$ *lexA71*::Tn*5*).

phenotype: altered patterns of *lacZ* fusion expression of a Mu d(*lac*) lysogen in colonies. The reason for its negative effects on Mu phage replication-dependent expression of the *lacZ* fusion during colony development is not clear. This particular insertion site sequence exactly matches the 9-bp consensus sequence (NGCTNAGCN) for Tn*10* insertion (9). Thus, this site is likely to be a hot spot for Tn*10* insertion.

Since *polA* is not a LexA regulon gene (40), it is predicted that the activation of the Rip pathway requires at least one other gene, the expression of which is under the control of LexA. Several genes encoding DNA replication and repair proteins are known to be regulated by the LexA repressor. One of these genes is *polB*, encoding DNA PolII (3, 11). Our result clearly rules out *polB* as the *rip* gene since the Δ*polB*::*spc* mutation had no effect on Rip activation. *recN*, *recQ*, and *ruvAB* genes are also under the control of LexA (10, 23, 33). The products of these genes are known to function in the RecF pathway of homologous recombination and recombination repair (22). Since the *recF*::Tn*3* mutation has no discernible effect on Rip activity, the products of *recN*, *recQ*, and *ruvAB* may have no role in this process if they require an active RecF protein for function. Several more SOS genes (summarized in reference 43) and DNA replication genes such as *dnaN* and *dnaQ* (13, 28) are also regulated by LexA; these genes remain to be tested.

It has long been known that *recA polA1* double mutants are inviable (8). On the basis of their experiments with a *recA718 polA12*(Ts) mutant which is temperature sensitive for growth on rich medium, Witkin and Roegner-Maniscalco (44) have speculated that a possible role of RecA protein is to perform recombinational repair of persistent gaps on the lagging strand that accumulate because of the Pol I defect. The rate of joining of nascent DNA fragments is considerably slowed in this mutant (34). We have found that the *polA25*::miniTn*10* mutant allele, which is most likely to encode a truncated DNA PolI, renders the mutant temperature sensitive when combined with *recA200*(Ts). This temperature sensitivity, however, can be suppressed by the *lexA*(Def) mutation when cells are replicating the chromosome by the *oriC* system (Fig. 5). This indicates that the defect in normal DNA replication due to the loss of the RecA and DNA PolI activities can be compensated by derepression of one or more LexA regulon genes.

We have not been able to determine whether *lexA*(Def) also suppresses the lethality of the *recA*(Def) *polA1* combination because we have been unable to construct *polA1 lexA*(Def) double mutants, and thus the combination of *polA1* and *lexA*(Def)::Tn*5* appears to be lethal (4a). Fijalkowska et al. (7) also reported that certain *polA* mutants are rendered nonviable under the conditions of constitutive SOS expression. The reason for this incompatibility is not known. It is likely that the 3′→5′ exonuclease activity becomes pivotal when the SOS response is constitutively turned on, because *polA1* inactivates both polymerization and 3′→5′ exonuclease activities whereas *polA25*::miniTn*10spc* appears to impair polymerization activity only (4). *recB* and *uvrB* mutations are also lethal when combined with a defective *polA* gene (24, 25). Whether these lethal combinations are also suppressed by the *lexA*(Def) mutation remains to be seen.

The suppression of *recA*(Ts) *polA25*::miniTn*10* mutants by *lexA*(Def) is seen only when the *oriC* system of initiation is active. When chromosome replication is originated solely from *oriK* sites as in *dnaA*(Ts) *rnhA recA*(Ts) *polA25*::miniTn*10* at 42°C, the same mutation fails to suppress the temperature sensitivity. It is likely that the *oriK* replication system has a specific requirement for DNA PolI at the initiation step in the absence of RecA, which cannot be compensated by derepression by *lexA*(Def). This is a requirement that is additional to that in the elongation stage, which can be suppressed by *lexA*(Def). The accompanying paper (4) describes experiments to determine which of the three activities of PolI is required at initiation in the absence of RecA and discusses possible roles for DNA PolI in the Rip pathway.

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REFERENCES

1. **Biek, D. P., and S. N. Cohen.** 1986. Identification and characterization of *recD*, a gene affecting plasmid maintenance and recombination in *Escherichia coli*. J. Bacteriol. **167:**594–603.
2. **Bochner, B., H.-C. Huang, G. Schieven, and B. N. Ames.** 1980. Positive selections for loss of tetracycline resistance. J. Bacteriol. **143:**926–933.
3. **Bonner, C. A., S. Hays, K. McEntee, and M. F. Goodman.** 1990. DNA polymerase II is encoded by the DNA damage-inducible *dinA* gene of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **87:**7663–7667.
4. **Cao, Y., and T. Kogoma.** 1993. Requirement for the polymerization and 5′→3′ exonuclease activities of DNA polymerase I in initiation of DNA replication at *oriK* sites in the absence of RecA in *Escherichia coli rnhA* mutants. J. Bacteriol. **175:**7254–7259.

4a. **Cao, Y., and T. Kogoma.** Unpublished data.

5. **Casadaban, M. J., and S. N. Cohen.** 1980. Analysis of gene control signals by DNA fusion and cloning in *Escherichia coli*. J. Mol. Biol. **138:**179–207.
6. **de Massy, B., O. Fayet, and T. Kogoma.** 1984. Multiple origin usage for DNA replication in *sdrA* (*rnh*) mutants of *Escherichia coli* K12: initiation in the absence of *oriC*. J. Mol. Biol. **178:**227–236.
7. **Fijalkowska, I., P. Jonczyk, and Z. Ciesla.** 1989. Conditional lethality of the *recA441* and *recA730* mutants of *Escherichia coli* deficient in DNA polymerase I. Mutat. Res. **217:**117–122.

7a. **Fuge, E., and T. Kogoma.** Unpublished data.

8. **Gross, J. D., F. Grunstein, and E. M. Witkin.** 1971. Inviability of *recA* derivatives of the DNA polymerase mutant of De Luria and Cairns. J. Mol. Biol. **58:**631–634.
9. **Halling, S. M., and N. Kleckner.** 1982. A symmetrical six-base-pair target site sequence determines Tn10 insertion specificity. Cell **28:**155–163.
10. **Irino, N., K. Nakayama, and H. Nakayama.** 1986. The *recQ* gene of *Escherichia coli* K12: primary structure and evidence of SOS regulation. Mol. Gen. Genet. **205:**298–304.
11. **Iwasaki, H., A. Nakata, G. C. Walker, and H. Shinagawa.** 1990. The *Escherichia coli polB* gene, which encodes DNA polymerase II, is regulated by the SOS system. J. Bacteriol. **172:**6268–6273.
12. **Joyce, C. M., W. S. Kelley, and N. D. F. Grindley.** 1982. Nucleotide sequence of the *Escherichia coli polA* gene and primary structure of DNA polymerase I. J. Biol. Chem. **257:**1958–1964.
13. **Kaasch, M., J. Kaasch, and A. Quinones.** 1990. Expression of the *dnaN* and *dnaQ* genes of *Escherichia coli* is inducible by mitomycin C. Mol. Gen. Genet. **219:**187–192.
14. **Karran, P., T. Lindahl, I. Ofsteng, G. B. Evensen, and E. Seeberg.** 1980. *Escherichia coli* mutants deficient in 3-methyladenine-DNA glycosylase. J. Mol. Biol. **140:**101–127.
15. **Kelley, W. S.** 1980. Mapping of the *polA* locus of *Escherichia coli* K-12: genetic fine structure of the cistron. Genetics **95:**15–38.
16. **Kogoma, T.** 1984. Absence of RNase H allows replication of pBR322 in *Escherichia coli* mutants lacking DNA polymerase I. Proc. Natl. Acad. Sci. USA **81:**7845–7849.
17. **Kogoma, T., K. Skarstad, E. Boye, K. von Meyenburg, and H. B. Steen.** 1985. RecA protein acts at the initiation of stable DNA replication in *rnh* mutants of *Escherichia coli* K-12. J. Bacteriol. **163:**439–444.

18. **Kogoma, T., and K. von Meyenburg.** 1983. The origin of replication, *oriC*, and the *dnaA* protein are dispensable in stable DNA replication (*sdrA*) mutants of *Escherichia coli* K12. EMBO J. **2:**463–468.
19. **Kohara, Y., K. Akiyama, and K. Isono.** 1987. The physical map of the whole *E. coli* chromosome: application of a new strategy for rapid analysis and sorting of a large genomic library. Cell **50:**495–508.
20. **Lennox, E. S.** 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology **1:**190–206.
21. **Lloyd, R. G., and S. D. Barbour.** 1974. The genetic location of the *sbcA* gene of *Escherichia coli*. Mol. Gen. Genet. **134:**157–171.
22. **Lloyd, R. G., and C. Buckman.** 1991. Genetic analysis of the *recG* locus of *Escherichia coli* K-12 and of its role in recombination and DNA repair. J. Bacteriol. **173:**1004–1011.
23. **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.
24. **Monk, M., and J. Kinross.** 1972. Conditional lethality of *recA* and *recB* derivatives of a strain of *Escherichia coli* with temperature-sensitive DNA polymerase I. J. Bacteriol. **109:**971–978.
25. **Morimyo, M., and Y. Shimazu.** 1976. Evidence that the gene uvrB is indispensable for a polymerase I deficient strain of *Escherichia coli* K-12. Mol. Gen. Genet. **147:**243–250.
26. **Ogawa, T., G. G. Pickett, T. Kogoma, and A. Kornberg.** 1984. RNase H confers specificity in the *dnaA*-dependent initiation of replication at the unique origin of the *Escherichia coli* chromosome in vivo and in vitro. Proc. Natl. Acad. Sci. USA **81:**1040–1044.
27. **Prentki, P., A. Binda, and A. Epstein.** 1991. Plasmid vectors for selecting IS1-promoted deletions in cloned DNA: sequence analysis of the *omega* interposon. Gene **103:**17–23.
28. **Quinones, A., J. Kaasch, M. Kaasch, and W. Messer.** 1989. Induction of *dnaN* and *dnaQ* gene expression in *Escherichia coli* by alkylation damage to DNA. EMBO J. **8:**587–593.
29. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
30. **Shapiro, J. A.** 1992. Differential action and differential expression of DNA polymerase I during *Escherichia coli* colony development. J. Bacteriol. **174:**7262–7272.
31. **Singer, M., T. A. Baker, G. Schnitzler, S. M. Deischel, M. Goel, W. Dove, K. J. Jaacks, A. D. Grossman, J. W. Erickson, and C. A. Gross.** 1989. A collection of strains containing genetically linked alternating antibiotic resistance elements for genetic mapping of *Escherichia coli*. Microbiol. Rev. **53:**1–24.
32. **Subia, N. L., and T. Kogoma.** 1986. Concatemer formation of ColE1-type plasmids in mutants of *Escherichia coli* lacking RNase H activity. J. Mol. Biol. **189:**389–399.
33. **Survinton, C. E., and R. G. Lloyd.** 1982. Damage to DNA induces expression of the *ruv* gene of *Escherichia coli*. Mol. Gen. Genet. **185:**352–355.
34. **Sweasy, J. B., and L. A. Loeb.** 1992. Mammalian DNA polymerase β can substitute for DNA polymerase I during DNA replication in *Escherichia coli*. J. Biol. Chem. **267:**1407–1410.
35. **Torrey, T. A., and T. Kogoma.** 1982. Suppressor mutations (*rin*) that specifically suppress the $recA^+$-dependence of stable DNA replication in *Escherichia coli* K12. Mol. Gen. Genet. **187:**225–230.
36. **Torrey, T. A., and T. Kogoma.** 1987. Genetic analysis of constitutive stable DNA replication in *rnh* mutants of *Escherichia coli* K12. Mol. Gen. Genet. **208:**420–427.
37. **von Meyenburg, K., E. Boye, K. Skarstad, L. Koppes, and T. Kogoma.** 1987. Mode of initiation of constitutive stable DNA replication in RNase H-defective mutants of *Escherichia coli* K-12. J. Bacteriol. **169:**2650–2658.
38. **von Meyenburg, K., and F. G. Hansen.** 1980. The origin of replication, *oriC*, of the chromosome: genes near *oriC* and construction of *oriC* deletion mutations. ICN-UCLA Symp. Mol. Cell Biol. **19:**137–159.
39. **Walker, G. C.** 1984. Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. Microbiol. Rev. **48:**60–93.
40. **Ward, D. F., and N. E. Murray.** 1980. Construction and characterization of *Escherichia coli polA-lacZ* gene fusions. J. Bacteriol. **142:**962–972.
41. **Way, J. C., M. A. Davis, D. Morisato, D. E. Roberts, and N. Kleckner.** 1984. New Tn10 derivatives for transposon mutagenesis and for construction of *lacZ* operon fusions by transposition. Gene **32:**369–379.
42. **Wilson, G. G., K. K. Y. Young, G. J. Edlin, and W. Konigsberg.** 1979. High frequency generalized transduction by bacteriophage T4. Nature (London) **280:**80–82.
43. **Witkin, E. M.** 1991. RecA protein in the SOS response: milestones and mysteries. Biochimie **73:**133–141.
44. **Witkin, E. M., and V. Roegner-Maniscalco.** 1992. Overproduction of DnaE protein (α subunit of DNA polymerase III) restores viability in a conditionally inviable *Escherichia coli* strain deficient in DNA polymerase I. J. Bacteriol. **174:**4166–4168.
45. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33:**103–119.